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Monday, August 25th

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MOS01 - Fourier-Transform MS

Room 1
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Chairs: Yury Tsybin, Julia Chamot-Rooke

MOS01-01 **Keynote: 40 Years of Fourier transform mass spectrometry: progress and prospects**

Alan Marshall[1], Steven Beu[2], Greg Blakney[3], Tong Chen[3], Yu Chen[3], Christopher Hendrickson[3], Nathan Kaiser[3], Daniel McIntosh[3], John Quinn[3], Ryan Rodgers[3], Chad Weisbrod[3]
[1]Florida State University, [2]S. C. Beu Consulting, [3]National High Magnetic Field Laboratory

Introduction:
Fourier transform ion cyclotron resonance mass spectrometry offers the highest mass resolution and mass accuracy for compositional analysis of complex organic mixtures ranging from petroleum to proteins.

Methods:
This presentation will describe several recent advances that extend FT-ICR figures of merit by an order of magnitude: phase correction; three-term segmented mass calibration; distributed post-excitation ICR radius; ion funnels; programmed ion injection; dynamically harmonized ICR cell with 120 degree excitation and detection and segmented end caps; conditional signal averaging; and mass spectral segment extraction.

Results:
All of these techniques have been implemented into a test bed 14.5 T spectrometer, and are being duplicated for installation in a 21 T magnet in Summer, 2014. Representative applications will be presented. Work supported by NSF DMR-11-57490, NSF CHE-10-19193, Florida State University, BP/The Gulf of Mexico Research Initiative to the Deep-C Consortium, and the National High Magnetic Field Laboratory in Tallahassee, FL.

Novel Aspect
Highest magnetic field FT-ICR mass spectrometer

MOS01-02 **Novel mass analyzers for rapid high-performance FTMS**

Yury O. Tsybin, Konstantin O. Nagornov, Konstantin O. Zhurov, Anton N. Kozhinov
Ecole Polytechnique Federale de Lausanne

Increasing the speed of high-resolution FTMS is of a primary importance for many applications. Instrumentation-wise, the current approach to this problem is increasing magnetic and electric fields in ICR and Orbitrap FTMS, respectively. Recording frequency multiples is an alternative route which has recently been undertaken by us with quadruple frequency multiple ICR cell implementation. However, more disruptive technological innovations are needed to address this challenge. Here, we present the rationale, design, implementation, analytical characteristics, and application advantages of novel ICR cells with narrow aperture detection electrodes (NADEL) and allied signal processing maximizing these advantages.

NADEL ICR cells with varied excitation and detection electrode dimensions were manufactured and implemented in a commercial 10 T LTQ FT-ICR MS (Thermo Scientific). Both dipolar and quadrupolar ion excitation and detection schemes were realized. Original (Thermo Scientific) and a customized high-performance data acquisition systems were employed for transient acquisition. FT and non-FT signal processing of transients was performed with a custom-written Python framework.

The following NADEL ICR cell operational regimes were determined and characterized: (i) conventional, with an average radius of ion excitation (standard spectral bandwidth); (ii) enhanced, with ion excitation closer to the narrow aperture detection electrodes (increased spectral bandwidth). Conventional regime with reduced cyclotron frequency detection (standard FTMS regime) was found to provide comparable (mass accuracy, dynamic range) or improved (resolution, sensitivity) analytical characteristics in regard to Ultra ICR cell. Serendipitously, we have discovered a stable and reproducible conventional regime with unperturbed cyclotron frequency detection. The obtained analytical benefits include improved mass accuracy and sensitivity. Enhanced regime differs significantly from the conventional one by increased spatial resolution, which translates into increased frequency resolution when acquiring transients with increased spectral bandwidth. Preliminary application of advanced signal processing approaches to analysis of such transients shows about 10-fold gain in resolution.

In addition to providing high performance conventional FTMS, NADEL ICR cell-based FTMS aims to accelerate the FTMS by shifting it from narrowband transients to those with increased spectral bandwidth. The ultimate objective is to generate transients with sha-function-like components to maximize the yield from accelerating the high-performance FTMS. Finally, the success of NADEL ICR cell implementation in the dipolar excitation/detection scheme opens an attractive avenue for implementation of quadrupolar ion excitation/detection and beyond.

Novel aspect
Ion traps with narrow aperture detection electrodes accompanied by advanced signal processing demonstrate improved FT-ICR MS analytical performance, including throughput

MOS01-03 2D FT-ICR MS using non-uniform sampling (NUS) and advanced data processing. Application to human plasma triglyacylglycerols (TAG) analyzed by nano ESI/IRMPD

Christian Rolando[1], Fabrice Bray[1], Lionel Chiron[2], Marc-André Delsuc[2]
[1]Université Lille 1, [2]Université de Strasbourg

2D FT-ICR MS has received little attention relative to 2D NMR since its introduction in 1987. The main drawbacks of the original version of the 2D FT -ICR MS were: a loss of resolution caused by in ICR-cell CID, difficulty of processing data at full resolution FT -ICR MS and the intense noise due to ion fluctuation. In recent years, we have revisited 2D FT-ICR and presented solutions to these problems using methods of fragmentation without gas such as IRMPD and ECD [1], developing software that can handle several gigabytes files and introducing an innovative algorithm based on mathematical theory sparcity for noise reduction [2]. Acquisition takes about an hour in the order of a LC run. Here we present a procedure for speeding the acquisition.
We recorded the FT ICR mass spectra with optimized 2D pulse sequence we previously designed. In a first time non-uniform spectra of the sample were obtained by extraction of random values from the spectra uniformly sampled. Reconstruction by a new approach has been done with the NPK package which was adapted to the size of the FT- ICR data by implementing a 64-bit version.
So far, we acquired 2D spectra with quadrupole resolution in the first dimension and resolution FT -ICR in the second dimension. In the second dimension, the full resolution costs only a little additional acquisition time due to longer FID. In the first dimension ions can be used only once because they are irreversible fragmented to the opposite of NMR in which the nuclei can be observed again. The first resolution size is very time consuming as the quadrupole resolution requires the recording of two thousand spectra leading to an acquisition period of two hours. 2D and nD NMR used regularly non-uniform sampling and Maximum Entropy reconstruction in particular for the analysis of proteins. The theoretical gain of non-uniform sampling is 2 to the power dimension minus one. This gain can be used to reduce the acquisition time or to increase the resolution at constant acquisition time. We will present here the gain in resolution obtain with different sampling strategies (randomly, linearly or exponentially spaced) with an innovative algorithm based also on the mathematical theory of sparcity and on compressed sensing developed for large FT-ICR data.
The power of this improved design of 2D FT-ICR MS experiments is illustrated by the analysis of triacylglycerol neutral lipids (TAG) from human plasma. From the 2D spectrum, TAGs with a total carbon of 54 to 64 and insaturation numbers from 14 to 4 may be identified and their individual fatty acid composition determined. We also found oxidized TAGs that are difficult to elute by chromatography. Families of TAGS are easily distinguished on the 2D mass spectra thanks to neutral loss lines. In one 2D mass spectrum obtained from 25 µL of human plasma over 50 TAGs were identified.
[1] van Agthoven et al. Two-Dimensional ECD FT-ICR Mass Spectrometry of Peptides and Glycopeptides. Anal. Chem. 2012, 5589.
[2] Chiron et al. Efficient denoising algorithms for large experimental datasets and their applications in Fourier transform ion cyclotron resonance mass spectrometry. PNAS USA 2014, 1385.

MOS01-04 New developments in speeding up orbitrap mass spectrometry

Alexander Makarov, Jan-Peter Hauschild, Eduard Denisov, Amelia Peterson, Oliver Lange, Eugen Damoc, Mathias Mueller, Erik Couzijn, Konstantin Ayzikov, Andreas Wieghaus, Markus Kellmann
ThermoFisher Scientific (Bremen) GmbH

Novel Aspect:
Significant increase of analysis speed is enabled by combination of compact high-field Orbitrap analyzer with novel instrument control and signal processing

Introduction
Ever increasing speed of liquid separations enables increased throughput of LC/MS analysis that however could be realized only if spectral acquisition rate of a mass analyzer matches well with chromatographic peak width. Quality of spectra acquired could be preserved only if increase of the rate is accompanied by a matching boost of analyzed ion currents, especially for narrow mass windows typical for MS/MS analysis. This talk opens new territory for acquisition speed in Orbitrap mass spectrometry by combining novel ion-optical devices with appropriate pipelining of their operation and signal processing.

Methods
Thermo Scientific Q ExactiveTM Plus instrument was modified to include a compact high-field Orbitrap for higher scan speeds. Ion transmission during narrow-band precursor isolation was improved by using a segmented hyperbolic quadrupole mass filter. Instrument control software was developed to ensure minimum scan overheads and possibility to work with ultra-short scan durations. Samples containing standard calibration mixtures, small- to medium-size proteins and HeLa protein digests were used for performance characterization of the instrument in infusion and nanoLC/MS mode.

Results and conclusions
Implemented improvements enabled an increase of acquisition rate in excess of 20 spectra/second with a transient duration of 32 ms. Reduction of fill times allowed for ion storage was compensated by an increase of ion transmission at low isolation widths. Meanwhile, optimal shape of transmission windows improves quantitative accuracy and supports segmented scans and sophisticated methods of data-independent acquisition. Improvements of identification rate were demonstrated for HeLa samples.
Even further increase of acquisition speed is possible using ultra-short transients (e.g. 8 and 16 ms). Somewhat counter-intuitively, it is the analysis of mid-size proteins (e.g. antibodies) that show improved signal-to-noise ratio at such rates.

This effect is explained on the basis of peculiarities of protein detection in the Orbitrap analyzer.
Discussion of future improvements relates to resolving power for shorter transients that are known to drop in FTMS proportionally to the transient duration. It is shown that alternative signal processing methods (e.g. filter diagonalization method) promise to alleviate this reduction but in reality are able to deliver significant advantage over the existing eFT (enhanced Fourier Transform) signal processing only at very high signal-to-noise ratios of ion peaks (in the range of 100s-1000s).

MOS01-05 Non orbital electrostatic traps and MR-TOF

Anatoly Verenchikov[1], Viatcheslav Artaev[2], Mikhail Yavor[1], Vasily Makarov[1]
[1]Mass Spectrometry Consulting, MSC-CG, [2]LECO Corp

Introduction
FT traps - ICR and orbital traps - deliver high resolution and continue improving mass accuracy. However, at 1E+5 to 1E+6 charge limit, and acquisition time in a seconds scale do limit their charge throughput. Lorentzian peak shape in FT traps does limit isotopic abundance sensitivity, requiring yet higher resolution. TOF MS is a fast analysis method, but limited to lower than FT resolution. The presentation describes novel method - a hybrid between FT and TOF, aiming for higher throughput and higher selectivity of MS analysis.

Methods
To extend the charge throughput and the data acquisition speed, a range of novel electrostatic traps was invented, based on extended two-dimensional multi-reflecting electrostatic analyzer, and on the hybrid FT-TOF acquisition methods. In-house ion optical simulation programs were used for analysis and optimization of the space charge throughput, for optimizing the analyzer aberrations, and for developing ion injection schemes. Encoding and decoding signal processing methods were developed and simulated based on experimental and theoretically generated spectra. Experiments were carried out in multi-reflecting TOF analyzer with encoded frequent orthogonal pulsing and with axial trap pulsed converter.

Results
Ion optical scheme of electrostatic multi-reflecting analyzers provide aberration limited resolving power over 1 million for most of the pulsed ion sources and converters. Estimated charge throughput as 1E+4 ions/s for coaxial E-trap, 1E+5 ions/s for ICR, and 1E+6 ions/s for Orbitrap vs 1E+10 ions/s flux from ion sources. Proposed cylindrical E-trap is expected to provide 1E+8 ions/s throughput, and proposed multiplexed planar E-traps are expected to provide 1E+9 ions/s. Using short image current detectors or sampling ions onto TOF detector, it is expected to accelerate acquisition to 10-20 ms at 100,000 resolution. The hybrid FT-TOF methods, like open trap (TOF with variable number of reflecting cycles), or MR-TOF with frequent encoded pulsing are capable of fast (1ns) signal detection at 20-30% duty cycle. However, those methods are limited to relatively sparse spectra, e.g. generated various tandems. Best combination of resolution, speed and dynamic range is expected in MRTOF analyzer with radial RF trap converter and recently improved TOF detector. Experiments prove combination of 1-2 ms acquisition speed, high (30-50%) duty cycle, and R=100K-200K resolution in two types of instrument: one with orthogonal and frequent encoded pulsing and second with axial trap converter.

Conclusions
Hybrid FT-TOF systems are projected to improve speed, sensitivity and dynamic range at high resolution compared to both FT traps and MR-TOF MS.

Novel Aspect
range of electrostatic traps and ion injection methods for improved charge throughput and speed, hybrid FT-TOF methods of data acquisition and analysis, multiplexing methods.

MOS02 - Synthetic Macromolecules

Room 2
Level 0

Chairs: Anna Crecelius, Ulrich Schubert, Gérard Hopfgartner

MOS02-01 Keynote: MALDI-TOF mass spectrometry as a powerful tool for the structure elucidation of complex ploymers

Harald Pasch[1], Nadine Pretorius[1], Helen Pfukwa[1], Karsten Rode[2]
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MALDI-TOF MS is an important tool for the analysis of synthetic polymers and for the elucidation of their molecular structure. Using this method, information on molecular masses and oligomer distributions can be obtained. Knowing the total mass and the mass of the repeating units, masses of endgroups can be calculated. Copolymer compositions can be analyzed and the method can be used for the characterization of polymeric additives [1].
The power and depth of information of MALDI-TOF MS can be enhanced further when the method is combined with liquid chromatographic (LC) pre-fractionations. LC can be optimized to be selective regarding molar mass (SEC), chemical composition (e.g. gradient HPLC) or functional groups (e.g. LCCC). Since complex polymers are distributed in more than one direction of molecular heterogeneity, a pre-fractionation can be conducted with regard to molar mass by SEC the resulting fractions being analyzed with regard to chemical composition or functionality by mass spectrometry. Alternatively, a chemical composition fractionation can be conducted by HPLC the fractions then being analyzed regarding oligomer distributions by mass spectrometry. In the present paper, the principal experimental approaches for the combination of LC and MALDI-TOF MS will be discussed. Taking the analysis of complex polyesters and polymer-clay nanocomposites as examples it will be shown that the combination of LC and MS enhances the depth of structural information significantly. As a second topic, the combination of MALDI-TOF MS with collision induced dissociation (CID) experiments will be addressed. The collision of macromolecules with a collision gas causes the degradation of the parent ion and the formation of some characteristic product ions that are unique and include particular information about

the structure of the parent ion. In the present paper, a number of applications of MALDI-CID-TOF will be discussed including the analysis complex alkylene oxide polymers, polyisocyanates and higher molar mass tannins. It will be shown that MALDI-CID-TOF studies can provide a more detailed insight into the topology of endgroups, the sequence distribution of copolymers and the architecture of polyflavonoids as compared to conventional MALDI-TOF studies.

MOS02-02 CID versus activated EPD for the characterization of PAMAM dendrimers

Aura Tintaru[1], Marion Girod[2], Rodolphe Antoine[2], Jérôme Lemoine[2], Philippe Dugourd[2], Laurence Charles[1]
[1]Aix-Marseille University, [2]University of Lyon

Introduction

Due to the high symmetry of dendritic structures, presence of defective by-products in a dendrimer sample is very difficult to assess by NMR. In contrast, MS is a perfect tool to address this issue since individual species can be readily distinguished. Structural imperfections can be further investigated in tandem mass spectrometry. Typically, dendritic impurities can be structurally characterized by monitoring, in their MS/MS spectrum, any deviation to the dissociation behavior of the perfect dendrimer used as a reference. However, depending of the activation process implemented, dissociation reactions might not always be usefully informative. Here, dissociation reactions induced by collisional activation and by activated electron photo-detachment were compared for a poly(amido)amine (PAMAM) dendrimer bearing hydroxyl terminations.

Methods

Collision-induced dissociation (CID) experiments were performed on deprotonated PAMAM molecules produced in the gas phase upon electrospray and using a triple quadrupole mass spectrometer. In activated electron photo-detachment (activated-EPD) experiments, doubly charged molecules were first produced in negative-mode electrospray ionization, then oxidized into radical anions upon electron photo-detachment using a 260 nm laser wavelength (VIS/UV tunable laser OPO), and further activated by collision in a quadrupole ion trap.

Results

CID data obtained for [M-H]- showed successive losses of 115 Da and 114 Da neutrals. These main reactions proceed from the deprotonated branch of the dendrimer and would first consist of 1,4-proton transfer from the CH2 situated in the a- position of the amide nitrogen atom to thenegatively charged oxygen atom, releasing the first 115Da neutral. In the second step, another 1,5-proton transfer proceeds with the elimination of the second 115Da neutral, yielding to a NH2 terminated branch, able to release thus a charge-assisted mechanism 114Da neutral. Subsequently, the charge will be located on another oxygen atom and this 3 step mechanism could be repeated as many times as HO-terminated branches the dendrimer has. Thus, CID data allows monitoring the number of intact branches.
MS/MS spectra obtained in activated-EPD strongly differ from CID data, due to the peculiar reactivity of odd-electron species, [M-2H]-°, produced upon EPD. Thus, fragmentation is shown to proceed via H-abstraction reactions from the methylene groups placed on α- position of the N-atoms and CO groups situated all along the dendrimer arm, followed by a 1,6- or 1,5-proton transfer which would further induced the homolytic cleavage of C-amine nitrogen bond. Afterwards, depending on whether the cleavage has been done, entirely parts of the dendrimer are released. Analyzing the intensity but also the absence of the different product ions from the MS/MS spectra, we could assume that the reactivity of CH2 groups strongly depends on its chemical environment.

Conclusion

Activated EPD conjugated with CID experiments could be used for PAMAM structural characterization.

Novel Aspect

The potential of activated EPD could be explored to obtain new insights in the dendrimers structural characterization.

MOS02-03 Atmospheric pressure solid analysis probe with ion mobility-mass spectrometry as a new powerful tool for the characterization of complex industrial mixtures

Caroline Barrère[1], Marie Hubert-Roux[1], Carlos Afonso[1], Amandine Racaud[2], Pierre Giusti[3]
[1]Normandie University, [2]TOTAL Marketing Services, [3]TOTAL Refining & Chemicals

Introduction

The use of atmospheric solid analysis probe (ASAP) in combination with ion mobility – mass spectrometry (IM-MS) has been shown to be very efficient for the analysis of complex mixtures such as petroleum compounds. Thanks to a combination of charge exchange and proton transfer processes, ASAP source allows an efficient ionization of a large range of molecules. However, IM-MS is a post-ionization bi-dimensional separation with a high peak capacity. This combination is applied here to the analysis of complex industrial mixtures such as polymer blends, formulated motor oil and polymers.

Methods

All experiments were performed using a SYNAPT G2 HDMS instrument (Waters corp., Manchester, UK) equipped with an ASAP probe. ASAP glass capillary tube was dipped in the liquid or melted sample and fixed to the ASAP probe holder before introduction in the ionization source. A nitrogen flow of 1200 L h-1 heated at 650°C was used for sample thermal desorption.

Results

The first series of samples analyzed by ASAP-IMMS corresponded to mixtures with known composition. An engine oil composed of two base oils and various additives has been studied. The analysis of separated compounds has permitted to identify their preferred ionization process and possible in source fragmentation before mixture study. As expected, molecular additives have been detected as protonated molecules or molecular ions with more or less fragmentation. In the case of polymeric additives, characteristics pyrolysis products were observed, as already reported. Despite the

molecular diversity of the sample, the complex ion distribution was resolved in the two dimensional drift-time vs m/z plot obtained by IM-MS. Exploring the way of complex polymeric samples characterization, different polymer blends composed of polyester and polyethylene, were studied. Their analyses show that ASAP source allows pyrolysis products of each polymer to be produced. However, polyethylene ions were clearly separated from the polyester ones in the drift-time vs m/z plot.
Others examples involving commercial polymers, such as polypropylene, of unknown compositions will be presented.

Conclusions
This work illustrates clearly the potential of the combination of ASAP ionization method with IM-MS for the analysis of complex industrial samples. Indeed, ion mobility spectroscopy could be regarded as an efficient separation technique that increases the peak capacity an dynamic range of analysis for industrial samples.

Novel Aspects
ASAP-IMMS strategy allows comprehensive study of complex industrial samples.

MOS02-04 MS/MS of incompletely and fully condensed POSS with different substituents – folding and unfolding routes.

Thierry Fouquet[1], Laurence Charles[2], David Ruch[1]
[1]Public Research Centre Henri Tudor, [2]Aix Marseille University

Introduction
Despite an increasing number of articles dealing with mass spectrometry as an efficient tool for the characterization of POSS structures or polymeric systems bearing POSS as monomer or even composite systems including various functionalized POSS, the tandem mass spectrometry behavior of these polyhedral cages have been poorly reported in the literature [1]. Here are proposed the very first fragmentation rules for different POSS structures bearing naked silanols (incompletely condensed structures), unclosed cages and fully condensed octahedrons with different substituents.

Methods
Fully condensed POSS(OSiMe3), POSS(OSiHMe2) and incompletely condensed POSS(OH)x (x=2,3) with naked silanols were commercially available. POSS(PrNH2)8 and incompletely condensed POSS(OSi(iBuMe2)x (x=2,3) were synthesized in the framework of this study. POSS compounds were produced in the gas phase as ammonium adducts and product ions generated upon CID were accurately mass measured in an orthogonal acceleration TOF mass analyzer.

Results
Incompletely condensed POSS(OH)x achieve their maximum condensation under CID through water releases, further eliminating butene/propene neutrals until a critical structure is yielded. Sequential charge-remote transfers of H/Me groups from one substituent to a neighboring one lead to the complete folding of the POSS(OSi(iBuMe)2)x cages and the production of polysiloxane side chains at one apex. These oligomeric substituents are then detected as fragments and the completely folded POSS expulsed as neutral [2]. In contrast, all the fully condensed precursor ions follow an unfolding pathway under CID. Presence of eight propylamine substituents induce sequential dehydration of POSS(PrNH2), ultimately leading to its complete unfolding into a poly(silazane) skeleton. Similar opening of the octahedron substituted with OSiMe3 and OSiHMe2 accounts for product ions generated during their CID. Sequential charge-remote transfers of H/Me lead to a linear co-oligomeric polysiloxane chain composed of randomly distributed linear and cyclic monomers. All peaks observed in CID could hence be accounted for by applying the dissociation reactions typically occurring in protonated polysiloxane-like oligomers recently proposed in the literature [3].

Conclusion
Both pendant groups and structure of the POSS cages were found to greatly influence their MS/MS pathways. While a fully condensed structure is able to unfold to produce a linear copolymeric skeleton as main dissociating species, incompletely condensed structures undergo a folding mechanism – the folded structure being detected as ion or released as neutral.

Novel Aspect
As stated in the introduction, tandem mass spectrometry behavior of POSS was never reported in the literature. We propose here the first fragmentation pathways for several POSS with both folding and unfolding mechanisms.

References
[1] T. Fouquet et al. Rapid Commun. Mass Spectrom. 2012, 26, 765.
[2] T. Fouquet et al. Rapid Commun. Mass Spectrom. 2014, in press
[3] T. Fouquet et al. Int. J. Mass Spectrom. 2011, 306, 70.

MOS02-05 Segregation in dried droplet polymer sample spots examined by MALDI imaging MS

Steffen Michael Weidner[1], Gabriel Stefan[1], Clemens Schwarzinger[2], Ulrich Panne[1]
[1]Federal Institute for Materials Research and Testing (BAM), [2]Kepler-University Linz

Sample spot preparation using the dried droplet technique still represents the predominant method in MALDI-MS analysis. However, after evaporation, instead of a homogenous area, often rings are formed. This could lead to a separation of matrix and polymer, which might have a significant impact on the reproducibility of the spectra. Moreover, polymer segregation while drying strongly affects a reliable molecular mass determination.[1, 2] Several effects could contribute to segregation, like capillary flow, Marangoni flow, diffusion and others. Their influence on the ring formation was investigated by imaging techniques.

Methods
Optical microscopy, MALDI imaging mass spectrometry (MALDI MSI) and IR imaging spectroscopy were applied to monitor segregation in dried droplet MALDI polymer sample spots. Different polymers, matrices, salts, solvents and sample concentrations usually applied for the analysis of synthetic polymers were tested.

Preliminary Data
Depending on the solvent, different effects predominantly contribute to the ring formation. A fast solvent evaporation favors segregation by capillary flow and leads to ring formation by separating matrix from polymer. Using slowly evaporating solvents Marangoni forces counteract to capillary flow. Here, an additional segregation of single polymer homologous was observed. This strongly affects a reliable molecular mass determination. The matrix concentration also seems to be very important. Our MALDI Imaging data clearly show that segregation in drying droplets is predominantly caused by the matrix transport, whereas the segregation of polymer seems to be only secondary. The use of common matrix concentrations (10 mg mL-1) in almost every case leads to a ring formation. In contrast to that, the application of matrix solutions with significantly higher concentrations prohibits a separation of matrix and polymers.

Conclusions
The ring formation in dried droplet sample MALDI spots, accompanied by a segregation of matrix and polymer, can be easily avoided using suitable solvents combined with higher matrix concentrations.

Novel Aspect
MALDI Imaging MS was used for monitoring different segregation effects in dried droplet polymer sample spots.

[1] S. Weidner, P. Knappe, U. Panne. MALDI-TOF imaging mass spectrometry of artifacts in «dried droplet» polymer samples. Anal Bioanal Chem 2011, 401, 127.
[2] S. M. Weidner, J. Falkenhagen. Imaging mass spectrometry for examining localization of polymeric composition in matrix-assisted laser desorption/ionization samples. Rapid Communications in Mass Spectrometry 2009, 23, 653.

MOS03 - Mass Spectrometry Instrumentation

Room 3
Level 0

Chairs: Matthias Frank, Günter Allmaier

MOS03-01 Keynote: Analysis of viruses, VLP-antibody complexes and vaccines by means of nano ESI combined with differential mobility analyzer and bionanoparticles collection

Guenter Allmaier[1], Victor Weiss[1], Marlene Havlik[1], Peter Kallinger[2], Martina Marchetti-Deschmann[1], Wladyslaw Szymanski[2]
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Introduction
For the physico-chemical characterization of bionanoparticles as virus-like particles (VLPs), VLP-antibodies complex, vaccines and recombinant antibodies, besides functional parameters usually methods as the imaging techniques SEM or AFM in different modes as well as far it is feasible ESI MS and as the separation techniques SEC, analytical ultracentrifugation, AF4 as well as MALS or DLS are applied showing pros and cons. Here, we want to present a technique, based on nano electrospray ionization (nESI) with charge reduction to singly charged species combined with an analyzer (separation device) operated at atmospheric pressure, which is mainly targeted to analyzed nanobioparticles with molecular masses beyond 100 kDa or sizes above 5 nm.

Methods
The nESI unit is integrated with a charge reduction chamber (incorporating a Po-210 source or a soft X-ray tube generating a bipolar atmosphere) and coupled to a nano differential mobility analyzer (nDMA; an ion mobility-based separation device) connected to condensation particle counter (CPC; allowing detection on the single particle level without any bias towards the chemical nature of the particle) or an electrostatic sampler for subsequent investigations (AFM, DotBlot etc.). This instrument is called macroIMS (ion mobility spectrometry), a.k.a. gas-phase electrophoretic mobility macromolecular analyzer (GEMMA) or scanning mobility particle sizer (SMPS). The second device is a home-built macroIMS device with an additional nDMA run in parallel (PDMA, parallel DMA) allowing the simultaneous size monitoring in an analytical nDMA and after size-separation in a “so-called” preparative nDMA collection of size-selected bionanoparticle fractions.

Results
The characterization of viruses as human rhino virus, selected VLPs, vaccine particles, gelatin-nanoparticles, liposomes, recombinant antibodies and VLP-antibody fragment complexes by means of nESI connected to a nDMA and a CPC will be demonstrated. Based on the determined sizes of the spherical bionanoparticles the molecular mass will be calculated and compared. Furthermore the off-line combination of SEC will be demonstrated for vaccines. Size-separated bionanoparticles were collected on different surfaces (e.g. mica, copper grid or nitrocellulose) for subsequent analysis. This will be also shown with PDMA approach.

Conclusion
Finally this approach will be presented to demonstrate the closing of the gap between ESI QRTOF mass spectrometry and aerosol micrometer particle physics.

Novel Aspect
Nano ESI with charge reduction (by Po-210 or soft X-ray irradiation) to form single-charge bionanoparticles (intact virus, vaccine particles or VLP-antibody complexes), followed by size (from 2.5 to 150 nm) separation with a differential mobility analyzer and chemical nature-independent detection.

MOS03-02 Precision mass spectrometry on short-lived nuclides: new methods and results

Lutz Schweikhard
University of Greifswald

High-precision mass measurements of short-lived nuclides are routinely performed by Penning trap mass spectrometry at several online facilities worldwide. The mass values provide valuable input data for the study of many fundamental questions such as the structure of atomic nuclei, the limits of the chart of nuclei with respect to the proton and neutron driplines and the region of superheavy elements, as well as the simulation of the stellar nucleosynthesis of elements. Two techniques introduced recently improve considerably the accessibility of nuclides with respect to half-life, production rate and "contaminating" isobars:
1) Phase-Imaging Ion-Cyclotron-Resonance (PI-ICR) mass spectrometry makes use of a position-sensitive ion detector to determine the location of an ion in the Penning trap after a well-defined excitation of the ion motion followed by an excitation-free period. The cyclotron frequency follows directly from the free accumulation of phase of the ion motion.
2) By use of two ion mirrors the Multi-Reflection Time-of-Flight (MR-ToF) technique extends the drift length by orders of magnitude from the one-meter dimension of the instrument up to several kilometers. Nevertheless, the total flight time of the keV ions is only a few milliseconds.
ad 1: In contrast to the conventional ToF-ICR method, where the ICR frequency is determined by repeatedly monitoring the ions' time of flight from the trap to detector after rf-excitation and axial ejection, the PI-ICR method developed at SHIPTRAP provides valuable data from just a few ion counts. Furthermore, its precision is five times higher than that of ToF-ICR MS. Moreover, it is not Fourier limited since not an amplitude as a function of frequency is measured, but instead the phase is determined. Thus, the resolving power exceeds that of alternative methods by a factor of 40. PI-ICR MS proved its applicability in a measurement of the 129Xe-130Xe mass ratio with the experiment time reduced by an order of magnitude compared with the ToF-ICR technique. This will allow ppb-level measurements of nuclides with half-lives well below a second (S. Eliseev et al., Phys. Rev. Lett., 110, 082501 (2013)).
ad 2: ISOLTRAP at CERN/Geneva has been extended with an MR-ToF section between the RFQ trap for ion bunching/cooling and its two Penning traps (for preparation and precision measurements). At first the MR-ToF section has been applied as a mass separator. Isolation from the orders-of-magnitude more abundant 82Rb allowed the first direct mass measurement of 82Zn, which is of significant interest for modeling the neutron-star crust, a proposed site of r-process nucleosynthesis (Wolf et al., Phys. Rev. Lett. 110, 041101 (2013)). Secondly, the MR-ToF MS was used to determine the masses of the neutron-rich 53Ca and 54Ca, which confirmed the presence of a new neutron shell at N=32 and the importance of three-body interactions (F. Wienholtz et al., Nature 498, 346 (2013)).
With these developments the frontiers of precision mass spectrometry of short-lived nuclides is further pushed to more exotic species. The latest results of the present experiments from both experimental setups will be reported.

MOS03-03 A new primary ion beam source for secondary ion mass spectrometry using vacuum electrospray of ionic liquids

Yukio Fujiwara, Naoaki Saito
National Institute of Advanced Industrial Science and Technology (AIST)

Secondary ion mass spectrometry (SIMS) is a powerful technique for characterizing the surface and interface compositions of inorganic and organic materials. In recent years, polyatomic or cluster ion beams have revolutionized the capability of SIMS. For instance, they can significantly enhance molecular secondary ion yields, enabling molecular imaging of organic and biological materials. For further improvement in SIMS analysis, it is required to develop a new cluster ion source capable of producing massive ions at higher current density with a smaller beam spot size.
From the viewpoint of generating such massive ions, vacuum electrospray of ionic liquids is expected to have a great potential; for instance, it probably improves beam-transport efficiency, thereby increasing current density as well as beam current. Ionic liquids represent room-temperature molten salts consisting of a cation and an anion. Since they have a negligible vapor pressure and a high ionic conductivity, it is possible to electrospray them in a vacuum. A wide variety of ionic liquids has been synthesized commercially in recent years.
Among these, we tested N,N-diethyl-N-methyl-N-(2-methoxyethyl)ammonium bis(trifluoromethanesulfonyl) amide (DEME-TFSA) and 1-ethyl-3-methyl imidazolium bis(trifluoromethanesulfonyl) amide (EMI-TFSA). We investigated beam characteristics generated by vacuum electrospray of these ionic liquids. We electrosprayed each ionic liquid in vacuum and measured the transient response of a beam current. Transient response analysis enabled us to study m/z distribution of charged species in the beam, thereby showing that m/z values of EMI-TFSA charged droplets were smaller than those of DEME-TFSA. We also confirmed that the m/z values of charged droplets diminished with decreasing flow rate. Also, we demonstrated that a stable target current was continuously generated. Then, we performed SIMS analysis using an ionic-liquid primary ion beam generated by vacuum electrospray, thereby demonstrating that SIMS analysis was performed using the ionic-liquid primary beam.
Obtained results showed that vacuum electrospray of ionic liquids is applicable to a primary beam source for SIMS.

MOS03-04 Gas flow in electrospray ionization/athmopheric pressure interfaces: simulation and experiment

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Introduction
In atmospheric interfaces of ambient ionization sources like electrospray ionization (ESI) ions are trasferred through a small capillary from ambient pressure to low pressure region. The particle transport is governed by electric forces, diffusion and by the flowing background gas. The gas flow can be laminar or turbulent and is strongly compressible. Its interplay with ions can strongly influence the efficiency of the source, which is however not fully understood.

Methods
Numerical and analyticalmethods are used to describe the gas dynamics for a given geometry. The resulting flow field is used as an input to simulate the dynamics of ions. The simulation is performed by the software SIMION using the SDS package as well as with self-written numerical software tools. The latter aims at treating the space charge effects by first principles. The theoretical findings are validated experimentally in a simple current measurement setup of a transfer capillary of variable geometry to define the gas flow.

Results
We find that a well-designed gas flow has a strong and positive influence on the ion transmission. The considerations lead to an interface with high transmission rate by optimizing the inlet of a transfer capillary. The numerical results are compared to experiment, explaining the central experimental findings with good agreement. A maximal transmission current is observed, which can be attributed to space charge effects.

Conclusions
Gas flows at high pressure are important in ion transport and deserve higher attention. Space charge effects only become important for high currents and can be suppressed efficiently by an improved extraction and collimation at the inlet using gas flows. Better understanding of the interplay of space charge and gas flow is the key to high current ion sources.

Novel Aspect
A high transmission atmospheric pressure interface for nano-electrospray is constructed. Very high transmission rates are found. It is studied in simulation considering electric forces, space charge and fluid flow.

MOS03-05 Development of high mass resolution tandem time-of-flight (TOF) mass spectrometer applicable to High Energy Electron Transfer Dissociation (HE-ETD)

Shigeo Hayakawa[1], Ryuji Fujimoto[1], Masanobu Sogi[1], Hirofumi Nagao[2], Naruaki Imaoka[2], Michisato Toyoda[2], Yasushi Shigeri[3]
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Introduction
Electron capture dissociation (ECD) and electron transfer dissociation (ETD) are widely used in protein biochemistry and proteomics for identifying and characterizing proteins The usefulness of ECD and ETD is associated with N-Calpha bond cleavage to form c- and z-type ions with no loss of labile post-translational modification groups. However most of the ECD and ETD experiments have been equipped FT-ICR or ion trap instrument. Due to long accumulation time of ms order in the trap-type mass spectrometer, mechanistic feature of the electron transfer dissociation cannot be unambiguously investigated. In order to investigate ETD processes in single collision condition and shorter than a few micro-sec, a time-of-flight tandem mass spectrometer applicable to HE-ETD with high mass resolution has been developed.

Methods
Ions are generated by an electrospray (ESI) ion source. The ions travel along an RF ion guide system and are orthogonally accelerated toward MULTUM-S II [1], which can mass-select in high resolution by increasing the number of cycles. Precursor ions are selected an ion gate before a collision cell which is filled with a target gas. The precursor and fragment ions are mass-analyzed in high resolution by a quadratic-field ion mirror [2]. The ions are detected multichannel plate detector.

Results
Figure 1 shows the developed TOF/TOF instrument. The ions pulsed by the orthogonal acceleration and the potential lift, has 4.5×q keV kinetic energy in the ground potential where q is the charge number. The precursor ions are selected by selecting the ejection timing from the MULTUM-S II and the timing of the ion gate pulse before the collision cell. The total flight time and half width of the doubly protonated Bradykinin after the thirty times turn in MULTUM-S II were 510 micro-sec and 20 nano-sec, respectively. The ability to isolate monoisotopical ions was achieved. HE-ETD spectra of doubly protonated peptides using a Cs target showed N-Calpha bond cleavages.

Conclusions
A TOF/TOF instrument has been developed, in which precursor ions are mass-selected by multiturn TOF in high resolution and the product ions are also mass-analyzed in high-resolution by a quadratic-field ion mirror. HE-ETD spectra using a Cs target were measured by this instrument.

Novel Aspect
The time-of-flight tandem mass spectrometer developed can be applicable to HE-ETD with high mass resolution. ETD process can be investigated in single collision condition and shorter than a few micro sec. High-energy dissociation processes by both collisional activation and electron transfer can be measured in one MS/MS spectrum.

Keywords
High energy electron transfer dissociation (HE-ETD), Multiturn time-of-flight mass spectrometer (MULTUM-TOF), Quadratic field ion mirror, Alkali metal target, N-Calpha bond cleavage.

References
[1] S.Shinma, et al., Anal.Chem. 82, (2010) 8456-8463.
[2] M.Toyoda, et al., Rev.Sci.Instrum., 78 (2007) 074101 1-9. H.Nagao, et al., J.Mass Spectrom., 16 (2010) 397-406. H.Nagao, et al., Eur.J.Mass Spectrom., 16 (2010) 551-556.

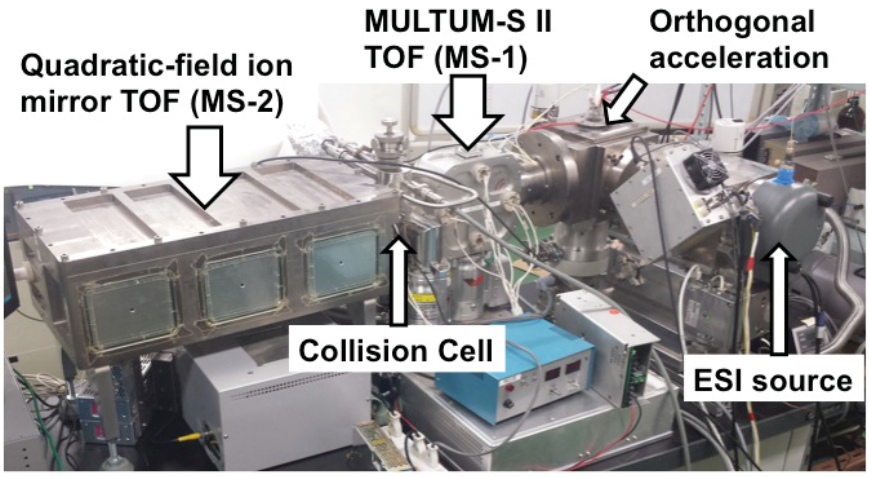

Figure 1. A photograph of high resolution TOF/TOF instrument for high-energy electron transfer dissociation (HE-ETD) experiment.

MOS04 - Aerosol MS and Atmospheric Science

Room 4
Level 0

Chairs: Urs Baltensperger, Renato Zenobi

MOS04-01 **Keynote: Molecular characterization of atmospheric aerosols by high-resolution mass spectrometry**

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Understanding the molecular composition and fundamental chemical transformations of organic constituents of atmospheric aerosols during their formation and aging is both a major challenge and the area of great uncertainty in atmospheric and environmental research. Particularly, little is known about fundamental relationship between the chemical composition and physicochemical properties of organic aerosol (OA), their atmospheric history, evolution, and the impact on the environment.
Analysis of OA samples using soft-ionization methods combined with high-resolution mass spectrometry (HR-MS) analysis provide detailed information on the molecular content of OA that is pivotal for improving the understanding of their complex composition, multi-phase aging chemistry, direct (light absorption and scattering) and indirect (aerosol-cloud interactions) effects on atmospheric radiation and climate, health effects. The HR-MS methods can detect thousands of individual OA constituents at once, provide their elemental formulae from accurate mass measurements and structural information based on tandem mass spectrometry. Integration with additional analytical tools, such as chromatography and UV/Vis absorption spectroscopy, makes it possible to further separate OA compounds by their polarity and ability to absorb solar radiation.
This presentation will feature a summary of recent research projects focused on the detailed chemical characterization of OA, conducted in the groups of the co-authors. We will describe contemporary HR-MS methods, review recent applications in field and laboratory studies of OA, and explain how the information obtained from HR-MS methods can be translated into an improved understanding of OA chemistry

MOS04-02 **Simultaneous gas- and particle-phase measurements using a chemical ionization high-resolution time-of-flight mass spectrometer**

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The organic fraction of atmospheric aerosol is often dominant and consists of thousands of compounds partitioning between gas and particle phases and undergoing chemical reactions in both. A detailed understanding of this complex system is of importance for the assessment of both climate and health effects of atmospheric aerosols. Chemical ionization mass spectrometry (CIMS) with its selectivity and high sensitivity and has been used for quantitative measurements of atmospheric gases for many years; only in the last decade this technique has been expanded to the particle phase.
A new Filter Inlet for Gases and Aerosols [FIGAERO, Lopez-Hilfiker et al., 2014] allows simultaneous measurements of the chemical composition of both gas and particle phase compounds, the latter via thermal desorption. Coupled to a high-resolution time-of-flight CIMS (HRToF-CIMS), it was deployed at two field sites to investigate atmospheric processes of biogenic emissions: Monoterpene oxidation products and new particle formation events were studied in the boreal forest in Finland in spring 2013. Monoterpenes, isoprene and anthropogenic pollutants were significant precursors for organic aerosol (OA) in a mixed forest in Alabama measured during the Southeast Atmosphere Study in summer 2013. Acetate, selective towards carboxylic acids, and iodide, less selective and providing minimal fragmentation during ionization, were employed as reagent ions. Laboratory tests were conducted to provide information on sensitivity, ionization processes, fragmentation patterns, and artifacts of both reagent ion schemes.
At both locations, 100's of organic compounds were observed in the gas and particles. Continuous observations show the diurnal variability and the influence of meteorology on gas-particle partitioning. The mass spectra were dominated by monoterpene and isoprene oxidation byproducts, including carboxylic acids, hydroxy hydroperoxides, and organic nitrates. Compounds with 16 – 20 carbons and 6 - 9 oxygens, assigned to dimers of monoterpene oxidation products, were detected at both locations in both the gas and particle phase, albeit different diurnal patterns. These represent some

of the first ambient measurements of those dimers in the gas phase, believed to play a crucial role in organic aerosol formation and usually thought to be formed in the particle phase.
This new inlet coupled to HRToF-CIMS provides intriguing new possibilities to explore organic and inorganic compounds in the atmosphere in both the particle and gas phase. Detailed structures in thermal desorption signals yield information on the volatility of individual compounds and reveal a contribution from thermal decomposition of large molecular weight organics or oligomers with implications for partitioning measurements and model validation.
Lopez-Hilfiker, F. D., et al. (2014), Atmos. Meas. Tech., 7 (4), 983-1001.

MOS04-03 Characterization of organic trace species in gaseous and particulate emissions of a ship diesel engine fueled with diesel and heavy fuel oil

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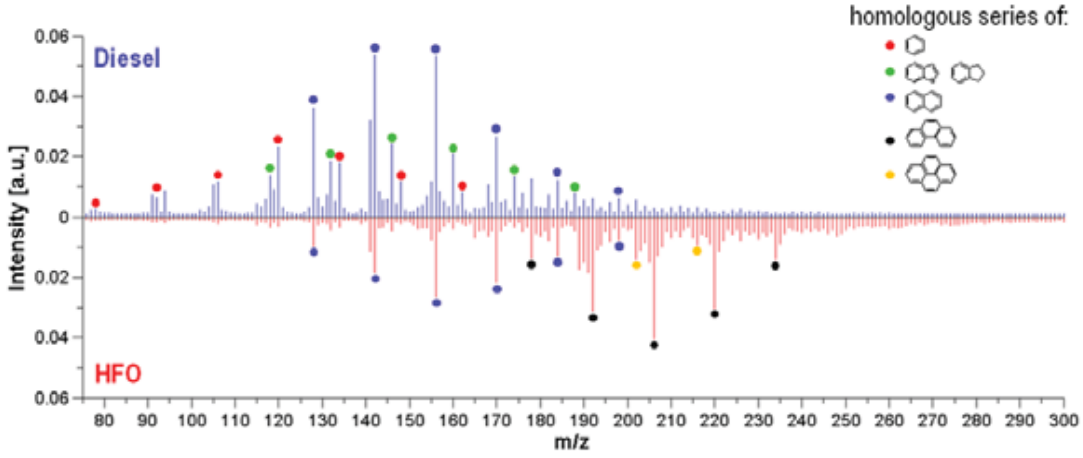
Introduction
Organic trace compounds in gas phase and particulate matter emitted with the exhaust from ships constitute a significant contribution to the anthropogenic environmental burden and pose a considerable health risk to humans in coastal regions. Nevertheless, there is only limited knowledge on the current and expected characteristics of such emissions. Various mass spectrometric techniques have been employed to investigate the organic signature of ship diesel engine emissions, comprising online monitoring of gaseous trace contaminants and characterization of organic contents of emitted particles.

Methods
Emissions from a one-cylinder ship diesel research engine have been investigated. Two different fuels, diesel distillate (DF) and heavy fuel oil (HFO) have been used. Filtered exhaust gas was monitored online by resonance-enhanced multiphoton ionization time-of-flight mass spectrometry (REMPI-TOFMS), which is selective for (poly)aromatic compounds (PAH), and single photon ionization time-of-flight mass spectrometry (SPI-TOFMS).In addition, particulate matter from the exhaust was sampled on quartz filters and its organic content was subsequently investigated by thermal desorption at defined temperature steps hyphenated to both mass spectrometric methods.

Results
Gaseous PAH patterns of diesel fuel were dominated by alkylated naphthalenes, when the fuel was switched to heavy fuel oil, larger PAH such as phenanthrenes and pyrenes became prevalent, while one- and two-ring aromatics were less abundant (see Figure, which depicts averaged PAH emission pattern). Time resolved concentrations could be monitored for several hours, and all aromatic and aliphatic hydrocarbons showed a quite similar time signature for DF combustion, exhibiting high emission peaks at the engine start, and slightly increasing concentrations at cruising conditions. Methanthiol could be monitored solely with HFO.
In DF particulate emissions consisted almost solely of homologue series of phenanthrene and pyrene, while the PAH content of heavy fuel oil particles was shifted to higher molecular species, at least up to m/z 400. Even at higher desorption temperatures in the HFO-derived particulate emission there were still masses up to m/z 400 of low-volatile, but thermally stable aromatic structures.

Conclusions
Online gas phase monitoring reflected the more volatile constituents of DF being present in high abundance, whereas HFO yielded more low volatile aromatic emission products, many of which are transferred to the particulate phase. Differences in signatures between DF and HFO are linked to mixing effects, leading to suboptimal combustion conditions for DF at cruising conditions. Especially volatile PAH are mainly carried over to the exhaust as unburned fuel content.

Novel Aspect
Online monitoring of organic trace contaminants in the exhaust of a ship diesel engine operating with heavy fuel oil and diesel along with the characterization of the organic fractions of the mitted particles.



MOS04-04 Molecular characterization of secondary organic aerosol from the green leaf volatile 3-Z-hexenal and related precursors

Mohammad Safi Shalamzari[1], Ariane Kahnt[2], Reinhilde Vermeylen[2], Tadeusz E. Kleindienst[3], Michael Lewandowski[3], Willy Maenhaut[4], Magda Claeys[2]

[1]University of Antwerp (Campus Drie Eiken), [2]University of Antwerp, [3]US Environmental Protection Agency, [4]Ghent University

Introduction

Much information is available about secondary organic aerosol (SOA) formation from terpenes and isoprene, that is aerosol formed by oxidation and transformation in the gas or particle phases. However, information about SOA formation from green leaf volatiles (GLVs), an important class of biogenic volatile organic compounds (BVOCs), emitted into the troposphere when plants are wounded or attacked by insects, is very scarce. 3-Z-hexenal is an important GLV formed in green leaves from the cell membrane unsaturated fatty acid α-linolenic acid by the combined reaction of lipoxygenase and hydroperoxide lyase enzymes. The focus of the study is on the characterization of 3-Z-hexenal-related organosulfates (OSs) with a MW of 226. In addition, attention is given to MW 212 OSs, which are structurally similar to the 3-Z-hexenal-related OSs but for which the BVOC precursor could not be pinpointed. Both the MW 212 and 226 OSs are present in ambient fine aerosol from K-puszta, Hungary, a rural site with a mixed coniferous/deciduous vegetation, at a substantial relative abundance that is comparable to that of isoprene-related OSs.

Methods

SOA from 3-Z-hexenal was produced in a 14 m3 Teflon-coated stainless steel chamber in the presence of acidic seed aerosol and its OS composition was compared with that of fine ambient aerosol collected from K-puszta, Hungary, during a warm 2006 summer period, containing biogenic SOA from different BVOCs. Two different liquid chromatography (LC) techniques were applied to the polar OSs: the first technique uses a trifunctionally bonded C18 stationary phase, whereas the second one is based on ion-pairing C18 LC with dibutylammonium acetate as reagent. With regard to MS techniques, use was made of negative ion electrospray ionization, high-resolution MS to determine the accurate mass, as well as linear ion trap MS to obtain detailed structural information.

Results

Using detailed interpretation of the MS data, the MW 226 OSs were structurally characterized as sulfate esters of 3,4-dihydroxyhex-2-enoic acid, whereas the MW 212 OSs were assigned to derivatives of 2,3-dihydroxy¬pent-2-enoic acid. Characteristic product ions include the bisulfate anion [HSO4–] and ions formed through loss of SO3, CO2 and H2O from the deprotonated molecule.

Conclusions

The green leaf volatile 3-Z-hexenal and (a) related plant volatile(s) are potential precursors for SOA through formation of polar organosulfates, which are of climatic relevance because of their capacity to increase the hydrophilic properties of aerosols. The formation of the MW 226 OSs is tentatively explained through photooxidation of 3-Z-hexenal in the gas phase resulting in an alkoxy radical, followed by a rearrangement, and subsequent sulfation of the epoxy group in the particle phase.

Novel Aspect

Organosulfates from the green leaf volatile 3-Z-hexenal have been characterized at the molecular level. SOA formation from 3-Z-hexenal through polar organosulfates is reported for the first time.

MOS04-05 New soft ionisation ultra-high resolution mass spectrometry methods for characterizing the organic fraction of atmospheric particles

Markus Kalberer[1], Peter Gallimore[1], Stephen Fuller[1], Ivan Kourtchev[1], Paddy Szeto[1], Anna Fee[1], Yongjing Zhao[2], Steven Cliff[2], Anthony Wexler[2], Peng Lin[3], Jinazhen Yu[3]

[1]University of Cambridge, [2]University of California, Davis, [3]University of Science & Technology, Hong Kong

Introduction

Organic compounds in atmospheric particles play an important role in climate change and human health from air pollution. Organic particle, their sources and atmospheric processing such as aerosol-cloud interactions are poorly understood. This is partly due to the lack of understanding of particle chemical composition. Organic aerosols are composed of hundreds to thousands of compounds, posing a significant analytical chemical challenge.

Methods

Aerosol particle chemical composition is characterized by ultra-high resolution MS to cope with the many hundreds of compounds in a sample. Complementary information from negative and positive mode electrospray ionization (ESI) (Lin 2012) and direct infusion ESI-MS versus HPLC-ESI-MS is explored. The advantage of using nano-electrospray compared to conventional ESI with respect to ion suppression in the presence of inorganic salts is demonstrated.
In addition, two different (semi-) online ionization techniques were developed: an extractive ESI (EESI) source (Gallimore 2013) for direct online aerosol analysis and a direct liquid extraction surface analysis (LESA) method (Fuller 2012) to characterize archived rotating drum impactor samples.

Results

The chemical composition of aerosols from various sampling locations and laboratory experiments is characterized by the methods described above. They reveal a large amount of additional information obtained by combining positive and negative ionization data. It will be shown that nano-ESI is less prone to ion suppression than conventional ESI and we demonstrate the synergies obtained from combining LC and direct infusion analyses (Kourtchev 2013).
The direct analysis methods show that soft ionization methods are capable of determining the composition of organics quantitatively with minimal fragmentation and detection limits in the nanogram per m3 air concentration levels.

Conclusions
These studies show that new, soft ionization MS techniques allow for a significantly improved characterization of organic aerosol composition, sources and evolution over time, compared to conventional ionization techniques.

Novel Aspect
We developed a range of novel soft ionization MS techniques to improve the chemical characterization of organic particles relevant to urban smog and climate change.

Literature
Fuller S.J. et al., Anal. Chem., 84, 9858−9864, 2012.
Gallimore P.J. and Kalberer M., Environ. Sci. Technol., 47, 7324−7331, 2013.
Kourtchev I. et al., Environ. Sci. Technol., 47, 4069−4079, 2013.
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MOS05 - Nucleic Acids

Room 5/6
Level 3

Chairs: Daniele Fabris, Eric Forest

MOS05-01 Keynote: Mass spectrometry for nucleic acids biophysics

Valérie Gabelica
Inserm/Université Bordeaux (U869)

There is now increasing evidence that specific nucleic acid structures modulate gene expression levels both at the transcriptional and at the translational level. In particular, G-quadruplex (G4) structures are attractive targets for anticancer strategies, since several studies showed that their stabilization by ligands caused proliferation arrest, telomere deprotection and changes in gene expression. Understanding the structure-function relationships in G4 DNA and RNA in order to target them requires innovative biophysical tools to probe the general and specific features of the structures adopted by a wide variety of sequences, their macromolecular assemblies, and their interactions with drugs.
Current biophysical assays for ligand binding to G-quadruplexes include melting assays, fluorescence displacement assays, and direct titrations monitored by spectrophotometry, surface plasmon resonance, or isothermal titration calorimetry. These in-solution methods however suffer from the fact that the signal reflects the weighted average contribution of all species simultaneously present.
This presentation will survey native mass spectrometry and ion mobility spectrometry approaches that can be used as biophysical tools to probe small molecule ligand interactions with G4 structures. First, MS is uniquely well suited to detect and quantify G4-drug interactions in a direct binding assay. With mass and intensity measurement, one can characterize the binding affinity and specificity of ligands to a variety of targets. We will also highlight how some details, such as cation binding, can also give insight into nucleic acid follding, ligand binding mode, and ligand-induced changes in folding. Finally, ion mobility spectrometry is a key method for studying the conformational space of the nucleic acids, and ligand-induced conformational changes.

MOS05-02 Ligand binding to DNA G-quadruplexes studied by ESI-MS from potassium solutions

Adrien Marchand, Valérie Gabelica
Inserm / Université Bordeaux

Introduction
A commonly used electrolyte in electrospray mass spectrometry (ESI-MS) of biomolecules is ammonium acetate (NH4OAc). Although some nucleic acid structures such as duplexes require only physiologic ionic strength to be properly mimicked in ESI-MS conditions, some other nucleic acid structures such as DNA G-quadruplexes also depends on direct binding of specific cations. For example, G-quadruplexes are mainly stabilized in cells by potassium cations. Here we studied ligand binding to G-quadruplexes in potassium solution. We show the effect of ligands on the number of potassium ions bound to the G-quadruplex, which are linked to changes in the structure of the target G-quadruplexes.

Methods
Recently we developed ESI-MS compatible conditions that allow to observe DNA G-quaduplexes with K+ ions specifically bound between G-quartets (Marchand and Gabelica, J. Am. Soc. Mass Spectrom., accepted). In that sample preparation method, NH4OAc is replaced with trimethylammonium acetate (TMAA) and the solution is doped with KCl at concentrations up to 1 mM. The trimethylammonium ion is too large to intercalate between G-quartets, where only K+ ions bind. Native mass spectrometry was used to obtain the stoichiometries of each complex (i.e. number of DNA strands, of ligands and of potassium ions). Circular dichroism was chosen to obtain information on the G-quadruplexes topologies in solution.

Results
Ligands were found to displace potassium cations to different extents. Among the tested ligands some of them were totally displacing one potassium ion in the human telomeric G-quadruplexes: 360A and PhenDC3: when theses ligands are added in the solution and interact with the G-quadruplexes containing 2 K+, the final stoichiometry is of one DNA strand with one ligand and one potassium ion. In contrast, the TrisQ ligand displaced least the potassium cations. Thanks to the circular dichroism we could show that 360A and PhenDC3 were displacing the equilibrium between G-quadruplexes topologies to antiparallel forms. TrisQ on the other hand was inducing the formation of a hybrid form.

Conclusions
Thanks to the new methodology we could propose new structural models for these complexes: 360A and PhenDC3 interact with the G-quadruplex disrupting one quartet and therefore ejecting one K+. The two remaining quartets are in a syn-anti stacking and lead to an antiparallel CD signal. It is the first time that G-quadruplexes binders were found to partially disrupt the structure and this result is important to understand the binding mode and in the development of new drugs.

Novel aspect
First study of ligand binding to G-quadruplexes in the presence of the physiologically relevant potassium cation.

MOS05-03 Formation and dissociation of the tetramolecular DNA i-motif by the sequences d(XnC4Ym) in the gas- and solution-phase

Xinhua Guo, Yanwei Cao, Yujiao Qin, Shang Gao
Jilin University

Introduction
Cytosine-rich DNA strands can self-assemble forming variety of four-stranded structures called i-motifs. These are characterized by C•C+ base pairs from one pair of parallel strands that alternately intercalate with C•C+ base pairs from another parallel pair such that the intercalating base pairs are roughly orthogonal and the pairs of parallel strands are anti-parallel. Here, we using ESI-MS, CD and UV spectroscopy demonstrate that non-C bases located at the end of the DNA sequence d(XnC4Ym) (X and Y represent thymine, adenine or guanine, and n, m range from 0 to 2) significantly influence on the formation of the tetra-stranded DNA i-motifs. The kinetics and intermediate during formation and dissociation of the tetramolecular complexes are also reported.

Methods
Sample preparation: All DNA strands are prepared in ammonium acetate–acetic acid buffered at pH=4.5
Mass spectrometry: An ESI-Q-TOF (micrOTOF-Q II, Bruker, Germany) mass spectrometer was used for determination of gas phase DNA complexes.
CD spectroscopy: CD spectra were obtained by a Jasco J-810 CD spectrometer in wavelength range of 230 - 320 nm.
UV spectroscopy: Heating and cooling curves were measured at 265 and 295 nm respectively by using a SHIMADZU UV-2550 spectrophotometer equipped with a S-1700 temperature controller.

Results
All DNA strands form tetramolecular complexes in the gas phase and show characteristic CD spectra indicating the formation of the i-motif structures in both gas phase and solution. Figure 1 gives comparative abundance percentages of each strand obtained by CD spectroscopy and mass spectrometry suggesting that a non-C base residue located at 5′ end favors formation of the four-stranded structures, with T > A > G for imparting stability in the gas phase and with exception in solution.
Moreover, singly, doubly and triply stranded ions were detected during strand association and upon collision induced dissociation of the four-stranded (dT2C4)4 (Figure 2) suggesting a molecule by molecule reversible formation and dissociation kinetic process. UV absorption melting and cooling cures reveal overlapping stand dissociations and associations consistent with such complex kinetics.

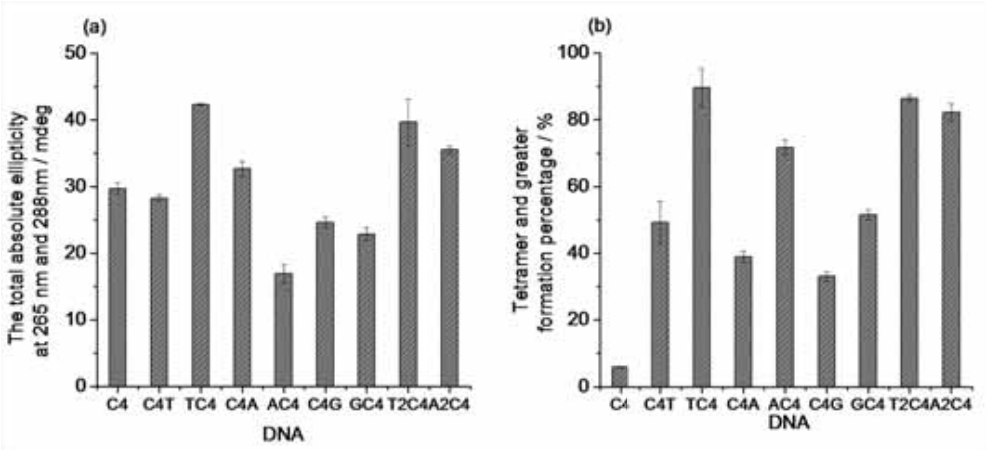


Figure 1. (a) Total CD absorbance of the strands at 265 and 288 nm, (b) tetramer and greater formation percentage of the strands

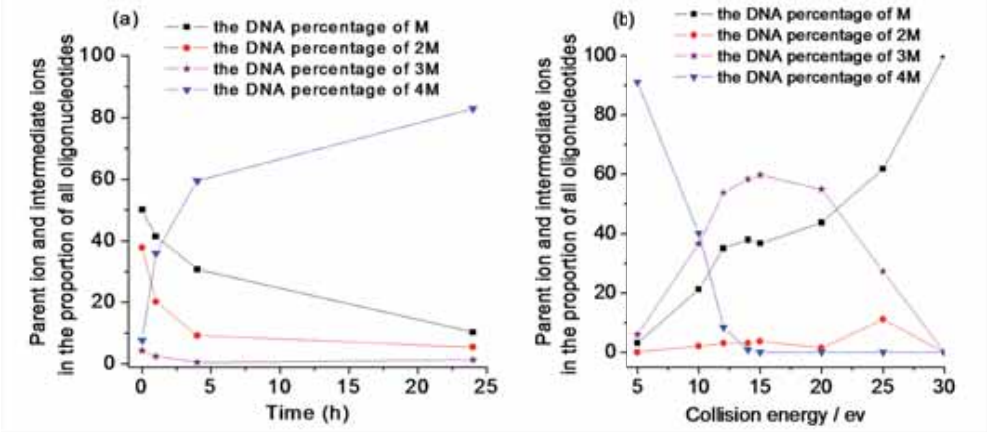


Figure 2. (a) Abundant percentage of each strand occupied on all oligonucleotides vs. annealing time, (b) abundant percentage of each strand occupied on all oligonucleotides vs. collision energies.

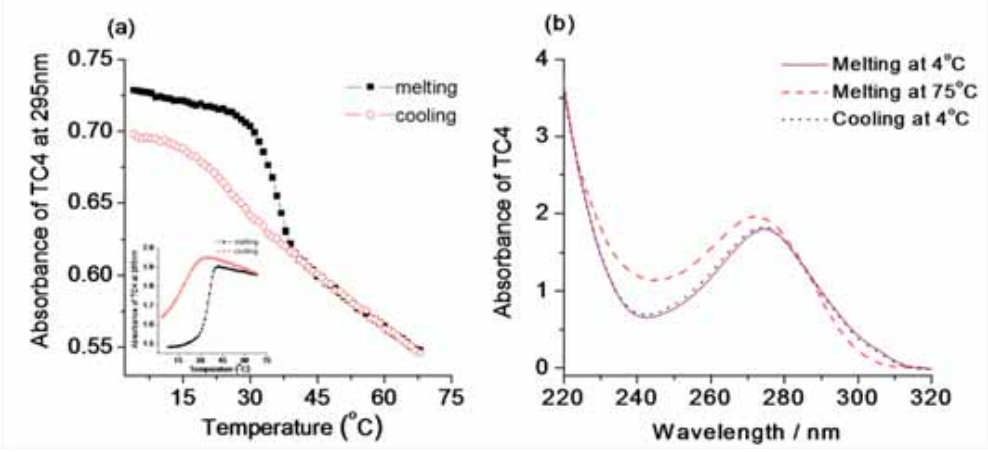


Figure 3. (a) Melting and cooling profiles of the $[d(TC_4)]_4$ at 265 nm (insert) and 295 nm; (b) UV scan curves of $d(TC_4)$ under different temperature.

Conclusion
Present work provided important information on bases and kinetic factors affecting on the formation of i-motif structure.

Novel Aspect
Detection of intermediate of i-motif during formation and dissociation

MOS05-04 **Non-standard gas-phase fragmentation of short, highly charged oligonucleotides**
Rahel Eberle, Stefan Schürch
University of Bern

Introduction
Short, structurally modified or unmodified oligonucleotides are relevant in the therapeutic antisense approach. They hybridize to a specific mRNA target, mediate its degradation and thus achieve gene downregulation. Tandem mass spectrometry is a sophisticated analytical tool for the comprehensive characterization of short oligonucleotides. The gas-phase dissociation of DNA is characterized by cleavage of the 3'-C-O bond in conjunction with the loss of the adjacent nucleobase. We found that pyrimidine nucleobases can follow a Retro-Diels-Alder (RDA) mechanism upon collision-induced dissociation (CID), resulting in the abstraction of a NCO- moiety. So far, this dissociation pathway has not been reported for oligonucleotides longer than dimers.

Methods
Unmodified and modified DNA oligonucleotides were systematically investigated by ESI coupled to a LTQ Orbitrap XL (Thermo Fisher Scientific) mass spectrometer. All experiments were performed in the negative ion mode with a potential of -600 V to -800 V applied to the nanoelectrospray needle.
For MS/MS characterization, the precursor ion of the highest charge state was selected and subjected to CID.

Results
The product ion spectrum of the fivefold negatively charged d(CCGGTT) gives evidence for the loss of a negatively charged fragment with a mass of 42 Da, which was attributed to the abstraction of an isocyanate (NCO-) ion. The origin of the isocyanate ion was identified by MS3 experiments on the primary [M-NCO-] fragment. Results indicated that the isocyanate ion originates from the 3'-terminal thymine. Comparison of various sequences revealed that a thymine nucleobase is more prone to NCO- loss than a cytosine and that a high charge state is a prerequisite for this prominent dissociation pathway.
Furthermore, as the loss of a H2O molecule was found to coincide with NCO- abstraction, the dideoxyoligonucleotide CCGGTT# lacking the terminal 3'-hydroxy group was subjected to CID. The product ion spectrum of the fivefold negatively charged precursor revealed no further loss of water, giving evidence for the terminal 3'-OH group constituting the exclusive source of H2O.

Conclusions
The expulsion of NCO- from pyrimidine nucleobases represents a prevalent dissociation channel for highly charged oligonucleotides up to 12-mers. Results of the study reveal the prerequisites for such decomposition and provide information about the underlying gas-phase dissociation mechanisms.

Novel Aspects
Knowledge of the origin of fragment ions constitutes a prerequisite for the unequivocal interpretation of tandem mass spectra and, consequently, assists the development of therapeutic antisense oligonucleotides.

MOS05-05 MS-based elucidation of RNA structures

Matteo Scalabrin, Papa Nii Asare Okai, Sugyan Dixit, Yik Siu, Daniele Fabris
The RNA Institute, University at Albany

Introduction
The elucidation of RNA 3D structure represents an important endeavor in structural biology, which has been pursued by a variety of experimental and computational approaches. We have been developing strategies based on chemical crosslinking and structure-specific nucleases to obtain the wealth of spatial constrains necessary to support full-fledged molecular modeling operations.

Methods
Substrates ranging from small (~20 nt) stemloops to larger (~380 nt) RNA constructs were treated with nitrogen mustard (Sigma) and other crosslinkers developed in house. Modified/unmodified substrates were digested with nucleases that attack specific nucleotides (e.g., RNase A and T1), single-stranded regions (e.g., Mung bean and S1 nuclease), or double-stranded structures (e.g., RNase V1 and RNase III). Hydrolytic products were characterized on a Thermo Scientific Orbitrap Velos mass spectrometer.

Results
We have previously shown that bifunctional crosslinkers and structure-specific nucleases represent effective tools for recognizing base-pairing interactions that define the higher-order structure of nucleic acids. Crosslinked conjugates are typically characterized by digesting modified/unmodified substrates prior to mass mapping/sequencing. Owing to the frequency of susceptible targets, nucleotide-specific RNases tend to produce numerous small products with identical base composition, which can easily match multiple different regions of the substrate. We investigated the possibility of combining crosslinking probes with structure-specific nucleases to minimize the ambiguities associated with product degeneracy. Indeed, we found that these enzymes provided larger hydrolytic products that could be unambiguously assigned to specific domains. For example, we applied this strategy to a 363-nt construct replicating the 5'-untranslated region (5'-UTR) of the HIV-1 genome. When samples treated with nitrogen mustard were digested with RNase T1 (G specific), numerous conjugated products were observed, which consisted of relatively short oligonucleotides (up to ~3-4 mer) bridged by the crosslinker. The majority of such conjugates could not be unambiguously assigned to a certain region of 5'-UTR. In contrast, RNase III (double-stranded specific) generated larger digestion products (up to 30 mer)

corresponding to unique regions. In particular, we observed a G15-G42 crosslink in the TAR domain and G310-G325 in the stem loop 3, which were consistent with putative secondary structures proposed for 5'-UTR. Additionally, the G119-G190 and G310-G315 crosslinks suggested long-distance interactions between distal domains, which may define the tertiary structure and global topology of this critical section of viral genome.

Conclusions
This work demonstrated the benefits of combining structure-specific nucleases with crosslinking probes to identify unique interactions that determine the architecture of complex RNA structures.

Novel aspects
Improved MS-probing strategies to obtain valid spatial constrains.

MOS06 - Clinical Applications and Screening

Chairs: Yoshi Wada, Ruedi Aebersold

Room 1
Level 1

MOS06-01 **Keynote: Direct mass spectrometric analysis of mucosal membranes – experimental approaches and applications**

Zoltan Takats, Nicole Strittmatter, Julia Balog, Frank Huang, James Kinross, Sacheen Kumar, Trevor Hansel, Emrys Jones
Imperial College London

Introduction
While recent advent of ambient MS provided new means for in-situ and imaging analyses and led to the development of real-time, in-vivo MS characterisation of tissues, there are no methods available for minimally invasive testing of mucosal surfaces including the associated microflora. Human mucosa-associated microbiome has been recently demonstrated to play a key role in the pathogenesis of localised (cancer, chronic inflammatory disease) and systemic (hypertension, diabetes, obesity) conditions. While the microbiota interacts with the host mostly via production of a variety of metabolites, currently there is no method available for the in-situ metabolic profiling of mucosa. The envisioned methods will presumably fill this gap, by providing a technique for the diagnosis of a wide variety of diseases ranging from acute infections through cancer to dysbiotic conditions of the microflora leading to chronic illnesses.

Methods
In-vivo testing of mucosal surfaces was performed by using Rapid Evaporative Ionization Mass Spectrometry (REIMS) in a non-invasive or minimally invasive fashion. Dedicated electrode geometries were developed for the selective sampling of mucus layer and mucosal surface. The bipolar electrodes were connected to distant mass spectrometric device (Xevo G2-S Q-TOF, Waters, Wilmslow, UK) using either direct aerosol introduction or a Venturi air jet pump-based aerosol transfer setup. The atmospheric interface of the instrument Special experimental setups were designed and built for respiratory, upper gastrointestinal (GI), lower GI and urogenital applications. In addition to REIMS, Jet Desorption Ionization (JEDI) has also been implemented for in-situ mucosal testing using similar experimental setups. Databases were created by the REIMS and DESI analysis of cultured microorganisms, mucus samples and ex-vivo tissue specimens.

Results
A database featuring more than 10,000 bacterial strains was created using clinical isolates and a comprehensive identification system was developed. The identification system was demonstrated to provide sub-species level identification for common pathogens. In case of mucosa-associated bacterial communities the system can still deliver genus-level identification and semi-quantitative data on the composition of the community. The system was tested successfully using colon and gastric mucosa/mucus samples with RT-PCR validation. The REIMS and JeDI probes were also employed for the in-vivo testing of mucosal metabolism, with particular emphasis on inflammations with different etiology. Mucosal eicosanoid (prostaglandin, leukotriene, etc.) profiles were found to show excellent correlation with the types of inflammation and also the responsiveness of the patient to various anti-inflammatory therapeutic regimens both in case of upper respiratory and lower GI inflammatory diseases.

Conclusions
A multimodal direct MS method approach was developed for the point-of-care identification of pathogens and stratification of patients with chronic inflammatory diseases.

MOS06-02 **Individualized tissue analysis for EGFR-dependent phosphoproteomic signature in non-small-cell lung cancer**

Yi Ting ET Wang[1,2], Chia-Feng Tsai[1], Chih-Chiang Tsou[3], Pei-Yi Lin[1], Sung-Liang Yu[2], Szu-Hua Pan[2], Pan-Chyr Yang[1,2], Alexey Nesvizhskii[3], Yu-Ju Chen[1]
[1]Academia Sinica, Taipei, [2]National Taiwan University, Taipei, [3]University of Michigan, Ann Arbor

The abnormal protein kinase activity with its corresponding change in protein phosphorylation states has been implicated in tumor formation and cancer progression. The discovery of aberrant phosphorylation can lead to the design of kinase inhibitor, such as gefitinib targeting mutated EGFR for cancer treatment. A sensitive quantitative phosphoproteomic signature analysis is helpful to find such tissue signature for different EGFR-dependent cancer subtypes, which may subsequently facilitate the identification of drug target candidate.We applied an informatics-assisted label-free quantitationstrategy combined with IMAC strategy to analyze the personalzied proteome as well as phosphorylation-mediated signaling networks and their interplay in lung cancer tissue. We aim to discover tissue signatures associated with specific lung cancer mutations on EGFR, including Del19-EGFR and L858R-EGFR and wild-type EGFR in 31 NSCLC paired tumor tissues and their adjacent normal tissue. A total of 7100 proteins and 20458 unique phosphopeptides were identified. For each normal and tumor tissue sample, each phosphopeptides or proteins abundance are normalized by ranking process for statistical analysis. The differential phosphoproteomics patterns can be grouped by ANOVA into differentiation of tumor and normal tissue samples as well as cancer tissue subtypes. Unsupervised statistical analysis of the phosphorylation expression pattern identified some kinases and phosphorylation substrates among different patient groups. In conclusion, this individualized phosphoproteomic screening could provide the opportunity to identify phosphorylation signature of individual patient with different disease phenotype for potential drug targets study in the future.

Novel aspect
Demonstrate the label-free quantitation method for individualized phosphoproteomics analysis and provide a NSCLC cancer subtype signature.

MOS06-03 Screening of biological samples by SWATH acquisition and processing by high resolution reference spectra

Stefan König, Thomas Wüthrich, Stefanie Salzmann, Wolfgang Weinmann, Susanne Nussbaumer
IRM Bern

Introduction
Fast and accurate screening is an important step for further decisions regarding possible treatments, possible quantification or identification of the detected compounds in body fluids. Up to date, immunoassays are the most common techniques for screening in clinical environment, even if these biochemical tests exhibit some significant disadvantages such as cross reactivities or lack of sensitivity for specific compounds. Based on the latest generation of QqTof mass spectrometers which are capable of acquiring complete MS and MS/MS spectra at a scan rate of 20 Hz and mass accuracy below 5 ppm in combination with core shell HPLC columns new screening methods were developed.

Methods
Urine samples were analyzed on a QqTOF instrument (5600 TripleTof, AB Sciex, Concord, CA). All samples were diluted with a mixture of water / acetonitrile / formic acid / ammonium formate (97.5 / 2.5 / 0.1% / 5.0 mM) and three internal standards were added (d3-EME, d3C13-Tramadol, d3-THC). The prepared samples were injected onto several different core shell columns in order to achieve fast and comprehensive screening of the samples.

Results
Human Urine samples from the last few months were analyzed in data independent SWATH acquisition mode (sequential window acquisition of all theoretical fragment ion spectra). The MS/MS scan parameters were optimized and the following parameters were found to be most favorable for significant library hits: scan range from 50 to 950 Da, SWATH windows of 25 Da, scan time of 35 msec for each SWATH window and collision energy of 35 eV ± 15 eV (collision energy spread). The samples were analyzed by different HPLC gradients. As internal standard three different labeled compounds were added (d3-EME, d3C13-Tramadol and d3-THC) in order to verify injection and the reproducibility of the HPLC gradients. The obtained data were processed against a high resolution and high accuracy reference library. This home-built library currently contains about 1000 spectra which are relevant for clinical drug screening. The acquired data set from different methods were compared in regards to library hit rates, signal to noise ratio and the overall sensitivity will be discussed in detail.

Conclusions
Data independent scan methods allow for non-discriminating investigations on any biological sample as long as the compounds can readily be ionized. In addition, the complete information of the acquired samples is archived and can be re-processed on a later stage in order to search for new compounds.

Novel Aspect
A data independent drug screening method for biological samples by QqTof instruments was developed which is based on Swath acquisition and accurate mass processing.

MOS06-04 Ultrasensitive detection and quantitation of neuroactive steroids using a post-activation ion-molecule reaction mediated by lithium

Scott A. Shaffer, Shunyan Mo, Kristina M. Deligiannidis
University of Massachusetts Medical School

Introduction
Neuroactive steroids (NAS) have been implicated in a variety of disorders including epilepsy, multiple sclerosis, neurodegeneration, brain injury, depression, schizophrenia, pain and anxiety. Several NAS are potent modulators of the GABAA receptor and can alter the excitability of the central nervous system. Gold-standard measurements of NAS by gas-chromatography mass spectrometry (GC-MS) require chemical derivatization to decrease polarity and increase volatility. Our motivation was to develop an assay to quantify NAS that minimizes sample preparation. We present a novel non-derivatizing LC-MS/MS assay to quantify a panel of NAS in plasma from women classified either as low or high risk to postpartum depression during the perinatal period. The 57 cohort study measured plasma concentrations from up to four gestational age time points and 3-9 weeks post-partum.

Methods
The NAS panel consisted of progesterone, deoxycorticosterone, allotetrahydroxycorticosterone, epimers 5α- and 5β-dihydroprogesterone, epimers allopregnanolone and pregnanolone, and pregnenolone. Calibrants and benchmarks containing deuterated internal standards were prepared in charcoal-striped human plasma, extracted by methyl tert-butyl ether, and reconstituted for LC-MS/MS in mobile phase containing 2 mM lithium acetate. NAS were separated using a Waters NanoAcquity UPLC using a 150 µm ID C18 column configured to a Thermo Orbitrap Velos Pro mass spectrometer. NAS were ionized to (M+Li)+, subjected to in-source CID, and further activated using HCD fragmentation. The ion-molecule reaction product, (M + Li + H2O)+, was used as the basis for the quantitative assay.

Results
We optimized an ion-molecule reaction producing (M + Li + H2O)+ cations from (M + Li)+ precursors in the HCD cell/C-trap of an Orbitrap mass spectrometer. Previous LC-MS studies in our laboratory using lithium cations quantitated NAS to plasma concentrations of 200 pg/mL i.e., 12 pg on-column. Presently, we improved the limit of quantitation of NAS to 7.8 pg/mL (62 fg on-column), while also preserving NAS S/N ratios >30. By comparison, detection limits for established GC-MS methods employing derivatization and electron-capture detection vary widely, but common values range 0.2 to 2 pg on-column. Moreover, the described ion molecule reaction is highly selective for C-21 progesterone metabolites over both C-24 bile acids or C-18 estradiol, making the assay both selective and sensitive for NAS. The post-partum depression study measured over 223 samples in duplicate from 57 cohorts. As expected, NAS levels were

highly elevated during pregnancy and dropped by over 2 orders of magnitude following childbirth. Coefficients of variation (CV) averaged 5.4 – 8.9% for each of the analytes. NAS benchmarks, measured at three concentrations (0.4, 8.0, and 40 ng/mL; n = 114), gave inter-assay values that ranged 2.1-12.9% (% difference) for accuracy and 4.1-18.3% (CV) for precision.

Conclusions
A sensitive and selective assay for NAS is described with promising applications to clinical measurements.

Novel aspect
A nanoflow LC-MS/MS assay for NAS capable of lower detection limits than GC-MS methods.

MOS06-05 **New approaches to multiplex newborn screening of lysosomal storage disorders by tandem mass spectrometry**
Frantisek Turecek, Mariana Barcenas, C. Ronald Scott, Michael Gelb
University of Washington

Introduction
Lysosomal Storage Disorders (LSD) comprise a group of over 40 degenerative diseases with a combined incidence of 1 in every 5,000 to 7,000 newborns. For over a decade our group has been developing individual enzyme assays based on tandem mass spectrometry for the detection of lysosomal storage disorders using dried blood spots (DBS) in the format compatible with the workflow in newborn screening laboratories. We report on current efforts that are focused on developing multiplex assays to diagnose disorders grouped into 2 groups. One consisted of 6 disorders, which were Krabbe (GALC), Gaucher (ABG), Niemann-Pick (ASM), Fabry (GLA), Pompe (GAA) and Mucopolysaccharidosis I (MPS I). The other group consisted of 5 additional disorders, which were MPS II, IV, and VI, and Neuronal Ceroid Leukodystrophies (NCL I and NCL II) using flow injection introduction into the mass spectrometer.

Methods
Mass spectrometric measurements were performed on Waters Xevo and Quattro Micro tandem quadrupole mass spectrometers. A punch of a DBS is incubated overnight in an appropriate buffer with an assay cocktail containing enzyme substrates and internal standards. After work up, the samples are flow-injected into the mass spectrometer and selected dissociation channels are monitored.

Results
New enzyme substrates were designed and synthesized to facilitate work up , increase the ionization efficiency, and control ion fragmentation. The new substrates for MPS I, II, IV and VI incorporate an aminophenol linker that was shown by theoretical calculations to increase the product gas-phase basicity and thus increase the ionization efficiency by a factor of 7-10. Enzyme activities were measured for DBS produced from normal individuals, confirmed affected patients, and quality control standards QC Low, Medium, and High. The data showed substantially increased ratios of product signals compared to blanks. For example, the specific activities for MPS I, II, and VI relative to blanks

increased 5.6, 2.0, 19.8 fold with the new substrates when compared to the original ones. These increases were due to a combination of factors such as lowered blanks and increased specific activities.

Conclusions
Owing to new developments and successful pilot programs, tandem mass spectrometry has become the method of choice for newborn screening of lysosomal storage disorders

Novel Aspect:
New substrates for tandem mass spectrometric detection of enzyme activities in dried blood spots for large-scale newborn screening.

MOS07 - Imaging MS – Instrumentation

Room 2
Level 0

Chairs: Ron Heeren, Markus Stöckli

MOS07-01 **Keynote: Expanding the usefulness of secondary ion mass spectrometry for biologically relevant measurements**
Christopher Anderton[1], Donald Smith[2,3], Franklin Leach[1], Ron Heeren[3], Ljiljana Paša-Toli[1]
[1]PNNL, [2]FOM Institute AMOLF, [3]National High Magnetic Field Laboratory, Florida State University

Secondary ion mass spectrometry (SIMS) routinely offers the ability to gain chemical and spatial information about biological samples. While the use of an ion beam for desorption and ionization of surface molecules in SIMS measurements typically allows for chemical maps with greater spatial resolution than laser-based MS imaging approaches, the excessive energy of the primary ions yields extensively fragmented surface molecules. This makes identification of parent molecules from the detected secondary ions a nontrivial endeavor. The development of cluster primary ion sources helped reduce fragmentation of the surface molecules and extended the applicability for biologically focused SIMS-based measurements. Enhanced secondary ion yields of higher molecular weight species generated from cluster sources unlocked the possibility to detect and identify lipids, metabolites, and other small biomolecules more

readily. However, the broad diversity of molecules within biological samples, where a multitude of species are often contained in a nominal mass unit, requires a paradigm shift in mass analyzers to allow SIMS to be a useful method for mapping species of interest. Recently, we have developed a Fourier transform ion cyclotron resonance (FTICR) SIMS instrument coupled with a C60 primary ion source, which can provide greater mass resolving power (m/m50% >3,000,000) and mass accuracy (<1 ppm) than conventional mass spectrometers used in SIMS measurements. While high mass accuracy often facilitates identification and classification of species of interest, tandem mass spectrometry capabilities are proven to be essential for confident identification of detected species. Here, we demonstrate the unique utility of the C60 FTICR-SIMS platform to achieve high mass accuracy, high mass resolving power, and tandem MS SIMS-based analyses employed on a variety of complex biological surfaces.

MOS07-02 A comparison of DESI and MALDI ionisation on an oa-TOF MS for tissue imaging experiments

Emmanuelle Claude, Mark Towers, James Langridge
Waters Corporation

Introduction

Mass spectrometry imaging (MSI) is gaining importance in clinical, omics and pharmaceutical research areas due to the significant technological improvements achieved over the past decade. There are several techniques that have been developed to ionize molecules directly from tissue. Initially matrix assisted laser desorption ionization (MALDI) was used, but in the last few years, other techniques like desorption electrospray ionization (DESI) have been applied to tissue imaging.

In this study, we will present data comparing and contrasting the two ionisation techniques for MS imaging of consecutive tissue sections. We will discuss the strengths and weakness of having both ionisation techniques on the same MS platform.

Methods

Experiments have been carried out on consecutive tissue sections from pig liver and patient biopsies. The sections were produced using a cryotome and deposited on a standard microscope slide which was preserved at -80C degrees until analysisby mass spectrometry.

For MALDI experiments, a SunCollect nebulizing spray device was used to evenly apply 9-aminoacridine (9AA) solution (10 mg/mL in ethanol/water, 70/30 v/v) in several coats. For DESI experiments, the tissue was directly mounted into the DESI source from the freezer, with no sample preparation or pre-treatment required. Data were acquired using a SYNAPT G2-Si mass spectrometer. When the MALDI source was in operation, a solid-state diode-pumped ND:YAG laser with a repetition rate from 100Hz up to 2.5 KHz was used. DESI experiments were carried out using the Prosilia source, directly mounted onto the mass spectrometer.

Results

Experiments were carried out in negative MS mode of acquisition with the integrated tri-wave ion guide optics used to separate ions by ion mobility in the gas phase. The mass range of acquisition was 100-1,000 Da, suitable for the detection of lipid species. Both sets of MS imaging data were processed and visualized using High Definition Imaging (HDI) 1.2 MALDI software.

Initial results in negative mode show that both techniques generated good intensity of lipid related ions from the direct analysis of tissue from pig liver. Common lipids were detected in both DESI and MALDI ionisation techniques. By contrast, unique lipids were also observed for both ionisation techniques. Correlation of MALDI and DESI images showed similar spatial locations of lipid species, as might be expected, but differences in spatial resolution were observed and will be discussed.

Further results will be presented comparing and contrasting the two ionisation techniques from a range of tissue samples.

Conclusion

The combination of DESI and MALDI ionisation options on the same platform adds increased capability for the study of tissue molecular composition.

Novel Aspect

Study of DESI and MALDI ionisation technique on the same mass spectrometer for imaging applications.

MOS07-03 MALDI-MS imaging with a synapt G2-S mass spectrometer: improving the lateral resolution to ~7 µm and the sensitivity for lipid analysis by use of novel matrices

Hans Kettling, Simeon Vens-Cappell, Jens Soltwisch, Alexander Pirkl, Johannes Müthing, Klaus Dreisewerd
University of Münster

Introduction

Mass spectrometers from the Waters Synapt G2 family are widely employed for MALDI-MS Imaging (MALDI-MSI). By default, these instruments provide a lateral resolution of ≥50 µm, which is, however, far off the often desired cellular resolution. Here we demonstrate the first MALDI-MSI analysis with a lateral resolution of ~7 µm using a modified Synapt G2-S HDMS mass spectrometer (no oversampling was used). Among others, a dithranol matrix [1] was used which provides particularly uniform microcrystalline matrix coatings. We moreover introduce a set of novel MALDI matrices with condensed phenols that produce a low ion background and particularly high signal intensities for endogenous lipids in both the positive and negative ion modes.

Methods

Mouse brain slices of 15-20 µm thickness were prepared using a cryotome. Matrices were dissolved in CHCl3:MeOH (2:1) and sprayed onto tissue using a custom-made semi-automated pneumatic spray system. Uniform matrix layers with mean crystal sizes of <3 µm were produced this way. The ion source of the Synapt G2-S instrument was amended with electromagnetic valves and pressure gauges that are controlled by custom-made software, as well as apertures that confined the pressure regions. This allows adjusting the pressure in the sample region between 0.04-4 mbar.

Results

The default Nd:YAG-laser of the instrument was used. Laser beam shaping included the use of a beam expander (x4), a circular 35 µm-wide aperture for spatial mode filtering, and mounting a lens with a short focal length of 40 mm inside the ion source. This improved the effective laser spot size (determined by inspecting the ablation craters) by almost one order of magnitude to ~7 µm. Elevating the ion source pressure to about 0.7 mbar was, moreover, found to increase the lipid ion abundances by up to a factor of 5 for some matrices (e.g., dithranol). Analyte signal intensities, number

of lipid species detected (including complex glycolipids such as gangliosides) and reduction of background were even more enhanced upon using a set of condensed phenols as novel matrices, which were previously not described for use in MALDI mass spectrometry. Comparison of the obtained ion distributions with H&E-stained slices (prepared after washing off the matrix) allowed differentiation of small-sized brain areas with dimensions in the low ten µm-range.

Conclusion
A true lateral resolution of ≤10 µm (without oversampling) can be obtained with a front-end Synapt ion mobility instrument upon adopting a few laser beam shaping steps. Enhanced performance characteristics for the MALDI-MSI analysis of lipids can be achieved upon elevating the buffer gas pressure in the ion source. The use of novel matrices furthermore extends the range of complex lipid species detectable from tissue. We speculate that efficient collisional cooling enhances MALDI-MSI at ultralow spot sizes and that further refinements will in the future enable use of laser spots with diameters below 5 µm.
[1] Le CH, Han J, Borchers CH (2013) Anal Chem 84:8391-9398

Novel Aspects
First report on MALDI-MS imaging with a lateral resolution of ~7 µm using a commercial Synapt G2-S mass spectrometer.

MOS07-04 Low temperature plasma mass imaging (LTP-MSI): do-it-yourself instrumentation and applications in biology.
Robert Winkler
CINVESTAV Unidad Irapuato

Introduction
Biological mass spectrometry imaging (MSI) is becoming a central tool in life science research. For the ideal method, no o little sample preparation should be necessary prior to measurement, in order to reduce both, manual handling effort and artifacts. Hence, the use of ambient ionization technologies is getting increasingly popular. We focused our development efforts on low temperature plasma (LTP) ionization, because of the wide range of biomolecules which is detectable, and because the instrumentation is simple to adopt for different mass analyzers.

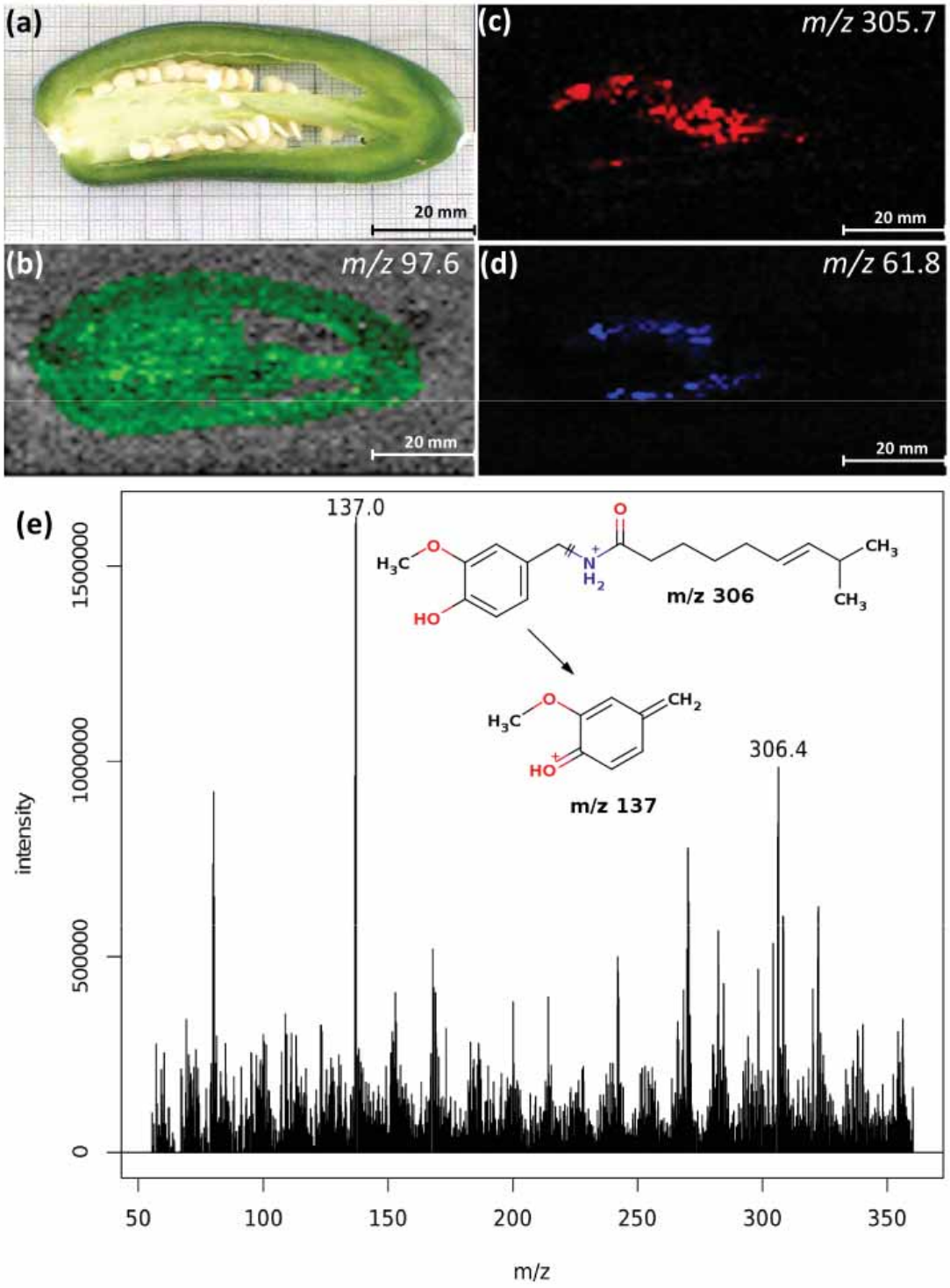
Methods
We developed a LTP-MSI system from low-cost commercial components. The diameter of our LTP beam is adjustable. The temperature of the plasma can be varied, to optimize the measurement according to the volatility of target compounds [1]. The sampling robot is built with Phidgets (http://www.phidgets.com), which are connected through an USB port to a standard computer. Our control software OpenMZxy (http://www.bioprocess.org/openmzxy) is open source and may be adjusted for individual needs [2].

Results
Diverse organic molecules such as alkaloids, terpenes, sugars, alkanes and amino acids can be ionized and measured using the LTP ion source. The complete LTP-MSI prototype can be built with less than 1,000 USD budget. Imaging biological tissues such as plant leaves or fruits revealed the distribution of molecules with a spacial resolution of about 1mm, which is sufficient for macroscopic samples. Interestingly, substances which are not easily accessible with other ionization methods, such as volatiles and highly hydrophobic compounds, are detectable with LTP ionization. Therefore, LTP-MSI is not only cost-efficient, but also supports the discovery of previously invisible molecules.

Conclusions
The building of a custom LTP-MSI system is manageable and the ambient ionization source can be mounted to various mass analyzers. Due to the ionization characteristics of the LTP source, yet unknown compounds and (semi)-volatiles can be detected. Altogether, LTP-MSI provides an attractive solution for biological imaging.

Novel Aspect
DIY solution for upgrading existing mass analyzers with ambient imaging; low temperature plasma mass imaging (LTP-MSI) of biological tissues.

References
[1] Martínez-Jarquín S, Winkler R. Design of a low-temperature plasma (LTP) probe with adjustable output temperature and variable beam diameter for the direct detection of organic molecules. Rapid Commun Mass Spectrom 2013;27:629–34.
[2] Maldonado-Torres M, López-Hernández JF, Jiménez-Sandoval P, Winkler R. “Plug and Play” assembly of a low-temperature plasma ionization mass spectrometry imaging (LTP-MSI) system. J Proteomics 2014.



MOS07-05 **High performance platform for atmospheric pressure high resolution MALDI mass spectrometry imaging**

Bernhard Spengler, Sabine Guenther, Andreas Römpp, Karl-Christian Schaefer, Oliver Schulz
Justus Liebig University Giessen

Introduction

Recent improvements in MALDI mass spectrometry imaging (MSI) are reported. High spatial resolution atmospheric pressure MALDI MSI on high mass resolution instruments has become a valuable histological tool providing molecular and topological information on biomolecules from various types of samples (1). High mass resolution allows to differentiate thousands of different compounds in complex plant or animal samples. High mass accuracy furthermore allows to identify many of these compounds based on determination of elemental composition. For structural characterization and identification, MS/MS analysis is performed directly on tissue and is evaluated in combination with topological information from MS/MS images.

Methods
Suitable matrices for positive or negative ion mode were homogeneously deposited on tissue sections by means of a high-resolution matrix-preparation robot (SMALDIPrep, TransMIT GmbH, Giessen, Germany). A high-resolution atmospheric-pressure MALDI ion source (AP-SMALDI10, TransMIT GmbH, Giessen) was used for imaging (1). The step size was set between 5 – 25 µm depending on targeted features. The source was coupled to a Q Exactive Orbitrap mass spectrometer (Thermo Scientific GmbH, Bremen), set to a mass resolving power between 50,000 and 140,000 at m/z = 200. Internal calibration was performed using a matrix ion signal as a lock mass, resulting in a mass accuracy of typically better than 3 ppm. In MS/MS mode, ion fragmentation during imaging measurements was performed by collisional activation in the HCD cell of the mass spectrometer. Images were created using our MIRION software. METLIN database search based on accurate mass and MS/MS data was used to identify compounds.

Results
Instrumental improvements led to a number of advantageous new features in high spatial resolution MSI. Reproducibility of the automated matrix preparation robot was improved, based on evaluation of gas dynamic theory of the spraying process and on in-depth analysis of matrix layer properties. Transmission of ions from the imaging source into the mass spectrometer was improved by optimization of electric fields and source geometry. The control software was improved for faster imaging scan rates. Visualization modes were extended by adding new algorithmic features to the image generation software package MIRION. Molecular images can now routinely be generated with a imaging bin width of $\Delta m/z = \pm 5$ ppm.

Conclusions
Molecular images of highest analytical quality were obtained with the improved instrumental setup. Examples from imaging plants, insects and mammalian tissue show the high reproducibility, high image contrast, low image noise and high information contents of the method.

References:
(1) Römpp, A, Spengler, B. (2013), J. Histochemistry and Cell Biology, 139, 759-783.

Novel Aspect
Improvements in instrumental properties and methodological procedures result in a new level of mechanistic insights into biochemical processes.

MOS08 - Carbohydrates

Room 3
Level 0

Chairs: Catherine Costello, Leopoldo Ceraulo

MOS08-01 Keynote: Automated, detailed glycan analysis by LC/MS for biotherapeutics and integrated biology

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Alterations in glycosylation are common in physiological and pathological processes as well as in the production of recombinant therapeutics. Glycan structures are, in the first instance, controlled by genes, however the complex pathways, systems and epigenetic factors that regulate their expression provide a further mechanism for fine tuning physiological responses by diversifying the glycans and the functions of the proteins to which they are attached.
Understanding the impact of genomic and post-genomic factors on glycosylation is a relatively underexplored field and requires new high throughput technologies that can match the output from other –omics platforms.
A robotic platform to release and label glycoproteins in a 96 well plate format has been developed as a front end to glycan separations technologies including HILIC and RP HPLC, MS and capillary electrophoresis, chip technologies and on-line combinations of these techniques, particularly LC/MS. Experimental data bases for these technologies that aid data interpretation are open source (http://glycobase.nibrt.ie/tools.html). The software and databases are now incorporated into Waters UNIFI 1.7 which presents reports that are GMP compliant. The software enables the detailed, quantitative structural assignments from HILIC profiling and exoglycosidase array digestions (which provide monosaccharide sequence and linkage (gu values)) to be directly compared with orthogonal on line MS technologies. This combines the advantages of both technologies and providing composition and fragmentation data in the same screen as the HILC data enabling confident, confirmed detailed assignments.
This talk will discuss the technology and pharma application as well as our recent attempts to explore an integrated glycobiology approach to cancer using these new technologies to generate both detailed glycan analysis and screening of large (>5,000) of samples.

MOS08-02 CE-ESI-MS/MS as a tool in protein glycosylation analysis

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The combination of capillary electrophoresis (CE) and ultrahigh resolution time of flight (TOF) mass spectrometry forms a powerful analytical platform for the analysis of biomolecules. CE is known for its high separation efficiency and sensitivity, especially in combination with innovative sheathless interfaces. We investigated the potential of sheathless CE-ESI-MS/MS for the analysis of protein glycosylation. For this purpose, we analysed digests of different glycoproteins: immunoglobulin (Ig)A, IgG and IgE, and prostate specific antigen (PSA). The digests were performed using various specific and nonspecific proteolytic enzymes. The CE-ESI-MS/MS analysis revealed separation of glycopeptides mainly on the basis of peptide moieties as well as degree of sialylation. Next to N-glycopeptide clusters, IgA1 hinge region O-glycopeptides were also readily detected.
Sialic acids are often found at the distal end of the glycan chain, serving as a binding site for human lectins, toxins and pathogens. A growing body of evidence shows that the linkage of sialic acids on glycoproteins is an important

Unpublished Figure

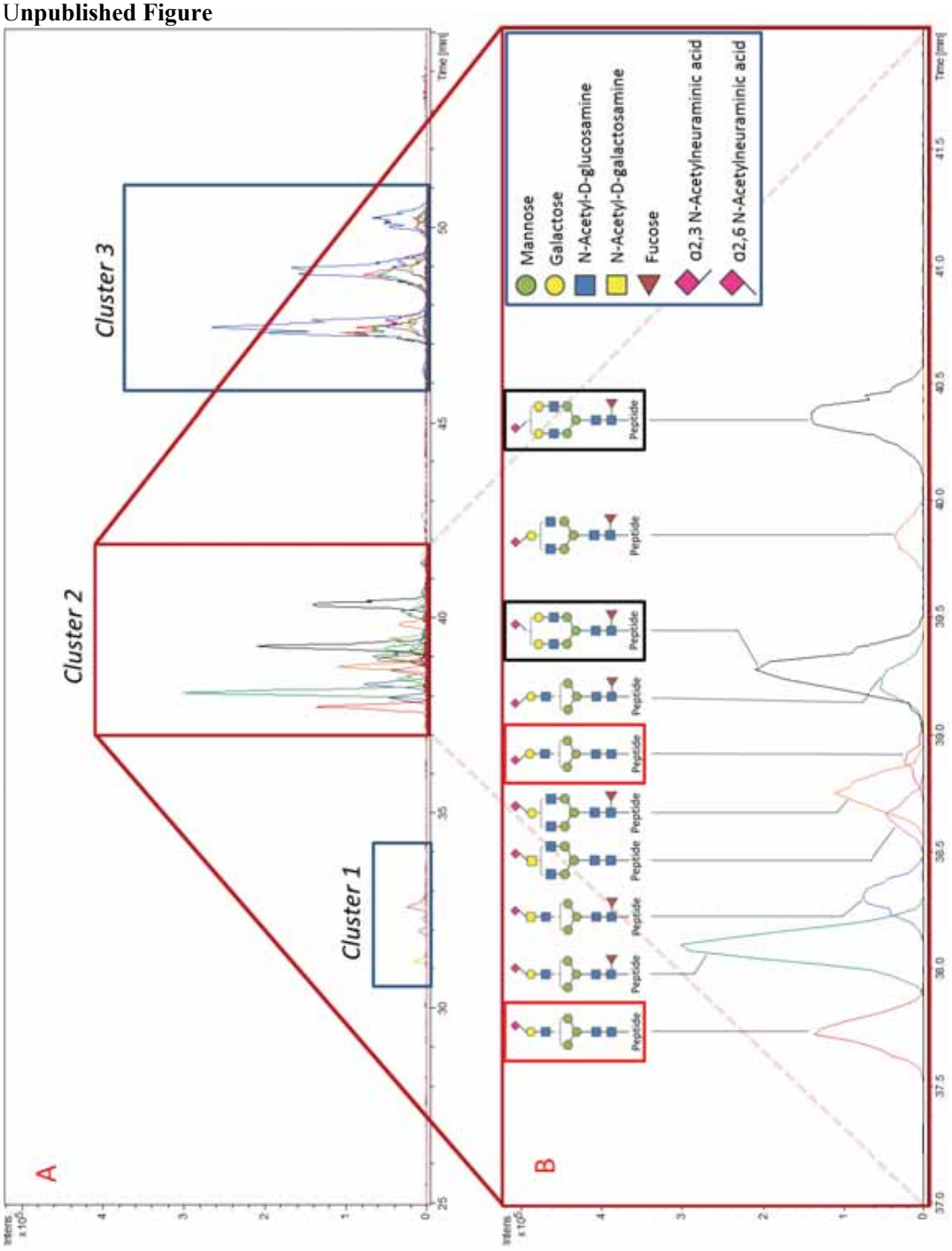

Figure 1: CE-MS analysis of PSA glycopeptides

A: CE-MS separation of non-, mono- and di-sialylated glycopeptides into three different clusters .

B: Extracted ion electropherograms of the most abundant glyco-compositions from the second cluster in Fig.1A, demonstrating a clear distinction between the different linkages of sialic acids (α2,3 vs α2,6).

maker of disease progression [1]. Interestingly, for PSA we observed isomer separation of sialylated glycopeptide species, suggesting that our CE method separates α2,3 and α2,6 sialic acid linkage isomers. The analysis of IgG Fc glycopeptides confirmed the separation of α2,3-sialylated glycopeptides, derived from recombinant IgG, from α2,6-sialylated glycopeptides, derived from human plasma IgG.

Taking all observations into account, we believe that our CE-ESI-MS/MS platform has great potential for studying glycosylation of proteins.

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MOS08-03 Xylan oligosaccharide mass profiling method for identification of *Arabidopsis thaliana* with altered O-acetylation in glucuronoxylans

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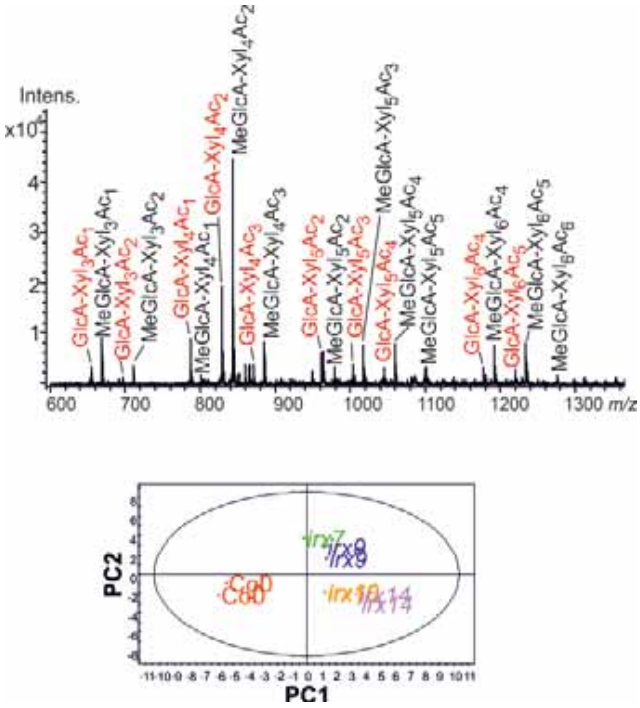
Mass spectrometry (MS) is a powerful tool for the biosynthetic studies of plant cell walls. The method requires only minute amount of oligosaccharides and is capable of elucidating the structures of heterogenic wall polysaccharides, such as hemicelluloses. O-acetylglucuronoxylans (AcGX) are the most abundant hemicelluloses present in flowering plants. The AcGX backbone is formed by β(1→4) linked xylopyranosyl (Xylp) residues, some of which carry α(1→2) linked (4-O-methyl)glucopyranosyluronic acid ((Me)GlcA) and acetyl residues at the 2-O/and 3-O positions. In the dicot species, AcGX reducing end is linked to a tetrasaccharide sequence (β-D-Xylp-(1→3)-α-L-Rhap-(1→2)-α-D-GalpA-(1→4)-β-D-Xyl). Isolation of xylans for structural studies is usually carried out in an alkaline solution resulting in concomitant deacetylation. Enzymatic hydrolysis releasing structural oligosaccharides for MS analysis was exploited to study O-acetylation in AcGX of Arabidopsis thaliana xylan biosynthesis mutants.
The xylan OLIgosaccharide Mass Profiling (OLIMP) method encompassing glycoside hydrolase (GH) family 10 endoxylanase hydrolysis and atmospheric pressured-matrix assisted laser desorption ionization-ion trap mass spectrometry (AP-MALDI-ITMS) detection was developed. The AP-MALDI-ITMS offers the advantage to analyze the mass and structure of carbohydrate in one system. GH10 endoxylanase cleaves the AcGX backbone into substituted



xylooligosaccharides (XOS). As the action is hindered by the side groups, endoxylanase can be used as a selective tool to liberate acetylated XOS directly from plant tissues, and the AcXOS fingerprint spectrum is acquired by MS detection. In this study, the % intensities of the mass peaks were compared and further on analyzed in Principal Component Analysis (PCA) to identify the Arabidopsis mutants with altered O-acetylation in AcGX.
The analysis of Arabidopsis xylan mutants defective in reducing end sequence or backbone synthesis, irregular xylem (irx)7, irx9, irx14 and irx10, clearly showed that acetylation was pleiotropically reduced in irx7 and irx91. In addition,

the analysis of double and triple reduced wall acetylation (rwa) mutants defective in cell wall polymer acetylation revealed that xylan acetylation was reduced in rwa3rwa4 and rwa1rwa3rwa42; indicating RWA3 and RWA4 are responsible for AcGX acetylation. Besides, the analysis of (Me)GlcA (gux1gux2) and acetylation (rwa1rwa3rwa4) deficient mutant showed that the glucuronidation was taking place before acetylation of xylans1 and the methylation of GlcA residues was affected by the reduced 3-O acetylation2, respectively; suggesting the interplay between processes involved in decorating the AcGX backbone.

Novel aspect
The xylan OLIMP combining GH10 endoxylanase hydrolysis and AP-MALDI-ITMS detection is a selective and sensitive tool for studying the acetylation of O-acetylglucuronoxylans in a semi-high throughput manner. The method is suited to analyze large numbers of samples.

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MOS08-04 Meaning and consequence of the competitive presence of the hydrogen bond and salt interactions on the dissociation orientation of deprotonated adducts in ESI

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Introduction

ESI/MS and MS/MS have become widely popular in metabolomics. The coupling with liquid chromatography is very useful for the analysis of complex mixtures of metabolites characterized by a large structural diversity. In ESI, adduct ions (as non-covalent, NC) often accompany the molecular species with eventually, product ions. Such NC species are generally stabilized by hydrogen bonding (HB) as ion-dipole (Idp) complexes easily cleaved by collisions. However, in several cases, salts (ion-ion interactions) may be produced from aggregates involving ion-pairing agents. Importantly, salt formation occurs only from zwitterion structure. Such species dissociate through covalent bond cleavage(s) without affecting the ion-ion interaction. This leads to misinterpretation of CID spectra assuming that «adduct ion» is covalently linked.Here, we are interested in NC complexes stabilized by competitive HB and ion-ion interactions.Our study is focused on NC complexes formed between various basic agents (BA) as amines or amino acids (R or K), and phosphorylated monosaccharide isomers (sugar phosphates, SP).

Methods

Non-covalent complexes between mono- and/or bisphosphorylated fructose and glucose isomers were analyzed using low- and high- resolution mass spectrometry. Sequential MSn spectra were recorded on a LTQ-Orbitrap XL (Thermo Electron Corporation, Germany) equipped with an ESI source in negative mode. These experiments were completed using QTRAP 5500 instrument (ABSciex, Canada). Resonant and «in axis» collision-induced dissociations were performed under various excitation energies.

Results

Various isomeric adduct anions+ based on the formal [BA+SP-H]- and [BA+SP-2H+Na]- species were analyzed under various CID conditions. Significant differences in the corresponding CID spectra were observed according to the excitation mode, isolation and/or activation conditions. The CID spectra display diagnostic ions from both HB and salt forms proving their coexistence and thus, that of canonic and zwitterion monomer forms. More interestingly, the [AA+PS-2H+Na]- amino acid adduct ions dissociate by direct sugar covalent bond cleavages and water release in addition to the competitive formation of [SP-H]- (with [SP+Na-2H]-) and [AA-H]- (with [AA+Na-2H]-) monomer ions.

Conclusion

Adduct ion fragmentation behavior reflects the dependence of the NC complex species distribution on the partner structure. The competitive stability of the HB and salt forms in ESI shows the role of the thermochemical control on desolvation process due to mainly the ambident character of sugar phosphates and amino acids. From the analytical point of view, these experiments highlight how structural elucidation of metabolites from CID spectra can be affected by the presence of adduct ions constituted by ion-ion interactions instead of HB or Idp interactions.

Novel aspect:

Production of the hydrogen bond and salt complex populations under thermochemical control.

MOS08-05 Improved glycopeptide analysis using acetonitrile enriched sheath gas and oxonium ion dependent ETD

Kristina Marx, Andrea Kiehne, Markus Meyer, Pierre-Olivier Schmit
Bruker Daltonics GmbH

Introduction

MS analysis of protein glycosylation is a major topic. Glycopeptide analysis is of particular interest in research areas as detailed information about glycan, peptide and glycosylation site is needed in order to understand relationships between structure, location and function. Generally, biological samples are complex mixtures of proteins and glycoproteins, further challenging the complete analysis of glycopeptides. An efficient combination of collision induced dissociation (CID) and oxonium ion triggered electron transfer dissociation (ETD) allows for extensive protein sequence coverage and detailed glycopeptide profiling in one run.

Methods

The Sigma UPS standard consists of 48 human proteins (MW 6- 3 kDa) with different post-translational modifications including glycosylation. The standard was reduced, carbamidomethylated and digested with trypsin. The tryptic peptides were separated on an UltiMateTM 3000 nanoRSLC with an Acclaim PepMap C18 column. An ion trap (amaZon speed ETD, Bruker Daltonics) equipped with a CaptiveSpray source. Spectra were acquired in “Fragment Triggered ETD” mode using Enhanced Resolution. Acetonitrile-enriched nitrogen was used as sheath gas to enhance the glycopeptide intensities and charge states. Data processing was performed by ProteinScape 3.1 SW. First, glycopeptide CID spectra were filtered out and searched against CarbBank database supported by the integrated search engine GlycoQuest. Afterwards, ETD spectra were used for glycopeptide sequencing.

Results

The main challenges in glycopeptide analysis in complex mixtures are the low concentration and the reduced ionization efficiency compared to non-glycosylated peptides. A significant improvement of glycopeptide detection sensitivity can be achieved by acetonitrile-enriched sheath gas. For the identification of non-glycosylated peptides as well as for the characterization of the glycan moiety of glycopeptides low energy CID in the ion trap is applied. The obtained spectra were submitted to a database search for protein identification via Mascot, and the glycopeptide CID spectra were searched against CarbBank using GlycoQuest in order to identify the glycan moiety. ETD is mandatory for the sequencing of the glycopeptide backbone, so all glycopeptides are additionally subjected to ETD. The ETD reaction is only triggered if oxonium ions have been observed in the former CID spectrum (“Fragment Triggered ETD”). This acquisition method allows for an efficient analysis of glycopeptides in complex mixtures.

Conclusions
ETD and CID for enhanced information in glycopeptide analysis

Novel Aspect
Glycopeptide intensity boosting and oxonium ion dependent ETD for efficient glycosylation site profiling in medium complex samples.

MOS09 - Environment – Biological Systems Interactions

Room 4
Level 0

Chairs: Kristin Schirmer, Thomas Hofstetter

MOS09-01 **Keynote: Deciphering the chemical language of insects by mass spectrometry**

Joanne Yew
Temasek Life Sciences Laboratory

The social behavior of insects is largely controlled by lipid pheromones, chemical messages that are used to communicate with members of the same species. The pheromone profile of individual insects is dynamic and can reflect changes in the environment, life history, and social experience. Thus, by eavesdropping on the chemical language of insects, we can gain insight into how behavior and ecology shape and are shaped by pheromones. Moreover, pheromone ligands can be used to control the reproduction of disease-bearing insects and agricultural pests.
Gas chromatography MS (GCMS) is the most widely used analytical method for insect pheromone analysis, many of which are lipids. While GCMS provides some structural information, analysis time can be long and little information can be gained about spatial expression. Here, I describe the use of alternative, complementary MS methods for direct pheromone analysis from single, intact insects.
UV-laser desorption/ ionization (UV-LDI) MS and Direct Analysis in Real Time (DART) MS are used to analyze the cuticular lipid profile of intact and, on occasion, live insects, including numerous Drosophila species, Sepsidae flies, and butterflies. Intact insects or dissected body parts are analyzed directly in the mass spectrometer, allowing rapid chemical profiles to be obtained with little sample preparation and no chemical matrix. In addition, both methods are able to detect higher molecular weight and more polar molecules, compounds which can be difficult to ionize under standard GCMS conditions
The use of MS characterization in parallel with genetic manipulation and behavioral assays has led to the identification of novel pheromones classes including an array of Drosophila sex-specific triacylglycerides with unconventional structures. In addition, the fine spatial resolution of UV-LDI MS (100-200 µm) resulted in the discovery of secretory glands and detection of pheromone profile changes specific to different body parts. Recent experiments using MS imaging have allowed in-situ visualization of a pheromone production organ in Drosophila. Lastly, new lipid-related genes involved in the biochemistry of pheromone synthesis have been identified using an MS-based screen.

Novel aspects
Direct MS analysis of intact and live insects has led to the discovery of novel lipid pheromones and identification of pheromone-secreting glands.

MOS09-02 **Characterization of plant glycated proteome and its changes during ageing and under environmental stress conditions**

Andrej Frolov[1], Elena Lukasheva[2], Dominic Brauch[1], Tatiana Bilova[1], Juliane Mittasch[3], Carsten Milkowski[3], Natalia Osmolovskaya[2], Ludger Wessjohann[4]
[1]Universität Leipzig, [2]Saint-Petersburg State University, [3]Martin Luther University Halle-Wittenberg, [4]Leibniz Institute of Plant Biochemistry

Introduction
Protein glycation is formed by reaction of reducing sugars with amino groups and commonly accompanies thermal food processing. Further oxidation of resulting Amadori compounds (glycoxidation) yield advanced glycation end-products (AGEs) known for their pro-inflammatory effects in humans. However, glycation may occur also before thermal treatment, i.e. during the life time of crop plants. In this context, it is important to know, if glycoxidation is increased in crop plants affected by environmental stresses and ageing.

Methods
The models of high light and metal stress were established with Arabidopsis thaliana and Brassica napus, respectively. The leaves and roots were harvested before stress application and in multiple points throughout the stress period. Soluble proteins were isolated, digested with trypsin and the digests were analyzed by LC x LC-LIT-Orbitrap-MS/MS, using boronic acid affinity chromatography or HILIC as the first dimension and RP-nanoUPLC as the second one. Modified peptides identified by database search were quantified by label-free nanoUPLC-ESI-Orbitrap-MS approach.

Results and Discussion
Both control and stressed A. thaliana and B. napus plants displayed rich patterns of glycated and glycoxidated proteins, representing mostly regulatory pathways, protein and nucleic acid metabolism. For plants, this information, as well as exact modification sites, is reported here, to the best of our knowledge, for the first time. In B. napus the product pattern dominated with triose- and tetrose-derived early glycation products and Nε-carboxymethyllysine (CML), methylglyoxal-derived hydroimidazolone (MGH) and argpyrimidine. However, glyoxal-derived hydroimidazolone (Glarg)-modified peptides were more abundant in A. thaliana plants. Glycated products related to multiple intracellular proteins clearly accumulated during the plant ontogenesis. However, though levels of glycation were increased under stress conditions in comparison to controls, only certain AGE types showed higher abundance in those plants.

Conclusion
Plant protein glycation and glycoxidation patterns are characterized for the first time. These patterns undergo qualitative and quantitative changes during plant ontogenesis and under stress conditions.

Novel aspects
The patterns of plant glycation are characterized for the first time. Ageing and plant stress increase degree of protein glycation and change the pattern of modified proteins

Keywords
Tandem mass spectrometry, glycation, glycoxidation, AGEs, environmental stress, A. thaliana, B. napus

MOS09-03 Metabolomic approaches to assess neurotoxic effects of Imidacloprid on the freshwater snail *Lymnaea stagnalis*

Sara Tufi, Marja Lamoree, Pim Leonards
Free University of Amsterdam

Introduction
Imidacloprid is one of the most used neonicotinoid insecticides worldwide. This class of insecticides acts as nicotinic acetylcholine receptors (nAChRs) agonists on the post-synaptic membrane leading to impaired nerve impulses. Even though Imidacloprid has been specifically designed for insects, neurotoxic effects have also been reported in other invertebrate species.In the Netherlands concentrations of Imidacloprid are frequently exceeding water quality norms and in addition, a significant decline in macro-invertebrates in Dutch surface waters has been reported. Due to the extensive use of Imidacloprid in agriculture, concerns have been raised about increasing effects in non-target species affecting ecosystem functionality. Therefore, we investigated the effect of Imidacloprid exposure on the metabolome of the central nervous system (CNS) of the pond snailL. stagnalis.

Methods
The freshwater snails L. stagnalis have been exposed to different concentrations of Imidacloprid (0.1, 1, 10 and 100 µg/L) for two and ten days. After the exposure the snails were sacrificed and the CNS dissected. A biphasic chloroform/methanol/water mixture has been used to extract the hydrophilic and lipophilic fractions which have been analyzed by a cross platform metabolomic approach based on hydrophilic interaction liquid chromatography (HILIC) and gas chromatography (GC). A targeted analysis of the main neurotransmitters, their precursors and metabolites has been employed in order to focus on neuronal metabolic pathways. Multivariate data analysis has been applied to find correlations between time and exposure concentrations to elucidate the mode of action of Imidacloprid in Lymnae stagnalis.Significant differences in metabolites levels were determined using one way analysis of variance (ANOVA).

Results
This study sheds light on the mode of action of Imidacloprid, highlighting the involvement of different metabolic and neuronal pathways. Potential biomarkers of exposure were found already at the lowest exposure concentration, which was a realistic environmental concentration. These results were compared with responses observed in a acetylcholinesterase activity assay, showing that metabolic chemical endpoints are more sensitive for detection of toxicant exposure of freshwater snails.

Conclusions
The targeted and untargeted metabolomic approaches have been successfully applied to understand the metabolic alteration of L. stagnalis caused by Imidacloprid exposure.

Novel aspects
Metabolic perturbation and biomarkers of exposure were observed at environmentally relevant concentrations. Our approach lead to the identification of more sensitive biomarkers than currently used endpoints to assess neurotoxicity.

MOS09-04 Cocktail approach for microsomal CYP450 phenotyping using UHPLC-QTOF

Dany Spaggiari[1], Laurent Geiser[1], Youssef Daali[2], Serge Rudaz[1]
[1]Université de Genève, [2]Hopitaux Universitaires de Genève

Dioxygenation is an important biochemical reaction that often initiates the mineralization of recalcitrant aromatic contaminants such as nitroaromatic explosives, chlorinated solvents, and polycyclic aromatic hydrocarbons in the environment. To date, the extent of contaminant dioxygenation in contaminated soils and aquatic systems, is assessed from changes in pollutant stable isotope ratios using compound-specific isotope analysis (CSIA). Because such dioxygenations are often accompanied by additional oxidative side-reactions, isotopic analysis of the contaminants impedes the derivation of pathway-specific kinetic isotope effects (KIEs). The evaluation of the more indicative isotope ratios of the hydroxylated product, however, is not feasible using the popular analytical approaches with gas chromatography coupled to isotope ratio mass spectrometry.
Here, we present an alternative analytical method to determine carbon isotope fractionation in oxidation products such as substituted catechols and benzyl alcohols with which pathway-dependent KIEs could be derived. Analyte separation was achieved by reversed-phase liquid chromatography using a purely aqueous eluent with fast temperature gradients up to 160°C. The separated compounds were converted online to CO2 in a wet oxidation interface (Finnigan LC IsoLink, Thermo Scientific) coupled to an isotope ratio mass spectrometer.
Method quantification limits ranged between 9.0 and 10.5 nmol of injected carbon for different (methyl) catechols and nitrobenzyl alcohols. Validation of dioxygenation KIEs was carried out based on the dioxygenation of nitrobenzene to catechol by nitrobenzene dioxygenase (NBDO) where substrate isotope fractionation was measured by GC/IRMS

while substrate and product isotope fractionation was accessible by LC/IRMS. The agreement of C isotope enrichment factors from substrate and product isotope ratios confirmed our approach, which was subsequently applied to study the pathway-specific KIEs of 2-nitrotoluene to multiple oxidation products.
Our study is the first to apply LC/IRMS for the investigation of stable isotope fractionation in organic pollutants and their polar oxygenation products as a complementary approach for the determination of pathways-specific KIEs.

MOS09-05 **Compound-specific isotope analysis of dioxygenation products by LC-IRMS**
Sarah Pati, Jakov Bolotin, Hans-Peter Kohler, Thomas Hofstetter
Eawag, Swiss Federal Institute of Aquatic Science and Technology

Introduction
Cytochromes P450 (CYPs) are the major phase I metabolic enzymes involved in the oxidative biotransformation of xenobiotics. The activity of CYPs has high inter-individual variability due to genetic polymorphisms and/or environmental factors (e.g. diet, drug therapy, toxic agents, etc.) which are part of the individual phenotype. Some of the major isoforms of the CYP superfamily involved in the metabolism of marketed drugs are recognized as highly polymorphic. According to the type of allelic variant affecting these CYPs, genetic polymorphism can significantly alter their metabolic activity, thus modifying the clinical response and/or increasing the risk of drug-drug interactions (DDI). For these reasons, phenotyping approaches are essential to evaluate and/or anticipate the CYPs activities.

Methods
A cocktail mixture was elaborated to increase the throughput of in vitro phenotyping studies by monitoring several CYPs activities in a single test. The cocktail comprised 8 CYP-specific probe substrates to simultaneously assess the activity of the most important CYPs, namely 1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1 and 3A subfamily. After cocktail incubation in optimized conditions with human liver microsomes (HLM), the substrates and their metabolites were analysed by a generic LC-MSE method using ultra-high-pressure liquid chromatography coupled with electrospray ionization quadrupole time-of-flight (QTOF) mass spectrometry (MS).

Results
This cocktail approach was applied to generate the CYP phenotypic profile of different allelic variants of HLM and results were in full agreement with CYPs genetic polymorphisms. Moreover, with an appropriate spiking of recombinant CYPs, it was possible to selectively modify the intrinsic activity of the polymorphic HLMs. In addition, this approach was successfully used to enhance the background understanding of the behaviour of xenobiotics or toxic agents on CYPs activities. In this context, the developed cocktail approach was applied to assess the impact of several phytochemicals and insecticides on microsomal CYPs activities.

Conclusions
The presented cocktail approach could be applied to correlate the metabolism rate with genetic polymorphisms of HLMs. In addition, it successfully highlights the impact of several compounds. Therefore, the proposed approach could be used to evaluate any potential DDI and/or toxicological effects.

Novel Aspect
Reliable qualitative and quantitative data of the incubation mixture were simultaneously acquired, producing an overview of the phase I biotransformation routes for each probe substrate in HLM. Considering that one CYP can be involved in the formation of several metabolites, a more precise CYPs phenotypic profile could be generated using QTOF-MS. In addition, the HLM spiking approach offers the possibility to mimic in vitro a specific microsomal CYP-phenotypic profile opening a new potential access to personalized medicine investigations and/or to anticipate phenomena.

MOS10 - Microbes and Viruses

Room 6
Level 3

Chairs: Günter Allmaier, Robert Hettich

MOS10-01 **Keynote: Rapid characterization of microorganisms by mass spectrometry: what can be learned and how**
Catherine Fenselau
University of Maryland

MALDI time-of-flight mass spectrometry and MALDI ion trap mass spectrometry have been developed as fieldable systems for rapid detection of unprocessed microorganisms in the battle field. Recently this direct approach has been extended for use in clinical microbiology in hospitals. Automated data processing has a critical role in both of these applications, and both library matching and proteomic bioinformatics have been developed to identify bacteria based on their mass spectra. This presentation will include an overview of the evolution of biomarkers observed directly from unprocessed bacteria using various ionization techniques and analyzers. Current limitations and challenges will be defined, and a prospectus will be presented.

MOS10-02 **Top-down mass spectrometry probes immune evasion by pathogenic *Neisseria meningitidis***
Julia Chamot-Rooke[1], Joseph Gault[1], Christian Malosse[1], Marie-Cecile Ploy[2], Catherine Costello[3], Guillaume Duménil[4]
[1]Institut Pasteur, [2]Limoges Université Hospital, [3]Boston University Medical School, [4]INSERM

Introduction
Throughout the bacterial kingdom, pathogen survival is linked to successful and effective escape from the immune system. This may be achieved by varying the structure or presence of surface exposed molecules on the bacterial surface. For Neisseria meningitidis (Nm), the etiological agent of cerebrospinal meningitis, important surface structures shown to be key to virulence are type IV pili (Tfp). Tfp are long and filamentous appendages that protrude from the bacterial surface. They are essentially composed of a single protein subunit or major pilin, PilE. In a previous work on

Nm reference strain, we showed that pilE can be highly posttranslational modified [1,2]. Here, we extend our study to a wide number of previously uncharacterised clinical isolates. We show, using top-down mass spectrometry, that PilE posttranslational modifications (PTMs) are tightly linked to bacterial immune evasion.

Methods
PilE proteins were purified from N. meningitidis clinical strains collected from patients with evidence of meningitis. Top-down experiments were performed either on a 12T solariX FT-ICR equipped with a hollow dispenser cathode or on an LTQ-Velos Orbitrap. For MS/MS experiments, ions of interest were submitted to ECD or ETD.

Results
High Resolution Mass profiling of all PilE proteins indicated that they were consistently found as multiple proteoforms and to carry an unprecedented number of PTMs. An in-depth investigation of a number of strains revealed that a classical bottom-up methodology was fundamentally unable to achieve full proteoform characterisation. Top-down mass spectrometry was therefore required and employed to achieve complete proteoform characterisation, highlighting the presence of multiple and multisite glycosylation of PilE [3]. Using the PTM localisation data provided by top-down mass spectrometry, molecular modelling of pilus fibers was performed and showed that the fiber surface is covered by glycans. Combined with the analysis of the genomic context of our meningococcal isolates, our results show that top-down mass spectrometry is a very efficient tool to completely map PTMs on bacterial proteins involved in virulence and probe their role in bacterial immune escape.

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2. Gault et al., J. Mass Spectrom., 48, 11, 1199 (2013)
3. Gault et al., Proteomics DOI: 10.1002/pmic.201300394

Novel aspect
First time top-down mass spectrometry is used on clinical samples as a tool to probe bacterial immune evasion

MOS10-03 Improvement in bacterial strain differentiation by MALDI-TOF MS profiling by using microwave-assisted enzymatic digestion

Zbynek Zdrahal, Ondrej Sedo
Masaryk University

MALDI-TOF MS profiling is routinely used in microbiologyfor identification of bacteria on the basis of their peptide/protein profiles. However, the discriminatory power of the method is not always sufficient in certain caseswhich require distinguishing of individual strains, as closely related strains can yield very similar MALDI-TOF mass spectra.
Strains of Staphylococcus aureus, Staphylococcus haemolyticus, and Bacillus subtilis (two ecotypes) were subjected to standard sample preparation procedure involving formic acid/acetonitrile extraction and intact protein profiling by MALDI-TOF MS. As a novel method variant, the bacterial cells were digested by trypsin under microwave irradiation for 2 min prior to the MALDI-TOF MS profiling. The evaluation of the discriminatory power of the method was carried out on the basis of cluster analysis.

The MALDI-TOF mass spectra obtained by the standard method did not provide signals enabling distinguishing between most of Staphylococcus strains belonging to the same species. Also the Bacillus subtilis strains were not differentiated on the basis of their ecotypes. The number of strain-specific signals was significantly increased by the novel method involving accelerated digestion. The analyses of S. aureus strains carried out in weekly intervals confirmed the ability of the method to distinguish between closely related strains repeatably. The robustness of the method was verified by distinguishing S. haemolyticus strains digested in three different commercially available microwave ovens. Although the B. subtilis ecotype discrimination was not absolute, significant improvement in the strain clustering, in comparison to the standard method, was also obtained.
Microwave-assisted digestion of bacterial cells by trypsin was found to improve differentiation of bacterial strains. The developed methodical variant represents a rapid, simple, and effective way to modulate the discriminatory power of MALDI-TOF MS profiling.

Acknowledgement
The authors thank to Ines Mandić-Mulec, Polonca Štefanič (University of Ljubljana) and Michiel Vos (University of Exeter) for preparation of Bacillus samples. This work was supported by project CEITEC - Central European Institute of Technology (CZ.1.05/1.1.00/02.0068) and by European Social Fund (CZ.1.07/2.3.00/20.0189).

MOS10-04 Discrimination of cyanobacterium *Microcystis aeruginosa* by MALDI-MS and analysis of its genetic diversity

Liwei Sun[1], Hiroaki Sato[2], Masanobu Kawachi[3], Xiwu Lu[1]
[1]Southeast University, China, [2]National Institute of Advanced Industrial Science and Technology, Japan, [3]National Institute for Environmental Studies, Japan

Microcystis aeruginosa, one of the most prevalent bloom-forming cyanobacteria, which produce hepatotoxic microcystins, has become a serious problem. Efforts employing gene technologies have revealed that there are high genetic diversity and clonality in M. aeruginosa. Also, both toxic and non-toxic colonies coexist in nature. Many attempts have been made to develop a rapid typing method to distinguish toxic M. aeruginosa from non-toxic strains, even employing microcystin genes themselves as markers. However, genetic similarity and toxicity are not always correlated. A higher resolution typing method is required to overcome the problem.
Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is beginning to play an important role in the rapid identification of microorganisms. Ribosomal proteins were demonstrated as effective biomarkers to identify microorganisms from species to strain levels in our previous studies. In this study, MALDI-MS was applied in identification of M. aeruginosa strains in order to investigate its genetic diversity and distinguish the toxic and non-toxic strains.

The type strain of M. aeruginosa, NIES 843, in which the whole chrome was already sequenced, was employed to study the sample preparation procedures and optimized conditions to observe ribosomal proteins as major peaks. A list of 54 ribosomal proteins was first made by calculating their molecular weight from their gene sequences registered at the NCBI database (http://www.ncbi.nlm.nih.gov/).
Three pre-treatment methods were compared: intact cell, cell lysis and 70S ribosomal subunit by ultracentrifugation. Although significant peaks were detected from intact cell and cell lysis samples, they could not be attributed to ribosomal proteins. On the other hand, 31 peaks from the ribosomal subunit samples matched the calculated molecular weight, therefore they were identified as ribosomal proteins. From these, 15 ribosomal proteins, which are Intelligible and without post-translation modification, were finally selected as biomarkers.Consequently, the 70S ribosomal subunit by ultracentrifugation was regarded as optimum pre-treatment method.
Twenty-five strains of M. aeruginosa were then measured by MALDI-MS. Based on the 15 ribosomal protein biomarkers, different ribosomal protein types were observed from their spectrum, suggesting the high genetic diversity of the M. aeruginosa and the high resolution of the MALDI-MS method in identifying M. aeruginosa at strain level. Furthermore, polygenetic analysis based onunweighted pair-group method with arithmetic means (UPGMA) method and different ribosomal protein types demonstrated that, these strains could be grouped into two groups, in which toxic strains and non-toxic strains seemed dominant, respectively. However, more evidence is needed to support the conclusion and explain the sporadic strains in each group. This research is the first report for the discrimination of cyanobacteria by MALDI-MS.

MOS10-05 Deep quantitative proteomics to reveal regulatory mechanisms that govern carbon metabolism in mycobacteria.

Marc Moniatte[1], Tarun Chopra[2], Romain Hamelin[3], Florence Armand[3], Diego Chiappe[3], John D. McKinney[2]
[1]EPFL SV PTECH PTP, [2]EPFL, Laboratory of Microbiology and Microsystems, [3]EPFL, Proteomics Core Facility

Introduction
Mycobacterium tuberculosis (Mtb) is the etiological agent of the disease tuberculosis. Studies aimed at characterizing its metabolic features within the host have hinted at fatty acids being an important carbon source utilized by the bacterium in vivo. Utilization of fatty acids requires considerable re-wiring of metabolic networks. A comprehensive knowledge of the mechanisms responsible for this adaptation would enable better understanding of the organism's physiology and also aid in development of novel drug therapies for tuberculosis treatment. Mycobacterium smegmatis (Msm) is used as a model system for Mtb, due to their similarity in basic cellular processes and non-pathogenicity of this species of mycobacteria. Here, we present a deep quantitative proteomics approach that provides a global view of how the Msm metabolic networks adjust to utilization of fatty acids as a carbon source.

Methods
M. smegmatis mc2155 were cultured on M9 minimal media supplemented with either glucose, acetate or propionate as carbon sources. Acetate and propionate are the immediate downstream products of fatty acid β-oxidation.
Protein extracts (10 µg) were digested with trypsin and subsequently labeled by a dimethyl labeling approach. Labeled peptides were pooled in 1:1:1 ratio and were fractionated by SAX (StageTip) into 6 fractions. After desalting, all fractions were analyzed by LC-MS/MS using a 235 min shallow gradient on a QExactive instrument. Four biological replicates were analyzed. Stringent criteria were used for identification and quantification. Only proteins present in 3 out of 4 replicates were used for quantification.
Quantitative data analysis was performed using MaxQuant and homemade tools and scripts.

Results
Two-dimensional liquid chromatography and mass spectrometry of isotopically-labeled peptides identified a total of 3,067 proteins with high confidence. This number corresponds to 44% of the predicted Msm proteome and includes most of the predicted metabolic enzymes. Compared to glucose-grown cells, 162 proteins showed differential abundance in acetate- or propionate-grown cells. Amongst these, acetate-grown cells showed higher abundance of proteins that could constitute a functional glycerate pathway. Gene inactivation experiments confirmed that both the glyoxylate shunt and the glycerate pathway are operational in Msm. We also demonstrate carbon source-dependent differential abundance of proteins that have not yet been functionally characterized.

Conclusion
Quantitative proteomics-based insights into glucose, acetate, and propionate assimilation in M. smegmatis suggest significant plasticity of metabolic networks.

Novel aspect
Deep quantitative proteome analysis reveals several novel features of carbon assimilation in M. smegmatis, indicating a richer metabolic capacity of this saprophytic species of mycobacteria.
Keywords: metabolism; mycobacteria, proteomics

TOS11 - Targeted and Quantitative Proteomics

Room 1
Level 1

Chairs: Paola Picotti, Markus Stöckli

TOS11-01 **Keynote: Dynamic signaling interactomes in health and disease**

Anne-Claude Gingras
Lunenfeld-Tanenbaum Research Institute at Mount Sinai Hospital / Department of Molecular Genetics, University of Toronto

Introduction
Kinases and phosphatases coordinate critical cellular decisions, including whether to grow and divide, to differentiate into a specific cell type, or to die. They must respond to environmental cues and transmit precise signals. Deregulation of the phosphorylation balance is implicated in multiple diseases, including cancer and neurodegenerative diseases. This deregulation can involve mutations directly in the kinase or phosphatase proteins, changes in their splicing patterns, or may involve expression modulation; all these events can lead to network rewiring. Since kinases and phosphatases frequently associate with regulators, scaffolding molecules and substrates, a possible outcome of response to a cue, or a mutation or splicing alteration (besides modulation of intrinsic catalytic activity) is a change in these physical interactions.

Methods
In the past several years, we have developed proteomics methods to monitor these regulated interactions for key signaling molecules. These include a coupling of affinity purification (AP) with Selected Reaction Monitoring or with the data independent acquisition approach SWATH. We recently introduced (Lambert et al., Nature Methods, 2013) a normalization strategy to automatically calculate fold change and confidence in the regulated interactomes. With our collaborators, we have also been developing software tools to perform identification from SWATH data, leading in a more efficient utilization of the instrument time, while increasing sensitivity in the detection of the regulated interactions.

Results
We have harnessed the AP-SWATH approach to probe the dysregulation of kinases and phosphatase interactions induced by mutations, and to analyze the consequences of pharmacological treatment on the interactions established by signaling proteins. We will discuss these approaches in the context of cancer and vascular disease.

Conclusions
Coupling affinity purification to MS2 level quantification such as SRM or SWATH accelerates the study of the consequences on interactions of point mutations, splicing alterations or drug treatment. Current ongoing efforts include an improved selection of the cancer-associated mutants to be profiled, using a structural-based strategy.

Novel aspect
Quantitative proteomics platform optimized for the analysis of interaction proteomics dynamics

TOS11-02 **The impact of biochemical background on quantification ranges of data-dependent, directed and targeted proteomics strategies**

Alexander Schmidt, Manuel Bauer, Erik Ahrne, Anna Baron, Timo Glatter, Anna Santamaria, Erich Nigg
Biozentrum, University of Basel

Introduction
Over the last years, directed and particularly targeted mass spectrometric workflows have gained momentum as alternative techniques to conventional data-dependent acquisition (DDA) LC-MS/MS approaches. By focusing on specific peptide species, these methods allow hypothesis driven analysis of specific protein sets of interest and have shown to be especially suited to monitor low abundant protein species within complex mixtures. Despite their growing popularity, so far, no study has evaluated the three approaches in terms of quantification, detection and identification limits in complex samples.

Methods
Here, we systematically compared the performance of conventional DDA, directed and various parallel reaction monitoring (PRM) approaches using a hybrid linear ion trap - orbitrap MS platform as well as selected reaction monitoring (SRM) using a triple quadrupole instrument. We assessed the limits of identification, quantification and detection for each method by analyzing a dilution series of 20 unmodified and 10 phosphorylated synthetic heavy labeled reference peptides, respectively, covering six orders of magnitude in peptide concentration with and without a complex human cell digest background.

Results
We found that while without background all methods performed very similar, targeted methods demonstrated to be at least 5-10 times more sensitive than directed or DDA methods when analyzing complex samples. In particular, higher stage fragmentation (MS3) of the neutral loss peak using a linear ion trap increased dynamic quantification range of some phosphopeptides up to 100-fold. We illustrate the power of this MS3 PRM approach for monitoring phosphopeptides by successfully quantifying 9 phosphorylation sites of the protein kinase MAD1 over different cell cycle states from non-enriched pull-down samples.

Conclusions
This is the first study that systematically assessed the performances of the most popular DDA and data independent acquisition (DIA) MS approaches currently used in the proteomics field. Particular for phosphopeptide analysis in complex samples, large performance differences were observed and high-resolution HCD and higher stage fragmentation based PRM approaches were identified as most sensitive approaches. We are convinced that with the increasing number of known phosphosites or other posttranslational modifications (PTMs) and the relatively low-cost of synthetic heavy reference peptides with modification homologues, such extensive phosphosite or PTM monitoring studies can be applied to any protein of interest that can be enriched from a complex protein sample without the need for modification specific enrichment steps. The results will help users to select the most suitable LC-MS methods for their studies and define reasonable expectations for future proteomics experiments.

Novel Aspect
Triple-stage fragmentation based parallel reaction monitoring extended the dynamic quantification range of phosphosite specific MS assays up to several orders of magnitude compared to standard SRM/PRM analysis.

TOS11-03 Quantitative proteomic analysis by variable SWATH acquisition of differentially expressed proteins in monocyte-derived dendritic cells

Ying Zhang[1], Dario Bottinelli[1], Aivett Bilbao[1], Bandar Alghanem[1], Frédéric Nikitin[2], Markus Müller[2], Frédérique Lisacek[2], Jeremy Luban[3], Caterina Strambio De Castillia[3], Emmanuel Varesio[1], Gérard Hopfgartner[1]
[1]University of Geneva, [2]Swiss Institute of Bioinformatics, [3]University of Massachusetts

Introduction
Immature dendritic cells (DCs) generated by in vitro culturing of peripheral blood monocytes are functionally activated with lipopolysaccharide (LPS). Proteomics studies on these cells can facilitate the understanding of the biological process involved from a molecular point of view. Unlike the data-dependent acquisition (DDA) approach for MS/MS analysis which selects in real time precursors for fragmentation, SWATH acquisition method collects fragment ion spectra for all the precursors contained in predetermined isolation windows over an defined m/z range and the whole chromatographic elution range. Here, we used label-free quantitation method, combined with SWATH mass spectrometry acquisition techniques, to determine the difference of the MDDCs proteome profiles between before and after the LPS activation.

Methods
Tryptic digest of MDDCs peptides were analyzed on a TripleTOF 5600 MS coupled to a 2D+ NanoLC-Ultra system (AB Sciex, Eksigent). MS/MS acquisition in DDA mode was performed by selecting the top 10 most intense precursor ions for subsequent MS/MS analysis and the total cycle time is 1.1 sec. SWATH acquisition method covers the precursor m/z range of 350-1250 Da. A set of 36 overlapping windows was constructed with a total cycle time of 2.5 sec. SWATH data were analyzed by Skyline and Spectronaut software.

Results
Preliminary data showed that the reproducibility of the quantitative result obtained from the same sample by SWATH method is higher than DDA method (an average CV of 10% v.s. 21%, respectively, for five repeated measurements). To analyze the protein sample of MDDCs before and after the LPS treatment, a spectral library containing 2312 protein groups and 18401 distinct peptides was constructed based on the result of the 2D-LC-MS/MS analysis of the pooled sample. According to the distribution of these peptide precursor ions within different m/z range, variable isolation window widths were designed for the subsequent SWATH analysis with the aim to equalize the population of precursor ions included in each window. Compared with the results obtained by SWATH analysis with fixed window width, this method gave an average of more than 20% more confident peptide identifications. Quantitative proteomics analysis of the LPS-treated MDDCs revealed a total number of 84 proteins which were differentially expressed with more than two-fold difference compared to the control. Bioinformatic analysis of these proteins revealed that most of them were involved in the metabolic process and immune system process.

Conclusions
SWATH acquisition method provides better quantification reproducibility than DDA approach. With customized variable isolation window widths, more proteins can be identified in a complex biological sample by SWATH acquisition method compared to the one with fixed window width.

Novel Aspect
Quantitative proteomics analysis of MDDCs by SWATH acquisition method with customized variable isolation window widths.

TOS11-04 Comprehensive proteomic analysis of 3D human liver and cardiac spheroids for drug toxicity investigation

Nathalie Selevsek[1], Jonas Grossmann[1], Paolo Nanni[1], Claudia Fortes[1], Patrina Gunness[2], Jens Kelm[2], Ralph Schlapbach[1]
[1]Functional Genomics Center Zurich (FGCZ), Zurich, [2]InSphero AG, Zurich

Introduction
Drug-induced cardiotoxicity and hepatotoxicity are currently the main reasons for market withdrawal of drugs and exclusion of drugs in clinical phases. Better models than in vitro 2D cells and animal subjects are needed for testing new drugs before entering human clinical trials. Recently, 3D cell spheroids have shown to be suitable for testing chronic exposure toxicity compared to 2D cells, due to their longer lifespans and greater stability. Besides, their 3D architecture display more organ-like function than conventional monolayer cell cultures. To demonstrate the relevance of the spheroids for investigating toxicity of drug compounds at the proteome level, we analyzed by shotgun proteomics protein digests extracted from 3-D hepatocytes and cardiomyocytes to deliver a first view in sample complexity and

protein dynamic range of the human liver and cardiac proteome. In parallel, we applied label-free quantitative approach for the profiling of protein abundances from 3D liver spheroids treated with different concentrations of acetaminophen.

Methods
Proteins were extracted from cardiac and liver spheroids using the high-intensity focused ultrasound in biochemical triplicates. After the cell lysis, protein extracts were subjected to filter aided sample preparation (FASP) for protein digestion. The peptides were analyzed on an Orbitrap Fusion instrument in data dependent mode.MS/MS spectra were searched using Mascot and validated using Scaffold.

Results
We demonstrated that very little starting cell material (i.e 12 spheroids) were required to map out a large fraction of the liver and cardiac proteome in single MS injection. Furthermore, high reproducibility in protein abundances was obtained between biochemical replicates and across all tested acetaminophen concentrations for the liver samples. Thus we generated quantitative profiles for several thousands of proteins in response to acetaminophen treatment, revealing hundreds of proteins affected by that drug. Several protein pathways were identified, providing a better understanding in the mechanism of drug toxicity.

Novel aspect
In conclusion, the results demonstrated that proteomic analysis of 3D humanliver and cardiac spheroids are very suitable for investigating drug toxicity and can be applied to other 3D tissue models.

TOS11-05 **Multiplex quantification of microbial and plant protein toxins in complex matrices by immuno-extraction and high resolution targeted mass spectrometry**
Mathieu Dupre, Francois Fenaille, Cecile Feraudet-Tarisse, Patricia Lamourette, Herve Volland, Stephanie Simon, Christophe Junot, Virginie Brun, Francois Becher
CEA

Introduction
The development of fast, sensitive and specific detection methods of protein toxins remains a major challenge to react efficiently to terrorist threat. Today, although the analytical strategies traditionally rely on very sensitive immunological methods, mass spectrometry constitutes an attractive complementary approach thanks to exact mass measurement and characterization capacities leading to unambiguous identification. In the context of the rapid detection of bioterrorism agents, we developed an immuno-LC-MS/MS method monitoring a high number of peptides for the multiplex quantification in complex matrices of ricin, epsilon toxin and staphylococcal enterotoxin B (SEB), considered as top-priority agents by the CDC. Using labelled standards, high sensitivity and reproducible quantification was achieved through the parallel reaction monitoring (PRM) mode available on the quadrupole-Orbitrap high resolution instrument.

Method
Samples were incubated with magnetic beads coated with toxin-specific antibodies. On-beads trypsin digestion was accomplished before by LC-MS/MS analysis. Peptide separation was achieved in 25 minutes on a C18 column with a linear gradient of 0.1% formic acid in acetonitrile. Using the PRM mode, 24 peptides were monitored for the three toxins, whereas absolute quantification was performed by isotope dilution i.e. labeled peptides for ricin and epsilon toxin and labeled protein for SEB (PSAQ standards).

Results
Preliminary in-depth characterization of the targeted proteins revealed the necessity to monitor a high number of peptides per protein to ensure maximum sequence coverage and minimize the risk of false-negative results. Indeed, both most sensitive peptides and those with potential amino acid substitutions were taken into account. For instance, up to 9 peptides could be detected for ricin at a concentration of 1 ng.mL-1. Detecting a high number of peptides with enough sensitivity in a multiplexed assay implies that each step of the sample preparation and analysis needs to be carefully optimised. Multiplexed immunocapture followed by on-beads digestion was developed and optimized to obtain the highest recovery of the three toxins in targeted complex food and biological matrices. Regarding the mass spectrometry detection, we used the PRM mode to take into account all fragment ions from a common precursor at high resolution. Normalized collision energies were optimized, and C-trap accumulation times were thoroughly adjusted for ion isolation by the quadrupole

Conclusion
Detection of the three toxins proved to be linear in buffer from 0.5 to 100 ng.mL-1, while their lower limits of quantification were estimated in the ng.mL-1 range. The whole process is currently applied to the quantitative analysis of toxins spiked into milk, orange juice, urine and plasma samples.

Novel Aspect
Multiplex quantification of protein toxins in complex matrices by immuno-extraction and high resolution targeted mass spectrometry (PRM mode).

TOS12 - Lipidomics

Room 2
Level 0

Chairs: Andrej Shevchenko, Eric Forest

TOS12-01 **Keynote: Natural variation of a signalling lipid**
Markus Wenk
National University of Singapore

Current mass spectrometry-based lipidomics aims to comprehensively cover wide ranges of lipid classes. We introduce

a strategy to capture phospho-monoester lipids and improve the detection of long-chain base phosphates (LCB-Ps, e.g. sphingosine-1-phosphate). Ten novel LCB-Ps (d18:2, t20:1, odd carbon forms) were discovered and characterized in tissues from human and mouse, as well as in D. melanogaster and S. cerevisiae. These findings have immediate relevance for our understanding of sphingosine-1-phosphate biosynthesis, signaling and degradation (Narayanaswamy et al 2014, Anal Chem. 2014 Mar 18;86(6):3043-7).

Understanding better the fundamentals of natural variation in lipidomes as well as specific recognition of individual lipid species are the scientific aims of SLING, the Singapore Lipidomics Incubator. This centre is a global magnet for collaborating parties in lipidomics – from academia and industry – delivering new technologies and intellectual capital. SLING organizes the international Singapore Lipid Symposium (ISLS), a major symposium in lipidomic research in Asia Pacific and 'i c lipid', an intensive immersion course in mass spectrometry based lipidomics (http://www.lipidprofiles.com/index.php?id=139)

TOS12-02 **Novel oxysterols in mouse and man**

William Griffiths, Peter Crick, Anna Meljon, Yuqin Wang
Swansea University

Introduction

Oxysterols are oxygenated forms of cholesterol or its precursors. They are formed in the first steps of cholesterol metabolism. Analysis of oxysterols is challenging on account of their poor ionisation properties and low abundance compared to background lipids. We have developed an LC-MS(MSn) methodology for high sensitivity oxysterol analysis which we call enzyme-assisted derivatisation for sterol analysis (EADSA).

In-born errors of cholesterol biosynthesis and metabolism, where an enzyme in the metabolic pathway is defective, result in debilitating diseases such as Smith-Lemli-Opitz Syndrome (SLOS), Cerebrotendinous Xanthomatosis (CTX) and Oxysterol 7α-Hydroxylase Deficiency (O7AD). Here we describe the effective diagnosis of these disorders from μL quantities of serum/plasma. Detailed analysis of plasma from these patients and from appropriate transgenic animals reveals a raft of novel oxysterols not normally observed in healthy individuals.

Methods

Oxysterols were extracted into ethanol, separated from excess cholesterol and other hydrophobic lipids by solid phase extraction and treated with cholesterol oxidase to introduce a 3-oxo group in the A-ring. Reaction with Girard P reagent results in the C-3-GP-hydrazone which is cationic. After removal of excess derivatisation agent by a second SPE step the oxidised/derivatised oxysterols were ready for analysis by LC-MS(MS2).

LC separation was on a C18 Hypersil Gold column (50 x 2.1 mm 1.9 μm) with a methanol/acetonitrile/water gradient. MS and MSn analysis was on an LTQ-Orbitrap. Quantification was by stable isotope dilution MS.

Results

Using the above methodology we have discovered and quantified a number of novel oxysterols in mouse and man including: 7α-hydroxydesmosterol, 7,8-epoxycholesterol, 12α-hydroxycholesterol, 24R-hydroxycholesterol, 7α,24-dihydroxycholesterol, 7α,(25S)27-hydroxycholesterol, 3β,7α-dihydroxycholest-(25S)-5-en-27-oic acid. Identifications were made by comparison to authentic standards, where available, or by retention time, exact mass and MSn spectra.

Conclusion

We have discovered a number of "new" oxysterols. At present it is unknown whether they are important in the aetiology of disease or just a consequence of it. It is likely that many of these metabolites are formed in shunt mechanisms as a result of a build up of metabolic intermediates. The discovery of elevated levels of some of these oxysterols may be used in disease diagnosis.

Novel Aspects

Discovery of novel oxysterols and routes of sterol metabolism.

TOS12-03 **Lipidomic characterization of tumor tissues using LC/MS, SFC/MS, MALDI-MS and multivariate data analysis**

Michal Holčapek[1], Eva Cífková[1], Miroslav Lísa[1], Vitaliy Chagovets[1], David Vrána[2], Jiří Gatěk[3], Bohuslav Melichar[2]
[1]University of Pardubice, [2]Palacký University, Olomouc, [3]Tomáš Bata University in Zlín

Introduction

Lipidomic analysis is challenging due to enormous complexity of lipid structures. The mass spectrometry (MS) plays a key role in the quantitative lipidomics either without separation (shotgun ESI-MS or MALDI-TOF) or in separation coupling with MS (mainly LC/MS and SFC/MS). Each modality can bring complementary information, which can be integrated in one overall methodology. The goal of this work is the development of complementary nontargeted and targeted MS based method for the comprehensive characterization of biological samples, which is applied for the characterization of changes caused by the tumor growth in comparison with surrounding healthy tissues.

Methods

Sample preparation: total lipid extracts obtained by chloroform – methanol – water extraction according to modified Folch or Bligh-Dyer.

HILIC-HPLC/MS: column Spherisorb Si (Waters), 1 mL/min, 40°C, gradient of acetonitrile / 5 mM aqueous ammonium acetate.

SFC/MS: UPC2 instrument (Waters) using UPC2 column, separation temperature 60°C and gradient of methanol as a modifier, Synapt HDMS G2Si instrument (Waters) using ESI ionization was used for IM-MS experiments.

MALDI-MS - LTQ Orbitrap XL (Thermo Scientific), 9-aminoacridine - 10 mg/ml in isopropanol/acetonitrile (60/40), laser energy 15 μJ.

Data analysis: Progenesis QI (Waters) software for lipidomic data followed by the multivariate data analysis using O2PLS method in SIMCA 13.0 (Umetrics).

Results
Comprehensive lipidomic analyses of tumor tissues and surrounding normal tissues from several clinical trials (breast, kidney and lung cancer) were performed using optimized HILIC-HPLC/ESI-MS, SFC/MS, MALDI-Orbitrap MS methods. Individual lipid classes were quantified based on the addition of single IS and response factors for each class related to the IS. Statistically significant differences in average concentrations were observed several classes of polar lipids (PI, PE, LPE, SM, LPC, etc.). Detailed analysis of lipid species inside above mentioned classes was performed using relative abundances of deprotonated molecules in the negative-ion ESI mode or protonated molecules in the positive-ion ESI mode followed by MS/MS experiments. Multivariate data analysis using orthogonal 2 projections of latent structures (O2PLS) enables a clear differentiation of tumor and normal tissues based on changes of their lipidome.

Conclusions
The statistically significant lipidomic differences were described for different types of tumor tissues (e.g., breast, kidney, lung) in comparison with surrounding normal tissues of the same patient obtained after the surgery.
This work was supported by ERC CZ project No. LL1302 (MSMT, Czech Republic).

Novel Aspect
Combination of UHPLC/MS, SFC/MS and MALDI-MS followed by the multivariate data analysis is used for detailed lipidomic characterization of cancer tissues.

TOS12-04 Malarial parasite development: lipidomic analysis of the *P. falciparum* life cycle in human erythrocytes

Todd W. Mitchell[1], Simon H.J. Brown[1], Phuong Tran[2], Alexander G. Maier[2]
[1]University of Wollongong, [2]Australian National University

Introduction
Development of the malaria parasite P. falciparum in human erythrocytes is linked with substantial changes of the parasite lipid profile. Lipid metabolism, which is nearly non-functional in normal erythrocytes, is elevated in the parasite to allow for the considerable morphological changes that occur during development within the human host. Substantial changes in both structural and storage lipids are observed, with necessary lipid synthesis precursors recruited from both the erythrocyte membrane and plasma. An ATP-binding cassette (ABC) transporter, gABCG2 is expressed predominantly in the gametocyte (sexual) stages of the parasite lifecycle and may play role in lipid transport during these stages. Here we aim to determine (i) development-associated changes in the lipid profile of P. falciparum infected erythrocytes, and (ii) the affect of gABCG2 knockout on these profiles.

Methods
In order to investigate these changes, we have optimized methods for lipid extraction directly from human erythrocytes (107 cells per group, i.e. uninfected erythrocytes, trophozoites and the five stages of gametocyte development). Lipids were extracted by bi-phasic methods utilizing methyl tert-butyl ether, and the extracts analyzed using a hybrid triple quadrupole linear ion trap mass spectrometer (AB Sciex QTRAP 5500) equipped with an automated chip-based nano-electrospray source (Advion NanoMate). Targeted precursor ion and neutral loss scans were used to identify and quantify 154 individual lipid species. Quantified lipids were normalized to cell count, allowing comparisons between morphologically different developmental stages.

Results
Quantification of ten lipid classes, including cholesteryl ester, free cholesterol, diacylglycerol, triacylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol and sphingomyelin was performed. Preliminary analysis indicates a build-up of storage lipids including diacylglycerol, triacylglycerol, and cholesteryl ester during gametocyte maturation, as well as an increase in free cholesterol. Glycerolphospholipids change substantially during development, with a notable reduction in the phosphatidylcholine to phosphatidylethanolamine ratio. Principle component analysis of lipid profiles clearly separates uninfected erythrocytes, trophozoites and all five stages of maturing gametocytes. Knockout of gABCG2 had little effect on trophozoites but significantly effected lipid levels in gametocytes.

Conclusions
The lipid profile of P. falciparum infected erythrocytes changes substantially during development and is distinct for each of the five gametocyte stages. gABCG2 appears to play a significant role in lipid transport during gametocyte development and may represent a new drug target in the treatment of malaria.

Novel aspect
The first data implicating gABCG2 as an important molecule in the sexual development of the human malarial parasite P. falciparum.

TOS12-05 Identification and immunomodulatory functions of novel galactosylceramides from gut commensal microbe *Bacteroides fragilis*

Sungwhan Oh, Dennis Kasper
Harvard Medical School

Introduction
Contribution of commensal microbiota to host health is of considerable interest. However, molecular effectors and mechanisms controlling these processes remain to be elucidated. Here, we have carried out lipidomic profiling of commensal bacteroidales, many of which are major symbionts of human gut. Novel sphingolipid species have been further investigated for their immunomodulatory functions in vitro and in vivo.

Methods
Total lipid extracts from multiples species of order Bacteroidales, including genus Bacteroides, Prevotella and Porphyromonas were prepared by modified folch extraction. Sphingolipidomic profiles were acquired by RP-HPLC-MS/MS (Agilent 1100/Thermo LTQ XL). Among identified sphingolipids, glycosphingolipid from B. fragilis have been further fractionated and purified for chemical analysis, structural assignment and in vitro/in vivo studies.

Results
Many of Bacteroidales species synthesized multiple classes of sphingolipids, such as ceramide, phosphoethanolamine-ceramide and glyco-ceramides, with distinct structures when compared to mammalian sphingolipids. Among species we have analyzed, only Bacteroides fragilis produced unique alpha-galactosyl ceramides (BFaGCs), which resemble the structure of known prototypic CD1d ligands. These BFaGCs are also identified in the gut of B. fragilis-monocolonized mice. BFaGCs have antagonistic activity to CD1d-mediated natural killer T cell (NKTs) activation and subsequent pro-inflammatory responses both in vitro and in vivo. BFaGCs antagonized interleukin-2 production by NKT cell in APC-NKT coculture and interferon-gamma and IL-4 production by prototypic CD1d ligand in vivo. Furthermore, oral administration of BFaGC to mice at 3 to 7 days after birth suppressed gut NKT cell proliferation and protected animals from NKT-mediated colitis in adulthood.

Conclusion
Bacteroides fragilis synthesizes unique alpha-galactosylceramides in the host gut, which regulates NKT cell proliferation and protect host from excessive inflammatory responses.

Novel aspects
This is one of the first reports that species-specific lipid mediators from commensal microbe can modulate host immune development and inflammatory responses.

References
An D, Oh SF, Olszak T, Neves JP, Avci FY, Erturk-Hasdemir D, Xi L, Blumberg RS, Kasper DL. Sphingolipids from a symbiotic microbe regulate homeostasis of host intestinal natural killer T cells. (2014) Cell. 156: 123-33.

TOS13 - Gas-Phase Ion Spectroscopy

Room 3
Level 0

Chairs: Jos Oomens, Julia Chamot-Rooke

TOS13-01 Keynote: Spectroscopy of ions in aqueous nanodrops

Evan Williams, Sven Heiles, Richard Cooper, Matthew DiTucci, Satrajit Chakrabarty, Terrence Chang
University of California, Berkeley

Introduction
The effects of ions on protein structure were first reported over 125 years ago by Franz Hofmeister, who ordered salts on their propensity to stabilize or destabilize native structures. This Hofmeister ion series is fundamentally important in many different areas owing to its remarkable correlation to many different physical properties. Despite extensive investigations using many different methods, a unifying mechanistic description of this effect remains elusive. Is this a result of direct ion-protein interactions, or is it an indirect affect of ion-water interactions affecting the protein environment? Detailed information about how water orients around ions, and how ions affect the hydrogen-bonding network of water itself can be obtained from spectroscopy of hydrated gaseous ions.

Methods
Experiments are performed using a 7.0 Tesla Fourier-transform ion cyclotron resonance mass spectrometer that has an ion cell that is surrounded by a copper jacket, the temperature of which is controlled either by resistive heating or cooling by a regulated flow of liquid nitrogen. Ions are formed by nanoelectrospray ionization using borosilicate capillaries that are pulled to an inner tip diameter of ~1 micron. Spectroscopy is performed using tunable IR radiation from an OPO/OPA laser system pumped by the 1064 nm fundamental of a Nd:YAG laser. First-order photodissociation rate constants are obtained from the ion abundances, and are corrected for frequency dependent laser power and for blackbody infrared radiative dissociation.

Results
Guanidinium salts disrupt native protein structure and are widely used to investigate various aspects of protein structure. The hydration of guanidinium by water was probed by measuring IR photodissociation spectra of gaseous clusters with up to 100 water molecules attached. For small clusters, hydration is not isotropic but becomes isotropic at large cluster size. These results show that guanidinium has an inner shell coordination number of three, the smallest of measured for an untagged ion in the gas phase. Comparisons to spectra of other hydrated ions show that guanidinium is weakly hydrated with water interactions occurring in the plane of the ion. The unusual hydration properties of guanidinium provide new insights into why this ion is an effective protein denaturant. Results for other ions show that some ions can affect the hydrogen-bonding network of water to long distance. Detailed studies indicate that some ions reside at the surface of small aqueous nanodrops whereas others are fully solvated. Results for model systems show factors that can make solvation of neutral functionalities preferential over charge sites.

Conclusions
Infrared photodissociation spectra of cations and anions show that some ions can affect the hydrogen-bonding network of water molecules to long distance, and these results provide new insights into how water orients around ions and how different ions affect protein structure.

Novel Aspects
Infrared photodissociation spectroscopy of gaseous hydrated ions provides new insights into the properties of these ions in water and their affect on protein structure.

TOS13-02 **Two-dimensional photofragmentation mass-spectrometry of cold ions.**

Oleg Boyarkine[1], Vladimir Kopysov[1], Alexander Makarov[2]
[1]EPFL, [2]Thermo Fisher Scientific

Spectroscopy of protonated biomolecules provides benchmarks for calculations of intrinsic three-dimensional structures of these large species and for their identifications [1-7]. We employ UV-laser photofragmentation, combined with high mass-resolution detection by an Orbitrap mass-spectrometer to measure fragmentation mass-spectra in function of UV wavelength for cryogenically cooled, protonated peptides. The measured 3D spectra (fragmentation yield vs UV wavenumber and m/z) are analyzed using Singular Value Decomposition algorithm [8], disentangling each 3D spectrum to a few pairs of UV spectrum + mass-spectrum. These pairs become specific and detailed "fingerprints" of distinct families of similar conformers, while the whole 3D spectrum constitute an unique identity of an ion. We demonstrate a use such 3D identity for distinguishing isobaric peptides.

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TOS13-03 **Gas phase reactions of seleniranium ions results in Pi-ligand exchange in competition with electron transfer**

George N. Khairallah[1], S. Fern Lim[1], Benjamin L. Harris[1], Philippe Maître[2], Richard A. J. O'Hair[1], Jonathan M. White[1]
[1]University of Melbourne, [2]Université Paris-Sud

Intro:
Seleniranium ions (RSe+) are important reactive intermediates that are involved in a wide range of useful transformations in organic synthesis. Nucleophilic attack at the selenium atom of a seleniranium ion yields an alkene and an active selenium electrophile, where this process results in π-decomplexation. This step is shown to be reversible and subsequent recomplexation results in reformation of the seleniranium ion. In this work, multistage mass spectrometry experiments, on a LTQ-FT high resolution modified mass spectrometer, are used to investigate the mechanism of olefin-to-olefin RSe+ transfer in the gas phase.

Experimental:
Ion-molecule reactions (IMR) were performed using a Finnigan LTQ-FT mass spectrometer that was modified to allow the introduction of neutral reagents, such as volatile alkenes, into the ion trap with the helium bath gas. Ions formed in the linear ion trap resulting from ion-molecule reactions and/or CID were transferred to the FT-ICR cell for accurate mass measurements.
Ab initio calculations were performed using the Gaussian 09 software package. Structures were optimized at the B3LYP/6-311++G** level of theory.Reported structures represent the lowest-energy conformation and transition state has been confirmed via intrinsic reaction coordinate scans.

Results:
Electrospray ionisation (ESI) on β-selenyl amines [C14H15NSe] in the positive ion mode yields the ammonium ions [C14H16NSe]+, which were mass selected and then subjected to collision-induced dissociation (CID) to yield the ion with the general formula [C14H1380Se]+ (m/z 261) confirmed via HRMS and proposed to be the seleniranium ion (RSe+). To provide further support for this assignment, gas-phase infrared spectroscopy experiments with a free electron laser (FEL) were conducted at CLIO (Centre Laser Infrarouge d'Orsay). The results obtained show a good qualitative match between the observed and computed IR spectra, confirming that m/z 261 corresponds to a seleniranium ([Ph-Se-CH2-CH-Ph]+) ion and not any other isomer (e.g.; selenonium ion ([Ph-Se-C(Ph)(CH3)]+)).
Ion-molecule reactions (IMR) with alkenes in the ion-trap mass spectrometer were employed to determine the reactivity of (RSe+) and the potential of π-ligand exchange. Thus upon the reaction of [C14H13Se]+ with cyclohexene, formation of a product ion at m/z 239 was observed. This latter ion is consistent with formation of a new seleniranium ion [C12H1580Se]+ by direct olefin to olefin transfer of the PhSe+ moiety (eq. 1). DFT calculations support the experimental observations.

[C14H13Se]+ + C6H10 --> [C12H15Se]+ + (C8H8) (eq. 1)

Conclusions :
The seleniranium ion was synthesized in an ion trap mass spectrometer and its identity confirmed via HRMS and IR spectroscopy. Ion-molecule reactions have shown that olefin-to-olefin RSe+ transfer in the gas phase is possible.

Novel aspect:
ESI-MS was used to generate a seleniranium ion in the gas phase and study its reactivity.

TOS13-04 Conformational equilibrium of single and double protonated 1,4-diamine-2-butenes by IRMPD spectroscopy

Thiago C. Correra, Lucas C. Ducati, José M. Riveros
University of São Paulo

Polyamines like, spermidines, spermines, and their unsaturated counterparts, have important roles in cell growth and the putrescine metabolic route, acting as a modulator of normal and pathological cell growth.1 Among these species, the diaminobutenes were shown to have enhanced efficiency as enzime inhibitors,2 and were proposed as a vector for cancer therapy and imaging.3

Despite the biological role of these diaminobutenes and recent investigations of their saturated analogs,4 no studies were carried out to identify their conformational equilibrium under different protonation conditions, aspect directly related to their biological role.

For these reasons we evaluated the protonation sites and preferred conformations of 1,4-di(dimethylamino)-2-butene 1 and 1,4-di(ethylamino)-2-butene 2 (Figure 1) single and double protonated ions, formed under ESI conditions, by IRMPD spectroscopy at the 600 1800 cm-1 range. The experiments were carried out at the FT-ICR at the FELIX facility and supported by B3LYP/6-31+G(d,p) calculations.

The IRMPD spectrum of single protonated 1 (Figure 2) shows bands at 1646, 1468 and 1362 cm-1 that are in agreement with the olefin backbone vibrations predicted by theory. Two other bands at 889 and 820 cm-1 were assigned to the asymmetric NR3 and symmetric HN+R3 stretches. The lack of bands at 1586 cm-1, where the N-H+-N bending motion was estimated to be present, suggests not only that the trans configuration 3 is more abundant in solution, but also rules out the protonation at the olefin that would lead to isomerization to the most stable cis structure. The cis stabilization (~ 8 kcal mol-1) was shown to be consequence of the H+ bridging the NMe2R groups. The spectra obtained for 1 protonated by either H+ or D+ are virtually identical and show that no bands related to the N H+-N bending are present.

The IRMPD spectrum for the double protonated 1 shows only 4 distinguishable bands (Figure 3). The absorption at 1375 cm-1, which was assigned to the NH+ bending mode based on calculations and isotopic effect experiments, suggests that the protonation takes place at the N atom. Besides that, the band at 970 cm-1 is in agreement with the out of the plane motion of the trans olefin hydrogens predicted at 977 cm 1. This also suggests that the cis configuration is not observed. The symmetric structure 6, where both nitrogen atoms are bridged by a H+ and the other H+ binds to the olefin, was also ruled out because the calculations carried out for this species predict absorptions in the 1100-1300 cm-1 range that were not observed.

These results show that the trans configuration is more abundant in solution and that the preferential nitrogen protonation prevents the cis-trans isomerization to happen.

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IMSC 2014

Conformational equilibrium of single and double protonated 1,4-diamine-2-butenes by IRMPD spectroscopy

Thiago C. Correra*, Lucas C. Ducati, José M. Riveros

Chemistry Institute, University of São Paulo, Brazil

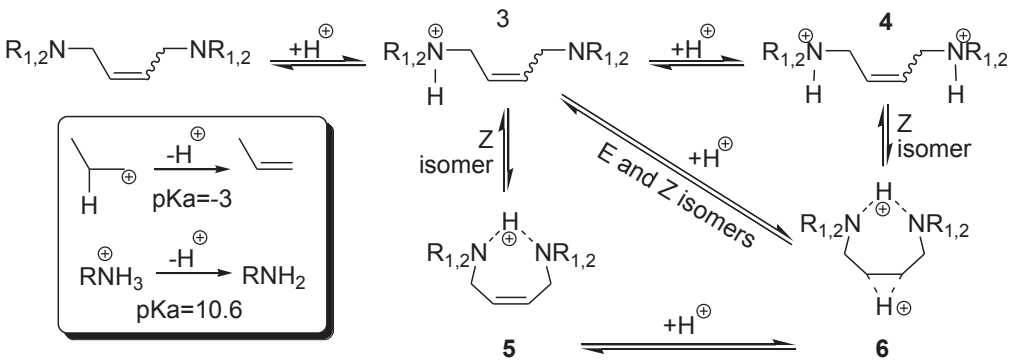

Figure 1. Possible protonation site equilibrium for diprotonated 1-4,diamines-2-butenes R_1,R_2 = Me,Me **1**, Et,H **2**. Insert show pKa values for protonated terminal alkenes and alkylamines. Compound 2 has shown similar behaviors and will not be addressed here due to the limited size of the abstract.

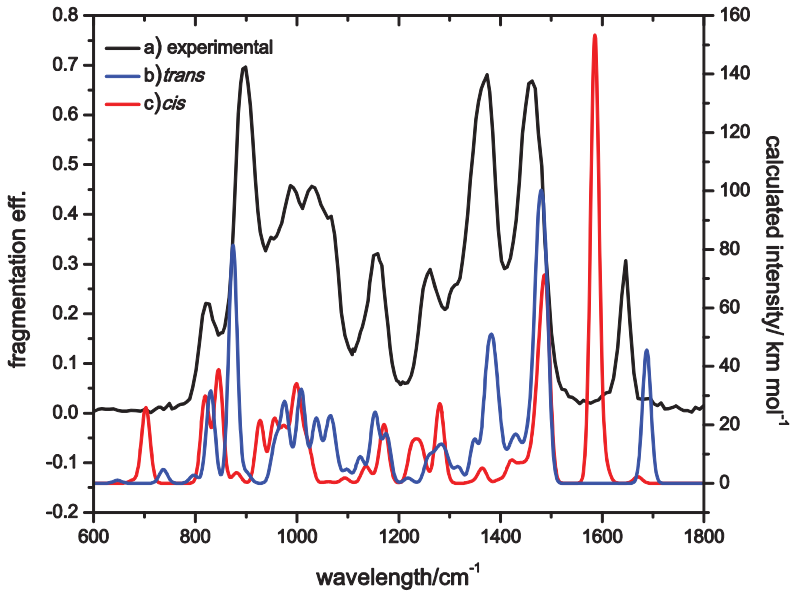

Figure 2. IRMPD spectrum of the single protonated compound **1** (**1**-1H$^+$). a) experimental spectrum, b) *trans* isomer and c) most stable *cis* isomer.

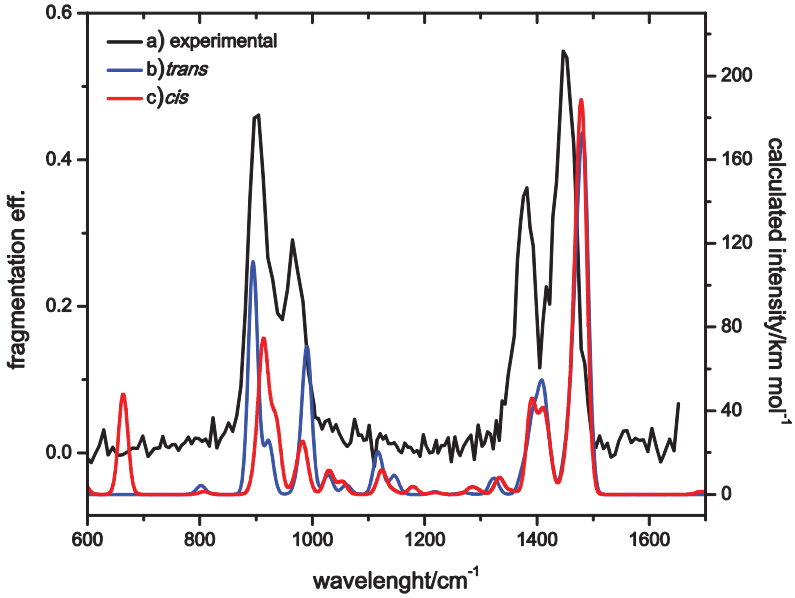

Figure 2. IRMPD spectrum of the double protonated compound **1** (**1**-2H$^+$). a) experimental spectrum, b) *trans* isomer and c) most stable *cis* isomer.

TOS13-05 **Laser spectroscopic investigations of dichlorofluorobenzenes by REMPI and MATI spectroscopy**

Sascha Krüger, Jürgen Grotemeyer
University of Kiel

Introduction

Ion spectroscopy is an essential method for investigation of ionic molecular species and their vibronic structure. Substitution of one or more hydrogen atoms in the benzene ring leads to significant change in electron density distribution. Previous research showed that certain halogen substitution pattern of benzene leads to strong activity of formally forbidden modes due to vibronic coupling. The vibronic structure in the first excited state (S1) was investigated by means of Resonance Enhanced Multi Photon Ionization (REMPI) spectroscopy, the ionic ground state (D0) by Mass Analyzed Threshold Ionization (MATI) spectroscopy. The experimental findings are supported by quantum chemical calculations.

Method

REMPI is a Multi Photon Ionization technique taking advantage of the resonance enhancement principle. At first the molecule is cooled down with regards to its degrees of freedom via a supersonic expansion. Detecting the mass-selected ion-signal while the wavelength of the laser is scanned yields vibrational spectra of excited states. In a two-color experimental setup the ionization-laser is irradiated with a fixed wavelength while the excitation-laser is scanned. REMPI provides information on the structure of electronically excited molecules and yields very exact excitation energies (±2 cm−1)

MATI is a special discipline of the REMPI technique and closely related to ZEKE- (ZEro Kinetik Energy)-spectroscopy. In contrast to REMPI the goal is to faciliate spectroscopy on molecules in Rydberg states. Rydberg states are highly-excited states that form bands just under each series limit. An electric-field puls ionizes Ryberg molecules and accelerates them into a Time of Flight mass spectrometer. The so called prompt-ions which were also formed during the laser excitation process are previously separated by an electric spoiling field that accelerates them opposite to the direction of flight. MATI provides information on the structure of ions and yields very exact ionization energies (±7 cm−1).

Results
The findings from the research provided very accurate values for the electronic excitation and ionization energies (IE) of Dichlorofluorobenzenes. Furthermore a detailed vibrational analysis could be conducted for the first time. This analysis advances the understanding of vibrational modes active during excitation/ionization. The REMPI spectra show unusual high activity of symmetry forbidden out-of-plane modes at low frequencies. Several cases of violation of $\Delta v = 0$ propensity rule were observed in the MATI spectra.

Conclusions
During excitation/ionization molecules are able to undergo geometric distortion along the eigenvector of certain vibrational modes. The experimental findings and calculations indicate a geometrical distortion during excitation and ionization. The present study brought new insight on the impact of heterohalide substitution to the vibronic structure of the benzene ring as well in S1 as in D0 state.

Novel Aspect
Geometry distortion during excitation and ionisation

TOS14 - Detectors and High-Mass MS

Room 4
Level 0

Chairs: Renato Zenobi, Günter Allmaier

TOS14-01 Keynote: High mass detection in imaging mass spectrometry

Ron M.A. Heeren, Anne Bruinen, Tiffany Porta, Shane Ellis
FOM-AMOLF

Introduction
Mass spectrometry based imaging (MSI) has matured to a high throughput tool in biomedical research. MALDI-MSI is routinely used to study protein and peptide distributions on tissue sections for the analysis of molecular signalling processes in various diseases. Studies have demonstrated excellent diagnostic and prognostic value that can be directly translated to the clinic. It is key to employ a multimodal biomolecular imaging approach combined with quantitative analytical proteomics and metabolomics. Innovations in the field target breaking boundaries in resolution, sensitivity and speed. The development of novel approaches such as microscope mode imaging combined with innovative detector technology and cluster based SIMS is one of these development areas. Here we demonstrate how the novel pixelated detectors can extend the molecular weight range that can sensitively be imaged.

Methods
Experiments were performed an Ultraflex III MALDI TOF/TOF instrument (Bruker Daltonik GmbH, Bremen, Germany) equipped with a Smartbeam® 355 nm Nd:YAG laser system and a Physical Electronics Triple Focussing Time-of Flight (TRIFT) optimised for stigmatic ion imaging and equipped with a C60+ primary ion source. A 2x2 array of Timepix detectors positioned behind dual microchannel plates was employed for ion detection and imaging. Each Timepix chip contains a 256x256 array of pixels (55 µm pitch) that can each record the impact position and TOF of an impinging ion per laser/ion beam pulse.

Results
The results of the implementation of a pixelated detector array with extremely high charge sensitivity on a MALDI-equipped linear time-of-flight mass spectrometer or a q-ToF MS system are described. This coupling is shown to allow a significant increase in detection efficiency for large macromolecules (i.e., intact antibodies) having m/z values up and above 400,000 and thereby providing a means to make the detection of large macromolecules more accessible in MALDI-mass spectrometry. In addition, the direct imaging capabilities of this detector are shown allow visualisation of mass-dependant spatial distributions in the MALDI ion cloud and the effect the ion optics exerts on the ion cloud. Such capabilities are demonstrated to provide new insights into dynamic chemical phenomena occurring in the ion source, ion optics and allow the visualization of molecular signals of disease.

Conclusions
High molecular weight detection is demonstrated to benefit from the application of parallel detection. Active pixel detectors are demonstrated to enhance the dynamic range in imaging MS experiments

Novel Aspect
High molecular weight detection with TimePix parallel detection systems.

TOS14-02 Development of a time and position sensitive ion detector for a stigmatic imaging mass spectrometer

Yosuke Kawai[1], Hisanori Matsuoka[1], Hisanao Hazama[1], Jun Aoki[1], Michisato Toyoda[1], Yowichi Fujita[2], Yukiko Ikemoto[2], Yasuo Arai[2], Kunio Awazu[1]
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Introduction
A stigmatic imaging mass spectrometer, which obtains ion images with a spatial resolution on the order of several microns at a time, has been developed recently [1]. For a stigmatic measurement, an ion detector is required to measure two-dimensional positions and flight time of individual ions simultaneously. Currently, the available detector that

satisfies this requirement is a delay line detector. Since, however, a delay line detector has been developed for the measurement of a low rate ion collision event, the position detection of ions can fail in a case where multiple ions impinge on the detector at the same time. Using matrix-assisted laser desorption/ionization method, the laser power has to be minimized in order to reduce the impingement rate of ions, leading to a long measurement time of about one hour to obtain an entire mass spectrometric image. To solve this problem, we have started to develop a time-resolved pixel array detector which can detect multiple ion signals by using a Silicon-On-Insulator (SOI) technology [2].

Methods

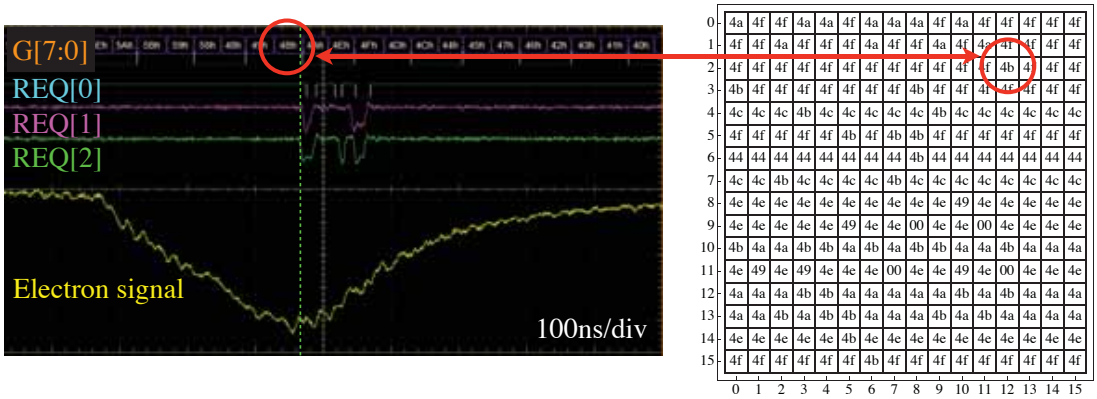
The prototype of the detector consists of 16 x 16 pixels with a size of 64 x 64 μm2 and a 8-bit grey code counter located outside of the pixel array. Each pixel has an electrode pad which receives ions, and under the pad a complementary metal oxide semiconductor (CMOS) circuit which detects ion signals and measures a flight time. Transistors formed on an electrical insulator with a SOI process have less parasitic capacitance relative to a conventional bulk CMOS process, and therefore the high speed response to the signals can be realized. When an ion arrives at the one electrode pad, the

signal is detected by the discriminator and the time stamp generated by the grey-code counter is stored in the 8-bit memory at the moment the signal exceeds a certain threshold value. Maximum resolution of the time information is 10 ns when the counter runs at 100MHz clock. By executing this process at each pixel independently, a multi hit detection can be achieved.

Experimental Results

The basic response of the prototype detector to the ion signal has been evaluated with the laser desorption ionization source. One of the results is shown in Fig. 1. The detector is located behind the micro channel plate (MCP) and irradiated by the stream of the secondary electrons emitted when the ions impinge on the MCP. Left panel shows the response of the detector running at 25MHz clock. G[7:0] is the time information generated by the counter and REQ[2:0] is the output signal of the discriminator of each pixel. Right panel shows the time stamp read out from the memory in each pixel. It is confirmed that the stored time stamp agrees with the counter value at which the signal is detected by the discriminator.

Fig.1. (Left) The response of the detector to the electron injection. (Right) The time stamp read out from the memory in each pixel.



Reference

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TOS14-03 Factors that affect transmission of high mass MALDI ions in a multi-quadrupoles rectilinear ion trap mass spectrometer

Wen-Ping Peng, Avinash A. Patil, Szu-Wei Chou, Pei-Yu Chang
National Dong Hwa University

Introduction

We develop a multi-quadrupoles rectilinear ion trap (RIT) mass spectrometer to study the transmission of high mass matrix-assisted laser desorption/ionization (MALDI) ions. MALDI ions were measured both at axial and radial directions with charge detectors. In axial direction,the ions are transmitted from the ion source to three quadrupole ion guides and a RIT mass analyzer. In radial direction, MALDI ions are transmitted and stored in the RIT and then scanned to acquire the mass spectra. In principle, the quadrupole plays as a high pass mass filter when operating in RF-only mode. However, ions pass quadrupole ion guide only when they are in limited m/z range with a fixed RF frequency and amplitude. This results in the loss of high mass ions. Lowering the RF frequency and raising the rf amplitude can help guide high mass MALDI ions. The flight time of MALDI ions are also measured by synchronization of a charge detector and a laser Q-switch. This measured flight time can be converted to the kinetic energy (K.E.) of MALDI ions.

Methods

The instrument comprises a MALDI ion source, a home-made square quadrupole (Q1), a 90-degree bent square quadrupole (Q2) and a short square quadrupole (Q3) to guide ions to a RIT mass analyzerwith front and back end-cap lenses for ion trapping. One electron multiplier assembly and a charge detector are fixed on the lateral sides of RIT for radially ejected ion detection. The other charge detector is placed behind the endcap lens to quantitate MALDI ions transmitted on axial direction.

Results
C60 ions were guidedwith RF amplitudes from 60 to 100Vp-p and frequenciesat 540 kHz, 540 kHz, and 530 kHz individually in Q1, Q2, and Q3.TheRF amplitude of RIT was set at 100Vp-p andthe frequency was 500 kHz. The number of C60 ions were measured from 25000 (0.5V) to 250,000 (4V) per pulse by an axial charge detector. It was found the rf frequency must be tuned low to 300 kHz (Q1), 300 kHz (Q2), 250 kHz (Q3), and 200 kHz (RIT) and the trapping potential was set at 80-150 Vp-ptoguide insulin B (MW~3495Da) ions. The rf amplitude of three guiding quadrupoles (Q1, Q2 and Q3) was further increased to 300Vp-p to guide cytochrome C (MW~12327Da) ions.The numbers ofinsulin B and cytochrome C ions are 100000 (2V) and 75000(1.5V) per laser pulse respectively. The mean flight time of both positive and negative ions is 0.35 ms and 0.62 ms respectively. This mean flight time can be used to calculate kinetic energy. The kinetic energy of C60, insulin B and cytochrome C are 6.2 eV, 30 eV and 106 eV respectively.

Conclusions
Ions intensity decreases as mass increases which implies that the ion transmission of high ions in quadrupoles becomes challenging. To guide high mass MALDI ions, the radio frequency and amplitude play a crucial role in ion transmission.

Novel Aspect
Examining key factors in transmission of high MALDI ions in a multi-quadrupoles RIT mass spectrometer

TOS14-04 A novel freestanding ultra-nanocrystalline diamond membrane for protein mass detection using MALDI-TOF-MS

Diana Hildebrand[1], Robert Blick[1], Hyun-Cheol Shin[1,2], Jonghoo Park[3], Zlatan Aksamija[4], Hyunseouk Kim[2]
[1]University of Hamburg, [2]University of Wisconsin-Madison,[3]Kyungpook National University, Daegu, Korea, [4]University of Massachusetts-Amherst

Introduction
Today biomolecule detection via Matrix-assisted-laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is usually carried out by secondary electron multiplier based detectors (e.g. MCPs). The secondary electron emission yield depends on the velocity of the incident ions causing a remarkable decrease of the detection sensitivity for slowly drifting large ions, such as intact proteins. Therefore, MALDI-TOF-MS-analysis remains restricted to the analysis of biomolecules with a molecular weight (MW) below 50 kDa. To overcome these limitations we introduce an improved ion detection principle by employing freestanding ultra-nanocrystalline diamond nanomembranes for the analysis of intact proteins.

Methods
The boron doped diamond nanomembranes (thickness: 100 nm, area: 400x400 µm2) are fabricated from diamond-on-insulator (DOI) material by optical lithography, followed by straightforward etching methods. The ion-detector consists of the ultra-thin diamond nanomembrane, an extraction gate, a microchannel plate and an anode. The number of electrons emitted by phonon-assisted-field-emission (PAFE) is amplified by MCPs and collected by an anode. A real time oscilloscope performs signal recording after low pass filtering. The detector is placed at the end of the flight tube of a MALDI-TOF-MS (Voyager-DE STR, Perseptive Biosystems). The applicability of the diamond nanomembranes as a field emitter was validated by ion detection in a MALDI-TOF-MS analyzing different proteins (Insulin, Cytochrome C, Apomyoglobin, Aldolase and Bovine Serum Albumin; Concentration: 10-100 µM; Matrix: Sinapinic acid).

Results
Using the diamond nanomembrane detector all of the analyzed proteins where detected, convering a broad mass range from 5.7 kDa to 66 kDa. Mass spectra of Cytochrome C (12,4 kDa, 100 µM) show overall similar peak intensities to those for Insulin (100 µM). Similar results where achieved for the detection of the proteins in 10 µM concentration including Apomyoglobin (16,9 kDa), Aldolase (39.2 kDa) and Albumin (BSA 66.4 kDa), showing the independency of the signal intensity on protein mass. The mass resolution (FWHM) obtained for Insulin (m/Δm=350) and Cytochrome C (m/Δm 438), respectively, for Apomyoglobin (m/Δm=892), Aldolase (m/Δm=1186) and Albumin (m/Δm =1443) increased with increasing MW.

Conclusions
Our data demonstrate the feasibility of the diamond nanomembrane detector-equipped MALDI-TOF-MS for protein analysis over a broad mass range. The results by the diamond-based detector outperformed those obtained from SiNx- and SOI-, and MCP-detectors. Ion detection by the nanomembrane-detector only depends on the kinetic energies of the impinging ions, providing ion-mass independent signal intensities. We expect further data demonstrating that this technology is a powerful tool for macro biomolecule (protein/complex) analysis such as virus capsids and protein aggregates.

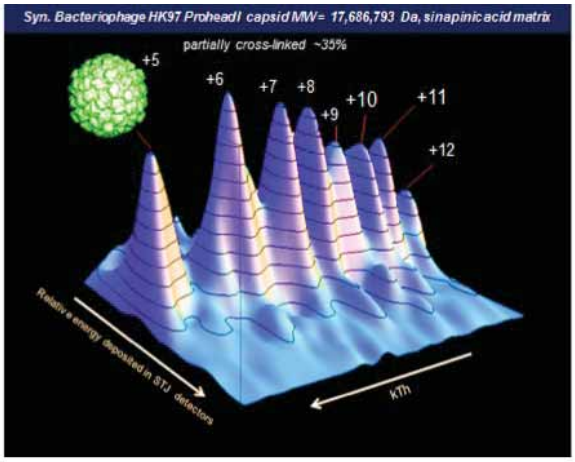
Novel Aspects
Detection of large proteins by MALDI-TOF-MS by phonon assisted field emission using a freestanding diamond nanomembrane-based ion-detector.

TOS14-05 Heavy ion mass spectrometry using STJ cryodetectors -- from Ferritin to the +1 charge state of bacteriophage HK97 capsid at 13MTh

Mark Bier[1], Logan Plath[1], David Sipe[1], Jonathan Feldman[1], Robert Duda[2], Hendrix Roger[2]
[1]Carnegie Mellon University, [2]University of Pittsburgh

Introduction
In 2003, Comet Corporation in Flamatt, Switzerland launched the first commercial superconducting tunnel junction (STJ) cryodetector based heavy ion mass spectrometer, the Macromizer™. This instrument was capable of detecting MDa masses and allowed the determination of low charge states by collision energy measurements. The Macromizer instrument is a matrix-assisted laser desorption ionization (MALDI) mass spectrometer with a time-of-flight (TOF) mass analyzer. The Macromizer was discontinued in 2005. In 2006 we acquired the first determination of Head II

capsid of the T7 bacteriophage HK97 at 13 MDa (ASMS 2007), expanded ProHead I at 17.7MegaDa, a multimeric capsid complex at least 26 MDa (ASMS 2008) and the capsid of the T9 bacteriophage D3 (16.6MDa) by distinguishing between the individual charge states. This talk showcases some of the STJ data that we have collected over the last seven years.



Heavy Ion Mass Spectrometry using STJ Cryodetectors -- from Ferritin to the +1 Charge State of Bacteriophage HK97 Capsids at 13MTh

Methods
A MALDI-TOF mass spectrometer equipped with a STJ cryodetector (MacromizerTM ; Comet AG) was used to obtain all data. A nitrogen laser at 337 nm was used for MALDI from a variety of matrix mixtures mixed with 0.1% TFA or AA and the acceleration voltage was 15 or 20kV. The bacteriophage HK97 capsids were made from 420 identical proteins (gp5). HK97 Head II (13 MDa) is the cleaved and cross-linked mature version of Head I which is approximately 17 MDa. To achieve the 13MTh (+1) result, the source code was modified. Laminin was dialyzed and mixed with sinapinic acid.

Results
Heavy ion complexes that we have weighed include biomolecules such as IgM (1MDa), ferritin (900kDa), laminin (800kDa), von Willebrand factor (220kDa to 1+MDa) and synthetic nanoparticles such as quantum dots (~1MDa), 5nm gold particles and more recently, multiply charged single-walled carbon nanotubes (3-5MDa). The observation of the +1 charge state at 12.9 MTh from HK97 Head II has shown that the instrument is capable of detecting ions at this mass range, which will be necessary to weigh larger nanoparticles such as whole virion of HK97 (MW ~45MDa), a goal of this work.

Conclusions
There are clear advantages to this STJ cryodetectors technology, but it has its limitations. Since typical MALDI requires highly organic preparations and because it is more energetic than ESI, there is always the potential for fragmentation on the plate, during ionization or in the flight tube. The primary advantage of STJ detection is the improved signal response of heavy ions above 150kTh and more importantly, improved detection into the single and double digit MTh m/z range while still distinguishing between charge states. Additionally, multimeric multiply charged species are observed in the spectra that are revealed by the STJ results that are not identified in the typical MALDI TOF mass spectrum with a MCP.

Novel Aspect
Remarkable STJ cryodetection macromolecular MS data, which includes the 13MTh HK97 at the +1 charge state. Attempts to weigh the HK97 whole virion at ~45MDa.

TOS15 - Effect-Directed Analytical MS

Room 5
Level 3

Chairs: Marc Suter, Olivier Laprévote

TOS15-01 Keynote: Towards higher throughput in effect-directed analysis

Marja Lamoree
Free University of Amsterdam

Introduction
In the past decades, Effect-Directed Analysis (EDA) – in which chemical analytical techniques are combined with biological/toxicological assays to direct the chemical analysis and identify environmental contaminants capable of causing adverse effects – has developed into a promising tool for investigative analysis. Research in this field was rekindled in the late nineties and focused on the identification of compounds that caused estrogenic effects in fish. By the implementation of assays covering other (endocrine disruption) endpoints than estrogenicity, such as (anti-) androgenicity and thyroid hormone disruption, the scope of EDA was widened. When problems related to the relatively low throughput and low identification success rate can be addressed, EDA may find its application in various fields, such as investigative water/sediment quality monitoring to support the corresponding policies, in human and environmental exposomics, etc.

Methods
Various toxicological endpoints have been used in our EDA studies, such as in vitro (anti-)androgenicity, thyroid hormone disruption and in vivo zebrafish embryotoxicity. To enhance the throughput of EDA, we used microfractionation into 96 well plates using 1D and 2D LC-techniques, thus facilitating the connection with most common bioassay formats. Mass spectrometric detection and identification were done using accurate mass data from high resolution mass spectrometers such as the LTQ Orbitrap and Time-of-Flight MS. For data analysis, both library searching with tailor made libraries and de novo identification strategies were applied.

Results
In this presentation, examples of successful EDA studies will be given revealing the identity of (emerging) pollutants in sediments, surface waters and biota samples such as polar bear plasma. Compounds that could be identified were organophosphate flame retardants, musk fragrances, steroidal compounds, oxygenated PAHs, hydroxylated PCBs and various herbicides. Through microfractionation and high resolution mass spectrometry, an improved bioactivity-to-identity correlation was obtained, leading not only to higher throughput but also to a higher identification success rate.

Conclusions
The application of accurate mass spectrometric techniques in environmental analysis has shown to facilitate the identification of unknown toxicants to some extent, but a major breakthrough in the identification of (known) unknowns has yet to come. In the near future, improved data analysis and web and database searching are expected to contribute further to the development of EDA as a tool for the identification of toxicants.

Novel aspects
The multidisciplinary approach of EDA, combining in vitro and in vivo bioassays, 1D and 2D LC fractionation and HR-MS for identification of toxicants has demonstrated to allow higher throughput and increase the number of successful identifications of toxic compounds.

TOS15-02 Identification of emerging pharmaceutical pollutants and human metabolites in urban wastewater treatment plants using effect-directed analysis

Caroline Gardia-Parège[1], Marie-Hélène Dévier[1], Nicolas Creusot[2], Selim Aït-Aïssa[2], Hélène Budzinski[1]
[1]EPOC-LPTC, [2]Inéris

Introduction
Wastewater treatment plants (WWTP) are the main source of endocrine disrupting compounds (EDCs) released in aquatic environment. Considering the large diversity of compounds and their possible transformation products, many EDCs are currently unknown. In this study, both an influent and an effluent of urban WWTP which shown in vitro biological activities, were subjected to effect directed analysis (EDA) in order to identify molecules responsible for measured activities.

Methods
Investigation was conducted on a representative urban WWTP (influent and effluent). After biological assessment of samples, the extracts were fractionated by HPLC using C18 column. 40 fractions were collected and bio-tested using in vitro reporter cell-based bioassays (estrogen, androgen and pregnane X receptors). In the most active fractions, the identification of compounds was performed by target chemical screening, based on LC-MS/MS, and non-target screening, based on LC-QTOF.

Results
Biological profile of crude wastewater extract indicated the presence of estrogenic, PXR-like activities and a strong androgenic activity. Target chemical analyses in raw influent extract showed the occurrence of high concentrations of natural steroids including androgens and corticoïds. These compounds could explain a part of the observed activities. In order to identify other active molecules in wastewater sample, EDA approach was performed on extracts of both influent and effluent. For estrogenic activity, the biological profile of the fractionationated effluent was the same as the influent except for one fraction. This result suggests the possible presence of transformation product produced by the treatment process. To identify the active compounds a LC-QTOF system was used. Our identification strategy allowed generating a list of suspected compounds including drugs, their metabolites, phytoestrogens and biliary metabolites. A selection of compounds was purchased and the confirmation of both structural identification and biological activities is still under process and will be presented.

Conclusions
In this work, WWTP samples were shown to present estrogenic and androgenic activities and a part of these activities could be explained by natural steroids detected by target chemical analysis. Considering the thousands of compounds present in crude extracts, EDA allowed to simplify samples focusing only on the active fractions and so to simplify the EDCs identification process. This approach combined to LC-QTOF has currently allowed establishing a list of non-target candidate compounds that could explain observed biological activities.

Novel aspect
EDA associated with Liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer (6540 LC-QTOF Agilent technologies®) to simplify complex environmental sample and to identify non-target EDCs.

Acknowledgements
The French Ministry for Ecology, Sustainable Development and Energy, the Aquitaine Region and the European Union (CPER A2E project) are acknowledged for their financial support. Europe is moving in Aquitaine with the European Regional Development Fund.

TOS15-03 Online LCxLC-ToF MS for effect-directed analysis in effluent and surface water

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Introduction
The environment is contaminated with a large number of compounds that may impact organisms in, e.g., surface waters. Due to the increasing complexity of the mixture of compounds occurring in the aquatic environment, there is a need to apply powerful and innovative separation techniques coupled to high resolution mass spectrometry (HRMS) for the identification of emerging contaminants. We have developed and implemented a comprehensive two-dimensional liquid chromatography (LCxLC) system linked to a 96-384 well fraction collector, a UV detector and a high resolution time of flight mass spectrometer (ToF-MS) to support the identification of emerging toxicants in Effect-Directed Analysis (EDA). The LCxLC system is interfaced by an Agilent 2-position/4-port duo valve andhyphenated with a splitter to a UV detector combined with a fraction collector and to ESI-ToF MS (EDA-EMERGE project, EU contract 290100). The collected fractions will be tested for developmental toxicity using a high throughput zebrafish embryo toxicity (ZFET) assay.

Methods
The system was validated using environmental standard compound mixtures of e.g. PAHs and triazine, organophosphate and carbamate pesticides, to characterize the chromatographic system, the stability of the retention times, recoveries of collected fractions, separation efficiency etc. Secondly, the hyphenation of LCxLC with HR-ToF-MS was optimized, including splitter settings, transfer of data files between different software packages, background subtraction using instrument software tools, etc. Finally, the optimized LCxLC-UV-ToF-MS system was used for the analysis of large volume and passive sampler extracts of waste-, surface and drinking water samples (project sponsored by the BE-Basic Foundation). The ZFET assay was conducted to direct the further confirmation of active fractions.

Results
The LCxLC-ToF MS system was optimized and validated for separation of compounds in the large volume and passive sampler extracts of water samples of various origin. Identification of the most important peaks was performed and a relationship with the observed activity in the bioassay was established.

Conclusions
We have demonstrated that a significant enhancement of the peak capacity facilitates the rapid identification of toxic compounds by online LCxLC-ToF MS and high throughput toxicity testing, leading to a comprehensive chemical characterization of environmental water samples, including toxicological evaluation of the identified compounds.

Novel aspects
This is the first report of comprehensive LCxLC coupling with high resolution mass spectrometry combined with high throughput ZFET testing, applied in environmental Effect-Direct Analysis.

TOS15-04 Exploring the performance of a nontarget screening workflow on known environmental contaminants

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Novel Aspects
First assessment of a nontarget screening workflow domain for environmental contaminants
Liquid chromatography-high resolution mass spectrometry (LC-HRMS) is a mature technique for the identification of contaminants in environmental samples. However, the performance of evaluation procedures for LC-HRMS data in nontarget screening approaches has not been widely assessed so far. Thus, a known set of environmental contaminants were used to test whether (i) the peaks of these compounds could be detected by chromatogram and spectral data processing of spiked water extracts, and (ii) to which extent LC retention and MS fragmentation prediction allowed to find the correct compound from database-derived lists of candidate structures for its molecular formulas.
The test compound list contained 78 micropollutants, while 26 aliquots of a pristine surface water extract were spiked at different compound and matrix concentrations using a Design of Experiment approach. After analysis by LC-HRMS (reverse phase LC on a C18 column, coupled to a LTQ Orbitrap XL instrument) parameters settings of the data processing software MZmine 2.10 were optimized to detect maximum target peaks from the samples and compared to a manual evaluation. The candidate structure lists for each compound were received from ChemSpider based on the molecular formula. Candidate selection was performed using MetFrag and the Chromatographic Hydrophobicity Index (CHI). MetFrag is a combinatorial fragmenter applying a bond disconnection approach, ranking the candidates according to the agreement between predicted fragmentation compared to the experimental mass spectrum. The CHI approach predicts the LC retention behaviour based on Linear Solvation Energy Relationships. For evaluation we (i) compared the match of experimental and predicted MS fragmentation and retention behaviour for the correct structure and (ii) compared to which extent correct structures are excluded by the two prediction methods. A comparison of the MetFrag scores obtained for the correct candidate structure shows that MetFrag performs well in many cases, which was improved by using the results from two different fragmentation techniques (HCD and CID). Retention prediction performed well for around 80% of the neutral compounds and 70% of the steroid compounds. The CHI model does not account for electrostatic interactions and thus CHI values of 66% cationic compounds at the LC conditions (pH 2.6) were overestimated, but were quite close to 33% more hydrophobic compounds, suggesting that in these cases an application of the method is possible. Based on the results of this study, it is possible to make some general statements for which types of structures and application of the methods is useful and in which cases the user has to be cautious with the results obtained. In general, anticipating the domain for nontarget screening workflow can improve the interpretation of nontarget screening results.

TOS15-05 Direct mass spectrometry-to-bioassay correlation for rapid identification of toxic pollutants in water using high-throughput effect directed analysis

Jeroen Kool[1], Willem Jonker[1], Marja Lamoree[1], Corine Houtman[2], Timo Hamers[1], Wilfried Niessen[1], Govert Somsen[1]
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Introduction

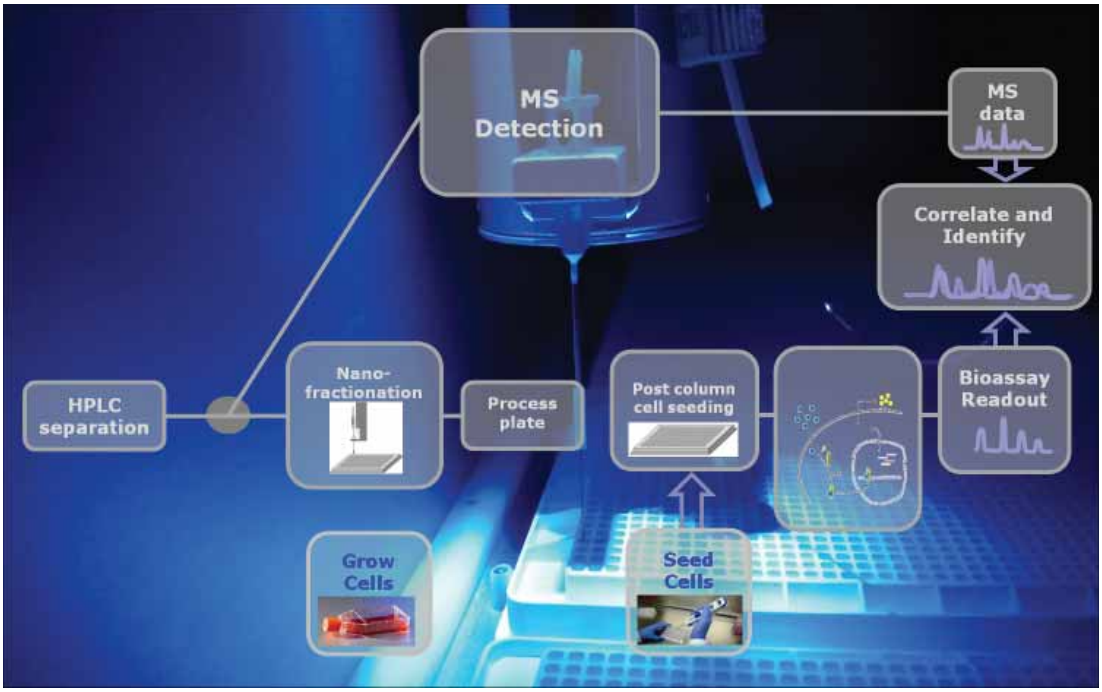
All kinds of contaminants emerge in the aquatic environment. Numerous bioassay surveys have demonstrated the presence of unknown compounds with toxic activities. Identification of toxic compounds is currently done by effect-directed analysis (EDA). Environmental samples showing toxic activity are fractionated with liquid chromatography into a limited number of fractions. Each fraction is retested for toxic activity using a bioassay. Ultimately, the "hot fractions" are analyzed with mass spectrometry to identify the toxic compounds. EDA studies often fail to identify the responsible toxic compounds, because the obtained fractions are still too complex for chemical identification. A second drawback is that current EDA methods are labor intensive and as such not suitable for routine monitoring.

Methods

We demonstrate a rapid and sensitive platform involving nanofractionation for high-resolution identification of toxic compounds in the environment, mainly focusing on water. The efficient combination of liquid chromatography with direct bio-assaying and parallel mass spectrometry enables us to screen many water samples in short timeframes. Nanofractions are subjected to a functional assay for estrogenic and anti-estrogenic compounds, and bioassay chromatograms are plotted and overlaid with MS data obtained in parallel.

Results

We developed a robust nanofractionation methodology capable of rapidly collecting very small LC fractions onto high density well plates for subsequent high resolution (mammalian) cellular assaying with parallel compound identification by MS. We identified bioactive pollutants in water samples by using accurate peak shape correlation of reconstructed bioassay chromatograms and extracted ion chromatograms. Besides accurate masses found for the bioactives, additional confirmation was obtained by MS/MS analysis, and in case of known pollutants, also by re-analysis of the corresponding standards. We used the estrogen receptor as end point for bioactivity assessment, but other endocrine disrupting targets can be used as well. Successful preliminary data for androgen receptor bioactivity assessment with parallel MS based identification of bioactives will also be shown.



Conclusions

The high-throughput EDA methodology developed opens up the possibility to rapidly and sensitively pinpoint and identify bioactive pollutants in the environment after standard sample pre-treatment.

Novel aspects

The combination of high-resolution nanofractionation followed by (mammalian) cell-based bioassays together with accurate bioactivity-to-identity correlation using parallel MS analysis. This enables cumbersome and time-consuming conventional EDA methods to be transformed into a more advanced and routine analysis strategy.

TOS16 - Labeling Strategies and Quantitative Biomolecule Analysis

Room 1
Level 1

Chairs: Paola Picotti, Marc Suter

TOS16-01 **Keynote: Measuring protein synthesis and breakdown using stable isotopes and mass spectrometry**

Dwight Matthews
University of Vermont

Scientists have been developing methods to measure in vivo synthesis and breakdown rates of protein since it was determined in the 1930's that protein was dynamic and not static. The earliest methods used isotope ratio mass spectrometry and stable isotopes, but these tedious methods were replaced with the introduction of radioisotopes. It was not until development of new mass spectrometry instruments in the 1970's that there was a resurgence in the use of stable isotope tracers. Scientists also realized the importance of understanding the turnover rates of individual proteins, rather than whole tissue protein turnover. The introduction of ESI and LCMS/MS instrumentation revolutionized proteomics, but almost all work was centered on identifying and/or quantifying relative abundances of individual proteins. It would take several years after introduction of modern bottom-up proteomic methods before scientists would realize what scientists as early as the 1930's knew: protein abundances are regulated by the rates of synthesis and breakdown of individual proteins, and that is what we should be measuring. Because ESI-LCMS/MS methods are specific for individual proteins, this technology is ideal to measure protein kinetics using stable isotope tracers. A number of different approaches have been developed, but very few are concerned with turnover rates not of specific proteins, but of specific protein posttranslational states. The latter is particularly important to understand, but measurements of individual protein posttranslational states require specialized protein fractionation methods in conjunction with ESI-LCMS/MS. The other important variable is the rate of individual protein turnover. LCMS/MS methods remain limited in determining low enrichment levels of stable isotopes, which is typically found in slower turnover proteins that will incorporate limited enrichment of stable isotope tracers during the time course of the experiment. Thus, we are faced with improving our ESI-LCMS/MS methodology with a specific focus on determining small enrichment increases of stable isotopes in proteins. These method move away from global measurements of proteins and protein turnover and focus on directed highly specific measurements of selected proteins. The current state-of-the-art of determining stable isotope enrichments in peptides from proteins by ESI-LCMS/MS will be discussed.

TOS16-02 **Using Selective Reaction Monitoring (SRM) mass spectrometry to unmask regulatory feedback loops controlling adipogenesis**

Robert Ahrends[1], Mary N. Teruel[2]
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Background
Due to modern lifestyle changes, obesity has a worldwide impact on human health. The obesity epidemic is now recognized as one of the most important public health problems facing the world today. Understanding adipogenesis is crucial to understanding obesity; failure of adipogenesis was shown to be a key factor in the development of diabetes. In earlier work using single-cell imaging, we demonstrated that there is a distinct decision made during the time course of adipogenesis. Thereby positive feedbacks loops between PPARg and other transcription factors (TFs) in the differentiation network areregulating this decision. We identified a positive feedback loop between PPARg and C/EBPb that plays a critical role in regulating adipogenesis. Since multiple feedback loops with different timing and strengths can sharpen the decision process and control the number of cells which are differentiating, we wanted to gain a better understanding of how many other proteins could be involved in the decision process.

Objective
The objective of this work is to search for feedback loops that could play a key role in the commitment decision.

Methods
Using Selected Reaction Monitoring (SRM) mass spectrometry combined with perturbations, we analyzed OP9 to detect peptides of TFs which can serve as probes. We validated these probes with isotopically coded internal peptide standards and established a SRM library of transcriptional key regulators. These probes were subsequently used to quantitatively profile different stages of adipogenesis to obtain time courses of different TFs. To achieve our major goal to elucidate the TF control network in more detail, we furthermore searched for hidden feedback loops in the differentiation system. To do so we chemically manipulated the activity level of PPARg and its potential feedback partners individually. If a protein was a component of one or several feedback loops and was experimentally manipulated, all the other components of feedback loops associated with this protein should display a relative change in abundance and vice versa.

Results
We developed a SRM methodology to monitor the concentration changes of TFs during adipogenesis. Using this SRM methodology together the perturbation of PPARg, we were able to validate known feedback loops (C/EBPa, C/EBPb) and to identify several new feedback Loops.

Conclusions
PPARg is the master regulator of adipogenesis. To successfully differentiate preadipocytes into adipocytes, its activity needs to be tightly regulated by a network of feedback loops. Overall, the study provides a new SRM MS-based method to uncover novel feedback loops regulating TFs.. Based on this method, we have identified 7 new proteins which are fundamental regulators of PPARg and the fat cell commitment decision.

TOS16-03 **Assessing the variability of ^{15}N metabolic labeling-based proteomics in mouse brain and plasma**

Giuseppina Maccarrone[1], Michaela D. Filiou[1], Magdalena Soukupova[1,2], Christiane Rewerts[1], Christian Webhofer[1,3], Christoph W Turck[1]

[1]Max Planck Institute of Psychiatry, [2]University of Glasgow, [3]Sandoz Biopharmaceutical

Introduction

In vivo15N metabolic labeling is a valuable tool for quantitative proteomics and hypothesis-free identification of molecular biosignatures and has been successfully applied to mouse models of cancer and psychiatric disorders. However, the variability of 15N metabolic labeling proteomics workflows has not been assessed in mice. Here, we address technical and sample preparation variability in mouse plasma and brain.

Methods

Plasma and brain specimens from 15N-labeled and unlabeled 56 days old male mice were mixed at a 1:1 ratio and technical variability was assessed by measuring the 15N/14N plasma and the 15N/14N brain samples three times each by nanoLC-ESI-MS/MS (technical replicates). The contribution of sample preparation to overall method variability was assessed by measuring three different brain and plasma samples derived from the same 15N/14N protein extract but processed independently for MS analysis (sample preparation replicates).

Results

LC variability evaluation by comparing peptide RTs across brain and plasma technical replicates revealed an average RT correlation coefficient of 0.96 for plasma and 0.98 for brain. In sample preparation replicates of brain and plasma the average RT correlation coefficient was 0.95 and 0.93, respectively. The technical variability for log2(15N/14N) ratios for replicate pairs in plasma and brain revealed correlation coefficients of 0.91 and 0.78, respectively. The average log2(15N/14N) ratio correlation coefficient in brain sample preparation was 0.76, markedly lower than the corresponding plasma sample preparation coefficient which reached 0.84.

Conclusions

Taken together, the comparison of the plasma and brain data revealed that sample type influences the variability of the 15N metabolic labeling workflow results in an LC-independent manner.

Novel Aspect

This is the first time that the 15N metabolic labeling workflow variability is assessed in mouse specimens. By evaluating method repeatability in mouse plasma and brain we provide an experimental design framework for 15N metabolic labeling proteomics applications in mouse models, allowing an accurate interpretation of the acquired data.

TOS16-04 **A novel SWATH-MS platform for comprehensive characterization of the epigenetic histone modifications**

Joerg Dojahn[1], Dietmar Waidelich[1], Sibylle Heidelberger[1], Quentin Enjalbert[1], Antonio Serna[1], Francesco Brancia[1], Sahana Mollah[1], Sean Seymour[1], Eric Johansen[1], Benjamin A. Garcia[2]

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Introduction

Histone post-translational modification (PTMs) sites regulate gene transcription, thus making reliable quantification a high priority in epigenetic studies. Quantification of histone PTMs is performed by shotgun proteomics favoring discovery of novel/low level PTMs, or targeted analyses (SRM) of known PTM sites. Each workflow has strengths and weaknesses for PTM quantification. Data independent acquisition (DIA) for comprehensive data generation combined with targeted data processing has recently been demonstrated to provide very high quality quantitative data. The advantages of this approach for targeted PTM quantification include no upfront assay development, quantitative data on all analytes and no dynamic exclusion of isobaric peptides. In this study, we develop a SWATH™ acquisition platform for quantitating histone.

Methods

Histones were acid extracted from HeLa cells. A custom-made synthetic heavy labeled modified peptide library was spiked into the sample. SWATH™ acquisition of the sample was performed using a QqTOF system and a chip based LC system operated at various flow rates. The same sample was also analyzed on an Orbitrap system coupled to a similar nanoLC system. Replicate analysis was performed for these samples to obtain quantitation statistics. Data was processed using either commercial or in-house software.

Results & Conclusions

Results from both the asynchronous non-treated and butyrate treated samples run in a normal data-dependent mode (DDA) was used to generate a spectral ion library for the histone samples. A key issue in analyzing histone peptide mixture is the presence of various isoforms of peptides, primarily from PTMs on the same peptide, but localized on different amino acids. Quantitation of these isoforms in a data-dependent mode using MS1 quantitation has been very challenging due to co-elution of isobaric forms. The peptide, GKGGKGLGKGGAKR from histone H4 is found to be acetylated at 4 sites: Lys2, Lys5, Lys9 or Lys13. Extracted ion chromatogram (XIC) of its precursor mass of m/z 768.9465 resulted in 2 peaks and a third that partially overlapped. This made it difficult to distinguish and quantify each isoform based on MS1. However, with SWATH acquisition, all the MS/MS fragments are collected in one data set, so unique/combination of MS/MS fragments of each isoform were extracted post-acquisition and used for sequence assignment and quantification even if they co-eluted. Based on the SWATH analysis, the peak at 39.2 min was identified as acetylated Lys 2, while Lys5 and Lys9 coeluted at 41.7min and partially overlapped with Lys13 peak at 42.1min. Replicate SWATH analysis resulted on average a 10% CV for the peptide fragments, quality rivaling an MRM approach. We will present results from more of these histone isoforms and also the comparison of the quantification of the SWATH platform versus data-dependent mode analysis on an Orbitrap mass spectrometer. This work illustrates the advantage of using SWATH analysis for characterization of histone modifications.

Novel Aspect
Using SWATH™ acquisition for unambiguous sequence assignment and quantification of isobaric histone peptides

TOS16-05 **MeCAT - New possibilities of protein analysis and quantification**
David Benda, Gunnar Schwarz, Michael W. Linscheid
Humboldt-Universität zu Berlin

Introduction
The need for reliable protein quantification methods steadily increases, mass spectrometry (MS) forms an integral part of protein quantification. Therefore, stable isotopes for protein labeling including ICAT and iTRAQ are used progressively. The isotope label is introduced with a specific reagent, while different isotopically labeled reagents are used for different samples. The mass differences of labeled samples are then detected by MS to get qualitative and quantitative information. As a further quantification methodology, we developed MeCAT (Metal Coded Affinity Tagging). MeCAT uses chelate complexes of lanthanides for relative and absolute quantification. For the later, elemental mass spectrometry can be employed.

Methods
Model proteins are labeled with the MeCAT-IA reagent, which contains a cysteine-reactive group and harbors a lanthanide ion for quantification. In order to separate labeled proteins from other components a HPLC separation coupled online to ESI-MS was used. Further fragmentation experiments with labeled proteins were performed, including infrared multi photon dissociation (IRMPD) and higher-energy C-trap dissociation (HCD). Both yielding characteristic fragments of the labeling group. Relative quantification was performed with differentially labeled samples using ESI-MS, ESI-MS/MS and ICP-MS as reference. Additionally, first experiments of assessing labeled peptides in MALDI-Imaging were conducted and a new cleavable linker including the MeCAT-reagent was synthesized.

Results
The investigated model proteins cover a mass range of about 14 to 67 kDa in the unlabeled state and of about 20 to 92 kDa in the completely labeled state. The completeness of the labeling was assessed by mass spectrometry. In further experiments, the fragmentation behavior of labeled proteins was investigated. Here, the fragmentation techniques IRMPD and HCD were applied. In addition, several differentially labeled proteins were detected simultaneously in order to verify the possibility of relative quantification. All five differentially labeled proteins could be detected and subsequently fragmented. These results can be used for determining the concentration of proteins or peptides relatively to a labeled internal standard protein with known concentration or to determine the relative amount of the same protein from different samples. Finally, this method was applied to assess the amount of HSA in a human blood serum sample. The results of the relative quantification were confirmed by ICP-MS experiments.

Conclusions
It was shown that all four different model proteins could be labeled completely. Hence, an important requirement for reliable quantification of proteins was fulfilled and that certain labeled proteins can be used as an internal standard for the relative quantification by MeCAT-IA.

Novel Aspect
In addition to protein quantification in biological samples like HSA in blood serum, MeCAT can be used as part of a cleavable reagent and in MALDI imaging.

TOS17 - Protein Phosphorylation and other Post-translational Modifications

Room 2
Level 0

Chairs: Jesper Olsen, Ruedi Aebersold

TOS17-01 **Keynote: Mapping high resolution kinase-substrate network**
Andy Tao
Purdue University

Protein kinases and their substrates represent the largest signaling network that regulates protein-protein interactions, subcellular localization, and ultimately cellular functions. Here we introduce two complementary proteomic strategies to map high resolution kinase-substrate network. In the first method, novel direct substrates of protein kinases were identified through Kinase Assay LInked Phosphoproteomics (KALIP), which combines a sensitive stable isotope labeled kinase reaction with quantitative phosphoproteomics. The in vitro kinase reaction is carried out in a highly efficient manner using a pool of proteins derived directly from cellular components and O18-labeled ATP. The resulting newly phosphorylated proteins are then isolated and identified by mass spectrometry. A further comparison of these in vitro phosphorylated proteins with those derived from endogenous proteins isolated from cells in which the kinase is either

active or inhibited reveals new candidate protein substrates. The KALIP strategy was applied to identify novel direct substrates of multiple kinases, including Syk, a protein tyrosine kinase with duel properties of an oncogene and Erk1/MAPK3, a serine/threonine kinase involved in both physiological and pathological cell proliferation.
In the second strategy, termed fluorescence complementation-mass spectrometry (FCMS), direct upstream kinases are identified based on bimolecular fluorescence complementation to stabilize kinase-substrate interactions, followed by mass spectrometric analyses. We chose cAMP response element-binding protein (CREB) and several known CREB kinases as a model system to verify the method. A human kinases collection was constructed. Two truncated mutants of CREB-VN were constructed, with or without the kinase inducible domain (KID) and expressed, along with the kinase-VC collection, in SILAC culture cells to identify known and novel CREB kinases.

TOS17-02 **Characterization of N-linked glycans from vaccine antigens: the CYD tetravalent dengue vaccine**

Jean Dubayle, Sandrine Vialle, Manon Fradin, Bruno Guy, Olivier Adam, Philippe Talaga
Sanofi Pasteur

Dengue disease affects more than 230 millions people every year in inter-tropical areas, causing approximately 25 000 deaths, mostly children. Dengue viruses (DENV) are enveloped Flavivirus that are transmitted via the bite of Aedes mosquitoes. The DENV particle is made up of three structural proteins; among these, the envelope protein (E) is the major surface glycoprotein, responsible for virus attachment and fusion1. Human dendritic cells (DCs) are targets of DENV, and infection is mediated in part by the binding of DENV to DC-specific ICAM3-grabbing non integrin. Recent work has shown that N-glycosylation can influence the virus growth cycle, and terminal mannosylation of E-protein is essential for infecting DCs2. In addition, these membrane protein N-linked oligosaccharides are differentially processed by enzymes in insect and mammalian cells. The future Sanofi Pasteur tetravalent dengue vaccine ChimeriVax™ (CYD) is composed of four chimeric viruses based on the backbone of the attenuated yellow fever 17D vaccine (YFV 17D), and expressing structural antigens of each of the four dengue virus serotypes (E and prM).
Characterization of the N-linked glycans from the E-protein from CYD will be presented. Due to the low level of N-glycosylation (~5%) and the difficulty to purify large amounts of E-protein, we developed sensitive methods like MALDI-TOF, nanoLC-ESI-MS/MS and HILIC after fluorescent labelling. N-glycosylation profiling of the E-protein and site-specific determination of N-glycans were performed.
Evidence for mannosylation of E-protein were obtained for all serotypes. The data clearly demonstrate that E-protein glycoforms are able to interact with DC-SIGN receptor which, to date is the most important DENV receptor. Our results support in vitro studies, comparing the immunological consequences of infection with the CYD dengue viruses versus their wild type parent viruses by investigating the infectivity of CYD dengue viruses 1 to 4 in human monocyte-derived dendritic cells. In addition, these data are really very important for further studying the role of N-glycans in the virus-host cell interaction for Dengue and increase understanding of virus entry mechanisms.

References

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TOS17-03 **Characterization of N-terminal acetylated proteins in *Pseudomonas aeruginosa* PA14 strain**

Julie Hardouin, Tassadit Ouidir, Frédérique Jarnier, Pascal Cosette, Thierry Jouenne
University of Rouen

The vast majority of the proteins encoded in any genome naturally undergo a large number of crucial N-terminal modifications that may affect the protein status, fate and function. For prokaryotes different modifications can occur: Nα-acetylation (NTA), initiator methionine excision (NME)... In contrast to eukaryotes, NTA reports for prokaryotic organisms remains scarce and there are few acetylated proteins in bacteria [1]. Pseudomonas aeruginosa PA14, a Gram-negative bacterium, is a well-known opportunistic, nosocomial pathogen that causes chronic infection in immunocompromised individuals. This work deals with the Nα-acetylation modification characterizations of the proteins of Pseudomonas aeruginosa PA14 by proteomic approaches [2].
The Nα-acetylated peptides contribute to a rather limited fraction of the peptide mixture after protease digestion. Thus, to identify Nα-acetylated peptides, two approaches were used. First, after digestion, Nα-acetylated peptides were enriched using CNBr-activated Sepharose resin. Secondly, proteins were fractionated according to their pI in solution and then digested. All the peptides were analyzed by nanoLC-MS/MS (LTQ Orbitrap Elite). Due to the high number of peptide identifications, several bioinformatic developments were necessary to automatically analyze this high data number.
We succeeded to identify 117 Nα-acetylated proteins (112 new bacterial modified proteins). Low overlap was observed with these two methods highlighting the need to use different approaches to increase the number of identified modified proteins. Nα-acetylated peptides showed higher frequency of Met, Ser and Ala at the first position. We used a label free quantification (spectral counting) to determine the abundance of each form (acetylated vs non-acetylated). The free peptides were mainly more abundant than the Na-acetylated peptides, except for 4 proteins. We also investigated proteins acetylated on their iMet. Amino acids with a small radius of gyration are generally recognized by methionine aminopeptidases. Here, interestingly, some peptides with small penultimate residues kept their iMet and were acetylated.
To our knowledge, this study is the first report of a global proteomic approach to identify N-terminally acetylated proteins in bacteria. These results provide the most extensive data set of Nα-acetylated proteins (117 Nα-acetylated proteins) for this bacterial species and greatly enlarge the Nα-acetylated bacterial protein database. This modification is a more frequent event in bacteria (2%) that it was believed, but its characterization is a real challenge due to its low abundance. The characterization of the N-termini of prokaryotic proteins appears necessary to establish a database of modified proteins and to advance hypotheses about the role of these modifications in the bacterial processes.

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TOS17-04 **Monitoring dynamic protein phosphorylation on intact proteins by native MS on an orbitrap EMR**

Michiel van de Waterbeemd[1], Philip Lössl[1], Violette Gautier[1], Fabio Marino[1], Masami Yamashita[2], Elena Conti[2], Arjen Scholten[1], Albert J.R. Heck[1]
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Introduction

Phosphorylation on Proteins influences their activity, conformation, oligomeric state and/or binding of interaction partners. Although investigation of protein phosphorylation by bottom-up peptide analysis is extremely powerful, it does not directly provide data on the distribution and relative abundances of the different phosphoisoforms. Making use of a modified Orbitrap Exactive Plus with extended mass range, we designed a native MS setup to qualitatively

and quantitatively investigate phosphorylation of intact biomolecular assemblies and the structural re-arrangements accompanying them. We tested our approach on two systems; the cGMP-dependent protein kinase (PKG), that auto-phosphorylates upon cGMP or cAMP binding, and the aurora kinase A (AurA), that phosphorylates its interaction partner protein aurora borealis (HsBora).

Methods
Kinase reactions were carried out in presence of ATP and MgCl2 with a 1000-fold molar excess of cAMP or cGMP (PKG) or with a twofold excess of Bora (AurA). Samples were subsequently buffer exchanged to 150-500 mM ammonium acetate pH 6.8 and diluted to 0.5-5 µM protein concentration. The masses of all proteoforms were determined from them/z values of consecutive charge states. To obtain phosphorylation rates, the intensity weighted average number of phosphorylations was calculated and plotted against time.

Results
When PKG is analyzed in presence of cGMP or cAMP but without Mg-ATP, a stable 153 kDa homodimer with 2–4 cGMP molecules or 0–2 cAMP molecules bound was detected, reflecting the protein's preference for cGMP over cAMP. In presence of Mg-ATP, all these PKG/cyclic nucleotide complexes become progressively phosphorylated over time as is evidenced by a shift of the phosphoisoform distribution to higherm/z. The highest number of phosphate incorporations that can be accurately assigned is 11 for cAMP- and 5 for cGMP-containing complexes. In total we detected more than 33 different proteoforms of PKG, comprising the different nucleotide loaded complexes.
To prove that this approach is also applicable to monitor heterophosphorylation we investigated the interaction of the 33 kDa kinase AurA and a 17 kDa N-terminal fragment of hsBora. When incubated with Mg-ATP, hsBora becomes increasingly phosphorylated with a maximum of five phosphorylations being detected. Additionally, a 1:1 complex of AurA and Bora was identified. The complex becomes more abundant with longer incubation time and shows the same trend of progressive phosphorylation as unbound hsBora.

Conclusions
Owing to the high resolving power of the modified Orbitrap instrument, we were able to obtain baseline resolution of multiple phospho-isoforms of PKG, hsBora and AurA as well as to monitor changes in the phospho-isoform distribution over time. Moreover, different cyclic nucleotide-bound states of PKG and the 1:1 AurA/hsBora complex could be detected, illustrating the ability to detect both covalent and non-covalent changes in a native setting with this Orbitrap mass spectrometer.

Novel Aspect
Monitoring intact phosphoproteins by Native MS on an Orbitrap EMR provides direct evidence for the phosphorylation stoichiometry and abundance.

TOS17-05 **Characterization of unusual post-transitional modifications in antibodies and related molecules**
Patrick Schindler, Thierry Besson, Michèle Coulot, Edwige Fongue, Karen Vincent, Damien Begue, Patrick Graff, Francis Bitsch
Novartis

Introduction
The IgG's consists of covalently linked light and heavy chains with a size of about 150 kDa. If in-depth analytic has to be performed several strategies can be used, the bottom-up, the top down and the mid down approaches.
They have all their strengths and weaknesses, but the mid down approach is particularly useful for the characterization of post-translational modifications (PTMs) of antibodies and related molecules. Indeed, the latter approach consists of cleavage by selective chemical reaction (e. g. reduction) and/or limited digestion resulting in the production of larger parts which can then be separately measured and eventually further characterized, either by top-down or bottom up approaches.

Methods used
Mid down and bottom approaches, IdeS, trypsin and (limited and complete) Lys-C digestions, UPLC-ESMS and MSe, MALDI-MSMS and EDMAN sequencing.

Results and conclusion
We have successfully integrated this mid-down approach into our characterization workflow, mainly by using the IdeS enzyme. The latter cleaves specifically human IgG's and does not need to be adapted to each molecule to avoid over-digestion, as it is the case for the other enzymes generally used, e. g. pepsin, papain or Lys-C. We will show its usefulness through several examples such as the characterization of O-linked glycosylations and hydroxyprolines.

Novelty
Identification of a PTM, hydroxyproline, which was never before reported as being present on antibody-related molecules

TOS18 - Ion-Molecule and Ion-Ion Reactions in the Gas-Phase

Room 3
Level 0

Chairs: Peter Armentrout, Gianluca Giorgi

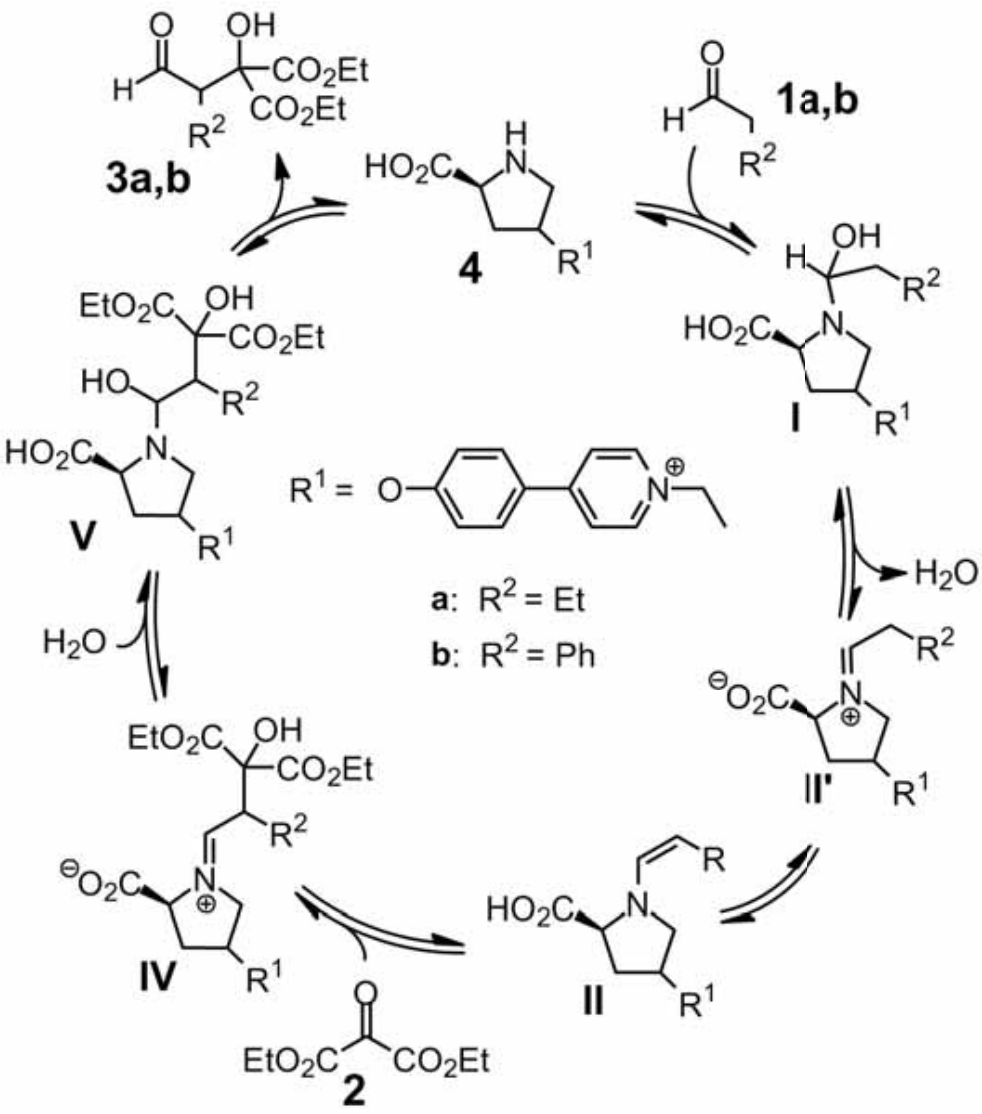
TOS18-01 **Keynote: Non-covalent molecular recognition as probed by tandem mass spectrometry**
Mary Rodgers
Wayne State University, Detroit

Nature makes use of a wide variety of noncovalent interactions including metal ion binding and hydrogen bonding to enable molecular recognition of various ions and molecules. The balance and interplay of many noncovalent interactions are generally needed to control structure and facilitate conformational change such that variations in the local environment (e.g., pH, metal ion concentration) can be used to fine tuning structure, and thereby alter function

in biological systems. Selectivity in molecular recognition processes generally requires both energetic and structural (size and shape) contributions to the binding. The thermochemistry of noncovalent ion-molecule complexes has been examined by measuring quantitative bond dissociation energies using guided ion beam tandem mass spectrometry techniques, whereas the structures of these complexes are probed using electronic structure calculations. The methods used are briefly reviewed and several examples of the types of information and insight that can be obtained from such structural and thermodynamic information are discussed. The binding of metal and protonated organic cations, including amino acids, to various crown ethers are reviewed and the trends elucidated on the basis of both electrostatic and structural contributions to the binding. Noncanonical base pairing has been examined for natural and modified nucleobases, with both the tautomeric form of the nucleobase(s) and the electron donating / withdrawing character of substituent(s) shown to be important contributors to the binding. Implications of such noncovalent molecular recognition for analytical applications and biological systems are discussed.

TOS18-02 **Using a charge-tagged proline-based organocatalyst for mass spectrometric mechanistic studies**

Johann Alexander Willms, Rita Beel, Martin L. Schmidt, Christian Mundt, Marianne Engeser
Rheinische Friedrich-Wilhelms-Universität, Bonn



Enantioselective organocatalysis has become a major research topic in organic chemistry.In this field, proline has proven to be an effective catalyst for a great variety of organic reactions, in most cases with either enamines or iminium ions as key intermediates. We have developed the synthesis of a charge-tagged L-proline-based organocatalyst 4 with the aim to elucidate mechanisms of organocatalyzed reactions by detecting transient intermediates via ESI-MS.
Our catalyst is composed of conventional L-proline with an 1-ethyl-pyridinium-4-phenoxy–substituent in 4-position. The stiff phenyl linker has the function to keep the charge tag preferably separated from the catalytic centre, so that the catalytic properties are not disturbed.
Because of its fixed charge, the signal abundance of the catalyst - and every intermediate to which the catalyst is bound - is strongly enhanced in ESI-MS compared to all neutral species in a reaction mixture, even if the particular species are present in very low concentrations.
As model reaction for the mechanistic studies we chose, among others, the direct proline-catalyzed asymmetric aldol reaction of the aldehydes 1a and 1b with diethyl ketomalonate 2 found by Jørgensen et al.

Methods
The MS experiments were performed with an Apex IV FT-ICR mass spectrometer equipped with an Apollo ESI source. To analyse the reaction directly after mixing the substrates, solutions of the reactants were fed into a PEEK mixing tee, diluted on-line by a second mixing tee and promptly introduced into the mass spectrometer. Different reaction times could be achieved by changing either the flow rate of the syringe pumps or varying the length of the capillary connecting both mixing tees.

Results
In the course of our MS investigations of the organocatalyzed reactions between the aldehydes 1a,b with carbonyl compound 2, the markedly enhanced ESI response factor of catalyst 4 enabled us to detect two intermediates of the proposed catalytic cycle shown in the scheme. In both cases the initial enamine intermediate II and the assumed iminium intermediate III could be detected as major signals in the mass spectrum in addition to the signal of the free catalyst. All Species were characterized by exact mass and MS/MS (in source CID). We were also able to obtain kinetic diagrams of both reactions showing the rise of enamine II during the first seconds after initiating the reaction with a subsequent increase of intermediate III.
Moreover the charge-tagged reaction intermediates were used to study their intrinsic reactivity in the gas phase of the ICR-cell.

Conclusion
Our charge-tagged organocatalyst is a suitable tool for the examination of L-proline-catalyzed reactions by ESI-MS. Further experiments with different organocatalyzed reactions will be performed in the near future.

TOS18-03 Electrospray mass spectrometric study of the metal triflates used as catalysts in their interaction with organic ligands: isomer recognition

Jean-François Gal[1], Claudio Iacobucci[2], Lionel Massi[3], Sandra Olivero[3], Elisabet Dunach[3], Francesco De Angelis[2]
[1]Université Nice Sophia Antipolis, [2]Università dell'Aquila, Italy, [3]Université Nice Sophia Antipolis, France

Introduction
Metal trifluoromethanesulfonates (triflates M(CF3SO3)n) are considered as Lewis «superacids», and such salts have remarkable catalytic properties. The variety and the complexity of their action is challenging for the synthetic chemist. Quantitative measures of the interaction of these Lewis acids with organic ligands would be useful for the rationalization of their catalytic effects. Under specific conditions, electrospray ionization mass spectrometry (ESI-MS) can afford such information [I. Monfardini, et al. Chem. Commun., 2010, 46, 8472-8474].

Methods
The ESI-MS method is based on the formation of positively charged adducts, using nitromethane as solvent. The adducts are formed by displacement of one anion in the salt M(CF3SO3)n by neutral Lewis bases, such as phosphoryl compounds, amides, ketones, alcohols. The reactions most often observed involved two neutral Lewis bases (LB) displacing one anion from the salt, for example:
In(CF3SO3)3 + 2 LB --> [In(CF3SO3)2(LB)2]+ + CF3SO3-
Such adducts are often the base peak in the ESI(+) mass spectra of mixtures of neutral ligands and metal triflates.
When two different ligands are in competition, the relative intensities of the ionic species containing the three different

permutations of ligands are related to their respective affinities for the metal center. A quantitative affinity parameter was obtained by modeling the effect of the ligand concentrations on the signal intensities [J.-F. Gal et al. J. Am. Soc. Mass Spectrom. 2012, 23, 2059-2062]. By combining relative affinities for various pairs of Lewis bases, a scale of relative ligand affinities for a given metal salt was generated.

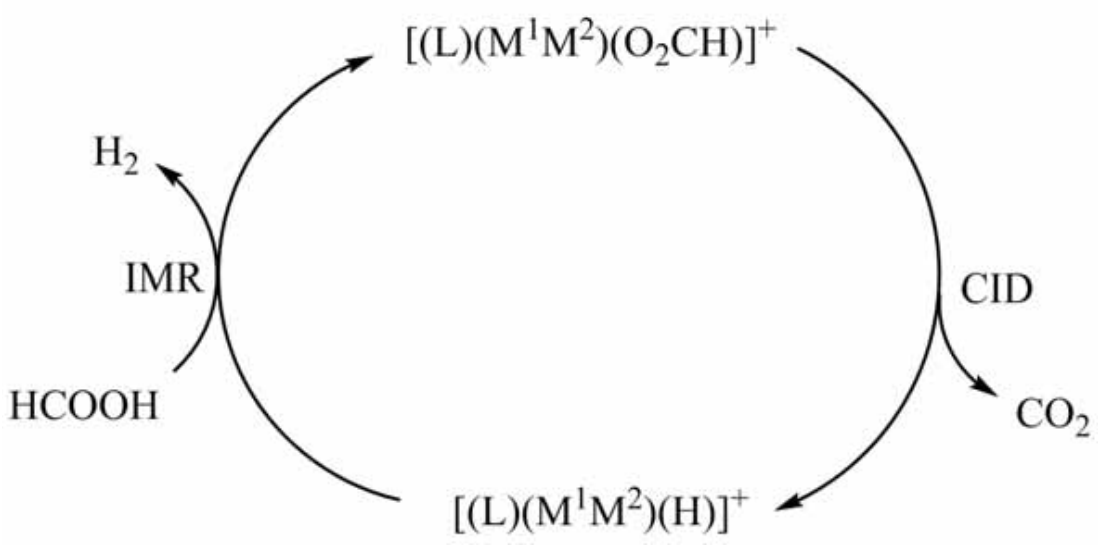
Results
This method is sensitive enough for quantifying the affinity difference between isomers of certain ligands. We therefore investigated the basic concept of ligand competition to develop a new stereoisomer recognition technique. Under carefully chosen conditions, it was possible to quantify enantiomers. From these results, it is also expected to get a better understanding of non-linear effects in asymmetric synthesis under organometallic catalysis.

Conclusions
The ESI mass spectra of a solution of metal triflate M(CF3SO3)n and a Lewis base LB produce essentially a singly-charged positive ion [M(CF3SO3)n-1(LB)2]+. The ligand competition between two different LBs for the metal center can be used for the recognition and quantification of stereoisomeric molecules. The method is also applicable to enantiomers under specific conditions.

Novel Aspect
Stereoisomer recognition and quantitation is possible using a ESI-MS ligand competition method. Enantiomers may be also distinguished.

TOS18-04 **Selective decomposition of formic acid into H_2 and CO_2 catalyzed by coinage metal hydride cluster ions**

Athanasios Zavras, George Khairallah, Jonathan White, Richard O'Hair
The University of Melbourne



Introduction

Hydrogen is of considerable interest as an alternative fuel source. Formic acid has attracted considerable interest as a hydrogen storage medium and efforts have been directed at developing catalysts for its selective decomposition into H2 and CO2. Coinage metal (Cu, Ag and Au) nanoclusters (CMNCs) have attracted significant attention due to their fundamentally interesting architectures, and unique properties with applications in areas such as catalysis. Electrospray ionization mass spectrometry (ESI-MS) has emerged as a valuable tool to monitor the solution and gas-phase reactivity of metal nanoclusters.. Here we have uncovered a gas phase cluster for the selective decomposition of formic acid, which has prompted us to explore the formation and reactivity of these clusters in the condensed phase.

Methods

Condensed phase clusters for ESI-MS analysis were typically prepared in a 10 mL glass vial and further diluted to concentrations of 50 – 100 μmol. This solution was injected at a sample flow rate of 5 μL. min-1 into the Finnigan ESI source of a LTQ-FT Hybrid Linear Ion Trap (LIT) Mass Spectrometer. In the CID experiments, the parent ion was depleted to a relative abundance of less than 20%.. Ion–molecule reactions (IMR) were performed using an LTQ-FT that was modified to introduce a gaseous mixture containing He and formic acid into the LIT. Ions resulting from IMR and/or CID were transferred to the FT-ICR cell for accurate mass measurements. Crystals suitable for structural characterization via X-ray crystallography were prepared by slow liquid diffusion.

Results

ESI-MS has been applied to survey the identity of phosphine protected metal formate clusters. A common cluster was observed with the following stoichiometry, [(L)(M1M2)(O2CH)]+ where: L = phosphine ligand; M1 or M2 = Cu, Ag or Au. CID of [(L)(M1M2)(O2CH)]+ results in [(L)(M1M2)(H)]+. IMR of formic acid with [(L)(M1M2)(H)]+ is an exothermic process, which regenerates [(L)(M1M2)(O2CH)]+ and releases H2. The combinations of various metals and ligands have a pronounced affect on the rate of reactivity highlighting a synergistic effect of having mixed metals, which leads to an increase in the rate of reaction. Additionally, the steric bulk of the ligand has a marked effect on the rate of reaction.

Conclusion

Formic acid has been shown to react with [(L)(M1M2)(H)]+ to generate H2, the rate of which is dependent on steric and electronic factors. The temperature dependence of [(L)(M1M2)(O2CH)]+ to generate [(L)(M1M2)(H)]+ in the condensed phase could provide critical insight into nanocluster formation via hydride intermediates.

Novel Aspect

ESI-MS has been applied to investigate the catalysis of formic acid in hydrogen on metal clusters.

TOS18-05 **Ion/ion reactions: new chemistries for metal ion removal, oxidation of peptides, and esterification in the gas phase**

Scott McLuckey, Alice Pilo, Carl Luongo, Joshua Gilbert, Jiexun Bu
Purdue University

Introduction

The advent of ionization methods that form multiply charged ions from polyatomic molecules has allowed for the exploration of ion/ion reactions as a new class of chemical reactions in analytical mass spectrometry. In this presentation, we emphasize the use of novel reagents that allow for the selective removal of metal ions when both metal ions and protons are present, the selective oxidation of methionine and alkylated cysteine residues in peptides, and the esterification of acidic sites via the use of 'onium' ions.

Methods
All of the data underlying the findings described in this talk were collected in either three-dimensional quadrupole ion traps or linear ion traps (LITs) operated in the presence of a background gas at 1-10 mtorr. In some cases, data were collected using hybrid triple quadrupole/LIT or quadrupole/LIT/TOF instruments.

Results
Most anionic reagents used previously tend to remove protons when mixtures of excess metal ions and protons are present on an analyte ion. In fact, even when the excess charges are nominally present as metals, most anionic reagents tend to remove protons if there are hydrogens present in the molecule. We have recently explored the use of weakly associating anions, which are often used as the anions in ionic liquids, as reagents for cation removal. We find that the superacid anion derived from a carborane salt reacts exclusively by metal ion removal from cations. These results, as well as those derived from the use of other superacid anions, will be described.
We have recently learned that the periodate anion can oxidize methionine, and to a lesser extent tryptophan, residues in gaseous peptide ions. Evidence for this selective ion chemistry will be presented along with the use of this chemistry for the oxidation of cysteine residues that have undergone reduction and alkylation.
We have also recently learned that tetraalkylammonium and trialkylsulfonium cations react with carboxylate, sulf(on)ate, and phosph(on)ate groups via alkyl ion transfer. This process gives rise to gas-phase esterification. We will describe the observed chemistry and provide an analytical example of its use in the analysis of phosphatidyl choline lipids.

Conclusions
The reactions described here represent some of the latest selective reaction types that have been shown to be possible with gas-phase ion/ion reactions. They enable novel analytical strategies to be developed for mixture analysis, disulfide mapping, and lipidomic applications.

Novel Aspect
Selective metal ion removal from gas-phase cations, selective oxidation of methionine and alkylated cysteine residues, and selective gas-phase alkyl ion transfer to anionic sites.

TOS19 - Microfluidic Devices and Nanotechnology

Room 4
Level 0

Chairs: Hubert Girault, Detlef Günther

TOS19-01 **Keynote: Recombinant protein QC and disease diagnostics using chip integrated affinity MALDI strategies**

Thomas Laurell
Dongguk University, Seoul, South Korea

Solid phase based affinity extraction integrated with matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) analysis offers a route to rapid, sensitive protein and target specific analysis and potentially multiplex biomarker analysis. By integrating nanovials with perforated bottoms in a silicon chip with a footprint of a standard MALDI target, a MALDI plate, ISET (Integrated Selective Enrichment Target) with custom specific functionality is achieved1. The nanovials can be loaded with an arbitrary chromatographic matrix allowing for the design of application specific MALDI assays. All fluid handling is processed on the ISET, employing an under pressure to the ISET backside, pulling the fluids through the solid phase packed nanovials. The nanovial array follows standard microtitre well plate formats and is hence fully compatible with standard laboratory robotics. The optimised design4 of the perforated nanovial outlet enables the use of a wide range of chromatographic matrices and thus broadens the ease of application specific MALDI-target design. The ISET platform has been employed to proteomic sample preparation and phosphopeptide analysis2,3. More recently the ISET platform has been adapted to automated sample processing where cobalt activated microbeads packed in the ISET nanovials enriched recombinant expressed His-tagged proteins in a quality control assay5. Using antibody activated microbeads in combinatio with the ISET opens the route to array based immuno MALDI MS. A critical issue in this respect is the relative high abundancy of the affinity probe on the microbeads in comparison to eg. low expressed proteins in a plasma sample. When performing on bead digestion of the captured biomarkers in the ISET commonly high background interference from digested antibody fragments limits the detection level. Recent work has now addressed this problem and will be further discussed. The microscale format of the solid phase extraction nanovials in the ISET lends itself very well to upstream miniaturised sample processing. In this perspective the use of capillary based acoustic trapping (acoustic tweezer) for noncontact processing of functionalised microbeads in affinity specific extraction of Angiotensin I in blood plasma has recently shown to be a powerful sample preparation tool in combination with ISET followed by MALDI MS6. This platform has also been implemented in a clinical application for rapid bacteria enrichment and purification in blood from positive blood culture flasks followed by MALDI MS bacteria biotying.

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TOS19-02 **When ambient ionization meets miniature ion trap mass apectrometer: chemistry, instruments and applications**

Zheng Ouyang, Yue Ren, Linfan Li, Xiaoyu Zhou, Ran Zou, R. Graham Cooks, Yu Xia
Purdue University

Introduction

The future application of mass spectrometry (MS) outside the traditional analytical laboratories has been prophesied with small systems operated with simple procedures. At Purdue University, we have taken an approach of combining ambient ionization with miniature ion trap mass spectrometers for developing complete and self-sustained analytical systems of small size, low power, and most importantly, friendly for non-expert users. In this presentation, we will report a series of advancements we have recently made in this field.

Methods

The miniature mass spectrometers use linear ion traps (LIT) retain the MS/MS capability and use discontinuous atmospheric pressure interfaces (DAPI) to transfer the ions generated in air. The gas dynamics associated with the DAPI operation has been studied using the electro-hydrodynamic simulation tools developed in-house. Different configurations of the DAPI inlet to the vacuum manifold have been recently studied for obtaining a 10-time improvement of the ion transfer to trap. A set of ambient ionization methods have been developed, with a focused effort for development of disposable sample cartridges for future point-of-care (POC) applications using miniature MS systems.

Results

Following the demonstration of Paper Spray for direct quantitation, the Extraction Spray was developed to take advantage of the stability in signal of the nanoESI and RSDs better than 10% was achieved for quantitation of amitriptyline in blood samples using Mini 12 desktop analytical system. The newly developed Slug Flow Microextraction nanoESI incorporates liquid-liquid extraction facilitated by slug flows with direct ionization by ESI. Real-time derivatization was performed and LODs of 0.2, 0.7, 0.6, 0.8 ng/mL were obtained for (5α-androstan-3β, 17β-diol-16-one), epitestosterone, 4,6-cholestadien-3-one, and stigmastadienone, respectively, in urine samples of amounts below 10mL. On-cartridge assay for enzymatic functions has also been explored with demonstration for cholinesterase in human whole blood. In addition to the analysis of biofluid samples, direct analysis of tissue sample using miniature MS systems has also been developed using Mini 12 and various extraction ionization methods. On-line Patenò-Büchi (P-B) reactions facilitated by UV irradiation has also been implemented to identify the locations of C=C bonds in the lipids.

Conclusions

The combination of the spray-based ambient ionization and miniature DAPI-LIT mass spectrometer is promising for development of complete analytical systems of small size for POC and similar applications. High precision quantitation can be achieved with simple operations and the implementation of real-time chemical reactions could significantly improve the sensitivity and the selectivity of the analysis.

Novel Aspect

Integration of ambient ionization and miniature mass spectrometers, on-cartridge reactions, and demonstration for a broad range of applications.

TOS19-03 **Microfluidics lipidomics using a novel integrated mass spectrometry technology**

Giuseppe Astarita, Angela Doneanu, Jim Murphy, Jay Johnson, James Langridge, Robert Plumb
Waters Corporation

Introduction

Lipidomics aims to measure the wide array of lipid species in biological samples to offer a better understating of their roles in health and disease. The need for a fast, comprehensive and sensitive analysis of the hundreds of lipid species challenges both the chromatographic separation and mass spectrometry.
Here we used a novel microfluidics platform—the ionKey/MS System— which integrates the UPLC separation into the source of the mass spectrometer. The iKey contains the fluidic connections, electronics, ESI interface, heater, e-cord, and the 1.7 um particles for fast and robust analysis. Inserting the iKey simultaneously engages both electronic and fluidics connections eliminating the need to make manual connections, delivering exceptional reproducibility and simplifying the user experience. Such integrated platforms are suitable for lipidomics analyses with performance comparable to analytical scale LC-MS analysis.

Methods

Lipids were extracted from mouse plasma. Untargeted analysis were performed with ionKey/MS system comprised of an ACQUITY UPLC M-Class, the ionKey source and an iKey C18 130 Å, 1.7µm particle size, columns (Waters Corporation, Milford, Massachusetts, USA). Flow rates were 2-3 µl/min. MS detection was conducted using a Synapt G2-S HDMS and a Xevo TQ-S (Waters Corporation, Manchester, UK) operated in both negative and positive ES modes. Progenesis QITM informatics solution and TargetLynxTM Application Manager were used to analyze the data.

Results

We identified and quantified over 200 lipid species belonging to various lipid classes including phosphatidylethanolamines (PE), lyso PE, phosphatidylcholines (PC), lyso PC, ceramides (Cer), sphingomyelins, hexosylceramides, lactosylceramides and cholesterol esters. Lipids were measured over approximately five orders of dynamic range. Lipids were separated according to acyl chain length and number of double bonds, allowing to separate isomeric species.

Conclusions
The novel ionKey/MS system leads to highly efficient LC separation of lipid molecules. Chromatographic results were equivalent to using analytical-scale columns [1-4], bringing considerable advantages: 1) >200x decrease in solvent consumption, making it convenient for the arge-scale analysis and screenings of hundreds or thousands samples; 2) >10x increase in sensitivity, which could facilitate the detection of low abundance metabolites; 3) low volumes injection (e.g., 0.2 µl), which makes it ideal when sample limited studies or when multiple injections are required.

Novel aspects
A novel microfluidics platform — the ionKey/MS — for fast and robust lipidomics analyses with considerable reduction in solvent consumption and increase in sensitivity. Potential applications include large-scale lipid profiling and low-abundance lipids analyses in biological materials.

TOS19-04 Membrane-assisted isoelectric focusing device as a micro-preparative fractionator for two dimensional shotgun proteomics

Mohammad Pirmoradian[1], Bo Zhang[1], Konstantin Chingin[1], Juan Astorga-Wells[1], Thorleif Lavold[2], Roman Zubarev[1]
[1]Karolinska Institute, [2]Biomotif AB

Capillary isoelectric focusing fractionation of proteins and peptides from complex samples has emerged as an attractive technique in many protein studies. Running isoelectric focusing at high loading capacity is challenging in terms of both focusing and mobilization. Recently we have introduced a novel online multi-junction capillary isoelectric focusing fractionator (OMJ-CIEF) for the separation of proteins and peptides in solution. In the current study, we optimized the OMJ-CIEF as a micro-preparative device for fractionation by pI of complex peptide mixtures. To fully utilize the analytical performance of the improved device, a novel algorithm was developed for pI calculation of identified peptides and post-translational modifications based on the elution time information.
The 10 cm separation capillary in OMJ-CIEF is made of PEEK with OD 635 µm, ID 395 µm. Multi-section voltage divider was assembled by connecting serially seven 1 MW, 1 W resistors. The two terminal electrodes of the divider were attached to the DC power supplier, and intermediate connections between the resistors were inserted into the vials of the OMJ-CIEF instrument by means of a platinum wire. Separation of yeast proteome digest was performed by stepwise elution of peptide zones in conjunction with the repeated refocusing of remaining peptides. 18 fractions were collected and UPLC-MS on each fraction (3µg) was performed by 8 cm analytical column and analyzed by Orbitrap Q Exactive.
Pharmalyte effect on the LC-MS experiment was studied by comparing the number of identified peptides from the yeast proteome digest spiked with different concentrations of Pharmalyte, from 0% (control) to 5%. Identification was not affected by Pharmalyte concentrations lower than 0.5%, which was used in OMJ-CIEF separation experiments.
In order to establish a stable linear electrical field during the experiment, we made a resistor-based voltage divider. The divider ensures the same voltage drop across each section. This improves the separation performance, avoids generation of hot spots, as well as preserves the effective length of the column.
To reduce the peak broadening of focused zones during their mobilization, we applied a stepwise refocusing procedure. After the focusing step, the first anode zone is eluted from the column, while the rest of the zones are subjected to refocusing within the column. Such refocusing not only sharpens the broadened peaks, but also improves the focusing of late-eluting basic polypeptides, which is a challenge in IEF performed at wide pH range.
In this study, we investigated the pI shift due to two important and well-studied PTMs, deamidation of Asn and phosphorylation on Ser, Thr or Tyr. To calculate the pI shift for each peptide, the exact focusing position of unmodified and modified species of the peptide were calculated. By means of fitting a curve, pI of each peptide was estimated. The difference between the pI values of modified and unmodified peptide represents the pI shift due to modification.
Our results indicate the high potential of modified OMJ-CIEF as a first dimension in proteomics analyses with automated workflow.

TOS19-05 Ultrafast peptide decomposition by superheating

Matthias O. Altmeyer[1], Pavel Neuzil[2], Andreas Manz[2]
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Introduction
We present a surface-controlled water microreactor, reaching temperatures up to 240°C at atmospheric pressure. This microfluidic device is used for the first time to selectively fragment peptides for identification by mass spectrometry (MS). With a single-stage MS we achieved the same information and quality as with a state-of-the-art multi-stage MS machine.
Tandem MS (MSMS) analysis is currently one of the most powerful and reliable methods to identify peptides and proteins, or for de novo sequence elucidation. The key point of this method is the fragmentation of the peptide backbone. In an ideal case it results in a ladder fragmentation from which the amino acid sequence can be deduced, but this method requires expert knowledge.

Methods
Here, we report the usage of superheating conditions in microfluidics for selective peptide fragmentation. Sample peptides were heated in a virtual reaction chamber (VRC) on a thermal cycler chip or in a capillary to temperatures up to 200°C without boiling. Sample superheating resulted in decomposition of the peptides while the degree of fragmentation correlated to heat exposure.
We have performed two sets of experiments: peptides were dissolved in water and

1. sample was spiked into a droplet of mineral oil, both dispensed on a hydrophobically coated microscope cover slip. The glass was placed on a micromachined silicon chip integrated with heater/sensor for temperature control (figure 1a).
2. or sample was pumped through the fused silica capillary with internal diameter of 50µm passing across the heated substrate (figure 1b).
In both cases we have exposed the sample to heat pulse at 120°C, 160°C, or 200°C for 10s.

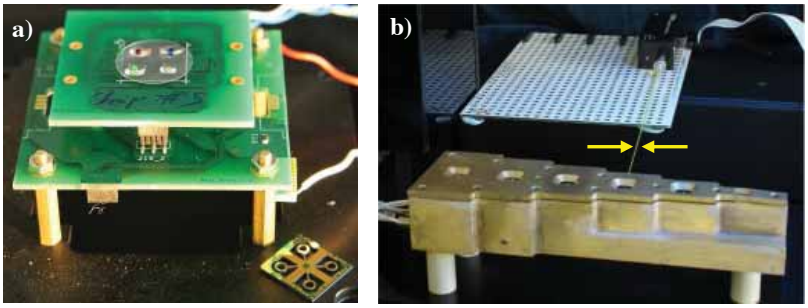

Figure 1. The two superheating systems. Samples are replaced by dyes for visualization. a) VRC on a FAS17 coated microscope cover slip on top of the thermal cycler chip; a second chip is placed in the lower, right corner. b) Capillary (arrows) on a custom-build brass superheating device.

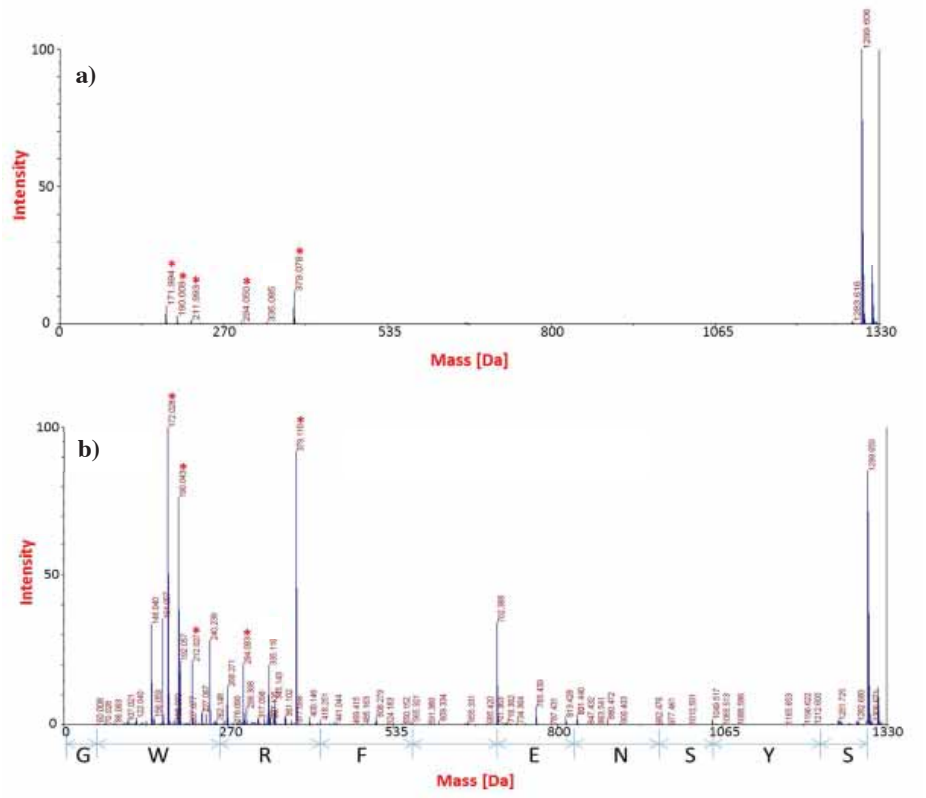

Figure 2. MS spectra from 100µM ACTH1-10 before and after superheating. a) Spectrum of ACTH1-10 before superheating. b) Spectrum of ACTH1-10 after superheating to 160°C for 10s. The arrows indicate mass differences which correspond to the loss of single amino acid (labelled below the spectrum). Asterisk indicate matrix-peaks.

Results

The backbone fragments of the sample after decomposition was monitored by MALDI- and ESI-MS. We used peptide ACTH1-10 (Adrenocorticotropic hormone, sequence: SYSMEHFRWG, monoisotopic mass [M+H]+: 1299.558) to demonstrate feasibility of this method. First we analyzed its MS spectrum without any heat treatment (figure 2a). Next we have optimized the superheating conditions for fragmentation which turned out to be at 160°C. The entire amino acid ladder sequence (complete y-ion series) of ACTH1-10 was confirmation, demonstrating a sequence-specific fragmentation pattern (figure 2b).

Conclusion and novel aspects

Our device expands single stage MS machines to allow fragmentations as in tandem MS machines. Therefore, the superheating-based fragmentation tool we introduced in this contribution makes enzymatic-hydrolysis based approaches redundant.

TOS20 - Imaging MS - Applications

Chairs: Olivier Laprévote, Markus Stöckli

Room 5
Level 3

TOS20-01 **Keynote: MALDI molecular imaging of proteins, metabolites and drugs for preclinical and clinical research**

Axel Walch
Helmholtz-Zentrum München

In the last years, Matrix-Assisted Laser Desorption/Ionization (MALDI) imaging mass spectrometry ("MALDI imaging") has emerged as promising technique for combined morphological and molecular tissue analyses. It enables a spatially resolved, unlabeled and multiplexed analysis of different molecule classes, ranging from small molecules (e.g. drugs) to proteins, molecules of cell metabolism, lipids and other analytes, directly in tissue sections. Molecules can be imaged in the histological context of tissues, and therefore, molecular profiles can be allocated to specific cell types. MALDI imaging has proven to provide novel and clinical relevant information in a variety of different biomedical questions, with focus in oncology and inflammatory diseases. While several studies so far have worked on a proteomic level, of increasing interest is also the ability of MALDI imaging to visualize the spatial distribution of drugs and their metabolites in tissues, which are valuable for drug development and efficacy studies in animal models and even in individual patients.
This presentation will give an update on the application of MALDI imaging in preclinical and clinical research.We discuss the use of MALDI imaging in clinical proteomics and put it in context with classical proteomics techniques for tissue analysis. In the research area of gastrointestinal disorders MALDI imaging has already been used to address several questions of upper- and lower gastrointestinal diseases, which will be briefly presented. We also highlight a number of upcoming challenges for personalized medicine, development of targeted therapies and diagnostic molecular pathology where MALDI imaging could help.
Because of its practical simplicity and ability to gain reliable information even from smallest tissue amounts, which may also originate from endoscopic biopsy sections, we believe that MALDI imaging might have the potential to complement histopathological evaluation for assisting in diagnostics, risk assessment, or response prediction to therapy.

TOS20-02 **Identification and spatial localization of proteins from mouse brain tumor using a combination of MALDI imaging and LC-MALDI**

Arndt Asperger[1], Michael Becker[1], Daniel Feldmann[2], Jennifer Ide[2], Mark Marchionni[3], Nathalie Agar[3], Charles Stiles[3]
[1]Bruker Daltonics GmbH, [2]Brigham and Women's Hospital, HMS, Boston, [3]Dana-Farber Cancer Institute, HMS, Boston

Introduction
A large body of knowledge about spatial localization of proteins in different types of tissue has been accumulated by using MALDI MS imaging. Very often identification of the proteins with interesting localization requires more efforts and time than MALDI imaging experiments. One workflow that achieves spatial localization and protein identification correlates MALDI imaging data with bottom-up proteomics data from LC-MALDI analysis of peptides from a second digested section. This work focuses on applying this workflow to study treated and untreated tumors in mouse brain.

Methods
Two consecutive coronal tissue sections of mouse brain (tumor treated, tumor untreated, healthy) were simultaneously subjected to on-tissue proteolytic digestion by nebulizing trypsin solution followed by incubation at 37˚C for 2 hours. One of the two tissue slices was covered with 0.1% TFA, incubated for 15 minutes, and collected by pipette for LC-MALDI-TOF/TOF analysis of the tryptic peptides. The other tissue slice was sprayed with 2,5-DHB for MALDI imaging at 100 µm spatial resolution. The list of identified peptides, grouped by protein, from the LC-MALDI dataset was correlated to the peak list from MALDI MS imaging dataset using ImageID utility in FlexImaging software.

Results
The combined workflow was used to identify and obtain spatial localization of proteins in mouse brain tumor treated with temozolomide, untreated tumor control and healthy mouse brain control tissue sections. On average, 649 proteins were identified and matched to peptide imaging data using this workflow in a single experiment. LC-MALDI-TOF/TOF identification part of the workflow yielded 741 proteins in untreated tumor control tissue, 568 proteins in treated tumor tissue and 649 proteins in healthy control tissue. Certain proteins are identified and localized in the tumor region of untreated tumor control tissue. One example of such protein is voltage-dependent anion-selective protein 2. This particular protein was not identified in the treated tumor tissue or the healthy control tissue. Other proteins are localized in both treated and control brain tumor tissues. We are working on in-depth comparison of localization and identification data from untreated brain tumor control tissue with treated tumor and healthy tissues.

Conclusions
Using combined imaging and LC-MALDI workflow to determine spatial localization and identify proteins in treated and untreated mouse brain tumors

Novel Aspect
Identification by proteins localized in tissue by MALDI Imaging

TOS20-03 **High spatial and high mass resolution of metabolite analysis using AP-MALDI MSI**

Dhaka Bhandari, Andreas Römpp, Bernhard Spengler
JLU Giessen

Introduction
In the post genomic era, a major challenge is to explain interactions of metabolites in an organism on the molecular level. Mass spectrometry imaging (MSI) can provide spatial information about a specific analyte within a complex biological system. MSI has some clear advantages, such as no requirement of prior knowledge of targeted analytes

(untargeted analysis), providing specific and precise identification of hundreds of compounds in a single experiment, at the same time keeping the spatial information (multiplexed analysis). In contrast to classical histology, the interpretation of MS images is user-independent. Utilizing high spatial resolution (< 10 µm) and high mass accuracy (< 2 ppm mass error) we investigated metabolites and natural products in plants (Brassica napus, Triticum aestivum, and Glycyrrhiza glabra) and insects (Paederus riparius and Harmonia axyridis).

Methods
Tissue sections were obtained after optimizing various techniques. 2,5-dihydroxybenzoic acid (DHB) matrix was uniformly deposited using a high-resolution matrix-preparation robot (SMALDIPrep, TransMIT GmbH, Giessen, Germany). Samples were scanned and ions were generated by a high-resolution atmospheric-pressure MALDI ion source (AP-SMALDI10, TransMIT GmbH, Giessen). The step size was between 5 – 25 µm depending on the targeted spatial features. The source was coupled to an orbital trapping mass spectrometer (Exactive or Q Exactive Orbitrap, Thermo Fisher Scientific GmbH, Bremen), set to a mass resolving power of 50,000 to 140,000 at m/z = 200. METLIN database search based on accurate mass, in combination with tandem mass spectrometry was used to identify compounds.

Results
All spectra generated in imaging measurements were acquired with a root mean square of mass error (RMSE) of better than 3 ppm. Molecular images with a bin width of $\Delta m/z = \pm 5$ ppm were generated. Various metabolites like amino acids, carbohydrates, glucosinolates, phenolic compounds, flavonoids, lipids and glycosides were identified and visualized from a single experiment at 10 µm pixel size. Certain compounds, spatially resolved with 5 µm pixel size, were found to be specific for different regions of the wheat seed. Natural products like glabrene and isoviolanthin showed different distributions in Glycyrrhiza glabra root sections. Whole insect imaging of Paederus riparius was performed at 20 µm spatial resolution. The defensive compound pederin as well as its metabolite pseudopederin were prominent in the fourth abdominal segment, where the reservoir of the defensive gland is located. Additionally phospholipids specific for brain, nerve cord, egg, ovary, gut were identified and visualized.

Conclusions
Wheat seed metabolites were visualized at 5 µm step size. Whole insects were imaged at 20 µm step size, demonstrating organ-specific molecular signals. Various section preparation methods were optimized to prepare insects and plant organs. The MSI images show high molecular specificity at cellular dimensions, due to the availability of high mass accuracy, high mass resolution and high spatial resolution.

Novel Aspect
High-resolution MSI in mass and space to describe biochemical processes.

TOS20-04 Towards quantification based MS imaging: filling the gap between MALDI MS imaging and tissue microproteomics

Isabelle Fournier, Jusal Quanico, Julien Franck, Maxence Wisztorski, Michel Salzet
Université Lille 1

Introduction
MALDI MSI reseals high potential for clinical studies looking for biomarkers discovery or deciphering physiopathological mechanisms. Such an application of MALDI MSI requires the possible identification of the protein markers detected. Proteins identification in MALDI MSI remains a difficult task because of the specific strategies used. Despite many efforts for developing direct identification at the level of tissues by in situ MS2, it was shown that only major proteins were identified whatever Bottom-Up or Top-Down strategies used due to ion suppression effects.

Results
To overcome this limitation we have developed new approaches involving on tissue micro-digestion followed by Liquid Microjunction micro-extraction (LMME) followed by Shot-Gun proteomics. The LMME strategy has demonstrated to be powerful for high confidence identification of proteins of various abundances from small tissue area. LMME was then included in the MS imaging workflow in order to combine imaging and identification data. In this context we have been able to identify an average of 1500 unique proteins in high confidence from each area. The identification data can then be back correlated to MALDI MSI if both are recorded under FT MS conditions, and allow by mass correlation to identify most of the digestion peptides observed. We then worked to reduce the size of the studied area down to one MSI image pixel dimension (currently 150 µm). However, we need again to increase the number of proteins both identified and imaged. To fulfill this gap between conventional proteomics and MALDI imaging we searched for providing image reconstruction based on identified proteins quantification. We have recently developed a strategy allowing for micro-dissection of the tissues by mounting the sample on a parafilm-covered glass slide. The parafilm support allows the facile excision of millimeter-sized pieces that can then be subjected to conventional proteomics techniques. Using this strategy, 1140 proteins were identified from millimeter-sized regions on a rat brain tissue section. This approach was extended to the level of a whole tissue section by cutting it in regular raster of pieces. Applied to one rat brain section allowed identification and quantification by spectral counting of proteins. Their distribution was mapped by plotting the spectral count of the protein on each piece and correlating the pieces to their original locations on the tissue.

Conclusions
Such a new imaging approach eliminates the need for back-correlation and provides a novel way to image proteins that is not limited by ion suppression effect and provides access to the localization of a much larger number of proteins than in conventional MS imaging approach.

Novel Aspects
We now combine LDI with droplet capture in automatized mode and obtained from 50 µm laser spot the proteome and its quantification of thousand proteins per spot opening the door of the novel MS imaging: Quantification based MS imaging.

TOS20-05 TLC-MALDI-FT-ICR-MS coupled to imaging mass spectrometry – A unique approach to first identify then subsequently map parasite specific lipid markers in vivo.

Berin Boughton[1], Mark Condina[2], Daniel Sarabia[3], C. Dean Goodman[3], Geoffrey I. McFadden[3], Ute Roessner[1]
[1]Metabolomics Australia, University of Melbourne, [2]Bruker Daltonics, [3]University of Melbourne

Thin Layer Chromatography (TLC) coupled to Matrix Assisted Laser Desorption Ionisation Mass Spectrometry (MALDI-MS) offers the ability to couple a rapid and versatile separation technique with routine MALDI-MS analysis providing an effective strategy for the analysis of lipid mixtures.[1] Due to the varying detectabilities of individual lipid classes using MALDI, separation of complex lipid mixtures using TLC allows a fast separation technique, with the ability to analyse multiple samples (eg. control and infected) at the same time and eliminate 'memory' effects, prior to analysis using MALDI Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS). Upon TLC separation, results from the hyphenated TLC-MALDI approach can then be used to inform subsequent in vivo Imaging Mass Spectrometry analyses.

To demonstrate this combined approach specific lipid compositions and changes within hosts infected by protozoan parasites were examined using the model organism Plasmodium berghei (malaria). Examination of the arthropod life stages has lagged behind those in the mammalian host where little is known of the fundamental lipid biology occurring during oocyst formation and sporozoite maturation within the malaria life cycle.[2]

The complement of lipids were extracted from both control mosquitoes and those infected with P. berghei using the standard Folch extraction method. Lipid extracts were then separated by TLC using optimised separation and matrix application conditions prior to MALDI analysis on a Bruker 7T SolariX FT-ICR-MS. Using this approach, parasite specific markers were first identified, then interactions of parasite and host were subsequently investigated in vivo. Individual infected mosquitoes were cryo-sectioned, mounted on slides then MALDI matrix applied. Prepared sections were then analysed using MALDI-FT-ICR-MS to determine the distribution of parasites within the arthropod host.

We show the combination of TLC-MALDI with Imaging Mass Spectrometry to be a useful tool to first determine specific markers that can then be used to inform subsequent imaging analysis. Further, we show the first mass spectrometric imaging analysis of malaria (P. berghei) infections within the mosquito host.

1.Fuchs et al., Chromatographia, 2009, 69, S95-S105.
2.Angrisano et al., International Journal for Parasitology, 2012, 42, 519-527.

PL04: Plenary Lecture - Curt Brunnée Award

Room 1
Level 1

Dimitris Papanastasiou - Chair: Catherine Costello

Ion transport through the atmospheric pressure interface and operation of ion optical systems immersed in fast transient gas flows have received significant attention as part of a greater effort to enhance sensitivity in Mass Spectrometry (MS) instrumentation. Ion motion in transient flows at speeds comparable or exceeding the speed of sound is explored by means of a diverse set of experimental tools and numerical methods developed specifically to evaluate, further improve and extend the functionality of the atmospheric pressure MS interface. Advanced exercise of Particle Tracking Velocimetry (PTV) to visualize low pressure gas flows, the conceptualization and characterization of the aerolens, a novel ion optical element designed to laminarize supersonic jets, and also revisions introduced in existing ion-molecule collision models to better approximate realistic conditions have been successfully performed and presented in greater detail. In addition, the combination of gas flow visualization technology, gas dynamics computations and ion optics simulations have greatly facilitated the development of a novel Differential Mobility Spectrometer (DMS), ultimately constructed and operated in the fore vacuum region of a mass spectrometer. High performance DMS analysis is demonstrated in subsonic laminarized low pressure gas flows for the first time. A new direction for intermediate pressure ion optics design is proposed by considering the gas dynamics phenomena observed in the fore vacuum region of a mass spectrometer.

WOS21 - New Ionization Techniques

Room 1
Level 1

Chairs: Frantisek Turecek, Silvia Catinella

WOS21-01 **Keynote: The development and future of spray ionization techniques**

Brian Chait
Rockefeller University

WOS21-02 **Development of surface acoustic wave nebulization**

David Goodlett[1], Scott Heron[1], Sung Hwan Yoon[1], Yue Huang[1], Shivangi Awasthi[1], Tao Liang[1], Erik Nilsson[2], Lisa Leung[1], Robert Ernst[1], David Kilgour[1]
[1]University of Maryland, [2]Deurion

Recently we reported surface acoustic wave nebulization (SAWN) (S. Heron et al. Anal. Chem. 2010, 82, 3985-3989) as a novel approach for the generation of multiply-charged ions for mass spectrometry from a planar surface. SAWN is softer than electrospray ionization (ESI) (Huang et al. JASMS 2012; Yoon et al. Anal Chem 2012) and is easily integrated with both digital microfluidics (DMF) and molded microfluidics due to its planar nature. Standing wave (SW) SAWN has been shown to increase sensitivity to comparable levels to ESI. We hypothesize that this is due to an expected decrease in droplet size. Here, we report on the effects of high frequency (HF) SW SAWN chips operating at a frequency of 20 and 30 MHz to further improve SAWN sensitivity.

It has been shown previously (J. Ju et al. Sensors and Actuators A: Physical. 2008, 570-575), that SW devices produce smaller more homogeneous distributions of droplets than the progressive wave (PW) devices that we originally reported on (Heron et al. Anal. Chem. 2010). Using Doppler phase velocimetry we measured the size and distribution of droplets produced by the PW SAWN design. While the majority of droplets were below 10 microns in diameter a smaller distribution of much larger (up to 100 microns) droplets were also produced and these made up the majority of the sample volume.

We hypothesized that this phenomenon was responsible for the poor sensitivity of the PW SAWN device because larger droplets require significantly more time or higher temperatures to adequately desolvate before detection by the mass spectrometer. In an attempt to improve sensitivity we designed a series of new SAWN IDTs based on use of standing waves to nebulize liquid. These new designs were placed in the same detector and we showed that droplet size reduced with a distribution of droplets that was more homogeneous. As a result the ion sensitivity improved 3-orders of magnitude and pushed the detection of peptides on SW chips down to low femtomoles on chip. We are now analyzing the effects of higher operational frequencies to see if this will affect the pinch off on the droplet surface in a way that will further reduce the size of the droplets and lead to an even greater increase in sensitivity. We have also describe our efforts to couple DMF separations to SAWN-MS.

WOS21-03 **Charged droplet beam source for secondary ion mass spectrometry using nano electrospray in vacuum**

Satoshi Ninomiya, Lee Chuin Chen, Yuji Sakai, Kenzo Hiraoka
University of Yamanashi

Introduction

Secondary ion mass spectrometry (SIMS) has been used for the analysis of inorganic materials and also organic molecules. One of the most serious problems in SIMS is its low ionization efficiency for organic molecules.The higher ionization efficiency has been observed when primary cluster ion beams such as C60+ and Bi32+ are used, and an improvement of sensitivity for imaging mass spectrometry for real-world biological samples has been reported. However, ionization efficiency in SIMS is still not enough for imaging with high spatial resolution (<1 micron). Therefore, to improve the performance of the SIMS imaging there is a strong need to develop novel primary ion beam sources. Electrospray droplet impact (EDI) method based on atmospheric-pressure electrospray technique has been developed as a new massive cluster ion beam source. As EDI can provide much higher ionization efficiency than other probes, EDI may be a promising technique for imaging mass spectrometry. However, the current EDI method lacks adequate beam focusing and thus beam density. Therefore, it is not practical for the use as a primary beam for SIMS imaging.

Methods
To solve the fundamental problems for EDI as a primary ion beam, we propose a new method of producing a high-brightness charged-droplet beam. A new charged droplet beam source was based on the electrospray generated in vacuum. For vacuum electrospray, the size of the initial droplet may be assumed to be maintained after being sprayed at the tip of electrospray emitter because of rapid evaporative cooling of the ejected droplets. In this study, the m/z distributions of the charged droplet beams were measured with a conventional time-of-flight technique.

Results
The m/z distribution of the charged droplet beam for vacuum electrospray was measured by the time-of-flight technique by using 30 µm inner diameter emitter. Maximum m/z value was evaluated to be around 106. This m/z value was much larger than those generated by atmospheric pressure electrospray (m/z: 10000~50000). The key factors that determine the size distribution of the charged droplets are the emitter size and the flow rate of the electrospray liquid. The results obtained using narrower capillaries with inner diameters of 1-30 µm will be presented.

Conclusions
The m/z distributionsof the charged droplet beams generated by vacuum electrospray depended on inner diameter of electrospray emitters. The vacuum electrospray technique could be expected to be a high-intensity massive cluster beam source, and we are now evaluating the performance of the vacuum-type electrospray droplet beam gun using surface analysis instruments.

Novel aspects
The characteristics of the charged droplet beams generated by vacuum electrospray were investigated.

WOS21-04 Matrix-free desorption/ionization induced by neutral cluster impact for soft analysis of complex (bio-)samples

Michael Durr[1], Andre Portz[2], Markus Baur[3], Christoph Gebhardt[4]
[1]Justus Liebig University Giessen, [2]JLU Giessen, [3]HS Esslingen, [4]Bruker Daltonics

Introduction
Desorption and ionization induced by neutral clusters (DINeC) can be employed as a soft and matrix-free method for transferring surface-adsorbed biomolecules into the gas phase. Using neutral clusters with polar constituents such as SO2, the impacting clusters do not only provide the energy necessary for desorption but also serve as a transient matrix in which the desorbing molecule is dissolved during the desorption process. As a consequence, desorption and ionization of oligopeptides and smaller proteins can proceed at comparably low energies of the impacting clusters and without any fragmentation [1]. High ionization efficiency was observed [2]. In this contribution, we show high-efficiency desorption/ionization of intact phospho- and glycopeptides by means of DINeC; complex biosamples with a multitude of constituents or high salt concentration were analyzed.

Methods
Desorption and ionization was induced by neutral SO2 clusters with a mean size of 103 to 104 molecules seeded in a pulsed He beam. The desorbed ions were accumulated in an ion trap prior to mass spectrometric analysis. Samples were prepared by drop casting the respective aqueous solution on Si/SiO2 substrates.

Results
Using a combination of DINeC and ion trap mass spectrometry, femtomol sensitivity was achieved for standard oligopeptides such as angiotensin II or bradykinin and no fragmentation was observed [3]. The signal of the intact molecules (M+H)+ is predominant even in the case of phospho- and glycopeptides, and typical fragments were observed only in low abundance. Samples with a multitude of components as obtained from realistic biotechnological processes such as tryptic digest of proteins were also successfully analyzed. Peptide mass fingerprint analysis was applied for the evaluation of the respective spectra with very good sequence coverage and protein score. When compared to ESI or MALDI, a substantial number of the unique peptides which were identified with DINeC were not detected with the other methods. Notably, even in the presence of a large excess of salt in the original solution clear spectra of the intact biomolecules were detected. The results are correlated to the very properties of the DINeC process. The method was furthermore successfully applied to a variety of different classes of molecules such as lipids, dye molecules, and pesticides.

Conclusions
DINeC is shown to be a very soft desorption/ionization technique as it allows, e.g., for the intact desorption/ionization of phospho- and glycopeptides. The method is especially suited for the analysis of samples from realistic bioprocesses as it can be applied to complex samples with a large excess of salt in the solution.

Novel aspects
Intact desorption/ionization of phospho- and glycopeptides with high efficiency; application of DINeC to complex samples; mass fingerprint analysis of DINeC spectra; desorption/ionization in the presence of large amounts of salt

References
[1] C. R. Gebhardt, et al., Angew. Chem. Int. Ed. 48, 4162 (2009).
[2] B.-J. Lee, et al., Rapid Commun. Mass Spectrom. 27, 1090 (2013).
[3] M. Baur, et al., Rapid Commun. Mass Spectrom. 28, 290 (2014).

WOS21-05 **UV-LDI- and MALDI-mass spectrometry augmented by UV-laser postionization: coupling a wavelength-tunable OPO-laser (213-400 nm) to a synapt G2-S mass spectrometer**

Jens Soltwisch, Hans Kettling, Marcel Wiegelmann, Klaus Dreisewerd
University of Münster

Introduction
Only a fraction of molecules that are transferred into the gas phase upon laser desorption/ionization (LDI) or matrix-assisted laser desorption/ionization (MALDI) are also ionized. Typical figures for the MALDI ion yield are in the range of 10-5-10-3. Photoionization of the desorbed neutral molecules is a means of potentially increasing the ion yields for small molecules. Generally, this requires the use of wavelength-tunable laser radiation adjusted to the chromophoric system of the analyte compound. Here, we present the first example of using UV-laser postionization (PI) on a hybrid MALDI QTOF-type mass spectrometer that was operated with elevated cooling gas pressure. An OPO laser providing a wide wavelength range from 213-400 nm was used for postionization. We show that under these conditions the analysis of small molecules is substantially enhanced, a feature that can be exceedingly useful for general MALDI-MS applications as well as for MALDI-MS imaging.

Methods
A MALDI Synapt G2-S (Waters) was employed as mass spectrometer. The MALDI ion source was modified to enable the use of two external lasers. The first – either a Nd:YAG-laser (λ=355 nm) or a N2-laser (λ=337 nm) – served for generation of neutral gas phase molecules and direct LDI/MALDI ions. Their beams were applied via the default port of the ion source. The beam of the second optical parametric oscillator laser (OPO, GWU Lasertechnik, τ~5 ns) was coupled via a second port and intersects the (MA)LDI plume closely above the sample surface (parallel to the sample plane). Distance of the PI beam to the sample surface, its width, and the time delay between the two laser pulses (adjusted to values in the microsecond range) were precisely controlled. The MALDI ion source was moreover modified to enable adjustment of the buffer gas pressure up to values of 4 mbar [1].

Results
First results that were obtained just before the abstract submission deadline demonstrated the high potential of the method. Ion signals for numerous compounds, notably including lipids, that were prepared for standard MALDI-MS or UV-LDI were found to be strongly increased, partly by orders of magnitude. Using the method in combination with imaging mass spectrometry, moreover, enabled the detection of numerous additional compounds from tissue slices. In this contribution, status as derived until the conference and perspectives of this timely method will be presented and discussed.

Conclusions
The use of UV-laser postionization in combination with a QTOF-type mass spectrometer opens new avenues for general laser mass spectrometry and MS imaging applications. The operation principle of the MALDI Synapt instrument, including a fine vacuum ion source for rapid collisional cooling after ionization and a decoupled mass analyses renders this platform particularly suitable for analysis of postionized molecules.

Novel Aspects
First report on the use of UV-laser postionization on a MALDI Synapt G2-S instrument demonstrating novel analytical possibilities.

[1] H. Kettling, S. Vens-Cappell, A. Pirkl, J. Müthing, K. Dreisewerd; this conference.

WOS22 - Cell Biology and Cellular Pathways

Room 2
Level 0

Chairs: Anne-Claude Gingras, Gérard Hopfgartner

WOS22-01 **Keynote: Quantitative interaction proteomics for epigenetics**

Michiel Vermeulen
Radboud University Nijmegen

Epigenetic modification patterns are dynamically being established, maintained and removed from the genome during differentiation and they help to create cell-type-specific gene expression profiles. Regulatory proteins can be recruited to these modifications to exert their function. The specific binding of these so-called chromatin 'readers' therefore significantly contributes to the biological function of each individual epigenetic modification. Our lab is using state-of-the-art quantitative mass-spectrometry based proteomics technology to identify chromatin readers for epigenetic histone and DNA modifications. We characterize the (dynamic) complexes that these readers form, we study their biology in (differentiated) stem cells and in different model organisms and we investigate their potential deregulation in cancer. In my lecture I will give an overview of current projects in the lab related to these topics.

WOS22-02 **Characterisation of human cell lines using rapid evaporative ionization mass spectrometry**

Nicole Strittmatter[1], Anna Lovrics[2], Emrys A. Jones[1], Ottmar Golf[1], Kirill A. Veselkov[1], Gergely Szakacs[2], Zoltan Takats[1]
[1]Imperial College London, [2]Hungarian Academy of Sciences

Introduction
Human cell lines represent a particularly important model to investigate human metabolism, infectious diseases and cancer. Characterisation of cancer development and metabolism might lead to the detection of new targets for anti-cancer drugs. For this purpose the NCI60 cell line panel was created, comprising 60 extensively characterised cell lines of nine different organs. However, comparably little information is available about the lipid composition of the NCI60 cell lines. In this study, recently developed rapid evaporative ionisation mass spectrometry was used to profile the lipid composition of cell lines. REIMS yields spectra mainly containing signals complex cell membrane lipids and has found application for obtaining tissue IDs and as identification tool for microorganisms.

Methods
Cells were cultured in T75 flasks using RPMI-1640 medium and collected at 80 % confluence. Cells were subsequently spinned down and supernatant discarded. Without any further sample pre-treatment, approximately 0.5mg of intact cells were placed between two handheld electrodes and rapidly heated up by RF alternating current. An electrosurgical generator in bipolar mode was used as an RF power supply. The produced aerosol was directly introduced into a Thermo Exactive mass spectrometer using the inherent suction of the vacuum system. Spectra were acquired in negative ion mode in the mass rangem/z150-2000. The sampling time per cell line was approximately 3-5s. The obtained REIMS spectral profiles were analysed using supervised and unsupervised multivariate statistical methods.

Results
REIMS proved capable of distinguishing individual cell lines of the NCI60 panel based on their spectral fingerprints. Good correlation with organ type of origin was found especially for melanoma, leukaemia and renal cell lines. The correlation between the REIMS profiles and different protein expression profiles as published in the NCI60 database

was investigated using in-silico correlation analysis methods. Strong correlations were observed especially for those factors influencing lipid synthesis as in case of mutations in the MAPK/ERK-pathway. The constituents of the REIMS profiles obtained from cell lines were characterised for the molecular information present and compared to spectra of primary tumours of the same organ type. No effect on the phospholipid profiles was observed due to IPTG presence or absence in the culturing medium.
The specificity of a REIMS-based tool to characterise the lipidome of cell lines was further investigated in case of mycoplasma infection and plasmocin treatment for HEK and HeLa cell lines and the influence of epithelial-mesenchymal transition for several colon cancer cell lines. In addition, the effect of cell cycle stages on the phospholipid composition was identified for mouse embryonic stem cells.

Novel aspect
Direct profiling of cell lines using REIMS gives characteristic spectral profiles within seconds.

WOS22-03 The production pipeline of the MHC peptidome
Arie Admon[1], Dmitry Bourdetsky[1], Lilach Gutter-Kapon[1], Elena Milner[1], Ilan Beer[2], Eilon Barnea[1]
[1]Technion - Israel Institute of Technology, [2]IBM Haifa Research Laboratory

Cellular protein degradation can be followed by large-scale analysis of their degradation products as Major Histocompatibility Complex (MHC) peptides. Furthermore, the MHC peptidome is a rich source of antigens for development of cancer vaccines. We performed large-scale immunopeptidome analyses, using dynamic-stable isotope labeling technology in tissue culture (dynamic-SILAC), followed by immunoaffinity purification of the MHC molecules, extraction of their bound peptides and capillary chromatography and tandem mass spectrometry on Orbitrap mass spectrometers of the purified peptides. Using specific inhibitors and analysis of the dynamics of synthesis of the diverse cellular proteins and their derived MHC peptides we were able to shed new light on the proteolytic pathways that lead to the formation of these immunopeptidomes. The more interesting effects were observed by inhibiting the proteasomes with inhibitors, such as epoxomicin and bortezomib (Velcade), which affected in a complex manner the rate of synthesis of the cellular proteins and of their degradation, and formation of MHC peptides. While (as expected) the proteasome inhibitors reduced the rates of degradation of many cellular proteins, they increased the degradation (and synthesis) rates of others. Correlating between the rates of production of the source proteins and their derived HLA peptides suggests that the contribution of the proteasomal proteolysis to the production of the HLA immunopeptidome should be reevaluated with more emphasis on the vesicular degradation pathways. Furthermore, the analysis suggest that the production of HLA peptides is derived significantly from newly synthesized proteins, many of which are defective ribosome products (DRiPs) and short lived proteins (SLiPs), which were defined in this study. This is in contrast to contribution from old proteins, which finished their functional life times in the cells (retirees).

WOS22-04 A sentinel protein assay for the quantification of cellular process activities using PRM and DIA
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Introduction
We developed a novel proteomic screening approach that provides a system-wide, quantitative snapshot of the activity status of a variety of cellular processes, simultaneously. The approach is based on the concept of sentinels which are biological markers whose change in abundance characterizes the activation state of a given pathway or functional module in a cell. Sentinels can be specific proteins, phosphorylation sites or degradation products. By assembling a panel of these sentinels that can be targeted in a single LC-MS run a snapshot of active pathways and functional modules in the sample can be generated. The strategy breaks with the current trend of constantly increasing the number of proteins measured thereby making the analysis of large numbers of samples more feasible.
To target the sentinels in the protein assay, here, we performed parallel reaction monitoring (PRM) and data independent acquisition (DIA) methods to study the effect of osmotic stress in yeast.

Methods
Yeast cells were sampled under normal conditions and various conditions such as osmotic stress, rapamycin treatment, amino acid and nitrogen starvation and entrance into stationary phase. Extracted protein lysates were digested and analyzed by nanoLC-Q Exactive Plus MS using PRM and DIA. To aid alignment and peak detection, iRT peptides were spiked into each sample and DIA runs were analyzed with Spectronaut and PRM runs with SpectroDive.

Results
Based on literature evidence and computational prediction, we selected a panel of 309 sentinels that covers 182 different cellular processes to probe the physiology of yeast cells under different conditions.
Here, we tested different MS acquisition methods for the measurement of a subset of the sentinel assay. In total 157 sentinels, covering 99 processes, were analyzed in a single LC-MS measurement per replicate and condition using PRM (targeting 311 peptides) and DIA.
By PRM we could detect more than 90% of the sentinels in at least one condition. Due to the lower sensitivity of the DIA method some of the lower abundant peptides could not be detected or quantified in DIA. However, we could extend the list of sentinel proteins and extract their abundances post-acquisition.

Conclusions
The sentinel assay precisely quantified the activity of almost a hundred different cellular processes using PRM in a single LC-MS run, thereby providing a rapid, system-wide snapshot of the physiology if yeast cells across the different conditions. Rather than obtaining a large amount of complex and redundant data as when striving for the highest numbers of identifications, the sentinel assay provides a condensed and information-rich snapshot. The obtained data recapitulating many known, but also reporting new responses, substantiated the generic applicability of the sentinel assay approach.

Novel Aspect
Sentinel protein assay for a quantitative snapshot of the activity status of a variety of cellular processes using DIA and PRM approaches.

WOS22-05 Quantifying 14-3-3 protein interaction and phosphorylation dynamics with SWATH mass spectrometry

Ben C. Collins, Christina Ludwig, Ludovic C. Gillet, George Rosenberger, Hannes L. Röst, Anton Vichalkovski, Matthias Gstaiger, Ruedi Aebersold
ETH Zurich

Introduction
Protein complexes and protein interaction networks are essential mediators of most biological functions. Complexes supporting transient functions such as signal transduction processes are frequently subject to dynamic remodelling. Currently, the majority of studies on the composition of protein complexes are carried out by affinity purification and mass spectrometry (AP-MS) and present a static view of the system. For a better understanding of inherently dynamic biological processes, methods to reliably quantify temporal changes of protein interaction networks are essential. Further, dynamic remodeling of protein complexes in signaling systems is frequently dependent on phosphorylation events and methods to concurrently quantify these changes are required. SWATH MS is a data independent acquisition method which relies on the targeted extraction to provide highly complete quantitative data in perturbed systems.

Methods
Affinity purifications of 14-3-3β from HEK293 cells stimulated with IGF1 in a time course were analysed SWATH mass spectrometry (AP-SWATH). Quantitative data were extracted in a targeted fashion using the OpenSWATH and Skyline softwares.

Results
The consistent and reproducible quantification of 1,967 proteins across all stimulation time points provided insights into the 14-3-3β interactome and its dynamic changes following IGF1 stimulation. Quantitative changes in 14-3-3β interacting proteins clustered in to 5 distinct time profiles, 2 of which were strongly related to the activity of basophilic kinases such as AKT. To our knowledge this study represents the largest reported interactome for a single bait indicating that at least 2.8 % of the proteome is engaged by 14-3-3β containing scaffold dimers, and that a substantial portion of these are regulated after IGF1 stimulation. In addition, an ongoing reanalysis of this system with respect to phosphorylation dynamics and their correlation with protein interaction changes is providing further insight into the dependence of protein interactions on phosphorylation in this system.

Conclusions
We have established AP-SWATH as a tool to quantify dynamic changes in protein-complex interaction networks in perturbed systems.

Novel Aspect
The targeted analysis of AP-SWATH data provides a highly complete quantitative picture of signaling complexes undergoing remodeling in response to stimulation. Iterative targeted extraction of the data is providing additional information on phosphorylation dynamics which can be correlated with protein interaction dynamics to gain further insight.

WOS23 - Top-down Proteomics

Chairs: Julia Chamot-Rooke, Yury Tsybin

Room 3
Level 0

WOS23-01 **Keynote: A version of the human proteome project that embraces quantitative top down MS**

Neil Kelleher
Northwestern University

Proteomics technology has advanced at a breathtaking rate since the development of soft-ionization techniques for mass spectrometry-based molecular identification over two decades ago. Now, the large-scale analysis of proteins (proteomics) is a mainstay in nearly all aspects of biological research including clinical research, where there is a high premium placed on molecular diagnostics and personalized medicine. While peptide-based proteomic strategies (bottom-up proteomics) were developed and optimized early and represent the gold-standard at present, we are now beginning to understand the limitations to bottom-up technology, namely the inability to characterize and quantify intact protein molecules from a complex mixture of digested peptides. To overcome these limitations, numerous labs have taken a whole protein-based approach to proteome research where intact protein molecules are the subject of analysis and full characterization and quantification can be facilitated. A discussion of these 'top-down' techniques and how they have been, and will likely be, applied to biological research of clinical relevance is the topic of this talk with a focus on how top-down proteomics impacts a new, cell-based version of the human proteome project (Kelleher, N.L., "A cell-based approach to the human proteome project", J. Am. Soc. Mass Spectrom., 23, 2012, 1617-24). With the unprecedented precision offered by mass spectrometry-based proteomics, both peptide- and whole protein-based strategies are poised to be used as complementary techniques to characterize complex disease phenotypes in the 21st century.

WOS23-02 **Sequencing of native protein complexes**

Mikhail Belov[1], Neil Kelleher[2], Alexander Makarov[1]
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Introduction
Native mass spectrometry (MS) is becoming an important integral part of structural proteomics and system biology research. The approach holds great promise for elucidating higher levels of protein structure; from primary to quaternary. This requires the most efficient use of tandem MS, which is the cornerstone of MS-based approaches.

Methods
All experiments were performed with the modified Q Exactive mass spectrometer using direct ESI infusion in a nano-flow regime. Several important hardware modifications included a novel front-end interface encompassing an orthogonal ion injection into the radio-frequency (RF) field of a dual ion funnel interface; a bent flatapole with an axial electric field, which ensures efficient ion transmission at low incoming ion flux; a higher mass-to-charge (m/z) quadrupole analyzer capable of selecting precursor ion species at m/z over 20,000; reduced frequencies on the RF multipoles of the mass spectrometer; an increased gas intake into the HCD cell, which enables a pressure increase in the Orbitrap analyzer and an image current preamplifier with improved linearity.

Results and Conclusions
Our pseudo-MS3 approach for obtaining peptide-level fragment ions from protein complexes invokes two spatially separated fragmentation steps within a high resolution Orbitrap mass spectrometer. During the MS2 step, collisional dissociation of the complex of interest to the constituent monomer subunits was performed in the elevated pressure region prior to a mass selection device. In the following MS3 step, the derived monomer subunits were subjected to m/z selection using a quadrupole analyzer and further dissociated to peptide-level fragment ions in the High Energy Collision (HCD) cell filled with an inert gas. The fragment ions were then injected into the Orbitrap for high-resolution high-mass-accuracy detection. We have found that successful MS2 activation of larger protein complexes, such as 14-mer GroEL complex, requires nearly complete complex desolvation and fine balance of collision energy and pressure in the elevated pressure region. Collisional activation of the protein subunits was carried out at the reduced pressure in the HCD cell to enable higher resolution detection. We have successfully applied MS3 strategy to detection and identification of heteromeric complexes, such as GroEL-GroES (14:7 mer) and a hexamer of Toyocamycin Nitrile Hydratase (TNH) at molecular masses of 870 kDa and 86 kDa, respectively. In experiments with GroEL-GroES complexes, we have observed dissociation of both types of subunits of the heteromeric complex, resulting in release of the highly charged heptameric rings of both GroES and GroEL, which was then isolated and sequenced to constituent peptide-level fragments at high mass resolution and mass accuracy.

Novel aspect
Novel multi-step selective fragmentation approach for sequencing large protein complexes

WOS23-03 **Top-down native ETD yields conformationally selective fragment patterns**

Albert Konijnenberg[1], Frederik Lermyte[1], Jonathan Williams[2], Jeff Brown[2], Frank Sobott[1]
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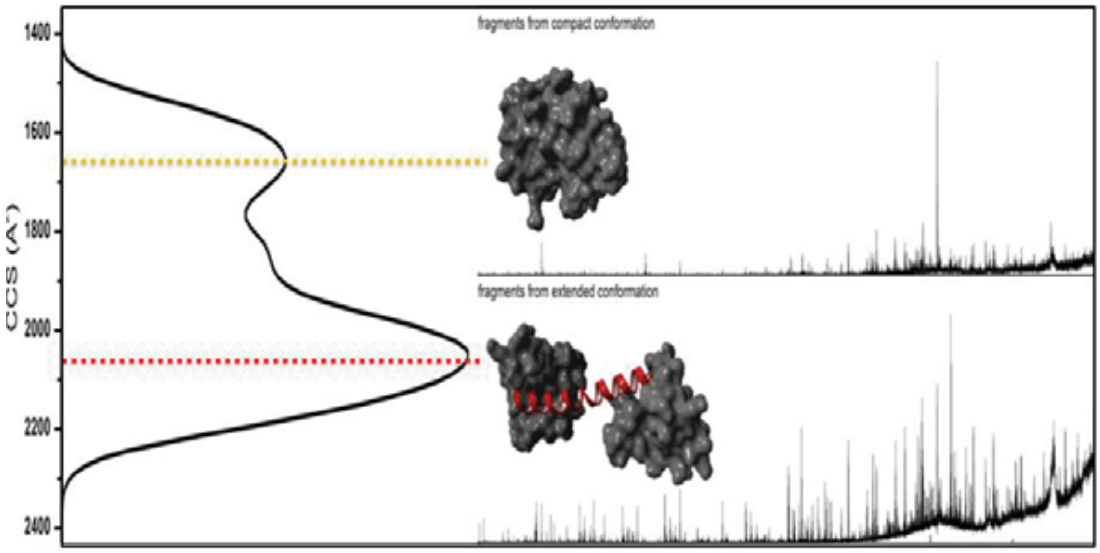
Although top-down fragmentation can provide crucial information about a protein, such as uncovering its sequence and possible PTMs, top-down CID experiments often result in loss of modifications and in poorly interpretable spectra. The specific and non-ergodic nature of ECD and ETD fragmentation provides an excellent alternative. Recently several research groups performed top-down ECD on native proteins and protein complexes, suggesting that the compact nature and lower charge states of these species do not necessarily hinder the fragmentation process.
Here we performed top-down ETD fragmentation of native proteins and complexes, ranging from small globular proteins (β-lactoglobulin, calmodulin) and intrinsically disorder proteins (IDPs) such as α-synuclein, RS12 and β-casein, to protein complexes such as concanavalin and alcohol dehydrogenase. All experiments were performed on a commercially available Waters Synapt G2 HDMS Q-TWIMS-TOF instrument, equipped with an ETD source. We then

correlate the observed ETD fragmentation patterns to the structural properties of the investigated proteins, specifically the collision cross section, the solvent accessible surface area and the b-factors from known solution structures.
Top-down ETD yielded good sequence coverage (up to 65%) for a range of proteins and protein complexes under native mass spectrometry conditions. We found that the efficiency for top-down ETD on native proteins is strongly dependent on the charge state of the precursor selected. We then used the data obtained to explore the possibility of native top-down ETD as a structural tool to investigate protein structure in the gas phase.



We show that there is a close correlation between the ETD fragmentation observed and the structure of the proteins investigated. By correlating the ETD fragments with the solvent accessible surface area, we can use top-down fragmentation to probe the solution phase structure of proteins and protein complexes. When we combined ETD with ion mobility we were able to obtain conformation specific fragmentation patterns: for example for the compact and dumbbell structure of calmodulin, which were in close agreement with the solvent accessible surface area. This validates the use of top-down ETD as a valuable tool for structural investigations of protein structures in the gas phase. We will also show how top-down ETD can be used to probe disorder in intrinsically disordered proteins and how this data can be correlated to the sequence of the IDPs investigated. The combination of ETD and ion mobility not only provides information on both a low resolution level - in the form of collision cross sections – but as well as on a residue specific level, where it shows the solvent exposed residues.
For the first time, we report the combination of ion mobility with top-down ETD on native proteins to obtain conformational specific ETD fragment patterns, which can be correlated with their solution phase structures.

Figure conformational specific ETD fragments obtained for the compact (globular) and extended (dumbbell) structures of calcium free calmodulin. Dotted lines indicate theoretical CCS for these structures.

WOS23-04 Combining low- and high-resolution top-down mass spectrometry for hemoglobin disorder diagnosis

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Hemoglobin (Hb) is a blood tetrameric protein. Healthy adults have ~97% HbA, ~3% HbA2 and<1% HbF, which are composed of two alpha and two beta, delta or gamma chains, respectively. Hb disorders can be divided into two groups: Hb variants (structurally abnormal Hb) and thalassemias (deficient synthesis of one globin chain). Hb disorder diagnosis is currently based on a combination of methods: hematological tests, protein analyses (chromatography, electrophoresis) and molecular biology. We aimed to establish a two step top-down mass spectrometry (MS) assay suitable for clinical laboratory practice that would simplify protein analysis step. First, an automated workflow is used for the identification of the most common Hb variants (HbS, HbC, HbE, HbD-Punjab, HbO-Arab) and for the quantification of HbA2 and HbF. Second, if a rare Hb variant is detected, a semi-automated workflow based on high-resolution (HR) MS allows the characterization of the mutant.
In the first step, Hb extracts (from healthy subjects and carriers of HbS, HbC or thalassemia) were analyzed in an integrated system composed of a nanoLC hyphenated to a 3D-ion trap with electron transfer dissociation (ETD) capabilities. The system is fully managed by an open access software, allowing automated data acquisition and interpretation for the identification of HbS, HbC and the quantification of HbA2. In the second step, seven rare Hb variants were analyzed. Hb samples were directly infused in an ESI-QqTOF with ETD capabilities. Hb sequence analysis was performed with DataAnalysis and BioTools software (Bruker Daltonics).
Preliminary results show that the integrated system for most common Hb variant identification and Hb quantification confidently detected HbS and HbC. The relative quantification of HbA2 was achieved with a maximum CV of 4%.
For rare Hb variant characterization, HR ETD top-down MS was used to provide a comprehensive sequence information. Semi-automated identification was based on 10 abundant diagnostic ions well distributed along alpha and beta chain sequences. These ions allowed to rapidly determine the approximate mutation position, within 15 to 20 amino acids. The

exact mutation position was then determined using manual annotation of the fragment ions within these amino acids.
In conclusion, we establish an automated MS assay for the identification of the most common Hb variants and the precise quantification of HbA2. Work is in progress to include the detection of HbE, HbO-Arab and the quantification HbF. For rare Hb variants characterization, a semi-automated workflow was developed to map the mutation position within the chain. This information should be sufficient to guide molecular biology confirmation experiments.
The combination of these two MS methods for protein analysis should simplify and speed-up Hb disorder diagnostic process. A comparative study between this MS platform and the conventional diagnostic workflow is currently in progress.

WOS23-05 Extreme ultraviolet activation and fragmentation of peptide and protein ions

Alexandre Giuliani
Synchrotron SOLEIL / INRA

Introduction
The activation of a selected precursor ion is the essence of tandem mass spectrometry, a specific and advantageous feature of analytical mass spectrometry. The most widely used method for ion activation is known as collision induced dissociation (CID). The search for new activation methods to complement the arsenal of available techniques is a very active fields. Especially, since the beginning of the century, numerous methods have been used successfully, involving electron capture 1, electron transfer 2, UV laser activation 3-5, or collision with high energy ions 6.
We present here a new activation method based on the interaction of extreme ultraviolet (XUV) radiation with biological ions.

Methods
A glow discharge lamp has been coupled to a commercial mass spectrometer. The lamp is operated windowless and fed with rare gases, such as helium, neon.... It produces a radiation in the 8 eV (155 nm) to 40 eV (30 nm) photon energy depending on the nature of the gas and the discharge conditions. Model peptides and proteins have been nano-electrosprayed in both ion mode from methanol water solutions. The desired precursor ions have been selected and photoactivated and the product ion mass spectra subsequently recorded.

Results
Peptides and proteins target ions have been activated by the XUV radiation of the lamp. The product of the irradiation is photoionization for cations or photodetachment for anions. Abundant fragmentations into all kind of sequence ions are also produced. The fragmentation mechanisms are discussed for both positive and negative ion mode. The fragmentations patterns observed for the model polypeptides are compared to CID and to other activation methods from literature. The position of this new mean of activation towrds Electron Induced Dissociation (EID) and Electron Detachment Dissociation (EDD) is discussed.

Conclusions
A new setup based on XUV photon activation is presented along with the first results obtained on model peptides and small proteins. This system appears extremely versatile and possess very appealing characteristic for tandem mass spectrometry of proteins.

Novel Aspect
A new activation method is presented based on photon activation using energetic UV that could complement the arsenal of existing techniques.

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WOS24 - Trace Gas Analysis of Breath and Food Flavours

Room 4
Level 0

Chairs: Patrik Spanel, Gianluca Giorgi

WOS24-01 Keynote: Clinical breath (VOC) analysis - pearls and pitfalls

Wolfram Miekisch, Jochen Schubert
University Medicine of Rostock

Volatile organic compounds (VOCs) in the breath may provide fast recognition of physiological, metabolic and pathological conditions through a non-invasive window. Several VOC based biomarker sets have been proposed for clinical applications such as cancer screening, diabetes control and recognition of organ rejection, oxidative stress or lung diseases. Mass spectrometry based techniques such as GC-MS, PTR-MS or SIFT-MS enabled detection and identification of a large number of breath constituents down to the trace levels (pmol/L - pptV). Up to now more than 800 different VOCs have been reported as constituents of human breath. Many of these data available were obtained by means of sampling breath in bags or canisters, pre-concentration of large volumes, time consuming gas chromatographic (GC) separation and mass spectrometric (MS) detection.
During recent years smart combinations of available techniques and progress in analytical instrumentation could be applied to enable fast and reliable analysis at the bedside. Automated sampling systems enable fast and reliable controlled alveolar sampling. Improved (micro)extraction techniques for pre-concentration are requiring now not more

than a few cc of exhaled air. Multidimensional and fast GC technology progressed in the way that miniaturized devices yield reliable substance separation within a few minutes. In combination with mass spectrometry those techniques enable identification of hundreds of VOCs down to the pptV level. Breath resolved continuous monitoring of relevant compounds and - in parallel - potential confounders can be done by means of direct MS (e.g. PTR-TOF). Sensor systems with tremendously enhanced sensitivity enable measurements of single breath markers at the bedside. In a clinical setup combinations of these techniques can be used to identify potential VOC markers and monitor physiological, metabolic or interventional changes at the point of care without a relevant delay.
Current problems to be solved include basic knowledge on biomarkers and their origin and exhalation kinetics, on confounding parameters that may affect results and transformation of the scientific data into clinical application. The high number of detectable compounds can lead to coincidental correlations with e.g. the disease state if the number of independent measurements (patients) is too low. Prospective, blinded and randomized trials are mandatory if reliability, sensitivity, specificity and relevance of clinical tests are to be assessed. Clinical application of breath biomarkers therefore requires a combined effort of chemists, physicists, statisticians, engineers and physicians resulting in unequivocal identification of breath biomarkers, a basic understanding of their generation and distribution in the body and clear correlations between blood and breath levels. If this knowledge and medical science can be combined with improved PoC applicable analytical methods lifesaving therapy and non-invasive monitoring tailored to the individual needs of the patient could be started at the very onset of disease.

WOS24-02 Breath acetone to monitor life style interventions in field conditions: an exploratory study using proton transfer reaction mass spectrometry (PTR-MS)

Devasena Samudrala[1], Julien Mandon[1], Phil Brown[1], Frans Harren[1], Luc Tappy[2], Gerwen Lammers[3], Simona Cristescu[1]
[1]Radboud University, [2]University of Lausanne, [3]Radboud University Nijmegen Medical Centre

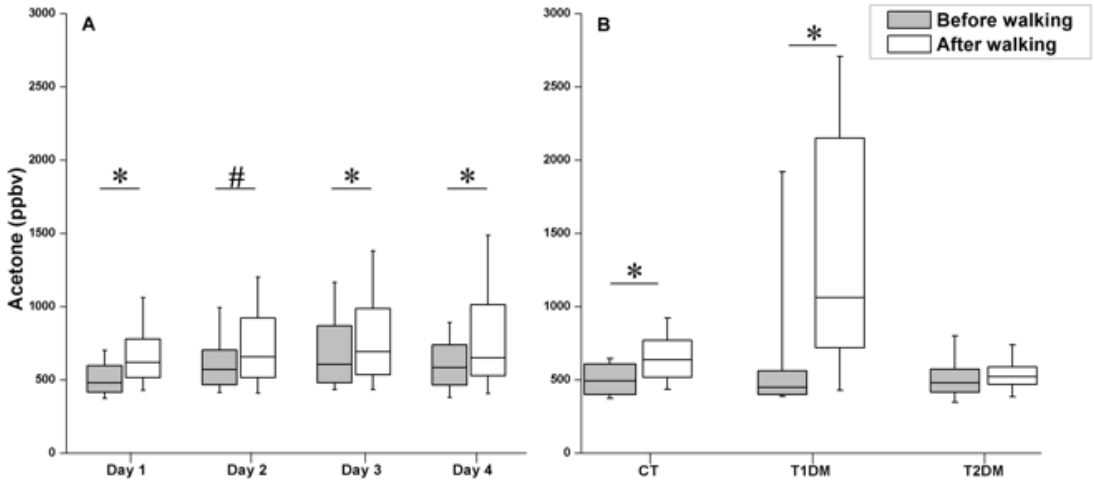


Figure 1

FIGURE 1 Breath acetone concentrations. (A) Breath acetone concentrations measured before and after the walk over 4 consecutive days for all participants. (B) Breath acetone concentrations measured before and after the walk in CT, T1DM and T2DM on the first day. Data are displayed as box plots showing the median, interquartile ranges (25 %, 75 %); whiskers indicate the 10 % - 90 % values. * $P < 0.05$, # $P < 0.1$.

Introduction

Obesity is associated with insulin resistance and type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease, and dyslipidaemia. A low fat oxidation rate, possibly related to impaired mitochondrial function, and intracellular accumulation of lipid metabolites, such as diacyl-glycerol and ceramides, may play a role in the pathogenesis of all these conditions. Diet and physical activity are important to control obesity and T2DM; both aim at promoting a negative energy balance and increasing lipid oxidation, thus alleviating tissue lipotoxicity. Their success rate in clinical practice is unfortunately low. Their effectiveness may be enhanced if health professionals and patients could rely on a sensitive marker of lipid oxidation to adjust diet and exercise on a day-to day basis. For this purpose, breath acetone concentration may be a suitable marker, since physiological variations of hepatic ketogenesis are known to occur during physical activity and energy restriction. Here, we present preliminary evidence than an activation of whole body lipolysis and hepatic ketogenesis induced by prolonged physical activity can be detected by monitoring breath acetone concentration in field conditions.

Methods

Twenty-three non-diabetic, 11 type 1 diabetic and 17 type 2 diabetic subjects provided breath and blood samples for this study. Samples were collected during the International Four Days Marches, in the Netherlands. For each participant, breath acetone concentration was measured using proton transfer reaction ion trap mass spectrometry (PIT-MS), before and after a 30-50 km walk on 4 consecutive days. Blood non-esterified free fatty acid (NEFA), beta-hydroxybutyrate (BOHB), and glucose concentrations were measured after walking.

Results
Breath acetone concentration was significantly higher after than before walking compared to before, and was positively correlated with blood NEFA and BOHB concentrations. The effect of walking on breath acetone concentration was repeatedly observed on all 4 days. Breath acetone concentrations were higher in type 1 diabetic subjects and lower in type 2 diabetic subjects than in control subjects.

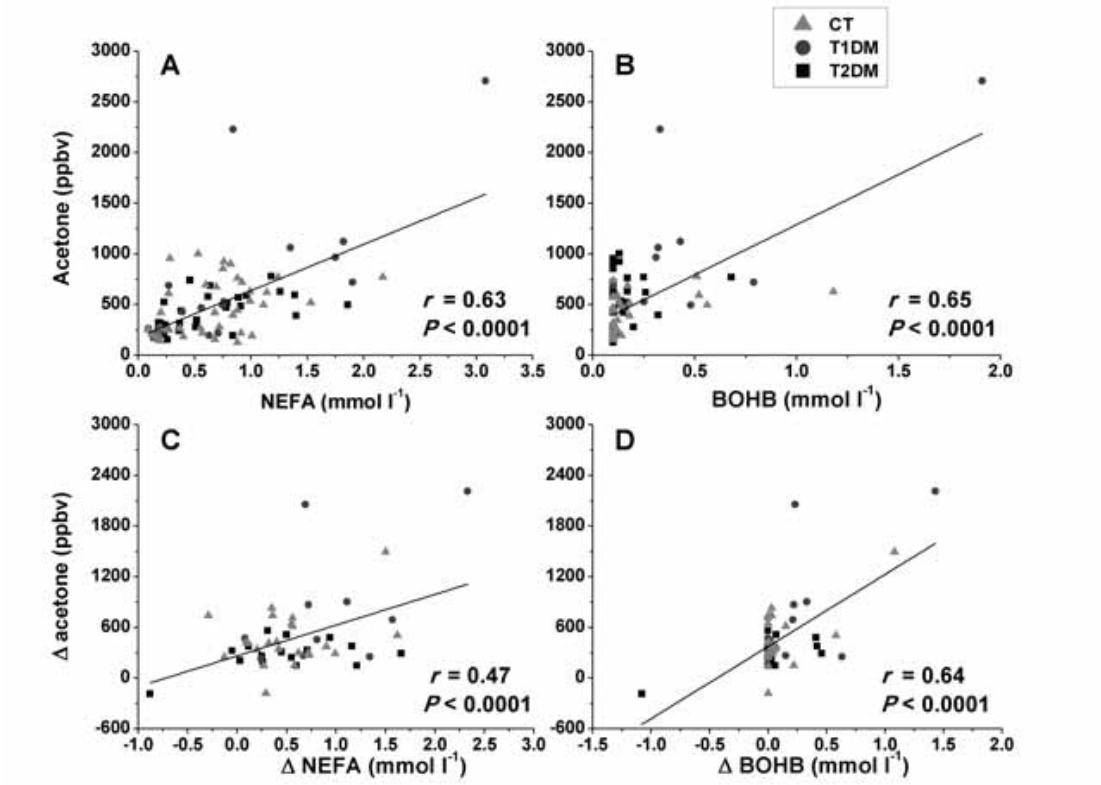


Figure 2

FIGURE 2 Relationships of breath acetone with NEFA and BOHB. Correlation of breath acetone (ppbv) with serum NEFA (mmol l-1) (A) and BOHB (mmol l-1) (B). Diference between post- and pre-walking NEFA (Δ NEFA, C) and BOHB (Δ BOHB, D) vs. changes in breath acetone (Δ acetone).

Conclusions
Breath acetone can be used to monitor hepatic ketogenesis during walking under field conditions. It may therefore provide real-time information on fat burning, which may be of use for monitoring lifestyle interventions.

Novel Aspects
Breath acetone measurements with PIT-MS are shown to be an indicator for monitoring lifestyle interventions in field conditions. Breath acetone allows for assessment of BOHB levels in blood to be monitored, providing real time monitoring of fat burning and hepatic ketogenesis.

Wednesday

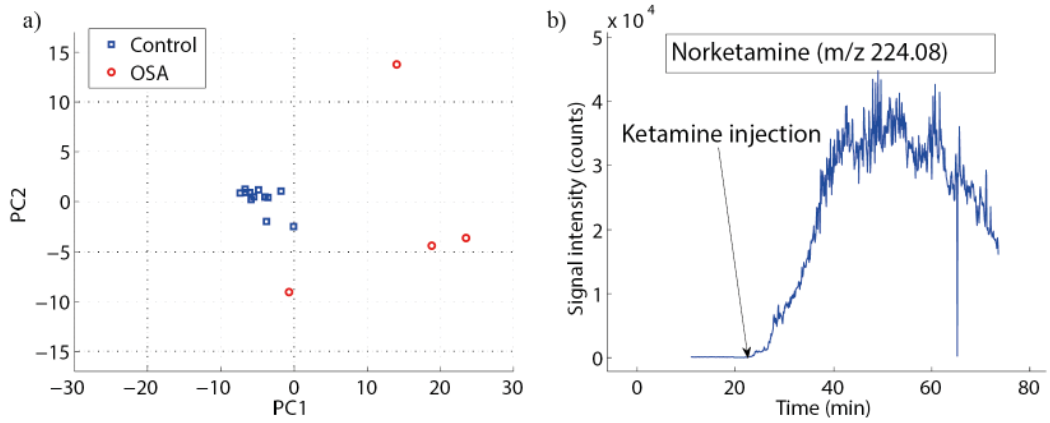
WOS24-03 *In vivo* exhaled breath analysis: adding to lung disease diagnosis and drug monitoring

Pablo M-L Sinues[1], Lukas Bregy[1], Robert Dallmann[2], Xue Li[1], Esther Schwarz[3], Yvonne Nussbaumer[3], Steven Brown[2], Malcolm Kohler[3], Renato Zenobi[1]
[1]ETH Zurich, [2]University of Zurich, [3]University Hospital Zurich

Introduction
Exhaled breath contains relevant metabolites that may reflect the biochemical activity within a subject. However, in contrast to other biofluids (e.g. plasma), the analysis of breath remains far less explored. Here we present some recent examples of how real-time breath analysis may contribute to the fields of disease diagnosis and drug monitoring.

Methods
We modified the entrance of a commercial quadrupole time-of-flight (Qtof) mass spectrometer to allow for the real-time analysis of breath via secondary electrospray ionization-mass spectrometry. We have studied i) differences between obstructive sleep apnoea (OSA) and case controls; ii) breath levels of ketamine and its main metabolites in mouse models.

Results
Diagnosis of OSA. We found a panel of discriminant features that allowed for the accurate prediction of disease/non-disease states. The figure (left, a) shows the score plot of principal component analysis of controls and OSA, suggesting a distinct breath signature for the latter.
Drug monitoring. The figure (right, b) displays the time trace of norketamine (the main ketamine metabolite) following injection of ketamine. Our method is in accordance with the literature on the mouse pharmacokinetics for ketamine, but provides much higher time resolution.



Conclusions
We conclude that the real-time mass spectrometric analysis of exhaled metabolites may contribute to address some of the most relevant clinical pharmacological problems, which are currently investigated through the analysis of body fluids other than breath.

Novel aspect
In vivo monitoring of exhaled compounds related to OSA and ketamine

WOS24-04 **PTR-TOF-MS characterization of roasted coffees (*C. arabica*) from different geographic origins**

Sine Yener[1], Andrea Romano[1], Luca Capellin[1], Tilmann Maerk[2], Flavia Gasperi[1], Luciano Navarini[3], Franco Biasioli[1], Pablo M. Granitto[4]
[1]Fondazione Edmund Mach, [2]Leopold-Franzens University Innsbruck, [3]Illycaffé S.p.A., [4]CIFASIS, French Argentine International Center for Information and Systems Sciences, UAM (France) / UNR–CONICET (Argentina)

Introduction
The taste and aroma of high quality coffee can vary considerably among samples from the same species and variety grown in different regions. In addition to the fact that geographic origin is embedded in coffee quality, marking of origin for product differentiation is highly demanded for traceability, authentication, and marketability purposes. In this study, we developed a mass spectrometry based set-up for the high-throughput characterisation of food samples. The aromatic profiles of six roasted C. arabica coffees (Brazil, Ethiopia, Guatemala, Costa Rica, Colombia, India) were analysed by Proton-Transfer-Reaction-Time of Flight-Mass Spectrometry (PTR-ToF-MS) to characterise aromatic profiles of coffee powders and brews.

Methods
Commercially available medium roasted C. arabica coffees were used for the experiment. Coffee brewing was performed by steam pressure coffee extraction in a stove-top coffee maker known as "moka" in Italy. The headspace measurements of coffee powder and brews were performed by a commercial PTR-ToF-MS 8000 instrument connected to a multipurpose autosampler. The proton transfer reaction was controlled by drift voltage (550 V), drift temperature (110°C), drift pressure (2.30 mbar) and E/N=140 Td. Multivariate data analysis techniques were applied in order to visualize data and classify the coffees according to origin.

Results
The results showed that the volatile compositions of coffees were highly influenced by the geographic origin of the coffee beans. Significant differences were found among volatile concentrations of coffee powders and brews. Tentative identification of mass peaks aided characterisation of aroma fractions. Principal component analysis allowed separation of coffees according to origin both for powder and brew. Some mass peaks were increased in the brew whereas decreased maybe be due to the lower solubility of aroma compounds in the brew or degradation of them by hot water.

Conclusions
Six C. arabica from different geographical origins were successfully classified by their volatile profiles in powder and brew. PTR-ToF-MS spectra of the coffees contained almost five hundred mass peaks and the high mass resolution allowed the tentative identification of diverse volatile compounds useful for aroma fingerprints and origin discrimination.

Novel Aspect
PTR-ToF-MS has been used for the first time for the rapid classification of the origin of ground roasted coffee powder and brew.

WOS24-05 **Evolved gas analysis by single photon ionization–mass spectrometry: a tool to distinguish different types of coffee**

Michael Fischer[1], Sebastian Wohlfahrt[1], Janos Varga[1], Mohammad Reza Saraji-Bozorgzad[2], Georg Matuschek[1], Thomas Denner[3], Ralf Zimmermann[1]
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Introduction
In this study, the applicability of thermogravimetry (TG) coupled to single photon ionization time-of-flight mass spectrometry (TG-SPI-TOFMS) for evolved gas analysis (EGA) of coffee was demonstrated. Coffee is a chemically well-known complex food product of large scientific and commercial interest.

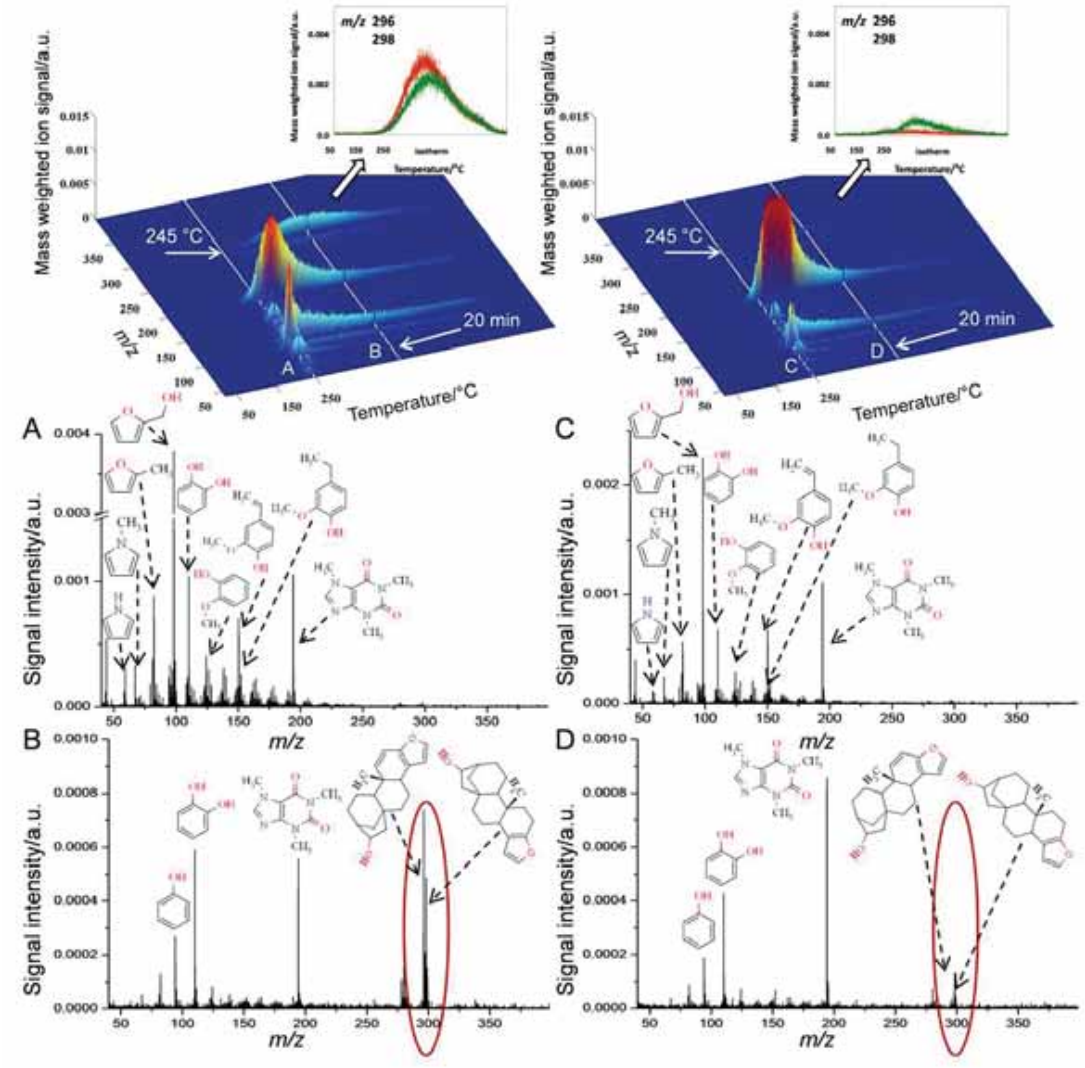
Methods

A simultaneous thermogravimetry/differential scanning calorimetry device (STA) was coupled to single photon ionization time of flight mass spectrometry (SPI-TOFMS) for evolved gas analysis (EGA). Thermal resolution with thermogravimetric signal (TG) is delivered by STA. On-line coupled EGA with SPI-TOFMS retains the thermal information for a chosen heating program from the STA and substantiates these with correlating mass spectra. The application of vacuum ultraviolet (VUV)-photons (8-12 eV) for soft ionization, allows almost fragment-free ionization. Thus, it becomes possible to interpret mass spectra of complex matrices, like natural products evolving simultaneously several molecules, without an additional separation step. The TG-SPI-TOFMS on-line coupling offers the possibility to track subset mass traces during one STA run. Focusing on material depended mass traces, differentiation of organic matrices is obvious[1].

Results

The roasting process and roasting experiments can be done laboratory-based on the micro scale using single beans in TG-SPI-TOFMS experiments. The gases evolved during the simulated roasting process can be analyzed by comparing the gained mass information with the literature. As known, Arabica as Robusta contains the diterpene Cafestol, but only the Arabica coffee bean contains Kahweol. The masses of those dehydrated diterpenes (m/z 296 and m/z 298) can be tracked by EGA during one cycle. The figure shows the results of the simulated roasting process of a single Arabica coffee bean (left column) and an individual Robusta (right column) coffee bean. The mass spectra B and D after 20 minutes allow discrimination between Arabica and Robusta coffee. The mass spectra at 245 °C (A, C) reveal different composition ratios.

Further experiments revealed the possibility of detecting Kahweol as a characteristic of the Arabica coffee also in powders and blends (roasted and grinded).



Conclusions

The application of TG coupled to Single Photon Ionization - Time-of-Flight Mass Spectrometry for simulated coffee roasting experiments (individual coffee beans) and grinded coffee powders, gave promising results and valuable insights in the chemical composition of the evolved roasting gases.

Novel Aspect
TG-SPI-TOFMS was applied to simulate the roasting process of single green coffee beans in the laboratory. Almost fragment-free ionization allows the interpretation of evolved gases' chemical composition.

1. Fischer M, Wohlfahrt S, Saraji-Bozorgzad M, Matuschek G, Post E, Denner T et al. Thermal analysis/evolved gas analysis using single photon ionization. J Therm Anal Calorim. 2013;113(3):1667-73. doi:DOI 10.1007/s10973-013-3143-y.

WOS25 - Nanomaterials in MS, Nanomaterials Characterization

Room 5/6
Level 3

Chairs: Hui-Fen Wu, Laurent Fay

WOS25-01 Keynote: Nanomaterial-based affinity mass spectrometry for the analysis of biomolecules

Yu-Chie Chen
National Chiao Tung University

Introduction
Due to the high surface-area-to-volume ratio and ease of surface modification, nanomaterials have been widely used for enrichment of target analytes present in complex samples. Importantly, such nanomaterial-aided affinity methods are highly compatible with mass spectrometry (MS). While functional nanomaterials are used as affinity probes for the target molecules, they also assist ionization during the MS analysis. Among these functional nanomaterials, metal oxide-based nanomaterials have gained considerable attention because they absorb light in the ultraviolet range, have favorable dielectric properties, and they show affinity to several types of functional groups. In this talk, I will discuss the use of metal oxide nanostructure-based materials in MS.

Experimental
Iron oxide magnetic nanoparticles (Fe3O4 MNPs) were prepared using the co-precipitation method. The surface of the generated Fe3O4 MNPs was coated with a thin layer of titania or alumina through sol-gel reactions. During the synthesis process, the precursors of metal oxides were hydrolyzed in acidic an condition, which was followed by the steps of condensation and polymerization. The generated sol-gel was either coated on the surface of the Fe3O4 MNPs or a suitable substrate to generate metal oxide (titania or alumina) coated films. Samples were prepared in the buffer of appropriate pH before conducting the enrichment experiment.

Results
Metal oxides have high affinity toward certain biomolecules such as phosphorylated peptide/proteins and histidine-tagged peptides/proteins. In this work, metal oxide-coated Fe3O4 MNPs were used as affinity probes for these target species. Identities of the target species conjugated to MNPs were verified by MS. Because of the light absorption capacity of metal oxide such as titania and iron oxide, the nanoprobes could then also assist ionization of the captured analytes in laser desorption/ionization (LDI)-MS. Interestingly, the metal oxide-based nanoprobes also interact with cells – for example, bacteria – through metal-ligand chelation. Thus, the nanoprobes enable efficient capture and concentration of small numbers (e.g. a few thousands) of target bacterial cells present in complex samples. The fingerprint mass spectra of the targeted bacteria – representing the identities of the bacterial strains were acquired by MS. In a follow-up study, the possibility of combining metal oxide nanomaterial-based affinity approaches with electrospray ionization (ESI) has been explored, and the new results will be discussed during the talk.

Conclusions
The results of this study demonstrate that metal oxide nanostructure-coated Fe3O4 MNPs can be used as affinity probes and can assist ionization in MS. This approach has several advantages – most importantly, high sensitivity and short analysis time. Overall, the presented nanomaterial-based sample processing approaches are highly compatible with LDI-MS and ESI-MS.

Novel aspect
The ability of metal oxide-based materials to assist sample treatment and ionization in MS analysis is demonstrated.

WOS25-02 Novel metal oxide nanomaterials for global phosphoproteome

Yu Bai, Liping Li, Linnan Xu, Huwei Liu
Peking Univeristy

Introduction
As one of the most common post-translational modifications, protein phosphorylation is of great importance in regulating various biological processes. Therefore, effective analytical methods for phosphoprotein are in urgent demand for further phosphoproteomic research. Mass spectrometry (MS) is considered as a powerful tool in the detection and identification of proteins. However, for phosphoproteomic analysis, effective enrichment approaches prior to MS analysis are often required due to serious signal suppression caused by non-phosphorylated species. Among these approaches, metal oxide affinity chromatography (MOAC) has become more and more popular in recent years. Different metal oxide material surface properties often lead to different enrichment performance in sensitivity, specificity or phosphoproteome coverage. Therefore, the exploration of novel MO nanomaterials for comprehensive phosphoproteomic analysis with enhanced sensitivity is still attracting considerable interest.

Methods
In a typical experiment, the peptides were diluted to a certain concentration by binding buffer (50% ACN, 0.2% TFA). Then the suspensions of those nanomaterials were added into 200 μL diluted peptides for selective enrichment. The mixed solutions were vibrated at room temperature for at least 30 min. After centrifugation, the supernatants were removed. After washing three times, the remained nanomaterials were redispersed. After centrifugation the supernatants were mixed with matrix (DHB 20 mg/mL, 50% ACN, 1% H3PO4) for MALDI-MS analysis.
All MALDI-ToF MS measurements were performed on a Bruker Daltonics Ultraflex I mass spectrometer.

Results and conclusions
In our study, binary SnO2-ZnSn(OH)6, ZnSn(OH)6, SnO2 nanospheres, and other novel nanomaterials have been synthesized and characterized. Those synthesized MO nanomaterials present different characteristics in phosphopeptide enrichment. SnO2 shows preferential detection for single phosphopeptides in most cases, while ZnSn(OH)6 presented impressive enrichment for multi-phosphopeptides in our work. This opens the door to solve the challenging work of multi-phosphopeptide detection by MOAC, and indicates that binary materials with flexible properties are promising for creation of complementary approaches for large-scale phosphoproteomic studies. A one-step and template-free synthesized uniform SnO2 nanospheres have large surface area and highly active surfaces for the effective binding of phosphopeptides, indicates that developing feasible methods for the synthesis of high-quality metal oxides should be considered in the screening of efficient and applicable MOAC affinity probes in target proteomic research.

Novel Aspect
Novel metal oxide nanomaterials have been synthesized with effective phosphopeptide enrichment, which are promising for creation of complementary approaches for large-scale global- and targeted- proteomic research.

WOS25-03 Characterization of nobel metal nanoclusters and nanocages on atomic scale by ESI-Q-TOF mass spectrometry

Elina Kalenius[1], Hannu Häkkinen[1], Maija Nissinen[1], Pia Bonakdarzadeh[1], Kari Rissanen[1], Kaisa Helttunen[1], Tanja Lahtinen[1], Kirsi Salorinne[1], Jukka Hassinen[2], Robin Ras[2]
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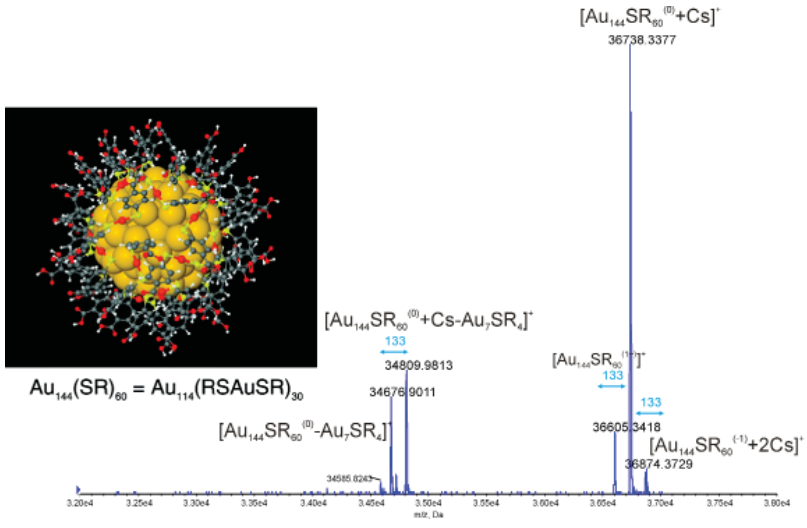
Small nobel metal clusters and metal cages have recently arouse interest originating from their intrinsic properties regarding electronic and 3-D structures, optical absorption and emission, potential in chemical/catalytic reactivity and storage of highly active chemicals. In their chemical and physical properties metal clusters/cages often show sharp size-property dependence, which sets high demands on their atomic level characterization. Even though, the synthesis methodology for nanoclusters has developed recently and their structures can be quite well predicted,the reality is often different and their synthesis might result in heterogeneous products which are demanding to study on atomic scale. As the size of a molecular system grows, their atomic scale characterization and structure elucidation becomes more demanding and the number of applicable techniques decreases in conjunction with their practical application.
Mass spectrometric analysis, in particular ESI-MS, has several features, which are advantageous in characterization of metal clusters and cages. The technique is sensitive, soft, solution-infused gas-phase technique. As a result, only rather small amounts (on ng level) of the analyte are required for the analysis of these relatively labile system and their characteristics can be studied in solvent free conditions.
ESI-Q-TOF mass spectrometry (ABSciex QSTAR Elite) was used for characterisation of various noble metal clusters and cages. These labile compounds are generally soluable in organic solvents and easily ionized. The m/z values, which they produce are can be either relatively high due to high molecular weight and low charge states (e.g. Au144(SR)60) or they can produce fragile inherently multiply charged molecules composition of multiple coordinating components (e.g. [MxLy(A-)z]n+ cages).
We have recently characterized, by using ESI-Q-TOF mass spectrometry, several Au and Ag thiolate-stabilized metal clusters and Pd/Pt coordination cages on atomic scale. These include neutral, anionic and cationic, singly and multiple charged nanoclusters stabilized with various ligands and their size varies from few kDa (e.g. Au25(SR)18) up to ~ 36 kDa (e.g. Au144(SR)60). Despite of obvious benefits, mass spectrometric analysis the clusters is still relatively rare and most mass spectra are obtained by MALDI-MS. ESI-TOF MS, however, offers several benefits, such as 1) higher mass accuracy and resolving power, 2) possibility to use different solvent systems including nonpolar solvents and 3) higher tolerance for heterogeneity, all of which are crucial for to analyse large metallic clusters and cages on atomic level.



WOS25-04 **Analysis of organic surface modifications of manufactured nanomaterials by thermogravimetry coupled to MS (TGA-MS)**

Per Axel Clausen, Vivi Kofoed-Sørensen, Yahia Kembouche, Brian Hansen, Asger W. Nørgaard, Keld Alstrup Jensen
National Research Centre for the Working Environment

Introduction

Manufactured nanomaterials (MNM) are often chemically surface modified in order to tailor their physical-chemical properties for specific applications. These surface modifications may influence their toxicological and environmental properties, but are often trade secrets. Therefore, procedures are needed for reliable analysis of unknown surface modifications. A limited number of publications describe quantitative methods and few describe use of mass spectrometry (MS) despite of its advantage. Here we present an approach, based on thermogravimetric analysis (TGA) coupled to gas chromatography (GC) and MS.

Methods

TGA of a series of MNM was performed using a Netzsch STA 449 F3 TGA coupled to a Varian CP-3800 GC and a Varian 1200 L triple quadrupole MS with both electron impact (EI) and Townsend discharge ionization (TDI). The GC was equipped with a short piece of deactivated capillary column as transfer-line to the MS, but was intended for future experiments on trapping simultaneously released degradation products of surface modifications and subsequent chromatographic separation with an active column. EI was used when the TGA gas was N2 and TDI when the gas was air. Samples for TGA varied from 10 mg for inorganic MNM to 120 mg for carbon nanotubes (CNT). The TGA was heated 50-1150 °C at rates 5-20 °C/min in dry N2 or air. The TGA flow was 25 ml/min and the flow to the MS was adjusted with a needle valve to fit a suitable pressure in the ion source. The temperatures in all transfer lines and GC were 230 °C. An array of other techniques was used to validate and confirm the TGA-MS measurement qualitatively and quantitatively. These techniques included thermal solid phase extraction (TSPE), GC-MS, matrix assisted laser desorption ionization time-of-flight MS (MALDI-TOF-MS), combustion elemental analysis of C, H, O, N (CEA) and acid-base titration (ABT).

Results

Results of two examples of MNM are given here, namely a coated silver nanoparticle (NP) and a CNT functionalized with carboxylic acid groups. TGA of the silver NP in air showed decomposition in several steps, which indicated the coating was a complex organic molecule. The total weight loss was 17 w%. TGA-MS showed that both 2-pyrrolidone and N-vinyl-2-pyrrolidone were released. This indicated that the coating was poly N-vinyl-2-pyrrolidone and this was confirmed by MALDI-TOF-MS. Further, the mass of the coating was confirmed by TSPE and GC-MS. TGA-MS of the carboxylic acid functionalized CNT showed decomposition in several steps with one early large step, which was decarboxylation releasing CO2 corresponding to 0.28 mmol COOH / g CNT. This amount was confirmed by both CEA and ABT.

Conclusion

TGA-MS appear to be a powerful tool for identification and quantification of unknown surface modifications of nanomaterials.

Novel aspects

This is the first general approach using TGA-MS for analysis of surface modifications on manufactured nanomaterials.

WOS25-05 **A new ICP-TOF-MS and new capabilities for the analysis of micro- and nanosamples**

Olga Borovinskaya[1], Sabrina Gschwind[1], Bodo Hattendorf[1], Martin Tanner[2], Detlef Günther[1]
[1]ETH Zurich, [2]Tofwerk AG

A growing interest in the elemental analysis of very small sample masses such as microdroplets, nanoparticles1 or cells2 has created a high demand for the further development of the ICPMS instrumentation. Simultaneous detection over the entire mass range and high temporal resolution are key parameters for the measurement of very short transient signals (200 µs- 500 µs) produced, for instance, from individual particles. Therefore, an ICP time of flight mass spectrometer (TOFMS) was developed, which can acquire and readout full mass spectra at the µs time resolution.3 The specific characteristics and analytical performance of the new ICPTOFMS are presented in this work.
This report describes the capabilities of the new instrument in combination with discrete microdroplet sample introduction for the elemental analysis of single microdroplets, multi-component nanoparticles and laser-generated aerosols. The use of this unique combination allowed for the mass quantification of three types of nanoparticles from the same suspension. The results of this quantification and some important aspects related to different sample introduction systems are discussed. Additionally, a new microdroplet-based approach for the quantitative elemental analysis of non-aqueous liquids, nanoparticle suspensions and laser-generated aerosols is presented and evaluated for selected applications.

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(2) Bendall, S. C.; Simonds, E. F.; Qiu, P.; Amir, E.-a. D.; Krutzik, P. O.; Finck, R.; Bruggner, R. V.; Melamed, R.; Trejo, A.; Ornatsky, O. I.; Balderas, R. S.; Plevritis, S. K.; Sachs, K.; Pe'er, D.; Tanner, S. D.; Nolan, G. P. Science 2011, 332, 687-696.
(3) Borovinskaya, O.; Hattendorf, B.; Tanner, M.; Gschwind, S.; Gunther, D. J. Anal. At. Spectrom. 2013, 28, 226-233.

WOS26 - Metabolomics

Chairs: Oliver Fiehn, Olivier Laprévote

Room 1
Level 1

WOS26-01 **Keynote: From MS data to systems biology applications in medicine – with specific emphasis on metabolic disorders and their co-morbidities**

Matej Oresic
Steno Diabetes Center

Primary obesity is associated with several cardiometabolic co-morbidities including non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes. However, the specific underlying mechanisms linking the expansion of adipose tissue to these co-morbidities are unknown.
In our research we applied mass spectrometry (MS) based metabolomics for the analysis of molecular lipids and polar metabolites, respectively. Specifically, we applied novel bioinformatics approaches for modelling of metabolomics data. We found that acquired obesity is associated with remodelling of membrane lipids in the adipose tissue. The remodelling may help maintain biophysical properties of lipid membranes, however at the cost of increased vulnerability to inflammation. We also found that the lipid molecular network behind the membrane lipid remodelling is amenable to genetic manipulation. In another study, we identified and validated a serum lipid signature which can be used in the estimation of liver fat and diagnosis of NAFLD, and is also predictive of type 2 diabetes. In another study, we applied the genome-scale human metabolic model and integrated it with two independent human experimental settings to study NAFLD. We identified a systemic shift of liver metabolism in NAFLD towards reduced flexibility at the network level, i.e., high liver fat markedly hampers the ability of the liver to adaptively regulate metabolism to meet excessive demands on basic liver functions.
Our studies show how comprehensive MS-based metabolomics approaches together with computational modelling at molecular pathway and biophysical levels may provide deeper insights into the mechanisms of complex multifactorial diseases.

WOS26-02 **Advanced LC-HRMS and GC-MS based methods for metabolomics of *Fusarium* head blight on wheat**

Rainer Schuhmacher, Christoph Bueschl, Maria Doppler, Bernhard Kluger, Nora Neumann, Alexandra Parich, Benedikt Warth, Marc Lemmens, Gerhard Adam, Rudolf Krska
University of Natural Resources and Life Sciences Vienna

Introduction
The combination of stable isotope labelling (SIL) with MS based metabolomics enables the improved untargeted study of plant microbe interactions at systems level. Here, a novel SIL assisted metabolomics approach was developed and applied to investigate the food and feed safety relevant plant disease Fusarium head blight (FHB) on wheat. The current understanding of FHB is still scarce and deserves in depth study to develop improved FHB prevention strategies in the future. In this study, the effect of F. graminearum and its major mycotoxin deoxynivalenol (DON) on the metabolome of 6 defined wheat genotypes differing in two major resistance QTLs (Qfhs.ndsu-3BS & Qfhs.ifa-5A) against FHB was studied in detail.

Methods
In view of the major bottlenecks of LC-HRMS based untargeted metabolomics approaches, we present a novel powerful 13C SIL assisted workflow for the automated detection of hundreds of secondary wheat metabolites. To extend the coverage of the wheat metabolome even further, a GC-MS approach for more than 130 polar, primary metabolites, was used in addition. To this end, a two-step derivatisation and data processing with the MetaboliteDetector software was applied. Two parent- and 4 near isogenic wheat lines (NILs), differing in the resistance level against FHB, were treated with either (U-13C) DON, F. graminearum or water as a control. The study was carried out with the aim to investigate both the metabolisation of DON and global metabolic changes in wheat as a function of time (0, 12, 24, 48, 96 h after treatment), the treatment and the presence of the FHB resistance QTLs.

Results and Discussion
Application of the SIL-assisted LC-HRMS approach allowed the detection and annotation of 7 novel DON biotransformation products, highlighting glucose and glutathione associated pathways. Furthermore, for the first time, the RP-LC-HRMS-accessible part of the wheat metabolome was reliably estimated to consist of ca. 500-700 metabolites (ESI+mode), all of which truly assignable to the investigated biological sample. In addition ca. 60 GC-MS derived metabolites were consistently found throughout the tested wheat samples. GC-MS results suggested that the wheat carbohydrate metabolism and the TCA cycle were significantly affected by DON & Fusarium treatment. Most importantly amino acids and related amines were significantly altered with elevated levels of the shikimate derived phenylalanine, tyrosine, & tryptophan. While the metabolic route, velocity and extent of DON-metabolisation differed significantly among the tested NILs, no QTL specific difference was observed with respect to the primary metabolites.

Innovative aspects
A novel and powerful 13C SIL assisted workflow for significantly improved untargeted LC-HRMS based plant metabolomics research was developed. SIL assisted automated data processing is used for highly efficient two dimensional data filtering and allows, for the first time, the reliable global annotation of the metabolic composition of complex organisms such as wheat. The approach is also excellently suited to facilitate global internal standardisation and efficient validation of the complete analytical workflow.

WOS26-03 Combination of double isotopic labeling and high resolution mass spectrometry: a novel method for untargeted fungal metabolic profiling

Emilien Jamin[1], Patricia M. Cano[2], Souria Tadrist[2], Pascal Bourdaudhui[2], Michel Péan[3], Laurent Debrauwer[4], Isabelle P. Oswald[2], Marcel Delaforge[5], Olivier Puel[2]

[1]INRA, MetaToul-AXIOM-MetaboHub, [2]INRA, ToxAlim UMR1331, Toulouse, [3]CEA, DSV, IBEB, CNRS, Aix-Marseille Université, Saint-Paul-les-Durance, [4]INRA, ToxAlim UMR1331, Platform MetaToul-AXIOM, Toulouse, [5]CEA Saclay, iBiTec-S, SB2SM and URA CNRS 8221, Gif sur Yvette

Characterization of fungal secondary metabolomes has become a great challenge in the last decades due to both the emergence of fungal threats, and the industrial interest of many natural products. In view of this, the aim of the present study was to develop an approach to characterize fungal secondary metabolome.
Aspergillus fumigatus (NRRL 35693) and Fusarium graminearum (PH1) were grown on wheat grains (Triticum aestivum) with different isotopic enrichments: (i) naturally enriched grains, (ii) 97% 13C, and (iii) 53% 13C / 97% 15N. Extract of each culture was then analyzed by HPLC coupled to a LTQ-Orbitrap mass spectrometer equipped with ESI or APCI. Metabolites were characterized using the Antibase database, then annotated by MS/MS experiments, and identified by comparison with standards when available. In vitro characterized metabolites were finally monitored in samples infected in field, by triple quadripole mass spectrometry.
Wheat grains represented the only source of carbon and nitrogen for fungal growth. Therefore, produced fungal secondary metabolites were either unlabeled (naturally enriched cultures), singly labeled (13C cultures) or doubly labeled (13C/15N cultures). This allowed discrimination of fungal metabolites against non-biological compounds which remained unlabelled in the three substrates. Fungal origin was further confirmed by analysis of a blank 12C wheat extract (without fungus). The use of a 50% 13C enrichment for the 13C/15N wheat substrate resulted in a specific isotopic pattern which enabled the specific detection of fungal metabolites. Furthermore, the m/z comparison of a same metabolite detected in the different cultures as non-labeled, fully 13C labeled and doubly 13C/15N labeled, led to the unambiguous determination of the number of carbon and nitrogen atoms. This was facilitated by our in-house developed software "MassCompare", which determines the only possible chemical formula for each metabolite. This approach was successfully applied to the detection and identification of 20 of the known metabolites of A. fumigatus, and the identification of one new Fumigaclavine. Moreover, isotopic labeling prevented false metabolite identification due to post-synthesis degradation. Finally, this protocol was applied to F. graminearum, for which only a few secondary metabolites have been described. 30 unknown metabolites could be characterized including 2 new Fusaristatins never described before. Further analyses of wheat spikelets infected in planta with F. graminearum revealed the production of many of these new metabolites in fields.
The method can now be applied to other fungal metabolomes, and new metabolites can now be isolated to perform toxicological studies, offering great possibilities for the discovery of new drugs or toxins.
Novel aspect: specific detection and identification of fungal secondary metabolites by HPLC-HRMS analyses of multi-isotopic enriched samples.

WOS26-04 Deciphering *de novo* induction of novel biomarkers in mycobiome interactions by MS-based metabolomics and microNMR

Jean-Luc Wolfender[1], Samuel Bertrand[1], Jeroen Jansen[2], Nadine Bohni[1], Olivier Schumpp[3], Katia Gindro[3]

[1]School of Pharmaceutical Sciences, EPGL, [2]Radboud Universiteit Nijmegen, [3]Swiss Federal Research Station Agroscope Changins-Wädenswil

Introduction

Plants and fungi possess inducible pathways that are activated in responses to stress that can produce highly bioactive defense compounds upon elicitation. Natural products produced by these pathways need to be identified and investigated further for an evaluation of their biological properties and for a fundamental understanding of the chemical events that trigger various interactions between micoorganisms. In this respect metabolomics represents an ideal approach to highlight biomarker induction in complex microorganism crude extracts.

Methods

In this context the confrontation of various strains of human and plant pathogen fungi have been studied by an MS-based metabolomics approach that take advantages of UHPLC-TOF-MS fingerprinting and high resolution metabolite profiling for an efficient localization of stress-induced biomarkers. New chemometric algorithms that serve at the detection of MS features in complex mixture of fungal metabolomes have been devised. The de novo structure identification of novel stress-induced biomarkers was assured by subsequent LC-MS targeted microisolation and microflow NMR analyses.

Results

Various sampling, MS-based and data mining strategies were compared to highlight at best de novo metabolite stress-induction at the confrontation zone between microorganisms. Comparison of replicates obtained from miniaturized solid media co-culture produced in the 12-well plate format were found to be the best compromise for generating reproducible series of samples for further metabolite profiling. In some of the co-culture studied novel sulfated polyketides were evidenced in others quinonic pigments were highlighted to be strongly induced. No clear correlation between confrontation morphological patterns and metabolite induction could be made. In all cases however significant induction mechanisms were evidenced at the molecular level thanks to the advanced data mining approaches applied. De novo structure identification of the targeted metabolite was be performed by HRMS and micro NMR after careful sample enrichment and chromatographic gradient transfer and MS-directed purification.

Conclusions
The HRMS based metabolomic approach devised enabled an efficient detection of significant metabolite inductions in interacting mycobiomes. The proposed approach is generic and can be applied to other types of microorganisms that can grow on solid media. New metabolites possibly produced though activation of cryptic biosynthetic pathways were highlighted.

Novel Aspect
Exploration of myco- or microbiome interactions will become essential in future to further understand the ecology of such complex microorganism communities that can have impact in both health and chemical ecology issues. Our approach can reveal biomarkers that can be key in such interactions.

Acknowlegments
This work was supported by the Swiss National Science Foundation Sinergia Grant CRSII3 127187, which was awarded to J.-L. W. and K. G.

WOS26-05 GC-MS based metabolite profiling as a means to hybrid performance prediction in winter wheat
Andrea Matros, Jochen Christoph Reif, Yusheng Zhao, Guozheng Liu, Hans-Peter Mock
IPK-Gatersleben

Introduction
The project aims at the development of accurate and robust hybrid prediction methods using a vast genotypic and phenotypic dataset with metabolite profiles of wheat lines and their crosses. Here a mapping population comprising 1604 hybrids and their 135 parental winter wheat lines is used to predict their combining abilities for a number of grain yield- and quality-related traits by means of their genetic fingerprints using the Infinium iSelect 9K wheat array and their metabolite profiles as determined by gas chromatography (GC) combined to mass spectrometry (MS).

Methods
We will present our approach for the untargeted metabolite profiling of selected lines of the mapping population. Field trials have been conducted for all the aforementioned lines. For metabolite profiling ten flag leaves were harvested from three individual environments at three different developmental stages for selected lines, each. Untargeted GC-MS profiling was performed to cover a wide range of substances. Extraction protocols and GC-MS analysis techniques were optimized for wheat flag leaf samples. N-alkane standard was used to adjust retention time shifts, and a mixed reference sample as well as labelled glucose was used to adjust ion intensity shifts during measurements.

Results
Our workflow for data processing, feature extraction, relative quantification, and statistics will be presented. Initial results from biometrical models for prediction of hybrid performance with metabolites as predictor variables will be discussed.

Conclusions
The obtained results demonstrated the usefulness of metabolite fingerprints as a means to predict the phenotypic performance of winter wheat hybrid combinations. The prediction values reached were lower than the ones obtained by using solely the genotypic data set as predictor variable.

Novel Aspects
Metabolite profiling as a new means in quantitative genetics approaches.

WOS27 - Small Molecules – Data Acquisition and Analysis

Room 2
Level 0

Chairs: Thomas Hankemeier, Silvia Catinella

WOS27-01 Keynote: Beyond the elemental composition: computer-assisted identification methods in a high resolution era
Robert Mistrik
HighChem

Introduction
High resolution mass spectrometers, coupled with high- or ultra-performance hyphenated techniques, allow the detection of thousands of compounds in complex samples; however their efficient and reliable identification is still a major bottleneck that is hindering progress in various scientific fields. There is also a growing concern that even those compounds reported as positively identified are in fact incorrect annotations confused either with structural isomers displaying similar fragmentation patterns, or even with structurally unrelated isobaric compounds sharing common elemental composition. Some emerging computer aided annotation programs are likely to contribute to the inaccuracies, since they often apply purely combinatorial bond-breaking logic, although small molecules definitively do not fragment in a uniform manner and often undergo complex rearrangements.

Methods
Despite the growing number of reported "automated" identification methods, the most reliable method for compound identification is still library searching. However, with the advent of high resolution multi-stage spectral libraries, this technique is facing a whole new array of unexpected methodological challenges that will be discussed along with new possibilities to share spectra in a publicly accessible internet cloud (mzCloud).
Even though many reported computer-assisted methods for "de novo" identification did not hold the promise they might have hoped for, there are functional ways that can assist in the identification of a vast number of unknowns, which will be presented. Those methods are based on an understanding of fundamental ion behavior and utilize an extensive database of fragmentation knowledge published in mass spectrometric literature over five decades (Mass Frontier), rather than relying on combinatorial methods or molecular formula calculations.

Additional new developments are exploiting the combination of library searching methods, both spectral and structural, with computational techniques like quantum chemical methods, precursor ion fingerprinting, fragment ion search and others, an overview of which will be given.

Results
A new high resolution database of MS/MS and multi-stage MSn spectra of more than 1,300 compounds has been developed. Spectra and spectral trees were acquired at various collision energies and precursor m/z, using CID and HCD, in both positive and negative mode. Each raw mass spectrum was filtered and recalibrated giving rise to additional filtered and recalibrated spectral trees that are fully searchable. Novel spectra processing and search algorithms, along with high-resolution specific parameters, have been implemented.
If a compound cannot be found in a library, the recently developed precursor ion fingerprinting offers an alternative approach resorting to the apparatus of quasi equilibrium theory, according to which the decomposition pattern of a given ion is determined by its structure, internal energy, and the energy deposited during the activation.

Novel aspects
Chemically-robust methods for identification of compounds using high resolution mass spectrometry

WOS27-02 Data-independent vs data-dependent fragmentation analysis for comprehensive screening of polar organic substances in environmental samples using LC-ESI-Orbitrap

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Introduction
The acquisition of fragmentation spectra is an essential tool for confirmation of target compounds and the most important method to generate structural information of non-target compounds. The data-dependent approach (DDA) is the most common way to generate fragmentation spectra using high-resolution MS, where the masses of interesting parent ions are placed on a predefined list. Advantages of this approach are well-defined, parent ion specific and reproducible fragmentation spectra. But the number of masses for which MSMS-spectra can be acquired is limited and unknown substances are triggered rarely. An alternative approach to the data dependent fragmentation is the data-independent analysis (DIA) where all ions of the full scan or pre-defined ranges of parent ions are transferred to the collision cell. Advantages of this method are that all present ions are fragmented and the fragment ions exactly follow the peak shape of the parent ion. But the precursor ion is not defined and no individual collision energy can be chosen. In order to perform a broad screening for hundreds of targets and non-targets in environmental samples, we evaluated the benefits of the DIA compared to those from DDA.

Methods
The investigations were done using several standard and matrix samples containing up to 400 target compounds with different concentrations and measured on a Thermo Q-Exactive mass spectrometer. The results of an established top 5 data-dependent experiment (1 x full-scan with 140'000 R and 5 x DDA with 17'500 R) were compared with varying data-independent experiments: starting with a full-scan fragmentation (all-ion experiment), the isolation range for the data-independent fragmentation was systematically reduced by introducing step by step 2 to 8 consecutive isolation sections (1 x full-scan with 140'000 R and 1 to 8 x DIA with 17'500 R). The resulting spectra were studied with respect to the number of present fragments, the limits of detection of confirming fragments, matrix effects, interferences and cycle times.

Results
While for the all-ion experiments (isolation window = full scan) the fragmentation was often incomplete with many missing fragments, the results could significantly be improved by splitting the isolation window to several, but smaller sections. Much better fragmentation results could be achieved for a large number of substances and across all matrices. In the various DIA experiments, more isolation windows resulted in an increase of selectivity and sensitivity while interferences of similarly fragmenting substances could be avoided. Finally, with the introduction of just five MSMS fragmentation sections, a similar performance could be achieved compared to DDA spectra for most of the investigated substances

Conclusion
The investigations yielded a comprehensive data-independent method for both targeted and non-targeted substances to be used for routine measurements of environmental samples. Further ideas and improvements to generate comprehensive datasets using the Orbitrap-technology will be discussed.

Novel aspects
Optimization of data independent MSMS fragmentation for Orbitrap using varying isolation windows

WOS27-03 Application of MALDI imaging to analyze glycosyl flavonoids from plant tissue, a method to localize and differentiate isomeric compounds by MS/MS data.

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Introduction
Flavonoids are one of the most important classes of secondary metabolites present in the human diet with nutritional and pharmacological benefits. There is ongoing interest in understanding the main physiological and ecological functions of flavonoids for plants. In addition, the tissue distribution of a specific compound can suggest its functions and it can be propose by MALDI Imaging. This technique is a powerful to investigated accumulation sites for specifics targets.

Recently, it has been applied in plant analyses, but usually by MS data. However, unreliable data can be produced due to nonspecific method (MS data), since plant tissues accumulate isomeric secondary metabolites, such as the flavonoids vicenin-2 and tiliroside in leaves of the Brazilian Arnica or hesperidin and rutin in leaves of citrus. These isomers can only be distinguished based on differences on the fragmentation pathway and diagnostic fragment ions must be recognized and used to produce the images.

Methods
A mix matrix of CHCA and DHB (1:1, 10 mg mL-1) added NaCl solution (0.25 mg mL-1) was applied on tissue by ImagePrep station. Transversal sections from fresh leaves and their petioles were cut at a thickness of 20 µm, which were stuck with double-sided tape to ITO slides for MALDI analysis. Imaging data were obtained from MS and MS/MS in positive and negative (without matrix) ion modes with the parameters: 110 ns PIE, 1000 Hz laser frequency, reflector mode, 500 shots and the ions were accelerated to 19 kV in the LIFT cell for MS/MS analyses.

Results
Recently our group published some initial data of MALDI-MS/MS imaging showing specific compartmentalization of homologous secondary metabolites. The possibility to work with transversal section from leaves give important informations about the plant physiology. For the isomeric mixtures we observed that vicenin-2 was accumulated in the epidermal cells on the adaxial side of leaves of the Brazilian Arnica, producing a top layer. Thus, we clearly demonstrated a specific accumulation of a glycosyl flavonoid on top of the leaf and acting as a chemical UV light barrier. The same protocol was applied to differentiate the accumulation sites of hesperidin and rutin in citrus species. Again the both isomeric structures show the same protonated molecule formula, but the MS/MS data exhibit different fragment ions. Imaging construction-applying MS/MS afforded the real tissue distributions of hesperidin and rutin in leaves and petioles of citrus species. Therefore, the proposition of important fragments ions was performed, which is relevant to construct specific and reliable images.

Conclusion
In summary, this work shows a method to differentiate flavonoid isomers compartimentalization by MALDI-MS/MS imaging. This novel aspect to investigate the gas phase dissociation reactions of isomeric natural products to generate MS/MS information looking to constructed MALDI imaging can be an important strategy to investigate secondary metabolites in complex mixtures as intact leaves and other plant tissues, producing data more reliable of tissue distribution of the compounds.

WOS27-04 Supersonic gas jet shift with respect to the radio-frequency quadrupole axis for increasing efficiency of environmental chemical analysis by mass spectrometry

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Russian Academy of Sciences

One of the basic problems of mass spectrometric analysis of the samples of natural origin is an input of an analyzed gas mixture from area of elevated pressure into a vacuum part of the instrument. Routine practice of sampling includes bulky system of differential pumping and does not provide sufficient sensitivity of the analysis. We have proposed an original way to overcome this difficulty by using the supersonic gas jet through relatively long channel, the free path length of the incoming gas atoms being comparable with radius of the channel [1]. The gas jet directed through the high-performance ion source with electron ionization and radio-frequency quadrupole (RFQ) into time-of-flight mass-

analyzer with orthogonal injection of ions (ortho-TOFMS) increases essentially transmission of the ions. However propagation of this jet strictly along RFQ axis affects sensitivity and leads to detection of only small part of the ions contained in the jet. Diminishing the sensitivity is likely to be due to formation of the ion clusters inside the gas jet of high density at RFQ exit.
For elimination of these difficulties, controllable displacement of the axis of the gas jet with respect to the axis of RFQ at the entrance of the ortho-TOFMS was realized.
The displacement of the gas jet by 2 mm with respect to the RFQ axes at the end of RFQ is shown to increase the ion current detected by a factor of 30 as compared with 1 mm displacement.
For the first time obvious evidence of static accumulation of ions (without pulse switching of electrode potentials) have been obtained at pressure of about 10-4 Torr of residual gas in the quadrupole. Resonant excitation by rotation of accumulated ions around the quadrupole axishas shown not high enough selectivity of the ion elimination: FWHM resolution is estimated to be about 10. Simulated resolution must be essentially higher, i.e. more than 1000 at 10-4 Torr for the residual gas density in quadrupole and that of about 100 for the estimated density of a gas stream near its axis. This result appears to be caused by influence of an electrostatic field of the accumulated ions.
Efficiency of gas jet displacement inside RFQ to obtain high enough ion current capable of recording was demonstrated. Further experiments are necessary for providing optimal conditions of selective ion accumulation and collision induced dissociation in the proposed gas dynamic interface at the entrance of ortho-TOFMS.
Non-uniform gas filling of RFQ ion guide/trap with shifted supersonic gas jet is a new feature of developed system.
The work is carried out in the framework of the Program 9 of Basic researches of Presidium of the Russian Academy of Sciences.
V. V. Raznikov, V. V. Zelenov New way to build a high-performance gas-dynamic interface to produce and transport ions into a mass analyzer. Int. J. Mass Spectrom. 325-327 (2012) 86-94.

WOS27-5 **Comparison of the qTOF and orbitrap configurations for the global metabolomic profiling on the example of the *Pseudomonas aeruginosa* endometabolome.**

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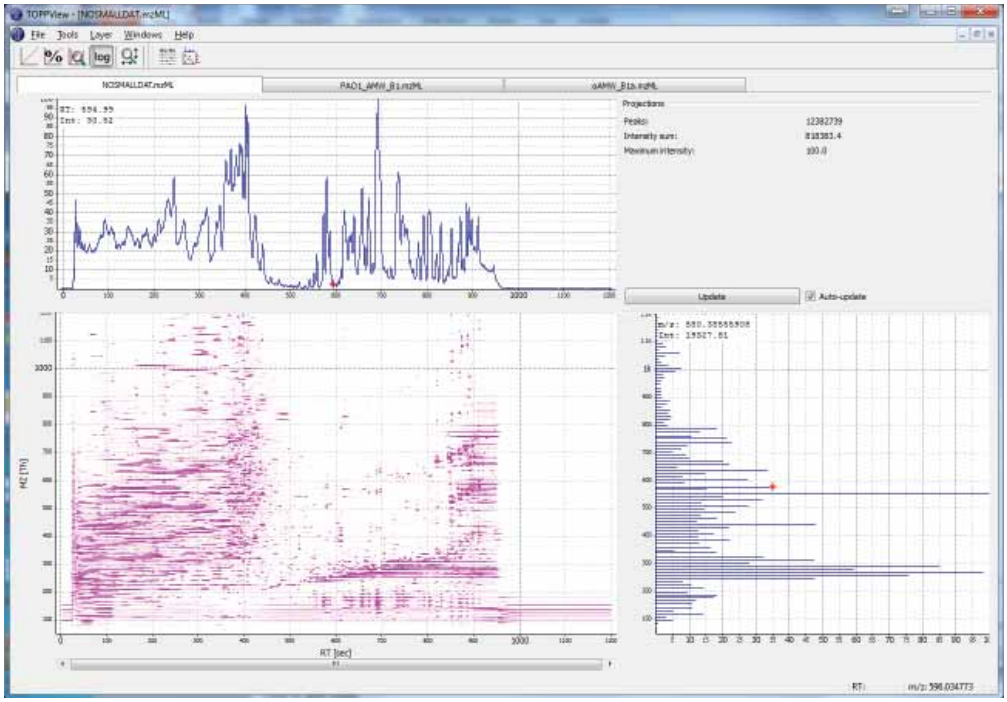
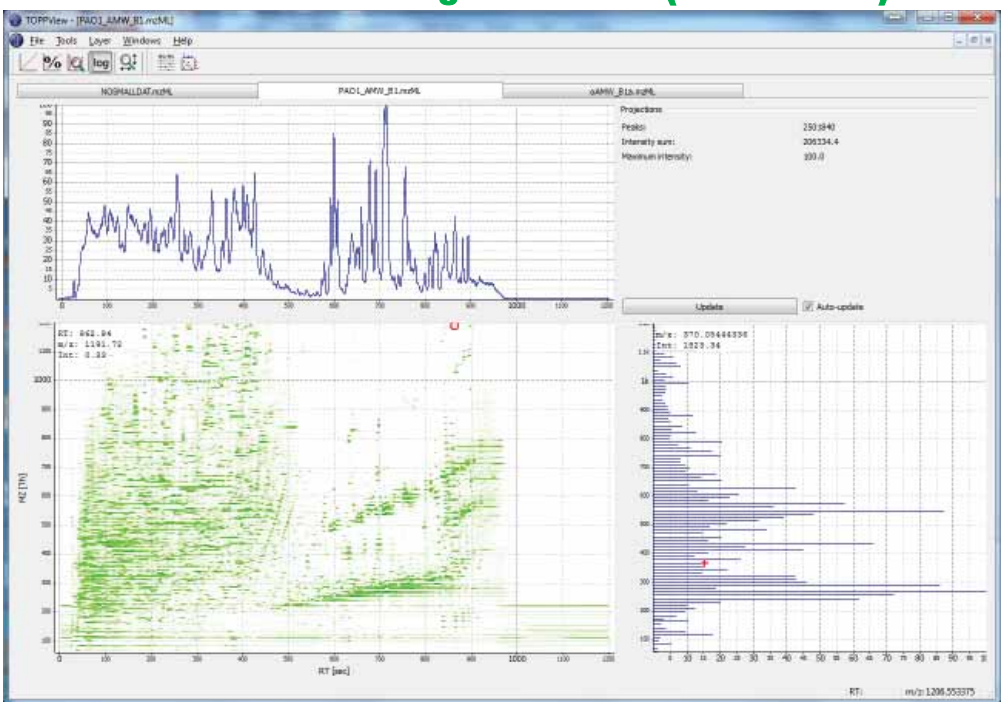
Introduction

Global metabolomics discovery workflows are used as a starting point of any investigation on the samples with unknown metabolite content. Recent advancements in the mass spectrometry technology led to the wide spread application of qTOF and Orbitrap configurations in proteomics, lipidomics and metabolomics. At the same time despite rigorous scientific discussion surrounding application of Orbitrap and qTOF MS in metabolomics profiling there is little data available comparing their performances. Thus we decided to fill this gap and conducted systematic comparison of these 2 configurations in both positive and negative on a serially diluted samples using available Orbitrap and qTOF equipment.

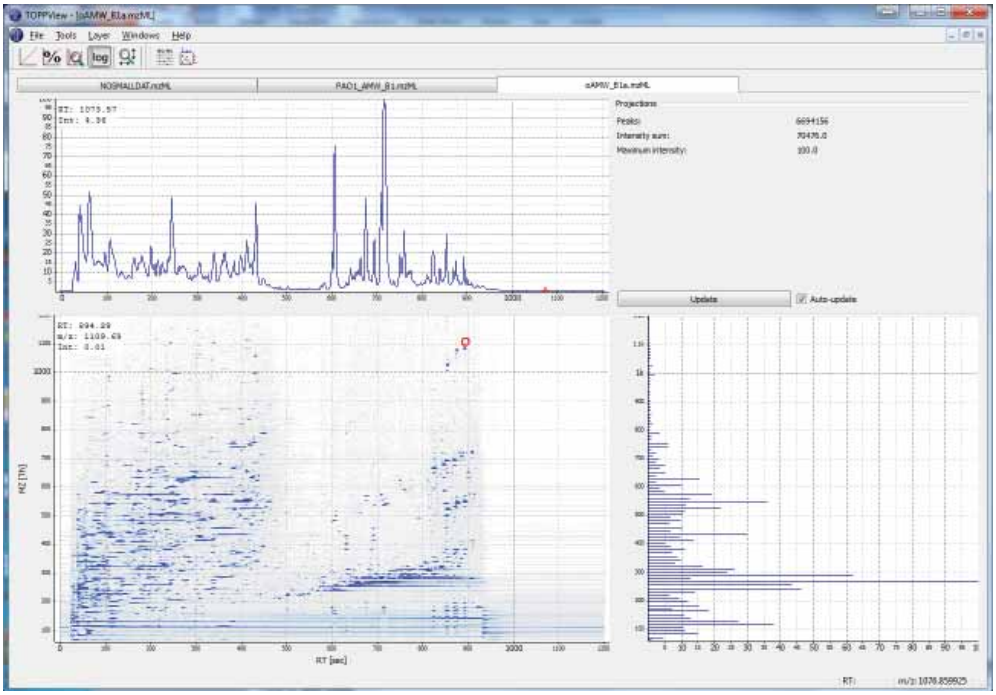
Waters XEVO G2 (PVE mode)



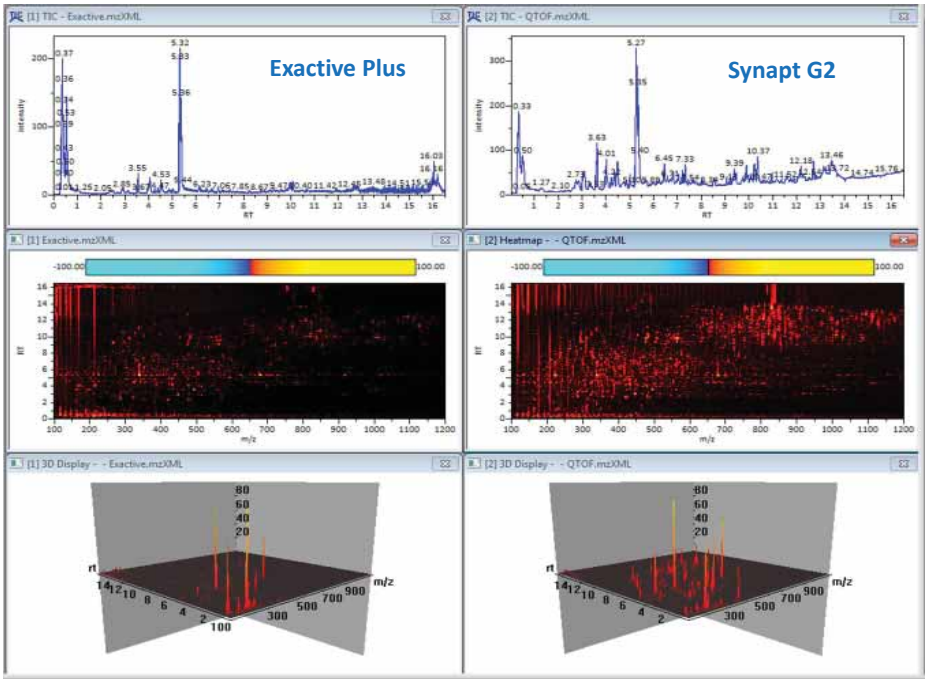
Agilent 6540v(PVE mode)



Orbitrap Velos PRO (PVE MODE)



Exactive Plus vs Synapt G2 (NVE MODE)



Methods

Metabolic extracts from the P. aeruginosa biofilm samples grown in the stationary mode were used as a model system. Metabolites were extracted using MeOH/AcN/H2O mixture, lyophilized and reconstituted in 10 mM Ammonium Acetate buffer and serially diluted four consequtive times. Samples were separated using identical chromatographic conditions (using the same LC column, solvent and gradient) and detected by Agilent 6540, Waters Xevo G2 qTOF and Orbitrap Velos Pro in both positive and negative mode. To avoid any bias in data processing the resulting LC-MS profiles were analyzed by the XCMSonline, mzMine2, and Genedata MSX softwares.

Results

Our data consistently showed distinct differences in the LC-MS profiles produced by the qTOFs and Orbitraps for P.aeruginosa metabolite extracts from biofilm cells. In particular, measurements in both positive and negative mode for serially diluted samples and consequent analysis by all three softwares revealed significantly bigger number of detected, deisotoped and declustered features for both qTOFs in comparison to the Orbitrap Velos Pro. For example HMDB search of the data from the 10 times diluted sample acquired by the Waters Xevo G2 in the positive mode resulted in

the putative assignments of 1031 metabolites, while the same sample acquired by the Orbitrap Velos Pro resulted in 431 putative assignment with 175 overlapping IDs. Re-analysis of the previously published data comparing performance of the Exactive Plus with Synapt G2 in the negative mode on the example of Arabidopsis metabolome confirmed our current observations.

Conclusions
Results of the current evaluation and re-analysis of the previously published data (Glauser et al) indicates on the significant advantage of the use of the qTOF over Orbitrap systems used in this comparison both in terms of the detected features and most importantly their putative identifications. Our data are in accordance with previously published results in proteomics by Cristobal et al (Analyst, 2012,137, 3541) showing almost double amount of unique peptide and protein group identification by the TripleTOF 5600 compared to the Orbitrap Velos for the shorter 23 min gradients.

Novel Aspect
To the best of our knowledge this is the first systematic comparison performance of the qTOFs and Orbitrap Velos Pro systems for global metabolomics profiling of the serially diluted samples detected in both negative and positive mode.

WOS28 - Biomolecular Conformation in the Gas-Phase and in Solution

Room 3
Level 0

Chairs: Lars Konermann, Julia Chamot-Rooke

WOS28-01 Keynote: Protein structure and folding in the gas phase

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University of Innsbruck

Introduction
Protein structure and folding in the complete absence of solvent has recently become a new focus of gas phase ion chemistry research for two major reasons. First, while it is known that native protein folds are determined by the sequential arrangement of the constituent amino acid residues, the delicate balance between external factors and intrinsic determinants of protein structure, stability, and folding is only poorly understood. Second, while native mass spectrometry, in which proteins and their assemblies are electrosprayed from non-denaturing solutions, is increasingly being used for biostructural characterization, the extent to which solution structure is retained in the gas phase remains unclear. From this perspective, the following research questions present itself, 1) What is the intrinsic stability of a native protein fold? 2) Under what conditions, for how long, and to what extent can solution structure be preserved in the gas phase? 3) Can isolated proteins fold to form native structures in the complete absence of solvent?

Methods
For probing of gaseous protein ion structures, and monitoring kinetic folding profiles, we used electron capture dissociation (ECD) and native ECD (NECD) on a 7 T Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with an electrospray ionization (ESI) source and a hollow dispenser cathode for ECD.

Results
ECD and NECD of horse and tuna heart Cytochromes c showed that their native folds spontaneously disintegrate on a timescale of 400 ms after desolvation. Consistent with its smaller number of possible salt bridges, tuna heart Cytochrome c unfolds faster than horse heart Cytochrome c. For both proteins, folding in the gas phase after extensive structural annihilation is far slower than folding in solution, and shows strikingly different kinetic profiles, despite their almost identical native folds. Gas phase folding of the three-helix bundle protein KIX, whose native fold can be preserved after transfer into the gas phase for >4 s, proceeds on a similar timescale, but surprisingly, is faster for more highly charged ions.

Conclusions
Once a native protein fold is lost after desolvation, folding in the gas phase can produce more compact structures, but these bear no or little resemblance to the original fold. Fast folding, on a ms to ms timescale, or even the formation of structural elements involving ~10 or more residues neighboring in sequence, is highly unlikely in the absence of hydrophobic interactions. Salt bridges can stabilize native folds after transfer into the gas phase, and their formation appears to be the driving force for protein folding in the absence of solvent.

Novel Aspect
Salt bridges play a major role in both stabilizing protein solution structure after transfer into the gas phase and as a major driving force in protein folding.

WOS28-02 Discovering a new subunit for an old complex by native mass spectrometry

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Weizmann Institute of Science

Introduction
The COP9 signalosome (CSN) is a multi-subunit complex involved in an array of biological process due to its role in regulating the ubiquitin/26S proteasome pathway. The CSN is conserved throughout evolution, from fungi to humans. In higher organisms, the complex is composed of 8 subunits, termed Csn1–Csn8. We recently discovered, by combining two MS based methods that involve the analyses of both the individual subunits and the intact complex, that the Myeloma-Overexpressed Gene 2 (Myeov2) protein, is the ninth subunit of the CSN complex.

Methods
We have isolated the endogenous CSN complex from human erythrocytes and analyzed its composition by an approach we recently developed to expose the subunit variability of protein complexes. Our method relies on denaturing the protein complex, and separating its constituent subunits using a monolithic column prepared in-house on a nanoUPLC system. Following elution from the column, the flow is split into two fractions, using a Triversa NanoMate robot. One fraction is directed straight into an on-line ESI-QToF mass spectrometer for intact protein mass measurements, while the rest of the flow is fractionated into a 96-well plate for subsequent proteomic analysis. The composition and heterogeneity of subunit composition is then exposed by correlating the subunit sequence identity with the accurate mass. By applying a single-step affinity-purification approach, we characterized in a similar manner the CSN complex isolated directly from HeLa cells. In addition, the composition and stability of both the erythrocyte and cell line derived CSN complexes were characterized on a QTOF instrument modified for high mass measurements under conditions that allow maintaining non-covalent complexes intact.

Results
Results obtained from the nanoUPLC coupled MS set-up indicated that in addition to the known consistent subunits of the CSN complex (CSN1-8), a small 6.2 kDa polypeptide, identified as Myeov2, repeatedly co-purified with the complex. Myeov2 was also identified in proteomic analysis of both a cross-linked complex and an extracted band from native-PAGE separation, leading us to propose that Myeov2 is an integral part of the CSN complex. Native mass spectrometry analysis of the endogenous CSN indicated that it is extremely stable in comparison to a recombinant complex lacking the Myeov2 protein, suggesting that this protein contribute to the stability of the CSN.

Conclusions
Our MS based results strongly suggest that the 6.2 kDa Myeov2 is and integral subunit of the CSN complex. These observations were than supported by FRAP experiments as well as co-immunoprecipitation and genetic manipulation cellular assays. Intrestingly, although a 25 kDa isoform of Myeov2 exists, unlike its smaller counterpart it does not appear to be part of the CSN complex.

Novel Aspect
MS analysis enabled us to identify a new subunit for the highly characterized CSN complex, which despite more than two decades of research escaped previous identification.

WOS28-03 Stability of the B2B3-beta crystallin heterodimer to increased oxidation by radical probe and ion mobility mass spectrometry

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[1]University of Sydney, [2]Yokohama City University, [3]University of New South Wales

Introduction
Crystallins are water soluble proteins in the lens of the eye of humans and other vertebrates that play a vital role in helping it to retain its transparency important to vision. They possess different N and C-terminal extensions that have been implicated in promoting self-association and interactions with other crystallins. These complexes help the subunit proteins to shield themselves from oxidative degradation and damage when exposed to reactive oxygen species. Long-term oxidative damage leads to the development of cataract, a leading cause of blindness worldwide.
β-crystallins have also been associated with the development of human cataract. Two basic β-crystallins, so-called βB2 and βB3, have been shown to associate and form a heterodimer whose structure has been probed by radical probe and ion mobility mass spectrometry. Here these methods are employed to study the stability of that structure following increased exposure to hydroxyl radicals.

Methods
Solutions of βL-crystallin in ammonium acetate and bicarbonate buffer containing the βB2B2-heterodimer were exposed to hydroxyl radicals over increased timescales by means of adjusting the flow rate of the solution as it passes through an electrospray discharge source. The average collision cross section of the oxidized heterodimer at increasing exposures was studied by ion mobility mass spectrometry and the levels of oxidation within peptide segments at each condition was also measured after proteolysis.

Results
The results demonstrate that the heterodimer can withstand limited oxidation through the incorporation of an average of some 6 oxygen atoms per subunit protein without any appreciable change to its average collision cross section and thus conformation. These results are in accord with the oxidation levels and timescales applicable to radical probe mass spectrometry (RP-MS) based protein footprinting experiments. Following prolonged exposure, the heterodimer is increasingly degraded through cleavage of the backbone of the subunit crystallins rather than denaturation such that heterodimeric structures with altered conformations and ion mobilities were not detected. However, evidence from measurements of oxidation levels within peptide segments, suggest the presence of some aggregated structure involving C-terminal domain segments of βB3 crystallin across residues 115-126 and 152-166.

Conclusions
Increased exposure of the βB2B2-heterodimer results in oxidative damage primarily through backbone cleavage over structural denaturation. Evidence for an aggregated structure involving part of the C-terminus of βB3-crystallin has been found through analysis of oxidation levels at the local level.

Novel Aspect
First application of Ion Mobility in concert with Radical Probe and Ion Mobility Mass Spectrometry to study oxidative damage to proteins and its impact on the structure of their complexes.

WOS28-04 Investigating the effects of ligands on nucleic acid structure and dynamics by IMS-based approaches

Dan Fabris
The RNA Institute, University at Albany

Introduction

The new classes of functional RNAs discovered in recent years are rapidly revolutionizing our understanding of non-protein coding sequences –the vast majority of the human genome. The observation that their function depends in large part on their ability to interact with other cellular components has keenly asserted the need for effective approaches to investigate the effects of ligands on structure and dynamics. For this reason, we are developing concerted strategies based on ion mobility spectrometry (IMS) to assess the impact of binding interactions on nucleic acid conformation.

Methods

All materials were purchased from commercial sources, or prepared according to standard molecular biology procedures. Nucleic acid constructs were extensively desalted by ultrafiltration or ethanol precipitation. Typical 5 mL samples containing 0.5 mM of analyte in 150 mM ammonium acetate and 10% 2-propanol were analyzed by nanospray ionization in negative ion mode. All MS determinations were carried out on a Waters (Milford, MA) Synapt G2 HDMS IMS, which was modified by adding a heated-tube element to minimize the desolvation energy.

Results

We assessed the potential of IMS in the investigation of structure/dynamics of nucleic acids by considering a broad range of ligands, including different cations, drug-like molecules, proteins, and antisense oligonucleotides. A direct comparison between systems that contained structure-defining versus catalytic Mg2+ demonstrated the capacity of detecting conformational changes associated with the creation of specific coordination sites and differentiating their characteristic signatures from the effects of non-specific binding and ionic strength. The ability of intercalators to induce helix unwinding was investigated by matching experimental data with predictions obtained from ad hoc topology modeling. Complex RNA systems, such as the HIV-1 packaging signal and the 5'-untranslated region (5'-UTR) were employed to study the structural rearrangements induced by the viral nucleocapsid (NC) protein –a well-known nucleic acid chaperone– and a tiled series of antisense oligonucleotides designed to cover the entire RNA sequence. The results have shown that IMS determinations can detect the conformational changes associated with the elimination of long-range pairing interactions that define the global fold of large RNAs. This outcome suggested also a possible new mechanism of action for antisense therapeutics, which may not involve degradation of target sequences, but rely instead on conformational blockage of catalytic and riboswitch regulatory elements.

Conclusions

This work has demonstrated excellent correlations between experimental results and expected effects of ligand binding on nucleic acid conformation. This observation supports the enormous potential of IMS in the investigation of structure/dynamics and the development of new classes of nucleic acid therapeutics.

Novel aspect

Discrimination of coordinated vs. diffuse metal binding; helix topology determinations; detection of antisense-induced conformational changes.

WOS28-05 Conformational dynamics of cellobiose dehydrogenase probed by structural mass spectrometry

Alan Kadek[1], Roland Ludwig[2], Petr Halada[1], Petr Man[1]
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Introduction

Cellobiose dehydrogenase (CDH) is an enzyme involved in the lignocellulose degradation. Being the only currently known extracellular flavocytochrome, CDH is unique from the point of view of its molecular architecture. It is a monomeric glycoprotein consisting of two domains connected by a flexible linker. The combination of a haem with an FAD molecule within a single protein gives CDH the ability to exchange electrons with a variety of protein partners, thus making it attractive for research in the fields of biocatalysis and biosensors. To date, however, only the structures of the two separate domains have been solved and the structure-function relationship for the whole protein remains undescribed. Therefore we use structural mass spectrometry methods to shed light on the structural organization as well as on the dynamics of the whole CDH protein in solution.

Methods

CDH from Myriococcum thermophilum was produced in yeast and purified to homogeneity. Characterization of its post-translational modifications was done using specific enzymatic digestions and subsequent MS/MS measurements of the resulting peptides. Conformational dynamics of the CDH under various conditions was then studied by hydrogen/deuterium mass spectrometry (HXMS). Native electrospray ionization coupled with ion mobility (IMMS) was used to study the global conformation of the intact CDH.

Results

In the present study we focused on the pH-induced conformational changes within the CDH molecule, as the protonation is deemed to be crucial for the regulation of interdomain contacting and thus for the function of the enzyme. We performed experiments at pH 5.5 (active state) and pH 7.5 (inactive state). Native ESI with IMMS have shown that, although the global changes are not very extensive and the system as a whole is highly dynamic, at the higher pH the conformation of CDH is more open, which results in the loss of enzymatic activity.
We optimized HXMS experimental conditions, which due to the CDH's disulfides and compact fold require 3M guanidine / 0.5M TCEP for complete digestion. HXMS of CDH performed at the two pH values have shown protein backbone de-protection for CDH in the active state. These changes were located on both the domains on the edge of their interaction interface proposed by molecular modeling.

Conclusions
Our findings have shown both global and local conformational changes in the CDH molecule depending on the pH of the solution, which seem to be consistent with our working theory of the CDH functioning. Experiments currently in progress are aimed at uncovering whether the local conformational changes of CDH are induced by the domain-domain interaction or if they are primarily caused by pH and thus facilitate the domain-domain interaction.

Novel aspect
Utilization of a combination of structural MS techniques to study interdomain conformational dynamics of a highly flexible protein.

WOS29 - Ambient Ionization and Miniaturization

Room 4
Level 0

Chairs: Zheng Ouyang, Renato Zenobi

WOS29-01 **Keynote: Ambient MS in motion: 3D robotic sampling, dynamic ionization, and microplasmas**

Facundo Fernandez[1], Rachel Bennett Bennett[1], Ezequiel Morzan[2], Jacob Huckaby[1], Maria Eugenia Monge[3], Rosana Alberici[4], Prabha Dwivedi Dwivedi[1], Joel Keelor[1], Martin Paine[1], Joshua Symonds[1], Thomas Orlando[1], Henrick Christensen[1]
[1]Georgia Institute of Technology, [2]Universidad de Buenos Aires, [3]CIBION-CONICET, [4]Thomson Mass Spectrometry Laboratory, UNICAMP

Introduction
In this presentation we showcase several examples of on-going efforts where we exploit the unique characteristics

of ambient mass spectrometric analysis that enable open air, direct ionization of target analytes on native surfaces and complex 3D objects. In contrast to «classical» ambient analysis which is typically performed in a static fashion (i.e.placing the sample at a fixed location respect to the ion source), we demonstrate a «dynamic sampling» procedure based on programing a dynamic probe trajectory in the gap between the ion source outlet and the mass spectrometer inlet, thus exploiting the thermal and flow velocity gradients that exist in this region in space. We also present a 3D surface sampling approach that combines features of both DART and PESI for probing non-planar surfaces through a machine vision system commanding a robotic arm. A new generation of miniaturized plasma ion sources that can be used for low-power ionization in field applicationsviaeither proton transfer or VUV photoionization mechanisms is also presented.

Methods
A quadrupole-time-of-flight mass spectrometer (microTOF Q-II, Bruker) equipped with a gas-ion separator tube interface (GIST, IonSense); a single stage time-of-flight mass spectrometer (AccuTOF, JEOL); an Orbitrap mass spectrometer (Exactive Plus, Thermo); and a triple quadrupole mass spectrometer (G6410AA, Agilent) were used. Microplasmas were built in-house either through a microfabrication procedure or a metal-insulator-metal automated approach. Robotic Plasma Probe Ionization (ROPPI) experiments were conducted by coupling a DART ion source (IonSense) to a home built PESI-type sampling device mounted on two different robotic arms: (a) a KUKA KR5 sixx R650 robot or (b) a Universal Robots UR5 six axis arm.

Results
Dynamic sampling, when coupled to ambient plasma ion sources, was shown to enable differential analyte desorption. This generated a transient characteristic profile for each target compound. By comparing the profiles of ion pairs, assignments could be made between precursor and fragment ions without the need for an ion isolation step. Three dimensional objects, such as model patterned spheres, produce, and clothing were effectively investigated with ROPPI MS, leading to the first automated ambient surface analysis tool for non-planar samples. Finally, experiments with various microplasmas operated in both AC and DC modes coupled to a new type of electrothermal vaporizer and operated with various discharge gases were carried out for the detection of contaminants of importance during manned space missions. The obtained detection limits met or were lower than those required for ensuring the safety of the space crew.

Conclusions
Ambient MS has matured but not plateaued, with many still-unmet challenges that can gain from the same characteristics that have made this field exciting from the very beginning.

Novel Aspect
Ambient MS is used for probing 3D surfaces in an automated mode and in a dynamic sample introduction mode. Miniature plasma ambient ion sources are also presented.

WOS29-02 **Reactions in DART source and analysis examples - mechanism study**

Shuying Liu[1], Hongmei Yang[1], Yang Wang[2], Qing Yu[3]
[1]Changchun Institute of Applied Chemistry, [2]Changchun University of Chinese Medicine, [3]Wenzhou Medical University

Introduction
Direct Analysis in Real Time (DART) refers to an atmospheric-pressure ion source, which is based on the interaction of long-lived excited-state neutral atoms or molecules with analytes and atmospheric gases. The DART ion source is operated by exposing the samples to a dry gas stream. However, the ionization mechanism of DART ion source is rather complicated. It is assumed that some complex processes depend on the proton affinity, gas phase acidity and the ionization energy of an analyte as well as the DART gas used and the presence of the make-up solvents.

Methods
Ar and He were used as DART gas to compare the different cases, and suitable make-up solvents were used to enhance the analysis sensitivity. DART ion source coupled with quadrupole time-of-flight mass spectrometry was applied to investigate the different types of saccharides in order to get high resolution MS data and element composition for the ion interested. Analysis of saccharide and glycoside were performed using in situ derivatization DART-MS.

Results
1. Ar-DART and He-DART analysis of labile compounds
Helium DART-MS analysis of labile compounds usually generates prominent fragmentation. Argon DART-MS yields significantly less energetic ions than conventional He-DART and is able to produce the intact molecular ions with little or no fragmentation. Intentionally doping a makeup solvent at the exit of the DART ion source can result in 1-2 orders of magnitude increase in detection signals.
2. TMAH Assisted DART-MS Analysis of glycosides and oligosaccharides
Glycoside and oligosaccharide were difficult to be ionized directly by DART due to their low volatility and proton affinity. Tetramethylammonium hydroxide (TMAH) was used to assist the ionization of analytes, and the signals of methylated analytes were detected. The procedure is easy to handle, and spectra can provide structural information in DART-MS.
3. Analysis of Saccharides by He-DART
Gas temperature had a significant effect on signal intensity in DART spectra, which is related to thermo-desorption of the analyte from sample surface. With the increase of a sugar chain, a higher gas temperature was needed for saccharide ionization. Non-covalent dimers and cross-ring cleavages were detected in the spectra of glucose and eight glucose-containing disaccharide isomers. Based on the mass spectra, disaccharide isomers with different linkage positions can be differentiated in both positive and negative ion mode.

Novel Aspects
1.The coupling of Ar-DART with makeup solvents provides a powerful tool to analyze the labile analytes.
2.Rapid DART-based determination of saponins is facilitated using in situ derivazation.
3.DART-MS provides us a new approach to discriminate the native disaccharide isomers.

WOS29-03 Halo-shaped flowing atmospheric pressure afterglow for ambient desorption/ionization mass spectrometry

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Introduction
Ambient desorption/ionization mass spectrometry (ADI-MS) has become an important addition to the analytical repertoire since its inception in 2004 with DESI and the subsequent commercial availability of the DART source in 2005. The primary attraction of ADI-MS is the avoidance of most, if not all, sample pretreatment, which allows rapid analysis of samples while retaining the sensitivity and specificity of mass spectrometry. However, analysis of some samples can benefit from coupling to sample-introduction methods such as laser ablation, which offers superior spatial resolution for solid samples. Here, a novel geometry of the flowing atmospheric pressure afterglow (FAPA) source, termed the halo-FAPA, is shown capable of ambient analysis, as well as facile coupling to a wide variety of sample-introduction techniques.

Methods
The h-FAPA construction, shown in Figure 1a and 1b, consists of a stainless-steel inner capillary (1.59 mm O.D., 1.00 mm I.D.) inside an outer capillary (3.18 mm O.D. 2.67 mm I.D). A thin ceramic insulating sleeve (2.20 mm O.D., 1.59 mm I.D.) surrounds the inner capillary, leaving a 1-2 mm gap for the discharge to form. Two separate helium flows were utilized, 0.6 L/min through the outer capillary and 0.3 L/min through the inner capillary. Negative 300 volts was applied to the inner capillary and current was limited to 30 mA, which was sufficient to produce a full toroidal plasma. Several sample introduction techniques were explored, including laser ablation, sample nebulization, droplet generation and head space sampling.

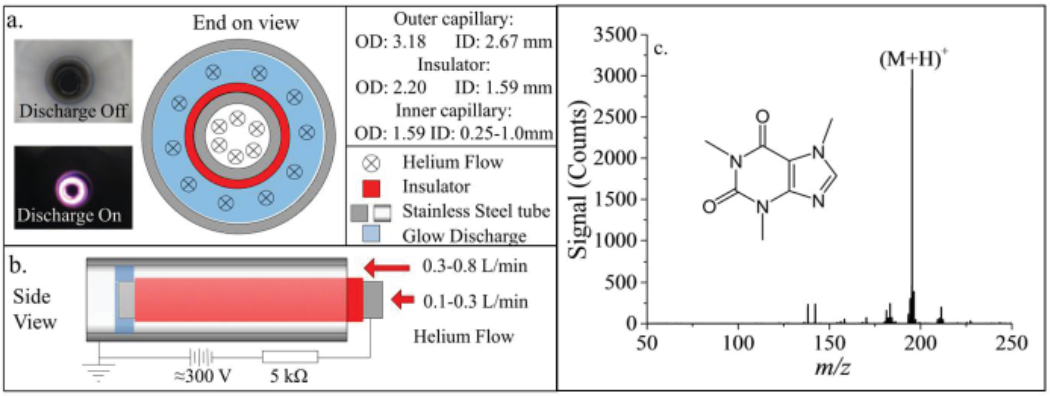

Figure 1. Schematic diagram of the h-FAPA: (a) end-on view and (b) side-on view, showing typical operating characteristics. c) Mass spectrum from a single laser-ablation event of caffeine deposited on a glass slide; approximately 25 ng of material were removed.

Results:
Direct comparisons to our conventional pin-to-capillary FAPA revealed an increase in sensitivity for ambient analysis, as well as droplet introduction. Laser ablation demonstrated the ability of the h-FAPA to desorb and ionize analyte from an aerosol. Figure 1c shows the mass spectrum from a single laser shot of caffeine deposited on a glass slide. This spectrum represents approximately 25 ng of caffeine and demonstrates the excellent sensitivity of the source. Several applications and fundamentals of a novel geometry are explored where the inner capillary is replaced with the inlet for a mass spectrometer.

Conclusions
A novel concentric-geometry atmospheric-pressure glow discharge was successfully used for ambient mass spectrometry and easily coupled with a variety of sample introduction techniques. Sensitivity was improved over previous designs of the FAPA source and a new sampling geometry improved ambient analysis reproducibility.

Novel Aspect
A novel geometry atmospheric-pressure glow discharge (APGD) is used for ambient mass spectrometry and several sample introduction methods are explored; a unique geometry is introduced in which the central channel is replaced with the capillary inlet of a mass spectrometer; this arrangement provides geometry-independent analysis.

WOS29-04 Progress on pocket mass spectrometer development

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Korea Basic Science Institute

Field portable mass spectrometer in a pocket size is of very interest especially in the area of military use, nuclear safeguards inspection, and space exploration, etc. However, a portable mass spectrometer for military use requires very low detection limit of trace chemical agents in the air in ppb range, while the required capability for nuclear safeguards mass spectrometer is very high mass resolution enough for verification of uranium isotope ratio in the field. Another requirement for space exploration is that a mass spectrometer must be able to survive in a plasma environment such as sprite or elves discharge.
For detection of chemical agents in the air in the ppb range, the target sample gases should be separated from the abundant molecules such as nitrogen, oxygen, water, and carbon dioxide. Precisely controlled swing of adsorption/desorption with pulsed sampling directly in the field is intriguing. A high mass resolution in the mass range of UF6 requires an accurate and stable RF ramp voltage. For survival of an electronic device in a plasma environment or in a glow discharge pressure range such as mesosphere or Martian surface, any of the operation voltages may not exceed the gaseous breakdown voltage. Very low voltage operation of an ion trap mass spectrometer with multiple frequencies would be one of the solutions. A recently produced digital-digital synthesizer chip enables one to instantly switch the frequency to half, keeping the ramp voltage low enough to avoids gaseous discharge breakdown. Various efforts for the development of a pocket mass spectrometer in this laboratory will be discussed.

WOS29-05 Miniaturised laser-based mass spectrometer for *in situ* investigation of planetary bodies

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University of Bern

Introduction
For many scientific questions in planetary science knowledge of the chemical and isotopic composition are important information since planetary formation and evolution leave a signature in the composition. Therefore, development of sensitive mass spectrometers for in situ investigation of the elemental and isotopic composition of planetary surface material are of high interest to current space research, for the selection of suitable samples to be brought to Earth by a return spacecraft, as well as for in situ scientific investigation.

Methods
The miniaturised laser-mass spectrometer (LMS) size is about 120 mm x Ø 60 mm (height x diameter). It uses laser ablation to vaporise, atomise and ionise solid samples and a time-of-flight (TOF) mass spectrometer (MS). Femto-second (fs) laser pulses are focused to a spot of 5–10 µm to remove and ionise surface material. The produced ions enter into mass analyser where they are dispersed according to their mass to charge ratio in the TOF-MS and detected by a fast multi-anode ion detector. Sensitive and accurate element and isotope measurement can be performed within 5–50 seconds with the detection of almost all elements (and their isotopes) with concentrations down to 10 ppb. By probing many locations on the sample, chemical mapping of a surface is also possible.

Results
The choice of laser parameters e.g., pulse duration, pulse energy, geometric shape, and wavelength, and type of mass spectrometer is critical to achieve optimal conditions for stoichiometric production of ions from the sample. With the help of a number of calibration standards we established relative sensitivity factors (RSC). Our RSCs are close to unity indicating that efficiency of ion generation in ablation process and its detection are optimal for quantitative elemental analysis. Almost every element is detected by LMS, with an abundance down to 10 ppb. The LMS mass resolution is in the range of 600–1500, which is not sufficient to resolve isobaric interferences, but allows for measuring well-resolved mass spectra of elements and their isotopes. In isotope analyses a typical accuracy and precision at ‰-level is achieved for concentration larger than 50 ppm. For example, 207Pb/206Pb ages with an accuracy in the range of tens of millions of years can be achieved for representative minerals.

Conclusions
The miniaturised LMS with the fs-laser ablation ion source is very promising for in situ elemental and isotopic analysis in space research. In particular, the fs laser allows for the standard-less LMS operation.

Novel Aspects
The development of LMS reached a level where its performance is comparable to established laboratory instruments (e.g., TIMS, LA-ICP-MS, and SIMS), yet at a size compatible with employing it on a spacecraft landing on a planetary body. One of the major contributing factors is the use of the fs-laser for ablation and ionisation.

WOS30 - Geology, Astrophysics and Space Exploration

Room 5
Level 3

Chairs: Roland Thissen, Detlef Günther

WOS30-01 Keynote: High precision mass spectrometry in a cometary coma: first results from the Churyumov Gerasimenko comet nucleus exploration

Kathrin Altwegg[1], Urs Mall[2], Björn Fiethe[3], Johan deKeyser[4], Jean-Jacques Berthelier[5], Henri Rème[6], Steve Fuselier[7], Tamas Gombosi[8], Peter Wurz[1], Martin Rubin[1]
[1]University of Bern, [2]MPS, [3]TUB, [4]BIRA, [5]LATMOS, [6]IRAP, [7]SwRI, [8]University of Michigan

In situ mass spectrometry in space has its own challenges. Not only has the mass spectrometer to be lightweight and energy efficient, but it also has to withstand a broad temperature range, high vibration levels during launch and a wide variety of pressures. Autonomous operation over long times, immunity to cosmic rays and high compression of data are other prerequisites for successful instruments on board spacecraft. The science goals for each mission are unique; the targets of the missions are much diversified, ranging from solar wind ions with high energies to the terrestrial ionosphere with neutrals, negative and positive ions and to planetary atmospheres with neutral densities well below 106 cm-3. It is clear that this range of species, densities and energies cannot be covered with one single type of mass spectrometers. The probably most advanced instrument currently flying aboard a spacecraft is the ROSINA (Rosetta Sensor for Ion and Neutral Analysis) instrument on board the ESA Rosetta spacecraft currently encountering comet 67P/Churyumov-Gerasimenko. Comet 67P/C_G is a short period comet and a size of roughly 4 km in diameter. On its way around the Sun the outgassing rate will increase by several orders of magnitude up to 1027 s-1. Rosetta will follow the comet from almost 4 AU through its perihelion at 1.3 AU and out again for more than 1 ½ years. The closest distances of just a few kilometers to the comet will be reached during the delivery of the lander Philae in November. Rosetta will, at the time of the IMSC, be less than 100 km from the cometary surface, but still outside of 3 AU from the Sun. It's about the time when the cometary atmosphere should be denser than the spacecraft background. The cometary atmosphere consists mostly of water and CO/CO2. However, it is known that comets have quite a diversified organic part in their coma, both as volatiles and as dust. Furthermore, isotopic ratios in water and other molecules can give very strong indications on the formation process of cometary, and therefore solar system material.
ROSINA actually consists of two mass spectrometers: RTOF (reflectron time of flight), a time of flight instrument and DFMS (double focusing mass spectrometer), a classical instrument in a Herzog-Matauch configuration. Both sensors have their own strengths and limits. However the suite of instruments, including the cometary pressure sensor (COPS) is very well adapted to the task ahead: namely a thorough investigation of the elemental, isotopic and molecular abundance of the volatile part of the cometary coma and its evolution along the orbit of the comet around the Sun.
In this talk we will give a short overview on mass spectrometry in space and present the ROSINA instruments, the challenges and the results so far.

WOS30-02 Formation of negative ions from "water group" positive ions at high collision energies: implications for the ionosphere of Titan.

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Introduction
The Saturnian magnetosphere is dominated by neutrals originating from the icy satellites and ring system. These neutrals are ionised in the inner and middle magnetosphere producing a plasma composed of protons, H2+, and so-called "water group" ions O+, HO+, H2O+ and H3O+. [1] The plasma is quite rapid, varying from ~ 30 km s−1 near Enceladus at 4 RS to 170 km s−1 near Titan at 20 RS. [1] A precipitation of O+ ions with energies of 1-4 keV was found at Titan by the Cassini CAPS spectrometer.[2] It has been speculated [3] that a few percent of O+ flux is converted to O− ions that may play a role in the Titan's negative ion chemistry, which was found unexpectedly rich, [4] but is still rather poorly understood [5].
The objective of this study was to measure the cross-sections for the formation of O− and HO− ions by charge reversal processes of O+, HO+, H2O+ and H3O+ ions in collisions with major constituents of Titan's atmosphere.

Methods
The experiments have been done on the modified ZAB2-SEQ mass spectrometer consisting of magnetic (B) and electrostatic (E) analysers. The ions were accelerated to 8 keV and mass-selected by B. The electron transfer processes occurred in the collision cell located between B and E. The negative ions formed in the cell were analyzed by E.

Results
In case of HO+, H2O+ and H3O+ ions, only the O− and HO− were detected. The cross-section values decrease for the studied targets in the order C2H6 ~ C2H4 > C2H2 ~ CH4 > N2 > H2. It follows from our results that formation of O− and HO− mostly takes place sequentially in two consecutive single electron transfer collisions and that the total relative cross sections correlate with EI cross sections. Differences in the [O−]/[HO−] ratios were observed for the different targets.

Conclusion
Our results show that the oxygen containing negative ions as well as the neutral species can be formed by the high-energy collisions of positive "water group" ions impacting the Titan's upper atmosphere. These species may interact with the components of the Titans atmosphere in many ways and can, for example, initiate numerous ion/neutral-, neutral/neutral-, and collision-induced unimolecular reactions.

Acknowledgements
This work was supported by the Czech Science Foundation (project 14-20915S).

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WOS30-03 ***In situ* exploration of solar system bodies: the potentiality of an orbitrap based mass analyser**

Christelle Briois[1], Laurent Thirkell[1], Roland Thissen[2], Alexander Makarov[3]
[1]LPC2E, [2]IPAG, [3]Thermo Fisher Scientific (Bremen) GmbH

Mass spectrometry is a powerful analytical technique that has been employed for years in space exploration to characterise the chemical composition of solar system bodies and their environment. Space exploration is dealing more and more with astrobiology, with a particular interest in environments that could bring information about prebiotic chemistry. Because of the harsh constraints imposed on the space probe instruments, their mass resolution is quite limited compared to laboratory instruments, sometimes leading to significant limitations in the treatment of the data collected with this type of instrumentation. The highest mass-resolving power (m/Dm) currently achieved in space is about 9,000 FWHM (Full Width at Half Maximum) at m/z = 28 with the DFMS (Double-Focusing Mass Spectrometer) of ROSINA (Rosetta Orbiter Spectrometer for Ion and Neutral Analysis) experiment onboard the current Rosetta cometary mission, dedicated to gas analyses of the comet 67P/Churyumov-Gerasimenko [1]. As m/Dm increases, several new plateaus of chemical information become accessible. Therefore, future in situ solar system exploration missions would significantly benefit from High Resolution Mass Spectrometry (HRMS).
Over the past decade, laboratory mass spectrometry has been revolutionized by the emergence of ultra-high mass-resolving power instruments. In 2000, a new concept avoiding magnetic or RF field was developed with Fourier Transform Mass Spectrometry (FT-MS): the FT Orbitrap MS [2,3]. This technique is envisaged for developing new-generation space instruments.
Since 2009, 5 French laboratories (LPC2E, LATMOS, LISA, IPAG, CSNSM) involved in the chemical investigation of solar system bodies formed a Consortium to develop HRMS for future space exploration, based on the use of the Orbitrap technology [4]. This development is carried out in the framework of a Research and Technology (R&T) development programme partly funded by the French Space Agency (CNES). The work is undertaken in close collaboration with the Thermo Fisher Scientific Company, which commercialises Orbitrap based laboratory instruments. A prototype is under development at LPC2E and a m/Dmof 100,000 FWHM has been obtained at m/z = 150 for a background pressure of 10-8 mbar.

The R&T activities in France are currently concentrating on the core elements of the Orbitrap analyser that are required to reach a sufficient maturity level for allowing design studies of future space instruments. We are indeed pursuing, within international collaborations, the definition of several instrument concepts based on the core elements that are the subjects of our R&T programme. In this talk, we briefly discuss science applications for future orbitrap-based HRMS space instruments. We highlight present results of our R&T programme.

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WOS30-04 **The study of leak detection for spacecraft with quadrupole mass spectrometer**

Rongxin Yan
Beijing Institute of Spacecraft Environment Engineering

Nowadays, helium mass spectrometer technology is widely used in the leak detection of spacecraft. This technology can effectively solve leak problem of single system. As the development of spacecraft, multi-system leak detection is needed. The multi-system leak detector on spacecraft by mass spectrometer has been developed and it has been used in the new spacecraft successfully.
The apparatus of leak detection for spacecraft with quadrupole mass spectrometer are consisted with the cumulative chamber, cycle sampling device, store vessel, pressurized gas equipment and leak rate calibration equipment. After pressured different inert gases into spacecraft, the spacecraft is sealed into the cumulative chamber. The gases in the cumulative chamber are forced to mix equally and pumped into the store vessel. After the gases leak out from the spacecraft into the cumulative chamber and cumulate in a day. The gases in the cumulative chamber and the gases in the store vessel are alternating analyzed by the quadrupole mass spectrometer. The ion currents are got by turns. The calibration gases are injected into the cumulative chamber. The gases of the cumulative chamber and the gases of the store vessel are analyzed again. The leak rates of spacecraft are calculated. The gases of different time in cumulative chamber vessel are comparatively measured with the gases of store vessel. Therefore, the problem of repeat and reliability of quadrupole mass spectrometer in long time is avoided. The problem of quadrupole mass spectrometer calibration is avoided by injecting the quantitative calibration gases into the cumulative vessel. The ion currents of calibration gases are the same level as the ion current of the gases in cumulative chamber by controlling the quantity of injecting the calibration gases. The problem of the linearity of quadrupole mass spectrometer is also avoided.

In the atmosphere, the minimal He concentration can be test is 13×10-9, CF4 concentration is 10×10-9, Ne concentration is 3×10-6, SF6 concentration is 0.3×10-9, Kr concentration is 10×10-9. For the collection chamber with 100 m3 volume, in 24 hours, the minimal He leak rate can be test is 1.5×10-6 Pam3/s, CF4 leak rate is 1.1×10-6 Pam3/s, Ne leak rate is 3.5×10-5 Pam3/s, SF6 leak rate is 3×10-8 Pam3/s, Kr leak rate is 1.1×10-6 Pam3/s.

WOS30-05 The exploration of space atmosphere composition by a miniature magnetic sector spectrometer

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Science and Technology on Vacuum & Cryogenics Technology and Physics Laboratory,Lanzhou Institute of Physics

A miniature magnetic sector mass spectrometer is independently developed by Lanzhou Institute of Physics. The mass spectrometer consists of three subsystems: (1) physics unit, (2) electric control unit, (3) high voltage power. The physical unit consists of an ion source, a magnetic sectorield analyzer and ion detector. The electric control unit consists of switching pre-regulator subassembly, digital sweep control circuit subassembly, sweep high voltage power supply subassembly, low voltage power supply subassembly, signal acquisition and processing subassembly, collection and data compression subassembly, electron multiplier high voltage power supply, and emission control subassembly. Two specific trajectories are selected to cover the mass ranges, 1~12u, 6~90u. The mass spectrometer's overall size is 170mm long, 165mm wide, and 165mm tall, it weighs 4.5kg, and its power consumption was measured to be 18 W. It was able to attain a minimum detectable ionic current 10-13 A. The mass spectrometer have two work modes, the first mode designed to measure the neutral gases, the second mode designed to measure ionosphere, the filament went out in second mode, the neutral gases cannot be ionized.

In the November, 2012, the mass spectrometer was carried by XX-1 satellite, which the satellite is located on the sun synchronization orbit. During more than a year, the mass spectrometer has explored the space atmosphere compositions in the satellite orbit and gas-emited from satellite, while it has detected the charged ions and abnormal ion current in space. The mass spectrometer has explored peaks at 1, 2, 4, 12, 13, 14, 15, 16, 17, 18, 28, 29, 32, 33, 44, 46u, which the 2, 4, 12, 18, 44u are H2+, He+, C+, H2O+, and CO2+ ion peak, respectively. The 13, 14, 15, 17 ion peaks are some organic fragment, and the 28 ion peak is possible to N2+ and CO+, and 16 is possible to O+ and organic fragment, and 46 is likely to NO2 and organic fragment.

Nowadays, the miniature magnetic sector spectrometer still works in space, the space atmosphere compositions and charged ions distribution will be deeply studied in future.

Novel aspects

A miniature magnetic sector mass spectrometer is independently developed,

detected the atmosphere compositions in the satellite orbit and gas-emited from satellite.

PL05: Plenary Lecture - SIMS Imaging

Room 1
Level 1

Dae Won Moon - Chair: Olivier Laprévote

Secondary ion mass spectrometry (SIMS) provides molecular specific information but for 2D imaging, SIMS needs specimens in general, to be frozen and dried for analysis in vacuum. For 3D imaging, specimens can be sputter profiled by recently developed gas cluster ions. To complement the distortion due to cryosection and sputter profiling, we have been trying to develop a multimodal mass and non-linear optical imaging methodology of various cells and tissues for cardiovascular and neuronal studies. In this presentation, multimodal SIMS and CARS imaging studies on aortic sinus in mouse atherosclerotic plaques and olfactory bulb, and nematode C. elegans are reported.
For time-of-flight (TOF) secondary ion mass spectrometry (SIMS) imaging, 30 keV Bi3+ ions for 2D mass imaging and 30 keV Ar1000 cluster ions for depth profiling were used to analyze various tissues such as aortic sinus in mouse atherosclerotic lesions, C. elegans, and mouse olfactory bulb. As a complementary non-linear optical imaging, coherent Anti-Stokes Raman Scattering (CARS) were used to get 3D lipid imaging down to ~50 μm with 300 nm spatial resolution in-vitro or ex-vivo.
For cryosectioned aortic sinus in the advanced stage of mouse atherosclerotic lesions, consistent SIMS and CARS images were obtained regarding to the lipid molecular SIMS imaging and lipid CARS imaging in the atherosclerotic plaques. Analysis showed that increased cholesterol palmitate may contribute to the formation of a necrotic core by increasing cell death.
For C. elegans, lipid CARS imaging was obtained for live C. elegans but for SIMS imaging, C. elegans were dried with water and sputtered with 30 keV Ar1000 cluster ions to get sectioned 2D SIMS images. Molecular specific SIMS imaging for lipids, neurotransmitters, and pheromones with complementary CARS lipid imaging were used to investigate the difference of molecular distributions in wild-type and various mutant C. elegans.
For a cryosectioned mouse olfactory bulb, SIMS imaging showed different distributions of lipid molecules and neurotransmitters which is consistent with the olfactory bulb structure of glomerulus, mitral cell layer, and granule cell layer. SIMS imaging was used to study the changes of neurotransmitters in an olfactory bulb upon odorant stimulus.
In conclusions, multimodal mass and non-linear optical imaging provides a practically useful platform to investigate cells and tissues for new biomedical understanding of cardiovascular and neuronal systems. New challenges for non-cryo tissue section, plasma/fs laser based ambient mass spectrometry for live cell membrane mass imaging, and super-resolution CARS will be discussed.

ThOS31 - Biomarkers and Diagnostics

Room 1
Level 1

Chairs: Silvia Catinella, Gérard Hopfgartner

ThOS31-01 **Keynote: Mass spectrometric profiling strategies for population phenotyping**

Matthew R. Lewis[1], Jake Pearce[1], Anthony Dona[1], Robert Plumb[2], Ian Wilson[1], Rachel Shaw[1], Robert Glen[1], Zoltan Takats[1], Elaine Holmes[1], Jeremy Nicholson[1]
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Metabolic phenotyping applied to molecular epidemiology offers a powerful means for discovering molecular biomarkers and metabolic pathways that underlie disease risk in human populations. Modern molecular profiling experiments are larger in scale than ever before, continuously challenging the limits of detection for subtle disease-related patterns. The combination of mass spectrometry based profiling in both untargeted and quantitative modes coupled with mathematical modelling to extract molecular fingerprints of biological specimens continues to provide a proven framework for identifying and characterising disease signatures. However, application to the new scale of research requires advancements in both technology and methodology, meeting challenges posed by the analytical and temporal stability of LC and MS measurements. Re-evaluation of the analytical system as a whole has allowed the design of a fit-for-purpose MS-based platform for profiling and semi targeted / quantitative assays that can achieve a high degree of metabolome coverage while accommodating thousands of samples per year. Emphasis on measurement precision across samples, sample batches, and full experiments allows for data basing of results and potential future meta-study among large cohorts. This vehicle for analysis is being applied to key epidemiological studies such as GRAPHIC, LOLIPOP, CombiBio, AIRWAVE, together yielding an excess of 10,000 subjects for metabolic characterisation in relation to multiple disease states. Here the emerging pipeline for MS-based biomarker discovery via large scale analysis will be presented with discussion of analytical constraints, limitations, compromises, and achievements, with examples drawn from a selection of clinical and epidemiological studies.

ThOS31-02 **Automated dried blood spot instrumentation coupled to HPLC-QqQ mass spectrometry – A vitamin D and E case study**

Götz Schlotterbeck, Timm Hettich, Stefan Gaugler, Irene Wegner
FHNW

Introduction
Automation is an ongoing trend in analytical chemistry including sample preparation, measurement and data processing. In the clinical setting high-throughput and fully automated analytical solutions are of special interest. Dried Blood Spot (DBS) analysis is an alternative method of sampling bio-fluids, where e.g. a blood sample is spotted on a filter paper and dried. However for routine applications of DBS like newborn screening (NBS) programs, punching of discs from the DBS card into microtiter plates is still the standard procedure in NBS laboratories all over the world. In this case study we present a complete automated workflow optimized for measurement of vitamin 25-hydroxy vitamin D3, vitamin D3, α-, γ-tocopherol and α-tocopherol acetate in dried blood samples by DBS-LC-QqQ mass spectrometry.

Method
About 15 µL capillary blood was spotted on Ahlstorm Bioanalysis 226 cards and dried for two hours. The dried cards were transferred to a Camag DBS-MS 500 extraction system. The blood spot were automatically extracted with methanol/water (77/33, v/v) with an extraction volume of 30 µL and a flow rate of 30 µL/min. The separation of the different vitamins was achieved in 10 minutes with a Zorbax SB C8 column (2.1 x 50 mm, 3.5 µm) and step gradient (77 - 95 % methanol plus 5 mM ammonium formate). The flow rate (Agilent 1200 SL HPLC system) was set at 0.5 mL/min and the column was operated at 40°C. To ionize the vitamins the ESI source parameter were set to 250 °C drying gas with 10 l/min flow rate, 50 psi nebulizer pressure and 4000 capillary voltage. All compounds were detected in positive mode with multi reaction monitoring experiment with one qualifier and one quantifier by a dwell time of 150 ms at each transition on an Agilent 6410 QqQ system.

Results
The method was validated for 25-hydroxy vitamin D3 following ICH guidelines. The limit of quantification (LOQ) of the approach was 15 ng/ml for 25-hydroxy vitamin D3, which is the main biomarker for a person's vitamin D level and 1 ng/ml for α-tocopherol, which reflects the vitamin E level. In a small study with fifteen healthy volunteers the fully automated DBS-LC-MS/MS application was tested. Vitamin D3 levels were found for about 40 % of the individuals below recommended levels. Increase of vitamin D3 levels were observed after exposure to sun and a diet rich in fatty fish (salmon).

Conclusion
The automated on-line extraction process was optimized for the vitamins D and E from dried blood spots with LC-MS/MS analysis. A thorough investigation of critical parameters including type of filter cards, extraction flow rate, extraction volume, rinse solvent between the extractions for ultra-low carry over, gradient conditions of chromatography and matrix effects for the ionization process was performed. We could demonstrate that automated on-line extraction of dry blood spots in combination with direct LC-MS/MS analysis can be readily applied in a high-throughput mode with the Camag DBS-MS 500 system.

ThOS31-03 Biomarker MS assays for small cell lung cancer: exploring molecular imprinted polymer potential in clinical proteomics.
Cecilia Rossetti[1], Abed Abdel Qader[2], Trine Grønhaug Halvorsen[1], Börje Sellergren[3], Léon Reubsaet[1]
[1]University of Oslo, [2]Technical University of Dortmund, [3]University of Malmö

Introduction
Mass spectrometry (MS) methods are highly requested in clinical proteomic, providing sensitive, reproducible and specific biomarkers assays. Such precise quantification tools need to be selective to analyze complex clinical samples where occurrence of high abundant protein and low biomarker expression limits the dynamic range. In addition matrix effect causes large variations and has a negative impact on sensitivity. By combining high-resolution MS and new strategies in sample clean-up, such as immunoextraction techniques, the above mentioned drawbacks can be circumvented.
An alternative approach intended to mime antibody specificity is represented by Molecular Imprinted Polymers (MIP), synthetic materials with affinity recognition sites widely used in sample preparation of small molecule analysis. Their application to protein and peptide enrichment is still unestablished, but it has generated wide interest since MIPs represent cheap and robust alternatives to antibodies whose costly, time-consuming production notably increases analysis costs.
Among biomarker serum assays with relevance in disease management, Pro Gastrin Releasing Peptide (ProGRP) determination resulted to have diagnostic and prognostic significance for Small Cell Lung Cancer (SCLC).
Absolute quantification of ProGRP was achieved employing immunocapture-MS by bottom-up approach, determining a signature peptide (NLLGLIEAK) highly specific for ProGRP ADDIN EN.CITE ADDIN EN.CITE.DATA 1. Based on such strategy this study intended to evaluate the potential of MIP application in ProGRP analysis.

Methods
Novel MIP selective for NLLGLIEAK were used in solid phase extraction (MISPE) and extracts were analyzed by liquid chromatography-tandem MS.

Results
A comprehensive selectivity study was accomplished to determine MIP retention mechanism of different peptides. Peptide standard solutions were used in extraction protocol optimization and MIP selectivity was further tested on samples with increased complexity. MIP were highly specific in retaining ProGRP signature peptide and in allowing the removal of other peptides from extracted samples. Selectivity was also confirmed in presence of other SCLC biomarker and in spiked serum analysis. Real sample extracts resulted to be cleaner after MISPE protocol which provided the depletion of many interfering peptides enabling ProGRP quantification. These results indicate MIPs real potential in enhancing method selectivity and overcoming MS issues related to complex matrix analysis.

Conclusions
MIP extraction is a promising enrichment technique in targeted proteomics: excellent selectivity is combined with robustness and reusability as well as fast and cheap production.

Novel Aspect
Use of MIP in biomarker quantification is an innovative strategy which could further represent an effective breakthrough in proteomic assays for clinical diagnostics.

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ThOS31-04 Ovarian cancer: hunting biomarkers by mass spectrometry Imaging and tissue proteomic

Michel Salzet[1], Fatou Benoit[1], Eric Leblanc[2], Isabelle Farré[2], Denis Vinatier[3], Maxence Wisztorski[1], Isabelle Fournier[1]
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Introduction

Early diagnostic and disease management is one of the most important challenges facing modern medicine, which is particularly relevant in cancer. The lack of effective assays measuring multiple blood-based biomarkers is absent in many types of cancer. Moreover, transforming a biomarker into a useful clinical diagnostic test is a complex process, which starts with identification, proceeds through validation. Identification can be carried out by various means (gene arrays, purification procedures, proteomics), that focus on observed changes of the marker correlated with the disease progression, either in the solid tumour or in a body fluid. As the markers are identified within extracts or in a non-spatial context, further validation is always required. Several choices are then available, such as establishing specific antibodies, using protein microarrays or including more refined techniques such as tissue laser micro-dissection. A major new alternative that combines both biomarker identification and validation in a single step is now possible at the tissue level with the development of MALDI Mass Spectrometry Imaging coupled to tissue micro proteomic.

Results

We present here data obtained on ovarian cancer (OVC) using such a technology. Many questions are being raised about the potential mechanisms of ovarian cancer origin and progression. Our findings reflect that a global molecular profile is more associated with the pathological states observed in serous ovarian cancers compared to the benign stage, based on principal component analysis (PCA) and Hierarchical clustering (HC) analyses of MALDI MS profiling studies. We also identified several biomarkers related to immune response modulation at early stages of the disease like the Cter part of PA28, "Reg Alpha" and HLA-G. Based on our data we investigate more deeply the serous ovarian cancer origin, its escape, its resistance and invasion strategies. We established that OVC cells establish an immune-tolerant environment and shown that alternative proteins from alternative ORF can be retrieved and acts as modulators of BRCA1 gene. We then demonstrate that PACE4 is implicated in OVC development and confirm that serous ovarian cancer derived from Mullerian cells issued from fallopian tube.

Conclusions

We established from 500 patients that the Cterm part of PA28 is a specific maker of serous OVC. It can be found at very early stages of the pathology and is a good marker for tumor resistance. We have discovered a tumor target PACE4 and found a strategy to reactivate macrophage in dormancy into the tumor. From our study we have now 7 novel biomarkers for serous OVC e.g. C-term Reg alpha, MUC 9, HLA-G, 4 alternative proteins. All of them are now under validation through multi-center analyses

Novel Aspects

We developed then a novel mass spectrometry imaging technology based on enzymatic digestion of FAB part of anti-rag alpha in order to follow this antibody into the tumor in prospective of therapy.

ThOS31-05 Discrimination of metastasis from breast and pancreatic cancer by MALDI imaging

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Introduction

The therapy of metastatic cancer in organs origin relies on the accurate classification of the tumor. The identification of the primary site of a metastasis is currently based on clinical information, morphology, immunohistochemistry and may include DNA/RNA analysis. This process is complex, expensive and may not lead to unambiguous results. Here we test if MALDI imaging allows the differentiation of pancreatic vs. breast cancer, and if the found classifiers are applicable to identify the tumor of origin in liver metastases and to identify potential marker proteins.

Methods

All tissues samples were formalin fixed paraffin embedded (FFPE). 96 breast and 92 pancreatic biopsies in a tissue microarray from different patients were included. Additionally, 6 liver metastases originating from breast or pancreas carcinomas were used for proteomic classification. All samples were subjected to deparaffinization and antigen retrieval followed by spray-application of trypsin and incubation at 37 ºC for 2 h. Matrix was deposited by the same sprayer prior to the MALDI imaging. Multivariate statistical analyses were applied to detect putative classification markers. The peptides were extracted with water and identified using off-line nanoLC-MALDI and Mascot. IDed peptides were matched with the image data to determine the protein localization.

Results

Cancer related spectra from 29 breast and 32 pancreas tumor samples were used as training set to build a classification model by a support vector machine algorithm (SVM). An SVM classifier based on 17 peptide signals was able to discriminate breast from pancreas carcinomas with high recognition capability (100%) and a cross validation of 99.2%. This classifier was applied to 2 test sets incl. 67 breast and 60 pancreas primary carcinoma patients. The SVM model classified breast and pancreas carcinoma with an accuracy of 83.4%, a sensitivity of 86% and a specificity of 77%.
We applied the model to classify 3 breast and 3 pancreatic liver metastasis samples. Classification results were concordant to the histopathological diagnosis made by pathologists. Identified proteins include heat shock protein beta-1(HSPB 27), heterogeneous nuclear ribonucleoprotein A1 (ROA1), heterogeneous nuclear ribonucleoproteins A2/B1 (ROA2), filamin A and SH3 domain-binding glutamic acid-rich-like protein (SH3L1).

Conclusions
MALDI imaging on FFPE samples originate in the valid determination of cancer types.

Novel Aspect
Classification of tumor of origin in metastatic cancer by MALDI imaging

ThOS32 - Ion Mobility MS

Room 2
Level 0

Chairs: Dave Clemmer, Günter Allmaier

ThOS32-01 **Keynote: An (ion mobility) mass spectrometry based framework to understand protein structure**

Perdita Barran[1], Rebecca Beveridge[1], Cait MacPhee[2], Kamila Parcholarz[2], Sophie Harvey[2]
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Introduction
In the last ten years mass spectrometry (MS) coupled with electrospray ionisation (ESI) has been extensively applied to identify proteins and elucidate stoichiometry of protein complexes, often without the need for labels. Because desolvated species are affected by solvent conditions such as pH, buffer strength and concentration, ESI-MS is an appropriate method by which to consider the range of conformational states that proteins may occupy including natively folded, disordered, denatured and amyloid.
Rotationally averaged collision cross sections of the ionised forms of proteins, provided by the combination of mass spectrometry and ion mobility (IM-MS), are also instructive in exploring conformational landscapes in the absence of solvent. The mass and conformer selected ions can also be subjected to dissociation (CAD, ECD, SID, UVPD) which can delineate the structure further. Here we ask "If the only instrument that you had to analyse a protein was an ESI ion mobility capable mass spectrometer, what information could you obtain about its structure"?

Methods
19 different proteins, both monomeric and multimeric, ranging in mass from 2846 Da to 150 kDa were selected and analyzed in IM-MS instruments under different solvent conditions considered.

Results
We distinguish which of these proteins are structured (melittin, human beta defensins, truncated human lymphotactin, cytochrome c, holo haemoglobin-α, ovalbumin, human transthyretin, avidin, bovine serum albumin, concanavalin, human serum amyloid protein and immunoglobulin G) from those that contain at least some regions of disorder (human lymphotactin, N-terminal p53, α-Synuclein, N-terminal MDM2 and p53 DNA binding domain) or denatured due to solvent conditions (ubiquitin, apo haemoglobin-α, apo haemoglobin-β) by considering two experimental parameters; Dz (the range of charge states occupied by the protein) and DCCS (the range of collision cross sections that the protein is observed in).
We provide a simple model which allows the prediction of the theoretical smallest and largest possible collision cross sections based on the volume of the amino acids in the sequences, and we compare these calculated parameters with the experimental values. Consideration of the occupancy of conformational states (based on the intensities of ions in the mass spectra) allows us to qualitatively predict the potential energy landscape of each protein.

Conclusions
This empirical approach to assess order or disorder has more accuracy than theoretical methods based on the amino acid sequences for the chosen systems, and could provide an initial route to characterisation. We present an optimised methodology to determine if a given protein is structured or disordered under ESI solution conditions, providing a basis for ab initio mass spectrometry based structure determination.

ThOS32-02 **Structural and energetics studies of iron porphyrine complexes by ion mobility mass spectrometry and collision induced dissociation**

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Introduction
Iron porphyrin is important in transfer and storage of dioxygen in hemoglobin and myoglobin.1 Metal porphyrines tend to aggregate via different bindings, and such aggregations affect dioxygen functions.2 Gas phase studies help understanding the structures and intrinsic binding properties of metal porphyrin oligomers. Combined ion mobility mass spectrometry and computational chemistry was used to explore the structures of monomers, dimers, and trimmers of different charges. Breakdown diagram of CID activation and RRKM fitting provides insights into thermochemical values.

Methods
Gas phase ions were electrosprayed from Fe(III) meso-Tetra(4-sulfonatophenyl) porphine–chloride (FeTSP+4H+Cl) in water/methanol mixture by Synapt G1 mass spectrometer. Gas phase ions were directed into the ion-mobility cell where species were separated based on their drift times. After IMS, ions were fragmented by collision-induced dissociation (CID) in ion-transfer cell. Experimental cross-section was measured based on calibration set of Hamilton.3 Optimized conformations were calculated at the PM6 level.

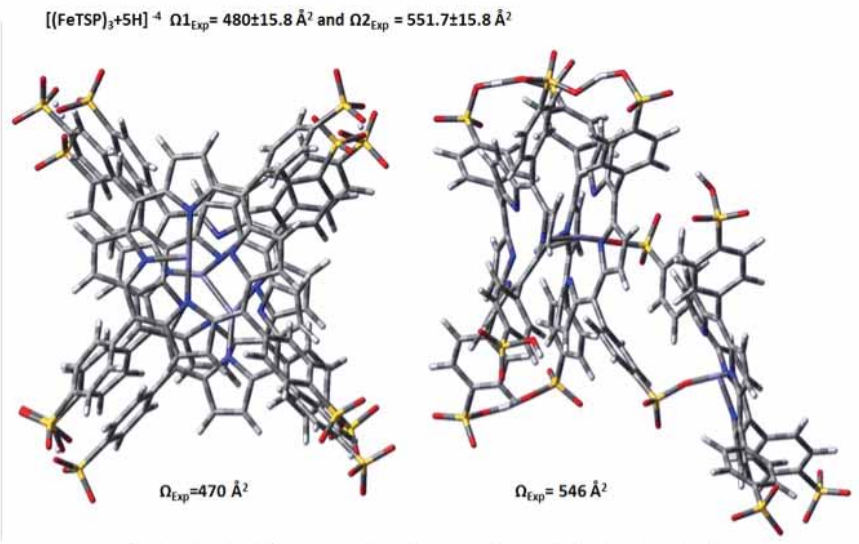

Fig 1. Calculated structures for trimer species optimized at PM6 level

Results

Dimer and trimer species with different charge states were observed. Different possible structures were optimized at the PM6 level for dimers. -2 and -3 charged dimers, [(FeTSP)2+4H]-2 and [(FeTSP)2+3H]-3, experimental cross sections agreed well with theoretical cross section of the structure I, bridged structure, where irons interact with sulfonic group. For [(FeTSP)2+H]-5, experimental and theoretical cross sections could not distinguish between isomers I and II. In isomer I, two monomers connect via iron-iron interaction. By CID, [(FeTSP)2+H]-5 dissociated to monomers of -2

and -3 charges while losses of sulfonic groups occur in [(FeTSP)2+3H]-3. The critical energy E0 for [(FeTSP)2+H]-5 dissociation (by RRKM modeling) was found to be higher than that for [(FeTSP)2+3H]-3. The dimer with larger charge dissociates in lower critical energy due to columbic repulsion effect and goes to charge separation channel giving monomers of -3 and -2 charges. For trimer species, two T1 and T2 peaks were seen in time arrival distribution, indicative of two different isomers. Cross sections of the optimized structures shown in fig 1 agreed with T1 and T2 experimental cross sections. These two isomers showed the same fragmentation channels, leading to [(FeTSP)2+4H]-2 and [(FeTSP)+H]-2. The RRKM fitting also obtained similar E0 for dissociation of two isomers of [(FeTSP)3+5H]-4.

Conclusion

A correlation between charge states, structures and energetics of dissociation of iron porphyrin oligomers was found.

Novel Aspects

- Experimental study of iron porphyrine oligomers energetics
- Detection of two different isomers for trimer

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Thursday

ThOS32-03 Choosing the right buffer gas in ion mobility spectrometry: the effect of ion-neutral interactions

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Introduction

In analytical chemistry, choosing the right buffer gas in ion mobility spectrometry (IMS) is analogous to choosing the right column in liquid chromatography. In both cases the right medium achieves maximum separation of the analytes present. However, recent developments in IMS and MS technology and the availability of commercial mass spectrometers equipped with IMS-based separation features lead to increasingly more applications where the focus of IMS is not separation but interpretation of the IMS "retention time" in terms of molecular structure. But the details and underlying physics establishing the connection between the collision cross section and molecular structure are often ignored leading to poor choices of experimental conditions and errors in data interpretation. Here we discuss the effect of ion-neutral interactions in IMS experiments and how this relates to the choice of an appropriate buffer gas.

Methods
Experimental data were obtained on two different IMS-MS setups, one including a 2-meter drift tube filled with 5 or 13 torr of nitrogen or helium, respectively, and the other employing a temperature-variable shorter drift cell held at pressures in the 1-4 torr range and operated in a temperature range from 80 to 600 K. The PSA method employed here to evaluate cross sections theoretically takes into account not only the orientation-averaged projection cross section, but also effects caused by the buffer gas-ion interaction (superposition and temperature effect) and scattering effects caused by concave parts of the ion shape.

Results
We present results for the small peptide tetraglycine and the 8.6 kDa protein ubiquitin in various charge states and various conformations. We compare cross sections measured in helium with those in nitrogen and evaluate how the cross section changes as a function of temperature for both biomolecules and both buffer gases. Theory is used to rationalize the data and to predict how various factors scale with molecule size. The interaction effect is quantified and found to significantly contribute to the cross section in all experiments presented here, but it is especially pronounced for experiments in nitrogen. We demonstrate the significance of the PSA method and present how our free on-line PSA calculator can be accessed by the research community.

Conclusions
An ion-neutral collision in IMS is significantly different from the collision of two hard spheres, not only because the particles are not necessarily spherical but also because they are not hard. The deviation from a hard-sphere potential is readily evident in temperature-variable IMS experiments and it depends strongly on the choice of buffer gas. Strong interaction effects obscure data interpretation with respect to molecular structure. Interaction is minimized in helium buffer gas.

Novel aspect
Quantify the effect of ion-buffer gas interaction in IMS for helium and nitrogen gases.

ThOS32-04 FAIMS-HRMS as a novel tool for in-depth analysis of crude oil

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Introduction
The complexity of crude oil imposes a difficult task on its analysis. Modern ultrahigh resolving mass spectrometers are capable of addressing the problem by enabling the analyst to unambiguously determine the molecular formula of a detected compound. Relying on mass resolving power alone on the other hand is too short-sighted.
For a thorough understanding of crude oil and its behavior the mere knowledge of molecular formulae of its constituents is far from enough. Additionally there is a need to distinguish between the different isomeric compounds present. This involves the differentiation between compounds of different classes - possibly addressed using traditional chromatographic techniques or chemical modification prior to analysis – but also between chemically similar constituents. For the latter

other means of separation have to be used, with FAIMS being one possible candidate. Therefore a FAIMS-system was modified to allow for the use of a photoionization source to analyze non-polar crude oil constituents.

Methods
Samples were dissolved in mixtures of methanol and toluene at final concentrations of 250-500 ppm before measuring using an Orbitrap Elite equipped with a FAIMS unit that was operated with a modified source housing to allow for APPI ionization. While acquiring mass spectra on the Orbitrap analyzer at a resolution of 480.000 at m/z 400 the compensation voltage was ramped at intervals of 1 V.

Results
The standard operation mode for the original FAIMS unit is electrospray. While ESI is an excellent ionization method for polar components it is less useful for less polar crude oil mixtures. Therefore we constructed a newly designed APPI source for the FAIMS unit.
The original FAIMS design is focussing on better ion transmission while reducing the separation capabilities. For a better separation changes on the geometry of the FAIMS design are necessary. Therefore, different electrodes have been implemented which will be shown here.

The usability of APPI-FAIMS-HRMS to analyze crude oil was first evaluated with mixtures of standard components. While using narrower electrode gaps lead to smaller distributions, this was accompanied by a significant drop in signal intensity, so that first spectra of real samples were recorded using the default electrode arrangement. Thus spectra of unpolar crude oil constituents like hydrocarbons were successfully recorded after ionization by VUV radiation and separation using the modified FAIMS unit.

Conclusions
While first steps were successfully undertaken to analyze crude oil using APPI-FAIMS-HRMS there are still some points that need attention. Especially the ion transmission has to be carefully balanced against means to improve the resolving power of the FAIMS separation. This is especially true for analytes of low abundance or ionizability that might otherwise not be detected.

Novel aspect
APPI-FAIMS-MS is here first employed to analyze crude oil on an ultrahigh resolving mass spectrometer.

ThOS32-05 **Ion mobility separation of star- and linear-shaped poly- and oligothiophenes – limits and possibilities to determine 3D structures**

Martina Marchetti-Deschmann[1], Max Kosok[2], Daniel Lumpi[3], Ernst Horkel[3], Guenter Allmaier[2], Martina Marchetti-Deschmann[2]
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Introduction
Organic electronic (OE) thin film devices have gained raising interest due to the variety of possible applications. Poly- and oligothiophene based compounds have a long history in the field and star-shaped compounds were found to exhibit better solubility and film-forming properties than their linear counterparts. The 3-dimensional structures of substrates are important for semiconducting and light emitting. The potential of Ion Mobility Separation Mass Spectrometry (IMS-MS) to predict the structure of such thiophenes is of interest in this study.
High-resolution (HR) mass spectra were acquired for poly- and oligothiophenes after (matrix assisted) laser/desorption ionization (LDI) on two instruments. IMS of isobaric compounds was optimized and experimentally deduced collision cross sections (CCS) were compared to theoretical calculations.

Methods
A SYNAPT HDMS and a SYNAPT G2 HDMS instrument (Waters, Manchester, UK), both equipped with a MALDI ion source (Nd:YAG lasers, 355 nm, 1 kHz repetition rate, 2 ns pulse width, pulse energy < 50 µJ), were used for HR MS. Samples (m/z 322.13-1233.39) were applied on stainless steel targets in THF either neatly or mixed with nitroanthracene (3 mg/mL in THF). All spectra were recorded as accurate mass data in V- and W-mode with Angiotensin II or a triarylthiophene as lock masses. Polyalanin was used for mobility calibration covering the collision cross-section and drift time (DT) range of the samples. Molecular structures were also theoretically calculated.

Results
Spray-based ionization techniques are difficult to apply to apolar molecules of higher mass. Using MALDI showed that the intact desorption of [M]Ÿ+• is feasible for polythiophenes, but especially in the lower molecular weight (MW) range matrix clusters make analyte assignment difficult. The substances' high UV absorption maxima allowed the application of LDI confirming in combination with HRMS structure identity and purity. The separation of isobaric star-shaped analytes differing only in the position of substituents was possible after optimizing TWIG parameters of the IMS cell. We furthermore show that Fluor substituents significantly influence the DT compared to CH3 residues, showing shorter DTs despite much higher MW. We also demonstrate the strength of the latest generation TWIG to separate gas-phase conformers allowing confirmation of sample purity. We also demonstrate the limits of IMS for isobaric, linear polythiophenes. Theoretical calculations for CCSs are compered to experimental data to corroborate experimental findings.

Conclusions
(MA)LDI in combination with HR IMS MS helps to confirm molecular identity of newly designed oligothiophenes confirming structure heterogeneity and allowing assumptions about relative 3D structures.

Novel Aspects
IMS separation of [M]Ÿ+• after LDI of oligo- and poylthiophenes (MW 322.13-1233.39), comparison of theoretical and experimental DTs and CCSs

ThOS33 - Data Analysis – General

Room 3
Level 0

Chairs: Pietro Franceschi, Yury Tsybin

ThOS33-01 **Keynote: Central dogma of proteomics provides identification of protein targets, action mechanisms and cellular death pathways of small molecule drugs**

Roman Zubarev
Karolinska Institutet

Phenotypic screening is a "black-box" approach to drug discovery, where small molecule libraries are screened in cell-based assays with a readout linked to a disease-relevant process (e.g., apoptosis of cancer cells). A serious bottleneck in this procedure is the discovery of the protein drug target and its mechanism of action (MoA). Thus the deconvolution of the underlying targets and MoA represents a great unmet need in drug discovery.

Here we describe a new method of providing this information using untargeted proteomics. The method is based on the Central dogma of proteomics, postulating that the greatest changes in protein abundance (or in occupancy of posttranslational modifications) are directly linked to the mechanism of biological action. Here we measure the late-apoptosis proteome change in cancer cell lines induced by action of a panel of anticancer drugs. The method capitalizes on recent developments in proteomics that made possible deep-proteome analysis (≥5000 identified proteins) in a reasonably short time (3-4 h with a label-free method and 1 h with TMT-10 labeling). This "high content proteomics" enabled quantitative cross-comparison of the proteomes of several cell lines treated with multiple drugs, with adequate controls and multiple replicate analyses.
Three cell lines (A375, H1299 and HCT116) were treated with 5-fluorouracil (5FU), methotrexate (MTX), paclitaxel (PCTL), doxorubicin (DOXO) and tomudex (TDX). In total, >5000 proteins were identified with FDR<1%. Label-free quantification data was obtained for 4168 protein groups identified with at least two unique peptides and showing non-zero abundance across all cell lines, treatments and controls. For each cell line, drug treatment and quantified protein, two parameters were calculated: regulation Reg (the abundance ratio between the treated and untreated sample), and specificity Spec (the ratio of Reg for a given drug and median Reg for other treatments). For each drug and protein, the (TYMS), the best-known target of 5FU. For PCTX, the first two positions occupied by beta-tubulins, the known targets of PCTX. For TDX, the first two candidates were TYMS and DHFR, again the correct answer. DOXO acts primarily

via DNA intercalation, and its protein target was found to be cell-line specific.
Mapping 50-100 top candidates on protein networks revealed the MoA. For 5FU, a large cluster representing ribosome was discovered, in agreement with previous findings. Mapping most abundant proteins and performing supervised principal component analysis revealed that the mechanisms of cellular death in 5FU and TDX are different, while technically both these drugs attack the same protein target TYMS. Subsequent experiments with other cell lines and drugs (e.g. camptothecin and SN-28) confirmed the validity of this approach.

ThOS33-02 **New approaches for optimizing the FTMS resolution in proteomics**

Marc-André Delsuc
IGBMC Gie CERBM

The precise detection of the isotopic pattern in the FTMS signal is very important, as it provides an easy measure of the charge state, but gives also access to the determination of the monoisotopic mass. However, obtaining the isotopic pattern is increasingly difficult for large molecules and large charge states as typically found in proteomics research.
Reaching this goal in FT-MS means generally improving the resolution by increasing the length of the transients, and reducing altogether the sensitivity of the measure and the throughput of the measure. In this study, we explore the conditions to obtain the isotopic resolution, and in particular present a new processing approach that allows a substantial improvement of the resolution for a given transient.
The resolution of the Fourier transform of a time series is limited in frequency by the duration of the series. In term of FTMS, this means that long transients have to be acquired. The Fourier transform step can be optimized by the use of adapted apodisation windows and zero-filling, however these actions are limited and the resolution cannot be extended over a limit related to the so-called Heisenberg–Gabor limit.
We use here a different approach, based on the compressed sensing general scheme, which allows overcoming this limit. This is obtained with the additional hypothesis that the MS spectrum is sparse and composed of sharp lines. A new algorithm, called RECITAL (standing for Resolution Enhancement by Iterative Algorithm) has been developed. It is implemented in an inverse problem approach; based on the Fast Fourier Transform scheme, it presents fast convergence and short execution times.
Transients of highly charged high-mass ions typically display large amplitude. These beats create a non-stationary signal and the density of information along the transient is thus varying rapidly. We also investigate the optimal transient duration associated with the presence of these beats, and how the isotopic resolution can be obtained in the presence of these beats.

The different approaches are first tested on simulated datasets, and then applied on different protein samples, such an isolated protein in native and denatured state, as well as mixtures of proteins, more typical of top-down approaches. Examples are taken both in FT-ICR and Orbitrap spectrometries, similarities and differences are outlined. In all cases, we show the improvements afforded by RECITAL when compared to the optimized Fourier transform. Finally, we propose optimal acquisition protocols for reaching the isotopic resolution in the shortest possible time, for a given protein sample.
From this study, we show that a new algorithm combined to optimized acquisition protocols can maximize the amount of information in a short period of time. This study should have a strong impact in top-down studies, antibody analysis, search for post-translational modification or in the study of large complexes

ThOS33-03 **Dynamically harmonized FT-ICR cell. Further characterization and new potential applications**

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Introduction
Recently more than 10 million resolving power at m/z 700 have been demonstrated on 4.7 T instrument in magnitude mode of Fourier transform spectra processing [2]. This level of resolution is high enough to resolve fine structure in peptides mass spectra to get information on atomic composition of ions [3]. Attempts were undertaken to increase dynamic range of FT ICR instruments and reduce measurement time and to apply this new technology to solve challenging analytical problems.

Methods
Experiments were performed using the recently introduced dynamically harmonized analyzer cell on instruments with different magnetic field strength (4.7-12 T) having different types of ion transfer optics (DC lenses and RF –multipoles). For computer simulations PIC based supercomputer code was used which takes into account ion-ion and ion-image charge interactions (capacity matrix method). For time domain signal processing different types of parameter estimation methods were used. Peak identification in mass spectra of extraterrestrial samples were performed using Compass DataAnalyses software (Bruker Daltonics).

Results
Further progress in implementation of dynamically harmonized FT ICR cell ("ParaCell") will be reported. Time domain signals obtained using this type of cell show very slow decay in the time range of minutes. They were carefully investigated using signal processing technique different from Fourier transform- parameter estimation methods.
Results are presenting for FDM processing of experimentally obtained spectra and theoretical spectra of two types- computer synthesized sum of exponentially decaying cosines with frequencies corresponding to fine structure frequencies and ab initio simulated spectra from the ensembles of ions giving fine structure.
The approach was used also to demonstrate the possibility to increase multiplexing in the NeuCode SILAC method suggested by Josh Coon group [4] by using FT ICR.
Comparison of performances of FT ICR equipped with "ParaCell" and Orbitrap in application to neutron coding method of multiplexing have been made by supercomputer simulations of mass spectra. Dynamic range and mass accuracy

distortions caused by ion clouds interaction have been evaluated using supercomputer simulations of FT ICR spectra based on Particle in Cell Algorithm.
Organics of extra-terrestrial origin have been analyzed at a resolving power of around 10 million and mass accuracy close to ppb. Atomic composition was determined by isotopic fine structure for unique identification of mass peaks.

Conclusions
FT ICR technology at current state of development has characteristics - resolving power and mass accuracy, unreachable by other mass spectrometry techniques and has a great potentials of applications to analyses of fine structures both natural and artificially made in the frame of neutral coding methods.

Novel Aspect
The first demonstration of extraordinary characteristics of dynamically harmonized ICR cell on samples of extraterrestrial origin

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ThOS33-04 Evaluation of spectral accuracy in triple quadrupole instruments

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Spectral accuracy can be defined as the capability of a mass spectrometer to provide isotopic patterns which are in agreement with the expected isotope distribution of a molecule. This concept has gained importance in the last few years because of the requirements for spectral accuracy in metabolic studies with enriched isotopes, for the identification of natural compounds in combination with exact mass determinations and for Isotope Dilution Mass Spectrometry. Current methods for the evaluation of spectral accuracy are based on the measurement of M+1/M and/or M+2/M ratios for natural abundance compounds and the comparison of those ratios with the expected values assuming a natural distribution. However, these procedures do not take into account problems such as the lack of purity of the measured mass cluster or tailing of the mass spectrometer at adjacent masses, particularly for quadrupole based instruments.
We have developed three alternative methods for the evaluation of spectral accuracy which can be applied to tandem MS instruments. The first method takes into account the purity of the mass cluster and the tailing of the mass peaks at adjacent masses and is based on the measurement of the whole isotope distribution for a natural abundance compound and the fitting of the observed distribution by multiple linear regression. This method can be applied both to single and tandem instruments. The second method is based on the increase of the bandpass of the first quadrupole to transfer the whole isotope distribution to the collision cell and the measurement of the whole isotope distribution of the product ions in the third quadrupole. This is particularly useful for the measurement of accurate isotope distributions of peptides at the (M+2H)2+ and (M+3H)3+ ions. Finally, the third method employs chlorinated and/or brominated compounds, such as PCBs and PBDEs and takes into account that the most probable fragmentation reaction is the loss of two halogen atoms from the molecular ion. In this way, and by selection of suitable precursor and product ions, the measured isotope composition is no longer dependent on natural abundances but on pure mathematical combination probabilities.
We have applied the three alternative methods for the evaluation of spectral accuracy of two triple quadrupole instruments coupled to liquid and gas chromatography respectively. The first method was applied for β2-agonists, PCBs and cellular metabolites both by LC and GC coupling, the second method for β2-agonists and peptides by LC-MS/MS and the third method for PCBs and PBDEs by GC-MS/MS. In all cases it could be demonstrated that both instruments tested provided high spectral accuracy with relative errors lower than 2%. Of special interest is the third method developed as the measured isotope distributions are independent from the natural abundances reducing the uncertainty of the theoretically calculated isotope distributions. The application of these procedures to metabolomic studies with isotopically labelled glucose will be highlighted.

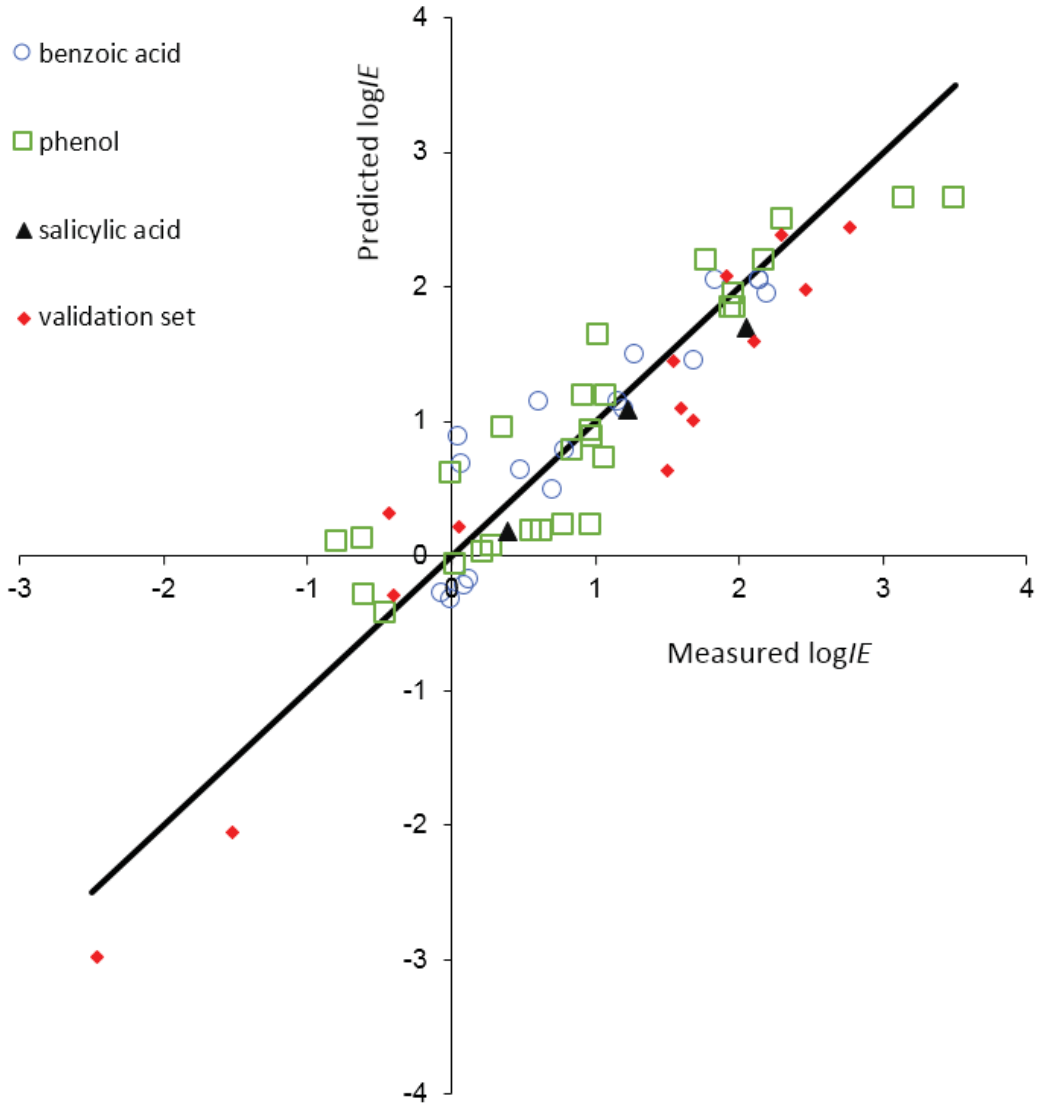
ThOS33-05 Predicting concentrations of small molecules without standard substances in LC/ESI/MS via ionization efficiency scales

Anneli Kruve, Jaanus Liigand, Piia Burk, Karl Kaupmees, Riin Rebane, Koit Herodes, Merit Oss
University of Tartu

Introduction
In contrast to significant success achieved recently in identifying unknown peaks in mass spectra in bio-samples, little has been achieved in predicting concentrations of the newly identified compounds, when standards are not available. The recently presented ionization efficiency (IE) scales for ESI and APCI sources can be used for this purpose. It has been proven that using these scales relative IE of a molecule in ESI source can be predicted by two simple parameters – ionization degree of the molecule in the solvent and degree of charge delocalization in the formed ion – which can both be easily computationally estimated.

Methods
The ESI ionization efficiency (logIE) scale has been measured with two types of methods – relative (based on ratio of ion signals of two compounds simultaneously present in the infused solution) and direct (based on comparison of calibration graph slopes). IE scales in different solvents were anchored to each other via absolute measurements of benzoic acid (ESI negative) and tetrapropylammonium (ESI positive) ESI/MS signals.
The COSMO-RS computational method was used for calculating the relevant molecular properties: pKa, WAPS, etc. Multilinear regression models were compiled for predicting logIE from the molecular parameters. Over fitting was prevented by including only statistically significant parameters.

Results
For both positive and negative ESI ionization altogether more than 120 compounds (training set) ranging for 5 orders of magnitude of IE have been measured. The intermediate precision of the logIE values does not exceed 0.26 logIE units and the pooled standard deviation over all assigned logIE values is 0.14 logIE units. The APCI scale ranges over 3 orders of magnitude and shows similar consistency.
As an example, the best logIE prediction model obtained for negative ESI was:
logIE=(1.04+/-0.34)+(2.23+/-0.34)•α+(-0.51+/-0.04)•WAPS•105
(α – dissociation degree, WAPS – charge delocalization parameter in anion)
For positive ESI mode an analogous model was achieved.
Models were validated with the validation compound set (structurally significantly different from the ones used for model building) for which the experimental logIE values were measured only after the validated model was built. As an example, the RMSD value for negative IE scale of the validation set – indicating the average disagreement between measured and predicted values – was found to be 0.48, sufficient to predict the ionization efficiency – and thus also the approximate concentration relative to some known compound – for previously unknown compounds.



Conclusions
Scales for both positive and negative ESI ionization in several solvents have been established and validated. With these steps we have acquired sufficient knowledge to estimate the concentration of compounds for which calibration with standards is impossible or impractical.

Novel Aspect
Universal model for predicting MS responses from molecular properties was developed.

ThOS34 - JMS Award Symposium

Room 4
Level 0

Chair: Michael Linscheid

ThOS34-01 A novel, ultrasensitive approach for quantitative carbohydrate composition and linkage analysis using LC-ESI ion trap tandem mass spectrometry

Kathirvel Alagesan[1], Daniel Varón Silva[1], Peter Seeberger[1,2], Daniel Kolarich[1]
[1]Max Planck Institute of Colloids and Interfaces, [2]Freie Universität Berlin

Introduction
Glycosylation is one of the most complex yet common post translational modifications, which drastically enhances the functional diversity of proteins and influences their biological activity. Identification and characterisation of glycans is an important step in correlating glycan structure to its biological function. Structural assignment of glycans (glycomics) and their compositions based on MS analyses is often based on taking well-studied glycosylation pathways for granted. Nevertheless, many monosaccharide building blocks are indistinguishable by mass alone and detailed linkage information is also not easily obtained by MS/MS analyses. In particular when studying glycosylation from less well studied organisms such as prokaryotes, monosaccharide analysis is indispensable to profile the composition of complex carbohydrates present in glycoconjugates.

Methods
Carefully designed sequential permethylation and reductive amination steps prior and after acid hydrolysis enable separation and differentiation of the various monosaccharides and their respective linkage positions. Separation of various derivatised monosaccharides was achieved using Reversed Phase – Liquid Chromatography (RP-LC)-ESI-MS/MS. In addition, absolute quantitation is accomplished including a set of internal standards, thus providing qualitative and quantitative information on the monosaccharide residues present in a specific sample.

Results
Various derivatised monosaccharides alditols were identified based upon their retention time along with mass spectrometric detection. The resulting fragment ions observed in the MS/MS spectra arise from the cleavage of methyl groups are diagnostic for the each substitution pattern. These differences allowed establishment of 'signature fragmentation patterns' for each derivatised monosaccharide alitol analysed. Furthermore, we have established fragmentation pattern library for various biologically important N-glycans for automated linkage analysis.

Conclusion
The method reported herein has a limit of detection of ≤ 250 fmol for all monosaccharides analysed and is sensitive to as low as 12 pmol of initial analyte, which now allows to perform both, glycomics and monosaccharide analyses from low µg amounts of initial glycoprotein. The present method reported here does provide more confidence in correlating the structure and stereochemistry of monosaccharides present in any type of glycoconjugates, thereby increasing the confidence in glycan structure assignments obtained from glycomics experiments.

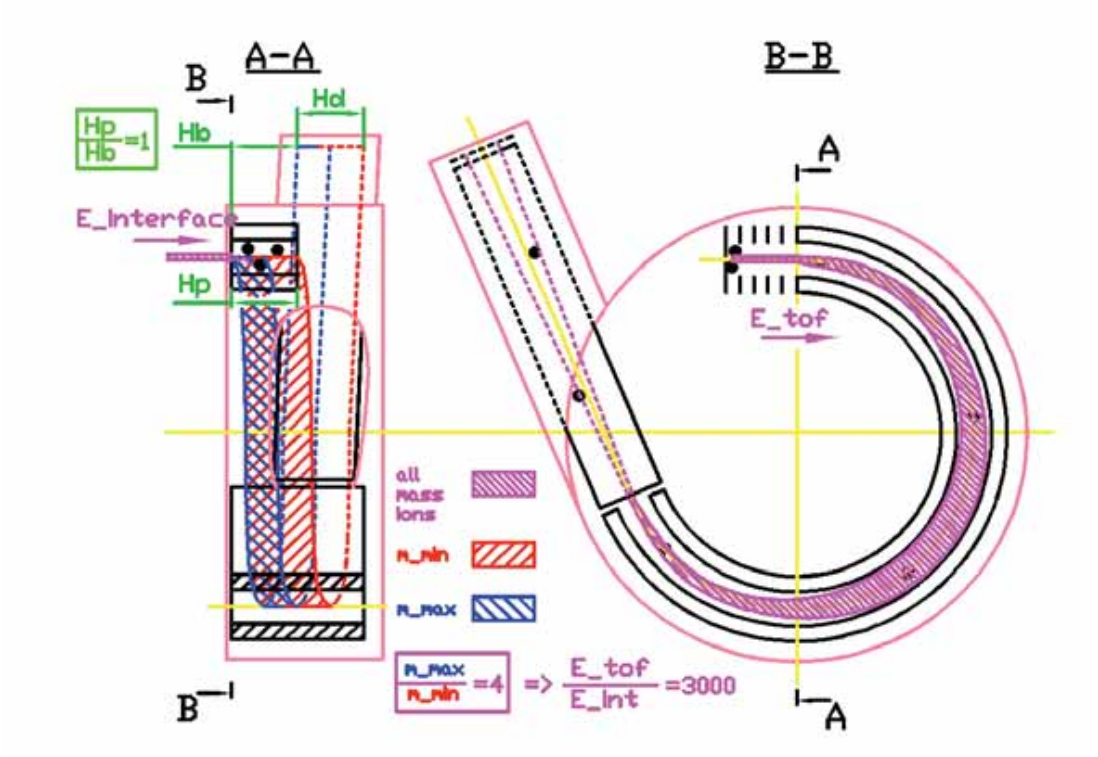
Novel Aspect
The methodology reported describes for the first time, a simple and sensitive method using Reversed Phase – Liquid Chromatography (RP-LC)-ESI-MS/MS for unambiguous identification and linkage determination of various monosaccharides (including N-acetylneuraminic acids).

ThOS34-02 Method of duty cycle enhancement for orthogonal accelerator TOF MS with axial symmetric mass analyser, connected with drift tube IMS

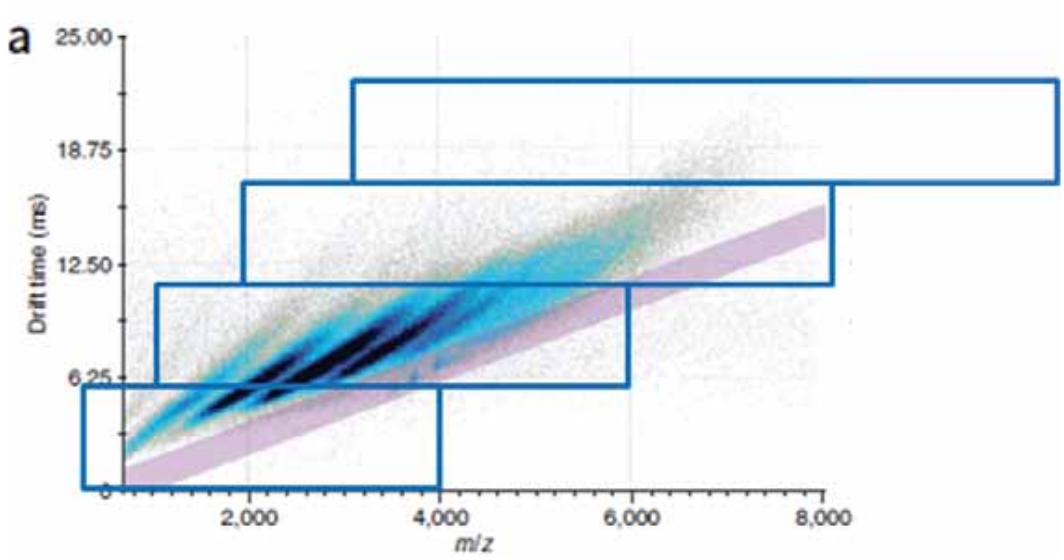
Denis Chernyshev, Alexey Sysoev, Sergey Poteshin
National Research Nuclear University MEPhI, Linantek Ltd

On the basis of recently developed ion mobility mass spectrometer [1,2], including a drift tube ion mobility spectrometer and an orthogonal acceleration electrostatic sector time-of-flight mass analyzer, a method of duty cycle enhancement was developed for orthogonal accelerator.
Axial symmetric mass analyser allows one to increase ratio of push region height of orthogonal accelerator and height of mass analyser, excluding detector's height, to 100%, because detector and push regions are divided by space, in contrast to mass reflectron. It allows one to reach 100% duty cycle for heavy ions in oaTOF MS, that is impossible in case of reflectron mass analyser, as reported in [3]. But mass discrimination of duty cycle is still remained that leads to significant losses of middle-weighted and light ions – almost to 99% lost.
New method is developed that allowes achieving 100% duty cycle for ions of all masses. At first, it demands to refuse usual TOF mass spectra timing, confined by consecutive orthogonal accelerator push impulses. And define it by TOF of the fastest and the slowest ions of interested mass. Consequently, to exclude intersection of spectra, difference between these times must be smaller than period of push pulses. As a result, condition for ratio of ion's kinetic energies in orthogonal directions was derived as a function of interested mass range.
On example of developed TOF MS it's showen that only small ratio of masses can be realized, like 4-5. But mass-mobility correlation, K~m-2/3, in fact, allows to divide mass-mobility spectrum for numerous mass spectra with rather small ratio of masses, like 4-5, that was empirically proofed. This all allows to reach 100% duty cycle of oaTOF MS for all masses, in contrast, for example, to 1-25% duty cycle for 20-890 Da, respectively. In fact, this is example of effect, known as "whole is greater than the sum of its parts". Limits of method's application are discussed. Among them are simultaneous existence of different ion's charge states, dissociation in interface.

Trace of ion trajectories in oaTOF MS with 100% duty cycle for all mass ions in case of ratio of interested masses equal to 4:



Example of division of mobility-mass spectrum into regions with ratio of masses equal to 4:



Spectrum from:
Ruotolo B.T. et al. Ion mobility-mass spectrometry analysis of large protein complexes. // Nat. Protoc. 2008. Vol. 3, № 7. P. 1139–1152.

Novel aspects
use of mass-mobility correlation for duty cycle enhancement for orthogonal accelerator IMS/oaTOFMS.

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ThOS34-03 **Membrane inlet mass spectrometry for in-field security applications**
Stamatios Giannoukos[1], Boris Brki[1], Neil France[2], Stephen Taylor[1]
[1]University of Liverpool, [2]Q Technologies Ltd

Introduction
European Union border security faces major challenges due to threats from terrorism and criminal organizations. On-site chemical detection and monitoring of compounds related to homeland security applications, civil defence and forensics is still limited with existing mainstream instrumentation. One possibility to complement existing instruments is through membrane inlet mass spectrometry (MIMS). Portable MIMS instruments have been used in this work for detection of human presence and illegal substances.

Methods
Our experiments were carried out, using two different types of portable mass spectrometers; a quadrupole mass spectrometer (QMS) and a linear ion trap mass spectrometer (LIT-MS) each coupled with a polydimethylsiloxane (PDMS) sampling probe.MIMS is a simple and powerful instrumental method for chemical analysis, ideal for field operations. It offers high sensitivity, selectivity, rapid and accurate analysis, with no or limited sample preparation. MIMS operating principle is relied on pervaporation separation through polymer membranes. In our study a membrane sampling inlet probe was connected with our MS systems allowing selective permeability of volatile organic compounds (VOC) in gas phase while blocking high abundance air molecules. At the same time, VOCs could pass through the membrane to the ion source for fragmentation and then to the mass analyzer for separation and finally to the detector for detection and further identification.

Results
We have examined the usage of MIMS for the chemical detection of VOC emissions from human exhaled breath and skin as chemical signs of human life in a confined space such as a shipping container. During tests with volunteers of both genders, a plethora of VOCs was detected, with emphasis given to CO2 emissions, acetone, isoprene and carboxylic acids. Moreover, we have also used MIMS to detect chemical odour signatures emitted from drugs, explosives and chemical weapons. The experiments used simulant compounds with similar physical and chemical properties to the parent substances in actual conditions, breakdown products, compounds that have been found in the headspace area of the parent compounds and precursors. Standard gas mixtures were prepared in dilution bottles and MIMS linearity and detection limits (low ppb levels) are demonstrated using our instruments and will be presented. Field experiments with real samples of targeted compounds were also undertaken.

Conclusions
The MIMS experiments led to the conclusion that the detected substances can be used as characteristic odour markers (alarms) of threat detection (i.e. illegal human trafficking, illicit transportation of drugs, explosives, chemical warfare agents) in border checkpoints.

Novel aspect
Chemical investigation of characteristic odors emitted from human body, narcotics, explosives and chemical warfare agents using a portable MIMS QMS and a new built in-house LIT-MS for security and forensics applications.

ThOS34-04 Nucleation and chemical reactivity of mixed aerosol particles: new approach based on mass spectrometric detection

Jozef Lengyel
Academy of Science of the Czech Republic

Atmospheric aerosols have attracted prime attention due to their influence on climate and atmospheric chemistry. One of the most famous example is the ozone hole where the polar stratospheric clouds (PSCs) play a key role in ozone depletion process [1]. The PSCs consist essentially of the nitric acid hydrates that can be formed via uptake of NOX species to ice aerosols and following chemical reaction. We have investigated the uptake processes of molecules on pure as particles [2], as well as the mixed water-nitric acid clusters [3]. In the presented work the mixed clusters are investigated by two different mass spectrometric techniques providing information on the nucleation, chemical composition, size and reactivity of these aerosols. In both experiments the species are produced by supersonic expansion of saturated nitric acid vapor seeded in a buffer gas, and mass spectra are investigated by a reflectron time of flight mass spectrometer. However, the different ionization methods are implemented to provide complementary information: electron ionization, and special method of photoionization after sodium pickup which relies on photodetachment of weakly bound solvated electron generated in the cluster from Na. The universal electron ionization proves the generation of the small aerosols, however, they are not seen in Na-doping experiment due to a fast charge-transfer reaction between the Na atom and HNO3 (see figure below). This intriguing result is also confirmed by the ab initio calculations. This implies that only aerosols containing at least one acid molecule are generated, i.e. the acid molecule serves as the nucleation centre in the expansion. The mass spectra also suggest that at least four water molecules are needed for HNO3 acidic dissociation. The unique combination of these two methods introduces a new tool for studying chemical reactivity of molecules in aerosols with solvated electrons. In the second part new experiments will be presented concerning pickup and chemical reactivity of various NOX and other atmospherically relevant species (e.g. freons) deposited on ice. The mass spectrometry is also coupled with photochemical studies in our experiment exploiting ion imaging techniques.

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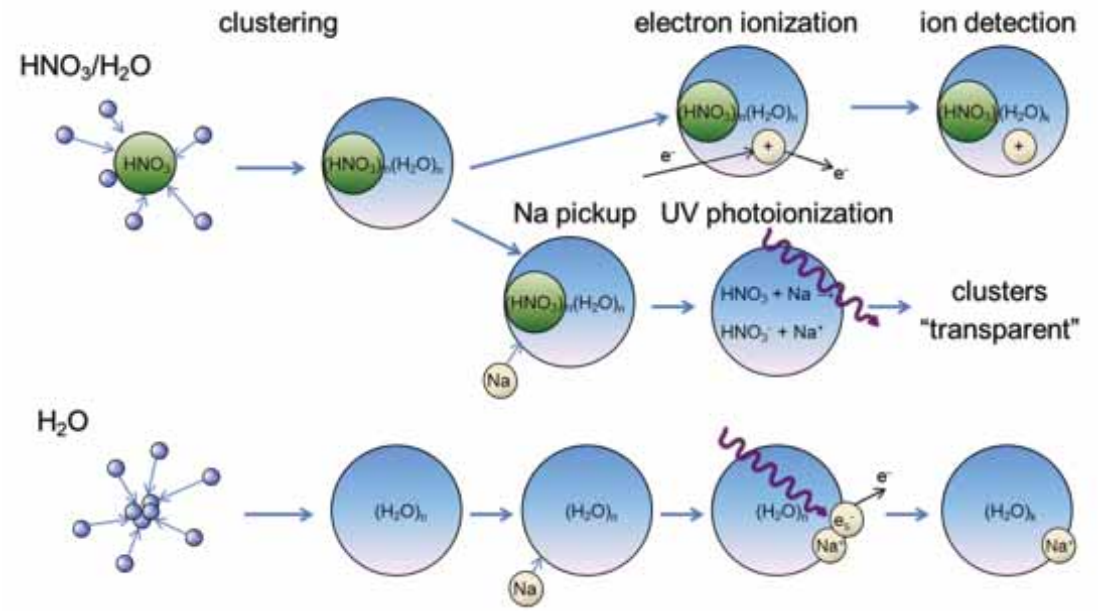

ThOS34-05 **Synthesis and reactions of atomically precise clusters**

Michael Wleklinski[1], Anyin Li[1], Zane Baird[1], Depanjan Sarkar[2], Qingjie Luo[3], Soumabha Bag[1], T. Pradeep[2], R. Graham Cooks[1]
[1]Purdue University, [2]Indian Institute of Technology Madras, [3]University of Pennsylvania

Introduction

Metal nanoclusters and nanomaterials are typically produced by laser ablation of pure metals or chemical reduction of metal salts. The resulting nanomaterials are used a variety of applications including surface enhanced Raman spectroscopy and heterogeneous catalysis. Nanoclusters can be studied in the gas phase via ion/molecule reactions or put down onto a surface for catalysis studies. In this study, electrospray of metal salts (with selected anions) is followed by thermal release of the anion to give the noble metal cations. As an extreme example, silver fluoride upon thermal heating gives both cationic and anionic silver clusters. Understanding the differences in reactivity between cationic and anionic metals is of interest.

Methods:

An electrospray ion source using nitrogen as the nebulizing gas (105 psi) was coupled to a coiled steel heating tube (200 – 250 °C). The metal ions emerging from the tube were sampled on-line using a Thermo LTQ mass spectrometer (Thermo Scientific Inc., San Jose, CA) operated at a capillary voltage of 140 V and a tube lens voltage of 240 V. After production in the thermally heated loop, the metal ion population was allowed to react at atmospheric pressure with various reagent (such as ozone, organic amines, and anhydrides) vapors.

Results

MISSING !!!

ThOS35 - Elemental and Isotopic MS, ICP-MS General, Cultural Heritage and Archaeology

Room 5
Level 3

Chairs: Ryszard Lobinski, Detlef Günther

ThOS35-01 **Keynote: Interested in the determination of molecules with a heteroatom in a complex matrix – why not use ICP-MS for speciation and bioimaging?**

Jörg Feldmann, Johannes F Kopp, Andrea Raab, Eva M Krupp, Dagmar S Urgast
University of Aberdeen

Introduction

Target molecule analysis is routine for triple quadrupole MS instruments even in a complex matrix. If the molecule in question is not entirely known and no standards are available, de-novo identification often relies on the isotopic fingerprint and the accurate mass of the compounds. If the task is the identification of compounds with a certain heavy heteroatom, it is difficult to screen the entire data set of a qTOF or Orbitrap for those compounds containing for example sulphur or selenium. Here maybe the isotopic fingerprint can be used to write an algorithm for molecules containing sulphur or selenium. Their quantification is however problematic without having a standard available. On the other hand Elemental mass spectrometry such as inductively-coupled plasma mass spectrometry (ICP-MS) can easily quantify those molecules containing heteroatoms even if those heteroatoms do not generate isotopic pattern such as arsenic and also allows for better detection limits. ICP-MS can be used as a detector for HPLC in the same way as ESI-MS, and in fact both techniques can be simultaneously coupled online in parallel. Using MALDI-MSI can give useful maps of target molecules in thin sections in order to identify hot spots within one thin section. When however thin sections from different tissues or different animals should be compared a more quantitative method is needed. This quantification is possible when bioimaging using MALDI-MSI can be combined with laser ablation (LA)-ICP-MS.

Methods
In this lecture it will be demonstrated that the use of the hyphenated technique HPLC-ICP-MS/ESI-MS can be used for de-novo identification of natural products containing a heteroatom (i.e. Arsenolipids) and that even their quantification can be done in a complex matrix without having those compounds available as standards. The extracts of marine samples such as algae, fish and whales will be analysed and screened for arsenolipids using RP-HPLC coupled online to ICP-QQQ-MS and simultaneously to ESI-Orbitrap MS. In a second example it will be demonstrated that the overlaying of molecular and elemental maps MALDI-MSI and LA-ICP-MS can show the redistribution of haem as well as of iron in a mouse kidney during an infection by pathogens.

Results:
Novel arsenolipids such as AsHC, AsFA, AsPL could be identified and quantified using this technique in whale brain and liver and in seaweeds, while the dual use of MALDI-MS and LA-ICP-MS could unravel that a mouse reacts with nutritionally immunity towards an infection fromCandida albicans.

Conclusions
de-novo identification and quantification of molecules containing a heteroatom and the quantification of elements with spatial resolution in the micrometer scale in tissues is only possible if ICP-MS is used.

Novel Aspect
Simultaneous online and off-line use of two complementary mass spectrometers for chromatography and imaging gives qualitative and quantitative information at the same time.

ThOS35-02 Novel coupling of separations with laser desorption elemental and molecular mass spectrometry

Jan Preisler, Iva Tomalová, Antonín Bednařík, Pavla Foltynová, Viktor Kanický, Tomáš Vaculovič
Masaryk University

Introduction
Mass spectrometry (MS) provides fast, sensitive and specific detection for liquid phase separations. In combination with laser desorption sample introduction/ionization techniques, it offers also advantages of off-line coupling: archiving and independent optimization of the separation and MS steps. We demonstrate two new approaches with applications in today's omics research, specifically metallomics and proteomics.

Methods
The first approach employs substrate-assisted laser desorption inductively coupled plasma (SALD ICP) MS1 and matrix-assisted laser desorption/ionization (MALDI) MS for off-line element and molecule detection of separation effluent. Species are separated by capillary electrophoresis (CE) coupled to a sub-atmospheric fraction collector via a liquid junction interface. The effluent fractions are collected on a custom-designed Au-coated polyethylene terephthalate glycol (PETG) sample target. The separation record is then covered with MALDI matrix and analyzed consecutively by MALDI MS and SALD ICP MS.
The second approach is based on diode laser thermal vaporization (DLTV) ICP MS.2 Here, samples are deposited on paper with preprinted rectangles or a line, dried and vaporized with a diode laser, which induces pyrolysis of the paper. The generated aerosol is then carried into the ICP MS. The technique was combined with ascending thin layer chromatography (TLC) on cellulose/aluminum sheets. A strip cut out of the chromatogram was scanned with the diode laser in a simple laboratory-built tubular chamber.

Results
Using the first scheme, both molecular masses of protein apoforms and metal distribution and quantity were obtained from a single CE record of rabbit-liver metallothionein complexes. Supplementary information on the complexes was also provided by MALDI MS when two different matrices at acidic and neutral pH were be applied alternately at the deposited fractions.
The second concept was proved on TLC – DLTV ICP MS of a four-cobalamin mixture. All cobalamins were separated; the detection limits surpassed optical detection by ~3 orders of magnitude.

Conclusions
Similarly to nebulizer ICP and electrospray, SALD ICP and MALDI offer comprehensive detection for microcolumn separations coupled to MS in metalloproteomics. Unlike the on-line interfaces, effluent splitting or repeating the separation for each of the detection modes is not necessary.
The second approach aims at simplicity and low cost. It employs a simple chamber with the minimal dead volume to reduce turbulent flow and wash-out time. The method is adequate for elemental speciation of simple mixtures and presents an alternative to HPLC coupled to nebulizer ICP MS.

Novel Aspect
Coupling of two new laser desorption sample introduction techniques with column and planar chromatography. New targets for combined element and molecule detection. Simple tubular cell for DLTV ICP MS.

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Acknowledgement
We acknowledge the grants GAP206/12/0538, CZ.1.05/1.1.00/02.0068 and CZ.1.07/2.3.00/30.009.

ThOS35-03 **Investigation of the pharmacological behavior of novel platinum(IV)-based anticancer agents by means of ICP-MS and LA-ICP-MS**

Sarah Theiner[1], Hristo P. Varbanov[1], Petra Heffeter[2], Walter Berger[2], Alexander E. Egger[3], Markus Galanski[1], Bernhard K. Keppler[1]
[1]University of Vienna, [2]Medical University of Vienna, [3]ADSI-Austrian Drug Screening Institute GmbH, Innnsbruck

Platinum(IV) complexes as potential anticancer agents offer some advantages over their platinum(II)-based congeners, applied in clinics. Pharmacological tuning by modification of the axial ligands combined with the higher kinetic inertness to substitution reactions turns platinum(IV) compounds into promising chemotherapeutics for oral administration.
In the current study, we investigated the in vitro and in vivo anticancer activity in murine models as well as the tissue distribution of two novel platinum(IV)-based anticancer drug candidates in comparison to satraplatin,the first orally active platinum-based cytostatic in clinical evaluation.
Platinum accumulation in tissues of mice after p.o. and i.p. treatment with the drug candidates of interest was determined using microwave-assisted acid digestion followed by quantification with inductively coupled plasma-mass spectrometry (ICP-MS). In this context, ICP-MS provides an ideal analytical tool to track metal-based compounds in the organism due to its sensitivity at the sub µg g-1 level. In addition, the spatially resolved platinum distribution in tissue sections of tumor and kidney was obtained using LA-ICP-MSI and correlated with corresponding histological structures.
Platinum accumulation in tissues differed, depending on the compound, the treatment regimen, the route of administration and dosage. Interestingly, the two novel platinum(IV) anticancer drugs exhibited similar in vivo anticancer activity after oral treatment although the in vitro cytotoxicity and the platinum concentration in tissues and serum differed significantly. Unexpectedly, the clinically-tested drug satraplatin proved to be inactive in vivo in the used model.
Concluding, we demonstrate, that prediction of the in vivo anticancer activity of Pt(IV) anticancer agents is limited, if solely cell culture experiments, platinum accumulation in tissue samples or serum platinum levels are considered.

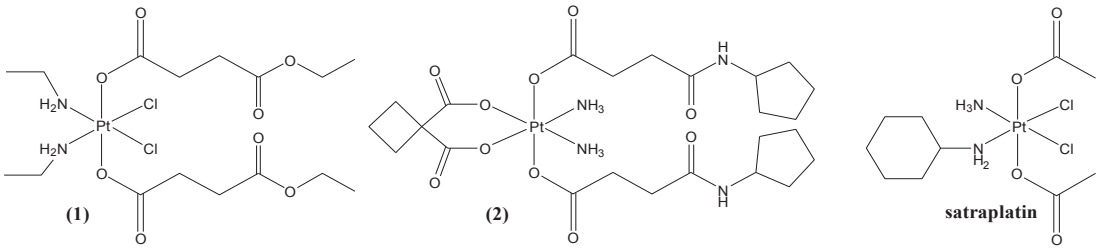

Figure 1. Structural formulas of the platinum(IV) compounds under investigation

ThOS35-04 **Precise quantification and isotope ratio measurement of boron in U_3Si_2-Al nuclear fuel by ICP-TOF-MS**

Abhijit Saha, V. G. Mishra, Dipti Shah, S. B. Deb, M. K. Saxena, B. S. Tomar
Radioanalytical Chemistry Division, Bhabha Atomic Research Centre

Uranium-silicide (U3Si2) containing low enriched uranium (LEU< 20% 235U) dispersed in aluminum (Al) matrix is the most widely accepted fuel for the conversion of highly enriched uranium (HEU> 90% 235U) to LEU in research and test reactors.1-3) As Si is always accompanied by boron (B), the latter can affect the neutron economy in the reactor core. Since the isotopic composition of natural B varies by -30 to +60‰ depending on the geological source,4,5) it becomes essential to determine its concentration along with isotope ratio in the fuel to arrive at the overall neutron poisoning effect of B. In this work a clean and reagent-free pyrohydrolysis (PH) extraction technique6-9) was employed for quantitative separation of B from U3Si2-Al matrix. About 0.5 g of U3Si2-Al samplewas pyrohydrolyzed at a furnace temperature of 1170-1220K and the distillate was collected in 10 mL of 200 mM NH4OH absorbing solution which was then analysed by ICP-TOF-MS. A schematic diagram of the pyrohydrolysis setup is depicted in Fig. 1. A comparative study on the time dependent recoveries of B from U3Si2-Al by the two gases viz., argon and oxygen are shown in Fig. 2, and consequently oxygen as the carrier gas with 75 min of PH time was selected for further experiments. In order to achieve the best possible instrumental precision on simultaneous quantification and isotope ratio measurement of B, optimization of the acquisition time with various concentrations of B solutions, as shown in Fig. 3(a) and (b), and measurements of both long and short-term stabilities, as shown in Fig. 4(a) and (b), were carried out. Therefore 50 s acquisition time was selected and the (10+11)B/7Li ratio (lithium as internal standard) was employed to generate the calibration curve for quantification of B. In order to account for and correct the variation in 10B/11B ratio due to mass bias effect and short-term fluctuations, Common Analyte Internal Standardization10,11) technique was employed as follows:

$$(10B/11B)std/sam/(10B/11B)det = K[\{(6Li/7Li)std - (6Li/7Li)det\}/(6Li/7Li)det] + 1$$

Three U3Si2-Al samples were analyzed by the proposed method and the results were compared with the ones obtained by an established spectrophotometric method12. The results of these exercises are given in Table 1. Agreement between the results at 95% confidence interval validates the proposed methodology. The accuracy of the method in the isotope ratio determination was validated by pyrohydrolyzing a mixture of pure U3O8 (free from B) and NIST SRM 951, boric acid and analyzing the distillate by ICP-TOF-MS and the results are given in Table 1.The precision on 10B/11B ratios obtained by the proposed method are better by a factor of 10 compared to the conventional mass bias correction approach. PH based simultaneous quantification and isotope ratio measurement of B by ICP-TOF-MS in U3Si2-Al nuclear fuel has been reported for the first time in the literature. The merits of the developed method are reagent-free sample treatment; generation of no liquid waste, sample obtained for analysis is as clean as water.

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Tables

Table 1: Analysis of U_3Si_2-Al and synthetic U_3O_8 + NIST SRM 951($^{10}B/^{11}B = 0.2473\pm0.0002$)

Samples	Amount of B found ($\mu g\ g^{-1}$)	$^{10}B/^{11}B$ ratio (applying CAIS)	$^{10}B/^{11}B$ ratio (by conventional mass bias correction using SRM measurement)
U_3Si_2-Al(I)	2.24±0.08 (2.2±0.1)	0.2522 ± 0.0006	0.254±0.007
U_3Si_2-Al(II)	2.15±0.10 (2.1±0.1)	0.2520 ±0.0005	0.252±0.008
U_3Si_2-Al(III)	1.95±0.08 (2.0±0.1)	0.2544 ± 0.0007	0.252±0.005
Syn mix (I)	-----	0.2471± 0.0005	0.246±0.006
Syn mix (II)	-----	0.2473 ± 0.0004	0.248±0.005

spectrophotometric results of B are given in the parenthesis; NIST certified $^{10}B/^{11}B = 0.2473\pm0.0002$

Figures

Fig. 1: Schematic diagram of pyrohydrolysis setup

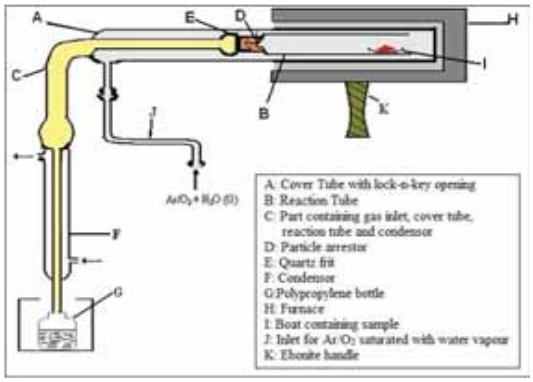


Fig. 2: Recovery of B from U3Si2-Al with time in presence of O2 and Ar

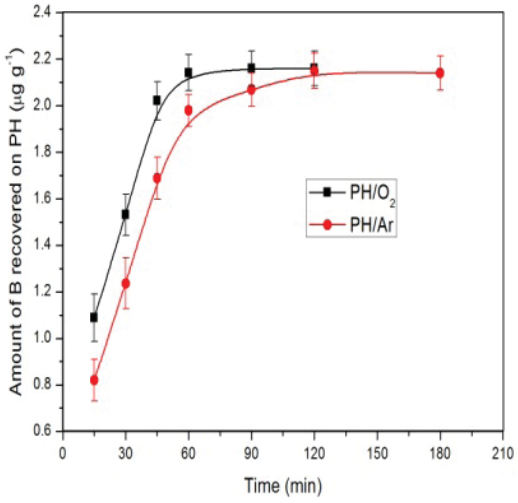


Fig. 3(a): %RSD vs Time for 5 µg L^{-1} B

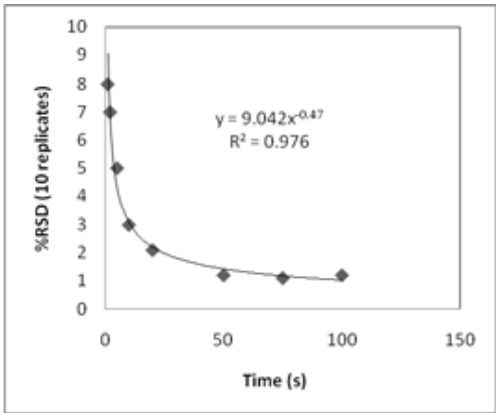


Fig. 3(b): %RSD vs Time for 500 µg L^{-1} B

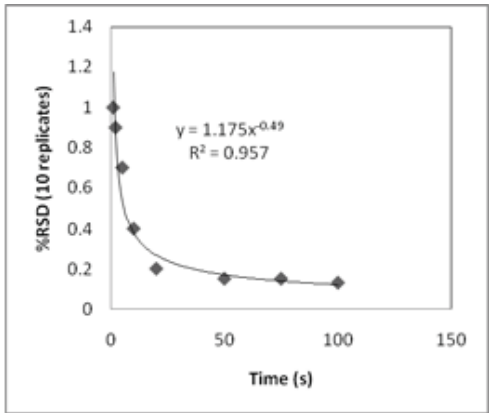


Fig. 4(a): Long- term stability of ICP-TOF-MS

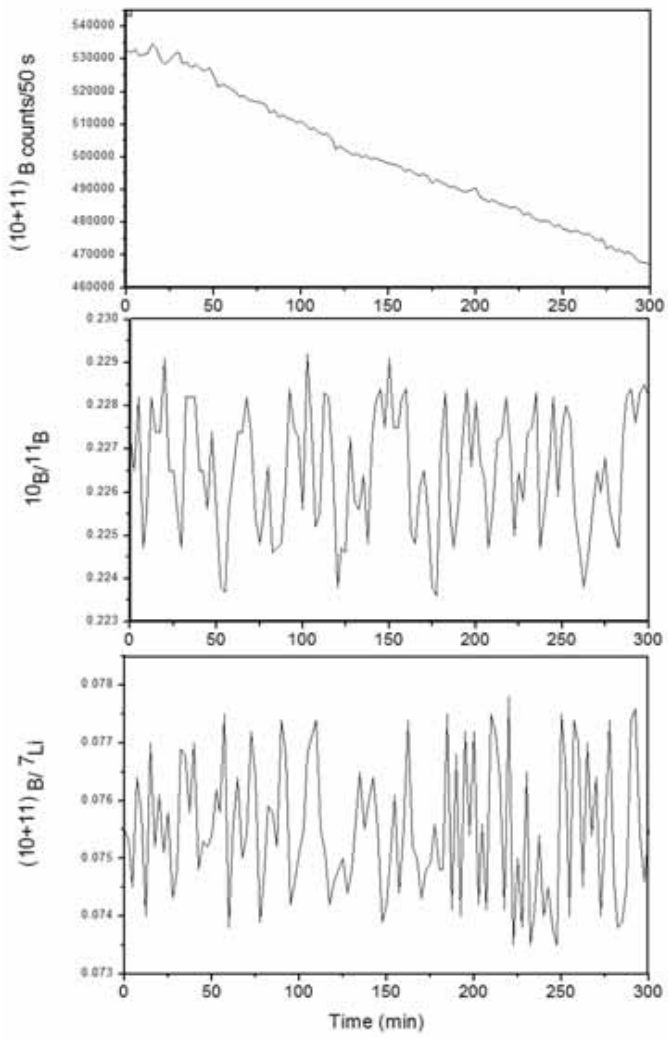
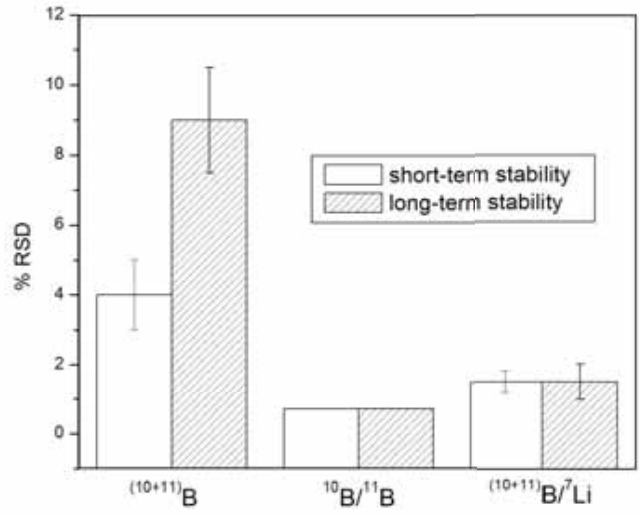


Fig. 4(b): Comparison of %RSD between long- and short-term stability of various responses



ThOS35-05 **Isotope ratio mass spectrometry for the study of catalytic reactions of transformation hydrocarbons**

Olesya Krol, Vladimir Doronin, Alexandr Belyi, Vladimir Drozdov
IHP SB RAS

Introduction

The development of oil refining processes promotes to produce the high quality fuel with improved properties. At the same time, technology modernization is impossible without fundamental research of the reaction mechanism. This is the aim of this work. The study of the stages and directions of the catalytic reactions is possible using reagents with labeled atoms. The method of isotope ratio mass spectrometry (IRMS) is investigated of the reactions joint transformation of hydrocarbons on the different catalytic systems.

Methods

The hydrogen transfer reactions between the hydrocarbons (the donors and acceptors of the hydrogen) were carried out on the zeolite catalysts in the conditions of cracking. The cyclohexane-H12 and cyclohexane-D12 – hydrogen donors, 1-hexene and 2-methylthiophene – hydrogen acceptors were used as reagents.

The catalytic experiments were carried out on aluminaplatinum catalysts in the conditions of catalytic reforming to establish the possibility of involvement of light hydrocarbon gases in joint transformation with C5+ hydrocarbons. The n-butane (with labeled carbon atom 13CH3-C3H7) and n-hexane were used as model mixture.

The quantitative analysis of the stable isotopes of hydrogen (D иH) and carbon (13Си12С) in the reagents and products was determined by method IRMS. The experiments were carried out on the isotopic mass spectrometer DELTA V Advantage (“Thermo Fisher Scientific”) in the configuration with gas chromatograph via pyrolysis or combustion reactor and with an interface ConFlo IV for online continuous flow of carrier gas helium. The individual conditions of chromatographic separation of the components were choose for each reaction. The isotopic composition of elements (δ, ‰) was calculated relative to the international standard VSMOW for hydrogen, and VPDB for carbon.

Results

According to the isotope analysis the high content of deuterium in the pentanes and hexanes was ascertained as are the main products of the reaction of hydrogen transfer. This allowed to assume the directions of the hydrogen transfermechanism between donors and acceptors of hydrogen.

The results of IRMS analysis confirm the coupling effect in the transformation of n-butane with C5+ hydrocarbons, that shows the actual possibility of joint processing of hydrocarbon gases with a gasoline fractions.

Conclusions and Novel Aspect

The use of reagents with labeled stable isotopes allowed to establish the main stages and directions of transformationhydrocarbons with a differents catalysts and conditions. Thereby, the catalytic reactions were investigated by the method IRMS and the optimal conditions of processing were selected to produce of high quality fuel.

ThOS36 - Advanced MS in Food and Nutrition

Chairs: Michel Nielen, Laurent Fay

Room 1
Level 1

ThOS36-01 **Keynote: Advanced MS-methods to study toxic secondary metabolites in food crops**

Rudolf Krska, Bernhard Kluger, Christoph Büschl, Michael Sulyok, Franz Berthiller, Gerhard Adam, Marc Lemmens, Rainer Schuhmacher
Universität für Bodenkultur Wien

Introduction
Despite huge research investments on mycotoxins and other toxic secondary metabolites of fungi and plants, prevention, control and proper exposure assessment remain difficult and agricultural and food industry continue to be vulnerable to problems of contamination. In the last few years, "mycotoxin analysis" has continuously developed from the target analysis of individual mycotoxins to metabolite profiling and metabolomics of (ideally) all secondary metabolites that are present in food crops and which are involved in plant-fungus interactions.

Methods
Recognising significant gaps in the current knowledge in prevention and control of mycotoxins in food crops, a highly interdisciplinary approach has been pursued which is based on data obtained from novel mass spectrometric methods. These include new occurrence data on multiple mycotoxins including their masked forms in foods. An untargeted metabolomics strategy for the detection of biotransformation products of mycotoxins using liquid chromatography – high resolution mass spectrometry (LC-HRMS) has also been reported. This methodology is based on in vivo stable isotopic 13C-labelling and subsequent measurement of biological samples by full scan LC-HRMS. It enables to study the metabolisation of mycotoxins by plants in order to improve the molecular understanding of resistance mechanisms of fungi and plants, respectively. A recently developed GC-MS based metabolomics approach was used to describe the response of the wheat primary metabolome upon treatment with the most prevalent Fusarium mycotoxin deoxynivalenol (DON).

Results
An LC-MS/MS "dilute and shoot" method for the determination of 331 fungal and bacterial metabolites was optimized and validated for four different food matrices. The applicability of the developed untargeted metabolomics approach was demonstrated through the simultaneous determination of (new) masked mycotoxins including the glutathione-mediated detoxification products of the Fusarium toxin DON which have been discovered by us recently.

Conclusions
This paper summarizes trends and amazing new findings in the area of multi-toxin testing of food and feed using latest state-of-the-art MS-methods and stable isotope labelling assisted metabolomics. The discovery of a novel masked mycotoxin (ZEN-16-glucoside) and of the glutathione-mediated detoxification products of the Fusarium toxin DON demonstrated the power of the employed MS-based approach to identify novel metabolites in food crops.

Novel Aspect
New occurrence data of (novel) masked toxins in food crops were revealed by employing a recently developed and validated LC-MS/MS multi-analyte method covering 331 metabolites. Within an untargeted metabolomics approach using in vivo13C labelling, novel metabolites originating from fungal and plant metabolism have been identified : The LC-HRMS based approach revealed novel DON-metabolites in wheat deriving from the gluthathione pathway whereas the GC-MS-based data revealed upregulated compounds in the wheat metabolome which are known to play a major role in plant defense after treatment with mycotoxins.

ThOS36-02 **Improved precision of measured isotope ratio through peak parking and scan-based statistics in IDMS of small organic molecules**

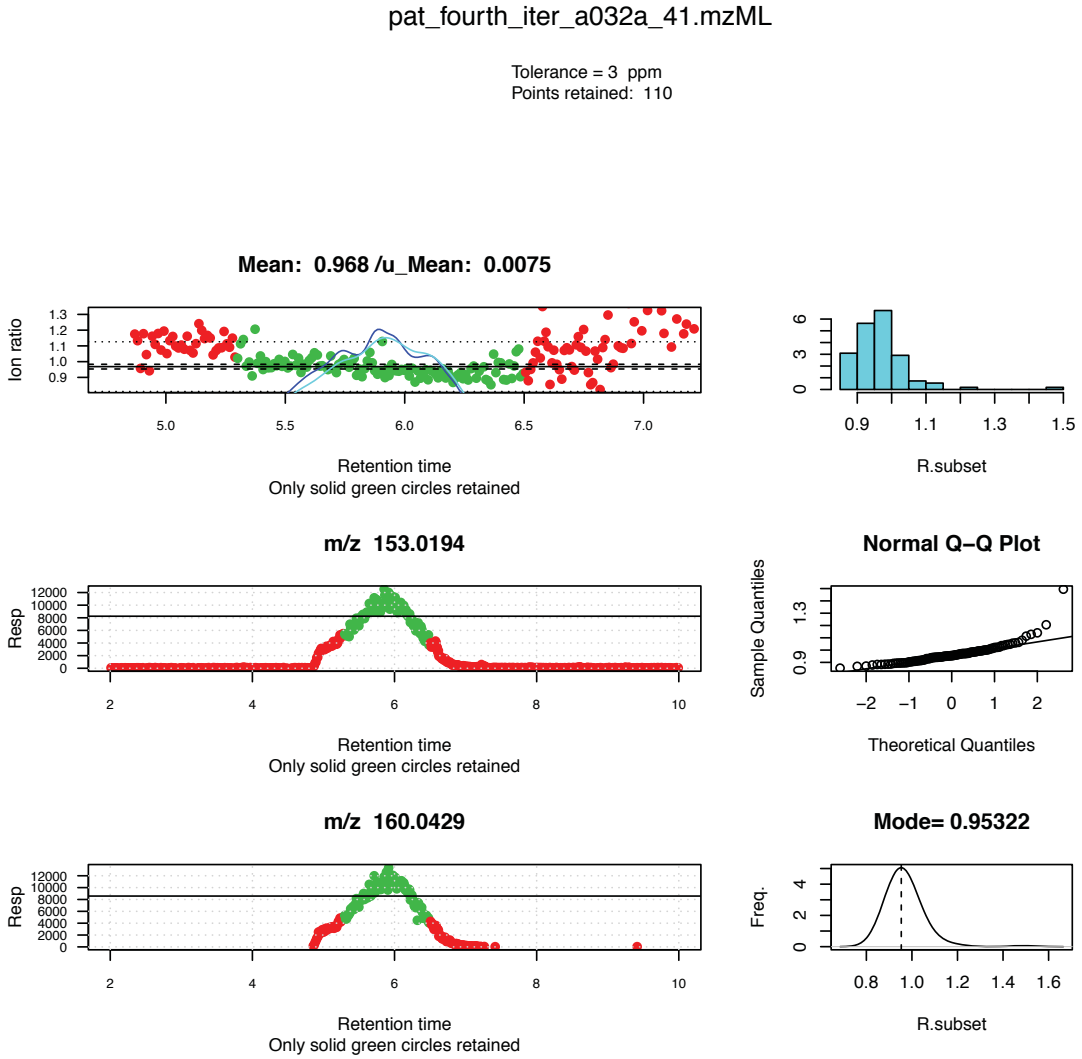
Andreas Breidbach
EC-JRC-IRMM

Introduction
To assess measurement capabilities it is essential to know the best representation of the «true value» of an analyte in a test material. For that isotope dilution mass spectrometry is a valuable tool because it can deliver bias-free estimates with high precision. In this work we show a novel approach to improve this precision.

Methods
Sample: Patulin, a mycotoxin, and its isotopologue were extracted from 2 mL naturally contaminated apple juice (40 µg/kg) spiked with 13C7-patulin (Romer Labs). A Supelclean LC-Ph SPE tube (3mL, Supelco) was used to retain the analytes which were eluted in a 1 mL fraction and injected as is.
Instrumentation:A) TSQ Quantum Ultra in SRM mode (Patulin: m/z 153→109; Isotopologue: m/z 160→115), B) Orbitrap Elite (both Thermo Scientific) in full scan mode (m/z 75-200, R: 60000). Separation: Hypercarb 100 x 2.1 mm, 3 µm analytical column (Thermo Scientific) with mobile phase MeOH/H2O/Trifluoro ethanol (800/199/1) and ionization by negative ESI. For peak parking (Orbitrap Elite) column effluent was diverted through a 100µL sample loop using the MS' divert valve. As soon as the analyte peak was centred in the sample loop the loop was switched in-line with the MS and its content was infused at 50 µL/min.
Evaluation:Isotope ratios for the TSQ data were calculated by dividing the patulin peak area through the isotopologue peak area. The Orbitrap Elite data was converted with «msconvert» (proteowizard.sourceforge.net/) to mzML format and read into the statistical software «R» (www.r-project.org/) using package «mzR» (www.bioconductor.org/). With "R" isotope ratios of patulin (m/z 153.0193) over isotopologue (m/z 160.0428) were calculated for scans containing both signals (tolerance ±3 ppm). Isotope ratios inside a specified time range were averaged for within-run statistics (Fig. 1).

Results
SRM acquisition with consecutive scan events for patulin and its isotopologue, integration of extracted ion chromatogram peaks, and dividing the patulin by the isotopologue peak areas resulted in one isotope ratio per run and a mean of 0.832 with an RSD of 9% (N=5).
The scan-based statistical approach applied to the Orbitrap Elite data eliminates some of the sources of variation inherent to the SRM approach. By calculating the isotope ratios from intensities measured within one scan variabilities like spray stability, peak integration, etc. did not figure in. Through the slow infusion >100 isotope ratios were averaged. A mean of means of 0.9782 with an RSD of 0.9% was calculated for 3 consecutive runs.

Conclusions
Through peak parking in a sample loop with slow infusion and scan-based isotope ratio calculations a significant 10-fold improvement in precision could be realized. This is of importance for estimation of a "true value" of contamination in food. We anticipate that this approach is useful for other analytes as well.



Novel Aspect
Scan-based isotope ratio statistics for improved precision.

ThOS36-03 **GC-APCI-MS/MS to enhance sensitivity for residues and contaminants analysis in food and biological fluids**
Emmanuelle Bichon, Ingrid Guiffard, Marc Bourgeois, Ronan Cariou, Philippe Marchand, Anaïs Vénisseau, Fabrice Monteau, Bruno Le Bizec
LABERCA-Oniris

A large scale of organic compounds is interesting the scientific community, based on their associated chemical hazard and potential occurrence in the environment. Some of them are already regulated in the field of food safety, such as Persistent Organic Pollutants which are still monitored in food and biological tissues worldwide to prevent any crisis and follow the human exposure. Over the last decade, other contaminants, such as food contact materials and brominated flame retardants, have been paid growing attention, evidencing them as a new potential chemical risk for human. In parallel, scientists pay more and more attention to natural steroids concentrations in biological fluids (serum, urine) in order to detect endocrine disruption at very low levels, particularly in children. For all these compounds, the analytical strategies imply in general gas chromatography coupled to mass spectrometry measurement, either in high resolution or on a triple quadrupole analyser, in electronic impact in any cases. This ionization is the most effective one but give a huge amount of fragments. In order to improve the specificity of the measurement in increasing the molecular

ion intensity, a more gentle ionization could be envisaged with the atmospheric pressure chemical ionization (APCI). In our work, we have compared the measurements carried out on the APGC-XevoTQS system (Waters) with those implemented on GC-EI-HRMS (Jeol 800D) and GC-EI-MS/MS (Agilent 7000). As an alternative, we have investigated an analytical strategy combining high chromatographic resolution with weak energy ionization with main aim to provide specific and sensitive signals to be further used in MS/MS. GC-APCI-MS/MS (QqQ) offered a perfect combination to this end. Ionization conditions (dry/wet, corona discharge), interface physical parameters (temperature, gas flow) and different derivatization reactions (when required) were investigated. Chromatographic (resolution, peak shape, noise) and spectrometric (specificity, sensitivity, repeatability) performances have been optimized and will be discussed in regard with standard analytical approaches currently used by analysts for residues and contaminants in various complex matrices.

ThOS36-04 The power of ion mobility-mass spectrometry for increased selectivity in food analysis: "a new beginning for collision cross section"

Séverine Goscinny[1], Michael McCullagh[2], Dave Douce[2], Vincent Hanot[1], Gauthier Eppe[3], Edwin De Pauw[3], John Chipperfield[2]
[1]Scientific Institute of Public Health, [2]Waters Corporation, [3]University of Liège-Mass Spectrometry Laboratory

Introduction

Nowadays, full scan mass spectrometry is an accessible technology. On one hand, significant improvements in assay performance are occurring rapidly in this instrumental areas e.g. Time-of-Flight analysers (ToF), have in addition to unlimited m/z range, very high sensitivity, increased mass accuracy and acquisition speed, high percentage of ion transmission but also ease of instrument set-up. On the other hand, laboratories have to perform and report under time-constrained conditions, sample treatment has become more generic with a concomitant increase in the complexity of full mass spectra. Consequently, data interpretation is becoming more complex. Ion mobility is known to be a powerful analytical tool for the separation of complex samples and have been extensively used for characterization purposes. Here, we propose to apply this technique to a whole new domain by using Collision Cross Section values as an additional identification parameter with high-resolution mass spectrometry methods.

Methods

For the assays, UPLC-HDMSE experiments were performed on a Synapt G2-S using a series of standard solutions, spiked matrices and real samples. The sample treatment comprises the extraction of the targeted compounds followed by a simple dilution step (for pesticides analysis) or mild purification (for food additives analyses) prior to analysis. Mass spectra are collected from m/z 50 to 1200 with an acquisition rate of 5 spectra per second. The mobility cell calibration is carried out using polyalanine solution during instrument setup. CCS values were generated from the standard solutions and inserted into a scientific library within a new scientific information system.

Results

The method was developed after thorough investigation of the behaviour of the targeted compounds in the mobility chamber. Parameters such as repeatability, intermediate reproducibility, instrument-to- instrument reproducibility, and matrix effect on the generated CCS values were studied. Based on those results, we are able to demonstrate how the there is no more than 2% difference between the CCS values generated by injecting reference standard solutions and CCS values obtained during an analysis with the presence of the matrix or just with calibration solutions, or other analytical conditions such as mobile phases and flow rate. With this 2% CCS difference used as new parameter in the identification process, we can confidently avoid false positives and false negatives while keeping the tolerance parameter for measured mass and retention time unexpectedly wide. The strategy used is different to current analytical trends, which tightly close down the tolerance windows during identification step.
In summary, with specific applications in food analysis, the identification power of CCS will be revealed while the proposed methodology remains simple and gives accurate results.

Novel aspect

Presentation will illustrate the advantages and a novel way of utilising ion mobility to enhance confidence in routine food analysis.

ThOS36-05 Non-target and unknown screening of food samples using accurate mass LC-MS/MS screening techniques

Ashley Sage, Jianru Stahl-Zeng, Harald Moeller, Detlev Schleuder, Jean-Pierre Lebreton
AB SCIEX

Introduction

Liquid Chromatography coupled to tandem Mass Spectrometry (LC-MS/MS) is a powerful analytical tool for the analysis of polar, semi-volatile, and thermally labile compounds of a wide molecular weight range, such as pesticides, veterinary drugs, mycotoxins and other food residues and contaminants. Mass analyzers based on triple quadrupole technology operated in Multiple Reaction Monitoring (MRM) mode deliver highly selective and sensitive quantitative results and are therefore well established for multi-target screening and quantitation of food contaminants. However, the use of triple quadrupole based mass analyzers limits the number of compound to quantify and identify. In addition there is an increasing demand for retrospective non-target (unknown) data analysis to identify unexpected food residues and contaminants. High resolution and accurate mass instruments are capable of performing targeted and non-targeted screening in a single LC-MS/MS run.

Experimental

Here, a generic QuEChERS procedure was used to extract residues and contaminants from fruit and vegetable samples. Extracts were subsequently analyzed by LC-MS/MS using the AB SCIEX TripleTOF® system operated in high resolution accurate mass MS and MS/MS mode. No prior knowledge of the compounds were known and no tuning of the instrument was performed. A generic C18 reversed phased UHPLC method was used to perform the separation of the components.

Results & Discussion
Non-Target compounds were identified based on non-target peak finding and sample control comparison to separate chemicals of interest from matrix components. Accurate mass MS and MS/MS information was used to empirically calculate molecular formulae. Found molecular formulae war searched against ChemSpider to find matching structures. Structures were automatically compared against the MS/MS spectrum and theoretically fragmented to tentatively identify the detected compound. Data processing was performed in MasterView™ software was allows quick processing and intuitive data review. The results shown will highlight the importance of modern software algorithms and workflows to aid in the identification of target and unknown compounds alike.

ThOS37 - Hyphenated Techniques – Applications

Room 2
Level 0

Chairs: Gérard Hopfgartner, Marc Suter

ThOS37-01 Keynote: Digital microfluidic sample processing for separations and mass spectrometry

Aaron Wheeler
University of Toronto

Separations and mass spectrometry are critically important analytical techniques that are useful for a wide range of applications. But a limitation for both techniques is the requirement of laborious, multi-step sample preparation prior to analysis. This has led to great interest in the microfluidics community in the development of automated, integrated techniques to serve as a front-end complements to separation systems and mass spectrometers. Most of this work has relied on the conventional microfluidic device format of planar devices with enclosed microchannels, but a new format known as «digital microfluidics» has recently become a popular alternative. In digital microfluidics, droplets are manipulated electrostatically on open devices (with no channels) bearing an array of electrodes covered with a hydrophobic insulator. Digital microfluidics has significant advantages for sample preparation, including the capacity to handle both liquids and solids (with no risk of clogging), and the ability to address each droplet individually (allowing for absolute control over all reagents). In this talk, I will review a number of methods recently developed using digital microfluidics for processing samples upstream of mass spectrometry and separations, highlighting systems for in-line monitoring of organic synthesis and for in-line and off-line analysis of dried blood spot (DBS) samples. I propose that these examples and others suggest that the combination of digital microfluidics with separations/mass spectrometry may be useful for a wide range of applications in laboratory science.

ThOS37-02 Online SPE-nano-LC-HRMS for analysis of polar organic micropollutants in environmental samples: method development, validation and applications

Michael Andrej Stravs[1], Jonas Mechelke[1], Heinz Singer[1], P. Lee Ferguson[2], Juliane Hollender[1]
[1]Eawag, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland, [2]Duke University, Durham, USA

Introduction
In traditional environmental LC-MS, chromatography and mass spectrometry is often preceded by solid phase extraction (SPE) of large sample volumes (100 mL - 1L), which is tedious and time-consuming. With online SPE, the extraction step can be automated, reducing workload, but still requiring large sample volumes (e.g. 20 mL). In contrast, in the field of proteomics, nano-LC-MS has been widely adopted for its high sensitivity even with very small sample volumes.
The goal of this study was to combine online SPE with nano-LC-MS in order to achieve high sensitivity in small environmental sample volumes, and to take advantage of the reduced matrix effect from efficient ionization at the nanospray MS interface. The method should be applicable for the analysis of 40 polar organic micropollutants with different physical-chemical properties and their transformation products in phytoplankton matrix as well as freshwater. This allows to perform biotransformation experiments in high throughput in multiwell plate format.

Methods
In the online SPE enrichment step, a microscale cartridge packed with chromatography grade (5 µm particles) polymeric sorbent material (Oasis HLB or a mixture of sorbents) is loaded with 10 - 100 µL of aqueous sample with a flow rate of 10 µL/min. In the elution step, the cartridge is eluted with pure organic solvent at a flow rate of 120 nL/min. The eluate is diluted with aqueous solvent for refocusing on the chromatographic column (Atlantis dc18, 100µm ID, Waters). Chromatography is then performed at sub-µL/min flow with a 10-95% organic phase gradient. Ionization at a nanoflow electrospray interface is followed by detection on a Q-Exactive high resolution mass spectrometer. Variations during the development process and their merits are discussed.

Results
Different online SPE set-ups, loading and elution conditions, and SPE materials were tested and optimized to reduce carryover, enable matrix elimination, and maximize recovery for the range of compounds. The final method was characterized in nanopure water, in biological matrix (Microcystis aeruginosa cell lysate) and in surface water, and more general applicability was tested with wastewater effluent samples. Low ng/L detection limits were achieved, which is in the range of current online SPE-LC-MS methods, and remarkable given that the used sample volume (50 µL) is very low even after scaling for chromatographic dimensions. Matrix suppression was very low under the tested conditions. The use of isotope-labeled internal standards was essential for accurate quantification. Robustness and reproducibility are discussed and application to algae matrix is presented.

Conclusions
A general purpose nano-LC-HRMS method applying proven concepts from online SPE and analytical LC-MS with modern nanoflow LC has been developed. It outperforms established systems especially where low sample volume is a critical variable.

Novel aspect
This work is a novel attempt at the implementation of a nanoflow system for a broad-range, general purpose nano-LC-MS system for use in environmental analytics.

ThOS37-03 Characterization of bioactive peptides from snake venoms by LC-MS coupled to bioactivity assessment via at-line nanofractionation

Marija Mladi[1], Janaki Iyer[2], Martine Smit[1], Wilfried Niessen[1], Govert Somsen[1], Manjunatha Kini[2], Jeroen Kool[1]
[1]Free University of Amsterdam, [2]National University of Singapore

Introduction
Snake venoms comprise a myriad of bioactive peptides and low-molecular-weight proteins which can cause multiple physiological reactions when administered to a prey organism. The diversity of effects involving varying mechanisms of action, together with the high target-specificity of each single constituent, make snake venoms very attractive materials for the discovery of new drugs for the treatment of different diseases. Screening, purification and characterization of relevant bioactive compounds from snake venoms is a challenging and often laborious task. This study describes the development of an advanced integrated analytical approach for the activity profiling and identification of venom constituents targeting the cardiovascular system.

Methods
The proposed methodology encompasses a liquid chromatography (LC) separation of crude venoms followed by parallel mass spectrometry (MS) detection and high-resolution fractionation onto 384-well plates. After freeze drying, the fractions are subjected to a bioassay of choice. In the present study, the ability of the venom nanofractions to modulate activity of angiotensin converting enzyme (ACE), thrombin and factor Xa was assessed. For the wells showing bioactivity, the parallel MS data reveal the masses of the corresponding compounds. Nano-LC-MS2 analysis is subsequently performed on the content of the designated nanofractions in order to identify the active peptides and proteins. Depending on the molecular size of the detected bioactives, in-solution trypsin digestion precedes the nano-LC-MS2 analysis. Amino acid sequences are determined using either protein database search engines (e.g. MASCOT) or de-novo sequencing.

Results
The nanofractionation and bioassay conditions were optimized maintaining LC resolution and achieving good read-out sensitivity. The developed integrative analytical approach was successfully used for the fast screening of snake venoms for compounds affecting ACE, thrombin and factor Xa activity. Parallel accurate MS measurements provided correlation of observed bioactivity to peptide/protein masses. After performing the bioassay, the analysed nanofractions in the well plates were subjected to nano-LC-MS2 analysis directly or after in-solution digestion. This resulted in identification of a number of interesting proteins with activity towards a specific cardiovascular drug target.

Conclusions
Accurate LC-MS and LC-MS2 combined with bioactivity assays using nanofractionation as linking technology allows fast screening and identification of snake venom proteins for pharmacological activity towards preselected drug targets.

Novel aspect
1) LC-MS hyphenated to at-line bioactivity determination using a nanofractionation approach for screening of snake venoms for proteins with a desirable pharmacological effect. 2) Direct protein sequencing by nano-LC-MS2 after in-well trypsin digestion of bioactive nanofractions.

ThOS37-04 Analysis of oligosaccharides in complex samples using MS-based techniques

Leon Coulier, Wibo van Scheppingen, Jort Gerritsma, Rob van der Hoeven
DSM Biotechnology Center

Introduction
The analysis of carbohydrate oligomers in complex samples, like biomass hydrolysates or food matrices, remains a challenging task. Common techniques for analysis of small molecular weight carbohydrates are based on liquid chromatography, i.e. anion exchange chromatography or ion-moderated partition chromatography, coupled to detection techniques that give little chemical information, like refractive index detection (RI) or pulsed amperometric detection (PAD). The lack of reference compounds and the complexity of the mixtures of carbohydrate oligomers require the need of more specific detection methods. In addition, common HPLC-based techniques for carbohydrates are relatively slow and a significant improvement in speed is necessary when dealing with large amount of samples.

Methods
High Performance Anion Exchange Chromatography (HPAEC) is coupled to Mass Spectrometry (MS) using a desalting interface. Separation was carried out on a Dionex BioLC system using a Carbopac PA20 column and a NaOH/NaOAc mobile phase gradient. After post-column desalting of the mobile phase using an Dionex ASRS desalting membrane the eluting compounds are detected by MS on a Thermo LTQ ion trap mass spectrometer using electrospray ionization in the positive ionization mode.

Results
To fulfill these needs several mass spectrometry (MS)-based techniques have been set-up in our laboratory for analysis of complex mixtures of oligosaccharides. These methods include High Performance Anion Exchange Chromatography (HPAEC)-MS, Hydrophilic Interaction Chromatography (HILIC)-TOF-MS and MALDI-TOF-MS. These techniques have in common that MS is used for detection and in combination with MS/MS this gives valuable information on the elemental composition, structure of individual oligosaccharides like building blocks and in some cases even linkage-information. Furthermore none of these techniques require extensive sample preparation, like derivatization. These three techniques all have their advantages and disadvantages, which will be demonstrated in this presentation.

It will be shown that the combination of the different MS-based techniques can be of great help in elucidating complex mixtures of oligosaccharides in complex biological background, i.e. biomass hydrolysates, in an industrial setting.

Conclusions
The combination of the different MS-based techniques can be of great help in elucidating complex mixtures of oligosaccharides in complex biological background, i.e. biomass hydrolysates, in an industrial setting.

Novel aspect
Coupling of HPAEC to MS and applied to complex biomass samples in an industrial setting.

ThOS37-05 Monoclonal antibodies complete primary structure and biosimilarity assessment in a single analysis by sheathless capillary electrophoresis-mass spectrometry

Rabah Gahoual[1], Jean-Marc Busnel[2], Johana Chicher[3], Lauriane Kuhn[3], Phillipe Hammann[3], Alain Beck[4], Yannis Nicolas François[1], Emmanuelle Leize-Wagner[1]
[1]CNRS – UMR7140, University of Strasbourg, [2]Beckman Coulter Inc., [3]University of Strasbourg, [4]Centre d'immunologie Pierre Fabre

Monoclonal antibodies (mAbs) are meeting an important success as therapeutic treatments and represent currently one of the fastest growing area in drug development. Patents on some approved mAbs are going to end giving the opportunity to produce "copies" referred as biosimilars. Regulation agencies requires for approval to demonstrate the chemical similarity between a candidate biosimilar and the original mAbs. mAbs are highly complex glycoproteins, displaying a wide range of microheterogeneities so adapted analytical techniques may be a huge asset for development/approval of biosimilars. A sheathless CE-MS platform was used to develop a CE-ESI-MS/MS method allowing fast and robust characterization of mAb primary structure and assess biosimilarity between 2 mAbs samples.
Samples were analyzed using a CESI-MS platform coupled to a 5600 TripleTOF mass spectrometer. mAbs samples, digested in-solution by trypsin, were analyzed using transient isotachophoresis followed by CE-ESI-MS/MS. Separations were performed in bare-fused silica capillaries in background electrolyte 10% acetic acid. Peptides, posttranslational modifications (PTM) and glycosylations were simultaneously characterized using the CESI-MS/MS data. Two approved mAbs and respective candidate biosimilars were considered.
CESI-MS/MS allowed to obtain 100% sequence coverage for mAbs samples while systematically up to 90% y/b ions were identified. It is a first that entire protein amino acid (AA) sequence could be characterized using a sole enzyme for mAbs digestion and one injection for peptides characterization. Note AA sequence characterization could be performed only through digested peptides without miscleavages. Same dataset also enabled to precisely characterize and estimate occurrence levels of all PTMs "hotspots" used to monitor product stability. Data were used to structurally characterize and estimate relative abundances of glycosylations. It was possible to characterize up to 16 N-glycans for all samples. Also two different glycosylation sites could be characterized with site dependent glycoform relative quantification established for cetuximab. Glycoform profilling established proved to be in agreement with other techniques like CE-LIF or MALDI-MS. Using CESI-MS/MS, approved mabs were compared to their candidate biosimilar. Trastuzumab data comparison allowed to distinguish trastuzumab from its candidate biosimilar by a difference of one AA and dissimilarities in glycoforms distribution. For cetuximab, the candidate biosimilar had, complete similarity in AA sequence while expression of toxic glycoforms were pointed out again in a single run.
Proposed methodology proved its capacity to characterize simultaneously on several aspects mAbs primary structure while allowing to determine/disconfirm biosimilarity between 2 samples.
Complete mAbs primary structure characterization (AA sequence, PTMs including glycosylations) and biosimilarity assessment in a single injection using CE-ESI-MS/MS.

ThOS38 - Non-Covalent Interactions

Chairs: Albert Heck, Leopoldo Ceraulo

Room 3
Level 0

ThOS38-01 **Keynote: Combining advanced native MS techniques for the characterization of non-covalent complexes**

Sarah Cianferani
CNRS - IPHC - LSMBO

Mass spectrometry is generally understood as "molecular mass spectrometry" with multiple applications in biology (protein identification using proteomic approaches, recombinant protein and monoclonal antibody characterization). An original and unexpected application of mass spectrometry emerged some twenty years ago: the detection and the characterization of intact biological noncovalent complexes called native MS. With recent instrumental improvements, native MS has emerged as a valuable MS technique for intact noncovalent protein assemblies' characterization, reaching a high level of reliability within the last ten years. Since recently, native MS also benefits from high resolution instrumentations, opening new doors for even more detailed characterization. Native MS provides high content information for multiprotein complexes characterization, including the determination of the binding stoichiometries or oligomerization states, site-specificities, relative affinities and in some cases dynamics. Recent developments of ion mobility / mass spectrometry instruments (IM-MS) provide a new additional level for MS-based structural characterization of biomolecular assemblies allowing size and shape information to be obtained through collisional cross section measurements.
Here I will present a representative panel of different applications of native MS, IM-MS and high resolution native MS with a focus on protein/ligand systems and protein therapeutics assemblies (antibody/antigen systems).

ThOS38-02 **Opposing charges in ESI-MS of non-covalent complexes explain many observations**

Rachel Loo, Joseph Loo
UCLA David Geffen School of Medicine

Introduction
It is common to describe the ESI-MS 8+ charge state of protein X as reflecting X with 8 protonated basic sites, rather than as X with; e.g., 10 or 11 protonated basic sites and 2 or 3 deprotonated acidic sites, although these are often indistinguishable. This bias is propagated in common explanations for the reduced stability of higher charge states; i.e., like-charge repulsion. If opposing charges contribute, we might instead attribute stability differences in protein noncovalent complexes to a greater number of salt bridges in the lower charge state.

Methods
Measurements were performed on FT-ICR and QTOF systems. Observations were also assembled from 20 years of literature reports.

Results
As solution ions traverse high electric fields in droplet liquid jets, they undergo intermolecular charge transfer and redistribute charge intramolecularly. Charge migrates from protonated residues to carboxylate anions to neutralize or form salt-bridges. Rattling an ion via gas phase collisions nudges residues into additional or rearranged bridges. This view suggests that ion pairs within gas phase proteins need not be limited to those annotated by NMR or xtallography, and that they may be more relevant to gas phase structure and dissociation of noncovalent complexes than previously thought.
Collisionally-activated noncovalent multimers typically dissociate asymmetrically by ejecting a single subunit bearing a large percentage of excess charge, typically rationalized as reflecting a subunit's unfolding, enabling it to depart with more charge. We propose that high dissociation barriers are circumvented by heterolytic ion-pair scissions that inherently partition charge asymmetrically. In other words, 14+ homodimers could produce 10+ and 4+ monomers by cleaving each of 3 Lys-Glu bridges into Lys+ and Glu-.
Should lower charge state complexes have a larger number of salt bridges (reflecting a greater number of opposing charges), we would predict that low charge state proteins would tend to be more compact, due to the additional restraints limiting their ability to expand when activated. We would expect that higher charge state complexes would be more likely than low to dissociate symmetrically, because they would have fewer ionic bonds to cleave. Because supercharging increases analyte charge, in part, by reducing opposite charges, supercharged complexes should release products with charge densities most similar to the precursor, as they should have the fewest ion pairs. All of these behaviors are observed experimentally. Moreover, collisional activation can add or rearrange salt bridges, to explain the unusual contractions observed by ion mobility for low charge states complexes.

Conclusions
Ramifications on the mechanism of ESI, charge state distributions, supercharging, and asymmetric/symmetric dissociation of noncovalent complexes are considered and found to explain many behaviors observed experimentally.

Novel Aspect
A new elaborated ion-evaporation mechanism for ESI explains many observations and provides explanations for asymmetric dissociations of noncovalent complexes.

ThOS38-03 Automated non-covalent mass spectrometry in drug discovery: improved screening of protein-ligand interactions

Rebecca Burnley[1], Hannah Maple[1], Olaf Scheibner[2], Maciej Bromirski[2], Mark Baumert[3], Mark Allen[3], Ricahrd Taylor[1], Rachel Garlish[1]

[1]UCB Celltech, [2]Thermo Fisher Scientific, [3]Advion Ltd

Introduction

Non-covalent or native mass spectrometry holds considerable promise for the routine screening of ligand libraries for binding to a target protein. Direct infusion of a protein sample in the presence of ligand from an aqueous buffer, using nano-electrospray ionisation and 'gentle' MS source conditions, allows detection of the protein-ligand non-covalent complex. In the context of drug discovery programmes, this technique can represent a fast, orthogonal technique for identification or validation of binding 'hit' compounds. Despite the attractiveness of this approach, there remain challenges with regards to the throughput, limit of detection of binding, and breadth of applicability to different samples. Here, the capabilities of an OrbitrapTM mass spectrometer for the analysis of protein-ligand interactions were investigated.

Method

Typically, a purified recombinant protein target at 10 µM is challenged with ligands at 10-100 µM in ammonium acetate, and analysed by nano-ESI. Observation of a peak, above a certain threshold, resulting from protein-ligand complex, indicates a 'hit'. Signal intensity is used to estimate relative abundance of different species, and ligand titrations can be used to estimate binding affinity. Here, model protein-ligand systems, as well as drug discovery targets, were analysed on the Exactive Plus EMR mass spectrometer, equipped with a TriVersa Nanomate chip-based device for automated sample introduction. Transport multipole and ion lens voltages, dissociation energies and pressure in the Orbitrap were optimised.

Results

Non-covalent interactions between proteins and ligands, with KDs from nM to mM, were retained as expected, in agreement with data from complementary techniques. Glycoforms of a highly glycosylated drug target could be baseline resolved, and binding of low MW fragments to this protein was observed. Challenging a protein with a mixture of ligands allowed those binding to be identified as hits.

The spectra obtained showed good spectral resolution and peak shape, resulting from efficient desolvation/ declustering. Additionally, sensitivity was found to be considerably improved compared to time-of-flight instruments.

Conclusion

Native MS can be used to identify hit compounds binding to target proteins. Furthermore, information regarding the stoichiometry, specificity and relative strength of the observed interaction can be obtained. The ability to achieve good spectral resolution for native MS of protein-ligand complexes increases the reliability of measuring the amount of binding, especially for low MW ligands and weakly binding ligands, as well as allowing more complex samples or mixtures to be analysed. This, together with improved sensitivity, can enhance the applicability and usability of native mass spectrometry for protein-ligand screening applications.

Novel Aspect

This automated MS platform improves quality, accuracy and sensitivity of the direct analysis of protein-ligand interactions.

ThOS38-04 Novel application of ion mobility and high resolution mass spectrometry to characterise ligand binding to a DNA aptamer

Chris Nortcliffe[1], David Clarke[2], Pat Langridge-Smith[2], Perdita Barran[1]

[1]University of Manchester, [2]University of Edinburgh

Introduction

Song et al1 outlined development of a DNA aptamer for the antibiotic kanamycin for use in testing of illegally treated milk. In their study they employ a gold nanoparticle assay to deliver a colour change when kanamycin is present. The DNA aptamer has a consensus sequence within it leading to a hairpin conformation. The aim of this work is to determine structural information about the kanamycin:DNA complex in the gas phase using a variety of MS techniques focusing on how the aptamer binds to the ligand.

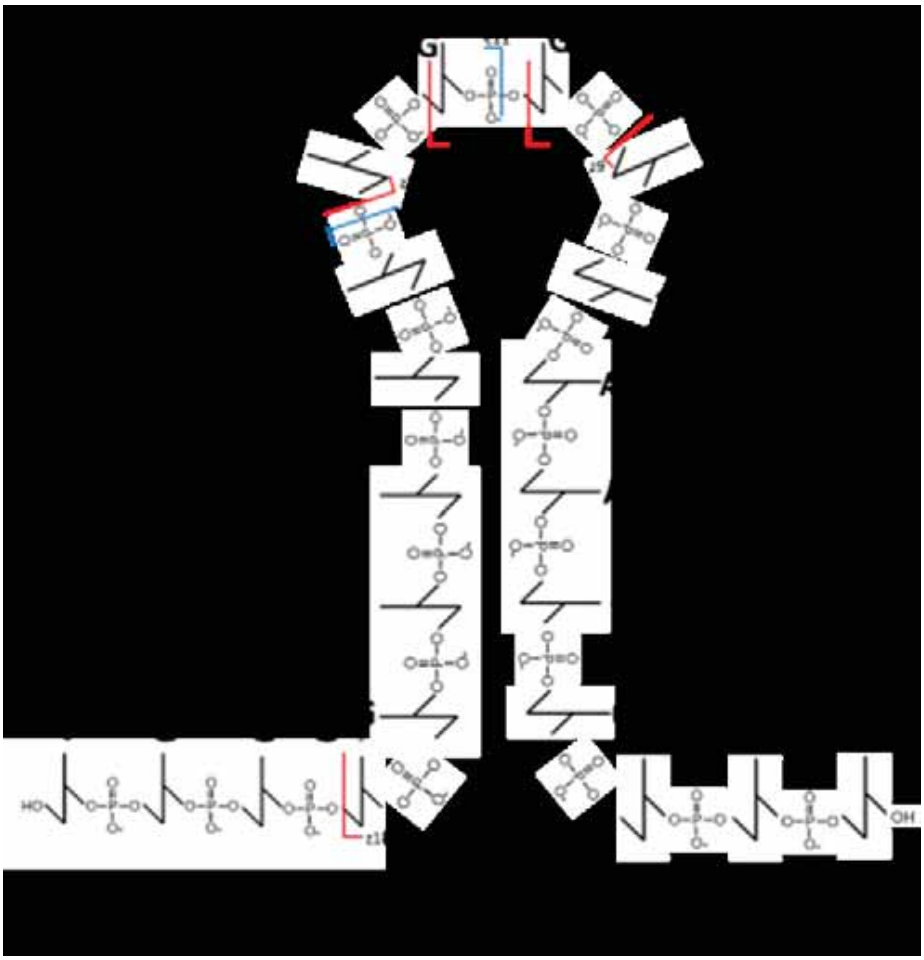
Methods

We here employ CID fragmentation using a 12T Bruker Solarix FT-ICR Mass Spectrometer. Two ion mobility MS techniques were utilised:, Drift time Ion Mobility on an in-house modified Q-Tof instrument, and Travelling Wave ion mobility on a Waters Synapt-G2. HDX-MS experiments were performed on a Waters Synapt-G2 S and Dynamx Software.

Results

CID fragmentation of the DNA aptamer provided excellent sequence coverage with most fragmentation sites being found in the hairpin fold. Fragmentation of the kanamycin: DNA complex showed more fragments of the bound complex associated with the 3' side of the DNA aptamer.

Ion Mobility provides complementary data on the Collision Cross Sections of the bound and unbound complexes with only a very small change in size upon binding supporting the theory that the structural change is minimal.



Conclusions
The combination of techniques provided complementary structural information. During fragmentation 7 fragments were observed with kanamycin still bound. Fragmentation mainly occurs in the hairpin region of the aptamer with 5 of the fragments on the 3' side of the molecule. This suggests that the interaction is stronger with the 3' side of the aptamer. The ion mobility data was carried out using both DT and TW Ion Mobility both of which gave complementary cross-sections. There is very little increase in cross-section upon binding of kanamycin implying there is not a large structural change and therefore the double stranded binding region is not interfered with.

Novel Aspect
Ion Mobility Mass Spectrometry has been applied to DNA systems before2–4, however not on a DNA aptamer system and not with a small molecule guest. Also the addition of High Resolution Top-Down fragmentation data and HDX provides a rich and varied base of information for obtaining structural information purely from MS techniques.

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ThOS38-05 UV-MALDI-MS analysis of non-covalent complexes with a 6-aza-2-thiothymine-matrix: effect of wavelength and fluence on the detection of the complexes

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Institute for Hygiene, Biomedical Mass Spectrometry, University of Münster

Introduction
The analysis of non-covalent complexes by MALDI-MS poses a challenge. Factors that all can result in their unintended dissociation are the acidic character of most MALDI matrices, the requirement of analyte-matrix co-crystallization and the thermal load during the overall MALDI-MS process. Best results are frequently obtained with the near pH-neutral 6-aza-2-thiothymine (ATT) matrix. Interestingly, this compound exhibits a peak absorption close to 265 nm, far off the standard MALDI wavelengths of 337 and 355 nm. Here, we investigated the influence of the laser wavelength in the range of 213-380 nm and that of the laser fluence on the ion generation. The data reveal distinct regions in which maximum intensities of the non-covalent complexes are obtained.

Methods
Human gastrin I (Pyr-GPWLEEEEEAYGW) and either kemptide (LRRASLG) or a protein kinase C substrate (VRKRTLRRL) were used as receptor-ligand systems to form non-covalent peptide/peptide complexes. Samples were prepared from aqueous solutions using the dried droplet method. MS experiments were performed with an oTOF-mass spectrometer that is equipped with an optical parametric oscillator laser (OPO; versaScan, GWU-Lasertechnik) providing tunable laser light with 5 ns pulse duration. The elliptical laser spot size on the target was ~350x700 µm². Ion signals were recorded for excitation wavelengths between 213-380 nm (step size: 10 nm) and by varying the laser fluence. In addition, the formation of streptavidin and biotinyl-glucagon complexes was studied at distinct laser wavelengths.

Results
The wavelength course of the threshold fluences for the total ion count (including analyte- and matrix-derived molecular and fragment ions) follows the inverse of the optical absorption curve. Consequently, the lowest threshold fluences and highest overall ion yields were found close to the optical peak absorption of about 265 nm. In contrast, for non-covalent complexes optimal ion yields (relative to the TIC or the monomers) are obtained in narrow wavelength-fluence bands that are found along the declining slopes of the absorption curves of the ATT matrix around 240 and 330 nm respectively. The major reason for this finding is probably the reduced thermal energy content in the MALDI plume at these excitation conditions. The Streptavidin-Biotin complex shows a weaker dependence on the wavelength. Probably this is a result of the high affinity (dissociation constant ~10-15 M). To our knowledge this is the first detection of an intact complex of Streptavidin and Biotin with UV-MALDI-MS.

Conclusion
For non-covalent peptide/peptide complexes optimal MALDI conditions are found if the ATT matrix is excited at wavelengths of low matrix absorptivity. Therefore overall data provide important hints on how MALDI mass spectrometry of non-covalent complexes can be optimized.

Novel Aspects
First comprehensive study investigating the wavelength-fluence dependence of MALDI-MS with an ATT matrix for detection of specific non-covalent peptide-peptide complexes. First intact MALDI-MS detection of a complex of streptavidin and biotin.

ThOS39 - Informatic Tools for MS

Room 4
Level 0

Chairs: Thomas Hankemeier, Ruedi Aebersold

ThOS39-01 **Keynote: Highly sensitive feature detection for LC-MS-based metabolimics**
Oliver Kohlbacher
University of Tübingen

LC-ESI-MS permits a quick and cost-effective screening for metabolitesand is increasingly becoming popular in clinical applications.
Sensitive and reliable detection as well as accurate quantification of metabolites is required in particular for large-scale studies. We present a novel algorithm for quantifying small molecule features
[Kenar et al., MCP, 2014, 13(1):348-59]. It combines a sensitive mass trace detection with an efficient feature assembly algorithm based on a machine-learning model for recognize metabolite isotope cluster.
Compared to other algorithms, we can demonstrate a higher sensitivityand excellent linearity of the quantification.
The algorithm is available as part of the OpenMS software package. It has recently been integrated into Thermo Fisher's Compound Discoverer platform.

ThOS39-02 **Illuminating the 'dark matter' of mass spectrometry**
Steve Stein[1], Gary Mallard[1], Xinjian Yan[1], John Halket[2]
[1]NIST, [2]King's College

Introduction
The identities of a large fraction of components observed in mass spectrometric analyses of complex materials are unknown. This lack of information is a concern for 'discovery'-type studies where not all analytes of interest are known in advance (metabolomics, for example). We present a means of dealing with this problem by building spectral libraries derived from unidentified spectra. In this approach, spectra from a material are collected, clustered and processed to generate a library of good quality consensus spectra of unidentified species. This library can re-identify these components in other studies as well as store the spectra for later analysis and identification. Applications of this method to both GC/MS and LC/MS-MS are presented.

Methods
GC/MS: Methods were developed with the unidentified spectra from the widely-used AMDIS program applied to over 5,000 TMS-derivatized pediatric urine samples. Low quality spectra were rejected by a variety of filters, including retention index matching and occurrence in two columns of different polarity.
LC/MS: The NIST 'Proms' progam perceived, quantified and annotated co-eluting ions as adducts, fragments, contaminants and alternate charge states. Spectra of co-eluting ions related by their precursor m/z (adducts and fragments) were annotated. Precursor ion purity and signal strength were among the filters used to reject low quality spectra.
a) Proteomics: Spectra not identified as peptides in shotgun proteomics studies of tryptic digests of tumors (from NIH/NCI/CPTAC experiments) served to create libraries of unidentified spectra.
b) Metabolomics: Spectra of e-coli metabolites that did not match the NIST MS/MS library were used to create libraries.

Results
GC/MS: A library of nearly 400 good quality unidentified spectra was created. This include common derivization artifacts, patient-specific drug metabolites and hundreds of apparently endogenous metabolites. The library re-identified over five times the number of spectra as used for building the library.
LC/MS (Proteomics): Ion-trap spectra from three highly-fractionated tumor analyses were used to build a spectral library of 3,806 unidentified ions. About 10% of these were identified with alternate search engines and some 1,000 of them were found in similar tumor samples.
LC/MS (Metabolomics): A recurrent library of some 2,000 e-coli metabolite spectra was generated. The number of recurrent spectra was over five times the number of identified ions. Annotation was added to spectra whose precursor m/z and expected major fragments matched known, but unidentified metabolites.

Conclusions
Libraries of good quality, unidentified spectra show promise as a general method for collecting, reusing and identifying the 'dark matter' of GC-MS and LC-MS/MS.

Novel Aspect
Building and using libraries of recurring, unidentified spectra in LC/GC-MS experiments.

ThOS39-03 enviMass 2.0 – A workflow for fast spill and trend detection of micropollutants in aquatic systems using LC-HRMS data
Martin Loos, Matthias Ruff, Juliane Hollender, Heinz Singer
Eawag, Swiss Federal Institute of Aquatic Science and Technology

Introduction
Organic trace contaminants have become increasingly detectable in river systems, posing potential risks to humans and biota. This rise can be attributed to both a steady increase in the emission of anthropogenic substances and an advance in their detection from new analytical methods. Consequently, a number of river monitoring stations have been equipped with liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) as a method of choice to comprehensively detect micropollutants and their transformation products. However, analysis has largely focused on compounds known or suspected to occur. Moreover, no automatisation for the extraction of temporal intensity profiles and the detection of critical temporal trends has been specified. Finally, little use has to date been made of the information in such profiles to group LC-HRMS signals of the same compound or to uncover common temporal patterns.

Methods
A data mining strategy is proposed to reveal concentration changes of micropollutants caused by riverine spills and discharge events. First, a novel algorithm is used to robustly extract ion chromatograms and peaks from temporal sequences of LC-HRMS data. Following steps of mass recalibration, retention time alignment and a first quality control (QC), a swift clustering technique is applied to extract temporal intensity profiles from these peaks. The profiles of internal standard compounds are then utilized for an accurate intensity normalization and a second QC filter. Benefitting from a new isotope pattern calculator, these normalized profiles are subsequently (1) screened for known and suspected micropollutants, (2) annotated for their presence in blank measurements and (3) grouped for isotopologues, adducts and homologue series, as aided by the intensity correlation between profiles. Discriminatory profiles from these groups are finally ranked for critical trends and aggregated.

Results
The data mining strategy has been tested on a unique data set of >600 LC-HRMS measurements of Rhine samples, revealing trends of international concern that would have remained undetected by targeted analysis alone. We present case studies for production intermediates, pharmaceuticals and solvents of industrial origin. The workflow is freely available (R-package enviMass v2.0) and provides an user-friendly interface.

Conclusion
Given high-frequency LC-HRMS monitoring data, the implemented workflow identifies critical temporal trends of riverine micropollutants. While hinting at common anthropogenic pollutant sources and emission patterns, this fully automatized workflow prioritizes unknowns for subsequent identification.

Novel aspects
enviMass provides an overdue tool to reveal critical micropollutant dynamics at the short time frames needed to react upon emissions, incorporating a joint assessment of known and unknown compounds. Adapted to complex matrices and large data sets by a multi-stepped data reduction, the information redundancy inherent to LC-HRMS data is minimized for this task.

ThOS39-04 MassTrees to study the evolution of the influenza virus and detect antiviral resistant strains
Kevin Downard, Kavya Swaminathan
University of Sydney

Introduction
Phylogenetic trees represent a mainstay in biology to display and infer the evolutionary relationships among various biological species based upon similarities or differences in their physical and/or genetic characteristics. A full phylogenetic analysis requires the comparison of a wide range of gene sequences including the presence or absence of particular genes. In the case of the influenza virus, reassortment processes result in progeny viruses that contain genes with different evolutionary histories. This necessitates a large amount of gene sequencing.
We have conceived and implemented a new approach and algorithm with which to construct phylogenetic trees using numbers (i.e. masses) rather than letters or words (i.e. sequences). Lists of peptide masses can be used to construct phylogenetic trees and these "mass trees" used to trace the evolutionary history of organisms. This affords a significant time saving given that mass spectral data of protein digests can be recorded within a fraction of a second.

This presentation will describe the basis and advantages of mass trees, demonstrate that they are congruent with sequence based trees, and illustrate their potential for studying the evolution of the influenza virus and identifying antiviral resistant strains.

Methods
Translated, non-redundant full-length neuraminidase gene sequences were obtained from the NCBI Influenza Virus Resource Database and the FluSim algorithm was used to generate theoretical monoisotopic masses for protonated tryptic peptide ions across each sequence. The MassTree algorithm was used to generate phylogenetic trees from the sets of mass values using a mass error tolerance of 5ppm and applying a distance score to compute the number of matching mass values within each set. The trees were mid-point rooted, viewed and coloured with Archaeopteryx software. Experimental mass spectral data was fit onto such trees and the mass trees and conventional sequence trees were compared using the Compare2Trees and MAST algorithms.

Results
Mass trees were employed to study the evolution of influenza neuraminidase across all subtypes (N1-N9) in human and animal hosts. The mass trees were shown to be congruent with sequence based trees and correctly charted the evolutionary history of 2009 human pandemic influenza viruses of animal origin. The trees were also shown to be able to resolve antiviral resistant from sensitive strains. Furthermore, experimental mass map data recorded for a circulating strain is correctly positioned onto a mass tree so as to quickly establish its evolutionary history and identify whether it is resistant or sensitive to the antiviral inhibitor oseltamivir.

Conclusions
Mass trees offer a viable alternative with which to characterise and chart the evolution of influenza viruses without gene or protein sequencing. They are expected to find wider application for evolutionary studies of organisms more generally.

Novel Aspect
Charting the evolutionary history of organisms with mass data obtained by mass spectrometry without the need for gene or protein sequencing.

ThOS39-05 Spectviewer, a software for mass spectrometry imaging

Jean-Pierre Both[1], Maxence Wisztorski[2]
[1]CEA, [2]Université Lille 1

The talk will be devoted to describe the evolution of the software Spectviewer dedicated to mass spectrometry imaging. It was developed first during a European project (COMPUTIS) and then during a project funded by the French National Agency for Research. I will describe the main aspects of the software covering the data treated by the software: Maldi-Tof in Analyze format, Tof-Sims data in the export file format of Ion-Tof (.grd), Maldi Orbitrap and imzMl format initiated during the Computis project.
The software can do peak extraction and deisotoping on the whole image or on region of interest (ROI) After peak extraction on the whole data set data is compacted on a reduced peak list image and peaks exported in a csv file. Then various methods to gain insight into the structure of the images, such as correlation analysis between peaks images of pixel spectra or by clustering. Data can be clustered by grouping pixels in spatial components (spectral proximity) or by grouping peaks which are colocalized. Some examples of clustering of large data files (several Gb) will be illustrated and how it can help in the extraction of small features.
The last and new developments concern proteins identification with peak extraction on ROI. The context of the study is the impact of benzalkonium chloride on rabbit eye. I will show some preliminary results of direct protein and peptide identification on the fly on the ms imaging data by direct coupling with fasta files. Comparison with results obtained by LCMS/MS in rabbit lens will be discussed and some results obtained near the optical nerve will be shown were biological signification of proteins involved can comfort identification. The unification of chromatogram processing and mass imaging data processing will be illustrated.

ThOS40 - 2D and 3D Analysis and Imaging of Inorganic, Organic, and Biological Materials

Room 5
Level 3

Chairs: John Vickerman, Detlef Günther

ThOS40-01 Keynote: 2D and 3D imaging of inorganic, organic, and biological samples

John Fletcher
Department of Chemistry and Molecular Biology, University of Gothenburg, Sweden

Secondary ion mass spectrometry (SIMS) provides the unique capability of chemical imaging at high spatial resolution in combination with extremely high surface sensitivity. The (primary) ion beams used to probe the sample surface can be focused to produce sub-micrometre spot sizes and the gradual erosion of the sample by the ion beam can be used to monitor the chemical composition of the target as a function of depth – depth profiling.1 Historically there have been two drawbacks related to SIMS analysis of organic, including biological, samples.

1. Low sensitivity to higher mass (> 200 Da.) molecular species
2. An inability to generate depth profiles of molecular species.

The introduction of cluster and polyatomic ion beams, especially C60, provided a route to overcoming these drawbacks creating a whole new discipline of molecular depth profiling and 3D molecular imaging.2-3 The introduction of C60 inspired the development of new instruments capable of fully exploiting the properties of these beams.4
Despite the progress made with the introduction of C60 there is a continuing need for improved sensitivity and recently new avenues have been explored further improving the capabilities SIMS. A particular area of advance has been the implementation of gas cluster ion beams (GCIBs). GCIBs were originally developed for semi-conductor surface processing but have been adapted for use as ion beams in SIMS, predominantly for use as etching guns in dual beam depth profiling experiments where a different ion beam is used for analysis.5-7

A prototype, high energy (40 keV) GCIB has recently been tested on our J105 – 3D Chemical Imager instrument (Ionoptika Ltd). The use of higher energy gas cluster ions resulted in an increase in higher mass signal from biological samples with improved imaging capability. The state of the art in terms of technological developments for SIMS will be described and the application of these new advances will be demonstrated for 2 and 3D molecular imaging of biological samples on a cellular scale.
The outlook for future developments and applications of SIMS for biological imaging will also be discussed.

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ThOS40-02 **Cellular scale imaging of cancer drugs using a stigmatic MALDI imaging mass spectrometer**

Hiroki Kannen[1], Hisanao Hazama[2], Jun Aoki[2], Michisato Toyoda[2], Tatsuya Fujino[3], Yasufumi Kaneda[2], Kunio Awazu[2]
[1]Osaka univirsity, [2]Osaka University, [3]Tokyo Metropolitan University

Introduction

Recently, the combined therapy using chemotherapy and photodynamic therapy (PDT) has been proposed for anticancer resistant cancer cells. PDT is a less-invasive cancer treatment using a photosensitizer selectively accumulating in a tumor tissue and a light source for excitation of the photosensitizer. The distributions of these drugs are important to evaluate the efficacy of the combined therapy. However, in conventional scanning IMS using matrix-assisted laser desorption/ionization (MALDI), the spatial resolution is limited by the laser spot diameter to about 10 µm. Therefore,

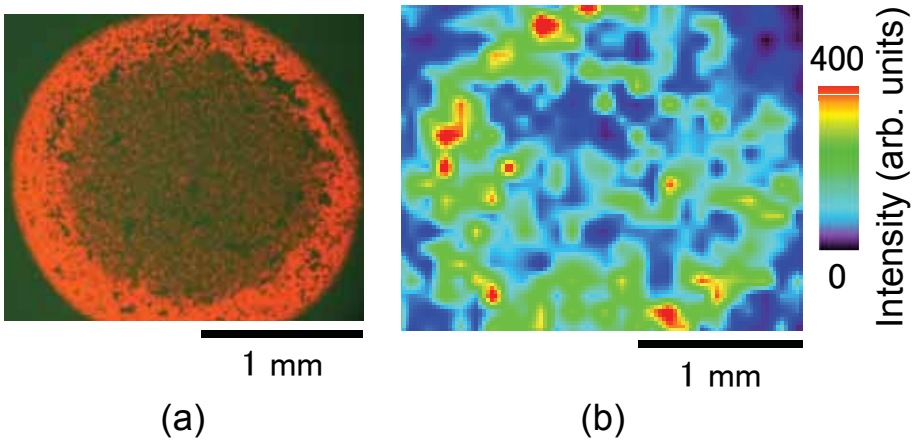
we are investigating cellular scale imaging of the cancer drugs using stigmatic MALDI IMS to observe the distributions of multiple drugs simultaneously. In this research, sample preparation methods for stigmatic IMS of cancer drugs have been investigated.

Materials and Methods

PC-3 cells were used as cancer cells, and protoporphyrin IX (PpIX) and docetaxel were used as the photosensitizer and the anticancer drug, respectively. In the sample preparation for imaging PpIX administered in the PC-3 cells, PpIX was administrated at a concentration of 10 mM. A suspension of the cells was spotted on a glass slide coated with indium tin oxide and gold. Then, a matrix, a-cyano-4-hydroxycinnamic acid was spray-coated onto the cells. In the sample preparation for imaging docetaxel, docetaxel was administrated to the PC-3 cells at a concentration of 50 mM. The mixture of 6-aza-2-thiothymine (ATT) and zeolite (NaY5.6) at the same weight ratio was used as a zeolite matrix (ATT/NaY) and spotted on the PC-3 cells. Mass spectra and ion images were obtained using a time-of-flight mass spectrometer (Voyager-DE-PRO, Applied Biosystems).

Results

Figure 1 shows a fluorescence microscope image and a mass spectrometric image of PpIX obtained from a dried droplet of the suspension of the PC-3 cells administered PpIX, and PpIX was successfully detected from the PC-3 cells. For ionization of docetaxel, the signal intensity of docetaxel increased to about 13 times by mixing the zeolite with the organic matrix ATT. Moreover, docetaxel at a concentration of 1 mg/mL was detected using the zeolite matrix and was not detected using ATT only. As the next step, ion images of docetaxel will be observed by spray-coating of the zeolite matrix.



Conclusion

An image of the photosensitizer, PpIX, was successfully obtained from the PC-3 cells. Docetaxel administered into the PC-3 cells were detected using the zeolite matrix in the droplet method. Cellular scale IMS will be performed using a stigmatic imaging mass spectrometer, MULTUM-IMG2, developed in the Osaka University.

ThOS40-03 **High resolution MALDI imaging of tryptic peptides in fresh frozen and FFPE tissue**

Katharina Huber[1], Pegah Khamehgir[2], Bernhard Spengler[2], Andreas Römpp[2]
[1]Justus Liebig University Giessen, [2]Justus Liebig University

Introduction

Mass spectrometry imaging is the method of scanning a sample of interest and generating an image of the intensity distribution of a specific analyte signal. The direct detection and identification of intact proteins in MS imaging is difficult due to limited mass range and fragmentation efficiency. On-tissue digestion of proteins and detection of resulting peptides can overcome these limitations. We focus on optimizing the spatial resolution and reliability of peptide identification. In the current study we have adapted our protocol to formalin fixed paraffin embedded (FFPE) tissue.

Methods

A series of washing steps was applied to tissue sections for fixation and to remove salts and lipids. Different deparaffinization and antigen retrieval steps were evaluated for FFPE tissue sections. Subsequently trypsin solution and matrix (dihydroxybenzoic acid) were deposited on the tissue with a home-built spraying device. MS images with 25 to 50 µm pixel size were acquired with an atmospheric pressure scanning microprobe matrix assisted laser desorption ion source coupled to a Q Exactive mass spectrometer (Thermo Fisher Scientific GmbH, Bremen) [1].

Results and conclusion

Tryptic peptides were identified by matching imaged m/z peaks to peptides which were identified in complementary LC-MS/MS measurements of an adjacent tissue section. All MS measurements were based on accurate mass (< 3 ppm RMS).A coronal mouse brain section was measured at 50 µm pixel size. Peptide peaks were detected on tissue with a mass resolution of R=80000 (@m/z700). Spatial features which consist of only one to two cell layers (ependyma) were detected. The sensitivity could be significantly improved compared to previous experiments and 450+ tryptic peptides corresponding to 100+ proteins were identified. Multiple peptides (up to 20) which show the same spatial distribution were detected for each protein.

A whole body section of an infant mouse and human gastric cancer biopsy sections were imaged at a spatial resolution of 50 µm. This resulted in highly resolved MS images, which indicates that our protocol is applicable for a wide variety of tissue types.

In attempt to further increase spatial resolution, a coronal mouse brain section was imaged at a pixel size of 25 µm. The resulting ion images of tryptic peptides showed excellent correlation with myelin and H&E staining. A FFPE horizontal mouse brain section was imaged with a spatial resolution of 50 µm and yielded highly detailed MS images of tryptic peptides. Results in terms of spatial information and number of identified proteins were comparable to fresh frozen tissue samples.

[1] Römpp and Spengler (2013) Histochemistry & Cell Biology 139 (6):759-783.

New aspects

Reliable identification of tryptic peptides at 25 to 50 µm pixel size for wide variety of tissue types (including FFPE tissue).

ThOS40-04 **Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry**

Bernd Bodenmiller[1], Charlotte Giesen[1], Hao Wang[2], Denis Schapiro[1], Andrea Jacobs[1], Bodo Hattendorf[2], Peter Schueffler[2], Daniel Grolimund[3], Joachim Buhmann[2], Simone Brandt[4], Zsuzsanna Varga[4], Peter Wild[4], Detlef Günther[2]
[1]University of Zurich, [2]ETH Zurich, [3]PSI, [4]University Hospital Zurich

Introduction

Tissues are complicated assemblies of multiple interacting cell types that communicate with each other to achieve physiological states. In cancer, malignant cells and cells of the tumor microenvironment (TME) facilitate tumor reprogramming, progression, and drug resistance. For an understanding of the underlying processes it is important to comprehensively investigate the components and their relationship within the TME. This necessitates imaging approaches that can simultaneously measure dozens of biomarkers to define cell types, their functional and signaling states, and spatial relationships.

Methods

For highly multiplexed tissue imaging at subcellular resolution, we have coupled immunohistochemical (IHC) methods with high resolution laser ablation and mass cytometry. In mass cytometry, rare earth metals are used as reporters on antibodies. Analysis of metal abundances using the mass cytometer allows determination of biomarker expression. In the approach presented here, tissue sections were prepared for antibody labeling using IHC protocols. Rare-earth-metal isotope tagged antibodies were selected to target proteins and protein modifications relevant to breast cancer. After antibody staining, the sample was positioned in a laser ablation chamber developed by Wang et al. (1) to minimize aerosol dispersion for high-resolution, high-throughput and highly sensitive analyses. The tissue was then ablated spot by spot, and the ablated material was transported by a mixed argon/helium stream to the CyTOF mass cytometer. After data preprocessing, the 32 transient, isotope signals were plotted using the coordinates of each single laser shot, and a high-dimensional image of the sample was generated. Single-cell features were computationally segmented and the single cell marker expression data were extracted for downstream bioinformatics analyses.

Results

Imaging mass cytometry provides high-dimensional analysis of cell type and state at subcellular resolution to study tissues and adherent cells. The novel imaging approach enabled the simultaneous visualization of 32 proteins and protein modi¬fications, with the potential to map up to 100 markers on a single tissue section with the availability of additional isotopes. Application of imaging mass cytometry to breast cancer samples allowed delineation of cell subpopulations

and cell-cell interactions, highlighting tumor heterogeneity and enabling new routes to patient classification. As such it has the potential to yield novel insights of the TME by exploiting existing large collections of FFPE tumor samples and associated clinical information.

Conclusions
Imaging mass cytometry complements existing imaging approaches and will support the transition of medicine toward individualized molecularly-targeted diagnosis and therapies.

Novel Aspect
Highly multiplexed imaging of tissue biomarkers at subcellular resolution.
(1) Wang HA et a. Anal Chem. 2013 Nov 5;85(21):10107-16.

ThOS40-05 Inspecting the anticancer drugs cisplatin and NKP1339 in tissue sections by LA-ICP-MSI

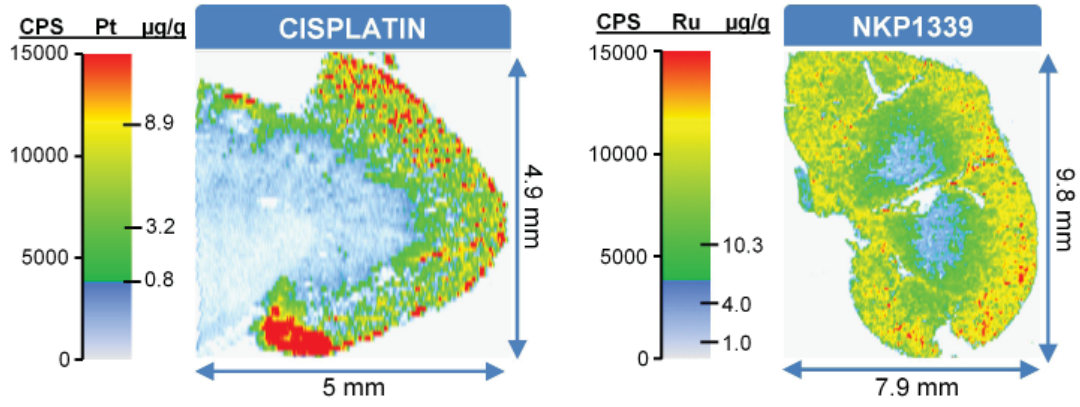
Alexander Egger[1], Christoph Kornauth[2], Sarah Theiner[3], Petra Heffeter[2], Günther Bayer[2], Werner Haslik[2], Bernhard Keppler[3], Robert Mader[2], Christian Hartinger[4]
[1]Austrian Drug Screening Institute GmbH, Innsbruck, [2]Medical University of Vienna, [3]University of Vienna, [4]University of Auckland

Introduction
Platinum-based anticancer drugs such as cisplatin or oxaliplatin are invaluable in cancer treatment. However, they may cause severe necrosis in the case of extravasation, thus requiring surgical intervention. So far, Pt distribution in damaged tissue has not been investigated and knowledge thereof may improve patient care.
Additionally, developing a new first in class anticancer drug, to supplement the spectrum of therapeutical options, resulted in promising investigational anticancer compounds based on ruthenium, such as sodium trans-[tetrachloridobis(1H-indazole) ruthenate(III)] (NKP1339).
Laser ablation inductively coupled plasma mass spectrometry imaging (LA-ICP-MSI) is a powerful analytical tool to visualize metal-based compounds in tissue section. Thus, it was applied

- to determine the spatially-resolved Pt distribution in resectates originating from Pt-based extravasation in clinical anticancer treatment.
- to compare the biodistribution of cisplatin and KP1339 in viscera obtained from an animal experiment.

Methods
We report careful method development, which included the preparation of matrix-matched calibration standards, independently prepared control samples, the comparison of internal standards such as 115In or 13C and the use of solution-based ICP-MS to validate quantitative data obtained by LA-ICP-MSI.
Subsequently, mice were treated with NKP1339 (50 mg/kg for 18 h) or cisplatin (15 mg/kg for 24 h) to determine spatially-resolved organ distribution (quantitatively) in tissue sections of liver, kidney, spleen and muscle.
The clinical samples were obtained from patients receiving cisplatin or oxaliplatin and required surgical intervention due to extravasation of the drug. The Pt concentration in tissue was determined in a combined manner: by solution based ICP-MS for quantification and by LA-ICP-MSI for recording biodistribution in various tissue types.
(Stained) tissue sections supported interpretation of the elemental distributions.

Results
Ruthenium and platinum were enriched in the cortex of the kidney in comparison with the medulla, whereas homogeneous distribution of both compounds could be observed in liver and muscle. In spleen, areas of high and low concentrations of the metal were recorded.
Regarding the patient samples, the outstanding sensitivity of LA-ICP-MSI allowed tracking Pt in connective tissue, fatty tissue, muscle tissue and nerve tissue at low concentrations up to four weeks after application of the drug.



Conclusions and Novel Aspects
NKP1339 and cisplatin exhibit similar distribution patterns in kidney, though their side effects in clinical use differ: NKP1339 is well tolerated, whereas cisplatin is nephrotoxic. Consequently,prediction of side effects solely by metal/drug distribution is limited.

SE03 - Conference Dinner

Bâtiment des Forces Motrices (www.bfm.ch)

FOS41 - Gas-Phase Ion Fragmentation Mechanisms

Room 1
Level 1

Chairs: Gianluca Giorgi, Leopoldo Ceraulo

FOS41-01 **Keynote: Surface-induced dissociation: characterization of an activation method for large complexes**

Vicki Wysocki, Royston Quintyn, Yang Song, Jing Yan, Lindsay Morrison
Ohio State University

Introduction

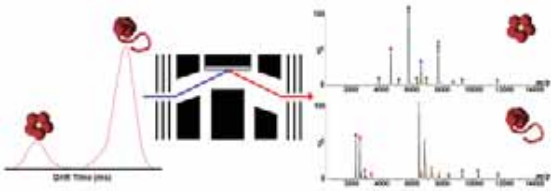
Surface-induced dissociation is an activation method that effectively fragments large protein and nucleoprotein complexes. The method involves collision of ions into a target surface, typically a fluorocarbon or hydrocarbon self-assembled monolayer of alkanethiol on gold. More thorough characterization of its capabilities is desirable in this time period after its initial potential has been realized. In the work presented here, the influence of source activation and overall charge is examined for a number of complexes with difference structural features (charge density, interface area, subunit flexibility), building from CID results reported by the Robinson group.[1]

Methods

Experiments are performed on a Waters Synapt G2 or G2S QTOF with ion mobility. Concentrations are typically 1-20 uM of complex in 10-200 mM ammonium acetate. triethyl ammonium acetate is used to reduce charge and m-nitrobenzyl alcohol is used for supercharging. A surface-induced dissociation device is available both before and after the traveling wave ion mobility cell. CID and SID can be compared under the same source conditions.

Results

Data for a number of dimers (with and without reduced disulfide bonds), tetramers, pentamers and hexamers have been collected as a function of source activation and charge state. Ion mobility is used to characterize collision cross sections of precursor and product ion structures. Although CID spectra of source-activated ions are very similar regardless of extent of source activation, consistent with monomer unfolding that is typical in CID results, SID, in contrast, provides unique spectra for ions of a given source activation. Collapsed structures are not necessarily folded – SID spectra indicate unfolding of one monomer may occur even in compact "collapsed" structures. Charge is also an important determinant of structural characterization by SID, with better substructure characterization obtained for SID reduced charge complexes in many cases.



Conclusions

Surface-induced dissociation provides excellent characterization of protein and nucleoprotein complexes, especially when cone voltage and precursor charge state are kept low

Novel Aspect

SID spectra provide substructure and are shown to be sensitive to structural changes induced by source CID or by unfolding assisted by high charge.

[1] Hall, Z., Hernández, H., Marsh, Joseph A., Teichmann, Sarah A., Robinson, Carol V.: The role of salt bridges, charge density, and subunit flexibility in determining disassembly routes of protein complexes. Structure. 21, 1325-1337 (2013)

FOS41-02 **CO_2 incorporation in hydroxide and hydroperoxide containing water clusters - unifying mechanism for hydrolysis and protolysis**

Einar Uggerud, Mauritz Ryding
Department of Chemistry, University of Oslo

Introduction

The temperature and pH dependent distribution of CO2 between the gaseous and aqueous states is critical for such diverse functions as cell respiration and the climate regulating balance between the amount of CO2 present in the atmosphere and dissolved in the oceans. Measurements1 have indicated that the reaction CO2(aq) + OH−(aq) ↔ HOCO2−(aq) is the dominating one at higher pH. The purpose of this work is to better understand the mechanism of this reaction at different levels of hydration.

Methods

CO2 was reacted with size-selected cluster ions, OH−(H2O)n (n = 2–12). For comparison purposes, the same measurements were performed on HO2−(H2O)n. The experiments were performed using a quadrupole–time-of-flight mass spectrometer (QTOF2, Micromass/Waters). The clusters were produced using ESI at atmospheric pressure and room-temperature. A quadrupole mass filter selected a single cluster type, which then reacted with CO2 (approx.

1.0×10−5 mbar) in the collision cell. Products were analysed in the TOF unit. The experimental measurements were supported by quantum chemical calculations (QCC) at the B3LYP/6-311++G(2d,2p) and Gaussian-4 theory compound method levels.

Results
The OH−(H2O)n clusters were found to react faster with CO2 for n = 2,3, while for n > 3 the HO2−(H2O)n clusters are more reactive. Insights from QCC revealed a common mechanism in which the decisive product-forming step of n > 2 clusters starts from a pre-reaction adduct where OH− and CO2 are separated by a H2O or H2O2. A proton transfer from the separating molecule to OH− then effectively moves the core ion motif next to the CO2 molecule. A covalent bond is formed between CO2 and the emerging new core ion in concert with the proton transfer. For larger clusters, additional H2O in-between OH− and CO2 requires successive proton transfers to bring about formation of the pre-reaction complex, after which the product formation is accomplished according to the concerted mechanism.

Conclusions
The higher reactivity of HO2−(H2O)n vs OH−(H2O)n for n > 3 is likely a consequence of the unfavourable kinetics associated with a multistep mechanism.

Novel Aspect
A novel mechanism is suggested for the reaction OH− + CO2, also applicable to bulk water and thereby to CO2 uptake in oceans. Furthermore, this mechanism avoids intermediate H2CO3 by combining the CO2 hydrolysis step and the protolysis step into one. The general mechanistic picture is consistent with low enthalpy barriers and that the limiting factors are largely of entropic nature.

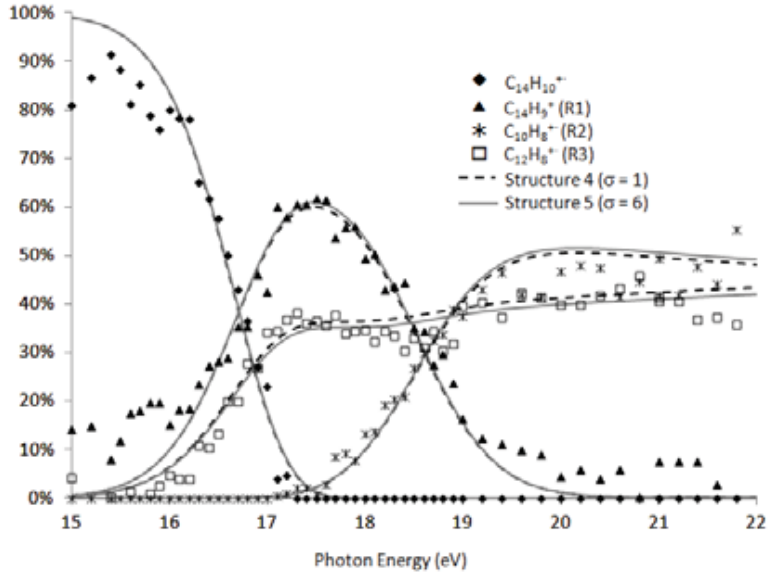
References
1. B. R. W. Pinsent et al., T. Faraday Soc., 1956, 52, 1512-1520.

FOS41-03 Post-collision internal energy distributions and PAH ion fragmentation

Paul Mayer, Brandi West, Alicia Sit, Sabrai Mohammad
University of Ottawa

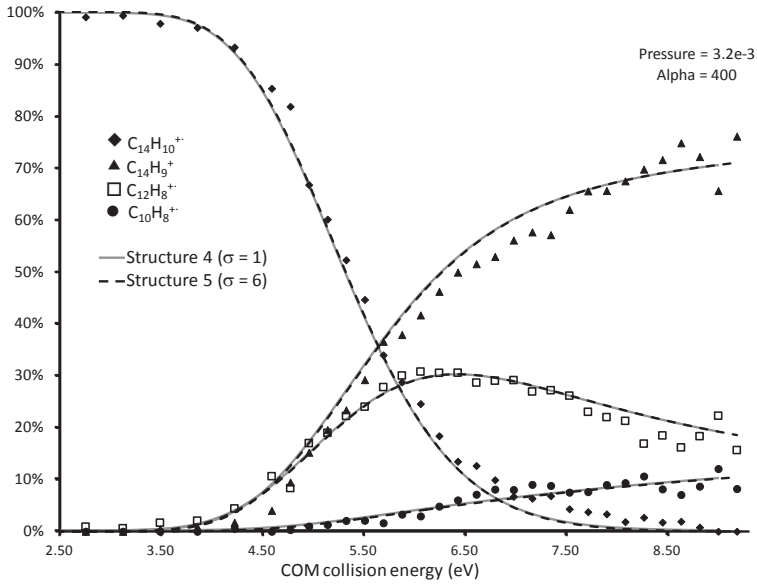
The dissociation of the naphthalene, anthracene and pyrene radical cations has been studied using two different methods; imaging photoelectron photoion coincidence spectrometry (iPEPCO) and atmospheric pressure chemical ionization – collision induced dissociation mass spectrometry (APCI-CID). We have previously published the iPEPICO results for napthalene and pyrene, and this is the first report of the iPEPICO results for anthracene. Two competing reactions were investigated in each case, H loss and C2H2 loss. For ionized anthracene, an attempt was made to assign structures to each fragment ion, although there is still room for debate for the structure of C12H8+· (between cyclobuta[b] naphthalene and biphenylene ion structures) which is discussed in the paper. The results from the iPEPICO fitting of the dissociation of ionized anthracene are: E0 = 4.28 ± 0.30 eV (-H) and 4.20 ± 0.30 eV (-C2H2) while the Δ‡S values (in J K-1 mol-1) are 12 ± 15 (-H) and either 7 ± 10 (using cyclobuta[b]naphthalene ion fragment in -C2H2) or 22 ± 10 (using the biphenylene ion fragment in -C2H2). Modeling of the APCI-CID breakdown diagrams required an estimate of the post-collision internal energy distribution, which was somewhat arbitrarily assigned in this study to a Boltzmann distribution (effective temperature) that increases with centre-of-mass collision energy. One goal of this work was to determine if such a distribution would satisfactorily reproduce the iPEPICO results. In then end, it did, with the APCI-CID results being similar: the E0 (in eV) values are 4.55 ± 0.10 (-H) and 4.18 ± 0.30 (-C2H2) while the Δ‡S values (in JK-1mol-1) are 13 ± 2 (-H) and either -1 ± 6 (using cyclobuta[b]naphthalene for -C2H2) or 12 ± 3 (using biphenylene for -C2H2). Agreement between the iPEPICO and APCI-CID results for all three ions will be discussed in light of the simple approximation made to the post-collision internal energy distribution.

iPEPICO breakdown diagram and fitting illustrating the effect of using structure 4 (black broken line) versus structure 5 (solid grey line) for the calculation of $C_{12}H_8^{+\bullet}$.



Friday

APCI/CID breakdown diagram and fitting illustrating the effect of using structure 4 (black broken line) versus structure 5 (solid grey line) for the calculation of $C_{12}H_8^{+\bullet}$. Pressure and corresponding alpha value are also given.



FOS41-04 **Peptide fragmentation: energetics, structures, and mechanisms**

Peter Armentrout
Department of Chemistry, University of Utah

Introduction

A wide range of experimental and theoretical studies are providing information about the mechanisms by which cationized peptides fragment, which is particularly valuable for protein sequencing applications. A detailed understanding of the mechanisms requires the energetics that control competition between channels. Theory predicts such energetics, but few experimental studies yield accurate absolute values. We utilize guided ion beam tandem mass spectrometry (GIBMS) to acquire the energetics of peptide fragmentations and have provided benchmark thermochemistry for assessment of theoretical mechanisms. In this talk, I will emphasize our results for the protonated tripeptides, GGG, GGA, and GAG. The combination of accurate reaction energies and quantum chemical calculations allow detailed mechanistic and structural information to be obtained.

Methods

A GIBMS is used to study the energy-resolved collision-induced dissociation (CID) of protonated GGG, GGA, and GAG as induced by collisions with Xe. These complexes are formed in an electrospray ion source that thermalizes the ions. Data are analyzed using models that account for multiple collisions, internal and kinetic energy distributions, lifetimes for dissociation, and explicit competition.

Quantum chemistry is used to calculate the energies for rate-limiting transition states for the fragmentations. Geometry optimizations and vibrational frequencies are performed at the B3LYP/6-311+G(d,p) level followed by single point calculations at the MP2(full) and B3LYP levels using a 6-311+G(2d,2p) basis set. Reaction mechanisms are explored using relaxed potential energy surface scans and intrinsic reaction coordinate calculations. All calculations utilize the Gaussian 09 suite of programs.

Results

H+GGG, H+GGA, and H+GAG all decompose to generate b2+ ions as the lowest energy process. These thresholds have been measured using threshold CID and agree well with theoretical calculations of a tight transition state for formation of protonated aminomethyl-5-oxazolone and its methylated analogue. For H+GGG and H+GAG, the neutral product is glycine, whereas H+GGA yields alanine (A) such that the y1+ fragment (protonated A) competes favorably with the b2+ ion in this case. The energetics and competition observed can be modeled and thermochemistry extracted, again in reasonable agreement with theory. Additional primary decomposition channels are being analyzed for all three systems. Interesting variations associated with the simple methyl substitution are being elucidated.

Conclusions

Comparison of experimental and theoretical energies identifies the structures of the b2+ ions and provides key thermochemical information for the first time. Such data can be used to predict the distribution of products under alternative excitation conditions.

Novel Aspect

Detailed experimental energies for peptide fragmentation remain rare yet such data in combination with theory provide unprecedented detail regarding the mechanisms and structures of cationized peptide fragmentation.

FOS41-05 **Distinction of alpha and beta forms of substituted glucose by tandem mass spectrometry and ion mobility spectrometry**

Laurence Charles[1], Guillaume Moreira[1], Aura Tintaru[1], Paola Posocco[2], Maurizio Fermeglia[2], Catherine Lefay[1], Didier Gigmes[1], Sabrina Pricl[2]

[1]Aix-Marseille University, [2]University of Trieste

Introduction

One way to enlarge the range of cellulose applications, limited by inherent poor mechanical properties, consists of grafting synthetic polymer chains using a "grafting from" process. Cellulose is however difficult to solubilize in organic media. Solvents such as N,N-dimethylacetamide (DMA) with 5 to 10 wt% of lithium salts should be used to circumvent this limitation, and grafting polymerization has thus to be optimized in this medium. Nitroxide-mediated polymerization (NMP) is a technique of choice to control the length of the grafted synthetic segment. In NMP, an alkoxyamine has first to be coupled with the substrate, via addition onto an acrylate moiety for example. Acroylation of hydroxyl groups is hence the very first step of the whole process and was optimized in preliminary experiments using glucose as a model substrate. While using mass spectrometric techniques to structurally characterize acrylated glucose species, unexpected results were obtained for the fully substituted glucose molecule depending on the solvent used in the reaction medium and were further investigated from a fundamental viewpoint.

Methods

Acroylation of hydroxyl functions of glucose was performed in DMA or THF (supplemented or not with a lithium salt), using different amounts of acryloyl chloride to obtain products with different substitution degrees. MS and MS/MS studies were performed using an ESI-QTOF instrument (QStar Elite, AB Sciex), with either lithium or ammonium adducts selected as precursor ions in CID experiments. Ion mobility spectrometry (IMS) was performed with a Synapt G2 instrument (Waters), using polyalanine for collision cross section (CCS) calibration. NMR measurements were performed on a Bruker Avance 500 MHz instrument.

Results

In contrast to data obtained for lithiated molecules, different MS/MS spectra were recorded for the ammonium adduct of fully acrylated glucose (Glu-5A) depending on the solvent (DMA vs THF) used in the synthesis medium. Apart from sequential elimination of acrylic acid neutrals observed as the main reaction in both cases, additional dissociation processes were exclusively observed when the activated molecule was synthesized in THF. Based on relative amounts of the alpha and beta forms of glucose as a function of the solution polarity, as determined by NMR measurements, as well as MS/MS experiments performed on mobility-separated species, anchimeric assistance was proposed to account for the additional product ions detected during dissociation of the alpha form.

Conclusions

Due to the distinct conformations they adopt when adducted to ammonium upon electrospray ionization, the alpha and beta forms of fully acrylated glucose prepared in THF could be separated in ion mobility spectrometry, allowing specific CID reactions to be assigned to the alpha form after IMS-MS/MS experiments.

Novel aspects

IMS and MS/MS distinction of alpha and beta forms of substituted glucose.

FOS42 - Forensics and Doping

Room 2
Level 0

Chairs: Laurent Bigler, Olivier Laprévote

FOS42-01 **Keynote: New trends in mass spectrometry in forensic pharmacology and toxicology**

Thomas Kraemer

Dept. of Forensic Pharmacology and Toxicology, Zurich Institute of Forensic Medicine, University of Zurich

Forensic pharmacology and toxicology encompasses the measurement of alcohol, drugs, medicaments and other substances in biological specimens and interpretation of such results in a medico-legal context. Highly sophisticated mass spectrometric equipment is a prerequisite for successful forensic casework in the modern forensic lab. While confirmation of immunoassay results is easily done by simple hyphenated MS techniques, more sophisticated MS techniques are needed for the very specific problems in forensics: Postmortem samples which can be anything from fresh to severely putrefied can cause surprising matrix effects. Sometimes only alternative matrices are available (hair, nails, oral fluid, respiratory air). In the last years, about 300 new illegal drugs entered the market, for which reference standards are rarely available. Mass spectrometry has been used to encounter all these problems. Postmortem tissue samples are investigated by MALDI-MS imaging (MALDI MSI) techniques using multiple reaction monitoring mode for screening and MS/MS or MS3 for confirmation. Consumption behavior of illegal drugs can be monitored in single hair using MALDI MSI. Intake of drugs or medicaments can be detected even in exhaled air employing the most sensitive LC-MS/MS equipment. High resolution mass spectrometry allows for identification of new drugs. Finally, simultaneous QUAL/QUAN analysis using liquid chromatography high resolution MS and employing new Data Independent Acquisition (DIA) approaches (MS/MSALL with SWATH Acquisition) will make forensic toxicology fit for future.

FOS42-02 **Screening for anabolic steroids in sports: new strategy based on the direct analysis of phase I and phase II metabolites by LC-MS/MS**

Georgina Balcells[1], Argitxu Esquivel[2], Oscar J Pozo[2], Jordi Segura[2], Rosa Ventura[2]
[1]Bioanalysis Research Group, IMIM-Hospital del Mar, [2]Bioanalysis Research Group, Antidoping Control Laboratory

Anabolic androgenic steroids (AAS) are the most frequently reported doping substances in sports. AAS are extensively metabolized and mainly excreted in urine as phase II metabolites. Some of the recently reported long-term phase II metabolites are not detectable using the current screening conditions based on hydrolysis and analysis of the released phase I metabolites. Liquid chromatography-tandem-mass spectrometry (LC MS/MS) allows for the direct detection of AAS conjugates. The objective of this study was to develop a sensitive and comprehensive screening method based on LC-MS/MS to directly detect phase II metabolites (glucuronides and sulphates) and metabolites excreted unconjugated in urine.

A screening method for the detection of 26 analytes (6 unconjugated metabolites; 13 glucuronides and 7 sulphates) was developed. The method consisted on a solid phase extraction followed by LC MS/MS analysis using a triple quadrupole instrument and electrospray ionization. Ionization and collision induced dissociation (CID) were studied in-depth for each group of compounds using different mobile phase solvents. Once separation and MS/MS conditions were optimized, a selected reaction monitoring method was used to monitor at least two specific transitions for each analyte. Validation of the method was performed in terms of selectivity, detectability and limit of detection (LOD), carryover, extraction recovery and within-day precision.

Ionization of analytes depended on their structure. In positive mode, metabolites with either a Δ4 3 keto or Δ1 3 keto function ionized as [M+H]+ regardless of their Phase II status (unconjugated, glucuronides and sulphates). Additionally, glucuronides lacking this feature might be also ionized by adduct formation as [M+NH4]+. In negative mode, both glucuronides and sulphates formed the ion [M H]-, resulting either from deprotonation of the acidic group or the sulphate moiety. A MS3–like strategy based on the CID of in-source fragments was also needed for the detection of some metabolites. Mobile phases containing water and acetonitrile with ammonium formate and formic acid gave the best results in terms of signal intensity, chromatographic resolution and signal to noise ratio. The extraction procedure gave recoveries above 83%. Intra-day precision was better than 25% and LODs were normally in the range 0.5 2 ng/mL. Selectivity was evaluated by the absence of interfering compounds in 10 blank urine samples. No carryover effect was observed. The method was applied to the analysis of AAS administration urine samples (stanozolol, methyltestosterone, metandienone, nandrolone, boldenone) confirming the suitability of the strategy.

The developed method is compliant with WADA requirements and represents a cost-effective approach that improves the detection capabilities of AAS as it includes phase II metabolites not detectable using the current screening strategy and, also, it could readily incorporate new long-term phase II metabolites described in the future.

FOS42-03 **New technologies to help facing new challenge for growth promoters' detection**

Laure Beucher, Gaud Dervilly-Pinel, Berengere Marais, Stephanie Prevost, Fabrice Monteau, Bruno Le Bizec
LABERCA ONIRIS

B-adrenergic agonists are synthetic arylethanolamine compounds used for therapeutic purposes for their bronchodilatator, tocolytic and cardiotonic properties. However, their illegal use as growth promoters in food producing animals has been widely observed. Because of their well-documented adverse effects on human health (food poisoning, cardiovascular and central nervous diseases), their use as growth promoters has been forbidden over the past decades in many countries including European Union Countries (1996) and China (2002).). To ensure consumer's protection, controls are performed in Europe according to the Directive 96/22/EC amended by Directives 2003/74/EC and 2008/87/EC. Highly sensitive and specific analytical methods for quantification and confirmation of trace residues in rearing animal tissues have been implemented. The analysis of these compounds is generally achieved using liquid chromatography (LC) associated to electrospray ionization mass spectrometry (Q-q-Q in MRM mode). Such strategy however fails when facing new b-agonist agents or new ways of application (administration of low dose cocktails). Therefore alternative analytical strategies have to be developed. Among the latest innovation, travelling-wave ion mobility separation (TWIM) coupled to Quadrupole-Time-of-Flight mass spectrometer has been investigated to tackle major issues such as co-eluted compounds, related ion suppression, closed mass-to-charge ratios and sensitivity limits. With this new technology, ions are separated based on the number of rolling back events they do, due to pulse height and gas pressure in TWIM cell. So this separation allows a 3D separation when coupled with ultra-high pressure LC and high resolution mass spectrometry. Species would be separated according to their retention time, their mass-to-charge ratio AND their mobility time (or drift time), and related to both their charge states and their spatial arrangement (sizes/shapes). Smaller ions collide less frequently with the buffer gas compared with larger ions, thus crossing the mobility cell faster. Ions are then defined by a specific collision cross section (CCS). The CCS could therefore be used as new identification point for confirmation purposes and provide additional confidence in the assignment b-agonists species. In addition, the use of TWIMS provides an orthogonal mode of separation which enables clean-up of spectral information for better both specificity and sensitivity of the detection. In this work, we have evaluated the strategy for different introduction modes (Direct injection, LC and Supercritical Fluid Chromatography), different eluting conditions (various solvents and pH) and different matrices (urine, meat, retina and feeds) for improved b-agonists detection. Results will be presented showing the matrix effect on ion mobility separation and the interest in terms of sensitivity, specificity and repeatability of the Ion-Mobility mass spectrometry results.

Keywords:
B-adrenergic compounds, food safety, ion mobility separation

FOS42-04 Analysis of sexual assault evidence by ambient mass spectrometry: a statistical comparison between DESI-MS and EASI-MS

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Introduction
Desorption electrospray ionization mass spectrometry (DESI-MS) and easy ambient sonic spray ionization mass spectrometry (EASI-MS) were employed in the forensic analysis of chemical compounds found in condoms and relative traces as well as condom-doped fingerprints, and their analytical performances compared. An effective discrimination of these traces would be highly beneficial in forensic investigations.

Methods
DESI-MS and EASI-MS spectra were acquired using a Thermo Scientific (San Jose, CA, USA) LTQ mass spectrometer equipped with a lab-built automated DESI ion source. Each condom was positioned around an aluminum plate of appropriate size and analyzed. Classification was carried out by two multivariate chemometric techniques: linear discriminant analysis (LDA), and soft independent modeling of class analogy (SIMCA). Condom-doped fingerprints were blotted on glass, paper and metal surfaces after handling the condoms. DESI and EASI imaging analyses were performed at high resolution (100µm) and with ad-hoc parameters, in order to reduce the total analysis time.

Results
Statistical analysis of data obtained from mass spectra only allowed to discriminate between condom brands/types with very high prediction abilities (100% with EASI-MS and 94% with DESI-MS). Imaging experiments showed a much lower sample degradation of DESI respect to EASI. Quality of images is comparable, with DESI offering a slightly higher quality.

Conclusions
Discrimination between condom brands and types is possible with high prediction percentages using both DESI-MS and EASI-MS. Although the difference is only small, EASI-MS allows for a higher discrimination respect to DESI-MS. For this reason EASI-MS should be preferred in the analysis of condom traces, while DESI gives more advantages in imaging analyses in terms of sample preservation and image resolution. The absence of any sample preparation technique avoids contamination, with the possibility to perform successive analyses on the same sample with other techniques.

Novel Aspect
We report the first statistical comparison of two different ambient ionization techniques for the analysis of condoms. This is also the first application of EASI-MS for the analysis of this kind of evidence and the second application of EASI-MSI. These results represent a good starting point for future investigations and on-field trials. In future, improvement in analytical performances of already existing portable mass spectrometers will allow to perform analysis directly on the crime scene.

FOS42-05 Unambiguous differentiation of explosives-related isomers using electrospray high-resolution mass spectrometry

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Introduction
Explosives detection and identification are important themes in the environmental and forensic sciences. Nitroaromatic compounds can be found in the areas surrounding training ranges, firing points, impact locations, contaminated fields and former ammunition plants; they can contaminate lakes, groundwater, as well as soil. However, accurate identification of isomeric nitroaromatic compounds, as explosives, remains a challenging task for current analytical methods. Electrospray multistage mass spectrometry (ESI-MSn) combined to high resolution mass spectrometry (HRMS) provides a powerful tool for structural characterization and differentiation of isomeric compounds.

Methods
The explosives-related compounds: 2,4,6 trinitrotoluene (TNT), 2,4 dinitrotoluene (2,4-DNT), 2,6 dinitrotoluene (2,6-DNT), 2 amino-4,6 dinitrotoluene (2A-4,6 DNT) and 4 amino-2,6 dinitrotoluene (4A-2,6 DNT) were analyzed using ESI and LTQ-Orbitrap XL mass spectrometer. Accurate mass measurements, including those of product ions, were performed at high resolution using a 7T Fourier transform ion cyclotron resonance mass spectrometer SolariX Qq-FT-ICR. Activation of deprotonated molecules was carried out using Sustained Off-Resonance Irradiation Collision-Induced-Dissociation (SORI-CID) and resolving power: 140.000 FWHM at m/z 400.

Results
From accurate mass measurements performed using FT-ICR/MS, unexpected results were found for the deprotonated TNT and 2,6-DNT precursors, that showed a first 30 Da loss corresponding to CH2O instead of the expected isobaric NO• loss. Theoretical calculations were performed and confirmed the proposed mechanism for the CH2O loss. To our knowledge, this is the first time that the CH2O loss is described for deprotonated TNT and 2,6-DNT prepared in API mode (ESI or APCI). Moreover, 2,4-DNT showed a diagnostic main fragment ion at m/z 116, allowing the unambiguous differentiation between 2,4- and 2,6-DNT isomers. For both 2A-4,6 DNT and 4A-2,6 DNT isomers, through the use of MS3, the amino-substituted isomers can be differentiated based upon an OH• loss for 4A-2,6 DNT vs. a NO• loss for 2A-4,6 DNT.

Conclusions
This new finding was established by accurate mass measurements at high resolution giving a new insight into dissociation processes of deprotonated TNT, DNT and A-DNT isomers. Notably, 2,6 DNT gave mainly the fragment ion at m/z 151 by an unexpected first loss of CH2O instead of the usual isobaric NOŸ. By contrast, 2,4 DNT gave m/z 116 by consecutive [NO•+H2O+OH•] losses. On the other hand, 2A-4,6 DNT yielded consecutive losses of two NO• neutrals whereas its isomer, 4A-2,6 DNT, underwent consecutive losses of NO• and OH•. Detailed MSn characterization of explosives has thus served to enable differentiation of each isomer, and clarify a previously ambiguous decomposition pathway.

Novel Aspect
The unexpected CH2O loss instead of isobaric NO• loss was unambiguously elucidated and all isomers of substituted TNTs were differentiated.

FOS43 - Environmental Analysis

Room 3
Level 0

Chairs: Marja Lamorée, Marc Suter

FOS43-01 Keynote: Wastewater-based epidemiology: the analysis of human biomarkers in sewage

Kevin Thomas, Yeonsuk Ryu, Jose Antonio Baz Lomba, Christopher Harman, Katherine Langford, Malcolm Reid
NIVA

Introduction
Europe consumes around 360 kg of cocaine every day. This was estimated by the quantitiative analysis of benzoylecognine in Europe's wastewater. A metabolite of cocaine, benzoylecogine is an exogenous urinary biomarker of cocaine use that is collected by sewer networks along with the biomarkers of other drugs. Use of these exogenous biomarkers has become widespread in the estimation of spatial and temporal drug use. Similarly sewage also contains the excreted biomarkers of endogenous human metabolism that directly reflects the exposure and stressors placed upon an entire contributing community. The quantitative measurement of these specific biomarkers in sewage from communities potentially allows the averaged patterns of factors related to lifestyle, disease and environment to be used for the assessment of community health. Key to this is the development of specific and accurate analytical techniques to quantify the individual biomarkers in wastewater. A good biomarker for wastewater-based epidemiology needs the following attributes:

- Specificity – the biomarker must be a specific marker of the factor under investigation (i.e. be produced exclusively by the drug, disease or stress) and not formed exogenously by, for example, microorganisms in the sewer system.
- Stability – the biomarker must be stable within the sewer system and must not partition extensively to solids
- Detectability – the biomarker by be present in sewage at sufficiently high concentrations to me accurately measured
- Sensitivity – the biomarker must be excreted at sufficiently high levels to observe significant differences between ´normal´and ´stressed´communities.

Results
The human biomarkers determined thus far in sewage are summarized in table 1. Robust biomarkers are available for both legal and common illegal drugs . New psychoactive substances pose a specific challenge since there are so many possible compounds, and stable urinary biomarkers may not be able for all. Evaluating novel biomarkers for NPS is possible using a combination of in silico techniques alongside suspect screening approaches . Health biomarkers pose an even larger challenge since there are so many to choose from, yet little is known about their suitability for monitoring in wastewater. Pharmaceuticals may be suitable exogenous markers for specific diseases, such as cancer, or responses to particular environmental stressor, such as hayfever. Determining the collective oxidative stress through the analysis of isoprostanes has been proposed as a biomarker for a general community-wide health assessment , although their analysis in wastewater is not trivial and low levels present in raw sewage suggests that other alternative biomarkers may be required for such an assessment to be effective.

Conclusions
Wastewater based epidemiology through the quantitiative analysis of specific human biomarkers in sewage has the potential to allow the assessment of factors that affect entire communities.

Novel Aspect
Novel approach for the assessment of community level for new biomarkers for the community assessment of factors such as environment, health, lifestyle and diet.

FOS43-02 Suspect and non-target screening of lake sediments: approaches to identify records of organic contaminants in complex matrix

Juliane Hollender, Aurea C. Chiaia-Hernandez, Emma L. Schymanski, Heinz P. Singer
Eawag, Swiss Federal Institute of Aquatic Science and Technology

Introduction
Lake sediment cores provide a valuable record of historical contamination. So far new analytical techniques such as high resolution mass spectrometry (HRMS) have not yet been applied to extend target screening to the detection of unknown contaminants in complex sediment matrix. The goal of the study was to explore whether suspect and non-target screening methods developed for water samples could also be viable for sediment.

Methods
A combination of target, suspect and non-target screening using LC-ESI-HRMS/MS was performed on extracts from sediment cores from two Swiss lakes. A suspect list of 840 compounds was compiled from consumption data of pesticides and pharmaceuticals and refined using the expected method coverage and a combination of automated and manual filters on the resulting measured data. Non-target identification efforts included enviMass filtering, the in silico methods MOLGEN-MS/MS and MetFrag as well as additional consideration of retention time/partitioning information and the number of references.

Results
In a comprehensive target analysis on average 16 of 200 compounds were detected in both lakes with the most prominent substance classes corresponding to personal care products, pesticides, and biocides. For suspect screening, candidates with masses between 120 to 1000 Da showed a mass defect relatively similar to the sediment matrix which made the identification of suspect candidates challenging. The automated data processing with ExactFinder (peak shape and intensity, blank subtraction, retention time) allowed the elimination of 80% possible suspect candidates, while visual inspection of chromatograms yielded 27 exact mass matches for further analysis. A score based on the number and intensity of matching predicted fragments using MetFrag reduced the number to 13. Finally, the biocide chlorophene, the pharmaceutical flufenamic acid and the pesticide lufenuron were confirmed, while 5 other candidates were rejected with reference standards.
For non-target screening, EnviMass proved a useful first step as background subtraction and noise removal eliminated up to 60% of the picked peaks. The non-target candidates contained between 9-18% peaks with isotopic pattern. Candidates with a distinct isotope signal and with a mass defect between -200 to 0 mDa were prioritized for further elucidation. The combination of MOLGEN-MS/MS and MetFrag helped to identify the bacteriostatic agent hexachlorphene and the mothproofing agent flucofuron.
Based on the results further chlorophene congeners were screened in the sediments, resulting in the tentative identification of dichloro- and bromochlorphene. The temporal pattern of the pollution between 1950 and 2010 was reconstructed for all suspect and non-target compounds.

Conclusions
This study demonstrates that complementary application of target, suspect and non-target screening can deliver valuable information despite the matrix complexity and provide records of historical contamination in two Swiss lakes with previously unreported compounds.

Novel Aspects
Combination of in silico tools for structure elucidation and confirmed identification despite complex sediment matrix.

FOS43-03 Influence of extraction pH upon the FT-ICR MS profiles of water samples from the Athabasca oil sands region

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Introduction
There is a strong dependence upon petroleum as a source of energy and as a precursor to compounds used in manufacturing, the food industry, medicine, and research. It has become increasingly necessary to turn to less conventional sources of oil, such as the oil sands found in Canada. An alkaline hot water extraction process can be used to obtain the bitumen from the oil sands, which can then be upgraded to synthetic oil. Approximately three barrels of water are consumed for every barrel of synthetic oil produced, but the water cannot be discharged into the environment after use. This oil sands process water (OSPW) must be stored in large tailings ponds and contains organic components from the oil sands. With the growing importance of the oil sands industry, there is an accompanying need to develop and utilize a wide range of analytical tools for environmental monitoring.

Methods
Samples of OSPW were collected from Alberta, Canada. Traditional sample preparation procedures have typically involved the acidification of the solution prior to extraction, but this can result in bias against basic organic components prior to analysis. To study the effects upon the sample profiles, acidic and basic extraction procedures have been compared using the OSPW sample. A 12 T solariX Fourier transform ion cyclotron resonance mass spectrometer was used for the subsequent characterization of the complex mixtures. Positive-ion and negative-ion electrospray ionization (ESI) and atmospheric pressure photoionization (APPI) experiments were performed.

Results
Following compositional assignments, sample profiles were generated; plots of contributions as a function of compound class were used to create summaries for the sample composition, while plots of double bond equivalents (DBE) versus carbon number for selected compound classes provide more detailed information. When compared to the basic extraction procedure, the acidic extraction process resulted in the observation of components with higher oxygen contents, including sulfur-containing components which also incorporated oxygen. This was reflected in both the ESI and APPI data. ESI experiments are suitable for characterizing the most polar components within the complex mixture, while APPI led to the observation of a greater range of heteroatom-containing components, including compounds with a higher sulfur or nitrogen content.

Conclusions
The choice of extraction procedures influenced the sample profiles, particularly with respect to the range of heteroatom-containing compounds observed and the oxygen content. As profile comparisons are important for environmental forensics, the study highlights the need for further development of sample preparation methods for complex environmental samples.

Novel Aspect
Ultrahigh resolution mass spectrometry to compare sample profiles for an environmental complex mixture following a new sample extraction method and a traditional extraction method

FOS43-04 Tracing genotoxic disinfection by-products after medium pressure UV water treatment using nitrogen labeling and mass spectrometry

Annemieke Kolkman[1], Dennis Vughs[1], Kirsten Baken[1], Bram Martijn[2]
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Introduction
Advanced oxidation processes, are becoming important barriers against organic micro pollutants (e.g. pharmaceuticals, pesticides) in (drinking)water treatment. Some studies indicate that medium pressure (MP) UV/H2O2 treatment leads to a positive response in the AMES fluctuation mutagenicity tests. This response appear to be removed after granulated activated carbon (GAC) filtration. It is hypothesized that the formed mutagenic products result from the reaction of photolysis products from nitrate with natural organic material (NOM). Unraveling the identity of the nitrogenous by-products (N-DBP) is a first step in a proper risk assessment. In this study we present an innovative approach to detect and identify formation of N-DBP by MP UV water treatment, based on labeling with stable isotopes and high resolution mass spectrometry.

Methods
Artificial water consisted of ultrapure water, Ponylake NOM and nitrate. Nitrate was added as 14NO3- and as 15NO3-. Samples were treated with MP UV using a collimated beam set-up. Blank samples, e.g. without MP UV treatment, were also prepared. Experiments were performed in triplicate. Solid phase extraction with OASIS HLB cartridges was used to concentrate the water samples. Tthe extracts were analysed using liquid chromatography coupled to an Orbitrap mass spectrometer. The raw data files were analysed using advanced software tools (Sieve, Thermo Scientific) for statistical differences between the treated and untreated samples. In addition, the extracts were analysed by Ames fluctuation test.

Results
The MP UV treated water samples showed a positive response in the Ames fluctuation test. These extracts were screened using high resolution mass spectrometry (MS). Differential analysis showed that after MP UV treatment, nitrogen containing products were formed. The incorporation of 1, and also 2 nitrogen atoms originating from nitrate, in the formed products was shown. In total 80 N-DBPs were detected that are formed after MP-UV treatment. All N-DBP were detected at low concentration, namely between 1-150 ng/L bentazon-d6 equivalents. The summed concentration of all N-DBPs was about 1 µg/L bentazone-d6 equivalents. For 3 products the structure of the N-DPB was elucidated

and confirmed with chemical standards. Screening for the identified N-DBPs in water samples from different parts in the actual drinking water production chain showed a correlation between the total concentration of the formed by-products and the response in the Ames fluctuation assay.

Conclusions
We have developed an innovative approach, based on stable isotope labeling and high resolution MS, to detect and identify low concentrations of mutagenic N-DBPs.

Novel aspects:
- Formation of a plethora of N-DBPs after MP UV treatment is shown in artificial water.
- The results obtained with the bioanalytical (Ames fluctuation) and chemical tools showed a good correlation.

FOS43-05 High resolution mass spectrometry based metabolomics: a new tool to detect and characterize emerging pollutants in water and food matrices.

Jerome Cotton[1], Fanny Leroux[2], Simon Broudin[2], Bruno Corman[2], Jean-Claude Tabet[3], Céline Ducruix[2], Christophe Junot[4]
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Introduction
Food trade globalization and extensive use of pesticides and drugs in agriculture and farming has increased the risk of food contamination and fraud. This has become a significant public health problem resulting in strengthened controls from the health authorities. However, the analytical methods used to detect pollutants are limited to targeted approaches towards expected contaminants, which prevent the possibility of finding unexpected or emerging contaminants. High resolution mass spectrometry (HRMS) based metabolomics associated with data mining can address this issue through monitoring of targeted compounds, but also to highlight and identify unexpected compounds. Part of the research described was carried out under the AgriFood GPS project funded by Bpifrance (http://agrifoodgps.sharepoint.com).

Methods
Standards and reagents were purchased from Sigma-Aldrich, VWR International, and Carlo Erba reagents. Analyses were performed using a Nexera LC-30AD liquid chromatographic system (Shimadzu) coupled to an Exactive mass spectrometer (ThermoScientific), and also a CTC-Transcend liquid chromatographic system coupled to a Q-Exactive mass spectrometer (ThermoScientific), both fitted with an electrospray source, with the resolution set to 30000 and 70000 (FWHM at m/z 400) respectively. Elution was performed in gradient conditions with mobile phases composed of water and acetonitrile containing 0.1% formic acid on a Xterra column (Waters). Data treatment was achieved with XCMS R package, statistical analysis with SIMCA P12, and signal annotation was performed using public metabolite databases and an in-house spectral database.

Results
A HRMS-based metabolomic approach was first developed and implemented on honey samples as a proof of concept study. A list of 83 pollutants (55 pesticides and 28 antibiotics) of interest was established. A liquid-liquid extraction method and a UHPLC-HRMS method were developed and validated in order to (i) detect these 83 pollutants, (ii) indicate whether their concentrations in the honeys are above or below regulatory limits, and (iii) detect a wide range of chemicals present. Our results on a panel of 76 honey samples showed that at least one of the targeted pollutants was detected in 74 of 76 honeys, and that an average of 5 xenobiotics were found per honey, with concentrations below the regulatory limits. Multivariate statistical analyses performed on analytically relevant features enabled to discriminate honey samples according to their floral origin. The data mining procedure also highlighted the presence of 22 unknown chlorinated xenobiotics in honey samples. One of these compounds was present in 4 lavender honeys and was formally identified as 2,6-dichlorobenzamide (a metabolite of dichlobenil, a herbicide that was widely used in lavender plantations before 2010).

Novel Aspect
Innovative use of HRMS-based metabolomics to perform multiplexed analyses of emerging pollutants and identify unexpected xenobiotics in food matrices.

FOS44 - Very Large Biomolecules and Structural Biology

Room 4
Level 0

Chairs: Michal Sharon, Eric Forest

FOS44-01 Keynote: Mass spectrometry and very large biomolecules

Albert Heck
Utrecht University

Around for more than a century the analytical technique of mass spectrometry is blooming more than ever, and applied in nearly all aspects of the natural and life sciences. In the last two decades mass spectrometry has become routine for the high-throughput analysis of peptides, lipids, glycan and other biomolecules. However, also intact proteins and even complete protein complexes can nowadays be analyzed, enabling MS to penetrate the field of structural biology. Here, I will describe the emerging role of mass spectrometry with its different technical facets in structural biology, focusing especially on the analysis of viruses, dynamic protein assemblies and therapeutic antibodies. I will describe how mass spectrometry has evolved into a tool that can provide unique structural and functional information about viral protein and protein complex structure, conformation, assembly and topology, extending to the direct analysis of intact virus capsids of several million Da in mass. Mass spectrometry is now used to address important questions in virology ranging from virus structural topology to how viruses assemble.

I will also describe recent developments in mass spectrometry technology that have allowed us to analyze intact native proteins and protein complexes using Q-ToF and Orbitrap mass analyzers with very high sensitivity and mass resolving power, and how that has enabled us to profile how our immune systems gets triggered by antibodies via the Complement pathway. Prospectively, I will describe what I think will be the contribution of this breakthrough for the future of proteomics.

FOS44-02 Probing protein structural transitions in complex biological backgrounds and on a large scale

Paola Picotti[1], Yuehan Feng[1], Giorgia De Franceschi[2], Abdullah Kahraman[3], Martin Soste[1], Andre Melnik[1], Paul Boersema[1], Patrizia Polverino De Laureto[2]
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Introduction
Protein structural transitions in response to environmental or genetic factors, such as those associated to allosteric regulation or misfolding, can strongly impact protein function, with drastic consequences on the physiology of cells. However, monitoring protein structures on a large scale and in a biological environment has so far not been feasible. We introduce a novel MS-based approach based on proteolytic probes and targeted proteomics tools to quantitatively analyze protein structural rearrangements in a complex proteome. We applied it to the quantification of in vivo protein structural transitions in the proteome of yeast subjected to a nutrient perturbation and used the data to shed light on the complex regulation of yeast metabolism.

Methods
Our method relies on limited proteolysis (LiP) of a proteome extract, under conditions where the proteolysis sites are dictated by the structural features of the protein substrates. To apply LiP to a complex proteome the approach exploits: 1. a double-digestion step that generates peptides amenable to bottom-up MS analysis and 2. the background-filtering capabilities of selected reaction monitoring (SRM) to probe LiP-patterns in complex matrices. To validate the approach (which we term LiP-SRM), we used different conformational states of the model proteins alpha-synuclein (a-Syn) and myoglobin (Mb) spiked into complex proteome extracts. We then applied it to proteome extracts of S. cerevisiae cells grown in media containing glucose or ethanol as the sole carbon source, to detect structural transitions induced by the metabolic shift.

Results
LiP-SRM applied to a-Syn and Mb resulted in the extraction of conformational markers that cover the complete sequence of the two proteins. Quantification of such "conformotypic peptides" enabled evaluation of the conformational change of the respective protein region. The results for Mb and a-Syn from cell extracts recapitulated data obtained by NMR analysis of the purified proteins, thus validating the approach. We then applied LiP-SRM in an unbiased manner to probe the structural changes of the yeast proteome upon the metabolic transition. We probed the structural features of >1,000

proteins and detected novel stimulation-induced transitions for ~300 proteins. Our analysis suggested a highly modular nature of carbon metabolism, with certain metabolic branches preferentially controlled transcriptionally and others regulated by enzyme conformational changes, including known and novel cases of allosteric regulation.

Conclusions
The approach allows probing of both pronounced and subtle conformational transitions of proteins with a resolution of ~10 amino acids directly in complex biological matrices.

Novel aspects
The approach opens new avenues in biological research, such as the possibility of monitoring protein structural transitions for components of a complete pathway, identifying ligand-protein interactions and, in general, detecting novel regulatory processes based on protein structural rearrangements.

FOS44-03 Radical probe mass spectrometry for high throughput protein footprinting

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Introduction
Radical probe mass spectrometry (RP-MS) is an important technique for the structural analysis of proteins and their complexes. High fluxes of oxygen-containing radicals induce the oxidation of amino acid side chains of proteins within milliseconds according to their solvent accessibility. Mass spectrometry is used to measure the oxidation levels to enable a three-dimensional map of a protein's surface to be constructed. The electrospray discharge source has proven to be a versatile method for RP-MS and, when coupled to on-plate deposition, ozonolysis can help facilitate high throughout applications. This work focuses on the application of ozonolysis as an oxidation source compared to the electrical discharge method.

Methods
Ozonolysis was performed with an ozone generator introducing an ozone/oxygen mixture at variable rates and voltages into aqueous solutions of peptides and proteins. In comparison, electrical discharge experiments were performed in an ESI source by increasing the needle voltage (from 4 to 8 kV) with oxygen was used as the nebulizer gas.

Results
A series of peptides with diverse amino acid compositions were subjected to ozonolysis to reveal their order of reactivity. Under the same solution and reaction timescales, bradykinin was partially oxidized whilst substance P containing a highly reactive methionine residue was fully oxidized. Ozonolysis reactions were performed to achieve levels of oxidation within the ubiquitin protein ranging from 15-75% corresponding to increasing reaction time. Ozonolysis for

a series of model proteins including cytochrome c, lysozyme and apomyoglobin were performed and analysis of their proteolytic peptides by MALDI-FTICR-MS revealed oxidation of the amino acid side chains was in accord with their levels of solvent accessibility.

Conclusions
The oxidation of model proteins support ozonolysis as a versatile method for high throughput footprinting applications of RP-MS which can accommodate small solution volumes.

Novel Aspect
First application of ozonolysis to facilitate high throughput protein footprinting by Radical Probe Mass Spectrometry

FOS44-04 Rapid and direct MALDI-MS identification of pathogenic bacteria from blood via ionic liquid-modified magnetic nanoparticles

Hui-Fen Wu, Mukesh Bhaisare L., Hani Nasser Abdelhamid, Bo-Sgum Wu, Hui-Fen Wu
National Sun Yat-Sen University

A novel method for pathogenic bacteria identification directly from blood samples by cationic ionic liquid-modified magnetic nanoparticle Fe3O4@SiO2 (CILMS) was reported. The magnetic nanoparticles were prepared by co-precipitation and the core-shell Fe3O4@SiO2 nanoparticles were prepared by the sol-gel process, followed by the grafting of 3-chloropropyltrimethoxysilane that was reacted further with N-methylimidazole to form cationic ionic liquid-modified Fe3O4@SiO2magnetic nanoparticles (CILMS). The pathogenic bacteria were separated based on the electrostatic interactions among the negative charges of the cell membranes and the positive charges of the CILMS particles. CILMS is used directly without the need for any further apparatus and auxiliary chemicals. The separated cells were detected using matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS). The lowest detectable number of bacteria was 3.4×103, 3.2×103, and 4.2×103 cfu mL-1 for Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus, respectively. The bacteria affinities toward CILMS were investigated using transmission electron microscopy that revealed immobilization of the CILMS on the outer of cell membranes. The present approach offers a high sensitivity, fast, and simple method for the cell capture of the pathogenic bacteria. The current approach could be adapted to separate and identify the pathogenic bacteria from septicemic patients.

FOS44-05 **Structural analysis of protein complexes by chemical cross-linking and mass spectrometry**

Alexander Leitner, Florian Stengel, Thomas Walzthoeni, Ruedi Aebersold
ETH Zurich

Introduction

Chemical cross-linking in combination with mass spectrometry (XL-MS) has established itself as an important technology for the structural analysis of protein complexes. The improved sensitivity of the current generation of mass spectrometers and sophisticated bioinformatics tools for data analysis have recently provided insight into large assemblies including proteasomes, ribosomes and chromatin remodelers. In order to make XL-MS more gener(ic)ally applicable and an accepted tool for structural biologists, we have developed an integrated workflow for the routine generation of structural information from protein complexes that includes a step-by-step protocol and open source, open access software (1,2).

Methods

Isolated protein complexes are preferentially cross-linked using two differentially isotope-labeled variants of a homobifunctional reagent such as the lysine-reactive disuccinimidyl suberate (non-labeled reagents can be used as well). Following enzymatic digestion, the resulting peptide mixture is enriched for cross-linked peptides using size-exclusion chromatography. LC-MS/MS analysis is carried out on Orbitrap mass spectrometers. Tandem mass spectra are assigned using the dedicated software xQuest and statistically validated with xProphet (2). The result is a list of validated XL contacts that can be used for direct interpretation or as input for hybrid structural modeling methods.

Results

We have recently applied XL-MS to study the assembly of two chromatin remodelers, INO80 (3) and SWR1 (4), and to the mammalian mitochondrial ribosome (5). In combination with available cryoelectron microscopy data, cross-linking allowed the positioning of subunits within these large complexes and provided additional insights into their architecture and function. Even in the absence of high-resolution structures derived from X-ray crystallography, the combination of multiple low resolution techniques is highly beneficial, as we could demonstrate by the integration of XL-MS and ion mobility spectrometry-MS analysis of intact protein assemblies (6).
In addition to highlighting these applications, we will present results from ongoing projects where our focus is on the development of complementary XL chemistries and the addition of a quantitative dimension to XL data, enabling the study of conformational transitions and rearrangements within protein complexes.

Novel aspect

Integrated workflow for cross-linking/MS; routine application to large protein complexes

References

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FOS45 - Single Cell MS

Room 5
Level 3

Chairs: Bernd Bodenmiller, Renato Zenobi

FOS45-01 **Keynote: Single cell pheno-functional proteomics by mass cytometry**

Scott Tanner, Olga Ornatsky, Vladimir Baranov, Dmitry Bandura
Fluidigm Canada Inc.

The central goal of cell biology is to understand the development, function, and interaction of multiple cell types in an organism. It is now accepted that heterogeneity exists in even small cell populations. Thus genomic and proteomic analysis of an ensemble of cells gives average values which can be misleading for each single cell. It is the cell-specific behavior that contributes to the systems biology of the ensemble. The cell differentiation markers on the surface of each cell identify the phenotype, and the translational modification of the intracellular proteins provides information on the functionality/signaling of that particular cell. Most often, the pheno-functional markers are not the most abundant proteins, and so immunologic (or other affinity reagent) staining beneficially reveals the presence and abundance of the specific targets of interest.
Flow cytometry, employing fluorescent tags and measured by optical fluorescence, has been the mainstay of single-cell proteomic investigation. These analyses become exponentially more complicated as the number of target proteins increases, yet many markers are required to effectively distinguish cell populations together with cell function. Mass cytometry has overcome the "fluorescence limitation" by using stable isotopes of biologically rare metals as tags that are read by atomic mass spectrometry.
An overview of the specific adaptations that allow Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) to answer the pressing challenges of multidimensional flow cytometry are presented, together with the translation and expansion of fluorescent labeling technologies to element tagging. Approaches to enhancing both of these core mass cytometry technologies (instrumentation and reagents) will be presented.
Recording several dozens of protein signatures of each of up to 1,000 cells per second generates massive multidimensional data. The translation of the raw mass spectrometry data into cell-specific cytometry information will be described, with an overview of approaches to multivariate unsupervised (or minimally supervised) data analysis.

Flow cytometry is inherently a cell suspension analysis technique, and mass cytometry has by now demonstrated its promise of expanding the multiparameter ability to assay complex samples, such as blood and other body fluids. However, extending these single-cell analysis capabilities to tissue samples has, until now, required cell dispersion methods that undoubtedly modify the signaling and disrupt the three-dimensional cell interactivity. Recent work [1,2] has shown the feasibility of multiparameter protein assay with single-cell imaging of FFPE tissue sections that, in the authors' opinion, presages yet another revolution in single-cell biology analysis.

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FOS45-02 Absolute quantification of proteins and protein modifications on the single-cell level

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Introduction

Analyzing the content of individual cells represents an important direction to unravel cell-to-cell variability. Single-cell analysis also plays an important role in systems biology, as it supports to comprehensively learn regulatory (signaling) networks. To unravel the complexity of signaling pathways and their interplays, we need high-throughput, quantitative and multiparameter single-cell technologies. Mass cytometry, which is based on atomic mass spectrometry, now allows for single-cell network analysis using antibodies. However, due to unknown antigen-binding site occupancies and non-specific bindings, their absolute copy numbers cannot be directly determined. This is a major caveat, as only absolute copy numbers allow to describe the networks structure, reaction rates and signaling thresholds that commit cells into defined disease states.

To enable the absolute quantification on the single cell level in mass cytometry, we combine it with targeted proteomics, aiming to study in a quantitative and temporal fashion the most relevant cellular signaling network behaviors, including the MAPK, AKT and JAK/STAT pathways.

Methods

Cells of interest are grown and split. One half is cross-linked, permeabilized and labeled with antibodies conjugated to pure isotopes of defined mass. Those cells are then nebulized into single-cell droplets and introduced into the mass cytometer (CyTOF). Here, mass and abundance are determined of the isotopes bound to the cells via the antibodies.

In parallel, the other half of these cells are lysed; extracted proteins are digested by trypsin into peptides. Selected peptides, which represent proteins and protein phosphorylation sites under investigation, are quantitatively measured by targeted proteomics (SRM). AQUA peptides (standards) are used to determine absolute copy numbers of endogenous peptides. This value can be used to calibrate the mass cytometry signal, which in turn allows determining the copy number per single cell.

Results

Important features to study complex signaling pathways and their perturbation\change upon stimuli are i) single-cell analysis in a multiplexed manner, ii) absolute quantification of molecule copy numbers. After selecting proteins and phosphorylation sites which cover multiple signaling pathways, we developed a panel of antibodies for CyTOF analysis. Together, SRM assays were developed and validated for the absolute quantification of the same proteins/phosphorylation sites. By analysing our biological markers by both CyTOF and SRM, we observed the same expression level and similar regulation pattern upon certain stimuli. Moreover, the use of standards in the SRM assays allowed us to calculate the average absolute copy number of each protein per cell population, which can be matched with the single-cell data obtained by CyTOF to extrapolate the copy number per single cell. Using this method, single-cell CyTOF measurements can now provide absolute copy number information to study regulatory signaling networks.

Novelty

This integrated CyTOF-SRM approach will significantly advance the state-of-the-art in the quantitative single-cell analysis.

FOS45-03 Profiling of algal populations with single-cell MALDI-FT-ICR mass spectrometry

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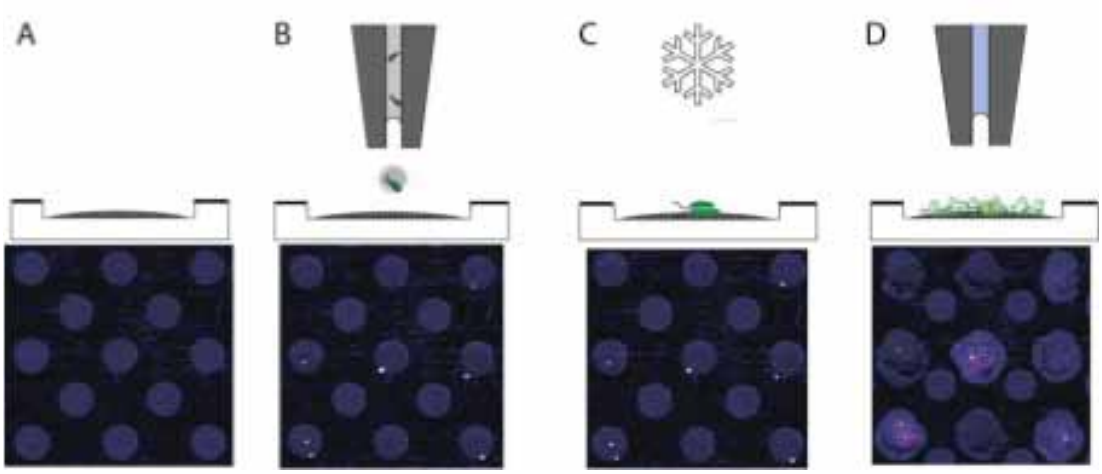
Introduction

Studying microbial populations at the single-cell-level is essential for understanding heterogeneity in these highly complex biological systems. Their investigation can reveal a whole new level of information and improve our understanding of how microbial communities work. We are currently developing a new approach for profiling of microbial populations using a high-density microarray (A) for matrix-assisted laser-desorption ionization – mass spectrometry (MALDI-MS).

Methods

Chlamydomonas reinhardtiicells are spotted onto a high-density microarray (B). Confocal fluorescence scanning locates the auto-fluorescent chlorophyll in the cells on the array. Cell lysis is achieved by fast freezing using liquid nitrogen (C) and matrix (DHB) crystallization from an organic solvent mixture (D). The photosynthetic pigments help to visualize the analytes during the sample preparation procedure.



Results
The array size of 1430 wells gives approximately 500 single cell events due to the stochastic distribution of the cells in the wells. The contactless piezo-spotting device completely prevents cross-contamination and gives no false positives (= MS signal with no cells) and few (around 50 events) false negatives (= cell but no signal). Relative peak intensities can be used to relatively quantify lipids and pigments detected in high-resolution MALDI-FT-ICR spectra. Chlorophyll fluorescence is orthogonally detected with fluorescence spectroscopy to cross-validate mass spectrometry.

Conclusions
The system developed shows the potential of MALDI-MS for high-throughput screening of single-cells. The new protocol can be used to characterize populations and extract biological information such as lipid profiles that is not accessible by conventional fluorescence based methods.

Novelty aspect
High-throughput single-cell MALDI-FT-ICR method to measure cell-to-cell heterogeneities in microbial populations coupled with orthogonal fluorescence readout.

FOS45-04 **Near-field laser ablation sample capture for mass spectrometry imaging**
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Introduction
New tools are needed to bring large biomolecule mass spectrometry imaging to the single cell size regime. Current mass spectrometry imaging techniques can obtain excellent spatial resolution for atoms and small molecules, but imaging of larger molecules is obtained at the expense of spatial resolution. In particular, laser-based methods such as matrix-assisted laser desorption ionization (MALDI) using conventional laser focusing configurations are limited to a resolution of 10 to 100 µm. The goal of this project is to image large biomolecules with mass spectrometry at better than 1 µm spatial resolution using near-field laser ablation sampling. We are using an atomic force microscope (AFM) to both to image as well as to focus the laser energy for sample ablation with the captured material analyzed by mass spectrometry.

Methods
The central component of the laser ablation sample transfer system is an AFM stage that is used to image the sample and keep the tip at a distance of approximately 10 nm from the surface for ablation. The metal AFM tip acts as an electromagnetic antenna that allows for ablation with a spot size much smaller than that for a laser focused with a conventional lens. A 355 nm laser is focused onto the tip and the fluence is set below the far-field threshold. The ablated material is captured on a surface, in a liquid droplet, or transferred via suction to a microfluidic system. The ablation region is imaged using the AFM.

Results
Near field laser ablation capture of small and large molecular standards was performed for optimizing the AFM and laser ablation configuration and estimating the efficiency of material removal and capture, which is critical in achieving acceptable detection sensitivity. It was found that irradiation nearly parallel to the sample surface yielded the best results. Ablation from sample thin films resulted in the removal of material from a 1 to 2 µm diameter and 200 nm deep spot. Small molecules were ablated and captured on a flattened 100 nm silver wire that is suspended over the target using a mechanical stage to adjust the position to 300 µm above and 100 µm away from the tip. After capture, the wire was attached to a MALDI target and ionized by LDI. MALDI matrix materials 4-nitroaniline and anthracene and rhodamine dye were detected in this manner. The peptide angiotensin II was ablated and captured then dissolved in a saturated matrix solution and detected using MALDI. Electrospray following ablation capture and vacuum assisted capture are also being tested.

Conclusions
Near-field laser ablation sample transfer has been demonstrated with off-line LDI and MALDI mass spectrometry of the captured material. The results demonstrate that quantities sufficient for mass spectrometry detection using MALDI or electrospray can be collected at submicrometer resolution using an AFM and near field ablation.

Novel Aspect
Near field AFM-directed laser ablation transfer for mass spectroscopy of biomolecules.

FOS45-05 Detection of microbial resistance markers in clinical samples using MALDI mass spectrometry

Omar Belgacem[1], Philippa Hart[2], Emmanuel Wey[3], Indran Balakrishnan[3]
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Introduction
In 1975, Catherine Fennselau's Group reported that gram negative bacteria could be analysed using a Mass Spectrometer. It was in 1996 (Krishnamurthy 1996, Claydon 1996) that the use of MALDI for a range of bacteria appeared as a method of choice for the identifications of pathogens. This technique is now accepted in major microbiology laboratories and was granted FDA clearance for gram(+) and gram(-) in August 2013. So far, using MALDI, only the Identification of a given Bacteria has gone through sorrow clinical evaluation. Several studies are now focusing on the detection of resistance markers. In our study, we report the use of MALDI in conjunction with fast enzymatic digestion and Liquid Chromatography for the detection and characterization of peptide biomarkers for β-Lactamases belonging to Ambler Classes A, B and C in laboratory and clinical strains of E. coli.

Methods
Clinical Isolates were obtained from the Bio-bank facility at the department of Medical Microbiology at the Royal Free Hospital NHS Foundation Trust. Further strains of E.coli were obtained from the National Collection of Typed Cultures (Public Health, UK). Bacteria were grown aerobically on Columbia blood agar and Luria-Bertani medium. The periplasmic compartments of whole bacterial cells were extracted and proteins were reduced, alkylated and digested using trypsin. Tryptic peptides were separated using nano-LC prior to MALDI-TOF-MS analysis. MS/MS were performed using HE-CID (20 keV).

Results
Using this LC-MALDI method, peptide sequences of proteins within the periplasmic compartment of whole bacterial cells were successfully identified. The majority of these proteins were identified as those known to be present in the periplasm. Amongst the periplasmic proteins identified were several classes of antibiotic resistance proteins: mainly TEM-1, CTX-M, Amp-C and carbopenameses such as ZIM and NDM-1. Initial experiments were performed on laboratory isolates of E. coli. In these cases, the resistance enzymes present were carried on a cloned plasmid. The results showed the ability of our method to discriminate at a species level and at a strain level where the only difference between strains was the carriage of a modified antibiotic resistance carrying plasmid. Further experiments were then performed using clinical isolates. In this case it was possible to detect peptide sequences for resistance enzymes in clinical strains where multiple resistance mechanisms were present: an aminoglycoside modifying enzyme and Beta-lactamases of different ambler classes. All results were confirmed and validated through comparison with results obtained using conventional methods employed in routine clinical analyses.

Conclusion
The next step in this project will be to increase the number of "markers" of antibiotic resistance, as well as to reduce the overall speed of analysis. We are confident that this method can be dramatically simplified.

Monday, August 25th

PS00-01 / Francis William Aston: Postcards from Switzerland
Kevin Downard
University of Sydney

MPS01 - Fourier-Transform MS

11:00-15:00
Poster Exhibition, Level -1

MPS01-01 / Hydrothermal liquefaction of biomass model compounds: characterization study by FTICR-MS
Annamaria Croce[1], Stefano Chiaberge[2], Tiziana Fiorani[2], Ezio Battistel[3], Pietro Cesti[2], Samantha Reale[1], Francesco De Angelis[1]
[1]Università degli Studi de L'Aquila, [2]eni, [3]eni versalis

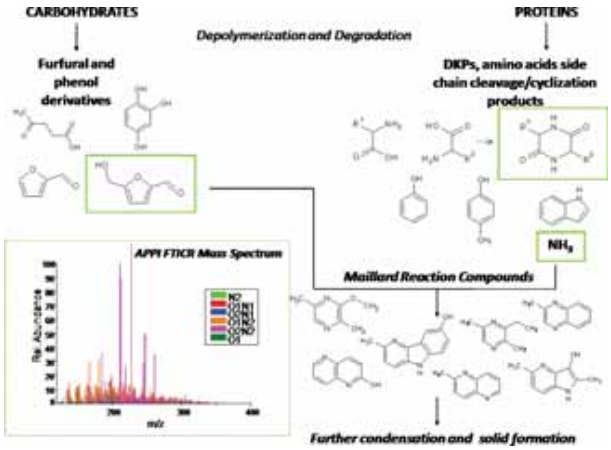
Introduction
The increasing demand for energy as well as related environmental concerns, and the predicted shortage of fossil fuels, are strongly pushing for search of new sources of liquid fuels (bio-oils), as suitable alternatives to fossil fuels. A valuable technology to produce bio-oils is the hydrothermal liquefaction (HTL) process of wet biomasses (mainly composed of carbohydrates, lipids and proteins), that allows their direct use without any pre-treatment. The resulting bio-oil is a complex mixture of organic compounds, produced by a series of competing and consecutive reactions occurring within the biomass itself.[1] Based on the observation of the complex composition of the bio-oil[1,2], we carried out a thorough study on the HTL treatment of model compounds. In particular we used carbohydrates (glucose, cyclodextrin, cellulose), protein model compounds (glycine, BSA), and binary/ternary mixtures of cellulose/BSA/triglycerides.

Methods
Aqueous solutions of model compounds were treated at different times under HTL conditions in a stainless steel reactor. Solvent extraction of the filtered resulting mixture gave the bio-oil, which was then characterized by GC-MS and FT-ICR-MS coupled with an APPI ion source.

Results
Furfural and phenol derivatives, as well as aliphatic compounds were identified by GC-MS analysis of bio-oils from carbohydrates, while APPI-FT-ICR-MS investigation allowed to determine high molecular weight compounds. Investigation of protein based bio-oils revealed diketopiperazines (DKPs) as major compounds (belonging to the O2N2 class) and other degradation products. As to the binary mixtures, despite DKPs were the most abundant compounds, other minor classes related to the Maillard reaction were detected (mainly N2 and O1N2 classes).

Conclusions
In addition to the mechanistic and analytical results, an important fallout of our study is the possibility of designing HTL operating conditions for obtaining low heteroatom content bio-oils, being this highly desirable in a technologically advanced biofuel.

Novel aspect
The FTICR-MS approach was applied for the first time to model compounds subjected to a HTL process, thus offering a molecular level description of the main conversion pathways which take place during the process.



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MPS01-02 / Coulomb-Interaction-Induced Effects on FT-ICR Mass Spectral Peak Shape: A Many-Particle Simulation Using GRAPE
Makoto Fujiwara, Naohisa Happo, Koichi Tanaka
Hiroshima City University

Introduction
In Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), ion motion trapped in the FT-ICR cell is essentially a superposition of three modes: cyclotron motion, magnetron motion and z motion. However, in practice, 103 to 106 different m/z ions are simultaneously trapped and excited in the cell and the ion cloud distribution consisting of each m/z ion ensemble and its temporal variation are quite complicated. Moreover at high ion density, the Coulomb interactions between ions considerably influence the ion cloud distribution. To analyze the Coulomb-interaction-induced effects by many-particle simulation we have introduced a special-purpose computer board (MD-One/E, Gazogiken) for many-body problems [1]. The board was combined with a single processor PC. The computer board makes it possible to calculate the summation routine of the ion-ion Coulomb interaction force components in high speed. Thereby in the case of 1,000 simulation particles the computation speed became 10 times as fast as a single processor only. Following the MD-One/E, a special-purpose computer GRAPE-6 (for "GRAvity PipE") has been employed and the computing configuration has been further accelerated.

Methods
On the above accelerated configuration with GRAPE-6, the influence of Coulomb-interaction-induced degradation of excited coherent ion cyclotron motion on FT-ICR mass spectral peak shape was investigated by many-particle simulation. The

simulation particles were equally divided and assigned to each of m/z 130.0 and 130.1 ions. Ion excitation was performed by frequency sweep at a magnetic field of 7 T and the ion spectra were simulated as a function of total ion population initially trapped in the FT-ICR cubic cell.

Results
From analysis of temporal variations for the ion cloud distributions during detection period it has been found that the coherent ion cyclotron motion of m/z 130.0 ion (lighter weight ion) cloud is out of phase earlier than that of m/z 130.1 ion cloud with increasing initial ion population. However, under certain conditions it has also been found that the spectral peak shape obtained form m/z 130.1 ion cloud is distorted earlier than that from m/z 130.0 ion cloud. Further we extracted respective ICR signal components resulting from m/z 130.0 and 130.1 ion clouds. In comparison between the two components, it has been found that the component from m/z 130.1 ion cloud is more significantly modulated than that from m/z 130.0 ion cloud.

Conclusions
The simulation results suggest that the relationship between the Coulomb-interaction-induced degradation and the spectral peak shape is quite complicated.

References
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Novel Aspect
Correlation between Coulomb-interaction-induced degradation of excited coherent ion cyclotron motion and FT-ICR mass spectral peak shape was investigated in detail by many-particle simulation using a special-purpose computer GRAPE.

MPS01-03 / Ion trap with a superposition of linear high frequency and homogeneous static electric fields

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RGRTU

Abstract
Ion traps are the effective devices for the mass separation, confinement and charge particles transportation. Ion traps type of Penning и Kingdonwith the static fields are used as the working cells in the high resolution MS with Fourier transform (FT-MS). Using RF linear and Paul ion traps as cells for FT-MS is limited by the large induced RF signal on the detector.

Methods
For overcoming of the specified technical problem we offer a new linear trap. The linear ion trap consists of two planar electrodes, one of which - discrete. Electric fields created by electrodes are the superposition of DC uniform field and quadrupole RF field. In result the ion trajectories have positive coordinates. In this regard the trap is called as a MONOTRAP like the monopole mass filter. The offered design of an ion trap when one of electrodes is grounded, will allow lowering significantly level of RF induced hindrances on the detector. The ion trap operation has been modeled numerically. It was realized homemade the ion trap with size 2x4x12 mm, one plane electrode contains 20 metal strips.

Results
By means of balancing of fields on two flat electrodes it was succeeded to suppress experimentally the induced signal on the detector to level of 100 dB. Numerical modeling of the ion motion in the field of the trap show the possibility of the input, capture and confinement of ions in the mass range Mmax/Mmin=5, with the initial energy dispersionWmax/Wmin=3 and initial angle dispersion α=30 . Here Mmax and Mmin , Wmax and Wmin are an maximal and minimal mass number and the ion energy consequently.

Novel Aspect
The offered ion trap so cold as MONOTRAP is a development of the linear quadrupole trap and using the transform Fourier method for identification ion mass on the base of the ion frequency oscillation spectra. This approach allows forming field compositions with various potential space-time distributions for improvement and development of new types of devices for ion confinement, mass separation and ion transportation of ions.

MPS01-04 / Creation and Injection Device for a 3D RF Ion Trap operated in Fourier Transform Mode applied to Fission Gas Release Analysis

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Introduction
When a 3D Radio-Frequency ion trap operates with a Fourier transform mode, the optimal dynamic for the detected image signal is obtained with a coherent motion of the ion cloud and with large amplitude of each ion motion.
Typically, the ion cloud can be rendered coherent by dipolar dc excitation [1] or by combining Stored Waveform excitations [2] prior confinement for mass-analysis. Moreover, the initial confinement conditions in position and velocity obtained with the SIFIM injection mode and by using an initial phase of the confinement RF voltage equal to pi directly lead to the two required criteria to have an optimal dynamics [3,4].
The SIFIM injection mode uses an external ion source. The ions are introduced into the ion trap by means of switched static voltages. They are submitted to a repulsive static potential in the trap where they are stopped before reaching the centre. The dispersion of initial confinement conditions is directly linked to the potential energy distribution in the ion source.

Method
An Ion Source-3D Ion Trap coupling is studied by means of Charged Particle Optics (CPO) Software. It uses a Boundary Element Method to calculate the potential at any point of the volume that makes do a specific electrode surface mesh to have the best accuracy for the ion trajectory computations.
Homemade LabView softwares have been developed (1) to extract data from CPO files, (2) calculate the ion trap acceptance ellipse in the phase space and (3) the position and velocity distributions of confinable ions.

Results
The improvements obtained with the new design of the creation-injection device are presented. The results concern the number of injected and confinable ions, the position and energy distributions at the beginning of confinement in the axial and radial directions. These results are applied for fission gas release analysis, i.e. low mass (1-6 u) high-resolution analysis and Kr and Xe isotopic high-sensitivity detection.

Conclusions
We find out potentials values applied to the source, lenses and entrance end-cap of the trap that satisfy optima of the image signal dynamic, further detected in mass analysis stage, for low masses as well as Kr and Xe isotopes.

Novel Aspect
The aim of this study is to take into account the influence of the

radial direction, the injection and confinement capability of low masses with a new design of the creation and injection device for a 3D RF quadrupole ion trap.

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MPS01-05 / FT-ICR MS for measurement of initial velocities of ions formed in MALDI process

Iva Tomalová[1], Vladimir Frankevich[2], Renato Zenobi[2]
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The spread of initial velocities of ions formed in matrix-assisted laser desorption/ionization (MALDI) is known to affect the mass resolution and calibration of time-of-flight mass analyzers. It also provides an important characteristic of the ion formation process. A novel approach employing internal MALDI source Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS)1 was used to investigate the initial velocities of multiple analyte and MALDI matrix ions.

This approach exploits a long (10 – 20 cm) field free region for measuring the initial ion velocity. No ion optics are placed in the ion flight path. An open cylindrical FT-ICR cell is used to trap and detect ions after variable delay times. The delay time corresponding to the signal intensity maximum and the known sample target-to-cell distance allow the mean initial ion velocity to be calculated. The placement of the MALDI target in the magnetic field (4.7 T) enables the detection of all ions with various angular velocity components.

Five peptides with molecular weights covering the range of 750 – 2900 Da in DHB as a MALDI matrix were used to investigate the dependence of initial ion velocity on the molecular weight. A decrease in the ion velocity with increasing molecular weight values was observed. Furthermore, simultaneous analysis of multiple matrix and analyte ions provided clear evidence of this dependence. The data correspond well to the results published by Spengler and Kirsch.2

Novel aspect

initial velocities of ions formed in MALDI were measured using a novel approach employing an internal MALDI source FT-ICR MS

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MPS01-06 / Characterization of Fractionated Pinewood Slow Pyrolysis Oils by Ultrahigh-Resolution FT-ICR Mass Spectrometry

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Introduction

Wood-based pyrolysis oil is one of the most promising liquid biofuels to substitute fossil fuels in the future. The chemical composition of pyrolysis oil is very complex and currently only limitedly known. By using ultrahigh-resolution Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry, coupled with negative-ion electrospray ionization (ESI), it is possible to characterize acidic, polar heteroatom-containing compounds present in pyrolysis oils. In addition, pyrolysis oil fractionation, based upon solvent solubility, could assist to resolve the chemical functionalities of the identified species in more detail.

Methods

The pyrolysis oil was produced from unbarked pine wood pieces (Pinus sylvestris). Slow pyrolysis experiments were carried out at the BioSampo Training and Research Centre (BTRC) of Kouvola Region Vocational College. The oil was collected within the temperature range of 315–360 °C. The resulting oil was biphasic with clearly separated aqueous and oily phases. Both phases were analyzed separately. In addition, the whole oil (both phases mixed together) was fractionated into five subfractions, based on solubility in water, ether and dichloromethane (DCM). All mass spectrometry measurements were performed with a Bruker APEX-Qe 12-T hybrid quadrupole FT-ICR mass spectrometer equipped an Apollo-II ESI source.

Results

The studied pinewood pyrolysis oil contained mainly Ox heteroatom class compounds (O2–O15). The compounds in the classes O2–O4 comprised mainly different fatty acids and hydroxy fatty acids as well as some resin acids. Following solvent fractionation, these extractives were concentrated into the subfraction of water-insoluble–DCM-soluble compounds. Phenolic derivatives, resulting from the lignin degradation, were found in every subfraction with high abundance. In addition, a lot of different sugars, mainly in the “sugar” fraction (subfraction of entirely water-soluble compounds), were observed.The main difference between the oily and the aqueous phases was that the resin and fatty acids as well as pyrolytic lignin were concentrated into the oily phase. There were clear differences between different subfractions, but separation was not quantitative as many compounds were present in all subfractions.

Conclusions

Solvent fractionation of pyrolysis oil should selectively direct the species into different subfractions according to their solubility. Based on the current results, however, solvent fractionation does not seem to work quantitatively, likely because of the highly complex chemical nature of the pyrolysis oil. Ultrahigh-resolution ESI FT-ICR MS was shown to be especially good in fingerprinting high-molecular weight compounds, such as extractives, lignin degradation products and sugars, of the slow pyrolysis pinewood oil.

Novel aspect

Solvent fractionation combined with ESI FT-ICR MS to characterize slow pyrolysis oils

MPS01-07 / Rapid and selective analysis of sulfur-containing species in crude oils by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry

Xuxiao Wang, Wolfgang Schrader
Max-Planck-Institut für Kohlenforschung

Introduction

The demand for affordable energy drives keeps the focus on fossil-based materials such as crude oil [1] Containing a high abundance of heteroatoms in crude oils is disadvantageous and deleterious to upgrading processes. A better understanding of crudes in petroleum is necessary [2]. However, crude oil is the most complex natural mixture available, ultra-high-resolution mass spectrometry such as FT-ICR MS [3] that is capable of reaching the necessary resolutions and accuracies is the method-of-choice for crude oil analysis. The combination with online reaction and ESI FT-ICR MS is a successful approach to rapidly selectively determining the distribution of sulfur species in crude oil.

Methods

A heavy bitumen was reacted with a series of organic reagents (ethyl iodide, C2H5I and ethyl triflate C2H5OTf) in dry 1, 2-dichloroethane (DCE) for 1min at RT. The samples were prepared and introduced into ESI FT-ICR MS. Mass spectra were recorded using a 7 T FT-ICR MS equipped with an ESI source. External mass calibration was performed using a tune mix solution from Agilent.

Additionally, a mixture of sulfur-containing standard compounds (dibenzothiophene, DBT) was reacted with C2H5I and C2H5OTf in dry DCE for 12h at RT. The yield was determined by ¬1H NMR. ¬1H NMR analysis was performed on Bruker Advance 300 NMR spectrometers. Chemical shifts of 1H spectra are reported as in units of parts per million (ppm) downfield from SiMe4 (δ 0.0) and relative to the signal of chloroform-d (J = 7.264, singlet).

Results and conclusions

ESI is the method of choice for analysis of polar compounds. Nevertheless, most of components in crude oil are non-polar. Therefore, we developed a derivatization method for crude oil analysis that makes the use of ESI feasable for crude oil samples. This initial derivatization reaction was now optimized by comparing different derivatization reactants to compare reactivities with standards and heavy crude oil samples. The use of different derivatization agents allows to selectively activate different functionalities, such as sulfur species. These pre-formed ions then can easily be analyzed by using ESI. Here, we compare the different reaction conditions from different derivatization reagents (C2H5I and C2H5OTf). These results show that the combination of chemical modifications with ESI FT-ICR MS is a successful approach to a selective analysis of the less-polar sulfur species in crude oils.

Novel aspects

Combination with online reaction and ESI FT-ICR MS is a successful approach to rapid selective analysis of sulfur species in crudes.

Literatures

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MPS01-08 / Petroleum and Its Fractions: Exploring the Saturated and Aromatic Hydrocarbon Composition by APCI(+)-FT-ICR-MS

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Introduction

Due to its complexity, petroleum chemical characterization is a huge great challenge. So far, FT-ICR MS via ESI is the technique of choice to resolve this complex mixture due to its ultra high resolution and selectivity to polar compounds. However the less polar compounds are commonly analyzed by conventional gas chromatography (GC) and by mass spectrometry using electron ionization (EI) or chemical ionization (CI) with a mass limit of ca. 500 Da.
The analysis of high MW non-polar hydrocarbons is challenging by atmospheric pressure mass spectrometry techniques. The lack of an API technique for it has limited the ability of mass spectrometry to examine the less polar fraction of crude oils. Based on previous studies about the use of pentane as solvent in atmospheric pressure chemical ionization (APCI), we decide to explore and compare the HC chemical composition of petroleum fractions using pentane APCI by ultra-high resolution MS with the objective of investigating the composition and molecular distribution of these samples.

Methods

Samples of crude oil from contrastanting API gravity were fractionated by SARA. The saturated and aromatic fractions were dissolved in pure pentane and analyzed by APCI(+)-FT ICR MS. The results were processed using the PetroMS software. Additionally, the hydrocarbon composition identified by ultra-high resolution MS was compared with the volatile hydrocarbons detected and identified by GC-MS.

Results

The samples were analyzed by APCI(+) FT-ICR MS and was possible to ionize non-polar high MW hydrocarbons by using pentane as the adictive. The results show that pentane doping allows a charge-transfer reactions followed by H loss generating [M–H]+ as the most abundant and intact stable ions readily identified with the ultra-high resolution of FT-ICR MS without the use of any derivatization and with no significant fragmentation (rather than H loss). The spectra were processed using PetroMS software in which the most abundant attributed class found in samples was the HC class. The DBE and carbon number distribution for class HC of each fraction were evaluated and the profiles obtained for petroleum samples with different API gravity were compared. For the saturated fraction the DBE found was in the range of 2 to 22 and the range was from C20 to C90.

Conclusions

APCI(+) using pentane as the dopant allows the investigation of HC and aromatic composition of SARA fractions of crude oils by classical petroleomic approaches. Abundant [M-H]+ ions were produced with typical profiles whereas the APCI distillation process permits the detection of relatively heavy HC up to C90. Other dopants for improve sensitivity are being investigated.

Novel Aspect

The viability of high molecular weight non-polar hydrocarbons analyses for contrasting API gravity petroleum samples via APCI(+) FT-ICR MS for Petroleomic studies is demonstrated.

MPS01-09 / Laser Ablation for Introducing Internal Calibrant for High Performance Liquid Chromatography/Fourier Transform-Ion Cyclotron Resonance (HPLC/FT-ICR)

Hung Su, Yu-Min Huang, Jentaie Shiea
National Sun-Yat Sen University

Introduction

Accurate mass measurement is crucial for unambiguously identifying an organic compound. Fourier transform ion cyclotron resonance (FT-ICR) is capable to provide such an analysis by recording a mass spectrum containing the ion signals of analyte and calibrant for internal calibration. The analysis is usually achieved by externally mixing the analyte with the calibrant solution following by electrospray ionization. However, as the analytes are introduced into the ESI source through high performance liquid chromatography (HPLC), it is difficult to mix the calibrant with the analyte for accurate mass measurement. In this study, an interface was developed to efficiently mix the calibrants with the analytes for HPLC/ESI/FT-ICR analysis.

Methods

The interface for introducing the calibrant into the HPLC/ESI/FT-ICR for internal calibration is comprised of: (1) a pulsed UV laser beam – to desorb calibrant from the solution flowing out of a syringe, (2) a three-way tee – to connect the sample solution flowing out of a HPLC column to a capillary and an electrode, and (3) a high voltage power supply - to induce ESI from the sampling solution flowing out of the capillary. The high voltage was applied to the sample solution by inserting an electrode to one arm of the three-way tee.

Preliminary Data

The calibrant used for internal calibration was introduced into the HPLC/ESI/FT-ICR through laser desorption followed by post-ionization in the ESI plume. Electrospray ionization was induced from the sample solution flowing out of the three-way tee. The calibrant solution (i.e., tuning mix or sodium formate) flowing out of a syringe was continuously desorbed by irradiating the solution at the exit of the syringe with a pulse laser beam. The desorbed calibrant molecules moved upward, joined in the ESI plume, and were ionized by interacting with the charged solvent species in the ESI plume. The analyte together with the calibrant ions were subsequently detected by the FT-ICR attached after the ESI source. The system was applied for high resolution analysis of a mixture of triglycerides and phospholipids. It was found that sodium formate ions contain known masses over a wide mass rang and provide enough ion peaks for internal calibration of all analytes. The proposed method showed a mass measurement error ranging from -0.85 to 0.24 ppm for lipid samples.

Conclusion

The calibrant for internal calibration was successfully introduced into the HPLC/ESI/FT-ICR system for lipid analysis through laser desorption.

Novel Aspect

Internal calibration of HPLC/ESI/FT-ICR was achieved by continuously delivering calibrant into the ESI plume through laser desorption

MPS01-10 / TreeRobot: A new software for automated acquisition of MSn spectral trees on Orbitrap hybrid mass spectrometers

Robert Mistrik[1], Juraj Lutisan[1], Jakub Mezey[1], Tim Stratton[2], Lukas Najdekr[3], Silvia Vlckova[4], Vladimir Patoprsty[4]
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Introduction

The appearance of tandem spectra may be strongly dependent upon the applied experimental conditions which considerably complicate identifications of unknowns through comparison to reference spectra. In the area of small molecules, the recently established concept of spectral trees addresses the spectra reproducibility problem by integrating multi-stage tandem spectra of numerous precursor ions acquired using various experimental conditions into a single tree record.

Methods

We will present a novel Orbitrap hardware/software platform for the informed acquisition of comprehensive MSn spectral trees for incorporation into a reference database. With chemical structure in hand and using real time algorithmic noise and artifacts filtering, all unrelated potential precursor ions are dynamically excluded. Real time optimization of instrument parameters and duration takes into account the dependency of ion yield on sample flow rate, the absolute breakdown curves of precursor ions, the S/N ratio of precursor scans, and the effects of varying collision energies on the resulting fragmentation efficiency to balance the need for quality spectra and to minimize the acquisition of useless data, while collecting as many characteristic spectra as possible.

Results

To collect comprehensive spectral trees containing as many energy resolved product ion spectra as possible, an advanced MSn acquisition software (TreeRobot) autonomously controls the Orbitrap MS system by making real time decisions in both a data dependent and data independent fashion.

Knowing the identity of a reference compound, not only the protonated, deprotonated or singly charged, an ion is automatically selected as the precursor from the full scan, however precursor ions at subsequent stages are selected from spectra filtered in real time using algorithms based on structural information. Product ion spectra are acquired from abundant precursor ions emerging at optimal collision energies determined from the break-down curves derived from energy resolved measurements at previous tandem stages.

The presented platform must deal with two primary limitations: first, acquisition time, which can without constraints exceed 5 hours per compound depending on the molecule size and, second, the sample amount. To limit both the duration and sample consumption, additional intelligent real time procedures are applied to prevent unnecessary acquisitions and to terminate the apparently “dead” MSn branch automatically.

Spectral trees of human metabolites, pharmaceuticals and environmental contaminants collected using the free of charge TreeRobot platform will be presented and their superiority over the data acquired using traditional systems will be demonstrated in compound identification examples. The presented spectral trees will be deposited into a publicly available database (mzcloud.org) where they will be available for review.

Novel Aspect

The novel automated acquisition of high resolution, energy resolved, hierarchically dependent spectral trees using algorithmic real time decision logic.

MPS01-11 / Vitreomics by ESI (+) FT-ICR-MS

Júlio César Santos Júnior[1], Carla Giane Loss[1], Pedro Carlos Mollo Filho[2], Ruggero Bernardo Felice Guidugli[2], Eduardo Morgado Schmidt[4], Marcos Albieri Pudenzi[3], Marcos Nogueira Eberlin[3], Nelci Fenalti Höehr[1]

[1]Department of Clinical Pathology, School of Medical Sciences, University of Campinas — UNICAMP, Brazil, [2]Team of Forensic Medicine West, Medico-Legal Institute, Police Technical Scientific Superintendence — SPTC, Brazil, [3]ThoMSon Mass Spectrometry Laboratory, Institute of Chemistry, University of Campinas — UNICAMP, Brazil

Introduction

Omics is an emerging science that uses most advanced analytical techniques to characterize and quantify molecules in biological samples. Its application in forensic science is extremely important, mainly when the focus is in forensic toxicology. Different strategies and alternative samples are being applied in forensic toxicological analyses. Vitreous humor is composed by 99% of water, with the remaining 1 % made up of sugar, salts and proteins. It is also subjected to less contamination and bacterial degradation due to the protected environment inside the eye. Therefore is an important and sometimes exclusive target.

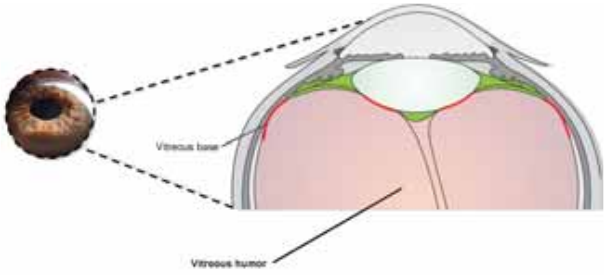
Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) provides ultra high resolution and accuracy in mass analysis and its accurate m/z values lead to the exact molecular composition. In this work we evaluated the complete composition by direct FT-ICR-MS analysis of vitreous humors of decomposing bodies with varying post-mortem periods (1-7 days) to function as a important tool forensic toxicology.

Methods

The vitreous humor samples analyzed (cases) (5 bodies) were classified into no decomposing bodies with 3 days post-mortem period (A3NDB) and decomposing bodies A1 (1 day post-mortem period), A2 (2 days post-mortem period), A3 (3 days post-mortem period) and A4 (7 days post-mortem period) collected between June and September of 2012. For the analysis in the positive ion mode, 10 µL was injected through a Advion TriVersa NanoMate® Robotic system. The m/z were then monitored by FT-ICR-MS in a LTQ-FT Ultra (Thermo Scientific, Bremen, Germany) mass spectrometer.

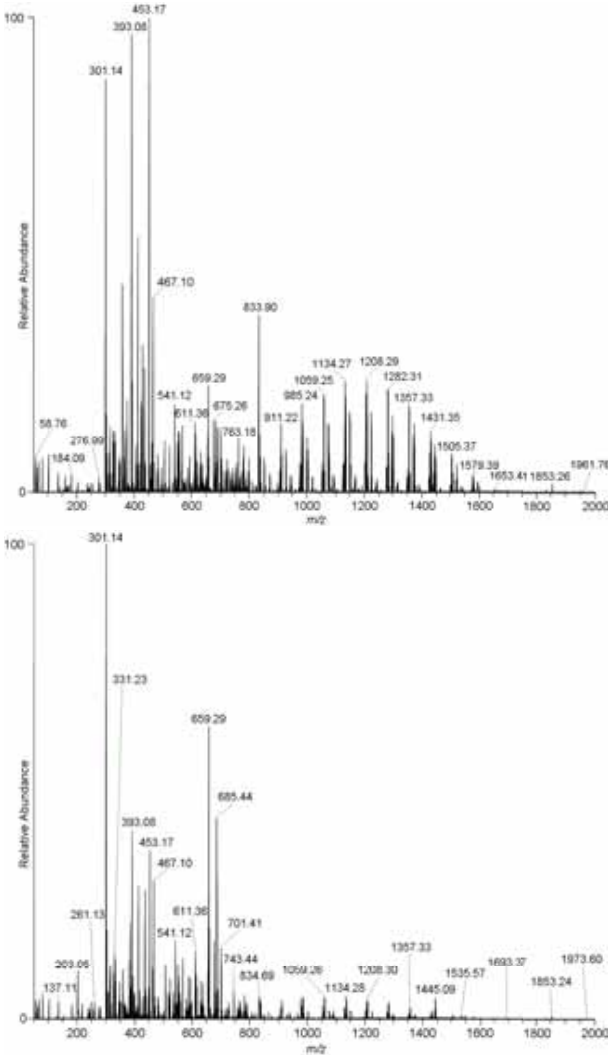
Results

Via ESI(+) FT-ICR-MS, the profiles of intact samples of vitreous humors were obtained. Such profiles were considerable similar for samples of the same period, but varied greatly due to different (bio)chemical composition in different post-mortem periods.



Conclusion

Although profiles of the vitreous humors samples as revealed by ESI(+)-MS monitoring showed great variability in (bio)chemical composition as a function of post-mortem period, due to intense putrefactive phenomena activities, which also promote metabolite degradation, still compounds could be both identified and accurately confirmed as well as actual vitreous humors collected from decomposing bodies with different post-mortem times via direct, rapid and separation-free ESI(+)-FT-MS analysis. The ultra high resolution and accuracy of the MS analysis eliminated isobaric interferences and permitted unequivocal detection. Direct analysis by ESI(+) FT-ICR-MS analysis of human vitreous humors proved therefore to provide a reliable toll in toxicology and forensic investigations



Novel Aspect

At the best our knowledge, the correlation of (bio)chemical profile at different post-mortem periods allied to forensic toxicology is extremely promising and innovative, since there are many unanswered questions as well as this is the first work in the literature dealing about this subject.

MPS01-12 / Comparing LDI-FT-ICR and LDI-TOF/TOF Mass Spectrometry to Characterize Vacuum Residue of Colombian Crude Oils

Enrique Mejía-Ospino[1], Jorge Orrego-Ruiz[2], Rafael Cabanzo[1]

[1]Universidad Industrial de Santander, [2]Instituto Colombiano de Petróleos

Introduction

Laser desorption ionization (LDI) was performed using two different mass analyzers: Fourier transform ion cyclotron resonance (FT-ICR) and time of flight tube (TOF/TOF) to study fractions (condensed) of two vacuum residue cuts in three distillation ranges, IBP-603ºC, IBP-645ºC, and IBP-687ºC [1]. FT-ICR and TOF spectra of each of these fractions are quite different. The results presented here show that LDI is effective in ionizing heavy fractions of crude oils independently of the mass analyzer. High double-bond-equivalent (DBE) compounds were

obtained in agreement with the presence of condensed structures in vacuum residues [2-3].

Methods

Samples

Two vacuum residua (VR) were fractioned by short path distillation process [1]. Each VR was distillated into three ranges, IBP-603ºC (C2R and C2G), IBP-645ºC (C4R and C4G), and IBP-687ºC (C6R and C6G), getting one condensed and one residue for each cut, namely; three condensed and three residues. The condensed samples were used in this study.

Mass spectra

Each condensed sample was analyzed in the LDI (+) ion mode on a SolariX FT-ICR Mass Spectrometer (Bruker Daltonik GmbH, Bremen, Germany) equipped with a 15 T refrigerated actively shielded superconducting magnet (Bruker Biospin) and on a UltrafleXtreme MALDI-TOF/TOF Mass Spectrometer (Bruker, Daltonic).

Table 1. Average mass calculated using LDI-FT-ICR and LDI-TOF/TOF

Sample	Average Mass (LDI-FT-ICR)	Average Mass (LDI-TO/TOF)
C2R	476.84161	531.09104
C4R	544.21002	552.40271
C6R	684.80114	528.61659
C2G	613.51250	574.29054
C4G	695.48318	583.83720
C6G	701.97611	611.00205

References

[1] Zuñiga-Liñan, L.; et al. J. Pet. Sci. Eng. 2011, 78, 78–85
[2] Marshall, A. G.; Rodgers, R. P. Acc. Chem. Res. 2004, 37 (1), 53−59
[3] Rodgers, R. P.; McKenna, A. M. Anal. Chem. 2011, 83 (12), 4665−4687
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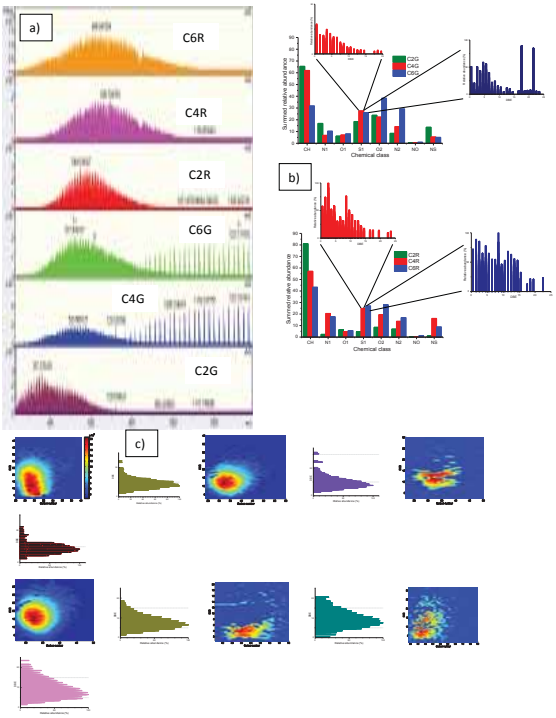

Figure 1. a) LDI-FT-ICR Mass spectra of three cuts of two vacuum residue of Colombian crude oils; b) Distribution of chemical classes observed and c) DBE versus carbon number (contour plot to the left) and DBE distribution plots (bar graphs to the right) for CH class compounds observed.

Results and discussion

Figure 1a shows LDI-FT-ICR mass spectra and Figure 2 shows LDI-TOF/TOF spectra of six samples of three distilled of each vacuum residues. The resolving power of the LDI-FT-ICR spectrum was about 250000 at m/z 500, while with LDI-TOF/TOF system it was only possible to reach a resolving power of 25000 at m/z 500. More than 12000 peaks with S/N ratios over 5 were observed for each LDI-FT-ICR spectrum and about 4000 peaks were observed on each LDI-TOF/TOF spectrum.

The results obtained with LDI-FT-ICR allowed detailed structural analysis of the three cuts of each vacuum residue but do not using LDI-TOF/TOF. The average masses, calculated from each fraction using FT-ICR and TOF/TOF system, are listed in Table 1. These results show that the ions detected are dependent of the mass analyzer. LDI-FT-ICR mass spectrum is a broader distribution that are shifted to higher masses, at least in heavy cuts (C4 and C6), while LDI-TOF/TOF produced distributions centered on lower molecular weight peaks.

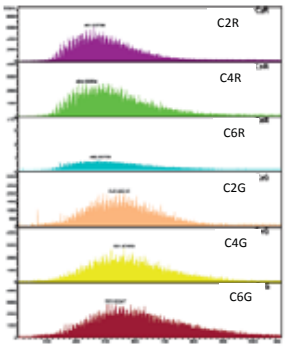

Figure 2. LDI-TOF/TOF Mass spectra of three cuts of two vacuum residues of Colombian crude oils

Conclusion

The results shown in this study demonstrate that LDI FT-ICR mass spectrometry allows characterizing vacuum residues fractions to the molecular level. LDI-FT-ICR can be effectively used to study CH, N1, O1, S1, O2, N2, NO and NS chemical class. However, due to its low resolving power, LDI-TOF/TOF allows only to determine average mass of vacuum residue fractions. Both methodologies are highly sensitive to compounds with aromatic groups (DBE >6).

References

[1] Zuñiga-Liñan, L.; et al. J. Pet. Sci. Eng. 2011, 78, 78–85
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MPS01-13 / Instantaneous frequency theory and applications in FTMS

Oleg Yu. Tsybin[1], Anton N. Kozhinov[2], Konstantin O. Nagornov[2], Yury O. Tsybin[2]

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Time-dependent frequency components in transients manifest themselves as non-linear features in frequency (mass) spectra, e.g., as magnetron sidebands. What is the true nature of generation of signals with time-dependent frequencies? How they can be accounted for in spectral analysis and instrument development to maximize FTMS analytical characteristics? Here, we present a theory of ion motion in FTMS that considers ion motion frequencies dependent on time (instantaneous frequencies). We then apply the described theory to develop algorithms for ion excitation and ion detection, and to establish a relationship of ion motion parameters and the analytically-constructed mass spectra. Finally, we validate the developed analytical approaches by comparison with SIMION calculations and experimental measurements.

Based on a theoretical analysis of charged particles' trajectories in an electromagnetic field we derived the differential equations that describe non-linear dependencies of phase and instantaneous frequency on time for ion motion in ion trapping mass analyzers,

ICR and Orbitrap FTMS. We then developed methods for solving these differential equations and solved them in linear and non-linear fields for ion oscillations along radial, azimuthal, and axial directions. We compare the obtained results with those derived with SIMION modeling of ion motion in an open-ended cylindrical ICR cell installed in a 10 T magnet and experimental results obtained with 10 T LTQ FT-ICR MS, Orbitrap FTMS.
The obtained analytical solutions describe explicit dependence of ion motion on time and initial values of ion radius and azimuth, as well as radial and azimuthal velocities, in harmonic electrical fields. General solutions of the derived differential equations are found in non-harmonic electric fields. For the first time, ion RF excitation is taken into account while deriving the post-excitation solution of ion motion. Algorithms for transient generation are derived for diverse detection electrode geometries, boundary conditions, initial parameters of ion motion, and the corresponding ion trajectories. Obtained transients correlate with those generated with SIMION and experimentally.
Overall, the obtained results improve the general understanding of FTMS and shall be useful for its further hardware and signal processing development. Particularly, we aim to optimize the calibration equations in FTMS and improve the achievable mass accuracy.

Novel aspect
Theory of ion motion with phase non-linearly dependent with time and its application to ion excitation and detection in FTMS

MPS01-14 / High-performance FT-ICR MS at unperturbed cyclotron frequency

Konstantin Nagornov, Anton Kozhinov, Konstantin Zhurov, Yury Tsybin
Ecole polytechnique fédérale de Lausanne

Modern FT-ICR MS is based on detection of reduced cyclotron frequencies of ions. This is primarily due to a trapping electric field which is essential for confinement of ions inside the ICR cell located in a homogenous magnetic field. The radial component of this trapping electric field shifts the unperturbed ion cyclotron frequency resulting in detection of trapping field-dependent reduced cyclotron frequency. Ergo, the trapping electric field detrimentally impacts the quality of the resultant mass spectra, limiting resolving power and mass accuracy for FT-ICR measurements. If the radial component is eliminated, an attractive possibility of measuring the unperturbed cyclotron frequency would be realized.Here, we demonstrate improved performance of FT-ICR MS with a customized ICR cell that enables direct detection of unperturbed ion cyclotron frequency.
A hybrid 10 T FT-ICR mass spectrometer (Thermo Scientific) was equipped with a narrow aperture detection electrodes (NADEL) ICR cell based on the design of an open-ended cylindrical ICR cell (Ultra cell). Transient signals were recorded in MIDAS format at 1-5 MHz sampling frequency using the advanced software interface of the built-in data acquisition system. To acquire transients at 5-100 MHz sampling frequency, an external data acquisition system was used (National Instruments). Data analysis was performed using the framework pyFTMS developed in house.
Preliminary data includes an extensive study of the operational regime where the trapping electric field is compensated within the NADEL cell via application of DC offset potentials to excitation and detection electrodes. For a wide range of trapping potentials the measured peaks correspond to the unperturbed ion cyclotron frequencies even for the most complex, petroleomics-grade, samples. Mass accuracy, as a function of number of charges, trapping potential, and acquisition time was studied for this compensated regime. General mass accuracy enhancement for peptides (standard deviation (sd) <0.1 ppm; mass range of 300 - 3000 m/z; external calibration), proteins, and petroleum (sd<0.05 ppm; 300 - 800 m/z; internal calibration) mass measurements was achieved for the compensated regime with a standard FT-ICR MS calibration procedure. The enhancement of the dynamic range for petroleum measurements was achieved for high trapping potentials of up to 10 V. Additionally, approaches that allow for circumvention of digital limitation issue and enhancement of FT-ICR mass measurements will be discussed.
The NADEL ICR cell is able to operate in the regime where the radical component of a trapping electric field is compensated in a wide range of trapping potentials, leading to direct detection of unperturbed cyclotron frequency. The FT-ICR MS performance with improved analytical characteristics is demonstrated on samples of proteomic and petroleomic origin.

Novel Aspect
ICR cells with narrow aperture detection electrodes allow for direct detection of unperturbed ion cyclotron frequencies and thus improved mass accuracy and sensitivity for MS applications.

MPS01-15 / Design of a Permanent Magnetic Orbital Trap Mass Analyzer

Chuan-Fan Ding, Chongsheng Xu, Xianzhong Ding, Haiyang Yang
Fudan University

Introduction
FTICR mass spectrometer relies on high intensity and highly homogeneous magnetic field which is conventionally generated by expensive superconductor magnet. Although there are recent reports on ICR device using permanent rear earth magnets, such device does not give superb mass resolving power due to its limited magnetic field strength. With limited magnetic flux, it is wise to create an annular space with high magnetic field intensity for ion trapping and mass analysis, rather than whole space filled with uniform magnetic field in which ions are resonantly excited.

Methods
An annular magnetic field region can be created using permanent magnets. Without using cyclotron resonance excitation, ions which are generated outside of the magnetic region can be injected tangentially into the annular orbital flight path in the magnetic field. All ions need to be accelerated to certain momentum before injection so that they can fly in the orbits with similar radius. There are focusing electrode arrays surround the flight path to restrict the ion motion in the axial direction and these electrodes are also play the role of image charge pick-ups. Totally 12 pairs of pick-up electrodes are used and they are grouped to provide 12 fold frequency of ion's cyclotron frequency, therefore an 12 times mass resolving power may be received for a certain time and magnetic field strength.

Results
Simulation for designing the focusing electrodes was carried out with SIMION 8. Static periodical focusing and static quadrupole focusing are compared and it was found that the static periodic focusing gives better isochronous performance. Preliminary result shows that a resolving power over 10,000 has been achieved with only 6000 Gauss magnetic field and a transient about 10ms at mass 400 Th.

Conclusions
Orbital motion of ions in annular space of magnetic field generated by permanent magnet gives alternative way of FTMS without using cyclotron resonance excitation. Good mass resolution can be achieved by proper design of focusing electrodes and making use of multiple pick-ups along the circular orbit.

Novel Aspect
New FTMS, generate high resolving power with low cost magnet.

MPS02-01 / Analysis of PFOS in Plastic Products by LC-MS/MS and the Assessment of Uncertainty

Tian Yuping, Cheng Tao
Shanghai Institute of Measurement and Testing Technology

Introduction

In the past few decades, as an important additive, PFOS (Perfluorooctane sulfonate) has been added to plastic products widely. According to the assessment by the OECD (Organization for Economic Co-operation and Development) in 2002, PFOS is one kind of persistent pollutant organic compound, and can be accumulated in the human body. It is difficult to detect PFOS in plastic products, since PFOS has many derivatives, such as perfluorooctane sulfonate.

Methods

Here we used the Liquid chromatography-tandem mass spectrometry (LC-MS/MS) to analyze the PFOS. First, the plastic materials were smashed by a liquid nitrogen frozen grinder, then extracted by Methanol, cleaned up with SPE cartridges, concentrated or diluted, filtered with a 0.45μm Nylon membrane, injected into the instrument, separated by column, detected with MRM mode, finally been quantified by the external standard. Additionally, we established the fishbone diagram to evaluate the uncertainty of the detected results, to ensure the results accurate and reliable.

Results

The method was used to analysis PFOS in different plastic matrices, such as polypropylene(PP) ect. The ions at m/z499.0,m/z98.7,m/z79.7 were used in the MRM mode to collect and process the data. At the same time, the measurement conditions, such as the voltage of cone, the energy of collision, the dry gas temperature ect., have been optimized.

The system has been successfully calibrated across a working range of 0.005 to 0.5μg/mL, by a coefficient of determination 0.998. The repeatability of nine injections of 0.005μg/mL standard was excellent, with % RSD values 4.2.

The fishbone diagram was established, which was based on five aspects to affect the analysis results, showing in figure 1. According to the evaluation, the expanded uncertainty value of the detected result was 8%.

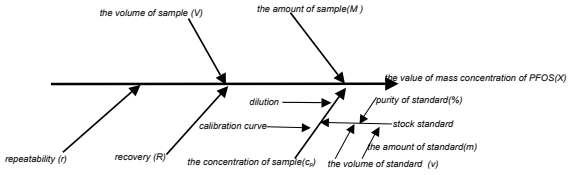

Figure 1. established the fishbone diagram

Conclusions

The results presented here demonstrate the effectiveness of this analytical technique to detect PFOS in plastic products, the expanded uncertainty of the detected results is less than 10%, the results met the requirements of the Directive.

Novel Aspect

We present an excellent analytical approach for the fast and simple analysis of PFOS in various types of complex plastic matrices using LC-MS/MS, and establish fishbone diagram to evaluate the uncertainty of the detected results, to ensure the results accurate and reliable.

MPS02-02 / One step further in the folding of multiply charged sodium cationized polylactides: ion mobility mass spectrometry and molecular modelling

Julien De Winter[1], Vincent Lemaur[1], Kirsten Craven[2], Jérôme Cornil[1], Philippe Dubois[1], Philippe Dugourd[3], Timothy Jenkins[2], Pascal Gerbaux[1]
[1]University of Mons, [2]Waters, [3]University Claude Bernard, Lyon I

Introduction

Ion mobility - mass spectrometry (IM-MS) was recently introduced as an additional tool for the mass spectrometry characterization of synthetic polymers. In particular, it was demonstrated that introducing IM as an ion chromatography step between the ion source and the mass analyzer can yield a more complete view of the polymer samples by: (i) allowing the separation between isomeric and isobaric ions; and (ii) increasing the dynamic range of the mass analyzer by sequentially introducing ions in the analyzer. The main objective of this work is to deeply characterize the three-dimensional structure of polymer ions using an original IM-mass spectrometer and a commercial one.

Methods

In the present study, the three-dimensional structures of multiply charged sodium cationized polylactides (Na+PLA), produced using Electrospray Ionization (ESI), were probed by combining experimental results from ion mobility measurements on a "Home-made" IM-MS instrument (linear drift tube) and a commercial one, Synapt G2 from Waters (the only IM-MS instrument currently commercially available, TriWave technology). Moreover, to give credit to our observation, theoretical data obtained by force-field calculations have been done.

Results

An in-depth study of PLA.Na+ has been realized on a linear drift tube allowing to get the real averaged Collisional Cross Section (CCS). This study also leads to the observation of interesting behavior of multi charged ions. Indeed, for singly cationized PLA oligomers, the cross section was shown to increase with the size of the oligomer following the relation ΩAvg=k.n2/3, i.e., the typical size dependence expected for spherical objects. In contrast, for poly-cationized PLA oligomers, this trend was not observed anymore. For doubly and triply cationized oligomers, the average collision cross section increases faster than for mono-cationized oligomers, then reaches a plateau and finally follows the trend observed for the mono-cationized oligomers.[1]

Conclusion

In the context of this poster, we intend to highlight the presence of this plateau and to explain its origin on the basis of theoretical calculations. Finally, a qualitative comparison between data recorded on the Home-made machine and the commercial one is presented.

Reference

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MPS02-03 / Comprehensive Analysis of Extractables from Rubber Stoppers used in Medical Devices and Pharmaceutical Container Closure Systems

Andrew Feilden[1], Kate Comstock[2], Amalendu Sarkar[3]
[1]Smithers Rapra, [2]Thermofisher Scientific, [3]Qure Medical

Novel Aspect

Comprehensive analysis of extractables from rubber stoppers used in medical devices and pharmaceutical container closure systems.

Introduction

Rubber and plastic are widely used in medical devices and pharmaceutical container closure systems. Extractables & leachables (E&L) assessment of these materials is an integral part of the submission for approval of new drug product and medical devices. E&L are potential risk to patients; it is important to identify E&L above certain levels so as to assess them toxicologically.
This study demonstrates a comprehensive workflow for medical grade rubber stopper extractable analysis using multiple techniques: HR-LCMS, GCMS, and ICPMS, also data process using novel software and database searching.

Methods

Four different medical grade rubber stoppers were extracted using de-ionized water and IPA utilizing reflux extraction and the Bucci Speed extractor.
Sample solutions were analyzed using HR-LCMS system consisting of Thermo Ultimate3000 UHPLC and Q Exactive bench-top Orbitrap Mass spectrometer. The high resolution full scan MS and data dependent MS/MS data were collected with polarity switching.
The data were processed by Thermo Scientific Compound DiscovererTM, Mass FrontierTM7.0, and mzCloudTM spectral database.
GCMS analyses were conducted on Thermo Scientific Trace ultra GC, Triplus RSH autosampler, and ISQ single quadrupole MS.
Elemental analyses were carried out on Thermo Scientific iCAP Q ICP-MS with KED mode setting.

Result

The high resolution full scan and ms/ms data acquired from Q Exactive were processed using Mass Frontier and Compound Discoverer for components extraction and structure elucidation.
Major component structures were confirmed through mzCloud database searching. The putative structures of minor components were proposed based on ms/ms fragments using the "fragment and mechanism" feature in Mass Frontier.
GCMS analyses were performed in full scan data acquisition mode to identify unknowns. The peaks detected were identified using the NIST library search. GCMS identified volatile and semi-volatile components which complement LCMS results.
ICPMS analysis focused on USP <232> elements and other elements; the results indicated that the extraction solutions are clean: all elements are at acceptable levels or below LOD.

Conclusion

This study demonstrated a comprehensive workflow for extractable analysis using LCMS, GCMS, ICPMS, and data analysis software.The workflow utilizes unique features of each system to ensure the separation, identification, and characterization of extractables in complex extraction samples. With this workflow, confident and complete extractable and leachable analysis can be accomplished.

MPS02-05 / Characterization of thermal degradation products of polymer material by reactive pyrolysis-GC/MS using EI ionization method and PI ionization method

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Introduction

Poly(buthylene terephterate) (PBT) has been widely used in automobile components because of their excellent mechanical and electrical properties and also high thermal stability. PBT is gradually degraded under the usage environment by external factors such as heat and oxygen, which would cause changing the color and decreasing of the mechanical strength. Therefore, it is important to analyze the structural changes during thermo oxidative degradation and figure out the relationship between material function and chemical structure from the perspective of quality control of final products. Reactive pyrolysis-gas chromatography mass spectrometry (reactive Py-GC/MS) is the one of the powerful techniques to analyze the chemical structure of condensed polymers involving PBT. The resulting pyrolysis products can be detected as methylated forms by selective decomposition of polymer chain in the presence of tetramethylammonium hydroxide (TMAH).
In this work, thermally treated PBT has been studied by reactive Py-GC/MS Because molecular weight determination of pyrolysis products would be difficult only used by electron ionization (EI), we also attempt to introduce photo ionization(PI) to obtain the both information about chemical structure and molecular weight.

Method

PBT dumbbell-test pieces were used for the thermal accelerated test. Thermal degradation samples with several time conditions (up to 400 h) were prepared in air. Each test piece was shaved into thin films by a rotary microtome (Leica Microsystems, RM2265). A stainless cup containing about 20 mg of the shaved sample together with 4 ml of TMAH (25wt% MeOH solution) was introduced into a pyrolyser (Frontier Lab, PY-3030D) heated at 420 °C. The pyrolysis products were analyzed online by a gas chromatograph/mass spectrometer (Agilent technology, 7890GC /JEOL, JMS-Q1050GC).

Result & Discussion

Pyrolysis products of the initial PBT sample on the pyrogram could be assigned both by EI and PI mass spectra. In the pyrograms of the PBT sample thermally treated at 180 °C, some component peaks changed in intensity depending on the treatment time. Based on the mass spectra obtained by EI and PI method, these peaks were assigned to the products originating from anti-oxidant, biphenyl type cross-linking and acid anhydride structures. The peaks of anti-oxidant component disappeared in the early degradation period before around 100 h. Alternatively, the other degradation products appeared prominently after around 250 h. It was suggested that the rapid thermo-oxidative degradation progressed in the PBT sample caused by the loss of anti-oxidant components. Mechanical strength decreased rapidly at the same timing. Thus, the amounts of these components seem related to the change of material function and it would be possible to use as an index of the thermo-oxidative degradation.

MPS02-06 / Sequencing of Copolymers using Mass Spectrometry

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Introduction

Synthetic polymers are of a great interest in medicinal-drug delivery systems; however, this necessitates smart polymers to achieve the goal. Thus, the sequencing of polymers has started to elucidate the detailed structure of polymers. Synthetic polymers result in more complex spectra in comparison to peptides due to these polydisperse nature, which include different chain lengths and sequences. In 2007, Thalassinos et al. presented a software for the determination of end groups in homopolymer MS/MS spectra. Furthermore in 2011, Baumgärtel et al. reported first results of the application of a software for de novo identification of fragmentation mechanisms of homopolymers. Copolymers have an even more complex spectra in comparison to homopolymers. In 2012, Weidner et al. developed the software MassChrom2D, a fraction dependent method using liquid chromatography hyphenated offline to MALDI enabling 2D copolymer compositions plots.

Methods

MALDI-ToF mass spectra were obtained using an Ultraflex III TOF/TOF mass spectrometer (Bruker Daltonics) with trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene] malononitrile as matrix in reflector and sodium chloride as doping agent. The instrument was calibrated prior to each measurement with an external PMMA standard from PSS Polymer Standards Services GmbH. Multiple computational methods for the evaluation of different parameters have been used for the copolymers.

Results

A new method is established to provide the analysis of linear copolymers, which is independent of their composition and polymer class. A statistical model was proposed for the polymerization process and to reduce the model fitting to high-dimensional numerical optimization and NP-hard combinatorial problems.

Conclusions

A simple model for the polymerization as well as a complex method for estimating the model parameters from MS and MS/MS spectra is proposed. Quantification of the abundances of every linear copolymer chain after optimization of certain parameters has been performed. Currently, the synthesis of copolymer libraries and additional experiments are conducted to demonstrate the plausibility of the computational results. The libraries will also help to explore the feasibility limits of the method. Furthermore, chemical questions will be correlated to a statistical model and supported by interpretable visualizations.

Novel Aspect

Sequencing of copolymers with mass spectrometry with computational methods

MPS02-07 / Evaluation of the thermal degradation of poly (butylene terephthalate) by high-resolution MALDI-TOFMS combined with Kendrick mass defect analysis

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Introduction

Poly(butylene terephthalate) (PBT) is an engineering plastic with good balance of mechanical and electrical properties and, being able to withstand use at high temperatures, is widely used in automobile components, such as connectors. At the processing temperature, thermal and hydrolytic degradation may take place. The detection of degradation products at the early stage is important to predict their life-time accurately.
Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has recently gained wide popularity in polymer research because of its high sensitivity and capability of detecting large molecules, even in complex mixtures. In the study of the degradation of PBT, the data acquired by MALDI allowed remarkably highly informative, yielding precise information on the structure and end groups of molecules originated in the oxidation process [1].
We have developed a new method for evaluating the thermo-oxidative degradation processes of PBT used in automobile components for the purpose of the quality management. In the present study, Kendrick mass defect (KMD) analysis derived from high-resolution MALDI mass spectra [2] was introduced to depict the formation of degradation products at the early stages of PBT degradation.

Experimental

Industrially synthesized and pelletized PBT sample was used in this work. The dumbbell-test pieces of PBT were thermo-oxidized at 180 ºC in atmospheric air for 50 to 400 hrs. Samples shaved to thin films by rotary microtome were dissolved in HFIP and mixed with DHB matrix. High-resolution MALDI-TOF mass spectra were recorded using a JMS-S3000 (JEOL Ltd.) TOF mass spectrometer with a spiral ion trajectory.

Result and discussion

The chemical structures of the heated PBT samples were tentatively assigned by referring to the published report [1] and verified based on the accurate mass observed by high-resolution mass spectra. The main components of initial PBT were cyclic oligomers and three types of linear chains with different end group combinations i.e. alcohol/alcohol, alcohol/carboxyl, and carboxyl/carboxyl terminus. As for the degraded samples, the ratio of the oligomers having carboxyl/carboxyl end groups relative to cyclic oligomers increased with time. On the other hand, the tensile strength and average molecular weight had decreased considerably after 200hrs.
The plots of KMD analysis based on the accurate mass spectra revealed the formations of several minor products, of which amounts were changed with time. These products would be possible used as an indicators in the early stages of thermo-oxidative degradation of PBT.

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MPS02-08 / Electrospun Nanofiber Surface Assisted Laser Desorption/ Ionization Mass Spectrometry

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Introduction

Nanostructured materials, which are capable of absorbing laser energy and trap analytes in the nanostructure for ease of energy transfer, have been used as effective assisting materials in surface-assisted laser desorption/ionization mass spectrometry (SALDI-MS). Electrospinning of polymers allows the preparation of nanofibers with characteristics such as large surface area-to-volume ratio and high porosity with small pore size. However, the composition and design of the nanofibers are required further optimization and investigation. We herein investigate the feasibility of using organic and inorganic-based electrospun nanofibers as the SALDI assisting materials. In this study, 1-amino-2-naphthol-4-sulfonic acid (ANS), a compound has good absorption capacity in the region of ultraviolet-visible, was incorporated in water-soluble poly(vinyl alcohol) (PVA) for the generation of electrospun nanofibers. The electrospun nanofibers were used as the assisting materials in SALDI-MS analysis. Additionally, sol-gel derived electrospun nanofibers generated from electrospinning was also investigated.

Experimental

Nanoifibers made from the mixture of PVA MWave= ~41,240 Da) and ANS (MW= 239 Da) was prepared by electrospinning with an applied voltage of 15.6 kV, collection time of 60 min, and the tip-to-collector distance of 10 cm. When preparing inorganic-based electrospun nanofibers, metal oxide sol-gel solution was prepared initially followed by electrospinning process to generated nanofibers.

The morphology and chemical composition of the as-spun nanofibers were analyzed by emission scanning electron microscopy and Fourier-transform infrared (FTIR) spectroscopy, respectively. All the mass spectra were obtained from a Bruker Daltonics Autoflex □ mass spectrometer.

Results

The as-spun pristine PVA and PVA/ANS revealed average diameter of 440 ± 127 and 365 ± 68 nm, respectively. The decrease of average fiber diameter might be due to the addition of ANS which caused the decrease of PVA concentration. The generated PNA/AVS electrospun nanofibers were used as the SALDI assisting materials. The results showed that small molecules such as glucose and amino acids were observed in the SALDI mass spectra. However, we also noticed that a series of ions derived from carbon clusters with a 12 atomic mass unit difference accompanied with the appearance of the analyte ions. Thus, we further investigated the resultant nanofibers by SEM after SALDI-MS analysis. We found that the shape of fibers was no longer remained. Grain particles were observed in the SEM images. Presumably, the nanofibers were destroyed during laser irradiation.

Conclusions

Our results showed that organic-based electrospun nanofibers can be used to assist SALDI MS analysis. However, some background ions derived from the laser irradiation product were observed. Thus, inorganic based electrospun nanofibers performed better ass assisting materials in SALDI-MS analysis.

Novel Aspects

Organic and inorganic electrospun fibers were explored as SALDI assisting materials.

MPS02-09 / Production of doubly charged species during solvent-free MALDI of small synthetic polymers

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Introduction

MALDI is very popular for ionization of synthetic polymers prior to their mass analysis since it yields a single mass distribution of singly charged species, and hence more simple mass spectra as compared to electrospray ionization (ESI). As a result, there is less severe demand on mass spectrometric resolution, ensuring the m/z measurement accuracy required for end-group analysis but also that of relative peak heights used in calculation of molecular weight distribution parameters. In addition, still in great contrast to ESI, insoluble synthetic polymers can also be subjected to MALDI-MS by using a solvent-free sample preparation procedure. Using orthogonal acceleration TOF for improved mass resolution in conjunction with ion mobility spectrometry, some small synthetic polymers were however evidenced to be generated in the gas phase as doubly charged species, particularly when using the solvent-free version of MALDI sample preparation.

Methods

Tested synthetic polymers were poly(ethylene glycol) (PEG, Mn 2000 Da), polycaprolactone (PCL, Mn 3000 Da), poly(methylmethacrylate) (PMMA, Mn 1980 Da), polystyrene (PS, Mn 2000 Da), and poly(4-vinylpyridine) (P4VP, Mn 1700 Da). Solvent-free samples were prepared by grinding a matrix/polymer(/salt) mixture in the solid state. Solvent-based MALDI samples were prepared according to the classical dried-droplet method. MALDI-MS and IMS experiments were conducted with a Waters Synapt G2 mass spectrometer with a laser emitting at 355 nm.

Results

Beside a major single cationization mode, PEG was found to readily adopt a +2 charge state upon solvent-free MALDI, while P4VP and PCL did to a lesser extent. In contrast, this phenomenon was not observed for PMMA and PS. PEG was further scrutinized using different alkali salts as the cationizing agents. The size threshold for PEG chains to interact with two cations increased with the alkali size while the abundance of doubly charged adducts was observed to decrease from Li to K, with no signal obtained for Rb and Cs. In solvent-free MALDI, abundance of doubly lithiated species were on average 50% higher as compared to those generated from samples prepared using the dried-droplet preparation. All these data were obtained using DCTB as the matrix and supplementary experiments are currently performed to study any influence of the matrix in this process. Results obtained in ion mobility experiments suggested that conformation of the MALDI doubly charged PEG adducts was similar to that of homologous ions generated upon electrospray ionization.

Conclusions

Thanks to the high resolving power of orthogonal acceleration TOF analyzer and the extended dynamic detection range enabled by ion mobility spectrometry, generation of doubly charged species could clearly be evidenced during MALDI of small polymers. Molecular organization in MALDI samples will further be studied using solid-state NMR to account for their different propensity to generate doubly charged species as a function of their preparation.

Novel aspects

Production of multiply charged species from low mass synthetic polymers in solvent-free MALDI

MPS02-10 / ETD as an alternative fragmentation technique to CID for the characterization of polytetrahydrofuran and polycaprolactone

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Introduction

The characterization of synthetic polymers can be performed by single-stage mass spectrometry (MS) but tandem MS is required for more detailed information. The main technique used to perform tandem MS analysis is collision-induced dissociation (CID). However, in the last decade, other fragmentation techniques have been developed such as electron-capture dissociation and electron-transfer dissociation (ETD). In particular, ETD mechanism involves the transfer of an electron to an isolated precursor ion, which generates an exited radical ion species that rapidly undergoes radical-induced cleavages. The goal of this work was to evaluate the information produced by ETD from a polyether, a polyester and their copolymer, and to compare the results with those generated by classical CID.

Methods

All MS/MS experiments were carried out using a LTQ-Orbitrap XL (Thermo-Fisher Scientific) equipped with an electrospray ionization (ESI) source. The MS scan was performed in the FT cell recording a window between m/z 300 and 4000. The resolution was set to 30 000. Collision gas was He. Fluoranthene was used for ETD experiments. Solutions of polymer (10-4M) were infused in H2O/CH3OH 50/50 (v/v) in the presence of Li acetate at a flow rate of 3µL/min.

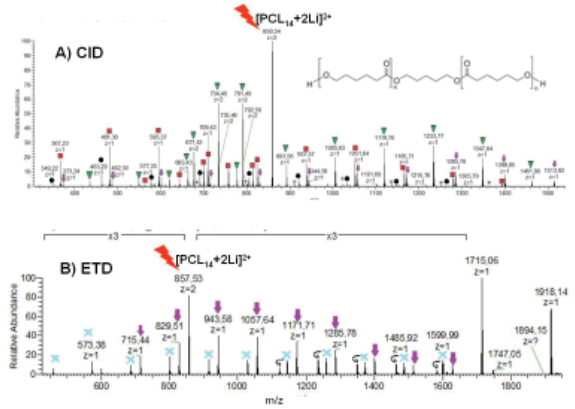

Figure: A) CID and B) ETD MS^2 spectra of the $[PCL_{14}+2Li]^{2+}$ ion from the Polycaprolactone diol 14mer (m/z 856).

Results

CID and ETD mass spectra were recorded from various lithium adducts (various charge states) from two homopolymers, polycaprolactone diol (PCL) and polytetrahydrofuran (PTHF), and one copolymer triblock (PCL-PTHF-PCL). As CID of PCL led to mass spectra of increasing complexity from precursors of higher charge states (2+, 3+), corresponding ETD mass spectra remained relatively simple. CID mass spectra displayed product ions series with multiple charge states whereas ETD, thanks to different fragmentation pathways, led mainly to singly-charged fragment ion series (see Figure). The CID of PTHF also led to complex mass spectra for the higher charge states (2+, 3+, 4+). However, under ETD conditions, precursor ions of PTHF, were prone to charge reduction, either by fluoranthene anion addition, or by electron transfer. ETD only did not provide any fragmentation. Nevertheless, a combination of ETD followed by CID (hybrid excitation) gave surprisingly from a 4+ precursor only one fragment ion series (2+). The CID of the triblock copolymer yielded ions series resulting from the fragmentation of the PCL block. Comparison of CID and ETD mass spectra recorded from the same precursor showed that in both cases, the PTHF block was not involved in the fragmentations, only PCL parts were fragmented. ETD spectra were also simplified compared to CID since all the fragment ions were singly-charged ions.

Conclusions

The ETD technique applied to PTHF and PCL analysis can be used as a complementary method to CID. ETD provides additional fragmentation pathways and/or tandem mass spectra simplification.

Novel Aspect

Gas-phase fragmentation of synthetic polymers using Electron Transfer Dissociation.

MPS02-11 / TOF-SIMS / MALDI-TOF combination for the molecular weight depth profiling of a polymeric bilayer

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Introduction

Depth profiling of polymeric-based complex architectures is a still rapidly growing-up and competitive field, since no technique could still provide all the informations mandatory for a deep understanding of the material composition. Secondary Ion Mass Spectrometry (SIMS) is the most widely used technique as it provides nm depth-resolved data about inorganic, organic and polymeric multilayers. In common case, this technique provides a fragmented vision of a multilayer, with no molecular information. On the other hand, MALDI-MS have traditionally been considered as a powerful technique for polymer analysis but limited to the bulk. Wesdemiotis and coworkers have recently developed the Surface-Layer MALD (SL-MALDI), a surface sensitive technique which was successfully applied to probe the surface concentration of polymeric blend with a depth resolution found less than 2 nm. Following the global trend of etching/analysis steps distinction, this investigation aims at coupling a TOF-SIMS etching step, a SL-MALDI analysis and Nanoscratch (NS) depth measurements to investigate an original molecular weight depth profile.

Methods

A reference bilayer made of the same polymer with different molecular weights (a polystyrene PS3.7k onto a PS1.8k) have been elaborated by spin coating and floating to mimic a molecular weight gradient of a unique polymer. Craters were produced into this reference bilayer using a TOF-SIMS device, while SL-MALDI-MS analyses were conducted into the so-formed craters to evaluate the distribution parameters of the polymeric layer.

Results

A direct TOF-SIMS profiling conducted using a Cs+ ion beam lead to the detection of fragments from carbonated layers without any data about their MW, making the fine polystyrene bilayer architecture indistinguishable. Spots of various depths were hence created using the same Cs+ ion bombardment for different sputtering times, and further mass-analyzed by SL- MALDI-MS, to evaluate their MW. Briefly, matrix and salt were mixed together in a solvent free process and gently sprinkled into the craters. Such analytical methodology allows MALDI-MS spectra to be recorded from the very first molecular layers (a few nm). In our case the depth resolution was slightly degraded to 40 nm due to our specific elaboration procedure for the reference bilayer. Such resolution was nevertheless sufficient to evaluate the distribution parameters for a given crater and plot these molecular weight as

a function of the physical depth of the craters as found by NS.

Conclusions
As the depth resolution of both etching and analysis steps are below a few tens of nanometers, we expect this original off-line coupling to be of high potential for the analysis of molecular and molecular weight gradients with a broad range of applications, e.g. in the field of the polymer light-emitting diodes-

Novel Aspect
An off-line combination of ion etching and LASER analysis allowed a molecular weight depth profile to be plotted as a function of the physical depth instead of the usual sputter time. To the best of our knowledge this original coupling has never been reported in the literature.

MPS02-12 / Copolymer characterization by combination of MALDI and pyrolysis GC-MS
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MALDI is now one of the technique of choice in structural characterization of polymers especially for the study of end groups and molecular weight determination. However characterization of copolymers by MALDI is still challenging due to the complexity of obtained spectra and optimization of MALDI analysis conditions. Pyrolysis GCMS is an older technique but still relevant for copolymer characterization especially for high molecular weight compounds as there is no limit in mass range for the use of this characterization. Indeed, it enables ratio determination of each monomer and identification of random or block copolymer.

This work will present a combinative study of different kind of copolymers by these two techniques
MALDI :
- Optimization of sample preparation and especially choice of matrix
- Determination of end groups and molecular weight
- Determination of individual length distribution for each monomer

Pyrolysis GCMS :
- Optimization of pyrolysis temperature for structural characterization
- Identification of pyrolysis product characteristic of each monomer
- Identification of specific pyrolysis products in case of random copolymers

The different data obtained by these two techniques will be compared, it will show the complementarity of the methods and the benefit to combine these two techniques to obtain a detailed image of copolymeric system.

MPS02-13 / Detailed insight into tyramine cross-linking in hyaluronan-based hydrogels
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Contipro Pharma a.s.

Introduction
Tyramine-based hydrogels have been widely studied as biomaterials for various biomedical applications, like tissue engineering and drug delivery systems. In particular, chemical cross-linking systems using enzymatic reactions have various advantages, such as biocompatibility, in-situ forming and easy control of reaction rates under mild conditions. The structural changes arising from tyramine (TYR) cross-linking were proposed for many times in literature. However, direct evidence confirmed only the formation of C-C diTYR and failed to explain other new signals appearing in NMR spectrum of cross-linked TYR-based derivatives.1 The aim of this work was to get a detailed insight of TYR-based modified derivatives after cross-linking and elucidate the structural changes formed.

Methods
Experiments were carried out using Waters Synapt mass spectrometers equipped with electrospray and Bruker Avance III 500 MHz was used to measure NMR spectra. TYR-based HA derivatives were prepared as described by Wolfova et al.2 and hydrogels prepared with various amounts of H2O2 and HRP were studied. TYR-based modified oligosaccharides used as standards were isolated by two-dimensional chromatography in an off-line mode.

Results
Purified TYR-based modified oligosaccharides were used to study the reaction mechanism of TYR cross-linking. Detailed evaluation of mass fragmentation and NMR spectra identified both, C-C and C-O coupled positional isomers. Both couplings were present also in hydrogels prepared by adding low amount of cross-linking reagents. C-C coupled diTYR was formed predominantly; the appearance of both forms in mixture is about 5:1.
Except from that, unknown ions corresponding to the formation of 'tri- or moreTYR' were present in MS spectra when higher amounts of cross-linking reagents were added. In this case, meta position of TYR was activated and the connection between three or more TYRs was formed. In comparison with hydrogels prepared with 10-times lower amounts of cross-linking reagents, the reaction proceeded up to the fifth stage, i.e. six TYRs were coupled into one molecule.

Conclusions
Mass spectrometry and NMR spectroscopy was used to characterize the cross-linking of TYR-based modified hyaluronan. Except from C-C and C-O coupled diTYR, the formation of "tri- and moreTYR" was also confirmed. The data are in agreement with fluorescence of hydrogels prepared with various H2O2 and HRP additions. When mostly diTYRs are present in hydrogels, the intensity of fluorescence increases; while it decreases when higher amounts of cross-linking agents are added thus the connection between three and more TYRs is formed.

Novel Aspect
Mass spectrometry was successfully applied in elucidation of structural changes formed in tyramine when cross-linked with H2O2 and HRP.

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MPS02-14 / Ion mobility spectrometry-mass spectrometry (IMS-MS) analysis of polyamidoamine (PAMAM) dendrimers

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Introduction

Polyamidoamine (PAMAM) dendrimers are described as very promising drug delivery platforms for the emerging nucleic acid therapeutics based on RNA interference.[1,2] They are believed to form non-covalent complexes with guest molecules via their numerous primary amine end-groups and to release them according to the proton sponge effect theory, stating that both their conformation and size expand when electrostatic repulsions between protons located onto buried tertiary amines increase as they experience a strong pH decrease while entering the endosome of targeted cells.[3,4] Techniques working on gas phase species are particularly efficient for the study of non-covalent complexes since useful information such as complex stoichiometry, structural details and conformational features can be obtained by mass spectrometry (MS), collision-induced dissociation (CID) and ion mobility spectrometry (IMS) experiments, respectively.

Methods

PAMAM dendrimers (generation 0 to 3) were investigated employing the combination of TWIMS and electrospray ionization mass spectrometry (ESI-MS). Ion mobility (IM) was utilized to evaluate the collision cross-sections (CCS) of the ions of perfect dendrimer structures. IMS-MS experiments allowed important information to be obtained about the conformation of dendritic structures in the gas phase. Moreover, different wave height (WH) and wave velocity (WV) values were employed to observe the effects of these parameters on the results obtained by IMS experiments.

Results

As a preamble to study conformational aspects of PAMAM-RNA complexes, changes in the conformation of naked PAMAM dendrimers are monitored here as a function of their mass and their protonation state, using travelling wave ion mobility spectrometry (TWIMS).

Conclusions

Preliminary experiments performed on two small PAMAM dendrimers have shown a significant shape extension as the charge state increases but also that the extent of this swelling phenomenon strongly depends on the dendrimers architecture.[5] More relevant higher generations (up to G3) ethylenediamine-core PAMAM are scrutinized here. TWIMS experiments were performed to evaluate CCS values of protonated species generated by ESI. Molecular modeling studies are currently ongoing to provide theoretical values to be compared to experimental CCS data.

Novel Aspect

Ion mobility spectrometry-mass spectrometry (IMS-MS) analysis of polyamidoamine (PAMAM) dendrimers for the detailed characterization of their structural details and conformational features.

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MPS02-15 / Resolving Ionization processes of polyamides in ESI-MS by ESI-IMS-MS

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Here we present first experiences with the analysis of structural features for a variety of synthetic polyamides such as: branching, copolymer structures, and inter/intra molecular reactions. Moreover the capability of ESI-IMS-MS in the assessment of these crucial parameters will be discussed. As such, we focus here on the ionization and structural analysis of polyamides in a newly developed trapped ion mobility spectrometer (TIMS).
First, ionization efficiency and behavior of polyamides in ESI-MS were investigated on an Agilent MSD TOF, utilizing the conventional electrospray source. All ESI-IMS-MS measurements were performed on a Bruker impact, modified with an TIMS ion funnel. In the TIMS analyzer ions are radially confined via rf trapping voltages while axial trapping is the result of a balance in the force of a flowing gas and a positional dependent DC potential. By scanning the DC trapping potential ions are eluted according to mobility in a fashion which allows accurate determination of mobility and collision cross section in a number of gases. High molecular weight Polyamides are not soluble in standard ESI solvents, therefore samples where dissolved and directly infused using pure formic acid. Despite the high abundance of protons in the solution, it was observed that the ionization process resulted in multiple charges with combinations of protonation and sodium adduct formation. This leads to a complex spectrum which is difficult to interpret. By introducing an additional dimension of separation in the form of ion mobility, the analysis of the polyamides shows a clear separation of overlapping charge states and different consistencies of protonation and sodium adduct formation. The use of TIMS improves the interpretation of the complex mass spectra obtained for the polyamides. In addition, special structural features, like cyclic polymers originating from ring closure reactions can be easily observed in the mobiligram.
Overall, here we present the separation of different ionization effects and molecular features of polyamides which can be distinguished via separation in the mobility dimension prior to mass analysis. Further study is needed to determine the structure of other inter/intra molecular reactions like the aforementioned cyclization. In addition investigation into the use of TIMS for higher molecular weight polymers where spectral complexity is further increased is envisioned in the near future.

MPS02-16 / Plasma-Enhanced Chemical Vapour Deposition investigated by Atmospheric-Pressure MALDI High-Resolution Mass Spectrometry using matrix pre-coated substrates

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Centre de Recherche Public - Gabriel Lippmann

Introduction

Plasma-Enhanced - Chemical Vapor Deposition (PE-CVD) is a method of choice for the elaboration of functional coatings. As the functionality of the coating may depend on the molecular integrity of the repeat unit in the plasma polymer, the understanding of the molecular structures and the control the elaboration processes require advanced characterization techniques. Plasma polymers tend to be highly cross-linked and strongly attached to the substrate; their characterization thus requires a combination of techniques such as XPS or FT-IR, which provide a global

composition of the coating, but lack of molecular selectivity. This work focuses on the development of a method for the investigation of PE-CVD by Atmospheric Pressure - Matrix-Assisted Laser Desorption/Ionization coupled to High Resolution Mass spectrometry (AP-MALDI HRMS).

Methods
Two kinds of PE-CVD coatings were produced from plasma-polymerized DiEthylAllylPhosphate (ppDEAP) and AminoPropylTriEthoxySilane (ppAPTES). The plasma polymers were subsequently submitted to Atmospheric Pressure - Matrix-Assisted Laser Desorption/Ionization (AP-MALDI PDF+, Masstech). The analysis of those AP-MALDI produced ions was achieved by High Resolution Mass Spectrometry (Orbitrap Elite, Thermo), taking benefit of its high mass accuracy, and MSn capabilities for the unambiguous identification of plasma-induced polymerization products. The choice of the matrix and the sample preparation were studied. We tested solvent-based or solvent-free matrix deposition/sublimation at the surface of the plasma polymer coatings as well as PE-CVD on matrix pre-coated substrates.

Results
This analytical approach led to the evidence of intact linear oligomers of ppDEAP and partially crosslinked oligomers of ppAPTES in controlled plasma process conditions, providing a way to produce plasma-induced polymers at atmospheric pressure mimicking those obtained by conventional wet-chemistry. The method provides fruitful information for the understanding of the growth mechanisms of plasma polymers. Matrix pre-coated substrates, which has recently been studied for biological tissue imaging for lipids and proteins, is presented here as an efficient approach to identify oligomers produced during plasma-induced polymerization in PE-CVD processes.

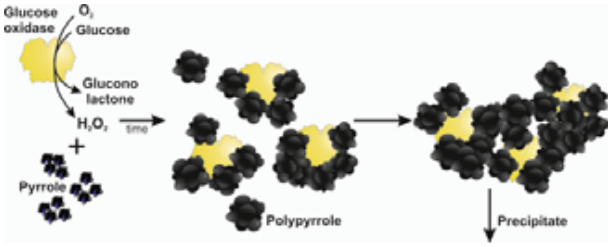
Conclusions
In combination with other techniques commonly used in the field of organic materials characterizations, AP-MALDI Orbitrap is demonstrated as a method of choice to gain access to growth mechanisms in PE-CVD processes used for the elaboration of functional coatings.

Novel Aspect
To the best of our knowledge this original approach has never been reported in the literature.

MPS02-17 / Application of stable isotope method for the nanocomposites investigation

Andrius Garbaras[1], Lina Mikoliunaite[2], Anton Popov[2], Almira Ramanaviciene[2], Vidmantas Remeikis1, Arunas Ramanavicius1
[1]Center for Physical Sciences and Technology, [2]Faculty of Chemistry, Vilnius University

The stable isotope ratio mass spectrometry method was applied for the determination of the stoichiometry of complex nanoparticles consisting of enzyme and conducting polymer. These nanoparticles were based on biocomposite consisting of enzyme glucose oxidase wrapped within conducting polymer polypyrrole. The formation of the biocomposite was evaluated using dynamic light scattering method in time. The consistent enlargement of the biocomposite was noticed as well as the relative decrease of the amount of spare glucose oxidase molecules in the polymerization solution. The polymerization of pyrrole was observed by UV-visible spectrometry and the optimal monomer concentration was determined. Isotope mixing model was applied for the evaluation of the constitution of biocomposite. The nitrogen isotope ratio approach was applied more successfully in comparison to the carbon approach for the determination of biocomposite stoichiometry.



MPS02-18 / Determination Method of VOCs in Accelerated Aging Polypropylene by Thermal Desorption-Gas Chromatography/Mass Spectrometry

Shuqi Sun, Ying Zhang, Jin Wang, Yang Song, Song Chen
SINOPEC Beijing Research Institute of Chemical Industry

Polypropylene (PP) is widely used in industry and daily life due to its high productivity and excellent properties. But like other polymers, polypropylene depredates in the atmospheric environment. For example, the wavelengths of sunshine over 290 nm could lead to the degradation and cause discoloration or chalking. Besides of sunshine, there are many factors that would course degradation, such as temperature, humidity, oxidation, and so on. Degradation process of polymers often take years, but researchers found methods to accelerate that process and to evaluate the aging behavior in lab. Accelerated lighting tests are often performed by a xenon lamp because its wavelength is quite similar with sunlight.
Chemical and physical properties of polymers change during the aging process. This study investigated the volatile organic compounds (VOCs) emitted from aging PP materials accelerated by a xenon light to imitate outdoor exposure.
In this study, PP were cut into small pieces and put into an apparatus that designed to collect the VOCs emitting from PP materials under the xenon lamp irradiation at temperatures of 60°C. The Tenax tubes could be fixed on the apparatus and the VOCs would be absorbed for a further analysis. Thermal desorption-gas chromatography/mass spectrometry (TD-GC/MS) had been applied to the determination of volatile compounds emitted from accelerated aging PP materials every other day.
During the TD analysis, the stainless steel tube was heated at 90°C for 3 min, the emitted volatile substances was cooled in the cold trap after carried there by helium stream. Then those substances absorbed in the cold trap were rapidly heated to 280°C. The target compounds were evaporated and then isolated in the gas chromatographic separating column and detected by the mass spectrometer. Chromatographic separation was carried out on an HP-5MS column with a temperature program, and the mass spectrometric detection was operated by a quadrupole mass spectrometer with an electron impact ion source. The mass spectrums of each chromatograph peak obtained from the TD-GC/MS method were searched in the NIST Mass Spectral Library to obtain the qualitative results.

The results indicated that the VOCs from aging PP were composed of alkanes, alkenes, oxygen compounds like ketones and aldehydes, and BHT was also detected. With the increasing of the illumination time, the concentration of the VOCs changed while the species did not within a month.
A novel apparatus was designed to collect the VOCs emitting from PP materials under the xenon lamp irradiation by a Tenax tube. TD-GC/MS method had been applied to the determination of VOCs in months.
The apparatus comprise a container, xenon light, a heating system and a timing system. The most novel aspect is that the stainless

steel with Tenax sorbent could be fixed on the top and a pump was involved for the adsorption.

Graphical abstract

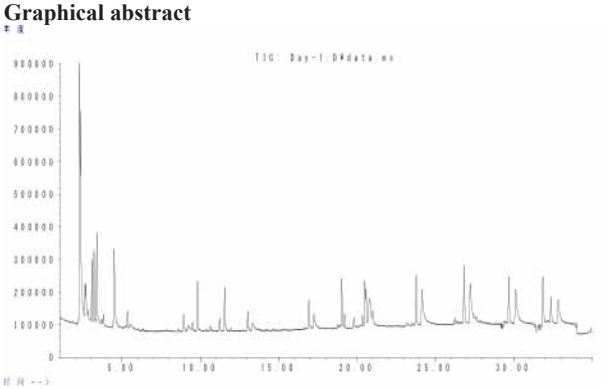


Fig.1 TIC of TD-GC/MS day 1

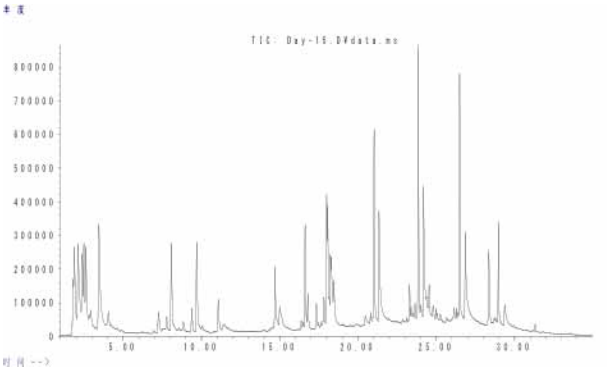


Fig.2 TIC of TD-GC/MS day 15

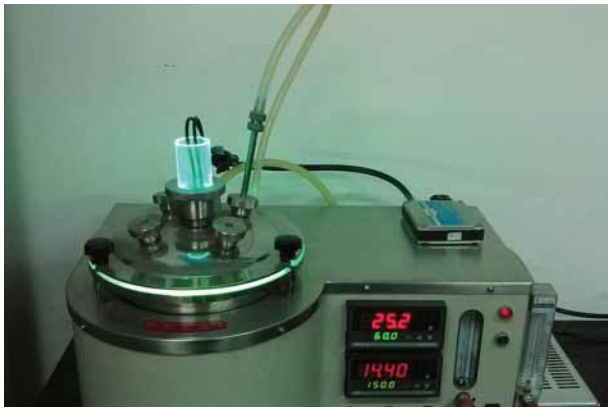


Fig.3 the VOCs collecting apparatus with a the xenon lamp

MPS02-19 / Real-time Characterization of Polymers by Using Thermogravimetry Coupled with An Ambient Mass Spectrometry

Jentaie Shiea, Ruei-Hung Hung, Min-Zong Huang
[1]*National Sun-Yat Sen University*

Introduction

Thermogravimetric analysis (TGA) coupled to mass spectrometry (MS) has been used to characterize the evolved gaseous fragments from materials subjected by thermal decomposition. Electron impact/chemical ionization (EI/CI) is commonly used for traditional TG-MS. However, the reliance on a high vacuum and production of fragment-rich spectra are critical problems of the technique. Ambient mass spectrometry (AMS) is a solution for these problems as it is a simple but useful technique that generates reactive species for direct interaction with analytes under ambient conditions, where useful molecular information can be obtained through soft ionization mechanisms. In this study, TG-AMS was used to provide direct and on-line detection of evolved gases for the analysis of different polymers and materials.

Methods

A commercial TG unit with a platinum crucible was used for study. A transfer system was developed to connect the TG unit to an ambient ionization source based on electrospray ionization (ESI) and/or atmospheric pressure chemical ionization (APCI) mechanisms. The ions were detected by an ion trap mass spectrometer. Thermoplastics and thermosets including PE, PS, PEG, PMMA and copolymers were used as samples.

Results

Preliminary results indicate that different kinds of polymers could be distinguished based on the molecular and fragment ions detected by TG-AMS. The mass spectra of polymer recorded at different temperatures clearly show characteristic polymer ions with mass range from m/z 50 up to 2000. For example, PMMA 625 and PEG 600 were distinguishable by different mass range of molecules and repeating molecular ion series that were 100 Da and 44 Da apart, respectively. The aforementioned neutral polymers were also collected and characterized using MALDI-TOF, where results from TG-AMS and MALDI-TOF analyses yielded similar mass spectra.

Conclusions

TG-AMS technique was successfully developed for the real-time characterizing polymers. The time-resolved information of molecules and fragments released from samples at different temperature was simultaneously obtained by TGA-AMS.

Novel Aspect

Thermogravimetry coupled with ambient mass spectrometry (TG-AMS) was applied for the real-time characterization of different polymers.

MPS02-20 / Investigation on asphaltene composition in crude oil in Saudi Arabia using mass spectrometry

Abdullah Aldawsari, Zeid Alothman, Ahmed Yacine Badjah
King Saud University

Asphaltenes are a complex class of components extracted from petroleum, which mainly consist of polyaromatic compounds with various functionalities. In mass spectrometry, electrospray ionization has proved to be a very useful technique to ionize macromolecules. It is a soft ionization technique which allows observation of the molecular ion and may produce multiply charged ions, effectively extending the mass range of the analyzer. In this work, the asphaltene fraction was extracted from a Saudi crude oil. The extracted fraction was injected to a tandem mass spectrometer by infusion, using either positive or negative electrospray ionization, in the mass range m/z 50 to 2000.

In the full scan spectrum obtained in ESI+ mode, a large number of peaks are observed in the mass range m/z 50 to 1196, with a series of consecutive fragments between m/z 450 and 900. A characteristic ion is detected at m/z 1196; its isotopic ratios correspond to a C78 formula with 10 sulfur atoms. The peak cluster between m/z 450 and 900 shows a higher ion at m/z 664 with 44 carbon atoms and 4 sulfur atoms.

Both precursors and daughters of the main observed peaks were investigated in both positive and negative modes. In ESI+ mode the fragmentation of some main ions shows a series of characteristic fragments corresponding to a repeated loss of a 56 amu fragment, which could be a C4H8 group. Another interesting observation was related to the formation of multiple charged ions

in positive ESI mode. While the presence of an ion at m/z 1329 indicated a single protonation, the presence of an ion at m/z 665 could be explained by a double protonation of the same molecule. In negative electrospray MS, the spectrum of the asphaltene sample showed much less fragments than that obtained with positive ionization. The base peak was recorded at m/z 369, while its main precursors were the ions 413, 827 and 1117. The fragmentation of the m/z 369 ions gave mainly two daughters at m/z 169 and 219. The results obtained in this work showed that ESI mass spectrometry could be a useful tool for characterization and investigation of highly complex mixtures such asphaltenes.

MPS03 - MS Instrumentation

11:00-15:00

Poster Exhibition, Level -1

MPS03-01 / Simultaneous FAIMS detection without scanning compensation voltage

Yuichiro Hashimoto, Masao Suga, Hideki Hasegawa, Hiroyuki Satake
Hitachi, Ltd.

Introduction

Since field asymmetric ion mobility spectrometry (FAIMS or DMS) is a powerful tool for separating ion species under atmospheric pressure, it has been widely used as both a standalone ion mobility detector and the pre-filter of a mass spectrometer. When a high-voltage asymmetric waveform at a radio frequency combined with a compensation DC voltage is applied between the two electrodes of a FAIMS device, only ion species with a specific mobility will pass through the electrodes. In the conventional configuration, the scanning time of the compensation voltage reduces the detection cycle, resulting in a tradeoff between the scanning time and the separating resolution. We developed a novel FAIMS device that can detect ion species with different compensation voltages simultaneously.

Method

We developed a prototype LC detector that consists of an ESI ion source, a pair of FAIMS separation electrodes, and an IonCCD detector purchased from OI-Analytica[1]. One of the two FAIMS electrodes is a normal stainless-steel electrode to which an asymmetric AC voltage with a 1.3-MHz frequency is applied. The other electrode is a ceramic plate thinly coated with conductive material of TiN. Applying voltages between both ends of the conductive electrode can create different compensation voltages at each position. The IonCCD detector has a 2126-pixel CCD array (24-µm pitch) that has been modified to directly detect positive and negative ions under atmospheric pressure. Ions transmitted from the FAIMS separation electrodes are detected by the IonCCD in a position-sensitive manner.

Results

We used a methanol solution of reserpine to evaluate our prototype. First, produced ions were bent orthogonally to prevent droplets and large particles from going directly into the following FAIMS separation electrodes. The separation area of FAIMS was 25 mm wide and 15 mm long. The different compensation field was created for each inlet position by applying a voltage of DCV between both ends of the conductive electrode perpendicularly to the ion's travelling direction. A CV spectrum was obtained every 100 ms without scanning CV voltages. When 8 V was applied as DCV, the FWHM of the reserpine ion peak was 4.3 mm, which corresponds to the compensation voltage of 1.38 V. From the evacuation flow rate, we estimated the transit time between the 15-mm electrodes to be about 2.5 ms. We feel this resolution is comparative to a different FAIMS at the same transit time of 2.5 ms. Dependency against the evacuation flow rate shows the trade-off relationship between the resolving power and ion transmission; ion transmission degrades at a high resolving power. The signal intensity versus the sample concentration showed that FAIMS separation improved the signal to background ratio compared with that without FAIMS separation.

Conclusions

We developed a novel DMS detector. This detector achieved simultaneous acquisition of CV spectrum in position sensitive manner using IonCCD.

[1] O.Hadjar, et al., J Am Soc Mass Spectrom. 2011, 22(4), 612–623.

Novel aspects

We developed a novel FAIMS device that can detect ion species with different compensation voltages simultaneously.

MPS03-02 / The Effective Potential of a Radio Frequency Linear Quadrupole Ion Trap

Donald Douglas[1], Alexander Berdnikov[2], Nikolai Konenkov[3]
[1]University of British Columbia, [2]Institute for Analytical Instrumentation RAS, [3]Ryazan State University

Introduction

The method of averaging ion trajectories is useful in the description of an ensemble of ions which move in radio frequency (rf) quadrupole fields. This approach is widely used for the theoretical description of the operation of ion traps. It leads to an "effective potential" for ion motion at low values of the Mathieu parameter q, where ions oscillate in a harmonic potential with a well depth D(q). However there is a problem with the potential well depth, D(q), for q>0.4. The behavior of the dependence of D(q) at higher q is presented in this report. The method developed here can be applied to quadrupole operation at any value of the Mathieu parameters . It is shown that the effective potential well depth, D, is given by the well-known relation (where V0 is the amplitude of the applied rf voltage, pole to ground) up to the stability boundary at q=0.9080.

Methods

The calculation of the well depth is based on the Mathieu equation instead of the standard averaging technique. Because the quadrupole potential is quadratic, time averaging of all the harmonics of the oscillation frequencies is possible and in this work all harmonics are considered, using a rigorous mathematical background.

Results

The results show that the potential well depth (i.e. the acceptable range of initial kinetic energies of the particles which are trapped) should be clearly separated from the potential well size (i.e. the acceptable range of initial coordinates). While for the potential well depth is equal to up to the stability boundary at q=0.9080, the potential well size decreases with increasing strongly to zero as (here is the field radius of the quadrupole and is the boundary of the stability zone).

Novel Aspect

With the introduction of a new concept of the averaging technique, the effective potential can be applied to quadrupole operation at any value of the Mathieu parameters .

MPS03-03 / Mass Selectivity with Dipole Excitation of Ions ina Linear Quadrupole Ion Trap with Round Rods

Donald Douglas[1], Nikolai Konenkov[2]
[1]University of British Columbia, [2]Ryazan Stae University

Introduction

The effects of using round rods on the peak shape and resolution of a linear quadrupole ion trap with mass analysis by resonant dipolar excitation for ion ejection are discussed. The dipolar excitation of ions is studied by means of the excitation contour, the fraction of ions ejected as a function of the trapping q value. The rod set geometry is characterized by the ratio of rod radius, , to field radius, . Using round rods generates field distortions described mathematically by additional higher-order spatial harmonics. The aim of this work is to study the dependence of peak shape and resolution on the higher harmonics with different ratios r/r0.

Methods

The spatial harmonics composition generated by round rod sets was calculated with a line of charges method which describes the electric potential by means of an analytical infinite series. To characterize the ion collective motion in weakly nonlinear quadrupole fields with dipolar excitation, an ion trajectory averaging method was used. The ion trajectories are calculated by the numerical integration of the ion motion equations with well-defined initial conditions for creation of an excitation contour. The resolution was determined from the contour.

Results

The study of the effects of the main spatial harmonics on the dipolar excitation process shows that the addition of weak nonlinear fields may improve the mass resolution of the resonant dipolar excitation. At r/r0=1.13 the resolution is the same as for a perfect quadrupole field. Considerable improvement of the resolution with relatively small tails take place in the region r/r0=1.14-1.16. Moreover, in this region the resolution R0.5 depends more strongly on the excitation time n measured in cycles of the trapping radiofrequency as R0.5 6.64n in comparison with an ideal quadrupole field where R0.5 1.94n. The addition of a single hexapole harmonic increases the resolution a factor of 1.6 over that of an ideal quadrupole field.

Conclusions

The optimum geometry for mass analysis with ion ejection, r/r0=1.14 -1.16 differs from the optimum geometry for conventional mass analysis for a mass filter, r/r0=1.128.

Novel Aspect

Introduction of the new concept of the excitation contour for dipolar excitation allows determining the main characteristics such as the peak shape, the resolution, the required excitation amplitude and the excitation time for different rod set geometries.

MPS03-05 / Planar Discrete Electrode Systems for a Creation superposition of DC and RF Electric Fields

E. V. Mamontov, E. Y. Grachev, V.S. Gurov, V.N. Dvyinin, V.V. Zhuravlev, A.A. Dyagilev
RGRTU

Abstract

The ion optical planar system for the creation of the linear ion trap is presented. The planar system with discrete electrodes is used for generate the superposition of the uniform DC and quadrupole RF fields.

Methods

The numerical modeling for the transformation of the continuously DC and RF fields into discrete field distribution is used. Electrode potential distribution is formed by the capacity and resistive voltage dividers.

Results

On the base of the numerical modeling of the required DC and RF field distributions the homemade linear ion trap with the plane geometry is manufactured. Discrete electrodes with sizes of 100 mm are put on ceramic plates with bilateral metallized coverings. The plane electrode consists of 50 parallel metal strips with width 0.2 mm. Continuous metallization of other surface of the plain electrode is divided into two parts P1 and P2 with an opposite phase RF applied to them potentials. Between strips and surfaces of P1 and P2 the parallel system is formed by 50 capacitor dividers of RF voltages. Surfaces of P1 and P2 of the plates have a triangular form.Distribution of the DC potential on discrete elements is created by a high-resistance resistive divider.

Novel Aspect

The method of the formation of DC and RF electric fields with variations of spatial distributions of RF and the DC potentials is proposed. At the heart of this method is the transformation of continuous distributions of potential on electrode borders into the discrete potentials. For practical realization linear quadrupole ion trap the original distributed capacity divider is constructed.

MPS03-06 / Separation of Isomers with Proton-Transfer-Reaction Mass Spectrometry: Selective Reagent Ionization and FastGC Inlet

Christian Lindinger[1], Lukas Märk[1], Lukas Fischer[1], Matteo Lanza[2], Kostiantyn Breiev[2], Alfons Jordan[1], Eugen Hartungen[1], Gernot Hanel[1], Jens Herbig[1], Simone Jürschik[1], Philipp Sulzer[1], Tilmann D. Märk[2]
[1]IONICON Analytik GmbH., [2]IONICON Analytik GmbH. / Universität Innsbruck

Introduction

Proton-Transfer-Reaction Mass Spectrometry (PTR-MS) is a well established trace gas analysis method in the fields of environmental research, food and flavor analysis, process monitoring, and many more. However, one drawback of this extremely sensitive direct injection technology is a somewhat limited selectivity. High mass resolution Time-Of-Flight (TOF) based PTR-MS instruments are capable of separating isobaric compounds but still cannot distinguish between isomers because of the identical exact mass. Here we present two approaches to solve this problem.

Methods

PTR-MS has originally been designed for being operated with H3O+ as reagent ions. Recently we introduced an advanced instrumental design (so-called Selective-Reagent-Ionization Mass Spectrometry (SRI-MS)) that allows us to switch the reagent ions between H3O+, NO+, O2+, Kr+ and Xe+. SRI-MS changes the ion chemistry within the drift tube, which can lead to different product ions for isomeric analytes.
Furthermore, we developed an integrated FastGC inlet system for PTR-MS instruments, which enhances selectivity even more. The instrument can be easily switched between «normal» direct injection and FastGC sample introduction mode via a multiport valve. Because of the sophisticated resistively heated column even the latter mode can be considered as near-real-time.

Results

We analyzed two isomeric «novel psychoactive substances»

with SRI-MS, namely 4-methylethcathinone (4-MEC) and N-ethylbuphedrone (NEB). With H3O+ as reagent ions only the (indistinguishable) protonated molecules are observed at m/z 192 (C12H18NO+); after switching to any of the alternative reagent ions characteristic fragment ions are detected with C4H10N+ (m/z 72) being the most abundant product ion for 4-MEC and C5H12N+ (m/z 86) for NEB (compare figure). This example serves to illustrate how SRI-MS can be used to distinguish isomers.
In order to test the FastGC inlet we introduced p- and o-xylene into the PTR-MS instrument. In «normal PTR-MS mode» both isomers appear on the same m/z and are not distinguishable. After switching to the FastGC mode and monitoring the respective m/z of xylene, p- and o-xylene get separated according to their retention times and are quantifiable independently. Another advantage of the FastGC mode is that compounds with concentrations so high that direct inlet PTR-MS analysis would be derogated can be «gated», i.e. they elute the column at different times than the compounds of interest, thus making sample dilution obsolete.

Conclusions
Both approaches presented here improve the selectivity of PTR-MS. With SRI-MS, as well as with the FastGC inlet we could successfully separate isomeric compounds, which would have not been possible with conventional PTR-MS (even with high mass resolution).
We gratefully acknowledge financial support from the FFG (Wien, AT) and the European Commission's FP7 under Grant Agreement Number 287382.

Novel Aspect
We introduce two very effective methods for increasing the selectivity of PTR-MS.

MPS03-07 / Exploring Impact of Dynamic Accumulation for Improving MS/MS Quality of QqTOF Data
Joerg Dojahn, Dietmar Waidelich, Sibylle Heidelberger, Quentin Enjalbert, Antonio Serna, Francesco Brancia, Christie Hunter
AB Sciex

Introduction
Recent innovations in QqTOF instrumentation has resulted in a large increase in MS and MS/MS acquisition speed providing deeper coverage of complex proteomes. Some workflows, such as iTRAQ® reagent quantitation or PTM characterization, benefit more from higher spectral quality than traditional data-dependent workflows. Here, a QqTOF acquisition strategy that uses precursor intensity to adapt the MS/MS accumulation time (dynamic accumulation) was explored for its utility in improving these proteomic datasets.

Methods
Analysis of complex protein digests was performed using nanoflow LC/MS analysis on a TripleTOF® system. Data collection was done in data dependent mode with prototype acquisition software to explore a range of acquisition rates and precursor intensity combinations for optimal coverage and spectral quality. Protein identification data was processed using ProteinPilot™ Software and results assessment was performed using Excel tools. A number of areas of improvement were investigated, impact on MS/MS quantitation for iTRAQ reagents, effect on number of acquired spectra and therefore subsequent processing time, impact on protein identification rates, and impact on the generation of SWATH™ acquisition spectral ion libraries. Ecoli lysate was labeled with 8plex iTRAQ® regents and mixed with equal loading in all channels. The sample was analyzed using three different acquisition strategies and the identification yields were characterized for both ID and quantitation.

Results & Conclusions
The dynamic accumulation approach provided a small increase in total protein/peptide identifications and significant improvements in the quantitation quality. The median reporter ion intensity was shifted higher by 34%, and the variance of protein ratio distributions was reduced (16% improvement in quality). The peptide variation about the protein was constant across the peptide intensity range, indicating improved quantitation of lower signal peptides.

Novel Aspect
Improving proteomic results using dynamic accumulation of TOF MS/MS spectra

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MPS03-08 / Advanced bioparticle accelerator
Szu-Hsueh Lai, Jung-Lee Lin, Chung-Hsuan Chen
Genomics Research Center, Academia Sinica, Taipei, Taiwan

Introduction
Currently, only electron and atomic ion accelerator are available. However, biomolecular ion accelerator has seldom been reported. In this work, we demonstrate the combination of Q-TOF and multi-steps acceleration for high throughput and high kinetic energy primary ion beam production. This could provide significant contributions to applications of large biomolecules top-down research for structural identification in the future.

Methods
When laser irradiates sample (like protein) through MALDI process, ions are desorbed from the surface and trapped by our homemade frequency scanned quadrupole ion trap. Similar to Q-TOF techniques, we apply positive and negative pulsed voltages on each end cap of ion trap respectively. Then ions are ejected from the trap and focused by einzel lens. After that, a series of square-shaped electrodes were wired to two independent switches in an alternating fashion and a sequential series of the selected pulsed voltage (e.g., 20 kV) are applied stepwise in synchronization with the movement of the ions. Calculations for the travel time of specific ions were used as the first approximation for the pulsed voltage on each acceleration plate. These programmable pulses between two adjacent electrodes are applied to increase the kinetic energy (up to 120kV by 6 steps) as an accelerator of the selected ions.

Results
The preliminary results are listed:
1. The facility was designed, assembled, and successfully tested.
2. A standard protein—cytochrome c was used to test and optimize for the performance. A level of 10^4(ions/shot) of ion flux was achieved.

Conclusions
We successfully integrate Q-TOF and multi-steps acceleration as a bioparticle accelerator. Also, a standard protein—cytochrome c was tested.

Novel Aspect
We gave a first demonstration of bioparticle accelerator which is much useful for large ions fragmentations.

MPS03-09 / Mechanism of Loss Occurrence of Ions Injected into Ion Guide Electrodes and Quadrupole Mass Spectrometer based on the Simulation of Transmission Efficiencies

Kiyomi Yoshinari[1], Yasushi Terui[2]

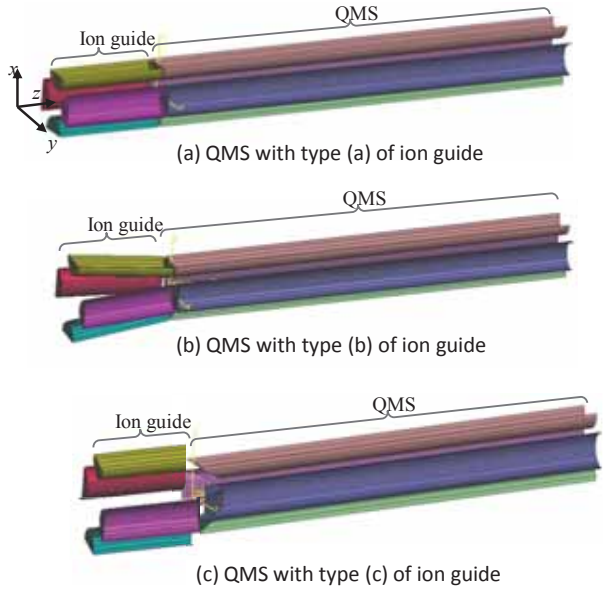
[1]Hitachi, Ltd., Hitachi Research Laboratory, [2]Hitachi High-Technologies Corporation

Introduction

Radio frequency quadrupole mass spectrometers (QMSs) have recently come into clinical use due to their compact size and rapid analysis ability. These QMSs require low loss of sample ions since biomedical samples are usually limited in quantity. Ion guide electrode system is usually equipped in front of a QMS to ensure low ion loss when ions are injected into the QMS.

Method

A typical ion guide electrode system consists of four rod electrodes, as shown in Fig.1 (a). In order to clarify what causes ion loss generation when ions are injected into QMS, we developed an original simulation code, "PISA-QMS", to analyze ion trajectories in the fringing field. In PISA-QMS, the three-dimensional electric field and ion trajectories are analyzed using BEM and Shanks' method, respectively. In this study, we calculated the ion trajectories in three types of ion guide systems, as shown in Fig. 1 (a), (b), and (c). Ion guide (a) is a normal type in which the inter-electrode spacing 2r0 and rod radii are the same as the QMS's. In ion guide (b), the inter-electrode spacing decreases continuously from 4r0 to 2r0 in the direction of ion traveling. In ion guide (c), the inter-electrode spacing of the ion guide is 4r0 and the QMS's rod end has a taper shape. We calculated the trajectories of 100 ions distributed initially within 0.5 mm of the central axis of the four-rod electrode system and with a 3 eV injection energy in three cases of ion guide, (a)–(c), using PISA-QMS.



(a) QMS with type (a) of ion guide

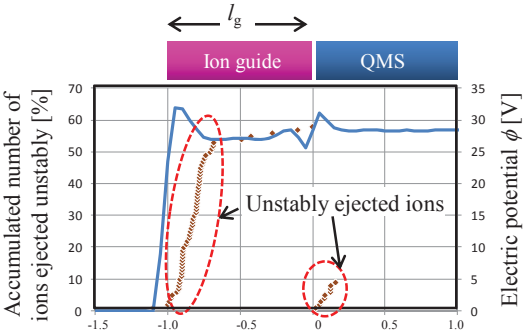
(b) QMS with type (b) of ion guide

(c) QMS with type (c) of ion guide

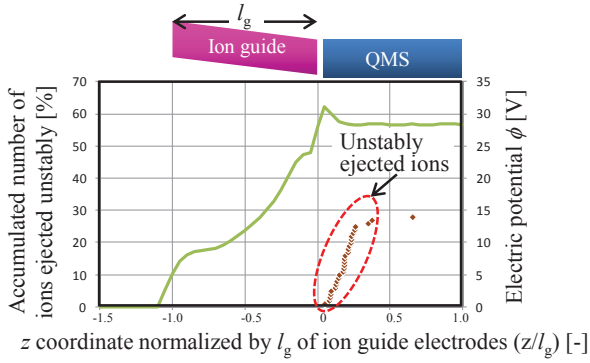
Fig.1 QMS equipping ion guide system

Results

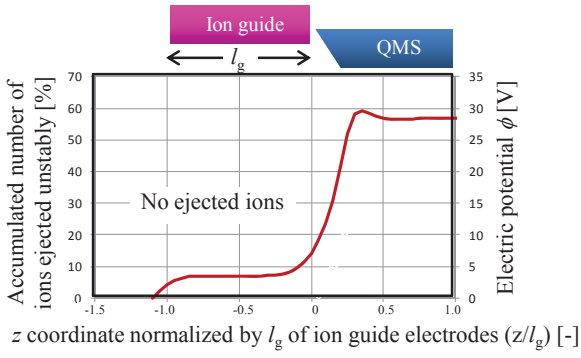
The simulation results for ion guides (a)–(c) are shown in Fig. 2 (a)–(c), respectively. The transmission efficiency of the ions successfully transmitted through the ion guide and QMS was 33□ in the case of ion guide (a). This result was then used to clarify the relationship between the position in the z-coordinate of unstably ejected ions and the electric potential distribution, as shown in Fig. 2 (a). We found that □1□ of unstable ions were ejected at the entrance of the ion guide, where the electric potential exceeded that inside the ion guide by □□□iIn ion guide (b), the increase □□□of electric potential disappeared and no ion was ejected at the entrance. □owever, ion loss was generated at the entrance of QMS, where the electric potential exceeded that inside the QMS by □□□q1In the case of ion guide (c), the transmission efficiency was obtained as 100□ while the excess of electric potential □ □□q2□decreased in comparison with □□□q1because of the taper shape of the QMS electrodes.



(a) QMS with type (a) of ion guide



(b) QMS with type (b) of ion guide



(c) QMS with type (c) of ion guide

Fig.2 The relationships between the position in z-coordinate of ejected ions unstably and electric potential distribution in cases of ion guide (a) - (c)

Conclusions

We clarified that the increase of electric potential □□□at the entrance of an ion guide or a QMS is what causes the ion losses. By reducing □□□the transmission efficiency of the injected ions should significantly improve.

New aspect

We clarified what causes the loss of ions injected into ion guide electrodes and QMS. This knowledge was then used to deriveanew ion guide electrode system for high transmission efficiency.

MPS03-10 / Improvements in Shotgun Proteomics Using a Benchtop Quadrupole High-Field Orbitrap.

Tabiwang N. Arrey, Eugen Damoc, Kai Scheffler, Markus Kellmann, Thomas Moehring, Kerstin Strupat
Thermo Fisher Scientific

Introduction

The ability to sequence and identify a large number of proteins from mid- to high complexity samples has significantly increased with advances in biological mass spectrometry. The widely used workflow applied in shotgun proteomics is based on the proteolytic digestion of a complex protein mixture such as a whole cell lysate and analysis of resulting peptides by liquid chromatography coupled to a tandem mass spectrometer. The success of shotgun experiments is highly dependent on the sample preparation, the performance and applied methods parameter settings of the chromatography and mass spectrometry systems as well as data mining capabilities [1,2]. Here, we evaluated the performance of a benchtop quadrupole high-field Orbitrap for shotgun proteomics.

Methods

Lyophilized proteolytic HeLa lysate was dissolved in 0.1 % TFA and separated on the Thermo Scientific Ultimate 3000 nano LC using different gradient lengths of 30, 60, and 120 minutes, and subsequently analyzed on the Thermo Scientific Q Exactive Plus mass spectrometer. The instrument was operated in data-dependent acquisition mode sequentially selecting and fragmenting per scan cycle the top 20 most intense precursor ions from a full MS spectrum. The acquired raw files were analyzed using Thermo Scientific Proteome Discoverer™1.4 software with the SEQUEST HT® search algorithm. Proteins were identified applying a false discovery rate of 1% and 5% (strict/relaxed) and filtered for peptides with high confidence, peptide mass deviation of 10 ppm and peptide rank one.

Preliminary Data

For our experiments a proteolytic digest of HeLa lysate was used to evaluate the mass spectrometer's performance. Different gradients were used to determine the number of proteins identified in different separation times. Comparisons were performed by evaluating the total number of identified peptides and proteins with the same gradient length on an existing platform (Q Exactive Plus). As expected the total number of identified peptides and proteins continuously increased with increased gradient lengths. Compared to the Q Exactive Plusresults from triplicate runs of each gradient length, shows at less 20 % increase in identified peptides and proteins for shorter gradient and less 10 % for longer gradients.

Novel Aspect

In-depth evaluation of a Benchtop Quadrupole High-Field Orbitrap for shot-gun proteomics

Reference

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MPS03-11 / Experimental and numerical study of a two-mirror multireflectron

Anastassios Giannakopulos[1], Dmitry Grinfeld[1], Igor Kopaev[2], Alexander Makarov[1], Michael Monastyrskiy[2], Michael Skoblin[3]
[1]Thermofisher Scientific, [2]General Physics Institute of Russian Academy of Science, Moscow, Russia, [3]Institute for energy problems of chemical physics, Russian Academy of Science, Moscow, Russia

Introduction

In the multi-reflection traps the ions are confined between a pair of electrostatic mirrors, making multiple reflections in each mirror successively, and the travel length may reach several kilometers being only restricted by the residual gas collisions. The electrostatic multi-reflection traps, optimized to acquire isochronous properties [1-3], offer a lot of opportunities for the time-of-flight mass spectrometry, with the mass resolving power approaching that of the Fourier transform mass spectrometric methods, but winning in the analysis time and sensitivity.

Methods

One of the simplest ion traps of this type comprises two identical axisymmetric ion mirrors facing each other and leaving a field-free region in-between. Each mirror contains four cylindrical electrodes whose geometries and voltages are optimized to eliminate most critical time-of-flight aberrations. As a result, the ion oscillation period is practically independent of energy in a wide range $\Delta\varepsilon$, the error being as small as $O(\Delta\varepsilon 4)$. The second-order position/angular aberrations also vanish.
The ions, after preliminarily stored and cooled in a RF quadrupole trap, are ejected by an accelerating electric field, strong enough to suppress the turn-around temporal spread. A system of deflectors steers the ion bunch into the multi-reflection trap. Upon multiple oscillations, the mass-separated ion bunch is deflected out of the trap to impinge on a time-resolving MCP detector.

Results

A mass resolving power of 80,000 is achieved at 100 oscillations. Nevertheless, our experiments show significant deterioration of the mass-resolving performance by the space charge, which restricts the maximum number of ions capable of being analyzed in one run. The theory and computer simulations reveal that the most critical space-charge interactions have the resonant nature and occur between ions of the same sort or between those with close masses, e.g. different isotope states. The experimental and simulation analysis of the self-bunching and coalescence effects in the trapped ion bunches is presented and discussed.

Novel aspects

Experimental and numerical study of ion motion in an isochronous multi-reflection time-of-flight analyzer. Mechanisms of self-bunching and coalescence are discussed.

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MPS03-13 / Characterization of a new high resolution ion beam imager to improve ion beam analysis in mass spectrometers

Jim Bupp, Bruce Laprade, Matthew Breuer
Photonis USA

Introduction

Ion optics modeling software is often used to design and predict the ion path within a mass spectrometer. The conventional method for aligning a beam typically consists of scanning the ion beam over a Faraday cup or electron multiplier, integrating the current, and adjusting the settings to produce the highest signal. However, ion modeling does not take into account many other factors within the mass spectrometer, meaning than ion loss within the instrument occurs frequently.

PHOTONIS has introduced a new high resolution ion beam imager that provides real-time video analysis or static photographs, allowing the user to visualize the location of any charged particle at any critical point within the mass spectrometer. This enables the instrument designer to characterize the ion beam at several points along the ion path for significantly less ion loss.

Methods

PHOTONIS has combined two proven technologies into a single unit for efficient ion modeling. A large 75mm Microchannel Plate (MCP) assembly is paired with a Nocturn CMOS low-light, high resolution camera. The resulting instrument is immune to sudden light damage, yet provides a highly detailed image of an instrument's ion beam. The test images were captured using FrameGrabber software on a standard PC allowing real-time analysis or storage for further evaluation. The test image can be captured at extremely low light levels at speeds to 100 frames per second at SXGA resolution (1280x1024 pixels) for high resolution analysis. Tests were done on a quadruple mass filter to identify proper focus and orientation, to characterize proper detector alignment, and to identify objects that could block the ion beam's path.

Results

Testing on a quadrupole mass filter shows the new ion beam profiling unit can clearly show whether an ion beam is properly or improperly focused, or whether it is tending toward positive or negative mode. It can also clearly identify whether the ion beam is properly aimed at the detector or focused. This paper will show actual image examples of such ion beams.

Conclusion

The ion beam profiler provides significant insight beyond traditional ion optics modeling software. It can clearly show alignment with the detector, whether a beam is focused, and other characteristics that may interfere with an efficient ion analysis. The characterization of the ion beam can then be optimized for a more efficient transport based on the imaging from this instrument.

Novel Aspect

A new ion beam profiling instrument combines a high resolution, low light digital CMOS camera with a large microchannel plate assembly to provide real-time video analysis of the ion beam path through the mass spectrometer, allowing the instrument manufacturer to optimize the ion path.

MPS03-14 / multi Resonant Frequency Excitation (mRFE) Ejection Mass Analysis on Quadrupole Ion Trap Systems

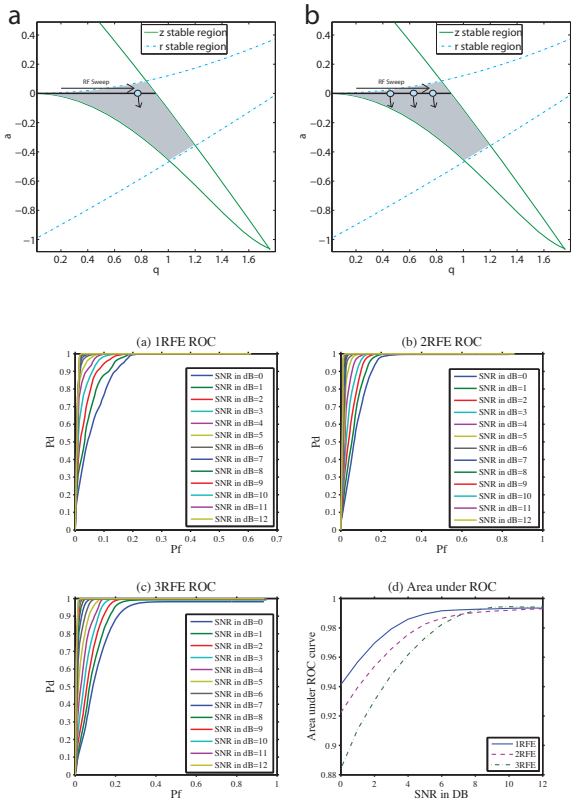
Evan Chen[1], Michael Gehm[1], Ryan Danell[2], Mitch Wells[3], Jeffrey Glass[1], David Brady[1]
[1]Duke University, [2]Danell Consulting, [3]FLIR Systems

Introduction

Several multiplexed mass spectrometry (MS) experiments have been demonstrated over the past three decades, and usually the multiplexed measurement can provide SNR and throughput gain. Conventional sampling strategy follows the Nyquist-Shannon sampling theorem, and it often that the conventional acquired data are compressed digitally to ease the data storage and transmission. Compressive measurement extends this approach by implementing the compression in physical data acquisition layer. Under certain sampling constrains, exact signal recovery from sub Nyquist data is possible. A broad array of compressive sensing techniques in MRI and optical imaging have been demonstrated during the last 10 years. In this abstract from[1], we present a multiplexed and compressive mass analysis approach for quadrupole ion trap mass spectrometry-multi Resonant Frequency Excitation (mRFE) ejection. This mRFE approach divides the mass spectrum into sub-mass ranges and detects the mass spectra of these sub-ranges in parallel to speed up the mass analysis process.

Method

Conventionally, trapped ions gets ejected by creating instability hole on the stability diagram and then sweep the Vrf (trapping RF voltage) to measure ion with different m/z sequentially. Here, we proposed mRFE (m is the number of instability holes) mass ejection method, where we create a set of instability holes where ions can get ejected in parallel. The mass ejection schematic is shown in Fig.1.

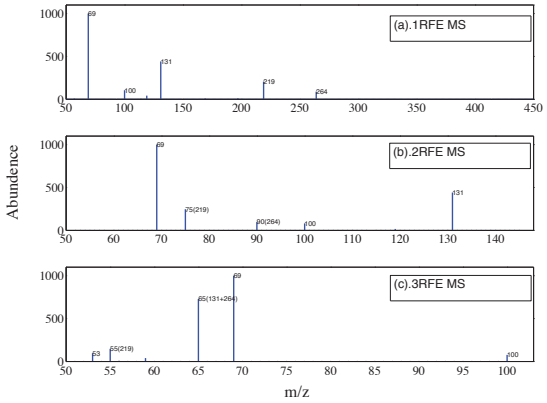


Result

The experimental mRFE FC-43 result is shown in Fig.2 [1]. (a) is the conventional 1RFE mass spectrum. (b) is the 2RFE mass spectrum, where the first sub-segment mass spectrum m/z 50--146

Monday

does not change, and the second sub-segment mass spectrum m/z 147--425 overlaid on top of the first-segment. m/z 219 becomes 75 and m/z 264 becomes 90. (c) is the 3RFE mass spectrum, similarly, the conventional mass spectrum is divided into 3 sub-segments and overlapped on top each other.
A simulation study based on FLIR 500 chemical library and a simple nearest neighbor in l2 sense classifier has shown that mRFE spectra is still capable for classification, as shown in Fig.3 [1]. At extremely low SNR conditions, 1RFE performs slightly better than mRFE. At SNR levels above 8 dB, there is no difference between 1RFE and mRFE classification performance.



Conclusions
We have successfully demonstrated a new compressive and multiplexed mass analysis approach, mRFE, on a QIT system. The approach reduced the mass analysis time 3 to 6 fold. 2RFE and 3RFE mass spectra were shown to be suitable for direct compound classification.

Novel Aspect
We brought the recent advancing computational and compressive sensing concepts into QIT mass spectrometer the first time. This proposed mRFE technique provides practical advantages for situations where the target mass spectrum is sparse and extended over a large mass range.

Reference
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MPS03-15 / Manipulating Alkali Metal Ion Distribution in MALDI with Sample Preparation Protocols as Revealed by Dual-Polarity Time-of-Flight Imaging Mass Spectrometry
Yin-Hung Lai, Hsun Lee, Yi-Sheng Wang
Genomics Research Center, Academia Sinica

Introduction
Alkali metal ions are common and important impurities in MALDI. Although they reduce the quality of mass spectra by complicating the spectral features and reducing the abundance of protonated species, they are essential primary ions to ionize carbohydrates. For the best sensitivity of mass spectrometer especially for carbohydrates, it is important to understand and control the distribution of alkali metal ions. The spatial distribution of alkali ion adducts of matrixes, carbohydrates, and proteins prepared with various sample preparation methods were thoroughly investigated. The change of alkali metal ion distribution with the composition of the samples was observed. Furthermore, by changing temperature of substrate, the ion distribution could be controlled and the heterogeneity of ion distribution could be minimized.

Methods
The spatial distribution of alkali metal ion in various MALDI samples was investigated by a synchronized dual-polarity time-of-flight mass spectrometer (DP-TOFMS). Simultaneous positive and negative ion detection was achieved by two linear TOF mass analyzers which were installed in a V-shape above the sample surface. Different strategies including changes of solvent constituent, dry condition, and temperature of the substrate were conducted to manipulate the distribution of alkali metal ion adducts.

Results
When pure matrix, 2,4,6,-trihydroxyacetophenone (THAP) was examined, the sodium ion adduct of THAP mainly distributed in the rim; there was little sodium ion adduct in the inner region of the sample spots. Interestingly, the sodium ion adduct of matrix equally distributed in a wide range of region when analytes were incorporated. Moreover, sodiated carbohydrates are predominantly distributed in the rim in the conventional MALDI condition. Heterogeneous distribution of sodiated carbohydrates retards the analytical performance and reproducibility of the mass spectrometric analysis.By regulating temperature of the substrate, a homogeneous distribution of sodiated carbohydrates was obtained.

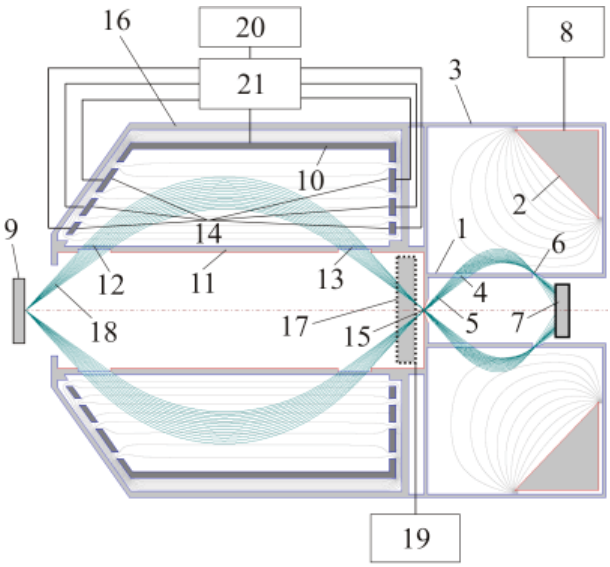
Conclusions
The result suggests that the alkali ions may form complexes with analytes, and their final distribution may depend on factors such as the sodium affinity, hydrophobicity, and polarity of analytes. As a consequence, the sensitivity of the MS for proteins and carbohydrates depends critically on the sample composition, drying condition, and temperature of the substrate. Owing to the simultaneous positive and negative ion detection and imaging capabilities provided by DP-TOFMS, the possible alkalization mechanism of analytes was analyzed. This work is helpful to the optimization of the sample preparation protocol for improving the sensitivity of mass spectrometry especially for carbohydrate analyses.

Novel aspect
Alkali ion adducts distribution in MALDI samples was manipulated by changing dry conditions and sample composition. Heterogeneous distribution of alkali ion adducts was minimized and the sweet-spot effect was settled.

MPS03-16 / High-aperture energy and mass spectrometer of ion fluxes
Victor Gurov, Michael Dubkov, Andrey Trubitsyn
Ryazan State Radio Engineering University

Simultaneous energy and mass analysis of secondary ions sputtered from a solid surface, allows studying the fundamental characteristics of the material undetectable by another methods. Unlike the secondary ions mass-spectrometry (SIMS) which provides data on the chemical and phase composition of the surface and subsurface layers, the additional analysis of the ion kinetic energy is a source of information on the physical characteristics (atom binding energy, electronic work function etc.) of the research object. The latest method of research is sometimes called the secondary ions energy and mass spectrometry (SIEMS).
SIEMS is a unique tool to study features of the nanoscale inorganic matter. The main limitation here is the lack of sensitivity of the measurements due to low luminosity (about percentage from 2π) of the commonly used diagnostic tools.

Isotrajectory mass-spectrometers with angular focusing [1] can provide high luminosity (about 10% from 2π).
The main advantage of ion optics systems, designed on the principles of a new science direction «Izopath optics» [2], is the full absence of chromatic aberrations. It is the precondition of developing of electronic microscopes with the high level of the spatial resolution.
The other advantage of izopath ion optics systems is the dispersion on masses of the pulse ion flows (bunches of ions). However classical mirrors investigated in the monography [2], for example cylindrical, have revealed their unpleasant feature - the absence of angular focusing. The design of axi-symmetrical izopath mass spectrometers with angular focusing of the second order near central angle 42° are offered and numerically studied in this paper to provide high level of sensitivity. On the basis of the computations the conclusions on accessibility of the mass resolution at level 500 at aperture ratio 10-20 % from 2π are drawn.
For the energy analysis stage classic cylindrical mirror energy analyzer (CMEA) is placedat the input of a mass spectrometer to provide the energy resolution less than 1% under luminosity of about 10% from 2π. Since the centralfocusing angle of the second order in CMEA also is 42 °, the devices considered here are perfectly compatible in space (Fig. 1) [3].



1 – inner cylindrical electrode of mass analyzer, 2 – outer conical electrode of mass analyzer, 3 – shield of mass analyzer, 4 – input window of mass analyzer, 5 – secondary ions bunches, 6 – output aperture of mass analyzer, 7 – ions detector, 8 – power supply unit of mass analyzer, 9 – target, 10,11 – outer and inner cylindrical electrodes of energy analyzer, 12,13 – input and output window of energy analyzer, 14 – correcting rings of energy analyzer, 15 – intermediate hole diaphragm, 16 – shield of energy analyzer, 17 – ion ow interrupter, 18 – continuous ow of secondary ions, 19 – high-voltage pulses supply unit, 20 – power supply unit of energy analyzer, 21- voltage divider.

Fig. 1. Ion optic scheme of the energy and mass spectrometer.

The device for ion flow interruption is placed between the stages of energy analysis and mass analysis before the intermediate hole diaphragm. This device for interruption may be done either of retarding or deflecting type that provides indeed rate of ion bunches of short duration to the input of the isotrajectory mass spectrometer.

References

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MPS03-18 / Experimental setup for the recognition of chiral metal clusters

Kathrin Lange
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Size-dependent chemical and physical properties of finite material aggregates are one of the most important investigations in modern science especially with respect to their relevance for heterogeneous catalysis [1].
Over several decades the aim of our group has been to control chemical reactions by changing cluster size, chemical composition and dimensionality of metal and metal oxide clusters [2]. Thus, it is possible to design cluster catalysts with specified chemical activity, stereoselectivity and enantioselctivity [3].
Chirality is one of the basic principles of nature. It has a huge impact on asymmetric catalytic reactions like the hydrogenation of β-ketoesters with Ni catalysts [4] or the Suzuki-Miyaura coupling reaction which is catalysed by palladium nanoparticles [5]. These reactions make use of chiral metal nanoparticles to control the reaction by directing its enantioselectivity. They can provide an enantiomeric excess of the product molecule of 90%.
The potential for asymmetric catalysis with size selected chiral metal clusters is immense.
This work describes at first a vacuum system to produce, characterise and perform reactions with chiral gas phase metal clusters. The cluster source is similar as that used previously in other experiments within the group [6]. It is believed that any population of chiral metal clusters produced within such a cluster source will be a racemic mixture of both enantiomers. Thus a method to enable the identification of enantiomers must be implemented. In addition, time-of-flight mass spectra of achiral organic molecules on niobium clusters will be presented.

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MPS03-19 / Advances in signal dependent detector optimization for coeluting peaks in triple quad MS

Felician Muntean, Barry Nesmith, Zicheng Yang, Ed George, Desmond Kaplan, Yann Hebert
Bruker Daltonics

Introduction

Dynamic range is a major requirement in quantitative mass spectrometry today and triple quadrupoles are usually the analyzers of choice. A triple quad detector needs to support the wide dynamic range and this work describes a novel technique to enable this. The MRM-specific Extended Dynamic Range (EDR) technique described here extends the range of an ion detector by several orders of magnitude and allows simultaneous optimal

detection of co-eluting compounds.

Methods
Experiments are performed on triple quadrupole mass spectrometer systems, both LCMS (EVOQ, Bruker Daltonics) and GCMS (SCION, Bruker Daltonics) equipped with channeltron type electron multiplier detectors and fast high voltage switching power supplies (ITT Excelis). The EDR method consists of adjusting detector voltage according to ion signal intensity of each MRM scanlet and applying the correct gain, as determined by a previous gain/voltage calibration. Polypropyleneglycol (PPG) solution in methanol is used by infusion in the LCMS system to perform the basic, preliminary testing.

Results
Detector calibration was performed for gains between 102 to 106 vs detector voltage, to yield a total detection range of ~108. Detector dynamic range is probed by infusing PPG 1000 solution in the LCMS system, negative ion mode, and monitoring simultaneously large ions formed by the acid added to the solution and small background ions. Simultaneous detection of 9.7e8 Counts Per Second (Cps) for the SIM ion at m/z 60 and 1.2e2 Cps for the SIM ion at m/z 727 is observed with excellent signal to noise, demonstrating 7 orders of magnitude of simultaneous ion detection range. Separate runs with each of the ions optimized individually resulted in signal levels identical to the ones with EDR within measurement errors. In contrast, when a single detector voltage was used for both ions, if the ion with the lower abundance was optimized, then the ion with the greater abundance saturated the detector and was under-detected; whereas, when the greater abundant ion was optimized, the small ion was not-detected.
Further experiments on a complete GCMS system indicate excellent linear response for 6 orders of magnitude between 100 fg and 100 ng on-column injections of Octafluoronaphtalene. Preliminary results on the LCMS system show over 5 orders of very good linear response between 2 ppt and 500 ppb of Testosterone, with room for further range extension on both ends.

Conclusions
Preliminary results demonstrated that scanlet-specific EDR allows optimal detection of simultaneous SRM ions that differ by 7 orders in magnitude. Additional experimental results will be presented, which include calibration curves of coeluting large and small MRM compounds.

Novel Aspect
Controlling detector gain dynamically and independently for each SIM or SRM, to achieve wide dynamic range for coeluting comp.

MPS03-20 / Development of a Quadrupole Ion Trap Mass Spectrometer for Spectroscopic Characterization of ETD/CID Generated Peptide Fragments using FELIX

Jonathan Martens, Josipa Grzetic, Giel Berden, Jos Oomens
FELIX Facility - Radboud University Nijmegen

Introduction
Electron transfer dissociation (ETD) and electron capture dissociation (ECD) are recently developed methods to induce fragmentation in multiply charged peptides. These techniques produce unique fragmentation patterns that contrast peptide dissociation resulting from collisional or multiple-photon activation. The very fast and nonergodic nature of the dissociation mechanism preserves information about post-translational modifications in the mass spectrum. In contrast to common observations of b- and y-type ions in CID/IRMPD MS/MS spectra, ETD/ECD spectra generally feature c- and z-type fragment ions, offering complementary sequence information. While the many appealing aspects of the technique have resulted in widespread developments, a complete description of the dissociation mechanisms involved is still under development. Recently, ion spectroscopy has been used to definitively assign the structures of peptides and their fragments generated in CID mass spectrometry experiments. Using this technique, we are able to examine the structures of fragments generated by ETD/ECD and thus gain valuable insight into the associated dissociation mechanisms.

Methods
ETD/ECD involve the association of nearly thermal electrons with isolated peptide ions. For ECD, this involves the direct capture of an electron and requires trapping of such electrons, most easily accomplished in an FT-ICR. ETD occurs through the transfer of low energy electrons to the peptide ion from a radical anion (fluoranthene). ETD can easily be accomplished in a quadrupole ion trap mass spectrometer, thus greatly expanding the practical accessibility of the technique. We have developed such an instrument with optical accessibility to the trapped ion population for irradiation with the intense, tunable light of the infrared free electron laser, FELIX. We have modified a Bruker AmaZon ion trap with a ring electrode having two 3 mm holes in the ring electrode to allow for the entrance and exit of the IR beam.

Results and Conclusions
Recently, the first results using FELIX have been obtained and the performance of the instrument will be discussed. Introduction of the holed ring electrode has insignificantly affected the performance of the trap in terms of both trapping efficiency and mass resolution. Initial photo-dissociation experiments using both the tunable light of FELIX and a continuous wave CO2 laser suggest that the size and distribution of the ion population allows favourable overlap with the incoming light beam. Initial results involving the spectroscopic characterization of a series of ETD fragments from small, multiply charged peptides will be demonstrated.

Novel Aspects
Demonstration of Bruker AmaZon quadrupole ion trap for IRMPD experiments at FELIX. Spectroscopic characterization of ETD peptide fragments.

MPS03-21 / Greater than 10X signal gain in magnetic sector mass spectrometry via aperture coding

Evan Chen[1], Zach Russell[1], Jason Amsden[1], Ryan Danell[2], Scott Wolter[3], Charles Parker[1], Mike Gehm[1], Brian Stoner[4], Jeffrey Glass[1], David Brady[1]
[1]Duke University, [2]Danell Consulting, [3]Elon University, [4]RTI International

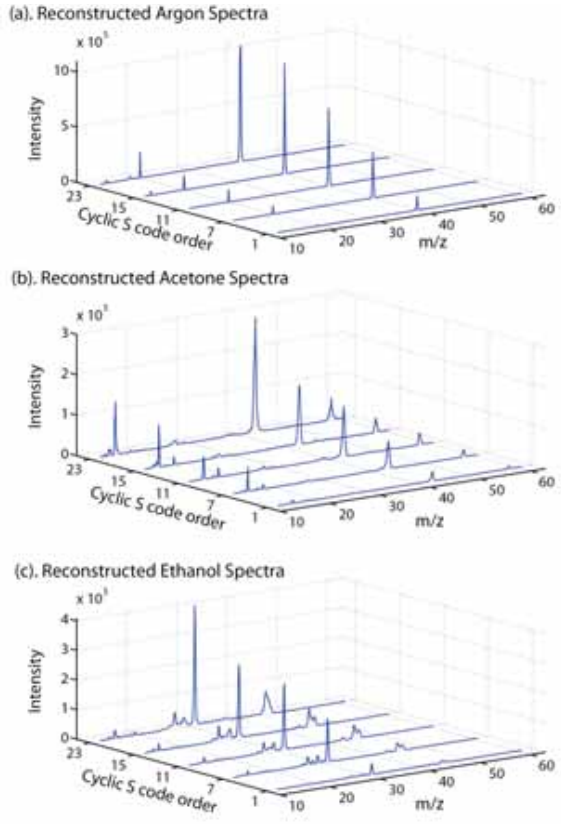
Introduction
Mass spectrometers based on magnetic sector mass analyzers while noted for their high mass accuracy and relatively low power requirements, suffer from large size and weight to achieve desired performance characteristics. Miniaturization of mass spectrometers and magnetic sector instruments in particular will enable high performance handheld and portable instruments. However, miniaturization often leads to a reduction in instrument throughput and signal to noise in order to meet the same resolution and performance metrics of a larger instrument.
In this abstract, we describe the application of spatially coded apertures to magnetic sector mass spectrometry. Similar to optical spectroscopy, application of spatially coded apertures to magnetic sectors yield a 10X increase in instrument throughput while maintaining equivalent resolution to a single slit after

spectral reconstruction. This results in an increase in signal to noise ratio and lower detection limits, which will enable magnetic sector miniaturization.

Methods

The simple mass spectrometer constructed to demonstrate the benefits of aperture coding is composed of an electron impact ion source, an aperture, a 90°magnetic sector, and a multichannel plate with phosphor screen as a detector. The apertures used are Cyclic S patterns with order of 1, 7, 11, 15, and 23. A schematic showing the aperture patterns and mass spectrometer geometry is shown in REF _Ref385339330 \h * MERGEFORMAT Figure. Since this mass spectrometer system is multiplexed measurement system, an accurate mathematical forward model and reconstruction algorithm are employed to reconstruct the mass spectrum from the image recorded at the detector. The forward model has the form of

Where is m/z, is the image recorded at the dectector, is the desired mass spectrum, is the ion spatial intensity at the coded aperture, is the coded aperture transmission function describing input aperture, is a kernel describing propagation through the spectrometer for a specific, is the central ion beam acceleration voltage, and B is the magnetic field. Using the maximum likelihood estimation method we can estimat , the mass spectrum, based on the image recorded at the detector and the forward model above.



Results

REF _Ref385340334 \h * MERGEFORMAT Figure a-c shows the reconstructed mass spectra from Argon, Acetone, and Ethanol spectra with various coded apertures of increasing complexity. The S-23 aperture yields >13x increase in throughput. Furthermore, increasing aperture order does not effect the widths of the mass peaks indicating that the resolution stays the same, independent of aperture order.

Conclusions

Using a simple magnetic sector mass spectrometer, we have demonstrated the benefits of aperture coding including increase in instrument throughput while maintaining resolution. Our results show a greater than 10X improvement in throughput without change in resolution. In future work, these concepts will be applied to other mass spectrometer geometries.

Novel Aspect

Using concepts from optical spectroscopy, we have demonstrated the use of coded apertures in magnetic sector mass spectrometry the first time.

MPS06 - Clinical Applications and Screening

11:00-15:00

Poster Exhibition, Level -1

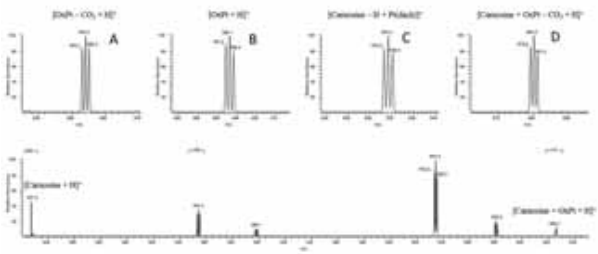
MPS06-01 / Carnosine and its Complexes with Pt-based Anti-Cancer Drugs: A Mass Spectrometry, Computational Modelling and In Vitro Cell Bioassay Study

Eslam Dabbish[1], Claire Camp[1], Ahmed Youssef [2], Asma Amleh[2], Helen Reid[1], Barry Sharp[1], Tamer Shoeib[2]

[1]University of loughborough, [2]American University of Cairo

β-alanyl-L-histidine (Carnosine) is a naturally occurring dipeptide found in different body organs and is concentrated in the cell cytosol. Carnosine and the related dipeptides, N-Acetylcarnosine (NAC) and β-alanyl-N-methylhistidine (anserine) have been reported to have many biological activities. Several electron rich sites within carnosine and the related dipeptides allow these dipeptides to interact with protons or free circulating metal ions within the body as well as with metal containing drugs such as the platinum based anticancer drugs cisplatin and oxaliplatin. In addition to studying the protonated form of the three dipeptides, their possible interaction with the anticancer drug oxaliplatin is of particular interest in this study.

Mass spectrometry, computational molecular orbital calculations and in vitro cell bioassay were used to investigate the interaction of carnosine and the related dipeptides with proton and oxaliplain drug. The mass spectrometric study were conducted using a triple quadrupole, a linear ion trap and a high resolution Fourier transform mass spectrometers, all employing the positive mode electrospray ionization to generate ions. For computational work, structures were optimized by means of density functional theory (DFT).



MS2 spectrum of the entire isotopic envelope of the ion [Carnosine + OxPt + H]+ generated at 15 ev in the lab frame and isolated from the full scan spectrum of a (2:1) molar mixture of Carnosine and OxPt in a (1:1) (v/v) water/methanol solution as obtained on the LTQ without allowing for incubation time. The sections of the spectrum shown under "x10" and "x50" signify the magni cation of the signal by 10 and 50 fold respectively for clarity. The signals assigned as [OxPt – CO2 + H]+, [OxPt + H]+, [Carnosine – H + Pt(dach)]+ and [Carnosine + OxPt – CO2 + H]+ are each expanded and normalized to 100% in inserts A through D respectively for clarity.

In vitro studies on hepatocellular carcinoma HepG2 cells

suggests that carnosine may inhibit the cytotoxic action of OxPt most likely through the formation of complexes that are less cytotoxic than OxPt alone. The identification and proposed fragmentation pathways for the collision-induced dissociation (CID) of the protonated species of the three dipeptides will be presented. Most species observed were unambiguously assigned. The Plausible fragmentation mechanisms that account for all the experimental results will be presented and are supported by the use of deuterated solvents and DFT calculations. Mass spectrometry evidence of the interaction of OxPt with carnosine, NAC and anserine will be presented. Most species observed were unambiguously assigned and compared to their theoretical isotopic patterns. Common CID fragmentation products of the protonated complexes of each of the three dipeptides examined with OxPt will be reported. Density functional calculations at B3LYP/LANL2DZ were used to obtain structural information and relative free energies of different isomers of the observed precursor [Carnosine + OxPt + H]+ both in the gas phase and in solution as well as to probe its fragmentation, highlighting plausible fragmentation mechanisms that account for all the experimental results. The proton affinities for carnosine, anserine and NAC were calculated to be 248.7, 246.6 and 247.5 kcal mol-1 respectively at B3LYP/6-311++G(2d,2p) level of theory.
The first hypothesis driven investigation of the role of the naturally abundant cytoplasmic dipeptide ligand carnosine in OxPt detoxification is presented. An in-depth investigation of the complexes formed between the drug and the ligand and the CID fragments generated is discussed.

MPS06-02 / Acoustic trapping for bacteria typing in blood culture with MALDI-MS

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Introduction

Identification of bacteria species with MALDI-MS has emerged as a new standard in clinical practice for microbial pathogen diagnosis. This approach is used in more than 800 microbiology labs around the world, and more than 20 million analyses were performed last year.The current standard protocol for sepsis involves overnight culturing in blood culture flasks to increase the bacteria concentration, followed by manual sample preparation in form of selective-lysis and several cumbersome centrifugation steps.

Methods

A novel approach to sample preparation for MALDI-MS bacteria typing from blood-culture flasks, that uses microfluidic acoustic trapping instead of standard centrifugation is demonstrated, figure 1.

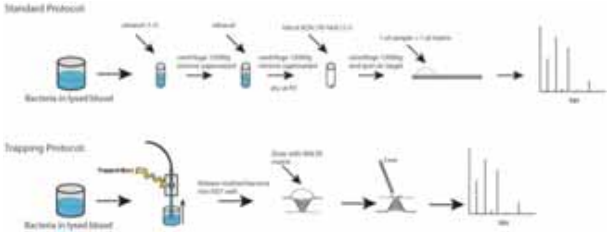

Figure 1. The manual Sepsityper protocol (top) in comparison with the acoustic trapping-based extraction, where bacteria are captured, washed, and lysed on larger seed-particles in an acoustic trap (bottom). Further, a solid phase extraction (SPE) step in an ISET microchip is performed to concentrate and purify analytes before MALDI-MS readout. All the assay steps in the trapping scheme (b)can be performed by a pipetting robot in an automated fashion.

Results

Escherichia coli was spiked in whole blood and cultivated in clinical blood culture flasks. At a concentration of approximately 107 bact. /mL the presence of bacteria was detected by the incubator. The red and white cells in the blood were selectively lysed using a lysis buffer from the Bruker-Sepsityper kit, 20 µLof the lysate was directly aspirated in an acoustic trap arranged in a glass capillary (200x2000 µm2 cross-section). The trapped bacteria was preloaded with a small number of seed-particles allowing for acoustically trapping of the bacteria and subsequent aspiration and dispensing of MilliQ water enabled efficient removal of plasma proteins. The trapped bacteria were then lysed by aspiration of 70% formic acid . The lysate (containing the bacterial proteins) was released into the previously presented ISET SPE sample preparation platform packed with 10 µm RP beads, in order to decrease the sample volume, concentrate and purify the bacterial proteins, as well as form a crystal for the MALDI-detection. The bacteria were successfully identified with MALDI-MS as E. coli using the proprietary Bruker-Biotyper software correlating the obtained spectra with the reference database. The average score for the acoustic trapping protocol was 2.19±0.09, as compared to 1.98±0.08 for the identical strain with the standard protocol

Conclusions

The presented microfluidic technology could replace three centrifugation steps and provide successful typing of bacteria from blood culture.

Novel aspect

The value of an automatable microfluidic assay that can improve bacterial identification using only a very small sample is huge, as clinical microbiology laboratories are in dire need of fast and reliable methods to analyze bacteria in order to provide the correct choice of infection therapy.

MPS06-03 / The Role of Plasminogen Activation System in Periodontal Tissue Destruction: Differential Proteomic and bioinformatic analysis in Two Experimental Periodontitis

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Introduction

Periodontal diseases, a complex multifactorial disease, results in a loss of support of teeth, and may also be a significant impact to general health. Accumulating evidences have provided insight into mechanisms of periodontal inflammation by performing experimental animal models to establish a cause-and-effect relationship between bacteria and the initiation of periodontal disease. To our knowledge, a systematic proteomic approach has not been applied within the periodontal tissue by using appropriate animal model. Therefore, proteomic approaches were used for the identification of certain novel protein(s) and the interaction networks of these novel proteins under controlled conditions, such as using experimental periodontitis model.

Methods

Two common used animal models, ligature and local LPS injection, were utilized for disease induction. The differential expression of proteins from periodontal tissues during the evolutional changes of periodontal destruction were investigated

by proteomic approach. Furthermore, the role of selected protein(s) will be analyzed through bioinformatic approaches.

Results
Within the limitation of this study, twenty-nine differentially expressed proteins
were found in ligature-induced mode, and twenty-eight differentially expressed
proteins were found in LPS-injected model. We found differentially expressed proteins expressed in both ligature- and LPS-induced experimental periodontitis models. In addition, from the bioinformatic analysis and critical references reviewed, the "plasminogen activation system" may probably play a promising role during periodontal destruction.

Conclusions
To better understand the intrinsic mechanisms of periodontal disease progression, systematic proteomic-based approach within the periodontal tissue would be a valuable approach to discover novel proteins under specific circumstances or response to certain factors.

Novel Aspect
We will be the first to determine the differential proteome profiles, and interaction networks of candidate protein(s) associated with the progression of periodontal disease. This approach may convey huge potential to inform paradigm shifts in our understanding of periodontal diseases and provide useful information to the monitoring of disease activity, evaluate therapeutic modalities and discover new therapeutic strategy in future.

MPS06-04 / Pharmacokinetic-Pharmacodynamic Study of Subcutaneous Injection of Nandrolone Decanoate Using Dried Blood Spots (DBS) Blood Sampling coupled with LC-MS/MS

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Introduction
Steroid pharmacokinetic-pharmacodynamic (PK/PD) studies requires frequent and prolonged blood sampling for which the participants need make repeated visits to the clinics. Therefore, a simple and minimal invasive mechanism to obtain blood samples which can be conveniently collected by patients themselves is highly desirable. We adapted the DBS technology for quantitation of testosterone (T) and nandrolone (ND) using ultra performance liquid chromatography with tandem mass spectrometry method (UPLC-MS/MS). The method was employed for a clinical study to investigate PK/PD of subcutaneous (SC) injection of nandrolone decanoate.

Methods
Healthy eugonadal men (n=8, age 31 ± 10 (SD) year) were administered 100 mg ND in 2 mL arachis oil vehicle by sc injection into a single subdermal abdominal site. Capillary blood obtained by finger prick was dried onto filter paper, recording the exact sampling time, before and daily for 21 days. Venous blood was sampled before and at weekly intervals to also store serum and spot onto filter paper. After storage at room temperature, the whole DBS underwent elution with 50% methanol:water followed by MTBE. The organic layer was evaporated, dried and reconstituted with mobile phase before injection on to LC-MS/MS. Calibration standards and quality controls (QCs) were made from separate lot of artificial blood. The LC-MS/MS method was validated according to FDA guidelines. The within-day precision was 2.5-4.4% (accuracies, 95-108%) and the between-day precision was 4.9-13.5% (accuracies, 100-106%) at all QC levels. The LOQ was 50 and 156 pg/mL for T and N, respectively (50 µL blood sample). All extraction recoveries and process efficiencies were between 96-107% with the matrix effect recoveries between 96-102%.

Results
Serum N rose to a peak of 2.50 ± 0.25 (SEM) ng/mL at a median (range) of 6 (4-13) days causing a reduction in serum T from 3.50 ± 0.57 ng/mL at baseline to a nadir of 0.38 ± 0.13 (SEM) ng/mL representing 89 ± 3% maximal suppression at a median (range) of 8 (5-16 days). In simultaneously sampled capillary and venous blood, there was a high correlation with serum N (Passing-Bablok r=0.956 & 0.964, respectively) as well as for serum T (r=0.948 & 0.992) respectively. Serum N and T concentrations were estimated with adjustment for capillary blood sample volume and hematocrit to define peak (N) or nadir (T) time and concentration.

Conclusions
This study demonstrates that (a) DBS sampling coupled with LC-MS/MS steroid assays can achieve intensive time sampling for PK/PD studies in the community without requiring frequent clinic visits, venesection or frozen serum storage and (b) androgen esters in an oil vehicle can be delivered predictably by SC injection avoiding the need for medically supervised deep IM injections.

Novel aspects
Accurate quantitation of T and ND eluted from whole DBS has been established for application to a clinical study involving injectable androgens.

MPS06-05 / Selective detection and quantitation of complementarity-determining regions of monoclonal antibodies for the development of therapeutic drug monitoring by MS

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Introduction
Many monoclonal antibodies (mAb) drugs will be now proceeded in preclinical and clinical trial, and precious blood concentration is one of the good indicator for drug activity. Therefore, therapeutic drug monitoring (TDM) based dosing strategy is an important matter for cancer therapy. For antibody drug TDM by MS, minimizing peptide complexity while maintaining specificity of the target protein is an important strategy. We report a novel method for the Fab-selective proteolysis to identify and quantify mAb by the limiting protease access to mAb, which we have named nano-surface and molecular-orientation limited (nSMOL) proteolysis.

Methods
Antibody drug was purified and immobilized on Protein G resin (pore: 100 nm), and limited proteolysis was performed by immobilized trypsin/lysyl endopeptidase mixed enzyme on the surface of nano particles (diameter: 200 nm). The generated peptides were collected and confirmed peptide sequences by MALDI-QIT-TOF MS. MRM quantitation were performed by LC-ESI triple quadrupole MS.

Results
nSMOL proteolysis is an entirely novel solid-solid proteolysis: (1) high-probability of protease-substrate contact by increasing the surface area; (2) Fab region is oriented outward to the solution; and (3) limiting protease access to the substrate making

use of the particle and resin pore diameter difference. nSMOL proteolysis enables highly efficient and quantitative detection of CDR peptides while decreasing the peptide numbers of the analytical target without antibody denaturation. Calibration curves of Trastuzumab peptides demonstrated linearity within 20% relative standard deviation for each MRM transition between concentrations of 1 ug/ml and 500 ug/ml in plasma.

Conclusions

To the best of our knowledge, strategy for CDR peptide-selective preparation in antibody proteolysis has never been investigated to date. The nano-surface and molecular-orientation limited proteolysis presented in this report is a novel method for the effective proteolysis and analysis of antibody CDR-derived peptides through the proteolysis by limiting protease access to the substrate. By using nSMOL proteolysis, direct identification and quantitation of CDR peptides are made possible by high-throughput MS independent of a variety of monoclonal antibody. This method possesses wide dynamic range and good linearity sufficient to measure Trastuzumab blood concentration as indicated in its pharmaceutical interview form. nSMOL proteolysis has the capability to overcome these two issues in the clinical MS development, reproducibility in preparation and minimization of the complexity, and can be proposed as a novel methodology capable of stable and repeated analyses in clinical and pharmaceutical fields. nSMOL approach can further be applied to uncovering interactions of protein complexes. Furthermore, quality control, animal study and clinical trials based on nSMOL with MS may aid accelerate the development of many biopharmaceutical proteins and drug formulations.

MPS06-06 / Characterization of Bacterial Fatty Acids by MALDI spiral-TOFMS Combined with Kendrick Mass Defect Plot Analysis

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Introduction

Mycolic acids, one of the long carbon-chain fatty acids, have been traditionally used as chemotaxonomic markers of the suborder Corynebacterineae such as Mycobacterium, Nocardia, Gordonia, Rhodococcus, Dietzia, and Corynebacterium. The distribution of mycolic acids is heterogeneity in carbon-chain lengths, functional groups, and the number of double bonds, and mycolic acids are classified into subclasses based on their functional groups. The distribution of mycolic acids characterizes each bacterial species, and analysis of them is important. TLC, HPLC, and GC-MS are generally used for analysis of mycolic acids. However, these traditional methods require long time and cumbersome prior treatments for sample preparation. We have been analyzed total fatty acid fraction which containing mycolic acids without prior purification of mycolic acids by MALDI spiral-TOFMS. The high mass-resolving power enables to identify mycolic acids in total fatty acids. The distribution of carbon-chain lengths and peak intensities of each mycolic acid subclass can apply for features of species. In addition, we have proposed the contour maps of total mycolic acids for visualization. In this study, we applied Kendrick mass defect (KMD) plot analysis to characterize the mycolic acids of suborder Corynebacterineae.

Methods

MALDI mass spectra were obtained by using a MALDI spiral-TOFMS (JMS-S3000, JEOL, Tokyo, Japan). Following reagents were used, DHB as a matrix reagent; NaI as a cationization reagent; PMMA as a standard reagent. For obtaining accurate mass, internal calibration was performed. To assign KMD plots corresponding to the mycolic acids, KMD plots for mycolic acids were constructed using calculated masses based on the compositions of each putative mycolic acid.

Results

Mass spectra obtained by conventional MALDI-TOFMS cannot be used for KMD plot analysis because of insufficient mass-resolving power. However, the mass resolving power of the newly developed MALDI spiral-TOFMS could stand up to application of KMD plots. We could obtain mass spectra by using the MALDI spiral-TOFMS and construct KMD plots easily. Each plot corresponding to the mycolic acids was overlapped with plots obtained by calculation using each composition of putative subclass.

Conclusions

KMD plot analysis doesn't need peak assignment and allows us to visualize the distribution of mycolic acids in bacterial samples. KMD technique could be used as a practical method for the characterization of mycolicacids.

Novel Aspect

Mycolic acids were characterized easily by MALDI spiral-TOFMS with KMD analysis.

MPS06-07 / Assessment and Identification of Acylated Peptides from Poly(alpha-hydroxyl ester) Microspheres by LC-MS-MS

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Introduction

In recent years, there has been an increasing interest in peptide and protein delivery systems in order to facilitate their controlled delivery and sustain therapeutic efficacy. Microspheres of biodegradable Poly (D,L-lactide-co-glycolide) (PLGA) has been extensively used for prolonged release of bioactive peptides. However, one of the issues in peptide delivery using PLGA formulations is the formation of peptide adducts as a result of acylation with lactic and glycolic acids, which could have potential detrimental effects, such as loss of activity, immunogenicity and toxicity.

Mass spectrometry is the method of the choice for determining chemical modification of biopharmaceuticals. The strength of this method is not only the ability to detect traces of minimally modified bioactives in the presence of unmodified compound, but also the ability to identify the exact site of modification.

In our first study, the acylation adducts of octreotide acetate released from PLGA microspheres were identified and compared with the same peptide that was released from poly(lactic-co-hydroxymethyl glycolic acid) (PLHMGA) and from poly(lactic-co-glycolic-co-hydroxymethyl glycolic acid) (PLGHMGA) microspheres. In our second study, goserelin peptide, which lacks known sites of acylation like the N terminus and primary NH of Lysine, was assessed for acylation in PLGA microspheres.

Methods

Peptide loaded microspheres were prepared by double emulsion solvent evaporation technique (W1/O/W2). Microspheres were characterized (size, morphology) and the in vitro release study was performed in PBS buffer at 37˚C. At predetermined time points, the suspensions were centrifuged and the concentration of peptide was measured in each supernatant.

In order to analyze the molecular weight of released (acylated) peptide, LC-MS was performed with electrospray ionization in positive ion mode, and an ion-trap mass spectrometer. To scrutinize the structural information and localize the actual modification site, LC-MS-MS was performed on the acylated adducts.

Results
Our data demonstrate that less acylated octreotide adducts were formed in PLHMGA and PLGHMGA microspheres than in PLGA. Moreover, besides the N terminus and primary NH of lysine in octreotide, we have found that the primary OH of threonine in the end group of octreotide was also subjected to acylation. Surprisingly, in goserelin, arginine was also acylated. We propose that acylation in goserelin follows by ring closure to reach a stable cyclic form.

Conclusions
The LC-MS-MS could be applied as a powerful tool for determining the peptide acylation in microspheres. N terminus peptide is the most susceptible site of acylation in octreotide. Intrestingly, besides on the expected lys and N-terminus, acylation can also be detected at OH of the end-terminal threonine in octreotide, and on the guanidine group of goserelin.

MPS06-08 / Use of on-line mass spectrometry for understanding dissolution processes of oral dosage forms
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Dissolution is one of the routine quality control tests carried out in the pharmaceutical industry. The dissolution test may be used as a surrogate for the in-vivo performance of the product (e.g. tablet) and/or to demonstrate batch to batch consistency. The extent of drug release is normally determined using UV measurement at a limited number of timepoints. In some cases this simple approach is not satisfactory. For example some products contain two or more drugs, and they may be present at very different levels. In other cases we are interested in understanding the dissolution mechanism – for example how do the dissolution rates of the different drugs compare, and how do they relate to that of a soluble filler which lacks a UV chromophore?
We have explored the potential of MS as a selective on-line measurement tool by using it to determine the dissolution rates of three drugs and lactose from the same tablet in the same experiment.
The potential of the approach is further enabled by the recent emergence of small footprint mass spectrometers – which can be easily co-located with the dissolution equipment and offers the potential for both routine testing system and investigative research. However the dissolution media traditionally used contain non-volatile buffers (for example phosphates) that are not routinely interfaced to a mass spectrometer, which is a potential limitation
A small footprint mass spectrometer has been coupled to a dissolution experiment to investigate the applicability of this method for dissolution monitoring. Sample dilution and preparation for MS was achieved using a sampling make-up flow from a high pressure pump and a Mass Rate Attenuator (MRA). The MRA samples a small aliquot from the flow from the dissolution bath and passes this into the make-up flow prior to analysis in the mass spectrometer.
The dissolution of a tablet containing three active ingredients and lactose as an excipient has been monitored using this instrumental set-up. Three different biologically relevant dissolution media (Simulated Gastric Fluid, Fasted State Simulated Intestinal Fluid and Fed State Gastric Fluid) have been successfully interfaced and no impact on the MS performance was observed as would be expected if the dissolution media were pumped directly into the MS without dilution The different sampling make-up flows have been observed to have an effect on the sensitivity with different dissolution media and must be optimized during method development.
Differences could be observed in the dissolution profiles monitored by MS for tablets showing visually different dissolution performance (ie increased time for full dissolution).
The Proof of Concept of interfacing dissolution testing to mass spectrometry has successfully been achieved and the rate of release of the components can be individually plotted; this has not previously been reported. As may be expected for on-line MS suppression/enhancement effects will need to be fully understood before this can be used as a release test but can currently be used to investigate dissolution processes

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MPS06-09 / Comparison of EI and CI based GC-MS analysis of leachables from Dental Polymer-Based Restorative Materials
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Objectives:
Polymer-based dental restorative materials contain allergenic methacrylate monomers and other components that have shown cytotoxic, genotoxic and estrogenic effects in vitro. Several studies report that these substances leach from cured materials in vitro and are also detected in saliva after restorative treatment. Previously used GC -MS methods for identification and quantitative analysis of these organic leachables are performed using MS instruments with EI ion source. Extensive EI-fragmentation provide spectra with no or low abundance of molecular ions and fragments with low m/z ratio only. In order to develop more selective methods for the identification and quantitation of substances from resin-based dental materials based on GC-MS we have used CI that results in spectra with high abundance of molecular ions and fragments with high m/z ratio that allows more selective quantitative SIM (Selected Ion Monitoring) analysis. To further enhance selectivity and chromatographic properties compounds with –OH groups were derivatized and analysed as their corresponding TMS (Tri Methyl Silyl) ethers.

Methods
The compounds were analysed by use of a Waters Quattro Micro GC-MS/MS system equipped with a Rxi-5ms column (30m x 0,25 mm i.d., film thickness 0,25µm). Previously identified leachables were used as reference substances: CQ, DMABEE, HEMA, HMBP, MEQH, TEGDMA, BHT, TEEGDMA, MMA, EGDMA, DEGDMA, Bisphenol-A and TPSB. Compounds with OH-groups were analysed underivatized and also after derivatization with MSTFA (N-Methyl-N-trimethylsilyl-trifuoroacetamide) or BSTFA (N, O-bis (Trimethylsilyl)-trifluoroactamide). All samples were analysed in full scan mode with EI and CI ionization. The obtained spectra provided basis for further SIM analysis.

Results
Chemical ionization gave much higher yields of molecular ions, particularly for the monomers. MEQH, Bisphenol A, HEMA and HMBP were completely derivatized by use of both MSTFA and BSTFA. BHT was not fully derivatized neither by MSTFA nor BSTFA. Dilution series of each of the reference standards were used to determine the Limit of Detection (LD) and the Lower Limit of Quantification (LLOQ). The developed methods have been applied in analysis of dental restorative materials.

Conclusion
The developed methods provide selective quantitative analysis and more reliable identifications of leachables from dental restorative materials. The results shows that an array of methods should be applied in order to fully characterize the leachables from dental materials. Some compounds must be analysed as their native compound, others as their TMS-ethers. CI analysis

confirmed a higher selectivity for some substances, for others EI should be applied.

Novel Aspects
The biocompatibility of different dental restorative materials is partly dependent upon the amount and nature of leachables. The described methods provide a useful tool to characterize the organic substances eluted from polymer-based dental restorative materials.

MPS06-10 / Developing a human cancer diagnostic system: Overview of the system construction

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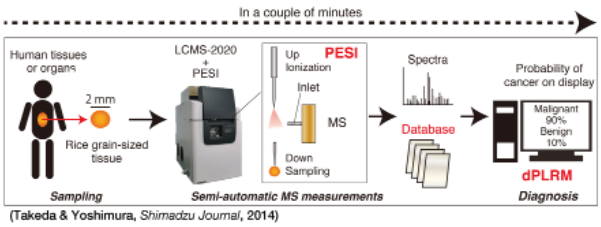
Introduction
In clinical settings, rapidness and less-invasiveness are very important requirements for diagnostic procedures, as they lessen the anguish and anxiety of patients. While recent advancements in imaging techniques enable us to diagnose the patients with minimal invasion, they are essentially indirect methods that do not depend on the identification of substances specific to certain class of diseases. Furthermore, pathohistological diagnosis is a kind of Supreme Court in medicine; it needs experienced pathologists and usually takes a week to draw a final diagnosis. On the other hand, blood chemistry is able to provide us with molecular information, though it requires laborious pretreatments of specimens lasting for a day. To circumvent these drawbacks of conventional diagnostic methods, we are developing a mass spectrometry-based on site cancer diagnosis system equipped with a learning machine.

Methods
Single quadrupole mass spectrometer (Shimadzu LC-MS 2020) is replaced its electrospray module with the Probe ElectroSpray Ionization (PESI) unit. The PESI-MS automatically collects very small amount of samples (~pL) from tissues placed on the special cartridge, and ionizes them at optimal condition. All the acquired mass spectra of specific m/z window are used for constructing a database for each class of cancer. This database is referred to by the learning machine called dual Penalized Logistic Regression Machine (dPLRM), when new cancer specimens are measured. By feeding the obtained spectral data into the dPLRM, it gives probability of cancer by implementing the multidimensional comparison of spectra with database. In this study, we used specimens from both hepatocellular carcinoma and renal cell carcinoma.



(Takeda & Yoshimura, *Shimadzu Journal*, 2014)

Results
We obtained over 95% of coincidence of diagnoses by PESI-MS/dPLRM with those by pathohistological ones in both classes of cancers.

Conclusions
Our new diagnostic system gave diagnosis of cancer paralleled with those by pathologists. Moreover, it takes only a few minutes to obtain the results on a probability basis, and it does not require any sample pretreatments that reduce the original information of specimens.

Novel Aspects
We have developed a novel cancer diagnostic machine that is characterized by two unique techniques; one is a very low invasive sampling and ionization method called PESI, and the other is machine learning called dPLRM. By putting them together into single quadrupole mass spectrometry and optimizing the condition of measurements, we achieved very high level of diagnostic performance to hepatic and renal tumors.

MPS06-11 / Extractive Analysis and Tissue Profiling using Flowprobe Mass Spectrometry

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Introduction
Rapid screening of histological samples is useful to ascertain disposition of endogenous and exogenous compounds of interest. The collected information about drug penetration, metabolite distribution, or infection and expression profiles is helpful for diagnostic, developmental, and various other applications. Mass spectrometric imaging facilitates this data acquisition through workflows that retain spatial registration of data while obviating the need for derivatization and synthesis of isotopically labeled compounds. The Flowprobe, a device for continuous spatially discrete extraction and ionization, in tandem with a high resolution mass spectrometer was used to quantitatively profile and image various tissue samples.

Methods
Healthy and infection compromised tissue samples from organs were cryosectioned to various thickness depending on application and related workflow. These were thaw mounted onto glass microscope slides or Prosolia Teflon ringed Aqua-96 Omni-slides where solutions of drugs of interest had been pre-spotted. Liquid microjunction surface analysis was performed using the nMotion software to control a Flowprobe System mounted on a Thermo Exactive Orbitrap mass spectrometer. Data sets were acquired in full scan mode covering a window of m/z 100-1200 enabling simultaneous acquisition of small molecule and lipid signals. Data were analyzed using Firefly 2.2 for Thermo software as well as BioMAP 3x.

Results
Solvent systems were optimized for sensitivity and stability of flowprobe extraction. Chicken liver, rat brain, and tuberculosis infected rabbit lung tissues were characterized focusing on through-tissue extraction; neurotransmitter, drug, metabolite, and lipid profiling; and compound distribution imaging. By Flowprobe analysis conducted by sampling from discrete spots on the liver tissue, quantitative and linear signal was acquired with through tissue extraction of drugs, indicating potential for standard integrated workflows. In analysis of the brain and compromised lung tissue biopsy sections, extraction a series of array based ion maps of drug and lipid distributions were produced by Flowprobe surface sampling entire tissue sections at 650 µm spacings, as well as spot profiles focusing on a series of necrotic lesions, tumorous, and uninvolved tissue.

Conclusions
Flowprobe analysis enabled the observation of differential localization of drugs and lipids, corresponding with studies based on traditional dissection and extraction of tumorous regions from

inside the lungs. Through the analysis of biopsy sections taken at various times after treatment, pharmacokinetic as well as spatial information indicated drug uptake and accumulation in regions of interest.

Novel Aspect
Method development for controlled, rapid, and sensitive spatial profiling of compounds in tissue using liquid microjunction surface extraction.

MPS06-12 / Optimization and application of UHPSFC-MS/MS method for screening of doping agents

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Introduction
Supercritical fluid chromatography (SFC) has only become competitive with current LC approaches recently, with the introduction of modern SFC platforms. Due to the properties of supercritical fluid and the possibility to apply high flow-rates, the analysis time can be substantially decreased in comparison with LC procedures, while maintaining or increasing the separation efficiency, especially when using sub-2-µm particles, known as ultra-high performance supercritical fluid chromatography (UHPSFC). Successful coupling of SFC with MS has already been described. However, the use of SFC-MS for the analysis of biological materials has been reported scarcely. The reason was probably the insufficient quantitative performance of the old SFC-MS platforms. Implicitly, also the information on detailed optimization of MS conditions and SFC mobile phase for coupling with MS and the evaluation of matrix effects is missing in scientific literature.

Methods
Two separation approaches ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) and ultra-high performance supercritical fluid chromatography tandem mass spectrometry (UHPSFC-MS/MS) were optimized in details and subsequently applied for the analysis of 110 doping agents in urine. The two methods were compared in terms of sensitivity, linearity and matrix effects.

Results:
In the first step, both UHPLC-MS/MS and UHPSFC-MS/MS method were optimized in terms of mobile phase composition, ESI source conditions and make-up solvent for UHPSFC-MS/MS. As a mobile phase, 0.1 % formic acid and CO2/MeOH based mobile phase with the addition of 2 % water and 10 mM ammonium formate were found to be the most generic conditions for UHPLC-MS/MS, and UHPSFC-MS/MS, respectively. Ethanol was chosen as the make-up solvent used for interfacing UHPSFC with MS/MS.
Under these conditions, 110 doping agents were successfully analyzed in urine with both methods. The evaluation of matrix effects at four concentration levels revealed generally their lower incidence in UHPSFC-MS/MS compared to UHPLC-MS/MS. While signal suppression was mainly observed with UHPSFC-MS/MS, signal enhancement was dominating in UHPLC-MS/MS, reaching extreme values in some cases, especially at low concentrations. The sensitivity of both techniques was adequate for screening purposes, and both techniques provided low enough LOQs in urine matrix (except bumetanide in UHPSFC-MS/MS), in agreement with the MRPL requested by WADA.

Conclusion:
With both techniques, a high efficiency separation and sensitive MS/MS detection enabled the analysis of all 110 doping agents with high throughput (7 minutes) and acceptable peak shapes in both ESI+ and ESI-, even for the most basic substances. Thus, UHPSFC-MS/MS was for the first time proven to be applicable for the anti-doping screening of urine samples.

Acknowledgement
The authors gratefully acknowledge the financial support of research projects of Charles University in Prague UNCE 204026/2012 and PRVOUK.

Novel aspect:
Application of UHPSFC-MS/MS for the analysis of biological materials and evaluation of matrix effects. UHPSFC-MS/MS for doping control analysis.

MPS06-13 / Development and validation of LC-MS/MS method for quantification of first line tuberculosis drugs and metabolites in plasma and application in clinical study

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National University of Singapore

Tuberculosis is second only to HIV/AIDS as the greatest worldwide killer due to a single infectious disease according to WHO statistics. Rifampicin (RIF) and isoniazid (INH), first line drugs for the treatment of tuberculosis, are known to cause hepatotoxicity as a serious adverse side effect. To further understand the pharmacokinetic parameters of these two drugs, we have developed and validated a rapid, sensitive and selective LC-MS/MS method for simultaneous quantification of rifampicin, isoniazid and their metabolites 25-desacetylrifampicin (DRIF), acetylisoniazid (AcINH) and isonicotinic acid (INA). Analytes were extracted from 20µl of plasma using solid-phase extraction (SPE) followed by chromatographic separation on Zorbax SB-Aq column (50mm × 4.6mm, particle size 5µm) using stepwise gradient elution of 5mM ammonium acetate and 90% acetonitrile with 0.1% formic acid. Baseline separation of all analytes was achieved in the total run time of 6 min. The analytes were detected under positive ionization mode by multiple reaction monitoring and quantification of analytes was performed by using deuterium-labeled internal standard. Excellent linearity ($r2 \geq 0.995$) was achieved for the analytes at different concentration ranges. The method was accurate (90 to 115%), precise (CV% < 14) and specific. Matrix effect was in the range of 93 to 111% except for INA (40 to 42%) while recovery from SPE was reproducible (CV% < 7.4) in the range of 60 to 86%. Post-preparative stability (24h, 6 ˚C autosampler) and freeze-thaw stability (3 cycles) were assessed with mean recovered concentration of > 85%. The method was successfully applied to a clinical study of healthy subjects to evaluate the effect of concomitant of INH on the pharmacokinetic parameters of RIF as well as the segregation of the subjects into slow or fast acetylators of INH. To the best of our knowledge, this is the first fully validated method with baseline separation of INH and its major metabolite AcINH, as well as INA as potential marker of hepatotoxicity. The methodology should enable a better understanding of the metabolism profiling and provides an alternative way of phenotyping the acetylation of INH.

MPS06-14 / Full Validation of a UPLC-MS/MS Method for Determination of an Anti-Allergic Indolinone Derivative and Application to Brain Drug Permeability Studies

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Introduction
Natural products are an important and unique source of lead compounds in drug discovery. In a previous study, we identified (E,Z)-3-(4-hydroxy-3,5-dimethoxybenzylidene)indolin-2-one (indolinone) from woad (Isatis tinctoria L., Brassicaceae) as a compound possessing histamine release inhibitory and anti-inflammatory properties [1]. Evaluation of the pharmacokinetic properties of the compound [2], and in particular, of its ability to cross the blood-brain barrier (BBB), is a prerequisite. However, quantification methods used to determine brain permeability of lead compounds in BBB models have typically not been validated. Hence, the reliability of such data has to be questioned. To overcome these shortcomings, we developed and validated a quantitative LC-MS/MS method for indolinone according to current industry guidelines [3,4]. The method was used to assess brain drug permeability of indolinone in several in vitro cell-based human and animal BBB models.

Methods
The LC-MS/MS quantification method for indolinone in Ringer HEPES buffer was validated according to EMA and FDA guidelines [3,4].

Results
The calibration curve of indolinone in Ringer HEPES buffer in the range between 30.0 and 3000 ng/mL was quadratic, and a weighting factor of 1/X2 was applied. Diluting samples up to 100-fold did not affect precision and accuracy. The carry-over did not impact the results. Indolinone proved to be stable for 3 hours at room temperature, and for 3 successive freeze/thaw cycles. The processed samples could be stored in the autosampler at 10°C for at least 28 hours. Moreover, indolinone was stable for at least 16 days in Ringer HEPES buffer when stored below -65°C. In the in vitro BBB models, indolinone showed apparent permeability coefficient (Papp) values higher than 20 x 10-6 cm/s.

Conclusions
The validation study demonstrates that our LC-MS/MS quantification method for indolinone is specific, selective, precise, accurate, and capable of producing reliable results. In the human [5] and animal in vitro BBB models, indolinone showed a high potential of brain permeation [6].

Novel Aspect
Full validation of a UPLC-MS/MS quantification method according to FDA/EMA guidelines in the context of brain drug permeability studies

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MPS06-15 / Optimization of laboratory MS/MS newborn screening of inherited metabolic diseases

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Introduction
The aim of our study was to optimize of the newborn screening (NBS) program for inherited metabolic diseases (IMD) to reach the aims of the Region4Screening (R4S) project, i.e. detection rate (DR) better than 1:3,000, positive predictive value (PPV) higher than 20% and false positive rate (FPR) lower than 0.3%.

Methods
1) Modification of current FIA-MS/MS method for NBS in order to increase the number of primary markers.
2) Development of new LC-MS/MS methods for second-tier determination of selected markers.

Results
In the pilot study we have expanded the present screening panel (10 IMDs) by including 20 additional IMDs (urea cycle disorders, tyrosinemia type I and disorders of methionine and propionate metabolism); primary markers arginine, citrulline, methionine, propionylcarnitine and succinylacetone were added.
During a testing period of 15 months 49,211 newborn samples were evaluated for urea cycle disorders, 40,892 for disorders of methionine and propionate metabolism and 3,788 for tyrosinemia type I. Employing LC-MS/MS methods for second-tier testing we determined total homocysteine (tHcy), methylmalonic acid (MMA) and methylcitric acid (MCA) concentrations in dried blood spot (DBS): tHcy 305 samples, MMA 163 and MCA 24 samples. One patient with cystathionine beta-synthase (CBS) deficiency was revealed. By including all 20 IMDs in NBS program the results of the pilot study predict DR 1:2,600; PPV 23% andFPR 0.19% which is in agreement with R4S target performance.

Conclusions
In summary, we suggest to extend the newborn screening panel in the Czech Republic from 10 up to 25 IMDs, including citrullinemia type I, argininemia, CBS/methylenetetrahydrofolate reductase deficiency, biotinidase deficiency and other 10 IMDs which could be detected secondarily.

Novel Aspect
In order to enhance efficacy of MS/MS newborn screening program the new rapid LC-MS/MS methods for simultaneous determination of tHcy, methionine and cystathionine have been developed.
Supported by MH CZ-DRO-VFN64165/2012; OPPK CZ.2.16/3.1.00/24012 and NT/12213–3 IGA by Ministry of Health of the Czech Republic.

MPS06-16 / Real time monitoring of the metabolic capacity of ex-vivo rat olfactory mucosa by PTR-MS

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Introduction
It has been evidenced that olfactory mucosa can metabolize inhaled odorous volatile organic compounds (VOCs) through various enzymatic mechanisms [1]. Moreover, it has been hypothesized

that the formed metabolites could be implied in overall aroma sensory perception. Thus, using headspace gas chromatography (HS-GC) [2], preliminary ex-vivo studies revealed the formation of a main metabolite (ethanol) when the odorant molecule ethyl acetate was injected in the gas phase in the headspace above a fresh explant of rat olfactory mucosa. Kinetics of apparition of the metabolite and concomitant disappearance of the main odorant could be measured. However, the HS-GC method did not allow to access to the data during the first five minutes of contact between the odorant and the mucosa, thus limiting the olfactory biological significance. A direct injection mass spectrometry method using PTR-MS, a technique dedicated to accurate detection of VOCs in real time, was therefore implemented.

Methods
A PTR-ToF-MS (Ionicon ToF 8000) instrument was used in standard operating conditions (H3O+ primary ions, E/N = 111 Td in the drift tube maintained at a temperature of 80°C and a pressure of 2.3 mbar) for all the experiments. A freshly dissected olfactory mucosa was placed in a 20 mL vial sealed with a Teflon-lined stopcock connected to the PTR-MS via a 3-way Luer valve. Known concentrations of gaseous ethyl acetate were injected in the headspace above the mucosa. The headspace was sampled for 1 sec to the PTR-MS at regular intervals during 5-6 minutes. Blanks of the empty vial, of the vial containing only ethyl acetate and of the headspace above the mucosa in absence of any VOC were recorded. All the measurements were done in triplicate.

Results
Depletion of ethyl acetate signal at m/z 89.060 in the headspace of the vial containing only the VOC was insignificant during the 6-min recording time and the only other ion detected was the McLafferty rearrangement fragment ion at m/z 61.028. The headspace above the mucosa was exempt of VOCs. Injection of ethyl acetate above the rat mucosa resulted in immediate apparition of ethanol at m/z 47.049 whose concentration increased continuously during recording time, while a continuous depletion of ethyl acetate signal was observed.

Conclusion
The obtained results demonstrate that an odorant molecule can be metabolized by an ex-vivo olfactory mucosa within seconds, producing an identified metabolite. These results afford new insights in the metabolic mechanisms at olfactory mucosa level. The PTR-ToF-MS technique appears to be a very promising method to investigate metabolism kinetics in real time.

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MPS06-17 / High Performance Liquid Chromatography-Tandem Mass Spectrometry method for quantification of 17-β-Estradiol in mouse plasma and brain

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17-β-estradiol (βE2) is one of the most important steroid hormones implicated in reproduction.Moreover, βE2 has an important role in the functioning of the brain and of the nervous system. This study aims at the design of a suitable analytical method for the identification and quantification of βE2 present at very low concentration in the mouse brain (around 1 fmol/mg) and plasma (around 0,01 fmol/µL).
LC/MS/MS (MRM mode) analyses were performed with a 5500 QTRAP (AB Sciex) instrument coupled to a LC system (Ultimate 3000, Dionex). Analyses of standard βE2 (Sigma-Aldrich) were achieved in the negative and positive modes, by direct infusion and/or LC coupling. Mouse brain and plasma fractions enriched in βE2 were prepared by Solid Phase Extraction (SPE) using a GX-271 ASPEC instrument (Gilson).
To improve the sensitivity of βE2 detection in mass spectrometry, different additives (NH4F, NH4Cl, CH3COONH4, HCOONH4, TEA, HCOOH, CH3COOLi) were tested at various concentrations (0.1%; 0.5%; 1%) in negative and positive ion modes by direct infusion. Two reversed-phase liquid chromatography (RPLC) columns were evaluated in the gradient elution mode, using the same additives.
The best sensitivity, matching βE2 concentration levels expected in mouse brain and plasma samples, was obtained in negative ion mode with methanol/water (9/1) containing 0.1% TEA. A MRM based RPLC-MS/MS method was developed on this basis, and used for the analysis of mouse brain and plasma samples after extraction on SPE cartridges.
Keywords: estradiol; mass spectrometry; MRM

MPS06-18 / Developing a human cancer diagnostic system: Validation of the system robustness

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Introduction
We are developing a mass spectrometry-based diagnostic machine. Single quadrupole liquid chromatograph mass spectrometer LCMS-2020 (Shimadzu) is used as a platform. We made manifold modifications on the mass spectrometer, such as replacement of ionization unit with Probe Electro Spray Ionization (PESI) module and installment of needle axis regulator. In order to realize the application of this system to clinical settings, we first validated the quality and stability of data acquisition in terms of differences among three devices. In addition, to further validate the stability of machine, we attempted to determine a package of calibrants that would be suitable for adjusting of our system.

Methods
(1) Modification of LCMS-2020: LCMS-2020 was modified to accommodate the PESI unit. Briefly, the ESI unit was replaced with PESI module. The needle axis was adjusted to the position where ion orifice is placed. Newly designed cassette was installed about 10 mm below the ion orifice.
(2) Sample handling and measurement: Human kidney tissues excised from cancer patients were used for constructing the database as wells validating the robustness of our system. A small piece of tissue, about the size of a grain of rice, is placed on a disposable cassette. After adding 30 mL of 50% Ethanol to the specimen, homogenization was performed and the extracted components were analyzed. The whole procedure was repeated on 10 different tissues by three different devices. The obtained data were analyzed through cross validation.
(3) Validating the stability of system by calibrants: As external standard, we chose PPGTs (Polypropylene, Triol Type 300, 700, 1500), which are commonly used as calibrants for LC-MS. Each compound was dissolved in 50% 2-propanol and added to the sample solution. As internal standard, we chose several species of phospholipids that are major components of bio-membranes.

Table 1 Diagnosis Result (Expressed as probability of cancer in percentage)

Pathological Diagnosis	Sample ID (#)	Device 1	Device 2	Device 3
Cancer	000-00434	70.9	99.6	100.0
	000-00452	100.0	100.0	100.0
	000-00454	100.0	99.9	100.0
	000-00458	100.0	75.5	100.0
	000-00460	100.0	98.6	100.0
Non-cancer	000-00431	0.0	0.0	0.0
	000-00437	0.0	0.0	0.0
	000-00451	0.0	0.0	0.0
	000-00453	0.0	0.0	0.0
	000-00461	0.0	0.0	0.0

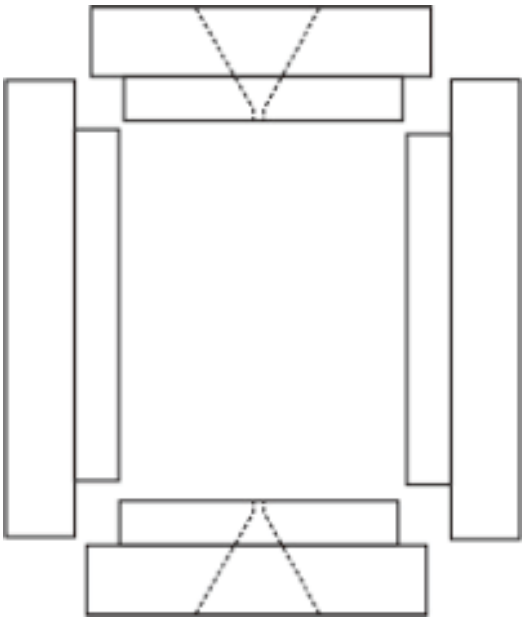

Figure 1. A cross section of a ladder shape ion trap mass analyzer.

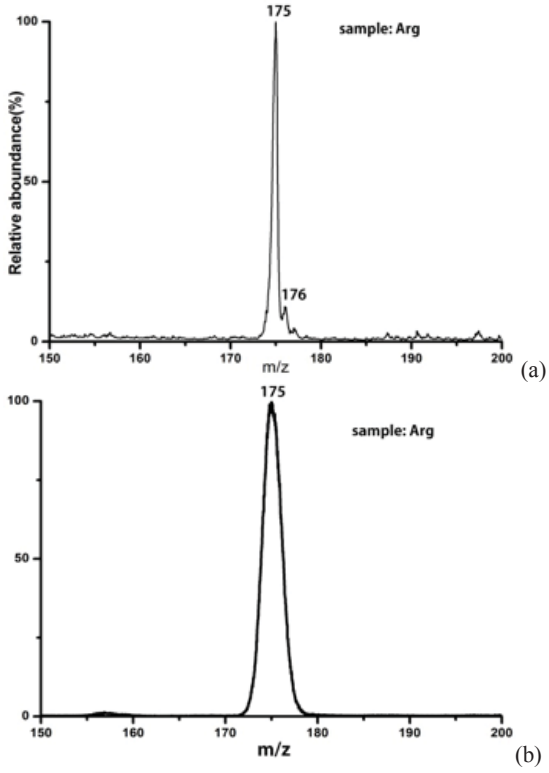

Figure 2 . The mass spectra of Arginine ion(m/e=175 Th). (a). Ladder shape ion trap; and (b). Rectilinear ion trap.

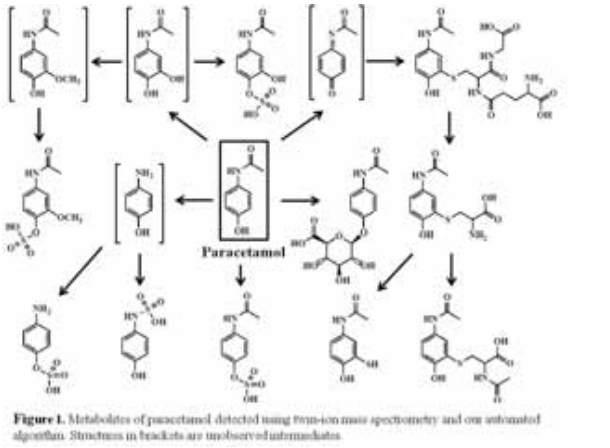

Figure 1. Metabolites of paracetamol detected using brain-ion mass spectrometry and our automated algorithm. Structures in brackets are unobserved intermediates.

Results

The modified LCMS-2020 equipped with PESI module worked well, by judging from the obtained spectra. As shown in Table 1, 13 out of 15 trials gave 98% or higher probability of cancer for the cancer specimens diagnosed by pathologists. In two cases, #434 (device1) and #458 (device2), we obtained lower probability from 70 to 75%, both of them was observed randomly. Concerning the non-cancerous case, all three devices consistently showed 0% of cancer probability. These figures indicate the robustness of our system in term of cancer prediction. Furthermore, we checked the performance of system by taking external and internal calibrants. In both cases, three devices revealed constant outcome in the patterns of spectra, the results suggesting the stability and robustness of systems.

Conclusions

Our system is a reliable method to assist the diagnosis of cancer as it gives stable results among different devices.

Novel Aspects

We have developed a novel cancer diagnostic system based on LCMS-2020, whose ionization part is replaced with PESI unit (PESI-MS). We validated the robustness of this system by comparing the diagnostic performance among three difference devices under various conditions.

MPS06-19 / Development of a mass spectrometric approach to study disorders of protein O-glycosylation

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Introduction

Glycosylation is the most common protein post translational modification and involves attachment of carbohydrate moieties to amino acid side chains. O-glycans are bound through the hydroxyl oxygen atom, typically in serine or threonine. Glycans coat all cell surfaces and are essential for life. However, we have little understanding of the glycan biosynthetic code because, unlike protein and nucleic acid biosynthesis, there is no template for glycan biosynthesis. Glycan biosynthesis is managed by a large number of glycosyltransferase enzymes (located in the endoplasmic reticulum (ER) and the Golgi) and is dependent on their expression levels, location and activity, each of which can change in response to environmental stimuli and cell differentiation, resulting in a range of glycan structures. Congenital disorders of glycosylation (CDGs), a family of rare genetic disorders, result from mutations in these enzymes or in the proteins that organize the enzymes, and can result in aberrant glycosylation. We are interested in studying glycan structures

expressed on a range of healthy cells and those with mutations in ER and Golgi proteins, in order to better understand the consequence of such mutations on glycan structure. Methods are therefore needed to solubilise and extract glycoproteins from cultured cells and to release and isolate their O-glycans for MS analysis.

Methods
We have developed a method for O-glycan release and isolation using a centrifugal filter as a reaction vessel, where O-glycans are released by base-catalysed β-elimination. The filter is centrifuged to retrieve the O-glycans, leaving the higher molecular weight proteins above the filter membrane. Use of centrifugal filters is very attractive in terms of sample handling, especially as a one pot reactor for small glycoprotein samples. The released glycans are permethylated for analysis by MALDI-MS.

Results
Our method works well for standard glycoproteins (e.g. fetuin, mucins). β-elimination is typically carried out overnight1 but exploration of microwave irradiation and sonication has enabled this step to be optimised to only 5 min of sonication, which also improved the MS data generated. Currently, we are working on applying the developed method to cultured cell models of CDG.

Conclusions
O-glycans can be released and retrieved in a filter device using optimised base catalysed glycan release, using sonication. There is potential for a one pot/one sample method by combining established protocols for N-glycan release and protein analysis with our O-glycan release from one sample, all in the same filter unit.

Novel aspect
Using methodology developed in the group2 for N-glycan release and isolation in a filter unit, we have developed a novel, simple and efficient protocol for O-glycan release and isolation, enabling a rapid and convenient read-out of the glycan structural consequences of CDGs using cellular models. Although β-elimination is well-accepted for releasing O-glycans, it has not before been carried out using sonication.

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MPS06-20 / Minimal labelling and low resolution selected reaction monitoring for accurate IDMS determinations by LC-ESI-MS/MS

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Introduction
The occurrence of matrix effects is relevant during LC-MS analyses, particularly when electrospray ionization (ESI) is employed. In LC-ESI-MS, matrix effect usually leads to a suppression of the analyte response due to co-eluting matrix constituents. When isotope dilution mass spectrometry is employed for quantification, the use of minimally 13C labelled analogues keeps the isotopic difference as little as possible with respect to the analyte overcoming matrix effects. Thus, analytical biases resulting from different behavior of both forms are minimized and accuracy, precision, and reliability of results correspondingly improved.

Method
The proposed method calculates the molar fractions of analyte and labelled analogue from the experimental mass spectra of the in-cell fragment ions obtained by selected reaction monitoring (SRM) in a triple quadrupole instrument. Resolution of the first quadrupole is reduced to the extent that, the whole parent ion cluster is transmitted to the collision cell as a prerequisite for monitoring accurate isotopic distributions of the molecular fragments. Applying multiple linear regression the analyte concentration can be directly obtained from the fragment-ion spectrum acquired for the sample without resorting to extra calibration runs as usually needed

Results
As a proof of concept this strategy is applied to the determination of seven β2-agonists in human urine and the protein biomarker Cystatin C in human serum. For the determination of β2-agonists we employed analogues labelled in one 13C atom whereas for the determination of Cystatin C we employed a proteotypic peptide labelled in two 13C atoms. The strategy was validated by analyzing Certified Reference Materials obtaining results in agreement with the certified values and precisions lower than 2.5% (RSD).

Conclusions
The transmission of the whole precursor ion cluster of each compound to the collision cell provides an increased sensitivity and the possibility of measuring the real isotopic distribution of the molecular fragment in the sample with a small number of SRM transitions. The accuracy and precision obtained by this new measurement procedure is comparable to those obtained by classical IDMS determinations.

Novel aspects
The combination of minimal 13C labelling, multiple linear regression and the decrease of resolution in the first mass analyzer provides accurate and precise quantifications of at sub ng g-1 levels. Isotope effects due to a different physicochemical behavior of the analyte and the labeled analogue are minimized. The decrease of the resolution of the first mass analyzer provides an increase of sensitivity and the accurate measurement of the isotopic distribution of the molecular fragment. Applying multiple linear regression we obtain from a single injection the molar fractions of analyte and labeled analogue in the sample without resorting to a methodological calibration graph saving time and expensive labeled reagents.

MPS06-21 / Interaction Analysis using SPRi-MALDI MS

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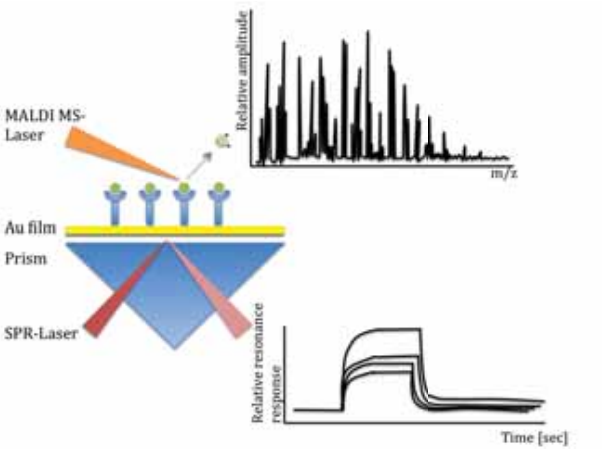
The investigation of new, high-throughput analytical techniques is important in clinical research and diagnostics, for more accurate and targeted determination of biomarkers. Surface plasmon resonance (SPR) is suitable to analyze biomolecules in a label-free fashion, and provides information on binding kinetics (kon/koff) and binding affinity in real time. Working in an array format provides rapid and high-throughput analysis of different interactions in parallel. The coupling of surface plasmon resonance imaging (SPRi) with matrix-assisted laser desorption/ ionization mass spectrometry (MALDI MS) enables a multiplexed detection and quantification of binding by SPRi on one hand, and on the other hand, characterization of interacting ligands on the molecular level with MALDI MS. [1]
We are investigating the binding specificity of various DARPins

for maltose binding protein (MBP). The advantage of SPRi-MALDI MS is the possibility to measure direct on the plate with no need of elution of the bound analyte. With MS we can even differentiate between protein isoforms.



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MPS06-22 / Full validation of UHPLC-MS/MS methods for the determination of kaempferol and 4-HPAA, and application to in vitro BBB and intestinal drug permeability studies

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Introduction

In rats, the flavonoid kaempferol induces anxiolytic activity after oral administration (p.o.), but not after intraperitoneal (i.p.) injection. However, i.p. application of its major metabolite 4-HPAA, formed by the intestinal microflora, induces behavioral changes.1 The systemic distribution and brain penetration of flavonoids and their metabolites are not fully understood, and validated quantification methods are mostly lacking. To evaluate the ability of kaempferol and 4-HPAA to cross intestinal and blood brain barriers (BBB), we screened these compounds in human and animal cell-based models using validated UHPLC-MS/MS quantification methods.

Methods

UHPLC-MS/MS methods were developed and validated according to international guidelines2.3. 13C-Labeled kaempferol served as internal standard (IS) for quantitation of kaempferol, and vanillic acid was selected as IS for the determination of 4-HPAA. BBB transport studies were performed with a validated human in vitro BBB model using hBMEC cells 4 and a rat triple.co-culture model.5 Intestinal transport studies were performed with Caco-2 cells using HBSS buffer as transport medium.

Results

Quantification range for both analytes was 20.0 to 2000 ng/ml, and the response curves were fitted with a quadratic curve with 1/X2 as weighing factor. Carry over was within acceptance criteria. Both analytes were stable in biological samples during sample collection and handling, during two weeks storage below -65°C, and after two freeze and thaw cycles. In the Caco-2 model, the apparent permeability coefficient of kaempferol from apical to basolateral side (Papp A→B), and from basolateral to apical side (Papp B→A) were 23.7×10-6 and 32.1×10-6 cm/s, while those of 4-HPAA were 2.3×10-6 and 4.0×10-6 cm/s, respectively. In the human BBB model, Papp A→B and Papp B→A of kaempferol were 37.3×10-6 and 29.1×10-6 cm/s, and those of 4-HPAA were 8.3×10-6 and 7.1×10-6 cm/s, respectively. In the animal (rat) BBB model Papp A→B and Papp B→A of kaempferol were 28.0×10-6 and 18.3×10-6 cm/s.6

Conclusions

The UHPLC-MS/MS assays were specific, selective, precise, accurate, and capable to produce reliable results. The permeability studies were indicative of high intestinal and BBB permeation of kaempferol, whereas its metabolite 4-HPAA showed low permeability in the three barrier models. Our data supports the described CNS effects of kaempferol, while the role of the metabolites needs to be elucidated in further studies.

Novel aspects

Use of UHPLC-MS/MS assays validated according to FDA and EMA guidelines for permeability studies of an important flavonoid and its major metabolite in various animal and human barrier models.

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MPS06-23 / A SISCAPA Immuno-MS Assay for Quantification of Soluble Transferrin Receptor in Human Serum

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Introduction

Transferrin receptors (TfRs) are membrane-bound carrier proteins to acquire iron. Elevated levels of TfRs may be associated with dysfunction of erythropoiesis due to iron-deficiencies or diseases such as hemolytic anemia and myelodysplastic syndromes. Soluble sTfR is a truncated, blood-circulating variant of the whole TfR molecule, and as such its measurement is of clinical importance. In contrast to the classic method evaluating the sTfR-level in patient's blood by ELISA assays, we present an alternative approach for automated quantification of sTfR in human serum based on parallel analyte enrichment in microtiter plates followed by MALDI-MS quantification.

Methods

A proteotypic peptide unique to sTfR was selected (GFVEPDHYVVVGAQR [295-309], MW=1672.844 Da) incl. a stable isotope standard (SIS) version (M+H+ = 1682.852 Da). To quantify sTfR in human serum, an automated SISCAPA workflow was used to digest the sample, enrich the endogenous and SIS peptides using a high-affinity rabbit monoclonal antibody (RabMAb) against the target peptide, wash and elute the peptides from the antibody, followed by automated MALDI measurement. The data were analysed by a software tool calculating the ratio of endogenous to SIS peptides from which the sTfR content of the sample was quantified.

Results
The adaptation of the SISCAPA-workflow to MALDI benefits from fully automated sample handling in on-target preparations and robust spectra acquisition (1-2% CV). To determine the endogenous level of sTfR in pooled plasma and the lower limit of quantitation of the SISCAPA-MALDI assay, two standard curves were generated. The first was a 12-point standard addition curve where a mixture of labeled (heavy) and unlabeled (light) peptide was spiked into pooled serum samples. The level at which the standard addition curve plateaus is representative of the endogenous level of the analyte. The second was a 12-point 'reverse' curve where the SIS peptide is titrated from 1 pmol to 0.5 fmol (1/2 dilution) while the synthetic light peptide was spiked at constant 500 fmol. Because the stable isotope standard is not endogenously present in the sample, the reverse curve elucidates a linear response from which the assay sensitivity can be determined. The experiment was performed with four technical replicates with a processing time of ~7 seconds per target spot. SW-based quantification calculated the average endogenous level of sTfR to be 185 fmol in 10 µl serum with 6 % CV at endogenous level. Using the reverse curve, the lower limit of detection was determined to be 4 fmol.

Conclusions
A sensitive, accurate, high-throughput and automated assay was developed for LC-free SISCAPA-MALDI quantification of sTfR.

Novel Aspect
MALDI used in a clinical imuno-Mass Spectrometric Assay

MPS06-24 / The novel iEndoscope for the rapid identification of gastrointestinal polyps and tumours in-vivo using rapid evaporative ionization mass spectrometry

Julia Balog, Frank Huang, Nima Abbassi-Ghadi, Laura Muirhead, Dora Perenyi, Sacheen Kumar, Zoltan Takats
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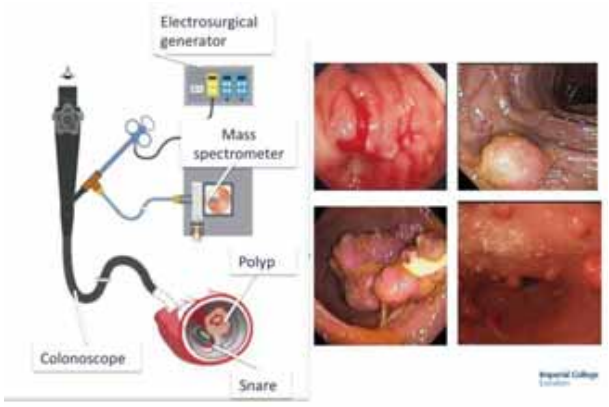
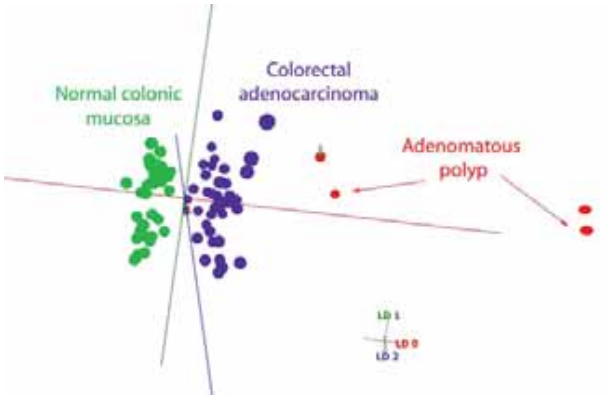
Introduction
In order to prevent the development of gastrointestinal tumours, endoscopic screening is routinely used. Colorectal cancers are still one of the leading morbidities in US and Europe, while gastric cancers have very poor outcomes, thus an early diagnosis is essential. Rapid Evaporative Ionization Mass Spectrometry (REIMS) is a technique used for in-vivo characterization of human tissue by mass spectrometric analysis of the aerosol released during electrosurgical dissection. The ionization technique can be combined with an endoscopic snare, allowing an in-situ, in-vivo sampling method within the patient. The aim of this study is to create and optimize an endoscopic and colonoscopic setup, and to test the method and classification algorithm in-vivo.

Methods
A commercially available endoscopic and colonoscopic snare is equipped with an extra T-piece in order to connect a 1/8 in. diameter PFTE tubing towards the atmospheric inlet of a mass spectrometer. The setup was optimized using pork colon and stomach model and used afterwards on human patients both in-vivo and ex-vivo. After acquiring spectra from the released surgical smoke, the workflow includes the construction of a tissue specific spectral database followed by a multivariate classification algorithm and spectral identification algorithm. Our aim is to separate healthy and cancerous tissue, to identify polyps containing cancerous cells, and to determine the condition of mucosa based on the REIMS fingerprint of each tissue type.

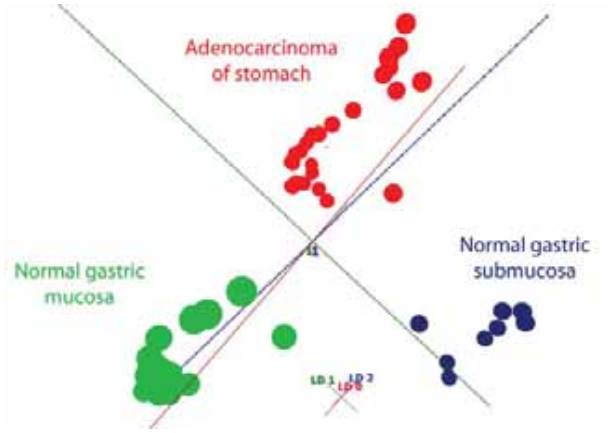
The novel iEndoscope for the rapid identi cation of gastrointestinal polyps and tumours in-vivo using rapid evaporative ionization mass spectrometry



1. Figure. The concept of iEndoscope. The surgical smoke generated during the removal of a polyp is transported to distant mass spectrometer and the acquired spectrum is compared to previously built tissue specific spectral database using multivariate statistics for classification.



2. Figure. Pseudo 3-dimensional LDA plot of ex-vivo colonic mucosa, adenocarcinoma and adenomatous polyp. Adenomatous polyps are clearly separated from both healthy mucosa and cancerous tissue.



3. Figure. Pseudo 3-dimensional PCA plot of human gastric tissue. Mucosal and submucosal tissue is clearly separated both from each other, and the tumour.

Results
Mass spectrometric data was collected after surgical or endoscopical resection of tissue and the histologically validated data was uploaded to our home-built database. Classification "models" were created from the full spectral data and was then used for the identification of unknown tissue. The iEndoscope

was used during the removal of polyps to determine if cancerous cells were present. The method was capable of differentiating between healthy mucosa, cancer, adenomatous polyp and different alterations (Crohn-disease, inflammation, etc.). Different parts of the gastrointestinal tract was sampled, however the structure of healthy mucosa was similar in all cases. Different layers of the colonic wall was also sampled, and a marked difference was revealed in the spectral pattern of mucosa, submucosa, smooth muscle and serosa in both colon and gastric.

Conclusions
The novel iEndoscope is a feasible technique for rapid identification of human tissue in-vivo during endoscopic interventions, as it can also be used as a "safety tool" giving a warning when submucosal region is burned. The technique can be adopted for laparoscopic interventions, widening the range of potiential surgeries, where our in-vivo, rapid identification method can be used. iEndoscope could significantly change the use of these tools allowing a broader range screening, and more precise identification of mucosal condition within one intervention.

Novel aspects
In-vivo tissue identification during endoscopic interventions, REIMS coupled with endoscope

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MPS06-25 / New Workflows for Identification and Profiling of Disulfide Bonds in Biopharmaceuticals
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Introduction
The spatial structures of biologics are crucial for their safety and efficacy and fundamentally determined by disulfide bonds (DSBs). DSB analysis is therefore indispensable and commonly performed by comparing peptide maps of reduced and non-reduced samples. As this approach requires the time-consuming preparation and analysis of multiple digests, we evaluated alternative approaches using ESI LC-MS³ and MALDI LC-MS³ and non-reduced protein digests. Disulfide bonds are initially cleaved either by ESI electron-transfer dissociation (ETD) on an ion-trap (IT) or by MALDI in-source decay (ISD), followed by collision induced dissociation (CID) of the released peptides in a second step.

Methods
Digests of α-lactalbumin and adalimumab were performed under optimized conditions to avoid disulfide scrambling and to obtain a good peptide yield by blocking free cysteines with N-ethylmaleimide. A combination of Lys-C and trypsin was used for proteolytic digestion at low pH of 5.8 in the presence of guanidine-HCl. The samples were separated using nano-LC followed by MS analysis. An ion-trap equipped with a CaptiveSpray ionization source and the capability to increase ion charge states was used in the ETD workflow, and a MALDI-TOF/TOF instrument was used in the ISD workflow. Software was developed to identify characteristic fragmentation patterns of disulfide-linked peptides in MS2 spectra and generate target lists for the MS3 step.

Result
A new MS3 experiment to profile DSB-peptides combined ETD-MS/MS with a targeted CID MS3 step. Instead of performing typical MRM experiments analyzing only few transitions, we acquire full scan MS3 spectra in the IT enabling simultaneous detection of many peptide fragments. With this targeted workflow, 7 of 8 expected adalimumab DSB-peptides were identified with average sequence coverage of 71%.
MALDI-ISD fragments corresponded to singly protonated peptides with reduced cysteines. For the identification of DSB-peptides, MS spectra were screened for triplets of the DSB-peptide and their two ISD fragments using the equation m/z (ISD1) + m/z (ISD2) – m/z (H2+H+) = m/z (DSB-peptide). All peaks were subjected to LC-MALDI-MS/MS analysis and Mascot database searches resulted in a sequence coverage of > 90 %. For adalimumab, all DSB-peptides with one disulfide bond were identified with this method.

Conclusions
Successful identification of disulfide bonds on biopharmaceuticals

Novel Aspects
MS^3 approaches in MALDI-TOF and ion traps for disulfide bon elucidation

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MPS06-26 / Mimicking Drug Metabolism by EC/MS
Agnieszka Kraj, Hendrik-Jan Brouwer, Nico Reinhoud, Jean-Pierre Chervet
Antec

Introduction
For almost two decades electrochemistry (EC) has been successfully coupled to mass spectrometry. The electrochemical cell is used as a reactor in which a controlled oxidation or reduction takes place prior to MS detection. The oxidation products show excellent agreement with cytochrome P450 reaction products in nature (e.g., liver), mimicking the enzymatic Phase I biotransformation (biomimetic oxidation). This purely instrumental approach is making the use of costly enzymes and the risk of non-specific reactions, obsolete. The reaction products are formed instantaneously in the EC cell allowing for direct coupling with MS and the measurement of short-lived compounds. Significant time and cost savings result using EC/MS compared to current in vitro (microsomes) or in vivo (rodents) approaches.

Oxidation of target compounds in an electrochemical cell is a complementary approach to traditional methods and delivers the oxidative metabolic fingerprint of the molecule in a very short time. Additionally, electrochemistry allows tracing the reactive metabolite conjugates with targets (e.g. proteins, glutathione) without matrix interactions in contrary to classical methods.

Methods
An electrochemical reactor cell (Antec, USA) was used for the oxidation of drug compounds. The cell potential was ramped from 0 to 3000 mV depending on the type of the working electrode (WE). The electrochemical reactor cell was connected directly to the electrospray source of a HCT plus (Bruker Daltonics, USA). The performance of the cell under elevated pressure was tested. For the formation of GSH adducts, i.e., Phase II reactions, a GSH solution was added after the EC cell using a mixing coil prior to the MS.

Results
We present oxidative metabolic profiles of different commercially available drugs. A new automated protocol for optimized oxidation of the selected metabolites will be shown.
Also a bulk electrolysis cell (the SynthesisCell) will be presented. Using the SynthesisCell any oxidation and reduction product can be generated in milligram quantities in a short period of time for example to synthesize reference material for NMR identification.

Conclusions
The data demonstrate that hyphenation of electrochemistry with electrospray mass spectrometry provides a versatile and user-friendly platform for rapid and cost efficient screening of target compounds (drugs, xenobiotics, etc.) in phase I and phase II metabolomics studies.

Novel aspects
Novel electrochemical reactor cell for the prediction of drug metabolism using Electrochemistry/MS

MPS06-27 / Quantitation of nucleosides and nucleoside triphosphates using LC-MS/MS in bioanalysis

Nerea Ferreirós[1], Dominique Thomas[2], Nikolas Herold[2], Oliver T. Keppler[2], Gerd Geisslinger[3]
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Introduction
Nucleosides are composed of a nucleobase and a five-carbon sugar. They can be phosphorylated to nucleoside mono-, di- or triphosphates. Nucleoside triphosphates (NTP) are the building blocks of DNA, if the sugar is 2'deoxyribose,or RNA if the sugar is ribose, respectively. The endogenous pools of NTP and the corresponding nucleosides are of interest in virus infections, oncology or in immune reactions, as activated immune cells rapidly proliferate. For the quantitation of NTP, most published methods require the addition of ion pairing reagents, like trifluoracetic acid, to the chromatographic mobile phases, which has the big disadvantage of reducing sensitivity. The aim of our study was to develop an analytical procedure for the simultaneous quantitation of all endogenous nucleosides and NTP. Due to the different polarity of the analytes, two LC-MS/MS methods for the determination of the studied compounds were developed: one for NTP and another one for nucleosides.

Table 1.NTP and nucleosides and the corresponding LLOQ and calibration ranges. ATP=adenosine-5'-triphosphate, CTP=cytidine-5'-triphosphate, dATP=2'-deoxyadenosine-5'-triphosphate, dGTP=2'-deoxyguanosine-5'-triphosphate, dCTP=2'-deoxycytidine-5'-triphosphate, GTP=guanosine-5'-triphosphate, TTP=thymidine-5'-triphosphate, UTP= uridine-5'-triphosphate;A=adenosine, C=cytidine, dA=2'-deoxyadenosine, dC=2'-deoxycytidine, dG=2'-deoxyguanosine, G=guanosine, T=thymidine, U=uridine.

Analyte	LLOQ [pg on column]	Calibration range [pg on column]
dATP + dGTP	10	10 - 400
dCTP + TTP	20	20 - 800
CTP + GTP	100	100 - 4000
ATP + UTP	500	500 - 20000
dA, dC, dG,	2	2 - 200
T	5	5 - 200
C, G	10	10 - 2000
A, U	100	100 - 20000

Table 1. NTP and nucleosides and the corresponding LLOQ and calibration ranges. ATP=adenosine 5' triphosphate, CTP=cytidine 5' triphosphate, dATP=2'-deoxyadenosine 5' triphosphate, dGTP=2' deoxyguanosine 5' triphosphate, dCTP=2' deoxycytidine 5' triphosphate, GTP=guanosine 5' triphosphate, TTP=thymidine 5' triphosphate, UTP= uridine 5' triphosphate;A=adenosine, C=cytidine, dA=2'-deoxyadenosine, dC=2' deoxycytidine, dG=2' deoxyguanosine, G=guanosine, T=thymidine, U=uridine.

Methods
The sample preparation included addition of the internal standards (stable isotopes of all 8 NTP and 4 nucleosides) and a simple protein precipitation with methanol. After centrifugation, the supernatant was divided and evaporated for the determination of nucleosides and NTPs. In both cases, the HPLC system consisted of an Agilent 1200 Series binary pump, degasser and column oven connected to a CTC PAL autosampler. Separation of the nucleosides was carried out in an Atlantis T3 column for 15 min, using 0.1% acetic acid and 10 mM ammonium acetate in methanol as mobile phases. The NTPs were separated in a BioBasic AX column using also a 15 min gradient program and two buffers. Buffer A (pH=5.6) consisted of 40 mL acetonitrile, 60 µL acetic acid, 1 mL ammonium acetate (1M) and 59 mL water while buffer B (pH=10.6) contained 30 mL acetonitrile, 0.3 mL ammonia solution (25%), 0.1 mL ammonium acetate (1M) and 69.6 mL water. For analysis, a hybrid triple quadrupole-ion trap mass spectrometer 5500 QTRAP (AB Sciex) equipped with a Turbo Ion Spray source operated in positive ion mode was used.

Results
Adequate separation of the NTPs was achieved due to a changing pH during the run without the need of ion pairing reagents. The lower limit of quantifications, calculated on the basis of the expected concentrations, are listed in table 1 together with the calibration ranges. All analytes showed good linearity and could be determined in samples containing around one million human T cells.

Conclusion
The described method can be applied to the combined determination of nucleosides and NTPs in different matrices, providing an insight into the endogenous levels of both nucleosides and NTPs. This may be helpful to monitor the drug efficacy of antiviral or immunosuppressive therapies.

Novel aspect
The described method is the first one which includes all endogenous nucleosides present in DNA and RNA. Levels of all endogenous NTPs can be determined without using ion pairing reagents.

MPS06-28 / Determination of Quercetin and its metabolites in rat plasma by ultra high performance liquid chromatography tandem mass spectrometry

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Introduction
Flavonoids are a large group of natural phytophenols which belong among the secondary metabolites distributed in plants. Quercetin is one of the most spread flavonoid found in vegetables and fruits. In vivo quercetin is metabolized into two groups of metabolites – glucosides and phenolic and aromatic acids. Quercetin is very important substance and because of its strong antioxidant effect and possible cardioprotective effect. It is frequently used in clinical practice.

Methods
Ultra high performance liquid chromatography – tandem mass spectrometry (UHPLC-MS/MS) method using Acquity UPLC and Quattro Micro triple quadrupole mass spectrometer was developed and validated for quantification of quercetin and its metabolites in rat plasma. The analytes were separated using the BEH Shield RP C18 (2.1 x 100 mm, 1.7 µm) analytical column and gradient elution with methanol and 0.1% formic acid. Analytes were ionized by electrospray (ESI) in positive/negative mode. Quantification of analytes was performed using selected reaction monitoring (SRM) using the precursor ions [M+H+] or [M-H-] and the corresponding product ions. Protein precipitation

was used for sample preparation. 100 µl of acetonitrile (ACN) were added to 50 µl of rat plasma. After 10 min, the sample was centrifugated for 10 min, filtrated and injected into UHPLC.

Results

First, columns with different sorbents including BEH C18, BEH Shield RP C18, BEH Phenyl, CSH C18, CSH Phenyl-Hexyl, CSH Fluoro-Phenyl were tested. BEH Shield RP C18 was chosen because of the best separation of analytes especially a critical pair of isomers tamarixetin and isorhamnetin. The mixtures of methanol and organic acids or buffers at different concentrations were tested as a mobile phase additives. Methanol in combination with 0.1% formic acid was chosen as the final mobile phase. Other additives affected the stability and response of analytes negatively. Subsequently MS conditions were tuned in positive/ negative ESI mode. The collision energy, dwell times and cone voltage were optimised for each analyte individually. Methanol and ACN were tested as a prepicipitating agents in several ratios with rat plasma. ACN was finally chosen in the rat plasma:ACN ratio 1:2. The method was validated in terms of linearity, limit of detection and quantification, accuracy, precision, selectivity and matrix effects.

Conclusion

Fast, selective and reliable method was developed, optimized and validated for determination of quercetin and its metabolites by UHPLC-MS/MS. Protein precipitation was optimized as a sample preparation step for rat plasma. The method was used for pharmacokinetic studies and for the explanation of metabolism and possible cardioprotective effect of quercetin and its metabolites in vivo.

Novel aspect

This method enables determination of complex spectrum of flavonoids. The compounds differed substantially in polarity, molecular weight and chemical structure, which is challenging in one-step sample preparation.

MPS06-29 / Structural characterization of in vitro metabolites of the new anticancer agent, EAPB0503, by liquid chromatography-tandem mass spectrometry

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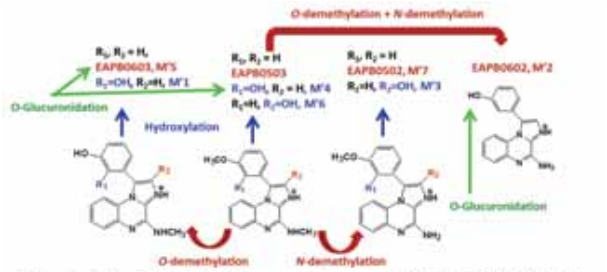
[1]Institut des Biomolecules Max Mousseron, [2]Faculty of Pharmacy

EAPB0503, belonging to the imidazo[1,2-a]quinoxaline series, is an anticancer drug with antitumoral activity against a variety of tumors. We have previously shown that this drug undergoesdemethylation and oxygenation reactions. In this paper, liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) was employed to assess the structures of unknown oxygenated metabolites of EAPB0503. EAPB0503 and its identified demethylated metabolites, EAPB0502 and EAPB0603, were incubated with human, rat, dog and mouse liver microsomes, as well as human, rat and dog hepatocytes. After separation on a C8 analytical column with a gradient elution of acetonitrile-formate buffer, positive ESI-MS/MS experiments were performed. To facilitate metabolite identification, the detailed fragmentation pathways of the parent compounds were first studied using high-resolution MS/MS. Additional hydrogen/deuterium exchange LC-MS/MS experimentswere used to support the identification and structural characterization of metabolites. Four hydroxylated metabolites were identified: M'4 and its demethylated derivative M'1 (OH in ortho position on the phenyl substituent in position 1), and M'6 and its demethylated derivative M'3 (OH on the imidazole ring at the C-2 position). Three phase II metabolites (Met A, EAPB0602 glucuronide; Met B, M'4 glucuronide; Met C, EAPB0603 glucuronide) were also evidenced. Elucidation of the metabolite structures was performed by comparing the chromatographic behaviors (changes in retention times), by measuring the molecular masses (mass increment), by studying the MS^2 spectral patterns of metabolites with those of parent drugs and for M'1 and M'4 by co-analysis with synthetic standards. The results of the present study provided important structural information relating to the metabolism of EAPB0503.



Phase 1 and phase 2 metabolites of the new anticancer agent: EAPB0503: LC-MS/MS analyses

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MPS06-30 / Determination of 2H-labeling of water in the interstitial fluid of rat brain using gas chromatography - quadrupole mass spectrometry

Anita Eberl[1], Reingard Raml[1], Denise Kollmann[2], Thomas Altendorfer-Kroath[1], Thomas Birngruber[1], Frank Sinner[1], Christoph Magnes[1]
[1]Joanneum Research - HEALTH, [2]Medical University of Graz

Heavy water (2H2O) can be used as a marker for the relative recovery of a cerebral open flow microperfusion (cOFM) probe. cOFM is a novel in vivo technique for continuous measurement of substance transport across the intact blood-brain barrier (BBB) (Birngruber et.al., Clin Exp Pharmacol Physiol 40: 864–871). 2H2O is well suited as marker substance, since it is a nonradioactive, stable compound and it does not show any adsorption effects on the surface of the probe. Additionally, it is possible to use 2H2O together with a study drug without interferences.

In the current study we present a fast and highly reproducible method to accurately measure 2H2O as a recovery marker for cOFM probes, using gas chromatography (GC) - quadrupole mass spectrometry (MS).

2H2O is injected intraperitoneally and it disperses uniformly into the whole body, whereas a heavy water content of up to 25 % of the total body water is nontoxic. Relative recovery was calculated by the ^{2}H-enrichment of cOFM samples versus the ^{2}H-enrichment of rat plasma at steady-state conditions. In the low concentration range, 2H2O behaves the same as H2O. Therefore the transport of 2H2O is limited only by the BBB permeability of water and the diffusion properties in the tissue. Conventionally, 2H-labelling of water is measured by converting the hydrogen of water to hydrogen and determining the 2H-labelling via isotope ratio mass spectrometry. In this study, 2H that is present in water was exchanged with hydrogen atoms, which are bound to acetone in a base-catalyzed reaction with KOH and acetone. After an incubation time of four hours, the 2H-labelling of acetone was directly measured from the headspace using GC-quadrupole MS under electron impact ionization. The small sample volume of only 5 µL and the lack of further sample preparation steps make this method a fast and hence highly competitive one. It was subjected to full validation to ensure reliability. 2H enriched to 3000 ppm in plasma showed accuracy values of 98% and a %RSD of 3.5 with n=8. A typical calibration curve was y =0.036x + 0.0038 with a coefficient of determination of 0.9994.

In summary, we were able to develop a fast and simple method using a conventional GC-quadrupole MS that yields highly reproducible results, allowing us to accurately measure 2H2O as a recovery marker for cOFM probes.

MPS06-31 / Quantitative Protein Measurement of Circulating Plasma Microparticles by Data-Independent nanoLC-MS2

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Introduction

Cells of the vascular system release a heterogeneous mixture of proteins, lipids, RNA, and cytoplasm components by way of spherical vesicles, e.g. microparticles (MPs) in the size range of 0.1-1µm. The shedding of MPs is induced by a variety of stress factors resulting in variable MP concentrations between health and disease. Furthermore, MPs have cell signaling and intercellular communication properties and interfere with inflammation and coagulation pathways. Therefore, MPs represent a repository of biological processes taking place in the vascular system. Today's most used analytical technology for MP characterization, flow cytometry, is lacking sensitivity and specificity. Our goal is the development of a highly reproducible, fast, and quantitative MP assay based on targeted data-independent (DIA) nanoLC-MS2.

Methods

MPs were isolated from 250µL of cell-free plasma and cleaned from abundant plasma proteins by repetitive centrifugation. MPs were morphologically controlled by cryo-TEM. MPs were in-solution digested with a combination of LysC and trypsin. In-solution digested MPs were subsequently analyzed with nanoLC-MS2 with data-dependent (DDA) or DIA acquistion on a QExactive instrument.

Results

MP pellets from 250µL plasma are invisible to the human eye. Staining of lipid membranes with sudan black enabled the visualization of MP pellets, as well as the training of the operator to avoid aspiration of those pellets during repetitive washing steps. Reproducible protein patterns on SDS-PAGE were achieved. MP preparations were characterized by cryo-TEM and showed the expected vesicle distribution.

MP protein profiles of 12 healthy volunteers were recorded by DDA nanoLC-MS2. A total of 981 proteins could be identified, with 916 detected in at least half and 668 in all samples (combined search results of MaxQuant and Easyprot at 1% PSM FDR). This data set is very rich in cellular, low abundant proteins, e.g. kinases, cell organelle and plasma membrane proteins. For instance, we detected 31 cluster of differentiation proteins enabling the generation of a cell profile of MP origin.

Despite reproducible protein patterns on SDS-PAGE, we encountered reproducibility problems of protein identification and quantification. High amounts of lipids might interfere during protein digestion. We therefore investigate on an improved digestion protocol aiming at CV's <25% for protein quantification. We also work on implementing a DIA method that allows for the "archiving" of each MP sample proteome for post-acquisition interrogation of peptide transitions from any target protein by Skyline.

Conclusions

The protein profiles of 12 healthy volunteers were very similar without differences between gender nor age. Removal of abundant plasma proteins is key for in-depth MP proteome analysis, e.g. targets like plasma membrane proteins, which mark MP origin. Intracellular proteins, e.g. from the cytoskeleton, can be used to quantify MP numbers, and levels of organelle proteins might point to causes of tissue damage.

Novel aspect

MP analysis by SRM like LC-MS2 instead of flow cytometry will improve the diagnostic use of MPs in vascular diseases.

MPS06-32 / Combining miniaturized Zebrafish bioactivity-guided fractionation with UHPLC-Orbitrap-MS and NMR dereplication for the early stage anticonvulsant's discovery

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Introduction

In natural product (NP) research, bioactive compounds are generally identified in complex natural extracts by bioactivity-guided fractionation. To improve the efficiency of the approach, bioassays can be coupled to HPLC or microfractionation method that can be directly correlated to LC-HRMS metabolite profiling for a rapid localisation and dereplication of the bioactive compounds. In this respect, a strategy has been developed for the rapid and efficient characterization of anticonvulsant NPs by combining an in-vivo behavioral zebrafish bioactive-guided fractionation, UHPLC Orbitrap-MS metabolite profiling and microNMR.

Methods

The approach is based on HRMS and MS/MS dereplication and monitoring of NP anticonvulsant compounds from traditional medicine plants.

The high resolution of an Orbitrap-MS and UHPLC separation were essential for a rapid and efficient detection and dereplication of complex spirostane-type steroidal saponins isomers that were found to occur at low concentration in the extract of interest. In parallel for the in-vivo biological profiling, the crude extract was fractionated in a 96 well plate and in each subjected to a particular zebrafish bioactivity assay to assess the activity of a single compound or a given zone of the chromatogram.

Results

HPLC microfractionation of the bioactive crude extract of Solanum torvum (a Solanaceae used traditionally against epilepsy), in combination with the in vivo zebrafish seizure assay, enabled the rapid localization of several bioactive compounds. These metabolites (steroid glycosides) were partially identified on-line by UHPLC-Orbitrap-MS but the presence of several isomers that could not been fully differentiated based on single HR-MS/MS involved NMR analyses to complete unambiguous identification. The high throughput post-column UHPLC-Orbitrap-MS monitoring enabled the precise localization of the active compounds among hundreds of fractions.

Conclusions

UHPLC-Orbitrap-MS in combination with HPLC biological profiling was found very efficient for the early identification of bioactive active compounds in complex plant extract and provide a rational explanation it's use to analyse traditional medicine plant that have potentially anticonvulsant properties.

Novel aspect

Bioactive-guided NP identification combining Zebrafish bioassay and UHPLC-Orbitrap-MS were found very efficient for the rapid determination of in vivo anticonvulsant properties of steroid glycosides at the microgram scale.

MPS06-33 / Metabolite monitoring in fed batch cell cultures using MALDI-TOF-MS

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Introduction

The analysis of intracellular metabolites is becoming an important task to routinely monitor biotechnological fed batch process reproducibility and performance. Nucleotide and amino acid concentrations perfectly document the fed batch culture performance. Traditionally, these metabolite levels are followed using liquid chromatography (LC) combined with UV detection. However the long LC-UV runtimes compromise the possibilities to regulate a running process via feedback. The MALDI-MS method presented here enormously reduces the time to analyze intracellular metabolites, and provides excellent robustness.

Methods

We used a commercial MALDI TOF instrument (5800, ABSciex, Germany) and a microarray sample target for mass spectrometry. The sample target consists of a coated and micro structured ITO glass slide. Hydrophilic spots on the otherwise omniphobic chip allow for fast, automated aliquoting and focusing of any organic solvent. Furthermore, a short extraction protocol was developed and used to monitor fed batch reactors.

Results

Adenosine-5´triphosphate was detected and quantified using an isotopcally labeled internal standard (13C15N-Adenosine-5´-triphosphate). The appearance of ATP is in good agreement with literature data. The di- and monophosphates of adenosine as well as guanisine, cytidine, uridine were also monitored. Any methodologically induced analyte fragmentation or hydrolysis was corrected for. Moreover, the recorded metabolite profiles were analyzed using statistical tools, e.g. principal component analysis.

Conclusions

The presented method has high-throughput capabilities and can be applied in an industrial environment. The detected metabolite profiles were cross-validated using HPLC-UV.

Novel Aspects

Development and implementation of a microarray for metabolite analysis of fed batch cultures using MALDI-MS. Fast and effective analyte extraction protocol combined with an analysis method including isotopically labeled internal standard.

MPS06-34 / Fast and simple sample preparation for ultra-fast screening of drugs in urine by LDTD-MS/MS

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Introduction

Toxicology laboratories generally use screening methods to obtain a semi-quantitative response for drug samples. Some screening techniques are fast but less specific and generate by far too many false positive results. Confirmation of those additional false positive samples is both time and cost consuming. The Laser Diode Thermal Desorption (LDTD) ion source coupled to a Mass Spectrometer offers specificity combined with an ultra-fast analysis for an unrivaled screening method.

Methods

The following 31 drugs were spiked in urine at 50, 100, 500 and 1000 ng/mL: Methamphetamine, Amphetamine, PCP, Imipramine, MDA, MDMA, MDEA, BZE, 6-AM, Codeine, Morphine, Hydrocodone, Hydromorphone, Oxycodone, Oxymorphone, 2-OH-ethylflurazepam, 7-aminoclonazepam, Alprazolam, Diazepam, Estazolam, OH-alprazolam, OH-triazolam, Lorazepam, Nordiazepam, Oxazepam, Temazepam, OH-midazolam, 7-aminoflunitrazepam, Chlordiazepoxide, Clonazepam and Flunitrazepam. The sample treatment include mix of 25 µL urine sample, 15 µL internal standard in methanol and 200 µL 0.1%TFA in acetonitrile. Samples are vortex and evaporated to dryness. 400 µL of a mixture of Hexane and Ethyl Acetate (1:1) is added to the vial and vortexes for sample reconstitution. After mixing, 4 µL are spotted onto the individual wells of the analyzing plate. Uses of buffer and b-glucuronidase addition are also evaluated with the same treatment.

Results

LDTD-MS/MS System operated in MRM mode allows rapid measurement of the all drugs desorbed simultaneously. Specific transitions are monitored for each drug to quantitate calibrator level. The APCI ionization is performed in positive mode. Analysis includes spiked drugs in urine, potentially interfering drug addition and 38 real samples. All compounds give linear response from 50 or 100 ng/mL to 1000 ng/mL. Potential cross reactivity is evaluated by monitoring all transitions while desorbing individual drug spiked at 1000 ng/mL. Cross reactivity between Codeine/Hydrocodone and Morphine/Hydromorphone are observed as they have same elemental composition and fragmentation pattern. For those isobaric compounds, confirmation method has to include separation. Drug concentrations in real sample were also evaluated in LC-MS/MS with a long gradient to separate each drug class. For each drug in real samples, correlation of the data generated by LDTD-MS/MS and LC-MS/MS, false positive and false negative results are evaluated. No false negative result was obtained for the drug analysis; however the benzodiazepine group requires b-glucuronide enzyme treatment for drug detection. This treatment generate chemical noise leading to some false positives results. Sample-to-sample run time of 9 seconds is achieved with the capability to simultaneously analyze 31 drugs.

Novel aspect

New sample preparation method for urine screening with Ultra High Throughput LDTD-MS/MS analysis

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MPS06-35 / Micro-Arrays for Mass Spectrometry (MAMS): Microarray Targets for Rapid Quantitative MALDI-MS

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Introduction

Novel micro-array targets were designed to improve quantification using MALDI-MS. The array is made of an omniphobic coated ITO/glass plate in the size of a microscope slide. Several hydrophilic spots in the micrometre size are arranged in parallel lanes (one lane per sample). Analyte samples are further not spotted to the target lanes, but automatically aliquoted over a lane in order to generate from each sample several identical replicates. Depending on the consistence of the sample, aliqouting is done by “dragging sample drops” in parallel over the lanes or just simply by a “rinsing-procedure” using a few microliters of the sample-matrix premix.

Methods

Microarray sample targets were fabricated using ITO glass slides (Sigma-Aldrich) and further coated with a layer of polysilazane (Clariant). Micro-array spots were then generated by scanning

pico-second laser ablation system. MALDI-MS analysis was carried out on an AB5800 MALDI-TOF-MS or on a Bruker solariX MALDI- FT-MS instrument. Aliquoting of aqueous samples was done using a metal sliding device.

Results
We tested our sample targets for simple peptide mixtures as well as for the quantification of antiretroviral drugs from complex matrices like lysates of peripheral blood mononuclear cells. Additional, we could show the usefulness of our targets for performing high-throughput metabolite analysis to monitor fed-batch cultures during their batch process. Furthermore, many samples like synthetic polymers, which have to be dissolved in highly organic solvent compositions, are difficult to pipette or to spot on conventional sample targets. Even with those samples, we could demonstrate the generation of homogeneous replicates, by using the MAMS aliqouting technique.

Conclusions
The q-MALDI micro-array targets allow an automatic generation of several identical replicates of one sample. Sample application for the whole chip is performed within seconds in a multiplexed style. Automatic aliquoting is possible for aqueous samples as well as for samples which are dissolved in highly organic solvents. Both, small spots as well as the increased number of replicates enable highly confident and accurate results using MALDI-MS.

Novel Aspects
We demonstrate a sample target with parallel lanes of microspots, in order to generate for each sample several identical replicates in the nanoliter volume range. This allows a confident and accurate quantification using MALDI-MS.

MPS06-36 / Screening Mycobacterium Tuberculosis Complex with Detonation Nanodiamond

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Introduction
Requests for direct diagnosis of mycobacterial disease are increasing. The detonation nanodiamond (DND) coupled with matrix-assisted laser desorption ionization (MALD-TOF) MS demonstrates the detection of early secreted cell filtrate protein 10 (CFP 10) from mycobacterium tuberculosis complex (MTBC) can reach the sensitivity, specificity, and positive and negative predictive values of 1.0, 1.0, 1.0, and 1.0, making it a suitable screening test for mycobacterial detection.

Methods
The DNDs were suspended in DI water at a concentration of 1 mg/mL. The DND solution was sonicated for 5 min before use. A 100 µL portion of DND solution (1 mg/mL) was put to 200µL filtered MGIT medium. After weakly vortexing for 60 min at room temperature, the protein-loaded DNDs were centrifuged at 13000 rpm for 3 min. The supernatant was removed, and the DNDs were additionally washed with DI water to remove residual contaminants. A 1.5 µL portion of saturated SA solvent was mixed with DNDs followed by depositing 0.8 µL of mixture solution on a plate, and then, the sample was analyzed by MALDI-TOF MS. The acquired mass spectra were analyzed by program. The frequency, intensity and m/z peaks were reported without prejudice.

Results
A total of 55 specimens from Buddhist Tzu Chi General Hospital (TCH) showed positive signals reported by the BACTEC MGIT 960 system, followed by DND MALDI-TOF MS analysis. Among the specimens identified by culture and biochemical methods, a total of 55 specimens were reported to contain MTBC strains. Results from DND MALDI-TOF MS showed that the sensitivity is 100%, the negative predictive value is 100%, the specificity is 100%, and the positive predictive value is 100%. The frequency and intensity of m/z 10662.1±8.1 is 100% and 100%. The frequency and intensity of m/z 5329.6±6.8 are 100% and 30%. Those two peaks are the cell filtrate protein 10 (CFP 10) and doubly charged peak of CFP 10. It is noted that the frequency of m/z 10108.9 and 5043 are 82% and 89% which may be used as markers.

Conclusions
This study has shown that a combination of 5 nm detonation NDs and MALDI-TOF MS analysis can easily detect secretory CFP-10 protein of MTBC from MGIT liquid culture medium. The early secreted CFP-10 protein can be used as a biomarker to differentiate MTBC from NTM. In a clinical trial, sensitivity, negative predictive value, specificity and positive predictive value can reach 100% with both m/z peaks at 10662.1 and 5329.6.

Novel Aspect
CFP10 marker and its doubly charge peak can be used to distinguish the MTBC

MPS06-37 / Evaluation of a Novel 96-well Filter Plate for the Effective Removal of Serum Protein and Phospholipids prior to LC-MS/MS Analysis

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Introduction
Extract cleanliness is very important when using LC-MS/MS analysis. When analyzing serum or plasma endogenous components such as salts, proteins and phospholipids are all present and can have a marked effect on instrument response in terms of ion suppression or enhancement effects. This variation in signal can lead to quantitation issues and method reliability problems. This poster evaluates the performance of a novel 96-well filter plate for the simultaneous removal of proteins and phospholipids prior to LC-MS/MS analysis.

Methodology
Protein removal was compared using various matrix:acetonitrile crash ratios from 1:1 to 1:6. Electrophoresis was performed using a NuPAGE Novex 12% Bis-Tris mini gel with MOPS SDS running buffer. Phospholipids and recovery and signal intensity for a variety of acidic, basic and neutral analytes were analyzed using a Waters 2795 liquid handling system coupled to a Quattro Ultima Pt triple quadrupole mass spectrometer. Analyte monitoring involved positive ion acquisition using electrospray ionisation in the MRM mode. Phospholipid analysis involved monitoring MRMs of the most intense phospholipid ions (selected from full scan, SIR and precursor ion monitoring), fragmenting to the common 184 product ion.

Results
Comparison of crash ratio demonstrated improved protein removal from the sample when using a 1:3 matrix:ACN ratio compared to 1:1 or 1:2. A rough protein quant of these samples (n=1) showed protein removal of approximately 94% for a

1:1 crash ratio but greater than 99% for a 1:3 ratio. Increasing the ratio to 1:6 did not improve protein removal significantly. Protein precipitated plasma samples were used as a benchmark to evaluate the degree of phospholipid removal. Matrix crash ratios of 1:3 and 1:4 were evaluated using ACN and 1% formic acid in ACN. 100 µL of plasma using both a 1:3 and 1:4 crash ratio demonstrated phospholipid free extracts. UPLC pressure response and metoprolol peak areas were then compared injecting 300 samples using this phospholipid depletion plates to a standard protein precipitation filter plate. The pressure trace and signal remained stable and horizontal for the phospholipid depletion plate. However, large pressure increases were observed for less than 100 samples from the protein precipitation plates. Recovery experiments for a variety of analytes with varying logP and pKa values demonstrated recoveries greater than 60%. These values are comparable to those generally obtained using these sample preparation devices. However, the overall signal responses obtained were much higher than those seen using other phospholipid removal plates.

Conclusion
Excellent removal of proteins and phospholipids were observed while demonstrating higher signal intensities and signal to noise compared to other phospholipid removal plates.

Novel Aspect
The evaluation of a novel 96-well filter plate for the elimination of proteins and phospholipids from biological matrices.

MPS06-38 / An analytical method (UHPLC-MS/MS) to determine the pharmacodynamic behaviour of the topically applied antiviral drug acyclovir

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Assessing the pharmacokinetics (PK) of topical drugs at their site-of-action in human skin is a challenging task. Usually only small amounts of the applied drug cross the first effective skin barrier, the stratum corneum, before reaching its intended site-of-action, the dermis. To investigate PK in human dermis in vivo, we have developed a minimally invasive, membrane-free sampling method called open flow microperfusion (OFM) that provides continuous access to the dermal interstitium. OFM is CE-certified for human use and is currently applied in preclinical and clinical studies. With a flow rate of 1 µl/min OFM provides diluted interstitial fluid for biochemical analysis. To exploit the information present in OFM samples, highly sensitive analytical methods are needed which can be used to analyse just a few microliter of sample and which are robust enough to be used in the highly regulated environment of clinical studies (Good Laboratory Practice). We report a sensitive and fully validated method to determine acyclovir in serum and OFM samples.
Acyclovir has been previously investigated to evaluate skin penetration of a topically applied cream in healthy volunteers using microdialysis sampling. It was not possible to detect acyclovir in these samples without pre-treatment of the skin. For sample preparation we used mixed mode (cation-exchange, reversed phase) µ-elution solid phase extraction. Different washing and elution solvents were tested to give maximum recovery of the analyte. The measurements were performed with reversed phase UHPLC- high resolution mass spectrometry in SRM mode. Acyclovir was determined in a range is between 0.1-100 ng/ml with an accuracy between 85%-115% and a precision <15%.
The developed method for acyclovir determination in serum and OFM samples offers improved limit of detection compared to previously applied techniques and is optimized for analysing OFM samples. It will be used in future experiments to investigate the pharmacodynamic behaviour of different acyclovir formulations in healthy human skin.

MPS06-39 / On tissue characterization of amyloidosis using MALDI mass spectrometry

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Introduction
Amyloidosis is a protein misfolding disorder in which soluble proteins aggregate as insoluble amyloid fibrils. Protein aggregates and amyloid fibrils cause functional and structural organ damage. Definitive diagnosis of amyloidosis is established by the demonstration of apple-green birefringence deposition with Congo red under polarized light microscopy on a biopsy sample of an involved organ (salivary glands, myocardium). Several proteins as light chain (AL), mutated or wild-type transthyretin (mTTR/ wtTTR) can be involved. Immunohistochemistry and genetic analysis is necessary to determine the exact nature of the misfolded protein. TTR, a homotetrameric 55KD protein from liver origin, constituted with 127 amino acids, is the most frequent protein encountered in cardiac amyloidosis. Our aim is to directly identify the nature of the major protein involved in the amyloid deposition, on paraffin included myocardial samples.

Methods
FFPE heart biopsies identified with amyloidosis by the gold standard method were used for the studies. Tissue sections of 12 µm were applied onto ITO (Indium Teen Oxided) coated conductive glass slides. Paraffin was removed by 3 baths of 5 minutes of clearify and lightly rehydrated with graded ethanol (100%, 95%, and 70°) before drying under desiccator. Antigen retrieval was performed before mass spectrometry analysis. Digestion was performed using trypsin ‘0.1 µg/µmol in 25mM ammonium bicarbonate for 1h30 hours at 37 degrees CHCCA (alpha cyano 4 hydroxycinamic acid was manually deposited on the tissue. MALDI mass spectrometry analysis were performed directly on tissue using a MALDI QIT TOF instrument (Shimadzu, Manchester, UK) or the MALDI 7090 TOF-TOF mass spectromer. MS and MS/MS analysis on the 7090 and MS and MSn analysis on the QIT-TOF instrument.

Results
MALDI profiles of heart tissue samples were obtained after tryptic digestion of the tissues. Major peaks were selected and then submitted to MS/MS on both 7090 instrument and resonance instrument. When needed MS3 was also performed on the MALDI QIT. Using these procedures cardiac actin and myosin were first characterized confirming the interest of such an analysis. The identification of thransthyretin using MS/MS analysis allows the characterization of amyloidosis directly from tissue sample.

Conclusions
A quick MS/MS analyses on tissue using high end MALDI TOF-TOF mass spectrometers allow the typing of amyloidosis. This result has clinical significance and could open the way for the use of MALDI for amyloidosis testing in hospitals.

Novel aspect
Direct typing of amyloidosis from FFPE tissue samples by in situ

MALDI analyses
First use of the new 7090 instrument for characterization of proteins in tissue
MS3 directly on tissue

MPS06-40 / An alternative approach for the analysis in clinical practice: determination of amphetamine and methadone in human urine by direct MEPS-MS/MS analysis

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Introduction
A speed and low cost of an analysis together with the regulatory approval are the most important requirements in clinical practice. Microextraction by packed sorbent (MEPS) may be considered an option for the optimal sample preparation technique due to easy automation, minimal requirements for the sample and elution solvent volumes, elimination of evaporation and reconstitution steps and possibility of coupling of sample preparation and injection into one step. The use of effective sample preparation step enabled for the elimination of chromatographic separation and thus a faster and cheaper method development and analysis of samples in clinical practice. The aim of this work was to develop and validate fast, sensitive and economically accessible method for the quantitative determination of amphetamine and methadone in urine samples using the microextraction by packed sorbent coupled directly with tandem mass spectrometry.

Method
The MS/MS triple quadrupole system with electrospray ionization operated in positive ion mode was used in this study. Quantification of all analytes was performed using selected reaction monitoring and deuterium labeled internal standards (amphetamine-d10 and methadone-d9). Suitable MS/MS conditions for the determination of amphetamine and methadone were found:capillary voltage 2500 V, extractor 1.0 V, RF lens 0.5 V, ion source temperature 120 °C and desolvation temperature 250 °C. The C8 MEPS sorbent and 100 µl of sample containing internal standardswas used for the extraction. The washing step was performed by 200 µl of water and 100 µl of 5% methanol. The analytes were eluted with 50 µl of 0.1% formic acid in methanol. The eluate was injected into MS/MS directly from the MEPS syringe.

Results
Developed MEPS-MS/MS method was validated. Standard and matrix calibration curves demonstrated good linearity (r2 > 0.997). Matrix calibration curves were linear in the range of 20 – 5000 ng/ml for amphetamine and 5 – 5000 ng/ml for methadone. Other validation data were as follows: method recovery (92 – 106%), method accuracy (88 - 109%), method precision (lower than 11%) and matrix effects (85 – 114%).

Conclusions
On-line MEPS-MS/MS method for the determination of amphetamine and methadone was developed. Due to meet validation requirements and advantages of MEPS such as the integration of sample preparation and injection steps into the one step, minimization of sample and elution volumes, easier automation and cheaper analysis, the MEPS-MS/MS method was found to be an appropriate combination for the analysis of huge number of samples in routine clinical laboratory.

Novel aspect
The work introduced an alternative approach suitable for the clinical bioanalysis, in which the combination of MEPS and mass spectrometry were employed without chromatographic separation.

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MPS06-41 / Strategies for development of high sensitive quantitative analysis

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Introduction
Nano and micro LC/MS have been applied routinely in the proteomic field, mainly for qualitative or relative quantitative analysis. In a bioanalytical environment – with a primary focus on absolute quantitation – accuracy, reproducibility, robustness and high throughput have been emphasized for many years. As a consequence, most bioanalytical labs still traditionally rely on LC methods with 2.1 mm ID columns (or even 4.6 mm ID columns). However with increased demand on added value, new concepts need to be introduced for robust quantification of much lower concentrations of analytes in biological matrices. Several approaches can be followed such as downscaling towards microLC to improve intrinsic sensitivity or 2D LC with trapping to increase loadability. However, throughput and adherence to quality criteria need to be safeguarded

Methods
An LCMSMS assay for quantification of pg amounts in plasma for midazolam was developed. MicroLC on a Eksigent microLC200 system was introduced in combination with a selective liquid-liquid extraction. A Halo C18 0.5 x 50 mm column (2.7 µ) was used at a flow rate of 50 µl/min. Also MRM3 on a API6500 was applied to improve both selectivity and sensitivity.
Whether this can be achieved with 2D LCMS (using a 1x50mm analytical column and a trapping column of 2.1x5 mm leaving out the liquid-liquid extraction, will be evaluated.

Results
For the midazolam assay, the approach with liquid-liquid extraction and microLCMS an LLOQ of 0.5 pg/mL was obtained. Other strategies (2D LC) still need to be executed to compare the performance.

Conclusions
An assay for quantitative analysis of low concentrations of midazolam in human plasma has been developed with a microLC approach. Alternative strategies can be envisaged to obtain similar sensitivities and performance but still need to be initiated at this moment.

MPS06-42 / Biotransformation of tyrosine kinase inhibitor - imatinib by high resolution mass spectrometry

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Introduction
Therapeutic drug monitoring is widely applied useful tool for treatment individualization in order to achieve optimal clinical response and avoid toxicity. Due to drug metabolizing enzymes

several bioactive or toxic metabolites are produced. Therefore determination of not only parent drug but also of metabolites may have clinical relevance and could be helpful for treatment adjustment. The aim of the work was profiling of imatinib metabolites in plasma of patients with chronic myeloid leukemia.

Experimental
Plasma samples taken from patients on treatment by imatinib (24h after dose of 400mg) were isolated by centrifugation of anticoagulated blood in EDTA tubes. Separation of metabolies was performed on Phenomenex Kinetex C18 column (100 x 2.1 mm; 1.7 µm) using UltiMate 3000 RS (Thermo Scientific) liquid chromatography. For detection Orbitrap Elite mass spectrometer (Thermo Scientific) based on exact mass measurement was used. Scan range of m/z 350 – 1200 was chosen and the resolution was set at 60,000 FWHM. MS/MS data were acquired in data-dependent strategy based on fragmentation of 25 selected exact m/z values of potential metabolites. Collision energy for CID was set at 35 eV. Dynamic exclusion duration of 3 s was enabled; mass accuracy was 5 ppm. Data were evaluated using Excalibur 2.2 SP1, MetWorks 1.3 SP3 and Mass Frontier 7.0 software.

Results
In plasma samples more than 90 potential metabolites in concentration range of 4 orders of magnitude were found. All metabolites were identified by exact m/z values and confirmed by MS2 and MS3 exact m/z fragmentation experiments. Due to complex structure of Imatinib fragmentations and electrochemical conversions in electrospray ion source were present. These phenomenones complicated interpretation of results and have to be involved for accurate identification of metabolites. Changes in profiles of several metabolisations between patients were followed.

Conclusion
High Resolution Mass spectrometry using Orbitrap principle is able to detect and confirm high as well as low intensive metabolites and its fragments. Tyrosine kinase inhibitor – Imatinib offers complex metabolite profile. It has diagnostic potential for individualized treatment of patients with adverse effects or resistance on treatment.

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Novel Aspect
The approach is able to detect and confirm identity of tens of metabolites by measuring high resolution scan and MS2 spectra.

MPS06-43 / Identification of a Novel Metabolite for The HIV treatment Tenofovir Disoproxil with LC-MS/MS

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Tenofovir Disoproxil Fumarate (TDF) is a nucleotide analogue reverse transcriptase inhibitor; the active form is tenofovir diphosphate. Whilst developing a rapid LC-MS/MS method to quantify systemic levels of the HIV drug treatments TDF, Emtricitabine and Efavirenz in human plasma a novel metabolite of TDF, not previously reported in humans was detected. The method was based on reversed-phase (RP) liquid chromatography (LC), on a Waters Acquity UPLC using a 2.1 mm x 100 mm HSS T3 Acquity column with detection via Multiple Reaction Monitoring (MRM) using a Waters XevoTQ-S tandem quadrupole mass spectrometer. Accurate mass experiments were performed on a Waters Q-ToF Synapt G2-S mass spectrometer.
Plasma samples were obtained from patients with a low viral load dose and analysed as below:
Mobile phases: A: water + 0.1% formic acid (FA), B: acetonitrile + 0.1% FA. Gradient: initial: 0.5% B, increasing linearly to 25% B over 1.3 minutes, increased to 80% B over 0.1 minute, held at 80% B until 2.2 minutes, increased to 95% B over 0.1 minute and held for 0.7 minutes, decreasing to 0.5% B over 0.1 minutes and held until 4 minutes to reequilibrate. Column temperature: 40°C, flow rate: 0.4 ml/min.
Transitions were measured in positive-ion mode with two recorded for each drug (quantitation and confirmation):
Tenofovir (protonated): 288.05-159.06 and 288.05-136.03
Emtricitabine (sodiated): 269.98 -151.97 and 269.98-140.98
Efavirenz (protonated): 315.999-244.02 and 315.999-168.07
When examining the tenofovir transitions (at retention time (RT) 0.94 minutes) an additional peak was seen eluting at a later RT, however, only in the early time point patient samples. It was hypothesised that a metabolite of TDF fragmented within the source of the mass spectrometer to form tenofovir, which was then detected and isolated by the first quadrupole and then fragmented, thus recording a response for the tenofovir transitions. Experiments were devised to identify the species.
Parent ion scans were performed on the fragments used for the tenofovir transitions, using the same chromatography conditions employed for the quantitative analysis; and the spectrum underneath the RTs observed in the original experiments were evaluated. Standards of tenofovir and TDF were obtained and MS/MS experiments were performed on a high mass accuracy MS. TDF and tenofovir were separately placed into blank plasma samples at 37°C and analysed with the LC-MS/MS method at set time intervals after addition. TDF gave a strong signal for the tenofovir transitions at a RT of 1.81 minutes. The novel metabolite produced a signal at a RT of 1.52 minutes. The signal of TDF decreased over time as the signal for the novel metabolite increased. The signal for tenofovir in plasma did not change over the course of the experiment.
A novel metabolite of TDF, previously not observed in humans, has been identified and disrupts the currently established mode of metabolism.

MPS06-44 / Benefits of High Resolution and Accurate Mass Instrument for therapeutic monitoring of baclofen and its metabolites in plasma and urine.

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Introduction
Baclofen (Lioresal ®) is a centrally acting muscle relaxant, prescribed in France for more than 40 years for the treatment of cerebral spasticity. It has recently attracted considerable attention as a potential medication for alcoholism. Currently, psychiatrists in Cochin center treat alcohol-dependent patients using high doses of baclofen (from 50 to 450 mg/day). It is therefore of interest to explain the variability in efficient dose observed in patients by monitoring baclofen and its major metabolites in plasma (desaminated and glucuronide metabolites). We describe a method to evaluate plasma and urinary concentrations of baclofen and a semi-quantitative evaluation of the metabolic ratio of such metabolites by liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). Tests were initially carried out on samples collected from patients treated in context of alcohol withdrawal and/or in cases of overdose for purposes of forensic toxicology.

Methods
Plasma and urine samples from patients were prepared using the following procedure: to 100 µL of plasma (or 100 µL of urine diluted 1/100), 10 µl baclofen-d4 (1 µg/mL) was added and then precipitated with acetonitrile. The organic layer was evaporated and suspended with 100 µL 80/20, water/acetonitrile (v/v, %). Chromatographic separation was achieved using an Accucore PFP column (100 x 2.1 mm , 2.6 µm, Thermo ScientificTM) using gradient elution with a mobile phase consisting of water and acetonitirile (both containing 0.1 % formic acid). Mass spectrometric analysis was performed on an Exactive Plus (Thermo ScientificTM) using electrospray ionization. Data were acquired in full scan in both positive and negative mode.

Results
The metabolites were not commercially available and for this reason, they were identified and confirmed using exact mass of the analytes and their fragment ions with a 5 ppm mass accuracy. The method was successfully validated for the quantitation of baclofen in urine and plasma with linearity going from 10 ng/mL to 2000 ng/mL. Precision and accuracy were evaluated using QC samples (n=3) and were below 15 %. The total extraction recovery was above 90 %. Baclofen concentrations in plasma varied from 24 to 1039 ng/mL. Desaminated and hydroxylated metabolites were detected in both urine and plasma (metabolic ratios measured at 35.10% and 8.55 % respectively). Baclofen glucuronide in urine was detected only in patients with elevated plasma concentrations (with a ratio in urine of 0.13%).

Conclusion
We developed a new validated LC-HRMS method allow the quantification of baclofen in plasma and simultaneously the monitoring of its phase I and II metabolites. Purpose of this method is to study the dose variability observed for patients receiving treatment in alcohol addiction.

Novel aspect
High resolution and Accurate mass instrument allow simultaneous identification and quantitation of baclofen and its metabolites within a single run.

MPS06-45 / A Validated High-throughput Assay for the Quantification of Amino Acids in Metabolic Phenotyping Studies

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Introduction
Ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) has shown great potential in metabolite phenotyping due to its sensitivity, reproducibility and speed. Metabolic phenotyping has largely relied upon global profiling techniques, offering the potential discovery of novel biomarkers. Targeted approaches, however, offer a more specific, sensitive and quantitative measure for selected compounds. Here we present a targeted UPLC-MS/MS method for the quantification of amino acids validated to support large-scale epidemiological studies. Current amino acid assays are mainly centred on optical detection, which necessitate complete resolution of all analytes for accurate quantification. With the specificity offered by tandem quadrupole MS/MS, a fast and robust method for high-throughput analysis has been developed.

Methods
The method employs derivatisation with 6-aminoquinolyl-n-hydroxysuccinimidyl carbamate followed by reversed-phase LC-MS/MS analysis. A three-day assay validation was performed to assess the precision and accuracy of the method and the robustness of the system for large-scale analyses.

Results
The development of a high-throughput, sensitive and reproducible method for the targeted analysis of amino acids in plasma/serum and urine is presented. Chemical derivatisation was performed to enhance reversed-phase chromatographic behaviour, increase the sensitivity and selectivity of detection and enable accurate quantification. The combination of derivatisation and reversed-phase chromatography allows reproducible separation of 35 amino acids in 6 minutes, significantly faster than current methods, including baseline resolution of critical isomeric pairs. Whilst optimised for the analysis of physiological amino acids, this assay also permits the detection of primary and secondary amine compounds through the generation of a characteristic fragment (m/z 171) from the derivatised molecular ion. Absolute quantification using LC-MS/MS operated in selected reaction monitoring (SRM) mode is demonstrated for the 20 coded amino acids with the use of stable isotope labelled internal standards.
Once optimized, a three-day validation assessing precision and accuracy was performed with three QC levels for 20 analytes. The method has been shown to be applicable to various biofluids including plasma, serum and urine from human and rodent sources. Stable isotope labelled standards were used to assess potential ion suppression/enhancement caused by the various matrices.

Conclusions
A LC-MS/MS assay has been validated to FDA accepted criteria indicating that it is robust, accurate and precise and suitable for large-scale epidemiological studies. The method was applied to a toxicology study, where consistent results were delivered for amino acid levels in biological fluids.

Novel Aspect
Development of a fast and reproducible UPLC-MS/MS method enabling high-throughput quantification of amino acids in large-scale epidemiological studies.

MPS06-46 / The Establishment of a General ESI-MS/MS Behavior of a Series of Antineoplastic Curcumin Analogues & The Formation of Unique [M-H]+ Ions During Photoionization

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Introduction
Curcumin analogues are novel antineoplastic agents designed by structural modifications of the natural product curcumin to improve potency and bioavailability. Tested curcumin analogues are 3,5-bis(benzylidene)-4-piperidones with various substituents on the aryl groups and the nitrogen atom. In this work, the ionization behavior of 14 compounds was investigated using various ionization techniques. In addition, the collision induced dissociation patterns of tested compounds were established to confirm their molecular structure and identify their diagnostic product ions for future qualitative/quantitative applications.

Methods
For single-stage MS analysis, samples were analyzed using various MS instruments equipped with different ionization techniques (ESI, APCI, MALDI & APPI). For tandem MS analysis, samples were dissolved in acetonitrile and injected into ESI-Qq-LIT-MS (4000 QTRAP) instrument using collision energy of 20-35 V and nitrogen as a collision gas.

Results
Single-stage MS analysis: curcumin analogues showed unusual ionization behavior during the positive ion mode MALDI- MS and dopant free APPI-MS in which a significant ion of [M-H]+ was observed along with the expected protonated [M+H]+ species. In contrast, [M+H]+ was only formed during ESI, APCI and dopant-APPI-MS. Both ions [M-H]+ and [M+H]+ were detected during MALDI-MS analysis even in the absence of matrix and/or solvent.
MS/MS analysis: curcumin analogues showed similar MS/MS dissociation behavior as the fragmentation was centerd on the piperidone ring of the 3,5-bis(benzylidene)-4-piperidone moiety. The presence of different substitutes on that moiety resulted in specific product ions for each curcumin analogue. Common fragment ions were identified allowing for the establishment of general MS/MS behavior. On the other hand, the side chain of curcumin analogues was fragmented in a unique fashion due to structural differences within the side chain. The fragmentation routes and the genesis of the product ions was confirmed via MS3 and neutral loss scans.

Conclusion
The unusual ionization behavior of curcumin analogues was related to the photon energy used during MALDI and dopant free-APPI-MS analysis. Two proposed mechanisms are probably involved in the [M-H]+ formation; (i) hydrogen transfer from the analyte radical cation (ii) hydride abstraction. The fragmentation of curcumin analogues followed the same pattern by breaking the piperidone ring resulting in the establishment of a general fragmentation pattern for all curcumin analogies. Unique product ions were also identified

Novel aspects
The formation of [M-H]+ ions was investigated; factors that contributed to such ion formation were identified. In addition, MS/MS behaviors of these novel compounds were established for the first time. This work is instrumental for future pharmacokinetic and metabolic studies of the tested antineoplastic agents

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MPS06-47 / Two dimensional capillary electrophoresis coupled with tandem mass spectrometry for determination of varenicline in urine matrices
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Introduction
Varenicline is a α2β4 nicotinic acetylcholine receptors partial agonist used in the treatment of the nicotine addiction.

Methods
A capillary electrophoresis analyzer EA-102 (Villa Labeco, Spišská Nová Ves, Slovakia), assembled in the CITP-CZE column-coupling configuration, was used in the work. CITP column = PTFE capillary tube, 800 µm I.D., 90 mm total length. CZE column = PTFE capillary, 300 µm I.D., 160 mm total length. Detection was performed on a triple quadrupole mass spectrometer (QqQ) Agilent 6410 Series Triple Quadrupole (Agilent Technologies, Santa Clara, CA, USA), operated with an ESI interface in the positive ionization mode. The hydrodynamically closed 2D-CE system was coupled with QqQ via an elution block with a short capillary transfer line (fused silica capillary, 75 µm I.D.). The transfer of separated zones from CE toward MS was performed using a spray liquid. The spray liquid was delivered to the elution block by syringe pump.

Results
The separation and performance parameters of the presented method were optimized with respect to achive the maximum detection signal and reproducibility of the peak area of varenicline. The optimum CITP-CZE conditions were: CITP – LE = 10 mM NH4Ac + 20 mM HAc (pH = 4,5), TE = 10 mM HAc (pH = 3,1), driving current 300 µA; CZE – BGE = 10 mM HAc (pH = 3,1), driving current 40 µA. The optimum composition of the spray liquid was methanol/water (50/50, v/v) with 0,1% (v/v) HAc and its flow rate was 2 µL.min-1. The optimized ESI-MS/MS parameters were: nebulizer pressure (15 psi); drying gas temperature (300°C); drying gas flow rate (5 L.min-1); capillary voltage (5000 V); fragmentor voltage (160 V); collision energy (18 eV). The performance parameters of the optimized method were evaluated for varenicline standard present in model water and model urine matrices. The MRM mode with the following transitions for varenicline was applied: m/z 212.1 → 169.1 (quantifier) and 212.1 → 183.0 (identifier). The optimized and approved method based on CITP-CZE-ESI-QqQ hyphenation was applied for the monitoring of varenicline concentration levels in human urine samples obtained in different time after oral administration of one tablet of the commercial drug Champix® to the healthy volunteer. The method was applied also for the monitoring of potential metabolic products of varenicline in human urine.

Conclusions
The presented CITP-CZE separation technique on-line coupled with ESI-MS/MS is a powerful tool for the analysis of unpretreated or diluted biological samples containing ultratrace concentration levels of ionic drugs and their ionic metabolic products.

Novel Aspect
This work demonstrated an analytical potential of new hyphenated method for the analysis of varenicline and its metabolite (2-hydroxyvarenicline) in unpretreated (diluted) biological matrix – urine.

Acknowledgements
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MPS06-48 / Study of UPF peptide-polyphenol conjugation by mass spectrometry
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Introduction
During the process of protection against oxidative stress, polyphenols are oxidized into reactive species. Reduced glutathione (GSH) is the major intracellular antioxidant in mammalian cells. It also eliminates the oxidation products of polyphenols by formation of peptide conjugates. Decrease in GSH levels and high grade oxidative stress are related to development of several pathological states including cardiovascular and neurodegenerative diseases, cancer and diabetes. The administration of GSH has limited effect due to rapid extracellular degradation and low cellular uptake. Therefore, novel non-toxic glutathione analogues with enhanced antioxidant activity have been designed. They are called UPF peptides. The aim of the present research was to investigate the conjugation of four novel glutathione analogues (UPF1, UPF17, UPF50 and UPF51) to selected polyphenols (quercetin, rutin, kaempferol, luteolin, apigenin, naringin and resveratrol) by different MS methods.

Methods
For the determination of these conjugates, sample preparation for conjugate formation and HPLC - ESI- MS and MALDI MS methods for conjugate detection were developed. Furthermore, the effect of GSH and UPF peptides on quercetin consumption on enzymatic oxidation with horseradish peroxidase (HRP) was determined spectrophotometrically.

Results
HPLC - ESI-MS and MALDI MS analysis showed the formation of glutathione and UPF peptide conjugates with flavonoids quercetin, rutin, kaempferol and luteolin. The mechanism of the UPF conjugation with polyphenols is analogous to glutathione. In addition, the formation of hydrated forms and bis-conjugates by enzymatic catalysis was observed. HPLC-MS analysis enabled the separation of hydrated forms and isomers, while more rapid detection was achieved with MALDI method. Determination of quercetin consumption showed, that UPF50 and UPF51 peptides inhibit HRP.

Conclusions
HPLC-ESI-MS and MALDI-MS are suitable methods for investigation of covalent interaction between UPF peptides and selected polyphenols.

Novel aspect
The formation of conjugates with novel UPF peptides

MPS06-49 / Abberant Glyosylation in Skin Tissue of Atopic Syndrome Patients

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Novel aspect
The first study to perform comprehensive, isomer-specific chromatographic profiling of human skin (stratum corneum) glycome by nano LC/MS

Introduction
Abnormalities of glycosylation are related to differential expressions of enzymes such as glycosyltransferases or glycosidases, and have been found in many human afflictions including autoimmune diseases and cancers. Therefore, the glycome has served as a valuable source of biomarkers. In this study we used nano-LC mass spectrometry to analyze and compare N-glycome of stratum corneum (outer-most layer of the skin tissue) of patients with atopic syndrome (n=30) and healthy control individuals (n=30). Glycans were comprehensively profiled and the changes in glycosylation were studied from individual samples.

Method
The samples were collected by stamping CuDerm tape discs 50 times at the inner arm flexures of each individual. Two tapes/tubes per individual were collected- one from the right inner flexure and the other from the left inner flexure. Proteins were extracted by submerging the tapes in phosphate buffered saline, subjected them to freeze thaw cycles and finally sonicating them. N-glycans were enzymatically released by peptide N-glycosidase F (PNGase F) from skin glycoproteins and enriched by graphitized carbon solid-phase extraction. The enriched glycans were quantitatively profiled by porous graphitized carbon nano-LC/MS. T-tests were used to identify significant differences between atopic syndrome cases and healthy controls.

Results
Human skin glycome were comprehensively characterized with trace amounts of tissue corresponding to 13µg proteins. In general, most glycans found in skin tissues in both control and patient group contain abundant high mannose type N-glycans and core glycan (Man3GlcNAc2). In addition, we found that high mannose type N-glycans are decreased in patient group while undecorated complex type N-glycans are decreased in control group.

Conclusions
Up to now human skin glycome profiling by nano-LC/MS has not yet reported. In this study, Based on qualitative and quantitative human skin glycome profiling, human skin samples (stratum corneum) from atopic syndrome patients (n=30) and healthy control individuals (n=30) revealed altered glycosylation trends. These findings may lead to the discovery of biomarkers for atopic syndrome and further functional analysis will provide a better understanding of the biological mechanisms of human skin tissue.

MPS06-50 / Characterization of phenolic compounds in Lycopus europaeus L. by HPLC-DAD-ESI-QTOF

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Introduction
Lycopus europaeus (commonly known as gypsywort), family Lamiaceae, has been used in folk medicine for many years for the treatment of hyperthyroid symptoms.It shows also antistaphylococcal activity and therefore represents a promising antibacterial agent. These effects are attributed to the presence of polyphenolic compounds. For further pharmaceutical use of L. europaeus, it´s important to identify and quantify compounds which are responsible for the above mentioned effects. It´s why the aim of our study was to investigate the composition and antimicrobial activity of water extracts of L. europaeus.

Methods
L. europaeus plants from the garden of medicinal plants (GMP) in Bratislava, Slovakia and from the slovak region Čertov (Č) were used. Lyophilised water infusion of dried leaves was used for the analysis.
HPLC (1260 Infinity) coupled to QTOF (6520) (Agilent, USA) was used. Analysis were carried out on a Hypersil BDS-C18 column (4.0×250mm, 5µm, Agilent Technologies, Germany) at 35°C and a flow rate of 0,4 mL/min. Gradient elution of water (pH 2.8 with HOAc) and MeCN was used.
Standards of caffeic acid, rosmarinic acid and luteolin-7-O-glucoside were purchased from Sigma-Aldrich (Germany).
Phenolic acids were quantified as rosmarinic acid (320 nm) and flavonoids as luteolin-7-O-glucoside (280 nm).

Results
4 flavonoids (quercetin-7-O-glucuronide, luteolin-7-O-glucuronide, apigenin-7-O-glucuronide, luteolin-7-O-glucoside), 6 phenolic acids (glucopyranosyl rosmarinic acid, caffeic acid, lithospermic acid, sagerinic acid, rosmarinic acid and salvianolic acid K) and 1 aldehyde (protocatechuic aldehyde) were identified according to their fragmentation mass spectra. Glucopyranosyl rosmarinic acid with [M-H] at 521,1357 and sagerinic acid [M-H] at 719,1638 haven´t been found in the genus Lycopus yet.
Rosmarinic acid (76,29 µg/mg – GMP resp. 52,65 µg/mg – Č) and luteolin-7-O-glucuronide (23,24 µg/mg – GMP resp. 29,32 µg/mg – Č) were the two most abundant compounds in both L. europaeus samples. Their content was from 10 to 25 times bigger than the content of other polyphenolic compounds. Analyses of

two different Lycopus samples showed their similar qualitative and quantitative composition.
Both extracts showed antistaphylococcal and antioxidative properties.

Conclusions
11 polyphenolic compounds were identified and 7 of them quantified. Rosmarinic acid and luteolin-7-O-glucuronide were the two most abundant compounds and seem to be responsible for the antimicrobial effect. Extracts of L. europaeus could be potentially used as antimicrobial agents in local applications.

Novel aspect
New phenolic profiles of L. europaeus were established. Two compounds were newly reported in genus Lycopus. A new potential antimicrobial agent was characterized.

Acknowledgments
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MPS06-51 / Multilevel characterization of therapeutic antibodies by CESI-MS

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Introduction
With the increase of the number of approved protein therapeutics in the market, comprehensive and reproducible characterization of these new generation drugs is crucial for the biopharmaceutical industry and regulatory agencies. One of the largest groups of biotherapeutics is monoclonal antibodies (mABs) possessing various post-translational modifications (PTMs) such as glycosylation and potential degradations during the manufacturing process that may affect efficacy and immunogenicity.

Methods
The exceptionally high separation power of capillary electrophoresis (CE) in conjunction with mass spectrometry fulfills multilevel characterization requirements necessary to reveal such modifications and degradations.

Results
In this presentation a comprehensive characterization example will be given for a representative monoclonal antibody Trastuzumab (Herceptin), illustrating the benefits of the integration of capillary electrophoresis (CE) and electrospray ionization (ESI) in a unified bioanalytical process (CESI) coupled with high resolution mass spectrometry. Both small and large peptides (3 – 65 amino acids) have been separated, identified with 100% sequence coverage and quantified, including degradative hotspots such as asparagine-deamidation, methionine-oxidation, glutamic-acid-cyclization, and C-terminal lysine heterogeneity using only 100 fmol of a single protease digest sample.

Conclusion
The low flow rate of the system (>20 nL/min) ensured maximized ionization efficiency and dramatically reduced ion suppression.

Novel Aspects
Special emphasis will be given to glycosylation microheterogeneity analysis to reveal potential in-source fragmentation mediated glycopeptide artifacts in view of the ADCC and CDC function assessment during the manufacturing process.

MPS06-52 / Comparison of fragmentation patterns of Q-TOF and Orbitrap accurate mass spectrometers for drug metabolism studies

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Information on metabolism data on drug candidates like the identification of physiologically relevant metabolites, defining metabolic pathways, and elucidation of metabolite structures have become increasingly important to the drug development process. In-vitro interspecies comparison studies are routinely performed to screen for qualitative and quantitative differences in the metabolism profiles between humans and animal species. For the structural identification of metabolites from these study types the interpretation of MS/MS fragmentation patterns of potential drugs and their metabolites is crucial.
Due to the developments in the last decade in the field of accurate mass spectrometry the metabolic modifications in drug metabolites can be determined more selectively by accurate mass shift and by the determination of the elemental composition. In addition to the availability of accurate mass, versatile fragmentation techniques are offered. In the poster the most common used methods like the CID (ion trap), the HCD fragmentation and the MSE method are compared in terms of obtained drug metabolism information for phase I and phase II metabolism.
According to the presented mass spectra in the poster, CID proves to be the first choice method rather for the phase II metabolites where the weak-bonded conjugates can remain intact during the fragmentation since it is a soft fragmentation method. For the phase I metabolites the HCD and MSE methods can provide more detailed structure information than CID because the lack of the low mass cut-off and the applied higher fragmentation energy that can result in fragments which cannot be generated by CID. Examples are showed from our drug discovery work that the combination of MSE and ion mobility can be an useful and easy screening method and it is able to distinguish co-eluting drug metabolites that show similar fragmentation patterns, too. The HCD fragmentation, especially its combination with ion trap is a tool to obtain deep structural information for the more important drug metabolites.
In our opinion the use of different analyzer technologies offers complementary ion manipulation possibilities to solve the problems arising in structural identification of drug metabolites.

MPS06-53 / Determination of Bleomycin A2 and B2 in plasma by HPLC-ESI-QTOF method

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Introduction
Bleomycin is one of the highly effective cytostatic antibiotics. It is isolated from the fermentation broth of Streptomyces verticillus. Bleomycin is present as a mixture of structurally related glycopeptide antibiotics, soluble in water.Bleomycin is applied to the treatment of lymphoma and testicular cancer. For better understanding of the mechanism of different bleomycin fractions acting, and biodistribution of bleomycin fractions in organism, a development of the powerful analytical method with the reliable identification and quantitation of bleomycin in pharmaceutical and biological matrices is of a high importance.

Methods
The chromatographic apparatus consisted of a LC Agilent Infinity System equipped with an Infinity 1260 gradient pump, a 1260 HiPals automatic injector, a column thermostat 1290, a photo-diode array detector Infinity 1290, Accurate-Mass quadrupole time-of-flight 6520 (Q-TOF) detector equipped with an electrospray ionization source and a computer with Mass Hunter software for data acquisition and processing (Agilent Technologies, USA). The chromatographic column ZIC-HILIC, 100x4.6mm with 5mm particles, was used. The column temperature was 30oC. The mobile phase flow was 0.4ml/min. The injection volume was 30ml.

Results
Zwitterionic stationary phases (ZIC-HILIC) was used as an innovative stationary phase for the HPLC separation of the major bleomycin fractions (A2, B2) intending to avoid the use of an ion-pairing additive in the mobile phase. The optimization of mobile phase was based on the selection of proper organic solvent and its concentration, ionic strength of the buffers, and pH of the water phase. The optimum composition of the mobile phases was methanol/ammonium formate (50mM, pH 6.2) (50/50 v/v). The optimized ESI-MS parameters were: fragmentor voltage (180 V); nebulizer pressure (40 psi); drying gas temperature (300°C); drying gas flow rate (12 L.min-1); capillary voltage (3500 V). The performance parameters of the optimized method were evaluated for standard of major bleomycin fraction (A2, B2) present in pharmaceutical and model plasma matrices. The HPLC-Q-TOF method was applied for the identification and determination of the bleomycin A2 and B2 fractions in the infusions Bleomedac aimed at an intravenous administration and also for the plasma sample (spiked one).

Conclusions
The HPLC-Q-TOF method was applied for the pharmaceutical and biological (plasma) samples. These method gives excellent selectivity for simultaneous determination of bleomycin A2 and B2 in plasma with a minimum sample pretreatment (direct injection of supernatant after methanolic precipitation of proteins).

Novel aspect
HPLC-Q-TOF method is a powerful tool for the separation, identification and determination of two major bleomycin fractions, A2 and B2 withou the need to use an ion-pairing additive in the mobile phase. These findings suggest that the method could be suitable for the clinical use, like the monitoring of bioavailability of the bleomycin A2 and B2 fractions in oncological patients.

Acknowledgments
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MPS06-54 / Mass spectrometry quantification of HER2 peptides in FFPE breast cancer tissues

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Introduction
The potential of protein quantification in formalin-fixed paraffin embedded (FFPE) tissues is high, as it would enable large retrospective biomarker studies using clinically annotated samples archived over the years. In order to assess the value of Selected Reaction Monitoring (SRM) as a technique for protein quantification in FFPE tissues, we developed an SRM assay to measure the human receptor tyrosine-protein kinase erbB-2 (HER2) in breast tumors. HER2 expression is routinely assessed in clinical pathology by immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH) as a marker of susceptibility to trastuzumab treatment.
We report the quantification of HER2 peptides in 40 archival FFPE tumors from women with invasive mammary carcinomas overexpressing HER2 and compare the agreement between SRM data and IHC or FISH data.

Materials and Methods
Suitable peptide candidates for building an SRM assay were identified using untargeted mass spectrometry (MS) on extracts from relevant cell lines (SKBR-3 and BT-474) and from FFPE breast tumors. Peptides were extracted from formalin-fixed material using a heat-induced antigen retrieval procedure. SRM methods based on isotope dilution with an internal reference standard were developed for the six best performing peptides, and were further analytically evaluated in terms of linearity, reproducibility and limit of quantification (LOQ). Peptides were quantified by SRM in a cohort of 4 x 10 breast tumors expressing different HER2 levels (FISH ratio: 0-2, 2-4, 4-10 and >10 respectively). The SRM data was compared with IHC and FISH data using ordinal logistic regression analysis.

Results
The untargeted MS approach identified 29 HER2 peptides. Six were selected as candidates for the development of an SRM assay based on the following criteria: unique to HER2, absence of methionines and satisfactory chromatographic performance. The six peptides showed a linear response over a calibration range of 0.012 to 100 fmol on column (R2: 0.99 - 1.00). LOQ ranged from 0.31 to 1.25 fmol on column, except for one peptide for which we were not able to determine the LOQ. The total peptide amounts quantified by SRM in 20µm thick tumor slices spanned over a range of 7.2 fmol to 4.7 pmol for the 40 samples. The six HER2 peptides were highly correlated with each other and SRM results matched with IHC and FISH data with misclassification rates of typically 30%.

Conclusions
SRM was used for the quantification of HER2 peptides in FFPE tissues, providing a similar tumor classification as IHC and FISH.

Novel Aspect
In most hospitals world-wide, FFPE is the preferred method for tissue preservation. To our knowledge, this is the first report of targeted MS protein quantification in clinical FFPE samples which uses a validated SRM assay and shows this level of agreement between HER2 assessed by SRM and by well-established clinical pathology methods. This report contributes to make FFPE clinical specimens accessible to SRM.

MPS06-56 / MALDI-TOF MS for Monitoring Drug Resistance in Hepatitis B Virus-Infected Patients during Antiviral Therapy

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Introduction
Minor drug-resistant variants may pre-exist in every patient infected with HBV. However, understanding the dynamic of genotypic evolution within HBV population requires the ability to accurately follow allele frequencies through time.In this study we used MALDI-TOF mass spectrometry (MS) for localization

and quantitative allele frequency detectionto investigate pre-existing HBV quasispecies and the genotypic evolution of drug-resistant variants during NA therapy.

Methods
Serum samples obtained from 42 chronic HBV-patients treated with lamivudine (24), entecavir (13) and tenofovir (5) monotherapy were analyzed by MALDI-TOF MS. The 37 selected HBV variants were determined in 4 separate iPLEX reactions upon initiation of treatment, at week 12, 24 and 48. The study group consisted of treatment-experienced (21) and naïve-patients (21).

Results
Resistant mutations in lamivudine-group were detected upon the treatment in 72 % (13/18) of naïve and in all experienced-patients (6) with the allelic frequency of 1.2 – 44% and 1.2 – 100% respectively. In entecavir-group resistant HBV-variants were identified in both groups (1.8 – 20%). All patients treated with tenofovir were experienced. We observed changes occurring in the HBV quasispecies within 48-weeks of NA-treatment. The percentages of resistant-variants notably increased relative to the baseline in the nonresponder patients, as well as new mutations appeared during NA-therapy (p=0.00058). In the case of responder group drug-resistant mutants disappeared in time.

Conclusions
These studies showed that there is a significant difference between the genotypic evolution of drug-resistant variants depending on the response to NA-therapy. Thepre-existence of natural resistance mutations in treatment – naïve patients is common.

Novel aspect
MALDI TOF MS is a powerful technique for determination of HBV resistant variants, including quasispesies. This assay can provide a valuable insight into the understanding of how resistance develops and enables early strategic decisions of antiviral treatment in the management of CHB.

MPS06-57 / Antiviral Potential of Catechins to Arrest Influenza Virus Infections with Confocal Microscopy, Molecular Docking and Mass Spectrometry

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Introduction
The benefits of tea to human health have long been recognized due to the strong association of tea with long life in many cultures. Catechins are a class of flavonoids present in the greatest abundance in the leaves of tea trees. Their antiviral properties have been the focus of recent interest including their potential to inhibit the propogation of the human immunodeficiency and the influenza viruses. Their ability to inhibit the influenza virus, in particular, has been stimulated by the rise in the number of circulating strains resistant to the current antiviral inhibitors. The mechanism of their mode of action, however, is not well understood. We have studied the potential of these compounds against circulating strains of influenza virus using a combination of sialidase activity assays with confocal microscopy, sialidase inhibition assays, computer-based molecular docking and mass spectrometry.

Methods
The antiviral properties of three catechins found in green tea, epigallocatechin-3-gallate (EGCG), epicatechin-gallate (ECG) and catechin-5-gallate (C5G), were studied following their addition to influenza infected MDCK cells employing a sialidase activity assay in conjunction with confocal microscopy. Companion molecular docking and the use of a mass spectrometry based assay developed in this laboratory were employed to study the nature of their binding to influenza neuraminidase.

Results
Treatment of infected MDCK cells revealed that the antiviral activity of all three catechins was optimal when added within 1h post infection. All three were shown reduce the sialidase acitivity of the viruses at concentrations above 50μM and completely at 150μM. Their IC50 values were approximately 30μM as determined by a plaque assay.
Companion molecular docking and mass spectrometry studies showed that they bind to the active site of influenza neuraminidase in the vicinity of the 430-cavity, a region adjacent to the 150-cavity targeted by oseltamivir and zanamivir that contains the majority of known resistance mutations. They therefore offer potential as the basis for newly designed antiviral inhibitors to influenza viruses that overcome these resistance limitations.

Conclusions
A combination of activity assays with confocal microscopy, sialidase inhibition assays, computer-based molecular docking and mass spectrometry show that catechins from tea leaves hold much promise as antiviral inhibitors to the influenza virus. Importantly they bind within the 430-cavity of influenza neuraminidase, remote from known drug resistant mutations, which render current antivirals ineffective.

Novel aspect
The antiviral potential and the molecular basis of the activity of catechins has been studied for the first time by a range of approaches including mass spectrometry.

MPS06-58 / Anthocyanidin Inhibitors Against the Influenza Virus by Mass Spectrometry

Kevin Downard, Kavya Swaminathan, Patrick Muller
University of Sydney

Introduction
Neuraminidase inhibitors are the only class of antiviral drugs currently approved for use against the influenza virus. They function by binding within the active site of the enzyme on the surface of the virus particle and in doing so inhibit its sialidase activity. Increased resistance of circulating strains to current antiviral drugs has prompted the development of inhibitors that bind in the vicinity of residue 430 (the 430-cavity). We have identified a class of flavonoids known as anthocyanidins that bind to this region of the enzyme. They constitute highly coloured pigments found in flowers and fruits, particularly berries. We have studied the potential of these compounds using a novel mass spectrometry based approach, developed in this laboratory, in combination with companion molecular docking and inhibition assays. We have examined the effect of substitutents on the phenyl ring on their binding to influenza neuraminidase.

Methods
Solutions containing the untreated and anthocyanidin-treated neuraminidase were resolved by native PAGE and non-binding peptide segments released through the addition of endoprotease to the blind excised protein band. MALDI mass spectra were recorded for the proteolytic products to identify the regions to which the inhibitors are bound. Companion molecular docking studies were conducted using the Autodock algorithm. Neuraminidase inhibition assays were performed in a standard 96-well plate using a modified form of a reported method. Neuraminidase activity was determined based on a spectrometric measurement of a released fluorescent substrate.

Results
The binding of three closely related anthocyanidins (cyanidin, pelargonidin and delphinidin) occurs within the 430-cavity of influenza neuraminidase as confirmed by a combination of mass spectrometry and molecular docking. Despite their similar structures, which differ only in the number and position of the hydroxyl substituents on the phenyl group attached to the chromenylium ring, subtle differences in their binding characteristics are revealed by mass spectrometry and molecular docking that are in accord with their inhibitory properties by neuraminidase inhibition assays. The cyanidin and delphinidin, with the greatest number of hydroxyl groups, bind more strongly and are better inhibitors than pelargonidin that contains a lone hydroxyl group at the 4' position.

Conclusions
Anthocyanidins hold much promise as starting scaffolds from which to develop new potential antiviral inhibitors to the influenza virus. Their different binding modes to current inhibitors, within the 430-cavity of influenza neuraminidase, provides a solution to drug resistant mutations now prodigious in the 150-loop and cavity region of the protein. This study demonstrates the sensitivity and power of a mass spectrometric based approach for screening the potential of such inhibitors at the molecular level, irrespective of the target protein with reasonable high throughput.

Novel Aspect
First molecular based investigation of the inhibitory potential of anthocyanidins against the influenza virus using mass spectrometry

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MPS06-59 / Development of an LC-HRMS-based metabolomic approach to study methicillin-resistant Staphylococcus aureus
Sandrine Aros-Calt[1], Bruno Muller[2], Celine Ducruix[2], Samia Boudah[1], Gaspard Gervasi[2], Christophe Junot[1], François Fenaille[1]
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Introduction
Antibiotic resistance often appears shortly after the introduction of a new drug as a direct consequence of its intensive use. Moreover, we are now running out of treatment options for many infections. Sometimes, confident identification of resistant strains can be difficult using conventional diagnostic techniques due to the lack of robust biomarkers of antibiotic resistance.Methicillin-resistant Staphylococcus aureus represents a major cause of hospital- and community-acquired bacterial infections. In this context, we have evaluated the usefulness of metabolomics as an approach to differentiate methicillin-resistant from susceptible S. aureus strains (MRSA and MSSA, respectively), and also to potentially highlight markers of antibiotic resistance for further detection purposes.

Method
Isogenic strains and a set of clinically relevant MRSA and MSSA strains were chosen for this proof-of-concept study. A sample preparation protocol was developed and consisted in a filter-based bacteria sampling, and a rapid quenching step followed by a rather harsh but artifact-free extraction procedure. Metabolomic analysis were performed by a robust liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) platform using an Exactive instrument (R=50,000 at m/z 200). Automatic peak detection was performed using the XCMS software, while compound identification was achieved using in-house databases. Finally, multivariate data analysis was used to highlight potential differences existing between resistant and susceptible strains.

Preliminary data
Little is known about intracellular metabolite pools in pathogens such as Staphylococcus aureus. In that aim, a specific sample preparation was designed and carefully optimized to obtain a reliable snapshot of bacterial metabolism. The quality of this development was monitored by the assessment of the Adenylate Energy Charge (AEC) corresponding to the quantification of AMP, ADP and ATP nucleotides using LC-HRMS and isotope dilution. In addition, both hydrophilic interaction liquid chromatography (HILIC) and pentafluorophenyl propyl (PFPP) columns were chosen for their complementarity and used to get the most exhaustive view of S. aureus metabolome by LC-HRMS.Thus, the metabolite pools of these strains were studied at different growth stages (early-, mid- and late-exponential growth phases) without any antibiotic exposure, which revealed a distinct behavior of resistant strains along the exponential growth phase. A few metabolites responsible for this discrimination were further identified using in-house metabolite databases.
To the best of our knowledge, this preliminary study is the first one providing clear evidence that metabolomics can differentiate clinically relevant MRSA and MSSA strains without antibiotic exposure.

Novel Aspect
LC-HRMS-based metabolomics enabled the distinction of methicillin-resistant Staphylococcus aureus from other methicillin-susceptible strains.

MPS06-60 / Analysing covalent protein-drug adducts: protein-melphalan adducts
Debbie Dewaele[1], Geert Baggerman[2], Frank Sobott[3], Filip Lemière[3]
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Many drugs form reactive electrophilic intermediates or metabolites in the body, often through metabolic activation. This has raised some questions because of the link between unspecific covalent binding of these drugs to proteins and possible adverse drug reactions (ADR). We want to develop specific detection and characterization techniques in order to get a better picture of the formation of these covalent protein-drug adducts. Improved understanding of this adduct formation and its effects could enhance drug efficacy and safety.
In this study melphalan, a reactive nitrogen mustard, is used as a test case for the formation of covalent adducts. In solution, melphalan can react quickly with the available nucleophilic groups of proteins via a nucleophilic substitution reaction. These nucleophilic groups include the N- and C-terminus of a protein and the thiol groups, amino groups, heterocyclic nitrogen atoms and carboxyl groups of the amino acid side chains. On a Synapt G2 HDMS Q-TWIMS-TOF we use complementary fragmentation techniques (CID, ETD) and ion mobility of intact (native/denatured) melphalanated model protein adducts to study their structure. The obtained top-down data will be complemented by bottom-up LC-MS/MS data from digests.
We present the analysis of in vitro incubation of melphalan with model peptides and proteins resulting in a variety of drug adducts. Interpretation of the different results makes it possible to predict the reactive alkylation sites on natively folded proteins. This gives us an insight into the chemical reactivity of this specific covalently binding drug compound towards proteins. Furthermore it opens the door to the development of widely applicable research strategies for the analysis of other covalent drug adducts.

MPS06-62 / Investigating Metal Binding and the Resulting Conformational Changes of Monomeric Alpha-Synuclein

Aimee Paskins, Eva Illes-Toth, Catherine Duckett, Caroline Dalton, David Smith
Sheffield Hallam University

Introduction

Oligomerisation and fibrillisation of α-synuclein into amyloid like aggregates has been implicated in the development of Parkinson's disease and other so-called synucleinopathies. In Parkinson's disease, aggregated α-synuclein forms deposits known as Lewy bodies in the substantia nigra pars compacta which are heavily phosphorylated and have been demonstrated to be metal rich in Cu, Zn and Fe. Phosphorylation is increased in synaptic enriched areas of the frontal cortex in the absence of Lewy bodies in early stages of the disease, suggesting this phosphorylation occurs before the formation of the amyloid aggregates. Alpha-synuclein is a 14.5 kDa intrinsically disordered protein capable of low affinity metal binding and is known to populate both an extended state and a more collapsed state linked to amyloid formation. This conformational state has been shown to be dependent on environmental conditions, including the presence or absence of a number of cofactors such as polyvalent metal ions, which modulate fibril formation. Little is known however, as to how post translational modifications affects the interaction of α-synuclein with these modulators and the subsequent conformation state of the full length protein.

Methods

Native and mutant α-synuclein were produced recombinantly in E.coli cells. Post-translational modification mimics (Ser87 to Asp and Ser129 to Asp) were introduced by site directed mutagenesis. Electrospray-ionisation-Ion Mobility Spectrometry-Mass spectrometry (ESI-IMS-MS) was performed to determine collisional cross sections and relative populations of the monomeric conformational states both apo and metal bound thus giving details of resulting structural changes. Collisional cross sectional areas were then compared with known model structures.

Results

We demonstrate that apo α-synuclein co-populates expanded conformational states in conjunction with a range of more collapsed conformations. Binding of divalent metal ions such as Cu2+ ions to the wild type protein shifted the conformational equilibrium towards the collapsed conformational states. Binding of Cu2+ to α-synuclein mutants containing post-translational modifications mimicking phosphorylation was also investigated and the resulting shift in conformational states determined and compared to the wild-type protein.

Conclusions

Results suggested a hierarchy in terms of α-synucleins binding affinity to polyvalent metals, with Cu2+ binding readily, followed by Zn2+, and no binding being observed for Fe3+. Cu2+ was shown to bind to alpha-synuclein in both its collapsed and extended conformations indicating that the binding site is sequence dependent yet binding induces the aggregation prone state.

Novel Aspects

This work highlights the link between post-translational modification of the α-synuclein monomer, and its affinity for the binding of polyvalent metal ions.

MPS06-63 / Tandem mass spectrometric characterization of a sugar-modified antisense oligonucleotide

Yvonne Hari, Stefan Schürch
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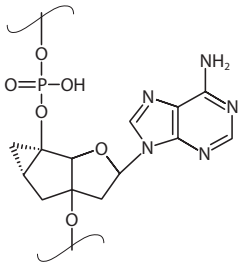
Introduction

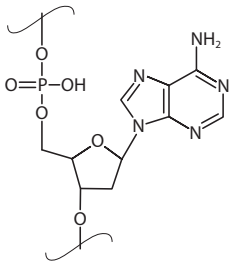
Chemical modification of the sugar moiety in nucleic acids is a promising approach for the design of novel antisense therapeutics. In tricyclo-DNA (tcDNA), an analogue currently evaluated for the treatment of muscular dystrophy, the deoxyribose is replaced by a three-membered ring system, which greatly enhances the resistance against degradation in the cell. The particular geometry of tcDNA gives rise to highly stable hybrid duplexes with complementary DNA or RNA relative to native nucleic acid duplexes, while simultaneously promoting the selectivity of the antisense oligonucleotide for the RNA complement.
While high biostability is a crucial property of antisense therapeutics, it renders the nucleic acid analogues inaccessible to biochemical sequencing techniques and necessitates the development of alternative analytical methods based on mass spectrometry.

Methods

All samples were analysed on a Thermo Fisher Scientific LTQ Orbitrap XL mass spectrometer equipped with a nano-electrospray source.



tcDNA



DNA

Results

Collision induced activation of tcDNA oligonucleotides gives rise to a-B- and w-ions, implying a DNA-like fragmentation mechanism. Despite extensive base loss, sequence coverage for a tcDNA 15mer could be attained by tandem mass spectrometry, which provides the basis for identification and quantification of the antisense oligonucleotide in medical diagnostics.
Additionally, the presented data on nucleic acid duplexes promotes mass spectrometry as an analytical tool for the evaluation of antisense properties of synthetic oligonucleotides. Tandem mass spectrometric characterization of tcDNA:DNA and tcDNA:RNA hybrid duplexes confirms earlier works on melting curves suggesting selectivity of tcDNA for complementary RNA over DNA. The gas-phase dissociation pathways of hybrid duplexes were found to include strand separation, backbone fragmentation, base loss, and the ejection of backbone fragments from the duplex, the last of which appears to be highly sequence-dependent.
For duplexes and single strands alike, base loss was found to be much more prominent from sugar modified nucleic acids than from their natural analogues. Mass spectrometric characterization of tcDNA is therefore expected to shed new light on the process and the conditions favoring base loss, thus providing new insights into the initiating event of backbone cleavage of DNA in the gas-phase.

Conclusions

The presented results promote mass spectrometry as a suitable tool for medical diagnostics of sugar-modified oligonucleotides and stress the potential of the technique for the study of antisense properties of synthetic nucleic acid analogues.

Novel Aspect
The presented work provides structural elucidation of a novel, promising antisense therapeutic and reflects on the influence of the sugar moiety on the gas-phase dissociation of DNA.

MPS06-64 / Ultra-Sensitive Quantitation of Exenatide with Micro-Flow LC Trap-and-Elute and High Resolution and Triple Quadrupole Mass Spectrometry Workflow
Houssain El Aribi[1], Jinyuan Wang[2], Daniel Warren[3], Anthony Romanelli[3]
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Introduction
Exenatide is a 39-amino-acid peptide, an effective medication to treat type-2 diabetes. Reported methods to quantify exenatide concentration in plasma include immunoassay and liquid chromatography mass spectrometry (LC/MS). LC/MS method has been chosen preferably due to the advantages in excellent selectivity, sensitivity, wide analytical range and reproducibility. To further extend lower limit of quantitation (LLOQ) to accurately study lower concentration exenatide in plasma samples (<100pg/mL), a micro-flow LC with high resolution accurate mass (HRAM) and/or Triple Quadrupole method was developed and demonstrated here with LLOQ at 10 pg/mL and excellent linearity, quantitation accuracy and precision, as well as low system carryover.

Methods
Plasma samples were prepared by immune-affinity or acetonitrile crash. Two micro flow gradient pumps were configured in this trap-and-elute workflow. A C18 trap column was used to trap the analyte from injected sample (50?L) then load to separate column. To reduce/eliminate carryover, wash solutions containing 1% trifluoroethanol was used. The TripleTOF® 5600 was operated in high resolution MS2 product ion scan mode monitoring the product ions of 833 m/z [M+5H]5+ (MS/MS for 6500). ESI sources (DuoSpray for 5600, and a newly designed micro flow source for 6500) with a 50?m ID electrode was used to couple micro-flow LC with mass spectrometer and 50?m ID PEEKsil tubings were used to plumb the whole system to minimize extra column volume.

Preliminary Results/Abstract
Comparing with the published best results where 100 pg/mL was determined as the LLOQ, 10 fold sensitivity improvement was observed on this ?UHPLC-MSHRAM platform configured with nanoLC 425 and TripleTOF 5600. The LLOQ of this method was determined at 10 pg/ml with 50uL injection. Wide linear dynamic range was observed from 10 to 2,000 pg/mL with excellent precision (%CV < 15% throughout the linear range), and accuracy within 89.5% to 112%. The ultra-sensitive quantitation workflow does not suffer from throughput and the total run time for each sample is 10 minutes. Carryover was a known issue for exenatide quantitation. And in this study, trifluoroethanol was introduced to effectively remove residue exenatide from sampling path as well as from separation column to maintain low system background and to ensure low level carryover. Compared with regular wash, the wash mechanism utilized in this method could reduce carryover by 87.5% and the observed carryover was at 0.17%.

Novel Aspect
Use of microflow UHPLC and advanced mass spectrometry for high throughput exenatide quantitation with significantly improved sensitivity

MPS07 - Imaging MS – Instrumentation
11:00-15:00
Poster Exhibition, Level -1

MPS07-01 / Macroscopic and microscopic spatially-resolved analysis of food contaminants using Laser Ablation Electrospray Ionization Imaging Mass Spectrometry
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[1]RIKILT, Wageningen University and Research Centre, [2]Wageningen University

Introduction
Recently, laser ablation electrospray ionization (LAESI) has been introduced as an ambient imaging mass spectrometry (IMS) tool. In contrast to alternative IMS techniques such as secondary ion mass spectrometry, matrix-assisted laser desorption ionization and desorption electrospray ionization, the desorption and ionization steps have been decoupled in the LAESI set-up. As a result LAESI does not require very flat surfaces, high precision sample preparation or the addition of matrix. Thanks to these features LAESI IMS may be the method of choice for spatially-resolved food analysis.

Methods
In this work LAESI full-scan accurate mass time-of-flight IMS has been explored for 2D and 3D macroscopic and microscopic imaging of pesticides, mycotoxins and plant metabolites on rose leafs, orange and lemon fruits, ergot bodies, cherry tomato and maize kernels from real-life sources.

Results
Accurate mass ion maps were obtained with an x-y spot center-to-center distance of 0.2-1.0 mm, together with co-registered optical images. The spatially-resolved ion maps of pesticides on rose leafs suggest co-application of registered and banned pesticides. Ion maps of the fungicide imazalil show that this compound is only localized on the peel of citrus fruits. However, according to 3D LAESI IMS some fungicide spots penetrate significantly deeper in the peel than others. Ion maps of different plant alkaloids on ergot bodies from rye show co-localization preferably on the edge and in the groove. The feasibility of untargeted IMS in food analysis is demonstrated by ion maps of plant metabolites in cherry tomato and maize kernels. In the tomato case traveling-wave ion mobility (IM) is applied to discriminate between different lycoperoside isomers; in the maize case quadrupole time-of-flight tandem mass spectrometry (MS/MS) is successfully used to elucidate the structure of a highly localized unknown.

Conclusion
The results obtained are very encouraging and it is envisaged that LAESI IMS will contribute to many scientific challenges in food science, food quality and safety, food security and in agriforensics.

Novelty
We exploited the unique features of LAESI IMS for the macroscopic and microscopic targeted and untargeted imaging of major food contaminants such as pesticides and mycotoxins and other natural substances. We consider this work as a significant original application of the known LAESI IMS technique and expect a major impact on future analytical research related to food integrity, food security and agriforensics, having a spin-off in plant research including plant metabolomics.

MPS07-02 / Molecular mapping of skin and biofilms by MALDI MS imaging

Boudewijn Hollebrands, Hans-Gerd Janssen, Christian Grun
Unilever R&D

Mass spectrometry imaging (MSI) has emerged as a powerful tool to study the spatial distribution and chemical identity of molecules present on a surface. Especially Matrix-assisted laser desorption/ionization (MALDI) MSI has been proven to be a powerful tool for the identification and localization of unknowns and is applicable to a wide range of (bio) molecules and samples. With proper sample preparation and measurement conditions it can be used to localize known compounds as well as to discover new analytes. Here, we present two recent applications of MALDI-MSI focussing on the uptake and distribution of endogenous and exogenous compounds in biological tissues.
In a first application, we use MALDI-MSI to better understand the dermal delivery of skin cream components into skin. In an ex-vivo experiment, skin creams were applied to the skin surface and the penetration of specific compounds present in the formulations was studied by imaging skin cross-sections. Skin penetration was visualised over time and simultaneously, skin morphology was studied by constructing detailed lipid distribution maps in a multimodal approach combining MALDI-MS2 imaging with bright field microscopy. The method enabled determination of the pathway and depth of penetration of various skin cream components.
In a second application, MALDI-MSI was used to look at the distribution of a surface-active biocide in biofilm material. It was found that the distribution of the biocide was a clear function of the exposure time. From the results of the different experiments it was concluded that MALDI-MSI allows reliable identification and sensitive detection of a wide variety of analytes in a diverse set of matrices and applications. This is, as far as we know, the first time that MALDI mass spectrometry imaging is applied to study biocide distribution in biofilms.
Together, the data demonstrate the great potential of MALDI-MSI to study the penetration of exogenous compounds into biological tissue.

MPS07-03 / Application of New Nanostructured Materials in the LDI-MS Analysis of Small Molecules

Robert Jirasko, Michal Holcapek, Jan Macak, Jan Gutwirth
University of Pardubice

The choice of suitable matrix for MALDI-MS analysis of small molecules, such as lipids, drugs and their metabolites has been recognized as an important task in last years. Several strategies to improve the performance of MALDI of small molecules were developed. Among them, the use of high molecular weight matrices, solvent-free matrix deposition, cationization agents, ion-less matrices or MS/MS strategies can be mentioned. The growing interest is registered in the development of matrix-free approaches, where nanoparticles or nanostructured surfaces are used instead of MALDI matrix.
In our work, we prepared various nanostructured materials by etching approaches and physical vapor deposition. Prepared materials were characterized by scanning electron microscopy. Mass spectra were measured using ultrahigh-resolution MALDI mass spectrometer LTQ Orbitrap XL (Thermo Fisher Scientific, Waltham, MA, USA) equipped with the nitrogen UV laser (337 nm, 60 Hz). The instrument was operated in both positive-ion and negative-ion modes in the normal mass range (m/z 100 - 1500). Tuning parameters were optimized individually for each used matrix and nanostructured material to achieve high and reproducible signal.
Testing and application of prepared materials for MALDI-MS analysis of drug and lipid samples and the comparison with standard matrix deposition approaches were performed. The model for evaluation of individual nanostructured materials and matrices was developed and applied for the analysis of selected small molecules. Particular matrices were scored based on the strength of extracted ion current, relative ratio of molecular adducts and fragment ions of particular small molecules.
The financial support of this project was provided by the Czech Science Foundation (No. P206/12/P065). M.H. acknowledges the support of the ERC CZ project No. LL1302 sponsored by the Ministry of Education, Youth and Sports of the Czech Republic.

Novel aspect
Application of new nanostructured materials for LDI-MS of lipids and comparison with selected matrices according to score values.

MPS07-04 / Dithranol is an Efficient Matrix for MALDI-MS Imaging of Glyco- and Phospholipids with High Lateral Resolution

Simeon Vens-Cappell, Hans Kettling, Jens Soltwisch, Johannes Müthing, Klaus Dreisewerd
University of Münster

Introduction
Mass spectrometry imaging (MALDI-MSI) of bioactive lipids directly from tissue slices has recently found an enormous interest. Here, we demonstrate that dithranol [1] constitutes a matrix that offers a particular high potential for the MSI analysis of phospho- and acidic glycolipids (including sulfatides and gangliosides) in the negative ion mode. The matrix produces uniform micro-crystalline sample preparations with average crystal sizes in the low µm-range and high analyte ion yields in both ion modes. MS experiments characterizing the performance of the matrix for the detection of gangliosides and SEM images characterizing the tissue coverage are presented. Examples demonstrating the MALDI-MSI analysis of mouse brain slices with lateral resolutions down to ~7 µm are shown.
[1] Le, C.; Han, J.; Borchers, C. Anal. Chem. 2012, 84, 8391-8398.

Experimental
Dithranol as well as GM1-containing ganglioside preparations (mouse brain) were dissolved in $CHCl_3$:MeOH (2:1 v/v). This solvent system ensures an efficient extraction of (glyco-) lipids from sprayed tissue. A custom-made semi-automated pneumatic spray system was used to produce uniform matrix layers with crystal sizes of a few µm. A Synapt G2-S HDMS mass spectrometer (Waters) was employed for the MS experiments. The MALDI ion source of this instrument was modified for operation at elevated cooling gas pressure of 0.7 mbar and for achieving an improved lateral resolution close to 7 µm (see contribution of Kettling et al. at this conference for details). MS experiments were performed in the negative ion mode.

Results
SEM and optical microscope images revealed that exceedingly uniform sample preparations with average crystal sizes in the low µm-range were obtained by spraying the dithranol matrix onto mouse brain tissue slices. Typically 10 bouts with 3 s lengths followed by 15 s drying intervals were employed at a sprayer-sample distance of 10 cm. The high extraction efficiency for plasma membrane lipids provided by the solvent system, the formation of uniform microcrystalline matrix layers, and the high ion yields obtained upon desorption/ionization render the dithranol matrix particularly suitable for high-resolving MALDI-MSI of endogenous lipids in tissue slices. We demonstrate this feature with selected examples of molecular images (including gangliosides, sulfatides, phosphatidylinositol) which were

obtained from different mouse brain areas. A minimum lateral resolution of about 7 µm was achieved in the MSI-analysis of mouse brain tissue.

Conclusion
There is an ongoing research effort to improve the lateral resolution of the MALDI-MSI analysis to a cellular level. The here presented combination of using a dithranol matrix, which produces uniform microcrystalline coatings upon spraying from aCHCl3/MeOH solution, and that of a modified Synapt G2-S mass spectrometer enables the generation of high-quality MALDI-MS images of glyco- and phospholipids with sub 10 µm-resolution on a highly-resolving QTOF-type mass spectrometer.

Novel aspects
First report on using a dithranol matrix for the sensitive analysis of plasma membrane lipids with a lateral resolution of ~7 µm.

MPS07-06 / Studies of an insect model for drug metabolism by Desorption Electrospray Ionization Mass Spectrometry Imaging
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Dept. of Pharmacy, University of Copenhagen

Introduction
An insect model based on the locust is tested for studies of drug metabolism. LC-MS studies of locusts injected with various drugs showed a high elimination rate of drug substances and their metabolites in the hemolymph. Desorption Electrospray Ionization (DESI) Mass spectrometry imaging (MSI) was applied in order to elucidate the metabolism, distribution and excretion of the drugs in the body of the locust.

Methods
LC-MS was performed on a Thermo QExactive Orbitrap mass spectrometer, equipped with a Dionex LC system. DESI-MSI was performed on a Thermo LTQ XL linear ion trap mass spectrometer, equipped with a custom built DESI imaging ion source 1.
Solutions of terfenadine were injected into the hemolymph of locusts. The animals were euthanized by freezing and sliced on a cryo-microtome. For improved sensitivity, images were recorded using the Displaced Dual-mode imaging method 2 which enables simultaneous recording of full-scan images and selected MS/MS images.

Results
A number of endogenous compounds were imaged in the locust, and their identities were confirmed by high-resolution MS and MS/MS. A large number of different metabolites present in the hemolymph were identified by accurate mass LC-MS, and three of the metabolites as a well as the parent drug substance were imaged by DESI-MSI. The parent drug substance was present in body in amounts high enough for detection in full-scan mode, whereas MS/MS was necessary for detection and imaging of the metabolites. The images show that the parent drug substance was not co-localized with any of the metabolites in measurable amounts. While the parent drug was present in large parts of the body and particular in the walls of the intestine, the metabolites were only detected in the intestines in lumps of unexcreted faeces. This was confirmed by removal of the intestine of an animal prior to cryo-sectioning, followed by imaging.

Conclusions
DESI-MS is applicable for whole-body imaging studies of drug substances and their metabolites in locusts. Even with the use of MS/MS, the sensitivity of MSI only enables detection of the metabolites in unexcreted faeces, whereas small amount of metabolites were also detected by LC-MS in the hemolymph.

Novel aspects
Mass spectrometry imaging of endogenous and exogenous compounds in insects; Insects as animal models in drug metabolism studies.

References
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2.C. Janfelt, N. Wellner, H.S. Hansen, S.H. Hansen. Displaced dual-mode imaging with desorption electrospray ionization for simultaneous mass spectrometry imaging in both polarities and with several scan modes. J. Mass Spectrom. 2013, 48, 361-366.

MPS07-07 / High throughput detection of directed evolution driven biotransformation reactions with liquid surface extraction analysis mass spectrometry
Cunyu Yan, Anthony Green, Jason Schmidberg, Lorna Hepworth, Sabine Flitsch, Perdita Barran
University of Manchester

The use of biocatalysts, both enzymes and also engineered whole cells, for producing the target chemicals such as pharmaceutical molecules and intermediates for chemical industrials presents significant advantages in terms of enhanced reaction selectivity, low cost and environmental friendly. The advent of recombinant DNA technology in the late 1970s and the development of directed evolution beginning in the 1990s, can generate popularized candidate enzyme mutants and may extend the narrow substrate scope for industrials, which need a quick, robust and specific approach for screening out the meaningful enzyme and substrate species for the further biotransformation improvement.
Dichloromehane extracts of CapW biotransform reactions incubated with esters and L-aminocaprolactam were evaporated and then redissolved in Acetonitrile/H2O (1/1) for pipetting (1 µL) onto a glass slide (Prosolia, Inc., Indianapolis, USA). The samples on the surface of the glass slide were analysed by liquid extraction surface analysis (LESA) using an automated, chip-based nanospray source (Advion, Ithaca NY, USA) coupled to a triple quadrupole mass spectrometer (TSQ Vantage, Thermo Scientific, San Jose, USA). The typical instrument parameters were set as follows: spray voltage (1.7 kV), N2 delivery gas (0.4 psi) and 40mM ammonium bicarbonate in Acetonitrile/H2O (4:1) as the extraction solvent system. 2 µL of solvent was used for each experiment with 1.5 µL being dispensed 0.4 mm above the surface and then delayed for 4.0 seconds followed by 2.2 µL asperating. The solvent forms a liquid-surface junction for extracting the components into the solvent, the sample was subjected to mass spectrometer through the nozzles on the nanospray chip. The mass spectrometer was optimised with the synthesized template compounds and multiple MRM transitions were set for monitoring the each reaction. Each sample was sprayed 1 minute under full scan mode for ensuring a stable spray current then switched to MRM mode for target final product monitoring.
LESA-MS/MS is proven to be a fast and sensitive method for screening the enzymes and their substrates for a specific biotransformation reaction. The system can reads chemical information on a solid surface directly. In the preliminary experiment, L-aminocaprolactam was chosen for screening esters which can form amides by catalysed with the enzymes of CapW, TmIU and SimL. Series of L-aminocaprolactam moiety containing template compounds were synthesized for the common fragment ions selection. Two diagnostic product ions dissociated from L-aminocaprolactam moiety, m/z 84 and m/z 112, were used to create MRM transitions for monitoring the reactions. 7 methyl

esters were tested using LESA-MS/MS. The MRM transitions which can be triggered by specific biotransformation samples were recorded and the candidate reaction samples were further tested by high resolution mass spectrometry combined with UPLC. 3-phenylpropanoate, methyl pyrazine-2-carboxylate may couple with L-aminocaprolactam under the catalysis of CapW and TmIU, results on other systems following further biological experiments will be also presented.

MPS07-08 / Comparison of Interleaved and Single Core Analog to Digital Converters and their Applications to TOF Mass Spectrometry

Andrew Glascott-Jones, Etienne Bouin, Marc Wingender, Francois Bore
e2v

Introduction

There are two ways to increase sampling rate of ADCs: time interleaving of "reasonably fast" ADC or building a faster ADC on a faster process. Each solution has of course drawbacks and advantages, we have developed both solutions for different applications. In this paper we compare the two approaches. The potential impact of these ADCs in Mass Spectrometry systems will be discussed.

Methods

Two ADC architectures will be examined, one using the single core approach the other using an interleaved method. The interleaved approach allows for higher sample speed but at the cost of increased levels of non-linearity. The single core allows for a low latency system with high bandwidth.
The EV10AS152A combines a 10-bit 3 GSps fully bipolar analog-to-digital converter chip, driving a fully bipolar Demultipler (DMUX) chip with selectable Demultiplexing ratio (1:2) or (1:4). A single core architecture is used.
In this section we introduce a Quad 12-bit 1.125GSps ADC, EV12AS350, platform for RF interleaving of four ADC cores at 1,125GSps on a single chip, based on a 160GHz 120nm SiGe BiCMOS technology.
Time domain interleaving requires many precautions or implies digital post-processing overhead. In this ADC we have combined the 160 GHz SiGe bipolar and the 120nm CMOS transistors advantages to provide a 4.5 GSps ADC platform which will make things much easier for system designers.

Results

The 5 GHz full power input bandwidth of the EV10AS152 allows the direct digitization of greater than 1 GHz broadband signals in the high IF region. The converter has performance of greater than 7 Effective Number of Bits (ENOB) up to 4GHz input frequency and the spurious free dynamic range is greater than 60dBc.
The EV12AS350 has been measured to have a bandwidth of 2.5GHz.Typically, the conversion of a 500MHz input signal gives the following results: The ENOB is 8.8 bit and the SFDR (Spurious Free Dynamic Range) is 75dBFs.

Conclusions

This paper will discuss the architecture and performance of two novel high speed ADCs suitable for TOF Mass Spectrometry applications.

Novel Aspects

The EV10AS152 has a very large input analog bandwidth (5GHz), this is useful in TOF instruments since the pulse broadening effect of the ADC can be minimized, improving the centroid calculation.
The EV12AS350 has a greater sample rate and better linearity which means that the accuracy of the centroid calculation will be less impacted by INL issues in the response of the ADCs and will have a greater intrinsic time precision.

MPS07-09 / A linear ion trap constructed with ladder shape electrodes

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Introduction

The performance of an ion trap mass analyzer relies on the electric field distribution inside the ion trapping region, and the electric field distribution relies on the geometry of its electrode shape. There were several reports on some simplified ion traps, such as Rectilinear Ion Trap and Cylindrical Ion Trap, and their performance could not be adjusted because of the fixed geometry of their electrode. The electric field inside an ion trap with ladder shape electrodes could be adjusted by changing the width and height of its ladder, so the performance of the ion trap could be optimized.

Methods

An ion trap mass analyzer which was built with four identical ladder shape electrodes was shown in Figure 1. There are two ladders of each electrode and their widths and lengths were adjusted for comparison. The electric field distribution insides the ion trap were theoretically simulated with SIMION 8. There are mainly quadrupole field plus some minor 12-pole and 20-pole components. The percentages of the 12-pole and the 20-pole components can be adjusted by changing the width and height of its ladder, so the performance of the ion trap could be optimized.
The performance of the ladder shape ion trap mass analyzer was tested. For the reason of comparison, the mass analysis property of a Rectilinear ion trap mass analyzer was also tested.
Results
Figure 2 shows the mass spectra of a Ladder shape ion trap, and a Rectilinear ion trap. The mass resolving power of the ladder shape ion trap is certainly higher than a rectilinear ion trap at the same ratio of X-direction and Y-direction electrodes.

Conclusions

Because of the property of an ion trap mainly depends on its electric distribution, better mass resolution can be achieved by proper design of ion trap electrode geometry.

Novel Aspect

New ion trap mass analyzer, which constructed with four ladder shape electrode, could have good performance of high resolving power.

MPS07-10 / iMatrixSpray - Open Source Sample Prep Solution for MS Imaging

Dieter Staab, Markus Stoeckli
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Despite MALDI mass spectrometric imaging being a rapidly growing research field, sample preparation remains the single biggest challenge to prevent an even broader application. In particular, the deposition of the matrix is a key step in this process and there is a wide variety of published solutions, which are suitable for different molecule classes or tissues. Based on our experience with many of these procedures, we built a novel and open system which would fulfill the key requirements of simplicity of use, robustness, reliability, homogeneity and most important high sensitivity in the MALDI MSI experiment. The device named iMatrixSpray will be release as free and open

software and hardware to allow production and modification by any interested party.
To prove the reproducibility of the system and the homogeneity of the spray, ink was sprayed on paper followed by the analysis of a digital scan of the sprayed ink. These repeated experiments resulted in reproducibility CV of better than 6% and the CV of the spray homogeneity being better than 5%. Also robustness of the system was developed and evaluated: after spraying 100 times a common used matrix solution on a full microtiter-sized plate without any intervention they spray performance was maintained without any clogging. The implemented washing protocol allows true push-button operation without the need for manual cleaning or priming the system.

Based on the obtained results we developed two optimized methods based on MALDI MSI readout for each iteration: One for matrix and one for internal standard deposition. The resulting protocols support the acquisition of MALDI MSI data which is in par in terms of sensitivity with a manual spray, but offering better robustness and homogeneity. By placing this system in the open domain, we stimulate use and further development of this system.

MPS07-11 / Electrostatic Spray Ionization (ESTASI) Mass Spectrometry Imaging of Thin-Layer Chromatography
Xiaoqin Zhong, Liang Qiao, Hubert Girault
EPFL

Recently, we have developed and patented a new ambient ionization method, namely electrostatic spray ionization, to ionize samples in a solution deposited on a plastic plate. In addition to the analysis of samples in solution, ESTASI is also ideally suited for Mass Spectrometry Imaging (MSI) because of its specific property of ambient in-situ ionizing dried samples on a surface or inside a porous matrix without much sample pretreatment.
Considering ESTASI has been successfully used as an interface between MS and various separation techniques (e.g. CE and gel IEF), one of the important applications of ESTASI-MSI is to profile samples separated on a Thin Layer Chromatography (TLC) plate, which is the first attempt for the imaging of TLC by ESTASI-MS.
In the presentation, the characterization and imaging of antibiotics and dyes separated on TLC plates with ESTASI-MS will be demonstrated to illustrate some of the practical application of the TLC-ESTASI MSI system. The favorable characteristics of TLC-ESTASI-MSI indicate advantageous applications in several areas such as food analysis, forensic sciences, environment monitoring, biochemistry, and etc.

MPS07-12 / In-depth identification of protein images by combining high mass resolution MALDI FTMS Imaging and high performance qTOF nLC-MS/MS
Matthias Witt, Sergej Dikkler, Matt Willets, Shannon Cornett
Bruker Daltonics

Introduction
Assigning protein identity to peaks in MALDI imaging can be challenging. Here we use a workflow where serial sections are digested under identical conditions; one section is imaged while the other is extracted and analyzed by LC-MALDI. A script matches identified proteins with corresponding peptide images. This approach can identify >500 proteins. In this study we examined if the number of proteins imaged and identified could be increased by combining high resolution MALDI-FTMS imaging with high-performance qTOF LC-MS/MS.

Methods
Serial sections of fresh-frozen rat brain and FFPE human kidney tumor were cut to 10 um and mounted as paired sections on ITO slides. The FFPE sections were deparaffinized in xylene followed by antigen retrieval in Tris buffer at 95 C for 25 min. All samples were then digested using the same protocol: Trypsin (200ng/ul in Ambic) was applied as an aerosol followed by incubation at 37C. HCCA matrix was applied as aerosol to one section for MALDI image analysis by either 7T FTMS (solarixXR, Buker). The companion section was extracted by covering with 0.1%FAd and the extracted tryptic peptides were analyzed by MALDI TOF/TOF (ultraflextreme, Bruker) or qTOF MS (impactHD, Bruker).

Results
FFPE human kidney tumor section was imaged using FTMS. The FTMS image dataset exhibits mass resolution in excess of 200k which allows for more than 3290 unique monoisotopic images, as many as 4-5 at a single nominal m/z, to be distinguished in the same m/z range, 700-2500. High performance qTOF analysis is an ideal complement to the images produced by the FTICR. From complex cell digests >4000 proteins have been identified using 4-hour gradients. Together these two technologies promise to greatly expand the range and throughput for identifying putative marker ion images.

Conclusions
New strategy for improving the number putative marker ions identified and mapped from MALDI imaging studies.

Novel Aspect
Combination of MALDI-FTMS imaging with LC-MS/MS ona QTOF for protein identification.

MPS07-13 / 3D MALDI Imaging of Mouse Heart after Myocardial Infarction

Soeren Deininger[1], Dennis Trede[2], Axel Walch[3], Peter Maass[4], Theodore Alexandrov[4], Arndt Asperger[1]
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Introduction
MALDI Imaging (MSI) is a spatially resolved, label-free analytical technique for direct analysis of biological samples which is maturing into a tool for 3D analyses. We integrate magnetic resonance imaging (MRI) and 3D MSI and apply it to a mouse model of myocardial infarction. The entire MS data set (1.2 Million spectra from 85 serial sections) was co-registered with MRI data and conventional histological images to construct a single 3D representation of the multi-modal data.

Methods
Myocardial infarction was induced in a C57BL/6J mouse by permanent ligation of the left anterior descending artery. On post-operative day 7, the mouse was sacrificed, the heart immediately explanted and stabilized in an ethanol-based fixative (PAXGene). MRI data was acquired and then the sample was embedded in paraffin, sectioned and mounted onto conductive glass slides. After de-paraffinization, low-res optical images were acquired and MALDI matrix was thereafter applied by vibrational vaporization. MSI analysis was performed on a MALDI-TOF (2-20 kDa, 25 um raster, 200 laser shots/pixel). After MS acquisition, all sections were stained with H&E and scanned with a high-resolution microscope scanner for detailed histological examination.

Results
The primary goal of this project was to apply a pipeline for multi-modal image analysis to a biologically relevant sample. In myocardial infarction, the penumbral zone, which surrounds the actual ischemic tissue, is of particular interest for evaluating the extent of damage caused by the infarction. We elucidated proteomic signatures specific to the different areas of the infarcted myocardium.
Our previously established registration method to reconstruct a 3D object from the stack of optical images proved insufficient for handling the fine morphological detail of the mouse heart. We refined the procedure and show a three-step process of initial stacking, rigid and elastic registration of the images. In addition, we show first results of reconstructing 3D data from high resolution microscopic images using a similar approach.

Conclusions
Description of a valid workflow for relevant clinical tissues in MALDI imaging and MRI

Novel Aspect
Detection of proteomic signatures of a myocardial infarct in 3D, using a spatially-aware peak-picking algorithm for MALDI Imaging data.

MPS07-14 / Mass spectrometric imaging of plant tissues using desorption nanoelectrospray ionization

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Introduction
Desorption nanoelectrospray ionization (nanoDESI; Ranc, V., et al., Chem. Listy 2007, 101, 524.) is a modification of well-known ambient ionization technique – desorption electrospray. NanoDESI was used for several applications, e.g. analysis of anthocyanins in red wine (Hartmanová L., e al., J. Chromatogr. A 2010, 1217, 4223.). The mechanism of ionization is still not completely described. Recently, we have introduced the fourth model of nanoDESI which is more robust and more user friendly. We have performed the first experiments of nanoDESI imaging as well.

Methods
Four models of nanoDESI have been developed since 2006 at Palacky University, Olomouc, Czech Republic.The experiments have been performed using an LCQ Deca ion trap (Thermo Finnigan, San Jose, USA) equipped with a home-made desorption nanoelectrospray ion source. The new moving device allowing motorized and controlled movement of sample was implemented to perform nanoDESI imaging. Rhodamine B was used as a testing analyte. Mildews on leaves of tomato and lettuce were used for nanoDESI imaging experiment - Bremia lactucae, cause of lettuce downy mildew, and Oidium neolycopersici, cause of tomato powdery mildew. A mixture of methanol : water 1 : 1 was applied as a spray liquid.

Results
The first experiments tested imaging of rhodamine B on rough glass. A photo of lines, a profile of total ion current and ion current at m/z 443 corresponding to rhodamine B confirmed potential of the device in mass spectrometry imaging (Figure). Applicability of this system was also evaluated by imaging of mildews on leaves. The leaves without and with mildew were compared. The leaves were not treated before experiment. Mass spectra acquired from leaf sections with and without mildew were significantly different.

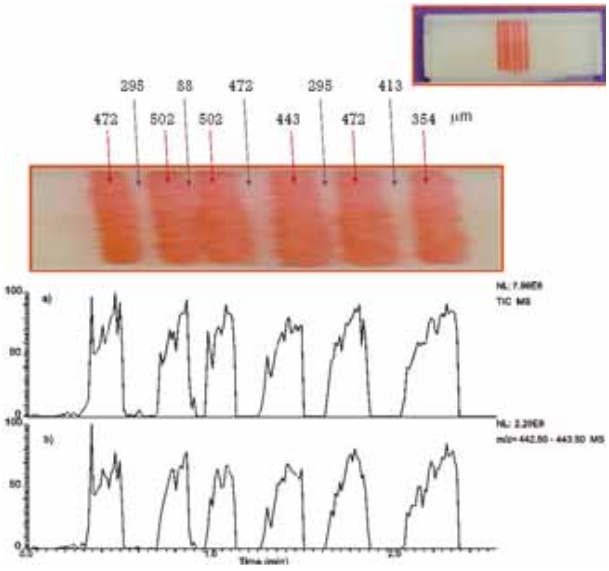

Figure Lines of Rhodamine B analyzed by nanoDESI with moving device, a) TIC, b) ion current of ion m/z=493 corresponding to Rhodamine B.

Conclusion
Desorption nanoelectrospray can analyze plant materials without sample preparation. New moving device for nanoDESI imaging allowed lateral resolution bellow 100 µm.

Novel Aspect
The new version of nanoDESI ionization allows mass spectrometry imaging and is useful for analysis of plant material without sample preparation.

The authors gratefully acknowledge the support by the Ministry of Education, Youth and Sports of the Czech Republic (project COST, LD13005).

MPS07-15 / Dependence of mass peak shape on r/r0 ratio in quadrupole mass analyser
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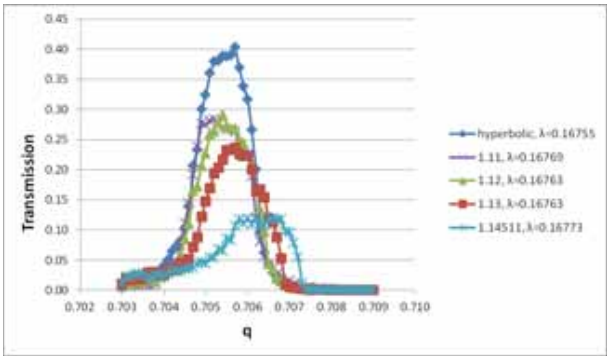
Introduction
The use of round rods in quadrupole mass analysers (QMA) arise a task of rods radius determination, to make best substitution of hyperbolic electrodes.

Earlier [1-4], a number of most successful QMA field radius to round rods radius (r/r0) ratios was indicated. In this work, a new view on peak shape vs. r/r0 ratio dependence, and possible ways to choose r/r0 ratio based on mass peak shape analysis are presented.

Methods
Using Boundary Element Method, potential distribution in electrode configurations of four rods inside round shield with various r/r0 ratios were calculated. After it, QMA spatial harmonics coefficients for each configuration were computed.
Peak shapes were determined by calculating ions trajectories considering realistic number of RF-field cycles and thirty calculated harmonic coefficients.
Trajectory calculations were performed by solving Mathieu equation, using Runge-Kutta–Nustrom–Dormand-Prince method.
It was proposed to assess field quality by harmonic coefficient a2, as a factor of "quadrupolarity", and k=sqrt(a12+a22+a32+...+aNmax2), as a factor of field "purity".
Harmonic coefficient a2 have nearest to 1 value at r/r0=1.112, while k reaches minimum at r/r0=1.1447. Consequently, the best r/r0 value should lie between these boundaries.



Results
Mass peak profiles for a number of r/r0 ratios were plotted. λ=a/(2q) parameter was chosen individually for each r/r0 ratio to obtain equal peak width at half height. Resulted profiles were overlapped at one drawing.
It was discovered, that approaching to lower boundary of r/r0 range increases transmission and improves peak low-mass boundary, but deteriorates peak high-mass boundary.
On the other hand, approaching to higher boundary of r/r0 range deteriorates transmission and peak low-mass boundary, but improves peak high-mass boundary. Peak tip shape becomes best at the middle of the range.

Conclusions
The choice of r/r0 ratio in the range from 1.112 to 1.1447 should be determined by tasks, which is to be solved by QMA, and by specified mass peak shape requests.
A simple averaging of high and low range boundaries gives compromise value of r/r0=1.12835, which agrees with previously obtained results [3-4].

Novel Aspect
Factors, that determine a choice of r/r0 ratio, were proposed. The influence of r/r0 ratio on peak boundaries and peak tip was considered.

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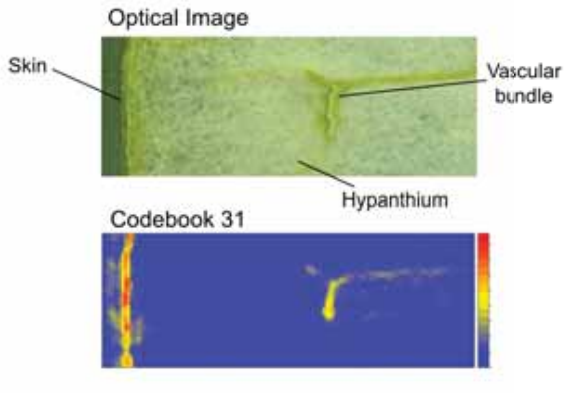
MPS07-16 / Self-organizing maps: A versatile tool for the automatic analysis of untargeted imaging datasets
Pietro Franceschi, Ron Wehrens
Fondazione E. Mach

Introduction
Mass Spectrometry Imaging (MSI) experiments constitute the ideal complement to metabolomics to investigate the spatial distribution of key metabolites. In spite of their caveats and limitations, they generate highly informative datasets, which are difficult to mine mainly due to their sheer size. In this contribution we illustrate how self-organising maps (SOMs) could be efficiently used to automatically analyze spatial information in MSI untargeted metabolomics datasets. In our approach, SOMs are used to identify a shortlist of m/z signals sharing a common, characteristic and interesting spatial distribution, thus labeling them as "biomarkers" for an area of the section. Additionally, the proposed algorithm can be used to process the raw data and extract high-resolution information on interesting ions.

Methods
Untargeted full scan (m/z 120 - m/z 700) imaging experiments were performed on apple (Golden Delicious) sections with a MALDI LTQ Orbitrap XL mass spectrometer with a resolution of 60.000. The CHCA matrix was deposited by using an ImagePrep station. Raw data were converted into the open CDF format and analyzed with a set of algorithms developed in R.

Results
The proposed algorithm has been applied to the imaging dataset collected on the apple section (Figure) to identify 42 characteristic spatial distributions. The one grouping the ions which show a high concentration in the region below the apple skin and in correspondence of one of the apple bundles is shown in Figure. The SOM algorithm associates to this spatial class a list of 35 ions. For 17 of these ions, it was possible to to associate them to secondary metabolites known to be present in apple in this specific area.



Conclusions
SOMs form a versatile tool for the untargeted analysis of high-resolution and high-accuracy MSI metabolomics datasets where they can be used to automatically identify spatial patterns and assess co-localization among different ions. This co-localization can be used to improve the chemical selectivity of imaging experiments, giving important tissue-specific information.

Novel Aspect
With the proposed algorithm, SOMs are used to associate the thousands of signals collected over the tissue to a limited number of characteristic spatial distributions. The ions belonging to the same spatial class are co-localized and they can be used in combination to mass spectra libraries and in-silico fragmentation engines to perform (partial) chemical annotation.

P.Franceschi, R Wehrens, PROTEOMICS Special Issue: Tissue Proteomics and Imaging Mass Spectrometry Volume 14, Issue 7-8, pages 853–861

MPS07-17 / Tissue Surface Properties Jeopardize Quantitative DESI Imaging of Organic Acids in Grapevine Stem
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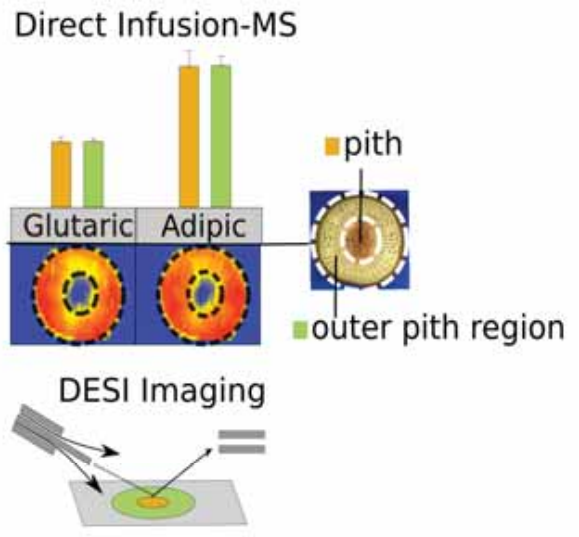
Introduction
DESI imaging has recently gain popularity as a tool to assess spatially resolved biological processes and to assist biomarker identification over unmodified sample surfaces, but how surface properties affect the output of DESI imaging experiments has not been investigated to date. We addressed this issue in a series of experiments which studied the distribution of small organic acids in grapevine stems. In our investigation, we compared the spatial distribution of endogenous and xenobiotic compounds obtained by DESI with the one resulting from the conventional analysis of the sections. The specific effects of the surface properties on the DESI detection of this class of compounds was also investigated by DESI profiling on different PTFE surfaces.

Methods
DESI imaging of endogenous and xenobiotic organic acids in grapevine stem was performed using a Thermo-Fisher Scientific LTQ Orbitrap XL mass spectrometer equipped with an OmniSprayTM ion source under negative ion mode with spatial resolution of 200 µm. Ion chromatography and direct infusion-MS were used to quantify the endogenous and xenobiotic organic acids in grapevine stems, respectively. DESI profiling of a mixture of organic acid standards was done with the same instrument on 3 PTFE surfaces with different pore size and porosity.



Results
DESI imaging showed that the distribution of malic (endogenous), glutaric (xenobiotic) and adipic (xenobiotic) acid were significantly different between pith and out pith region. This specific distribution was not confirmed by IC and direct infusion, which indicated a rather uniform distribution over the tissue section. DESI profiling results on the PTFE surfaces suggest that the local physical properties of the tissue surfaces strongly affect the ionization process as well as their relative quantitative detection.

Conclusions
Different surface properties within a structurally/biologically heterogeneous tissue can affect the quantitative detection of analytes resulting in MS images misrepresenting the true distribution of the analytes.

Novel Aspect
As in the case of MALDI, the outcomes of DESI imaging experiments could be affected by the local properties of the tissue sample. Experimental results, then, have to be carefully validated.

MPS07-19 / Development of new stigmatic imaging mass spectrometer and its application for surface analysis of high functional organic materials
Jun Aoki, Hisanao Hazama, Kunio Awazu, Michisato Toyoda
Osaka University

Introduction
Measurement methods of spatial distribution of molecules such as proteins and drugs at cellular-scale are required in many fields including pathology, pharmacology, etc. Recently, scanning type imaging mass spectrometry (IMS) with matrix-assisted laser desorption/ionization (MALDI) is intensively used for biomolecular analysis. However, the spatial resolution of scanning MALDI-IMS is limited by the laser focus diameter to about 10 - 100 µm. Therefore, we are developing a stigmatic MALDI imaging mass spectrometer, in which spatial resolution of sub-micron can be achieved irrespectively to the laser focus diameter.

Methods
The experimental apparatus for stigmatic imaging consists of MALDI ion source, a multi-turn time-of-flight mass spectrometer (MULTUM-IMG) and a time and position sensitive delay line detector. Ion distributions at the sample plate are magnified and

projected with the ion optical lens system onto the detector. The ion optical system of MULTUM-IMG satisfies the perfect spatial and temporal focusing condition, so that the spatial distribution of ions can be conserved after circulation.

Results
We applied this new apparatus to several practical applications, for example surface analysis of high functional organic materials. Conventionally, secondary ion mass spectrometer (SIMS) is mainly used for the surface analysis. However, in the case of analyzing the organic materials composed of large number of atoms, fragmentation of molecules caused by ion bombardment in the ionization process of SIMS prevents to acquire the information of original molecular structure. So soft ionization method, for example MALDI, is required for these analysis. By using our new stigmatic imaging mass spectrometer, soft ionization and high spatial resolution of 1μm can be realized simultaneously. We observed three samples of organic materials; organic light-emitting device (OLED), organic thin-film solar cell and high functional polymer for a coating material of smart-phone's screen. The molecules on the surface of these devices can be ionized without fragmentation, and observed as high spatial resolution images.

Conclusions
The distributions of organic materials were imaged with high mass and spatial resolution. This analysis technique is useful for development of the organic materials, by evaluating performance of efficiency and durability, deterioration process and manufacturing quality.

Novel Aspect
Molecules of high functional organic materials was ionized without fragmentation, and observed as high spatial resolution image by using new stigmatic imaging mass spectrometer.

MPS07-20 / A new ion beam based on water clusters for ToF-SIMS imaging

Nick Lockyer[1], Sadia Sheraz[1], Taylor Kohn[1], Irma Berrueta Razo[1], Andrew Barber[2], John Vickerman[1]
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Introduction
In secondary ion mass spectrometry molecular imaging the achievable resolution is limited not by the size of the primary ion beam, but by the secondary ion yield of the analyte. Hence there is great interest in improving secondary ion yields, including the development of new types of primary ion projectile. Cluster and polyatomic projectiles have largely replaced atomic ions for molecular ToF-SIMS analysis over the last decade. More recently massive gas cluster ion beams (GCIBs) have been developed for ToF-SIMS applications, most commonly based on Arn clusters (n=500-4000). Evidence to-date suggests that these Ar-GCIBs, like smaller polyatomics, provide enhanced molecular sputter yield, but do not significantly enhance the ionisation process itself. Separate studies have shown that the incorporation of water into the sample can increase the ionisation efiiciency in the SIMS process. We have therefore developed a GCIB source based on (H2O)n clusters to investigate whether we can combine these effects to bring about further increases in secondary ion yield.

Methods
We have developed an (H2O)n GCIB source (n=1-10000) using a supersonic jet expansion technology. The source currently operates up to 20 kV with a focused probe size of 10 micrometers. The sputter yields and secondary ion yields of (H2O)n+ primary ions have been compared, on a number of representative biological compounds, to Arn+ and C60+ to establish the relative performance characteristics. Experiments were performed on a J105 buncher-ToF SIMS instrument (Ionioptika Ltd, UK) operating with continuous primary ion beams.

Results
Under conditions of low (H2O)n+ fluence (1011 ions/cm2) secondary ions yields are enhanced by a factor ~4 compared to C60+ whereas under higher fluence the enhancement increases to a factor 10-50. Sputter yield values for (H2O)n+ are comparable to Arn+ and C60+ and follow the predictions of theory.

Conclusions
Our data is consistent with an enhanced ionisation efficiency for biological molecules under (H2O)n+ bombardment. The water-based GCIB source shows promise as a new ion beam for molecular SIMS imaging applications where spatial resolution is currently limited by sensitivity.

Novel Aspect
We have developed a novel water-based GCIB source for molecular SIMS imaging.

MPS07-21 / Identifying the distribution of chlorpromazine and its metabolites in mouse liver samples using a newly developed Imaging Mass Microscope: iMScope

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[1]Shimadzu corporation, [2]Shimadzu Europa GmbH

Introduction
Chlorpromazine is an antipsychotic medicine which sometimes causes side effects such as hepatopathy in case of long treatment. MALDI Imaging Mass Spectrometry (IMS) helps visualizing the distribution of bio molecules and drugs in organs. The previous data that we have shown using iMScope (a unique instrument coupling microscope and IMS) have helped to confirm basic specifications, but also to get several imaging data of human and mice organs with distribution of Chlorpromazine. Here, we demonstrate the use of a new version iMScope with higher spatial resolution and new statistical tools to study also metabolomics.

Methods
IMS analysis were performed using an atmospheric pressure (AP) MALDI-IT-TOF mass spectrometer (iMScope, Shimadzu). Dosed tissue sections were surgically removed from C57BL/6J (male, 8weeks of age) mouse liver 24 h after treatment with chlorpromazine (250mg/kg). Samples were frozen in dry ice acetone and, then sectioned at a thickness of 10 μm and transferred to an ITO slide glass (Sigma).

Results
The distribution of chlorpromazine and related metabolites could be clearly visualized in a mouse liver sample following a dose of 250mg/kg. As chlorpromazine is extensively metabolized in the liver and kidneys by cytochrome P450 isozymes CYP2D6 (major pathway), CYP1A2 and CYP3A4 both the hydroxlyated gluronic acid and demethlyated metabolites were also detected. Given the dose was 250mg/kg parent and metabolite drug were also found in blood vessels of the liver at a spatial resolution of 20um in addition to recognized markers of liver hepatopathy. Differential analysis of the control and dosed tissue samples also highlighted the influence of chlorpromazine on endogenous lipid distributions using integrated software tools over a mass range of 400-1000m/z in positive ion mode.
The iMScope approach helped to visualize not only the distribution of chlorpromazine and metabolites in tissue but also markers of liver

hepatopathy and differences in lipid profiles between control and dosed liver samples.

Conclusions
Imaging Mass Spectrometry is a powerful tool to visualize the distribution of drugs and matabolites in tissues. Bringing together optical microscopy and mass spectrometry on one platform can also be used in differential metabolomics analysis.

Novel Aspect
iMScope, an instrument coupling microscope and IMS, allow to visualize drugs and bio molecules successfully within biological tissue sections from drug dosed mice organs compared with control ones.

MPS07-22 / Effect of elastic collisions on state of an ion cloud confined in an RF quadrupole trap elucidated by the temporal invariance method
Yves Zerega, Aurika Janulyte, Jacques André
Aix-Marseille University

Introduction
When ions confined in electrodynamics traps collide with buffer gas, their spatial and energy properties are modified. As the knowledge of these properties at equilibrium is a great concern, the temporal invariance method, developed in the 80's by the authors for a 3D ion trap [1,2], is now improved and can be applied to other devices such as LIT.

Method
The temporal invariance method addresses the spatial and energy properties of confined ions subjected to collisions with neutrals at equilibrium.
This method states that the ion cloud recovers the same statistical properties for regular time interval Tm, a multiple of the RF confinement voltage. It is assumed a family of distribution functions representative of the ion state for the positions and velocities, functions characterised by both a shape and associated parameters.
Integral equation (with 12 integration variables) has to be numerically solved with a Monte-Carlo method, requiring trajectory computation only over 0 to Tm. It must be founded (1) if the function family is suitable, then (2) the associated parameters values. The previous works concerned the confinement in an infinite 3D ion trap, with centred Gaussian as distribution functions.

Results
The improvement of the variance reduction method using a control model of the trapping device allow one to reduce the number of ions (computation time) necessary to obtain a same accuracy. This method has been tested with the harmonic potential depth model as the control model associated to the 3D Paul trap, with trajectories computed by solving Mathieu equations.
The confinement of Cs+ ion subjected to collisions with He, Ne and Ar as buffer gas has been tested. We founded that, even with He, the distribution is not Gaussian: the tail of the distribution function is higher. That agrees with the results given by DeVoe, which have proposed a Tsallis distribution [3]. Hence, with a finite trap, an ion loss can occur by collision with the walls and the probability of dissociative collision increases with the collisional gas mass.
This time reduction is necessary to apply the method to more realistic devices. For instance, the program is being adapted to integrate the data coming from trajectories computed using CPO [4]: it has been tested for ions confined in a 3D ion trap truncated at 6r0 (quasi-pure trap).

Conclusions
The variance reduction method obtained by using a control model overcomes the computation slowness of the ion trajectories obtained for realistic confinement devices.

Novel Aspect
The use of a model control allows us to increase the efficiency of the Monte-Carlo computation strongly for light buffer gas atoms. The program is able to integrate the CPO trajectories computation for realistic Paul traps preluding future works for LIT.

Acknowledgements
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MPS07-23 / Development of a novel Two Channel Off-axis Ion Funnel Trap (TCOAIFT) for use in a FTICR Mass Spectrometer
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The Chinese University of Hong Kong

Introduction
The overall sensitivity of a traditional ESI interface is mainly limited by the ion transmission efficiency of skimmer cone and the ion trapping capacity of the hexapole. Although the several geometries of electrodynamic ion funnel, including conventional ion funnel (IF),[1,2] V-shape ion funnel (V-IF) and two-channel off-axis ion funnel (TCOAIF),[3] were developed to replace the skimmer cone to improve ion transmission efficiency, the ion trapping capacity of the hexapole becomes the an important factor for limiting the sensitivity of the instrument. Ion funnel trap (IFT) developed by Smith [5-6] could substantially improve the sensitivity and ion utilization efficiency for TOFand IMS.This presentation aims to report the development of a new version of ion funnel trap based on the two-channel off-axis ion funnel (i.e. TCOAIFT).

Methods
TCOAIFT uses a high-transmission grid on the terminus electrode of the drift section in the ion funnel channel (IFC) to form a trapping section.A pulse voltage was used to control the ion accumulation and ion ejection events. This device was use to replace the traditional skimmer cone and was used to trap and to transmit ions across the medium pressure region (i.e. 1-10mbar) in a Fourier transform mass spectrometer (FTMS). Several peptides and proteins were used to evaluate the robustness, sensitivity gain and ion trapping capacity of the TCOAIFT.

Results
TCOAIFT could enhance the robustness of FTICR instrument by reducing the contamination of the transfer ion optics.The ion signal intensities of the tested peptide samples using a newly cleaned ESI source could be sustained reproducibily over a week. Physically, the trapping volume of TCOAIFT was ~5 times of that of a hexapole ion trap. Experimentally, a factor of 3 times enhancement of the ion trapping capacity was obtained by TCOAIFT versus the hexapole ion trap under identical conditions. Two orders of magnitude enhancement in signal intensity for low concentration samples were demonstrated in TCOAIFT. The S/N ratios for angiotension II and substance p were enhanced by factors of 30-50. Collision-induced dissociation of peptides

and proteins could be achieved in TCOAIFT by elevating the rf-potential. The fragment ions derived in the conjoined region of the TCOAIFT could also be trapped efficiently.

Conclusions
TCOAIFT is a unique multi-functional device for efficient ion transmission, ion trapping and ion fragmentation in medium pressure.

Novel Aspect
Two channel off-axis ion funnel trap; ion trapping capacity; contamination reduction; collision-induced dissociation

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MPS07-24 / Improvements of TOS-SIMS mass spectrometry imaging: high spatial resolution combined with high mass resolution and high sensitivity for relative quantification
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CNRS ICSN

Introduction
TOF-SIMS using cluster ion source is now recognized as a powerful method for in situ chemical, biological and medical applications. TOF-SIMS imaging provides the incomparable advantages of a routine micrometer scale resolution with a simple sample preparation. However, it suffers from some limitations, such as lack of sensitivity and poor mass resolution when the primary ion beam is focused below 1 µm.
We propose to address these two limitations by implementing a delayed extraction and by summing the ion signal over several µm depths. Finally, we evaluated the repeatability of TOF-SIMS imaging in order to determine if relative quantification is possible on tissue sections.

Methods
A TOF-SIMS IV mass spectrometer (ION TOF GmbH) equipped with bismuth and argon cluster ion sources was used to record lipid ion images on rat brain sections.
For the repeatability study, three adjacent brain sections from four rats were successively analyzed. Each brain hemisphere was imaged separately and variability from left and right parts was determined. Mean data were then compared for the four animals.

Results
The delayed extraction can be implemented on TOF-SIMS instruments without instrumental modification. Using a focused ion beam of about 400 nm, the mass resolution has been measured as a function of the delay of extraction. In the positive ion mode, mass resolutions up to 10 000 and 9 000 are obtained for phosphocholine [C5H15NO4P]+ (m/z 184.1) and cholesterol [M+H-H2O]+ (m/z 369.4) ions, respectively. The optimized delay of extraction is similar in the positive and negative ion modes leading to mass resolution ten times higher than the ones described in the literature for the highly focused primary ion beam mode. This experimental value was compared to simulation data from SIMION software. This mode can produce well-contrasted ion images and differentiate Purkinje cells on brain sections.
Massive cluster ion sources of argon have recently been used for TOF-SIMS imaging on biological surfaces allowing organic depth profiling. We propose here a new way to enhance the sensitivity by one or two orders of magnitude, by simply adding the recorded surface images over several µm depths.
Finally, repeatability of TOF-SIMS imaging was evaluated over 4 different rat brains and 3 successive tissue sections. Coefficients of variation were always below 10% indicating that TOF-SIMS imaging is highly reproducible and allows relative quantification.

Conclusions
Delayed extraction and 3D-imaging using massive argon cluster allows increasing mass resolution and sensitivity both at least by a factor of 10. A repeatability of TOF-SIMS analysis better than 10% allows relative quantification of lipid species on tissue sections in two different states (healthy versus diseased samples …).

Novel aspects
Delayed extraction and 3D-imaging are used to improve mass resolution and sensitivity of TOF-SIMS. An excellent repeatability allows relative quantification on tissue sections.

MPS07-25 / Direct Ion Imaging with Active Pixel Detectors
Shane Ellis, Anne Bruinen, Ron M. A Heeren
FOM Institute AMOLF

Introduction
There exists a constant desire to increase the analytical capabilities of MSI. These capabilities are largely determined by the sensitivity and spatial resolution that can be achieved for a given instrumental setup. In conventional microprobe MSI spatial resolution is limited largely by the ability to focus the surface laser/ion beam to a sufficiently small spot and the size of the matrix crystals. One approach for decoupling the spatial resolution from the laser/ion beam spot size has been the use of microscope mode MSI. In this approach a position sensitive detector is employed and the ion optics designed such that the spatial distribution of ions striking the detector surface is a magnified image of their distribution on the surface. By decoupling the probe size from the spatial resolution, this approach promises significant improvements in both speed and sensitivity. Here we describe recent progress in the field of direct ion imaging MSI using the Timepix detector on two different TOF instruments employing both UV photons (MALDI) and C60+ primary ions (cluster SIMS).

Methods
Experiments were performed on both a Bruker Ultraflex III MALDI TOF/TOF and a Physical Electronics Triple Focussing Time-of Flight (TRIFT) designed for stigmatic ion imaging and equipped with a C60+ primary ion source. A 2x2 array of Timepix detectors positioned behind dual microchannel plates was employed for ion detection. Each Timepix chip contains a 256x256 pixel array (55 µm pitch) that each record the impact position and TOF of an impinging ion per laser/ion beam pulse.

Results
Implementation of the Timepix detector on the Ultraflex III allows for the efficient detection of highm/zions [1]. Although not designed for stigmatic imaging, the ability to visualise the focussing properties of the ion source allows the optics to be optimised for stigmatic imaging of a chosenm/zrange. This is demonstrated by analysis of a protein mixture spanningm/z 22000-66000 where a spatial resolving power of ~11-16 µm is demonstrated within a laser diameter of ~125 µm. Furthermore,

this geometry/detector combination also facilitates imaging of metastable ion decay along the flight path.
Results obtained using the TRIFT stigmatic imaging mass spectrometer equipped with a C60+ primary ion source for SIMS will also be presented. Here the advantage of high resolution SIMS analysis of complex surfaces with the softer cluster ion source is demonstrated and enhanced using the direct imaging capabilities of the Timepix detector.

Conclusions
These results highlight the capabilities of active pixel detectors to improve the spatial resolution and sensitivity of MSI. The decoupling of spatial resolution from the laser/ion beam size shows promise as a means of circumventing the inherent increase in analysis time with increasing spatial resolution encountered in conventional microprobe MSI. In addition, we have demonstrated for the first time the stigmatic ion imaging of intact proteins withm/zvalues exceeding 20000.

Novel Aspect
Direct imaging of ions on the detector surface of a linear-TOF using active pixel detectors.

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MPS08 - Carbohydrates

11:00-15:00
Poster Exhibition, Level -1

MPS08-01 / Plasma protein N-glycosylation profiling by LC-MS of glycopeptides after depletion of 14 high abundant proteins

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Human plasma is a complex matrix containing hundreds of proteins being potential biomarkers for major diseases and congenital disorders of glycosylation (CDG). Unfortunately, glycoproteomic studies of human plasma yields data most of the time dominated by high abundant proteins and information about low abundant proteins is suppressed. Therefore, a simple and high throughput preparation method is developed to reduce sample complexity by cleaning and depleting the sample of the 14 most abundant plasma proteins.
Fourteen high abundant proteins are captured from human plasma using camelid antibody domains immobilized on agarose beads dividing the sample in two fractions, one containing the abundant proteins and one containing the lower abundant ones. Glycoprotein fractions are subjected to proteinase treatment followed by HILIC enrichment of glycopeptides and LC-MS/MS analysis. The method is developed in such a way that its future robotization is possible.
Going further in the direction of automation of the analysis, a multipurpose software with graphic user interface (GUI) is being developed to improve data processing and automate assignment of glycopeptides as well as matching deglycosylated peptide MS/MS data.
We envision that this workflow, relying on protein depletion and LC-MS analysis of glycopeptides with software-supported structural assignment, will be valuable for glycoproteomic biomarker discovery of autoimmune diseases and cancer.
Keywords: Glycosylation, plasma, biomarkers, depletion, proteins, automation, bioinformatics

MPS08-02 / Characterization of biotherapeutic protein glycosylation with structure-specific LC/MS/MS

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Recent advancements in biotherapeutic protein design have underscored the importance of glycosylation towards optimal drug bioactivity. The presence/absence of certain glycosyl modifications and structural motifs, as well as their abundances in a biopharmaceutical preparation, can significantly affect drug stability and immunogenicity. However, the inherent structural diversity of glycosylation significantly hinders analysis. We propose isomer-specific LC/MS/MS screening as a method for rapid identification and structural elucidation of biopharmaceutical glycosylation. To aid this process, we have developed a database of diagnostic fragment peaks and expected peak abundances that analysts can use to quickly annotate MS/MS data in a user-independent manner.
Biotherapeutic glycoproteins including mAbs, recombinant proteins (such as EPO), and fusion proteins were prepared and analyzed. After removing salts and detergents, N-glycans were enzymatically released and purified by graphitized carbon solid phase extraction. Native N-glycans were comprehensively analyzed without any need for chemical labeling or derivatization. Chip-based porous graphitized carbon nano-LC/Q-TOF provided chromatographic isomer separation profiling and structural characterization. Accurate mass MS was used to compositionally annotate and profile the N-glycans, while CID MS/MS was used for structural elucidation. A database of diagnostic MS/MS fragment masses and expected abundances was developed and applied to identify and structurally characterize biopharmaceutical glycans.
To develop a CID MS/MS fragment database, archetypal glycans bearing common bioactive motifs were prepared from biotherapeutic glycoproteins and analyzed by isomer-sensitive nano-LC/MS/MS. LC gradients as well as MS and CID instrumental parameters were optimized. MS/MS spectra were screened for diagnostic fragments that could be used to predict the presence or absence of specific glycan modifications and/or structural motifs. Database entries include diagnostic fragment peaks for sialic acid O-acetylation, mannose phosphorylation, NeuAc vs NeuGc sialylation, etc. (For purposes of illustration, all m/z are given for positive mode, protonated MS/MS spectra.) Also contained in the database are diagnostic precursor ion losses for galactose-alpha-1,3-galactose. Finally, energy-resolved fragment peaks are specified for high mannose glycosylation, polylactosaminylation, core vs antennal fucosylation, etc.
The database was applied to structurally characterize glycans from biosimilars and biobetters produced by various cell-based expression systems. We were successful in identifying atypical and/or contaminant glycosylation on various types of biotherapeutic glycoproteins.

Novel aspect
A database of CID diagnostic fragments enables rapid LC/MS/MS screening and characterization of biotherapeutic glycosylation.

MPS08-03 / A microfluidic chip-based strategy for biopharmaceutical glycome analysis

Hyun Joo An[1], Serenus Hua[1], Gregory Staples[2], Youngsuk Seo[1], Myung Jin Oh[1], Rudolf Grimm[2]
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Glycosylation plays an important role in ensuring the proper structure and function of most biotherapeutic proteins. However, bioactive glycan modifications such as sialylation and phosphorylation add significant complexity to an already-diverse biopharmaceutical glycome. This diversity can greatly complicate glyco-analytical efforts during drug development, production, and regulatory approval. To address this issue, we have developed a novel microfluidic chip capable of online capture, enrichment, and LC separation of both sialylated and phosphorylated glycans. Biopharmaceuticals agalsidase beta and darbepoetin alfa (EPO) were selected as archetypal phosphorylated and sialylated glycoproteins, respectively. Following N-glycan release, digests are purified by PGC solid-phase extraction and injected onto a novel microfluidic LC chip. Injected glycans pass through three integrated chip components, described in sequence: 1) a two-layer enrichment column packed with titanium dioxide (TiO2) and porous graphitized carbon (PGC); 2) an analytical column packed with PGC; and 3) a nano-ESI spray tip. Following a single injection, neutral and phosphorylated/sialylated species are separately eluted in two consecutive chromatographic runs and characterized by mass spectrometry.

Initial validation of the chip design confirms that the two-layer enrichment column separates sialylated/phosphorylated and neutral glycans. Analysis is initiated by injection of a glycan mixture onto the two-layer enrichment column. Sialylated and phosphorylated glycans are trapped and enriched by the TiO2 layer of the enrichment column, while neutral glycans flow past the TiO2 layer and are trapped by the PGC layer of the enrichment column.

Following enrichment, a water/acetonitrile gradient elutes the neutral glycans from the PGC layer of the enrichment column onto the PGC analytical column, which further separates them prior to MS analysis. LC/MS data confirms that only neutral glycans are eluted during the initial chromatographic run.

Next, sialylated/phosphorylated glycans are eluted from the TiO2 layer of the enrichment column by a high-pH aqueous elution buffer. These glycans are subsequently trapped by the PGC layer. A water/acetonitrile gradient then elutes the sialylated/phosphorylated glycans from the enrichment to the analytical column for LC/MS analysis. LC/MS data confirms that only highly acidic glycans are eluted during the second chromatographic run. Over 95% sialylated/phosphorylated glycan recovery was achieved from the TiO2 layer of the enrichment column (as compared to a similar chip design with only a single-layer PGC enrichment column). Glycan abundances between the double- and single-layer designs were highly correlated ($R > 0.96$).

This chip-based strategy for biopharmaceutical glycan analysis increases chromatographic peak capacity, compresses dynamic range, enhances MS sensitivity, and improves glyco-analytical capabilities.

Novel Aspect

A microfluidic chip combines selective capture of sialylated and phosphorylated glycans (or glycopeptides) from complex mixtures with subsequent nano-LC/MS analysis.

MPS08-04 / Searching the interaction of carbohydrates with Z- and E-sinapinic in solid MALDI samples

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Successful application of MALDI-MS started with the introduction of commercial cinnamic acids as matrices. Rational design and synthesis of new cinnamics have been described too. Because the presence of a rigid double bond in its structure cinnamics can exist as two different geometric isomers, the E- and Z-forms. Commercial available cinnamics used as matrices are the E-isomer. As a new rational design of matrices, Z-cinnamics were synthesized and their properties as matrices studied. Their performance was compared with that of the corresponding E-isomer and classical matrices (DHBA, THAP, norharmane) for the analysis of carbohydrates. We demonstrated the good performance for Z-SA. Furthermore molecular modeling (DFT) of the optimized geometry and stereochemistry of E- and Z-forms suggested some factors governing the analyte-matrix interaction. This complementary tool showed that as consequence of the geometry change in the alkene rigid bond moiety, the stereochemistry of the matrix molecule changes dramatically and at molecular level the analye-matrix interaction changes too. In the first part of this study MALDI mass spectra were obtained in experiments conducted with sample prepared using the mixture and the thin layer method. Thus, to better understand the quite different performance of each geometric isomer as matrix the physical and morphological properties as well as the photophysics in solid state were studied. In order to get additional information about analyte-matrix interaction at molecular level in solid state, fluorescence and phosphorescence microscopy for solid surface scanning, solid flash-photolysis and MALDI-MS imaging of the same solid sample surface were complementary used. For sample preparation the mixture and sandwich method were used together with the called picotapa method because a picoL of analyte solution was loaded on the air-dried matrix layer. The picoL volume was manipulated with the Pressure Probe under microscope. Both, E- and Z-SA did not show fluorescence emission. For our surprise, when carbohydrates such as β-cyclodextrin or maltoheptaose were used as analyte, and sample was prepared by mixture method, fluorescence emission of the solid sample was detected. The new fluorescence observed was not homogeneously distributed all over the surface. As a clear ring with quite intense emission in its border when Z-SA was used and as few tiny weak florescent spots distributed at random on the surface when E-SA was used. The shape and bright of fluorescent image obtained depends on the matrix, and for each matrix the final image depends on the analyte too. The fluorescent image matched with the highest intensity analyte signals in the MALDI MS image.

Changes in the photophysics of cinnamic acids in solid state by carbohydrates correlate with cinnamics behavior as MALDI matrices.

MPS08-05 / Improving the quantitation of carbohydrates for metabolite profiling of biological extracts by GCMS.

Deshmukh Sandeep, Birkemeyer Claudia
University of Leipzig, Germany

Introduction

Carbohydrates and intermediates such as organic acids and sugar alcohols are central constituents in all forms of life. Apart from their nutritional value they serve as structural components and participate in various cellular processes. However, interference with matrix components often troubles to correctly quantify these metabolites in biological tissues. For example, the presence of phosphoric acid in biological samples was shown to decrease

the sensitivity of carbohydrate GCMS analysis. We anticipated to selectively remove phosphate and focused in particular its separation from biologically important organic acids; solid phase extraction (SPE) protocols were applied to screen anion exchange (AEX) sorbent materials for this purpose.

Methods
A mix of 13 different carbohydrates and organic acids in 1.5 mM phosphate buffer was applied to 17 silica- and polymer-based AEX-SPE cartridges. Formic acid with and without 100 mM sodium fluoride at different pH (pH 2.4 and 3.8 and 2% FA in methanol) was tested for compound elution. The eluates were driedin vacuoand subsequently treated with methoxyamine in pyridine followed by N-Methyl-N-trifluoroacetamide before GCMS analysis. The traditional approaches of phosphate metal precipitation and selective compound derivatization were compared to the results obtained with SPE. The final SPE protocol was applied to yeast cells to assess its potential use in GCMS metabolite profiling.

Results
Among the different SPE protocols tested on aqueous samples, polymer-based materials provided the best compromise between efficient phosphate removal and quantitative recoveries of organic acids compared to silica-based materials; in addition to that, polar organic solvents such as methanol were the best choice for satisfactory compound elution. Furthermore, non-volatile additives such as NaF proved to be incompatible with the subsequent protocol for GC derivatization.
In contrast to the SPE protocol, poor recoveries of organic acids were obtained by selective derivatization protocols; for precipitation of phosphate with metal salts recoveries of organic acids and carbohydrates were insufficient as well. Though phosphate could be removed to an acceptable extent, the non-volatile precipitate was again incompatible with the subsequent derivatization protocol for GCMS.
The final protocol was successfully applied to GCMS metabolite profiling of dried yeast cells.

Conclusions
Application of SPE successfully achieved selective phosphate depletion improving the detection of carbohydrates by eliminating the suppression effects in GCMS analysis.
The developed protocol can be used for GCMS metabolite profiling of biological matrices such as yeast.

Novel Aspect
For the first time, a protocol has become available for selective removal of phosphate as a frequent, often highly abundant compound in bioassays enabling accurate GCMS analysis of biologically important.

MPS08-07 / Mass spectrometric profiling of patatin glycoconjugates and their alterations with genotype variability

Erika Lattova[1], Adela Brabcova[2], David Potesil[1], Jan Barta[2], Zbynek Zdrahal[1]
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Introduction
Protein glycosylation in plants has a significant impact on physico-chemical properties and their biological functions. Most of plant N-glycans carry β1,2-xylose (Xyl) attached to the β-linked mannose residue on the common conserved Man3GlcNAc2 core and α1,3-fucose (Fuc) on GlcNAc reducing end. These saccharide epitopes are absent in mammalian glycoproteins and they are known to be highly immunogenic. Therefore using plants as the production factories for therapeutic proteins requires modification of their N-glycosylation pattern. Different methods and techniques can be used to study the structural characteristics of glycoforms to provide information. Among them, mass spectrometry is the most sensitive and powerful technique to differentiate glycoform fingerprints in patatins isolated from potatoes of different genotypes.

Methods
Patatin samples of selected potato species were digested with trypsin or PNGase A. To enhance sensitivity and improve structural characterization, a modified version on-target derivatization with phenylhydrazine (PHN) was applied. All fractions were analyzed principally by MALDI-TOF/TOF-MS instrument (UltrafleX III, Bruker) operated in the positive reflectron mode. Individual parent ions were manually selected for LIFT (MS/MS) experiments. MSn data were recorded on Orbitrap ELITE instrument (Thermo Fisher Scientific, Waltham, MA, USA) equipped with Nanospray Flex Ion Source using direct infusion.

Results
The data obtained from mass spectrometric analysis provided evidence for differences in profiles of patatin N-glycans. The typical glycan plant Man3(Xyl)GlcNAc2(Fuc) was dominant in the spectra of all samples and patatins isolated from species of Desiréeshowed solely the presence of this single structure. In proteins of other genotypes additional oligosaccharides were found. The second abundant structure corresponded to the complex glycan with an addition of GlcNAc residue - GlcNAcMan3(Xyl) GlcNAc2(Fuc). In some variants increased level of shorter fucosylated forms was observed. Two patatin variants, from genotypes of speciesSolanum stenotomumandSolanum andigenumexhibited also peaks corresponding to high-mannose structures (Man7-9GlcNAc2).

Conclusion
MS results showed that genotype variability can impact the glycosylation in patatins and if it is desired the glycan profile can be beneficially targeted and may have different biological function.

Novel Aspect
Mass spectrometric investigation of glycoconjugates towards enhanced quality of patatin potato cultivars.

Acknowledgments
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MPS08-08 / A nanoESI-QTOF MS approach for screening and sequencing of underivatized dextran chains containing up to 35 glucose repeats

Mirela Sarbu[1], Roxana Ghiulai[2], Alina Zamfir[3]
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Introduction
Dextrans are linear polysaccharides made up of repeating (α1-6)-linked poly-D-glucose; all have (α1-3) branches, and some also present (α1-2) or (α1-4) branches. The length of the dextran chains can vary, exhibiting molecular weights from 1,000 Da to 2,000 kDa. Dextrans have various clinical and pharmaceutical applications and uses. Being long chain polysaccharides,

structural analysis of dextrans in their native state by electrospray ionization (ESI) mass spectrometry (MS) is a challenging task. They exhibit poor ionization, yielding in the mass spectrum mainly singly charged ions, which hampers the detection of high molecular weight species.

Methods
Our protocol is based on high resolution MS performed on a hybrid quadrupole time-of-flight instrument (QTOF MS) with nanoelectrospray ionization (nanoESI). For nanoESI QTOF MS experiments, the sample used in this study as a model substrate was a polydisperse dextran mixture of an average molecular weight of 4,000 (Dex 4), which was prepared for MS by dissolving the dry sample in deionized water to a concentration of 5 pmol/µL. All mass spectra were recorded in the positive ion mode. MS/MS was performed at low ion acceleration energies using argon as the collision gas. The product ion spectrum represented a sum of scans acquired at variable collision energy (CID) within 30-80 eV range.

Results
By the developed and applied approach and using only pure water as a solvent, we were able to induce the formation of multiply charged molecules, from singly to quadruply charged ions related to chains containing up to 35 Glc repeats in their native state. Glc35 represents the longest chain ionized and detected by ESI without previous derivatization and/or separation prior to MS. Under carefully optimized conditions, we have detected over 100 ions corresponding to species containing from Glc2 to Glc35 repeats. By MS/MS employing CID, the ions at m/z 1,409.48, 1,107.35 and 1,438.47 assigned to [G17+2Na]2+,[G20+H+Na+K]3+and [G35+2H+Na+K]4+were sequenced and reliably characterized. The optimized fragmentation conditions induced efficient ion dissociation with high sequence coverage and ions diagnostic for the chain length. Hence, in this study MS/MS was successfully applied for the first time to fragmentation analysis of an underivatized chain containing 35 glucose repeats.

Conclusions
We reported here on the first development of a high resolution method for screening and sequencing of underivatized long chain polysaccharides. The method feasibility and benefits were demonstrated on a polydisperse mixture of long chains consisting of glucose repeats which was reliably characterized due to the beneficial combination between the high sensitivity and mass accuracy provided by nanoESI-QTOF MS.

Novel aspects
We proposed here a novel and straightforward method based on nanoESI-QTOF MS and CID MS/MS for structural analysis of long chain underivatized dextrans.

MPS08-09 / Preparation and Characterisation of Chito - Oligosaccharides by MALDI-TOF MS and Size Exclusion Chromatography (SEC-MALLS) for Biomedical Applications

Mariam Martin Mnatsakanyan, Emerson Ferreira Queiroz, Olivier Jordan, Gerrit Borchard, Jean-Luc Wolfender
University of Geneva

Chitosan is a biodegradable natural polysaccharide with extensive applications in biomedical industry (antifungal, drug delivery, gene delivery, surgical adhesives, etc.). Chitosan derived oligosaccharides (COS) depict even more unprecedented biomedical properties due to their chemical heterogeneity and thus can be regarded as a natural source for bio-functionality. The link between the biomedical properties of chitosan/COS and their chemical heterogeneity in molecular weight (Mw), degree of polymerisation (DP) and degree of acetylation (DA) is well postulated, thus making it necessary to define the "ideal" MW, DA or DP contributing to a given bio-functionality.
In this study, a mixture of COS with different DP for further screening as cholesterol binding candidates were successfully generated by acidic degradation of chitosan from different geographical regions and comprehensively characterised by analytical size exclusion chromatography with multi-angle laser light scattering detector (SEC-MALLS) and matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-TOF MS).
The Mw distribution measured by SEC-MALLS indicated decrease in Mw from ≈1.2+104 to 6.05+103 after 7 hours acidic degradation depicting depolymerisation of all tested original chitosan into their respective COS's. The degree of distribution of COS (DP) by MALDI-TOF was between 2 and 32 indicating that chitosan acidic hydrolysates were a mixture of dimers, trimers, tetramers, etc. The reproducibility of Mw measurements was shown by comparing SEC and MALDI-TOF MS analyses. Furthermore, ionisation and fragmentation pattern of oligosaccharides as a function of their DA was evaluated for the first time by MALDI-TOF and MS/MS analyses.
Overall, the usefulness of such multi-dimensional approach to chemical characterisation is enormous as each technique provided complementary and accurate information about the chemical heterogeneity of derivatised COS.

MPS08-10 / Automated Glycan Assignment Using Accurate Mass Measurement with a Calibrated Retention Time in Glucose Units

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Introduction
Glycan profiling is often performed using HPLC or CE with a fluorescence (FLR) detector. Identification of the glycans is done separately using a mass spectrometry. Each analytical approach has its advantages and limitations. We bridged the merits from both analytical techniques and developed an unique informatics tool, which allows direct LC/FLR/MS data acquisition and glycan identification.

Method
The N-linked glycans from the innovator/biosimilar candidate Infliximab were enzymatically released and labeled 2-aminobenzamide (2AB) tag. Glycans released from 10 µg protein were injected onto an analytical scaled hydrophilic interaction liquid chromatography (HILIC) column packed with sub 2 µm particles. The HILIC UPLC was directly interfaced to a FLR detector and a QTOF MS system in tandem without a flow splitter. The time offset between the two detector was automatically corrected. The scientific library utilized contains experimentally obtained GU values of 308 common N-linked glycans from serum and several biotherapeutic proteins including Infliximab.

Preliminary Results
For each N-linked glycan analysis, a retention time calibration standard, 2AB-labeled dextran ladder was run prior to the Infliximab N-glycan samples. The dextran ladder chromatographic peaks were used to generate a retention time curve, and this curve was automatically applied to all peaks from an «unknown» sample to generate a GU value based on the retention time. The accurate mass measurement was performed simultaneously for each chromatographic peak. Since the FLR and MS peaks were time aligned, both the GU value and accurate mass were combined and used to search the glycan identification from the scientific library. We demonstrated the utility of this analytical system by performing glycan profiling and comparability murine cell lines

respectively. Our preliminary results clearly indicated glycan profile differences between the innovator and biosimilar Infliximab, most likely resulting from the differing cell lines (murine vs. CHO cell) used for innovator and biosimilar production. While both mAbs have consistent primary structure, the N-glycan profiles show several clear differences. Each Innovator infliximab batch contains approximately 1% Gal (1,3)α- galactose and 4% NeuGC containing N-glycans; these structures were not observed within candidate biosimilar batches. In addition to the absence of potential immunogenic N-glycans present in the innovator, hybrid-type glycans were also largely absent from the biosimilar candidate. Besides glycan identification, relative quantitation and statistical analysis for each glycan component was also performed for multiple batches of the mAb.

Conclusion
This study shows a new integrated HILIC-LC/FLR/QTOF MS analytical system and informatics tool allows direct LC/FLR/MS data acquisition for glycan characterization and quantitation.
Novel aspect: Integrated HILIC-LC/FLR/QTOF MS analytical system with a built-in glycan scientific library for glycan analysis

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MPS08-11 / Negative Ion Mode ESI-LC-MRM-MS for Differentiation of Native Human Milk Tetra- and Pentaoses & Possible Application in Human-Milk-Typing

Marko Mank, Philipp Welsch, Bernd Stahl
Nutricia Research

Introduction
Within the complex composition of human milk, human milk oligosaccharides (HMOS) represent a major group of macronutrients. Interestingly, their functional impact on the developing neonate is far beyond nutrition as well comprising prebiotic, anti-infective or immuno modulatory effects. Differentiation between individual HMOS isomers such as neutral Lacto-N-Tetra- and Fuco-Pentaoses is still an analytical challenge but key to learn more about the functional role of these distinct structures e.g. in infant nutrition. Consequently, we aimed to develop an advanced µESI-LC-MRM-MS approach enabling reliable multi-compound HMOS analysis in human milk.

Methods
In a first step, different purified human milk Lacto-N-Tetraose- and Lacto-N–Fucopentaose-isomers were examined in negative ion mode by enhanced product ion (EPI) scanning on a 3200 QTRAP®-MS-system (ABSciex). Thereby, specific diagnostic HMOS fragment masses could be confirmed. They served to create a new µESI-LC-MRM-MS-application employing H2O-EtOH gradient elution via a 2.1x30 mm porous graphitic carbon (PGC) column (Thermo Scientific). Methodological applicability to diluted human milk samples was afforded by simple 3 kDa ultrafiltration prior to LC-MS HMOS analysis. The final method was tested with 4 pre-selected milk samples representing the 4 known human milk types as independently confirmed by high performance anion exchange chromatography (HPAEC).

Results
In the course of our EPI-MS experiments, we were able to confirm suitable diagnostic HMOS-CID-fragments for reliable differentiation of LNFP I, LNFP II, LNFP III, LNFPV, LNT and LNnT. Utilizing these diagnostic fragment masses in a new MRM-LC-MS-method, fast identification of 4 isobaric Lacto-N-Fucopentaoses (LNFPs) and 2 Lacto-N-Tetraoses (LNTs) in complex human milk samples could be achieved in parallel. Interestingly, four human milk samples, each representing one of the four known human milk types, could directly be distinguished from each other using our new method.

Conclusions
Our novel negative ion mode µESI-LC-MRM-MS method proofed to deliver unambiguous detection and distinction of isobaric Type I and Type II-core HM Tetraoses (LNT & LNnT) and 4 fucose-positional pentaose-isomers (LNFPI-V). Applicability of this concept to real world samples after minimal sample pretreatment was demonstrated and cross validated by HPAEC analyses. Since different human milk types exhibit characteristic patterns of individual LNFP- and LNT-isomers, rapid human milk typing may thus be facilitated.

Novel aspect
Identification of native constitutional HMOS isomers utilizing a novel µESI-MRM-LC-MS concept in negative ion mode. Taking advantage of highly diagnostic MRM transitions, structural assignment of HMOS is retention time independent and may be considered as useful for rapid human milk typing in future.

MPS08-12 / Bottom-up characterization of a monoclonal antibody Trastuzumab with sheathless CESI-MS coupled to the Orbitraps mass spectrometers

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Introduction
Trastuzumab is a humanized monoclonal antibody used to treat metastatic breast cancer. In the US, the patent on Trastuzumab expires in 2014, which opens up the opportunity for worldwide biosimilar competition. The current FDA guidelines for approval of biosimilars require rigorous characterization of candidate heterogeneity, including glycoforms and degradation products, whose analysis relies heavily on mass spectrometry (MS). Capillary electrophoresis (CE) is well suited to the separation of such species due to their differences in charge states and Stoke's radii. Recent advances in electrospray ion source technology have allowed the sheathless coupling of CE to MS, eliminating the dilution effects of prior sheathflow techniques, potentially allowing complete sequence coverage and glycoform characterization in single runs with reduced sample manipulation.

Methods
Trastuzumab was analyzed by bottom-up (tryptic digest) CESI-MS. CE was performed using a Beckman CESI 8000 High Performance Separation System in tandem with a Thermo Scientific Elite equipped with ETD or a Fusion Tribid® mass spectrometer. The CESI 8000 was equipped with OptiMSTM technology, which by operating in an ultralow flow regime, allowed for improved sensitivity and reduced ion suppression. The data was analyzed with Byonic, Peaks PTM 7.0 and Proteome Discoverer 1.4 software.

Preliminary data
For both digestions, preliminary results showed 100% coverage of the light chain using both Proteome Discoverer and Byonic software. Heavy chain peptide coverage was challenging due to several short amino-acid peptides for both bottom-up and middle-down, but 100% sequence coverage was still achieved. For example, short amino acid sequences from the tryptic digest, such as TISK and DELTK, were difficult to identify, but still successful. Various protein modifications such as carbamidomethylation, deamidation, oxidation and N-glycosylation were all identified. The results of Byonic analysis of both ETD and HCD spectra demonstrated many of

the common glycoforms, including the most abundant G0F and G1F forms and also the less abundant non-fucosylated and sialylated forms. Using extracted ion chromatograms for MS1 profiles, we were able to additionally identify other commonly observed glycopeptides when MS2 data were not available. For example, 14 glycopeptide variants of the commonly observed miscleaved peptide K.TKPREEQYNSTYR.V were identified using both MS1- and MS2-based strategies. The migration order in CE was highly reproducible among glycan collections of a specific glycopeptide over multiple injections and capillary columns, adding confidence to the assignments by MS1 profile alone. Additionally, we were able to identify more glycopeptides, including the G2F form using the Fusion instrument. Shorter CE-MS runs on the Fusion can be potentially used to reach a similar or better depth of structural characterization achieved using the Elite instrument.

Novel aspect
Comprehensive characterization of Trastuzumab sequence and glycoforms was accomplished by combining sheathless CE with HRAM mass spectrometry.

MPS08-13 / Sulphoglycomics Made Easy: a Simplified for Procedure for N- and O-glycomics
Poh-Choo Pang, Stuart Haslam, Anne Dell
Imperial College London

Introduction
Matrix-assisted laser desorption ionisation time of flight (MALDI-TOF) mass spectrometry has proven to be a robust tool for the fast, high-sensitivity analysis of primary structure of complex mixture of glycans originated from various biological sources such as cells, fluids, tissues, and organs. However, detecting and analysing sulphated glycans using MALDI-TOF is often a challenge. Herein, we present a simple modification of our glycomic procedure to purify and analyse sulphated glycans from biological samples.

Methods
The strategy relies on the separation of sulphated glycans during chloroform extraction after permethylation. A variety of biological samples were tested (mouse thymus, spleen, kidney and brain; mucin protein MUC5B). Each sample was subjected to glycan extraction and purification as described previously. The various glycan pools were then permethylated and subjected to chloroform extraction. Aqueous and chloroform fractions were then further purified before MALDI-TOF analysis.

Results
Pools of permethylated glycans were successfully separated in the water–chloroform mixture. Sulphated glycans were selectively recovered from the aqueous fraction, while neutral and sialylated glycans remained in the chloroform fraction.

Conclusions
By testing N- and O-glycans isolated from various biological sources, we demonstrated that this method permits the specific purification of sulphated glycans from very complex mixtures of molecules.

Novel aspect
An improved method for sulfoglycomics.

MPS31-02 / The influence of different sample collection methodologies on blood metabolomic phenotype LC-MS/MS profiles
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Introduction
Blood is one of the most used biological fluids for research, diagnostic, health and drug monitoring. Nevertheless up to now its collection is not always performed under standardized conditions. Especially if the aim is the targeted analysis of endogenous metabolites, aspects like anticoagulants or sample storage by dried blood spots (DBS) and dried plasma spots (DPS), become crucial for the comparability of samples in large-scale studies. The aim of this study was the methodic screening of 180 metabolites by the BIOCRATES p180 Kit in samples stored under the five most common conditions (heparin, EDTA, citrate, serum, DBS and DPS) in order to determine their comparability. All the samples were collected from the same person on the same time.

Methods
According to the AbsoluteIDQ® p180 Kit standard procedure 10 µL sample and 10 µL ISTD mixture were pipetted on a filter spot in the 96-well kit plate. After a derivatisation step on the filter spot in the well with PITC, the analytes were extracted with 300 µL methanolic solution. The extract was filtered through the plate into a deep well receiving plate under light centrifugation. Afterwards the obtained extracts were analysed by the UHPLC-ESI-MS/MS method and a FIA-MS/MS method comprised in the kit.

Results
Our study showed that the different methodologies used for blood sampling do not affect the technical feasibility of the AbsoluteIDQ® p180 Kit process and the MS/MS analysis. However differences in the metabolomic profile were found between anticoagulated and non anticoagulated samples. From the different compound classes measured in the study, concentration differences related to the different sample collection methodologies could be identified especially for acylcarnitines and some amino acids.

Conclusion
Our data show that blood sample collection methodologies have an impact on mass spectrometric analyses, especially through ion suppression and by the formation of coordination complexes. In terms of sample standardization in metabolomics and biomarker research this fact has to be kept in mind when designing large scale experiments with samples coming from different institutions.

Novel Aspect
Systematic monitoring of 180 metabolites comparing five different sample collection methodologies performed with samples all deriving from the same person.

MPS31-03 / Developing a cancer diagnostics system: Towards on-site multi purpose gadgetry

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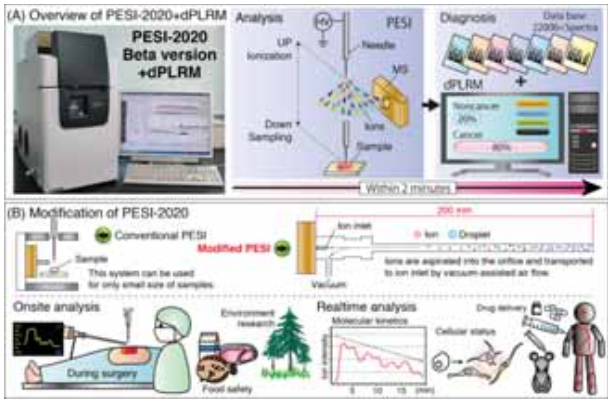
Introduction

Mass spectrometry becomes one of the most promising techniques in bio-medical fields over the last decade. We have developed a cancer diagnostics machine that present the clinical diagnosis of cancer on-site, by adapting an ambient ionization method and machine learning. While this technique does not require any kind of sample pretreatments, our current system cannot cope with the clinical requirements, where direct sampling from living human body is desired. In this presentation, we focus on the new system, where focal sampling in situ is realized.

Methods

Single quadrupole mass spectrometer (LCMS-2020; Shimadzu Co.) was directly connected to the probe electrospray ionization (PESI) module (PESI-2020). To establish the cancer diagnosis system, we employed the dual penalized logistic regression machine (dPLRM), which is a Bayesian inference-based learning machine that enables us to get a probabilistic prediction of cancer (Fig. A). This algorithm does not rely on the identification of molecular markers for cancer, as it comprehensively analyzes the whole spectra in certain mass windows. Furthermore, to optimize the PESI-2020 to on-site analysis, we improved the ion inlet by using a long tube made of stainless steel. The tube was connected to the mass spectrometer with vacuum system, which facilitated the uptake of ionized molecules. This modification enables us to place the ion orifice next to the specimens even if they were too large to install them on the sampling stage (Fig. B).



Results

We have analyzed surgically extirpated human renal cell carcinoma, hepatocellular carcinoma, gastric carcinoma and colonic carcinoma. Total of 22,770 spectra were used as a training dataset for dPLRM. To evaluate the applicability of PESI-2020+dPLRM to the cancer diagnosis, randomly selected blind test data were fed into the dPLRM. We obtained over 92 % coincidence to those diagnosed by the conventional pathohistological method. From another standpoint, to evaluate the ion detectability of modified-PESI, we analyze the mouse liver tissue. While elongation of ion transmission tube worsened the ion intensity, we achieved significant improvement by employing the vacuum system (6L/min). Under this condition, modified-PESI could obtain mass spectrum from living anesthetized mouse liver, strong enough for further analysis.

Conclusions

In this study, we validated the performance of PESI-MS-based diagnosis system. When analyzing the human cancers, we could obtain diagnostic outcome paralleled with pathohistological diagnosis. Furthermore, we confirmed the usefulness of modified-PESI system to living animal in situ. By using this machine, we will perform direct measurements on human body during surgical intervention. These results collectively show the possibility of future application of PESI–MS as an on-site diagnosis system in humans.

Novel Aspect

PESI-2020+dPLRM gave probabilistic diagnosis of human cancers, and diagnostic ability was approximately equal to the conventional pathohistological method. Moreover, we developed a modified-PESI system that uses a long stainless tube to collect ions generated at distance from the mass spectrometer.

MPS31-04 / Novel urine assay for predicting acute pancreatitis severity by MALDI-TOF mass spectrometry

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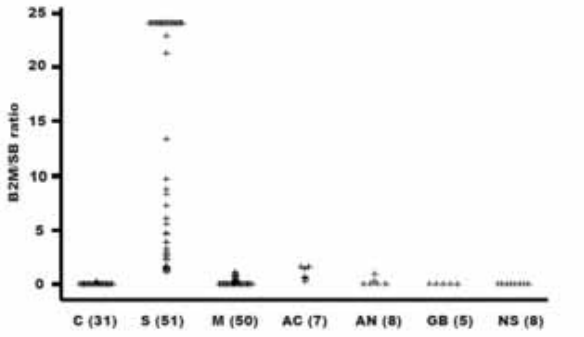
Introduction

The current methods for predicting the severity of acute pancreatitis (AP) are either complicated or universally unavailable. Similarly, most of the biomarkers of AP severity lack efficient sensitivity and specificity. Urinary protein profiling by MALDI-TOF mass spectrometry (MS) for predicting AP severity is advantageous owing to its easy sampling, high throughput, and low cost.

Methods

MALDI-TOF MS was used to rapidly acquire spectra in urine samples from AP patients. AP severity was determined by ratios of specific peaks on the MALDI-TOF spectra. Label-free quantitative proteomics by nanoLC-MS/MS was applied to the analysis of AP urine for analyzing the possible pathology of AP severity.



Results

Urinary protein MALDI-TOF MS profiling was used to classify AP patients into patterns S and M according to their signal intensity ratio of β-2 microglobulin (B2M) to saposin B (SB). Pattern S patients with B2M/SB ratio higher than 1.120 present a higher Ranson score, computed tomography (CT) grade, and longer hospitalization than pattern M patients with B2M/SB ratio of 0.040-1.102. Using B2M/SB ratio of 1.127, it is possible to predict severe AP with a sensitivity of 83.7% and specificity of 74.3%. The expression of urinary megalin, cubilin, and other proteins related to kidney injury and inflammation was found to vary in severe AP patients.

Conclusions:

B2M/SB ratio determined by MALDI-TOF MS can be used as an important and practical method to diagnose AP severity as well as to rapidly monitor AP progression. The proteomic study shows

that severe AP may cause enhanced urinary excretion of tubular proteins, which could be attributed to renal tubule injury.

Novel Aspect
We developed a novel urine assay based on MALDI-TOF for predicting AP severity.

MPS31-06 / Toward standardization of C-peptide measurement: Development of reference material and serum C-peptide measurement by isotope-dilution mass spectrometry
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Introduction
Human C-peptide which consists of 31 amino acids is secreted from the pancreas with the equimolar insulin to the blood. The measurement of C-peptide concentration in plasma is often performed to evaluate insulin secretion levels as clinical diagnosis. However, the results are not always comparable between laboratories and methods. To recalibrate and ensure the comparability, reliable calibrator and reference measurement method are required. In order to achieve standardization of C-peptide measurement, we have developed certified reference material (CRM) of C-peptide and measurement method as a candidate reference method by isotope-dilution mass spectrometry.

Results and Discussion
Development of C-peptide CRM:
C-peptide CRM was prepared as the lyophilized form of synthetic peptide having human C-peptide sequence. The certified values were determined by amino acid analyses. The sample was mixed with isotopically labeled amino acids, and quantified by pre-column derivatization LC-MS/MS and hydrophilic interaction chromatography (HILIC)-MS after acid hydrolysis. The certified values are (100 ± 5) mg/L as C-peptide and (102 ± 5) mg/L as total C-peptide (mixture of C-peptide, deamidated C-peptide, and pyroglutamylated C-peptide). This material is available as NMIJ CRM 6901-b.
Development of measurement method for serum C-peptide:
For measurement of serum C-peptide, a lack of sensitivity by LC-MS/MS analysis with conventional pre-treatment method is resulted in less reliable result. We have developed the immunoaffinity purification and chemical modification method for LC-MS/MS analysis. The immunoaffinity purification utilizes monoclonal antibody against human C-peptide that was immobilized on magnetic beads instead of conventional solid phase extraction. The purified C-peptide was N-terminally modified using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC). With this method, the LC–MS/MS peak area increased 23-fold compared with the conventional method. The limit of quantification was estimated to be 0.003 ng, which was lower than previously reported. By comparison with an established commercial immunoassay, this method using CRM 6901-b as the calibrant and isotopically labeled C-peptide as the internal standard showed high correlation (r2 = 0.9994) with the commercial immunoassay. The result shows that this method enables traceable analysis having a potential as reference measurement method.

Conclusions
The developments of both C-peptide CRM and the quantification method provide a platform for higher order reference method for standardization of serum C-peptide measurement.

MPS31-07 / Validation of UHPLC-MS/MS methods for the determination of kaempferol and 4-hydroxyphenylacetic acid in rat plasma, and application to pharmacokinetic studies
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Introduction
Kaempferol is a major flavonoid in the human diet and in medicinal plants, and has been shown to possess antidepressant properties when administered orally. The compound undergoes transformation into 4-hydroxyphenylacetic (4-HPAA) by the intestinal microflora [1]. However, the fate of the metabolite and its pharmacological effects in the body are largely unknown [2]. We here investigated the bioavailability of kaempferol and its metabolite 4-HPAA.

Methods
UHPLC-MS/MS methods were developed and validated according to international guidelines [3, 4]. 13C15-labeled kaempferol was selected as internal standard (IS) for quantitation of kaempferol, and 2H2-labeled 4-HPAA was selected as IS for quantification of 4-HPAA. To avoid matrix effects, phospholipids and proteins were removed via SPE (Waters Ostro 96-well plate) for analysis of kaempferol, whereas protein precipitation of bovine serum albumin (BSA) as a proxy matrix was used for 4-HPAA.
Kaempferol and 4-HPAA were administered intravenously (IV) in several doses (1-2-4 mg/kg) to rats. Blood samples taken from 0 to 12 h were analyzed by non-compartmental analysis using Phoenix WinNonlin (Certara, USA).

Results
Quantification range for both analytes was from 20.0 to 2000 ng/ml, and the response versus concentration data were fitted with a quadratic curve with 1/X as weighing factor. Carry over was within acceptance criteria (below 20%). Both analytes were stable in biological samples during sample collection and handling, during one month storage below -65°C and after three freeze and thaw cycles.
After IV application of 4 mg/kg, the clearance (CL) of kaempferol was high (4.06±0.18 L/h/kg), and the volume of distribution (Vd) was 0.4±0.03 L/kg. The terminal elimination rate constant (ke) and the terminal half-life (t½) were 10.35±0.41 h-1 and 4.2±0.003 min, respectively. The pharmacokinetic parameters of 4-HPAA were CL=1.04±0.08 L/h/kg, Vd=1.47±0.48 L/kg, ke=1.3±0.35 h-1 and t½=1±0.32 h.

Conclusions
The assays for kaempferol and 4-HPAA are specific, selective, precise, accurate, and capable to produce reliable results. The high CL, resulting in the high ke, and the short t1/2 suggest that kaempferol undergoes intensive metabolism. The relatively high Vd of its metabolite compared to the rat total body water content demonstrates a potential binding in a peripheral site, which allows 4-HPAA to exert a pharmacological effect [2].

Novel aspect
To avoid endogenous compounds from rat plasma as significant interferences, bovine serum albumin was used as appropriate proxy matrix for successful method validation for the metabolite 4-HPAA.

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MPS31-08 / Quantitative analysis of prostate specific antigen isoforms using immunoprecipitation and isotope dilution mass spectrometry

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Prostate specific antigen (PSA) is a widely used serum marker for prostate cancer (PCa). However, it has limited specificity for distinguishing early PCa from benign prostatic hyperplasia (BPH). More recently, promising data is emerging regarding proPSAs (including truncated proPSA forms, [-2], [-5], and [-7]) have been shown to be more cancer-associated than PSA. Multiple reaction monitoring mass spectrometry (MRM MS) has been frequently applied to measure low abundance biomarkers in tissues and biofluids, owing to its high sensitivity and specificity, simplicity of assay configuration, and exceptional multiplexing. In this study, we developed and optimized a method for an immunoprecipitation-based platform and MRM MS assay capable of sensitive and accurate quantification of proPSA in serum. Optimization was made in the immunoprecipitation and stable isotope dilution MRM MS workflow to achieve an adequate level of sensitivity. The efficiencies of immunoprecipitation and immunoaffinity depletion were also compared. The strategy we demonstrated that the target proteins can be enriched effectively; both limit of detection (LOD) and limit of quantitation (LOQ) of proPSA are able to achieve in sub nanogram/milliliter range, corresponding to a concentration that is 6-order lower than the concentration of the most abundant proteins in serum with good linearity. Furthermore, the simultaneous measurement of multiple biomarkers, including the mature and precursor forms of PSA can be performed in a single multiplexed analysis using LC-MRM MS. The strategy demonstrated here provides an attractive alternative for reliably measuring proPSA to improve the detection of PCa.

MPS31-09 / Evaluation of endocrine disruptors in H295R cells culture media with high-resolution mass spectrometry using a qualitative and quantitative steroidomic approach

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Introduction
The development of efficient methods to screen chemicals and evaluate their potential effect on human steroidogenesis is pursued by regulatory agencies. The aim of this study was to assess capabilities of high-resolution mass spectrometry for the simultaneous untargeted detection of steroids and quantification of selected key steroids in human adrenal H295R cells culture media to evaluate toxicity of triclocarban (TC), a suspected endocrine disruptor.

Methods
H295R cells were cultured according to the Organization for Economic Cooperation and Development guidelines and incubated 48 h with solvent control (DMSO) or TC at different concentrations (0.5, 1, 2.5, 5, and 10 µM; n=5 for each condition). The culture media of the incubated cells were precipitated and concentrated 20-fold using SPE HLB cartridges before injection in a ultra-high performance liquid chromatography (UHPLC, Acquity H-Class) coupled with high-resolution MS operated with an ESI source in positive mode (QTOFMS, MaXis 3G). Separation was performed in 16 min with an Acquity BEH C18 column (1.0 x 150 mm, 1.7 µm). Metabolomics data processing was performed using CoMet software (Nonlinear Dynamics); multivariate analysis was performed with Simca-P software (Umetrics).

Results
The analysis of steroids using MS techniques is not straightforward due to the low concentration exhibited in cells culture media. The sample preparation was devised to concentrate steroids in the most generic manner. Owing to the duty cycle time required to ensure adequate quantification performance, data acquisition was performed in TOFMS mode. Multivariate data analysis was performed using a chemical driven feature selection that filtered «steroid-like» compounds as a function ofm/zusing a tailored library composed of HMDB and Lipidmaps databases. In order to highlight and classify potential steroid perturbations using these selected features, two data analysis strategies were applied successively: Orthogonal-Partial Least Squares - Discriminant Analysis (OPLS-DA) and Correlation Analysis (CA). Among the 50 steroid-like compounds highlighted with OPLS-DA, 11 were identified with standard comparison and quantified. The 50 candidates were also investigated further using CA to distinguish early, medium and late decreasing steroids related to TC concentrations. Pregnenolone, 17α-hydroxypregnenolone and 11-dehydrocorticosterone demonstrated the earliest decrease while untargeted strategy highlighted 18 additional, yet unknown, features that exhibited medium and late decrease. The three earliest decreasing steroids correspond to upstream precursors in the steroidogenesis and are indicative of an early steroid biosynthesis disruption mechanism of TC.

Conclusion
A simultaneous screening and quantification method by UHPLC-TOFMS was developed for culture media analysis. This analytical strategy revealed key changes in steroidogenesis caused by TC. Further experiments will be performed for the screening of other potential endocrine disruptors.

Novel Aspect
Simultaneous untargeted qualitative and quantitative approach to perform steroid profiling with high-resolution MS to evaluate endocrine disruptors.

MPS31-10 / A validated quantitative LC-MS/MS method using isotopic MRM transitions to evaluate global ratios of modified cytosines

Makoto Tsuji[1], Hironori Matsunaga[2], Daisuke Jinno[3], Hiroki Tsukamoto[3], Naoto Suzuki[3], Yoshihisa Tomioka[3]
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Introduction
5-Hydroxymethylcytosine (5hmC) and 5-methylcytosine (5mC) represent important epigenetic modifications to DNA, and a sensitive analytical method is required to determine the levels of 5hmC in the genomic DNA of tumor cells or cultured cell lines because 5hmC is present at particular low levels in these cells. We have developed a sensitive LC-MS/MS method for quantifying 5-hydroxymethyldeoxycytidine (5hmdC), 5-methyldeoxycytidine (5mdC), and deoxyguanosine (dG) levels using stable isotope labeled internal standards, and used this method to estimate the global level of 2 modified cytosines in genomic DNA prepared from small number of cells.

Methods
Genomic DNA was hydrolysed and nucleosides were analysed with LC-MS/MS. Concentration ranges for 5mdC and dG is much higher than that for 5hmdC. MRM transitions for isotopologue (isotopologue-MRM) were used to quantify the 5mdC and dG levels because of the abundance of these nucleosides relative to 5hmdC. The use of isotopologue-MRM for the abundant nucleosides could avoid the saturation of the detector, and allow for all three nucleosides to be analyzed simultaneously without the need for the dilution and re-injection of samples into the instrument. The global ratios of modified cytosine nucleosides to dG were estimated following the quantification of each nucleoside in the hydrolysate of genomic DNA.

Results
The quantification limits for 5hmdC, 5mdC and dG were 20 pM, 2 nM and 10 nM, respectively. The limit of estimation for the global 5hmC level was less than 0.001% using 200 ng of DNA. Using this method, we found that MLL-TET1, which a fusion protein in acute myelogenous leukemia, did not produce 5hmC, but interfered with TET1 activity to produce 5hmC in cells.

Conclusions
Our analytical method is a valuable tool for further studies aiming at a deeper understanding of the role of modified cytosine in the epigenetic regulation of cells.

Novel Aspect
Isotopologue-MRM method may be a useful option for simultaneous quantification of abundant analytes and trace analytes. MLL-TET1 may acts as dominant-negative mutant form of TET1.

MPS31-11 / Biomarkers of autism spectrum disorders (ASD) based on the comparative analysis of the metabolite concentrations in saliva
Keiji Gamoh, Yoshinori Nishiwaki
Kochi University

Introduction
Autism spectrum disorders (ASD) are a behaviourally-defined group of neurodevelopment disorders characterized by impairments in social interaction and communication, and repetitive, overly focused behaviours. While the syndrome has been shown to be highly heritable, various theories have been presented suggesting both genetic and environmental factors, such as dietary and chemical exposures. Our interest was focused on the analytical chemical investigation of ASD based on a liquid chromatography/mass spectrometric (LC/MS) method. Salivary samples were used for the exhaustive analysis of biological metabolites. In the present study, we demonstrated the metabolic (exhaustive) analysis of the saliva of both ASD and controls using a reversed-phase separation mode and an electrospray ionization method of the LC/MS, in addition to the investigation of biomarkers of ASD based on a comparative analysis using multivariate statistics software, SIEVE.

Experimental
Saliva samples were provided from children of a primary school and a special needs school affiliated to Kochi University in Kochi city. Solid-phase extraction elutes of saliva samples were injected to the reversed-phased ODS column. The high resolution LC/MS system is composed by Accela (LC) and Orbitrap Exactive (Thermo Fisher Scientific). The flow rate was 0.2 ml/min with 10 µL injection. The ESI in positive/negative mode scanning in mass range 100-1,000 was optimized with the instrument settings. The multivariate statistics software SIEVEwas applied to the analytical data of the mass chromatograms to compare the analysis of the metabolite concentrations in saliva samples.

Results and Discussion
The distribution of m/z in the saliva sample chromatograms of autism groups andcontrols, which was statistically analyzed by SIEVE, was recorded. The detected compounds within the retention time tR= 1.5~2.5 mins were approx. 700 and the most of them had less than m/z 500. Statistical analysis by SIEVE of total ion currents, which were obtained from the saliva samples of autism groups and controls was also recorded. Over ten kinds of difference compounds were detected by SIEVE ($p<0.01$). Two compounds were detected as difference compounds and one of them was glutamic acid. The concentrations of glutamic acid, which were detected from autism groups and controls were compared: 1) Controls have significantly low concentration (p-value<0.005), 2) Average values of peak area of autism groups are about 3 times to controls, 3) Large variance of autism groups was observed. The differentially abundant metabolites in saliva between ASD children and controls were successfully detected by the combination of liquid chromatography/orbitrap-high resolution mass spectrometric analysis and SIEVE. One of them was identified as glutamic acid based on the data base of structural analysis software. The higher concentration of the glutamic acid of autism children than controls was significantly observed. Saliva might be used as a possible biological sample to determine an ASD marker based on the metabolomic analysis to etiologically elucidate ASD.

MPS31-12 / Comparison of three sample preparation methods for identification of bacterial pathogens from urine specimens by MALDI-TOF Mass Spectrometry.
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BioMérieux

Introduction
VITEK® MS is a MALDI-TOF (Matrix Assisted Laser Desorption Ionization – Time of Flight) mass spectrometer used for microbial identification. The spectral database aids identification through recognition of a unique fingerprint. Direct analyses on clinical samples such as blood cultures or urines can lead to better patient outcomes, optimized antibiotic therapy and cost reductions.

Methods
This study compares three urine-processing methods followed by MALDI-TOF MS analysis, which can reliably identify pathogens directly from urine specimens. The first approach involved a short urine culture on agar plates followed by a deposit of the bacterial lawn on MALDI-TOF target slides. The second approach consisted of filtering urines samples on a dual filtration system before deposition of the bacterial sediment on MALDI-TOF target slides. The third method is a well-described method based on differential centrifugation. Although being slightly longer, the short growth on solid medium is completely suitable for routine laboratory work. The two other methods are faster as performed directly from urines without culture. To compare the three methods, a blinded trial was performed with 19 clinical urine samples, respectively, in which bacterial counts were at least of 105 cfu/mL.

Results
The 5 hours culture on agar plates was found to be very efficient with the capacity to identify microorganisms in positive urines from 100 µL at 105 cfu/mL and to a less extent for urines at

103-105 cfu/mL. The biomass amplification on agar allowed to eliminate the defensins peptides that could negatively affect the identification by MALDI-TOF. The filtration solution generated fast and reliable identification results from 5 to 10 ml of urine sample with bacterial load ≥105 cfu/mL. The centrifugation approach was found to be less user-friendly and less efficient with the same urines panel presumably because it didn't remove the defensins peptides which peaks disturb the identification. Results from supplementary samples treated by each method independently confirmed these observations.

Conclusion

With a rapid turn-around time, the short growth and dual-filtration methods have the potential to optimize microbial diagnostics at the point of care level.

MPS31-13 / Chemical and biological study of human urinary biomarkers of dioxin exposure previously highlighted by metabolomics with high-resolution mass spectrometry

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As a reliable human biomarker of dioxin intoxication is missing, a metabolomic approach was carried out on urine samples of workers submitted to a severe dioxin occupational exposure. Untargeted analysis was performed with ultra-high pressure liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UHPLC-QTOF) and highlighted a dysregulation of steroids and bile acids. A subset of 24 biomarkers was further selected by the study of an acute dioxin poisoning; three of them were identified by comparison with authentic standards. The aim of the current study was i) to evaluate the 24 biomarkers in an independent cohort and, ii) to identify candidate biomarkers by purchasing available standards and synthetizing the missing products by in vitro metabolic reactions.

Urine samples were extracted on HLB cartridges and then analyzed with UHPLC-QTOF in ESI ion negative mode (Acquity UPLC & Xevo G2 QTOF, Waters). Features were selected with a "steroid" filter extracted from LIPID MAPS (conjugated steroid classes were selected) and submitted to multivariate data analysis (Markerlynx, Waters; SIMCA-P, Umetrics). Metabolic reactions of authentic standards were performed in human pooled liver microsomes (HLM) to generate potential matching candidates that were analyzed on the same instrument.

The 24 biomarkers obtained in the previous work were studied in an independent population exposed to the same toxic. Multivariate analysis separated a control group from this cohort that has lived around a waste incinerator with dioxin emission levels higher than the legal norm. The latter cohort had a long term low dose exposure in comparison to the previous cases of acute exposure, corroborating the 24 compounds as exposure biomarkers. A definite identification is necessary for a better understanding of toxic effects of dioxin.

The majority of the steroids excreted in urine are phase I and II metabolites. As numerous stereoisomers and structural isomers exist, their identification is challenging. Only a few steroids of the subset were commercially available. Among them, pregnanediol-glucuronide was successfully identified (retention time (RT) and m/z comparison). Other standards such as glycocholic acid didn't match the RT observed in real samples; additional investigations on isomeric structures are currently being performed.

When standards of potential biomarkers were not available as is, metabolism reactions were carried out in HLM on their postulated parent compound. Biosynthetized glucuronide conjugates of hydroxyandrosterone and hydroxyetiocholanolone (slightly chromatographically resolved isomers) were successfully generated and matched to the molecules detected in real samples. Additional syntheses with other isomeric precursors are under investigation to obtain the identification of the remaining postulated biomarkers.

In vitro metabolic synthesis and authentic standards allowed the identification of three further dioxin toxicity biomarkers; the biological validation is on progress.

Combining metabolomics with high-resolution mass spectrometry and metabolic approaches to discover and validate human biomarkers of dioxin exposure.

MPS31-14 / Preserve the integrity of tissue sample analytes by heat stabilization

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Denator

Introduction

The action of proteolytic and other protein-modifying enzymes rapidly change the composition of the proteome and post translational modifications (PTM) after sampling. Subsequent analytical results reflect a mix of the in vivo molecular status and degradation products and display increased inter-sample variation. Effective enzyme inactivation and standardization of sample handling eliminate this problem.

Methods

A heat-stabilization system has been used to generate rapid, homogenous thermal denaturation of enzymes to stop degradation in tissues. Comparisons were made to snap-freezing and inhibitors, and in time study manner, compared with different post-mortem intervals. Using mass spectrometry, Western blot, RPPA and activity assays, the protein and peptide content, including PTMs were examined.

Results

The results show rapid changes in phospho-states on a variety of different proteins detected only minutes after sample excision whereas in stabilized samples, levels remain unchanged during 2 hrs in room temperature. In three minutes post-mortem both proteins and endogenous peptides/neuropeptides, including PTMs, are subjected to substantial degradation. Conversely, amounts and identities of the detected proteins/peptides in stabilized samples show maintained integrity. Similarily, levels of pCREB, pGSK3β and pERK1/2 were unchanged for 2 hrs, whereas snap-frozen samples showed a dramatic decrease in levels after 10 min in room temperature.

Conclusions

Post sampling changes may distort our view of in vivo protein expression. Thorough suppression of enzymes e.g. proteases, lipases, phosphatases and kinases post sampling is important for the analysis results to reflect the in vivo status of the sample as closely as possible. Heat stabilization quickly and completely stops enzymatic activity by protein denaturation and thereby enables researchers to produce more relevant results.

Novel aspect

This approach may be of great help in disease research to differentiate true biomarkers from those found in any situation where cells are under stress.

MPS31-15 / SRM as a new efficient detection tool for the early diagnosis of the Lyme disease

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Introduction
Lyme disease is the most important vector-borne disease in the Northern hemisphere and represents a major public health challenge. It is caused by spirochaetes of the Borrelia burgdorferi sensu lato species complex, which are transmitted by ticks. The disease diagnosis is a real challenge but current techniques, which rely on bacteria culture and/or PCR, are still not efficient enough to ensure a rapid and valuable diagnosis. Therefore, we evaluated the power of SRM mass spectrometry as a new tool for the direct detection of Borrelia proteins in skin biopsies. We first optimized the method on a murine model, then we applied our strategy to infected human cutaneous samples.

Methods
As a first step, we identified Borrelia proteins in mouse skin samples infected by Borrelia burgdorferi ss by using a classical Ge-LC-MS/MS strategy. From this experiment we selected our proteins targets for the SRM approach. Proteins extracts were prefractioned onto SDS-PAGE and analyzed by LC-SRM mass spectrometry on a triple quadrupole in scheduled mode. We monitored 314 transitions corresponding to 66 peptides from 9 proteins. Finally we investigated direct LC-SRM analyses of protein extracts without gel prefractionation. For this purpose, we compared six protocols of sample preparation compatible with liquid digestion and direct injection in LC-SRM.

Results
After gel prefractionation (25 bands) and LC-SRM, we detected 10 peptides corresponding to 4 Borrelia proteins (OspC, flagellin, GAPDH and DbpA) in infected mouse skin samples. In particular we detected all the transitions corresponding to the six peptides followed for OspC, a protein essential in the early transmission to the vertebrate host. The GAPDH and DbpA peptides were near the LOD whereas the flagellin and OspC peptides were above the LOQ. By using an AQUA™ peptide of OspC, we estimated an amount at 600 fmol of this protein per mg of skin biopsy.
We applied the same strategy to three skin biopsies from human patients naturally infected by Borrelia afzelii. The detection of OspC was successful for all biopsies with a quantity of near 20 fmol /mg biopsy. The flagellin protein was also detected in one biopsy. After this proof of concept, we investigated a gel-free approach based on a liquid digestion of the skin protein extracts. The comparison of different protocols was performed on murine skin samples and led to the selection of a RapiGest™-based extraction. Finally we analyzed a human skin sample by using this shortened approach and we successfully detected OspC and flagellin.

Conclusion
These results constitute the first example of Borrelia detection in human skin samples by a SRM targeted approach.

Novel Aspect
This study constitutes the proof of concept of the ability of SRM for an early diagnosis of the Lyme disease.

MPS31-16 / Investigating Biological Variation in Human Hepatocytes of Phase I and II drug Metabolism Enzymes

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AB Sciex

Introduction
Measurement of drug metabolizing enzymes responsible for phase I and II biotransformations is a fundamental aspect of assessing drug-drug interactions, and evaluating drug safety and efficacy. In this work, we used SWATH Acquisition, a data independent acquisition method, to analyze protein expression levels of many of the enzymes involved in the drug metabolism.

Methods
A spectral ion library containing more than 2000 proteins was generated from data dependent analysis of a pooled sample. In the SWATH data generated using a TripleTOF® 5600+ system, an average of 1987 proteins, including 19 CYP proteins, 12 UGT proteins, and 7 GST proteins, were quantified across the 13 samples. The quantitative interpretation of SWATH data was achieved by automatic extracting characteristic fragment ions for each identified peptide from high resolution TOF MS/MS spectra. A set of protein/peptides of interest obtained from SWATH acquisition was then further analyzed with MRM using a QTRAP® 6500 System.

Results & Conclusions
Quantitative comparison of two phase II metabolism enzymes, EST1 - liver carboxylesterase 1 and EST2 - cocaine esterase, showed 40% variations across 13 samples. The relative intensities of 4 individual peptides of each protein showed very good agreement (<12% CV), which highlight the reproducibility of quantitation. Principal component analysis (PCA) was applied to discover proteins differing between the samples. Here, multiple proteins were found to be correlated, such as CYP3A4 and CYP3A5, which are two major phase I drug metabolism enzymes in cytochrome P450 superfamily. Previously published data also demonstrated the positive correlation between these two proteins [1]. Finally, very good correlation (<15% CV) was seen between the MRM and the SWATH acquisition data.

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Novel Aspect
Data independent acquisition for the quantitative profiling of a large number of proteins key in the investigation of drug metabolism.

MPS31-17 / Understanding Leishmania Life Cycle by MALDI-MS Profile and Chemometric Analysis

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Introduction
MALDI-MS is an important technique to microbial identification. There are plenty of work on bacteria, but few information for other microorganisms, such as protozoan [1]. Leishmania is responsible for the leishmaniasis, a neglected disease that causes 20,000 to 30,000 deaths and more than 1.3 million new cases per year. The clinical manifestations vary ranging from visceral to mucosal or diffuse cutaneous leishmaniasis. The former is caused by L. amazonensis, a species transmitted in the Amazon region [2]. Leishmania spp. life cycle involves promastigotes

that develop in the sandfly alimentary tract and amastigotes that replicate in mammalian host macrophages, requiring several physiological adaptations to these environments [3]. Here we compared L. amazonensis protein fingerprinting in amastigote and promastigote form, aiming to identify proteins and peptides unique to a particular life-cycle stage.

Methods
L. amazonensis cells were suspended in aqueous NaCl 0.9% and 1 mL was deposited in a MALDI plate, followed by 1 mL of matrix solution (CHCA in 50% acetonitrile and 2.5% TFA). The analysis was performed in an Autoflex III TOF/TOF mass spectrometer (Bruker, Bremen) using the FlexControl 3.3 software (Bruker Daltonics, Inc.). The spectra of 10 replicates of each form were compared by chemometric analysis in the software Pirouette 3.0 (Infometrix).

Results
L. amazonensis promastigotes and amastigotes MALDI-MS profiles showed significant difference by principal component analysis (PCA). The ions of m/z 6205, 6279, 6222, and 6296 exhibit the higher discrimination factors for amastigotes, whereas those of m/z 2133, 3180, 3593, 3923, 4241, 5025, 5110, 5831, 5854, 5875, 6352, 7184, 7342, 7841, 10026 are the main ions responsible for promastigote discrimination.

Conclusions
L. amazonensis protein profiles varied within the form. The diagnostic ions have been settled as stage specific and seem to be important biomarkers to the better comprehension of the complex biology of this parasite.

Novel Aspect
MALDI-MS fingerprinting offers a simple protocol to identify Leishmania life cycle biomarkers, accessing relatively low molecular weight proteins and peptides, whereas the majority of the current proteomic studies focuses on larger proteins. This is the first study of Leishmania life cycle by MALDI-MS fingerprinting.

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MPS31-18 / In vivo cytochrome P450 3A activity in a pregnant woman as measured by endogenous cortisol 6β-hydroxylation clearance

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Introduction
Cytochrome P450 3A (CYP3A) is involved in the metabolism of approximately 50% of all drugs currently prescribed to patients. We have previously reported a 1.5~3.2-fold day-to-day intraindividual variability in the CYP3A activity in healthy women (Drug Metab Dispos, 2013). CYP3A is under control of the nuclear pregnane X receptor (PXR) where estradiol acts as a ligand of PXR. During pregnancy, the increased plasma concentration of several hormones might play a significant role in modulation of CYP3A activity. Understanding the effects of pregnancy on CYP3A activity provides an indicator to adequately treat maternal disease and reduce the risk to the fetus. The aim of our study is to evaluate the effects of pregnancy on CYP3A activity using endogenous cortisol 6β-hydroxylation clearance (CLm(6β)) as a new index for in vivo CYP3A phenotyping in humans.

Method
Blood samples were obtained at 10:00, 11:00 and 12:00 each study day from a healthy woman prepregnancy, during 18-34 week gestation, and 16-55 week postpartum every 4 to 13 weeks. Urine samples were obtained for 2 h from 10:00 to 12:00. The study was approved by Tokyo University of Pharmacy and Life Sciences Human Subjects Review Board and written informed consent was obtained. Plasma concentration of cortisol was analyzed by GC-MS using [1,2,4,19-13C4]cortisol as an internal standard, followed by a bismethylenedioxy-3,11-dipentafluoropropionate derivatization procedure. The amount of 6β-hydroxycortisol in urine was analyzed by HPLC. Estradiol and progesterone in serum were measured by RIA.

Results
CLm(6β) was calculated as the amount of urinary excreted 6β-hydroxycortisol during 2-hour urine collection period divided by the corresponding AUC of cortisol. In this subject, the mean value for CLm(6β) for 40 days was 2.04±0.25 ml/min and 1.5-fold intraindividual variability of CYP3A activity was observed in the nonpregnant period. Plasma concentrations at the 34-week gestation were dramatically increased to 26600 pg/ml of estradiol and 182 ng/ml of progesterone and decreased to 12 pg/ml and 0.3 ng/ml at 16 weeks of postpartum, respectively. The mean plasma concentration of cortisol during gestation (218.7±44.8 ng/ml) was 3 times higher than that during the postpartum period (63.0±18.2 ng/ml).The mean value for CLm(6β) for 18-34 week gestation (3.02±0.38 ml/min) was 1.2-fold higher than 16-54 week postpartum (2.52±0.57 ml/min), but within intraindividual variabilities (1.5~3.2-fold) of CYP3A activity reported in our previous study. Further studies are necessary to clarify the effects of pregnancy on CYP3A activity.

Conclusion
There was a dramatically higher plasma concentration of estradiol during gestation, but the extent of CLm(6β) was a little higher during gestation than nonpregnancy and postpartum.

Novel Aspect
The time course data of CLm(6β) demonstrates a change in the CYP3A activity of the same individual throughout the pregnancy and the postpartum period.

MPS31-20 / Rapid identification of fungi of the genus Aspergillus using ribosomal protein biomarkers as observed by MALDI-MS

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Introduction
Aspergillus fumigatus and relating fungus cause a disease “aspergillosis”. The taxonomy of the Aspergillus species has been mainly based on their morphologic character and the ability of sexual reproduction, and therefore identification of fungal species has not been an easy task. Because susceptibility to antifungal agents is highly species related, rapid and accurate identification of fungi of the genus Aspergillus have been required. We have developed a new phylogenetic classification of bacteria species using MALDI-MS based on the variations of the amino acid sequences of ribosomal subunit proteins reflecting the molecular evolution. We have attempted to extend the ribosomal protein profiling by MALDI-MS to the taxonomic characterization of fungi. Ribosome of Eukaryote is composed of 78 kinds of

ribosomal subunit proteins together with 3 rRNAs. To make a reliable biomarker list, we have characterized ribosomal subunit proteins of several genome sequenced strains of A. fumigatus and relating fungus, and verified the translated amino acid sequences registered in public databases such as NCBI.

Experimental
Genome-sequenced strains, A. fumigatus Af293 and A1163 and relating teleomorphic species Neosartorya fischeri NRRL181 were used as the samples. Each strain was grown in PDB liquid medium at 37 °C for 4 days. The fungal mat was ground and ultra-centrifuged to obtain ribosome fraction. MALDI mass spectra of ribosomal subunit proteins were observed on an AXIMA CFR plus (Shimadzu, Japan) time-of-flight mass spectrometer. DNA sequences and translated amino acid sequences of ribosomal subunit proteins were obtained from NCBI and SwissProt via Internet.

Results and discussion
The calculated mass values of ribosomal subunit proteins of the genome-sequenced strain samples were checked by comparing with the observed mass by MALDI-MS. Surprisingly, only nearly half of subunit proteins were matched, even if considering possible post-translational modifications such as acetylation. The homology of amino acid sequences and mRNA sequences of other fungus suggested that incorrect amino acid sequences were caused by misidentification of intron/exon boundaries on the public databases. After a detailed manual inspection, the masses calculated from tentative correct amino acid sequences were verified by comparing with the observed masses. Finally, almost all of the ribosomal subunit proteins could be identified on the MALDI mass spectra. We have confirmed that several ribosomal subunit proteins are species specific. Therefore, ribosomal proteins would be useful biomarkers for rapid identification of the genus Aspergillus at the species level.

MPS31-21 / A novel index for assessing 3β-hydroxysteroid dehydrogenase activity in humans based on the measurement of dehydroepiandrosterone and androstenedione
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Introduction
3β-Hydroxysteroid dehydrogenase (3β-HSD) catalyzes the conversion of dehydroepiandrosterone (DHEA) to androstenedione (AD) in adrenals and gonads. The absence or decrease in the 3β-HSD activity results in the decreased synthesis of androgen. In general, non-classical 3β-HSD deficiency occurred in many hirsute women has been diagnosed using the serum AD/DHEA ratio as a marker of the adrenal steroidogenic responses to ACTH. However, the diagnostic criteria to differentiate the non-classical 3β-HSD deficiency from the polycystic ovary syndrome have not been clearly established. An accurate assessment of 3β-HSD activity is required for a diagnosis of non-classical 3β-HSD deficiency. The present study focuses on the metabolism of DHEA to AD in order to develop a novel index for assessing the 3β-HSD activity in humans.

Methods
Four healthy volunteers (subject 1, man; subjects 2−4, women) participated in the present study. The blood samples were collected every hour between 9:00 and 18:00. Trimethylsilylation (TMS) and pentafluoropropionate (PFP) derivatizations were used for the GC-MS analyses of plasma DHEA and AD (Yokokawa et al, J Chromatogr 2009), respectively. The plasma concentrations of DHEA and AD were measured by GC-MS (Shimadzu GC-MS QP2010) with stable isotope-labeled androgens as internal standards.

Results
The average plasma concentrations during the day in the four subjects were DHEA (4.55; 3.58; 6.79; and 3.95 ng/mL) and AD (0.53; 0.85; 1.55; and 0.97 ng/mL), respectively. The average AD/DHEA ratios were 0.12 (0.10−0.15; subject 1), 0.24 (0.17−0.33; subject 2), 0.23 (0.19−0.28; subject 3), and 0.27 (0.20−0.42; subject 4). The within-day inter-individual and intra-individual variabilities were observed to be 2.2-folds and 1.5−2.1-folds, respectively. A significant correlation between DHEA and AD was seen in all the four subjects; with their correlation coefficients ranging from 0.62 to 0.97. The slope on AD (y) vs DHEA (x) graph would correspond the extent of conversion of DHEA to AD, that is, 3β-HSD activity. The slope of the lines was higher in female subjects (0.141, 0.118, and 0.103) when compared to that of the male (0.081). Since the y-intercept (AD) corresponds the point at which there is no DHEA (x=0), the value can be regarded as the proportion of AD formed via routes other than 3β-HSD-mediated metabolism and/or the secretion process. This indicates that the conventional marker (DHEA/AD ratio)does not always reflect accurate activity of 3β-HSD in vivo in humans.

Conclusions
The present study demonstrates a significant correlation between DHEA and AD in plasma. Our method described herein serves as an index for assessing the 3β-HSD activity. However, further studies are required to clarify the accuracy of this method.

Novel Aspect
The present study proposes a novel index for assessing 3β-HSD activity in vivo in humans.

MPS31-22 / Ticagrelor effects on the adenosine pathway revealed by stable isotopes and mass spectrometry in a dog heart ischemia study
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Astrazeneca

Introduction
The aim of this study was to investigate the impact of ticagrelor on the metabolic fate of adenosine in the ischemic heart to explain the positive effect of ticagrelor beyond inhibition of platelet function, using stable isotope labeled adenosine and inosine locally administrated into the heart to exclude poorly controlled sources of adenosine (ATP)

Methods
Adenosine is a short acting endogenous substance released locally at sites of ischemia and trauma. Ticagrelor has been shown to inhibit adenosine cell uptake in-vitro. In this study we measure adenosine levels in the heart by micro dialysis before, during and after short periods of cardiac ischemia with and without ticagrelor or dipyridamole. An experiment was designed to allow investigation of locally administrated stable isotope labeled 13C10, 15N5-adenosine without interference from ATP metabolism. The adenosine and inosine stable isotope tracers were infused into the ischemic dog heart via the dialysate probe, also used to collect extracellular adenosine and its metabolites inosine and hypoxanthine. Endogenous and stable isotope labeled adenosine and metabolites were analyzed by liquid chromatography mass spectrometry.

Results
Inhibition of adenosine metabolism to inosine was observed when the adenosine transporter ENT-1 inhibitor dipyridamole

was co-administrated via the dialysate probe. Similarly, we also observed a dose dependent effect of ticagrelor-treated dogs on stable isotope labeled adenosine metabolism.. The kinetics of adenosine metabolism is unaffected by the ischemia itself as well as by elevated endogenous levels of adenosine released from ATP. Our results also indicate that conversion of inosine to hypoxanthine does not seem to involve ENT-1 since it was not affected by any treatment.

Conclusions
Our data support the growing amount of evidence that ticagrelor in addition to its P2Y12 antagonism also inhibit adenosine cell uptake through ENT-1 and thereby protect adenosine from its intracellular metabolism. The study design also shows the power of stable isotope labeling and mass spectrometry as a tool to explore pathways difficult to access by investigations of endogenously formed adenosine and metabolites.

Novel aspect
Dialysis probe technique in combination with stable isotope labeled metabolites and LC-MS/MS is a powerful technique to answer questions on metabolic mechanisms

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MPS31-23 / Detection of LPS modification as a biofilm signature using the VITEK® MS system
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Introduction
Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is commonly used as a tool for rapid identification of microorganisms in clinical and industrial fields. This technology may also prove to be of value for the detection of drug resistance mechanisms, biomarkers and virulence factors. Recently, a potential biofilm biomarker corresponding to a biofilm-associated palmitoylation of lipopolysaccharide (LPS) lipid A was identified by comparing biofilm and planktonic bacteria (Chalabaev et al., 2014; submitted). Here, we investigated whether this LPS structural modification could be detected and monitored using VITEK® MS Plus system (bioMérieux)

Methods
Bacterial cultures of Escherichia coli, Klebsiella pneumoniae, Serratia marcescens, Citrobacter koseri and Pseudomonas aeruginosa were monitored in trypcase soy liquid medium during several days. LPS lipid A was isolated from bacterial cells as described by El Hamidi et al. 2005. Extracts were then analyzed on VITEK® MS system, with linear negative acquisition mode. Instrument parameters were adjusted to optimize the detection in the mass range 1000 – 3000 m/z. 1µl of lipid A extract was deposited on the target and covered with the same amount of matrix 2,5-dihydroxybenzoic acid (DHB) suspended at 10 mg/ml in a 0.1 M solution of citric acid. Biofilm formation was analyzed from bacterial cultures by crystal violet staining method (Christensen et al. 1982).

Results
VITEK® MS analysis shows the possible detection of lipid A and modified lipid A peaks for all species tested. The ratio of peak intensities between the native lipid A and the modified lipid A forms were calculated, showing that lipid A palmitoylation increases during incubation time (Ratio at exponential phase < Ratio at 3 days < Ratio at 8 days) and correlate with biofilm formation as confirmed by crystal violet staining. By contrast, no lipid A palmitoylation was detected in liquid exponential phase growth of corresponding bacterial cultures.

Conclusion & Novel Aspect
This study demonstrates the potential of VITEK® MS for detection of a newly described widespread characteristic of Gram-negative bacteria biofilms. While sensitivity and specificity issues will need to be addressed regarding detection of palmitoylated lipid A in clinical settings (clinical studies in progress), the detection of this biofilm biomarker by MALDI-TOF MS could constitute a rapid and cost efficient approach to assist clinicians in evaluating the extent of biofilm-associated risk of medical device infections,by a wide diversity of microbial pathogens, therefore unnecessary preventive treatment of misdiagnosed biofilm infections.

MPS31-24 / The utilization of LC-MS/MS methods in diagnosis of cystathionin- β-synthase deficiency.
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Introduction
Cystathionine β-synthase (CBS) deficiency is a genetic disease affecting the metabolism of the sulfur containing amino acid homocysteine to cystathionine. Diagnostic hallmarks of this disease are grossly elevated concentrations of plasma total homocysteine combined with decreased plasma concentrations of amino acids cysteine and cystathionine, with varying elevations of plasma methionine, S-adenosyl methionine (SAM) and S-adenosyl homocysteine (SAH) levels. In our laboratory we employed a panel of LC-MS/MS methods enabling the diagnosis of CBS deficiency in patients with hyperhomocysteinemia by determination of metabolite profile and enzyme activity in plasma. Here we present the utility of these methods in diagnosis of CBS deficiency in a patient with hyperhomocysteinemia and show the effect of pyridoxine therapy on the metabolite levels and plasma enzyme activity.

Methods
All LC-MS/MS methods were carried out using positive electrospray ionization and selected reaction monitoring mode.
1) LC-MS/MS determination of amino acids (cystathionine, methionine) after derivatization with alkyl chloroformates (EZ:faast kit, Phenomenex, USA).
2) LC-MS/MS determination of SAM and SAH using chromatographic separation of the metabolites on a Hypercarb column filled with porous graphitic carbon stationary phase.
3) CBS enzyme assay in plasma using stable isotope substrate 2,3,3-2H serine. CBS activity in plasma was determined by LC-MS/MS analysis of the product of the enzyme reaction – 3,3-2H-cystathionine - after derivatization with alkylchloroformates ((EZ:faast kit)

Results
The determined levels in the patient's plasma before and after treatment with pyridoxine, respectively: total homocysteine (393 and 28 µmol/L, resp.) methionine (129 and 23 µmol/L, resp.) cystathionine (133 and 156 nmol/L, resp.) SAM (315 and 55

nmol/L, resp.) SAH (228 and 72 nmol/L, resp.) and CBS activity (27 and 120 nmol/hour/L)

The control reference ranges:
Total homocysteine (3.5-15 µmol/L) cystathionine (80-1000 nmol/L) methionine (12-40 µmol/L) SAM (30-160 nmol/L) SAH (15-80 nmol/L) and CBS activity (100-1000 nmol/hour/L plasma).

Conclusions
In a patient with hyperhomocysteinemia CBS deficiency was suspected. The diagnosis was confirmed after metabolite profile and CBS activity determination in plasma and by subsequent DNA analysis. This patient carries pyridoxine responsive mutation and the metabolite levels and CBS activity in plasma were normalized after the start of the pyridoxine therapy.
Novel aspect:
We present the usefulness of LC-MS/MS methods in diagnosis of CBS deficiency and in monitoring the effects of pyridoxine therapy on metabolite and enzyme activity levels.

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MPS31-25 / Improved Sample Preparation and HPLC/MS Analysis of Vitamin D Metabolites from Human Plasma

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Sigma-Aldrich

Introduction
The Analysis of Vitamin D metabolites has continued to be a topic of interest in current research, because those serve as biomarkers for possible disease states and for vitamin sufficiency. Vitamin D is present in two forms, vitamin D2 and D3. Important derivatives in the determination of vitamin D are the 25-OH vitamin D3 and D2. In 2006 the occurrence of epimeric forms of 25-OH vitamin D3 and D2 that are biologically inactive was discovered. Current ELISA methods cannot distinguish these forms of the vitamin D metabolites resulting in a reporting of a total 25-OH vitamin D. Additionally in LC/MS the 25-OH vitamin D3 and D2 cannot be resolved by mass spectrometry because they are isobaric.

Methods
In this work, an LC/MS method for the analysis of Vitamin D metabolites was developed to include dihydroxy metabolites along with the epi-homologs. Special focus of this development was the chromatographic resolution of the isobaric compounds. In addition, sample preparation techniques are evaluated to reduce the impact of biological matrix ionization effects.

The chromatographic method development consisted of a screening of different stationary phases such as C18, Cyano, Phenyl Hexyl and pentafluorophenyl phases.

Results and conclusions
This development resulted in a method for the direct quantitation of the isobaric metabolites 25-OH vitamin D3, 3-epi-25-OH vitamin D3 1-α-OH vitamin D3 along with 25-OH vitamin D2, 3-epi-25-OH vitamin D2. In addition, human serum samples were processed using standard protein precipitation techniques as well as using SPE with zirconia-coated silica particles for the comparison of matrix interference impact.
The resulting sample preparation and LC-MS method allows a robust and accurate quantitation of all the associated Vitamin D metabolites.

Novel Aspect
Robust and accurate quantitation of all the associated Vitamin D metabolites via LC-MS is presented.

MPS31-26 / A proteomic investigation into the molecular mechanism of HIV tat induced neuronal apoptosis

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IIDMM, UCT

HIV related neurocognitive disorders affect up to 70% of HIV patients with varying degrees of severity. While there has been a great deal of work suggesting various viral and host molecules and pathways which may ultimately result in neuronal apoptosis, there are no all-encompassing data to consolidate these findings and provide insight as to how they may all function together. To this end, we performed SILAC based quantitative proteomic analysis on HIV-Tat treated neuroblastoma cells. Isolated protein was fractionated by PAGE and analysed by nLC-MS/MS on the orbitrap velos. Using MaxQuant, we identified 2791 unique protein groups with quantitation by minimum two unique peptides. Using the student's t-test, we identified 482 differentially regulated proteins which were analysed using Ingenuity Pathway Analysis (IPA). Herein, we present direct proteomic evidence for the entry of HIV-Tat into neurons as well as kinase cascades which ultimately results in apoptosis via key HIV-dementia associated signaling pathways. We also provide evidence for several pathways resulting in known HIV-dementia and Alzheimer's disease pathologies which are certainly capable of contributing to the apoptotic phenotype and warrant investigation as therapeutic targets. Our findings are highly congruent with literature regarding key molecular features in HIV-dementia. Together, these data provide a proteomic map of cellular pathways leading to neuronal dysfunction which can be used to test the effect of potential treatments and inhibitors used to query the involvement of specific HIV-Tat dysregulated pathways.

MPS31-27 / Quantification of HER2 from FFPE Tumor Tissue using Targeted Mass Spectrometry (MS)

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Introduction
There is strong evidence that increased levels of the HER2 receptor in cancer positively correlates with a clinical benefit from targeted therapies such as trastuzumab. The current method of HER2 evaluation, immunohistochemistry (IHC), does not allow for absolute HER2 quantification, is subjective in nature and is prone to false-positives. Fluorescent in-situ hybidization (FISH) can be used instead of IHC to detect gene amplification of HER2, however gene amplification does not always result in overexpressed protein. Thus there is a need for absolute protein quantitation in formalin-fixed, paraffin-embedded (FFPE) tissue. We report the simultaneous quantitation of Her2 and other proteins in FFPE tumor biopsies using targeted mass spectrometry and present a comparison with other diagnostic methods, IHC and FISH.

Methods
We used trypsin digestion mapping of rHER2 to identify unique peptides for SRM development. Stable isotope-labeled peptides were synthesized as internal standards, and standard curves

were generated in a complex eukaryotic matrix (PC3 cells) to determine LOD, LLOQ, accuracy, precision and linearity of the assays. Seventeen gastroesophogeal cancer (GEC) cell lines were analyzed for Her2 expression in parallel with FISH/IHC. Expression thresholds were established for HER2+/HER2-; the sensitivity/specificity of the established cutoffs were then tested prospectively in FFPE biopsy tissues on 10uM FFPE slides.

Results
The chosen HER2 peptide had a LLOD of 0.150 fmol and a demonstrated linear range up to 25 fmol on column. HER2 MS on GEC cell lines revealed concordance with FISH (HER2:CEP17) ratio (R2=0.96). For 121 GEC FFPE cases, HER2 expression was seen in 69.4% of cases, with a range from 0.159 to 24671 fmol/ug. 8.2% of the GEC cases showed HER2 > 750 amol (10/121) - all were HER2 amplified as determined by FISH; no cases <550 amol/ug wereHER2 amplified. IHC/FISH results for cases with 550-750 amol/ug demonstrated a heterogeneous 'equivocal' zone, not unlike 'IHC 2+', which may require FISH confirmatory testing.

Conclusions
Targeted mass spectrometry was used for the simultaneous quantitation of Her2 and eleven other predictive/prognostic markers for cancer. A case study of a gallbladder cancer patient with amplified Her2 as detected by the OncoPlexDx SRM method will be presented as evidence of the diagnostic power of our assay.

Novel Aspect
OncoPlexDx is a CLIA-certified laboratory offering the first diagnostic test that uses targeted mass spectrometry for the simultaneous quantitation of multiple proteins in FFPE clinical tissue. We demonstrate the feasibility of absolute quantitation of Her2 in FFPE tumor biopsies and establish quantitative levels that correlate with gene amplification and treatment response.

MPS31-28 / Proteomic Analysis of Bence Jones Proteins Isolated from Urine Sample

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Introduction
Proteomic analysis of biological samples (tissues, cells, body fluids such as urine, plasma/serum) allows the discovery of disease biomarkers and understanding disease mechanisms. In kidney diseases, elevated amount of protein and change in protein profile of urine is observed. Immunoglobulin light chains (LC), also named as Bence Jones proteins (BJPs), exist in excessive amounts in disease states such as light chain deposition disease, light chain amyloidosis, and multiple myeloma. BJPs are causative agent in the progression of the renal failure and used as biomarkers of the mentioned kidney diseases. However, the severity of kidney diseases associated with the presence of BJPs in urine varies among patients. Therefore, proteomic analysis of BJPs is important for the detection and identification of those proteins and may play an important role in clinical diagnosis.

Methods
Proteins were isolated from the urine of patients with multiple myeloma disease. Electrophoretic analysis of samples was performed by using both one-(reducing and non-reducing) and two-dimensional (2D) gel electrophoresis. Following 2D-gel electrophoresis, relevant spots were analyzed with MALDI TOF/TOF MS. For molecular weight determination of native proteins, LTQ XL linear ion trap mass spectrometer was used in combination with liquid chromatography system.

Results
The clinical data indicated that although the patients suffer from same disease, the time required for complete renal failure varies among them. 1D-gel electrophoresis in reducing conditions showed that all the samples include immunoglobulin LC monomers. However, under non-reducing conditions, some of the samples include LC dimers in relatively high amounts. 2D-electrophoresis and MALDI MS/MS analysis revealed that proteins, which exist as monomers, are immunoglobulin light chain. The other proteins, which exist as dimer, are BJPs. The mass spectra acquired with on-line liquid chromatography revealed that the molecular weights of monomers and dimers are approximately 23 kDa and 46 kDa, respectively.

Conclusion
BJPs, obtained from different patients, have different tendency to exist as light chain monomers or dimers in urine. Mass spectrometric analysis enabled to identify proteins and to calculate their exact molecular weights. These results are well correlated with the clinical data. As the tendency of proteins in dimer form is increased, the renal toxicity of proteins is also increased.

Novel Aspect
Proteomic analysis of BJPs helps to understand the variations seen in the course of renal failure.

MPS31-29 / A Method for Isolating Free Thiol-Containing Proteins from Plasma

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Proteomic studies have as a goal to describe as many proteins as possible; however, even handling the newest and most powerful technologies, many researches are facing problems in covering all proteins that are expected to exist in a sample. Cysteine is one of least abundant amino acids in protein sequences. Moreover, it is present in important sites in proteins; active sites, regulatory sites, structure-stabilizing points and cofactor binding sites are involved with the function played by Cys residues. In this sense, methods and technologies targeted at Cys-containing peptides could add the data to fact database, which will be useful for investigating proteomes and may provide the new functional status of Cys-containing proteins. For this purpose, the hetero-bifunctional cross-linker [succinimidyl 6-[3'-(2-pyridylthio)-propionamido] hexanoate] (LC-SPDP) has been used to isolate proteins containing free-thiol groups, as it is capable of forming a disulfide bond with any free thiol group. The method has been tested for its usage in isolating papain, a cysteine protease from an extract of Carica papaya latex. The method turned out to be effective to isolate the free-Cys form (active form) of the enzyme from the extract, by the elution process with a reducing agent. Next, the method has been applied to human plasma to see whether or not the plasma contains free-thiol containing protein(s). Taking into account that a free-thiol in a protein is sensitive to oxidation, it is interesting to estimate the ratio of free-Cys form(s) to the oxidized one(s), which could reflect a physiological state rationalized with respect to susceptibility to oxidative stress. Since the plasma contains various low-molecular-weight compounds and high concentration of albumin and immunoglobulins, they have been removed with 10 kDa-MWCO ultrafiltration (Millipore) followed by treatment with a HiTrap Albumin & IgG Depletion column (GE healthcare). The resultant solution was applied to the present resin-LC-SPDP system. Through the test using commercially available human plasma, transthyretin (TTR) was observed as a major protein in the eluted fraction, which turned out to contain a single free Cys in the sequence (Cys10). This residue has been reported to form

a disulfide bond with some other reactive thiol compounds, such as sulfonate, cysteine, homocysteine, etc. Furthermore, 10-15% of the Cys10 are persistent in the reduced form. TTR has been deeply involved with some physical disorders and can be useful for diagnosis of cancer and amyloidosis process. In this sense, data about the ratio of the free Cys form to the oxidized ones may correlate with the physiological status or the propensity to develop some diseases as a disease-specific signature.

MPS31-30 / Plasma proteomic biomarker strategy in a porcine hepatectomy model

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Introduction

Partial hepatectomy (PHx) is a feasible procedure for the treatment of liver diseases. Monitoring liver function during and after surgery is crucial for the improvement of post-operative course. The pig has recently become increasingly relevant as a model organism for biomedical research because of the similarity in size, physiology, and genetics. Especially, in the study of liver disease, the analyses of plasma proteomic profiles would facilitate the understanding of the biological process, because many of the proteins in plasma are synthesized in the liver and abundance and structures of these proteins change in response to liver diseases. Here we adopted a strategy using magnetic beads based matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) to elucidate plasma proteomic biomarkers in a porcine 70% PHx model.

Methods

A 70% PHx was successfully performed for fifteen micro minipigs (MMPs) which were one to two years of age. Plasma and serum samples were collected at 0, 1, 3, 6, 24, 48, 72, 96, 120, 144, and 168 hours after operation from central vein (i.e. jugular vein). Serum samples from portal vein were also collected during an operative course. Pig plasma and serum peptides/proteins were purified with C8 magnetic beads and obtained spectra by MALDI-TOF MS. The spectra were analyzed with multivariate statistics and machine learning methods were applied using ClinProTools 2.2TM. Some of the peaks identified as useful classifiers were identified with liquid chromatography followed by nanoLC-MALDI-TOF MS analysis.

Results

The comparative analyses of the proteomic profiles of plasma versus serum, central vein versus portal vein were performed. Chronological distribution of plasma proteomic pattern was defined with machine learning method. By building a support vector machine classifier, an effect on plasma proteomic pattern of an administration of phosphodiesterase III inhibitor, olprinone, was detected with good cross validation accuracy around perioperative period. Of note, some peaks enabled an annotation of the stages from immediate early to recovery stages after hepatectomywhich retained the normal liver function defined by biochemical indices.

Conclusions

ClinProt system combined with a further peak identification process is a promising biomarker strategy to evaluate the postoperative liver function as well as to define effects of drugs in a porcine 70% PHx model.

Novel Aspect

With utilizing a porcine hepatectomy model, we have listed candidate peptides which can delineate post-operative course. Current strategy's strengths lie in its independence from target-specific reagents such as antibodies and probes.

Keywords

MALDI-TOF MS, proteomic pattern analysis, plasma, porcine hepatectomy model

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MPS31-31 / Analysis of vitamins D metabolites by isotope-dilution liquid chromatography – tandem mass spectrometry using enzyme-assisted derivatisation

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Introduction

The total serum concentration of 25-hydroxyvitamin D3 (25-OHD3) and 25-hydroxyvitamin D2 is currently used as an indicator of vitamins D status. Vitamins D insufficiency is claimed to be associated with multiple diseases, thus accurate and precise reference methods are needed. Here we present a novel enzyme-assisted derivatisation method for the analysis of vitamins D metabolites in adult serum utilising 25-[26,26,26,27,27,27-2H6] hydroxyvitamin D3 as the internal standard.

Methods

Extraction of 25-hydroxyvitamins D from 100 µL of serum is performed with acetonitrile. Cholesterol oxidase is used to oxidize the 3β-hydroxy group in the vitamins D metabolites followed by derivatisation of the newly formed 3-oxo group with Girard P reagent. Quantification is achieved by isotope-dilution liquid chromatography – tandem mass spectrometry.

Results

Extraction with acetonitrile results in an extraction efficiency of 102 % and equilibration with the internal standard is achieved within one hour. Standard addition experiments on adult human serum over the range of approximately 10-60 ng/mL of 25-hydroxyvitamin D3 gave a recovery of 102 - 106 %. Analysis of the NIST standard reference material 972, level 4, containing a high level of the epimer of 25-OHD3 (3-epi-25-OHD3), confirmed the complete selectivity of cholesterol oxidase toward the 3β-hydroxy group. The limit of quantification is <1 pg on-column. Intra- and inter-batch precision is currently being investigated. Finally, besides 25-hydroxyvitamin D3, 24R,25-dihydroxyvitamin D3 and other uncharacterised dihydroxy metabolites were detected in adult human serum.

Conclusions

In addition to being accurate and robust in quantifying the serum level of 25-OHD3, diagnostic MS3 fragment ions of m/z 189 and 205 aid the identification of vitamins D metabolites. Based on the obtained results the presented method can reliably quantify not only 25-OHD3 but vitamins D metabolites in human serum.

Novel Aspect

To our knowledge this is the first LC-MSn method relying on enzyme-assisted derivatisation for the analysis of vitamins D metabolites. The combined use of cholesterol oxidase and Girard P reagent allows for simultaneous analysis of multiple steroids. Girard P reagent gives characteristic fragmentation patterns in MS2 and structurally informative MS3 spectra, making the method highly selective.

MPS31-32 / Lipidomic and Proteomic Profiling and Imaging of Uveal Melanoma using MALDI-IMS-MS

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Introduction

Uveal melanoma (UM) remains the most common intraocular malignancy in adults, particularly amongst the Caucasian population1. It is known that over 90% of patients develop UM within the choroid region leading to a poor prognosis compared to UM cases that originated in the ciliary body or iris. Unlike cutaneous melanoma, UM metastases invade distant sites hematogenously. As a result metastases spread to the liver, possibly reaching the lungs, skin and bones. Imaging of removed organs and tissues is routinely performed to support clinical patient diagnosis.

Matrix assisted laser desorption ionisation ion mobility separation mass spectrometry (MALDI-IMS-MS) profiling and imaging was employed to investigate the tumour regions and adjacent human eye anatomy of patients with UM. UM sections included excised whole eye samples taken from both living and deceased individuals.

Multivariate statistical modelling including principle component analysis (PCA) and partial least squares discriminant analysis (PLSDA) obtained from lipid and peptide profiles enabled further insight into biological differences/similarities within the patient sample cohort.

Methods

After FFPE removal, lipid and on tissuetryptic digest images and profiles were performed on excised human eye sections. On tissue digestion was performed with trypsin (20µg/ml) solution containing Octyl-α/ β-glucoside. Tissue was incubated overnight 37°C/ 5% C02. Alpha-cyano-4-hydroxycinnamic acid (CHCA) was deposited using the Sun Collect spraying system. Peptide mass fingerprints, and MALDI Images were performed using the HDMS SYNAPT TM G2 system (Waters Corporation, Manchester, UK).

Results

Initial profiling experiments of tumour, choroid, choroid-tumour and lense areas produced varied spectra which aided the observation of tumour related proteins. Lipid and peptide MALDI images displayed diverse spatial distribution of many species throughout the various regions of the eye; choroid, cornea, retina, lense and UM tumour regions. High resolution images of the tumour mass displayed considerable variation between regions within the tumour tissue. Lipidomic PCA statistical analysis displayed separation of 3 main patient groups from the rest of the samples with PLSDA revealing the presence of lipids representative of sphingolipids and Lyso-phosphocholine, a phosphatidylcholine degradation product. The proteomic analysis of UM tissue included differences in actin and histones between each patient sample possibly indicating UM progression and disease status.

Conclusions

MALDI-IMS-MSI was able to display distinct regions relating to numerous species present throughout the whole eye sections and differences between certain proteins and lipids were readily observable via statistical modelling.

Novel Aspect

A lipidomic and proteomic approach using ion mobility profiling and imaging to investigate Uveal melanoma in human eye sections.

[1] Linge et al (2012) Invest Opthalmol Vis Sci 53. (8) 4634-4643

MPS31-33 / Mass Spectrometry-Based Biomarker Discovery: Quantification of Targeted Neuropeptides in Neuropathic Pain

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Neuropathic pain is a consequence of damage or disorder to the peripheral and/or central nervous systems, leading to allodynia, pain in response to non-noxious stimuli, and hyperalgesia with an increased pain responses to noxious stimuli. Tachykinin family, including substance P (SP), its precursor, metabolites and neurokinin A (NKA), play a central role in nociceptive transmission by interacting with specific receptors. Firstly, we used behavioral tests to demonstrate the animals were neuropathic. Then, we developed a LC-MS/MS method to quantify targeted peptides in collected brain and spinal cord tissues. Finally, we performed analyses to associate neuropeptide CNS tissue expressions with pain behavior results and the role of the TRPV1 receptor, a transducer of thermal stimuli.

This study used a chronic constriction injury rat model. Two behavioral tests were used before and after surgery to establish neuropathy behavior. Tissues were frozen and homogenized in a 0.25% TFA solution at a ratio of 1:5 (w/v). The samples were sonicated and large proteins were removed by precipitation. The chromatography was achieved using a 12 minutes linear gradient along with a Thermo BioBasic C8 100×1 mm column at a flow rate of 75µL/min. The mass spectrometer was coupled with the LC system using an electrospray ion source set to 4000 V in positive mode. The mass spectrometer operated in full scan MS/MS mode.

Behavioral tests demonstrated that 6 days following the surgery, the animals were neuropathic. The LC-MS/MS analyses revealed that spinal β-tachykinin58-71, SP and SP3-11 concentrations were significantly up-regulated in neuropathic animals ($p < 0.001$; $p < 0.001$ and $p < 0.05$, respectively). In contrast, the analysis of spinal SP5-11 concentrations in neuropathic animals revealed a significant down-regulation ($p < 0.05$). Interestingly, no significant differences in the spinal NKA concentrations were observed ($p > 0.05$). When compared to normal animals, the brain β-tachykinin58-71 and SP concentrations were significantly up-regulated in neuropathic animals ($p < 0.05$ and $p < 0.001$, respectively). This study shows that β-tachykinin58-71, SP and SP3-11 are closely related to pain behavior. Differential concentrations were also measured between TRPV1 null and WT mice tissues. SP and NKA were significantly down-regulated in TRPV1 null mice.

All results suggest that SP, NKA, β-tachykinin58-71 and C-terminal SP metabolites could potentially serve as drug efficacy markers in early drug discovery. Moreover, TRPV1-/- mice exhibited inferior SP and NKA concentrations in CNS. A deficit in thermal responses in TRPV1 null mice could be related to the down-regulations of SP and NKA. These findings are consistent with the analgesic effects of selected TRPV1 ligands administered into pain-induced animals. Therefore, the TRPV1 receptor could be an interesting target for the development of new analgesics particularly in a context of co-administration with an anti-inflammatory drug.

This study outlines the importance of substance P, its precursors and metabolites in pain transmission as well as a close link between the TRPV1 receptor and expression of SP and NKA.

MPS31-34 / Micro-heterogeneity of pyrrole-imidazole polyamides (PIPAs) which are the novel diagnostic agent and/or drug candidates using mass spectrometry

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Introduction

A polyamide containing N-methylpyrrole (Py) and N-methylimidazole (Im), designated PIPA, can bind to specific nucleotide sequences in the minor groove of double-helical DNA with high affinity and specificity, suggesting that PIPA blocks binding of transcription factors inhibiting gene expression [Dervan, P. B. (2001) Bioorg. Med. Chem., 9, 2215–2235]. Hence characterization of by-products as well as micro-heterogeneity have not been reported. The advantages of PIPA are stability (nuclease resistant), entering into cell nucleus without any delivery system and no-toxicities in animal experiments. In the present study we focused two targets, one was human TGF-β1 which is presently at the preclinical stages on renal diseases [Matsuda, H et al. (2011) Kidney Int. 79, 46–56], another was a tandem hairpin PIPA visualization for telomeres [Maeshima K. et al. (2001) EMBO J. 20, 3218-3228].

Methods

Fmoc-Py-OH and Fmoc-Im-OH as building blocks were prepared in-house. Amide bond formation was performed on an automated synthesizer, PSSM-8 (Shimadzu) or a manual synthesizer, PetiSyzer® (HiPep Labs.), by the Fmoc-strategy. On-line LC-MS, Agilent 1100, HCT ultra (Bruker Daltonics) with HiPep-Cadenza (3.0x150 mm, HiPep Labs.) or HiPep-Intrada (3.0x150 mm, HiPep Labs.) and LTQ Orbitrep Velos (Thermo Scientific) and Easy nLC II (Thermo Scientific) with a nano capillary column (Nikkyo Technos 0.075 x 110 mm) were used. MALDI-TOF-MS/MS used were ultraflex III and ultrafleXtreme (Bruker Daltonics).

Results

Generally production of PIPA requires sophisticated multistep reaction and purification procedures with large amounts of man power. In the chemical syntheses target molecules are generally known, thus de novo sequencing is not necessary, although in the stepwise solid-phase syntheses theoretically numerous deletion compounds can be generated. By-products often disturb ionization of the target compounds in mass spectrometry. Production economics strongly requires quality control during assembly. The difficulty of analyses are as follows: the most building blocks for the target PIPA are Im and Py which differ only 1 Da. Thus by-products have similar properties and many case HPLC-peaks are overlapped. In the large scale production efficiency of each acylation should be characterized to save the expensive building blocks (Py and Im) and labor in purification procedures.

Conclusions

PIPAs have functions for gene regulation through DNA binding and applied as novel chemical probes for diagnostics and/or therapeutics. Focusing on large scale production of PIPA, QC methods have been established.

Novel Aspect

Stability and characterization of by-products in synthetic PIPA were not reported previously. Even tiny amounts numerous oxidized compounds have been identified. While mass differences was 15.995 oxygen-adducts were indicated, which were envisaged at Py but not at Im residues. This is the first report to explore the micro-heterogeneity of PIPA.

MPS31-35 / The Clinical Metabolomics Facility (CMF) – accelerating translational research

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Introduction

Metabolic profiling based on ultra-performance liquid chromatography - mass spectrometry (UPLC-MS) has opened new avenues to discover candidate biomarkers in clinical samples. Clinical applications of metabolomics may not only provide a better understanding of pathophysiological mechanisms in disease, but also provide new predictive signatures of therapeutic treatments efficacy. Access to this technology is the major hurdle for its application in clinical research, because state of the art instrumentation and the required scientific expertise are often lacking in the clinical research setting. To provide high quality metabolomics services for clinical research, the Clinical Metabolomics Facility (CMF) was founded at the Bern University Hospital (Inselspital Bern) with the aim to strengthen interdisciplinary research by accelerating the discovery and validation of clinically relevant biomarkers and signatures.

Methods

The CMF offers the following analytical techniques: two dimensional UPLC-MS, gas chromatography - mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectroscopy. Non-targeted analyses are performed using a high resolution ion mobility time-of-flight mass spectrometer (Synapt G2-S HDMS, Waters) coupled to UPLC (Waters). Targeted metabolite analysis are performed on triple quadrupole mass spectrometers (TQ-S, Waters) in combination with UPLC (Waters) or GC-MS.

Results

Our metabolomics workflow has two major steps: the first consists in a non-targeted metabolic profiling based on an holistic approach to identify potential candidate signatures, e.g. for cancer, lung disease or diabetes in wide range of material (tissues, blood, breath condensates and cell lysates). The non-targeted metabolomics workflow consists of an hypothesis-generating experimental study design, sample collection, sample preparation and sample measurements. After alignment of the ion chromatograms, ion pattern are recognized and deconvoluted for label-free quantification. The metabolite candidates are identified against appropriate metabolite databases (e.g., METLIN, HMDB) by using the power of the high mass accuracy ($\leq$ 2 mDa). The generated structural hits are then filtered by accurate isotope ratio measurements ($\pm$ 2%) and fragmentation mass spectra. In a second step, the potential biomarkers discovered by the non-targeted approach are further validated by targeted metabolite analysis. Typical clinical projects illustrating our workflow will be presented and discussed.

Conclusions

The CMF at the Bern University Hospital (Inselspital Bern) provides an important link for accelerating translational research by bringing metabolomics and medical experts together.

Novel Aspect

A metabolomics platform devoted to translational research in a clinical environment.

MPS31-36 / MS based quantification of individual glycation sites in plasma proteins as potential type 2 diabetes biomarkers

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Introduction
Hyperglycemia of type 2 diabetes mellitus (T2DM) initiates intensive protein glycation, which refers to the non-enzymatic reaction of reducing sugars with amino groups. Resulting Amadori products are the first stable adducts of the Maillard reaction, which appear to be suitable for diagnostics and therapy control. Nowadays, glycated hemoglobin and serum albumin, which is highly sensitive to even low changes in glucose concentrations, are recognized as markers of hyperglycemia. However, only methods to determine the global glycation degree of these proteins have been established so far. Since the reactivity of glycation sites varies within proteins, characterization of individual glycation sites might provide sensitive biomarkers that are more suitable to diagnose diabetes, i.e. reliable information about a person's glycemic status. As the concentration of plasma proteins differs by several orders of magnitude and glycated species represent only a minor part of a protein, highly sensitive and specific analytical techniques are required to quantify individual modification sites.

Methods
Plasma samples obtained from T2DM patients and non-diabetic individuals were digested with trypsin, enriched for glycated peptides by boronic acid affinity chromatography (BAC), desalted by solid phase extraction (SPE), and separated by RP-HPLC coupled via an electrospray ionization (ESI) source to a triple quadrupole tandem mass spectrometer (ESI-QqQ-MS). Quantification relied on multiple reaction monitoring (MRM) of multiple glycation sites corresponding to plasma proteins using 13C, 15N-labelled peptides as internal standards.

Results
Based on a list of 40 glycation sites identified in our laboratory as prospective biomarkers using a small number of plasma samples taken from T2DM patients and healthy individuals [1], 30 glycated peptides detected with reasonable sensitivities were selected for a more detailed study. Thus, all peptides were synthesized as external standards and additionally 15 peptides were synthesized with 13C,15N-labelled amino acids as internal standards using established procedures [2-3].
All steps of sample preparation and analysis, i.e. tryptic digestion, BAC, SPE and RPC-MS/MS, were optimized for recovery, precision and each step was validated. Finally, all glycation sites were quantified in plasma samples obtained from five patients with diagnosed T2DM and age-matched healthy individuals (35-65 years). The levels of ten tryptic glycated peptides corresponding to different plasma proteins were significantly increased ($P < 0.05$) in diseased patients. Currently, the study is further extended using two cohorts of 50 samples each to judge the influence of glucose levels for all 30 glycation sites and compare their predictive values to N-terminally glycated hemoglobin (HbA1c) that is already used in clinical diagnostics.

Conclusions
The presented strategy provides sensitive quantification of prospective T2DM biomarkers to evaluate their clinical potential.

Novel aspects
A robust method to identify and quantify novel glycated peptide biomarkers for T2DM in human plasma was established.

MPS31-37 / Epigenetic effects of Benzo[a]Pyrene on placental histones: a new global MS-based profiling approach

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Introduction
Epigenetic mechanisms are highly dynamic and environment-sensitive. In particular, histone posttranslational modifications (PTMs) constituting the histone code can undergo disruption through a chronic exposure to ubiquitous pollutants. Among them, Benzo[a]Pyrene (BaP) is known for being responsible for alteration in gene expression. Early stages of the in utero development are particularly critical regarding the pollutant exposure. In fact, environmental pollutant-mediated modification of the fetal epigenome can impair the normal placental development and functionality, with consequences in the adult susceptibility to some diseases afterwards. Using machine learning algorithms and mass spectrometry of intact proteins, we developed a new global approach for sample classification based on histone PTMs pattern.

Methods
BeWo cells, derived from human choriocarcinoma, were either untreated or treated with 1 µM BaP, and intact acid-extracted histones were analyzed by UPLC-ESI-QTOF mass spectrometry. Raw data were preprocessed (XCMS package for R) and the resulting matrix was normalized prior to multivariate data modeling (SIMCA-13). Unsupervised and supervised classification methods, including principal component analysis (PCA) and orthogonal partial least square discriminant analysis (OPLS-DA), were applied to a training set (control vs BaP treated cells) for selecting differential forms of histones that substantially contributed to sample clustering. Classification model was validated using an external test set, and discriminant variables were selected for histone variant assignment.

Results
Unsupervised methods revealed two distinct sample clusters, showing that BaP induced changes in histone profiles. An OPLS-DA model was built using a training set of 20 samples and succeeded in discriminating 1 µM BaP samples from control. This was confirmed by applying the model to a 10 samples external test set. After relevant variables extraction, species corresponding to various modified histone forms were putatively identified on deconvoluted intact mass spectra. These identifications were then confirmed by MS/MS peptide analysis. Our global profiling approach, capable of preserving the combinatory aspect of histone PTMs, allowed us to discriminate samples according to their histone profiles. Besides, we have clearly identified the H2A.Z histone variant, usually associated with DNA double strand breaks.

Conclusions
UPLC-MS combined with pattern recognition applied to intact histones from BeWo cells exposed to BaP revealed changes in term of PTMs as well as variant composition. Moreover, results highlighted some specific markers of DNA damages.

Novel Aspect
To our knowledge, no previous study has dealt with MS-based pattern recognition strategy for characterizing BaP-mediated epigenetic effects in placental cells. This approach would serve as a new powerful tool for elucidating histone code changes occurring under toxicological conditions.

MPS31-38 / Nanoflow LC/MS for the Quantitation of Aristolochic Acid DNA Adducts

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Introduction

Since the time of Hippocrates, Aristolochia sp have been used as herbal remedies to treat a variety of human conditions and diseases. However, most species of Aristolochia produce the highly toxic aristolochic acids (AAs), which are both nephrotoxic and carcinogenic. Evidence that cancer could be ascribed to these acids rests on the isolation of DNA adducts from target tissues. Aristolactam-DNA adducts are derived from metabolites of AA-I and AA-II, and in both cases the deoxyadenosine adducts are dominant with smaller levels of deoxyguanosine adducts being formed. These adducts arise by the same metabolic pathways that are followed by most carcinogenic amines and nitro compounds in which the intermediate N-hydroxyamine metabolites (in this case, the N-hydroxylactams) are the purported pro-carcinogens. Surprisingly, the C-8 purine adducts are not observed, and only the products of adduction at the exocyclic amino groups of the purine nucleobases are found. We have developed a sensitive and specific assay for the quantitation of the aristolactam I (AL-I) adduct of deoxyadenosine in human tissues samples using nanoflow ESI/LC/MS.

Methods

DNA from renal tissue samples was spiked with a synthetic isotopically labeled internal standard (dA-AL-I-15N5) and digested enzymatically as previously described to release DNA adducts. Samples were chromatographed on a nanoflow HPLC system consisting of a Dionex Ultimate 3000 pump and autosampler. The HPLC column was a fused silica column (100 µm x 10 cm) bomb-loaded with Magic C18 (5 µm). Flow rate was approximately 300 nL/min, and column elution consisted of a gradient of 20% solvent B:80% solvent A increasing linearly to 50% solvent B:50% solvent A over 60 min. Solvent A was 2% acetonitrile in 0.1 % formic acid; solvent B was 98% acetonitrile in 0.1% formic acid. Column effluent was directed to a custom nanoflow ESI source mounted on a Thermo Instruments LTQ ion trap mass spectrometer. LC/(MS)3 methods were used for quantitation of the adduct as described previously using the transitions m/z 543 $(MH)^+ \rightarrow$ m/z 427 $(BH2)^+ \rightarrow$ m/z 292, 293, and 412.

Results and Conclusions

Nanoflow HPLC, coupled to an ion trap mass spectrometer, provides excellent peak shape and sensitivity for quantifying dA-AL-I adducts. The limit of detection was less than 50 fg of the adduct injected onto the column. Calibration curves were constructed over the range of 50 fg to 10 pg with excellent statistical correlation ($R2 \geq 0.99$). Our initial experiments have detected the dA-AL-I adduct in kidneys from mice treated with AA-I. Data are presented showing adduct levels measured at selected doses and for varying time points after treatment.

Novel Aspect

We have developed a quantitative assay using nanoflow HPLC coupled to ESI/MS which provides high levels of sensitivity, suitable for detection of the deoxyadenosine-aristolactam I adduct in human renal tissue.

MPS31-39 / A Systems Biology / Multi-Omics Approach for the Study of Heart Regeneration in Zebrafish

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Ideally for systems biology a multi-omics approach should be employed for a thorough understanding of disease states and disease progression. Genomics, proteomics, metabonomics and lipidomics all offer different information for the same question and should be combined. Here we have applied a multi-omic approach to studies on Zebrafish. Zebrafish have been studied extensively due to their ability to regrow tissue following trauma to the heart.

We report preliminary results of proteomics, metabonomics and lipidomics studies on the plasma of zebrafish who have had a small section of the heart removed (under anaesthetic) or a sham operation (anaesthetic only) vs. control samples. Due to the small size of the fish less than 20 µL of plasma was obtained. Of this 7 µL was taken for proteomic analysis and the remainder processed for metabonomics and lipidomics analysis (the latter via protein precipitation by a 1:3 dilution with 50:50 acetonitrile:isopropanol).

Protein extracts were proteolysed with trypsin and the resulting peptides separated over a 90 minute linear reversed-phase LC gradient using a Waters nanoAcquity UPLC and high resolution Q-ToF Synapt G2-Si. Metabonomics was performed using a 12 minute reversed-phase LC gradient using a Waters I-Class Acquity UPLC and Synapt G2-S. The extracted lipids were separated over a 20 minute reversed-phase LC gradient and acquired on the same instrument as the metabonomics data. Data were acquired using a data independent acquisition approach, whereby the collision energy was switched between a low and elevated energy state during alternate scans. Proteomic acquisitions also utilised ion mobility in the acquisition scheme. Analysis of technical replicates was performed in triplicate, randomised, with conditioning QC samples prior to analysis and spaced every 6 samples during analysis. Analysis was undertaken in both positive and negative mode electrospray ionisation.

The acquired data were processed and searched using Progenesis QI and dedicated protein sequence, small molecule and lipid compound databases. Initial multivariate PCA plots of the data demonstrate a clear separation between the plasma from Zebrafish that had undergone the actual operation and the control samples. The Zebrafish that had undergone the sham operation displayed a similar profile to the control samples. Identification of the species responsible for the separations is on-going.

The combination of data in this systems biology approach to the analysis of Zebrafish plasma should give real insight into the changes within Zebrafish who have undergone heart trauma, which may ultimately provide assistance within patients who suffer heart trauma in the human population.

MPS31-40 / Biomarkers probed in biological fluids by surface plasmon resonance imaging coupled to MALDI mass spectrometry in array format

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Introduction

The coupling of surface plasmon resonance (SPR) with mass spectrometry (MS) relies on two well-established techniques allowing the analysis of biomolecular interactions and bio-structural characterization respectively. The ability to affinity-based enrichment of specific ligands on the SPR biosensor

combined with their structural identification by MS leads to valuable applications expected in proteomics, bio-diagnostic, with the identification of biomarkers and protein variants related to diseases. The recent introduction of SPR in array format compatible with MS detection offers the advantage of a multiplexed SPR-MS analysis. Here we report the SPRi-MS analysis of protein biomarkers directly from a biological fluid (human saliva) using a functionalized biochip in array format, and without any need of sample pre-purification or spiking with the targeted biomarkers.

Methods
All SPRi-MS experiments were performed using a SPRi-Plex imager (Horiba Scientific- GenOptics) and a MALDI-TOF spectrometer Voyager-DETM STR (ABSciex). An adapted Opti-ToFTM MALDI plate allowed transfer of the SPRi biochip into mass spectrometer following SPRi experiment. MALDI matrix was directly spotted on the biochip, and mass spectra were obtained from distinct spots. Biochips were gold surfaces functionalized by a self-assembled monolayer of short polyoxyethylene carrying an N-hydroxysuccinimide group for immobilization of biomolecular receptors. Anti-α-amylase (Rabbit) and monoclonal anti-lysozyme (Mouse/IgG1) antibodies against human α-amylase and lysozyme, were grafted on the functionalized biochip. SPR running buffer was 10 mM ammonium acetate, pH 7.5.

Results
The SPRi-MS coupling was applied to the study of human saliva, a crude biological fluid. Our system led to successfully detection and structural identification of protein biomarkers such as amylase and lyzozyme. Human saliva samples were simply diluted in SPR running buffer before to be loaded onto an anti-α-amylase and anti-lysozyme antibodies biochip. SPRi signals were recorded indicating effective interactions between antigens contained in saliva and the grafted antibodies. Consecutively to the SPRi experiment, MALDI mass spectra were recorded directly from the biochip surface from each antibody spot, showing protein ions attributed to the corresponding specific antigens α-amylase or lysozyme. Finally, on-chip proteolytic digestion followed by MALDI-MS detection of peptides, confirmed the identification of α-amylase and lysozyme as specifically captured on specific antibodies spots.

Conclusion
The feasibility of an SPR-MS study directly carried out from biological fluids such as saliva by was demonstrated. The biochip surface functionalization made of short polyoxyethylene-based self-assembled monolayer was also compatible with on-chip digestion of specifically bound analytes, followed by MS detection.

Novel aspect
Real-world application of the SPRi-MS coupling.

MPS31-41 / Analysis of Cerebrospinal Fluid using High Resolution Mass Spectrometry

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Due to the fact that CSF is the only body fluid that is in direct contact with the brain it serves as an important source for biomarker discovery. Especially for disorders of the central nervous system, such as Alzheimer's Disease (AD) and Parkinson's Disease (PD), CSF can provide information enabling an early diagnose as well as monitoring disease progression and therapy strategies. However, the high amount of albumin, immunoglobulin and transferrin makes the identification and quantification of low abundant proteins, with a potential to be a suitable biomarker, challenging. In order to analyze the variation of protein abundances in CSF we present a label-free mass spectrometry (MS) strategy. The label-free solution is based on a highly reproducible and stable liquid chromatography (LC)-MS system. In comparison to other techniques, like those that use labelling, label-free LC-MS has a broad range of benefits, including reduction of protein loading, no costs for labelling reagent, increased sequence coverage per protein as well as increased overall proteome coverage. A label-free workflow consists of in-solution digest, LC-MS data acquisition and data analysis/evaluation.By using a three hour LC gradient and a high resolution orbitrap mass spectrometer we are able to identify over 600 proteins from 500 ng CSF. Hence, we can separate the digested peptides of non-processed CSF (in terms of non-depleted or pre-fractionated) to get a profound analysis of the CSF proteome. We also pursue alternative strategies concerning LC separation and MS method optimization for a much deeper analysis. These strategies will be applied to compare the CSF proteome profiles of healthy controls with AD and PD patients in order to identify potential biomarkers.

MPS31-42 / UHPLC-MS/MS Method for Monitoring of Neurotransmitters and Their Metabolites in Brain Microdialysates

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In vivo neurochemical monitoring using microdialysis sampling is important in neuroscience because it allows correlation of neurotransmission with behaviour, disease state, and drug concentrations in the intact brain. A significant limitation of current practice is that different assays are utilized for measuring each class of neurotransmitter. Dopamine (DA) and serotonine (5-HT) are major modulatory catecholamines in the brain involved in motor functions, mood, learning and reward. Furthermore, they play an important role in several psychiatric disorders such as addiction, schizophrenia, Parkinson´s disease, etc. A sensitive assay method was developed for a parallel, rapid and precise determination of DA, 5-HT and their metabolites (homovanillic acid (HVA), 3-methoxytyramine (3-MT), 3,4-dihydroxyphenylacetic acid (DOPAC), and hydroxyindolacetic acid (5-HIAA)) from brain microdialysis samples. The method consisted of a pre-treatment part, derivatization of neurotransmitters and their metabolites by 3-aminopyridyl-N-hydroxysuccinimidyl carbamate (= APDS, derivatization of amino group) and Girard reagent T (derivatization of carboxylic acid), and detection method UHPLC-ESI-MS/MS, where the selected reaction monitoring (SRM) mode was used for its extremely high degree of selectivity and the stable-isotope-dilution assay for its high precision of quantification. The developed method was characterized with high precision ≤ 13.6 % (determined as RSD) and acceptable accuracy ≤ 14.1 % (determined as RE). The method was tested on samples obtained from various brain centers (e.g. nucleus accumbens, prefrontal cortex) of rats after an acute drug administration. It was found that the developed assay could be applied for a simultaneous analysis of neurotransmitters and their metabolites (DA, HVA, 3-MT, DOPAC, 5-HT and 5-HIAA) present in in brain microdialysis samples and the time monitoring of their concentration changes on picogram level after acute administration of drug. The developed method for quantification of DA, HVA, 3-MT, DOPAC, 5-HT and 5-HIAA potentially expands the diagnostic methods in regards to neuropsychiatric and neurological disorders, where DA, 5-HT and their metabolites concentration levels are altered in comparison to physiological levels. The method may well contribute to a better understanding of the pathophysiology and pathogenesis of many neuropsychiatric disorders and in pharmaceutical research of new drugs to treat neurological diseases.

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MPS31-43 / Molecularly Imprinted Polymers Separation Combined with UHPLC-MS/MS: A Tool for Experimental and Clinical Diagnostics

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Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Further, some reactive oxidative species act as cellular messengers in redox signaling. Thus, oxidative stress can cause disruptions in normal mechanisms of cellular signaling. In humans, oxidative stress is thought to be involved in the development of many diseases. A new analytical method combining separation based on molecularly imprinted polymers (= MIP) combined with highly sensitive and selective LC-MS detection, for quantification of biomarkers derived from basic organism structure units was developed. The analytical procedure covers monitoring of 8-iso-prostaglandin F2a (= 8-isoprostane), o-tyrosine and 8-hydroxyguanosine as a group of oxidative stress markers present in various body-fluids (blood plasma, urine, exhaled breath condensate, cerebro-spinal fluid). 8-isoprostane is produced by non-enzymatic direct oxidation of arachidonic acid on the cell surface by oxygen radicals. It is generally well-established as the most significant biomarker of oxidative stress. Oxidation of amino acids and nucleic acids produces other markers of oxidative stress like o-tyrosine and 8-hydroxyguanosine. The separation method consisted in highly cross-linked polymeric phases extraction with predetermined selectivity for a single molecule / a group of structurally related molecules. In non-covalent imprinting, selectivity is introduced during preparation of the MIP. The present work offers also the comparison of MIP-separation and immuno-separation of particular biomarkers present in low concentrations in various biological matrixes. LC-ESI-MS/MS detection method operated in multiple reactions monitoring (MRM) mode was used for its exceptionally high degree of selectivity, and stable-isotope-dilution assay for its high precision of quantification. The developed method allowed unequivocal parallel determination of oxidative-stress biomarkers at the same run. The method was optimized and validated. The imprecision of the method ranged between 6 – 14 %. Finally, the method was tested on real samples collected from experimental animals as well as patients with different oxidative stress induced diseases.

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MPS31-44 / Nanoprobe-based affinity mass spectrometry for quantification and glycosylation profiling of liver cancer biomarkers

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Introduction
Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and the third most common cause of cancer death worldwide. However, the lack of sensitivity of current approaches to diagnose the disease at its early stage necessitates the development of more robust biomarkers for more sensitive detection of HCC. Thus, analytical strategies with high throughput multiplexed capability have to be developed for the quantification and verification of protein biomarker candidates identified in the discovery stage. In addition to concentration, altered glycosylation patterns may also provide better sensitivity for disease diagnosis.

Methods
In this work, we established an analytical workflow integrating nanoprobe-based immunoaffinity assay for selective serum protein enrichment and LC-MS/MS for sensitive protein quantification and glycosylation profiling. Nanoprobes were synthesized by conjugating antibodies of HCC biomarker candidates to Fe3O4 magnetic nanoparticles and were used to extract the target candidates from human serum. Protein quantification was achieved by coupling the nanoprobe-based enrichment with multiple reaction monitoring (MRM). Following protein enrichment and tryptic digestion, we also studied the glycosylation pattern of the target analyte by glycopeptide enrichment using HILIC spin column. Subsequent LC-MS/MS analysis and automated bioinformatics was employed to confidently identify the glycopeptide and glycan information.

Results
Using AFP as a model target analyte, analytical figures of merit for the nanoprobe-MRM method were evaluated. The nanoprobe-MRM method had good linearity (r2=0.9956 for interday experiments) and a limit of detection of 2.5 ng/mL. Furthermore, glycosylation profiling of AFP identified 9 glycopeptides and 5 glycoforms from only 20 mL cord blood sample.

Conclusions
We were able to develop and validate a nanoprobe-MRM method designed for HCC biomarker candidates, with figures of merit obtained that are within the limits required for bioanalytical method validation set by the US Food and Drugs Administration (US FDA). In addition, a nanoprobe/HILIC-based sequential enrichment for glycosylation profiling was also developed for highly confident glycosylation profiling.

Novel Aspect
A nanobrobe-based mass spectrometry workflow for protein quantification and glycosylation profiling is presented.

MPS31-45 / Targeted and non-targeted metabolomics in the research of chronic kidney disease

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Tasmania has a high occurrence of chronic kidney disease (CKD), which progresses to end-stage kidney failure (ESKF)

requiring treatment with dialysis or a kidney transplant. CKD is commonly accompanied by neuropsychiatric conditions and premature cardiovascular disease. The uraemic syndrome is the accumulation of compounds in the blood caused by lower excretion via urine due to kidney malfunction. Uremic molecules may hold the key to understanding and treating the predicted consequences of CKD.

Methods

The presented work investigates the metabolic picture of uraemia using mass spectrometry based metabolomics and targeted metabolite analyses. Biofluids were analysed by reversed-phase LC coupled to Mass Spectrometers: Thermo LTQ-Orbitrap XL; Bruker µTOF; Waters Xevo QqQ. Different data analysis methods were employed to suit the experimental design.

Results

Metabolomics approach was employed to find potential molecules of interest in serum or urine specimens collected from CKD and dialysis patients and also healthy controls. Putative uremic toxins were traced in serum and urine and related to the pathology of the individuals, to test the agreement with the current concepts in nephrology and also examine the rate of clearance of these molecules by hemodialysis. According to the findings in the preliminary stage, a set of target molecules was constructed for simultaneous LC-MS/MS analysis (Waters QqQ), based on possible relation to psychological state and also cardiovascular events. This dataset provides information on an important metabolic pathway affecting the observed pathology, and suggests points of intervention in chronic kidney disease patients.

Conclusions

LC-MS is a strong tool in the analysis of metabolites relevant to disease, especially when combining targeted with non-targeted approaches. However, the selected method should address the limitations of the instrument, the nature of the samples, and the experimental design. The extraction of valuable information also depends on the gathering of relevant metadata and complimentary pathology tests.

Novel aspect

Investigation of a human disease usually involves complex clinical work with chosen and sometimes limited chemical analysis. The multiple analytical approaches applied in our study expose different layers of the metabolic environment of the disease and provide a rich set of data for planning a wider clinical experiment.

MPS31-46 / Plasma levels of Tβ4 in cardiac patients and matched controls analyzed by LC-MS and ligand binding assay

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The 4.9 kDa peptide Thymosin βeta 4 (Tβ4) is ubiquitously expressed in all cells except red blood cells, and plays a role in endothelial cell migration, angiogenesis, corneal wound healing, and stem cell differentiation. The largest Tβ4 content is found in leukocytes and platelets. When platelets are activated large amount of Tβ4 isreleased locally. This leads to an increase in plasma levels and may serve as a biomarker of cell damage. Endogenous levels of Tβ4 were analyzed in samples from cardiac patients and matched controls at two time points four months apart. The levels of Tβ4 were analyzed both in a ligand binding assay (EIA) and by mass spectrometry methods (LC-MS vs LC-MS/MS). Tβ4 was measurable in all patients and controls. The level of Tβ4 showed a larger variation in patients versus healthy controls, 0.9 - 9.8 µg/mL vs 1.9 – 5.8 µg/mL, respectively. The median Tβ4 plasma concentration was 3.70 µg/mL in the patient group and 3.30 µg/mL in healthy controls at the first visit.The mean levels of Tβ4 was almost identical at both visits, 3.93 – 3.59 µg/mL in patients versus 3.37- 3.40 in controls. Sample preparation procedures were also evaluated and the results showed that different sample preparation protocols influence the Tβ4 plasma concentration, which may explain why the Tβ4 levels in the study samples were higher than previously reported.

Group (n)	Visit	Mean µg/mL	Median µg/mL	Range µg/mL	sd
Controls (n=46)	1	3.37	3.30	(1.9-5.8)	0.85
Patients (n=48)	1	3.93	3.70	(0.9-9.8)	1.88
Controls (n=44)	2	3.40	3.30	(1.5-5.5)	0.92
Patients (n=44)	2	3.59	3.45	(1.2-12.9)	1.87

MPS31-47 / Plasma metabolite profiling of chronic myeloid leukemia patients

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Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disorder caused by constitutively active BCR-ABL tyrosine kinase. The objective of this work was to evaluate the influence of tyrosine kinase inhibitors approved in the CML treatment to the overall metabolite plasma profile in the patients.

Methods

Plasma samples from healthy controls (n=15), patients before treatment (n=14), treated with hydroxyurea (n=7), imatinib (n=17), dasatinib (n=8) and nilotinib (n=9) were deproteinized and analysed by liquid chromatography (aqueous normal phase separation mode) coupled to tandem mass spectrometry (Qtrap 5500, AB Sciex, USA). The data acquisition was proceeded in both positive and negative multiple reaction monitoring mode and the metabolites were detected and quantified in MultiQuant 3.0 software. The statistical treatment of the data obtained involved both unsupervised (principle component analysis) and supervised (discriminant function analysis, orthogonal partial least squares discriminant analysis) methods using R programme language with statistics packages.

Results

About 140 metabolites were detected in CML patients plasma. The statistical approaches clearly separated patients before treatment and hydroxyurea treated group from the other groups under study.

Conclusions
The study of plasma metabolic profile in CML patients was accomplished. The results revealed differences in metabolite levels of the groups treated with tyrosine kinase inhibitors, patients before treatment and healthy controls.

Novel Aspect
First study concerning the influence of tyrosine kinase inhibitors to the plasma metabolic profile of CML patients.

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MPS31-48 / Application of an improved LC-MS/MS method for the measurement of steroid levels in supernatant of H295R cells to meet the criteria of OECD TG 456

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Endocrine disruption (ED) is a topic currently under scientific and political debate. In this context, much effort has been done to establish in vitro methodologies that could give reliable insights into the ED mechanisms of compounds without the use of animals. Amongst those methods, the steroidogenesis assay a regulatory accepted method (OECD TG 456 and OPPTS 890.1550) aims to identify compounds that interfere with the steroid hormone synthesis. It uses the human adreno-carcinoma cell line H295R, which harbors the genes encoding key enzymes for steroidogenesis. The assay determines changes in estradiol (E2) and testosterone (T) levels measured in the supernatant of treated cells. In contrast to most commonly used RIA or ELISA, analytical methods such as LC-MS/MS offer the potential to determine several hormones in parallel and largely avoid cross-reactivity of the test substance, as it could occur with the classical immunoassays.
Herein, we present a newly developed method that not only determines E2 and T in a single run, but also 12 of hormones involved in the sex-hormones biosynthesis. This improved method not only meets all quality criteria required under the OECD TG 456 with high efficiency, but it opens the possibility to better identify specific targets that can be disrupted by ED during steroidogenesis.

MPS31-49 / Performance investigation of a novel integrated microfluidics platform in high-throughput LC-MS MRM disease protein marker verification

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Introduction
Biomarker validation is technology challenged since it requires analyzing large numbers of samples with high-throughput, but requires high sensitivity, high resolution, large dynamic range and excellent selectivity. Targeted LC-MS based assays afford protein quantification with the reproducibility and throughput required in order to improve marker acceptance with MRM, using tandem quadrupole MS, being one of the enabling technologies. Here, the application of a novel microfluidics platform for quantification of marker peptides and proteins is presented, considering speed, sensitivity and selectivity.

Method
MS Qual/Quant mixture was spiked into an E. Coli such that loads for a 1 µl injection ranged from 32 amol to 40 fmol peptides in 100 ng E.Coli. The sample was injected 3 times at 4 different loadings and separated using 45 minute reversed phase gradients at flow rates of 1.2 µl/min. A Xevo TQ-S MS was operated at two different quad resolutions setting to monitor each peptide with three transitions. Data were analyzed using MassLynx and Skyline software.

Results
Important aspects when characterizing an LCMS system for validation experiments are retention time reproducibility of the eluting species, technical reproducibility of the monitored transitions and consistent quantitative measurement accuracy for each peptide. MRM transitions were inspected ensuring that a minimum of 3 peptides per protein and 3 transitions per peptide were detected and could be analyzed. Typical retention time reproducibility for the monitored peptides show a SD of 0.02min for peptide EGHLSPDIVAEQK and technical reproducibility for the three monitored transitions of Light (L)/Heavy (H) analogs of peptide GGPFSDSYR were calculated as y5 (L 3.3%, H 5.7%), y6 (L 0.8%, H 1.2%) and y7 (L 5.4%, H 2.9%).
Ratio measurements for peptide AVQQPDGLAVLGIFLK from Carbonic Anhydrase II, which is present in the mixture at elevated levels for the light analog are in good agreement with an expected value of 8.4 vs. 8.5±0.8 experimentally determined.In order to investigate the effect of interference from background matrix, the experiments were repeated at unit and elevated quadrupole resolution settings and measured ratios contrasted with the expected values from four different injection volumes, ranging from 0.1 to 1 µl. The results show that unit quadrupole resolution afforded sufficient quantitative accuracy, with precision not noticeably affected by quadrupole resolution. Limits of detection that can be achieved is shown with NLSVEDAA[R] from Catalase which is observed at 3.2 amol on column.

Conclusion
A novel microfluidics platform for quantification of marker peptides and proteins is a high throughput, sensitive and robust platform. Quantitation results are shown to provide good agreement with expected values and limits of detection at low amol level are achieved.

Novel Aspect
Novel integrated microfluidics device coupled to a XevoTQ-S Mass Spectrometer.

MPS31-50 / Non-target screening of mercapturic acids in human urine – comparison of different LC-MS approaches

Robert Bloch, Merle Plaßmann, Werner Brack, Martin Krauss
Helmholtz Centre for Environmental Research GmbH - UFZ

Pollution, lifestyle and other factors contribute to an internal chemical environment that affects biological functions of the human body. Glutathione conjugation is an important detoxification mechanism to eliminate reactive electrophiles formed by metabolism of environmental pollutants or internal biomolecules. Among other pathways glutathione conjugates are subsequently converted to mercapturic acids (MA) and renally excreted. This study aims at developing and comparing two novel methods to detect MA in human urine using a non-target approach. Both methods are based on the fact that all MA show a common neutral loss (NL) during mass spectrometric fragmentation.
Two mass spectrometers were used: A Thermo high-resolution LTQ Orbitrap XL and an AB Sciex QTrap 6500, both equipped with Agilent liquid chromatography systems. A full scan with

in source fragmentation was performed on the Orbitrap. The data was extracted and searched for NL with the R packages xcms and CAMERA. With the QTrap, a constant NL scan was performed directly and solely a peak picking step was necessary afterwards. For the assessment of matrix influence and software performance, MA standards were spiked into water and pooled urine samples. For evaluation purposes, the peak information was also extracted in a targeted way by using mass and retention time of the compounds.
From 16 MA standards, 11 were detected in the target procedure. Large molecules with several functional groups were not detected, possibly due to other fragmentation reactions than the investigated NL. In non-target mode, the detection of the standard compounds depended strongly on the parameter settings. In general, more additional unidentified peaks were found using the QTrap. Recovery experiments showed that the QTrap analysis is more influenced by matrix than the Orbitrap analysis. The recovery rates are in the range of 30% - 140% (QTrap) and 30% - 80% (Orbitrap).
Both methods have advantages and disadvantages. The Orbitrap was operated at a nominal resolving power of 100 000 and has a high mass accuracy of about 5 ppm over the whole mass range. Thus, the search for NL can be done with the exact mass difference, which makes the method much more selective than the unit mass resolution QTrap method. On the other hand, the constant NL scan in quadrupole instruments is more reliable than a software-based search for NL mass pairs. Additionally, a QTrap scan is very fast, allowing an UHPLC separation for enhanced sample throughput. With the evaluated non-target methods, urine samples of smokers and non-smokers will be analyzed and compared. Results will be presented at the conference.

MPS31-51 / UHPLC-QTOF Based Metabolomics for discovering Etiological Biomarkers of Liver Cancers Using Serum Samples from a Large Multinational Prospective Cohort

Dinesh Barupal, Augustin Scalbert, Talita Duarte-Salles, Magdalena Stepien, Isabelle Romieu, Mazda Jenab
International Agency for Research on Cancer

Introduction
The European Prospective Investigation into Cancer and Nutrition (EPIC) is a large study of diet and health having recruited over half a million (520,000) people from ten Western European countries with collection of detailed dietary and lifestyle information as well as biological samples at recruitment [epic.iarc.fr]. The main objective of this prospective cohort study is to investigate relationships between diet, nutritional status, lifestyle and environmental factors and the incidence of cancers and other chronic diseases. Mass spectrometry based metabolomics analysis of blood specimens from EPIC could enable identification of novel diagnostic markers and disease risk factors, as well as to provide new insights into potential biological mechanisms of how dietary and lifestyle factors relate to disease risk.

Methods
Archived serum samples (10uL) from a case-control study nested within the EPIC cohort were utilized for this study. Metabolites were extracted using a cold-methanol based protein precipitation method from a total of 546 samples, belonging to liver cancer cases and their matched control subjects (1:1). Agilent 1290 UHPLC with Agilent QTOF 6550 (ESI+) was used for data acquisition. A C18 RP-HSS T3 column (Waters) was used to separate the metabolites (Sol A: H20 (0.05% FA); SolB:MeOH (0.05% FA), runtime:12.5 mins. A pooled QC blood sample was injected every 20 samples. Raw data was processed using Agilent Qual software and R. MS/MS Spectra were acquired in Auto MS/MS and Targeted MS/MS using a narrow isolation width and matched against LipidBlast, HMDB, Metlin, MassBank and NIST MS/MS databases for identification of compounds. Statistical analyses were performed in R, SAS and Statistica software

Results
A total 1685 compounds were detected using the Agilent molecular feature extraction (MFE) algorithm across all the 546 samples. Up to 80% of these compounds were observed in the pooled quality control sample and out of them 90% showed high technical reproducibility (CV <10.0%), reflecting the minute inter-batch variation introduced by machine drift. Around 2000 MS/MS spectra have been collected on three collision energies for the compounds. Matching of these MS/MS spectra against spectral databases identified a range of compounds belonging to lipids, amino acids, bile acids, carnitines, di-tri peptides and aromatic compounds. The next steps in the analyses will be to compare alterations in metabolites between cases and controls using multivariable conditional logistic regression models and to assess correlations between metabolite profiles and dietary intake patterns.

Conclusions
A high resolution mass spectrometry based metabolomics workflow in a nested case control study within a prospective cohort study has been established for the objective of identification of novel diagnostic or lifestyle-related cancer markers.

Novel Aspect
Use of a Hybrid QTOF mass spectrometer in a large prospective cohort study to study cancer etiology and explore for novel diagnostic markers.

MPS31-52 / Applications of Mass Tags for Diagnostic Immuno Mass Spectrometry

Martina Lorey[1], Belinda Adler[2], Alain Rouleau[3], Marven El Osta[4], Hong Yan[2], Ville Jokinen[5], Rabah Soliymani[1], Patrick Ducoroy[4], Wilfrid Boireau[3], Simon Ekström[2], Thomas Laurell[2], Marc Baumann[1]
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Introduction and Aims
Mass tags are small reporter molecules which can be attached to biomolecules. They are photocleavable and thus detach from their carrier upon UV-laser irradiation during mass spectrometry (MS) analysis and are released in ionized form, allowing detection of the resulting mass peak. Due to the chemical structure of the mass tag it can serve as its own matrix and supersede additional treatment of the sample.
The major advantages of mass tags as a means of secondary detection are that there is no size limitation of the targeted biomolecule, no digestion of the targeted protein is needed, no matrix to assist ionization is necessary, and the targets' abundance compared to other biomolecules in the sample plays a minor role. Unlike fluorescence labels, mass tag reporter molecules don't exhibit quenching and thus allow true multiplex experiments.

Methods
We have developed microarrays on different surfaces for biomarker detection in biological fluids using a MS read out. For those we utilize coated silicon to covalently bind antibodies or substrates on the surface, and porous silicon to immobilize substrate or antibodies by adsorption. These microarrays allow rapid diagnosis for various purposes: from research and drug testing to differentiation between cancer types to provide «personalized» medicine.

Results
Different assays for biomarker detection have been created. In assays with covalently immobilized antibodies on coated-silicon and - gold surfaces specific antibodies against the desired biomarkers are immobilized in an array on the surface. The sample is either treated with mass tags to label all biomolecules present in the sample, or the desired biomarker carrying mass tags is spiked into pure blood plasma. After incubation and washing a rapid MS analysis shows on which spots the tag can be detected, thus stating whether the tagged substrate is captured by antibodies on that spot.
Quantification experiments utilizing Surface Plasmon Resonance (SPR) experiments gave insights about the correltation between mass peak intensity of the peak and amount of bound protein on the same spot when using covalently immobilized antibody arrays.
Furthermore, sandwich assays for biomarker detection have been used to improve the sensitivity of this new read-out method for microarrays on porous silicon surfaces.

Conclusion
We were able to create MS immuno-assays detecting prostate specific antigen, a prostate cancer biomarker with mass tags as photocleavable reporter molecules on porous silicon surfaces with clinically relevant sensitivity. Using the same read out we created assays for LAG3, a breast cancer marker, on coated silicon and coated gold surfaces and quantified our MS results using SPR.

Novel Aspects
We develop diagnostic microchips with a mass-tag read-out. These chips allow rapid diagnosis for various purposes, regardless the size or abundance of target molecules as well as multiplex screens.

PS00-01 / Francis William Aston: Postcards from Switzerland

Kevin Downard
University of Sydney

Introduction
Francis William Aston is best known for his discovery of the isotopes for several hundred elements by means of his mass spectrographs. Away from the Cavendish laboratory, Aston was a keen traveller and sportsman who particularly enjoyed annual visits to Switzerland to indulge in winter sports during the 1920s and 30s. An early member of the Great Britain Ski Club, Aston was a more than competent skier.
This presentation will describe Aston's adventures in Switzerland and will feature detail of his travel plans, those who accompanied him on the slopes and his activities, and include photographs and letters.

Methods
Historical research

Results
Aston made annual visits to Engelberg and Arosa in Switzerland during the 1920s and 30s. In Arosa he would stay at the Bellevue hotel or Hotel Kulm. He was sometimes accompanied by his sister Helen and on other occasions by fellow Trinity colleague and friend, mathematician John Littlewood. He also met other eminent scientists there including Hungarian chemist George de Hevesy and Danish physicist Neils Bohr. But Aston's travel arrangements didn't always go to plan as his letters to Hevesy reveal.
As well as enjoying cross country skiing, Aston's time away from the laboratory allowed him to socialise, crystallise his thoughts and gain inspiration for future experiments upon his return to Cambridge. He was not alone among the scientific community in seeking reinvigoration in the Swiss Alps. Physicist Erwin Schrödinger retreated to Arosa to help him recover from tuberculosis in the 1920s and it was here that he reportedly had an affair after becoming estranged from his wife. His rekindled love life and the fresh mountain air reportedly inspired some of Schrödinger's greatest contributions to quantum mechanics.
The presentation provides an intimate account of the life of Aston during the time of some of his greatest discoveries.

Conclusions
The presentation provides an intimate account of the life of Aston during his visits to Switzerland to indulge in winter sports and the importance of these visits to his subsequent research.

Novel Aspect
First dedicated presentation on Aston's time in Switzerland in concert with the staging of the IMSC conference in Geneva.

TPS11 - Targeted and Quantitative Proteomics

11:00-15:00
Poster Exhibition, Level -1

TPS11-01 / Substrate screening with MeCAT – A comparison of strategies for relative protein quantification

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Introduction
Due to the high dynamics of the proteome, quantitative analysis has become increasingly more important for the understanding of processes in living organisms. Nowadays, mainly methods are used, which are based on stable isotope labeling and subsequent analysis by mass spectrometry (MS). A new approach for quantification is the labeling with lanthanide chelate complexes. By using metal coded affinity tagging (MeCAT) these complexes are covalently bound to the thiol group of cysteine residues. [1] The quantification can then be performed either by molecular or elemental MS. [2,3] Here, we applied MeCAT for screening in a complex biological sample in order to identify potential substrates of the high temperature requirement protease A1 (HtrA1). The HtrA1 protease is of interest mainly due to its suspected tumor suppressor function. [4]

Methods
To meet the special requirements of a complex system, the substrate screening was performed using electrospray ionization (ESI)-MS and software-based data analysis. For the purpose of the screening, a cell lysate was divided into two aliquots. One aliquot was treated with the HtrA1 protease, whereas the other aliquot was only treated with buffer as a reference sample. Both aliquots were then labeled differentially with MeCAT and mixed. Separation of proteins was carried out by gel electrophoresis and liquid chromatography. In addition, the MeCAT based substrate screening was also performed by using inductively coupled plasma (ICP)-MS of mineralized gel bands and laser ablation (LA)/ICP-MS of two-dimensional polyacrylamide gels.

Results and Conclusions
To achieve the identification of potential substrates, the software-based data analysis was adapted to assess MeCAT labeled peptides. Thereby, the substrate screening was implemented successfully and potential substrates were identified and relatively quantified. In addition, it was demonstrated that a MeCAT-based substrate screening can also be performed using ICP-MS. ICP-MS analyses based on the mineralization of gel bands provided an absolute quantification of the proteins. Using laser ablation (LA)/ICP-MS, the proteins were additionally quantified relatively with high spatial resolution on two-dimensional polyacrylamide gels. ICP-MS-based results were then compared with the quantification by ESI-MS. The ESI-MS results were also further compared to stable isotope labeling by amino acids in cell culture (SILAC) experiments. Consistent results were generated with both MeCAT and SILAC.

Novel Aspect
Application of MeCAT on a complex biological sample. Comparison of orthogonal MS-techniques for quantification.

References
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TPS11-02 / Multiplexing TMT and SILAC for accurate quantification of mammalian proteomes in MS2 mode

Bogdan Budnik[1], Nikolai Slavov[2], Alexander van Oudenaarden[3]
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Despite the potential of Tandem Mass Tags (TMT) labeling to increase the throughput and accuracy of protein quantification, its accuracy and use remain limited by co-isolation interference. We developed experimental strategy that allows combining TMT and SILAC labeling for multiplicative increase in the number of samples that can be simultaneously quantified proteome-wide at MS2 level. We characterize the limits of TMT quantification and demonstrate comparable accuracy to that achieved by SILAC, with median fold-change error below 30 %. Combining SILAC and TMT labeling allows quantitation of whole organism proteomes1 with acceptable error and better coverage than TMT MS3-based quantification.

1. Slavov N, Budnik BA, Schwab D, Airoldi E, van Oudenaarden A (2014) Constant Growth Rate Can Be Supported by Decreasing Energy Flux and Increasing Aerobic Glycolysis. Cell Reports 7: 3 DOI: http://dx.doi.org/10.1016/j.celrep.2014.03.057

TPS11-03 / Global proteome changes in rat spinal cord associated with neuropathic pain

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Introduction:
Neuropathic pain (NP) is a major clinical syndrome caused by disease or dysfunction of the nervous system and is often mediated by neuronal networks in the dorsal spinal cord. Unfortunately, the underlying of biological mechanisms of neuropathic pain is still not well understood. We used quantitative proteomic techniques in order to quantify the changes on protein expression levels. Through submitting those protein expression profiles against existing protein pathway databases, the activated bio-functions or pathways were detected. The protein expression profiles and protein pathways that are involved in control and disease subjects likely reveal how the pain is regulated.

Methods:
Briefly, lumber spinal cord tissues of rat from pain model and two control groups were dissected into four regions (ipsilateral-dorsal; contralateral-dorsal; ipsilateral-ventral; and contralateral-ventral), then homogenized. Proteins were first extracted from homogenized rat spinal cord tissues and digested into peptides with our optimized sample preparation protocol. Then the digests of each group of rats were labeled with dimethyl isotope labels, mixed together and analyzed by nano-LC-MS/MS system, consisting of Agilent 1100 series HPLC and Thermo Fisher LTQ-FT Ultra mass spectrometer. Peptides and proteins were identified by Mascot against SwissProt database and quantification of identified proteins was performed through MSQuant. The quantified proteins with expression fold change were statistically evaluated and submitted against the existing database via IPA® searching engine from Ingenuity to identify the activated protein pathways affected by NP.

Result:
We have found a number of proteins have significantly different expression level in ipsilateral-dorsal spinal cord region between spinal nerve ligation (SNL) group and sham nerve injury (Sham) group. And few signaling pathways and ion channel proteins are strongly associated with our significant proteins. To further extend our understanding, the protein expressions in ipsilateral- and contralateral-regions of spinal cord were also compared in both Sham and SNL rat group.

Conclusions:
By quantifying the pain regulated proteins and together with protein pathway analysis, our result could explain what and how the proteins were regulated in different regions after the nerve ligation on the protein level. That is of high important for better understanding the underlying mechanisms of NP, and could provide neuropathologists potential biomarkers for further investigation.

Novel Aspect:
Dimethyl labeling-based quantatitive proteomics method was the first time applied on the study of global changes of protein expression in rat spinal cord. The proteins with significent alternated expression among different regions could reveal the pathophysiological changes in neuropathic pain.

TPS11-04 / Differential Proteomics of Monosodium Urate Crystal Induced Inflammatory Response in Dissected Murine Air Pouch Membranes by iTRAQ Technology

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Monosodium urate (MSU) crystals (NaC5H3N4O3 • H2O) are the stimulating agent of gout, which is one of the most common causes of inflammatory arthritis in men. The murine air pouch is a bursa-like space that resembles the human synovial membrane. Injection of MSU crystals into pouch induces an acute inflammatory response similar to human gout flare. The iTRAQ-based quantitative approach was used to identify differentially expressed proteins in murine air pouch membrane, and they can serve as potential biomarkers for the acute gout. The iTRAQ labeled tryptic peptide samples were fractionated by strong cation exchange (SCX), basic reverse phase chromatography or solution-IEF; following by LC-MS/MS analysis. A total of 951 proteins were identified and quantified from all combined fractions. Among them, 157 proteins exhibited ≤ 0.5 or ≥ 1.5 fold differences between MSU-induced sample and control sample. Ninety-eight up-regulated proteins were analyzed by Gene Ontology and found these differentially expressed proteins were associated with inflammation complement system, tricarboxylic acid cycle and HDL-mediated reverse cholesterol transport. S100A9 and cathelin-related antimicrobial peptide were further confirmed by using Western Blot analysis. These proteins were up-regulated in murine air pouch membrane sample and have correlation with inflammation and antimicrobial activity. Furthermore, pyruvate in TCA cycle was found could be a new signal in inflammation.

TPS11-05 / Optimization of workflow for targeted MS-based proteomic quantification of osteopontin in healthy and cancerous human breast tissues

Katarzyna Macur[1], Lars Hagen[2], Tomasz Ciesielski[3], Lucyna Konieczna[4], Jarosław Skokowski[5], Bjørn Munro Jenssen[3], Geir Slupphaug[2], Tomasz Bączek[4]
[1]Intercollegiate Faculty of Biotechnology University of Gdansk & Medical University of Gdansk, Gdansk, Poland, [2]Department of Cancer Research and Molecular Medicine and PROMEC, Proteomics and Metabolomics Core Facility, Norwegian University of Science and Technology, Trondheim, Norway, [3]Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway, [4]Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Gdansk, Poland, [5]Central Biobank of Tissues and Genetic Material Bank, Medical University of Gdańsk, Gdansk, Poland

Introduction
Osteopontin (OPN) is a protein associated with tumor aggressiveness, and is suggested to be a potential breast cancer biomarker. However, its levels typical for healthy breast tissues and tumors need to be established [1]. To address this issue we focused on the optimization of workflow, which includes OPN isolation from human breast tissue samples and its subsequent absolute quantification using a targeted proteomic approach, based on the multiple reaction monitoring mass spectrometry (MRM-MS).

Methods
The OPN levels were determined on the basis of its two characteristic peptides concentrations. A novel protocol for targeted isolation of the peptides from human healthy breast tissues and tumors was elaborated. Here, the enrichment of the analyzed OPN peptides was there achieved by capturing via anti-peptide antibodies (CAPA). Simultaneously, the MRM-MS method was developed and stable isotope labeled standards (SIS) [2] were used for their quantification.

Results
The developed workflow was tested on the pilot set of 8 normal breast tissue and 6 tumor samples. The data proceeded by PCA analysis indicated that the measured OPN concentrations show similar direction of changes as the clinically applied tumor development scale and the tumor infiltration stage.

Conclusions
The evaluated SISCAPA-MRM-MS method was successfully applied in OPN determination in the initial study on healthy and cancerous human breast tissue samples. Therefore, the method may be useful for further screening of larger number of clinical samples.

Novel Aspect
The elaborated workflow represents a novel approach for OPN quantification in normal breast tissues and breast tumors.

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TPS11-06 / Characterization of SIL Universal Antibody and SIL Human Proteins for Quantitative Mass Spectrometry

Pegah R. Jalili, James J Walters, Gordon Nicol, Kevin Ray, Anders Fridström
Sigma-Aldrich

For accurate protein quantification in the clinical setting, a prerequisite is that experimental variations in protein extraction, fractionation, enrichment, proteolysis and analysis need to be minimized. The ideal way of optimizing the reproducibility is to add a full length stable isotope labeled (SIL) recombinant protein, that is equivalent to the native target protein to the sample, at the initial stage of sample preparation. To this end, we have developed a stable-isotope-labeled universal monoclonal antibody (SILUMab) standard which contains conserved regions of IgG1 heavy chain and lambda light chain as well as SIL human proteins. A representative SIL human protein, APOA1, and SILUMab are characterized to show that they are similar to the native protein and can serve as excellent internal standards in quantitative MS workflows.

TPS11-07 / Metal labelling for quantification of post translational sugar modifications of proteins

Stefanie Ickert, Lena Ruhe, Gunnar Schwarz, Rene Becker, Sebastian Beck, Michael Linscheid
Humboldt-Universität zu Berlin

Introduction
Quantitative analysis has become more and more important in order to understand the dynamics of the proteome. Therefore, we have developed Metal Coded Affinity Tagging (MeCAT) [1] that uses chelate complexes of lanthanides for relative and absolute quantification with elemental mass spectrometry together with sequence elucidation of peptides and proteins by molecular tandem mass spectrometry. The aim of this present work is to extend the application of MeCAT to the quantification of post translational modified proteins, i.e. sugar modifications.

Methods
A MeCAT reagent harboring a lanthanide ion for quantification and also containing a dibenzocyclooctyne reactive group (DIBO) was used for quantitative labeling of Nacetylglucosamine. This reagent was coupled to post translational sugar modifications of proteins, i.e. ovalbumin (Gallus gallus), after treatment with Endoglycosidase H.
Furthermore, cysteine residues were labeled with the previously described MeCAT-IA containing another lanthanide ion. The labeled proteins were separated from buffer and excess of reagents by SDS-PAGE. After in-gel proteolysis, labeled peptides could be detected using HPLC/ESI-MS and identified with -MS/MS. In addition, ICP-MS of the labeled proteins was used for quantitative analyses, after mineralization of the gel protein bands.

Results
Endoglycosidase H was successfully utilized to deglycosilate the protein, except for one N-acetylglucosamine residue [2]. A linker with an azide reactive group could succesfully be attached to the protein for subsequent labeling using 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid with a dibenzocyclooctyne reactive group (Tb-DOTA-DIBO). All intermediates were characterized using ESI-MS/MS. Qualitative data could be obtained by relative quantification using the Tb-DOTA-DIBO as also the MeCAT-IA cysteine tag , both harboring different lanthanide ions, by HPLC/ESI-MS. Absolute quantification of sugar modification sides was done by ICP-MS and external calibration using standard salt solutions.

Conclusion
The combination of MeCAT-IA and Tb-DOTA-DIBO labeling enables a qualitative and quantitative analysis of unknown glycosylation sides using ESI-MS and tandem mass spectrometry. Furthermore, absolute quantification of selected glycosylations can now be achieved using the new Tb-DOTA-DIBO reagent in ICP-MS.

Novel Aspect
Quantitative and qualitative analysis of post translational sugar modifications of proteins using lanthanide containing metal labels.

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TPS11-08 / The investigation of detergent addition to tryptic digests for improving in-situ proteomic experiments.
Ekta Patel, Malcolm Clench, Simona Francese
Biomedical Research Centre, Sheffield Hallam University

Introduction
The incorporation of a detergent within trypsin digest protocols, has been previously described in a study of tumour tissue sections1 to improve the yield of tryptic peptides. In present investigations, a comparison between non-ionic surfactants and anionic surfactant-detergent blends is employed to further enhance protein enzymatic digestion from a range of substrates including fingermarks and fresh frozen tissue. A MALDI based bottom up proteomic approach is utilised to identify the species in-situ.

Methods
Fingermarks: Ungroomed fingermarks were deposited2 prior to in-situ enzymatic digestion with 0.5µL spots of 20µg/mL trypsin solution (reconstituted in 50mM ammonium bicarbonate, pH 8) containing either n-Octyl ß-D-glucopyranoside (OcGlu), n-Octyl, 1-thio-ß-D-glucopyranoside (OcThio), n-Decyl ß-D-maltoside (DDM), N-Octanoyl-N-methylglucamin (MEGA-8) orRAPIGEST in varied concentrations (0.5%-2%). Samples were subsequently incubated in a humidity chamber for 3 hours at 37°C.
Fresh frozen tissue: Following wash steps of ethanol and chloroform, 20µg/mL trypsin solution containing either OcGlu or MEGA-8 was deposited (seven layers) using the SunCollect automated sprayer (SunChrom, Friedrichsdorf, Germany). Samples were incubated in a humidity chamber for 4 hours at 37°C.
MALDI analysis: α-cyano-4-hydroxycinnamic acid (CHCA) with aniline in acetonitrile:water:TFAaq (1:1:0.1 by volume) was spotted (10mg/mL) onto the digest regions of the fingermarks or sprayed using the SunCollect (5mg/mL) onto the tissue sections and subjected to MALDI-MSP or MSI (HDMS SYNAPTTM G2 system, Waters Corporation, Manchester) analysis.

Results
Peptide mass fingerprints (PMFs) were evaluated for all detergents. Preliminary data from fingermarks and tissue demonstrated that at lower concentrations, DDM and MEGA-8 produced richer peptide profiles. It can be deduced that the «mass range selectivity» between the different detergents varies. Therefore in order to maximise coverage and boost the number of tryptic peptides produced across the entire mass range a mixture of all four non-ionic detergents (in varying concentrations) was utilised.

Conclusions
Although the addition of 0.1% OcGlu was previously reported to greatly increase peptide yield1, current investigations show that DDM and MEGA-8 produced richer peptide profiles thus enabling the detection of low-abundant proteins. Further work includes optimising the detergent mixture, whilst manual data interpretation and identification of peptides remains on going.

Novel aspect
The in-situ identification of proteins and peptides within fingermarks and tissue using novel detergents.

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TPS11-09 / Protein expression changes within the epidermis of living skin equivalent tissue observed across a time-course by MALDI-MSI using on-tissue digestion protocols.
Christopher Mitchell[1], Michael Donaldson[2], Simona Francese[1], Malcolm R. Clench[1]
[1]Biomedical Research Centre, Sheffield Hallam University, [2]Stiefel, A GSK Company

Introduction
Atopic Dermatitis (AD) is a chronic relapsing skin condition characterised by intense itching, dry skin and inflammation. The majority of patients present with a degree of skin dryness that reflects a decrease in water content and lipid levels in the skin. There is a strong association between eczema and with mutations in the filaggrin gene.
Living skin equivalent (LSE) models have been shown to model the activity of native skin to a high extent. In this study a bottom-up approach was employed to examine changes in protein expression in LSE over-time via MALDI-MSI. This will form the basis for modelling in-vitro disease conditions and observing skin treatment responses in future investigations.

Methods
Commercial LSE samples (LabSkinTM (Evocutis, UK)) were obtained and incubated for 4, 24 and 72 hours. After incubation, samples were washed with buffer solution and left to dry. Samples were snap frozen and stored at -80°C.
Cryostat sections were washed in 70% ethanol and then 90% ethanol for 1 min at a time. Slides were then submersed in chloroform for 5s to remove lipids.
Trypsin was dissolved in 50mM NH4HCO3 to give a 20µg/ml solution, (including 0.1% (10mM) Octyl-α1 β-glucoside)) and applied to the skin section using an automated sprayer. The sections were then incubated overnight (15 hours) at 37°C, 5% CO2 / 95% air. A matrix solution 5mg/ml αCHCA, (70% MeOH / 0.2% TFA solution with an equimolar amount of aniline) was then sprayed onto the tissue section surface using the automated sprayer.
Images were acquired on a Synapt G2 HDMS Mass Spectrometer (Waters, Manchester).

Preliminary Results
It was possible to identify a number of digested proteins and map their distribution across the epidermis of the skin model tissue sections at high mass accuracy, e.g. filaggrin, loricrin and involucrin. A spatial resolution of 50µm enabled the visualisation of the microenvironment of the epidermis, particularly the epidermal junctions e.g. granular layer. Significant changes in the

expression of the major proteins of the skin including keratin 1 m/z 1118.51 in the tissue sections were not observed.
Some noticeable changes for the filaggrin ion feature m/z 1535.3 could be observed. The distribution of the filaggrin ions were shown to be more compacted progressively with time; this was supported by multivariate statistical analysis. These changes can be associated with epidermal differentiation.

Conclusions
The MALDI images show the distribution of major peptides within skin equivalent tissue. These findings correspond well with that which is expected for native skin.

Novel Aspect
MALDI-MSI can be used to observe the process of epidermal differentiation in living skin equivalents across a time-course.

TPS11-10 / Unraveling the effects of Vitamin D on global protein expression in insulin producing cells by using SILAC in combination with 2D LC MS/MS

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Background
Experimental evidence indicates that vitamin D may play a beneficial role in pancreatic β -cell function. Global gene expression studies have shown that the active metabolite 1,25-(OH)2D3 modulated not only vitamin D-regulated genes, but also genes involved in ion transport, lipid metabolism and insulin secretion. The aim of the present study was to investigate the influence of vitamin D on the INS-1 cell proteome.

Materials and method
Stable isotope labeling by amino acids in cell culture (SILAC) in combination with high resolution 2D LC coupled to Orbitrap MS was used to quantitatively assess the impact of 1,25-(OH)2D3 on global protein expression in INS-1 cells. Proteome Discoverer computational proteomics platform (version 1.3, Thermo Scientific) with default settings was used to identify and quantify proteins. To determine the average change in protein abundance after treatment (fold changes), relative peak intensities of distinct peptides from each protein were used. The fold changes were calculated as ratios of the areas of the monoisotopic peaks of the light (treated) versus heavy (control) peptides after normalization. The cut-off for up- and down-regulated proteins was set at ≥ 2.0 and ≤ 0.5, respectively. To assign a functional category to each of the confidently identified proteins Gene Ontology (GO) and GO slim annotations were directly retrieved from the web service provided by Protein Center (http://webservice.proteincenter.proxeon.com) using Proteome Discoverer.

Results
LC MS/MS analyses of the fractionated samples resulted in over 5400 confident protein identifications. Of these, 31 were found differentially expressed in the presence of 1,25-(OH)2D3. Proteins whose expression levels markedly increased in the presence of 1,25-(OH)2D3 included proteins implicated in insulin granule motility and insulin exocytoses. Additionally, modulation of several membrane proteins, including transporters, receptors etc., suggest that 1,25-(OH)2D3 has also an impact on proteins regulating transport and homeostasis of ions. The function in pancreatic b-cells of wast majority of the vitamin D-regulated proteins identified in this study is presently unknown.

Conclusions
We reveal a number of novel vitamin D-regulated proteins which may contribute to a better understanding of the reported beneficial effects of vitamin D on pancreatic β-cells. Taken together, our findings should pave the way for future studies providing insights into molecular mechanisms by which 1,25-(OH)2D3 regulates protein expression in pancreatic β-cells.

Novel Aspect
SILAC in combination with high resolution 2D LC fractionation coupled to Orbitrap MS revealed a number of novel vitamin D-regulated proteins in INS-1 cell line.

TPS11-11 / Label free SRM-based relative quantification of antibiotic resistance in Pseudomonas aeruginosa isolates

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Introduction
Both acquired and intrinsic mechanisms play a crucial role in Pseudomonas aeruginosa antibiotic resistance. Many clinically relevant resistance mechanisms result from changes in gene expression namely multidrug efflux pumps overproduction, AmpC b-lactamase induction or derepression and/or carbapenem-specific porin OprD inactivation or repression. Changes in gene expression are usually assessed using RT-qPCR assays and require an accurate normalization. As an alternative, a label free mass spectrometry-based approach was evaluated in order to directly quantify proteins implied in antibiotic resistance.

Methods
Reproducible assays were developed using a conventional bore chromatography coupled with an electrospray mass spectrometer operating in Selected Reaction Monitoring (SRM) mode. The SRM method allowed, within a single run, the multiplexed detection of 25 proteins, including P. aeruginosa-specific peptides, MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY(-OprM) efflux systems, AmpC and OprD. Like normalization using reference genes for RT-qPCR, geometric averaging of P. aeruginosa-specific peptides allows accurate normalization necessary for relative quantification. Cutoffs for protein overproduction were established using thirty laboratory-derived mutants from the literature and verified using fifteen clinical strains. Concurrently, the same bacterial suspensions were analyzed by RT-qPCR. Antimicrobial disk susceptibility tests (AST) were done according to CLSI.

Results
Both SRM and RT-qPCR were able to identify resistance related to efflux pumps at high expression levels, AmpC derepression or decreased OprD expression.
For tripartite pumps, protein concentrations were globally in agreement with mRNA measurements but SRM results for MexAB-OprM were more accurate as measurement of a two-fold

change proved to be more significant.
Without induction, both AmpC protein and ampC mRNA predicted piperacillin/tazobactam or ceftazidime resistance. Highly reduced OprD production predicted resistance to carbapenems. Predictions of imipenem and meropenem resistance/susceptibility were, respectively, 86% and 91% in agreement with AST. One very major error with imipenem and one major error with meropenem were observed.

Conclusions
We demonstrate that SRM works as well as RT-qPCR. The mass spectrometry-based approach can be adapted to analyze complex resistance mechanisms by taking advantage of each contributory effect. As multidrug resistance of P. aeruginosa involves several multiple resistance mechanisms, this multiplexed method should allow a more targeted choice of antimicrobial treatment, in particular when efflux pump inhibitors will become available.

Novel aspect
SRM offering multiplexing and quantitative capacities is particularly suited to perform an accurate normalization of a bacterial sample as soon as surrogate microorganism-specific peptides are selected.

TPS11-12 / Comparison of data-independent and data-dependent proteomic analysis of human cells on a Thermo Scientific Q Exactive Plus instrument

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Introduction
Quantitative mass spectrometric method termed data-independent analysis (DIA) gained attention as a highly multiplexed version of targeted proteomics. We present a novel DIA method called hyper reaction monitoring (HRM) implemented on a Thermo Scientific Q Exactive Plus instrument. Using the improved resolution and speed of the newest generation of mass spectrometers, the method holds promise to combine the identification power of data-dependent analysis (DDA) and quantification ability of selected reaction monitoring (SRM).

Methods
1 µg of the HEK-293 tryptic digest were measured in technical triplicates using an Easy-nLC 1000 connected to a Thermo Scientific Q Exactive Plus mass spectrometer. The samples were separated on self-made 30 cm and 50 cm analytical columns packed with 3 µm and 1.9 µm Bruker Magic C18AQ beads respectively. A two hour gradient from 5 % to 35 % acetonitrile with 0.1 % formic acid at 300 nl/min was used.
For DDA acquisition, a modified method from Kelstrup CD, et al.J. Proteome Res. 2012 was used. The full MS scan range was from 400 to 1200 m/zwith the resolution of 70k using 1x106 ion target and 120 ms max. injection time. The top 12 most intense ions (8≥ z ≥2) per full scan were fragmented (2 m/z isolation window) and scanned with 17.5k resolution with mass range from 200 to 1800 m/z using 5x105 ions and 60 ms max. injection time. For DIA (HRM) acquisition, one full MS scan was followed by 19 DIA windows (for 30 cm column set-up) or 9 DIA windows (for 50 cm column set-up) to cover the mass range from 400 to 1200 by sequential acquisition. The full MS scan was recorded in mass range from 300 to 1800, with resolution of 35k, fill time of 120 ms and 5x106 ion target. The DIA segments were recorded using 35k resolution, automatic fill time and 3x106 ion target. The DIA isolation window ranged from 30 Th to 222 Th for 19 window method and 62 Th to 316 th for 9 window method. The DIA isolation window was calculated based on predetermined ion flux during the chromatographic separation with the 2 Th windows overlap.
The DDA acquisitions were analyzed and spectral librarys were generated by MaxQuant 1.3. The HRM runs were analyzed with Spectronaut 5.0.

Results
With the 30 cm column, the DDA acquisitions revealed 33,071 average quantifiable IDs while the HRM runs showed 34,386. For the 50 cm column set-up the DDA acquisitions revealed 36,869 average quantifiable IDs while the HRM runs showed 37,359. The median coefficient of variation for DDA was 8.75 and 11.15 while for DIA was 7.63 and 10.8 using the 30 cm and 50 cm column set-up respectively.

Conclusions
The HRM method analyzed using Spectronaut outperformed DDA method under the same conditions in terms of coefficient of variations, reproducibility and the number of quantifiable IDs.

Novel Aspect
Novel data-independent method (HRM) implemented on a Thermo Scientific Q Exactive Plus that combines identification power of DDA and quantitation ability of SRM is described.

TPS11-13 / A targeted proteomics study of lipid synthesis pathways in stromal stem cells

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Lipids are essential for energy homeostasis, organ physiology, and numerous aspects of cellular biology like building membranes, morphology changes for the specific cell function and lipid signaling. The peroxisome proliferator-activated receptors (PPARs) are nuclear fatty acid receptors that have been implicated to play an important role in obesity-related metabolic diseases such as hyperlipidemia, insulin resistance, and coronary artery disease [1].
Over the last years, PPARγ became of great interest because upon its activation it has great impact on many different biological processes in different tissues like bone formation, blood clotting and inflammation besides its main function of lipid storage, adipocyte differentiation and glucose homeostasis [2].
We want to investigate the regulatory effect of PPARγ with regard to enzymes involved in metabolic pathways particularly the enzymes of the lipid metabolism, to investigate how the metabolism itself can modulate PPARγ.
To achieve this aim we activated PPARɣ in a stromal stem cell model using Rosiglitazone, a PPARɣ-selective agonist. By the use of LC/ESI SRM we first screened and identified the enzymatic players in the pathways and will then use isotope coded peptides for the validation of our probes and the absolute quantification of targeted proteins to further investigate this pathway applying a different set of perturbations.
Our preliminary results show an up regulation of proteins which are involved in the glycolysis and pentose phosphate pathway upon Rosiglitazone treatment. A first closer look at those regulated proteins revealed them as potential downstream targets of PPARɣ.

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TPS11-14 / Characterization of an Improved Ultra-High Resolution QTOF for Proteomics Applications

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Introduction

In shotgun proteomics it is desirable to identify and quantify a large number of peptides from complex samples like human plasma samples or whole cell lysates in fast LC separations. Complexity and concentration range, however, pose a great challenge to the MS instrumentation in terms of sensitivity, resolution and dynamic range. Several hardware modifications of a bench-top QTOF were evaluated addressing these performance aspects.

Methods

To test of these modifications, different complex tryptic digests (Escherichia coli, Saccharomyces cerevisiae, human plasma) were mixed with stable isotope labeled peptides or digests of standard proteins at known concentrations over several orders of magnitude. Samples were analyzed with nanoUHPLC and a CaptiveSpray ion source connected to the modified QTOF. For peptide identification and quantitative analysis the MaxQuant software package was used (Nature Biotechnology 26, 1367 - 1372 (2008)).

Results

For higher sensitivity at fast acquisition speed, ion extraction from the collision cell into the orthogonal acceleration of the TOF-analyzer was improved by using a novel collision cell design. Increased resolution without changing the effective flight path could be achieved with a modified reflectron. In addition, a faster detector (reduced width of individual ion signals) led to further improvements in resolving power.
Using an optimized detector digitizer combination, a threefold higher dynamic range was observed. However in complex samples, the dynamic range is also limited by the capability of the instrument to resolve nearly isobaric compounds. The performance improvements were analyzed in a label-free quantification experiment, evaluating in particular the number of quantifiable peptides over the entire dynamic range. As a defined model system for complex proteomics samples, a mixture of 48 standard proteins spanning a concentration range of five orders of magnitude (Universal Proteomics Standard, UPS-2, Sigma) was spiked into samples of 500ng E. coli digest at a concentration of 1:2. During separation in two hour gradients, the UPS-2 peptides could be quantified based on the MS full scans at levels from 500fmol down to the low attomole range. Similar analyses with background samples of higher complexity (S. cerevisiae) and wider dynamic range (human plasma) were carried out to further evaluate the benefit of the novel hardware features.

Conclusions

Improvements to several hardware components allow quantification of complex proteomics samples with very high dynamic range

Novel Aspect

Increased dynamic range and speed in a QTOF system enables improvement of quantitative and qualitative proteomics.

TPS11-15 / High quantification efficiency in plasma targeted proteomics with a Q-TOF platform

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Introduction

Targeted proteomics for biomarker validation requires improved methods to precisely quantify specific subsets of peptides in complex proteomes. These tasks have traditionally been performed on triple quadrupoles, applying MRM and stable isotope labeled standards (SIS). This approach requires the knowledge of the ion transitions and time-consuming method development. Furthermore, in highly complex biological samples, such as plasma, a wide dynamic range needs to be covered with high sensitivity. High resolution systems, such as QTOF, are now able to address these limitations, while providing comparable selectivity and post-analysis data mining.

Methods

Freeze-dried plasma tryptic digests were spiked with 43 SIS peptides and re-suspended in 0.1% TFA. In total 7 samples, with SIS peptides over a 104-fold concentration range, were measured in 4x injections. Peptides were separated on a Pepmap pre-column and separated on a 25cm, 2µm PepMap UHPLC column in a 60 min gradient. A CaptiveSpray source was used as an interface with an impact QTOF, operated in the high resolution extracted ion chromatogram (HR-XIC) or middle-band CID modes. All results were processed in Skyline software.

Results

High quantification efficiency was reached for the target peptides in both the MS-based method (HR-XIC) and a middle-band CID quantification method. The fragment ion information of the latter method also provides qualitative output in the same run. The system setup enabled reproducible quantification with an average CV for peak areas at 4.5%. Retention times varied ± 15 s over a 60 min gradient. The maximum linear range of the assay was 10^4 for both approaches. Nevertheless, quantification on MS signals using HR-XIC revealed a higher dynamic range, on average, compared to quantification on MS/MS signals using data-independent middle-band CID. For both approaches, a broad quantification range could be covered for natural plasma peptides ranging from 180 ug/ml to 230 ng/ml for targeted HR-XIC and 210 ug/ml to 360 ng/ml for middle-band CID.
Results clearly show that concentrations obtained for natural plasma peptides from measurements on a UHR-Q-TOF system using HR-XIC or middle-band CID mode are comparable to the reference literature values. Thus results demonstrate capability of the tested discovery platform for targeted proteomics.

Conclusions

Using bbCID methods, targeted plasma proteomics showed very reliable results across a wide range of cocentrations

Novel Aspect

Targeted plasma proteomics on a benchtop UHR-Q-TOF system

TPS11-16 / Sortase A-mediated site-specific immobilization of peptides and proteins for interactome analysis by LC-MS/MS

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Introduction

Affinity purification-mass spectrometry(AP-MS) experiments are being increasingly used to study protein complexes and to identify protein-protein interactions. However, identification and quantification of low-abundant interaction partners is frequently

challenging because of the limited dynamic range of LC-MS/MS. The bait protein or antibody is often the dominant protein complicating the detection of less abundant interaction partners. Covalently crosslinking the bait to the matrix may facilitate MS analysis. However, most common approaches lead to randomly oriented crosslinks which may cause loss of functionality of the bait protein. Here we made use of sortase A-catalyzed transpeptidation to site-specifically attach peptides and proteins to agarose matrices, subsequently used for interaction analysis.

Methods
GGGRGGVEGGC(acyl acceptor) was synthesized by solid-phase peptide synthesis and covalently coupled on activated agarose beads (SulfoLink, Pierce). Sortase A and bait proteins (acyl donor) containing the C-terminal LPVTGGSG motif were expressed in E. coli. Sortase A-based ligation reactions were performed using different acyl acceptor/acyl donor and sortase A/acyl donor ratios.Coupling efficiency was measured by MALDI-MS using the 13C5,15N1-labeled reference peptide GSLGSSGALPV*TGGGR. Pull-down experiments were performed using "heavy" and "light" labeled Jurkat T cells (SILAC) and LC-MS/MS analysis (Orbitap Elite).

Results
Firstly, in order to prepare an immobilized acyl acceptor peptide which can be generally used in pull-down experiments, the peptide GGGRGGVEGGC containing an N-terminal GGG-motif was synthesized and covalently linked via a stable thioether bond to iodoacetyl-activated agarose beads. Subsequently, we studied the efficiency of enzyme-mediated ligation with regard to bait immobilization. Optimized and robust reaction conditions were found. Between 1 and 10 nmol of bait could be covalently linked to 20 µL agarose beads. Finally, to examine whether the "sortase approach" can be successfully utilized for interactome analysis, we report peptide-protein and protein-protein interaction studies for the T cell adapter protein ADAP and PRS-mediated interactions of the CD2BP2 GYF domain, respectively. The results compare well to conventional pull-down experiments, but the site-specific immobilization reduces the bait protein background and thus, facilitates the identification of binding partners.

Novel aspect
Site-specific immobilization of bait via sortase A-mediated ligation improves protein interaction analysis by SILAC/LC-MS/MS

TPS11-17 / Monitoring of H3K56ac level in cancer cell lines during cell cycle by SRM

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On the level of the chromatin activity of gene expression is orchestrated by the posttranslational modifications of histones. Histone acetylation generally impairs nucleosomal stability. Nucleosomes containing acetylated lysine 56 on histone H3 (H3K56ac) remain stable. Instead, this modification promotes nucleosome disassembly in the process known as nucleosome breathing. Then the DNA is more susceptible to interaction with nuclear proteins. H3K56ac is a marker of newly synthesized nucleosomes during DNA replication and reparation or high nucleosome turnover in active gene promoter site in yeasts. In contrast to yeasts, in mammalian cells the H3K56ac is catalyzed by dissimilar histone acetyltransferases with much lower efficiency. Role of H3K56ac in nuclear processes and carcinogenesis in mammalian cells is still unknown. Ambiguous and distinct results in the H3K56ac research are caused by the antigen rarity in combination with cross-reactivity of antibodies. We focused on the regulation of H3K56ac during cell cycle and compared its level in mammalian cells by mass spectrometry based method.
HeLa and HL60 cells were synchronized and used for histone isolation at different times and cell cycle stage (G1-0h, S-3h, G2-6h, G2/M/G1-9h, G1-12h after double thymidine blocks release). For mass spectrometry analysis were isolated histones prepared by Filter aided sample preparation (FASP) method. During sample preparation proteins were digested overnight by ArgC protease. We employed nanoLC Eksigent 425 coupled with QTRAP 6500 SRM for method development and further samples analysis. As targeted peptides for level evaluation of H3K56ac were monitored non-acetylated YQKSTELLIR and acetylated YQ[K(Ac)]STELLIR and their appropriate heavy forms. Moreover, peptide without known modifications from histone H3 (YRPGTVALR) was measured to normalize for the amount of H3 present in different samples.
SRM method was developed and successfully applied to H3H56ac level monitoring. We were able to distinguish levels of acetylated form in different cell cycle stages and in different cell lines.

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TPS11-18 / Increasing depth of coverage in data independent acquisition

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Introduction
Data independent acquisition (DIA) strategies have been used to increase the comprehensiveness of data collection while maintaining high quantitative reproducibility. In DIA, larger fixed-width Q1 windows are stepped across the mass range in an LC timescale, transmitting populations of peptides for fragmentation, and high resolution MS and MS/MS spectra are acquired. Previous work has shown that using more narrow Q1 windows can improve peptide detection and increase sample coverage. Here both method and instrumentation advancements will be explored to continue to increase depth of sample coverage.

Methods
The MS analysis was performed on a modified quadrupole time of flight instrument equipped with an ADC detection system. DIA data collection was done using SWATH™ acquisition with prototype acquisition software to explore a variety of acquisition strategies. The DIA data was interrogated using a comprehensive yeast spectral library created from many data dependent experiments. Results assessment was performed using Excel tools.

Results & Conclusions
Original work exploring variable window size and more narrow windows demonstrated that increasing the number of total Q1 windows from 24 to 60 windows provided an increase in confident peptide detections with good quantitative reproducibility of ~15%. To enable higher sample loads, the dynamic range of the detection system was extended by switching from a TDC based detection system to an ADC based system on a modified TripleTOF system. At the higher sample loads, we next applied increasingly narrow Q1 windows during SWATH acquisition to

continue to improve the S/N in MS/MS. The number of windows was extended from 60 to 100 windows across the 400-1250 precursor m/z range while maintaining a cycle time of 3.2 secs. This provided a 20% increase in confident peptide detections with 20% or better CVs across replicates. Further optimization of longer chromatography and investigations of impact on other proteomes will be discussed.

Novel Aspect
Improving depth of coverage of DIA results using multiple workflow strategies

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TPS11-19 / Highly selective protein proteolysis using aptamer immobilized polymer supports for mass spectrometry based proteomics

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Introduction
In proteomics studies, proteins are initially digested by specific proteolytic enzymes and then the digest mixture is analyzed using a mass spectrometer. However, traditional in-solution digestion suffers some drawbacks, such as autolysis of protease, long analysis times and lack of control. There have been developed variety of digestion methods to improve protein digestion performance. In this study, to obtain digestion products of specific proteins at very low concentrations that peptides cannot be observed when solution digestion is applied, aptamers were attached to the amine functionalized polymeric surfaces to capture their specific targets and supply a convenient media for improving protein digestion performance.

Method
Aptamer immobilized surfaces was obtained by covalent attachment of targeted protein aptamers via -SH functional groups at 5'end onto the amine functionalized polystyrene divinyl benzene (PS-DVB) using Sulfo-succinimdyl-4-(N-maleimidomethyl)cylohexane-1-carboxylate (Sulfo-SMCC) crosslinker at pH 7.2 (20 mM Tris-HCl buffer). The very low amount of lysozyme and/or thrombin containing solution (~0.007 pmol/µL) was added onto the aptamer attached solid surfaces. Then, proteins on the surface were digested using trypsin enzyme. Peptide fragments were analyzed by MALDI-TOF-MS (Voyager DE PRO, Applied Biosystems).

Results
In this study, in solution trypsin digestion method was applied using aptamer attached polymer for enrichment and efficient proteolysis of targeted proteins in proteomics applications. Thus, diffusion rate of trypsin molecules were maintained for efficient proteolysis of proteins at very low concentrations. Then, digest solutions were analyzed by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). MS signal pattern of the digest obtained from aptamer mediated proteolysis were found to be similar with conventional in solution digestion MS signal pattern. However, it was observed that aptamer immobilized surface has some important advantages such as digestion capability of proteins at very low concentrations (~0.0007 pmol/ µL).

Conclusion
The presented aptamer immobilized solid supports is a promising method for highly selective protein digestion. In addition, the negatively charged phosphate groups on immobilized aptamers improved the digestion efficiency of enzymes by attracting them via protonated ammine groups on basic amino acids of trpysin molecules. Therefore autolysis of trypsin may be minimized by electrostatic interactions between aptamers and trypsin causing surface oriented mobilization of trypsin molecules in solution. The immobilized aptamer within the microbioreactor coupled with MALDI-MS is a promising strategy for efficient protein digestion and peptide mapping in many bioanalytical applications and proteomics.

Novel aspect
Highly selective trypsin digestion approach for proteins at very low concentrations using aptamer immobilized polymer.

TPS11-20 / Amyloid beta peptide quantification via Direct Infusion - Mass Spectrometry

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Alzheimer's disease (AD) is the most common form of dementia in modern society. Pathological hallmarks of this neurodegenerative disease are senile plaques, which are mainly composed of amyloid beta (Aβ) peptides and neurofibrillary tangles. A primal role in AD pathogenesis is attributed to Aβ peptides that are under focus as an AD biomarker for early disease diagnosis. The peptides are found in plasma and in cerebrospinal fluid (CSF), while particularly the concentration of Aβ 1-42 is known to be reduced in the CSF of AD patients due to the deposition of this peptide in the brain. However, the low endogenous concentrations and the extremely hydrophobic characteristics of Aβ peptides make the identification and quantification challenging. The high aggregation tendency is especially a problem for liquid chromatography (LC) based methods particularly with regard to carry over effects. Here we present a LC independent Aβ quantification method using direct infusion mass spectrometry (MS). Analyses were performed on a novel quadrupole-orbitrap MS (Q Exactive) that facilitates quantification on the precursor level and by parallel reaction monitoring (PRM). First, synthetic peptides were used for method development in respect to reproducibility and sensitivity. Identification of Aβ peptides is possible in the low pico molar range. In addition, linearity of intensity and concentration is detected over three orders of magnitude. Subsequently, CSF samples were analyzed with the direct infusion MS method. Therefore, the CSF was processed in order to reduce the sample complexity and to achieve Aβ enrichment. By applying direct infusion MS, problems with hydrophobicity and carryover of Aβ peptides can be avoided since no chromatographic separation is used before MS analysis. Furthermore, measurement time was reduced to a few minutes. In summary, our strategy provides a fast, high throughput, selective and robust method for absolute quantification of Aβ peptides.

TPS11-21 / The Optimization of host-cell protein detection using data-independent SWATH-MS

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Introduction
Analysis of the Host Cell Proteins of Biotherapeutics by MS provides a logical way to complement and improve assays developed with other techniques. Earlier studies relied on proteomics techniques, were sometimes selective, and mostly required some pre-knowledge of the HCPs, and considerable expertise. Routine, time-effective HCP analysis would provide industry cost and time benefits for biotherapeutics development. We present routine methodology for unbiased and comprehensive HCP analysis. A standard instrument platform is used to detect and quantify a number of contaminant proteins in the presence of highly abundant product protein with unambiguous evidence for identity and concentration. Routine chromatography provides a robust methodology that is complete within a few hours.

Methods
Monoclonal mouse IgG1 digests were spiked with a commercial six-protein digest mixture at relative concentrations between 5 and 500 ppm. Samples were analyzed using a 30-minute RP LC gradient at 20 µL/min. MS-based identification was achieved using a data dependent acquisition method of one TOFMS survey scan followed by 20 MSMS spectra of 50 msec each. Triplicate SWATHTM data-independent acquisitions were performed using a 20Da Q1 window width to produce high-resolution MSMS chromatograms of every fragment ion of every precursor between m/z 400 and 1200. IDA data were searched using ProteinPilotTM Software and these results informed a peptide library used to extract fragment ion chromatograms from the SWATHTM data using PeakView® Software

Preliminary Results
Quantitative analysis was performed via peptide fragment ion chromatogram extraction using the SWATH™ Acquisition tool in PeakView® Software. For each of the model HCPs, three to four peptides were used for SWATH quantitation. The MSMS information is used for quantitation, rather than just confirmation of peptide identity, providing superior accuracy compared to MS1-based quantitation. In all cases, protein-level quantitation reflects summation of the individual peptide signals. Four peptides from the antibody itself was used for quantitation. Despite up to a 100,000 fold difference in abundance between the product and the contaminants, the quantitation of both HCPs and antibody was highly reproducible. Quantitation of the antibody serves as an internal loading control, allowing normalization of the HCP signals, enabling the comparison of inter-day HCP levels with high quantitative accuracy. This eliminates the need to dedicate a system to HCP analysis alone for a number of days. Variations in antibody response across samples run on different days were low. CVs were measured for each protein and concentration level. At the higher load levels, ≈200 ppm, CVs were all between 3-7%, and at the lowest level, ≈10 ppm, the average CV was only 7%. These results indicate extremely high quantitative reproducibility, independent of protein identity, suggesting broad applicability for the unbiased quantitation of HCPs down to low ppm levels.

Novel Aspect
Reproducible HCP quantitation down to 10 ppm in a single 30 minute gradient without using nanoflow or multidimensional chromatography.

TPS11-22 / Determination of cystatin C in human serum by isotope dilution mass spectrometry using mass overlapping peptides

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Introduction
Cystatin C is a low molecular weight protein used as alternative marker to creatinine for the determination of glomerular filtration rates. The accuracy of present routine assays must be evaluated with reference procedures. Mass spectrometric methods have been scarcely applied for the determination of Cystatin C and Isotope Dilution Mass Spectrometry (IDMS) has not been applied yet. There is a need of developing SI traceable reference methods in human serum samples to tackle the lack of standardization of protein biomarkers like Cystatin C for clinical chemistry.

Methods
We propose a peptide-based IDMS approach that uses Mass Overlapping Peptides (MOPs) minimally labeled in 13C. Molar fractions of labeled and natural abundance peptides are directly obtained from the experimental mass spectra of the in-cell fragment ions obtained by selected reaction monitoring (SRM) in a triple quadrupole instrument. Resolution of the first quadrupole is reduced so that the whole parent ion cluster is transmitted to the collision cell. So, the concentration of the natural abundance protein can be directly obtained from the fragment-ion spectrum acquired for the sample without resorting to extra calibration runs as usually needed.

Results
We accurately measured the isotopic composition of fragment ions of the target peptide. We characterized the labelled peptide in terms of isotopic enrichment and concentration using three different mass spectrometers. We demonstrated that degradation of added peptide standards occurs during protein denaturalization or reduction or alkylation of cysteine residues and that the addition of the labelled peptide just before the proteolysis step provides better precisions. Quantitative recoveries performed in reagent blanks and in a Cystatin C free serum sample ruled out the occurrence of spectral interferences. We obtained a good agreement between the experimental and the certified values during the analysis of the certified Reference Material ERM-DA471/IFCC.

Conclusions
The combination of MOPs, decreased resolution in the first mass analyzer and multiple linear regression tries to improve the current accuracy and precision of absolute quantifications of low levels of proteins in real samples. The procedure is able to measure precise and accurate isotope distributions and can be applied for the measurement of low µg g-1 levels of Cystatin C in serum samples with satisfactory results

Novel Aspect
Isotope effects due to a different physicochemical behavior of the analyte and labeled analogue are minimized using MOPs. Decreasing the resolution of the first mass analyzer the whole precursor ion cluster is transmitted to the collision cell increasing sensitivity and measuring the real isotopic distribution of the molecular fragment. From a single injection we can obtain the molar fractions of the natural and labeled peptide in the sample without resorting to a methodological calibration graph saving time and expensive labeled reagents.

TPS11-23 / Identification and quantification of low abundance host cell proteins in a high purity monoclonal antibody

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Waters

Introduction
A major portion of biopharmaceuticals today are produced by recombinant DNA-technology using a well-selected host cell system. Host cells express a large number of their own proteins that can easily contaminate the recombinant protein drug. Even after sophisticated purifications steps, low levels (1-100 ppm) of host cell proteins (HCPs) may still remain in the final purified biopharmaceutical. Because HCPs can sometimes elicit an immunogenic response, regulatory guidelines mandate that they are identified and quantified in order to protect patient safety. Mass spectrometry-based assay have been recently developed for identification and quantification of HCPs in biopharmaceuticals [1-2].

Material and Methods
A concentrated (100 mg/mL), highly-purified murine monoclonal antibody (mAb) was obtained from National Institute of Standards and Technology (USA). The sample was denatured, reduced, alkylated and digested with trypsin. The mAb digest was spiked with known concentrations of four protein digests including yeast

alcohol dehydrogenase (ADH), rabbit phosphorilase b (PHO), bovine serum albumin (BSA) and yeast enolase (ENL), used as internal calibration standards for quantitative measurements based on the peak area of the top three peptides to each protein [3]. The peptide mixture was fractionated by RP chromatography at high pH (pH 10) in the first dimension, followed by an orthogonal separation at low pH (pH 2.5) in the second dimension, in a ten-step fractionation experiment [1-2]. As peptides eluted from the second dimension, a quadruple time-of-flight mass spectrometer was used to detect the peptides and their fragments by alternating collision cell energy between a low and elevated energy state. The data was searched against a mouse database (16,600 proteins) to identify and quantify the low abundance protein impurities (HCPs) present in the mAb.

Preliminary Data
The amounts of the spiked-in protein digests loaded on the first dimension column were 2,000 femtomoles ADH, 400 femtomoles PHO, 100 femtomoles BSA and 20 femtomoles ENL.
2D-LC RP/RP separations provided enhanced dynamic range and the multiplexed low energy/high energy data collection allowed for reproducible detection of low-level HCP peptides. Four HCPs were identified in the mAb sample and their concentrations were in the range of 200 - 20 ppm. Results of the LC/MS assay (total HCPs concentrations expresses in ng HCPs/mg protein or ppm) were compared with ELISA measurements.
Our findings indicate that the 2DLC/MS assay can be used as a generic method for quantitative HCP analysis in the biopharmaceutical industry.

References:

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Novel Aspect
A 2DLC/MS-based assay enables identification and quantification of low-abundance HCPs in high-purity biopharmaceutical preparations.

TPS11-24 / Enhanced Performance and Robustness in Peptide Quantitation Using a Newly Developed Triple Quadrupole Instrument

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Introduction
Sample complexity and low concentration of some biomarkers are the main challenges in the multiple reaction monitoring (MRM)-based biomarker verification methodology. Consequently, the development of MRM-based methods with stable isotope-labeled standards peptides in biological fluids has focused on improving method sensitivity and increasing the dynamic range by lowering detection limits. Furthermore robustness and reproducibility are essential for using these assays in high-throughput clinical research environments.
This work demonstrates the sensitive and robust determination of human plasma peptides using a newly designed Triple-Quadrupole-Mass-Spectrometer. Improvements include a new optimized Q1 ion transfer optics and a novel ion detector that uses a high voltage conversion dynode with low noise characteristics.

Method
Sensitivity was assessed using both a standard peptide in a simple matrix as well as a plasma protein assay using commercially available kits. The kits provide standard plasma digest and stable-labeled synthetic peptides for 40 proteins present at different concentrations in plasma. Both a daily QC kit and a monthly calibration curve kit were used. The QC samples were interspersed between sets of hundreds of injections of plasma digest (40 µg per injection) to assess the robustness of the system. All analyses were performed using a standard-flow UHPLC coupled to the QQQ mass spectrometer. The impact of the high voltage dynode was measured using 10, 15, 18 and 20 kV settings for tryptic peptides which generated high m/z product ions.

Preliminary data:
The sensitivity of the system for a standard peptide (LVN from bovine serum albumin) was excellent, thus allowing detection of 6.25 amol on-column with an average S/N of 21 and area RSD of 3.6% (n=6). Robustness was monitored for both area response and retention time stability. After more than 600 injections of plasma digest (40 µg per injection), area response remained stable with area RSDs of QC peptides lower than 15% The retention times were extremely stable (average of 0.46% RSD for all peptides across all runs) which allowed the weeks of analyses to be performed with no modification of retention time windows. The novel high voltage conversion dynode was found to increase the area response for higher m/z product ions by 20-50% depending on the product ion.

Novel aspect
Enhanced performance and operational robustness of UHPLC/QQQ system for peptide quantitation

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TPS11-25 / The Automated Optimization of Selected Reaction Monitoring Methods for Higher Sensitive Measurements of Peptides

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Introduction
Selected reaction monitoring (SRM) known as multiple reaction monitoring (MRM) in conjunction with stable isotope dilution has been employed as a standard workflow for peptide quantification in proteomics. In MRM-based proteomics quantification, four steps will be followed to design any MRM assay; the selection of candidate proteins, the identification of representative peptides, the best selection of MRM transitions pairs (precursor/fragment ion), and the optimization of the instrument parameters, in particular collision energy (CE). In general, there are three ways to select transitions in MRM assays: i) from shotgun proteomics experiments, ii) from proteomics MRM databases or iii) by generating spectral libraries from synthetic peptides. While with shotgun experiments and MRM database the building of MRM assays are straightforward, the optimization of MRM methods with synthetic peptides can be time intensive. In the present work, the selection of the optimal MRM transitions (selectivity and sensitivity) and the tuning of instrument parameters were performed using an automated workflow based on synthetic peptides, considering the acquisition parameters (i.e. charge state, CE) and resulting in the selection of most favorable transitions set.

Methods
MS/MS spectra of one hundred ninety three isotopically labeled (`heavy`) peptides were recorded on a QTRAP 5500 (AB Sciex) individually by flow injection analysis (FIA). MS2 spectra were acquired by stepping the CE voltage from 10 to 70 V (steps of 2

V) for precursor ions with charge states of 2+/3+ in most cases, and 4+/5+ for larger peptides using a batch builder script on Analyst 1.6 (AB Sciex). The predominant charge state of each peptide, fragment ion assignments, and CE profiles generation were obtained using in-house developed java-based software (SRMOptimizer).

Results
With the SRMOptimizer software, transitions and their optimized CE values were generated from MS/MS spectra library for the 193 synthetic peptides, in which transitions were selected considering three most intense b and y ions. In order to compare the transitions selections in this approach with other tool, we generate a transitions list for all peptides from SRMAtlas (HumanPublic_2012_02). Results show 60% of similarity between the two sets of transitions, taking into account the difference in the CE values due to the way of calculating or optimizing these values using SRMAtlas or SRMOptimizer software, respectively. To assess the relative effect of optimizing MRM transitions by this approach in a quantitative manner, the relative intensities of the two sets of transitions were compared. This approach shows about 2- 10 fold higher in the relative intensity, in particular for these peptides with low signal response.

Conclusion
This study is suggesting MRM assay development workflow based on synthetic peptides libraries that allow for more sensitive measurements. Moreover, the optimization of all fragment ions in the MS/MS library will help users to replace non-selective transitions in their MRM assay.

Novel Aspect
Developing an automated workflow to optimize MRM transitions for higher sensitive measurements

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TPS11-26 / Investigating the effect of protein degradation on the quantification of genetically modified soya using stable isotope labeling and mass spectrometry

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Introduction
Genetic modified (GM) soya, such as Roundup Ready Soybean,which contains the 5-enolpyruvlshikimate-3-phosphate synthase (EPSPS) gene from Agrobacterium tumefaciens CP4 confers resistance to herbicide glyphosate. The protein-based approach for detecting the level of GM soybean is based on the qualitative analysis of CP4 EPSPS protein that is only present in GM soya. The digests of CP4 EPSPS purified from soy seed were labeled with stable formaldehyde isotopes and analyzed by MS. The method was applied to investigate the degradation of the CP4 EPSPS protein by heat. The quantification of GM levels was performed using the degraded fragments of CP4 EPSPS. A strategy was proposed to quantify the GM levels for highly processed foods.

Method
Aliquots containing identical amount of the protein extract purified from GM soy seed by SAX column were heated at 95 °C for 0, 30, 60 and 120 min and at 121°C for 0 and 30 min. One aliquot without heating was used as an internal standard. For the quantification of CP4 EPSPS protein, all aliquots were separated by SDS PAGE. The digests of heated samples were labeled with dimethyl-H2 and those of reference samples without heating were labeled with dimethyl-D2. The dimethyl-H2 labeled samples were mixed with the equal amount of dimethyl-D2 labeled references. The temperature effects was quantified by analyzing the mixtures of isotopic labeled peptides using MALDI-TOF MS. The degraded peptides were also analyzed using LC-MS. The isotope labeled digests of heated samples without SDS PAGE separation were analyzed using LC-MS and peptide ratios were used to determine the levels of GM soya.

Results
The CP4 EPSPS peptide SFMFGGLASGETR [M+H]+ at m/z 1359 was used for quantification of CP4 EPSPS and the m/z values of the H- and D-labeled peptide were 1387 and 1391, respectively. The intensity ratio of the m/z 1387 to 1391 is directly proportional to the peptide ratio of the H- to D-labeled peptide. After being heated at 95 °C for 30, 60 and 120 min , the CP4 EPSPS protein had decreased by 20%, 35% and greater than 80%, respectively. The CP4 EPSPS protein decreased by greater than 80% when the sample was autoclaved at 121 °C for 30 min. Several peptides derived from degraded CP4 EPSPS quantified using isotope labeling and LC-MS. The GM levels of real food samples were determined using the proposed method.

Conclusions
The effect of protein degradation on the quantification of genetically modified soya was investigated using stable isotope labeling and mass spectrometry. The degraded peptides from CP4EPSPS were used to quantify the GM levels of foods.

Novel Aspect
The dimethyl labeling method combined with MS was applied to quantify the GM levels of highly processed foods.

TPS11-27 / Quantitation of MET using Mass Spectrometry for Clinical Application: Correlation with IHC and MET Gene Amplification in FFPE Tumor Tissue

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Introduction
Aberrant up-regulation and activation of MET has been documented in multiple kinds of cancer and several anti-MET agents are in late stage clinical trials. Currently, IHC of FFPE tissues is used to quantify MET expression; however, IHC suffers from antibody non-specificity and lack of quantitative resolution. Moreover, MET IHC is hampered by antigenic instability in FFPE sections, limiting its utility to recently cut FFPE sections. In contrast, the Liquid Tissue-selected reaction monitoring (LT-SRM) platform is quantitative, epitope independent, and allows for simultaneous quantitation of several targets from a single FFPE section. Increasing recognition of the importance of other biomarkers in cancers suggests that 'economic' testing of scarce samples will be necessary. Consequently, we sought to develop a MET quantitative assay within our multiplexed LT-SRM MS test.

Methods
We used trypsin digestion mapping of rMET to identify unique peptides for MS assay development. The assay was pre-clinically validated in 5 cell lines, and compared to a quantitative immunoassay measurement of MET protein. To assess the MET MS assay stability from archival FFPE sections, freshly cut FFPE tissue sections were immediately microdissected, processed and analyzed, while adjacent sections were processed and analyzed one year after cutting. We evaluated the expression level of MET in 130 FFPE GEC tissues. The correlation of SRM MET expression to IHC and FISH was assessed.

Results
Proteomic mapping of rMET was used to identify the optimal SRM peptide. The LLOD for this peptide was 150 amol. Validation of the MET MS assay on cell lines revealed concordance when compared to ECL. The MET LT-SRM assay demonstrated excellent temporal stability, defined as reproducibility of measurements in serial sections cut from 33 FFPE tumors analyzed one year apart. Analysis of 130 GEC FFPE tissues showed a broad range of MET levels (<150 amol/ug to 4669.5 amol/ug). A high correlation was observed between SRM MET expression and both MET GCN and MET/CEP7 ratio as determined by FISH (n=30; R2=0.8982). The IHC (H-Score) did not correlate well with SRM (n= 44; R2=0.5371) nor FISH GCN (n=31; R2=0.5093). A MET SRM level of >1500 amol/ug was 100% sensitive (95% CI 0.69-1) and 100% specific (95% CI 0.92-1) for MET amplification by FISH.

Conclusions
The OncoPlexDx multiplexed assay measures the absolute level of MET and the other targets in clinical tumor FFPE tissue with high precision. Compared to IHC, SRM provided a quantitative and linear measurement of MET expression, reliably distinguishing between non-amplified and amplified MET tumors at a cut-off point of >1500 amol/ug.

Novel Aspect
Our LT-SRM platform allows the quantification of MET and the other targets from clinical FFPE tissue, providing essential information that will help inform clinical decisions. We are currently running the assay in a CLIA-certified-CAP-accredited laboratory at OncoPlexDx.

TPS11-28 / Automated High Throughput Peptide and Protein MRM Optimization for Pharmaceutical Method Development
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Introduction
In order to support the expanding protein component of pharmaceutical companies' drug pipeline, the rapid development of sensitive and selective peptide bioanalytical methods are required. As an alternative to ligand binding assays (LBAs) LC-MS/MS methods are both sensitive and selective, have a wide dynamic range, and have been a staple in the quantitation of small molecule drugs. Choosing the best MRM for the peptide or protein is a challenge because multiple charge states are possible and the many product ion possibilities leave many MRMs to be screened. In addition manual tuning can be tedious, and optimizing via LC injections is time consuming when monitoring multiple MRMs per peptide and multiple peptides per protein.

Methods
DiscoveryQuantTM software was used with infusion to screen the charge state distribution of a small protein and to tune and optimize the trypsin digest of a larger protein in two different workflows. The QuickTune and FineTune experiments were used to identify product ions of the peptides, and then optimize ion optic parameters using MRM based methods. Secondly Skyline (MacCoss Lab) was used to assign the product ions to the tryptic peptides and used with the FineTune experiment to optimize the CE and DP assigned by Skyline. The optimized parameters from both of these workflows were used to create LC-MS/MS methods and the sensitivity of the methods were compared to a LC-MS/MS method constructed using unoptimized Skyline parameters.

Preliminary Results/Abstract
Fifteen peptides between 8 and 25 amino acids in length were observed in the digest mixture of E. coli BGAL. The product ion spectra were collected at several discrete collision energies using QuickTune followed by an MRM based optimization of DP, CE and CXP using FineTune. The optimized parameters were incorporated into an LC-MRM method and used to analyze the sample. A comparison was made to the results of an LC-MRM injection constructed using parameters from Skyline software (MacCoss Lab). There were gains in sensitivity and signal to noise ratio observed for the majority of peptides in the mixture, the average gain was >50%. Two of the peptides with the highest gains in sensitivity had a product ion found by automated software tuning that could not be assigned to the a, b, c or x, y, z ion type.
In the second workflow, Skyline software was used to predict the y ion spectrum of each of the fifteen peptides. These ion masses along with a CE and precursor ion DP were imported into DiscoveryQuantTM software and used for the basis of an MRM FineTune. These optimized parameters were incorporated into an LC-MRM method and compared to the unoptimized Skyline LC-MRM method. Again the majority of peptides showed a gain in sensitivity, the average gain was again >50%.
Using the charge state optimization workflow the charge state distribution of human insulin was illustrated under different LC conditions along with the product ion spectrum arising from each charge state.

Novel Aspect
An automated and high-throughput workflow for the tuning and optimization of therapeutic protein and peptide molecules using automated software tool.

TPS11-29 / Flexible and Multiplexed Targeted Quantification of Proteins by GeLC MS/MS
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Introduction
Biological research often focuses on the functional characterization of a limited selection of interesting proteins, whose abundances alter specifically in response to genetic, physiological or pharmacological interventions. For quantifying proteins, Western blots or ELISA are commonly employed yet they are impeded by the availability and specificity of antibodies as well as limited dynamic range and multiplexing capabilities. Protein quantification by mass spectrometry with chemically synthesized standards is laborious and inflexible for small scale discovery studies. Here we report the development of a fast, inexpensive pipeline for targeted multiplexed protein quantification that relies on the expression of isotopically labeled reporter peptides and GeLC-MS/MS, which may substitute Western blots and/or ELISA in selected biological and clinical applications.

Methods
We selected 31 reporter peptides (4 to 8 peptides per protein) who were most abundant in the tryptic digests of 5 common protein standards. A chimeric gene encoding these peptides flanked by Strep- and His-tags was chemically synthesized (Life Technologies), and expressed in a Lys, Arg dual-auxotroph E.coli strain in the media complemented with 13C15N-Arg and 13C-Lys (Silantes). Tryptic in-gel digests were analyzed by LC-MS/MS on a LTQ Orbitrap Velos (Thermo Fisher Scientific) using Mascot (Matrix Science) and Progenesis LC-MS (Nonlinear Dynamics) software.

Results
LC-MS/MS analysis of the tryptic digest of 41 kDa chimera protein confirmed that its full length sequence was expressed with >98% heavy isotope incorporation efficiency. Its trypsin cleavage was quantitative and peptide yields were the same as in the digests of corresponding standard proteins despite in the

chimera peptides were concatenated in an arbitrary order.
We evaluated the quantification capabilities by spiking 20 fmols to 25 pmols of an equimolar mixture of the same 5 proteins into a constant background of 50µg E.coli lysate and subjected them to SDS PAGE. Gel slabs corresponding to spiked proteins were excised along with bacterial background, combined with gel bands having equal amounts of isotopically labeled chimera, co-digested with trypsin and subjected to LC-MS/MS. The relative quantification of spiked proteins based on the abundance ratio of "light" and "heavy" peptide counterparts was accurate (R2=0.96) over a dynamic range from 2 fmol to 2.7 pmol of the injected target proteins and enabled their quantification at 0.17-1.94 ng amounts despite dominating (>104-fold) excess of background E.coli proteins.

Conclusion
Co-digesting gel separated isotopically labeled chimera with five native proteins provided technically simple and accurate means for their targeted quantification at the low femtomole range from total protein extracts.

Novel Aspect
Flexible multiplex targeted protein quantification by GeLC MS/MS.

TPS11-30 / Chemical hydrolysis-based middle-down proteomics

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Advances in high-resolution mass spectrometry (MS) and liquid chromatography (LC) allow practical implementation of middle-down proteomics (MDP) approaches for proteome characterization. Large peptides (>3 kDa) analyzed in MDP minimize the number of database-matched MS/MS spectra required for confident protein identification and increase the probability of localizing multiple post-translational modifications (PTMs) in a single MS/MS scan. Given the absence of a sufficiently specific protease for the generation of large peptides, we considered highly specific chemical reagents targeting the least frequent amino acid residues present in proteomes (Cys and/or Trp) to develop a chemical hydrolysis-based MDP platform.
Commercial protein mixtures were digested with 2-nitro-5-thiocyanobenzoic acid (NTCB) that cleaves at Cys; ando-iodosobenzoic acid and BNPS-skatole that cleave at Trp. The effect of reaction buffers, temperature, and cleaving agent-to-protein ratio were evaluated. Isoelectric point-based pre-fractionation was applied prior to reversed-phase liquid chromatography (LC) performed with either C8 or monolithic columns. MS analysis was carried out on a LTQ Orbitrap Elite FTMS (Thermo Scientific) running top-5/10 data-dependent MS/MS acquisitions using ETD, CID, or HCD. Data analysis used SEQUEST, MS-Align+, X!Tandem, and OMSSA, with manual validation.
First, a 7-protein mixture was used for assessing the efficiency of digestion protocols. The proposed digestion strategies allowed for identification of all proteins, with average peptide size of 5.5 kDa. Average peptide charge state spanned between 4+ for Cys and 9+ for Trp cleavage. High reproducibility of chemical hydrolysis was confirmed by multiple replicas. Efficiency of MS analysis was improved by modifying acquisition parameters along the chromatographic gradient, taking into account the wide range of peptide length and charge state distributions. Given the high number of cleavage sites identified and the simultaneous lack of observed reaction side products, the NTCB protocol was chosen for further optimization and application on large scale investigation of yeast and human proteomes. Bioinformatic survey indicates that unique peptides larger than 3 kDa generated by NTCB guarantee alone identification of 86.8 and 80.6% of total gene products forS. cerevisiaeandH. sapiens, respectively. Finally, a bioinformatic search strategy specific for chemical-based MDP, which accounts for the mass variation induced by a particular chemical agent (e.g., NTCB cleavage produces a +24.99 Da mass shift), is under development.
A robust chemical-based MDP workflow has been designed, comprising optimized digestion protocol, peptide fractionation, LC separation and MS analysis. Current work focuses on developed workflow validation in proteomics-grade experiments.

Novel aspect
Simplified and efficient protein chemical digestion for production of large (~5 kDa) peptides is proposed for middle-down proteomics.

TPS11-31 / Automated sample preparation workflows for MS-based proteomics applications

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Introduction
Mass spectrometry (MS) based bottom-up proteomics is based on the large scale identification of peptides, and depends upon proteins being efficiently converted to peptides by a protease of known specificity. The most common preparation methods are digestion in solution (ISD) or digestion of proteins separated on an SDS-PAGE gel, in-gel digestion (IGD). Both methods consist of a lengthy sequence of washing and chemical modification steps. To increase throughput and reproducibility, automation of these processes is highly desired. Contrary to other Omic-applications, proteomics analysis by LC-MS/MS remains time-intensive, making the measurement the rate-determining step in the pipeline. Thus the preparation of samples does not require a high- but rather a medium-throughput setup.

Methods
The normal benchtop methods for the IGD and the ISD were adapted to make automation in a robotic setup possible. In addition, we modified a standard CTC-PAL robot setup with a vacuum chamber that can be controlled by the robot's software and facilitates the removal of large volumes washing solvents required by IGD. In order to check for the quality and to exclude the possibility of cross-contamination during the automated digests, a number of mass-spectrometry driven assays were established. Using SILAC-labelled proteins (stable isotope labeling in cell culture) we were able to test for the different quality parameters.

Preliminary data
By using this pipetting robot tool we were able to achieve efficient and reproducible ISDs in a medium-throughput procedure. In experiments comparing directly the quality of the machine-driven digestion with a manual preparation, we were able to show comparable identification numbers with higher reproducibility of the digest in the automated setup. Furthermore, by integrating a vacuum chamber, we are able to perform the rigorous washing steps required for the efficient digestion of proteins embedded in acrylamide gel-slices (SDS-PAGE slices). Similarly to the automated ISD, MS based proteomics revealed that the automated procedure was not only more reproducible than the manual format, but in the case of the IGD it lead to more identifications and less background, too. In contrast to competing high-throughput liquid-handling robot systems, the modified PAL RTC systems are less expensive and provide more flexibility.

Novel aspect
Our solution provides a flexible and cost effective platform for proteomicssample preparation with high productivity and good reproducibility. Different digestion protocols can be realised on this platform.The medium-throughput achieved with our solution is ideally suited for labs processing dozens to a few hundred samples per week.

TPS11-32 / Comparison of different sample preparation strategies reveals quantification biases in gram-negative bacteria and human cells
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Introduction
The sensitivity, accuracy and robustness in quantitative LC-MS studies heavily rely on efficient sample preparation procedures. In particular absolute quantification studies critically depend on protocols that allow a maximum of protein solubilization and detection of fully cleaved peptides derived from efficient enzymatic digestion. Moreover, suboptimal sample preparation protocols will result in an underrepresentation of proteins and therefore cause erroneous absolute quantification.
In this study we evaluated the propensity of different one-step sample preparation strategies for protein solubilization and digestion efficiency and report on buffer specific quantification biases of subproteomes and complexes in Pseudomonas aeroginosa and Hek293 cells.

Methods
We performed protein extraction using different chaotropic (urea and guanidiniumhydrochorid) and MS-compatible detergent (RapiGest (RG), Sodiumdeoxycholate (SDC)) containing buffers in independent triplicate experiments. Following tandem LysC/trypsin digestion peptides were desalted and analyzed on a dual-pressure LTQ-Orbitrap. Evaluation of label-free quantification (Progenesis, Nonlinear Dynamics) results was performed by SafeQuant and significantly regulated proteins were then subjected to the functional annotation tool DAVID.

Results
Based on our comparative study we obtained strong differences between the buffer systems. The detergent based systems were superior in protein extraction and interfered less with proteolysis as more human and bacterial protein IDs with more fully cleaved peptides were observed compared to chaotropic buffers.
However, upon label-free quantification and DAVID analysis we obtained buffer specific enrichment of individual protein classes. Whereas high abundant protein complexes like ribosomes and human histones appear more abundant in the presence of Gua, detergents significantly enrich for different types of membrane proteins in both species. Although individual high abundant protein complexes and cytoskeleton proteins are detected with higher intensities using chaotropic salts, the detergent based buffers affect most of the proteins in the bacterial and human proteome with SDC having slight preferences over RG.

Conclusion
Solubilization and digestion buffer systems specifically enrich for individual protein classes, which cause buffer specific biases that would affect absolute protein quantification. The introduction of major biases can be counteracted by correction factors for the most affected protein classes.

Novel Aspect
In-depth assessment of the quantification biases introduced by different protein solubilization/digestion strategies and impact on composition of the detectable proteome.

TPS11-33 / A new method to control ratio distortion for isobaric labeling approaches
Erik Ahrné, Timo Glatter, Alexander Schmidt
Proteomics Core Facility, Biozentrum, University of Basel

Introduction
Peptide labeling with isobaric mass tags enables sensitive and precise multiplexed peptide/protein quantification. However the co-fragmentation of multiple peptides in a single MS/MS spectrum impairs the accuracy of this quantification approach. Important and systematic ratio suppression has been reported for iTRAQ and TMT labeling experiments and several methods, both bioinformatic and experimental, have been proposed to minimize ratio distortion.

Methods
This includes an algorithm, which detects co-isolated peptide signals in the MS1 spectrum, and subsequently estimate and adjust for co-fragmentation in the MS2 spectrum1. In another study, extensive fractionation, narrowing the precursor-ion isolation width as well as delaying peptide fragmentation to occur closer to the apex of the chromatographic peak have demonstrated to reduce co-fragmentation and ratio suppression2. Finally, triple-stage mass spectrometry (MS3) has been reported to dramatically improve quantification accuracy, but at the expense of significantly lower peptide and protein identification rates3. Here we propose a straightforward method using internal calibrants to globally correct the measured ratios from an isobaric mass tag experiment without losing proteome coverage.

Results
In agreement with the results reported in previous studies we observe a log linear correlation between TMT ratios and reference ratios for multiple different data sets4. Thus, modeling TMT ratios as a function of a set of known protein/peptide ratios spanning the full quantification range allows us to derive a correction model. We evaluate our approach under two scenarios; 1) where reference ratios are obtained from a separate MS1 signal based label-free experiment and 2) where heavy-labeled AQUA peptides (Thermo Fisher Scientific) are employed. Our global ratio correction method is thoroughly benchmarked on both TMT 6-plex and 10-plex datasets acquired from samples where two proteomes (Bartonella and Human) are mixed at fixed ratios.
The necessary data processing steps to obtain adjusted ratios are implemented in the SafeQuant software tool5.

Conclusion
Our ratio correction method requires limited additional experimental and bioinformatic efforts and is compatible with any isobaric labeling method and high-resolution LC-MS platform. The full quantification workflow offers an attractive alternative to MS1 signal based quantification (e.g. label-free or SILAC) providing high proteome coverage and discrimination between constant and altered protein abundances across two or multiple sample conditions while accurately measuring their magnitude.

1) Savitski, M. et al., Proteome Res 2013
2) Savitski, M. et al., Anal. Chem. 2011
3) Ting, L. et al., Nature Methods 2011
4) Mertins, P. et al., Mol. Cell Proteomics 2012
5) Glatter, T. et al., J Proteome Res 2012

TPS11-34 / Studying the effect of natural genetic variation on protein abundance in C. elegans

Kapil Dev Singh[1], Bernd Roschitzki[2], Mark Elvin[3], Gino Poulin[3], Basten Snoek[4], Jan E. Kammenga[4], Sabine Schrimpf[1], Michael Hengartner[1]
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Introduction

Complex diseases are caused by a combination of genetic, environmental as well as lifestyle factors, and are mostly polygenic in nature. To study the influence of natural genetic variation on the development of complex diseases, we are using Caenorhabditis elegans, a model organism that has orthologs of many human disease genes. We focus on cancer signaling pathways (Apoptosis, Notch, MAPK and Wnt). From the two highly divergent wild-type strains - Bristol N2 and Hawaii CB4856, 200 recombinant inbred lines (RILs) were generated. Transcriptome analysis of these RILs showed significant heritable variation in gene expression, but very little is known about variation at the protein level. We used Selected Reaction Monitoring (SRM) to determine the effect of natural variation on protein abundance. Our results indicate that for signaling pathways transcriptome variation tends to be higher than proteome variation.

Materials and Methods

From the above mentioned signaling pathways 156 core proteins with 377 proteotypic peptides (PTPs) were selected. Heavy labeled PTPs (hPTPs, from JPT technologies) were used as internal controls. By SRM triggered MS/MS, a consensus MS/MS spectra library was generated for 340 (90.2 %) peptides corresponding to 154 proteins. Based on this MS/MS library, 5 transitions per precursor per peptide were defined and further validated by spiking hPTPs into worm samples. To reduce complexity, samples were pre-fractionated prior to SRM by reversed-phase chromatography on an YMC-Triart C18 column at pH 11 on an Agilent 1100 HPLC system. SRM measurements were acquired on a TSQ Vantage mass spectrometer followed by data analysis using mProphet software, and protein significance analysis using R-package MSstats.

Results

From our initial experiment with two biological replicates of N2 and CB4856 and one sample of four RILs (WN31, WN71, WN105 and WN186; empirically selected based on the largest variation in transcriptome expression levels) we were able to quantify between 71 and 116 proteins. Forty-four proteins (mostly represented by 1 or 2 peptides) could be quantified in all samples with the mProphet software. These 44 proteins (represented by 114 PTPs) were measured additionally in 3 biological replicates of the above mentioned 6 lines, to allow statistical data evaluation and for further confirmation of the differential expression. Most of the proteins were non-significant and/or below fold-change cutoff, but 8 proteins (LIN-2, LIP-1, MEK-2, PSR-1, SIR-2.1, SPR-5, SUP-17 and SUR-7) showed significant differential expression. These 8 proteins (represented by 15 PTPs) were measured additionally in one sample of 50 core RILs to perform protein quantitative trait locus (QTL) mapping. Only for PSR-1 we found significant QTL which showed a weak trans-QTL on chromosome 2.

Conclusions

Based on our data we conclude that protein levels of the analyzed signaling pathway are under stronger evolutionary control than transcript levels.

Novel Aspect

This project is unique because it combines a targeted approach to analyze the effect of genetic variation on selected proteins with the use of protein QTL mapping in RILs.

TPS11-35 / There's a hole in my assay, dear ELISA, dear ELISA! Using targeted MS to detect gluten in beer that is invisible to ELISA.

Michelle Colgrave, Hareshwar Goswami, Crispin Howitt, Greg Tanner
CSIRO

Introduction

Gluten is a diverse class of proteins found in wheat, rye, barley and oats. Coeliac disease (CD) is estimated to affect ~70 million people globally, with the majority remaining undiagnosed. When CD patients ingest gluten, it triggers an inappropriate auto-immune reaction resulting in intestinal inflammation and damage. The only current treatment for CD and gluten intolerants is lifelong avoidance of dietary gluten, however, such diets are costly and often low in fibre and high in calories, which in themselves are health risks. The worldwide market for gluten-free products is predicted to grow by over 10% per annum to a value of more than US$6 billion in 2018. Gluten-free foods are commonplace, however, current methodologies (ELISA) do not accurately measure gluten as the antibodies do not detect all classes of gluten equally and assumptions are made in the calculation of gluten content.

Methods

Gluten, extracted from barley flour using optimised protocols, was subjected to enzymatic digestion by filter-aided sample preparation or directly digested in beer. The gluten peptide fragments were identified and characterised by high resolution LC-MS/MS on a 5600 TripleTOF (AB/Sciex) with proteins identified from the Magniolophyta subset of proteins from Uniprot-KB (ProteinPilot, AB/Sciex). An MRM-based approach (on a 4000 QTRAP, AB/Sciex) was explored for specific protein identification and quantification in food ingredients and products. Gluten in beer was quantified using the WHO-accepted ELISA that employs the Skerritt antibody raised against wheat gliadin.

Results

Prototypic peptides to act as biomarkers of gluten were selected based on the results of in solution digests of gluten extracts from barley flour. MRM analysis of both flour and beer revealed those peptides present in an unmodified form, an important step to verify that the selected gluten fragments will enable gluten quantification in commercial foods. In addition to examining whole beer, comprehensive profiling of the sub-30 kDa and sub-10 kDa fractions of beer were undertaken and revealed a significant amount of truncated and degraded gluten. Strikingly, beers that contained very high levels of B-hordein fragments in the sub-10 kDa fraction gave near-zero ELISA readings despite containing significant levels of gluten as judged by MRM-MS. When these beers were spiked into a beer that elicited a strong ELISA response, this response was suppressed in a dose-dependent manner. Furthermore, the suppressant was found to be present in the sub-3 kDa fraction, implying that the gluten fragments present in beer are responsible for false negatives seen by ELISA.

Conclusions

ELISA is suitable for measuring gluten in raw, unprocessed food precursors, such as flour. In beers, the presence of gluten fragments leads to suppression of the ELISA response. Targeted mass spectrometry holds promise as an alternative to the WHO-accepted ELISA technology.

TPS11-36 / Evaluation of candidate proteases for middle-down proteomics

Liana Tsiatsiani, Henk van den Toorn, Frank van Breusegem, A.F. Maarten Altelaar, Albert J.R. Heck
Utrecht University

Mass spectrometry-based proteomics is the most powerful technique for the study of proteins in complex samples such as cell or tissue extracts. Besides large-scale protein identification and quantification, characterization of post-translational modifications is also made possible. However, the two main routes in proteomics, bottom-up and top-down, suffer from limitations in protein sequence coverage and spectra deconvolution, respectively. For this, a third avenue, the middle-down proteomics, is emerging that similar to bottom-up proteomics is based on proteolytic digestion of proteins but employs longer than tryptic peptides (typically <3kDA) for protein assignment. Key challenges for middle-down proteomics are the robust generation of peptides in the range of 10kDa, efficient chromatographic separation of these peptides in complex mixtures and finally peptide fragmentation efficiency. In this study we focus on the sample preparation for middle-down proteomics and in particular on the proteolytic generation of long peptides. To this end, we evaluate METACASPASE-9 (MC9), a plant protease from Arabidopsis thaliana, which cleaves N-terminally to Arg or Lys residues but with a narrower specificity than trypsin.

We characterized the MC9 enzymatic activity using fluorescence-based activity assays, in silico and in vitro digestions of single protein substrates or Escherichia coli cell lysates. The digestion products were analyzed with LC-MS/MS and different fragmentation methods such as Higher-energy Collisional Dissociation (HCD), Electron-Transfer Dissociation (ETD) and Electron-Transfer/Higher-Energy Collision Dissociation (EThcD) using an Orbitrap Elite mass spectrometer. Digestions of Bovine serum albumin (BSA) with MC9 or trypsin showed that MC9 peptides are about 500Da bigger than tryptic peptides and 40% of those share the same sequence with tryptic peptides. This indicates that the two proteases generate partially different subsets of peptides. Furthermore, MC9 peptides increased BSA sequence coverage by 4% and were more frequently identified by ETD fragmentation.

In silico digestion of the E.coli proteome with MC9 or trypsin showed that the average peptide mass is about 6 and 2kDa, respectively. Subsequent experimental data showed that MC9 peptides had an average mass of 2kDa and were on average 400Da longer than the tryptic peptides. Compared to the mass range of identified tryptic peptides (0.7-6.5kDa) , MC9 peptide mass range was extended by 1kDa to 0.7-7.5kDa, irrespectively of the employed fragmentation method.

Besides its property to generate longer peptides than trypsin, MC9 activity remained high in denaturing conditions and its specificity was triggered by acidic residues proximal to the basic Arg or Lys. Such peptide sequences, which include adjacent basic-acidic residues, are often missed by trypsin and for this reason MC9 is superior in cleaving these sites. Based on the previous and given that protein phosphorylation sites also carry a negative charge, MC9 could be a beneficial enzyme in middle-down and phospho-proteomics.

TPS11-37 / Targeted proteomics approach to develop a bioassay detecting environmental glucocorticoids with zebrafish embryos

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Introduction

Synthetic glucocorticoids (GCs) are widely used in medicine. These compounds have been detected in the aquatic environment, and their predicted concentration range is 10-1000 ng/L (0.03-3 nM). GCs mimic cortisol, the natural stress hormone, by acting through the glucocorticoid receptor (GR) and altering related gene expression patterns. Potential adverse effects of GCs range from developmental toxicity to impairment of stress response, glucose metabolism and immunosuppression. The molecular mechanisms behind these phenomena are incompletely understood. This project investigates the effects of GCs on protein expression with the aim to derive specific and sensitive protein biomarkers for GC-like activity that can be used in effect-directed analysis (EDA), potentially identifying novel GR-active compounds. In our work, zebrafish embryos are used to investigate the effects of clobetasol propionate (CP), a highly potent GR-active compound.

Methods

Targeted proteomics analyses are conducted on tryptic digests from 30-40 zebrafish embryos exposed to 10 or 100 nM CP from 96-120 hours post fertilization. GR target genes were selected based on previous knowledge of GC-regulated genes and physiological processes. Their protein expression are analyzed by multiple reaction monitoring (MRM), a technology with a unique potential for reliable quantification of analytes of low abundance in complex mixtures. In MRM analysis, tryptic peptides are analyzed by LC-MS using a TSQ Quantum Ultra MS (Thermo Scientific). Up to 5 proteotypic peptides per protein were selected according to in silico prediction. The two most intense transitions and their ratios are then monitored. The relative abundance of each protein is quantified by comparing the results to the protein products of a known housekeeping gene, Rps18.Differential analysis will identify candidate proteins responding to CP exposure.

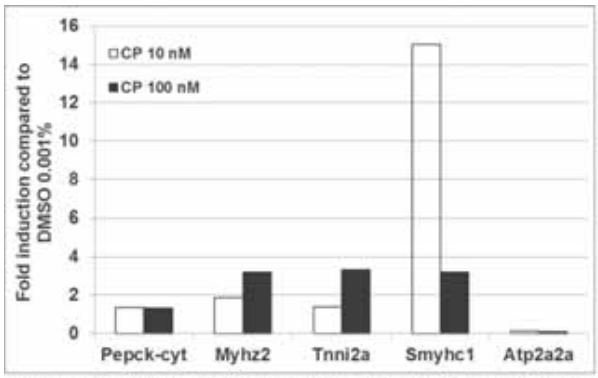

Figure: Relative protein abundance after CP exposure

Results

The MRM assays are under development, by now we have established them for 60% of the intended targets. In a trial experiment, 5 target proteins (Pepck-cyt, Myhz2, Tnni2a, Smyhc1 and Atp2a2a) were monitored in control and exposed samples (Figure). Smyhc1 and Atp2a2a showed strong changes in expression in exposed samples and therefore are potential biomarkers of GC exposure. Further experiments are ongoing.

Conclusions

The trial experiment already identified two potential protein biomarkers of GC exposure. After having assembled the full set of potential biomarkers, they will be validated by other GR-active

compounds, non-GR-active compounds and environ¬men¬tal water samples in order to prove their specificity and sensitivity.

Novel aspect
The search for novel protein biomarkers will increase our knowledge about the molecular response to GC exposure. The validated biomarkers will form the basis for a sensitive bioassay to be used in EDA to detect GC-like activity in water samples, potentially leading to the identification of unknown GR-active pollutants with EDA.

TPS11-38 / Mass spectrometry-based proteomics of amniotic fluid incident to normal, preeclampsia and polyhydramnion pregnancies

Ruta Navakauskiene[1], Ilona Zaikova[2], Sandra Baronaite[2], Audrone Arlauskiene[3], Dalius Matuzevicius[4], Dalius Navakauskas[4], Grazina Treigyte[2]
[1]Vilnius University Institute of Biochemistry, [2]Department of Molecular Cell Biology, Vilnius University Institute of Biochemistry, [3]Center of Obstetrics and Gynecology, Vilnius University Hospital Santariskiu Klinikos, [4]Electronic Systems Department, Faculty of Electronics, Vilnius Gediminas Technical University
ster, [4]Laboratory of Nematology, Wageningen University

Introduction
Amniotic fluid (AF) is a complex mixture and reflects the physiological status of the developing fetus. Many proteins presented in AF are of exceptional interest because their expression reflects physiological and pathological conditions of the fetus and/or pregnancy. Proteomics-based identification of biomarkers for fetal abnormalities and pregnancy complications in AF in the future may provide targets for therapeutic intervention, however most of them still need to be verified.

Methods
Proteins isolated from amniotic fluid of three different status of pregnancy - normal, preeclampsia and polyhydramnion, were fractionated by 2DE, visualized by Coomassie blue staining and all visualized proteins after in-gel digestion were subjected for identification with 4800 MALDI TOF/TOF™ Analyzer (Applied Biosystems). Computational analysis of 2DE gel images was used to define changes in expression levels of identified proteins between different cases of pregnancy. Gel images were analysed using originally developed software prototype with new pre-processing, alignment, segmentation and subsequent analysis algorithms. The method of gel image alignment exploits Multi-Layer Perceptron combined with SURF keypoint descriptors. Applied protein spot detection and segmentation algorithm is based on Watershed transformation combined with multiscale symmetrical feature detection.

Results
In our study we characterized proteins isolated from amniotic fluid obtained from normal pregnancy, developed preeclampsia and polyhydramnion. Around 60 proteins were identified by mass spectrometry analysis. Some proteins' expression level increased in preeclampsia cases in comparison with normal pregnancy. These proteins are: Apolipoprotein A participating in lipid transport; Clusterin associated with apoptosis and Alzheimer's disease; Retinol binding protein, taking part in the modulation of gene expression during development of a fetus; Fibrinogen binding chain that takes part in regulating various cellular processes and its gene mutations lead to several disorders; Transthyretin that play role in carrier of hormone and retinol binding protein, changes in its level could be associated with neurobiological disorders. Expresion of some identified proteins decreased in AF from preeclampsia and polyhydramnion: Lumican, Serotransferrin, Fibrinogen beta chain, Ceruloplasmin, Statherin and others. These proteins are important in cell and molecule migration, metabolic processes.

Conclusions
In the study new proteins typical for amniotic fluid of preeclampsia and polyhydramnion pregnancies were identified. These proteins are associated with development, signal transduction, metabolic processes. Proteomic tools could be valuable for discovering biomarkers to predict pregnancies risk.
Novel Aspects. In this study we focus ourselves on the specific AF proteins that could be biomarkers for fetal development and pregnancy-related disorders.

Acknowledgement
This research was supported by the Research Council of Lithuania (project No. MIP-033/2013).

TPS12 - Lipidomics

11:00-15:00
Poster Exhibition, Level -1

TPS12-01 / Solid phase extraction materials for the extraction of Phosphatidylethanol

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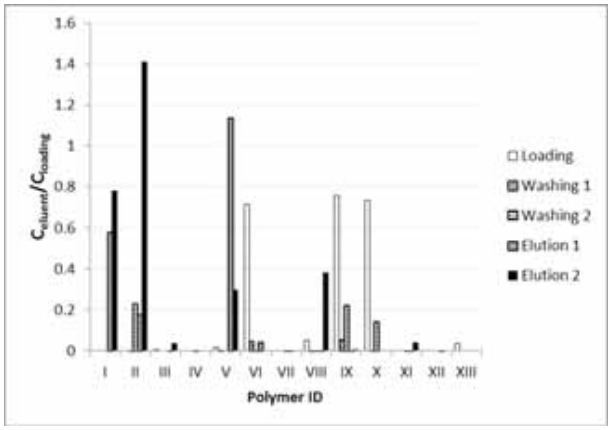
Background
Phosphatidylethanol (PEth) is a group of phospholipids formed by the action of phospholipase D in the presence of ethanol. As the elimination of PEth from the body is slow, and can be found even 14 days after the administration of ethanol, PEth has been suggested as an alcohol abuse marker. One reason for the limited use of PEth in clinical laboratories is the analytical methods employed for the extraction. The most common liquid-liquid extraction is time consuming and labor-intensive. Recently, SPE procedures for phospholipids were demonstrated as an effective alternative for the other methods. In the study, a library of SPE having polymeric nature and surface modified with different functionalities e.g. anion exchange, hydrophobic interactions and molecularly imprinted moieties screened against PEth.



Methods
We subjected PEth (Abcam, UK) to sample preparation on the library of SPE materials packed in 3mL cartridges (I.D=9 mm) and performed ESI-MS/MS analysis on a Sciex 3000 triple quadruple to monitor the fragmented ions (255.5, 218.5 Da) to quantify the extraction performance. SPE conditions: 100mg of

polymer beads were packed in 3mL SPE cartridges in triplicates. Loading - 1mL 4uM PEth (H2O); Wash-H2O/ACN 50:50+H2O/ACN 10:90; Elution-H2O/ACN 10:90,5% FA+ H2O/ACN 10:90 10% FA.

Results
Among different SPE materials tested, we highlight three materials (Fig.1) that showed binding to PEth: I,II and V. The polymers in question are of highly cross-linked materials (>80%). The cross-linkers used for the synthesis of these were di(ethylene glycol) dimethacrylate, Poly(ethylene glycol) diacrylate and trimethylolpropane trimethacrylate. The hydrophobic nature of these monomers and their presence in high concentration result in a hydrophobic surface on the polymer, providing affinity with the organic chains in PEth during loading. Although the washing protocol uses a high organic content and thus any PEth bound due to hydrophobic interaction should be washed off. When using polymer II, some removal was seen during washing due to the high concentration of acetonitrile, confirming this hypothesis. Polymer I and V have an amino-group ending modification providing an anionic exchange functionality which will interact with some deprotonated phospho groups in PEth. Given the nature of this interaction (electrostatic interactions) the binding is expected to be stronger and the condition for the elution (addition of FA) provided a good elution for these materials.

Conclusions
We found new SPE materials (I,V) which showed promising affinity towards PEth and optimized the SPE condition for those materials against PEth. Interestingly, we also found a hydrophobic material (II) eluting PEth in an ion-exchange fashion, the mechanism of this material is currently being investigated. The studies on the implementation of this method for the extraction of PEth from plasma and studies on the sensitivity optimization will be shown.

Novel Aspect
These findings will change the extraction limitations and increase the possibility of PEth analysis in the clinical laboratories due to the versatility of SPE method.

TPS12-02 / Effects of fatty acyl chain lengths, unsaturation degree, concentration and used matrix on phosphatidylcholine responses in MALDI-MS

Vitaliy Chagovets, Miroslav Lísa, Michal Holčapek
University of Pardubice

Introduction
Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is sensitive, fast and robust method for lipidomic qualitative analysis. The quantitative analysis with MALDI-MS is still rather challenging due to high demands for the sample uniformity and shot-to-shot signal reproducibility, which is strongly dependent on matrix, solvents and sample preparation. Another important problem of MALDI-MS quantitation is that the instrument response depends on the lipid type and composition.
Phosphatidylcholines (PCs) are essential and abundant constituents in biological systems. PC molecule is composed of polar phosphocholine head group, glycerol backbone and two nonpolar fatty acyl, alkyl or alkenyl chains. It is believed that the polar head group gives the main contribution to the mass spectrometer response and nonpolar chains have minor influence. Nevertheless, it is important to investigate these effects in more details for better understanding of lipids properties and for accurate quantitation.

Methods
Sets of PCs with fixed fatty acyl (14:0, 17:0 or 18:1) in sn-1 position and varied in length and saturation degree of fatty acyls in sn-2 position of glycerol backbone were synthesized. Samples with various concentrations of lipids were mixed with several MALDI matrices including DHB, 9-aminoacridine and diaminonaphthalene. Prepared solutions were studied with MALDI LTQ Orbitrap XL mass spectrometer (Thermo Scientific, Waltham, MA, USA).

Results
MALDI-MS investigation of synthesized PC sets revealed the dependence of the intensity of lipid-related peaks on fatty acyl lengths. The character of the dependence was changed depending on used matrices and concentrations of lipids. In case of DHB matrix, the dependence was not monotonic. Peak intensities increased with the chain length increasing for samples with higher lipids concentrations and decreased for lower concentrations. The fact of lipid peaks decrease for longer fatty acyl chains for samples containing lower lipids concentrations was common for different matrices, while the slope of this decrease was again not the same for various matrices. The weakest changes of relative peak intensities were observed for 9-aminoacridine matrix. The smallest shot-to-shot deviation of relative abundances was also obtained for 9-aminoacridine as a matrix.

Conclusions
The instrument response depends on fatty acyls composition of PCs. This dependence must be taken into account upon the quantitative MALDI-MS analysis of phospholipids. MALDI matrix must also be chosen with care to avoid distortion of estimation of lipid concentrations based on peak intensities.

Authors acknowledge the support of projects No. LL1302 (ERC CZ) and No. CZ.1.07/2.3.00/30.0021 (MSMT CR).

Novel Aspect
Comprehensive study of PCs composition and concentration influence on the response of mass spectrometer with Orbitrap mass analyzer is provided. The effect of MALDI matrix on the response is considered as well.

TPS12-03 / Separation of Triacylglycerol Regioisomers by Differential Mobility Spectrometry

Martin Šala[1], Miroslav Lísa[2], Larry J. Campbell[3], Michal Holčapek[2]
[1]National Institute of Chemistry, [2]University of Pardubice, [3]AB SCIEX

Nowadays ion mobility (IM) spectrometry attracts a lot of attention in analytical chemistry due to recent technical advances, which enable to conduct demanding experiments and develop new applications ranging from small molecules to biomacromolecules. The most popular combination is ion mobility - mass spectrometry (IM-MS), where IM could work either as the prefilter to remove the chemical noise or as another separation dimension for difficult analytical tasks, such as separation of isobaric species. Differential mobility spectrometry (DMS) is one variant of IM based on the use of two parallel conductive plates with applied asymmetric radio-frequency (RF) field, which is used in our work to investigate separation of triacylglycerol (TG) regioisomers.
Here, we present a new method for separating and quantifying TG regioisomers using DMS. Traditionally, the separation of TG regioisomers has been achieved by methodologies like silver-ion or chiral chromatography, which require long gradients (i.e., hours). An alternative MS-based method uses fragment ion ratios to estimate the determine abundances of TG regioisomers in a mixture. DMS separation of TG regioisomers can rapidly provide more definitive identification of such species.
An AB SCIEX QTRAP® 6500 system with SelexION™ was used for these analyses of standard solutions and real biological samples using the direct infusion analysis. Silver ions were added

to the solution to form adducts with TG molecules. MRM and MS/MS experiments of pure standards and their mixtures were conducted to confirm the identity of DMS-separated compounds. We have successfully separated mixtures of two regioisomeric pairs containing one (SOS/SSO) or two (SOS/SSO) double bonds with the addition of silver ions; S means stearoyl (C18:0) and O is oleoyl (C18:1Δ9). We examined a wide parameter space, including several organic modifiers, flow rates, temperatures and DMS residence times for the best separation, the highest sensitivity and the signal stability.

We present a new approach to determine TG regioisomers using DMS. The DMS-based separations yielded a marked reduction in the analysis time (i.e., minutes), compared to the traditional alternatives. In addition, it offers the possibility of another dimension in the separation of lipids in LC/MS analyses.

TPS12-04 / Determination of compositions and regiospecificity of two fatty acyl groups in phospholipids by using MALDI-TOF/TOF

Young Hwan Kim, Geul Bang
Korea Basic Science Institute

Introduction

Lipids with relative composition within the cell are dynamic because of numerous pathways involved in their biosynthesis and turnover. However, their mass spectral profilings were very complex and not quantitative due to the alkali metal adduction of lipid species with Na+ and K+ ions present in biological samples. Thus, the mass spectra of lipids ionized by ESI and MALDI indicate simultaneously sodiated, potassiated and protonated molecules of the same species. In this study, we determined the polar head group and two fatty acyl compositions of phospholipids by MALDI tandem mass spectrometry of the protonated, sodiated and deprotonated molecules. Especially, the regiospecificity of two fatty acyl groups in standard PLs could be determined from the intensity ratios of product ions due to the loss of each fatty acyl group. This empirical rule was applied to the determination of the regiospecificity of PCs in mouse brain.

Methods

The standard PLs were commercially purchased from Avanti Polar Lipids, Inc. Total lipid extracts were prepared by modified Matyash method after mouse brain extracted from mice under anesthesia. The binary matrix composed of 2,5-dihydroxybenzoic acid (DHB) and cyano-4-hydroxycinnamic acid (CHCA) in 70% methanol added with 1% of 250 mM piperidine and 0.1% TFA was used. The binary matrix is also added with 15mM sodium acetate. The MALDI mass spectra were acquired on an UltrafleXtreme MALDI-TOF/TOF mass spectrometer (Bruker Daltonics) with a 355 nm Nd:YAG laser (repetition rate 1,000 Hz) of Smartbeam II in reflectron mode and LIFT mode was used for tandem mass spectrometry.

Results

The high-energy CID tandem mass spectra of the protonated, deprotonated and sodiated molecules of standard PLs generated by MALDI provided the information about the lipid class, the compositions and regiospecificity of two fatty acyl groups, as shown in Figure 1. The regiospecific-product ions correspond to the [M+H-RCO]+ and [M+Na-59-RCOOH]+ in PC, [M+H-RCO]+ in PE, and [M-H-RCOOH]- in PA and PG, as summarized in Table 1. Based on this empirical rule and diagnostic ions, fatty acyl structures as well as class of each phospholipid species observed in MALDI profiling spectra (Figure 2) of mouse brain extract was determined, as summarized in Table 2.

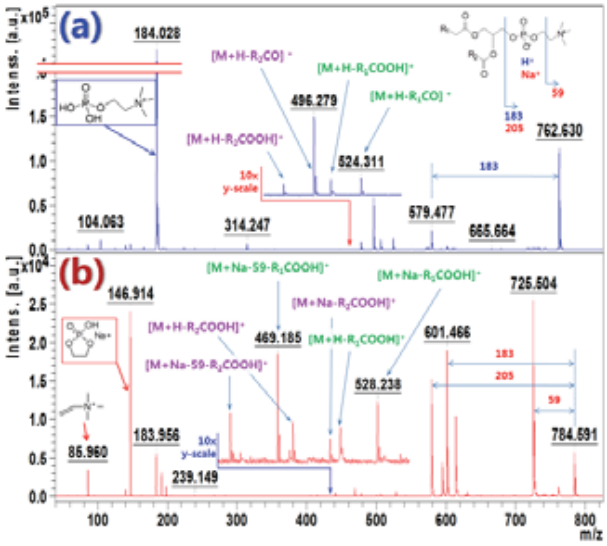


Figure 1. The MS/MS spectra of (a) $[M+H]^+$ (m/z 762) and (b) $[M+Na]^+$ (m/z 784) of 16:0/18:0-PC standard were obtained by using Ultraflextreme MALDI-TOF/TOF in positive-ion mode. These spectra show the product ions informative on specific polar head group the composition of two fatty acyl groups.

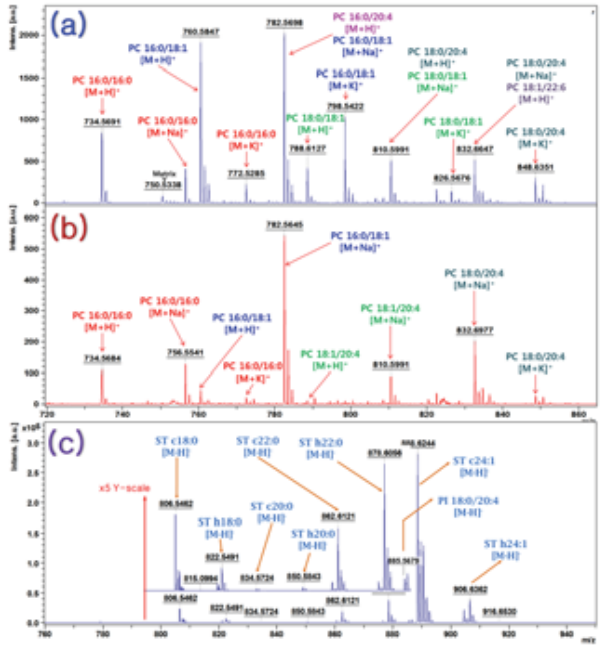


Figure 2. MALDI mass spectra of lipids extracted from mouse brain (a) without sodium acetate and (b) with sodium acetate on binary matrix in positive-ion mode using UltrafleXtreme. Sodium saturated matrix was used to simplify the mass spectral pattern of phospholipids. $[M+Na]^+$ ions were increased but $[M+H]^+$ and $[M+K]^+$ ions were decreased. On the other hand, (c) there was no effect according to sodium saturated matrix in the negative-ion mode .

Table 1. Intensity ratio of specific product ion pairs informative about the regiospecificity of two fatty acyl groups measured in triplicate.

Species2	Product ion ratio type	Ave. ratio	Species	Product ion Ratio type	Ave. ratio
16:0/18:1-PE [M+H]+	[M+H-R2CO]+/ [M+H-R1CO]+	2.07±0.08	16:0/18:1-PE [M+Na]+	[M+Na-44-R2COOH]+/ [M+Na-44-R1COOH]+	0.75±0.04
18:0/22:6-PE [M+H]+		2.22±0.14	18:0/22:6-PE [M+Na]+		0.94±0.13
16:0/18:0-PC [M+H]+	[M+H-R2CO]+/ [M+H-R1CO]+	4.47±0.97	16:0/18:0-PC [M+Na]+	[M+Na-59-R2COOH]+/ [M+Na-59-R1COOH]+	0.46±0.04
18:0/20:4-PC [M+H]+		3.74±0.51	18:0/20:4-PC [M+Na]+		0.61±0.16
18:0/22:6-PC [M+H]+		3.65±0.19	18:0/22:6-PC [M+Na]+		0.69±0.08
16:0/18:1-PA [M-H]-	[M-H-R2COOH]-/ [M-H-R1COOH]-	3.27±0.13	16:0/18:1-PG [M-H]-	[M-H-R2COOH]-/ [M-H-R1COOH]-	1.84±0.05
18:0/20:4-PA [M-H]-		6.67±1.14	18:0/20:4-PG [M-H]-		1.86±0.04
18:0/22:6-PA [M-H]-		9.38±2.41	18:0/22:6-PG [M-H]-		2.92±0.48

Table 2. Phospholipid and sphingolipid species identified from in-house "iLipid" database searching. Most of lipid species extracted from mouse brain were identified as phosphatidylcholine (PC) and sulfatide (ST) in the positive- and negative-ion modes, respectively.

Measured (m/z)	Adduct Type	Lipid species	Measured (m/z)	Adduct Type	Lipid species
734.5691	[M+H]+	PC16:0/16:0	806.5462	[M-H]-	ST c18:0
756.5523	[M+Na]+	PC 16:0/16:0	810.5419	[M-H]-	PS 38:4
760.5847	[M+H]+	PC 16:0/18:1	821.5446	[M-H]-	PG 40:6
772.5285	[M+K]+	PC 16:0/16:0	822.5491	[M-H]-	ST h18:0
782.5698	[M+Na]+	PC 16:0/18:1	834.5724	[M-H]-	ST c20:0
	[M+H]+	PC 16:0/20:4	835.5324	[M-H]-	PI 34:1
788.6127	[M+H]+	PC 18:0/18:1	850.5843	[M-H]-	ST h20:0
798.5422	[M+K]+	PC 16:0/18:1	862.6121	[M-H]-	ST c22:0
806.5557	[M+H]+	PC 38:6	878.6056	[M-H]-	ST h22:0
810.5991	[M+Na]+	PC 18:0/18:1	885.5679	[M-H]-	PI 18:0/20:4
	[M+H]+	PC 18:0/20:4	888.6244	[M-H]-	ST c24:1
822.6474	[M+H]+	PC o40:5	890.6434	[M-H]-	ST c24:0
826.5676	[M+K]+	PC 18:0/18:1	904.6233	[M-H]-	ST h24:1
832.6647	[M+Na]+	PC 18:0/20:4	906.6362	[M-H]-	ST h24:0
	[M+H]+	PC 18:1/22:6	Mass tolerance < 10 ppm		
848.6352	[M+K]+	PC 18:0/20:4			

Conclusions

The tandem mass spectrometry of the protonated and sodium-adducted molecules of lipid species provides the information about the compositions of two fatty acyl groups as well as the structure of a polar head group. Especially, the regiospecificity of two fatty acyl groups in PLs could be determined from the intensity ratios of two product ions due to the loss of each fatty acyl group. The isobaric lipid mixtures were also assigned into the identification of individual species with characteristic fatty acid-related product ions. In addition, the results of this study will be used to MALDI imaging mass spectrometry (IMS) of phospholipids directly on brain tissue section.

Novel Aspect

Determination of lipid class and two fatty acyl information such as the composition and regiospecificity by MALDI tandem mass spectrometry

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TPS12-05 / GLP-1's effects on palmitate-induced islet lipotoxicity investigated by targeted lipidomics

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Acute rise in free fatty acid (FFA) levels promote the release of insulin from insulin-producing beta-cells. In contrast, prolonged elevated FFA levels, which are observed in subjects with type 2 diabetes mellitus (T2DM), lead to beta-cell functional impairment and decline in insulin secretion. This latter process is referred to as lipotoxicity. Glucagon like peptide1 (GLP-1) has anti-diabetic properties including amelioration of lipotoxicity caused by FFA. Underlying mechanisms are still unclear, however.

In this study, targeted lipidomics using liquid chromatography/gas chromatography combined with mass spectrometry was used to investigate mechanisms of amelioration of palmitate-induced islet lipotoxicity by GLP-1. Lipids were extracted from human islets cultured in the presence of palmitate for prolonged time periods in the absence or presence of GLP-1.

The lipidomics analysis was focused on sphingolipids and FFAs. Fifty different lipidspecies were identified and quantified, including FFA (8), sphingomyelin (12), ceramide (11), dihydroceramide (9), glucosylceramide (5), and others (5).

We found that GLP-1 suppressed palmitate-induced elevation of several lipid classes including sphingomyelin, ceramide and dihydroceramide. Further analysis of the results showed that GLP-1 affected sphingomyelin lipid signaling pathways with associated reduction in islet apoptosis.

Our conclusion is that GLP-1 has an anti-lipotoxic effect, where sphingolipids are akey lipid class. It is anticipated that these novel findings may offer novel therapeutic approaches for the treatment of T2DM.

TPS12-06 / Off-line 2D HPLC of aliphatic hydrocarbons

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Aliphatic hydrocarbon mixtures are most often analyzed by gas chromatography/electron ionization mass spectrometry (GC/EI-MS), because of high chromatographic resolution, sensitivity and selectivity of the MS detection. However, number of detected compounds is limited by the functional range of the method, i.e. the technique is limited by number of carbon atoms or rearrangement of polyunsaturated hydrocarbons at high temperatures in the GC injector and/or column, respectively. Obviously, principally different analytical methods are required to broaden the range of molecular weights and thus to allow analysis of hydrocarbons with considerably longer aliphatic chains. Reversed-phase high-performance liquid chromatography (RP-HPLC) has a great potential for it, but on-line MS detection of hydrocarbons in RP-HPLC is insensitive, because of their low polarity and proton affinity. Moreover chromatographic resolution in RP-HPLC is significantly lower than in GC. Therefore off-line 2D chromatography based on RP-HPLC (1st dimension) and silver ion high-performance liquid chromatography (Ag-HPLC) (2nd dimension) was developed.

Hydrocarbons separated by RP-HPLC were detected by universal evaporative light scattering detector, which does not provide any structural information. Nova-Pak C18 column and acetonitrile/ethyl acetate were used as a stationary and mobile phase, respectively. Fractions were continuously collected each 30 s and analyzed by Ag-HPLC with atmospheric pressure chemical ionization (APCI) MS detection. Two ChromSpher Lipids columns connected in series and mobile phases containing hexane/toluene were employed.

In the first dimension hydrocarbons are separated by equivalent chain length. The retention times in Ag-HPLC (2nd dimension) increased with number of double bonds. Components with trans double bonds eluted ahead of cis isomers. [M-H]+, [M]+·, [M+H]+, [M+39]+ were abundant ions observed in the APCI-MS spectra. Relative intensity of ions strongly depends on mobile phase composition, number of carbon atoms and degree of unsaturation. Developed 2D-HPLC method allowed separation of complex mixture of cuticular aliphatic hydrocarbons from flesh fly Neobelieria bullata according to the chain length and double bond position.

Novel aspects: Combination of RP-HPLC and Ag-HPLC to off-line 2D HPLC/MS is powerful tool for analysis of aliphatic hydrocarbons.

Financial support from the Czech Science Foundation (Project No. P206/12/1093) and the Academy of Sciences of the Czech Republic (RVO: 61388963) is herewith acknowledged with appreciation.

TPS12-07 / A Single Run LC/MS/MS Method for Phospholipidomics: application to S. cerevisiae lipidome and marine lecithin

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The term lipidomics refers to the characterization of lipid molecular species and of their biological roles with respect to expression of proteins involved in lipid metabolism and function, including gene regulation. We have compared normal phase (NP) and reversed phase (RP) liquid chromatography coupled to mass spectrometry with the objective of finding the best compromise for a single run analysis of whole phospholipids.

LC/MS/MS (MRM mode) analyses were performed with a 5500

QTRAP (AB Sciex) instrument coupled to a LC system (Ultimate 3000, Dionex). Analyses were achieved in the negative (PE, PS, PA, PI, PG) and positive modes (PC).
Liquid chromatography parameters were adjusted in NP and RP conditions in order to separate reference compounds from PE, PS, PC, PI, PA and PG lipid classes in a single run in gradient mode. Normal Phase allowed separating all phospholipid classes according to their polar head groups, but repeatability and reproducibility were rather poor. Reversed Phase had a superior robustness and was found to be a satisfactory compromise, with a separation of phospholipid species according to unsaturation and additional selectivity for the separation of isomers.
This single run method has the potential for a wide applicability to lipid extracts from different origins. Two examples are presented: (i) the analysis of the variation of the yeast phospholipidome in cases where mutations affect enzymes involved in de novo synthesis or remodeling of phospholipids; (ii) the analysis of two lecithins from marine origin, one of them being enriched in PS after enzymatic modification.

TPS12-08 / Improving Lipid Profiling Performance using Micro Flow Liquid Chromatography and High Resolution Mass Spectrometry

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AB SCIEX

Introduction
Using metabolites as putative biomarkers we can study changes in relation to disease or physiological states using an application known as metabolomics. These changes often tend to be related to lipids, which form the largest subset of molecules in the metabolome. Therefore it is important to be able to identify and quantitate lipids and metabolites from biological samples. The application of microflow chromatography has yet to be assessed for this application and here we present the results when compared with traditional high flow chromatography.

Methods
This study was performed with an Eksigent microLC 200 µUHPLC system coupled to a TripleTOF 5600 system. Chromatographic separation was achieved on a CSH C18 column (1X50mm, 1.7µm) at a flow rate of 100 µL/min within 8 minutes. The MS system was operated in information dependent mode where up to 8 MS/MS experiments were triggered from the TOF survey scan to collect high resolution accurate mass MS/MS spectra for identification.

Preliminary data
Comparing with regular flow chromatographic results, significant sensitivity improvements were observed using the MicroLC 200 and the TripleTOF 5600 system. With optimized chromatographic components to minimize gradient delay volume and post column volume for micro flow applications, significant improvements in chromatographic performance were also observed. The average peak width reduced to nearly half compared with regular flow method which lead to larger peak capacity and reduced run time thus enabling high-throughput metabolomics workflows. An average of 24.4 fold improvement in TOF MS peak intensity was observed (ranging from 1.9 to 117X for different species) for the15 isotopically labeled internal standards assessed (22 monitored species including sodium and/or ammonium adducts). The resolution for lipid isomers was also noticeably improved which leads to more identified/confirmed biomarkers and more reliable quantitation results. Other benefits using microflow technology for metabolomics include dramatic reduction in solvent and waste handling cost (up to 92% less solvent usage and generated waste). Since retention time could be used as a key identification point for biomarker identification and confirmation, the chromatographic reproducibility was also evaluated with this setup.

Novel aspect
Use of micro flow UHPLC and high-resolution mass spectrometry with significantly improved sensitivity for high throughput metabolomics workflows.

TPS12-09 / Qualitative and Quantitative Analysis of Oxidized Fatty Acids by Information Dependent and Data Independent Strategies on a Quadrupole Time-of-Flight Hybrid Ins

Jean-Baptiste Vincendet, Cyrus Papan
AB SCIEX

Introduction
The qualitative and quantitative study of oxidized fatty acids from complex biological samples with mass spectrometry has been facing challenges due to the isobaric overlapping, in which the molecules share same formula composition but different in structures. To assess different strategies for oxidized lipids analysis, we developed and evaluated multiple targeted/non-targeted and information dependent/independent methods. Both qualitative and quantitative results were carried out to analyze oxidized fatty acids.

Method
Lipids extracts were directly infused into a QqTOF system by flow injection or online separated by a HFLC system using ZORBAX Eclipse XDB-C18 column (2.1 mm x 50 mm, 1.8 µm particle size) for different workflows. Four different experiments including information dependent acquistion (IDA), pseudo multiple-reaction-monitoring (MRM), MS/MSALL, and SWATH were performed with a QqTOF instrument.

Preliminary Results
We applied both direct infusion and LC strategies in our study. Among all mass spectrometry analysis approaches we assessed, SWATH and MS/MSALL are information independent experiments. For discovery, SWATH identified 45 oxidized fatty acids, whereas MS/MSALL experiment identified 34 species. Ion suppression from infusion based strategy is a main reason limited MS/MSALL to discover low abundant species. IDA experiment also identified 34 species. The reasons that IDA experiment did not perform as good as SWATH were: precursor ion masses of oxidized fatty acids were isobaric overlapped; many of the oxidized fatty acids were not based line resolved by chromatography; and the numerous small molecule interference ions in the same mass range of oxidized fatty acids. Moreover, IDA experiment cannot quantify oxidized fatty acis due to the intermittent acquisition of MS/MS.
Both SWATH and pseudo-MRM were LC based experiments. Their results were also comparable. However, SWATH overcomes some common limitations existing in the pseudo-MRM, such as relatively slow analysis rates and lacks the capability to dynamically refine or expand the measured lipids for extensive investigation. Overall, our results demonstrate that the information independent methods are more competitive in the study of oxidized fatty acids.

Novel Aspect
Establish of better workflow for qualitative and quantitative analysis of oxidized fatty acids.

TPS12-10 / HPLC /APCI-MS3 OF 1,2-DIOL diesters in vernix caseosa

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Diesters of long-chain 1,2-alkanediols (1,2-DDE) have been detected in the skin of several mammals. Human skin biosynthesize 1,2-DDE at its early stages of development. These lipids are thus found in vernix caseosa, a biofilm that progressively coats human fetus during the last trimester of pregnancy and also covers newborn babies. Vernix caseosa has distinct antimicrobial and wound-healing properties due to concerted action of protein, peptides and lipids. Our knowledge about vernix caseosa lipids is limited. The aim of this work is to comprehensively characterize one particular lipid class of vernix caseosa, 1,2-DDE.

Vernix caseosa samples were collected from healthy subjects immediately after the delivery. Total lipids were obtained by chloroform:methanol (2:1; v/v) extraction and the neutral lipid classes were isolated on semipreparative silica gel TLC plates using hexane:diethyl ether (93:7; v/v). 1,2-DDE were analyzed by non-aqueous reversed-phase HPLC with APCI-MSn detection (LTQ Orbitrap-XL, Thermo). Data-dependent scanning was applied to achieve MS2 and MS3 data allowing us to elucidate structures of the 1,2-DDE molecular species. 1,2-DDE standards were synthesized from fatty acid chlorides and long-chain 1,2-diols.

Full-scan APCI mass spectra of 1,2-DDE showed protonated molecules and cation-radicals $[M]^{+\cdot}$. Post-column infusion of ammonium formate yielded ammonium adducts. Collision induced dissociation of the ammonium adducts in the ion trap (MS2) promoted eliminations of neutral fatty acids. Further fragmentation (MS3) provided information about the diol chain. Positions of double bond were obtained from MS2 spectra of $[M+C3H5N]^{+\cdot}$ formed in the APCI source in the presence of acetonitrile. Good separation of 1,2-DDE was achieved in non-aqueous reversed phase system with two conventional C18 columns connected in series and acetonitrile/ethyl acetate gradient. The reconstructed chromatograms of 1,2-DDE from vernix caseosa typically showed several peaks with the same mass; they were explained by species differing in the number of methyl branches and partially also by the position of double bond. In general, 1,2-DDE eluted the column in the order of their equivalent chain numbers, i.e., by increasing chain length, which was effectively shortened by two methylene groups for each double bond.

We identified 2250 molecular species of 1,2-DDE in 141 chromatographic peaks. Saturated species formed 30% of 1,2-DDE; monounsaturated, diunsaturated and triunsaturated lipids accounted for 49%, 18% and 3% of 1,2-DDE, respectively. The most abundant 1,2-DDE were monounsaturated species combining C22 diol, C18:1 fatty acid and C16:0, C14:0 or C15:0 fatty acid.

Novel Aspect: The reversed-phase HPLC/APCI-MS3 method was developed and applied for the first comprehensive characterization of 1,2-DDE in vernix caseosa.

This work was supported by Czech Science Foundation (project P206/12/0750).

TPS12-11 / The lipid profile of follicular fluid undergoing ovary superstimulation by high throughput MALDI-MS

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Introduction

Treatment via superstimulation has been extensively used to increase cattle fertility and improve the quality of oocytes and embryos as well as follicular cells differentiation. Since phospholipids (PL) play a significant role in cell structure and proliferation and also dictate thermal phase behavior and several physicochemical properties of the embryos, it is crucial to investigate how superestimulation treatment may affect their composition in the ovary follicular fluid (FF). The PL profiles of bovine FF and the PL changes caused by superstimulation treatments are unknown. The objective of this study is thefore to gain insight into the effects of superstimulation with FSH (follicle-stimulating hormone) or FSH combined with eCG (equine chorionic gonadotropin) on the profile and abundance of PL from cows submitted or not to superstimulatory protocols.

Methods

The FF were recovered from Nellore superstimulated cows with FSH or FSH/eCG treatment and compared to the one from non-superestimulated cows (control group-C). The lipid profiles of the FF of the groups C (n=14), FSH (n=14) and FSH/eCG (n=14) were investigated by MALDI-MS. The total lipids were extracted by Bligh Dyer extraction liquid. The extract (1µL) was added to a MALDI plate. After drying, 1µL DHB matrix was added. Analyses were performed by a mass spectrometer MALDI-TOF/TOF BrukerAutoflex III in the mass range 700-900 m/z. The MALDI-MS data were statistically analyzed by principal component analysis using MetaboAnalyst 2.0 followed by the ANOVA of selected ions.

Results

The PL found in the samples were mainly represented by phosphatidylcholines (PC) and sphingomyelins (SM). The most abundant ions were [SM 16:0+Na]+ of m/z 725.6, [PC 34:2+H]+ of m/z 758.6, [PC 34:1+H]+ of m/z 760.6, [PC 34:1+Na]+ of m/z 782.6, [PC 36:2+H]+ of m/z 786.6, [PC 36:2+Na]+ or [PC 38:5+H]+ of m/z 808.6. These assignment were of the ions was based on MS/MS fragmentation. The experimental groups could be resolved by their PL profile through PCA. The lipid ion of m/z 758.6 [PC (34:2) + H]+, a polyunsaturated PC, was observed to be less ($P<0.05$) abundant (mean±std) in non-superstimulated cows (55.9±4.0) when compared with superstimulated cows by FSH (67.7±4.0) or FSH/eCG (66.8±3.7) treatments.

Conclusions

The profiles by MALDI-MS of PL of FF have been determined. They indicate that ovarian superstimulation modulates PL profile in bovine FF and suggests the PC (34:2) as a potential biomarker of the follicular fluid of cows undergoing ovary superstimulation.

Novel aspect

The PL profile of FF has been determined by MALDI-MS and its alteration after superstimulation has been elucidated.

TPS12-12 / Lipidomic Characterization of Kidney Cancer Tissues using HILIC-HPLC/ESI-MS

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Introduction

Kidney cancer is one of the most common tumors both for men and women. Renal cell carcinoma (RCC) represents more than 90% of kidney cancer cases. Similarly to tumors of other primary locations, early detection of RCC and subsequent therapy increases the chance of cure. Lipid metabolism plays an important role in the oxidative stress and is associated with kidney cancer risk factors. The principal function of kidney is the filtration of blood and the excretion of excessive compounds and metabolites from body through urine. The HPLC/MS characterization of lipidomic differences between kidney cancer tissues and surrounding normal tissues is the first step, which is then followed by the search for these lipids with different expression in body fluids of kidney cancer patients compared to healthy volunteers.

Methods

Total lipid extract: chloroform – methanol – water extraction according to the Folch extraction.
HPLC: column Spherisorb Si (Waters), 1 mL/min, 40°C, gradient of acetonitrile / 5 mM aqueous ammonium acetate.
MS: ion trap Esquire 3000 (Bruker Daltonics) with ESI ionization.
Data analysis: multivariate data analyses using O2PLS method in the SIMCA software version 13.0 (Umetrics).

Results

Comprehensive lipidomic analyses of kidney tumor tissues and surrounding normal tissues were performed using optimized HILIC-HPLC/ESI-MS method. Individual lipid classes were quantified based on the addition of single IS and response factors for each class related to the IS. Statistically significant differences in average concentrations were observed for phosphatidylethanolamines (PE), lysophosphatidylethanolamines (LPE), sphingomyelins (SM) and lysophosphatidylcholines (LPC) for twenty kidney cancer patients compared to surrounding normal tissues of the same patients. Detailed analysis of lipid species inside above mentioned classes was performed using relative abundances of deprotonated molecules [M-H]- for phosphatidylinositols (PI), PE and LPE species or [M-CH3]- ions for phosphatidylcholines (PC) in the negative-ion ESI mode, while SM and LPC were quantified using protonated molecules [M+H]+ in the positive-ion ESI mode. Multivariate data analysis using orthogonal 2 projections of latent structures (O2PLS) enables a clear differentiation of tumor and normal tissues based on changes of their lipidome.

Conclusions

The statistically significant lipidomic differences described for kidney tumor tissues of twenty patients compared to surrounding normal tissues of the same patients can help with early diagnosis and follow-up.
This work was supported by ERC CZ project No. LL1302 and project No. CZ.1.07/2.3.00/30.0021 (both from Ministry of Education, Youth and Sports of the Czech Republic).

Novel aspects

Characterization lipidomic differences between kidney tumor tissues and surrounding normal tissues using multivariate data analysis.

TPS12-13 / Lipidomics: Supercritical fluid chromatography/ion mobility – mass spectrometry as a tool for fast nontargeted analysis of lipids

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Introduction

Lipidomic analysis is challenging due to enormous complexity of lipid structures. Two well-established approaches are commonly used in lipidomics, such as shotgun and LC/MS. Targeted analysis using shotgun provides relatively fast and simple identification and quantitation of lipid species, but serious limitations are the ion suppression and the identification of isobaric and trace species. Nontargeted LC/MS provides more detailed information about lipidomic composition than shotgun, but it usually requires the combination of more chromatographic modes due to the complexity of samples which is time consuming. The goal of this work is the development of new nontargeted analytical approach using supercritical fluid chromatography (SFC) followed by ESI ionization and ion mobility-mass spectrometry (IM-MS) separation and identification of species for fast and comprehensive lipidomic analysis.

Methods

Standards of lipid class representatives containing oleic acid were used for the method development. Total lipid extracts from selected human tissues prepared by modified Folch procedure using chloroform – methanol – water extraction were used for the comprehensive lipidomic analysis. SFC experiments were acquired with UPC2 instrument (Waters) using UPC2 column, separation temperature 60°C and gradient of methanol as a modifier. Synapt HDMS G2Si instrument (Waters) using ESI ionization was used for IM-MS experiments.

Results

Individual parameters of SFC analysis have been carefully optimized to achieve a maximum number of separated lipid classes. Developed SFC method enables the fast separation of 16 nonpolar and polar lipid classes within 6 minute analysis including the partial separation of some species inside individual classes. Mass spectra with high mass accuracy and resolving power are acquired using ESI in both positive- and negative-ion modes for the identification of individual species. The composition of esterified fatty acids and polar head groups are characterized based on their characteristic fragment ions obtained from data independent analysis with high and low collision energy. IM is used as an additional separation dimension enabling the separation of lipid classes together with the separation of individual lipid species differing in acyl chain lengths and the number of double bonds coeluting in SFC. Developed SFC/IM-MS method is applied for the comprehensive analysis of lipid composition in real lipid samples. Obtained data from analyzed samples are processed using Progenesis QI software followed by the statistical analysis.

Conclusions

Fast SFC analysis is developed for the separation of nonpolar and polar lipid classes in one analysis followed by IM-MS identification of individual species. Developed SFC/IM-MS approach enables high-throughput nontargeted lipidomic analysis of complex lipid samples.
This work was supported by ERC CZ project No. LL1302 (MSMT, Czech Republic).

Novel Aspect

High-throughput nontargeted lipidomic analysis using fast SFC separation of lipids followed by IM-MS identification.

TPS12-14 / Mass spectrometry technique coupled with isotopic labeling as an useful tool in metabolism studies of lipid compounds

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Isotopic labeling is well-known technique to track the metabolism pathways of various compounds in living organism by precursors labeling. This method coupled with mass spectrometry becomes a powerful tool to study cell processes in details.
In this report we present two types of LC/ESI-MS applications coupled with isotopic labeling to study metabolism pathways of two different kinds of lipid compounds, occurring in all living organisms: dolichols and phosphatidylcholine (PC), by defined labeled compounds identification. The investigated compounds were labeled with 13C(dolichols) and 2H (phosphatidylcholine) isotopes and determined on the basis of Q1 and/or fragmentation spectra, recorded in the positive mode. Moreover, the characteristic fragmentation process for PC phospholipid class enabled to identify the labeled PC-containing compounds in phospholipid mixture by more selective MS mode - Neutral Loss scanning.
The polyisoprenoids, natural polymers, built of 5-100 and more five-carbon isoprenoid units, are produced in two metabolic pathways what has an influence on the isotopic labeling ratio. Thus the Q1 ESI-MS spectra of labeled polyisoprenoids are characterized by complex isotopic profile of investigated compounds indicating not only the presence of 13Catoms in the compounds structures but also the favorable metabolic pathway of polyisoprenoids, in this study - under osmotic stress.
The phosphatidylcholine belongs to the wide known and well described group of phospholipids. In completed labeled PC compounds analyses, the fragmentation spectra were recorded to confirm the presence of labeled choline part in the structures of investigated compounds. The fragmentation data give an opportunity to optimize Neutral Loss scanning, as an alternative MS mode to Precursor Ion scanning, only for labeled PC identification in a phospholipid mixture.
The demonstrated examples show the broad possibility of mass spectrometry technique in the identification of labeled compounds with various isotopic atoms and with the different ratio of isotopic labeling. The MS results obtained in both analysis enabled to postulate the favorable biosynthesis pathway of polyisoprenoids in A. thaliana plant cultivated under osmotic stress and establish the ability of L. dumoffii bacteria to utilize exogenous choline for PC synthesis.

TPS12-15 / MALDI Imaging of Rat Testis at 10µm Pixel Size and 470k Mass Resolution

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Introduction
Spatial and mass resolution are both important parameters in MALDI imaging. In many applications of MALDI imaging the spatial resolution must be lowered in favor of better mass spectra, however. Testis is a good model system for demonstrating the benefits of high spatial resolution, since it contains many small scale structures such as seminiferous tubules, interstitial space, smooth muscle layer lining. Spatially resolving these structures presents a better picture or the biology of the system. High spatial resolution can only be achieved with special sample preparation methods that minimize analyte migration. Here, we have used a previously published sublimation method to achieve 10um spatial resolution along with mass resolution of 470k using MALDI-FTMS.

Methods
Frozen rat testis sections were cut at 10µm thickness using a cryo-microtome and transferred onto ITO coated glass slides. Sections were then dried in vacuum. Sublimation was performed in a custom built device using a 152 mm inner diameter sublimation apparatus (Ace Glass, NJ, USA) exactly as described in [Chaurand et al. Mol Cell Proteomics. 2011 Feb;10(2):O110.004259] using 2,5-DHB matrix. The MALDI imaging measurements were performed on a 7T FTMS. Analytes were measured over the range of m/z 500-900 and using the minimum laser diameter setting. Lipids were identified based on their accurate mass by matching against the Lipidmaps.org database.

Results
The spatial resolution was sufficient to resolve anatomical features as small as 10um in the rat testis sections. Several ion clearly show sub-structures inside the seminiferous tubules. Notably, ion images at m/z 758.545 Da and m/z 788.607 show clear differentiation of the interstitial space from the smooth muscle layer lining the basal lamina of the tubules. The muscle layer has a thickness of less than 10µm and the interstitial space ranges from zero to 40µm. The mass resolution in the lipid m/z 700 - 900 was up to 470k. Without such high resolving power several phospholipid ion images would have been ambiguous due to overlap of isobaric ions, e.g., a pair of ions with a mass distance of only 3 mDa (at m/z 808.5819 and m/z 808.5849 Da). The first signal was found in the interstitial space, the second one in the lumen of the seminiferous tubules. The high mass accuracy of the FTMS allowed a tentative assignment of the molecular formulas of the two substances as [C44H84NNaO8P]+ and [C46H83NO8P]+ and their structures were tentatively assigned as phospholipids PC 36:2 and PC 38:5.

Conclusion
Detailed analysis of substructures in tissue with a lateral resolution of 10 um.

Novel Aspect
MALDI imaging with high spatial and high mass resolution by MALDI-FTICR

TPS12-16 / Nano-LC-MS/MS for the Quantitation of Prostanoids in Immune Cells

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Introduction
Prostanoids are endogenous lipid mediators derived from arachidonic acid exhibiting a large variety of functions including the inflammatory response. To investigate the role of prostanoids produced by murine mast cells and T lymphocytes, a highly sensitive quantitation method for the prostanoids prostaglandin E2 (PGE2), PGD2, 6 keto PGF1α, PGF2α and thromboxane B2 (TXB2)by means of nano-liquid chromatography-tandem mass spectrometry has been developed and validated according to FDA guidelines. The challenge in developing this method was the limited number of immune cells that could be isolated from murine tissue (40,000 mast cells) or blood (5,000 T cells). Current methods for prostanoid quantitation in cells require cell counts in the magnitude of 200,000 to 2,000,000. Thus, our aim was to develop a more sensitive method to measure prostanoid concentrations from a limited number of cells.

Methods
Mast cells were isolated from paws of mice after inducing an inflammation process by local injection of zymosan. In contrast, prostanoids generated by T lymphocytes were isolated from blood of two different groups of mice: one group had been sensitized with oxazolone and the other group was non-sensitized. Both groups where challenged with the allergen before taking the blood. The samples were extracted with ethyl acetate. An Eksigent nano-LC 2D Ultra system equipped with a C8 column was used for chromatographic separation. The LC-system was connected to a hybrid triple quadrupole – ion trap mass spectrometer 5500 QTRAP (AB Sciex) and a nanospray ion source. The mass spectrometer was operated in negative multiple reaction monitoring mode (MRM).

Results
A validation according to FDA guidelines was done successfully in terms of linearity, precision, accuracy, recovery, stability and lower limit of quantitation (LLOQ). The LLOQ were 75 fg on column for PGE2 and PGD2 and 112.5 fg on column for 6 keto PGF1α, PGF2α and TXB2, respectively. In the mast cells, we found that the production of PGE2 due to an inflammatory stimulus increases significantly stronger than the synthesis of the other prostanoids. In contrast, the T cells did not show any difference in prostanoid production, no matter if they were obtained from sensitized or non-sensitized mice.

Conclusion
The sensitivity, selectivity and reliability of the developed method make it suitable for the study of the multiple roles of prostanoids in physiological and pathophysiological processes. It is applicable to other cell types and areas of research where the cellular roles of prostanoids have to be further investigated, for example in cancer research.

Novel aspect
We describe the first nano-LC-MS/MS method for the quantitation of prostanoids in immune cells isolated from mice. The developed method provides a high sensitivity and requires very small sample volume.

TPS12-17 / Evaluation of oxidation products in fatty acid esters by easy ambient sonic-spray ionization mass spectrometry

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Introduction
Easy ambient sonic-spray ionization mass spectrometry (EASI-MS) is one of the new desorption/ionization techniques and has been successfully applied as a fast and direct method to evaluate the quality parameters of different substances The purpose of this study was to test the ability of EASI-MS technique to identify primary and secondary oxidation products such as hydroperoxides and dimmers in fatty acid methyl esters- FAME (biodiesel). .The identification of these compounds will be useful to quality control of this substances.

Methods
Commercial soybean biodiesel samples were submitted to different oxidation procedures varying the temperature (25, 40 and 110°C) and exposure to air. Samples were analyzed at different time intervals both during the induction period (IP) and in the advanced stages of oxidation by EASI-MS. 2 µL of sample was added directly onto a paper surface and mass spectra were acquired over a m/z range of 100-1000 using a singlequadrupole mass spectrometer (Shimadzu).

Preliminary Data
EASI(+)-MS showed that during the earlier stages of oxidation the major ions that changed were the hydroperoxides, that are recognized as primary oxidation compounds. Herein, the hydroperoxides are described as FAME(OO) and were detected as the [FAME(OO) + Na]+ ions of m/z 347, 349, and 351 and the respective [FAME(OO) + K]+ ions of m/z 363, 365, and 367 for linolenic, linoleic, and oleic methyl esters, respectively. Ions resulting from bishydroperoxides, denoted as FAME(2OO), were also identified as [FAME(2OO) + Na]+ ions of m/z 379 and 381 and [FAME(2OO) + K]+ ions of m/z 395 and 397 for linolenic and linoleic methyl esters, respectively. In addition, we observed that these ions increased moderately during the initial stages of oxidation and this trend was in agreement with the peroxide value. Furthermore, the major changes in the EASI(+)-MS profile occurred after the induction period (IP) due to the emergence of ions related to volatile compounds that are decomposition products of hydroperoxides (m/z 200-250) and, interestingly, due to also the formation of dimmers (m/z 600-700). Therefore, these secondary products of biodiesel oxidation can function as suitable markers of biodiesels with high levels of oxidation. Another parameter is about EASI(+)−MS applied in oxidation index (Iox), herein defined as the ratio between the sum of the relative abundances of [FAME(O) + K]+, [FAME(OO) +Na]+, and [FAME(OO) + K]+ ions of m/z 347, 349, 351, 363, 365, and 367 and the sum of the relative abundances of the non-oxidized [FAME + Na]+ ions of linolenic, linoleic, and oleic acids (m/z 315, 317, and 319).

TPS12-18 / Mutation of FOXN1 gene and changing in skin lipid profile: a lipidomic analysis in nude mice

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Introduction
Nude mice, commonly used as animals of laboratory, carry a spontaneous mutation affecting the gene Foxn1. Due to a failure in the development of the thymic epithelium, homozygous nude mice lack T cells and consequently experience an impairment of cell-mediated immunity. This mutation also induced a default of keratinization in the hair growth follicles. Hence, nude mice appear hairless. Skin plays an important role in the organism protection by providing a barrier between the body and the hostile external environment. Skin also prevents the loss of water and electrolytes. Lipids are key components of the skin. While, ceramides, cholesterol and free fatty acids are the main lipids encountered in the stratum corneum, phospholipids are almost present in the basal cells of the epidermis. Moreover, sebaceous glands and, to a less extent, keratinocytes produced, at the surface of the skin, a layer of lipids, constituted of ceramides, cholesterol and fatty acids.

Methods
Taken into account the hairless phenotype of nude mice and the key role play by lipids of the skin, we engage in a lipidomic study to search for possible changes in lipid composition of skin related to Foxn1 mutation. Skin biopsies were withdrawn at the inguinal fold of nude mice and BALB/c, grinded and extracted with an appropriate mixture of organic solvent. After being resuspended, lipid extracts were analyzed by liquid chromatography coupled to a high resolution mass spectrometer (UPLC-HRMS). Following preprocessing of the data, a table, listing peak areas associated to an unique m/z and retention timeversus samples, was generated and exported for multivariate data analyses such as principal

component analysis and partial least squares-discriminant analysis. Identification were performed using high resolution mass spectrometry data, comparison of retention time to standard lipids and mass spectrometry fragmentation study.

Results
Multivariate data analysis lead to an unambiguous separation between lipids extracts from nude and BALB/c mice biopsies. Our results indicate that mutation of Foxn1 leads to profound modifications in the lipid profile of skin in nude mice. Indeed, an increase in numerous diacylphosphatidylcholines, diacylphosphatidylserines, diacylphosphatidylethanolamines, diacylphosphatidylinositols and ceramides associated to a decrease in triacyl and diacylglycerides was exhibited in nude mice. 142 lipids involved in this change were successfully identified.

Conclusions
UPLC-HRMS experiences and multivariate statistical analysis were performed in nude and BALB/c mice. It reveals marked change of skin lipid profile induced by mutation of Foxn1.

Novel Aspect
At this time, no one has performed a study relating to the impact of Foxn1 mutation on the lipid composition of the skin.

TPS12-19 / Robust LC-MS/MS Analysis of CNS-derived GM1 and GM2 Gangliosides

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Introduction
GM1 and GM2 gangliosidosis are autosomal recessive lysosomal storage diseases resulting from reduction or loss of lysosomal acid β-galactosidase or β-hexosaminidase A activity, respectively, and are characterized by accumulation of these gangliosides in the central nervous system (CNS). Previously, ganglioside content in the CNS had been assessed by thin layer chromatography (TLC), a low throughput separation assay lacking sensitivity and specificity. To this end, we developed a high throughput LC-MS/MS method for GM1 and GM2 gangliosides to provide a robust, selective and sensitive method allowing for better understanding of the effectiveness of gene therapies for gangliosidosis.

Methods
Brain homogenates from wild type, β-gal KO, and HexA KO mice were spike with d3-labeled GM1 and GM2 (Matreya). The glycolipids were extracted by Folch, isolated using C18 SPE, and analyzed using a Kinetex C18 (1.7µm, 100Å) 2.1 x 50 mm UPLC column (Phenomenex) coupled to a Waters Quattro Premier XE operating in the positive ion electrospray mode. MRM transitions for GM1 (fatty acids: 16:0, 18:0, d3-18:0, 18:1, 20:0, and 20:1) and GM2 (fatty acids: 18:0, d3-18:0, 20:0) were monitored using their common fragment at m/z 290. Calibration curves of GM1 and GM2 (A¬¬¬vanti) were created and the area ratio of each ganglioside species was calculated to the corresponding internal standard.

Results
We determined that the base hydrolysis step employed in previous TLC methods has detrimental effects on the recovery of gangliosides from brain tissue. By omission of this step, we note a five-fold increase in sensitivity and a recovery greater than 98%. Calibration curves were evaluated by comparing spikes that were prepared in 100% and 5% tissue homogenate, and neat. Following analysis using the method of standard addition, the curves were determined equivalent and justifid neat standard curves. Following determination of micro-heterogeneity in GM1/GM2 fatty acids, we incorporated their measurement into an MRM method. Quantification of GM1 ganglioside in brain tissue gave measurable differences in the KO's as compared to wild type (WT). For example, a 13.5 fold increase in GM1 was observed in β-gal KO mice while HexA KO mice were only 0.8 fold over WT. By contrast, GM2 levels in HexA KO mice were 87 fold higher than WT levels as compared to a 2.5 fold increase over WT levels in β-gal KO mice. We also note a shift in the relative acyl chain composition when comparing GM1 in WT, β-gal KO and HexA KO mice. For example, the fraction of 18:0 and 16:0 acyl chains in GM1 were decreased in the β-gal KO as compared to the WT, while the 20:0 fraction was increased. Interestingly, this effect was not observed for the HexA KO mice.

Conclusions
The LC-MS/MS method developed will facilitate a better understanding of the effectiveness of gene therapies for gangliosidosis in preclinical models and will allow evaluation of human patient samples in future clinical trials.

Novel aspect
A quantitative ganglioside assay with improved sensitivity and specificity to other methods.

TPS12-20 / Imaging mass spectrometry for analysing changes in the brain lipidome during cerebral ischemia in mice

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Introduction
Imaging mass spectrometry is a new technique, which can be used for 2-dimensional imaging in tissue slices of different molecules, such as lipids and drugs. The technique can thus provide knowledge about the distribution and topographic location of specific lipids in the tissue of interest [1].
Desorption electrospray ionization (DESI) is an ambient ionization technique that can be used for mass spectrometry and works under atmospheric conditions, requires no sample preparation except freezing and slicing and works well for imaging. It has been shown that DESI imaging can be used for visualization of the distribution of various phospholipids in brain slices of neonatal rats with induced ischemia [2]. Phospholipids in the brain function as components of cell membranes but also as precursors for biological active signalling molecules.

Methods
7 - 8 weeks old C57BL/6 mice were subjected to permanent middle cerebral artery occlusion (pMCAO). Brains from mice, with 30 min. up to 24 hours post-surgical survival, were collected, frozen and cut into 30 micrometre thick cryosections. The changes in the lipidome of the sections were investigated by DESI imaging, which was performed on a Thermo LTQ XL linear ion trap mass spectrometer (Thermo Scientific, San Jose, CA, USA) equipped with a custom-built DESI imaging ion source [3].

Results
Initial studies show the accumulation of the sodium adducts of ceramide Cer(d18:1/18:0) and lysophosphatidylcholine (lysoPC) in the ischemic area including lysoPC(16:0) and lysoPC(18:0), which have been identified by tandem mass spectrometry. Further analysis is focusing on localization of other lipid species.

Conclusions
By use of DESI imaging we can detect the formation and degradation of selected phospholipid species in the developing infarct. This may help to understand the role of biological active lipids under inflammatory conditions.

Novel aspect
This project may help to identify the molecular mechanisms in the ischemia progression and formation of scar tissue.

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TPS12-21 / The Effective MALDI MS Matrixes and Additives for Ganglioside Analysis
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Introduction
A ganglioside is a glycosphingolipid with one or more sialic acids, and gagliosides play important roles in many biological processes such as immune response, cell-to-cell communication, and tissue differentiation. MALDI MS analysis of gangliosides is challenging because they are fragile and therefore significant in-source fragmented ions are produced. Recently, we found that one of the MALDI matrixes, 5-methoxysalicylic acid (5-MSA) provided minimal in source fragmentation of gangliosides and therefore gave the low detection limit. In addition, we further optimized ionization of gangliosides by employing various salt additive conditions. Our results showed that cationization of gangliosides through cesium ions in the positive ion modes have several advantages over other salt additives. First, heavy cesium ions spread mass spectral profiles of gangliosides over widerm/zranges and therefore reduced spectral complexity. Second, cesium adduct ions gave the better sensitivity than other adduct ions.

Methods
Brain GD1a extract were prepared in chloroform and methanol (1:1, v/v) with the concentration of 1.0 mg/mL. Organic MALDI matrix solutions (10 mg/mL) were prepared in 4:1 v/v MeOH/H2O (DHB, super-DHB (sDHB), MBT, and MSA) or 1:1 v/v ACN/0.1% TFA in H2O (CHCA). In case an alkali salt additive was added to a matrix solution, final concentration of a salt additive in a matrix solution was 20 mM. MALDI MS analysis was performed using ABI 4800 Plus MALDI-TOF/TOF analyzer (Applied Biosystems, Foster City, CA).

Results
We found that 5-methoxysalicylic acid (MSA) is the very effective matrix for MALDI MS analysis of gangliosides since MSA generated almost sialic acid (SA) loss-free ganglioside profiles with intense GD peaks. In the positive ion mode, we can easily examine the extent of the SA loss, but complex adduct ions made ganglioside profiles hard to interpret. Therefore, an effective additive for spectral simplification was needed. In order to find the effective salt additive for spectral simplification, various alkali chlorides were tested. Among salt additives tested, CsCl was found to be very effective since it was easy to identify ion species since mass spectral profiles of gangliosides were spread over wider m/z ranges. In addition, Cs generated the adduct ion form, [M - 2H + 3metal]+ more than any other alkali metals. This suggests that Cs may have a higher affinity to sialic acid residues of gangliosides than any other alkali metals.

Conclusions
In this study, salt additive effect on MALDI MS of gangliosides was investigated. We found that cationization of gangliosides through cesium ions in the positive ion mode not only reduced spectral complexity by spreading mass spectral profiles of gangliosides over wider m/z ranges, but also provided the better sensitivity than other adduct ions.

Novel Aspect
Novel MALDI matrix system for effective analysis of gangliosides in the positive ion mode

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TPS12-22 / Isotopically Labelled Girard Reagents for Multiplexed Analysis of Oxysterols and Cholestenoic Acids in Plasma and CSF
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Introduction
Oxysterols are bioactive intermediates in the bile acid synthesis pathways that are ligands to the nuclear receptor LXR and the G-protein coupled receptor EBI2. They have also been shown to promote breast cancer proliferation by modulation of the estrogen receptor. Analysis of oxysterols and cholestenoic acids in biological matrices is complicated by their low abundance (pg/mL to ng/mL), especially when compared to cholesterol (µg/mL to mg/mL). The lack of chromophore and poor ionisation profile in mass spectrometry present additional challenges. Some of these problems can be overcome by chemical derivatisation prior to analysis by either GC/MS or, more recently, LC/MS.

Methods
Our group has developed an analytical strategy based on oxidation with cholesterol oxidase followed by derivatisation with the Girard P reagent. We have termed this EADSA (enzyme-assisted derivatisation for sterol analysis). The Girard P derivative incorporates a permanently charged quaternary ammonium ion leading to greatly enhanced sensitivity in ESI-MS. Additionally, predictable fragmentation yields structurally informative MSn spectra that allow identification of up to 40 metabolites.
We have now expanded this method by incorporating isotope labels into the Girard P reagent. This allows the analysis of up to four samples in a single LC/MS run on the LTQ-Orbitrap. As well as making more efficient use of instrument time this improves accuracy of quantitation by allowing a direct comparison of the analytes of interest. We have applied this method to plasma and CSF samples both with and without prior hydrolysis of sterol esters.

Results
Here, we demonstrate the principle of multiplexed EADSA using a Girard P reagent incorporating five deuterium atoms. We present a series of validation experiments that show the method has good accuracy and precision for a wide range of metabolites. In addition, we illustrate our approach with the rapid identification of patients with a deficiency in oxysterol 7α hydroxylase (CYP7B1) which can cause severe cholestatic liver disease in infants and motor neuron signs in adults. We present a detailed analysis of both plasma and CSF from these patients, showing perturbed oxysterol and cholestenoic acid profiles. The biological significance of these changes along with possible treatment with bile acids is discussed.

Conclusions
The multiplexed analysis of oxysterols provides a powerful tool for lipidomics. The direct comparison of multiple samples

increases efficiency by making better use of instrument time, and improves accuracy by negating matrix effects. This method can be used for the rapid diagnosis of inborn errors in cholesterol metabolism.

Novel Aspects

· Validated method for the multiplexed analysis of oxysterols and cholestenoic acids

· Rapid identification and analysis of plasma and CSF from patients with a rare genetic disease

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TPS12-23 / Lipid visualisation and identification through collision cross section aided correlation of MALDI imaging and MS/MS fragmentation data sets.

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Introduction

Mass spectrometry imaging (MSI) is rapidly becoming an established technique within lipidomics research. Using MSI, a broad range and number of species can be visualised within a tissue section. However, subsequent identification can be extremely challenging. Lipids can be identified by extracting them from the same or consecutive tissue section and performing MS/MS. However, due to the large number of isobaric or near isobaric species, ambiguous identifications can be made when correlating peaks within the imaging data set to extracted lipids by accurate mass alone.

Here, we demonstrate the use of ion mobility to differentiate ions and calculate collision cross sections (CCS) that when utilised along with accurate mass, adds confidence to peak assignment between the imaging and extracted lipids, allowing accurate relation of fragmentation information from extracted lipids to imaging data.

Methods

Data were acquired using a MALDI SYNAPT G2-Si mass spectrometer incorporating a tri-wave ion mobility cell. CCS calibration was conducted prior to imaging a section of rat brain. During the imaging experiment, an external lock mass was acquired at set time points to maintain mass measurement accuracy. Post imaging, lipids were extracted from the same tissue and deposited onto a stainless steel target plate. A second CCS calibration was acquired and the extracted lipids analysed by MS/MS. Identifications were assigned to imaging information using accurate mass and calculated CCS values.

Results

Proof-of-principal experiments have been carried out using a thin section of rat brain. The brain section was produced using a cryotome and deposited on a standard microscope slide. A SunCollect nebulising spray device was used to apply CHCA evenly in several coats. Using polyalanine for CCS calibration, the CCS of ions present within the imaging data set were determined and derived. High mass measurement accuracy was maintained throughout the experiment by using red phosphorous as an external lock mass compound. A mixture of 2:1 v/v CHCl3/MeOH was used to extract lipids directly from the imaged tissue section, in quantities sufficient for MS/MS identification by MALDI. CCS was calculated for the extracted lipids and combined with high mass accuracy values. Peaks identified by MS/MS were correlated to the peaks present in the imaging data set using the mass and CCS values.

Further work will be done and presented to optimise the lipid extraction procedure and automated assignment of the identified lipids to the ion signals present in the imaging dataset.

Conclusions

Utilising a CCS calibration allowed for the correlation of lipids present within an imaging data set with those present in a lipid extract. This enabled accurate identification to be assigned to lipids within the imaging dataset, from MS/MS experiments carried out on the lipid extract. This increases confidence in structural assignment compared to using accurate mass alone.

Novel Aspect

Verifying the assignment of MS/MS identifications of extracted lipids to ions observed in tissue imaging datasets using ion mobility collision cross section measurements.

TPS12-24 / Higher resolution LC-MS and MS-MS analysis of lipid extracts using benchtop Orbitrap-based mass spectrometers and LipidSearch software

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Introduction

Identification of lipids by untargeted lipidomics requires sophisticated software with an extensive lipid database. In addition, overlapping isobaric and isomeric lipid ions must be resolved. We present a benchmark evaluation of the effect of mass resolution and scan speed on the number of lipid species that can be potentially identified in LC-MS experiments.

Methods

LC/MS/MS analyses of lipid extracts were performed using a novel benchtop quadrupole high field Orbitrap mass spectrometer. Total lipid extracts (Avanti Polar Lipids, Inc.) were separated via a 2.1 x 100 mm Ascentis C18 column using a Dionex 3000 RSLC chromatograph coupled to a quadrupole high field Orbitrap mass spectrometer operated in either positive or negative ion mode. High resolution accurate mass data were acquired using full scan MS at 15K, 30K, 60K, 120K and 240K (FWHM at m/z 200) mass resolving power and data-dependent MS/MS.

LipidSearchTM software (Thermo Scientific) was used for lipid identification through a database search of the accurate masses of precursors and the fragment ions predicted for each potential adduct form of the lipids in the database (> 1,500,000 entries).

Results and Conclusions

Analysis of lipid extracts obtained from bovine brain, heart, liver and yeast served as a benchmarking study to determine the resolution needed to separate overlapping isomeric and isobaric lipid species. The lipid extracts were commercially obtained in order to remove the variability of the lipid extraction from this experiment.

The number of lipid species identified in each different experiment was assessed at the sum composition (MS) and isomer (MS/MS) levels. At the higher resolution settings (120K, 240K), the number of lipid species identified were at least 20% more than at the lower mass resolution settings (15K, 30K). These results show that even with a relatively long (21 min) HPLC gradient significant overlap of the precursor ions may exist leading to a lower number of lipids identified. Thus, mass resolution as well as scan speed are very important factors in LC-MS based lipidomics experiments. This work provides a benchmark for the number of lipid species found in several commercially available lipid extracts allowing comparison of different instrumental strategies for untargeted lipidomics.

Novel Aspect

Analysis of lipids using a benchtop quadrupole high-field Orbitrap LC-MS at 240K mass resolution provides a benchmark for lipidomics requirements.

TPS12-25 / Structural studies on sphingolipids –a revisit with LIT MSn with high resolution mass spectrometry

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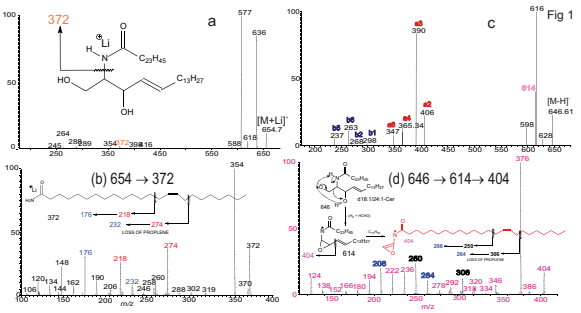
Introduction

Sphingolipids play important roles in membrane structure, interact with the extracellular matrix and neighboring cells, as well as participate in intracellular and extracellular signaling. Glycosphingolipids including α-glycuronosylceramides (GSL-1) from e.g., S. yanoikuyae, are important lipid antigens. In the epidermis, ceramides (Cer) comprise the major constituent of sphingolipids and are known to play diverse roles in the outermost layers of the skin including water retention and provision of a physical barrier. Sphingolipids are huge families consisting of numerous diversified structures. For example, epidermal ceramide contains seven classes (ceramides I-VII). Tandem mass spectrometry has been used for characterization of sphingolipids, but structural information is incomplete.

Methods

CID MSn mass spectrometry was conducted on a Thermo Scientific LTQ Orbitrap Velos with Xcalibur operating system. Lipids in chloroform/methanol (1/2) were infused (1.5 μLmin-1). The ESI needle was set at 4 kV, and temperature of the heated capillary was 300°C. Helium was used as the buffer and collision gas at a pressure of 0.75 mTorr. The MSn spectra were obtained with an optimized relative collision energy ranging from 30-40% and with an activation q value of 0.25, and activation time of 10 ms.



Results

In this talk, LIT MSn spectrometric approaches for complete structural characterization of sphingonoids, including ceramides and glycosphingolipids desorbed as [M-H[- and [M+Li]+ ions will be presented. For example, the MS2 spectrum of the ion of the [M + Li]+ ion of the d18:1/24:1-Cer at m/z 654 (Fig 1a) contained feature ions at m/z 372 from cleavage C2-N bond of LCB, leading to assignment of the 24:1-fatty acid chain and the sphingosine long chain base (LCB). Further dissociation of the ion at m/z 372 (654 → 372; Fig 1b) yielded the ion series locating the double position of the 24:1-fatty acyl group at C-15. The location of the double bond can also be deduced from MS4 on the ion of m/z 404 (646 →614→404; Fig 1d) arising from the corresponding [M – H]- ion of m/z 646 (Fig 8c). Using the same LIT MSn strategy, we are able to define the structures of ceramides that consist of α-, β-, or ω-hydroxy fatty acid substituent, and the GSL-I family that consists of sphinganine LCB with a double bond located at C12 to distinguish it from a sphingosine LCB containing GSL.

Conclusions

LIT MSn revealed structural details of sphingolipids, including the identities of LCB, sugar moiety (if present) and of the fatty acyl chain including the position of double bond and functional groups.

Novel aspect

High order LIT MSn provides structural details of sphingonoids, permitting differentiation of isomeric structures that are difficult to analyze using other analytical methods

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TPS12-26 / Application of MALDI-MS for rapid screening of lipid residues in archaeological pottery

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Introduction

Lipid analysis of food residues preserved in association with archaeological artefacts is a well-established method for obtaining information on the artefacts and their use.1 The detection of triacylglycerides (TAGs) is particularly important because TAG distributions provide one of the few ways of discriminating the species of animal fat, such as dairy and adipose fats.2 Current techniques include solvent extraction of lipids followed by derivatisation, GC and GC-MS, with high temperatures required to elute intact TAGs. Alternatively, LC-MS approaches have proved successful.3 In order to obtain the best results for high temperature (HT)GC-MS of intact TAGs, on-column injection is used. However, GC- and LC-MS analyses are time-consuming; for large ceramic assemblages, and where only a small proportion of extracts contain detectable levels of analytes, a medium-throughput screening technique to identify extracts for further analyses is highly desirable. Direct mass spectrometric methods can address this issue. In the work presented here, the suitability of one such direct method – MALDI-MS – is compared with that of on-column injection HTGC-MS for identifying TAGs in lipid extracts of archaeological potsherds dating to the late Neolithic period (ca. 4500 years before present).

Methods

Solvent extracted lipids from archaeological potsherds were analysed using MALDI-TOF/TOF-MS (Bruker ultraflex III) and on-column injection HTGC-MS (Agilent 7890A GC and 5975C MS).

Results

Analysis of the samples by HTGC-MS showed a range of lipids including intact TAGs and their breakdown products monoacylglycerides, diacylglycerides and free fatty acids. However, the intact TAGs gave only weak responses, with small peaks and a very narrow range of TAGs detected. In contrast, when analysed by MALDI-MS, the TAG signals were of good S/N and a much wider range of TAGs was detected. Tandem MS then allowed identification of the specific fatty acids present on each of the TAG species.

Conclusion

MALDI-MS is able to detect intact TAGs in total lipid extracts from archaeological potsherds with greater sensitivity than can HTGC-MS, and provides information about the fatty acids attached to the glycerol backbone of the TAGs. The MALDI-MS method is also rapid, taking around 1 min per sample (compared to ~40 min for HTGC-MS), making it suitable as a screening method to identify samples containing well-preserved lipid residues suitable for further analysis.

Novel Aspect

This method provides a new way to analyse lipid residues from archaeological artefacts, allowing better detection and greater structural characterisation of the TAGs present than current methods provide. Analysis of archaeological lipid extracts in this way can help more efficiently screen large ceramic assemblages, minimising unnecessary destruction of valuable cultural artefacts and ultimately allowing us to gain more knowledge of the diets and lifestyles of people in the past.

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TPS12-27 / Lipidomic analysis of spinocerebellar ataxia plasma samples

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Introduction
Lipids are involved in energy storage, cell signaling and membrane constitution. Their implication in diseases such as cancer, diabetes, neurological or inherited metabolic diseases has already been established. They thus are valuable candidates for biomarkers discovery. In this respect, we have developed a comprehensive lipidomic methodology based on liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). It was applied to the comparison of 101 plasma samples from patients with different progressive forms of spinocerebellar ataxia (SCA1, 2, 3 and 7), obtained from the FP7 European project Neuromics partners. SCA 1, 2, 3 and 7 belong to the group of polyglutamine expansion rare diseases (repetition of coding CAG), as is Huntington disease, and share common symptoms including changes in balance, movement, coordination, and speech. The importance of establishing biomarkers of polyglutamine expansion SCA is related to the high disease heterogeneity and lack of characteristic symptoms that leads to diagnostic, prognostic challenges and difficulties in making therapy decisions.

Methods
Plasma total lipid extracts were obtained by a Bligh and Dyer modified partition. Extracted samples were separated on a Kinetex C8 column connected to an Accela quaternary HPLC pump coupled to a Q-Exactive mass spectrometer recording in full scan mode at a mass resolution of 140 k fullwidth half-maximum (FWHM) at m/z 200. Reliable data processing tools were developed to handle LC-HRMS data sets. They include automatic peak detection and alignment, annotation based on exact mass, retention time, the presence of the 13C isotope and the relative isotopic abundance (RIA).

Results
From the 725 unique lipid species identified in these plasma samples, i.e. 430 glycerophospholipids, 180 glycerolipids, 79 sphingolipids, 33 sterols and derivates and 3 fatty acids, several lipids belonging to the same class were found increased in the SCA 7 samples. In particular ether and plasmalogen forms of 2 phospholipid classes (PC, PE) are significantly more abundant in SCA 7 compared to control plasma. Furthermore, a correlation has been highlighted in subgroups SCA 3 and 7 between the clinical score of severity SARA and areas of lipids identified as GM3 gangliosides species.

Conclusion
We have developed a robust methodology for lipid profiling using LC-HRMS coupled with integrated data processing tools and potential diagnostic and prognostic markers of the subtypes SCA 3 and SCA 7 have been highlighted. To confirm these results, two new plasma collections of these same patients, i.e. sampled respectively one and two years after the first sampling will be analyzed.

Novel Aspect
Untargeted lipidomic approach using UHPLC-HRMS, integrated data processing tools and spectral dataset to profile lipid species in human plasma.

TPS17 - Protein Phosphorylation and other Post-translational Modifications

11:00-15:00
Poster Exhibition, Level -1

TPS17-01 / Complementation of Ti-, Zr- and Fe-based PolyMAC for in-depth phosphoproteome analysis of B cell signaling

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Introduction
B cells are essential components of immune response, capable of producing specific antibodies through engagement of the B cell receptor (BCR). Though a number of studies have analyzed tyrosine phosphorylation after BCR stimulation, there is very limited information available about the overall changes in B cell phosphoproteome. Previously, we have introduced a novel soluble nanopolymer-based phosphopeptide enrichment approach termed PolyMAC-Ti (Polymer-based Metal ion Affinity Capture using titania), which has demonstrated superior enrichment capabilities due to the homogeneous reaction environment. Here, we expand it to two more metal ions – PolyMAC-Fe and PolyMAC-Zr – functionalized with iron or zirconium and each capable of enriching unique subsets of the phosphoproteome. We combined PolyMAC-Ti, PolyMAC-Zr and PolyMAC-Fe to quantitatively measure changes in the B cell phosphorylation after BCR stimulation.

Methods
We utilized SILAC approach for quantitative measurement of changes in phosphorylation after the engagement of BCR with anti-IgM antibody and Syk kinase inhibition. Various sets of samples were combined and fractionated using HILIC or RPLC chromatography under slightly basic conditions. Each fraction was split and enriched with PolyMAC-Ti, PolyMAC-Zr or PolyMAC-Fe in order to achieve a more complete coverage of the phosphoproteome. The enriched phosphopeptides were analyzed by nanoflow liquid chromatography tandem mass spectrometry (nLC-MS/MS). The MS spectra were acquired on an Eksigent ultra 2D LC system that is coupled to hybrid linear ion trap orbitrap mass strometer (LTQ Orbitrap Velos, Thermo Fisher). Data were searched and quantitated using the SEQUESTTM and MascotTM algorithms within the Proteome Discoverer software.

Results
The foundation of our novel approach lies in a water soluble and highly accessible polymer support for homogeneous phosphopeptide enrichment. PolyMAC reagents are synthesized from a polyamidoamine (PAMAM) dendrimer which is functionalized with metal ions to specifically capture O-phosphorylated peptides through bidentate chelation chemistry. An extensive comparison was made between PolyMAC-Fe, PolyMAC-Zr, PolyMAC-Ti and IMAC reagents in terms of the specificity, reproducibility and the type of phosphopeptides being enrichmed.
Our data revealed that PolyMAC-Fe, PolyMAC-Zr and PolyMAC-Ti are complementary in terms of phosphopeptide enrichment. Therefore, we have combined these enrichment

procedures with SILAC-based quantitation and large-scale fractionation to attain a more in-depth analysis of the B cell phosphoproteome. We were able to identify a larger percentage of multiply phosphorylated peptides than with PolyMAC-Ti alone. Overall, out of over 20,000 unique phosphosites identified, close to 20% were dependant on BCR signaling. These sites were further mapped in a variety of major signaling networks, some of which were further biochemically explored, offering more detailed information about B cell engagement and maturity.

Novel aspect
Combining differentially functionalized, homogeneous, fine-tunable nanopolymer technologies (PolyMAC) for highly efficient phosphopeptide enrichment.

TPS17-02 / Motif-Targeting Quantitative Proteomics for Absolute Phosphorylation Stoichiometry Measurement

Chia-Feng Tsai[1], Chia-Feng Tsai[1], Yi-Ting Wang[2], Hsin-Yung Yen[3], Chih-Chiang Tsou[4], Wei-Chi Ku[5], Pei-Yi Lin[6], Alexey I. Nesvizhskii[4], Chi-Huey Wong[3], Yasushi Ishihama[7], Yu-Ju Chen[6]
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Measurement on the phosphorylation event is often used as indicator for signaling pathway activation. Despite being very sensitive, conventional methods such as immunoassay or kinase activity assay only provides arbitrary ratio and requires control sample to compare the relative level of phosphorylation. The critical information on absolute amount of protein and extent of phosphorylation to alter signaling pathways is missing. Here, we developed a motif-targeting quantitative proteomic approach to calculate absolute phosphorylation stoichiometry on a large scale. This strategy only requires the measurement of a single ratio between phosphatase-treated peptides and recovered phosphopeptide from unphosphorylated peptides after kinase reaction. To our knowledge, this is the first approach to access the phosphorylation stoichiometry for large scale human proteome in single cellular status. The performance of this approach was evaluated to measure the absolute stoichiometry as well as to compare the phosphoproteomic alterations between the targeted-therapy (gefitinib) sensitive (PC9) and resistance (PC9/gef.) lung cancer cell lines. With reproducible quantitation result (standard deviation: ±6%), more than one thousands of phosphorylation sites and their phosphorylation stoichiometry were calculated for serine/threonine phosphopeptides and especially for high percentage of low abundant tyrosine phosphopeptides. This approach not only revealed the basal level of phosphorylation stoichiometry of the two lung cancer cell lines, but also differentiated the regulation at the protein level and more dramatic phosphorylation degree associated with drug resistance in lung cancer. The result also revealed that acidic phosphorylation motifs from CK2 had higher proportion (30%) of high phosphorylation stoichiometry (>70%) than those from MAPK and EGFR. Through network analysis, the measurement identified a substrate of CK2 as the potential drug resistant target to reverse the drug resistance in PC9/gef cells. We expect that this motif-based isotopic quantitative approach may be useful to study phosphorylation-mediated events from broad ranges of fundamental research to discovery-driven cancer research.

TPS17-03 / N- and O-acetylation of threonine residues in the context of proteomics

Christine Enjalbal[1], Jean-Baptiste Boyer[2], Alain Dedieu[2], Jean Armengaud[2], Gilles Subra[3], Pascal Verdié[3], Jean Martinez[3]
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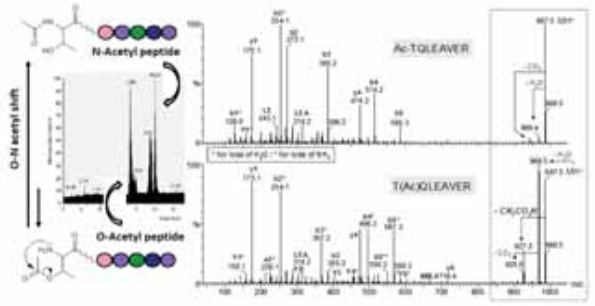
The detection of post-translational modifications (PTMs) of proteins is a matter of intensive research. Among all possible pitfalls that may lead to misidentifications, the chemical stability of modified peptides is scarcely questioned. Global proteomic studies devoted to protein acetylation are becoming popular. Thus, we were concerned about the intrinsic stability of O-acetylated peptides because of the O-N acyl transfer reactivity occurring when an amino moiety is present in the vicinity of the acylated hydroxyl group. Here, the behavior of isomeric O- and N-acetylated, N-terminal threonine–containing peptides was explored in a standard proteomic workflow. We demonstrated a strong chemical instability of O-acetylation, which prevents its detection.



TPS17-04 / Unexpected N-glycosylation occuring in hen eggwhite lysozyme at a non-consensus sequon analyzed by complementary LC-MS/MS based methods

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Bruker Daltonics GmbH

Introduction
N-glycosylation is expected to occur in proteins comprising the specific sequence motif NX(S/T), where X may be any amino acid with the exception of proline.
Hen eggwhite lysozyme (HEWL), however, represents a 14.4 kDa antibacterial enzyme that is lacking the sequence motif NX(S/T). Nevertheless, Trudel 1) et al., in 1995, proposed the existence of a minor N-glycosylated form of HEWL based on results obtained from ConA binding, SDS-PAGE separation, Edman sequencing and deglycosylation by PNGase F.
In this paper, we describe the detailed analysis of N-glycosylation in wildtype HEWL employing various state-of-the-art mass spectrometric methods. For the first time, our data allow to determine unambiguously the sequence region in which N-glycosylation occurs in HEWL as well as to profile the compositional heterogeneity of N-glycans modifying HEWL.

Methods
HEWL was digested in solution using trypsin as a cleaving enzyme. From the resulting tryptic digest, N-glycopeptides were enriched by ZIC-HILIC. Enriched N-glycopeptides were analyzed by LC-MS/MS engaging MALDI-TOF/TOF (Bruker ultraflextreme) and ESI-iontrap-ETD-MS/MS (Bruker amazon speed ETD) instruments, respectively. Furthermore, ZIC-HILIC enriched N-glycopeptides were enzymatically deglycosylated in 18O water using PNGase F. The resulting deglycosylated peptides were again analyzed by LC-MS/MS using MALDI-TOF/TOF and ESI-CID-MS/MS, respectively.

All N-glycopeptide MS/MS data were processed with Proteinscape 3.1 software (Bruker) featuring the GlycoQuest search engine for glycan identification and a dedicated classification algorithm for highly specific detection of N-glycopeptide candidate spectra in LC-MS/MS datasets.

Results

Data obtained from intact N-glycopeptides when analyzed by MALDI-MS/MS and ESI-ETD-MS/MS, respectively, provide evidence for N-glycosylation to occur in HEWL´s amino acid region [37-44], which comprises three asparagine residues (N37, 39 and 44). However, none of these sites is part of a known N-glycosylation specific sequon.

MS/MS analysis of N-glycopeptides after deglycosylation by PNGase F additionally confirms HEWL sequence region [37-44] to be N-glycosylated and suggests all three of the present Asn residues to be partially glycosylated.

Finally, our MALDI-TOF data provide a detailed composition profile of the N-glycans modifying HEWL comprising a variety of complex as well as high-mannose type structures.

Conclusions

Based on the LC-MS/MS data presented here, N-glycosylation is shown to occur in HEWL sequence region [37-44]. According to our data, all three Asn residues found in this region are partially glycosylated, although none of them is part of a known N-glycosylation specific sequon.

Novel aspects

First-time detailed LC-MS/MS characterization of low abundant N-glycosylation in HEWL occurring at a non-consensus sequon

1) J. Trudel, A. Asselin, Biochem. Cell Biol. 73: 307-309 (1995)

TPS17-05 / Peptide structure analyses using topological mass spectrometry

Yasushi Shigeri, Akikazu Yasuda

National Institute of Advanced Industrial Science and Technology (AIST)

Introduction

After 30 years of traditional endocrinology studies, matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry (MALDI-TOF MS) was developed to analyze the secretory granules directly in frozen sections. In our studies, we focus on recent studies of the post-translational modifications that have been found in pro-opiomelanocortin-derived peptides in fish pituitary, starting with a background on the application of mass spectrometry that has been used for these studies.

Methods

Fish, such as carp (Cyprinus carpio) and goldfish (Carassius auratus), were purchased from a local pet shop. A slice of pituitary, sectioned at 40 mm thickness using a CM1850 cryostat (Leica Microsystems, Germany), was placed on a MALDI sample plate and was rinsed using the matrix solution of a-cyano-4-hydroxycinnamic acid (CHCA) saturated in a solution of acetonitrile/water 50:50 (v/v) containing 0.1% trifluoroacetic acid (TFA) for removal of any excess salts present on the surface of sample. Fresh CHCA matrix solution was then added to the sample and dried. The MALDI-TOF mass spectra were acquired using a Microflex LRF mass spectrometer (Bruker Daltonics, Bremen, Germany). Structural determination with MS/MS experiments was performed on a LCQ Fleet (Thermo Scientific, MA).

Results and Conclusion

The precursor protein, pro-opiomelanocortin (POMC) undergoes extensive post-translational processing in a tissue-specific manner to yield various biologically active peptides involved in diverse cellular functions. The recently developed method of matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) for direct tissue analysis has proved to be a powerful tool for investigating the distribution of peptides and proteins. In particular, topological mass spectrometry analysis using MALDI-MS can selectively provide a mass profile of the hormones included in cell secretory granules. An advantage of this technology is that it is possible to analyze a frozen thin slice section, avoiding an extraction procedure. Subsequently, tandem mass spectrometry (MS/MS) has a profound impact on addressing the modified residues in the hormone molecules. Based on these strategies with mass spectrometry, several interesting molecular forms of POMC-derived peptides have been found in the fish pituitary, such as novel sites of acetylation in alpha-melanocyte-stimulating hormone (MSH), hydroxylation of a proline residue in beta-MSH, and the phosphorylated form of corticotropin-like intermediate lobe peptide (CLIP). Moreover, novel peptides have been discovered from frog skin.

Keywords

MALDI, peptide, post-translational modification, topological mass spectrometry analysis

References

1) Yasuda, A., Jones, LS., and Shigeri, Y. (2013) The multiplicity of post-translational modifications in pro-opiomelanocortin-derived peptides. Frontiers in Experimental Endocrinol., 4, article 186, 1-5

TPS17-06 / Effects of glycation on aspirin-mediated acetylation of human blood proteins.

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Introduction

During decades, the competition effect between aspirin-mediated acetylation and protein glycation was a matter of concern. However, the exact interplay between these two post-translational modifications is not well understood yet. Both acetylation and glycation occur at the primary amino groups of proteins and several efforts were done to explain the protective effect of aspirin over glycation. Nevertheless, the influence of previous protein glycation on the action of aspirin was never investigated. Of note, the beneficial effect of aspirin seems to be reduced in patients with diabetes mellitus, suggesting that protein glycation may impair the acetylation process of aspirin. A qualitative and quantitative analysis was here performed to 1) identify acetylated and glycated proteins, 2) quantify the level of acetylation and glycation, and 3) elucidate the common modification sites.

Methods

Plasma and red blood cell protein extracts of healthy volunteers were incubated with, 30mM glucose for 24h followed by 500µM aspirin for 30min. Qualitative and quantitative analysis were carried out by western blot and tandem mass spectrometry (MS) to assess the acetylation and glycation levels of the two blood compartments.

Results
Label free MS evidenced an increase in the acetylation level after glucose incubation by western blot and MS for both plasma and red blood cell proteins. Interestingly, a decrease of the glycation level was already present after only 30 min of aspirin incubation for several proteins. These modifications could be possibly due to conformational changes exerted by both glucose and aspirin, but this requires further investigations. A number of common amino-acid sites where both acetylation and glycation took place were also identified.

Conclusions
The present study assessed the extent of aspirin-mediated acetylation and glycation of plasma and red blood cell proteins. The mutual influence that takes place between these two modifications may be interpreted as the result of conformational changes induced by glucose and aspirin. This strategy will be applied to other blood compartments (platelets and leucocytes) and to diabetic patients in order to better understand the interplay between these two post-translational modifications

Novel aspect
The proposed analytical strategy is useful for the estimation of the extent of glycation and aspirin-acetylation on different biological samples.

TPS17-07 / Identification of viral phosphorylation in human immunodeficiency viruses

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Introduction
Human immunodeficiency virus (HIV) belongs to lentivirus family and causes AIDS while Simian immunodeficiency virus (SIV), as a counterpart, which infects in monkeys. The budding of virions occurs in lipid raft containing regions of the cell, and is dependent on the phosphorylation of viral proteins in the p6 region of Gag/Gag-Pol polyproteins. Based on this evidence, we hypothesized that multiple phosphorylation sites in these proteins may destabilize charges and promote electrostatic repulsion, leading to the activation of the viral protease and enhanced maturation of virions. To understand the mechanism, we first aim to identify the phosphorylation sites in Gag and Gag-Pol polyproteins and to characterize these phosphorylation sites in the maturation of virions and infectivity.

Methods
HIV-1 infectious molecular clones such as pNL4-3 and different HIV-1 clades reconstituted based on primary infections in stimulated PBMC cells are being prepared. SIV is purified from primary infected monkeys. The purified virions are then solubilized, trypsinized, and phosphopeptides are enriched using TiO2 and NTA-Fe methods. Then, the enriched phosphopeptides are analyzed using LC-MS/MS (TripleTOF 5600 ABI). Identification and assignment of phosphorylation sites are performed with Mascot, ProteinPilot, ScaffoldPTM based on Ascore and protein probability. In addition, in silico phosphorylation prediction tools including virPTM and NetPhos are used to compare to experimental results from LC-MS/MS.

Results and conclusions
In the preliminary results, we have identified 39 phosphorylation sites in Gag, Gap-Pol polyprotein and pvu with 99% confidence using localization of probability score in HIV-1. In the comparison with in silico phosphorylation site prediction tools, eight confident phosphorylation sites identified by mass spectrometry were also found in in silico predictions. We now further investigate these roles of phosphorylation sites and map the phosphorylation sites in SIV as well as extend our investigations in different HIV-1 clades, which eventually will help us to understand the primary HIV-1 infections and vaccine.

Novel aspect
39 phosphorylation sites were identified highly confident, which is uncovered by previous studies.

TPS17-08 / Discrimination between symmetry/asymmetry dimethylation on histone H4R3: their cell cycle dependent dynamics

Takeshi Kawamura[1], Yoko Chikaoka[1], Yuzo Yamazaki[2], Matthew Openshaw[3], Omar Belgacem[3], Kazuki Yamamoto[1], Tatsuhiko Kodama[1]
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Introduction
Histones are subjected to variable modifications, including acetylation, methylation, phosphorylation and ubiquitination. Combinatorial modifications on histones involve in epigenetic regulations through chromatin structure. Dimethylation on arginine of histones forms two isomers, which are modified by distinct enzymes and have antagonistic effects on transcriptional regulation and other biological outputs. Although we have reported that high-resolution PSD on MALDI-TOFMS has advantages in discriminating between symmetry and asymmetry dimethylation on H4R3 by using characteristic neutral losses, further challenges remained to establish a system to define biologically relevant changes. Here, we will report a feasible study of analysis of cell cycle dependent H4R3me2.

Methods
Histone (H4R3me2) was purified from HeLa S3 cells, which cell cycle was a synchronized. The combinatorial modifications on 1-23 aa in the isolated histone were determined using orbitrap LC-MS. Model synthetic peptides and isolated peptides from cell cycle-synchronized cells were analyzed using LC-MALDI (off-line). High resolution PSD experiment was performed with MALDI-7090 (Shimadzu/Kratos), incorporating an axial spatial distribution focussing (ASDF™) cell.

Results
As we reported previously, singly charged characteristic ions generated in high resolution PSD enabled to differentiate symmetry and asymmetry dimethylation. The PSD was able to analyze several hundred attomol of the synthetic peptides, a sensitivity of which was almost sufficient to detect the target peptide in the cell cycle-synchronized cells (~0.5 % of modifications on H4 tail). Moreover, since high resolving power of MS/MS enabled to avoid an overlap between the characteristic ions and unwanted fragment ions, intensities of the characteristic ions thought to be applicable to quantitative analysis. As expected, by using the PSD, we could determine the ratio of symmetric/asymmetric dimethylation in mixed synthetic peptides semi-quantitatively. We have already found that H4R3me2 elevated on early S-phase. By using the semi-quantitative approach and LC-MALDI system, we will report that a relevance between the biological event and a ratio of the isomer in the individual cell cycle peaks.

Conclusions
We have established an analytical approach to develop a system to semi-quantify H4R3me2. Especially, we determined the relative ratio of symmetry and asymmetry dimethylation in mixture after LC separation.

Novel Aspect
High resolution PSD on MALDI-TOFMS could be applicable to perform semi-quantitative analysis of symmetry and asymmetry dimethylated Arg in H4R3me2.

TPS17-09 / Simple and reproducible sample preparation for single-shot phosphoproteomics with high sensitivity
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Introduction
Post-translational modifications of proteins are important regulatory mechanisms in all biological systems. Phosphorylation is the most ubiquitous and well-studied modification and it plays a key role in all central physiological and pathophysiological pathways. Unfortunately, in-depth phosphoproteome analysis by quantitative mass spectrometry (MS) often requires large amounts of sample material, extensive fractionation, and many hours or days of MS acquisition time, increasing costs and limiting the number of samples that can be measured. In this study we have optimized sample preparation parameters for reaching comprehensive in-depth phosphoproteome coverage of up to 10,000 unique phosphopeptides in a single LC-MS run on the Q-Exactive Plus.

Methods
HeLa cells were starved overnight and serum stimulated for 15 minutes. For lysis, a traditional modified RIPA lysis buffer followed by acetone precipitation and resuspension in urea was compared to a simple single step guanidine hydrochloride (GndHCl) lysis. The comparison was evaluated both on the level of yield, phosphoproteome coverage and introduced in-vitro artefacts. Phosphopeptide enrichment was optimized by varying the ratio between TiO2 beads and sample (w/w). Furthermore, column lengths, LC gradients and MS acquisition parameters were optimized to find the best compromise between depth of phosphoproteome and instrument run time. Finally dilution series were analyzed to establish a measure of sensitivity.

Results
Compared to the RIPA lysis and acetone precipitation protocol that is optimized for mg of starting material we found the guanidine GndHCl based lysis method to work very well for a broad range of sample amounts (from low ug to 10's of mg). GndHCl lysis reduces sample preparation artefacts and thus the sample complexity without compromising the depth of phosphoproteome analysis. Simplifying the procedure with fewer steps in the protocol gave higher yield, minimized risk of sample loss, and increased reproducibility. As expected longer gradients and/or multiple enrichments increased the depth of the phosphoproteome by 10-40%, but at the cost of MS time. Analysis of phosphopeptide enrichments from varying amount of starting material ranging from 10ug to 1mg showed a strong correlation between sample amount and the number of identified phosphopeptides. The optimized phosphoproteomics protocol for low sample amounts allowed for identification of 2000 unique phosphopeptides from 30ug of starting material and up to 10,000 unique phosphopeptides from 3mg in a single LC-MS run.

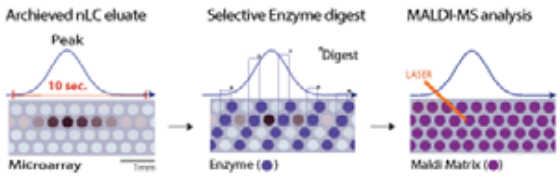
Conclusion
Optimized sample preparation, improves yield, reproducibility, and throughput while decreasing in-vitro artefacts and use of costly instrument time. Our optimized protocol enables studies of minute amounts of sample material, simplifies sample preparation, while maintaining maximum phosphoproteome coverage.

Novel aspect
Optimized high through-put and sensitive analysis of the phosphoproteome.

TPS17-10 / Micro-Arrays for Mass Spectrometry (MAMS): Self-Aliquoting Micro-Array Targets for nLC-MALDI-MS
Martin Pabst[1], Küster Simon[1], Jasmin Krismer[1], Robert Steinhoff[1], Rolf Brönnimann[2], Jens Boertz[3], Gerd Hayenga[3], Fabian Wahl[3], Petra Dittrich[1], Renato Zenobi[1]
[1]ETH Zurich, [2]EMPA Dübendorf, [3]Sigma-Aldrich

Introduction
We introduce a novel self aliquoting microarray sample target for nLC-MALDI-MS applications. The target is in the dimensions of a standard microscope slide and can be used for archiving of up to 2700 individual fractions from a nLC separation. Readout can be done by MALDI-MS as well as by fluorescence. The microarray target has an omniphobic surface with 2700 hydrophilic micro-spots in the micrometre size. The omniphobic surface confines the collected fractions to the predefined spot positions. Spot reservoirs can further be used for on-chip nanoliter reactions.

Methods
MALDI-MS analysis was carried out on an AB5800 MALDI-TOF-MS (AB Sciex) as well as on a SolariX MALDI-FT-MS instrument (Bruker). Chromatography was done on an Eksigent/Ekspert nanoLC400 (AB Sciex), using a RP C18 EASY-column (Thermo Fisher). Fractions from nanoLC were collected using a droplet spotting device as published recently by Küster et al., Anal. Chem. (2013).



Results
The self-aliquoting properties of the microarray target allow a manual aliquoting of matrix, internal standards or even enzyme solutions - by just using a simple mechanical sliding device, before or after fractionation of an nLC run. By combining the microarray technology with droplet microfluidics, the spots can be used also as nanoliter reaction cavities. Thereby, enzymatic or chemical reactions can be performed in distinct spots by an under oil spotting/reaction technology.
Our novel microarray target technology designed for nLC-MALDI-MS enabled furthermore the development of a new approach for the analysis of protein modifications. We demonstrated this by an in depth analysis of the human serum IgM glycosylation on the peptide level.

Conclusions
The self-aliqouting properties of the high-density microarray target allow a manual aliquoting of matrix and internal standards. Furthermore, the combination of microarray targets with droplet microfluidics allowed the development of a new approach for a straightforward identification of protein modifications.

Novel Aspects
We designed a (self-aliquoting) high-density micro-array platform, which can archive up to 2700 individual fractions from an nLC separation. A MALDI-MS readout can be correlated with on-chip enzyme digests.

TPS17-11 / Ion mobility mass spectrometry and MM conformational search of glycopeptides

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Novel Aspect

The interaction between the innermost GlcNAc and peptide backbone contributes to the compactness of glycopeptide ions in gas phase.

Introduction

Glycosylation alters the physicochemical properties and biological activities of proteins. During biosynthesis, N-linked glycosylation promotes folding and overall stability of proteins. In fact, physicochemical analysis indicated that the innermost GlcNAc contributes to the entire acceleration of folding and to two thirds of the native state stabilization of glycoproteins, while the remaining one third is derived from the next 2 saccharide units. These facts prompted us to investigate the interaction between protein and glycan in the vicinity of the attachment site by using ion mobility MS of glycopeptides and furthermore to compare the data with the conformations calculated by molecular mechanics (MM).

Method

Four different IgG subclass peptides EEQ(Y/F)NST(Y/F)R and their glycosylated species bearing a single GlcNAc at Asn were synthesized. Other GlcNAc-bearing peptides were obtained from six different glycoproteins by tryptic and endoglycosidase M digestion. Peptides with known CCS were derived from tryptic peptides of bovine apotransferrin, human hemoglobin and horse myoglobin.

The (glyco)peptide samples were analyzed by ion mobility MS using a Waters SYNAPT G2 system. The drift time of doubly protonated molecules of (glyco)peptides was measured and converted into CCSs by a theoretical equation.

The MM conformational searches (using MMFF94s force field) were performed by CONFLEX 7 (CONFLEX Co.).

Results

The amino acid sequences of glycopeptides from IgG1 and IgG2 are EEQYNSTYR (exact mass 1188.5) and EEQFNSTFR (1156.5), respectively. IMS MS of doubly protonated molecules indicated that IgG1 glycopeptide revealed smaller CCS compared with IgG2 while the molecular mass of the former was higher. Interestingly, this conformational feature was observed in the corresponding GlcNAc-bearing peptide, suggesting a specific interaction between the innermost GlcNAc and peptide backbone sequence. The CCS of IgG3 (EEQYNSTFR) was larger than IgG4 (EEQFNSTYR), in spite of the same molecular mass.

The CCSs based on MM were consistent with the observations described above, and the conformations included hydrogen bonds between GlcNAc and peptide backbone. In addition, the conformations of these four unglycosylated peptides were considerably different each other and those of the GlcNAc-bearing species were as well, indicating that the hydrogen bonding between glycan and peptide within glycopeptides ions was dependent on the peptide sequence.

Conclusion

In general, the CCSs of glycopeptides are smaller than those of peptides. Our findings from IMS MS and MM calculation suggested that the interaction between the innermost GlcNAc and peptide backbone contributes to the compactness of glycopeptide ions in gas phase.

TPS17-12 / Fully Automatable Multi-Dimensional Liquid Chromatography with Online Tandem Mass Spectrometry for Proteomics and Selected PTMs

Quan Quan, Henry Law, Samuel Szeto, Ivan Chu, Guohui Li
The University of Hong Kong

TPS17-15 / A fast and efficient 3-level method for characterization of N- and O-linked glycosylations

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TPS17-17 / Structure characterization and differentiation of biosimilar and reference products using unique combination of complementary fragmentation mechanisms

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Introduction

Biosimilars are subsequent versions of innovator biopharmaceutical products created after the expiration of the patent on the innovator product. The approval of a biosimilar product by a regulatory agency requires thorough characterization that demonstrates comparability with a reference product in quality, safety and efficacy. High resolution mass spectrometry provides accurate characterization of various protein properties including primary structure, type and location of post-translational modifications, and the detection of low abundant sequence variants or impurities. In this study, we developed a robust approach for comparability study of biosimilar and reference product.

Method

Three samples, an original drug, a recombinant variant and its biosimilar product were characterized and compared. Each sample was digested using trypsin after reduction and alkylation, separated on a C18 column and analyzed in triplicate using an Orbitrap Fusion Tribrid mass spectrometer with an EASY-ETD ion source. A unique HCD product dependent ETD method was used to obtain not only the amino acid sequence information for modified and unmodified peptides, but also location of modifications and structural information of glycans. The results were analyzed using PepFinder 1.0 software to identify peptides as well as the type, location and abundance of modifications including N and O-linked glycosylation.

Results

Peptide mapping results indicated 100% sequence coverage for all of the three samples. A five order magnitude dynamic range for identified peptide abundance was achieved, which allowed identification of modified peptides with less than 0.01% in abundance of the unmodified versions. Three major N-glycosylation sites were identified with a variety of different glycoforms for each site. Identity and relative abundances of the various glycoforms at N448 were consistent among all the three samples, with most of glycans on this site containing sialic acid. Glycoforms on N103 were similar between the biosimilar and the reference product, while the relative abundance profile was different. Glycans on N117 of original drug were primarily of high mannose type, which is substantially different from the type of glycans identified on the recombinant variant and its biosimilar products. Other major modifications identified and quantified include N-terminus pyroglutamate, C-terminus Lys loss, O-glycosylation at threonine, asparagine deamidation, methionine oxidation, as well as some sample preparation related modification such as formylation at the peptide N-terminus.

Conclusion
This new approach offers confident, efficient and comprehensive analysis, not only for biosimilar comparability study but also for lot-to-lot comparison of a same compound.

Novel aspect
Comparative characterization of biopharmaceutical products using unique combination of complementary fragmentation mechanisms and a new peptide mapping software.

TPS17-18 / Phosphoproteomic analysis of methanoarchaeon Methanohalophilus portucalensis FDF1T reveals diverse functions in methanogenesis and osmoadaptation

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Methanohalophilus portucalensis FDF1T is a halophilic methanoarchaeon isolated from solar salterns. This strain FDF1T exclusively utilize C-1 compounds such as methanol or methylamines as catabolic substrates for methanogenesis, and it has been used as a model organism for studying the osmoadaptive strategies through biosynthesis of osmolyte. Accordingly, cells were cultured in mineral medium containing 120 g L-1 NaCl and 20 mM trimethylamine as a sole carbon and energy source in this study to derive the phosphoproteome. Here, we presenta phosphoproteomics profile from M. portucalensis FDF1T using a gel-free and gel-based trypsin digestion combining the TiO2 phosphopeptide enrichment for the high-accuracy MS/MS analysis to conduct the site-specific protein phosphorylation for the regulatory networks of methanogenesis or osmostress adaptation in this methanoarchaeon. A total of 310 unique phosphopeptides along with 253 in vivo phosphorylated sites on 150 M. portucalensis FDF1Tproteins were identified, in which the phosphorylation sites are covered not only Ser/Thr/Tyr protein phosphorylation-based signaling with 54.9%/16.6%/14.6% but also His/Asp phosphotransfer-mediated signaling systems with 9.1% and 4.7%. Unexpectedly, these identified phosphoproteins were mostly involved in the single carbon metabolism for methanogenesis and osmolytes biosynthesis through the transmethylation from the oxidation of methylamines or the reduction of carbon dioxide. Interestingly, three distinct phosphorylated methylamine methyltransferases (MtmB, MtbB, and MttB) which involved in the methyltransferase system for initial methanogenesis were found to be incorporated the novel 22nd amino acid,pyrrolysine (Pyl), into their protein sequences. Additionally, phosphoproteins were also participated in many different cellular processes, such as the biosynthesis and metabolism of carbohydrate/protein/nucleotide, DNA or protein repair systems, and molecular chaperone systems. Furthermore, an in-depth analysis into glycine sarcosine N-methyltransferase (GSMT), including glycine N-methyltransferase (GMT) and sarcosine N-methyltransferase (SMT), through the point mutation and in vitro protein kinase phosphorylation at the identified phosphotyrosine sites, Tyr-169 and Tyr-177, revealed that its GMT activity was specifically up-regulated by Tyr-169 phosphorylation. In summary, our findings highlight the importance of protein phosphorylation in regulating a variety of essential metabolic processes for the halophilic methanoarchaeon to produce methane and accumulate osmolytesduring salty stress. So far, this is the first direct evidence to indicate that not only Ser/Thr/Tyr but also His/Asp phosphorylation plays a vital role in methanogenesis and osmoadaptation in the obligate anaerobic, halophilic, methylotrophic methanoarchaea.

TPS17-19 / Quantification of post-translational modifications of Histones in a single LC-MS/MS Analysis

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Max Planck Institute of Molecular Cell Biology and Genetics

Post-translational modifications (PTMs) of histones are important transcriptional regulators. Numerous PTMs are simultaneously present in histones in varying stoichiometric ratios and rapid quantitative characterization of the full modification pattern is however challenging. We have developed a method to detect and quantify more than 50 modifications in a single LC-MS/MS analysis that, in combination with genetic manipulations, leads to the assessment of the functional significance of histone modification marks.

Methods
After acidic extraction, histones were separated by SDS PAGE. Unmodified lysine residues were in-gel derivatized with propionic anhydride followed by digestion with trypsin. Then, the recovered peptides were again treated with propionic anhydride and subjected to LC-MS/MS analysis on a LTQ Orbitrap Velos.

Preliminary data
Chemical derivatization of free amino groupswith propionic anhydride hinders tryptic cleavage at the C-termini of lysine residues, reduces the charge state heterogeneity and increases chromatographic retention. Rapid and quantitative acylation of free α- and ε- amino groups with no noticeable side reactions was achieved by using an isopropanol / triethylamine mix. A novel hybrid stationary phase C-18 (TriArt, YMC Europe) enabled reproducible retention and quantitative recovery of very hydrophilic peptides, such as the di- and tri-methylated histone H3 peptide –TKQTAR- (K4), along with efficient separation of peptides.
We have validated the derivatization by quantifying the hydrophilic peptide -TKQTAR- from histone H3 in its methylated isoforms using isotopically-labeled synthetic peptides and achieved better than 10,000 dynamic range while the detection sensitivity was at the attomole range.

Novel aspect
Single LC-MS/MS analysis for reliable and sensitive quantification of post-translational modifications of histones

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TPS17-20 / Screening Method for SUMOylation Sites Using HCD on Orbitrap Mass spectrometer

Fu-An Li, Yu-Shing Cheng
INSTITUTE OF BIOMEDICAL SCIENCES

Introduction
Hsu et al. observed that dimethyl labeling provides a signal enhancement for the produced of a1 ions in the CID mode of Q-TOF instrument, which can be applied for identifying the N-terminal amino acid. HCD is a fragmentation technique in the LTQ-Orbitrap instrument, the dissociation takes place in an octopole collision cell (HCD cell) generating spectra similar to Q-TOF instrument. In our study, we observed that the phenomena of enhanced a1 ions that arised from dimethyl labeling also occurred in the HCD mode of Orbitrap Elite.

Methods
In this study, we use a standard SUMOylated peptide mixed with proteins mixture as the sample to demonstrate the method. The protein sample was digested to generate tryptic peptides, and then performed dimethyl labeling. Afterward, the dimethyl labeled peptides are subjected to LC/MS/MS analysis with Orbitrap Elite. The digested peptides were analyzed in data-dependent mode by

LC/MS/MS. The top five most intense precursor ions in each MS spectra were isolated for MS/MS analysis. In data process, we screened for SUMOylated peptides by monitoring two a1 ions from HCD spectra on LTQ-Orbitrap Elite.

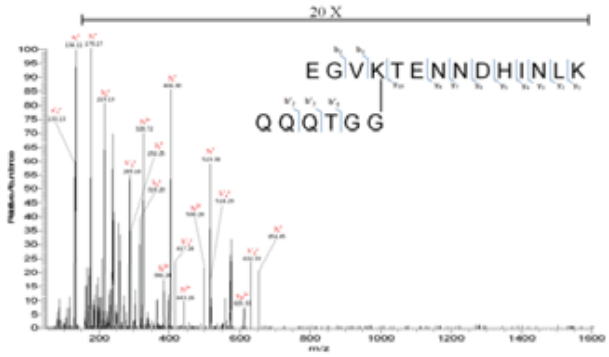

Figure 1. The HCD MS/MS spectra from SUMOylated peptides resulting from trypsin digestion of the di-SUMO-2. The precursor is 4+ ion at m/z 577.5682.

Results

For the optimized condition, the precursor ions were fragmented with HCD modes in normalized collision energy of 25%. Fragment ions were analyzed in the orbitrap at the resolution of 15000 at m/z 400. As the result shown in Figure 1, dimethyl labeled peptide exhibit the enhanced two a1 ion signals (m/z 133.13 and 134.11) corresponding to two N-terminal amino acids of SUMOylated peptide during HCD fragmentation.

Conclusions

The method can successfully trace the SUMOylated peptides from the protein mixture by HCD fragmentation of orbitrap.

Novel Aspect

Specific mass tag of methylated peptide increase the identification rate of SUMOylation sites by HCD on Orbitrap Mass Spectrometer.

TPS17-21 / High-resolution mass spectrometry for the screening and characterization of protein carbonyl-quenching activities

Mara Colzani, G. Vistoli, M. Carini, G. Aldini
Università degli Studi di Milano

Introduction

Reactive carbonyl species (RCS) are highly electrophilic compounds generated in the organism upon oxidative stress and implicated in the pathogenesis/progression of different oxidative-based disorders, such as diabetes, fibrosis and Alzheimer's disease. Carbonyl quenchers are nucleophilic compounds able to form unreactive adducts with RCS. Carbonyl quenching represents a promising strategy to reduce RCS concentration and to prevent their spontaneous and detrimental reaction with nucleophilic moieties of DNA, lipids and proteins [1]. In this work, we analyzed and compared the carbonyl quenching ability of different carbonyl quenchers and of natural extracts.

Methods

The in vitro quenching ability of carbonyl quenchers such as aminoguanidine, hydralazine, pyridoxamine and carnosine was tested on different RCS, including 4-hydroxy-trans-2-nonenal, methylglyoxal and malondialdehyde. The ability to prevent protein carbonylation was quantified by using an innovative approach based on high-resolution mass spectrometry and on ubiquitin, as model protein [2].

Results

The different RCS formed specific adducts on distinct nucleophilic residues of ubiquitin. Increasing amounts of carbonyl quenchers prevented the formation of protein adducts, as determined by calculating the UC50 values - that is the concentration required to inhibit ubiquitin carbonylation by 50%. Quantitative analyses showed different carbonyl quenching activities: carnosine efficiently quenched the 4-hydroxy-trans-2-nonenal, aminoguanidine was more active on methylglyoxal, pyridoxamine was particularly active on malondialdehyde, while hydralazine efficiently quenched all RCS. The reactivity of the tested quenchers towards pyridoxal (as endogenous aldehyde) was tested to estimate their selectivity: carnosine and pyridoxamine were highly selective.

The quenching ability of complex mixtures, such as natural extracts, was also tested, revealing the ability of green coffee bean extract and procyanidins from Vitis vinifera to prevent protein carbonylation.

Conclusions

The proposed analytical strategy was used to characterize and compare the carbonyl quenching ability of pure compounds and to detect the ability of natural extracts to prevent protein carbonylation. The analysis of the reaction products between RCS and carbonyl quenchers by high-resolution mass spectrometry led to the elucidation of the quenching mechanisms.

Novel Aspect

Our strategy, based on high resolution of mass spectrometry, represents an innovative platform to test the carbonyl quenching ability of pure compounds as well as of natural extracts.

References

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TPS17-22 / Sequential Phosphoproteomic Enrichment through Complementary Metal-Directed Immobilized Metal Ion Affinity Chromatography

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Methodologies to enrich heterogeneous types of phosphopeptides are critical for comprehensive mapping of the under-explored phosphoproteome. Taking advantage of the distinct binding affinities of Ga3+ and Fe3+ for phosphopeptides, we designed a metal-directed immobilized metal ion affinity chromatography for the sequential enrichment of phosphopeptides. In Raji B cells, the sequential Ga3+-Fe3+-immobilized metal affinity chromatography (IMAC) strategy displayed a 1.5−3.5- fold superior phosphoproteomic coverage compared to single IMAC (Fe3+, Ti4+, Ga3+, and Al3+). In addition, up to 92% of the 6283 phosphopeptides were uniquely enriched in either the first Ga3+-IMAC (41%) or second Fe3+-IMAC (51%). The complementary properties of Ga3+ and Fe3+ were further demonstrated through the exclusive enrichment of almost all of 1214 multiply phosphorylated peptides (99.4%) in the Ga3+- IMAC, whereas only 10% of 5069 monophosphorylated phosphopeptides were commonly enriched in both fractions. The application of sequential Ga3+-Fe3+-IMAC to human lung cancer tissue allowed the identification of 2560 unique phosphopeptides with only 8% overlap. In addition to the above-mentioned mono- and multiply phosphorylated peptides, this fractionation ability was

also demonstrated on the basic and acidic phosphopeptides: acidophilic phosphorylation sites were predominately enriched in the first Ga3+-IMAC (72%), while Pro-directed (85%) and basophilic (79%) phosphorylation sites were enriched in the second Fe3+-IMAC. This strategy provided complementary mapping of different kinase substrates in multiple cellular pathways related to cancer invasion and metastasis of lung cancer. Given the fractionation ability and ease of tip preparation of this Ga3+-Fe3+-IMAC, we propose that this strategy allows more comprehensive characterization of the phosphoproteome both in vitro and in vivo.

TPS17-23 / Combining bottom-up and top-down mass spectrometry to characterise the differential phosphorylation of human RIP2 kinase

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Introduction
Progress towards the characterisation of protein phosphorylation has been made by bottom-up proteomics, but analytical development is still necessary for charactering the different proteoforms of a phosphorylated protein.
Here we present the combined use of top-down and bottom-up proteomics to study the autophosphorylation of a human protein, the receptor-interacting protein 2 (RIP2) which has a kinase domain (RIP2-K). During bacterial infection, RIP2 relays the activation of NOD-like receptors to NF-κB production for an efficient immune response. Upon NOD-like receptors recruitment, RIP2 undergoes autophosphorylation and ubiquitination, which promotes the binding of downstream effectors. Determining the RIP2 autophosphorylation is essential for understanding its function.

Methods
All the experiments were carried out analysing the RIP2-K. To distinguish the autophosphorylation from the phosphorylation due the insect cell expression, three different samples were prepared:
(i) wild type (WT) RIP2-K was expressed carrying a His-tag.
(ii) RIP2-K was mutated in its active site (D146N), becoming unable of autophosphorylation.
(iii) Part of the mutant RIP2-K was subjected to in-vitro phosphorylation using the WT RIP2-K and ATP.
To study the different proteoforms (i.e., determining the number of phosphorylation) the samples were analysed intact by liquid chromatography coupled to ESI-TOF MS.
To identify the sites of phosphorylation, proteins were digested and the phosphopeptides purified using IMAC column and differential elution with distinct matrices (CHCA and DHB) prior to MALDI-TOF analysis. Moreover, the phosphoproteins will be also subjected to gas-phase fragmentation using a FT-ICR instrument coupled with chip-based electrospray ionization (TriVersa NanoMate).

Results
Regarding the analysis of intact RIP2-K, the kinase was present in different proteoforms carrying several phosphorylated residues. The in-vivo and the in-vitro phosphorylated proteoforms differed. This indicates that insect cell expression affected the RIP2 phosphorylation and that further experiments will be carried out for identifying the autophosphorylation sites.
Regarding the digested RIP2-K, we were able to identify several phosphopeptides. The differential elution with distinct matrices allowed us to fractionate the peptides and avoid signal suppression due to overlapping species.

Conclusions
Our preliminary results showed that it is necessary to specifically design samples to decipher the RIP2-K autophosphorylation and underline the importance of investigating intact and digested phosphoproteins.

Novel Aspect
Combining bottom-up and top-down mass spectrometry allows us to fully characterise different proteoforms of a human kinase. Our data show that insect cell expression affects the phosphorylation degree of RIP2-K.

TPS17-24 / Complete Post-Translational Modification Mapping of Pathogenic N. meningitidis Pilins Requires Top-Down Mass Spectrometry

Christian Malosse[1], Joseph Gault[1], Marie-Cecile Ploy[2], Catherine C. Costello[3], Guillaume Duménil[4], Julia Chamot-Rooke[1]
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Introduction
In pathogenic bacteria post-translationally modified proteins have been found to promote bacterial survival and evasion from the host immune system. In the human pathogen Neisseria meningitidis the protein PilE is the major building block of type IV pili, extracellular filamentous organelles that play a major role in pathogenesis. Previous reports have shown that PilE can be expressed as different proteoforms, each harbouring its own set of post-translational modifications (PTMs) and that specific proteoforms are key in promoting virulence [1]. Efficient tools that allow complete PTM mapping of proteins involved in bacterial infection are therefore strongly needed. We show here, using clinical strains, that top-down mass spectrometry is required to achieve this goal when more than two proteoforms are present simultaneously.

Methods
A clinical isolate of N. meningitidis strains obtained from a patient that had been hospitalised with evidence of meningitis was chosen for this study.Top-down experiments were performed either on a 12T solariX FT-ICR or a LTQ-Velos Orbitrap, using ECD and ETD as fragmentation techniques. For bottom-up experiments, samples were trypsin digestedand analysed on the Orbitrap mass spectrometer.

Results
FT-ICR MS profiling of PilE purified from the N. meningitidis 278534 strain revealed the presence of four major proteoforms. Comparison of the theoretical mass and the lowest mass major peak observed in the MS profile indicated a difference of more than 600 Da, showing that PilE could be highly post-translationally modified. Armed with mass profiling data and results from bottom-up experiments, we tried to assign peptide combinations, and thus PTMs, to specific proteoforms. Only the lowest and highest proteoforms could be fully characterized, pinpointing the severe limitations of bottom-up for complete proteoform mapping. To achieve this goal, a top-down ECD MS/MS approach allowing each proteoform to be investigated separately was therefore developed. Our study highlights the interest of using top-down mass spectrometry on clinical samples to fully characterize virulence proteins.
1. Chamot-Rooke et al., Science 331, 778 (2011)
2. Gault et al., J. Mass Spectrom. 11, 1199 (2013)
3. J. Gault et al., Proteomics DOI: 10.1002/pmic.201300394

Novel aspect
Comparison Bottom-Up/Top-down Mass Spectrometry for the complete PTM mapping of proteins involved in bacterial virulence

TPS18 - Ion-Molecule and Ion-Ion Reactions in the Gas-Phase

11:00-15:00
Poster Exhibition, Level -1

TPS18-01 / Efficient gas phase dehydrogenation reactions in MALDI mass spectrometry with novel nitroarene matrices or -additives

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Introduction
Previously, we showed that the cinnamic acid derivative α-cyano-3-hydroxy-4-nitrocinnamic acid has a high efficiency to dehydrogenate, fragment and/or oxidize a multitude of analyte classes in the gas phase when used as MALDI matrix [1]. In this contribution, the analyte dehydrogenation reaction is elucidated using quantum calculations of all involved species and by a series of ESI- and MALDI-MS and -MS/MS experiments. Further nitroarenes are introduced that partially show extremely high analyte dehydrogenation efficiencies. Possible application fields for these new MALDI compounds will be discussed.

Methods
Several nitrated α-cyanocinnamic acid (CCA) derivatives were synthesized according to [2]. Picric acid, trinitrobenzene solution as well as several (di)peptides were purchased from Sigma-Aldrich Co and GenScript.
Ab-initio calculations were performed with the software Gaussian 09 using the Møller-Plesset perturbation theory at the MP2(Full)/6-311+G(2d,2p) level of theory. All MS and -MS2 experiments were performed with a hybrid oTOF mass spectrometer (QStar Pulsar i, AB Sciex) equipped with a modified oMALDI 2 or a custom-build nanoESI ion source. A pulsed N2-laser (337 nm) was used for the MALDI-MS experiments.

Results
The dehydrogenation efficiency of the investigated nitrated CCA derivatives was studied using several peptides as analytes. Moreover, the effect of adding multiply nitrated additives such as picric acid to conventional MALDI matrices was investigated. Together, these results demonstrate that the abstraction efficiency of hydrogen atoms from analyte X-H bonds depends only on the number of matrix/additive NO2-groups. ESI-MS experiments allowed for identification of nitro radical cations as responsible species for analyte dehydrogenation.
Calculation of the energy levels of the involved matrix and analyte species clearly supports the assumed reaction pathway of two strongly exergonic analyte hydrogen abstractions to the nitro-oxygens resulting in a doubly hydrogenated nitro radical cation. This species is assumed to partially undergo a subsequent loss of water.
Analyte species whose two X-H bonds to be dehydrogenated are not directly connected but bonded by a vinyl group exhibit increased dehydrogenation efficiencies. This preference is probably caused by an optimized distance between analyte hydrogens and matrix/analyte nitro oxygens.

Conclusions
Peptides can undergo stabilizing intramolecular proton shifts upon dehydrogenation as well as intramolecular rearrangement reactions subsequently to fragmentation with strongly altered fragment ion abundances in dependence on the nature of the involved amino acids and fragmentation pathway. Therefore, using nitrated MALDI matrix or additives can enable an enhanced, rapid identification of amino acid residues.

Novel Aspects
Nitrated MALDI matrices and -additives enable efficient gas phase analyte dehydrogenation: pathways are elucidated and possible applications discussed.

TPS18-02 / Reactivity of Hydrated Monovalent First Row Transition Metal Ions M+(H2O)n, M = Cu and Zn toward C6H5Cl, C6H5Br, C6H5I and C3H7I

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Introduction
Hydrated singly charged metal ions M+(H2O)n in the gas phase can be considered as a model system for aqueous solutions of transient species. They have been studied in detail over the last two decades by a combination of experiment and theory [1]. The investigation of the reactions of M+(H2O)n (M = Cr, Mn, Fe, Co, Ni and Cu) with HCl shows the uptake of Cl by forming [MCl(H2O)n]+ [2]. In the present study, reactions of M+(H2O)n (M = Cu and Zn; n < 40) with C6H5Cl, C6H5Br, C6H5I and C3H7I were studied by Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry in the gas phase.

Methods
The experiments were performed on a modified Bruker/Spectrospin CMS47X FT-ICR mass spectrometer with a 4.7 T superconducting magnet. M+(H2O)n (M = Zn and Cu) ions were generated in a laser vaporization source using an Nd:YAG laser and transferred to the ICR cell. The reaction gases were introduced into the ultrahigh-vacuum region (p = 10-9-10-8mbar) through a needle valve at variant pressure. The reactions of interest were observed at different reaction times in the range of 0-120s to monitor reaction pathways.

Results
Depending on the metal and reaction gas different reactions occurred. Uptake of C6H5Cl, C6H5Br, C6H5I and C3H7I was observed for Cu(H2O)n+. At longer reaction times the uptake of more than two molecules was observed. But at longer reaction delays some of the molecules taken up evaporate. For Zn(H2O)n+ the reaction with C6H5Br, C6H5I and C3H7I proceeds by forming [ZnX(H2O)n)]+ (X = Br, I). By analysing the kinetics the reaction rate constants were determined for all observed reactions. Zn(H2O)n+shows a higher reactivity compare to Cu(H2O)n+. Also the reaction with C3H7I has a higher reactivity than the reaction with C6H5I.

Conclusion
Previous studies at our working group show the high oxidation potential of Zn(I). From the studied transition metal ions, only Zn(I) is oxidized by CH3CN, with formation of CH3CNH or CH3CHN and the metal hydroxide. An earlier study with NO shows the oxidation of zinc and iron by forming [MOH(H2O)n]+[3]. The new results show the oxidation of Zn(I) due to halogenated aromatic and aliphatic compounds. Presumably due to the large radius of Copper(I), up to three molecules are taken up by the clusters. However, as soon as all H2O molecules are

evaporated, black-body radiation induces the loss of the non-covalently bound ligands.

Novel Aspects
Investigation of the oxidation reaction of Zn(H2O)n+ by C6H5Br, C6H5I and C3H7I and determination of the reaction rate constants using FT-ICR.

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TPS18-03 / Revised and expanded scale of the Lithium cation basicities.

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Introduction
The gas-phase lithium cation basicity (LiCB) is defined as the negative of Gibbs free energy for the reaction (1), LiCB = -ΔrG:
B + Li+= [BLi]+ (1)
There are often significant differences between calculated and measured LiCBs, which exceed the experimental errors and the expected uncertainties of computational methods. In particular, it was observed that in the higher range of the LiCB scale (> 150 kJ/mol), the discrepancies between calculated and experimental values had a tendency to increase. High level quantum chemical calculations and new experimental measurements by equilibrium techniques were carried out to clarify the origin of these discrepancies.

Preliminary Results
Fourier transform ion cyclotron resonance (FTICR) mass spectrometry was used for the determination of equilibrium constant K of reaction (2) leading to relative lithium cation basicity (ΔLiCB) of two bases (ligands) L1 and L2:
[L1Li]+ + L2 = [L2Li]+ + L1 (2)
Li+ adducts were trapped in the presence of known pressures of L1 and L2. When a steady state is observed, the difference between the LiCB of L1 and L2 is given by ΔLiCB = RTlnK.
The mass spectra do not exhibit significant peaks other than the simple adducts L-Li+ and Li+-bound dimers. It was commonly accepted that the direct equilibrium measurement of ΔLiCB cannot be achieved in the presence of Li+-bound dimers. Nevertheless, we demonstrate the feasibility of equilibrium measurements by means of a kinetic model.
A scale of ΔLiCBs was established at 373 K for about 60 ligands between 115 kJ/mol and 240 kJ/mol. The ΔLiCB were combined to build a self-consistent scale, anchored to the absolute LiCB value of pyridine (146.7 kJ/mol).
A precise correlation with a slope close to unity between the present LiCB measurements and the previous ones was observed for the lower part of the scale (< 150 kJ/mol), but the upper part of the two scales diverged.
LiCBs were calculated at 373.15 K at the G2MP2 level for all ligands studied by equilibrium measurement. The average of the absolute deviation between the experimental and calculated LiCB is roughly 3 kJ/mol. The deviation is random with no systematic effect evident.

Conclusions
Equilibrium constant measurements of Li+ transfer were performed under carefully controlled conditions for establishing a LiCB(373 K) scale. We demonstrated that Li+ transfer equilibrium measurements in the gas phase can be performed in spite of the simultaneous formation of Li+-bound dimers. The lower basicity range of the previous FTICR LiCB(373K) scale, is in good agreement with the present measurements up to about 150 kJ/mol, but the upper part appears to be biased by a systematic contraction of the previous scale. The values above 150 kJ/mol of the new scale are in good agreement with high level calculations.

Novel Aspect
Li+ basicity equilibrium measurements in the high basicity range are reported, which were previously considered impossible because of dimer formation.

TPS18-04 / Characterization of Ammonium Nitrate Vapor with Flowing Atmospheric-Pressure Afterglow Mass Spectrometry

G. Asher Newsome[1], F. Lucus Steinkamp[2], Braden C. Giordano[3]
[1]Nova Research, [2]U.S. National Research Council, [3]U.S. Naval Research Laboratory

Introduction
Ammonium nitrate (AN) is an ionic solid commonly used as a fertilizer and in commercial blasting applications. Legitimate availability of the material makes it difficult to prevent its use in improvised explosives, requiring trace detection technology for counterterrorism efforts. Solid AN decomposes at room temperature into gaseous ammonia and nitric acid, both species that are present at trace concentrations in the atmosphere. The composition of the mixture is shown to be dependent on atmospheric conditions. Flowing atmospheric-pressure afterglow (FAPA) ionization mass spectrometry is used to analyze ammonium nitrate headspace vapor formed under different levels of controlled humidity, temperature, and air velocity over solids. An ion-molecule reaction scheme was implemented to detect ammonia.

Methods
A FAPA ion source was mounted in front of the inlet of an LTQ Orbitrap mass spectrometer. A cubical plastic enclosure was fitted around the FAPA and ion inlet with feed-throughs for mounts and supplies. A Miller-Nelson test atmosphere generator controlled the temperature and relative humidity of air in the enclosure. Solid ammonium nitrate was placed either in the source enclosure or in a separate atmosphere-controlled container with vapor products ported to the source enclosure. Gaseous carbon tetrachloride was doped into the FAPA discharge gas for ammonia detection.

Results
Ion chromatography has been used to confirm that characteristic ammonium nitrate headspace vapor consists of ammonia and nitric acid. The quantity and ratio of the gaseous products varied depending upon the conditions of the experiment.
Carbon tetrachloride added to FAPA discharge gas at 75 ppm produced abundant reagent ion signal from CCl3+• and other chlorinated organic ions. Ion-molecule reactions with the ammonia-rich AN headspace produced real-time analyte signal. Multiple species, including [CCl3+NH3 HCl]+, [C2Cl2N2H]+, [C3Cl2NH2]+, and [C3Cl3NH]+, were observed with a corresponding decrease in reagent ion signal.
FAPA ionization produced a molecular ion signal in the negative mode for nitrate at a constant background level. Pentaerythritol tetranitrate (PETN) was introduced to the afterglow via gas chromatography to simulate the presence of AN headspace, and

increased nitrate signal above background was observed from PETN degradation.

Conclusions
Varying humidity over AN solids produced no change in the amount of ammonia detected from the headspace in real time. Species collection and preconcentration may result in dependence measured by ion chromatography. Charring in the discharge cell was prevented by using CCl4 instead of chlorinated hyrdrocarbon, but some fouling caused signal reduction. Carbon tetrafluoride will be used to generate more abundant, monoisotopic ions with reduced source fouling.

Novel Aspect
Ammonium nitrate headspace vapor is characterized in controlled environmental conditions with ion-molecule reaction FAPA mass spectrometry.

TPS18-05 / Chemical Modification of Graphene via Reactive Landing of Hyper-thermal Molecular Ion Beams
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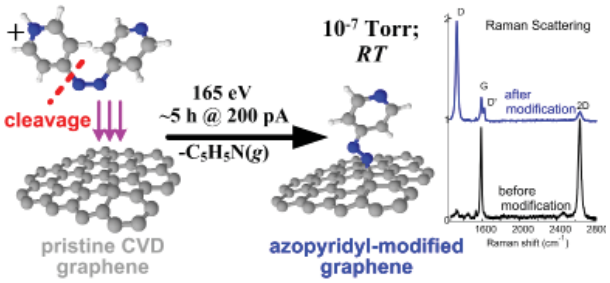
Max-Plank-Institute for Solid State Research

Introduction
The emergence of graphene as a 2D Dirac material has ignited a frenzy of study into its distinctive chemical, electronic, magnetic, optical and thermal properties, while broadly uniting scientific intrigue across multiple disciplines. Chemical modification of graphene presents a viable pathway for tailoring electronic properties such as band-gap and majority carrier type. Covalent functionalization also enables subsequent coupling, which is vital for molecular diagnostics and molecular electronics.

Methods
Hyperthermal ion beams (1-200 eV) present a unique approach to modification, and are readily formed by increasingly common methods such as electrospray ionization. In this work, an electrospray ion beam deposition (ES-IBD) system is employed to carry out the modification between graphene and an azypyridine compound in high vacuum. Raman Scattering and atomic force microscopy are used to characterize the pristine and modified surface.



Results
For collisions exceeding a threshold value of ~165±3 eV, molecular cation beams of 4,4'-azobis(pyridine) covalently attach to chemical vapor deposited graphene. The resulting azopyridyl-functionalized graphene is produced in 3-5 h, corresponding to an ion exposure of the order of a close-packed monolayer.

Conclusions
This route highlights a facile approach for the controlled modification of graphene and extends the scope of candidate species that would not otherwise react via existing conventional methods. The present study demonstrates a one-step non-destructive route to covalently functionalize chemical-vapor-deposited graphene using the controlled deposition of hyperthermal molecular ion beams of azopyridine.

Novel Aspect
Hyperthermal ion chemistry presents a unique solution to the challenge of producing controllably dense or ordered monolayers on graphene. This type of reaction and functionalization has not yet been reported, including the high precision over parameters such as deposition energy, coverage, and reagent purity.

TPS18-06 / The molecular and ionic vapor components over the LaI3 and the La-LaI3 system
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Introduction
The lanthanide halides are of interest for various modern technologies. It was established that iodides characterize a highest possibility to emit the ions that make them very attractive for focused ion beam technique. The thermodynamic data on molecular sublimation of these compounds have been investigated well while information on ionic components is very scanty. This work is concerned with mass spectrometric investigation of molecular and ion emission of the LaI3 and the La-LaI3 system.

Methods
The Sector magnetic mass spectrometer MI1201 with a Knudsen effusion cell was used. The combined ion source allows to analyze molecular components in electron ionization (EI) mode (the ionizing voltage of 50 eV was used) and charged particles in thermal ion emission (TE) mode. In this case the ions are extracted from the cell directly by a weak electric field (about 50-100 V/mm).

Results
The LaI3 was investigated in temperature range 860-1020 K in the IE and the TE modes. It was found that molecular vapor over this compound consists of LaI3 molecules giving La+, LaI+, LaI2+ and LaI3+ ions and dimeric molecules (about 4%) producing La2I5+ and La2I4+ ions. In the TE mode I–, LaI4–, La2I7–, La3I10– and La4I13– ions were found. The sublimation enthalpy of the LaI3 in monomer and dimer forms was found 292±9 kJ/mol and 358±15 kJ/mol respectively. These results successfully supplement literature values 300 kJ/mole for LaI3 and 380±17 kJ/mol for La2I6. The formation enthalpies of the LaI4– and the La2I7– ions calculated by the III law of thermodynamics are -883±24 kJ/mol and -1551±27 kJ/mol respectively. The $\Delta fH^{o}298$(LaI4–) is in a good agreement with quantum-chemical calculation using STDQ-MP4 method (-870 kJ/mol).
The vapor composition over La-LaI3 system in the temperature range 890-1300 K was represented by La+, LaI+, LaI2+, LaI3+, La2I3+, La2I4+ and La2I5+ ions (IE) as well as I–, LaI3–, LaI4– and La2I7– ions (TE). The LaI3– ion pointed out the formation of lanthanum diiodide in gas phase.

Conclusions
The molecular and ionic vapor species have been identified by the Knudsen effusion mass spectrometry. The sublimation enthalpies in the form of monomer and dimer molecules and the formation enthalpies of negative ions have been determined.

Novel aspect
The existence of LaI4–, La2I7–, La3I10– and La4I13– ions over LaI3 as well as their formation enthalpies were obtained for the first time. The LaI2 molecules over the La-LaI3 system has been have been observed at high temperatures.

The work was supported by the Russian Foundation for Basic Research (project №14-03-31021_mol_a) and grant of the President of the Russian Federation (project MK-6762.2013.3).

TPS18-07 / Spin-isomers in the gas-phase: reactivity of ferracyclobutadienes
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Introduction
Metallacyclobutadienes are often regarded as intermediates in alkyne metatesis reactions, although only a handful of this highly reactive species could be isolated and characterized. In the work group of Prof. Filipou from the University of Bonn a new class of ferracyclobutadienes [FeL3(C3R3)]+ (L = CO, PMe3; R = NMe2) has been synthesized. Their properties and reactivity in the highly diluted gas-phase of a FT-ICR mass spectrometer shall be discussed in work presented here.

Results and Discussion
Electrospray ionization is ideal for the cationic complexes under investigation. The fragmentation cascades of the intact complexes induced by CID or IRMPD start with the successive loss of the monodentate ligands until the «naked» 10-VE-ferracyclobutadiene is reached. The mass selected coordinately unsaturated ferracyclobutadienes show a very interesting reactivity towards small alkynes. Especially for the 12 VE-species, very complex kinetics are observed even after intensive thermalisation processes. They can be rationalized by presence of two spin isomers. A highly reactive species (triplet) reacts by C-H-activation to diverse follow up products, whereas the lesser reactive species (singlet) only shows the slow coordination of alkyne. Quantum chemical calculations confirm the presence of extreme small singlet-triplet gaps for the 12 and 14 VE species with differing reactivity patterns for the different spin states.

Experimental
The measurements were performed on a Bruker Apex IV FT-ICR with a 7 T magnet and Apollo ESI-source. The instrument is equipped with two pulse- and two leak valves. As CID-collision gas and for the thermalisation argon 5.0 was used. IRMPD was achieved with a CO2-Laser with max. 25 W. The reaction gases propyne, 1-butyne and 2-butyne were either introduced via a leak valve (Pressure in the range of single molecule reaction, < 10-8 mbar) or were pulsed into the cell. A typical experiment (MS6) starts with the accumulation of the ferracyclobutadienes in the hexapole of the instrument, fragmentation via IRMPD, mass selection of the desired coordinately unsaturated ferracyclobutadiene, thermalisation, re-isolation, variation of the reaction time in presence of the pulsed alkyne, excitation and detection. 16-500 scans were accumulated and reaction products were identified by exact mass and by fragmentation after further mass selection.

New aspect
Spin-isomers of new ferracyclobutadienes can be characterized in a FT-ICR due to their distinct reactivity.

TPS18-08 / Improving Usability of Gas-Phase Hydrogen/Deuterium Exchange Mass Spectrometry to Study Conformational Changes of Biomolecules
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Gas-phase hydrogen/deuterium exchange (HDX) is a fast and sensitive, yet unharnessed analytical approach for providing information on the structure in a complimentary manner to mass analysis. We and others have recently shown that labeling of heteroatom bound hydrogens of peptide and protein side chains can impart valuable information on biomolecular structure. Here we introduce a novel and easy method for fast and controlled HDX using a novel liquid HDX reagent, by saturating the flow gas in the skimmer region, and show its use for studying conformations of biomolecules.
We furthermore combine the setup with LC and for the first time with a chip-based automated nESI robot. Ion mobility and electron transfer dissociation were furthermore integrated in the workflow.
HDX was facilitated by saturating the N2 gas to the skimmer region by leading it over a container filled with a liquid HDX reagent. The setup was simple and did not require any replumbing of existing gas inlets or tubing. We tested the suitability of this modification on three generations of commercial Q-TOFs, which are particularly suitable for studying biomolecules.
The samples were injected in the mass spectrometer with nESI spray, and HDX was carried out immediately after desolvation in the skimmer region, giving access to study the solution-phase conformations as biomolecules have been shown to be able to retain their conformation in the gas-phase in tens of ms after desolvation.
To perform rapid HDX, we further investigated the capability of a highly basic liquid HDX reagent, aq. deuterated ammonia solution, to enrich a flow of N2 gas with ND3. The HDX capability of the setup and the new liquid reagent was compared to D2O, MeOD and EtOD solvents and pure ND3 gas. The new reagent showed superior labeling of biomolecules to the other liquid reagents and similarity to pure ND3 gas. Results from labeling peptides using our setup indicate that ND3 accounts exclusively for the deuteration, due to its high gas-phase basicity and the use of this liquid HDX reagent alleviates the need for a more complicated setup required for using pure ND3 gas.
Results show that our setup is able to study conformation and conformational changes of biomolecules. IMS and subsequent ETD was performed in tandem with the HDX setup, making it possible to elucidate detailed structural information. A successful interfacing of an chip-based nESI robot to the HDX setup allows a highly sensitive medium-throughput screening of biomolecules, with run cycles per sample of 50s.
Deuterium uptake of biomolecules was compared for solution and gas-phase HDX-MS, and shows that gas-phase HDX provide access to analytically useful heteroatom bound side-chain sites not accessed by solution HDX-MS. The observation that gas-phase HDX labels other heteroatom bound hydrogens in an almost orthogonal manner to solution phase HDX is analytically useful and provides new insights into fundamental knowledge of gas-phase HDX mechanisms.

TPS18-09 / Ion Intensity and Thermal Proton Transfer Reactions in Matrix-Assisted Laser Desorption/Ionization

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Introduction

Matrix-assisted laser desorption ionization (MALDI) is one of the important method in mass analysis for biomolecules. Generating the first ions remains the most controversial part of the ionization mechanism. Several mechanisms have been proposed to explain the mechanism of ion generation in MALDI.However, the effects of each mechanism in MALDI are difficult to determine because it is not easy to quantitatively measure the contributions of these mechanisms.

Methods

The ionization mechanism of ultraviolet matrix-assisted laser desorption/ionization (UV-MALDI) was investigated by measuring the total cation intensity (not including sodiated and potasiated ions) as a function of analyte concentration (arginine, histidine, and glycine) in matrix of 2,4,6-trihydroxyacetophenone (THAP) using time-of-flight mass spectrometer. Time-resolved fluorescence intensity was employed to investigate how analytes affected the energy pooling of the matrix. Thermal proton transfer model was used to predict the ion intensity as a function of analyte concentration.

Results

The total ion intensity increased up to55times near the laser fluence threshold as the arginine concentration increased from 0% to 1%. The increases were small for histidine, and almost no increase occurred regarding for glycine. The increases became small for all analytes at high laser fluence. No detectable energy pooling was observed for pure THAP and THAP/analyte mixtures. The increase of ion intensity can be explained by the thermal proton transfer model.

Conclusions

In contrast to the thermal ionization of the photoexcited matrix and energy pooling models, in which matrix ions must first be generated to initiate proton or charge transfers between matrix ions and neutral analytes, the thermal proton transfer model suggests that analyte ions can be directly generated from the thermal proton transfer reaction between the matrix and the analyte. Because the analytes used in this work did not affect the energy pooling rate, the energy pooling model cannot explain why the total ion intensity increased as the analyte concentration increased.

Novel aspect

Our findings indicate that thermally-induced proton transfer must play a significant role in MALDI processes.

TPS18-10 / Manipulating radical reactivity by charge polarity switching in gas phase distonic ions

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Introduction

Distonic ions possess a charge and radical centre separated by the molecular framework, and are used to model the reactivity of neutral radical intermediates in the gas phase. We have recently demonstrated that a remote negative charge in certain distonic ions can stabilize the radical by up to 20 kJ/mol.[1] Computational work has demonstrated that this effect is smaller in magnitude and opposite in direction in radical cations compared to analogous anions.[2] Therefore, radical reactivity in a distonic ion could be tuned by switching polarity. Herein, the effect of charge polarity on the reactivity of distonic arylperoxyl radicals is examined.

Methods

Distonic arylperoxyl radical ions were prepared using a linear ion trap mass spectrometer (LTQ, Thermo Fisher Scientific) that has been modified to enable (i) photodissociation of precursor ions using a Nd:YAG laser (Continuum Minilite II); and (ii) ion-molecule reactions by addition of neutral reagents to the helium buffer gas.

Results

Trimethylammonium (+NMe3) and trifluoroboron (-BF3) charge-tagged phenyl radicals were isolated in the ion trap by UV photodissociation of phenyl iodides. Prompt reaction of these distonic radical ions with dioxygen was observed independent of ion polarity with efficiencies of ca. 5%. Importantly however, the reaction products differed markedly between positively and negatively charged systems (see Scheme). While the positively charged phenyl radical combined with dioxygen to yield phenylperoxyl radicals in high abundance, the corresponding anion does not produce the peroxyl radical in isolable amounts. Rather, the latter phenylperoxyl radical rearranges to eject a formyl radical and yield a putative cyclopentadienone. To probe this phenomenon further insulate the radical from the charge, an tetrahydrocarbazole-based aryl iodide scaffold was prepared capable of forming both positive and negative ions. Deuterium labeling indicated that the charges reside on a remote carboxylic acid motif in each case (i.e., RCO2H2+ and RCO2-). Photodissociation of these precursor ions yields the aryl radical and subsequent reaction with dioxygen again revealed polarity-dependent product ion distributions. The arylperoxyl radical is observed only from the positively-charged phenyl radical precursor. This suggests that the remote charge perturbs the potential energy surfaces describing rearrangement and subsequent dissociation of phenylperoxyl radicals in different ways depending on the polarity. The generality of these observations to other radical types and other reactions are explored.

Conclusions

Radical reactivity is significantly perturbed by charge-tag polarity. Judicious selection of charge-tag moiety, and rigorous experimental and computational assessment is advised when deploying distonic radical ions as probes for neutral radical reactivity.

Novel Aspect

Distonic ions can show distinctive radical reactivity depending on the polarity of the charge.

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[2] G. Gryn'ova, M. L. Coote, J. Am. Chem. Soc., 135, 15392 (2013)

TPS18-11 / Poly-Anion Production in Penning and RFQ Ion Traps

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The number of electrons in a cluster affects its properties, e.g. its geometrical shape, ionization potential, polarizability, or

dissociation energy, making the charge state of a cluster a crucial parameter. Poly-anionic clusters are produced by sequential electron-attachment to cluster mono-anions stored in an ion trap. The poly-anion production is investigated in Penning and linear radio-frequency quadrupole (RFQ) traps at the ClusterTrap setup [1].

The range of anionic charge states produced with the electron-bath technique in a Penning trap is restricted by the upper mass limit of this trap. By installation of a cylindrical Penning trap with a 12-Tesla superconducting magnet, the mass and thus cluster-size range is enhanced by a factor of 20 compared to the previously used hyperbolic 5-Tesla Penning trap. In a parallel effort, a production method of poly-anions in a RFQ-trap has been developed. To this end, an electron beam is guided through an ensemble of cluster monoanions, stored in an RFQ- trap, which is operated as a 2- or 3-state digital ion trap (DIT).

For first experimental tests with the 12-Tesla cylindrical Penning trap, gold cluster mono-anions Aun-1, n = 330 – 350, have been exposed to an electron bath. As a result, higher negative charge states up to hexa-anionic clusters have been observed [1]. For comparison: At comparable trapping voltages the respective cluster size limit of the previous 5-Tesla hyperbolic Penning trap was about n = 60 and the maximum gold-cluster (negative) charge state was z = -3 [2].

At the RFQ-trap, di- and tri-anionic gold clusters have been produced by exposing mono-anions to an electron beam [3]. The conventional harmonic as well as the 2-state digital trapping voltages [4] affects the electron path through the trap, and thus the electron energy during attachment. However, the 3-state DIT allows time slots of zero-volt potentials to be implemented in the driving signal [5]. Thus electrons can pass through the trap unhindered and at well-defined energies. A new electron source with an energy distribution ~0.5eV is currently set up for electron-attachment studies.

We report about the lates developments with respect to both methods as well as their combination with the aim to reach even higher (negative) charge states.

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TPS18-12 / A Relative Comparison of Proton Affinities of MALDI Matrices Using Bacteriophage HK97 Head II Capsid

Mark Bier[1], Jonathan Feldman[1], Logan Plath[1], David Sipe[1], Robert Duda[2], Hendrix Roger[2]

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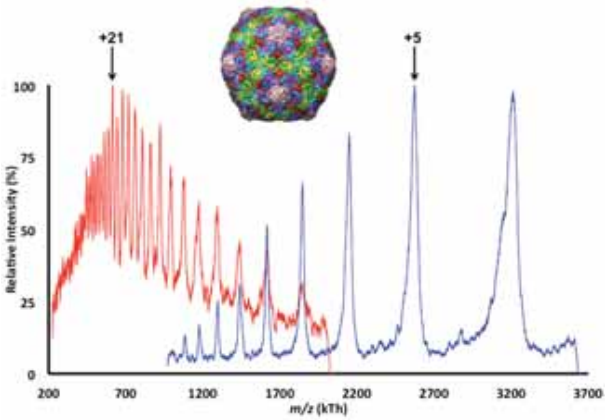
Introduction

Extensive studies have lead to the use of certain organic molecules as MALDI matrices. The use of matrices such as alpha-cyano-4-hydroxycinnamic acid (4HCCA), sinapic acid (SA), and 2,5-dihydroxybenzoic acid (2,5-DHB) has become routine in the analysis of biomolecules by mass spectrometry. The mechanism of charge generation in MALDI is the result of a gas phase proton-transfer reaction that occurs between matrix ions and analyte molecules in vacuo. The rate of proton transfer is a thermodynamic and kinetically controlled process of which proton affinities are used to semi-quantitatively describe. Head II from bacteriophage HK97 can be used to determine the relative proton affinities using the bracketing method.

Methods

Samples were analyzed by MALDI-TOF utilizing superconducting tunneling junction (STJ) cryodetection on a Macromizer™ (Comet AG, Flamatt, Switzerland). MALDI matrices were used with purities ≥98.0%. Typically, matrix solutions were prepared in 49.95/49.95/0.10% v/v acetonitrile/water/trifluoroacetic acid. Matrix solutions were mixed in varying ratios with HK97 bacteriophage Head II capsid ranging from 1:1 to 1:20 by volume and spotted on a stainless steel 96-well MALDI target. HK97 bacteriophage Head II capsid was prepared as described previously (J. Mol. Bio. 1995, 247, 618-635).



A Relative Comparison of Proton Affinities of MALDI Matrices Using Bacteriophage HK97 Head II Capsid

Results

MALDI-TOF measurements performed on HK97 bacteriophage Head II capsid using 4HCCA as matrix indicate a charge state distribution with a peak charge state of +21. Experiments show that using 4HCCA as matrix, charge states up to +52 can be resolved by MALDI-TOF-STJ MS. Using SA as matrix, a charge state distribution was observed to peak at +5. The observation of resolved mass spectral peaks at high charge state using 4HCCA and lower charge states using SA agrees with other researchers that a relationship between proton affinity (PA) and charge state distribution exists. Preliminary results of trans-3-indoleacrylic acid system indicates that there is a deviation from the literature value for PA of this matrix system as indicated by the charge state profile of the HK97 bacteriophage capsid mass spectrum. While direct quantitative determination of the proton affinities of the matrix species cannot be determined by these experiments, Head II from HK97 can provide an improved comparison of "matrix" PAs by using the bracketing method. Matrix PA refers to the gas phase plume above the MALDI plate and not to a specific protonated matrix ion.

Conclusions

A comparison of common MALDI matrices using HK97 Head II capsid allows for a relative determination of proton affinities of a specific matrix system. Head II provides an improved method of PA bracketing because of its ability to "super" charge and, thus, distinguish between two separate matrix systems.

Novel Aspect

An improved method for the determination of proton affinities in matrix:analyte MALDI systems is shown using HK97 bacteriophage capsid with use of bracketing.

TPS18-13 / Supramolecular mass spectrometry: association of MS methods to computational chemistry to access, at a molecular level, systems relevant to host-guest chemistry

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Introduction

Host-guest chemistry represents one of the major topics of Supramolecular Chemistry and concerns the design, the synthesis and the characterization of selective/specific receptors able to strongly entangle target molecules within dedicated cavities. Perfect associations between the host and the guest partners rely on the complementarities between their topologies and functionalities. Numerous spectroscopic methods are developed to investigate those non covalent associations with particular interests on the measurement of binding constants and the determination of the structure of the association. NMR and UV-vis spectroscopies are often used to investigate such systems. Nowadays, mass spectrometry has been demonstrated to be a valuable and elegant way of studying non covalent associations extracted from the condensed phase to gas phase by means of a soft ionization method, such as Electropray.

Methods

In the context of our investigation, the experiments are conducted by using conventional mass spectrometry methods such as ESI-MS and ESI-MSMS (CID). In addition, more sophisticated approaches such as energy-resolved CID, ligand exchange experiments and of course ion mobility mass spectrometry were implemented in our work to obtain an in-depth description at the molecular level of the energetics and structure of the gas phase non covalent associations. Beside the experimental part of the project, it is really important to obtain corresponding theoretical data such as optimized structures, energetics, isomerization and decomposition thresholds and collisional cross sections. This aspect of the project relies on the use of state-of-the-art computational methods such as molecular mechanics and dynamics approaches and DFT calculations.

Results

For the present communication, we would like to give an overall overview of the results that were obtained in our laboratory and that are related to host-guest chemistry with different receptors such as a chiral crown ether [ChemEuJ 2008], a bitopic cucurbituril that present homotropic allosterim capabilities [chempluschem 2013] and a chiral calixarene. Those examples were selected to demonstrate that the association of high level theoretical chemistry with state-of-the-art MS methods represents a powerful tool for investigating host-guest complexes from structural and energetic points of view. Such studies are important to further design specific receptors or sensors for targeted molecules.

Conclusions

The association between mass spectrometry methods and theoretical chemistry represents an elegant and efficient way of investigating hots-guest chemistry with a special emphasis of getting reliable data on energetics and structures.

Novel aspects

Numerous data are reported dealing with non covalent associations between large biomolecules and their ligands. Fewer studies are related to the host guest chemistry domain of research probably because of the availability of the systems to organic chemists nowadays but also since non specific associations are more likely to appear with small molecules than with bigger.

TPS18-14 / Dianions as strong gas-phase bases

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Multiply charged anions have been proposed as a route to create gas phase bases, potentially even stronger than the current strongest reported gas phase base, LiO- [1]. The additional charge(s) present on a doubly (or multiply) charged system leads to an increased proton affinity, but at the same time a reduced electron affinity. Computational studies probing the limits of gas-phase basicity concluded that to create multiply charged anions that are both highly basic, but have a positive electron binding energy «will be a challenge... even if multiply charged anions are considered [and] lithium hydroxide will be hard to replace at the top of the gas-phase acidity scale» [1].

In this contribution, we present experimental evidence for deprotonation of several neutral molecules by a dianion «superbase,» demonstrating that it is possible to generate dianions that behave as particularly strong gas phase bases. Using electrospray ionisation mass spectrometry, a series of dianions originating from phenylenedipropiolic acid have been generated in the gas phase and isolated within a linear quadrupole ion trap. Subsequent ion-molecule reactions between the doubly deprotonated species and neutral reagents of known gas phase acidity have allowed the relative basisity of the dianion species to be bracketed, providing evidence that one of the dianions generated possesses a proton affinity in excess of the methanide anion (416.7 kcal/mol) and is a good candidate for the strongest known gas phase base.

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TPS18-15 / Spectroscopic evidence for a gas-phase librating G-quartet/Sodium ion complex

Caterina Fraschetti[1], Laura Guarcini[1], Maria Montagna[1], Leonardo Guidoni[2], Antonello Filippi[1]
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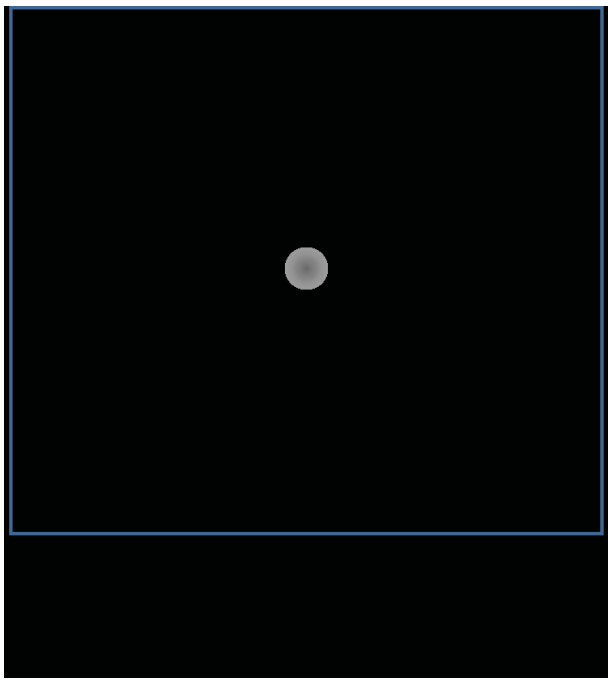
The G-quartet represents the elementary cell of the G-quadruplex, a more complicated structure crucial for several biochemical functions. Indeed the presence of guanosine rich motifs in telomeres, which are compact portions of DNA located in the terminal positions of chromosomes, has been largely studied through an enormous number of publications investigating both their physico-chemical and biological properties.1-3 Furthermore the organization of G-quadruplexes can be thermodinamically assisted by the presence of several metal cations.4 Despite the large body of evidences collected in condensed phases, the gas phase structure of M+-G4 aggregates has never been experimentally explored. The formation of M+-G4 species (M= Li, Na, K) has been observed in electrosprayed CH3OH/H2O solutions containing guanine in presence of the relative salts of alkali ions.5 The available computational results concerning Na+-G4 system seem to depend on the employed basis set, thus suggesting a complicated potential energy surface. On the ground of the multi-faceted computational descriptions, herein the first spectroscopic investigation of gas phase Na+-Gn (n=2, 4) conformational landscape has been performed by means of ESI-IRMPD technique, in the high frequencies region (2800-3700 cm-1) and in the low spectral region (900-2000 cm-1). The computational support arises from a combination of the static calculations and ab initio molecular dynamic (AIMD) simulation based on DFT approach, the latter performed with the aim to investigate the finite temperature "breath" of a very flexible aggregate, and to include the anharmonic effects on their vibrational properties.6 A good matching between the experimental measurements

and IR spectrum obtained by AIMD simulation pointed to the substantial adequacy of a dynamic description which includes the temperature effect and the anharmonic correction. In conclusions, the comparison between IRMPD spectrum of Na+-G4 complex and AIMD simulation, revealed the presence of two different metastable populations of conformers, separated by a free energy barrier easily accessible at room temperature.



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TPS18 - TPS20 - Imaging MS - Applications

11:00-15:00

Poster Exhibition, Level -1

TPS20-01 / Study of interactions between reactive gaz species and microorganisms by nano-resolution mass spectrometry imaging

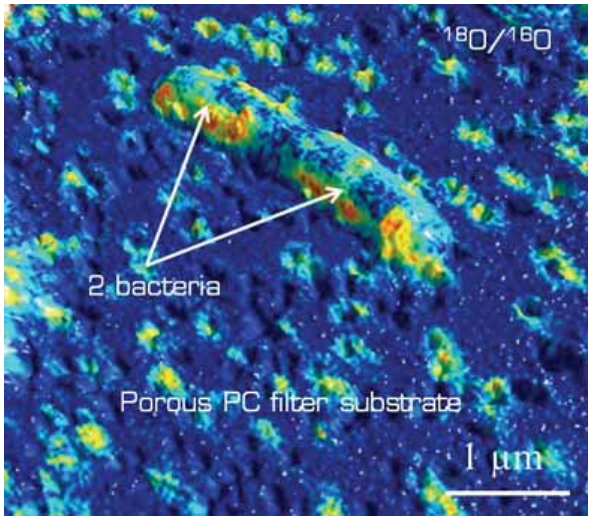
Audinot Jean-Nicolas, Nicolas Desbenoit
CRP-GL

By working with a plasma reactor under gas flow conditions, it is possible to obtain a reactive gaz which keeps high reactivity on materials surfaces. Nowadays, these plasma reactors are commonly used for decontamination applications by acting on various micro-organisms (bacteria, virus, …). The interaction mechanism of the reactive species with microorganisms and living tissues is currently one of the most active fields of research in the plasma community [1].

Several innovative characterization methods are currently developed in order to study thoroughly the modifications induced by the reactive species (e.g. Reactive Oxygen Species (ROS) on plasma-treated microorganisms. The method used in this work consists of determining the effect of hydrogen, oxygen and nitrogen coming from the plasma on Escherichia coli bacteria. In order to follow the treatment effect on bacteria, we used isotopically labelled 15N2, 18O2 and 2H2 containing-Ar gas mixtures to produce the plasma. To localize and quantify the amount of reactive species on the bacteria, we used the unique mass spectrometry technique allowing working at the cellular scale: the NanoSIMS50 [2-3].

Different exposure times (1, 5, 10 and 15 min) were used to treat the biological samples under different experimental plasma conditions. The NanoSIMS50 analyses deliver isotopic images of cellular structures and we measured gas fixation pixel by pixel in different intracellular areas for each individual E. coli. Once the acquisition done, we calculated the values of the isotopic ratio and the percentage of penetration of labelled gas for individual bacteria [4]. For example, an increase in isotopic oxygen incorporated into the structure as a function of the exposure time was observed, until a saturation time. These results were compared with other treatments performed with nitrogen and hydrogen labelled gases (15N, 2H).

Moreover, in order to go further in the understanding of degradation mechanisms, NanoSIMS and AFM are associated to combine morphological and chemical information on images of treated bacteria (Figure 1) [5-6].



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TPS20-02 / The important role of the matrix (application) in the MALDI MS Imaging procedure.

Marco Giampà[1], Thomas Patschkowski[1], Jan Kölling[2], Tim Nattkemper[2], Manfred Lissel[1], Michael Becker[3], Karsten Niehaus[1]
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The understanding of biological processes is important to differentiate, e.g., a healthy tissue from an non healthy tissue. The identification and characterization of new biomarkers are optimal elements to give a qualitative (chemical nature) and quantitative (spatial tissue distribution) differentiation.
Mass spectrometry is a valid tool to determine the chemistry of a tissue using the MALDI process (Matrix-Assisted Laser Desorption/Ionization) as ion source that allows for obtaining ions in the gas phase from a solid sample. These ions are analysed by mass spectrometry according to their m/z ratio and detected in every tissue's position.
This innovative analytic method is called Imaging Mass Spectrometry (IMS) which is a technique that simultaneously maps the spatial distribution of multiple biomolecules directly in a single tissue section generating an image of the distribution of selected ions with a defined resolution. As an instrument we use ultrafleXtreme MALDI TOF (Bruker ®). The matrix serves fundamental functions in the desorption and ionization process of ions from the tissue sample and then in Imaging results.
In this work, we used this technique to analyze the composition of the mouse heart tissue and to compare two different matrices 2,5 dihydroxybenzoic acid, (DHB) and α-Cyano-4-hydroxycinnamic acid (HCCA) applied to the tissue via a vibrational spray. Using HCCA as matrix in positive mode, imaging results are best as compared to use DHB. The signals are sufficient in intensity to do the molecular identification by fragmentation processes of precursor ions. This resulted in the information that the chemistry of the matrix is very important and therefore we rationally synthesized a new reactive matrix „Maleic Anhydride Proton Sponge (MAPS)" that forms positive adducts with long chain alkylic alcohols.
Moreover is also important how the matrix is applied onto the tissue. In fact, we compared two modes of application of the matrix: vibrational spray and sublimation. The matrix's application by sublimation has given us high spatial resolution images and then best biological information.
This work shows how the application's way and the chemistry of matrix could have effect onto contrast and resolution of images obtained by MALDI Imaging mass spectrometry. In fact, our future prospectives are the development of new matrices by rational chemical synthesis improving image contrast and development of new application modes for matrices such as sublimation thus improving the spatial resolution. Moreover, a close collaboration with the Biodata Mining Group (Faculty of Technology, Bielefeld) will be valuable in the development of new software for data analysis and visualization by cluster analyses. This will improve the interpretation of Imaging MS experiments.

TPS20-04 / Direct Analysis of Animal and Plant Tissues by LAESI-MSI and Ion Mobility for Mapping of Metabolites and Small Proteins

Matthew Powell, Peggi Angel, Brent Reschke, Callee Walsh
Protea Biosciences

Introduction
Mass spectrometry imaging (MSI) workflows for the direct analysis of biological samples at ambient pressures provide useful information for the localization of a wide variety of biomolecules within animal tissues and botanical samples. Due to the inherent complexity of the biomolecule populations within these samples, the detection of lesser abundance species can be challenging. The use of high resolution accurate mass (HRAM) mass spectrometry is one tool for improving the discrimination of mass spectral peaks for biomolecules in MSI studies. Alternatively, the use of ion mobility as a post-ionization separation tool can further increase the peak capacity and the confidence in metabolite identification for these workflows.
In this work, we combined laser ablation electrospray ionization mass spectrometry imaging (LAESI-MSI) with ion mobility for the direct analysis of animal and plant tissue samples. By selecting out sub-regions of the mass spectra based on drift times, we were able to improve the detection and mapping of a wide variety of metabolites and small proteins.

Methods
Animal tissue sections (normal mouse brain and human Alzheimer's brain tissue) were prepared by cryosectioning and thaw mounting onto standard glass microscope slides. Botanical samples (spider plant leaves) were mounted by scotch tape to glass slides. The mounted samples were placed matrix-free into a LAESI DP-1000 Direct Ionization System for analysis on a Waters Synapt G2S QTOF MS and analyzed using LAESI-MS with ion mobility. Data were collected in negative and positive ion mode using a mass range of m/z 50 to 1200 with MS and ion mobility-MS scans. Data was imported into ProteaPlot software for post-processing and image generation. DriftScope was used to select regions of interest based on different ion mobilities for selective ion mapping by drift time.

Results
Replicate analyses of the LAESI-MSI analysis of animal and plant tissues were performed with and without ion mobility. The use of drift time selection to create sub-spectra sets for mapping of LAESI-MSI data allowed the detection and mapping of a larger number of biomolecules, including small proteins. Several putatively identified metabolites and lipids were poorly mapped without ion mobility in human brain samples from Alzheimer 's disease patients and in mouse brain samples. However, with ion mobility engaged, these maps showed increased spatial resolution to the anatomical features of the brain tissue sections. Similarly, the use of ion mobility enabled the detection and localization of lesser abundant metabolites for spider plant leaves.

Conclusions
The combination of ion mobility with ambient pressure MSI techniques such as LAESI provides a powerful tool for detecting medium and lower abundant biomolecule species in complex biological samples. This workflow extends the utility of LAESI-MSI to allow the detection of a wide variety of metabolites, lipids, small proteins, and other biomolecules.

Novel Aspect
Combination of LAESI MSI with ion mobility separation to increase the detection and mapping of lower abundance metabolites and small proteins.

TPS20-05 / MALDI/LDI-FTMS imaging of intact plant tissues

Katsutoshi Takahashi
National Institute of Advanced Industrial Science and Technology

Introduction
Recently MALDI mass spectrometry imaging (MSI) has been a powerful tool to map spatial distribution of molecules on the surface of biological materials. Frequently MSI has been applied to animal tissue slices to map various biological molecules on the

slice. However, most recently, it has been also applied to plant tissue analysis. We've been developing high resolution and high accuracy mass spectrometer dedicated for mass spectrometry imaging of plant tissues and reported the successful MSI results coming from thin slice of young leaf of Arabidopsis thaliana.

Methods

Because it's been still difficult to make thin slices from tissues of small plants such as Arabidopsis thaliana, we tried to glue small intact tissues of plants such as leafs, roots or sprouts onto a small transparent ITO-coated slide glass instead. The intact tissues were then vacuum dried and matrix substance was applied by sublimation prior to mass spectrometry imaging experiment. The tightly focused UV-LASER beam was irradiated, inside in-house build vacuum ion source chamber, onto the matrix-coated sample surface and m/z of the produced ions were measured by commercial FTICR-MS (Bruker Daltonics Inc.; Apex-Qe-94T). We tried to irradiate UV-LASER beam onto no-matrix coated surface of the freeze dried plant tissues to obtain molecular ions by means of LDI process.

Result

300 pulses of Tightly focused UV-LASER (l=355nm, pulse width=1ns, 5.6microJ/pulse) was irradiated onto the surface of matrix coated / non-coated plant tissue to obtain single mass spectrum. The position of laser irradiation was raster scanned to get MS image of the specimen. The obtained series of mass spectra were converted to imzML format file, and the data-cube was analyzed using our in-house build software "LabMSI," which is freely available and runs on Windows and MacOS environments.

Molecular ions of various small metabolites including glucosinolates and anthocyanins were observed and their spatial distribution in the tissues was mapped in 20 micrometer space resolution with laser spot size less than 10 micron successfully. We tried to use various matrix substances such 9-AA, DHBA and CHCA in negatively charged ion mode and DHBA and CHCA in positively charged ion mode, respectively. Each matrix substance had different tendency to produce different kind of small metabolites, such as sugars, lipids, anthocyanis and other primary and secondary metabolites. Even without any matrix substances, molecular ions of small metabolites were obtained in LDI process inside the plant tissue. Indivisual ion spiecies were identified by means of the measured high accuracy m/z values. In our typical case, we succeeded to identify more than 10% of ions found on MSI averaged mass spectrum with making queries to METLIN server.

We applied the our method to plant tissues grown under the different physical and chemical stimuli environments, such as under the different color lights or under the different concentrations of plant hormon applied, to distinguish the metabolite distribution changes leaded by such stimuli.

TPS20-06 / Multimodal Imaging Mass Spectrometry for Probing Aβ-Plaque Pathology in Transgenic Alzheimer's Disease Mice

Jörg Hanrieder[1], Stina Syvänen[2], Andrew G. Ewing[3]
[1]Chalmers Univiersity of Technology, [2]Uppsala University, Uppsala, [3]Chalmers and Gothenburg University, Gothenburg

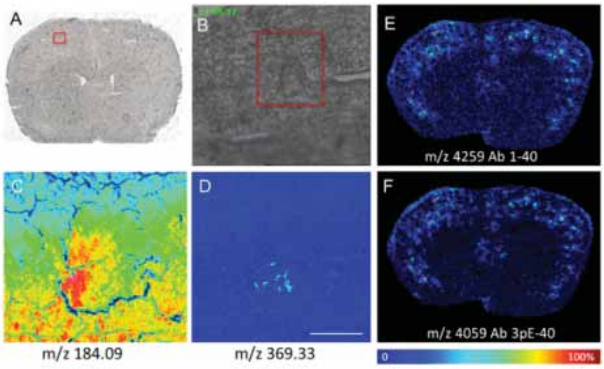
Alzheimer's disease is the most common neurodegenerative disorder affecting 12% over 65 (1). The exact mechanisms underlying AD pathogenesis are still not fully understood, significantly hampering the development of therapeutic treatment strategies. In AD, cognitive decline has been linked to formation of β-amyloid (Aβ) deposits as senile plaques as well as intracellular neurofibrillary tangles comprised of hyper-phosphorylated tau protein (2). The neuropathology in genetic- and sporadic AD is similar with respect to protein accumulation. The arctic and swedish mutation (ARCSWE) of amyloid precursor protein (APP) results in significant increase of neurotoxic Aβ peptides and fibrils (3). Changes in peptide truncation and plaque associated neuronal lipid species have been implicated with proteopathic mechanisms in AD (4).

The aim of this study was therefore to employ SIMS and MALDI based multimodal imaging mass spectrometry to probe Aβ plaque pathology in tgARCSWE mice with particular focus on associated neuronal lipid species and Aβ peptide truncation.

Transgenic C57BL/6-CBA-F1 male mice carrying the swedish double mutation alone (K670M, N671L) and the Arctic mutation (E693G) of human APP were studied. Lipid changes were examined in adult mouse brain (18 month) using ToF-SIMS and MALDI imaging on fresh frozen coronal cryosections at two different bregma (CPu and Hippocampus).

SIMS IMS revealed localizations of distinct lipid species in different brain regions in transgenic AD animals including sulfatides, triglycerides and cholesterol. Consecutive MALDI IMS identifies beta amyloid containing plaques as well as AD implicated Aβ peptide truncation (pyro-Glu). High resolution SIMS imaging performed in burst aligned mode, shows localization of phospholipids and cholesterol to these amyloid plaques in AD mice. This implicates a prominent role for cholesterol in promoting neurotoxic protein accumulation.

In conclusion, SIMS and MALDI based multimodal imaging is therefore a promising approach to interrogate chemical plaque pathology in Alzheimer's disease.



Annex 1: SIMS imaging of individual Aβ plaques (A, magnification B). SIMS analysis revealed localization of phosphocholine (PC) lipids to the plaque as indicated by the PC-headgroup (C, single ion image of m/z 184.09, size: 512x512µm). In addition, characteristic localization of cholesterol to the center of the plaque was observed (E). Subsequent MALDI imaging analysis of the same tissue section revealed the Aβ peptide chemistry in these plaques. Here, different truncations (E, Aβ1-40 and F, Aβ 3pE-40) were observed, including the 3-pyroglutamate truncation (Aβ 3pE-40), which is considered very neurotoxic and promotes extensively oligomerization. A manuscript with these results is currently in preparation.

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TPS20-07 / Development of Quasi-trapping Chemical Ionization Source with VUV Lamp for Online Mass Spectrometry

Keyong Hou, Ping Chen, Lei Hua, Haiyang Li
Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences

Novel Aspect

A quasi-trapping chemical ionization source with vacuum ultraviolet (VUV) lamp for online mass spectrometry was developed for high sensitivity online analysis.

Introduction

Single Photon ionization (SPI) is an attractive soft ionization method due to its high molecular ions yield and simple spectrum interpretation comparing to classic electron impact (EI) ionization in mass spectrometry (MS). The application of vacuum ultraviolet (VUV) lamp based SPI was limited due to low photon energy and poor photon flux density. Our group ever designed a chemical ionization source (DC-CI) using O2+ as reactant ions to ionize the analyte with IP higher than 10.6 eV by charge-transfer reaction. While, the sensitivity of DC-CI was only improved 60% comparing to SPI. The purpose of this article was to improve the sensitivity and extend the IP of VUV lamp simultaneously.

Methods

The QT-CI ion source consists of a commercial 10.6 eV krypton discharge lamp and an ionization region. The VUV lamp is set outside the ionization chamber and sealed with an O-ring. The ionization region comprises three tubular steel stainless electrodes: repelling electrode, focusing electrode, and skimmer electrode. A RF voltage is applied on the focusing electrode, and the three electrodes constitute a quasi-trapping region. The mass spectrometer used in the experiments was a home-made orthogonal acceleration time-of-flight mass spectrometer (TOFMS) with mass resolution of 6000 at m/z 100.

Preliminary Results

The IP range of analytes was extended to 12 eV, and the ion intensities of analytes were improved by 12-118 folds with different compounds comparing to SPI based on VUV lamp. The RF voltage accelerated the photoelectrons emitted from the electrode under photoelectric effect with VUV light, and the photoelectrons ionized the reactant gas O2, and then triggered the chemical ionization through ion-molecule reaction with reactant ion O2+. The simulation results showed that the mean total number of collisions (TNC) at QT-CI mode got a remarkable enhancement as high as 12 fold comparing to SPI for C3H7+ ion. Ion transmission efficiency in QT-CI mode was 2.4-fold to that in DC-CI. So,the quasi-trapping effect increased the collisions between O2+and analytes molecules, and enhanced the efficiency of chemical ionization,and the enhancement of signal intensity was mainly due to the quasi-trapping effect.

The QT-CI coupled with TOFMS was used to online analysis VOCs in water and atmosphere. The VOCs listed in the EPA method of To-14 and To-15 can all be ionized by the QT-CI source. Three order linear response curve for benzene in the concentration range of 10 ppb–10 ppmv was achieved with capillary inlet direct sampling. LOQ (S/N=50) of CHCI3 in water was 0.5 ppbv with membrane inlet sampling.

Conclusions

A new quasi-trapping chemical ionization source based on a VUV lamp was introduced for time-of-flight mass spectrometry. The IP range of analytes was obviously extended, moreover, the ion intensities of analytes were improved by 12-118 folds. The QT-CI TOFMS has the potential for online VOCs analysis with high sensitivity.

TPS20-08 / Visualization and quantification of brain metabolic fluxes of glucose in the awake mice by mass spectrometry

Yuki Sugiura
Keio University

Novel Aspect

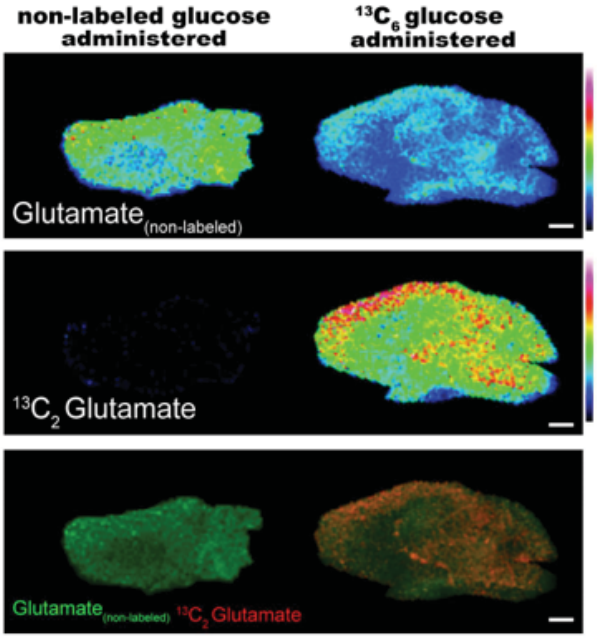
Imaging mass spectrometry of «fluxes» of glucose metabolic pathways in the brain was achieved by utilization of stable-isotope labeled glucose.

Introduction

Biotransformation of glucose in organs includes multiple pathways, while quantitative evaluation of percentages of its utilization for individual pathways and their spatial heterogeneity in vivo remain unknown.

Method

MALDI-imaging mass spectrometry (IMS) and capillary electrophoresis (CE)-MS based metabolomics combined with a focused microwave irradiation (FMW) for rapidly fixing tissue metabolism allowed us to quantify and to visualize metabolic fluxes of glucose-derived metabolites in the mouse brain in vivo. At 15 min after the intraperitoneal injection of 13C6-labeled glucose, the mouse brain was exposed to FMW, which can stop brain metabolism within 1 sec.



Visualization of cerebral cortex-selective 13C6-glucose metabolic ux into 13C2-glutamate in the sagittal section of the mouse brain. Shown are ion images for unlabeled and 13C2-labeled glutamate at signals of m/z 145 and 147, respectively, in the sagittal section of the mouse brain for both saline (left) and 13C6-glucose administered groups (right). While ions corresponding to unlabeled glutamate was detected in both examined groups, ions representing 13C2-glutamate was speci cally detected from brain sections of the 13C6-glucose administered group, demonstrating cerebral cortex-selective glucose –> glutamate metabolic ux. Scale bar: 1 mm.

Results

CE-MS quantification of metabolic intermediates containing 13C atoms revealed that a majority of the 13C6-glucose was diverted into syntheses of glutamate, lactate and UDP-glucose. IMS showed that regions rich in glutaminergic-neurons exhibited a large signal of 13C2-labeled glutamate. On the other hand, the mid-brain region was enriched with an intensive 13C6-labeled

UDP-glucose signal, suggesting an active glycogen synthesis. Collectively, application of the current method makes it possible to examine the fluxes of glucose metabolism in a region-specific manner.

Conclusion

Collectively, application of the current method makes it possible to examine the fluxes of glucose metabolism in a region-specific manner.

TPS20-09 / Brain distribution of selective serotonin reuptake inhibitors in male CD-IGS rats using MALDI-TOF mass spectrometric imaging

Julius Apuy, Yang Tang, Mehran Moghaddam
Celgene Corporation

Selective Serotonin Reuptake Inhibitors (SSRIs) are small molecules that can easily penetrate the blood-brain barrier in order to block the re-uptake of serotinin, a neurotransmitter, into the pre-synaptic nerve cells thereby increasing the extracellular levels of serotonin in the synaptic cleft for binding to the postsynaptic receptor. SSRIs are typically used as antidepressants in treating neurological disorders.

The tissue distribution of a series of marketed SSRIs (Celexa, Lexapro, Prozac, and Zoloft) in the rat brain by Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF-MS) provide insights about the similarity and differentiation in brain distribution of each SSRIs.

These compounds, that are known to preferentially partition into the brain to be efficacious, will be important in establishing the brain imaging infrastructure using MALDI-TOF-MS.

TPS20-10 / 2D and 3D analyses for the organic thin film using laser desorption ionization.

Takaya Satoh[1], Masahide Shima[1], Hironobu Niimi[1], Yoji Nakajima[2], Makiko Fujii[3], Toshio Seki[3], Jiro Matsuo[3], Junichi Osuga[4], Yoshihisa Ueda[1]
[1]JEOL Ltd., [2]Asahi Glass Co., Ltd., [3]Kyoto Univ., [4]JEOL (EUROPE) SAS

Introduction

The electrical devices composed of organic and inorganic materials such as organic light-emitting diodes (OLED) devices have been widely used. There are various techniques for surface analysis such as a scanning electron microscopy/energy dispersive x-ray spectroscopy (SEM/EDS), electron probe microanalysis (EPMA), X-ray photoelectron spectroscopy (XPS), time-of-flight secondary ion mass spectrometry (TOF-SIMS), etc.. However, the analytical techniques to obtain the molecular information on organic layers are limited. In this presentation, we will discuss about the possibility to apply the imaging mass spectrometry (IMS) and depth profiling using laser desorption/ionization time-of-flight mass spectrometer (LDI-TOFMS) as for analyzing organic layers.

Methods

Three types of OLED compounds thin films deposited on Si wafer were prepared,

(i) α-NPD/Si: α-NPD deposited on Si wafer with 600 nm thickness.

(ii) α-NPD/2-TNATA/Si: 2-TNATA was deposited on Si wafer with 700 nm thickness. The α-NPD was deposited above the 2-TNATA layer.

(iii) α-NPD/2-TNATA/Si(mesh): 2-TNATA deposited on Si wafer with 440 nm thickness. The 880 nm α-NPD was deposited making a mesh pattern of 55 lines per inch above the 2-TNATA layer. The IMS and depth profiling were performed with MALDI-TOFMS (JMS-S3000, JEOL). The α-NPD/Si was also measured with SEM/EDS (JSM-7001FTTLLV, JEOL), XPS (JPS-9010, JEOL) and TOF-SIMS (Ar gas cluster ion source developed in Kyoto Univ. was applied to JEOL's JMS-T100LP) for supplement measurements.

Results

The [M]+ ions of α-NPD with negligibly small fragment ions were observed from α-NPD/Si with LDI-TOFMS. In the case of the TOF-SIMS, not only [M]+ ions but also many kinds of fragment ions were observed. The LDI-TOFMS and TOF-SIMS had an advantage for organic compounds analysis compared to SEM/EDS and XPS which could only obtain elemental or chemical state information. The LDI-TOFMS has lower spatial resolution rather than TOF-SIMS, but clear mass spectrum obtained with LDI-TOFMS has the advantage in degradation analysis, which the measurements of minor components will be often important.

Depth profile analysis was further estimated with two layered thin film: α-NPD/2-TNATA/Si. The turnover of the ion intensities of α-NPD and 2-TNATA were understood by accession to boundary of two layers. The ionization region in depth direction was depended on the laser intensity. The several hundred nanometer layer structure was clearly observed in appropriate laser intensity.

Conclusions

There were advantages for analyzing multiple layered multiple layered organic thin films with LDI-TOFMS compared to existing surface analytical techniques. Further investigation about the 2D and 3D analyses by changing the laser condition using α-NPD/2-TNATA/Si(mesh) will be given in the presentation.

Novel Aspect

Mass spectrometry imaging and depth profile analyses on thin films of organic material in electronic devices were performed.

TPS20-11 / Methodology for precise understanding of drug imaging mass spectrometry using Mass Microscope

Shuichi Shimma[1], Satoko Osawa[1], Yukari Tsubata[2], Akihisa Sutani[2], Ryosuke Tanino[2], Takeshi Isobe[2], Akinobu Hamada[1]
[1]National Cancer Center Research Institute, [2]Shimane University Faculty of Medicine

Introduction

Conventional mass spectrometric analysis provides quantitative information on pharmaceuticals in normal and diseased tissues such as cancer. However, tissue extraction precludes determining information on their spatial distribution. To bring precise spatial distribution of molecules in specimens, imaging mass spectrometry (IMS) based on Mass Microscope was developed. Especially, cancer tissues have complicated morphology due to heterogeneity, therefore to perform IMS under the microscopic view with high sensitivity is essential. In order to understand the obtained ion distribution image precisely, the ion image and stained tissue sections should be comparable. To achieve this aim, we developed two-step matrix application procedure which can avoid tissue damage by matrix application and laser irradiation. Two-step matrix application can provide tissue staining using post-measured sections. In this presentation, we will provide the standard sample preparation procedure. Additionally, we will present quantification and data analysis method using erlotinib administered tumor section resected from mice xenograft model of non-small cell lung cancer (NSCLC).

Methods

Erlotinib, epidermal growth factor receptor (EGFR) tyrosine

kinase inhibitor, was analyzed in this study. Erlotinib was orally administered to mice bearing PC 9 and PC 14 (human NSCLC) at a dosage of 50 mg/kg/day for three days. The tumor tissue was resected at 180 min after treatment. The obtained tissue was quickly frozen in liquid nitrogen. We applied the two-step matrix application which performed spraying of α–CHCA solution after eight-minute α–CHCA sublimation. After performing IMS for erlotinib using MS/MS mode in Mass Microscope, the tissue section was stained using HE or EGFR antibody after removal of matrix layer. For quantification, we prepared serial sections and dissected interested regions using laser capture microdissection (LMD) system (LMD7000, Leica). After dissection, erlotinib was extracted from each small piece of tissue to validate the tissue distribution of erlotinib by LC-MS/MS system.

Results
Two-step matrix application method provided higher ionization efficiency sufficient to perform IMS in MS/MS mode as well as tissue surface protection for observation of stained post-measurement tissue section. According to the IMS results, erlotinib ion intensity in the necrosis region was higher than tumor region. This intra-tissue concentration was confirmed and in accordance with the quantification results using LC-MSMS. The post-measurement tissues were immunostained for EGFR, and we confirmed low expression of EGFR in the necrosis region.

Conclusion
In IMS for visualizing drug distribution in complicated tumor tissues, we found the necessity of IMS under microscope, precise quantification using the conventional system and tissue staining of post-measured tissues to compare ion images. To achieve those requirements, our developed procedures including matrix application were essential.

Novel Aspect
Combination of IMS using Mass Microscope and immunohistochemical analysis using post-measured samples reveals real distribution of pharmaceuticals in vivo.

TPS20-12 / Exploring the head and neck tumor in situ metabolome by MALDI FT-ICR MSI

Anna C. Crecelius[1], Lukas Krasny[2], Franziska Hoffmann[3], Günther Ernst[3], Dennis Trede[4], Theodore Alexandrov[5], Orlando Guntinas-Lichius[6], Ulrich S. Schubert[7], Vladimir Havlicek[2], Ferdinand von Eggeling[3]
[1]Laboratory of Organic and Macromolecular Chemistry, [2]Institute of Microbiology, [3]Core Unit Chip Application (CUCA), Institute of Physical Chemistry FSU and Institute of Human Genetics, [4]SCiLS GmbH, [5]Center for Industrial Mathematics, University of Bremen, [6]Department of Otorhinolaryngology, Jena University Hospital, [7]Laboratory of Organic and Macromolecular Chemistry (IOMC), Friedrich Schiller University Jena

Introduction
Head and neck cancer comprises of malignant tumors located in the oral cavity, the throat, and the upper aerodigestive tract. It is the seventh most common type of cancer in Europe. The typical histology of head and neck tumor is squamous cell carcinoma (HNSCC), which is fatal without radical therapy. Nevertheless, half of the patients develop a locoreginal recurrence in the next two years. Hence, the spatial molecular characterization is crucial to improve individualized therapy. In this study, the technique MALDI MSI, which facilitates the histopathological analysis and enables the simultaneous analysis of hundreds of molecular compounds in a single measurement has been applied to head and neck tumor to discover the spatial metabolome.

Methods
Four head and neck samples were analyzed in this study: Two HNSCC samples, one normal tissue sample and one benign adenolymphoma sample. Twelve µm thick sections were cut in a cryomicrotome at -20 °C and attached on ITO-coated glass slides. The matrix CHCA was sprayed onto the tissue using the ImagePrep device and analyzed in a Solarix 12 T FT-ICR mass spectrometer, using the positive ion mode with a m/z range of 200 to 1,500, and a spatial resolution of 50 µm. A consecutive section was always stained by H&E and co-registered with the MSI datasets. The software SCiLS Lab was used for processing the datasets by spatial segmentation where similarities of spectra were statistically determined and similar spectra were grouped into one cluster. The generated segmentation maps were overlaid on the corresponding histological images and validated by an experienced pathologist.

Results
The MALDI FT-ICR MSI analysis of HNSCC revealed that the metabolic expression is heterogeneous within the tissue. In order to allow a deeper analysis of this differentiation a new computational approach for analyzing the datasets in an easy and fast way was developed, in which the uncompressed datasets were reduced to groups of similar mass spectra by a clustering process. Each cluster was given a certain color and the resulting segmentation map was superimposed onto the H&E stain. In this manner all four tissue samples were analyzed and the majority of the cluster showed unique metabolic features, as determined by the comparison between the segmentation and the H&E images.

Conclusions
We successfully analyzed HNSCC samples by MALDI FT-ICR MSI. The huge datasets were processed by clustering all m/z signals according to their similarity, which enabled the fast and easy extraction of useful information. The obtained variation in the metabolic expressions could be attributed back in most of the cases to histological changes. Hence, the developed methodology has the potential to be extended to other types of cancer to bring to light further molecular characteristic, which up to now have not been easy accessible.

Novel Aspect
Spatial metabolomics of head and neck tumor by MALDI FT-ICR MSI

TPS20-13 / Evaluation of Distribution of Ingredients in Pharmaceutical Solid Dosage Forms using Time of Flight Secondary Ion Mass Spectrometry

Tatsuo Koide, Noriko Katori, Yukihiro Goda
National Institute of Health Sciences

Purpose
Providing information on the distribution of ingredients in solid dose pharmaceuticals by conventional analytical techniques has been difficult. However, chemical imaging techniques have made it possible to assess the distribution of ingredients and to use this information to monitor processes and quality control during pharmaceutical manufacturing. In this study, we investigated the feasibility of a chemical imaging technique with time-of-flight secondary ion mass spectrometry (TOF-SIMS) to analyze distribution of active pharmaceutical ingredients (API) and excipients in solid dosage forms.

Methods
Ethenzamide (Ez, as API), lactose (Lac, as an excipient), cornstarch (CS, as an excipient), and magnesium stearate (Mg-St,

as a lubricant) were mixed in a blender and the mixed powder was compressed into tablets. The content ratio of Ez:Lac:CS:Mg-St was 20:56:24:1. The particle size of Ez, Lac, CS, and Mg-St measured as D90 were 56, 163, 31, 15 µm, respectively. Planar distributions of the ingredients on the surface and the cross section of those tablets were measured using TOF-SIMS. TOF-SIMS data acquisition was performed using a PHI TRIFT V nano-TOF instrument (ULVAC-PHI, Inc.). The instrument employed a 30 keV Bi3+ ion source. Chemical images generated by TOF-SIMS were analyzed using a peak height method.

Results
The peaks at 166+, 365+, and 24+ m/z were used to generate the chemical images of Ez, Lac, and Mg-St, respectively. The spectrum of CS overlapped that of Lac because the two compounds are similar in chemical structure. Therefore, the peak at 85+ m/z was used to generate the image of the excipient (Lac and CS). By using these peaks, the distribution of ingredients was evaluated by TOF-SIMS. TOF-SIMS provided information on Mg-St distribution, which was not clearly detected by a vibrational chemical imaging method such as near-infrared spectroscopy. This suggests that a chemical imaging technique using TOF-SIMS could provide detailed information on smaller particles, which cannot be obtained using other imaging techniques. When we compared the number of Mg-St particles observed on the surface and those observed on the cross section, the number of particles on the surface was about 4 times greater than that of the particles on the cross section. In addition, when we compared the planar size of Lac particles observed on the surface with those observed on the cross section, the particles on the surface were found to be much smaller than those on the cross section. These results suggested that the smaller ingredient particles tended to fill in the gaps between larger ingredient particles on the surface of the tablet.

Conclusion
A chemical imaging technique using TOF-SIMS was found to be useful for analyzing the distribution of ingredients, especially for small particles in solid dosage forms.

Novel Aspect
A chemical imaging using TOF-SIMS providednovel information on small particles in solid dosage forms.

TPS41 - Gas-phase Ion Fragmentation Mechanisms

11:00-15:00
Poster Exhibition, Level -1

PS41-03 / Propane loss from diethylamines, investigation of the fragmentation mechanism using FT-ICR and sector-field experiments in combination with DFT calculations

Claus Gernert, Sarah Seulen, Jürgen Grotemeyer
Christian Albrechts Universität zu Kiel

Introduction
Dyes with diethylamino sidegroup, for example rhodamin B, show unusual losses of 44 Da. In the present work we investigated the fragmentation behavior of dyes using different model systems. With the resolving power of FT-ICR mass spectrometry and the advantages of sector-field measurements as well as DFT calculations we were able to get several hints for the basic mechanism.

Methods
All mass spectra have been recorded on an Apex III FT-ICR mass spectrometer with a 7.05 T superconducting magnet (Bruker Daltonik, Bremen, Germany) using different fragmentation techniques. A Nd:YAG laser (Inlite II, Continuum, Santa Clara, USA) was used with 355 and 532nm for photodissioziation experiments within the ICR cell as well as CID experiments with Argon as collision gas. For the DFT calculations Gaussian was used with a bvp86/6-311+g(df,2pd) basis set. ZAB measurements were done with a double focusing sector- field mass spectrometer with reversed-Nier-Johnson geometry (VG MM ZAB-2F).

Results
Different aniline derivatives as well as toluidine were used as model systems for the investigation of the reaction mechanism leading to the loss of 44 Da, which is a loss of propane. For this reason anilines have been modified to investigate the influence of charge and size of the molecule to the fragmentation mechanism. This loss can be explained by an unusual fragmentation mechanism, which can be either radical, concerted or a combination of both variants. In high resolving ICR mass spectra of the different anilines we observed several unusual radical fragments as CH3 or C2H5 depending on the size of the investigated molecule as well as neutral losses using different fragmentation techniques. Beside standard CID measurements a new experiment was developed using a Nd:YAG laser with different wavelength to perform PD experiments. Radical fragments were observed in ZAB measurements as well. B2E measurements showed that the origin of the loss of 44 Da has different precursor ions, unlike we expected. Charge influences and geometry changes were studied on Toluidine and Ms3 fragments from CID measurements and compared with the results of DFT calculations and ZAB B2E and MIKES experiments.

Conclusion
The observation of radicals in the mass spectra, which are always a violation to the even electron rule, leads to at least a radical part in the fragmentation mechanism generating a loss of 44 Da. A concerted mechanism cannot be declined using FT-ICR measurements. ZAB measurements on toluidine show different reaction pathways for the formation of propane, too. With the help of MIKES measurements it was possible to map the different pathways. Altogether the high resolution FT-ICR measurements, DFT calculations and the advantages of ZAB experiments turned out as a powerful combination in the reaction mechanism investigation.

Novel Aspects
Investigation of the fragmentation mechanism using FT-ICR and ZAB measurements in combination with DFT calculations.

PS41-04 / Fragmentation Reaction of Azo Dyes using High Resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

Martin Clemen, Jürgen Grotemeyer
Christian-Albrechts-Universität zu Kiel

Introduction
It has been shown that xanthene dyes, such as Rhodamine B, show interesting fragmentation reactions in a mass spectrometer. To understand the mechanisms of these reactions, other dye molecules with similar and some new structural properties have been investigated. In this work we present numerous azo compounds. Nevertheless fragmentations during mass spectrometry analysis of this group synthetic dyes has not been studied in excess. Our investigations concerning fragmentation pathways were carried out in a high resolution fourier transform

ion cyclotron mass spectrometer (FT-ICR-MS) using different dissociation methods.

Methods
The azo compounds have been synthesized by azo coupling reactions from aniline derivatives or by N-alkylation of 4-Aminoazobenzene or 2-Aminoazotoluene.
All mass spectra have been recorded on an APEX III FT-ICR mass spectrometer equipped with a 7.05 T magnet (Bruker Daltonik, Bremen, Germany). The samples were dissolved in MeOH/H2O and ionized using electrospray ionization.
Fragmentation of the molecular cations was achieved using sustained off-resonance irradiation (SORI) collisional induced dissociation (CID). Argon acted as collision gas.
Further, a Nd:YAG laser (Inlite II, Continuum, Santa Clara, USA) was guided into the ICR cell to do photo dissociation experiments, using second and third harmonics.

Results
A cleavage of the azo bonding is visible in all measurements. In the presented results we focus on the fragmentation pattern of the alkyl amino functions.
A loss of 17 mass units could be observed in mass spectra of all monosubstituted aminoazotoluene derivatives. This can be attributed to ammonia (NH3) due to the high mass resolving power. In the analyzed protonated form a quaternary amine (alkyl-NH2+-azotoluene) should be present. A rearrangement process of this species can lead to the observed elimination of ammonia. In this case the needed H-atom cannot be derived from the N-alkyl function.
The behavior of the disubstituted molecules differs. In this case we observe losses of alkylamine from the disubstituted form of aminoazotoluol. These losses can also be explained by the proposed rearrangement mechanism.
To verify this we introduced fully deuterated alkyl groups. For the monosubstituted form only loss of unlabeled ammonia could be observed. The disubstituted form shows loss of alkylamine containing only one of the two labeled sidechains. Participation of the methyl function in ortho position is very likely.
All observed fragments originating from the alkylated amino function can be build by losses of radicals or neutral molecules.

Conclusions
Finally we found interesting fragmentation reactions of azo compounds. Synthesizing deuterium labeled systems we were able to get a deeper insight into the mechanisms. For mono- and disubstituted alkylaminoazo derivatives our results are consistent with a rearrangement process.

Novel Aspect
Unusual fragmentation products of amino-azo-compounds were found in MS/MS experiments.

TPS41-05 / Photofragmentation spectra of halogenated methanes in the VUV photon energy range: the role of the halogen atom

Antonella Cartoni[1], Antonella Cartoni[1], Paola Bolognesi[2], Ettore Fainelli[2], Lorenzo Avaldi[2]
[1]University of Rome Sapienza, [2]CNR

Introduction
The emission of greenhouse gases (GHGs) and ozone depleting substances (ODSs) are among the major cause of climate change. Halogenated methanes are a class of compounds involved in these important processes. In order to assess their relevance as possible source of reactive species it is fundamental to study their fragmentation under controlled experimental conditions. Although several studies have been carried out to obtain thermochemical data with different radiation sources, to the best of our knowledge a comparative study of their VUV fragmentation patterns is not reported. The main goals of this work are: i) the study of the fragmentation spectra of CH2X2 molecules (X=F, Cl, Br, I) and the assessment of the role played by the particular halogen atom in the photofragmentation of these molecules; ii) a brief study of the series of chloromethanes containing one (CH3Cl) to four (CCl4) chlorine atoms to analyze the effect of the number of halogen atoms in the photofragmentation.

Methods
The molecular fragmentation mass spectra have been recorded using the photoelectron-photoion coincidence (PEPICO) technique together with a VUV rare gas discharge lamp and an effusive beam of the molecule under examination. The photon source is a rare gas discharge lamp used to produce several emission lines. The most intense linesare at 21.22 eV (He I), 16.67 eV (Ne I) and 11.62 eV (Ar I). The ion analyzer is a homemade Time of Flight Mass Spectrometer.

Results and Conclusions
The results show the peculiar behaviour of the CH2F2 molecule as the only dihalomethane that dissociates via the H-loss channel yielding CHF2+ ion and H atom, with relevant atmospheric implications. As far as the CH2I2molecule is concerned,this is the only dihalomethane that shows small but measurable signal due to I2+ ions probably formed from molecular detachment. The fragment ions associated to the loss of the halogen atom dominates the mass spectra of all the other halomethanes and it is the only intense fragmentation channel observed for CH2Br2 and chloromethanes, confirming the role of these compounds in atmospheric science as a source of reactive halogen atoms.

Novel aspects
This study allows to assess the effect of the halogen atom in the photofragmentation of halogenated methanes and give new insights on the chemical physics of these compounds.

Acknowledgments
Financial support from the Italian Government (MIUR FIRB 2010 RBFR10SQZI project)

TPS41-06 / Kinetic energy release and fragmentation pathways of substituted benzeneamines

Sarah Seulen, Jürgen Grotemeyer
Christian-Albrechts-Universität Kiel

Introduction
The loss of propane from a diethylamino sidegroup was shown for rhodamine B with electrospray ionization (ESI) and ICR-MS [1]. A possible reaction mechanism was proposed [1]. To gain further information about this fragmentation small molecules with diethylamino sidegroup and an aromatic system have been studied under different reaction conditions.

Methods
All spectra were recorded with a Micromass ZAB-2F from Vacuum Generators, a double-focussing sector field instrument with a reversed Nier-Johnson geometry. The samples were brought into the gas phase and ionized with electron or chemical ionization. Mass-analysed-ion-kinetic-energy-(MIKE)-spectrometry of the formed ions provided values for the kinetic energy release (KER). Information about the fragmentation pathways were obtained with MIKE-spectrometry and linked-scan techniques like B2E.

Results
Fragmentation reactions during measurements with a sector field instrument proceed on a significantly shorter timescale and with higher energies than measurements with a FT-ICR mass spectrometer. Besides it was possible to use electron ionization and chemical ionization to generate different ion species. Small molecules with diethylamino sidegroup and an aromatic system have been used to investigate the influence of the ion species on the fragmentation mechanism of the diethylamino sidegroup.
The studied diethylaniline derivatives showed a similar fragmentation mechanism after electron ionization. In most cases no propane loss from the molecular radical cation was observed. Instead the prominent elimination was the loss of a methyl radical followed by the loss of ethylene. For both fragments small KER values have been determined which indicates simple bond cleavages.
With chemical ionization a more complex fragmentation pattern was observed. In the MIKE-spectra of the [MH]+ neutral and radial losses occurred. The prominent loss of a methyl radical is a violation of the even electron rule. Additionally a loss of 44 Da (propane) was observed. The KER values for these propane losses were increased which is a hint for a rearrangement reaction. B2E linked-scan measurements were used to determine the precursor of the fragment ion that evolved from the 44 Da loss of the [MH]+. Different precursor ions were observed which leads to the presumption of different fragmentation mechanisms.

Conclusions
The MIKE- and B2E-measurements made it possible to map the fragmentation pathways of the different ion species which were found to be quite different. KER values for selected fragments indicate different fragmentation reactions and therefore give clues to reaction mechanisms.

Novel Aspects
Values for the kinetic energy release of alkyl fragments were determined for fragmentation reactions of alkyl substituted benzeneamine.

Literature
[1] J. Peters, M. Clemen and J. Grotemeyer, Anal. Bioanal. Chem., 2013, 405, 22, 7061-7069.

TPS41-07 / Exploring the structure and reactivity of metal complexes with ion mobility mass spectrometry and ion/molecule reactions.
Nicole Rijs, Maria Schlangen, Helmut Schwarz
Technical University Berlin

Introduction
Ion/molecule reactions are useful mass spectrometric tools for probing the intrinsic reactivity of metal catalysts with a variety of neutral substrates. However, normally one cannot easily distinguish isomeric species utilising a mass analyser alone, and a mixture of isomers can be present in a single MS signal. Ion-mobility spectrometry in tandem with mass spectrometry offers the potential to separate species based on their mobility, that is, their size, shape and charge distribution, thus allowing separation of isomers. This is of great advantage to the study of ion/molecule reactions with metal complexes, as one can probe the fundamental reactivity of isomerically "pure" species and also learn more about their structure.

Methods
Here, a Synapt G2 HDMS travelling wave ion mobility mass spectrometer (TWIMS-TOFMS), which is modified to allow for ion/molecule reactions or addition of co-gases, is used to probe the intrinsic reactivity of the metal complexes of interest. Thus, ionic metal complexes generated via electrospray ionization are manipulated using standard mass spectrometric analysis techniques (e.g. mass isolation and CID), along with ion-mobility separation and ion/molecule reaction. Isomer-separated species are reacted to shed light on their individual bimolecular reactivity, and paired with complementary electronic structure calculations to understand reaction mechanisms.

Results
Examples of several studies on metal based systems will be presented. Of particular interest are: (1) the non-innocence of the nitrogen carrier gas present in the ion-mobility cell, which can easily react with the open coordination site of many metal complexes, (2) the dramatic difference in ion/molecule reactivity uncovered for some isomer-separated species, (3) the ability to probe isomerization processes with energy resolved experiments, and (4) progress toward suitable metal complex calibrants for calculation of collisional cross sections. The DFT predicted reactivity of copper oxide, along with several other metal complexes, will be briefly discussed in light of the experimental results obtained.

Conclusions
Ion mobility mass spectrometry in combination with ion/molecule reactions was successfully used to probe the fundamental reactivity of a ligated copper oxide complex, along with several other ionic metal complexes. New insight into formation processes and the structure of species generated via ESI were gained, along with study of their individual bimolecular reactivity, resolved for the first time.

Novel Aspect
The use of modern ion-mobility mass spectrometry was successfully used to probe the structure and the uni- and bimolecular reactivity of isomer-separated metal complexes'.

TPS41-08 / Surprising fragmentation of N-substituted N-perfluoroacyl-amino acids
Nino Todua, Anzor Mikaia
National Institute of Standards and Technology

Introduction
Derivatives of amino acids and peptides are objects of serious studies by various mass spectrometry techniques starting from the late 1950th. The use of a combination of known derivatization methods leads to mixed derivatives, and interpretation and utilization of competing decomposition processes of these derivatives under mass spectrometry conditions are essential. The present study reports important ions in the EI spectra of N-alkyl(aryl)-N-perfluoroacyl-amino acids, their alkyl esters and anhydrides.

Methods
N-Alkyl-N-perfluoroacyl-amino acids (N-Alk-N-PFA-AA), their alkyl esters (N-Alk-N-PFA-AAAE) and perfluoroacyl anhydrides (N-Alk-N-PFA-AA-PFAA) were synthesized for Glycine, N-alpha-Methylglycine, L-Alanine, 2-Methyl-L-alanine, L-Phenylalanine, beta-Alanine, Serine, Valine, Threonine, Cysteine, Leucine, Isoleucine, Aspartic acid, Glutamic acid, Lysine and Methionine with the use of methyl iodide, trideuteromethyliodide, trifluoroacetic, pentafluoropropionic and heptafluorobutyric anhydrides as derivatization reagents. N-Phenylglycine, N-Benzylglycine, 2-Methyl-N-(4-methylphenyl)-L-alanine were the starting materials for the

syntheses of N-aryl-N-perfluoroacyl-derivatives. Commercially available 2H-, 13C- and 15N-Labeled amino acids were initial compounds for the synthesis of derivatives.

Preliminary data

Introduction of N-alkyl group does not change well established fragmentation patterns for alkyl esters of N-perfluoroacyl-amino acids under EI conditions. However, spectra of N-Alk-N-PFA-AA and N-Alk-N-PFA-AAAE show additional dissociation pathways leading to prominent peaks of ions type a, b and c.
The mass value of ion a increases by 1 Da in the spectra of 2-13C or 15N labeled compounds and by 14 Da in the case of 2-methyl homologues. Mass shift is also observed for N-CnD2n+1-analogs. Ion b originates from the M+. and its formation is a result of carbonyl oxygen migration to C-atom connected to amino-group and C-N bond dissociation. Peaks of resulting fluorinated nitrilium cations at m/z 110, 160 and 210 (CnF2n+1=CF3, C2F5, C3F7) are prominent in the spectra of N-methyl-N-PFA derivatives.
Ion c is a result of a migration of perfluoroalkyl radical to acidic carbonyl via a 6-membered skeletal rearrangement followed by C-C-bond cleavage.

Novel aspect

Nitrilium cations type a and b, and oxonium ion-radicals c are characteristic for N-alkyl-N-PFA amino acids, their alkyl esters and anhydrides, and can be effectively used for structure elucidation.

TPS41-09 / N-terminal Charge Derivatization for Discrimination between Leu and Ile in Peptides by High-Energy CID MS/MS Analysis

Masahiro Miyashita[1], Atsushi Kitanaka[1], Ayumi Kubo[2], Takaya Sato[2], Michisato Toyoda[3], Hisashi Miyagawa[1]
[1]Graduate School of Agriculture, Kyoto University, [2]JEOL Ltd., [3]Graduate School of Science

Introduction

De novo sequencing is carried out by interpreting mass differences between a series of peptide fragments generated by collision-induced dissociation (CID). However, fragmentation of peptides often gives complicated and incomplete product ion spectra, making de novo sequencing difficult. To overcome these problems, we previously introduced chemical modifications at the N-terminus of peptides to obtain more regular and informative fragmentation, and found that derivatization with 4-amidinobenzoic acid brought about the most effective fragmentation under low-energy CID (LE-CID) conditions. One of remaining problems in de novo sequencing is discrimination between Leu and Ile residues in peptides. The discrimination is possible based on differences in side chain fragmentation between Leu and Ile, which is observed under high-energy CID (HE-CID) conditions. However, this is observed only when basic residues, such as Arg or Lys, are present at the N- or C-terminal end, indicating that the discrimination is difficult for peptides without basic residues. It is known that charge derivatization at the N-terminal end facilitates the side chain fragmentation by HE-CID, but derivatization reagents reported so far contain a quaternary ammonium or phosphonium group. Since introduction of these groups having a “fixed charge“ results in reduction of number of mobile protons, it often hampers effective backbone fragmentation under LE-CID. In this study, we investigated the effect of N-terminal charge derivatization using structures with high proton affinity to obtain derivatization reagents that is effective both for Leu/Ile discrimination by HE-CID and for sequence determination by LE-CID.

Methods

Compounds having various basic moieties (pyridine, guanidine or amidine) were introduced into the N-terminus of peptides by a succinimide ester method or a coupling reagent (DMT-MM). Side chain fragmentation at the Leu and Ile residues was measured using two types of MALDI-TOF/TOF spectrometers (Bruker and JEOL). LE-CID MS/MS analysis was performed using an IT-TOF spectrometer (Shimadzu).

Results

As a result of HE-CID MS/MS analysis, side chain fragmentation at the Leu and Ile residues was observed after derivatization with Aba or 4-(guanidinomethyl)benzoic acid (Gmb), and the latter showed the most effective side chain fragmentation without affecting the backbone fragmentation under LE-CID conditions.

Conclusion

N-terminal derivatization with Gmb allows Leu/Ile discrimination by HE-CID as well as sequence determination by LE-CID. This approach may offer an efficient and reliable de novo peptide sequencing method.

Novel aspects

The novel chemical derivatization technique for de novo peptide sequencing, including Leu/Ile discrimination under HE-CID conditions, was demonstrated.

TPS41-10 / Crown ether/fullerene conjugates: ionisation, alkali metal ion affinities and dimerisation

Ina D. Kellner[1], Leanne C. Nye[1], Marc S. von Gernler[1], Jing Li[1], Manolis D. Tzirakis[2], Michael Orfanopoulos[2], Thomas Drewello[1]
[1]Friedrich-Alexander-University Erlangen-Nuremberg, [2]University of Crete

Introduction

The crown ether/fullerene (ce/f) conjugate is a classic in the development of making mass spectrometry useful for the analysis of fullerenes and their derivatives. As many fullerene derivatives are only ionised by difficult-to-control oxidation and reduction processes, Wilson and Wu synthesized the first ce/f conjugate with the express aim to better investigate fullerenes by electrospray ionisation [1]. Tzirakis and Orfanopoulos recently prepared a new type of ce/f conjugate in which the crown is attached directly via a C–C single bond to C60, which also attains a hydrogen atom as the second substituent to the former fullerene double bond [2]. The new conjugates are investigated here by ESI- and MALDI-MS.

Methods

ESI experiments were performed with a QIT- and a QTOF-MS and MALDI experiments were carried out with a reflectron TOF instrument.

Results and Conclusions

The formation of host-guest complexes of the crown ethers with alkali metal cations allows for easy observation in the positive ion mode, whereas the acidic nature of the fullerenyl hydrogen facilitates deprotonation and detection of the ion $[M-H]^-$ in the negative ion mode. In addition, the application of MALDI allows the formation of true molecular ions in both ion modes. The charge location on the resulting ions is established by product ion analysis following the decay of the activated ions.
A key aspect of this study is the post-source decay (PSD) of three differently sized ce/f conjugates metalated with five alkali metal cations at varying laser fluences. The resulting fragmentation dynamics provide a good estimate of the relative bond strength

between metal cation and crown ether moiety. As in the case of free crown ethers [3], the affinities for a given crown size were found to increase with the charge density of the metal ions.
In the positive ion mode in ESI a singly bonded fullerene dimer of the form (crown)–C60–C60–(crown)M+ is observed. Its formation during the electrospray process via a radical mechanism is confirmed by variation of the experimental conditions.

Novel Aspects
Five different ways to ionise the ce/f conjugates have been developed. Relative alkali metal ion affinities were established by MALDI-PSD experiments. The unprecedented formation of a singly bonded fullerene dimer during the electrospray process has been studied.

References
1. S. R. Wilson, Y. Wu; J. Chem. Soc., Chem. Commun., 9, 784-786 (1993).
2. M. D. Tzirakis, M. Orfanopoulos; Angew. Chem. Int. Ed., 49, 5891-5893 (2010).
3. M. T. Rodgers, P. B. Armentrout; Mass Spec. Rev., 19, 215-247 (2000).

TPS41-11 / Ligand-Sphere Chemistry and Sphere-Sphere Interactions of Negatively Charged Alkoxylated Fullerenes
Rolf W. Kirschbaum, Thomas Drewello
Friedrich-Alexander-University Erlangen-Nuremberg

Introduction
The addition of oxygen nucleophiles to C60 has been amongst the earliest methods of fullerene derivatisation. However, alkoxylated fullerenes are less well characterised than other fullerene derivates, as undirected multiaddition has often complicated the product assignment.
Only recently, efforts have been made to develop the direct addition of oxygen nucleophiles into a preparative approach, thereby demanding a better understanding of the underlying reaction mechanisms.

Methods
The reaction of C60 with alkoxides (using MeOH, EtOH, 2 PrOH or BnOH) in toluene/alcohol solution was followed by negative-ion ESI-MS. Product analysis involved collision-induced dissociation (CID) experiments using a quadrupole ion trap and a Q TOF mass spectrometer.

Results
The reaction leads to only few products including species of the type [C60(OR)n]− (n = 1, 3, 5) and a dimeric [C120O(OR)7]−. The CID behaviour is particularly interesting as the organic ligands are not only simply stripped off during activation, instead hydrogen transfer may occur to the fullerene in order to avoid unfavoured electronic configurations of the anions. The even-electron adducts [C60(OR)n]− do not only form odd-electron species (n = 0, 2, 4), but are accompanied by their hydrogenated even-electron derivatives [C60H(OR)n]−.
CID-based structure elucidation of the dimeric [C120O(OR)7]− ion reveals a furanoid-bridged C60 dimer with four ligands on the neutral and three ligands on the charge-carrying sphere, [(OR)4C60OC60(OR)3]−. The furanoid bridge is a frequently occurring feature in fullerene dimers, and the amazingly selective formation of the dimer follows the same addition pattern as in [C60(OR)5]−. The use of EtOH gives rise to additional and unprecedented fullerene aggregates of the type [C120(OEt)8]2− and [NaC120(OEt)8]−. Analysis reveals two directly connected fullerenes carrying four ligands and one charge(!) each. The sodiated species is the salt bridge-stabilised fullerene dianion.

Conclusions
The formation of alkoxylated fullerene anions in ESI is particularly selective. Depending on the nature of the alkoxy group, alkali metal ions can stabilise the bond between two negatively charged fullerne moieties (salt bridge) similar to the well known oxygen bridged fullerene dimers.
Hydrogen transfer to the fullerene occurs during CID of the anions in order to reach a favoured electronic configuration. This CID behaviour is observed for all identified fullerene species to a significant extent.

Novel Aspect
The CID behaviour of alkoxylated fullerene anions has been studied for the first time. Hydrogen transfer competes with ligand loss leading to favoured electronic configurations. Novel dimeric and trimeric fullerene derivatives were discovered and structurally elucidated by means of mass spectrometry.

TPS41-12 / The sodium ion affinity sequences of ligated fullerenes, PCBM fullerenes and trimetallic nitride endohedral metallofullerenes
Jakob Hitzenberger, Marc von Gernler, Thomas Drewello
Friedrich-Alexander-University Erlangen-Nuremberg

Introduction
In this study, the relative sodium ion affinities of fullerene derivatives are probed using the well-established kinetic method. Based on gas phase experiments of pairs of functionalized fullerenes a conclusive order of affinities is established. The fullerene derivatives under study included malonated fullerene and PCBM derivatives with different chain lengths and up to three ligands attached to C60 and partly also to C70. Trimetallic nitride endohedral fullerenes of the type [Sc3N@PC81BM] and [Lu3N@PC81BM] were also investigated.

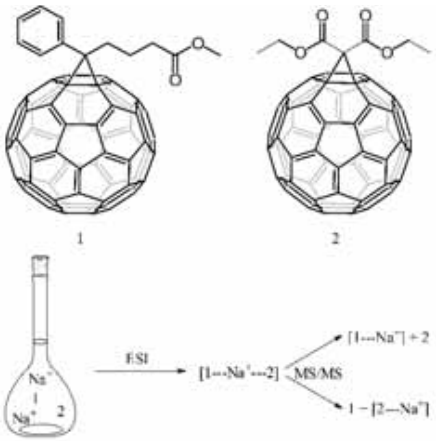


Figure 1: Experimental setup for the kinetic method with C_{60}-mono (1) and $PC_{61}BM$-mono (2).

Methods
Cooks' kinetic method has emerged as a powerful tool for the determination of thermodynamic affinities. This method can be applied to a wide range of systems and often works straightforwardly on a variety of different analyzers. The kinetic method is based on the competitive dissociation of hetero-dimeric complexes of two different ligands that are connected by a charge-

carrier, to which the affinity is to be measured. The affinity is evaluated through the competing dissociation of the adduct ion in MS/MS experiments. Properties like gas phase basicities and acidities or metal cation affinities are among the most popular quantities established by this approach.[1]

Results
The Na+ affinity rises with increasing oxygen content in the ligands. This is evident comparing PCBM and malonated species and by increasing the number of ligands. While the malonate adducts were isomerically pure, the PCBM bis- and tris- adducts were mixtures of isomers, which affected the conclusiveness of some of the PCBM data. In malonated fullerene derivatives, different ligands showed the expected shift in Na+ affinity as a function of the nature of the ligand and increasing fullerene size increased the affinity. Also for the two different endohedral complexes, their affinity appears to be influenced more by the C80 cage then by the nature of the endohedral trimetallic nitride cluster.

Conclusions
A conclusive order of relative Na+ affinities for a number of important fullerene derivatives has been established. The Na+ affinity is influenced the most by the nature and amount of the ligands and only to a lesser extent by the size of the fullerene, with no influence by the encapsulated guest.

Novel Aspects
The relative Na+ affinity of fullerene derivatives has been studied here for the first time. Unprecedented insight into the influence of nature and number of the ligands and the size of the fullerene has been obtained.

[1] R.G. Cooks, J.S. Patrick, T. Kothiaho and S.A. McLuckey, Mass Spectrom. Rev. 13(4) (1994) 278-339

TPS41-13 / Protection of Labile Phosphate Ester Groups by Metal Complexes Reveal Dependence on Charge and Size of Interacting Metals

Frank Kjeldsen, Simon Svane, Thomas,j.d. Jørgensen, Christine, J. McKenzie
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Introduction
Sequencing of phosphopeptides are hampered by the facile detachment of the phosphate group (e.g. as H3PO4), which complicate reliable determination of the phosphorylation site and peptide identification. We recently described the novel application of a digallium complex to reduce the otherwise facile detachment of H3PO4 from phosphopeptides during CAD fragmentation. In the present study, we aim at elucidating the reason behind this phenomenon of phosphate ester protection.

Methods
For each metal complex we evaluated the ability to reduce phosphate detachment, the selectivity towards phosphate in the presence of carboxylate anions and the thermodynamic constants for the interaction between metal complexes and phosphate. The selectivity of 2,6-bis((N,N'-bis(2-picolyl)amino)methyl)-4-tertbutylphenol (bpbp-) based dimetal complexes were evaluated by mixing 2.5 mM metal complex in 50% acetonitrile containing phosphoserine:serine in ratios of 1:1, 1:10 and 1:50 at RT for 2-3 h. The solutions were diluted to 2 µM before MS and MS/MS analysis (Orbitrap XL mass spectrometer, ThermoFisher) using resolving power of 30,000 in the positive off-line mode. Energy-resolved phosphate and backbone loss of metal complexes bound to FQpSEEQQQTEDELQDK in CAD were measured. Thermodynamic constants were obtained by isothermal titration calorimetry (VP-ITC microcalorimeter, MicroCal Inc.) at 30 °C.

Results
Complexes differing only by the identity of the metals (Ga2, In2, Fe(III)Fe(II), Zn2, Cu2, (VO)2, FeZn, FeGa, GaCu, GaZn, Co2) making up the binding site were used in this study. Nearly 100% selectivity towards the phosphate group in phosphoserine was found for the three complexes of [LGa2]5+, [LIn2]5+, and [LFe(III)Fe(II)]4+ even at 50 times excess of the competing serine amino acid. For the remaining lower charged metal complexes a lesser degree of selectivity for the phosphate group was found as the phosphate/carboxylate ratio decreased. CAD energy profiles for metal complexes with FQpSEEQQQTEDELQDK demonstrated a rate of phosphate loss that was >10 times larger than that of backbone cleavages for all complexes but the gallium complex. It was expected that complexes of the same charge produced roughly the same affect, but even the [LIn2]5+ complex lost abundantly the phosphate group from the peptide. Unexpectedly, the [LIn2]5+ complex was found to be retained on the peptide after the phosphate loss. Using both dephosphorylated and methylated phosphopeptide analogues showed that while [LIn2]5+ initially coordinates to the phosphate group it is re-coordinated to carboxylate groups upon CAD activation. This observation was not found with [LGa2]5+ probably because of the reduced bite angle of the Ga-complex. Interestingly, while conjugated to the methylated phosphopeptides, [LIn2]5+ reduced the phosphate loss to the same level as proven by the complex of [LGa2]5+.

Conclusion
Data support that charge and size of the metal complex is of paramount importance for the phosphate ester protection of phosphopeptides.

Novel aspect
Metal complexes reveal previously unknown feature of phosphate ester protection in CAD fragmentation of phosphopeptides.

TPS41-14 / Probing the Mechanism of One-pot Synthesis of the Benzopyranopyrimidines by ESI-MS

Davila Zampieri[1], Bruno R. Vilachã Ferreira[2], Pedro H. Vendramini[2], Bruno R. S. de Paula[3], Paulo J. P. Moran[3], Marcos N. Eberlin[2]
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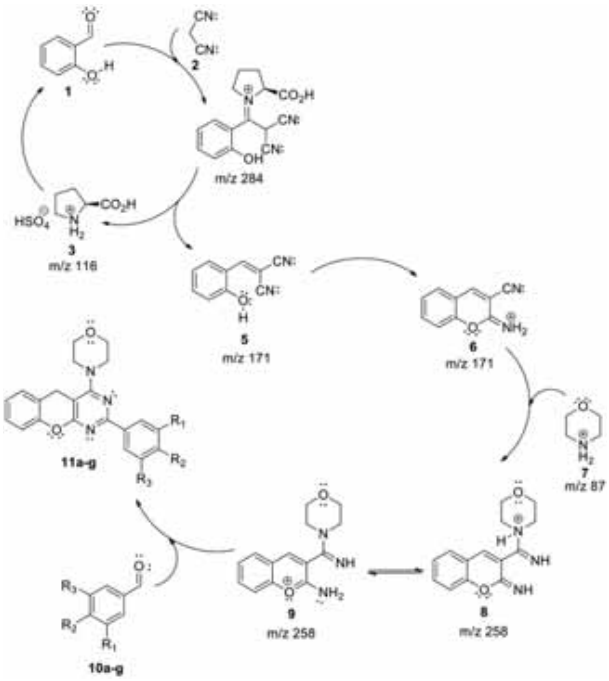
Introduction
Electrospray ionization mass spectrometry (ESI-MS) has become a technique of choice for mechanistic studies and high-throughput screening of homogeneous catalysis [1]. The development of ESI-MS has greatly expanded the range of molecules that can be analyzed, including those of high polarity, molecular mass and structural complexity [1].
Multi-component reaction protocol with environmentally benign solvents and catalytic systems is there for one of the most suitable strategies, which meets the requirements of green aspects of chemistry for developing libraries of medicinal scaffolds [2]. The condensed heterocyclic systems are of considerable interest not only because of their potential biological activity, but also due to their versatility as synthons in organic transformations [2]. This work describes the intermediates intercepted by ESI-MS of the one-pot synthesis of benzopyranopyrimides that promotes step and atom-economy, and avoid the use of chemicals auxiliary.



Scheme 1. Intermediates/products intercepted and characterized by ESI(+)-MS(MS).

Methods

A stirred mixture of salicylaldehyde 1 (2.0 mmol), malonitrile 2 (1.0 mmol), L-prolinium sulfate 3 (0.3 mmol) in ionic liquid 1000 µL. After 2 hours was added morpholine 7 (1.0 mmol) and substituted aldehydes (1.0 mmol). The resulting mixture was stirred at room temperature for appropriate times. Then the resulting mixture was treated with 1000 µL of acetonitrile with traces of formic acid. All intermediates and products have been characterized by ESI(+)-MS(MS) FT ICR.

Results

Our investigation began with the on-line monitoring by ESI(+)-MS(MS) "ion fishing" of the Knoevenagel condensation with 1, 2, 3 in ionic liquid, followed by subsequent Pinner reaction 5→6 (m/z 171) at room temperature. Next, the cyano group of intermediate 6 can be attacked by the amine 7 to produce intermediates 8 and 9 (m/z 258). Ion 9 is the key intermediates of the second reaction cycle. Amine 9 reacts with other substituted aldehydes 10a-g, having electron withdrawing and donors groups, leading to the formation of the benzopyranopyrimides 11a-g. (Scheme 1).

Conclusions

ESI(+)-MS(MS) monitoring of the second step, we have intercepted the intermediates m/z 258. The detection of this key intermediate made us suggested a new mechanistic proposal for the synthesis of the benzopyranopyrimidines.

Novel Aspect

Generally, mechanistic studies are done by ESI-QTOF-MS due to high resolution. However, the FT ICR is also useful tool for this purpose, leading to the characterization and elucidation of an intermediate not yet described in the literature for this sort of reaction.

[1] Eberlin, M.N. Eur. J. Mass Spectrom., 2007, 13, 19-28. [2] Rai, U. S.; Isloor, A. M.; Shetty, P.; Vijesh, A. M.; Prabhu, N.; Isloor, S. Thiadeeswaran, M. Fun, H-.K. Eur. J. Med. Chem., 2010, 45, 2695-2699.

TPS41-16 / Effect of Tyrosine Position on the Fragmentation Reactions of b3 Ions from Model Tripeptides

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Izmir Institute of Technology

Introduction

Upon fragmentation of singly- or multiply-protonated peptides, series of sequence informative N-terminal b and a and/or C-terminal y ions are mainly formed via amide bond cleavage. Further fragmentation of bn ions leads to the generation of the corresponding an ions via CO (28 Da) loss. They are generally appeared as the base peak in the dissociation of bn ions. Contrary to this fact, the a3 ions are rarely observed in the CID mass spectra of peptides. In the present work, we have systemetically examined the effect of tyrosine position on the fragmentation reaction of simple b3 ions via low-energy CID.Of particular interest, a possible formation of stable a3 ion is studied in the CID mass spectra of b3 ions.

Methods

The study utilized C-terminal amidated isomeric model tripeptides, namely YGG-NH2, GYG-NH2, and GGY-NH2. The CID mass spectra for b3 ions were collected using a LTQ XL linear ion-trap mass spectrometer. For constructing the breakdown graphs and the precursor ion scan experiments, a hybrid triple quadrupole/linear ion trap instrument-4000 QTRAP was used.

Results

The b3 ion CID-MS3 spectrum of GGY-NH2 shows a significant intensity for a3 ion at m/z 250 (approximately 30 %). In contrast, the relative intensities of a3 ions are not obvious (below 4%) for b3 ions of YGG-NH2 and GYG-NH2. The bulky nature of tyrosine residue's side chain, located at the C-terminal end, may stabilize the gas-phase structure of a3 ion. The breakdown graphs were constructed in order to evaluate the energy dependence of the products ions on the applied collision energy. Additionally, the m/z 136 ion (Yimm) is detected as a common peak in all b3 ion CID-MS3 spectra. Precursor ion scan experiments have been carried out to explore the probable sources for the formation of Yimm ion.

Conclusions

The fragmentation reactions of b3 ions from simple tripeptides have been clearly affected by the position of tyrosine residue within the sequence, in particular the intensity of a3 ion significantly enhanced when the tyrosine is positioned at the C-terminal. This aspect can be explained by stabilizing effect of tyrosine side chain, at least two glycine containing tripeptides.

Novel Aspect

The position of tyrosine residue in the tripeptide sequence plays an important role for observing a stable a3 ion through the fragmentation of b3 ions.

TPS41-17 / Collision induced decomposition of AOT5Yb2+: an unexpected intracluster rearrangement

Leopoldo Ceraulo, Serena Indelicato, David Bongiorno, Vincenzo Turco Liveri
University of Palermo

Recently we studied the self-assembling of sodium bis(2-ethylhexyl)sulfosuccinate (AOTNa), one of the most popular surfactant yielding reverse micelles in apolar solvents, by electrospray ionization mass spectrometry (ESI-MS), energy-resolved mass spectrometry (ER-MS), infrared multiphoton decomposition (IRMPD), ion mobility mass spectrometry and

molecular dynamics simulations (MD). Now we present a study of the singly positively charged aggregates resulting from ESI of solutions containing (AOT)3Yb by mass spectrometry (ESI/MS), tandem mass spectrometry (ESI/MS/MS) and energy-resolved mass spectrometry (ER/MS). The collision induced decomposition of the most abundant ion [AOT5Yb2]+ involves an intracluster rearrangement.

Methods

(AOT)3Yb solution were prepared by mixing appropriate amounts of an aqueous solution of Yb(NO3)3 with a 10-2 M aqueous solution of AOTNa. After a reaction time of at least 2 days, the precipitate was filtered, washed several times and vacuum-dried at room temperature.
ESI experiments in positive ion mode of (AOT)3Yb were made on q-ToF LC/MS system (Waters q-Tof Premier). Accurate mass measurements were obtained using Q-Exactive Thermo Scientific mass spectrometer.

Results

The structure, stability and fragmentation patterns of AOT-Yb and AOT-Yb-Na aggregates were studied by collision induced decomposition (CID) experiments. As representative species of AOT-Yb we studied [AOT5Yb2]+.
In order to establish the fragmentation behavior of AOT-Yb aggregates, several MS experiments were made using increasing values of collision energy.
The collision induced fragmentation experiments of [AOT5Yb2]+ have been performed by selecting the ion at m/z 2447 as precursor, that is the most abundant isotopic species of this cluster. At the lowest collision energy (laboratory frame energy 15 eV), the spectrum shows only a peak at m/z 1913, that remains the only fragment observed up to 25 eV and whose formation involves the loss of a 534 Da neutral species.
In order to achieve information on its elemental composition, the CID-MS/MS spectrum of [AOT5Yb2]+ ion (m/z 2447.00) has been recorded also on a Q-Exactive instrument. The accurate mass measurement evidenced a loss of a neutral fragment of 534.35894 Da, that agrees with the formula C28H54O7S (difference -0.2 ppm). This implies a whole loss of AOT-C8H17 as neutral specie. Two possible mechanisms that can rationalize this fragmentation process will be discussed.

Conclusions

The loss of AOT-C8H17 from [AOT5Yb2]+ ion and it is due to an intracluster rearrangement involving two AOT moieties. This behavior has been rationalized by two possible fragmentation mechanisms.

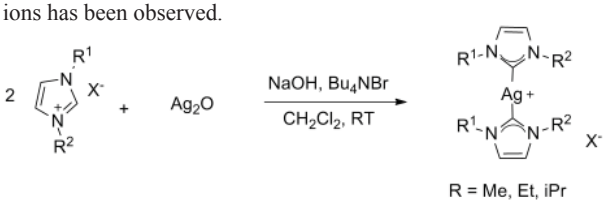
Novel Aspects

The loss of AOT-C8H17 has not been observed neither for AOTNa or AOT alkali metal ions, nor for AOT divalent cations aggregates. This could be due to both a particular geometric assembling of [AOT5Yb2]+ and a "catalytic" effect of the Yb3+ ion.

TPS41-18 / Synthesis and characterization of silver(I)-NHC complexes. Mass spectrometrical studies of relative bond dissociation energies.

Melanie Schmidt, Marianne Engeser
University of Bonn Kekulé-Institute for Organic Chemistry and Biochemistry

Dinuclear silver complexes ([Ag(MeIm)2]+SO4CH3- (MeIm ≙ 1,3 dimethyl imidazol 2 ylidene), [Ag(MeEtIm)2]+Br-, [Ag(EtIm)2]+Br-, [Ag(MeiPrIm)2]+Br-, [Ag(EtiPrIm)2]+Br-, [Ag(iPrIm)2]+Cl- and [Ag(iPrIm)2]+BF4-) have been synthesized starting from 1,3 dialkyl-imidazolium salts and AgO2 under basic phase tranfer catalysis conditions.They have been characterized by NMR-, UV/Vis- and IR- spectroscopy and ESI mass spectrometry. Most molecular structures were confirmed by X ray crystallography. A significant influence of different counter ions has been observed.



R = Me, Et, iPr

Heteroleptic complexes with two different ligands, prepared in situ by mixing solutions of homoleptic complexes, have been studied by ESI-MS/MS using CID and IRMPD. The fragmentation predominantly yields monoligated complexes.
Their relative abundances have been analysized with Cooks kinetic method to obtain relative bond dissociation energies. Every possible mixing combination was tested and a self consistent and very plausible stability trend depending on alkylchain length has been obtained.

TPS41-19 / Effect of Basic Amino Acid Residues on the Charged Separation Reactions of Doubly-Protonated Model Heptapeptides

Özge Görgün, Talat Yalcin
Izmir Institute of Technology

Introduction

Peptides that include basic amino acid residues have tendency to form multiply-protonated ions easily. Tryptic peptides generally contain lysine (K) or arginine (R) at their C-terminal end. It is known that multiply-protonated peptides mainly produce y ions. In here, a systematic study has been carried out using model peptides containing basic amino acids (H, K, R). Formation of complementary singly-protonated y and b ions, which called symmetric cleavage, was observed when the basic amino acid residue approaches to the C-terminal.

Methods

The study utilized two sets of model heptapeptides, namely XYAGFLV-NH2, YXAGFLV-NH2, YAXGFLV-NH2, YAGXFLV-NH2, YAGFXLV-NH2, YAGFLXV-NH2, XYAGFLVX-NH2 and XAAAAAA-NH2, AXAAAAA-NH2, AAXAAAA-NH2, AAAXAAA-NH2, AAAAXAA-NH2, AAAAAXA-NH2, AAAAAAX-NH2 where X is H, K, or R. All peptides are C-terminal amidated rather than free acids. The doubly-protonated ([M+2H]++) CID mass spectra were collected using a LTQ XL linear ion-trap mass spectrometer.

Results

The [M+2H]++ CID mass spectra of XYAGFLV-NH2, YXAGFLV-NH2, and YAXGFLV-NH2 (X = H, K, or R) comprises of mainly b7++ and b6+ product ions. However, the [M+2H]++ CID mass spectra have become more complex when the basic residues are getting closer to C-terminal position. In particular case, the formation pathway of b2+/y5+ complementary ions have become a dominant ions in MS/MS spectra. The similar fragmentation behavior is also recorded for six alanine containing heptapeptides. This behavior can be explained by coulombic repulsion of the charges.

Conclusion

In this study, mainly b7++ and b6+ ions have been observed for the peptides series containing basic residues closed to N-terminal position. By contrast, when the basic residues are getting closer to C-terminal position, b2+/y5+ complementary ions have become

prominent in MS/MS spectra. It can be concluded that, when basic amino acid residue approaches to the C-terminal, symmetric cleavage, was observed for all peptides series containing basic amino acid residues.

Novel Aspect

Elucidation of fragmentation mechanisms of doubly-protonated peptide ions containing basic amino acid residues namely His, Lys, Arg, on the model heptapeptides have been investigated.

TPS41-20 / Probing the Mechanism of Brønsted Acid Catalyzed Azlactone Ring Opening by ESI-MS

Bruno Ferreira[1], Adriane A. Pereira[2], Pedro P. de Castro[2], Amanda C. de Mello[2], Marcos N. Eberlin[1], Giovanni W. Amarante[2]

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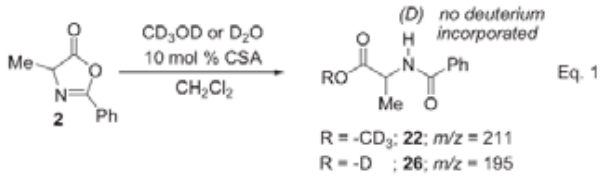
Introduction

Within the past decade, electrospray ionization mass spectrometry (ESI-MS) has rapidly occupied a prominent position for mechanistic studies due to its intrinsic advantages allowing for efficient "fishing" of multiple intermediates and products directly from a "real-world" solution.1

Azlactones rings have been largely explored because of their particularities: (i) they are protected amino acids, which can be further used in the synthesis of many natural products. (ii) they are excellent pronucleophiles providing complex structures after addition to electrophiles. Amarante and co-workers have explored the potencial of azlactones as pro-nucleophiles in the presence of Michael acceptors by using an organocatalytic approach.2



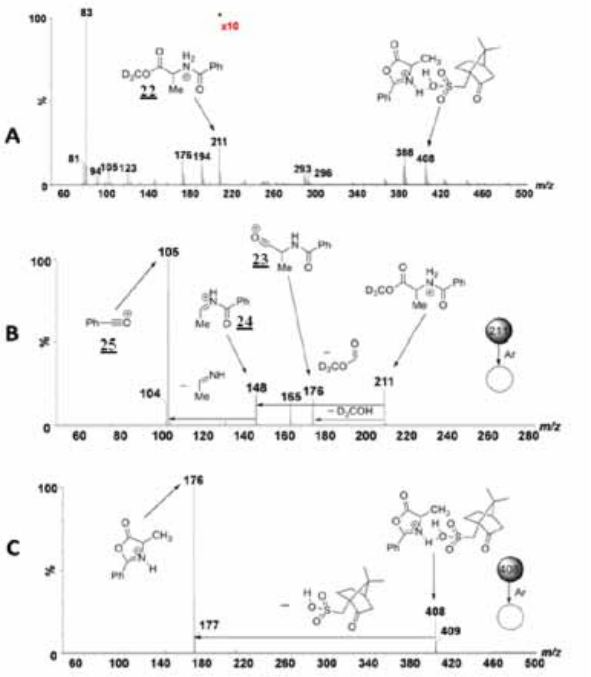
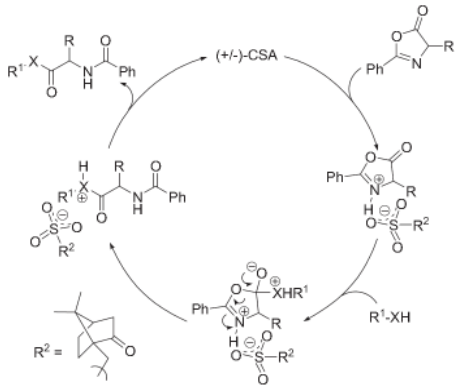


Fig. 2. (A) ESI(+)-MS of the azlactone **2** and CD_3OD reaction solution in the presence of CSA as catalyst; (B) ESI(+)MS/MS of the ion of *m/z* 211; (C) ESI(+)-MS/MS of the ion of *m/z* 408.



Scheme 3. Mechanism hypothesis for the azlactone activation following by nucleophilic attack.

Methods

The reactions of opening of the azlactone ring by lipophilic alcohols and amines were carried out using 0.2 mmol of azlactones, 0.02 mmol of CSA (10 mol%) and 0.21 mmol of nucleophile (0.2 M in azlactone).

Results

The (+/-)-camphorsulfonic acid (CSA) functions as an effective Brønsted acid catalyst for the azlactone ring via nucleophile attack. Two experiments were performed to intercept potencial intermediates involved in the catalytic cycle. First, after 5 min of reaction, an aliquot was taken from the crude mixture of azlactone 2 and CD3OD in the presence of 10 mol% of CSA, diluted in acetonitrile, and directly analyzed by ESI(+)-MS. An ion of m/z 211 corresponding to the final product 22 [22+H]+ was intercepted. Its CID showed fragment ions of m/z 176, 148 and 105, which were attributed to 23, 24 and 25, respectively. Another key species of m/z 408 corresponding to the association of the azlactone and the catalyst, that is, [2+CSA+H]+ was also intercepted. In the second experiment, D2O was used as the nucleophile. The two ions [2+CSA+H]+ of m/z 408 and [26+H]+ of m/z 195 were intercepted.3

Conclusion

Mechanism investigation via ESI(+)-MS/MS revealed azlactone activation by the catalyst CSA to form an ion pairing intermediate, following by nucleophilic attack. No H/D exchange occurs when D2O is used, suggesting that CSA is responsible for the protonation and de-protonation steps.

Novel Aspect

The key intermediates for the opening of the azlactone ring were intercepted and characterized by ESI(+)-MS(/MS).

1 D. Schröder, Acc. Chem. Res. 2012, 45, 1521.

2 Ávila, E. P.; de Mello, A. C.; Diniz, R.; Amarante, G. W. Eur. J. Org. Chem. 2013, 1881.

3 Pereira, A. A.; de Castro, P. P.; de Mello, A. C.; Ferreira, B. R. V.; Eberlin, M. N.; Amarante, G. W. Tetrahedron 2014, 70, 3271.

TPS41-21 / Laser-Induced Hydrogen Radical Removal in UV MALDI-MS Allows for the Differentiation of Polyphenol Isomers

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Suntory Institute for Bioorganic Research

Introduction

Polyphenols showed irregular peaks beside the deprotonated molecules in matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). We were interested in the MALDI ionization phenomena.

Methods
Dihydroxybenzoic acids (DHBs) and flavonoids and their glycosides were analyzed as polyphenol analytes. MALDI-MS and MS/MS spectra were acquired with an instrument of Ultraflex III MALDI-TOF/TOF mass spectrometry (Bruker Daltonics, Germany).

Results and Discussion
2,5-DHB is well known as an excellent matrix in MALDI-MS. In negative-ion mode MALDI-MS, a 2 Da reduced peak from DHB molecule was observed beside the deprotonated molecule ([M – H]–), suggesting that a hydrogen radical removal (H*) produced the irregular peak [M – H* – H]–. The property of phenolic hydroxy group causes the loss of hydrogen radical. We compared the structure isomers of dihydroxybenzoic acids (DHBs) and the the irregular peak [M – H* – H]– from 2,5-DHB was the most abundant among DHB isomers.[1] We predicted that the hydrogen radical removal from polyphenol compounds because they have phenolic hydroxy groups.
Negative-ion MALDI TOF MS and MS/MS spectra of flavonoid mono-O-glycosides showed the irregular signals that were 1 and/or 2 Da smaller than the parent deprotonated molecules ([M – H]–) and the sugar-unit lost fragment ions ([M – Sugar – H]–). The 1 and/or 2 Da mass shifts were generated with the removing of a hydrogen neutral radical (H*) and with the homolytic cleavage of the glycosidic bond such as [M – H* – H]–, [M – Sugar – H* – H]–, and [M – Sugar – 2H* – H]–. It was revealed that the hydrogen radical removes from the phenolic hydroxy groups on the flavonoids, not from the sugar moiety. The extents of the hydrogen radical removals depend on glycosyl positions. Flavonoid mono-glycoside isomers were distinguished according to their TOF MS and tandem mass spectra.[2]

Conclusion
Hydrogen radical removals occurred in negative-mode MALDI-MS of polyphenols because they were generated from phenolic hydroxyl groups. The hydrogen radical removals of DHBs and flavonoid mono-glycosides depended on their structures and the phenolic hydroxyl group positions. Their polyphenol isomers were distinguished according to their TOF MS and tandem mass spectra.

Reference
[1]Tohru Yamagaki, Takehiro Watanabe, Mass Spectrometry 2012, 1, A0005. DOI: 10.5702/massspectrometry.A0005
[2]Tohru Yamagaki, Takehiro Watanabe, Masaki Tanaka, Kohtaro Sugahara, J. Am. Soc. Mass Spectrom. 2014, 25, 88-94.

TPS41-22 / Structure and energy dependent ion isomerizations of folates detected using ER-IMS/MSn
Yayoi Hongo[1], Hiroyuki Koshino[1], Shunya Takahashi[1], Takemichi Nakamura[1], Takae Takeuchi[2]
[1]RIKEN, [2]Nara Women's University

Introduction
Folates are substances containing a conjugated form of pteroic acid with L-Glu. Folic acid is one of a typical folates and an important class of water soluble B-vitamins. Mass analyses are indispensable to determine and characterize folates in food and blood etc. The MS/MS feature of folates is also significant on the quantitative trace analysis using selective reaction monitoring and the detection of related compounds. However, the fragmentations of folates are not always explained by simple cleavages of the linear structures due to the ion isomerization during the collisional activation. In those cases, reliability of the structure elucidation using the MS/MS technique may be reduced. Recently, ion isomerization through the ion activation can be detected by commercially available Q-TOF devise, if the isomers were separated in the drift cell. We have conceptually presented and demonstrated a new technique, energy-resolved ion mobility tandem mass spectrometry 'ER-IMS/MSn', to probe changes of the ion shape (isomerization) with subsequent ion dissociation. The 'ER-IMS/MSn' potentially provides us additional information for the folate characterization with low energy CID.

Methods
We used Synapt G2 (Waters) to conduct the ER-IMS/MSn. In the experiment, a targeted precursor was isolated by a Q-pole and conducted collisional activation in CID cell prior to ion mobility separation with the physical ion shapes. Ion mobility separated ions can be activated subsequent CID.

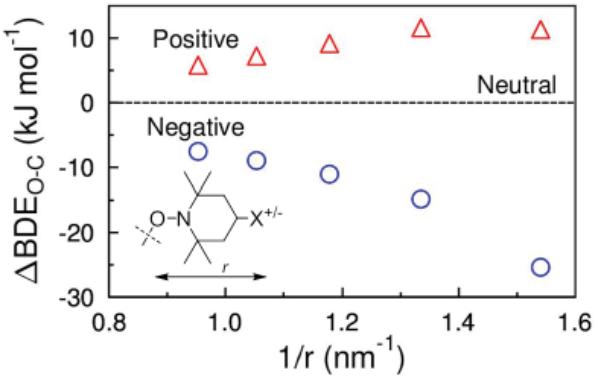
Results
The ER-IMS/MSn on the deprotonated folic acid ([M-H]- at m/z 440) gave the characteristic product ion at m/z 311 which was assigned to the loss of Glu unit through the rearrangement at the carbonyl group of p-aminobenzoyl unit. The isomerization of precursor depended on the laboratory collision energy settings. The fraction detected at the earlier drift time (smaller isomer) corresponding to the characteristic Glu loss via five-membered ring formation at p-aminobenzoyl glutamic acid. The larger size fraction appeared with increasing collision energy settings and fragmented into m/z 378 which was absent in the MS/MS spectrum of the smaller isomer. These observations indicated that the relatively stable ion structure of [M-H]- was favorable to rearrange. Furthermore, CO2 loss (m/z 396) from [M-H]- also resulted in isomer formations to succeed each different fragmentations.

Conclusion
Fragment ion peaks corresponding to such a small neutral losses provide less structural information in a conventional MS/MS, however the observation of isomerization can be a new means to characterize the molecular structure. Our new technique to acquire the structural information using ER-IMS/MSn provides an additional dimension to consider the ion fragmentation.

Novel Aspect
Fragmentation pathways including isomeric ion formation using ER-IMS/MSn were informative to characterize folates.

TPS41-23 / Probing the effect of charge location and polarity on energetics of gas phase distonic ions
David Marshall[1], Ganna Gryn'ova[2], Michelle Coote[2], Stephen Blanksby[1]
[1]Queensland University of Technology, [2]Australian National University

Introduction
Distonic ions possess a charge and radical centre separated by the molecular framework, and are used as gas-phase probes for the reactivity of elusive neutral radicals. Combining tandem mass spectrometry and high-level quantum chemical calculations, herein we assess the applicability of this comparison with reference to persistent aminoxyl radicals - commonly employed as antioxidant polymer stabilisers and mediators for controlled radical polymerisation. Specifically, the kinetic method is employed to evaluate the effect of an aminoxyl radical on the gas-phase acid/base behaviour of a remote charge-carrying moiety. These data uncover the cause, nature, and scope of an unprecedented charge-radical interaction.



Methods
Proton-bound hetero-dimers for kinetic method analysis were prepared by combining analyte pairs in methanol (3-10 μM) and infusing directly into the electrospray ionization source of a Waters QuattroMicro triple quadrupole mass spectrometer. Argon is used as the collision gas (3.0 ± 0.1 mTorr) in collision-induced dissociation experiments. Rigorous computational assessment was carried out at multiple benchmarked levels of theory, from DFT to high-level composite methods. The results are shown to be largely method-independent.

Results
Gas phase acidities (GPAs) of carboxylic acids are influenced by the presence or absence of a nitroxyl radical. The GPAs of the aminoxyl radical 4-carboxy-TEMPO (CT•) and analogous closed-shell alkoxyamines (CT-R) are determined by the kinetic method to vary by as much as 20 kJ/mol.[1] Proton-bound dimers of CT• and CT-R were subjected to collision-induced dissociation. Relative and absolute GPAs confirm the increased acidity of carboxylic acids in the presence of an aminoxyl radical, and by extension, the greater stability of aminoxyl radicals in the presence of carboxylate anions. The magnitude of stabilisation decreases as the carboxylate and aminoxyl are further separated by rigid spacers, but remains measurable beyond 8 Å. When the study is repeated on 4-amino-TEMPO and its alkoxyamine derivatives in the positive ion mode, the results obtained from the kinetic method confirm that a remote positive charge has a destabilising effect on aminoxyl radicals. However, the effect is smaller compared to that observed in analogous anions. Radical ion (de)stabilisation results from a superposition of conventional polar effects and an unprecedented through-space Coulombic interaction between the charge and polarisable aminoxyl radical. [2]

Conclusions
Certain gas-phase distonic ions exhibit lower proton affinities than closed-shell analogues. By corollary, an ostensibly remote charge manipulates bond dissociation energies elsewhere in the molecule (see Figure). Radical behaviour in distonic ions is tunable by charge polarity and proximity.

Novel Aspect
Distonic ions can exhibit distinctive radical behaviour depending on the proximity and polarity of the charge.

[1] G. Gryn'ova, D. L. Marshall, S. J. Blanksby, M. L. Coote, Nature Chem., 5, 474-481 (2013); [2] G. Gryn'ova, M. L. Coote, J. Am. Chem. Soc., 135, 15392-15403 (2013)

TPS41-24 / Heterolytic N-C Cleavage to the N-terminal Side of the Aminoketyl Radical in ECD/ETD
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Ecole Polytechnique Fédérale de Lausanne

Introduction
A plethora of mechanistic proposals exist that attempt to rationalize peptide and protein fragmentation patterns in ECD and ETD experiments. Given the widespread use of these two electron-based MS/MS techniques, elucidating and understanding their underlying mechanisms is clearly beneficial. Until recently, each of the major mechanistic proposals uniformly included C-terminal side aminoketyl radical fragmentation.

Methods
A set of model tryptic peptides, AnK (n=3-5) and G5K, was modelled computationally. Amber11 was used for replica exchange molecular dynamic simulations. Transition state structures and associated enthalpies were computed at B3LYP-dDsC/6-311++G(2d,p)//B3LYP/6-31G(d) level. Additional optimizations were done at the ωB97X-D/6-31G(d) level.

Preliminary results
In a proof-of-principle computational study (J. Phys. Chem. B, 2012, 116, 10807), we recently demonstrated that heterolytic N-terminal side cleavages are also kinetically viable (the "enol" pathway). Therefore, these cleavages were suggested to be charge driven, as opposed to radical driven, fragmentations of distonic radicals, contrary to other popular mechanistic proposals. Our more recent study, using a robust and unbiased search algorithm to identify low-energy conformers, reveals that both C- and N-terminal side cleavages persist in conformers that likely exist in experimental conditions. The relative C-/N-terminal ratios also appear unaffected by precursor ion energy level, lending additional credibility to its existence. Such findings are important for studies that attempt to rationalize relative product abundances in ECD/ETD mass spectra (e.g., J. Phys. Chem. A 2014, 118, 308-324). Finally, the removal of charge separation that accompanies heterolytic N-Cα bond cleavage in the enol mechanism is explored by examining several pathways involving proton transfers within the vicinal H-bonding network that stabilize the resultant ion-molecular complex (J. Phys. Chem. B, 2014, 118, 2985). Preliminary comparison of the A5K v G5K conformer datasets indicates that the transition state of the latter for the enol pathway is less stable and, hence, heterolytic backbone cleavage is less favourable. This is in line with the proposed transient formation of an unstable primary carbocation on glycine.

Conclusions
Our computational study of model tryptic peptide systems indicates that gas-phase fragmentation of radical peptide polycations via electron capture is likely governed by competitive radical driven and charge driven mechanisms. The latter is strongly dependent on the local conformation due to the role of proton transfers in peptide backbone fragmentation, with three common H-bonding motifs identified for all conformers under consideration.

Novel aspect
Study indicates the involvement of H-bonding networks in charge-driven fragmentation of distonic radicals.

TPS41-25 / Formation of benzyl carbanion in collision-induced dissociation of deprotonated phenylalanine homologues
Kanako Sekimoto, Natsuki Matsuda, Mitsuo Takayama
Yokohama City University

Introduction
Measurements of α-amino acids, the important constitutive units of various organic macromolecules, have been performed via collision-induced dissociation mass spectrometry (CID-

MS). It has been reported that CID of protonated amino acids can be interpreted simply by the loss of specific neutral species such as NH3, H2O and CO2H2, which depends on the various side chains. In contrast, deprotonated amino acids fragment irregularly to lose the above neutral species, although the aliphatics retain a characteristic preference for CO2H2 loss. Here we have investigated the fragmentation behavior of deprotonated L-phenylalanine (Phe) and its homologues, L-homophenylalanine (HPA) and L-phenylglycine (PG), using atmospheric pressure corona discharge ionization CID-MS, and found the crucial factors governing the fragmentations of deprotonated Phe homologes.

Methods
Mass spectra were acquired on a TSQ7000 triple-quadrupole mass spectrometer (Thermo Fisher Scientific). The corona discharge ion source used contained a needle with the radius of tip curvature of ca. 1 μm and metallic orifice plate in laboratory ambient air with relative humidity of 40–70 % at 298 K. The discharge conditions used were 3 mm in the point-to-plane electrode gap, 90° in the needle angle with respect to the orifice central axis, and -1.9 kV in the DC corona voltage. A ceramic micro-heater was placed between the needle and the orifice plate in order to vaporize condensed-phase analytes used. The collision gas and energy (lab.) for CID were argon at 2.2×10-3 torr and 5–25 eV, respectively.

Results and Conclusions
Deprotonated analytes [M–H]- (M: Phe HPA and PG) fragmented to lose unique neutral species, e.g., the loss of NH3, CO2, toluene and iminoglycine for [Phe–H]-; styrene and ethenamine/CO2 for [HPA–H]-; and CO2 for [PG–H]-. The observed fragmentation behavior leads to the following conclusions:
1. The principal factor governing the fragmentation behavior of the deprotonated Phe homologues [M–H]- is the stability of the structure of intermediates and/or product ions. That is, all the precursor ions [M–H]- dissociate to form a carbanion at the benzyl position, having a resonance-stabilized structure, via proton rearrangement through a transition state and/or via simple dissociation reactions.
2. If a given precursor ion [M–H]- is capable of generating a benzyl carbanion via both proton rearrangement and a simple dissociation reaction, the proton rearrangement proceeds preferentially as compared to the simple dissociation reactions.
3. If there are some different proton rearrangement pathways generating benzyl carbanions in the CID of a certain [M–H]- ion, the pathway originating from the position at which a proton can be released less energetically proceeds under lower collision energy conditions.

Novel Aspect
All of the fragmentations of deprotonated Phe homologues are attributable to the formation of benzyl carbanions having resonance-stabilized structures.

TPS41-26 / Study of small neutral losses and ion rearrangements on protonated bunodosine 391 and IAA(indole-3-acetic acid)-amino acid conjugates

Hiroyuki Koshino[1], Yayoi Hongo[1], Naomi Muto[1], Shunya Takahashi[1], Kohei Kazuma[2], Katsuhiro Konno[2], André J. Zaharenko[3]
[1]RIKEN, [2]University of Toyama, [3]Butantan Institute

Introduction
In the molecular characterization using low-energy CID, intense small neutral losses reduce the structural information of a target ion. Ion rearrangements also disturb the characterization of an original linear ion structure. Ion rearrangements were necessary to consider the mechanism to give neutral losses (CH2O2, NH3, CO) from protonated bunodosine 391, N-(6-bromo-1H-indol-3-yl)acetyl-L-histidine, which was isolated from the venom of the Brazilian sea anemone Bunodosoma cangicum.1 If the correlation between the chemical structure and its fragmentation pathways was revealed, the feature of small neutral losses formed by low-energy CID could be more informative for the molecular characterization.

Methods
Experimentally to elucidate the mechanism of fragmentation pathways on bunodosine 391, MS/MS and MS3 experiments were performed using several structural analogs and IAA(indole-3-acetic acid)-amino acid (Trp, Tyr, Phe, etc) conjugates were synthesized for analysis. The accurate MS/MS, MS3, and energy-resolved experiments were conducted with Q-TOF-MS (Synapt G2 HDMS, Waters).

Results
Protonated bunodosine 391 ([M+H]+, m/z 391) fragmented into m/z 156 and 110 assigned to protonated histidine and loss of CH2O2 from m/z 156, respectively. Sequential neutral losses to form [M+H-CH2O2]+ (m/z 345), [M+H-CH2O2-NH3]+ (m/z 328), and [M+H-CH2O2-NH3-CO]+ (m/z 300) were confirmed by the MS3 experiments. The fragmentation into m/z 328 and m/z 300 from m/z 345 required ion rearrangements from the original linear amide structure. We proposed a cyclic lactam formation to give the sequential losses of NH3 and CO as reported for γ-lactam.2 A coupling reaction between the indole and His units should be necessary to cyclization. To investigate the effect of imidazole ring to the coupling reaction, N-methyl-His analogs were examined as well as bunodosine 391. Small neutral losses were shifted to 14 Da larger fragments in the spectrum of 1-N-methyl-His analog, indicating that 1-N-methyl of His did not affect the pathways. By contrast, the MS/MS experiment on 3-N-methyl-His analog gave fragments at m/z 361 [M+H-CO2]+, indicating that methylation at 3-N of His unit changed the pathways to form small neutral losses.

Conclusions
The fragmentation pathways on protonated bunodosine 391 including small neutral losses and ion rearrangements were investigated by MS/MS and MS3 experiments using several structural analogs of bunodosine 391 and IAA-amino acid conjugates. We would like to propose the possible fragmentation pathways on bunodosine 391 to form neutral losses via cyclic ion rearrangements by 6-endo-trig cyclization like Pictet-Spengler reaction or pericyclic reaction like Diels-Alder reaction.

Novel Aspect
Mechanism of small neutral losses via ion rearrangements with cyclization on protonated bunodosine 391 was discussed.

1. A. J. Zaharenko et al., J. Nat. Prod., 2011, 74, 378-382.
2. K. M. Morgan et al., J. Org. Chem., 2014, 79, 517-528.

TPS41-27 / Collision induced dissociation of Erythrinian Alkaloids in ESI-MS/MS: experimental and computational studies

Thais Guaratini[1], Denise Brentan da Silva[1], Norberto Peporine Lopes[1], João Luís Callegari Lopes[1], Ricardo Vessecchi[2]
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Introduction
The alkaloid erythroidine was isolated from Erythrina americana, showing an important curare-like activity. Currently, it is established the action of the erythrinian alkaloids by inhibition

of a4β2 nicotinic receptor, which allowed the development of pharmaceuticals for memory functions. However, up to now, there are no ADME studies. LC-MS/MS analyses are the current methodology to investigate metabolism of drugs. The fragmentation mechanism can be crucial for structural elucidation of metabolites. Thus, the aim of this work was to define the fragmentation mechanism of protonated dienoids erythrinin alkaloids supported by computational methods.

Methods

Alkaloids were isolated fromE. verna. High resolution fragmentation analysis were performed in a triple stage quadrupole-Orbitrap (LTQ-Orbitrap-XL, Thermo-Scientific, USA). Conditions: capillary voltage 3270 V, tube lens voltage -138.71 V, capillary temperature 275 °C, He as collision gas in CID. DFT calculations and optimization of most stable cations geometries: Gaussian 03 suite of programs at B3LYP/6-31+G(d,p).

Collision induced dissociation of Erythrinian Alkaloids in ESI-MS/MS: experimental and computational studies

Thais Guaratini, Denise B. Silva, Norberto P. Lopes, João L. C. Lopes, Ricardo Vessecchi

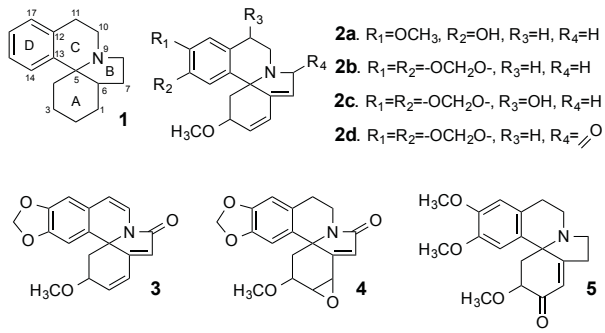

Figure 1: Basic moiety of erythrinian alkaloids (1). Analyzed compounds: erysovin (2a), erythralin (2b), erythrinin (2c), 8-oxo-erytralin (2d), 10,11-dihydro-8-oxo-erytralin (3), 1,2-epoxy-8-oxo-erytralin (4) and erytratidinone (5).

Results

Analysis of 2a and 2b confirmed the H2 mechanism in addition to elimination of 17u followed by 32u and 28u for 2a and 30u and 28u for 2b. The proposed mechanism for NH3 elimination requires no restriction for two internal E2 elimination and the theoretical calculations support the proposal. 2c can easily lose water affording a double bound between carbons 10-11, as the structure of the other analogue 3. In both cases it was not observed the mechanism of NH3 elimination by restriction of the second internal E2. Also, the other analogues investigated did not show this reaction, which was easier to explain due to the absence of H atoms at carbon 6. The ion [M-MeOH-NH3]+ is the precursor of other 2 major fragments observed in MS3 and MS4 spectra of 2a and 2b. High resolution data has confirmed a neutral elimination of CH3OH and CH2O, respectively and both product ions lose CO. In this case, a benzylic carbocation is necessary to initiate the dissociation mechanism. The first reaction involves the charge transfer of non-bounded electrons of the O atoms from the aromatic ring. The second reaction was a ring contraction losing CO. A different pathway formed other two minor ions in MS2 spectra. In MS3 and MS4 experiments it is clearly demonstrated the formation of the ions at m/z=151 (2a) and m/z=149 (2b) from the [M-MeOH]+ ion. The fragmentation initiates with a conjugation to stabilize the carbocation formed by CH3OH elimination from [M+H]+. H at C4 is transferred to the aromatic ring. The anquimeric assistance of N afforded one more canonic form that increased the ion stability, but at this moment a competition between the charge migration and an E2 elimination (driven by the charge) can result in the ion of ring D added by 2 carbons of ring C.

Conclusion and Novel Aspects

This is a first complete fragmentation map showing in detail each single fragmentation reaction of dienoids erythrinian alkaloids. This novelty will be useful for direct identification of metabolites in biological samples, helping future pharmacokinetics studies.

TPS41-28 / Gas-phase behavior of novel binuclear nickel(II) and cobalt(II) complexes with bridging phosphinato ligands under MALDI and ESI conditions

Vasily Babaev, Ekaterina Trofimova, Ildar Rizvanov, Dmitry Yakhvarov, Oleg Sinyashin

A.E. Arbuzov Institute of Organic and Physical Chemistry of the Russian Academy of Sciences

Transition metal complexescontaining two or more interconnected metal coordination centers are of high practical interest for different areas of applied chemistry, molecular biology and pharmacology. Mass spectrometry analysis is very attractive due its information content, sensitivity and a relatively small experimental time unlike the methods of X-ray crystal structure analysis and radiospectroscopy. Moreover, the application of mass-spectrometry for structural analysis of high spin state complexes is very promising due some limitations of nuclear magnetic resonance in case of paramagnetic samples.

The investigation of the structure and gas-phase behavior of new binuclear nickel(II) and cobalt(II) complexes of type [M2(μ-O2P(H)Ar)2(bpy)4]X2, where M = Ni, Co; X = Br-, Cl-; Ar = Ph, p-ClC6H4, Mes (2,4,6-trimethylphenyl), Tipp (2,4,6-triisopropylphenyl), Ant (9-anthryl); bpy = 2,2'-bipyridine (Fig. 1) is the main goal of the present study. Such knowledge can be an important tool for the fast identification of paramagnetic bi- and polynuclear complexes in solution and can be characterized as novel aspects for in situ monitoring of magnetically active molecules formation.

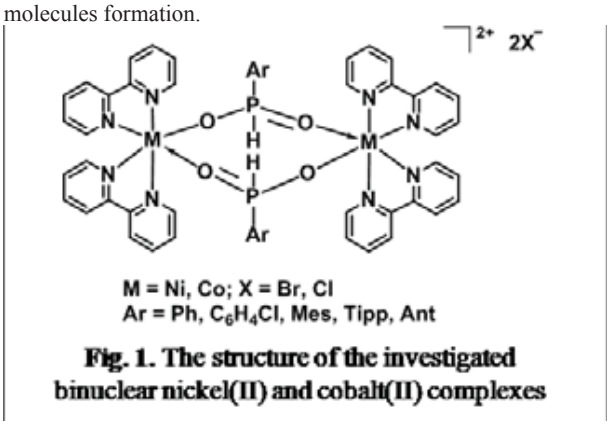

Fig. 1. The structure of the investigated binuclear nickel(II) and cobalt(II) complexes

The gas-phase behavior of nickel(II) and cobalt(II) complexes formed by {μ-O2P(H)Ar}- bridging ligandshas been investigated by ESI, LDI and MALDI mass spectrometry in positive and negative ion mode. According to our study the investigated complexes can be prepared in solution and characterized by ESI-MSwithout isolation as previously suggested[1].

Our experiments involved the investigation of solutions containing [Ni(bpy)2Br2] and corresponding phosphinic acid ArP(O)(OH)H in N,N-Dimethylformamide (1:1 molar ratio) injected into the mass spectrometer.While the molecular ion is not observed, the structural information, including the bimetallic nature of the species, was obtained from the peaks of fragment ions [M2(bpy)2LX2]+, [M2(bpy)2L2X]+, [M2(bpy)2L3]+, where L = {μ-O2P(H)Ar}-. The presence of the peak corresponding to the cation [M(bpy)2L]+ confirms the fragmentation of the complex molecules in experimental conditions.It is worth noting that

the ESI mass spectra were similar to those obtained with the complexes formed by crystallization [2, 3]. These signals are also observed in LDI and MALDI experiments.
Thus the observed regularities of the mass spectrometric behavior of binuclear nickel(II) and cobalt(II) complexes formed by {μ-O2P(H)Ar}- bridging ligands allow to identify in solution new complexes which can be applied as secondary building units for creation of new magnetically active materials based on transition metal complexes.

References:

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2. D. Yakhvarov, et al. Inorg. Chem., 2011, 50, 4553.
3. E. Trofimova, et al. Mendeleev Commun., 2013, 23, 135.

TPS41-29 / The use of fragmentation und retention patterns of BADGE, BFDGE and their derivatives for detecting related substances without reference compounds

Christoph Czerwenka, Anton Turkowitsch
AGES

BADGE and BFDGE are used in epoxy-based lacquers for internal coating of tins. If food is packaged in such tins migration of these compounds or their hydroxy and chlorine derivatives into the food may take place. To assess the potential transfer of these substances migration experiments are performed and the various compounds are measured in the obtained solutions. During the validation of a LC-MS/MS method for this purpose we noticed that a conversion of the various compounds into each other may occur, complicating the determination of recoveries. Moreover, for some derivatives no reference compounds were available, further impeding method validation.
Through studying the mass spectrometric fragmentation and chromatographic retention behaviours of the BADGE- and BFDGE-related substances for which reference compounds were available and subsequently deducing structure-related patterns we were able to propose chromatographic time windows and MS/MS transitions and conditions for the other derivatives. The line of deduction and the obtai9end postulates will be presented in this contribution.
The feasibility of this approach was confirmed by the observation of chromatographic peaks for BFDGExH2O within the suggested time window for the proposed MS/MS transitions during validation experiments. Further work will aim at testing the validity of the other postulates and establishing semi-quantitation for the substances for which no reference compounds are available.
In conclusion, an in-depth study of chromatographic and mass spectrometric data can help in analysing substances for which no reference compounds are available.

TPS41-30 / Monitoring the Oxidative Desulfurization Process of Organic Compounds by FT-ICR

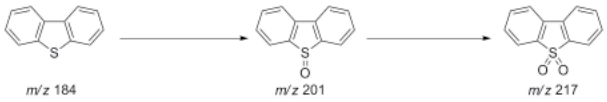
Pedro Henrique Vendramini[1], Marcos A. Pudenzi[1], Heliara D. L. Nascimento[1], Vanessa G. Santos[1], Elias Tessaro[1], Rosana C. L. Pereira[2], Wagner L. Bastos[2], Erica T. Morais[2], Davila Zampieri[1], Bruno R. Vilachã Ferreira[1], Marcos N. Eberlin[1]
[1]UNICAMP, [2]PETROBRAS R&D Center

Introduction

In the last decades, much attention has been paid to the deep desulfurization of fuels due to more stringent environmental regulations. Although hydrosulfurization (HDS) is efficient in removing thiols, sulfides and disulfides, it is difficult to reduce refractory sulfur-containing compounds such as dibenzothiophene (DBT) and its derivates to an ultra-low level. Therefore, the development of the alternative ultra-deep desulfurization processes, such as oxidative, is desired. The extraction of fuels using IL's to remove sulfur compounds has been reported recently.1
Thus, an alternative to this process is to use H2O2 in the presence of supported transition-metal, for example, vanadium (V), rhenium (Re), osmium (Os), tungsten (W) and ionic liquid (IL) as interface. The goal of this work is to study the oxidative desulfurization process mechanism in biphasic-systems (H2O2/IL) by mass spectrometry.2

Methods

The desulfurization experiment was conducted in a 25.0 mL two-neck flask equipped with a condenser. The mixture was heated from room temperature to 60 ºC in a oil bath with stirring and kept at 60 ºC for 48h. In a typical run, a model oil (using 20.0μg DBT-sulfur source, diluted in 1.0 mL of hexane. Next, was added 0.20% (v/v) of [BMIM][BF4] (IL), 5.0 μg of catalyst (V2O5) and 1.0 mL of H2O2 30% and the resulting mixture was stirred for 48h to 60 ºC.



Scheme 1: DBT oxidation mechanism

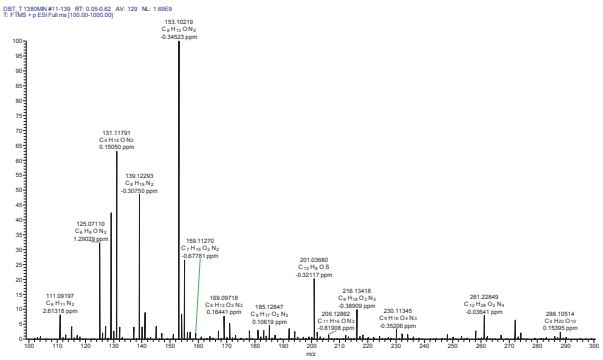


Figure 1: Full scan time at 24h

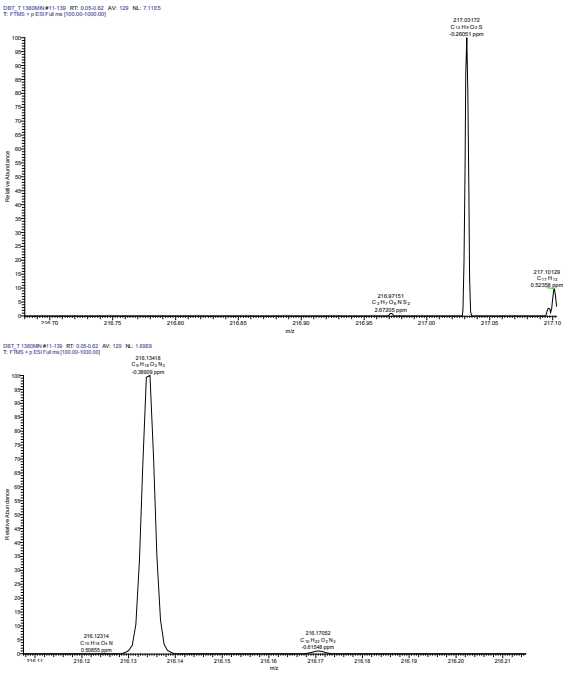


Figure 2: Zoom of the Spectra HRMS

Tuesday

Results
The reaction was monitored with two ionization sources, ESI and APPCI, for 48h. We do not observed DBT (sulfurated compound) by ESI technique. The APPCI ionization source was the ideal technique. We were able to visualize the oxidated intermediates (m/z 201 e m/z 217) by ESI(+)-MS.

Conclusion
The preliminary studies show that the key intermediates for the desulfurization process were intercepted and characterized by ESI(+)-MS.

Novel Aspect
Further mechanistic studies using charge-tagged reagents, major intensity of the intermediates, will be required to elucidate the details of the mechanistic pathways. In particular, we hope to observe specific intermediates during the reaction of tiophene in this same biphasic-system via [2+4] Diels-Alder.

1 Gao, H.; Guo, C.; Xing, J.; Zhao, J.; Liu, H. Green Chem. 2010, 12, 1220.
2 Zhou, M.; Meng, W.; Li, Y.; Wang, Q.; Li, X.; Zang, S. Energy Fuels, 2014, 28, 516.

TPS41-31 / A mass spectrometry and computational study of the competition between intramolecular SNAr and substitution of hydrogen reactions in the gas phase

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Institute of Organic Chemistry PAS

Introduction
Aromatic nucleophilic substitution (SNAr) and nucleophilic substitution of hydrogen reactions have been extensively studied due to their role in organic synthesis [1,2]. It has been proved experimentally that in condensed phase formation of σH adduct which leads to the substitution of hydrogen is almost always a much faster process that the formation of σX adduct which is an intermediate in the SNAr reaction. In the present work we attempted to test this reaction rates relation in the gas phase.

Methods
MS experiments: 4000 QTrap and Synapt G2-S HDMS spectrometers. A gas-phase intramolecular reaction of N-(2-X-5-nitro-phenyl)-N-methyl-acetamides (2, X = H, F, Cl) anions have been studied. Acetamides anions were generated by decarboxylation of the respective acids anions (1) [3]. DFT calculations were done using Gaussian 09 software package on PBE1PBE/6-311+G(2d,p)//PBE1PBE/6-31+G(d) level.

Results
Summary of the results of mas spectrometric experiments performed for acids anions 1 are shown in the following scheme.

Scheme
CID experiments performed for anions 2 show that they undergo elimination of H2O yielding nitroso compounds 4 (through σH adduct 3) and elimination of ketene molecule yielding nitroaniline anions 7. Additionally, in the case of fluorine derivative, HF elimination has been observed yielding SNAr reaction product 6 (through σX adduct 5). DFT calculations showed also that in the case of the fluorine derivative activation free energies of the σH and σX adducts formation are comparable, while for Cl derivative the formation of σH adduct is strongly favored. Activation free energy of the ketene elimination reaction is significantly higher but this reaction is a simple bond breaking process so its rate can be high providing an appropriate energy excess.

Besides the scope of this work it has been observed that the decarboxylation reaction of 1 is accompanied with the HX elimination yielding most likely SNAr product 8 which on turn decomposes to products 6 and 9.

Conclusions
It was proved by experiment and calculations that also in the gas phase formation of σH adduct is faster than the formation of σX adduct. Providing that the resulting σH adduct can undergo further transformations this reaction channel is preferred over the SNAr reaction proceeding via the formation of σX adduct.

Novel Aspects
For the first time direct competition between formation of σH and σX adducts in the gas phase, identified on the basis of their transformations products, has been observed. It is also the first observation of the H2O elimination from the σH adduct in the gas-phase.

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3. T. Bieńkowski, W. Danikiewicz; Rapid Commun. Mass Spectrom., 17, 697-705 (2003).

TPS41-32 / Identification of diketopiperazine b2-ions from deprotonated peptides

Jos Oomens, Josipa Grzetic, Jonathan Martens, Giel Berden
Radboud University

Introduction
Dissociation of (de)protonated peptides in a mass spectrometer is a process of considerable interest as all MS-based peptide sequencing methods rely on it. Deeper mechanistic insight into peptide fragmentation chemistry is anticipated to eventually improve scoring algorithms used in peptide identification. Knowledge on the molecular structures of sequence ions formed in the MS provides an important piece of information in this understanding and IR spectroscopy of the mass-selected peptide fragments has developed into a reliable method for structure identification in MS.
b-Type fragment ions are commonly believed to possess a five-membered oxazolone ring at the C-terminal side and this has indeed been verified by IR ion spectroscopy for various b-ions. However, alternative structures have also been identified for species containing specific residues. Some protonated peptides containing His, Arg and Pro have been spectroscopically shown to form b2-ions with a six-membered diketopiperazine ring structure. Peptides containing Gln and Asn in the second position were shown to form cyclo-imide b2-ions.
Here we investigate the influence of His and Pro on the structure of b2-ions in the dissociation of deprotonated peptides.

Methods
Deprotonated peptides are generated by direct infusion electrospray ionization in negative ion mode. Ions are accumulated in a linear hexapole trap before being injected into a 4.7 T Fourier Transform ion cyclotron resonance MS. Fragmentation is induced either by collision induced dissociation in the hexapole trap or by IRMPD using a 35-W cw CO2 laser. The fragment under study is isolated using a SWIFT waveform and then irradiated with the wavelength tunable output from the FELIX free electron laser. Plotting the extent of dissociation as a function of wavelength is then used to reconstruct an IR spectrum of the ion under study.

Results and preliminary conclusions
IRMPD ion spectra were recorded for deprotonated ProProPro and AlaHisAla and their b2 fragment anions. Comparison of the spectra for the b2 fragments with vibrational spectra calculated for possible candidate structures using density functional theory suggests that both fragments possess the unusual diketopiperazine structure instead of the common oxazolone structure. For the ProPro b2-anion, deprotonation occurs on one of the equivalent alpha-carbons of the diketopiperazine ring, forming an enolate anion. In contrast, the AlaHis b2-anion is deprotonated on the His side chain. For the ProPro b2-anion, an experimental spectrum recorded for the parent [Pro3 – H]– anion suggests that the amide bonds are in the cis-configuration, explaining an easily accessible pathway to the diketopiperazine fragment.

Novel Aspect
First observation of diketopiperazine b2 fragment ions from deprotonated peptides

TPS42 - Forensics and Doping

11:00-15:00
Poster Exhibition, Level -1

TPS42-01 / Simultaneous analysis for forensic drugs in human blood and urine using ultra-high speed LC-MS/MS

Toshikazu Minohata[1], Keiko Kudo[2], Kiyotaka Usui[3], Noriaki Shima[4], Munehiro Katagi[4], Hitoshi Tsuchihashi[5], Koichi Suzuki[5], Noriaki Ikeda[2]
[1]Shimadzu Corporation, [2]Kyushu University, [3]Tohoku University Graduate School of Medicine,, [4]Osaka Prefectural Police, [5]Osaka Medical Collage

Introduction
In Forensic Toxicology, LC/MS/MS has become a preferred method for the routine quantitative and qualitative analysis of drugs of abuse. LC/MS/MS allows for the simultaneous analysis of multiple compounds in a single run, thus enabling a fast and high throughput analysis. In this study, we report a developed analytical system using ultra-high speed triple quadrupole mass spectrometry with a new extraction method for pretreatment in forensic analysis. The system has a sample preparation utilizing modified QuEChERS extraction combined with a short chromatography column that results in a rapid run time making it suitable for routine use.

Methods
Sample preparation of human whole blood and urine was carried out by the modified QuEChERS extraction method (1).
Treated samples were measured using a Nexera UHPLC system and LCMS-8050 triple quadrupole mass spectrometer (Shimadzu Corporation, Japan) with MRM triggered automatic MS/MS data acquisition.
Samples were separated on a YMC Triart C18 (100x2mm, 1.9µm)at a column temperature of 40 °C for 15 min. A flow rate of 0.3 mL/min was used together with a binary gradient system; the mobile phase consisted of [A] 10mM ammonium formate in water and [B] methanol. (1) Usui K et al, Legal Medicine 14 (2012), 286-296

Results
The simultaneous analysis of drugs of abuse in clinical and forensic laboratories requires highly specific system. Conventional procedures to analyze drugs in complex matrices like whole blood involve tedious, time consuming, expensive, and complex steps, and possible sample loss and contamination problems are not unusual. The developed system in this study contained not only optimized MRM transition parameters with product ion scanning which is automatically triggered once an MRM exceeds a specified threshold, but also sample preparation utilizing modified QuEChERS extraction.
In this experiment, two different matrices consisting of human whole blood and urine were prepared and 18 drugs were spiked into extract solution. Calibration curves constructed in the range from 0.01 to 1 ng/mL for 12 drugs (Alprazolam, Aripiprazole, Atropine, Brotizolam, Estazolam, Ethyl loflazepate, Etizolam, Flunitrazepam, Haloperidol, Nimetazepam, Risperidone and Triazolam) and from 1 to 100 ng/mL for 6 drugs (Bromovalerylurea, Amobarbital, Barbital, Loxoprofen, Phenobarbital and Thiamylal).All calibration curves displayed linearity with an R2 > 0.997 and excellent reproducibility was observed for all compounds (CV < 12%) at low concentration level.
Moreover, the method is capable of simultaneously obtaining both qualitative and quantitative information in a single analysis. Acquisition occurs so rapidly that MS/MS scans and MRM measurements can be performed concurrently while maintaining quantitative accuracy.

Conclusions
The combination of the modified QuEChERS extraction method and high-speed triple quadrupole LC/MS/MS with a simple quantitative method enable to acquire reliable data easily.

Novel Aspect
Development of analytical system using ultra-high speed LC-MS/MS with a new extraction method for human whole blood and urine

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TPS42-02 / Rapid Screening of Adulterated & Counterfeit Products using a Bench-Top High Resolution Mass Spectrometer and mzCloud Database Search

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Introduction
Adulterated and counterfeit drugs pose a real danger to consumers. They may contain a structurally modified active pharmaceutical ingredient (API) or no API. Also some over-the counter natural supplements and recreational products contain APIs. These products are illegal and can cause harm to the public.
Presented here is a workflow for screening of illegal products using a bench-top Orbitrap mass spectrometer and a high resolution fragmentation spectral database search in mzCloudTM. UHPLC combined with HRAM data, and mzCloud database search provided a simple, effective screening tool for adulterated & counterfeit products. This workflow was successfully applied to a series of samples to detect analogue adulterants.

Methods
Samples were ground and dissolved in a mixture of methanol, water, and acetonitrile, followed by Vortex mixing, sonication, and filtration.
LCMS analysis was carried out on Ultimate3000 UHPLC system and a benchtop Orbitrap mass spectrometer in positive ESI mode. Full scan MS and MS/MS data were collected at resolutions 70,000 and 15,000 respectively.
The HRAM Data was searched against mzCloud spectral database using a novel high resolution search algorithm.

Results
HRAM full MS and MS/MS data was processed in mzCloud

spectral library: individual MS/MS spectra were input into mzCloud and searched against the reference library. The accuracy of spectral matches for potential hits was indicated by scores for three independent algorithms with the HighRes algorithm used for primary sorting.
Other criteria, such as m/z value, structure, and substructure, which allow a hit from a primary search to be used to expand into similarity searches based on features beyond fragmentation, are also available in mzCloud. In this study, through spectrum search, major components in the unknown samples were identified: Sildenafil, Sildenafil analogue I, Hydroxythihomosildenafil and Chloropretadalafil. Based on the primary hits found through mzCloud library searching, subsequent fragmentation ion searches (FISh) were performed on the initial sample data to detect additional potentially related minor components based on fragmentation similarity.

Conclusion
The preliminary results demonstrate that high resolution MS and MS/MS data and a fragmentation spectral library search, coupled with fragment ion searching for related components, enables unknown component identification and structure elucidation. This workflow increases the speed and confidence of API adulterant and unknown analysis.

Novel Aspect
Rapid Screening of Adulterated & Counterfeited Products using a Bench-Top Orbitrap MS and High Resolution Spectral Database mzCloud

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TPS42-03 / Highly sensitive analysis of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol in hair by micro-pulverized extraction and liquid chromatography/tandem mass spectrometry

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Introduction
A main metabolite of Δ9-tetrahydrocannabinol (THC), 11-nor-9-carboxy-THC (THC-COOH), is an effective marker to prove the intake of cannabis. In forensic cases, hair is often used as a retrospective evidence for drug use. According to recommendations of the Society of Hair Testing, the cut-off concentration of THC-COOH in hair for confirmatory test is 0.2 pg/mg, which is a very small value as compared with those of the other abused drugs. The general method to quantify THC-COOH at the cut-off levels is as follows: a hair sample is digested with an alkaline solution, THC-COOH is extracted with organic solvent under acidic condition, and pentafluoropropyl-derivatized THC-COOH is quantified by gas chromatography/tandem mass spectrometry (GC/MS/MS)in negative ion chemical ionization (NICI). However, the sample preparation is complicated and the instrumental conditions are limited. In this study, we developed a simple and highly sensitive analytical method for THC-COOH in hair using micro-pulverized extraction and liquid chromatography/tandem mass spectrometry (LC/MS/MS).

Methods
Drug-free hair samples (10 mg) were spiked with THC-COOH (0.2 pg/mg hair). The samples were treated by two procedures as follows: (i) After the sample was digested with 1 M NaOH aqueous solution, THC-COOH was extracted with hexane/ethyl acetate under acidic condition. (ii) After the sample was pulverized with a stainless steel bullet, THC-COOH was extracted with acetonitrile in the saturated solution of NaCl. Each extract was injected into an LC/MS/MS instrument in negative ion electrospray ionization.

Results
The procedure(i), which is generally used for the analysis of THC-COOH in hair by GC/MS/MS, was unsuitable for LC/MS/MS because the extracts had matrix components which interfered with THC-COOH peak. On the other hand, the procedure (ii) reduced the matrix effects and enabled the quantification of THC-COOH in hair at 0.2 pg/mg by LC/MS/MS.

Conlclusions
The sample preparation using micro-pulverized extraction is very simple and LC/MS/MS is easier to use than GC/MS/MS in NICI which requires the ion source replacement, the waiting time to stabilize the instrument, and the proper derivatization for highly sensitive detection of THC-COOH. This method would be used as the first choiceforthe analysis of THC-COOHin hair.

Novel Aspect
A simple and highly sensitive analytical methodfor THC-COOH in hair using micro-pulverized extraction and LC/MS/MS was developed.

TPS42-04 / Validation of dried-blood-spot analysis for the quantification of drug concentrations in capillary whole blood samples within targeted PAH therapies

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Introduction
Patients with pulmonary arterial hypertension (PAH) commonly receive an endothelin receptor antagonist (ambrisentan, bosentan, or macitentan), a phosphodiesterase-5 (PDE 5) inhibitor (sildenafil or tadalafil) or a combination of both [1]. Therapeutic drug monitoring (TDM) of these compounds in PAH patients allows the determination of individual drug concentrations and correlation with the clinical outcome of the patients. Because inter-individual variability of drug concentration is large, TDM might offer a way to tailor therapies appropriately.
The quantification of PAH drugs in plasma by means of LC/MS/MS techniques is well established in clinical drug trials [2]. An essential prerequisite for TDM is the analysis of trough level samples, which can be generated by patients using the dried-blood-spot (DBS) technique. Based on this technique, we developed extraction and LC/MS/MS methods for the quantification of PAH drugs in capillary blood.

Methods
Patient plasma, whole blood, and DBS containing at least one of the substances were processed by adding internal standards and subsequent extraction (protein precipitation or liquid/liquid extraction). After centrifugation, reducing the supernatant to dryness, and reconstitution with LC/MS/MS eluent, the extracts were subjected to the LC/MS/MS analysis [3], in multiple reactions monitoring mode.
Quantification was performed by linear regression of the calibration curves consisting of at least seven calibration samples and three quality controls and assessment of the peak area ratio from analyte to internal standard. To complete the validation we correlated venous whole blood, plasma, and DBS (capillary whole blood). Therefore samples obtained from healthy volunteers and patients exposed to these compounds were taken contemporaneously at several points in time.

Results
The lower limits of quantification for the DBS method were 5 – 10 ng/ml for tadalafil, sildenafil, ambrisentan and bosentan. All methods were validated according to FDA guidelines and fulfilled its standards [4]. The DBS technique was validated for a

hematocrit range of 30% to 50%. The within-day and day-to-day accuracy, the stability and precision were within a range of +/- 15% [4]. First experiments suggest that there is a constant factor for the plasma-DBS-ratio, facilitating future correlations.

Conclusion
Current evidence suggests that DBS samples reflect concurrent plasma concentrations and that TDM could hence be simplified and made more comfortable for patients by using DBS sampling techniques.

Novel aspects
For a TDM the DBS method is reliable and much more feasible than using whole blood. It is less invasive, yet capillary blood sampling using a lancet and filter paper is well known and established in different clinical tests.

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TPS42-05 / Evaluation of high-throughput automatic explosives trace detection systems using the dry transfer method

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Introduction
The threat of terrorism or criminal bombings has become a serious problem worldwide. Thus, detection technologies for hidden explosive devices are in high demand in order to maintain a safe society. However, commonly-used swab-sampling ETDs require human operations, which make 100% inspection relatively costly. Since auto-sampling ETDs would dramatically reduce the need for human operations, we've been developing the automatic explosives trace detection systems with a high-throughput of more than 1000 objects/hour. We previously reported a high-throughput automatic explosives trace detection system (ETD) which can detect trace particles explosives attached to the surface of the objects [2]. Our configuration using cyclone type separator enriched the concentration of explosives concentration by more than 50 times compared to that without the concentrator. Online connection between the particle heating unit and a mass spectrometer enabled high-speed detection within a few seconds; otherwise the conventional off-line heating method takes more than 10 seconds for controlling the temperature of the heating unit.

Methods
In our previous studies, we evaluated detection efficiencies using NIST standard reference materials for trace explosive detection (NIST SRM 2905 [2]), which was made with octadecylsilane-modified silica (C18) particles with a nominal size of 20 - 30 μm containing mass fractions of 0.01% and 0.1% explosives. As more realistic particle explosives particles, we prepared particle samples using dry transfer method [3]. In order to make a sample, a volume of the solution, proportional to the mass of explosive desired (1-10 μg), was deposited onto a Bytac coupon. After drying process, the explosives particles were transferred to glass slides or target objects such as plastics plates and synthetic leather sheets by rubbing the coupon against the object surface.

Results and Conclusions
Typical detection efficiencies of dry transfer samples ranged from 0.1 % to 1 %, which were much lower than those of the NIST materials of 10-20 %. We suppose that these low efficiencies come from the low detachment efficiencies of dry transfer particles from the target objects.

This work was supported in part by the R&D Program for Implementation of Anti-Crime and Anti-Terrorism Technologies for a Safe and Secure Society and Funds for Integrated Promotion of Social System Reform and Research and Development from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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Novel Aspect
High-throughput automatic explosives trace detection system was tested with dry transfer samples.

TPS42-06 / Development of a Novel Apparatus for Analyzing Minerals in a Single Strand of Hair

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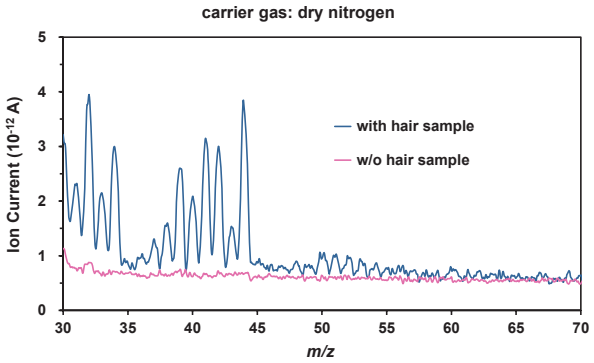
Introduction
Several progressive diseases such as breast cancer and Alzheimer are potentially diagnosable or predictable by analyzing trace elements contained in human hair. Since human hairs grow at the rate of about 1 cm per a month, a hair of 10 cm length contains the ten-month track record of mineral dynamics. A disorder of mineral dynamics in the human body can be traced back from the present to the past by testing a single strand of hair from its root to tip. Epidemiological studies have been carried out for hair samples donated from breast cancer patients and subjected to X-ray fluorescence analysis using synchrotron radiation at the Spring-8. (Chikawa, J., et al., J. X-Ray Sci. Technol., 2007, 15, 109-129.) Their studies revealed that the concentrations of trace elements such as calcium showed variation profiles unique to breast cancer, and signs of the disorder of mineral dynamics had been recorded in the hairs substantially long before the cancer onsets. The throughput and costs are challenges of their approach. A use of laser ablation mass spectrometry may enable trace elements analysis of a hair to be carried out not only in a lower cost but also in a shorter time. We are developing a compact apparatus of laser ablation mass spectrometry specialized for analyzing trace elements in a single strand of hair.

Methods
The apparatus consists of a quadrupole mass analyzer, a laser ablation system, a pneumatic transport system and a vacuum pumping system. The laser ablation system involves a sample stage equipped with a one-axis motorized actuator and an optical system for focusing a laser beam. A hair sample under an atmospheric pressure is contained in a quartz capillary which is connected to one end of a long capillary tube by using joint parts of minimized dead volumes and is placed on the sample stage, so that the laser exposure (ablation) point on the hair sample moves along the length of the hair according to a linear motion of the stage.

Results
Mass spectra obtained from hair samples of a healthy donor showed several prominent peaks in the range m/z 30 - 70, which were not observed in the control mass spectrum without hair sample (see Figure). The majority of these peaks are attributed to

organic fragment ions generated from a principal component of hair, keratin; however, some of them are most likely attributable to minerals, such as calcium at m/z 40. Selected ion monitoring of 5 elements (Mg, Cu, Fe, Cu, Zn) showed modestly steady signal responses during laser exposure of the hair sample at a movement rate of 0.05 mm/s. Further details will be presented at the conference.



Conclusions

Variation profile of hair minerals can be measured in minutes. The prototype apparatus needs further improvements on the sensitivity and reproducibility to be put into practical use.

Novel Aspect

A prototype of a compact clinical analyzer based on laser ablation mass spectrometry was developed for analyzing trace elements in a single strand of human hair.

TPS42-07 / Investigations on storage-induced changes of the red blood cell lipidome

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Since more than forty years, athletes use blood doping techniques to improve the oxygen delivering capacity of the blood by artificially increasing the number of circulating red blood cells (RBCs). While the misuse of recombinant human erythropoietin can be directly detected in blood and urine specimens by using different analytical strategies only indirect approaches such as the monitoring of blood parameters within the athlete biological passport or the detection of phthalates are currently available to provide sufficient evidence for autologous blood transfusions (ABT).

Generally, RBCs have a comparably simple structure and can be readily obtained in large amounts. Metabolic and structural changes are an ideal starting point for preventive doping research as biomarkers for RBC storage lesions might be used to provide direct evidence for the misuse of ABT following reinfusion in elite athletes.

The aim of this study was to identify new lipid biomarkers that can potentially be used to develop direct detection strategies for autologous blood doping and complement the already existing approaches. For that purpose, the RBC lipidome and its alterations in the course of 42 days of ex vivo storage have been investigated using both liquid chromatography-high resolution mass spectrometry (LC-TOF) and gas chromatography-high resolution mass spectrometry (GC-TOF).

While for LC-TOF measurements no extensive sample preparation besides a liquid-liquid-extraction was necessary as all lipid classes can directly be investigated here, samples for GC-TOF investigations were prepared by thin layer chromatography followed by saponification and finally derivatization.

Within this preliminary study blood samples from 4 subjects have been investigated to identify possibly new biomarkers. The samples for this study were collected in accordance with guidelines of the European Directorate for the Quality of Medicines & HealthCare as well as the Declaration of Helsinki.

TPS42-08 / MALDI MS Profiling and Imaging of Illicit Drugs in Fingermarks in tandem with Conventional Enhancement Techniques

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Introduction

Conventional enhancement of fingermarks provides ridge detail to be used for suspect I.D. The choice of enhancement technique is dictated by the substrate and the suspected composition of the marks on the surface. Matrix-assisted laser desorption/ionization mass spectrometry allows for fingermark ridge reconstruction whilst simultaneously providing chemical information on species contained within the residue, potentially providing additional intelligence1-3. The detection of illicit drugs following initial enhancement could therefore be integrated in the current fingerprinting workflow which could offer invaluable investigative leads and improve operational effectiveness.

Methods

Fingermark spiking - 50 µL of 10 µg/mL solutions of cocaine and methadone were deposited onto glass slides and allowed to dry. The fingertip was rubbed against the drug residue and marks were deposited onto the desired surface.

Conventional development – Fingermark enhancement techniques were applied either at West Yorkshire Police Laboratories (Wakefield, UK) or at crime scenes using the methods outlined in the 'Manual of Fingerprint Development Techniques'4.

Matrix application – The MALDI matrix (α-CHCA) was applied using either the SunCollectTM auto-sprayer (SunchromGmbH, Friedrichsdorf, Germany) (for MALDI MSI) or manual spotting (for MALDI MSP).

Instrumentation – Mass spectrometric analyses were conducted using an Applied Biosystems API «Q-Star» Pulsar i hybrid QTOF instrument (Concord, Ontario, Canada) incorporating a SPOT 10 kHz Nd:YVO4 solid-state laser (Elforlight Ltd.) or a Waters "Synapt"TM G2 HDMS ion mobility mass spectrometer (Waters, Manchester, UK).

Results

Fingermarks generated from drug spiked fingertips were enhanced using a range of conventional development techniques (including; powders, ninhydrin, vacuum metal deposition). In all cases, initial enhancement did not affect the ability to detect the drugs via subsequent MALDI MS analyses. The developed methodologies have also been used to confirm the presence of illicit substances in fingermarks recovered from crime scenes.

Conclusions

The ability to use MALDI MS in the detection of illicit drugs in fingermarks following initial enhancement could provide additional important intelligence in a forensic investigation. The application of the established methodologies to crime scene samples (obtained on attachment with Crime Scene Investigators (CSI's) from West Yorkshire Police (Leeds, UK)) proves the feasibility of this novel technology to "real" samples obtained in the field.

Novel Aspect

MALDI MS has been used to detect illicit drugs in fingermarks following the application of conventional fingermark enhancement techniques.

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TPS42-09 / Mass spectra of some benzodiazepine series drugs and their trimethylsilyl derivatives

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Introduction

Conversion of drugs to trialkylsilyl derivatives prior to mass spectrometry experiments is a common practice. Gas chromatography and mass spectrometry data is presented for partially and completely silylated drugs of benzodiazepine series; they are widely used for the treatment of anxiety disorders and remain as drugs of abuse as well.

Methods

Commercially available Lorazepam (I), Lormetazepam (II), N-Desmethylflunitrazepam (III), Temazepam (IV), Nordiazepam (V) and 7-Aminonitrazepam (VI) were objects of the study. Trimethylsilylation was carried out at 60°C with the use of BSTFA/1% Me3SiCl or MSTFA as silylating reagents. On-column derivatization1 of Lorazepam was performed to identify any thermal decomposition products since it is believed2 that Lorazepam undergoes complete decomposition in GC system prior to mass spectrometry analysis. Mass spectra of resulting trimethylsilyl (TMS) derivatives were recorded on GC-MS and GC-MS-MS instruments with quadrupole analyzers.

Preliminary data

Benzodiazepine skeleton is rather stable and M+• of (I)-(VI) under EI conditions mostly eliminate CO and HCO. However substituents, those are giving different benzodiazepines unique pharmacological properties, do determine their behavior under EI conditions. The nature of ortho-substituent at 5-phenyl and the ability of N(1)-C(2) to undergo keto-enol tautomerism (presence/absence substituents at N(1)- position) determine the fragmentation pathways. Functional groups at position 7 are eliminated as radicals and neutral species; their influence on existing fragmentation pathways is minimal.

The peak intensities of M+• and [M-CH3]+ ions in the spectra of TMS derivatives vary form 1% (bis-TMS-I) to 100% (bis-TMS-III); the base peaks in the spectra of (TMS-V) and (bis-TMS-VI) correspond to [M-H]+ ions, and in the case of (TMS-II) and (TMS-IV) – [M-CHO]+. Ions [M-Cl]+ exhibit high intensity peaks in the spectra of (bis-TMS-I) and [M-F]+ (TMS-III). All the above illustrate unique behavior of each representative of the same class of compounds under EI conditions; details are discussed.

The retention index values for TMS derivatives varies by 50-300 unites compared to their precursors. Analysis of chemical structure – retention index correlations are presented as well.

It is concluded that Lorazepam does not decompose prior the ionization chamber. Formation of N,O- and O,O-(bis-TMS-I) during on-column derivatization at T ≥150°C while “standard derivatization” yields just N,O-(bis-TMS-I) and spectra analysis allow determination of a fragmentation mechanism.

Novel aspect

Fragmentation pathways under EI conditions are determined. Methods for reliable identification of drugs of benzodiazepine series by GCMS are developed.

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TPS42-10 / Monitoring the distribution of drugs of abuse in longitudinal sectioned hair samplesby multi-modal mass spectrometry imaging

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Introduction

Hair testing is a powerful tool routinely used for the detection of drugs of abuse in toxicology and forensic applications. The analysis of hair is highly advantageous as it can provide prolonged detectability versus that in biological fluids and chronological information about drug intake based on the average growth of hair. However, current methodology routinely involves complex and time-consuming sample preparation followed by gas or liquid chromatography coupled with mass spectrometry. Mass spectrometry imaging has been employed to monitor the distribution of drugs of abuse and their metabolites throughout single longitudinal sectioned hair samples.

Methods

Longitudinal sections of hair samples were prepared with an in-house built device before mounting onto a conductive glass slide.Prior to analysis samples were gold-coated using a Quorum Technologies SC7640 sputter coater (New Haven, USA) equipped with a FT7607 quartz crystal microbalance stage and FT690 film thickness monitor to deposit a 1 nm thick gold layer.Samples were analyzed using a Physical Electronics TRIFT II TOF-SIMS (Physical Electronics, USA) equipped with an gold liquid metal gun tuned for 22keV Au+ primary ions. A Waters MALDI HDMS SYNAPT mass spectrometer (Waters Corporation, Manchester, UK) and BrukerUltraflex III (BrukerDaltonics, Bremen, Germnay) were also used to acquire mass spectra and images from hair surfaces.

Results

Scanning electron microscopy was performed to assess the quality of the longitudinal sections of hair samples. The images obtained from the sectioned hair sample showed the exposed medulla, cortex and a portion of the cuticle observed as a narrow layer surrounding the cortex.

Single longitudinal sectioned hair samples were coated with CHCA and analysed by MALDI-MS/MS imaging. The images showed the distribution of a product ion at m/z 182, derived from the precursor ion of cocaine at m/z 304. MetA-SIMS images of longitudinally sectioned hair samples showed the distribution of cocaine atm/z304, the major fragment atm/z182 derived from the neutral loss of benzoic acid and benzoylecgonine the major metabolite of cocaine atm/z290.In other hair samples other drugs such as methadone was observed atm/z310.

Conclusions

SEM imaging of longitudinally sectioned hair samples revealed the internal structure of the hair where the drug reported to be bound. MALDI-MS/MS imaging revealed a broad distribution of cocaine and its metabolites throughout the longitudinal sectioned hair samples, whilst high spatial resolution imaging using MetA-SIMS provided a more detailed distribution which enabled a more accurate chronology of drug usage to be obtained. The initial area examined was 2mm of hair, which based on the average growth rate of hair corresponds to 6 days. As the pixel size that can be achieved by high spatial resolution techniques such as TOF-SIMS is 1 µm, this corresponds to a 5 minutes time frame.

Novel aspect
Application of multi-modal mass spectrometry imaging to monitor the distribution of drugs of abuse in single longitudinally sectioned hair samples.

TPS42-11 / Semi-untargeted metabolomics approach based on precursor ion scan for metabolic studies: steroid metabolism as a proof of concept

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Fundació IMIM

Metabolic studies in doping control are focused on the detection and identification of as many metabolites as possible even those representing a low percentage of the dose administered might be useful in the doping control. The versatility of the triple quadrupole instruments allows the application of different open scan modes (precursor ion or neutral loss scan) capable to detect compounds sharing a common structure core by selecting common ions or loses. Using this approach all compounds related with the administered drug are monitored (semi-untargeted acquisition). For drugs having a non-natural feature (e.g. polyhalogenated drugs), the application of these open scan modes followed by a visual comparison allows for the easy interpretation of the results. However, when dealing with endogenous related compounds, such as anabolic androgenic steroids, the visual discrimination between actual metabolites and endogenously produced compounds is difficult.

In the last decade global profiling strategies such as metabolomics has emerged as powerful complements to the classical analytical methods. Metabolomics analysis aims to compare multiple biological groups in order to identify altered species. Despite its enormous potential the application of metabolomics-like approaches for metabolic studies is rather limited. In the case of endogenous related compounds, the application of such strategies for the evaluation of metabolic studies is an attractive option.

The aim of the present investigation is to develop an approach based on semi-untargeted acquisition by liquid chromatography coupled to tandem mass spectrometry and metabolomics analysis and to apply it in metabolic studies. Steroids were selected as a proof of concept due to the large number of endogenous related compounds which can interfere the visual selection of metabolites. Particularly, methandienone (MD) was chosen as a model compound mainly due to the large number of known metabolites. 10 basal samples and 10 samples collected after the administration of MD were analyzed using a precursor ion scan method of the common ions at m/z 77, 91 and 105 exhibited by all steroids. The results were processed with the XCMS approach which incorporates a nonlinear time alignment, matched filtration, peak detecting and peak matching. Subsequently, the data sets were analyzed using statistical approaches for the detection of the potential metabolites.

The uses of the developed method revealed 12 compounds as potential MD metabolites. 10 of these metabolites were identified as previously reported MD metabolites. The rest of metabolites are under investigation to evaluate their usefulness as biomarkers for doping control.

The developed semi-untargeted metabolomic approach has been shown as a powerful alternative for the evaluation of metabolic studies. This unexplored alternative might reveal previously unreported metabolites which can be helpful in the fight against doping.

TPS42-12 / Enhanced Confirmation Criteria for Reducing False Positive Rates (FPR) in Toxicology Screening using High Resolution, LC-QToF, Accurate Mass Analysis

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Introduction
Toxicological drug screening based on the measurement of exact precursor m/z values, isotopic pattern and retention time alone is unable to distinguish isomers or interfering matrix compounds. As well due to the large numbers of structural and stereoisomers for a molecular formula, false positives are common in real life screening. We describe the application of diagnostic ions (isotopes, adducts and fragment ions) for confirmation by rapidly alternating full scan ToF-MS acquisitions and broad-band Collision Induced Dissociation (bbCID) to significantly reduce the FPR and increase the degree of confidence in screening applications.

Methods
61 compounds covering several compound classes based on their relevance in post-mortem and routine drug screening were used in this study. After acetonitrile precipitation, urine and serum samples were spiked with toxicological mixes at 4 levels (10-500 ng/ml) and analyzed in a QTOF (impact HD, Bruker) in full scan and bbCID modes using a 14 minute RP-UHPLC gradient. In the bbCID mode, additional detection criteria mandated at least one diagnostic ion with a retention time difference <0.05 minutes. The data were compared to the number of expected identifications to assess the FPR. Authentic samples from two forensic laboratories were investigated using the same workflow.

Results
In full scan mode, all compounds were detected at all concentration levels in the spiked samples, no false negatives were encountered. A few additional plausible compounds were also detected such as caffeine, or degradation compounds such as cocaine in a mix with cocathylene. However, the full scan ToF-MS data produced 333 false positives in serum, i.e. more than the 274 expected identifications and 547 false positives in urine. These typically arose due to the low detection threshold of 750 counts for traces with high noise levels or from low intensity peaks within the ±0.5 min RT window. For the enhanced detection criteria, ToF MS and bbCID were used for confirmation. Here at least one diagnostic ion must be detected within a +/-0.05 min RT detection window. Diagnostic ions include bbCID fragment ions, isotopes and adducts. After applying the enhanced criteria, the false positives were completely removed with the exception of tramadol which cannot be removed as a positive in presence of o-desmethylvenlafaxine. These compounds have the same RT, identical mass and the same main fragment ions.

Conclusion
Applying the diagnostic ion concept for confirmation criteria virtually eliminates false positive findings..

Novel Aspect
Diagnostic ions provide an enhanced level of confidence of detection and identification.

TPS42-13 / A fast, reliable automated LC-MSn drug screening solution for clinical research and forensic toxicology

Andrea Kiehne[1], Birgit Schneider[1], Sebastian Goetz[1], Isabelle Buckle[2], Markus Meyer[1], Jürgen Kempf[3]
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Introduction

There is strong demand in both clinical research and forensic toxicology for automated, robust and sensitive analytical solutions to overcome the well-documented limitations of GC-MS, LC-UV and immunoassay technologies. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) combined with mass spectral library searching is an emerging identification strategy in this field of application. However, until now the adoption and successful implementation of this technology by non- MS experts has presented many difficult challenges. We describe an MSn workflow for a robust and accurate solution for the rapid detection, identification and reporting of drugs and drugs of abuse in biological specimens with unprecedented ease of use.

Methods

Serum sample preparation was performed according to a liquid-liquid extraction (LLE) protocol. After sample extraction (urine or serum), a fast 11 minute-binary UHPLC separation is deployed prior to mass spectrometric analysis in an ion trap. Drug and / or metabolite detection is confirmed by matching the generated MS, MS2 and MS3 spectra with retention time in both positive and negative modes to the Toxtyper spectral library containing the corresponding information for over 900 compounds of toxicological relevance.

Results

The automated screening approach revealed a high reproducibility of correct identifications and an overall high transferability between different laboratories. In conclusion the presented screening method offers a fast and reliable routine identification tool for clinical and forensic analysis. A subset of 200 compounds were defined and spiked into human serum matrix at 3 different concentration levels (low therapeutic, therapeutic and elevated). All compounds were combined into mixtures containing 5-10 substances each and then analyzed. Commonly used or miss-used benzodiazepines like diazepam could be successfully detected and identified at low therapeutic doses (low ng/ml range). Also low-dose benzodiazepines like flunitrazepam could be detected and identified in spiked serum samples.

Conclusion

The combination of MS^2/MS^3 spectra and retention time allows identification of drugs and metabolites at therapeutic levels and offers a fast and reliable routine identification tool for clinical and forensic analysis.

Novel Aspects

Fast drug screening in an ion trap based on special library

TPS42-14 / Novel sorbent coated samplers for Trace Chemical Detection by Solid Phase Microextraction Direct Analysis in Real Time (DART) Mass Spectrometry

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Solid Phase Microextraction (SPME) technologies have been used extensively with gas chromatography and liquid chromatography serving as a convenient means to isolate trace components from samples. We have utilized several different types of novel sorbent coated wire and wire mesh with ambient ionization in order to provide a means to complete rapid screening of samples prior to time consuming GC and LC analysis. The use of direct analysis in real time (DART) mass spectrometry for rapid determination of the composition of trace components in a sample is completed in seconds per sample.

For liquid samples we have established extraction protocols for detection of pesticides as contaminants and pharmaceutical products as adulterants in herbal supplements. The utility of elongated C-18, PDMS-DVB, anionic sorbent and cationic sorbent coated wires for these applications is that a small portion, typically 1millimeterm, of the 10 - 30 mm wire can be analyzed by DART-MS which degrading the sample. If a contaminant or adulterant is detected the remaining sample can be eluted from the wire using a small volume of solvent to permit subsequent analysis. Utilizing the screening process is enabled by using multiple wires and a 96-well format SPME wire holder with the robotic sample handler of the DART-SVP system.

For solid samples such as explosives and ignitable liquids dispersed on solids as encountered in arson investigations the utility of sorbent coated wire mesh for collection and retention of trace chemicals has been examined using SPME-DART. In these experiments the SPME wire mesh was placed in conventional trace evidence filter holders and attached to a field portable vacuum cleaner unit. Samples of explosive powders were placed in arson debris cans the lids of which were modified to permit evacuation of and entry of replacement filtered air. Direct analysis of the sorbent coated mesh after short 5 minute exposure times enables detection and characterization of the explosive seconds after analysis. As anticipated different sorbents collect different chemicals resulting essentially enabling a more complete characterization of the explosive. Comparison of the results of SPME-DART with conventional GC/MS results will be presented as a means to validate the method.

TPS42-15 / Analysis of doping agents using ultrafast LCMS/MS with scheduled MRM

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Introduction

Faster and faster, higher, further - doping accompanies sports for many centuries. But since the illegal substances could not be proved, a doping case was first discovered in 1812 - because the culprit was caught in the act.

Doping generally refers to the use of substances of prohibited substance groups, and the use of prohibited methods to improve performance. Especially in horse racing also terms such as negative doping, that is doping to defeat, are an issue. In the past the attitude "Allowed is, what is not found" predominated while nowadays even the slightest traces of doping agents can be detected in blood and urine. Thus, the analytical possibilities of the different labs are crucial for the detection of a substance.

Methods

With the following application example from the scope of horse doping the excellent sensitivity and the clear advantage of an ultra-fast MS technique is explained. Real samples from a horse doping laboratory were tested for various steroid hormones, in the form of free steroid or steroid esters, as well as prohibited neuroleptics, benzodiazepines and opioids. The samples were analyzed with Shimadzu's triple quadrupole mass spectrometer LCMS-8050 coupled to a NEXERA X2 UHPLC.

Results

Due to the high scanning speed of the LCMS-8050 it is possible to obtain data in outstanding quality and with no loss

in sensitivity even with a large number of MRMs. This could be shown impressively based on some experiments performed during application development. In further measurements, the reproducibility was, as a measure of good data quality, examined while a Synchronized Survey Product Ion Scan was carried out simultaneously using a standard solution containing doping agents.

Conclusion
Independent from the number of MRMs or simultaneously performed synchronized survey scans the LCMS-8050 coupled to a Nexera X2 system provides excellent sensitivity with high data quality in scheduled MRM mode with fast polarity switching (5 msec for the detection of positively and negatively charged analytes in one run.

Novel aspect
Combining a large number of MRM's with synchronized survey scans without loss in data quality

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TPS42-16 / Atmospheric Solid Analysis Probe-Mass Spectrometry (ASAP-MS) for rapid screening of drugs of abuse in biological fluids

Camilla Liscio, Bryan McCullough, Christopher Hopley
LGC

Novel aspect
A sample preparation-free (direct sample analysis) and ultra-rapid (seconds per sample) MS semi-quantitative method for the screening of drugs of abuse and metabolites in complex biological matrices

Introduction
Screening of drugs of abuse and metabolites in biological matrices is a frequent practice in a variety of scenarios (e.g. medical, occupational, sports participation). Hence, the existence of sensitive and comprehensive analytical methods is crucial to guarantee efficient and reliable detection of these target compounds. Immunoassays, rapid and relatively inexpensive, are the first-line screening approach for the abused drugs in bio fluid. However, due to some major limitations such as insufficient specificity and limited coverage of drugs, a second analytical technique (GC-MS or LC-MS), more expensive and time consuming, is usually required for drug confirmation1. On the other hand, performing on-site abused drugs screening could provide immediate scientific evidence and improve the quality of clinical management in emergencies. Direct analysis (DA) by ambient mass spectrometry (MS) permits rapid data acquisition (seconds per sample) bypassing time consuming and complex sample pre-treatment. Furthermore, a combination of this ambient desorption ionization technique with the emerging transportable mass-spectrometers could provide a straightforward and very powerful option for at-site analyses. This study investigated the performances of an Atmospheric Solid Analysis Probe-Mass Spectrometry (ASAP-MS) semi-quantitative method for the detection of an array of abused drugs and metabolites in urine in view of a potential application for on-site analyses.

Methods
A standard Micromass Z-spray source fitted with a corona discharge pin and a heated nebuliser Ion Sabre APcI probe was customised for atmospheric solids analysis probe (ASAP). The source was coupled to a Micromass Quattro Ultima QqQ MS for MS and MS/MS data acquisition. Synthethic urine (Surine™ Negative Urine Control, Sigma, UK) was spiked with different amounts of methanolic drugs standard solutions to obtain final drug concentrations in matrix between 5ng-5000 ng/mL. Urine was sampled on glass rods which were then directly introduced in the source to proceed with the analysis.

Results
ASAP-MS analysis was performed on an array of abused drugs and metabolites in urine. Limit of detection and quantification, linearity, dynamic range and repeatability/reproducibility were evaluated for all the analytes of interest.

Conclusions
A remarkably high-throughput ASAP-MS method was developed and tested for an array of abused drugs and metabolites in bio fluid. No time consuming and complicated sample preparation was required. Application of this technique coupled to transportable MS could lead to very promising outcomes for on-site drug screening.

1. Moeller KE et al. Mayo Clin Proc. 2008; 83:66-76

TPS42-17 / Development of method for GHRPs determination in urine with solid-phase extraction microplates

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Introduction
Currently, there is growing interest in peptide compounds as doping agents and dietary supplements. Amidst them, growth hormone secretagogues (GHS) occupies attention of anti-doping laboratories all around the world. One of the representatives of GHS is a group of synthetic peptides Growth Hormone Releasing Peptides (GHRP) which consist of 5-7 amino acids, part of them are unnatural or D-amino acids. GHS unspecifically bind with transmembrane G-protein-coupled ghrelin receptor, which stimulates Growth Hormone (GH) production from pituitary glands. Thereby they enhance muscle strength and activity and provide rehabilitative effect after injury. Thus, GHRPs are performance-enhanced drugs and unapproved for human consumption by FDA (U.S. Food and Drug Administration). Moreover, most of them has not undergone preclinical and clinical trials. All of listed features were the reasons for including them in the prohibited list regulated by World Anti-Doping Agency (WADA).Nevertheless, GHRPs available in the drug-markets and there are evidences, that elite and amateur athletes use this substance as doping.
In several laboratories around the world methods for GHRPs determination in human biofluids were developed and introduced in routine screening: by LC-MS (Cologne, Rome, Montreal and Gent) and by surface plasmon resonance method (Barcelona). In this report we describe modified UPLC-MS method for GHRPs determination in urine. In optimized method microplates are used instead of catridges for solid-phase extraction, which significantly increase the productiveness.

Method
To purity and enrich targeted analytes before UPLC-MS/MS detection mixed-mode anion exchange solid phase extraction (SPE) microplates (Waters) and Positive Pressure Manifold (Waters) were used. Sample preparation includes several stages: equilibration of mixed-mode anion resin, sample loading, washing from unspecific urine components and elution of compounds of interest. The eluate was diluted 2-fold by 2% water solution acetic acid and analyzed for the presence of prohibited compounds by Ultra Performance Liquid Chromatography (UPLC) coupled to the TSQ Quantiva triple quadruple mass-spectrometer (Thermo Scientific).

Results
SPE microplates were used to enhance productiveness of method and ability to use it as screening technique. Using microplates

allows to increase the number of urine samples from 12-24 (with conventional SPE manifold) up to 92-94 per session of sample preparation. Positive pressure manifold can be used for N2-evaporation. This allows to include thermolabile peptides and proteins in the list of analytes of the method in future without any lost due to adhesion or temperature denaturation during evaporating of samples.

Novel aspect
Using SPE microplates for purification of small prohibited peptides from urine samples during sample preparation allows to promote the productiveness of method. Exclusion of temperature evaporating stage expands the list of the peptide analytes. Developed method can be used in anti-doping control.

TPS42-18 / HPLC-ESI-MS/MS for the determination the alkaloid content of the stem bark of Tetrapterys mucronata, a Malpighiaceae occasionally used to prepare the ayahuasca

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Introduction
Ayahuasca is a psychotropic plant decoction that has a long cultural history of use (Carlini, 2003). The ayahuasca decoction is typically prepared using the cortex and stems of Banisteriopsis caapi (Spruce ex Griseb.) C.V. Morton (Malpighiaceae) and the leaves of Psychotria viridis Ruiz & Pav. (Rubiaceae) (Halpern, 2004). Tetrapterys mucronata Cav. (Malpighiaceae) is a plant used in some regions of Brazil in the preparation of ayahuasca. For assessing safety issues in the usage of ayahuasca methods for the profiling of putative toxic compounds is key. In this respect MRM quantification of several alkaloids of interest have been developed.

Methodology
Aqueous and ethanolic extracts were obtained from the stem bark of T. mucronata and were analyzed by HPLC-PDA-ESI-MS and UHPLC-TOF-MS metabolite profiling. The isolation of the main compounds of the ethanolic extract was performed by directly transferring the analytical HPLC conditions to medium pressure liquid chromatography (MPLC) separation. The quantification of these compounds was performed by HPLC-ESI-MS/MS in Multiple Reaction Monitoring (MRM) mode.

Results
This procedure resulted in the isolation of four alkaloids (1-4) from ayahuasca, together with two amides (5-6) and one phenylpropanoid glycoside derivative (7). The alkaloids (1-4) were quantified in the stem bark of T. mucronata and revealed the presence of significant quantities of bufotenine and 5-methoxy-bufotenine. The determination of the level of such compound usually not found in ayahuasca is key, because they are consumed as drugs and are prohibited substances in countries such as the USA, the UK and Australia.

Conclusions
The tryptamine alkaloids bufotenine and 5-methoxy-bufotenine are known for their toxic and hallucinogenic properties, the results obtained indicate that the consumption of this plant as an ingredient in ayahuasca preparations may present a risk to consumers. In countries such as Brazil, where the consumption of ayahuasca is legal, attention should be paid to the more stringent control of the identity of the plant material used in the preparation of ayahuasca to avoid the risk of intoxication.

Novel aspects
This study provides the first identification and quantification of the bufotenine and 5-methoxy-bufotenine in a plant occasionally used in the ayahuasca preparation.

TPS42-19 / Solid-Phase Micro Extraction Atmospheric Pressure Chemical Ionization Mass Spectrometry (SPME-APCI/MSn) and its Application in Forensic Toxicology

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Introduction
Direct, fast and reliable techniques for the analysis of different components are important tools in forensic toxicology. Atmospheric pressure chemical ionization mass spectrometry equipped with a direct inlet probe (DIP-APCI-MS) has been demonstrated being a fast method for the direct analysis of solid or liquid samples1. Unfortunately the DIP setup does not provide any access to volatile organic compounds (VOCs). Furthermore an enrichment of trace level compounds is not possible. Solid-phase micro extraction (SPME) enables the sampling of VOCs out of the gas phase as well as trace level compounds out of liquids. Several strategies of coupling SPME with MS have been described2. Here we present a solvent free thermal desorption of SPME fibers in the APCI source providing a sensitive analysis by utilizing an ion trap mass spectrometer.

Method
The experiments were conducted using a Bruker Amazon Speed mass spectrometer (Bruker Daltonik, Bremen, Germany) equipped with a DIP-APCI II source. The SPME fibers were obtained from Supelco (Supelco, Bellefonte, PA, USA). The SPME fiber is thermally desorbed directly in the APCI vaporizer. For the evaluation of the method, different SPME coatings, gas flows, desorption temperatures were tested and different analytes with relevance in forensic toxicology were investigated. The mass spectrometer settings were chosen as follows: Nitrogen dry gas flow rate and temperature: 2L/min and 300°C. Corona needle current: 4000nA; Capillary voltage 4kV. Spectra were recorded in positive and negative ion mode. Scan speed: 32500 m/z /s. Mass range: m/z 70 - 600. MSn-experiments were conducted up to MS3 using auto-MSn mode.

Results
In a first set of experiments amphetamine base was tested as a model substance for VOCs. Amphetamine base is an important tracer for the detection of illicit drug laboratories. SPME samples were taken from headspace out of a 2.5L bottle containing different amounts of amphetamine. The molecular ion of amphetamine (positive ion mode, m/z 136) could clearly be detected with good intensities. Additional information for the identification was delivered by MSn fragment spectra. In a second set of experiments, cocaine and caffeine were extracted from a liquid using SPME and subsequently analyzed. Both substances could be easily identified via their molecular ions (positive ion mode, cocaine m/z 304; caffeine m/z 195) and their characteristic MSn spectra.

Conclusions and Novel Aspect
A simple and solvent free method for the direct mass spectrometric analysis of organic compounds collected on SPME fibers has been developed. The analytes are desorbed and ionized in an APCI source with subsequent analysis of the ions with an ion trap mass spectrometer.

References
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(2) Deng et al. "Strategies for coupling solid-phase micro extraction with mass spectrometry" Trends in Anal. Chem. 55, (2014), 55-67

TPS42-20 / Controlling the abuse of cobalt in horses

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Cobalt is a well-established chemical inducer of hypoxia-like responses, which can cause gene modulation at the Hypoxia Inducible Factor pathway to induce erythropoietin transcription. It had found initial medical use for the treatment of anemia, but such use had long been discontinued due to its adverse effects. Cobalt salts are orally active, inexpensive and easily available. Hence, it is an attractive blood doping agent for enhancing aerobic performance. Indeed, recent intelligence and investigations of out-of-competition samples as well as findings in official post-competition samples have confirmed that cobalt was being abused in equine sports. Since cobalt is naturally occurring, thresholds are necessary to be established for controlling its abuse in the horse. In this study, population surveys of total cobalt in raceday samples (> 7400 urine and > 370 plasma) were conducted using ICP/MS. Based on these data, a urinary threshold of 75 ng/mL and a plasma threshold of 1.5 ng/mL are proposed for the control of cobalt abuse in raceday or competition samples. Results from administration trials with a range of cobalt-containing supplements showed that these common supplements could elevate urinary and plasma cobalt levels above the proposed thresholds within 24 hours after administration. It is therefore necessary to ban the use of cobalt-containing supplements on raceday as well as on the day before racing in order to implement and enforce the proposed thresholds. Since the abuse with huge quantities of cobalt salts can be done during training, and legitimate cobalt-containing supplements are allowed to be used during training, different urinary and plasma cobalt thresholds are required to control cobalt abuse in non-raceday or out-of competition samples. This can be achieved by setting the thresholds above the maximum urinary and plasma cobalt concentrations observed or anticipated from the normal use of legitimate cobalt-containing supplements. Urinary threshold of 2000 ng/mL and plasma threshold of 10 ng/mL are proposed for the control of cobalt abuse in non-raceday samples. In addition, definitive LC/MS methods to confirm the presence of cobalt in urine and plasma will be presented. This paper describes a comprehensive and effective approach for the control of cobalt abuse or misuse in horses. To further improve the control of cobalt abuse or misuse in equine sports, a database of cobalt basal values in samples collected from a significant number of untreated horses in different geographical locations should be established, and more administration trials should be conducted with other legitimate cobalt-containing supplements commonly used on horses in different countries. Such work would require further international collaboration.

TPS42-21 / Rapid analysis of active ingredients in different dosage forms of pharmaceutical products by Desorption Electrospray Ionization Mass Spectrometry (DESI-MS)

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Introduction

A direct, rapid and selective analysis technique is important for the screening of seized items of evidence in forensic toxicological laboratories, particularly for seizures consisting of a high number of different exhibits which often occurs in the field of designer drugs and counterfeit pharmaceuti¬cals. The ambient MS technique Desorption-Electro-spray-Ionization-Mass Spectrometry (DESI-MSn) is especially helpful for the screening of various pharmaceutical dosage forms like tablets, gels, patches and injectables because of its identification power and the possibility to analyze samples directly. The fast information provided by DESI-MS screening greatly facilitates the direction of the follow-up analysis strategy. Significant selectivity enhance¬ment and suppression of matrix components can be achieved by a proper choice of the DESI-solvent and the ionization parameters.

Method

Experiments were performed using a Bruker HCTplus ion trap mass spectrometer, equipped with a Prosolia OmniSpray DESI source. The desorbing solvent (acetonitrile/water (75:25)) was supplied at a flow rate of 3µL/min by a syringe pump. Different spray impact and collection angles and tip-to-surface distances of 2-4 mm were applied. DESI-MS spectra were obtained in positive and negative ion mode with a scan speed of 26000m/z per second (mass range 50-500m/z). Auto-MSn experiments were performed for unambiguous analyte identification.

Results and conclusions

Different dosage forms of illicit pharmaceuticals were directly analyzed by DESI-MS in positive and negative mode. In white seized tablets without further information about the active ingredients Clenbuterol and Metandienone were identified. Counterfeited PDE-5 inhibitors containing products like "Viagra®" and "Levitra®" were directly analyzed by DESI-MS and the main ingredients were identified by MS/MS. The coating of the tablets was removed in a small spot by application of the DESI-solvent jet for approximately one minute. In addition to the tablets also inadmissible products containing Sildenafil like oral jellies (e.g. "Kamagra") were directly analyzed by DESI-MS. The flexibility of DESI-MS for the analysis of a broad range of pharmaceutical products was further successfully demonstrated by the identification of esterified anabolic steroids in oil-based injectables that are frequently misused in sports doping (e.g. Sustanon®) and of active substances in transdermal matrix patches of forensic relevance (buprenorphin, fentanyl and testosterone). Altogether, surprisingly low interference of matrix compounds in the various pharmaceutical dosage forms concerning analyte ionization and detectability was observed.

The presented results have been achieved in the course of the co-operative R&D project MIME, funded by the German ministry of education and research in the National security research programme.

Novel aspects

Direct DESI-MS analysis of a broad range of pharmaceutical dosage forms as a new screening tool for police and customs units dealing with big and heterogeneous seizures.

TPS42-22 / Use of dynamic bilayer polymer coatings in the trace analysis of controlled substances by capillary electrophoresis-mass spectrometry

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Introduction

Trace analysis of controlled substances is important in forensic toxicology, e.g. the assessment of traces of illicit drugs in wipe or suction samples of potentially contaminated surfaces in order to establish evidence of a drug transport or a clandestine production. These samples typically exhibit complex matrices consisting of dust, oily constituents, plasticizers and other environmental chemicals. Existing methods for their analysis by GC-MS or LC-MS are often susceptible towards problematic

matrices and need complex sample preparation. The coupling of capillary electrophoresis with mass spectrometry via electrospray ionization (CE-ESI-MS) is a promising alternative, especially when dynamically coated capillaries are employed to reduce surface adsorption and analysis times.

Method
The setup consisted of an Agilent 7100 CE instrument and a Bruker HTCplus ion trap mass spectrometer coupled with an Agilent coaxial sheath-liquid ESI-sprayer. The sheath liquid (MeOH/H2O 1:1, 0.1% formic acid) was supplied by a syringe pump (Fisher Scientific) with a flow rate of 7 µL/min. The run buffer was made up of 200 mmol/L formic acid with 10% isopropanol. The separation voltage was set at +28 kV, capillary length was 70 cm and i.d. 50 µm. Nebulizer gas pressure was at 10 psi, dry gas flow 4 L/min and temperature 250°C. Two bilayer coatings, PB-PVS (hexadimethrine bromide/polyvinylsulfonic acid) and PB-PSS (hexadimethrine bromide/poly-4-styrene¬sulfonic acid), were compared to bare fused silica capillaries.

Results
The analysis time for the separation of a mixture of controlled substances including heroin, MDMA, methamphetamine, benzoylecgonine, cathinone derivatives such as MDPV, 3,4-DMMC, 4-MEC, methylone, butylone, methedrone, methcathinone and β-phenylethylamine as internal standard is shortened from 25 min to 8 min in dynamically coated capillaries. Additionally, the reproducibility of the migration time in the coated capillaries improves significantly compared to uncoated capillaries e.g. for heroin from 1.3 % to 0.13 %. In case of the PB-PVS coating a small amount of PVS (0.0025 %) has to be present in the run buffer to obtain a constant stability of the coating which had no adverse effects for MS detection (as ion suppression). The PB-PSS coating does not require a permanent addition of the anionic polymer in the run buffer at all and is stable for an extended time-frame. The method was successfully applied to extracts of wipe samples from forensic case work.

Conclusion
The application of bilayered polymer coatings in capillary electrophoresis coupled to mass spectrometry leads to shorter analyses times and better peak shapes. There is less adsorption of analytes and matrix to the capillary wall, a higher EOF despite of low pH buffers and almost no impairment of MS detection, resulting in a highly suitable concept for trace analysis of illicit drugs in complex sample matrices.

Novel aspects
Trace analysis of controlled substances in samples with complex matrices with dynamically coated capillaries in CE-MS. Application to new types of designer drugs (cathinone derivatives).

TPS42-23 / Isolation and mass spectrometric identification of new cannabimimetic designer drugs and related synthesis impurities in ´Spice´ products

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Introduction
Starting from 2008 herbal mixtures like ‘Spice’ were sold in many European countries mainly via internet. Although declared as incense, they are smoked by the consumers as a Cannabis substitute. Typically, the contained synthetic, cannabimimetic drugs, often amino alkyl indoles clandestinely produced in Asian countries, are immediately replaced by new designer drugs of the same class in case ofsubmission under the controlled substances acts. This situation has not only caused enormous problems for legislation and criminal prosecution, but also for the forensic laboratories with new synthetic substances surfacing every few weeks. Some of them are only marginally treated in the scientific literature and little is known about the various synthesis pathways of these substances necessitating a more intensive research regarding occurring side compounds in the named products.

Methods
For this work ´Spice´ products of four different brands from recent police seizures were used. Approximately 70g of each herbal product were extracted twice, first with 350 mL and again with 100 mL acetonitrile. The resulting extracts were evaporated to dryness and submitted to normal phase column chromatography to separate main active and side compounds in a preparative scale. MS-experiments were performed using a Dionex UHPLC coupled to a Bruker AmaZon speed ion trap mass spectrometer equipped with an ESI-source. For chromatography a 100*2.1 Kinetex 2.6 µm C18 column at 40 °C was selected. A stepwise gradient with two eluents based on water/acetonitrile/formic acid at a flow rate of 0.5 mL/min over 12 min was applied. ESI voltage was set at +4.5 kV. The MS was operated in “ultra scan” mode in the range of 70-600 m/z with a scan speed of 32500 m/z per sec. Auto-MSn experiments were performed for unambiguous analyte identification. Additionally, HR-MS experiments by Orbitrap-MS were performed for exact mass determination of selected fragments.

Results
The main active ingredients, new cannabimimetic designer drugs XLR-11, STS-135, APICA and 5F-PB-22, were separated from synthesis impurities and components of the herbal matrices. In other chromatographic fractions even low concentrated synthesis side products of the main active substances were isolated and structure elucidation performed by MSn experiments. In each analyzed herbal brand the chemical structures of at least 15 different trace components were found and verified by HR-MS and NMR measurements. Due to varying retention times and fragmentation pathways even positional isomers were distinguishable. In the case of APICA e.g. three different methylated species occurred in which the methyl group was either attached to the adamantyl moiety, the indole or the pentyl chain.

Conclusion
The identification of cannabimimetics and synthetic trace components in herbal mixtures by state-of-the-art MS techniques is an important factor to stay on track with the ongoing introduction of new psychoactive substances to the worldwide illicit drug market.

Novel Aspects
Preparative LC of cannabinoid synthesis impurities with subsequent identification and structure elucidation by ESI-MS/MS and ESI-HR-MS/MS experiments.

TPS42-24 / Detection of methasterone metabolites in human urine, elucidation of their glucuconjugates and excretion kinetics

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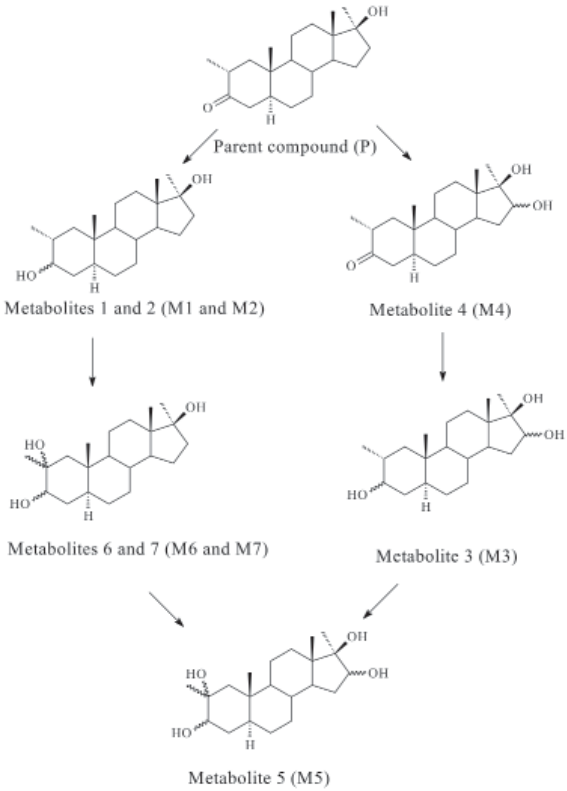
Androgenic steroids have been widely used doping agents in sports for their strength and weight gain properties. Metabolism studies are very important in order to determine the best targets for analysis, since normally unaltered drugs are excreted only for a brief period of time. Methasterone is very popular amongst bodybuilders and is easily bought online as a “nutritional supplement”. Its metabolism has been investigated in vitro, showing some metabolites which could possibly be formed by

human metabolism. However, this kind of study is unable to determine the phase 2 metabolism of the compounds, which is why human metabolism studies are still extremely necessary.
The main goals of the present work were to study the human metabolism and urinary excretion of methasterone and to determine the best analytical targets for its detection in toxicological, forensic and doping analyses.
Methasterone was administered to two male volunteers, urine samples were collected and post-administration samples were compared to their respective blank urines in order to allow metabolite detection. Samples were analyzed with and without enzymatic hydrolysis using beta-glucuronidase, in order to allow the determination of glucuconjugates. Sulphates and other phase 2 metabolites were searched for using mass spectrometric approaches such as neutral loss and parent ion scans using UPLC-ESI-MS/MS and the unconjugated metabolites were analyzed by GC-EI-MS after derivatization with MSTFA/NH4I/2-mercaptoethanol.
Seven metabolites were detected and identified in urine samples from volunteers who used methasterone. No metabolite peaks were detected for sulphate and cysteine and N-acetylcysteine conjugates. The excretion form of these metabolites was determined to be glucuconjugated, since no metabolite peaks were detected in the free fraction and the excretion kinetics was evaluated, revealing a long term excretion metabolite: 2ξ,17α-dimethyl-5α-androstane-2ξ,3ξ,16ξ,17β-tetrol. This metabolite was still detectable in the urine samples after 7 days, while the parent compound was only detectable until 48 hours since administration. This represents a great advantage in detecting the abuse of this compound in doping control, which is why this metabolite is therefore recommended as the analytical target for the detection of the use of this steroid.



TPS42-25 / Quantitation of 23 designer Cathinones in Urine by GC-MS-MS

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Cathinones, so-called as «legal highs», are closely related to amphetamines and have similar stimulating effects. Recently, these cathinone derivatives have been getting more popular among drugs users in Taiwan and seriously. The development of analysis method of cathinones demanded by Court is urgent. For the demand, we developed quantitative method for monitor 23 designer cathinones in urine samples by GC-MS-MS simultaneously. The samples were extracted by solid phase extraction (SPE) with Bond Elut certify 130 mg 3 mL column, deuterium labeled methylone, mephedrone, methdrone, 4-fluorometh-cathinone, methylenedioxypyrovalerone (MDPV) and diethylcathinone as the internal standards.

The development of the present study of cathinones include: (1) The detection limits of GC-MS-MS / MS2 for monitor the most commonly abused 23 cathinones were less than 200 ng/mL. (2) The determination coefficient range was 0.990~0.999, the limit of quantitation range was 1~5 ng/mL and inter-day precision range was 5%
~20% by confirmation method. (3) Within 50 authentic urine specimens collected from previously identified case for heroin and methamphetamine abuse from court, it was successfully illustrated that 3,4-Methylendioxycathinone (bk-MDA) and Methylone were identified in some cases.

The aim of this paper is to summarize the information about cathinones abuse in Taiwan and can be applied to cathinones abused cases. In the future, we will develop method for hair analysis.

TPS42-26 / Optimization and application of UHPSFC-MS/MS method in screening of doping agents

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Univerzita Karlova v Praze, Farmaceuticka fakulta v Hradci Kralove

Introduction
Supercritical fluid chromatography (SFC) has become competitive with current LC approaches only recently, with the introduction of modern SFC platforms. Due to the properties of supercritical fluid and high flow-rates, the analysis time can be substantially decreased in comparison with LC procedures, while maintaining or increasing the separation efficiency, especially when using sub-2-µm particles, known as ultra-high performance supercritical fluid chromatography (UHPSFC). Successful coupling of SFC with MS has already been described. However, the use of SFC-MS in the analysis of biological materials has been reported scarcely. The reason was probably the insufficient quantitative performance of the old SFC-MS platforms. Implicitly, also the information on detailed optimization of MS conditions and SFC mobile phase for coupling with MS and the evaluation of matrix effects is missing in scientific literature.

Methods
Two separation approaches ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) and ultra-high performance supercritical fluid chromatography tandem mass spectrometry (UHPSFC-MS/MS) were optimized in detail and subsequently applied in the analysis of 110 doping agents in urine. The two methods were compared in terms of method sensitivity, linearity and matrix effects.

Results
In the first step, both UHPLC-MS/MS and UHPSFC-MS/MS method were optimized in detail in terms of mobile phase composition, ESI source conditions and make-up solvent in case of UHPSFC-MS/MS. Concerning mobile phase, for UHPLC-MS/MS, 0.1 % formic acid and for UHPSFC-MS/MS CO2/MeOH based mobile phase with the addition of 2 % water and 10 mM ammonium formate were found to be the most generic conditions. Ethanol was chosen as the make-up solvent added for interfacing UHPSFC with MS.
Using these conditions, 110 doping agents were analyzed in urine using both methods. The evaluation of matrix effects at four concentration levels revealed generally their lower incidence in UHPSFC-MS/MS compared to UHPLC-MS/MS. While signal suppression was mainly observed with UHPSFC-MS/MS, signal enhancement was dominating in UHPLC-MS/MS, reaching extreme values in some cases, especially at low concentrations. The sensitivity of both techniques was adequate for screening purposes, and both techniques provided low enough LOQs in urine matrix (except bumetanide in UHPSFC-MS/MS), in agreement with the MRPL requested by WADA.

Conclusion
With both techniques, a high efficiency separation and sensitive MS/MS detection enabled the analysis of all 110 doping agents with high throughput (7 minutes) and acceptable peak shapes in both ESI+ and ESI-, even for most basic substances. Thus, UHPSFC-MS/MS was for the first time proven to be applicable for the anti-doping screening of urine samples.

Acknowledgement
The authors gratefully acknowledge the financial support of research projects of Charles University in Prague UNCE 204026/2012 and PRVOUK.

Novel aspect
Application of UHPSFC-MS/MS for the analysis of biological materials and evaluation of matrix effects. UHPSFC-MS/MS for doping control analysis.

TPS43 - Environmental Analysis

11:00-15:00
Poster Exhibition, Level -1

TPS43-01 / Effluent, surface, ground and drinking water analysis of classical and novel drugs used in cancer treatment: 5-fluorouracil and protein kinase inhibitors

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Introduction
Especially regarding the occurrence of drugs used in cancer therapy, very limited data are available, as these compounds are not part of regular monitoring programmes. Presumably, the sometimes rather extreme physicochemical properties of a number of these compounds make it difficult to quantitatively determine them at sufficiently low levels in environmental matrices. To add to the knowledge on the occurrence of some pharmaceuticals that are used in the treatment of cancer, we have developed and validated a method for the quantitative analysis of 5-fluorouracil (5-FU), which can be regarded as a classical anticancer drug that has been in use for decades, usually administered to patients in a clinical setting. In addition, we have focused on the more recently introduced protein kinase inhibitors (PKIs). These drugs are used more or less chronically as oral medication, without the need for in-hospital administration. In this poster, concentrations of 5-FU, imatinib, sorafenib, erlotinib and sunitib will be presented in effluents and surface water at various locations in The Netherlands.

Methods
For both compound classes, a solid phase extraction procedure and an LC-ESI-MS method was developed. For the PKIs imatinib, sorafenib, erlotinib and sunitib, an LOD of 0.01 ng/L was obtained. The LOD for 5-FU was 4 ng/L.

Results
In all surface water samples, the PKIs were measured at or just below the ng/L level. In ground and drinking waters, traces of the PKIs were encountered, usually well below 0.5 ng/L. The concentrations in the effluents were, as expected, the highest, with maximum levels of 12 ng/L.

Discussion
The classical anticancer drug 5-FU was not measured in any of the samples. This is most likely due to the relatively high LOD of 4 ng/L, which is well above the expected level of around 1 ng/L in European STP effluents (Johnson et al., 2013). Imatinib and erlotinib have been reported as High Production Volume Pharmaceutical (Howard & Muir, 2011). Both compounds are bioaccumulative, and imatinib has been classified as persistent. Occurrence, fate and effects of the PKIs need to be studied in more detail, taking their potential effects (after chronic exposure) on aquatic organisms into account. Occurrence of the PKIs in especially surface and drinking water sources most likely has a negative influence on the public perception of (drinking) water quality and safety.

Conclusions
To study the occurrence of a selection of anticancer drugs, a small survey was carried out using water samples of diverse origin that were kindly provided by The Waterlaboratory. For 5-FU, a method with a lower LOD needs to be developed. For the PKIs, a suitable method is available, revealing the presence at ng/L level of various PKIs in effluents of STPs and in surface waters. The PKIs were sporadically measured in very low concentrations in ground- and drinking water samples.
Novel aspects This is the first report of the occurrence of four selected anticancer drugs belonging to the PKIs in environmental water samples.

TPS43-02 / Development of new materials for passive samplers based on porous organogels followed by GC-MS analysis

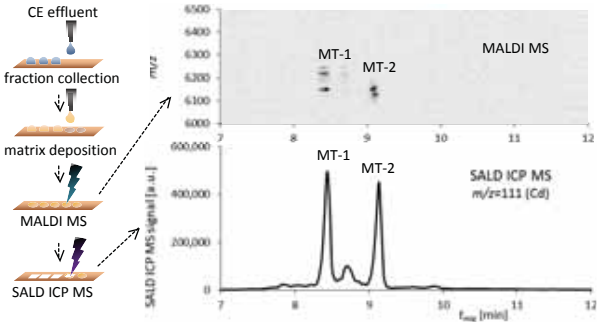
Eric Leroy[1], Jean-Christophe Garrigues[2], Sophie Franceschi[2], Emile Perez[2], Alexandra Ter Halle[2]
[1]Université Paul Sabatier-Service commun de spectrométrie de masse, [2]Université Paul Sabatier, Laboratoire des IMRCP, UMR CNRS 5623

Introduction
A new type of passive samplers made of porous organogel is under development. An organogel is a semi-solid system in which a liquid is immobilized by a three-dimensional network composed of self-assembled gelator fibers. In the laboratory we have developed an eco-friendly method to introduce and control the microporosity inside the organogel. Sugar crystals are used as water soluble templates to introduce the porosity.

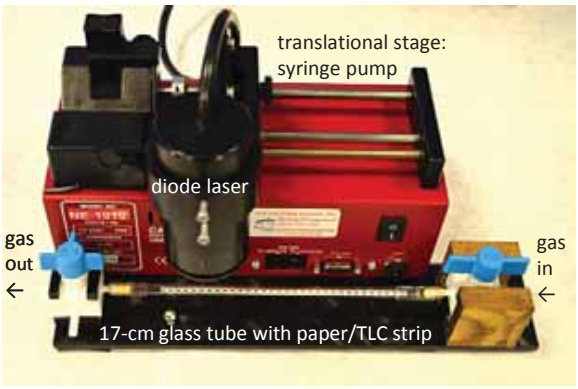
Methods
Porous organogel passive samplers were tested under laboratory conditions in pure water spiked with model compounds (Anthracene, Phenanthrene or the 16 priority HAP in mixture). The uptake was undertaken for 8 hours. For GC analysis the

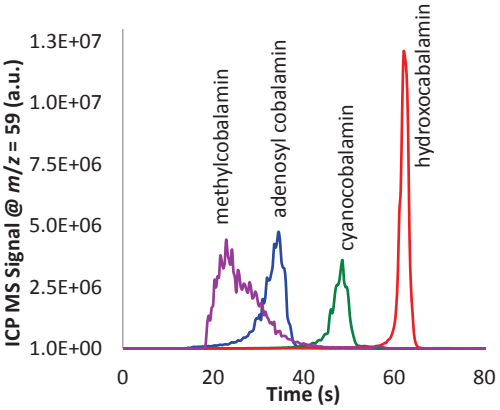
materials were simply removed from the aqueous solution, dried and dissolved in a minimum of MeOH (or CH2Cl2). The mixture was injected in GC-MS.

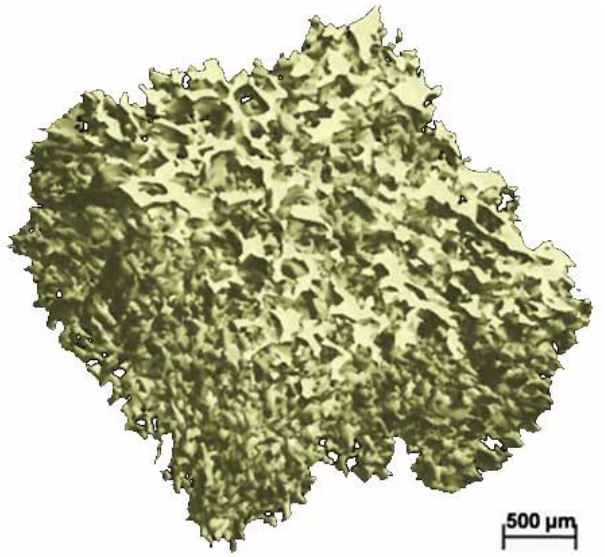
CE – MALDI MS/SALD ICP MS of metallothioneins



The chamber for DLTV (a) and TLC – DLTV ICP MS of four cobalamins







Results

Shown is a 3D microtomography reconstruction of a porous organogel prepared with caprylic/capric triglyceride (organic liquid) and 15w% of a gelator (12 hydroxystearic acid). The resulting material revealed interconnected open-pore structures with an effective porosity ranging from 56 to 65 %. Pore sizes ranged from 25 to 500 micron and could be modulated by varying the size of the grains in the template.

A large variety of organic liquid can be gelled ; consequently the sampler can be adjusted to the polarity of the organic pollutant to be sampled.

Conclusion

Pollutants diffuse much more rapidly into the immobilized liquid in the organogel due to the absence of a diffusion barrier compared to classical passive samplers. Due to the rapid exchanges with the surrounding media, this new type of materials should be considered as equilibrium passive sampler. Moreover the solubilization ability of the gelled liquid allows an important accumulation of the pollutants inside the material for an enhanced sampling.

Perspective

The rapid uptake of this kind of material could help our understanding on both thermodynamic and kinetic control on organic pollutant in the environment at very low concentration.

TPS43-03 / Occurrence of endocrine active substances in wastewater and river water collected from the aquatic environment of Taiwan

Pei-Hsin Chou[1], Yi-Po Yeh[1], Kuang-Yu Chen[1], Masanobu Kawanishi[2], Takashi Yagi[2]

[1]National Cheng Kung University, [2]Osaka Prefecture University

Introduction

Over the past few decades, environmental contaminants capable of binding to nuclear hormone receptors have drawn much attention owing to their ability to interfere with endocrine systems at trace concentrations. To protect human health and the environment, it is important to identify pollution sources and to determine environmental relevant concentrations of these endocrine active substances. In this study, liquid chromatography tandem mass spectrometry (LC−MS/MS) and bioassays were used to investigate the occurrence of endocrine active substances in the aquatic environment of Taiwan. LC−MS/MS is a powerful tool to quantitatively analyze endocrine active substances, and bioassays are useful techniques for evaluating the combined effects of a mixture of pollutants. The combination of LC−MS/MS with bioassay analysis is beneficial for identifying major contaminants and discovering potential emerging pollutants in the environment.

Methods

Sewage influent, treated effluent, and river water samples were collected from municipal wastewater treatment plants and several river systems in Taiwan. Water and suspended solids were separated by filtration and were further extracted using solid phase extraction and soxhlet extraction. The quantification of natural and synthetic endocrine active substances, such as estrone, 17β-estradiol, estriol, 17α-ethynylestradiol, 5α-dihydrotestosterone, bisphenol A, nonylphenol, triclosan, etc., was carried out using an Agilent 1260 HPLC system coupled to a Thermo Scientific TSQ Quantum Ultra triple quadrupole MS. Endocrine disrupting activities were measured by yeast-based reporter gene assays using recombinant strains co-transfecting with human androgen receptor and estrogen receptor.

Results
Natural estrogens, xenoestrogens, and xenoantiandrogens were often detected in wastewater and river water samples by LC−MS/MS. In addition, natural androgens were also found in sewage influent. Bioassay analysis showed that wastewater treatment processes were able to reduce endocrine disrupting activities, whereas estrogenic, antiestrogenic, and antiandrogenic activities were occasionally found in the treated effluent. Target compounds analyzed by LC−MS/MS only partially contributed to the endocrine disrupting activities found in effluent and river water samples, suggesting the presence of unidentified endocrine active substances in the aquatic environment of Taiwan.

Conclusions
The combination of LC−MS/MS analysis and bioassay assessment successfully revealed the occurrence of endocrine active substances in wastewater and river water in Taiwan. Experimental results showed that a variety of trace contaminants were found and significant estrogenic, antiestrogenic, and antiandrogenic activities were often detected. Further investigation will be undertaken to elucidate the mixture effects and to identify emerging endocrine active substances.

Novel Aspect
By using LC−MS/MS and bioassays, major endocrine active substances in wastewater and river water in Taiwan were identified. Also, the distribution of endocrine active substances in different river systems in Taiwan was also presented.

TPS43-04 / Identification of chemical structures of polyfluoroalkyl substances in fire extinguishing chemicals by using ultra high resolution mass spectrometry

Atsushi Yamamoto[1], Shiho Kitai[2], Yasuyuki Zushi[3], Shigeki Masunaga[4], Hideya Kawasaki[2], Ryuichi Arakawa[2]
[1]Osaka City Institute of Public Health and Environmental Sciences, [2]Kansai University, [3]National Institute of Environmental Studies, Japan, [4]Yokohama National University

Novel Aspects
Fire extinguishing chemicals before the regulation included several polyfluoroalkyl substances previously unknown to environmental researchers.

Abstract
Per- and Polyfluoroalkyl substances (PFASs) have been used in a wide range of products because of their versatility. However, a part of PFASs, such as perfluorooctane sulfonic acid (PFOS), has been recently substituted because their bioaccumulation potency and adverse health effects were reported. In the environmental analyses, to understand the environmental pollution by PFASs, target analysis by liquid chromatography/mass spectrometry (LC/MS) is commonly used. However, it is not enough to carry out target analysis alone because PFASs are present in various chemical forms. Recently non-target analysis with high-resolution (HR) MS was applied for the detection of unknown PFASs in consumer products and showed successful results [1]. Among various applications of PFASs, fire extinguishers are considered one of the most important applications. Fire extinguishers are equipped in parking facilities, airports, and industrial complexes to prevent petroleum fires. The fire extinguishing chemicals are spread over the surface of the flammable hydrocarbon and make air bubbles to hinder evaporation and re-ignition of the hydrocarbon. Although Japan's authority put regulations on fire extinguishers including PFOS in 2010, it is estimated that the contribution to contamination by PFOS is predominant, compared to other applications. Therefore discovery of marker substances for fire extinguishing chemicals has been anticipated. Here, we used HR-MS to identify the chemical structures of the fire extinguishing chemicals.
Dionex UltiMate 3000 and ThermoFisher Orbitrap Exactive were used as LC/MS in the present study. The products examined here consisted of fire extinguishing chemicals used before the regulation. Full scan and all ion fragmentation modes (25 eV) in electrospray ionization were used to acquire mass spectra.
The presence of several compounds with C8F17 chains was indicated from the extracted ion chromatograms of m/z 418.9734. At the same retention time as each peak in the chromatogram, the relative ions of C8F17– were separated from other ions by some criteria, such as peak shapes, difference of m/z from 418.9734, and isotopolog ratio. Consequently, the presence of chemicals with perfluorooctane sulfonamido group was indicated. Similar approaches were tiered to discover larger PFASs. Two PFASs with two C8F17 chains were finally identified.
LC/HR-MS was successfully applied to the identification of specific PFASs in fire extinguishing chemicals. The analysis of these PFASs in environmental research will show the contribution to PFOS contamination from fire extinguishing chemicals.

Acknowledgement
This work was financially supported by Grant-in-Aid of Japan Society for the Promotion of Science (26241026).

Reference
[1] Trier X., Granby K., Christensen J.H., J. Chromarogr. A, 2011, 1218, 7094-7104.

TPS43-05 / Determination of alkylphenol ethoxylate in textiles and leathers by NPLC and quadrupole orbitrap MS

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This paper presents a method for the determination of alkylphenol polyethoxylate (APEO) in textiles and leather by normal-phase liquid chromatography and quadrupole-orbitrap mass spectrometer of high sensitivity and resolution based on exact mass.
The ubiquitous presence of APEO in the environment as well as concern for endocrine disruption effects in biota caused by their degradation products (such as octyl- and nonylphenol) has raised interest in the environmental fate of these compounds.
The technique for normal phase liquid chromatography was completely separated various APEO from the short(2) to long(20) ethoxy chain length APEOs using acetonitrile and methanol as mobile phase. Quadrupole orbitrap mass spectrometer was used high resolution (R=70,000) to separate single/double charged ion by electrospray competition effect and also eliminated isobaric interference of small ethoxylate monomers of alkylphenols using the sensitivity of femtogram unit to qualitative analysis smaller monomer between 1~5 ng/mL. The difference value(delta (Δ) ppm) between exact mass and experimental mass was less than ±5 ppm, and the result for quantitative analysis was able to detect alkylphenol ethoxylate as ng/mL unit.

TPS43-07 / Quantification of TBT by GC-MS/MS in water samples at levels required by the WFD and stability studies of butyltin compounds by using a triple spike approach.

Andres Rodriguez Cea, Pablo Rodriguez-Gonzalez, J. Ignacio Garcia Alonso
University of Oviedo

Introduction
The European Water Framework Directive (WFD) requires for tributyltin (TBT) a limit of quantification (LOQ) of 0.06 ng L-1 in surface water samples. On the other hand, one of the

main problems when analysing organotin compounds in water samples is their stability. In fact, the preparation of a reliable water reference material has not been carried out yet. The study of the stability of TBT, DBT and MBT and their interconversions under different storage conditions (temperature, light, container or addition of stabilising chemicals, etc) is a necessary step before the preparation of any water reference material.

Methods
For quantitative purposes, a 250 mL water sample (0.06 ng L-1 of TBT) is mixed with an appropriate amount of the 119Sn-enriched butyltin mixture. After derivatization and liquid-liquid extraction in hexane, the analytes are injected into the GC-MS-MS operating in Multiple Reaction Monitoring (MRM) mode acquisition. The concentration of the natural butyltin compounds in the water samples is then calculated by Isotope Dilution Mass Spectrometry (IDMS) in combination with Isotope Pattern Deconvolution (IPD). For the stability studies, river water samples were mixed with a triple spike enriched solution and storage in glass, polypropylene or PTFE containers. Samples were kept under different temperatures (22ºC, 4ºC and -20ºC), in darkness or exposed to natural light. Acetic acid or HCl was added to some samples as stabilizing agents. After two weeks (short-term stability study) or four months (long-term stability study) samples were analysed by GC-MS-MS.

Results
The recovery (%) of TBT calculated for three independent water samples at a level of concentration of 0.06 ng L-1 was 126.6 ± 1.2. The LOQ for TBT, calculated as ten times the standard deviation of nine blanks turned out be 0.04 ng L-1 of TBT. The influence of the different storage conditions on the stability of TBT, DBT and MBT and their interconversions was observed by using the triple spike approach.

Conclusions
A procedure based on IDMS for the determination of TBT in water samples using GC-MS-MS operating in MRM meets the requirements of the WFD in terms of LOQ. The triple spike approach is a useful tool for the study of the stability of butyltin compounds.

Novel aspects
For the first time, the measurement of the isotope ratios for butyltin compounds in MRM has been applied for the quantification of these compounds in surface water samples and for the study of their stability.

TPS43-08 / Comparison of different mass spectrometric techniques for the determination of polychlorinated byphenyls by isotope dilution using 37CL-labelled analogues

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Introduction
Polychlorinated biphenyls (PCBs) are a group of ubiquitous contaminants considered persistent organic pollutants. Although the industrial use and production of PCBs has long been banned, the persistency of these compounds means they still represent a serious environmental problem. The determination of PCBs in environmental samples has been traditionally performed by GC/MS in combination with isotope dilution using commercially available 13C12 labeled internal standards. The recent synthesis of 37Cl-labelled PCBs allows the use of both molecular and elemental ionization sources for the accurate determination of PCBs in environmental and biological samples.

Methodology
A mixture of priority PCBs labelled in 37Cl has been synthesized in our laboratory to be used as isotopic tracer for the determination of PCBs in solid samples. The extraction of the natural abundance PCBs was carried out by focused microwaves in less than ten minutes. Multiple linear regression is applied to calculate directly, from the experimental mass spectra of the samples, the molar fraction of the isotopic tracer and the analyte, avoiding the construction of a methodological calibration graph.

Results
We compare four different mass spectrometric techniques coupled to Gas Chromatography: single quadrupole ICP-MS, triple quadrupole ICP-MS/MS, single quadrupole NCI-MS and triple quadrupole EI-MS/MS. The comparison was carried out by performing the reverse isotope dilution analysis of a mixture of 37Cl-labelled PCB standards and the analysis of two different Certified Reference Materials (sediment and mussel tissue) containing PCBs at the very low ng g-1 range.

Conclusions
Elemental ionization sources such as ICP and NCI provide chlorine specific detection but suffer from high background signals in complex solid matrices as sediments or biotissues. These matrices contain a large variety of chlorine compounds that complicate the chromatographic separation. However, the measurement of the isotopic composition of chlorine is facilitated by the elemental ion sources. On the other hand, the use of GC-MS/MS in the SRM mode has been shown to provide the highest level of accuracy and precision for PCBs determination by IDMS at ng/g levels in real samples.

Novel Aspects
The newly synthesised 37Cl-labelled PCBs enables the use of elemental ionization sources such as ICP or NCI because the isotopic label is in the heteroatom. Isotope effects are minimized this way in comparison with multiply labelled analogues in deuterium or 13C. The use of focused microwave assisted extraction reduces drastically the total analysis time and the use of organic solvents in comparison with more conventional procedures such as methods based on Soxhlet extractions. The application of multiple linear regression avoids the construction of a calibration graph decreasing thus the total analysis time.

TPS43-09 / Multi-component quantitative analysis of pharmaceuticals in the environment by UHPLC-MS/MS with on-line SPE

Anja Grüning[1], Julia Sander[1], Ute Potyka[1], Stephane Moreau[1], Mikael Levi[2]
[1]Shimadzu Europa GmbH, [2]Shimadzu France

Introduction
Pharmaceuticals comprise a group of emerging contaminants which have received considerable attention in recent years.Many common drugs can be found in the environment and sometimes even in drinking water. These drugs and their metabolites get into the waste water through excretion via the urine or feces and may reach surface water, groundwater and also drinking water after the passage in the sewage treatment plants. So far, conventional sewage treatment plants are failing to eliminate the Biodegradable substances completely. Many of these compounds are ubiquitous, persistent and biologically active with recognized endocrine-disruption functions. Paying attention to the hazardous nature of these compounds, there is a need to provide fast and sensitive multi-residue methods that are able to analysis multiple classes of compounds within one analytical procedure.

Methods
This study describes a novel multi-residue UHPLC-MS/MS method that utilizes an online SPE enrichment of the various compounds followed by a fast and optimized chromatographic gradient which results in excellent ng/L detection levels.

Results
With online SPE no further sample pre-treatment is necessary but the transition of the low pressure online SPE part of the analysis to the high pressure analytical part is difficult.

Conclusion
Using the benefit of the modular design of Shimadzu's Nexera X2 combined with the high speed values for MRM recording and the fastest polarity switching time of 5 ms on the Shimadzu LCMS-8050, the difficulties of analyzing various classes of compounds in different polarities during one single analysis in sufficient sensitivity could be overcome.

Novel aspect
Using the benefit of the modular design of Shimadzu's Nexera X2 and the high speed values of the LCMS-8050 to overcome the difficulties of low pressure online SPE coupled to an UHPLC.

TPS43-10 / Aerobic activated sludge transformation of methotrexate: identification of biotransformation products
Tina Kosjek[1], Noelia Negreira[2], Ester Heath[1], Miren Lopez de Alda[2], Damia Barcelo[2]
[1]Jozef Stefan Institute, [2]IDAEA-CSIC

Introduction
Among various classes of pharmaceuticals, cytostatic chemotherapy drugs are of particular environmental concern because they are potentially carcinogenic, mutagenic and genotoxic. Their fate in the environment is largely unknown, but they can contaminate wastewater treatment effluents and consequently aquatic ecosystems. This study describes the biotransformation of the cytostatic and immunosuppressive pharmaceutical methotrexate.

Methods
The susceptibility of methotrexate to microbiological breakdown was studied in a batch biotransformation system, in presence or absence of carbon source and at two activated sludge concentrations. The primary focus of the present study are methotrexate biotransformation products, which were tentatively identified by ultra-high performance liquid chromatography-quadrupole-Orbitrap-mass spectrometry (UHPLC-Q-Orbitrap-MS). Data-dependent experiments, combining full-scan MS data with product ion spectra were acquired, in order to identify the molecular ions of methotrexate transformation products, to propose the molecular formulae and to elucidate their chemical structures.

Results
Among the identified transformation products of methotrexate 2,4-diamino-N10-methyl-pteroic acid is the most abundant and persistent. Other biotransformation reactions involve demethylation, oxidative cleavage of amine, cleavage of C-N bond, aldehyde to carboxylate transformation and hydroxylation. Finally, a breakdown pathway is proposed, which shows that most of methotrexate breakdown products retain the diaminopteridine structural segment.

Conclusions
The findings suggest that MET is readily biodegradable and broken down to transformation products, of which nine were successfully identified. Q Exactive hybrid quadrupole - Orbitrap MS served as a powerful tool in structural elucidation of the transformation products, and was concurrently employed to obtain the quantitative information of methotrexate breakdown, all in a single run.
Even though our experiments attempted to mimic the biotransformation that occurs during real wastewater treatment, one needs to be aware that the formation of transformation products highly depends on the composition of a wastewater matrix and activated sludge, as well as on the concentration of the parent compound. Therefore, further investigations in this field are necessary, including studies on occurrence and effects of the identified compounds in sewage works and in the environment.

Novel Aspect
In total we propose nine transformation products, among them eight are described as methotrexate transformation products for the first time.

TPS43-11 / Determination of endocrine disrupting compounds in water samples by isotope dilution mass spectrometry
Neus Fabregat-Cabello, María Ibáñez, Juan Vicente Sancho, Antoni Francesc Roig-Navarro
Universitat Jaume I. IUPA

Introduction
Endocrine Disrupting Compounds are a group of exogenous organic compounds which are able to interfere with the normal function of the endocrine system. Their mechanisms are mainly based on mimic a natural hormone or by inhibit or stimulate the production of hormones. These compounds also form part of the so-called "emerging" pollutants, which are natural or synthetic compounds generated by industrial, agricultural and urban activities that have been detected in several environmental matrices over the past 10 years or so (U.S. Geological Survey 2013). Among them, alkylphenols and plasticizers still present a challenging determination due to their ubiquitous presences as contaminant as well as the method sensitivity required in order to accomplish the international regulations.

Methods
The developed methodology is based on the quantitative and confirmative determination of two alkylphenols (nonylphenol (NP) and t-octylphenol (OP)) and two plasticizers (bisphenol A (BPA) and di-2-ethylhexyl phthalate (DEHP)) in complex matrix water samples by UHPLC-ESI-MS/MS. The extraction is achieved by means of hollow fiber liquid phase microextraction (HF-LPME). Alternatively, the quantification method is based on Isotope Dilution Mass Spectrometry (IDMS) using the corresponding isotopically labeled analogues of each target analyte. The quantification is carried out by using isotope pattern deconvolution (IPD).

Results
A thoroughly study of the different contamination sources has been performed in order to establish the different contamination sources and maintain the blank levels. Afterwards, the developed methodology has been validated in in tap water and waste water (influent and effluent) at two levels.

Conclusions
We present here a new measurement method for the rapid extraction and accurate quantification for OP, NP, BPA and DEHP. The combination of both extraction and determination techniques have permitted to validate a HF-LPME methodology at the required levels by the European legislation.

Novel Aspect
Due to the low cost of HF-LPME and total time consumption, this

methodology is ready for implementation in routine analytical laboratories. As far as we know the selected plasticizers has never been determined by Isotope Pattern Deconvolution previously. This permits to obtain the concentration of each compound without the need to build any calibration graph, reducing the total analysis time. Besides, since this quantification is based on Isotope dilution, the matrix effects are also corrected.

TPS43-12 / Ease of Use and Low Detection Limits of a New Dry Sampler for Determination of Vapor Phase and Particulate Isocyanate Derivatives

Jens Boertz, Olga Shimelis, Emily Barrey, Michael Halpenny, Jamie Brown
Sigma-Aldrich

Introduction

Isocyanates are a main ingredient in the production of polyurethane (PUR) materials.
Exposure to isocyanates can put workers at risk for respiratory disorders like "occupational asthma" [DHHS (NIOSH) Publication Number 96-111 (1996)].

Methods

A new air sampling device allows sensitive and reliable determination coupled with convenient and safe use. It uses an impregnated media in the sampler to form stable isocyanate derivatives and permits the shipping of the sampler to the testing laboratory without the need for field desorption using hazardous solvents.

Results & Conclusions

The design and capacity of this sampler allows for sampling times of more than 8 hours. Low detection limits are obtained in the analysis of the urea derivatives by LC-MS-MS.

Both the isocyanate monomers and oligomers are assessed. The sampler works for aromatic monomers such as toluene diisocyanates, 4,4'-diisocyanato diphenylmethane and phenyl isocyanate as well as for aliphatic monomers such as isophorone diisocyanate, 1,6-hexamethylene diisocyanate and ethyl isocyanate. Additionally polymeric species are determined such as HDI-Isocyanurate, HDI-Biuret and IPDI-isocyanurate.

Novel Aspects

This work presents the results from a field testing of this sampler during a simulated spray paint coating. The air was quantitatively analyzed for monomeric isocyanates.

TPS43-13 / Evaluating the performance of advanced waste water treatment steps via quantitative screening of 483 micropollutants using SPE-LC-HRMS

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Introduction

Within coming years most large municipal wastewater treatment plants (WWTPs) in Switzerland will be upgraded with an advanced treatment step (ATS) to reduce the persistent load of micropollutants (MPs) into surface water. Assessing the performance of the upgraded plants requires the monitoring of several MPs. A comprehensive target screening approach was used to provide an overview of ubiquitous MPs in treated effluent and propose MPs appropriate for the monitoring purposes. The selection criteria include: presence in all varieties of municipal effluents, different removal degrees within the WWTPs and analyzable with an available, ready-to-use analytical method for cantonal and federal offices.

Methods

Wastewater samples were taken from 13 WWTPs (4 with and 9 without an ATS) for this study. The samples were spiked with 163 isotope-labeled internal standards (IS) and enriched by offline solid phase extraction (SPE) with a multi-layer extraction cartridge containing different adsorbents (Oasis HLB, Strata XAW, Strata XCW, Isolute ENV+, Envi-Carb). After elution and evaporation, the sample extracts were analyzed by liquid chromatography (XBridge 50x2mm, generic methanol-water gradient) coupled to a Q-Exactive high resolution mass spectrometer (HRMS) operated with an electrospray ionization probe. The samples were measured separately in positive and negative HR full scan mode (R 140,000@200m/z) followed by data independent MSMS experiments (Top 5; R 17,500@200m/z).

Results

The method enables the quantification of 483 MPs in untreated and treated waste water samples. The median limits of quantification (LOQs) were 25 ng/L for the influent of the WWTPs, 10 ng/L for the effluent of the biological treatment and 3 ng/L for the effluent of the ATS. Of the 483 MPs, 218 were found at least in one of the wastewater samples (mainly pharmaceuticals) while 64 substances were detected in all WWTPs. The elimination for these 64 ubiquitous MPs were calculated. Over 40% of these MPs were completely removed by the ATSs, while 40% of these MPs showed only partial elimination (30%-80% removal) and less than 20% were recalcitrant MPs (removal during ATS below 30%). Based on these eliminations 19 out of the 64 compounds were selected to monitor the removal efficiency of the ATS in WWTPs.

Conclusions

The ubiquitous occurrence of 64 compounds in waste water has been identified with the SPE-LC-HRMS method presented here. According to their elimination during biological treatment and ATS, 19 compounds have been proposed to monitor the performance of the ATS.

Novel aspect

A multi-compound SPE-LC-HRMS method capable of quantifying 483 emerging contaminants in influent and effluent samples of WWTPs for prioritization in future monitoring efforts.

TPS43-14 / Screening and Quantitation of Targeted and Non-targeted Environmental Pollutants in Water Samples

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AB SCIEX

Introduction

Pharmaceuticals and Personal Care Products (PPCP) are environmental contaminants of growing concern. In order to properly assess the effects of such compounds on our environment, especially their disruption of endocrine function in mammals and fish, it is necessary to accurately monitor their presence in the environment. The diversity of chemical properties of these compounds makes method development challenging.

Experimental

Here we present results of PPCP findings in water samples collected in different geographies and from different type of water, including drinking water, creeks, rivers, lakes, sea etc. All samples were analyzed by direct injection Liquid Chromatography coupled to tandem Mass Spectrometry (LC/MS/MS).

Results & Discussion
Two analytical methods were used. A method which enables the quantitation of PPCP at low ppt levels using Multiple Reaction Monitoring (MRM) and their identification using full scan MS/MS with mass spectral library searching using a hybrid triple quadrupole linear ion trap LC-MS/MS system (QTRAP® 6500). In addition a high resolution and accurate mass LC/MS/MS system (TripleTOF™ 5600+) was used to further explore collected samples for unexpected analytes. Data processing turned out to be the bottleneck of the general unknown screening methodology. New and advanced data processing tools where used to automatically identify unexpected and unknown pollutants.

TPS43-15 / Multiple Solid Phase Microextraction (m-SPME) Coupled with Ambient Mass Spectrometry (AMS) for Rapidly and Accurately Quantifying Trace Emerging Pollutants in p
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Introduction
Emerging contaminants are ubiquitous in aqueous samples. Prolonged exposure to these samples may cause adverse health effects via accumulation of toxins in the body. Ambient mass spectrometry (AMS) is known for its fast and direct sample analysis without pretreatment. However, quantitative analysis for most AMS techniques is still a problem because the sampling volumes are not in consistent. Since the amount of analytes extracted by the solid phase microextraction (SPME) fibers dependents on analyte concentration, this feature makes SPME a good tool to perform quantitative analysis for certain chemical compounds in aqueous solution. In this study, we have combined multiple SPME fibers with thermal desorption-electrospray ionization mass spectrometry (TD-ESI/MS) for quantitative study of trace emerging containments in water.

Methods
The SPME fibers clipped on a direct probe were dipped in the solution for sampling for 10 min. The fibers on the probe were then inserted in the preheated oven to desorb the analytes on the fibers. The desorbed analytes were carried by a nitrogen into an ESI plume to react with the charged solvent species for ionization. The analyte ions were subsequently detected by a mass spectrometer attached to the TD-ESI source.

Preliminary Data
The preliminary results showed that reproducibility (n=6) for quantification of 10 ppb bisphenol A (BPA), 4-tert-octylphenol (4-t-OP) and 4-n-nonylphenol (4-n-NP) was less than 4.0 %. The results of sensitivity test based on the ion signal of 4-n-NP (m/z 219) showed that the detection limit of 4-n-NP in aqueous solution was as low as 500 ppt. SPME/TD-ESI/MS was used to analyze the river water contaminated with BPA, 4-t-OP and 4-n-NP, a good linearity of quantification (from 2.5 to 500 ppb) was achieved for each analyte with R2 values greater than 0.997. Furthermore, the detection limit was dramatically improved when multiple SPME fibers (n=10) were used for extraction. The results showed that m-SPME/AMS was able to detect BPA in sub ppb level within 5 min.

Conclusion
The coupling of SPME for sampling and ambient TD-ESI/MS for desorption, ionization and detection was able to achieve a fast, sensitive and accurate quantification for certain emerging pollutants in water.

Novel Aspect
Accurate quantification of emerging pollutants in ppt level was achieved using multiple solid phase microextraction/ambient mass spectrometry (m-SPME/AMS).

TPS43-16 / Quantification of Arsenolipids in the Certified Reference Material NMIJ 7405-a (Hijiki) Using RP-HPLC-ICPMS and High-Resolution-ESMS
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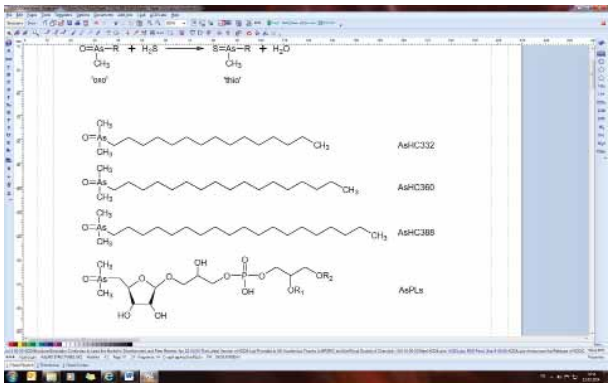
Arsenic-containing lipids (arsenolipids) are natural products recently shown to be widespread in marine animals and algae. Research interest in the area lies upon their possible role in the membrane chemistry of organisms, and, because they occur in many popular seafoods, their metabolism and toxicology. Progress has been restricted, however, because of the lack of standard compounds and of a quantitative method for their analysis. We report that the certified reference material NMIJ CRM 7405-a (Hijiki) is a rich source of arsenolipids, and we describe a method based on HPLC/ICPMS/ESMS to quantitatively measure seven of the major arsenolipids present.
Sample preparation involved extraction of the arsenolipids with DCM/methanol followed by a clean-up step with silica, and conversion of the (oxo) arsenolipids originally present to thio arsenolipids by brief treatment with H2S. Gradient elution using on a C8 reversed phase column revealed the presence of more than eight arsenolipids in this CRM. The two main groups of arsenolipids present were arsenic containing hydrocarbons (AsHCs) and arsenic containing phospholipids (AsPLs) shown in figure 1.
Compared to their oxo analogues, the thio arsenolipids showed much sharper peaks on reversed-phase HPLC, which facilitated their resolution and quantification. The compounds were identified and quantified by HPLC/ICPMS/ESMS, which provided both, arsenic-selective detection and high resolution molecular mass detection of the arsenolipids.
In summary, the concentrations of two arsenic-containing hydrocarbons and five arsenosugar phospholipids are reported in Hijiki NMIJ CRM 7405-a, which has been previously certified for total arsenic and inorganic AsV. The CRM is a readily available, characterised source of arsenolipids to assist research requiring the analysis of these new and fascinating marine natural products.

Figure 1: structures of As-hydrocarbons and As-phospholipids



TPS43-17 / Determination of organic pollutants families in environmental samples based on characteristic ions obtained by Electron Impact
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Introduction
One of the main steps for analyses of pollutants in environmental samples is identification and quantification.

In environmental applications the Mass Spectrometry involving EI ionization is of particular interest by producing a large variety of ions which lead to possibility of identification of compounds. For deducting the molecular structure from a mass spectrum usually the comparison of the unknown mass spectrum with mass spectra from databases, using computer techniques is used. However for identification of novel compounds supplementary data are necessary. The aim of this paper is to describe how structural information data can be obtained from explanation of ions arising in mass spectra based on mechanisms of fragmentation. In paper will be discussed a rapid and robust method for presentation of the families of pollutant in environmental samples, based on characteristic ion chromatograms, registered by GC/MS in EI mode.

Method

The fragmentation processes of the positive ions in ion source can be classified in two main categories: simple fission and Re-arrangement Processes. Simple fission mechanism involves the cleavage of an odd-electron aggregate to produce a positive ion and neutral radical. Rearrangements mechanisms involve elimination of a neutral molecule involving a cyclic intermediate and two electron shifts. The stereochemistry of the molecule allows the centers involved to come into close proximity so that the transfer of the hydrogen can occur.

Results

In the paper more common groups of organic pollutants detected in environmental samples are presented by ion chromatogram profiles, as: Saturated hydrocarbons (chromatogram to m/z=85); Lineal Alkyl Benzenes (LABs) (m/z=91 and m/z=218, 232, 246 and 260); Fatty acids (m/z=60); Methyl ketons and Trialkylamines (m/z=58); Triglycerides (ions R-C≡O+); Acyclic isoprenoids (m/z 183); Bicyclic sequiterpenoids (m/z 123); Tricyclic terpanes (m/z 191); Pentacyclic triterpanes (Hopanes) (m/z 191 and molecular ions); Polyaromatic Hydrocarbons (PAHs) and Sulphonated Polyaromatic Hydrocarbons (SPAHs)-Benzothiophenes (on molecular ions); Sterols (m/z 215 and m/z 213); Nonylphenol polyetoxylates (NPnEO, n=1) (m/z 193 and m/z 169); Linear alkylbenzene sulfonates (LASs)(m/z 91 and molecular ions to M=312, 326, 340).

Novel aspects

The present study lead to structural identification of novel compounds from families of organic pollutants detected in environmental samples. The molecular distribution of the isomers in samples can be described by the profile of few characteristic ion chromatograms.

TPS43-18 / Quantification of micropollutant degradation in the riverbank using a LC-HR MS screening method

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Introduction

Riverbank filtration (RBF) is a commonly applied method to produce high quality drinking water in Europe. The increasing load of organic micropollutants (MP) cause considerable conflicts with regard to maintaining drinking water quality criteria's. Thus, contamination of shallow groundwater is a substantial concern, especially in effluent-dominated streams and underlines the need for an improved understanding of fate and transport of organic micropollutants.

Methods

Surface-water to shallow-groundwater, to drinking water well transport of a broad setup of 528 emerging contaminants (log Dow (pH 7) between -5.7 and +5.6) was investigated at three different river sites in the western part of Switzerland (extension of analytical method presented by Otto et al.). The obtained samples were spiked with 163 internal standards (IS) and enriched by offline solid phase extraction using a multi-layer extraction cartridge with five different adsorber materials (Oasis HLB, Strata XAW, Strata XCW, Isolute ENV+, Envi-Carb) to enable the enrichment of micropollutants with a broad spectra of chemical-and physical properties. After elution and evaporation the sample extracts were analyzed by liquid chromatography (XBridge 50x2mm, generic methanol-water gradient) coupled to a high resolution mass spectrometry (Q-Exactive) operated with an electrospray ionization probe. The samples were measured separately in positive and negative HR full scan mode (R 140'000 @200m/z) followed by data independent MSMS experiments (Top 5; R 17'500@200m/z).

Results

Overall, 134 of the 526 MPs were detected in the sampled surface-, ground water, and drinking water well samples, including 70 pharmaceuticals (including 14 metabolites), 4 anaesthetic(including 2 metabolites), 32 pesticides (including 11 metabolites), 3 biocides (including 1 metabolite), 9 per-and polyfluorinated compounds, 5 food additives, 3 anti-corrosion agents (including 1 metabolite), 3 industrial chemicals, 3 personal care products, one additive and one tracer above the limit of quantification (LOQ). The LOQ varied only slightly for the two different surface waters, where additional spiking was carried out, i.e. the LOQ was below 10 ng/L for 79%, an 83%, respectively, and below 1 ng/L 23, and 25%, respectively. Riverbank filtration with varying MP removal potential ranging from 22, 41, and 60%, respectively, was observed at all three sampled sites. Nevertheless, the MP removal cannot solely be attributed to RBF, because dilution with other groundwater components may be an underestimated factor.

Conclusion

Out of the 134 detected MPs, concentrations of 37, 40, and 42 MPs, respectively, were removed during RBF at the three investigated sites. Apart from that, 24 compounds were present in all analysed samples, whereof no, or only slight degradation was observed for sulfamethoxazole, lamotrigin, and carbamazepine.

Novel Aspects

This study presents the application of a multi-compound screening method to evaluate the removal potential of RBF for a broad setup of organic micropollutants.

TPS43-19 / Analysis of electronics waste by GC x GC combined with high-resolution mass spectrometry: using exact mass information to explore the data.

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Introduction

Comprehensive two-dimensional gas chromatography (GC x GC) in combination with high-resolution mass spectrometry (HRMS) is a powerful tool for the analysis of complex mixtures. However, new software tools are required for the interpretation of the rich information content in GC x GC/HRMS data sets.
A dust sample from an electronics recycling facility was analyzed by using GC x GC in combination with a new high-resolution time-of-flight (TOF) mass spectrometer. Non-traditional Kendrick Mass Defect plots identified halogenated contaminants in the electronics waste sample. Database search results combined

with elemental composition determinations from exact-mass data were used to identity of (potential) persistent organic pollutants (POPs).

Method
Separations were carried out by using a Zoex ZX-2 thermal modulator GC x GC system installed on an Agilent 7890 gas chromatograph. The eluent from the GC x GC system was introduced into a JEOL AccuTOF GCV 4G high-resolution TOF mass spectrometer. JEOL Mass Center software was used for data acquisition. GC Image software with support for high-resolution data was used to view, process and analyze the two-dimensional GC data. Software tools developed at the Ontario Ministry of the Environment and GC Image identified families of halogenated contaminants.

Results
A composite mass spectrum was created by summing the mass spectra for all components in the GC x GC/HRMS analysis. Halogenated contaminants were readily recognized by their mass defects [1]. Nontraditional Kendrick mass defect plots were created by converting the measured IUPAC m/z to H/Cl mass scales corresponding to the mass of a chlorine atom minus the mass of a hydrogen atom. The nominal mass was plotted vs. the corresponding mass defect for each peak.
H/Cl mass = IUPAC mass x (34/33.96102)
Mass defect plots facilitated rapid identification of families of compounds that differ by the number of chlorine substituents. The KMD plots for H/Cl and H/Br are nearly identical, allowing us to view both Cl and Br substitutions in one plot.
Among the families of halogenated contaminants identified in this sample were chlorinated terphenyls, brominated bisphenol A, polybrominated biphenyl ethers (PBDEs) polychlorinated biphenyls (PCBs), and brominated benzenes. Individual contaminants were also identified, including an unusual halogenated amide that may have been used as a flame retardant.
[1] Karl J. Jobst, Li Shen, Eric J. Reiner, Vince Y. Taguchi, Paul A. Helm, Robert McCrindle, Sean Backus, The use of mass defect plots for the identification of (novel) halogenated contaminants in the environment, Anal Bioanal Chem (2013) 405:3289–3297

Conclusion
Software tools incorporating nontraditional Kendrick mass defect plots greatly enhanced the interpretation of the GC x GC-HRMS data.
This example illustrates the analysis of a complex sample using GC x GC, high-resolution MS and Kendrick mass defects. The combination of these techniques is a very powerful tool for detailed qualitative analysis.

Novel aspect
Comprehensive GC x GC with high-resolution MS and new software tools used to process complex data sets from environmental waste analysis.

TPS43-20 / Marine Microorganisms as a Source of Volatile Organic Carbons and Reactive Aldehydes

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It has been well documented that the degradation of volatile organic carbons (VOCs) in the troposphere results in a wide range of secondary pollutants that can have harmful impacts on human health and the environment. It has also been demonstrated that oxidation of these VOCs followed by their decomposition can lead to reactive aldehyde (RA) species (i.e. glyoxal, and methylglyoxal) that have been linked to the formation of secondary organic aerosols. The vast majority of research describing VOC and RA emissions focuses on anthropogenic and terrestrial biogenic sources. Minimal attentions has been given to marine microorganisms as a significant contributor of these molecules. Herein describes the release of VOCs and glyoxal into the gas phase from the peroxidation and subsequent degradation of phospholipids present in the sea surface microlayer (SSML) during an algal bloom.
Algal blooms were generated in a marine aerosol reference tank using seawater collected from the Scripps Institution of Oceanography pier (La Jolla, CA, USA), and was supplemented with F/2 media. SSML was collected with via glass plate technique, concentrated using solid phase extraction, and analyzed by LC-MS/MS in positive-ion data dependent modes on a Thermo LTQ-Orbitrap XL. Head space was trapped with a gas tight syringe or by solid phase microextraction, and analyzed on an Agilent 7820A GC / 5975 Series MSD system.
Phosphatidylcholines (PC) and their corresponding short chain oxidation products were specifically characterized due to their unique presence in eukaryotes. This allowed for a distinction between algal- and bacterial-derived phospholipids. These findings correlated well with data collected from the head space in which a series of VOCs (i.e. pentane, pentanal, hexane, and hexanal) were found. Scheme 1 outlines a potential cleavage pathway for a peroxidized polyunsaturated fatty acid chain of a phospholipid to yield the observed VOCs. Spectra were assigned for PA-PC (m/z 782), HPA-PC (m/z 798), and HOOA-PC (m/z 650), which correspond to structures (1), (2), and (6), respectively, of the heterolytic pathway. A total of 5 short chain products were found to derive from PAPC. Analogous molecules from SAPC were also identified. Additionally, Scheme1 shows how marine microorganism contribute to the formation of glyoxal. To demonstrate this potential, glyoxal was generated in vitro using linolenic fatty acid methyl ester and UV light.
This work demonstrates the potential for algal blooms to contribute significantly to the concentration of VOCs and RAs in the troposphere. It was shown that during a bloom cycle phospholipids are enriched at the air-water interface. The hydrophobic tails undergo lipid peroxidation followed by degradation into a series of short chain oxidation products. The nature of the cleavage products (i.e. VOCs and RAs) are dependent upon the site of oxidation and number of available bis(allylic) hydrogens in conjugation along the fatty acid chain.
Recently it was noted in the Journal of Geophysical Research that there is a «missing source» of glyoxal in the atmosphere. Marine microorganisms may represent that source.

TPS43-21 / Determination of Endocrine Disrupting Chemicals in Drinking Water at Sub ng/L Levels using Direct Injection and Triple Quadrupole Mass Spectrometry

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Introduction
The presence of endocrine disrupting chemicals (EDCs) in the aquatic system has raised concerns about the aquatic environment and its impact on human health. In sufficient concentrations, these chemicals can interfere with the endocrine system causing adverse health effects in an organism or its progeny. EDCs of different origins in aquatic streams may lead to the potential contamination of the drinking water system. As a result, EDC levels in municipal water supplies are regulated by several government agencies down to ng/L levels (EPA Method 539, EPA Method 1698).
In this work, we demonstrate how the increased sensitivity of the Agilent 6495 Triple Quadrupole LC/MS can be utilized to simplify the analytical workflow in tap water analysis.

Methods
A high-end triple quadrupole has been modified to enhance sensitivity in positive and negative ion modes. Improvements include an optimized Q1 ion transfer optics and a novel ion detector that uses a high voltage conversion dynode with low noise characteristics.
Tap water (Santa Clara, California) was spiked with androstenedione, equilin, 17-β-estradiol, estriol, estrone, 17-α-ethynylestradiol and testosterone at different concentration levels. 900 µL were injected into the system by direct injection. Chromatography was performed using gradient separation with ammonium fluoride aqueous solution and acetonitrile/methanol binary solution as mobile phases. The mass spectrometer was operated in MRM and fast polarity switching mode and two transitions were acquired per compound.

Results
The Agilent 6495 Triple Quadrupole LC/MS has a number of improvements to increase its performance and operational robustness. Enhanced sensitivity gives enhanced peak area response and improved peak area precision, which ultimately leads to lower detection limits compared to previous high-end designs. In order to test the sensitivity of the instrument and the feasibility of detecting hormones in drinking water at sub ng/L levels, target compounds were diluted in tap water using the working standard and the instrument detection limit (IDL) was assessed.
Linearity was evaluated for each target compound from 0.1 to 35 ng/L using external calibration approach. Correlation coefficients (R2) for calibration curves were higher than 0.99. Reproducibility was also studied by performing repeatability studies (n=5), expressed as area RSD%. Excellent precision and accuracy within 80 and 120% was achieved.

Conclusion
The increased sensitivity of the 6495 Triple Quadrupole enabled a streamlined analytical workflow with direct injection of tap water samples instead of time-consuming sample preparation procedures such as offline solid phase extraction. Furthermore, quantitative performance based on signal response precision was used to demonstrate the instrument's sensitivity with IDLs ranging from 0.02 to 0.78 ng/L

Novel aspect
High sensitivity quantitation and reliable confirmation of hormones was achieved in drinking water by direct injection and triple quadrupole mass spectrometry.

TPS43-22 / Non-target and post-target analysis of organic environmental contaminants in river sediments

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Introduction
Time-of-flight mass spectrometers (ToF MS) have gained popularity over scanning instruments for non-target and post-target analysis because full mass range spectra are acquired with minimal mass bias. This provides a number of advantages including the possibility of deconvolving chromatographic interferences using modern software. The selectivity of high resolution TOF MS (R > 25,000) further enhances the ability to isolate compounds, as well as to determine chemical formulae based on accurate mass measurements for the identification of a greater number of compounds. Non-target and post-target analyses were used to determine the occurrence of organic contaminants in river sediment samples to enhance the contamination profile compared to traditional targeted approaches.

Methods
Archived surface sediment samples collected from upstream and downstream of Niagara Falls, ON were analysed using high temperature desorption (CDS Analytical) , coupled to an Agilent 7890 GC and a Leco Pegaus high resolution time-of-flight mass spectrometer (GC-HRT). The MS data were collected in EI mode with a mass range from 45 to 650 m/z and a mass resolution > 25,000. Data were processed using Leco's ChromaTOF HRT software, which consisted of Peak Find for spectral deconvolution and library database searching using NIST 11 and Wiley 10. References were also used to compare contamination profiles between the upstream and downstream sampling locations.

Preliminary data
The objective of this investigation was to better characterize organic environmental contaminants in these surface sediment samples beyond the targeted list of legacy persistent organic pollutants (POPs) that were monitored by Environment Canada's Niagara River Upstream/Downstream Monitoring Program. The unique deconvolution algorithm in the ChromoTOF HRT software increased the number of identified peaks substantially in a non-targeted approach, which included isolating features that were not chromatographically resolved under very generic chromatographic conditions on a 30m Restek Rxi-5silMS column, 0.25mm i.d., 0.25µm film thickness. High resolution, accurate mass data allowed one to predict chemical formulas for non-target, unknown compounds and provided tentative identification. A number of halogenated POPs not measured previously in these samples were identified using post-target analysis by leveraging the hydrogen substituted for chlorine (Cl-H) scaled mass defect. The scaling factor 34/33.96102 for the ratio between the nominal and exact mass of Cl-H was used to differentiate halogenated compounds when plotting Cl-H Scaled Mass Defect vs Nominal Mass. A number of organic contaminants were measured in these samples that are not reported currently by the government monitoring program. This approach may be used for screening or identifying potentially new compounds for long-term monitoring.

Novel aspect
Non-target and post-target analysis of organic environmental contaminants in surface sediment for the identification of compounds not monitored routinely.

TPS43-23 / Determination of pcb and screening of environmental pollutants using simultaneous scan and MRM measuring of GC-MS/MS

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An analytical method was developed for a determination of PCBs and chlorinated pesticides, and a screening of environmental pollutants using a tandem quadrupole mass spectrometer (GC/MS/MS). The GC/MS/MS was operated in simultaneous scan and MRM measuring (scan/ MRM) to reduce an analysis time. For the determination of PCBs and chlorinated pesticides, the conventional method (isotope dilution method) was applied to the MRM data in order to obtain precise quantitation results. On the other hand, Automated Identification and Quantification System with a Database (AIQS-DB) was applied to the scan data for the screening of environmental pollutants. AIQS-DB allows an automatic identification and semi-quantitation of targets compounds without standard sample analysis. It was developed by Kadokami et al. for 1000 pollutants. The database includes retention indices, mass spectra, and internal calibration curves for pollutants. The pollutants are identified using the mass spectrum and retention time predicted by retention index and retention times of n-alkanes. Semi-quantitation is performed using internal calibration curve. The developed method was applied to river

water samples. PCBs were selectively detected and determined from the MRM data and 84 pollutant compounds were semi-quantitated from the scan data. The results demonstrated that the developed method is effective for the target analysis of PCBs and chlorinated pesticides, and the screening of environmental pollutants by only one analysis.

TPS43-24 / An analytical method for environmental pollutants using GCxGC-MS/MS with ultra fast mrm switching mode

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Generally, gas chromatograph mass spectrometer (GC-MS) is used for the analysis of environmental pollutants. The number of environmental pollutants dramatically increases these days, the number of analysis methods together with sample pretreatment methods largely increases. These methods require specialized experience and advanced technique for sample pretreatment and data analysis for environmental analysis. To resolve such complexity and difficulty, the analysis system for multitarget analysis with high separation ability and highly sensitive/selective detection has been developed. Comprehensive two dimensional gas chromatograph (GCxGC) and triple quadrupole mass spectrometer (MS/MS) were coupled, and the analysis of PCBs in environmental samples by the system has been investigated.
In GCxGC-MS, scan mode has been widely used for both identification and quantification of target compounds. However, separation by single MS is not sufficient to resolve interferences by matrixes and other pollutants in environmental samples. Selective and sensitive detection by MS/MS with multiple reaction monitoring (MRM) has resolved most of the interferences. The fast switching MRM up to 600 transitions / second using GCMS-TQ8030 (Simadzu Corporation) was suitable for multi targets analysis maintaining sufficient sampling points. Noise reduction by MS/MS has largely improved sensitivity, became over 30 times higher in tetra- and penta-CB at 0.1pg, and quantitative performances compared to GCxGC-MS.

TPS43-25 / Characterisation of ofloxacin's transformation products by UHPLC-HRMS after a photocatalytic treatment based on TiO2 nanofibers.

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Introduction
The photocatalytic degradation of ofloxacin (OFX) and deuterated ofloxacin (OFX-D3) were investigated under UVA irradiation using synthesised TiO2 nanofibers (TiO2-NF). Although TiO2 heterogeneous photocatalysis has shown to be an effective water treatment to remove organic micropolluntants, the recovery and improvement of the catalyser still remains a challenge. The aim of this work is to evaluate by UHPLC-HRMS the degradation kinetics and transformation products (TPs) of both, OFX and OFX-D3 after suspended and supported TiO2-NF photocatalysis. For a simple and fast data treatment of TPs generated across samples, computational and data filtering methods have been applied.

Methods
TiO2-NF were synthesised by electrospinning and thermal treatment under O2 atmosphere. Nanofibers were deposited on a glass filter. 50 mL solution containing 10 mg/L of OFX or OFX-D3 was photocatalytically treated under UVA light (λmax = 365nm, 1,7mW/cm2) for 240 min. For the kinetic studies, 400 µL aliquots samples were collected and analysed by UHPLC-ESI(+)-MS in SIM mode monitoring the protonated molecules [M+H]+. For the identification of the OFX TPs, all samples were analysed by UHPLC-HRMS and UHPLC-MS/HRMS in full scan in a quadrupole-Orbitrap (QExactive, Thermo Fischer). The generated raw data was converted to a *mzXML file for post-data processing. A (m x n) matrix was generated, where m = m/z values and the retention time of the diagnostic ions; and n = number of samples analysed. Based on the information obtained from the analysis of blanks and standards, a set of ions were identified as potential OFX and OFX-D3 TPs. A subset of these analytes was selected for MS/MS analysis in order to provide structural information.

Results
The application and combination of HRMS and MS/MS-HRMS allowed to detect and to confirm those TPs generated at low concentration levels. Data filters applied in this work resulted of great importance, as it was possible to reduce the original list of most than 3000 ions to a maximum of 200 candidate by-products. Moreover, multiple mass defect filters (MMDF) permitted the identification of 66 ions related to OFX structure, which resulted in small cleavages: –CH3, -F, CO2, oxidations in the piperazinyl ring, etc. Although most of the structures had lower MW than OFX, dimmers and more complex structures were also detected and identified.

Conclusions
TiO2 nanofibers have shown to be an effective way for the elimination of emerging organic micropollutants. As a consequence of OH· attack, different by-products were generated and identified by UHPLC-HRMS. Moreover, MS/MS provided structural information, which resulted of great importance for the elucidation of the different compounds generated.

Novel aspects
It is the first time where the use of a deuterated compound has been used not only for determining TPs but also for being able to suggest the reaction pathways taking place during the photocatalytical treatment.

TPS43-27 / Kinetic Parameters of the NO2 / Methane Soot Uptake for Tropospheric Modeling

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Long-term forecasting the chemical composition of the troposphere is possible on the basis of modern databases which contain kinetic parameters of both gas-phase and heterogeneous reactions. Carbonaceous aerosol including soot is one of the most abundant atmospheric particulate matter. The reactions of the soot polycyclic aromatic compounds with nitrogen containing trace gases release highly carcinogenic and mutagenic compounds. In contrast to the rate constants of gas-phase reactions, the uptake coefficients for heterogeneous reactions are non-elementary parameters. In general, they depend on a number of parameters including volume concentration of the reactants. Extrapolation of the laboratory data to the real troposphere which is characterized by much lower reactant concentration, variable humidity, and specific surface of the aerosol particles is one of the problems to use the uptake coefficient.
Kinetic studies of NO2 uptake on a soot deposited from the

methane flame were carried out using a coated-insert flow tube reactor combined with high-resolution, low-energy electron-impact mass spectrometer. The distinguishing features of the instrument are: a working resolution of 20,000, a mass range of m/z 1 – 1400, molecular beam sampling with phase-sensitive detection and a unique home-made ion source with electron bombardment from an indirectly heated LaB6 cathode. The specific surface area of soot, 40 ± 10 (2σ) m2 g-1, was determined using BET method.
For a number of NO2 concentrations (1×1012 - 2×1013), variable humidity (5×1012 - 1.8×1015 molecule cm-3) at ambient temperature, a set of the time-dependent uptake coefficients γ for the NO2 uptake on fresh soot coverings was determined. The time-dependent uptake coefficients were parameterized by $1/\gamma(t) = 1/\gamma 0 + c1 \times t$, the parameters γ0 and c1 being dependent on [NO2]. The only detected gas-phase products were NO (about 50%) and HONO. The work was supported by RFBR, grant No 13-05-00139.
On the basis of proposed kinetic model and using experimental data for the NO2 / methane soot uptake, Langmuir constant, the desorption rate constant and the rate constant for unimolecular heterogeneous reaction are determined. These parameters are necessary for more detailed tropospheric modeling.
The uptake of nitrogen containing trace gases on a fresh soot surface is considered as a consequence of elementary steps: reversible adsorption of the gas reactant, formation of a surface complex followed by its unimolecular decomposition and liberation of the products. Outcome of the model is extrapolation of experimental data upon NO2 uptake on a fresh soot to the troposphere.

TPS43-28 / Monitoring of glufosinate degradation in rumen fluid using liquid chromatography coupled with tandem-mass spectrometry

Jana Pisarčíková[1], Anna Kopčáková[2], Jaroslav Legáth[3], Peter Javorský[1]
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Introduction
Basta (glufosinate ammonium) is a broad-spectrum contact herbicide used to control a wide range of weeds in agriculture. It inhibits the activity of an enzyme, glutamine synthetase, which is necessary for the production of the amino acid glutamine and for ammonia detoxification in the plant tissues. Laboratory studies have shown that glufosinate is readily biotransformed to its metabolite 3-methylphosphinicopropionic acid (MPPA) by oxidative deamination in soil. An application of Basta on fields represents the risk of consumption of herbicide by grazing animals. Therefore, our in vitro experiment was designed to investigate the effect of Basta on ruminal bacteria and its potential degradation in rumen.

Methods
LC-MS equipment:Analyses were performed on LC system Ultimate 3000 (Dionex, USA) using Zorbax Eclipse® Plus Pursuit 5 PFP, Agilent Technologies analytical column of 250 mm x 2.0 mm (5µm). Column was kept at 40 ºC during the experiment. The mobile phase consisted of 10 mMammonium acetate-acetonitrile (90:10 v/v) at flow 0.6 ml/min. API 2000 triple quadrupole mass spectrometer (AB Sciex, USA) equipped with an electrospray ion source was used for carrying out the experiment. The mass spectrometer operated in both, positive and negative ionization modes.

Results
Basta spiked samples of rumen fluid in different concentrations were incubated for 48h and measured after derivatization with p-nitrobenzoylchloride in negative and positive ionization modes to confirm the formation of glufosinate derivate. Quantitative analyses were done via multiple reaction monitoring (MRM) in negative mode using fragment transitions with the highest sensitivity atm/z329→285 andm/z151→61 for glufosinate and MPPA, respectively. The separation was complete in 3 minutes. The calibration standards were prepared in rumen fluid blank sample to minimize matrix effect and calibration curves were constructed by plotting peak area vs. concentration and were linear over the range of 5-500 µg/mL (correlation coefficients 1/x were 0.998 for glufosinate and 0.992 for MPPA). The limits of detection (LODs) were 0.001 and 0.005 µg/mL with recoveries 92.5% and 113.0% for glufosinate and MPPA, respectively. The relative standard deviations (RSDs) were less than 5.9%.

Conclusions
Proposed LC-ESI/MS/MS method allows relatively easy, rapid, accurate and selective determination of glufosinate and its metabolite MPPA in rumen fluid after simple derivatization with p-nitrobenzoylchloride.

Novel aspects
Method for quantification of herbicide glufosinate and its major metabolite 3-methylphosphinicopropionic acid in rumen fluid matrix using LC/MS/MS.

Acknowledgement
Supported by NRL UVMF in Košice, Slovak Republic

TPS43-29 / Microfluidic electrochemical cell with MS detection as tool for the study of the biotransformation of perfumery compounds

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Chemicals interacting with living systems undergo to numerous transformations due to their participation to the complex network of biochemical reactions occurring in the organisms (metabolism). Biotransformation and bioaccumulation of xenobiotics are usually assessed by in-vivo assays however these methods are expensive, time consuming and raise ethical issues concerning the use of animal testing.
Redox enzymes like the family of cytochrome P450 (CYP) have a crucial role in the metabolism and are responsible for biotransformation of xenobiotic compounds via oxidation. This step is particularly important to evaluate the bioaccumulation of lipophilic (high log P) compounds such as the majority of the chemicals employed in fragrances. In this work, we use a microfluidic amperometric flow cell to mimic the action of the redox enzymes on different chemical compounds. This electrochemical cell (EC) is interfaced on-line with a triple quadrupole mass spectrometer (MS) for the detection of the metabolites deriving from the redox processes (mainly oxidation) occurring in the cell. The fine tuning capabilities of the electrochemical cell and the structural information provided by MS could predict if a compound will be easily metabolized and the possible metabolic pathways.
EC-MS was set up by interfacing a triple quadrupole instrument (AB SCIEX, 4000 Q-TRAP) with the an amperometric cell (Antec, Leyden) equipped with a potentiostat. In the initial phase the tuning and the detection parameters of the electrochemical cell and of the MS were tested on a model compound. Afterwards specific EC-MS methods were developed to study the oxidation of two well known ingredients in the flavour and fragrance industry. These compounds characterized by high log P values,

required a specific optimization regarding in particular the mobile phase. The information obtained by EC-MS was compared with the previous knowledge on the biotransformation of these compounds. Finally, the possibility of using EC-MS as screening tool for compounds libraries was also investigated on a set of Firmenich's chemical products.
EC-MS provides a simple and fast approach to study the degradation process of chemicals in the environment. In contrast of traditional in-vivo (laboratory animals) or in-vitro experiments (isolated liver cells, microsomes), by EC-MS it is possible to quickly produce and identify metabolites and to isolate intermediates which are normally too diluted.
The simulation of key biotransformation steps in a controlled and reproducible manner by electrochemical processes combined with the sensitivity and the structural information capabilities of MS, offers a new tool for the assessment of the environmental impact of fragrances ingredients.

TPS43-31 / Investigation of the effect of air-fuel-equivalence ratio on soot emission from a flame source

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[1]Helmholtz Zentrum München Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH), [2]Jing Ltd

Soot or black carbon is a strong light absorber 1 and is linked to adverse health effects among the exposed populations2. In this study, the High-Resolution Aerosol-time-of flight mass spectrometer (HR-TOF-AMS, Aerodyne Research Inc, USA), along with particle counters (Electical Low Pressure Impactor, ELPI, (Dekati, Finland), a Scanning Mobility Particle Sizer (SMPS, TSI 3080 (DMA), TSI 3025 (CPC), USA)), an optical black carbon analyzer (Aethalometer® AE33 (Magee Scientific, Slovenia)), and PAH measurement (In-situ derivatization thermal desorption Gas Chromatography-Mass Spectrometry, IDTD GC-MS) were used to investigate the soot formation from a Combustion Aerosol Standard (CAST, MiniCAST 5200, Switzerland). To vary the chemical and physical composition of the resulting soot emission, the air-to-fuel ratio (λ) was adjusted to 1.12, 1.04, 1.00, 0.96, 0.92, and 0.88 by regulating the mass flow of the quenching gas (N2) and oxidation air (compressed air). Figure 1 shows the resulting HR-TOF-AMS mass spectra of organic matter using an oxygen deficient or oxygen rich flame. A shift to high masses in an oxygen deficient flame mixture and a higher contribution of low mass fragments in oxygen-rich flame can be observed. An increase in oxygenation and a decrease in hydrogenation of the organic matter with decreasing λ is observed. This could be due to the cooling from the quenching gas which might have prevented the organic vapors from the hot exhaust from being reduced during soot formation. Results from the other measurement techniques used will be presented. This study provides a novel aspect in understanding the soot chemical and physical properties under different flame conditions.

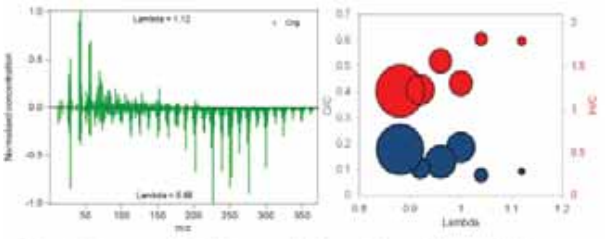

Figure 1. Mass spectra of (top left) oxygen-rich flame and (bottom left) oxygen-deficient flame. O/C and H/C (left) for the different λs. The size of the bubbles represents the standard deviation of the measurements.

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TPS43-32 / Comparison of electron ionization and vacuum ultraviolet photoionization of atmospherically relevant aerosol components using an aerosol mass spectrometer

Maarten Heringa, Jay Slowik, Andre Prevot, Urs Baltensperger, Patrick Hemberger, Andras Bodi
Paul Scherrer Institut

Introduction

The effects of atmospheric aerosols on the earth's climate, visibility and human health are highly recognised but poorly constrained. This is due to the complex composition of the organic fraction of the ambient aerosol and the difficulty to simultaneously detect a wide range of compounds with both high time resolution and chemical specificity. Aerosol mass spectrometry has provided important insights into organic aerosol composition but the speciation of compounds has been difficult due to the extensive fragmentation of the molecular ions. This interferes with the objective to identify the contributions of the numerous sources to the ambient aerosol which could be used as basis for possible future legislations.

Methods

The Aerodyne aerosol mass spectrometer (AMS) provides on-line characterization and quantification of the submicron non-refractory aerosol components, i.e., species that evaporate rapidly at 600 °C and 10-7 torr. After flash vaporization, the vapour of aerosol components are ionized by electron ionization (EI) at 70 eV and analysed by a time of flight mass analyser. EI enables robust detection of a wide range of compounds, however, interpretation of spectra from complex mixtures can be ambiguous since similar fragment ions are obtained from a wide array of compounds. To reduce fragmentation, the EI ion source was replaced by vacuum ultraviolet (VUV) photoionization. The X04DB synchrotron VUV beamline at the Swiss Light Source (SLS) was implemented in the ion source of the AMS by a direct coupling under high vacuum conditions. The VUV beamline can be operated in the higher harmonic free 5-21 eV energy range.

Results

The results of the comparison study for two different ionization methods, EI and VUV photoionization, for different atmospherically relevant aerosol components will be presented. Additionally, the implications of the modified AMS setup and data acquisition parameters will be discussed.

Conclusions

The potential of the SLS VUV beamline implementation into the AMS setup will be presented and linked to the characterization of ambient aerosols.

Novel aspects

Integration of VUV photoionization into the Aerodyne aerosol mass spectrometer provides new possibilities in the characterization of ambient aerosol. Characterization of atmospherically relevant aerosol components and tracer molecules of different aerosol sources can lead to a more reliable and detailed source apportionment and can give better insights in the aging of aerosol components.

TPS43-33 / Development and application of analytical method by GC/MS for urban dust analysis

Caroline Franco[1], Michele Fabri de Resende[1], Leonardo de Almeida Furtado[1], Taila Figueredo Brasil[1], Marcos N. Eberlin[2], Annibal D. Pereira Netto[1]
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Introduction

Urban dust (UD) is a complex mixture consisting of particles directly emitted on environmental surfaces and deposited atmospheric aerosol particles. UD originates from a number of natural and anthropogenic sources and contains different classes of molecules adsorbed. Among them, polycyclic aromatic hydrocarbons (PAH) are highlighted because most of them are carcinogenic. Once released into the environment, UD particles can be ressuspended leading to human exposition, contamination of soils and water bodies, leading to relevant environmental pollution.
The determination of PAHs in UD samples, usually involves several steps including purification and pre-concentration. This work describes the development and application of a high throughput method for PAH determination in UD using GC/MS.

Methods

UD samples were collected in 10 different sites of the city of Rio de Janeiro and Niterói, Rio de Janeiro State, Brazil, in February 2014. The selected sites represented distinct scenarios of pollution, vehicular traffic and urban use. PAH extractions were performed by ultrasonic extraction with dichloromethane. The extracts were concentrated using a rotary evaporator, filtered and analyzed by gas chromatography (Agilent 5890) coupled to a mass spectrometer (Agilent 5975), using a DB-17MS column (30 m x 0.25 mm x 0.25 µm). The injector and the transfer line were 350°C and 310°C, respectively. The GC temperature was: 60 °C (1min); heated at 40 °C min-1 to 300 °C and heated at 4 °C min-1 to 310 °C (kept for 10 min). PAH detection was performed by SIM after EI at 70eV. Molecular ions and two or more target or confirmation ions were used for PAH identification and quantification. Perdeuterated internal standards were employed for the internal standards method.

Results

The method developed showed high selectivity for PAH in UD samples and a complete separation of 16 PAH was accomplished in 29 min. Wide linear range (0.5 to 5000 µg L–1), good sensitivity (LOQ between 0.5 and 1µg L-1) and recoveries ranging from 62% to 108% were obtained. Because no clean-up steps were need, the method was easy to perform and showed a high throughput. PAH concentrations in the sampling sites ranged from < LOD to 10. 2µg L-1 in coastal areas, 5. 45 to 150 µg L-1 in residential areas and 23.6 to 3960 µg L-1 in high traffic intensity areas.

Conclusion

The developed method showed benefits for PAH determination in urban dust. It is fast a high throughput analysis, no sample pre-treatment and simple requiring. The results showed PAH distributions and of total concentrations that are compatible with to sample origin and particle sizes.

Novel aspect

Selective GC/EM method for detection of PAH in urban dust. An analytical method without pre-treatment. Characterization of PAH content of urban dust according to particle size and origin was attained.

TPS43-34 / Determination of monoterpene concentrations in the blood and urine using HS-SPME/GC/MS

Hiroaki Akutsu, Kazuhiro Sumitomo, Shin Kukita, Yoshiaki Sato, Shusei Fukuyama, Shinobu Osanai, Hiroshi Funakoshi, Naoyuki Hasebe, Masao Nakamura
Asahikawa Medical University

Introduction

We have reported that forest walking results in the modification of autonomic activity and a decreasing blood pressure. Conifer trees produce volatile organic compounds (VOCs) such as isoprenoids and terpenoids. Anti-inflammatory and anti-cancer effect of essential oils containing geraniol or limonene have been reported1). We have reported the identification of conifer-derived VOCs in the atmosphere of conifer forest and in blood and urine from the conifer forest walkers2).In this presentation, we have tried the determination of monoterpene (alpha-pinene, beta-pinene, 3-carene and D-limonene) concentrations in the blood and urine from the conifer forest walkers by headspace solid phase micro extraction / gas chromatography / mass spectrometry (HS-SPME/GC/MS).

Methods

Monoterpene stock solution containing 100mM of alpha-pinene, beta-pinene, 3-carene and D-limonene were prepared in DMSO,respectively. Monoterpenes standard solution were diluted to 500 pM, 5 nM, 50 nM and 500 nM to build the calibration curves. Forest walking was performed for 60 min in the afternoon at Tsubetsu forest on 7 th July, 2013. The blood and urine samples were collected after walking and kept cold and transferred to tight head space vials. A SPME 80 um fiber consisted of Carboxen/polydimethylsiloxane was used to detect monoterpened. Sample vial were preheated for 20 min at 55°C, then adsorption of monoterpenes onto SPME fiber were carried out for 20 min at 55 °C. The samples were then analyzed by GC/MS using a JEOL JMS-T100 GCv and column used was a DB-5MS 30 m × 0.25 mm × 0.25 um.

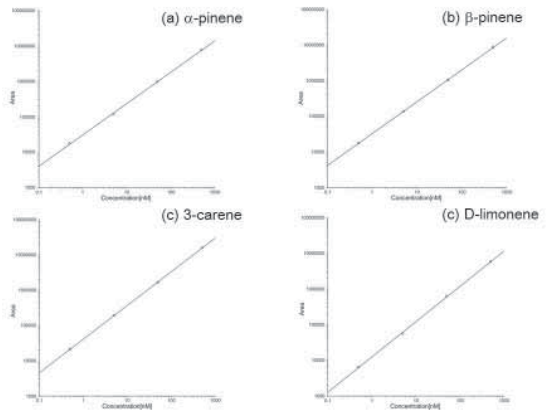

Fig.1. Calibration curve of monoterpens (α-pinene, β-pinene, 3-carene and D-limonene).

Results and Conclusion

Linearity of the calibration curves were ranged from 500 pM to 500 nM. The concentration of monoterpenes (alpha-pinene, beta-pinene, 3-carene and D-limonene) in the blood and urine were determined by comparison with individual standard curves that were prepared in the same concentration range. The concentrations of alpha-pinene in the blood and urine after forest walking were 13-26 nM and 5-22 nM, beta-pinene were 1-2.5 nM and 1-5 nM, 3-carene were 0.5-0.8 nM and 0.2-0.3 nM, and D-limonene were 10-58 nM and 3-10 nM, respectively.

References

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Novel aspects
Monoterpene concentration both in the blood and urine from conifer forest walkers were increased after walking.

TPS43-35 / Shotgun ecotoxicoproteomics of Daphnia pulex: biochemical effects of the anticancer drug tamoxifen at environmentally relevant concentrations

Patrice Waridel[1], Myriam Borgatta[2], Céline Hernandez[1], Laurent-Arthur Decosterd Decosterd[3], Manfredo Quadroni[1], Thierry Buclin[3], Nathalie Chèvre[2]
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Introduction
Ecotoxicology studies the impact of toxic compounds on ecosystem health and function at various levels (population, organism). With the development of "omics" technologies, such as proteomics, it is possible to unveil links between the effects of xenobiotics at the organism level and changes at the molecular level.Because micro-crustaceans, such as daphnids, are sensitive to chemicals and are key organisms in the food chain, they are particularly convenient for studying effects of xenobiotics in the aquatic ecosystem. Among pollutants released into the environment by human activities, residues of pharmaceutical agents have been identified worldwide in the aquatic environment, and are an increasing matter of concern because of their potential impact on ecosystems. In this context, the aim of this study was to find differences in protein expression resulting from acute and middle-term exposure of daphnid Daphnia pulex to the anticancer drug tamoxifen, using shotgun protein identification and quantification.

Methods
D. pulex neonates were exposed to two different concentrations of Tamoxifen during 2 or 7 days in duplicates. After cell lysis of frozen organisms, proteins were extracted, precipitated, digested with trypsin, and resulting peptides fractionated before LC-MS/MS analyses with a LTQ-Orbitrap XL mass spectrometer. Protein identification and label free quantification were carried out with MaxQuant 1.3.0.5. Statistical analyses were performed using local-pooled-error (LPE) estimates with multiple testing corrections. Protein annotations and functional analysis were performed with PANTHER (www.pantherdb.org).

Results
About 4000 proteins could be identified with a minimum of one unique peptide and quantified in at least one replicate sample. Considering both time points and tested concentrations, 189 proteins showed a significant fold change, most of the regulated proteins being observed at the highest concentration tested and for the longer exposure time. About one third of significant proteins were positively regulated, whereas two thirds showed decreased levels after tamoxifen treatment. The identity of regulated proteins suggested a decrease in translation, an increase in protein degradation and changes in carbohydrate metabolism as the major effects of the drug. Besides these impacted processes, which reflect a general stress response of the organism, some other regulated proteins play a role in Daphnia reproduction.

Conclusions
Our preliminary results show an impact of tamoxifen on Daphnia pulex at concentrations close to values which can be found in the aquatic environment. On-going investigations aim at detailing the biochemical processes involved in its toxicity for these micro-crustaceans.

Novel Aspect
Ecotoxicological assessment of tamoxifen on Daphnia pulex using shotgun proteomics.

TPS43-36 / Isotopic exchange mass spectrometry reveals molecular structure of Natural Organic Matter

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Institute for Energy Problems of Chemical Physics Russian Academy of Sciences

Introduction
Natural Organic Matter (NOM) represents one of the largest reservoirs of active carbon on Earth. It is present in terrestrial rivers, ocean, glaciers, peat lands and reflects changes in atmospheric chemistry, serves as electron acceptor for the anaerobic oxidation for some microorganisms and even protects Earth from 'snowball' state. Due to the extreme complexity NOM is poorly separating even on fractions and separation on individual species cannot be performed at all no matter what separation technique is used. In this study we present the methodology to obtain chemical and structural information about each molecule of NOM using isotopic exchange technique coupled to high resolution FT ICR MS.

Methods
Number of labile hydrogens (acidic and hydroxyl) was enumerated using in-ESI source H/D exchange as published in [Kostyukevich et. all AnalChem 2013 pp 5330-5334] and [Kostyukevich et. all AnalChem 2013 pp 11007-11013]. Oxygen exchange (16O->18O) was performed by dissolving NOM in H2(18)O and heating in sealed vial for 30 days at 95C. Exchange of aromatic hygrogens for deuterium was performed by treating DOM with deuterated TFA followed by extraction.

Preliminary Results/Abstract
We identified number of labile hydrogens in 450 species and number both of labile hydrogens and oxygens in 231 species in the NOM from Suwannee River. Compact visual representation of the obtained data was performed using 2D and 3D Van Krevelin diagram and Kendrick mass defect diagram which can be useful for classification of different NOM samples. The observed number of labile hydrogens in NOM molecules varies from 2 to 7 and increases with molecular weight. Also we observed that there are non-labile oxygens atoms in NOM molecules. Those atoms stems from ether group. The number of non labile oxygens atoms increases with molecular weight . We demonstrated that both information about labile hydrogens and labile oxygens can be useful together for the structural characterization of particular molecules in mixture. For example our results showed that molecule C14H18O8 (DBE=6) has 4 labile hydrogens and 1 non-labile oxygen. Thus we can conclude that four -OH groups and 3 labile oxygens attached to carbon with double bound are presented in the molecule. Considering possible structures (C14H18O8) and information above we found that the molecule structure is mostly close to flavon unit. This conclusion is reasonable as one of the main process of NOM production is biodegradation of plants.

Novel Aspect
An approach based on the Isotopic exchange and FT ICR MS was developed and used for structural characterization of NOM

PS00-01 / Francis William Aston: Postcards from Switzerland
Kevin Downard
University of Sydney

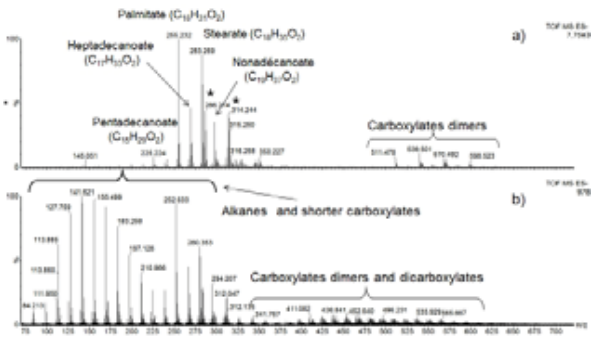
WPS21 - New Ionization Techniques

11:00-15:00
Poster Exhibition, Level -1

WPS21-01 / Direct Analysis in Real Time Mass Spectrometry (DART-MS) of irradiated magnesium stearate
Diane Lebeau, Ludovic Beuvier, Manon Cornaton, Muriel Ferry
CEA

Introduction
Magnesium stearate (MgSt) is used as lubricant in many industrial processes. In nuclear field, its behavior and its degradation mechanism under irradiation has to be better understood. Stearates are usually characterized by thermolysis or LC or GC chromatography. However, as fatty acid salts, MgSt cannot be easily solubilized in conventional solvents. This study presents the use of Direct Analysis in Real Time (DART) in combination with TOF analyzer for characterization of MgSt samples and their degradation products. The use of DART allows analysis to be done without any sample preparation, while TOF analyzer permits identification of products by accurate mass measurements. Different MgSt samples, irradiated at different doses and non-irradiated were studied to propose a degradation mechanism of MgSt under irradiation.

Methods
Gamma irradiations were performed in an industrial irradiator equipped with a 60Co source. Samples were exposed at room temperature under air. Three doses of irradiation were applied: 500, 1000 and 4000 kGy. A DART SVP ion source was interfaced with a LCT XE Premier for analysis of samples. The DART was operated in the negative ion mode at 150 °C, with ultrapure helium as the carrier gas at 80 psi. The grid electrode voltage was set to 3500 V. Samples were introduced manually by swirling a glass capillary tube in the stearate powder and inserting into the helium stream.



*DART mass spectra of non-irradiated magnesium stearate (a) and of irradiated magnesium stearate at 1000 kGy (b). * marked ammonium adducts*

Results
MgSt DART spectrum has shown that crude sample was not pure. Structures were proposed based on the mass spectrum. The mass error for molecules ions of all compounds are identified within maximum ± 20 ppm. Carboxylic acids with aliphatic chain lengths between C15 and C19 are identified as deprotonated ions. However, if carboxylates with odd number of carbons are detected only as [M-H]- ions, carboxylate with even number of carbons are also detected as aggregates with ammonium. Irradiated samples present more complex mass spectra. Under radiolysis, carboxylates with shorter aliphatic chain, alkanes and dicarboxylates are detected. Carboxylates can be formed by aliphatic chain breaking, or by bond breaking between the aliphatic chain and the carboxylate group and consecutive reaction with an oxygen molecule. Alkanes can be formed by decarboxylation. These molecules are observed at 500 and 1000 kGy but not anymore at 4000 kGy: these molecules can react to give products with higher oxidation degree, like carboxylic and dicarboxylic acids, these last being formed only at the highest dose.

Conclusions
These results show that DART source is a convenient ionization source for the study of degradation products of MgSt under radiolysis, in one step and without any sample preparation. A first degradation mechanism of MgSt irradiated at room temperature with gamma rays under oxidative atmosphere, up to doses as high as 4000 kGy can be proposed.

Novel Aspect
This strategy has helped to put in evidence the mechanism of degradation of StMg under irradiation.

WPS21-02 / Low-Pressure Dielectric Barrier Discharge Ionization Source for High Sensitive Analysis of Explosives
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Introduction
Threat of explosive attack against important infrastructure has been a serious problem. Mass spectrometer (MS) is one of reliable analyzers for explosive trace detections. For the use of MSs in on-site detection of explosives, their downsizing is required. To downsize MSs, a small pump must be used. However, using small pumps is leading to decrease in the sensitivity. To compensate such decrease, we previously developed a low-pressure dielectric-barrier-discharge-ionization (LP-DBDI) source. The LP-DBDI source achieves 10 times higher sensitivity compared to a conventional atmospheric pressure chemical ionization (APCI) source. In this study, we investigated usefulness of the LP-DBDI source for the detection of explosives (TNT, RDX, PETN and TATP) with a compact MS evacuated by small pumps.

Methods
The MS consisted of the LP-DBDI source, a quadrupole ion guide and a linear ion trap (LIT) mass analyzer. Samples were vaporized by heating and introduced into the LP-DBDI source. The LP-DBDI source consisted of a glass tube and inside and outside electrodes. By applying a high rectangular voltage to the electrodes, a low-temperature plasma was generated inside the glass tube. Ions generated in the LP-DBDI source were introduced into the LIT mass analyzer and then analyzed. This MS was evacuated with a diaphragm pump (20 L/min) and a turbo molecular pump (2.8 L/s, 35 L/s).

Results
TNT, PETN and RDX were measured in a negative ion mode, while TATP was measured in a positive ion mode. In the mass spectrum of TNT, molecular ion (M-) and fragment ions ([M-NO]-, [M-H2O]-) were observed at m/z 227, m/z 209 and m/z 197, respectively. The molecular ion peak was the most intense, suggesting that the LP-DBDI source achieved soft ionization. When PETN was analyzed, NO3 adduct ion ([M+NO3]-) was observed at m/z 377. On the other hand, in the case of RDX, molecular ion, fragment ions ([M-HNO2, NO2]-, [M-NO2]-)

and NO2 adduct ion ([M+NO2]-) were observed at m/z 222, m/z 129, m/z 176 and m/z 268, respectively. In the mass spectra of TATP, fragment ions ([TATP/3]+) and ([TATP/3+NH3]+) were observed at m/z 75 and m/z 91. The sensitivity of our instrument was sufficient to detect 500 pg of TNT, 5 ng of RDX, 5 ng of PETN and 25 ng of TATP in a signal-to-noise ratio of over 10. This sensitivity is roughly equal to that of a conventional MS with an APCI source using a larger turbo molecular pump (5.5 L/s, 55 L/s) than that our compact MS used.

Conclusions
We combined the LP-DBDI source with the compact MS. The sensitivity of that instrument for explosives was similar to that of a conventional MS with an APCI source, indicating that the LP-DBDI source would be the key technology for on-site detection of explosives.

Novel aspect
High sensitive detection of explosives by a LP-DBDI source.

WPS21-03 / Influence of the target plate material and sample layer thickness on LDI ionization efficiency for C60

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Introduction
In order to establish the validity of models thus far proposed for matrix-assisted laser desorption/ionization (MALDI) models, such as disproportionation, the "lucky survivor" model, cluster formation and exciton pooling, a basic LDI approach, based on variable thickness electrospray deposition of C60 (stable and ionizable in both negative and positive mode) on a range of different target plate materials, was chosen as starting point. The ion yield for pulsed ion extraction at different time intervals after pulsed laser irradiation was monitored by commercial MALDI-TOF MS instruments (Shimadzu AXIMA; Bruker Ultraflex III) for a range of different laser fluences. The presence of one or more ion yield maxima at different time intervals could be an indication for the number of ionization mechanisms contributing to LDI and the time frames in which they operate. Varying the target plate material and sample layer thickness can shed light on the sample influence itself and the interaction with the target plate material during ion formation in both negative and positive ionization mode.

Methods
An automated sample deposition setup, capable of high-throughput electrospray deposition, was developed. The sample application technique was first extensively tested to establish its robustness. Standard MALDI target plates were milled out to be subsequently fitted with target insets made of a variety of different materials, among them a range of metals, alloys and insulators.

C60 was deposited on the MALDI target insets and was subsequently analyzed using a range of different laser fluences and delayed extraction times in positive and negative reflectron mode. The resulting spectra were processed in an automated fashion by means of a MATLAB script, yielding an intensity profile accompanied by a standard deviation plot.

Results
Preliminary results for the positive ionization mode suggest the presence of two maxima for copper and aluminum targets, whereas stainless steel insets just show a single maximum ion yield. Insulated sample insets provide higher ionization yields in positive mode and all targets tested thus far in negative mode have shown a lower ion yield compared with positive mode.

Conclusions
The possible presence of two ion yield maxima for copper and aluminum insets, seems to indicate more than one ionization step is involved. The results also suggest that the heat insulating properties of the insulating insets could affect the ion production efficiency in positive mode.

Novel aspect
Systematic verification of target plate material influence and sample layer thickness on ion formation in (MA)LDI.

WPS21-04 / Direct Analysis in Real Time (DART) Mass Spectrometry - Benefits in Organic Synthesis

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Introduction
For a synthetic chemist the direct analysis of reaction mixtures is crucial to investigate the development of a reaction and optimize conditions in the quest for synthetic success. While mass spectrometry provides an array of tools to investigate reaction mixtures, they generally require sample preparation and optimized methods. Direct Analysis in Real Time (DART)-MS provides a way to directly investigate reaction mixtures without laborious sample preparation and optimization of runtimes, solvent mixtures, or choice of column types. Furthermore, the unique mode of ionization provides access to mass-ranges and polarity-types that are poorly covered by other MS-methods.

Methods
Herein, we like to present the versatility and robustness of DART-MS for common polycyclic aromatics synthesized in our lab. The corresponding reaction mixtures directly obtained from the reaction flask are subjected to an IonSense DART-SVP in positive mode coupled to a Shimadzu single quadrupole mass spectrometer LCMS-2020 without further sample preparation or knowledge of sample concentration. Apart from varying the temperature of the DART-beam, no optimizations were performed.

Results
This set-up allowed the development of reaction mixtures in our lab to be efficiently investigated. The measured samples are polycyclic aromatic compounds ranging from 200 to 1000 m/z. As no sample preparation or method-optimizations were required, the reactions could be monitored close to real time. While the DART-MS does not allow ratios of conversion to be determined as GC-MS or LC-MS would, it nevertheless allowed the formation of the desired target structure as well as the point of full conversion to be observed. Furthermore DART-MS provided confirmation of mass for structures that would otherwise only laboriously be confirmed using ESI-MS or MALDI.

Conclusions
Polycyclic aromatic compounds were rapidly identified during the organic synthetic process directly from the reaction mixture, using the DART source attached to a single quadrupole LCMS-2020.

WPS21-05 / Ionization efficiency model for multiple charged ions in negative mode ESI-MS

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Introduction

ESI-MS is increasingly the method of choice for determination of peptides. In order to use ESI-MS for quantification it is necessary to either have the standard substance or to know the ionisation efficiency (IE) of the peptide in the ESI source. We have previously developed a model for predicting ionisation efficiency of singly charged analytes. There are, however, many analytes (e.g. peptides), for which singly charged ions have too high m/z values for most mass analysers. For such analytes multiply charged ions are preferably used in MS analysis. It is known from the previous studies that IE for both negative and positive mode can be estimated by knowing the ionization degree of the molecule in solution and the extent of charge delocalization in the ion. There are no studies of IE of multiple charged ions in the literature.

Methods

Relative ionization efficiency scales were compiled for different molecules capable of forming ions with multiple charge in the negative mode ESI-MS. The analytes contained carboxyl, sulpho, hydroxyl and bromo groups. Those giving doubly charged ions were measured in both acidic (pH=2.68) and basic (pH=10.74) solvent.

Using COSMO-RS different parameters were calculated for all 30 measured molecules: aqueous pKa, logP (octanol-water), charge delocalization parameters (WAPS and Klamt parameters). Deprotonation degrees were calculated form pKa valued obtained from COSMO-RS calculations and from literature. The obtained parameters were correlated with measured ionization efficiencies and suitable parameters were chosen on the basis of this to develop a model for multiple charged ions.

Results

The ionization efficiency scale was compiled for both basic and acidic solvents, and for doubly and singly charged ions and one taking into account both. Based on initial results we see that molecules with certain functional groups (sulpho group and aromatic ring; bromide group) give doubly charged ions and some do not.

The range of the ionization efficiencies and their order depends somewhat on the pH of the solvent. In the basic solvent the range is about 2.5 units, in the acidic solvent about 4 units. There are more doubly charged ions in basic solvents and therefore the IE of doubly charged ions in such solvents are higher for most of the ions than for singly charged ions. In the acidic solvent most molecules are singly charged and the ionisation efficiencies for doubly charged ions is lower. All in all, the ionisation efficiencies are about one log unit lower in the acidic solvent for doubly charged ions. In the lower end there are some molecules where this tendency does not apply. On the other hand for several analytes the ionization efficiency of singly charged ions increases remarkably.

Conclusions

The first IE studies of doubly charged ions demonstrate that the pH of the solvent has a weaker effect on molecules with higher ionization efficiencies and stronger effect on molecules with lower ionisation efficiencies.

Novel Aspects

For the first time ionization efficiencies of multiply charged ions have been studied.

WPS21-06 / Cold Electron for Pulsed Ionization Mass Spectrometers

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The most common electron source for electron impact ionization (EI) is a hot cathode which creates a stream of electrons via thermionic emission from a metal surface. However, precise timing control of pulsed electron emission is impractical because the temperature response of a hot cathode is slow. In this paper, a secondary electron multiplier initiated by illumination of UV photons from a light emitting diode (LED) has been demonstrated as a pulsed electron source for sample gas ionization in an Ion Trap Mass Spectrometer.

Electron emission from a metal surface tends to become significant only for temperatures over 1000 K and the electron emission rates are sensitive to filament surface temperature, therefore the filament needs to be kept at a constant temperature for stabilizing electron emission even when the electron beam is not in use. However, in an ion trap mass spectrometer (ITMS), or a Time-of-Flight mass spectrometer (TOF MS), or a Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS), ionization of sample gases must occur in a pulsed mode. An electron gate control is well-established for electron ionization in an ion trap, in which a negative electric field blocks electron flow and switching to a positive field allows passing of an electron beam into the ionization region for a short duration. In a TOF MS, even shorter ionization pulses are required, such that high-cost laser ionization is commonly used rather than electron ionization since switching an electron gate in ns domain is not very achievable. A cold electron source using a secondary electron multiplier with UV LED developed in this paper solves this timing problem and shows many other advantages.

For feasibility test of the cold electron generation, a channel electron multiplier (CEM) and a micro-channel plate electron multiplier (MCP) were illuminated by UV photons from a LED. The MCP was the Miniature Advanced Performance Detector from Photonis Group, Model MicrotronTM and the CEM was ChanneltronTM Model 4504 from Photonis Group. Two UV diodes from Sensor Electronic Technology, Inc., with emission wavelengths of 300 nm (Model UVTOP295 TO18FW) and of 260 nm (Model UVTOP 255 TO18FW) were used. The emitted electron beam from the electron multiplier was focused and introduced into the center of the 4 mm ion trap.

Mass spectra of Benzene, Toluene, and Xylene using the cold electron beam are measured comparable to using hot filament cathode electron source. This results show that the cold electron source is very useful and practical for pulsed ionization mass spectrometers. In addition, the cold electron source displays a number of advantages such as low power consumption, no heating, stable and controllable pulsed electron beam

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WPS21-07 / Wavelength and Fluence Dependence of UV-MALDI-MS with 2,4,6-Trihydroxyacetophenone and Dithranol Matrices for the Analysis of Lipids

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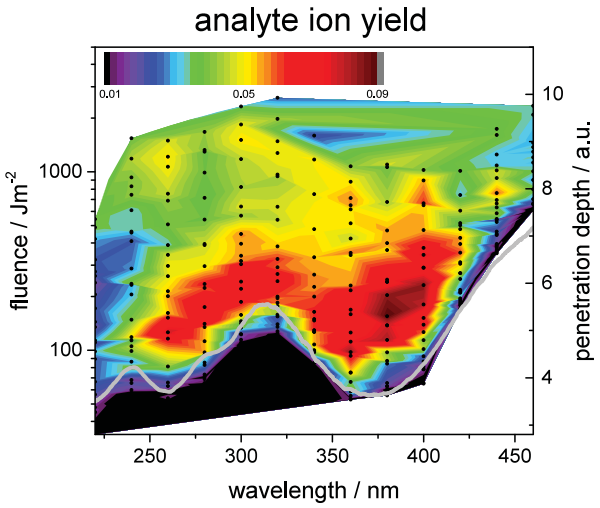
Introduction

Using a laser wavelength that corresponds to a high optical absorption of the matrix is a key element for sensitive UV-MALDI mass spectrometry. Here we investigated the influence of the wavelength and laser fluence for UV-MALDI-MS with two matrices that are particularly suited for the analysis of lipids: 2,4,6-trihydroxyacetophenone (THAP) has a peak absorption at ~290 nm in the solid state and shows a long-tailing red flank

extending to about 380 nm (50% peak absorption). Dithranol [1] exhibits a broad modulated absorption profile. Local absorption maxima are found at about 260 and 370 nm. Using a tunable laser we investigated a wide wavelength range from ~220–460 nm and recorded the signal intensities of phosphatidylglycerol (18:1/18:1) and phosphatidic acid (18:1/18:1) generated from the two matrices as a function of wavelength and laser fluence.

Methods

A prototype orthogonal TOF mass spectrometer, equipped with an oMALDI 2 ion source (AB Sciex), was employed. Tunable laser light (t~5 ns) was generated with an optical parametric oscillator (OPO; versa scan, GWU Lasertechnik). Wavelength and fluence dependent ion intensities are plotted in form of heat maps that allow for a particularly intuitive evaluation of the complex desorption and ionization processes.



Heat map of MALDI analyte ion yield as a function of laser wavelength and fluence. Matrix: dithranol; Analytes: PG (2x18:1)/PA (2x18:1); "ion yield": sum of quasi-molecular lipid ion signals divided by TIC; grey line represents the laser penetration depth (1/absorptivity of the matrix)

Results

Using this strategy various interesting effects are revealed. In the following the main results as obtained for dithranol are summarized (upon abstract submission, data for THPA were still recorded). Evaluating the overall ion yield (TIC) showed that the ion detection threshold fluence follows widely the inverse of the modulated absorptivity (in line with previous work [2]). Increasing the laser fluence highest TICs are obtained at the local maximum at 260 nm while a lower TIC is obtained at the second maximum of 370 nm, despite of an even higher absorptivity. This result points to an effect of the photon energy on the overall ionization process, an assumption that is supported by the wavelength-dependent ratio of protonated vs. radical matrix ions. Although also the highest signal intensities of protonated/sodiated lipids are found at 260 nm, the highest "ion yields" (signals of intact analyte ions/normalized to the TIC) are rather obtained at the long wavelength maximum at 370 nm. This points to the involvement of photon energy-dependent fragmentation as well as secondary ionization processes.

Conclusions

Recording MALDI data as a function of wavelength and laser fluence allows identification of optimal excitation conditions (that do not necessarily correspond to the standard MALDI wavelengths of 337 and 355 nm) and provides valuable insights into the MALDI mechanisms.

Novel Aspects

First detailed study on the influence of the laser wavelength on the UV-MALDI-MS performance characteristics.

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WPS21-08 / Super-Heated Electrospray Ionization Mass Spectrometry for Sub-Critical Aqueous Solution

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Introduction

To assist the desolvation of electrosprayed charged droplet, commercial ESI source is usually equipped with heating element for the drying gas. High temperature ESI can also be achieved by simply heating the ESI emitter. This has been done previously by several research groups either to improve the desolvation efficiency, or to study the conformation change of protein under thermal activation. However, the heating of electrospray ion source under atmospheric pressure is limited to the normal boiling point of the solution, which is 100 °C for water. The boiling takes place when the vapor pressure of the liquid at a given temperature equals the ambient pressure.

Methods

Here, we attempt to obtain a stable electrospray ionization of super-heated aqueous solution by performing the electrospray under super-atmospheric pressure (P > 1 atm). The ion source is pressurized with pure nitrogen to a maximum pressure of 11 atm, and it is coupled to a commercial mass spectrometer via a custom made ion transport capillary with i.d. of 0.25 mm. A booster pump with variable pumping speed is added to the pumping system to regulate the pressure in the first pumping stage at 1~1.3 Torr. The ESI emitter is embedded within a heating block.

Results

The liquid state of water and the stable electrospray can be sustained up to 180 °C (liquid temperature) by pressuring the ion source to ~ 11atm. The high pressure mass spectrometry is performed on several peptides and proteins to demonstrate its application in the temperature controlled thermally induced denaturation and dissociation. For proteins with liquid temperature < 100°C, the thermally induced denaturation results we obtained were similar to other previous reports. In addition to denaturation, we are able to extend the ESI-MS measurement to liquid temperature greater than 180°C to induce the thermal dissociation that is not commonly observed under <100°C liquid temperature.

Conclusions

We had developed an ESI ion source that electrospray the super-heated (sub-critical) solution directly under super-atmospheric pressure. This method may have potential use in the proteomics to supplement the present dissociation methods. Although the maximum achievable temperature in this study is not as high as those established methods, it can readily be extended to > 200 °C with some minor upgrades in the hardware to accommodate higher pressure.

Novel Aspect

Novel ESI ions source that electrosprays the super-heated (sub-critical) solution directly under super-atmospheric pressure.

WPS21-09 / A New Tool to Predict Thermal Desorption Efficiency Based on Molecular Functional Groups and the Chemistry behind it for High-Throughput LDTD-MS/MS Analysis

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Introduction

Mass spectrometry analysis performance lead laboratories to test high numbers of molecules which contain various structures. UPLC system is currently the gold standard for these analysis and compounds are separated on properties such as acid/base and polarity to achieve adequate separation and ionization. Uses of Laser Diode Thermal Desorption ion source reduces the analysis time to 8 seconds per sample compared to minutes by UPLC-MS/MS. Methodology for prediction of vaporization and ionization success relies on different properties than those of liquid chromatography.

Method

A panel of 250 compounds with various structural characteristics is analyzed in LDTD-MS/MS. 2 µL of pure solution at 2.5 µM is deposited in 96-wells plate and dried. Structural characteristics of all molecules are analyzed with Pallas™ software for the prediction of the local pKa of the functional group. Upon observations, application of additive substances on desorption plate are tested to validate the predicted vaporization behavior. The compounds are pooled in buffer solution and diluted 10x prior analysis. Quantitation is achieved for the different plate treatments.

Results

The chosen compounds cover acidic, basic, polar and non-polar types of molecules. A full scan spectrum of these molecules shows that they work in positive mode, negative mode or in both. Some compounds do not show molecular ions on the spectrum at all and their sensitivity can vary widely. Correlations between the vaporization efficiency and the predicted local pKa of functional groups were made. Functional groups such as COOH (carboxylic acid), R-CHOH and NH2 give calculated pKa values ranging between 3-5, 13-19 and 8-13 respectively. Functional group R-CHOH from glucuronide ring indicates the highest value at 18 and over. Application of a solution of EDTA as surface coating enhances the vaporization of acidic function. The effect is correlated to the value of the pKa. Molecules containing R-CHOH group are the most affected by the presence of coating. Similarly the basic compounds, with NH2 function, show enhancement in vaporization with coating, TETA (triethylenetetramine) in this case. Phenols functional groups do not produce effects on thermal desorption, even with a calculated pKa for functional group of 9.9 as shown by THC analysis. Main mechanism of the coating is to prevent strong binding of functional groups in the drying samples. Consequently, there is a reduction of the energy necessary to vaporize those molecules allowing desorption before degradation. Nevertheless, some compounds such as glucuronides with pKa>18 do not vaporize even in the presence of the coating. The ionization success rate using additives rose from 80% to more than 97% in this study. By examining the structure of the compound, it is possible to predict the coating solution necessary for an adequate desorption.

Novel aspect

First correlation between molecular structures and vaporization efficiency in LDTD ionization process.

WPS21-10 / Orbitrap mass spectrometer with LIFDI and ESI ion sources simultaneously installed on axis

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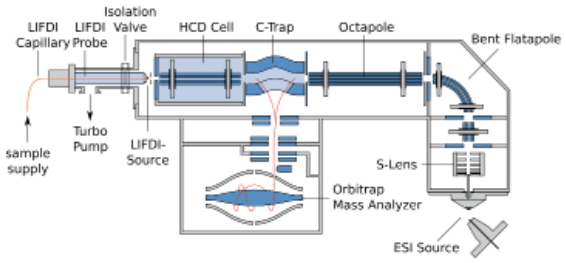
Introduction

This work presents an Exactive Orbitrap™ mass spectrometer with a standard ESI source at the front end of the instrument and an additional Liquid Injection Field Desorption Ionization (LIFDI)1)source at its back end. The LIFDI capability adds a complementary ionization technique for characterization of reactive compounds including organometallic samples which can not be accessed by ESI.2,3)

Methods

In a standard Exactive™ instrument, the back flange and the charge detector are replaced by a new flange with LIFDI source. LIFDI is able to generate intact molecular M+• ions of fragile molecules like transition metal complexes. The ions are introduced into the HCD cell from its back side through an aperture. Ions are trapped in the HCD cell and then transferred to the C-trap. Characterization of intact M+• ions and/or HCD fragment ions is performed in the Orbitrap analyzer.
The combined ESI - LIFDI setup allows a very quick change from one ionization technique to the other: The ion source of interest is switched-on, the other one switched-off and the respective tune parameters are loaded. An entire LIFDI analysis is performed within a few minutes even for samples sensitive to oxygen and/or moisture.



Results

This work presents high-resolution, high mass accuracy LIFDI spectra with intact molecular ions M+•of various reactive compounds like a Ruthenium complex for metathesis catalysis, an air- and light-sensitive luminescent Boron complex, and an air-sensitive Rhodium complex. ESI spectra as well as LIFDI spectra of PEG are shown for comparison of data produced by both ionization techniques.

Conclusion

The Exactive Orbitrap™ instrument with additional LIFDI source provides for characterization of highly reactive air/moisture-sensitive compounds without excessive experimental effort. Changeover from one technique to the other is quick and convenient.

Novel Aspect

LIFDI adds complementary soft ionization capability to the Exactive Orbitrap, making important classes of reactive air/moisture-sensitive compounds accessible for analysis.

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WPS21-12 / Nanoporous Complex Assisted Laser Desorption Ionization

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Novel Aspect
Ionization of absorbed small organic molecules in nanoporous metal coordination complex was observed by laser desorption method.

Introduction
Mass spectrometry (MS) is one of the most important structure characterization methods in organic chemistry. Among the practical ionization methods, MALDI is one of versatile and reliable method for various organic compounds including biopolymers. The compound is usually mixed in a solvent containing small organic molecules called matrix having strong absorption at the laser wavelength. Though the origin of ion procedure in MALDI is still not fully understood, the most acceptable ion formation mechanism involves gas-phase proton transfer to the sample from photo-ionized matrix molecules. The number of matrix molecules prevents the formation of sample clusters and minimize sample damages from the laser pulse to increase the efficiency of energy transfer from the laser to the analyte.
Recently one of our research groups reported that a single crystal of nanoporous metal coordination complex which consists of highly aromatic 2,4,6-Tri(4-pyridyl)- 1,3,5-trazine (TPT) and ZnI2 can absorb a variety of organic molecules, and they array in good order in the crystal. It was proved that X-ray crystallographic structure determination of the absorbed organic molecules could be available even for liquid or oily samples.
We hypothesized that this nanoporous complex also acts like the matrix to ionize analyte absorbed. In this case, idealized charge transfer will be occurred between aromatic ligand of the porous complex and analyte. This method makes it possible to ionize various organic compounds effectively by using minimum amount of the nanoporous complex.
According to the literature methods, the nanoporous metal coordination complex was prepared. The oily tocopheryl acetate (known as synthetic vitamin E) solution soaked in a single crystal of the nanoporous complex, and we definitely identified the analyte molecules existed in the crystal by single crystal XRD method. This crystal is grained on the MALDI plate, and irradiated by laser at the visible solid agglomerate observed by the monitor. In consequence, protonated molecular ion peak related to tocopheryl acetate and its deesterificated fragment were clearly observed.

Conclusion
Laser desorption ionization of organic small molecule by using nanoporous metal coordination complex was achieved. The quasi-molecular ions of tocopheryl acetate and some other aromatic small molecules absorbed in the nanoporous complex were detected in each mass spectrum.

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WPS21-13 / Active Capillary Dielectric Barrier Discharge Ionization: Investigation of the Ionization Mechanism(s)

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Introduction
Dielectric barrier discharge (DBD) based ionization methods for mass spectrometry are starting to be accepted as tools for routine analysis. Based on this principle, several new sources, geometries and applications have been published. However for new sources, e.g. the active capillary plasma source (Nudnova et al., Rapid, Commun. Mass Spectrom. 2012), which utilizes air instead of a noble gas, the mechanism of ionization remains poorly understood.

Methods
All experiments were carried out on an ion-trap mass spectrometer equipped with an advanced version of the active capillary DBD source first described by Nudnova et al.. For this study a series of tertiary amines (C2-C6) and isomers, as well as several analytes with different functional groups have been investigated to determine the selectivity and sensitivity of the source in comparison with a secondary electrospray ionization (SESI) source. All analytes were brought to the gas phase by a heat assisted nanospray evaporation system. As carrier gases air, N2 and CO2 were applied. The role of water on the ionization efficiency and the in-source fragmentation was also investigated.

Results
In terms of sensitivity, the DBD source used in this study was comparable with the SESI source with an absolute detection limit of about 300-500 ppt in MS and 1-50 ppt in MS2 mode. In terms of selectivity SESI provided higher ion counts for amines with shorter chain lengths, whereas the DBD source was more sensitive for longer alkyl chain length (≥ C4). In the absence of water (0.0% RH) still a significant amount of ions was produced with the DBD source. By introducing the sample dissolved in deuterated solvents (e.g. D2O, MeOD, EtOD, CDCl3, Benzene-D6), significant amounts of deuterated ions (M-D+) were observed. This proves that a different or an additional ionization pathway, other than proton transfer from H3O+, which is usually assumed for SESI, occurs for the DBD-ionization. When using N2 as the carrier gas, no difference in the ionization pattern was observed. However for dry CO2, M+O+H+, M+O2+H+, M+-H and M+CO2+H+ species were observed besides the M+H+ ions. For increasing humidity protonated species were exclusively observed as well as less in-source fragmentation. Furthermore, for all conditions and analytes investigated in negative mode, no analyte ions and hardly any chemical noise were detected.

Conclusion
Our results clearly show that the DBD source yields protonated ions even in the absence of water, but when water is present, it is preferred as H+-donor. Therefore the underlying mechanism does not necessarily require H3O+ as stipulated for SESI or APCI. Overall we suggest a “soft” electron impact based mechanism, where the positively charged intermediate analyte species are stabilized by proton abstraction from whichever surrounding molecule.

Novel Aspect
First report on the mechanism of the recently developed active capillary plasma ionization source.

WPS21-14 / Enhanced screening of environmental pollutants in complex matrices by GCxGC-TOF MS with variable-energy electron ionisation

Laura McGregor[1], Leonhard Pollack[1], Anthony Gravell[2], Praveen Kutty[2], Ian Allan[3], Nick Bukowski[1], Steve Smith[1], Graham Mills[4]
[1]Markes International, [2]National Resources Wales, [3]Norwegian Institute for Water Research, [4]University of Portsmouth

Two-dimensional gas chromatography with time-of-flight mass spectrometry (GCxGC-TOF MS) can provide highly sensitive detection and confident mass spectral identification of pollutants within complex environmental extracts. Nevertheless, the identification of individual compounds may be hindered by weak molecular ions or when similar mass spectral characteristics are evident across entire chemical classes. Select-eV ion source technology aims to combat this problem by allowing both hard and soft electron ionisation with no inherent loss in sensitivity. Select-eV provides enhanced molecular ions whilst retaining structurally-significant fragment ions, delivering both confident compound identification and increased selectivity. We show the potential of this technology for the analysis of both target pollutants and unknown chemicals in two different, complex extracts.

The routine monitoring of water quality is now a requirement of environmental legislation, such as the EU's Water Framework Directive. Often the cause of a poor water quality status is unknown and extensive investigative monitoring is needed to determine what chemical maybe responsible. Passive sampling devices (e.g. semi-permeable membrane devices (SPMD), LDPE and silicone rubber) are often used for this purpose. The samplers were deployed for several weeks in a polluted river course in the UK to effectively sequester large volumes of water and provide a concentrated, representative extract for analysis by GCxGC-TOF MS with Select-eV.

In a second study, explanted silicone breast prostheses obtained from patients over a wide age range were collected. Silicone oils in the prosthesis extracts were removed using a multi-step extraction procedure and the resultant extracts were analysed by GCxGC-TOF MS with Select-eV. This novel approach aims to better estimate the overall body burden of bio-accumulative substances and how this changes over time of exposure.

This presentation shows the suitability of this novel analytical platform for environmental investigations, using both target-focused studies as well as non-targeted routines for screening for the presence of emerging contaminants.

WPS21-15 / Sputtering and ionization of biomolecules induced by molecular cluster and noble gas cluster ion beams

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Introduction

Recently, an argon gas cluster ion beam (GCIB) has been applied as a projectile for time of flight-secondary ion mass spectrometry (ToF-SIMS) and improved the problem of molecular fragmentation and enhanced the emission of the molecular ion on SIMS. When an accelerated large cluster ion collides with a solid surface, multiple collisions at the shallow surface layer occur with numerous constituent atoms, improving the sputter yield and increasing secondary ion intensity. To apply this technique to the analysis of macromolecules in the practical bio-samples, however, the signal intensity of high mass molecular ions should be further enhanced. The secondary ion yield can be determined from the sputtering yield and ionization probability. The sputtering yield increases with the incident ion energy, but is a trade-off with molecular fragmentation. The properties of the bombarding primary ion beams and the chemical nature of the sample surface during a collision with the primary ion influence the ionization probability.[1,2] Selecting the appropriate primary ion species may flexibly modify the sample surface by primary particles, enhancing the secondary ion intensities.

Methods

We have developed a size-selected GCIB ToF-SIMS apparatus.[3] Large cluster ion beams of krypton (Kr), water (H2O), methanol (CH3OH), methane (CH4), as well as argon (Ar) are generated and used as the projectiles for SIMS. The samples were formed as thin films on a silicon substrate and no matrix agent was used. An aqueous solution of aspartic acid (1 mg/ml) was dropped onto a silicon substrate and freeze-dried in vacuum.

Results

For the bombardment of H2O, CH3OH and CH4 cluster ions, intensity of [M+H]+ ion is enhanced compared to that of Ar and Kr cluster ion. For the H2O and CH3OH cluster, [M+H]+ ion attached with several H2O or CH3OH molecules were detected.

Conclusions

The molecular clusters, which include protons, assisted the proton attachment to the intact molecules, and hence the intact ion intensity is enhanced. The lower energy per molecule is favorable to the proton attachment.

Novel Aspect

Kr, CH4, CH3OH and H2O cluster ion beams are generated and applied to the SIMS measurement of biomolecules. The molecular clusters including protons assisted the proton attachment and hence the intact ion intensity is enhanced. The lower energy per molecule of the projectiles is favorable to the proton attachment.

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WPS21-16 / Characterization of architectural differences of synthetic polymers using vacuum ionization-ion mobility spectrometry-mass spectrometry

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Introduction

Characterization of products from polymer synthesis is vital in determining composition and purity. Here, newly discovered ionization methods that rely only on a suitable matrix and vacuum have been successfully applied to drugs, peptides, and proteins are now extended to synthetic polymers. Branched poly(ethylene glycol) (PEG) polymers are ionized directly from surfaces using these novel vacuum ionization methods, laserspray ionization vacuum (LSIV) as well as matrix-assisted ionization vacuum (MAIV). When coupled with ion mobility spectrometry (IMS), architectural differentiation is demonstrated.

Methods

A Waters SYNAPT G2 with a commercial intermediate pressure-MALDI source was used for LSIV and MAIV with the extraction voltages removed and IMS enabled. MAIV was also conducted by introducing the dried polymer:matrix:salt mixture directly to the atmospheric pressure inlet aperture with minimal heat applied. Comparative ESI and MALDI were obtained using the same mass spectrometer. Branched PEG was purchased from NOF Corporation and cyclic and linear caprolactones were synthesized by the Grayson group as previously outlined

[Grayson, MRC, 2013; Macromol., 2011, Trimpin]. Linear PEG standards, salts, and matrices were obtained from Sigma. Over 30 matrices were tested to determine which produced the most abundant multiply charged polymer ions. Alkali metal salts and matrix were combined with polymer in a polymer:matrix:salt molar ratio of 1:500:10.

Results
Matrices that produced the most abundant multiply charged ions by LSIV were 2,5-dihydroxyacetophenone, 2-nitrophloroglucinol, and dinitrophloroglucinol and those for MAIV were 2-bromo-2-nitro-1,3-propanediol, 2-methyl-2-nitro-1,3-propanediol, 5-bromo-3-nitropyridine-2-carbonitrile, and 2-naphthol. Generally, lithium trifluoroacetate (TFA) salts produced the most abundant multiply charged branched PEG ions directly from the solid state. Multiple charging using MAIV and LSIV improved drift time separations with sigmoidal transitions, previously reported using ESI-IMS-MS [Clemmer, AC, 2008], recorded for the [M+2Li]2+, [M+2Na]2+, and [M+2K]2+ charge states for polymers of molecular weights between 2000 and 5000 Da. Gas-phase separations of isomeric blends consisting of branched and linear polymers as well as the development of strategies to differentiate between polymeric isomers, isobars, and gas-phase conformations using IMS-MS and MS-MS will be discussed.

Conclusion
Multiply charged polymer ions were able to be produced directly from a surface simply by application of matrix and addition of salt followed by direct introduction into the vacuum of the mass spectrometer where ions are produced with (LSIV) and without (MAIV) the use of a laser. Thus, potential applications of these vacuum ionization methods include better IMS separation and MS-MS fragmentation. Furthermore, vacuum ionization is potentially a powerful tool when analytical separations and cross section analyses are desired.

Novel Aspect
Polymers are ionized directly from surfaces by vacuum ionization methods and are separated based on differences in shape and charge of the gas-phase ions using IMS-MS.

WPS21-17 / Improvement of ionization yields in TOFSIMS using Optimized Charge Compensation and Matrix enhanced ionization

Nicolas Desbenoit, Gilles Frache
Centre de Recherche Public - Gabriel Lippmann

Introduction
Time-Of-Flight secondary Ion Mass Spectrometry is a widely recognized analytical technique for molecular characterization of surfaces. Due to the high-energy collision of primary ions on the sample surface, a significant rate of fragmentation is observed, and in some cases, the detection of intact molecular ions remains difficult. With the recent improvements based on the use of primary cluster ion guns (e.g. bismuth clusters), molecular ionization has been significantly improved, expanding the application of TOFSIMS from an elements/fragments mapping tool to a powerful molecular microscope used in various fields ranging from materials characterization to biological tissue imaging. Nonetheless, the yield of molecular ionization can be significantly modified depending on instrumental parameters and sample preparation.

Methods
In a first step, instrumental parameters require a careful optimization namely in terms of charge compensation in order to avoid electron induced fragmentation of the topmost molecular layer. Test molecules exhibiting a high tendency to in-source fragmentation in TOFSIMS were used to optimize a "sufficient" charge compensation. In a second step, the ionization yield is further enhanced taking benefit of the deposition of organic matrices commonly used in MALDI-MS. Finally, solvent free matrix deposition by sublimation is presented. Optimized matrix deposition allows Matrix-Enhanced SIMS to be applied to molecular imaging without sacrificing the lateral resolution.

Results
This optimized analytical approach led to the detection of molecules, which are not detected in conventional TOFSIMS experiments. Namely, oligomers with m/z up to several thousand can be detected thanks to matrix-enhanced ionization, thus extending the mass range of observable intact molecules in TOFSIMS. In addition, ME-SIMS tissue imaging allows for the visualization of (bio)-molecules of interest (e.g. lipids) with a higher signal or even for the detection of previously undetected ranges of molecules.

Conclusions
Matrix-Enhanced TOFSIMS combined with optimized charge compensation and appropriate solvent-free matrix deposition extends the range of detectable molecular ions. This allows for the detection of more selective molecular signals, in addition to generally observed less specific fragments.

Novel Aspect
Matrix-Enhanced TOFSIMS is demonstrated to enhance the accessible mass range in TOFSIMS. Matrix-Enhanced TOFSIMS with matrix sublimation is applied to the improved imaging of intact lipids in tissue sections.

WPS21-18 / Combination of Raman/LIF spectroscopy and laser ablation mass spectrometry

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Introduction
In recent years, hyphenated techniques have received increasing attention to approach complex analytical problems. The combination of different orthogonal analytical methods, such as laser ablation (LA)-MS and optical spectroscopy such as laser induced fluorescence (LIF) or Raman scattering, yields in complementary information of the same sample. The individual techniques reveal typical characteristics, which restrict their application: e.g. Raman spectroscopy displays structural information, however provides no information about the molecular mass. Mass spectrometry, on the other hand, allows for an accurate determination of the molecular mass, but lacks to provide structural information. To circumvent this problem tandem MS or modifications of the analyte are preferred methods, but they require a sufficient amount of material. Thus, a combination of LA-MS and optical spectroscopy could be highly beneficial for an unambiguous identification of complicated analytical samples since both methods provide complementary information: MS gives the exact molecular mass, while laser spectroscopy reveals the structural information.

Method
In the conducted feasibility-study, the combinations of multiple laser sources (from the ultraviolet to the mid-infrared wavelength range) and several selected ionization schemes, such as matrix-assisted laser desorption/ionization (MALDI), atmospheric pressure chemical ionization (APCI), atmospheric pressure photo ionization (APPI), electrospray ionization (ESI) and dielectric barrier discharge ionization (DBDI) were compared.
Eventually, a combined system for laser ablation and laser spectroscopy was coupled to an orthogonal time-of-flight mass spectrometer (API-HTOF, Tofwerk) with an atmospheric pressure

inlet. Radiation at 532 nm at repetition rates from 10 Hz to 200 kHz for both techniques was obtained by using the second harmonic of a shared diode pumped solid state (DPSS) laser (Blade IR 25, Compact Laser Solutions GmbH, Nd:YO4). A Czerny-Turner spectrograph (shamrock sr-303i, ANDOR Technology) was used to detect the emitted Raman and fluorescence light/radiation.

Results
Laser-induced fluorescence, Raman and LA-MS spectra of small, biologically relevant molecules (mass < 1kDa) could be observed. The orthogonal chemometrical datasets allow a structural and chemical identification of the observed species. The combination of a high repetition rate laser and fast spectroscopic/spectrometric instrumentation allows for a rapid scanning of the sample for future imaging studies.

Conclusion
With this contribution we demonstrate the performance of combining mass spectrometry and laser spectroscopic methods for the analysis of small biologically relevant molecules (mass < 1kDa). The instrument presented here offers a label-free, sensitive and specific detection of a variety of compounds in their native state.

Novel Aspects
Combination of laser spectroscopy and mass spectrometry in one instrument.
Fast laser source for rapid scanning applications.

WPS21-19 / The effect of the laser pulse duration in infrared free-liquid MALDI

Aleksandra Michalik[1], Toralf Beitz[2], Jens Riedel[1], Ulrich Panne[1], Hans-Gerd Löhmannsröben[2]
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[2]Physical Chemistry, University of Potsdam

Introduction
Infrared free liquid matrix-assisted laser desorption/ionization (IR-FL-MALDI) is a technique where an analyte is directly isolated from a liquid phase in a form of ions or ionic aggregates through laser desorption. Hence, it allows a direct coupling of liquid samples to gas phase ion detection schemes like MS or IMS. The desorption/ionization is typically performed with nanosecond pulsewidth lasers.1,2
The effective ion formation was found to vary strongly with the laser properties like wavelength and pulse energy. However, the influence of the laser pulse duration has not yet been studied. For this purpose IR-FL-MALDI was coupled to a drift time IMS accompanied by high resolution shadowgraphy, which was performed to have a better insight into the temporal response of the desorption process to different laser pulse widths.

Methods
The setup consist of a microdroplet source introduced coaxially to a homebuilt IMS. A solvent is introduced using a syringe pump at a constant flow rate of 20 µL/min. The ionization is performed using a wavelength tunable OPO laser with a pulse duration of 9 ns and a diode pumped solid state Er:YAG laser with a constant wavelength of 2.94 µm and tuneable pulse duration in a µs to ms range. All measurements were performed in the positive ion mode at a wavelength 2.94 µm. Subsequent ion mobility spectra were recorded with desorption laser pulse widths of 9 ns, 1 µs and 1ms, respectively. Corresponding high resolution shadowgraphy observation of the desorption process was performed on acoustically levitated droplet with a high speed camera and a laser driven light source for back illumination.

Results
The spectra obtained towards longer pulse widths expose an enhanced resolution and improved signal to noise ratio, but moreover, a decreased analyte drift time. These phenomena are connected to the irradiation procedure; in case of Er:YAG laser more mass is ablated throughout individual laser pulses, while the OPO laser results in a better temporal confinement of the formation of the ions. The shadowgraphy results allow a direct determination of the densities, the spatial distribution and the relative velocities of the ion clouds. A concise evaluation of the complementary results gives better understanding of the underlying processes and an experimental optimization of the ion formation.

Conclusions
Our primary investigation shows that IR-FL-MALDI can be performed with ns, µs as well as ms laser irradiation. Both methods give good results with a small advantage on the side of the Er:YAG laser. Higher pulse durations affect the drift time what is directly related to the ionization mechanism. This effect is related to the higher amount of ablated mass by Er:YAG laser as indicated with shadowgraphy.

Novel aspect
The performed experiments give novel insights into the ionisation mechanism of IR-FL-MALDI related to the irradiation with µs, ms laser pulses and its comparison to a traditional IR-FL-MALDI process which is still carefully examined.

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WPS21-20 / Improving the performance of an ultrasonic levitator coupled to API-TOF MS

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Introduction
In microfluidic approaches agglomeration and contamination occurs caused by the increased surface-to-volume ratio. To avoid these, levitation technique has been introduced; the acoustic levitation is one of the most promising techniques due to little requirements on samples. For MS analysis it is desirable to contactlessly remove matter from the droplet which is drawn into e.g. a ToF-MS which can be realized by laser desorption/vaporization.[1] The previous approach, however, still had some shortcomings. Thus, further improvements were made in several aspects. More flexibility concerning the solvent is provided by the use of different laser wavelengths. Stable levitation is essential; accordingly, new high frequency characterization of the levitating sound field and the levitated object has been carried out.

Methods
Levitation of droplets (µL-range) is performed by an improved version of our home-built levitator positioned in front of the MS inlet. Desorption is carried out by different lasers using different wavelengths. MS spectra are acquired by an orthogonal ToF-MS with an atmospheric pressure interface.
For further investigations on the droplet's behaviour in the acoustic field a high-speed camera is mounted in front of the trap to facilitate a visual observation. For shadowgraphy experiments a white light source diffused by milk glass is installed behind the levitator. For synchronized stroboscopic visualization of the sound field, the frequency doubled output of a Nd:YVO4 Laser at a synchronized repetition rate was used as a light source. Also new approach to quantitatively determine the resulting levitation

force exploiting the net repelling force between the sonotrode and the reflector is presented.

Results
The improved stability of the droplet in the new levitator can be rationalized by both, the sound-field diagnostics and high frequency stroboscopic imaging of the droplet. The new design can be shown to result in a lower amount of unwanted overtone vibrations. Laser pulse properties like duration and repetition rate are optimized on maximum spectral quality.
In former investigations a CO2-laser (λ=10.6 µm) has been utilized in combination with glycerol as a chromophore. It can be shown that higher vapour pressures increase signal intensities of protonated analyte species at same conditions and furthermore result in less pronounced solvent or solvent/analyte clusters. Because of higher vapour pressures it is preferable to exchange glycerol with water, methanol or acetonitrile but it is necessary to choose a different laser wavelength (λ=2.94 µm) for desorption.

Conclusion
An improvement in geometrical alignment of the trap has been undertaken as well as a choice of more typical solvents with a higher vapour pressures is now available. Moreover, lasers can be substituted according to the individual solvent absorption and desired laser pulse properties.

Novel Aspects
Optimized laser pulse properties, solvents with higher vapour pressures accessible, improved reflector positioning, high-speed observations of droplets in trap

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WPS21-21 / Possible Triplet Ionization Mechanisms in the UV MALDI Matrix 2,4,6 Trihydroxyacetophenone

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Introduction
Ionization pathways remain uncertain in many MALDI matrix materials. In this study, 2,4,6 trihydroxyacetophenone(THAP) was investigated, since it may have characteristics that differ from previously studied matrixes.

Methods
Static and time-resolved absorption and emission studies were performed on THAP in solution and in the solid state. The effect of purity and solid sample preparation method were investigated. The Coupled Physical and Chemical Dynamics (CPCD) model was extended to include intersystem crossing, phosphorescence and triplet-triplet reactions.

Results
THAP undergoes efficient intersystem crossing (ISC) after excitation at typical MALDI ultraviolet wavelengths. The rate of ISC and the efficiency of phosphorescence were found to be dependent on the morphology of the crystals fromed from different solvents in at least one case, indicating strong intermolecular interactions. The CPCD model is compared with the data, with particular attention to the possible role of triplet pooling reactions in ionization.

Conclusions
Triplet states and their reactions may be important for ion formation pathways in THAP. Since a variety of MALDI matrix materials are also found or expected to undergo efficient intersystem crossing, this may be a widespread phenomenon. The CPCD with triplet pooling is largely consistent with the available THAP data.

Novel Aspect
Evidence for new, potentially widespread, ionization pathways in MALDI are presented, along with corresponding theory and model.

WPS21-22 / Electrostatic Spray Ionization: a New Versatile Ambient Ionization Technique

Liang Qiao, Hubert Girault, Elena Tobolkina, Natalia Gasilova
EPFL, Switzerland

Introduction
A new and versatile ambient ionization technique has been developed since 2012 in our lab, and is named electrostatic spray ionization (ESTASI).1 During ESTASI, an electrode is placed behind an insulator and connected to a pulsating square wave high voltage (HV) source. Samples presenting on the insulator can be ionized for MS characterization. This technique has been used to develop different ionization devices, such as microchip emitters, micropipette emitters, and plates with or without wells for ionization from microdroplets.

Method
The pulsed HV for ESTASI (from 0 V to 9 kV, frequency 5 to 40 Hz) was generated by amplifying voltage square wave pulses with a high voltage amplifier or by an electric circuit with two synchronized switches and a direct current HV power source. The self-designed ESTASI ion source was coupled with a linear ion trap mass spectrometer (Thermo LTQ Velos). The spray voltage of the internal power source of the LTQ was always set as 0 during the experiments.

Results
Many applications with great analytical chemistry merits have been achieved with the developed ionization devices based on ESTASI principle. Fractions of peptides or organic molecules from capillary electrophoresis were collected on an insulating target plate, and then analysed by ESTASI-MS.1 The ESTASI was also used to interface gel electrophoresis and mass spectrometry, where the protein or peptide bands in a polyacrylamide gel after isoelectric focusing were directly ionized by ESTASI for MS analyses.2 The ESTASI was further developed for ambient mass spectrometry imaging of biomolecules or cells.3 In the combination with microfluidic techniques, water-in-oil droplets were analysed by ESTASI-MS though a spyhole of a microchannel.4 Despite the application in bioanalytical chemistry and life science, the ESTASI-MS was also used for fast characterization of fragrances to "sniff" out fake perfume and quantification of food additives.5

Conclusions
We have studied a new principle of soft ionization under ambient conditions. This technique is used for various ionization devices and applied for many applications in analytical and bioanalytical chemistry.

Novel Aspect
A new ambient ionization method that is highly versatile.

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WPS22 - Cell Biology and Cellular Pathways

11:00-15:00

Poster Exhibition, Level -1

WPS22-01 / Conformational Analysis of alpha-Synuclein in Membrane Systems Using Traveling Wave Ion Mobility Mass Spectrometry

Shin Jung C. Lee, Hugh I. Kim
POSTECH

Intro

a-Synuclein (a-Syn) is an intrinsically disordered protein as well as amyloidogenic protein which can form insoluble β-sheet like aggregate. Especially, this protein is known to be involved in transport of neurotransmitters by assisting the fusion of neural vesicles at synaptic terminal. Especially, the fibril formation of a-Syn is highly dependent on its' membrane interactions, but the interaction degree and conformational changes in membrane interface are not studied at the regional level of the protein. By combining electrospray ionization ion mobility mass spectrometry (ESI-IM-MS) with hydrogen deuterium exchange mass spectrometry (HDX-MS), we probe the structural properties of the intrinsically disordered protein in heterogeneous model membrane systems.

Method

First, we identified the helical conformation of a-Syn using trifluoroethanol (TFE) which can stabilize helix of proteins. Through circular dichroism spectroscopy and synchrotron small angle X-ray scattering (SAXS), solution-phase structural properties including secondary structural component, shape, and size were probed. Then, gas-phase structures were investigated using ESI-IM-MS. By analyzing ESI-IM-MS, membrane-induced helices of a-Syn were also studied in relevance with co-solvent induced helices. HDX-MS was utilized to probe the regional interaction of the protein with membranes.

Results

In CD spectra, the portion of helical conformation gradually increases with the addition of TFE. Through molecular dynamics simulations based on the experimental SAXS profiles, we found that helical propensity of solution-phase a-Syn can be maintained in gas-phase conformation probed by ESI-IM-MS. Also, the structural change induced by membranes with different polarities was investigated using ESI-IM-MS. With zwitterionic lipids, the helical propensity of a-Syn is more enhanced, therefore, its' interactions with lipids are probed by HDX-MS. With anionic lipids, electrostatic interaction between basic N-terminal of a-Syn and negatively-charged head groups is a major driving force to form helices. On the other hand, hydrophobic interaction is dominant by the insertion of hydrophobic central region of a-Syn into acyl tail region of zwitterionic lipids.

Novel aspect

By combining SAXS and ESI-IM-MS with molecular dynamics simulations, this study represents new approach for structural analysis of IDPs in heterogeneous membrane systems. Particularly, we obtained information for the protein-membrane interaction at the regional level as well as the structural characterization. Also, it provides insight for effects of zwitterionic lipids on the inhibition of a-Syn fibrillation. As zwitterionic lipids are dominant in the outer leaflet of neural vesicles which directly interacts with a-Syn, our result is expected to provide a more plausible mechanism for the physiological feature.

WPS22-02 / Understanding the molecular basis of (R/W)9 cell penetrating peptide (CPP) effect on the phenotype of EF cells, a model cell line for Ewing sarcoma.

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Introduction

Ewing sarcoma is a very aggressive bones and soft tissues cancer with a high metastatic potential. This sarcoma is initiated by a chromosomal translocation leading to the synthesis of a fusion protein EWS/FLI-1 which is a strong transcription activator modulating the expression of a large panel of genes. The phenotype of Ewing sarcoma cells is characterized by a striking loss of focal adhesion and a disorganized cytoskeleton, however the signaling pathways involved in this tumoral transformation are poorly understood. It was shown that (R/W)9 cell penetrating peptide (CPP), an amphipatic and polycationic peptide (sequence: RRWWRRWRR) has an actin remodeling activity in EF cells and decreases the ability of the cells to grow without anchorage (Delaroche et al, JBC 2010). Therefore we decided to use this (R/W)9 CPP as a tool to investigate Ewing sarcoma.

Methods

EF cells are a model cell line obtained by the transformation of 3T3 fibroblasts with EWS/FLI-1 oncoprotein. We chose to use a differential quantitative proteomic approach based on SILAC metabolic labeling to evaluate (R/W)9 CPP effect on EF cells. To begin with, we compare EF and 3T3 cells proteomes and then, we assessed the effect of an incubation with (R/W)9 peptide on EF cells proteins expression.

Results

We manage to identify about one hundred proteins whose expression is significantly modified after transformation of 3T3 fibroblasts with EWS/FLI-1 fusion protein. On the contrary, the incubation of EF cells with (R/W)9 peptide doesn't lead to an extended change in protein expression levels as only a few proteins undergoes a significant change of their expression.

Conclusions

Quite surprisingly the significant changes of EF tumoral cells phenotype observed after incubation with (R/W)9 CPP are not correlating with deep modifications in protein expression profiles: only a few proteins expression levels have been modulated. The tumoral phenotype reversion could thus be due to more subtle changes such as post-translational modifications of the proteins. We are thus currently investigating phosphorylation using a quantitative and phosphoproteomics approach. To see if the few changes in protein expression correlate with the observed phenotype we are also currently assessing the effect on the proteome of other CPPs which do not remodel the actin cytoskeleton.

Novel Aspect

First of all, to our knowledge, the molecular origins of Ewing sarcoma cells phenotype has never been studied by a global proteomic approach. Among the list of proteins that are significantly affected by the oncogenic transformation of 3T3 fibroblasts, some proteins such as caldesmon or tropomyosin,

known to play a role in actin dynamic, could be good candidates for further biochemistry experiments.
In addition very little is known about CPPs interacting partners and cellular fate. This study therefore also aims at evaluating the effects they could have on cells proteome, a field that has not been investigated yet.

WPS22-03 / ToF-SIMS analysis of osteoblast-like cells and their mineralized extracellular matrix on strontium enriched bone cements

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[1]Institute of Physical Chemistry, Justus Liebig University of Giessen, [2]Centre for Translational Bone, Joint and Soft Tissue Research, Technische Universität Dresden

Commonly used implants for therapeutic approaches of non-diseased bone do not sufficiently support the healing process of systemically altered bone e.g. osteoporotic bone. Since strontium(II) has been proven as an effective anti-osteoporotic drug [1] new types of strontium enriched calcium phosphate bone cements were developed for this purpose[2].
As osteoporosis is characterized by an imbalance of osteoblast and osteoclast activity [3] the influence of this newly generated strontium enriched biomaterials on the cellular behavior of osteoblast-like cells was investigated by time of flight secondary ion mass spectrometry (ToF-SIMS). ToF-SIMS was used to analyze whether there is a strontium uptake by osteogenically differentiated human mesenchymal stem cells (hMSCs) and whether strontium is incorporated into their mineralized extracellular matrix.
Therefore hMSCs were cultured in osteogenic differentiation medium for 21 days on the strontium enriched calcium phosphate cements S100 and A10 and for reference also on the pure calcium phosphate cement (CPC) and on a silicon wafer.
The distribution of strontium within the mineralized extracellular matrix synthesized by the osteoblast-like cells cultured on the cements was analyzed. Therefore SIMS mass images were compared to microscope images, which were obtained from the same spot of each sample. A higher intensity of the strontium signal could be detected in the region of the mECM, synthesized by cells cultivated on one of the Sr- enriched bone cement (S100) in comparison to the reference group.
Furthermore depth profiles of the osteoblast-like cells cultured on different biomaterials and substrates were performed. A uniform strontium distribution was observed within all investigated cells. But different amounts of strontium were found in cells cultured on different substrates. To compare the strontium concentration inside the cells an appropriate measurement procedure had to be found. Compared to the negative controls the strontium content in the cells cultured on the strontium enriched biomaterials was much higher.
A higher concentration of strontium inside the cells means that more strontium can take part in signaling pathways. As strontium is known for its beneficial effects on osteoblasts by promoting osteoblastic cell replication and differentiation, and reducing apoptosis [4] the strontium enriched calcium phosphate cements are promising implant materials for osteoporotic bone.

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WPS22-04 / LC-MS/MS as a tool to study polyamine flux

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Polyamines can be used as biochemical markers of cancer and many other pathophysiological conditions. Thus, more detailed understanding of the circulating flow of polyamines (polyamine flux) through the interconnected biosynthetic and catabolic pathways is a matter of interest. To study polyamine flux in detail, we developed a LC-MS/MS method to follow the label incorporation from the 13C,15N-labeled polyamine precursors, arginine, methionine and ornithine, into labelled polyamines. The method was used to study the polyamine flux of three distinct cell lines with different spermidine/spermine-N1-acetyltransferase (SSAT) expression levels. SSAT is the key enzyme in polyamine catabolism, and its overexpression has been linked with increased insulin sensitivity and protection against the negative effects of a high-fat diet in transgenic mouse.
Selected reaction monitoring method was used to follow the amount of unlabelled and labelled polyamines as well as the position of the label in the molecule. Calibration curves of unlabelled analytes were also used to measure 13C and 15N-labelled analytes. Deuterated polyamines with different m/z compared to labelled and unlabelled analytes were used as the internal standards.
The method overcame the limitations of the previous methods based on radiolabelled or fluorinated compounds. Moreover, the method enabled base-line separation of the various polyamine analogues and quantitation of the single or double labelled polyamines separately. With the developed method, de novo synthesis rates of polyamines could be monitored until the plateau phase in the intracellular label accumulation was reached. Monitoring of the dilution or accumulation of labelled polyamine pools made it possible to get insight to the dynamics of polyamine metabolism. The obtained results confirmed that increased SSAT expression is associated with accelerated polyamine flux.

WPS22-05 / Proteomic analysis of scaffolding protein interaction network by IP- GeLCMS/MS

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Introduction

Scaffolding proteins interact with multiple members of signaling pathways to organise their components in complexes and regulate signal transduction. The IQ motif containing GTPase activating protein 1 (IQGAP1) has been reported to associate with more than 90 different proteins. IQGAP1 is widely expressed in a variety of cell types in different organisms and coordinates many of the fundamental cellular activities. There are indications of IQGAPs involvement in diabetes, however, the role of IQGAP1 in one of the major target cells of insulin – adipocytes is unknown. The aim of this study is to characterize the protein interaction network of IQGAP1 in human adipocytes and elucidate its function and potential involvement in insulin signaling mechanisms.

Methods

Total protein fractions from control and insulin-stimulated cells were immunoprecipitated (IQGAP1-IP), analyzed with GeLCMS/MS (in-gel digestion followed by nanoflow reversed-phase liquid chromatography-mass spectrometry on LTQ Orbitrap Velos Pro) and the obtained data were used for identification and semi-quantification (spectral counting, SC).SDS-PAGE and immunodetection was used to verify both the GeLCMS/MS identification and SC-quantification.

Results
IP-GeLCMS/MSanalysis of primary human adipocytes revealed an extensive interaction network of IQGAP1-associated proteins. We were able to identify not only protein groups known in other cell types, but also a specific group of proteins involved in fatty acid metabolism. In insulin-stimulated adipocytes the association between IQGAP1 and myosin was significantly increased. The sensitivity of the Orbitrap allowed identification and quantification of many proteins below the detection limit of protein silver staining. Most of the GeLC/MS/MS results correlated well with SDS-PAGE/immunodetection verifications; however, due to e.g. immunoglobulins interference some proteins could not be immunologically detected.

Conclusions
We characterized a network of proteins associated with IQGAP1 in primary human adipocytes. Moreover, we were able to show an insulin dependent association between IQGAP1 and other proteins, which demonstrates the IQGAP1 involvement in insulin signaling. The combination of an MS-based approach to other types of analytical methods is of great importance for reliable results.

Novel aspect
This is the first MS-based characterization of IQGAP1-network and novel interaction partners were identified. This study was performed on cells from individual human subjects, which broadens the current knowledge of the protein map of adipocytes. Our research gives new insights into the insulin signaling mechanisms.

WPS22-06 / Identification of intracellular platin-protein complexes and their effect towards development of cisplatin resistance

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Introduction
Cisplatin (CDDP) is presently used to treat humans who suffer from head and neck, cervical, lung, gastric, bladder, testicular and ovarian cancers [1, 2]. But the effectiveness of CDDP is limited by two major drawbacks: toxic side effects and secondly intrinsic or acquired resistance of tumor cells to the drug. For CDDP, the platination of DNA is a crucial step for the development of the cytotoxic effect. However, less than 5% of the CDDP reacts with the nucleotides of the DNA [3]. For a long time it was believed that glutathione is the major cellular target of CDDP. Furthermore, resistant cells have often enhanced levels of glutathione and the CDDP-glutathione-complexes are no longer effective in tumor cells. Moreover, the addition of CDDP to plasma has shown that 95% of CDDP will be protein-bound after 24 hours [4]. A multiplicity of potential binding partners for CDDP exists within a tumor cell, and thus an increased intracellular formation of biologically inactive adducts with proteins or peptides consequently appears to contribute to resistance [5]. Therefore, the aim of our study is the identification of protein-adducts of CDDP in the human ovarian cancer cell lines A2780 and the CDDP-resistant subtype A2780cis as well as the characterization of the resulting reaction products.

Methods
For this purpose we used the fluorescent CDDP analogue carboxyfluoresceindiacetat-CDDP (CFDA-Pt) to enable the distinction between CDDP-binding and non-CDDP-binding proteins. We performed 2D-Gel electrophoresis (2-DE) and high-resolution mass spectrometry analysis (ESI-MS/MS) to identify CDDP-protein-adducts. Furthermore, we used in vitro incubation of CDDP with potential proteins and the characteristic CDDP-isotopic pattern in ESI-MS/MS to identify the potential CDDP-binding sites. In addition, we explored the effects of the siRNA-mediated knock-down of a protein binding CFDA-Pt in A2780 and A2780cis cells on cytotoxicity of CDDP and DNA-platination..

Results
To enhance the separation and resolution of acidic-neutral proteins as well as alkaline proteins, the 2-DE analysis in the pH ranges 4-7 and 6-10 is established. Furthermore, several CDDP-protein-adducts including members of the protein disulfide isomerase family, few heat shock proteins and the glucose-regulated protein 78 (GRP78) could be identified. GRP78 shows a differential expression and regulation after CDDP treatment in A2780 and A2780cis cells. After siRNA transfection a knock-down of GRP78 was achieved.

Conclusions
A strategy to detect and identify CDDP-protein-adducts using the fluorescent CFDA-Pt and ESI-MS/MS is established. The in vitro incubation of CDDP with GRP78 is the first step for the detection of CDDP-protein-adducts in more complex samples. Differential expression of GRP78 indicates a relevance of GRP78 for CDDP resistance in this cell line.

Novel Aspects
These are the first results for the detection of intracellular CDDP-protein-adducts.

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WPS22-07 / Sequestration by IFIT1 impairs translation of non-2'O-methylated capped RNA

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Viral nucleic acid serves as trigger of innate immune responses and at the same time is target of cellular antiviral defense mechanisms. Viruses that generate capped RNA lacking 2'O methylation at the RNA 5'end are severely affected by the activity of the antiviral cytokine type-I interferon. We employed an unbiased proteome-wide affinity purification-mass spectrometry based approach to identify murine and human proteins binding to capped RNA with different 5' end methylation status. Binding of selected candidate interactors, the interferon-induced proteins with tetratricopeptide repeats (IFIT), to capped RNA species was validated by biochemical approaches. We further determined the influence of IFIT1 on translation rates of cellular and viral proteins by pulsed stable isotope labelling in cell culture (SILAC) coupled to mass spectrometry as well as in vitro translation assays. We identified human and murine IFIT proteins as the only interferon stimulated proteins with higher affinity for unmethylated as compared to methylated capped RNA. Within the IFIT complex, IFIT1 had the ability to directly bind and discriminate RNA based on cap methylation states. Pulsed SILAC coupled to mass spectrometry as well as in vitro translation assays indicate that IFIT1 selectivity regulates translation of 5' unmethylated viral RNA in infected cells. Mechanistically, IFIT1 perturbed binding of the cellular cap-binding protein EIF4E to unmethylated but not to methylated capped RNA suggesting a competition mechanism.
Inhibition of viruses generating RNA lacking 2'O-methylation at the 5' cap is mediated by interferon-induced protein IFIT1. The selectivity of IFIT1 for viral RNA serves as potent antiviral

mechanism and at the same time allows progression of the antiviral program in infected cells.

WPS22-08 / Characterizing Mouse Thymus using Imaging Mass Microscope

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Introduction

The thymus is a multi-lobed organ composed of cortical and medullary areas surrounded by a capsule. T cell precursors enter the sub-capsular cortical areas, where they encounter networks of cortical epithelial cells and undergo proliferation. As they differentiate, they move from the cortex towards the medulla of the thymus. Targeting mouse thymus by imaging mass spectrometry (IMS) using matrix-assisted laser desorption ionization (MALDI) will clarify molecular arrangements without the need for target-specific reagents. In this study, we plan to visualize microenvironments of thymus using Imaging Mass Microscope especially from the standpoint of metabolism.

Methods

Normal ICR mouse thymus was obtained at 4 week of age as a control. One milli-gram of dexamethasone (DEX) per 20 g body weight of mouse was administered orally as thymic apoptosis inducing model. Ten micron thick frozen sections of mouse thymus were cut on a cryostat. The sections were transferred to conductive Indium-Tin-Oxide (ITO) coated glass slides for sagittal orientation and airbrush sprayer was used for matrix application. The MALDI measurement and image analysis was carried out on an iMScope, imaging mass microscope. MALDI measurements were done in a negative mode using 9-Aminoacridine (9-AA) as a matrix with a mass range of 300-900 Da. The lateral resolution for the MALDI imaging was set to 50µm. For the statistical analyses, the mass spectra were internally recalibrated on common peaks and normalized on the total ion count. The data was further analyzed by multivariate analysis such as principal component analysis (PCA), hierarchical cluster analysis (HCA) and Support Vector Machine (SVM).

Results

MALDI IMS in the negative ion mode using 9-AA as the matrix showed the presence of many signals including nucleotides in thymus tissues of mice. Among these peaks, there are reasonably good classifiers between cortex and medulla on the basis of PCA and HCA. It must be of particular interest to apply this dataset into DEX-induced apoptosis model of thymus, which reveals altered classifiers between medulla and cortex. Furthermore, SVM supports the concept that T cell movements between cortex and medulla were perturbed in an apoptotic model.

Conclusions

MALDI IMS of mouse thymus with 9-AA matrix yields several peaks including nucleotides which delineates between cortex and medulla. Multivariate analysis revealed some of nucleotides are good classifiers of cortex from medulla in normal thymus. These markers were altered in thymus of DEX-induced apoptosis model.

WPS22-09 / MudPIT analysis of Cucumis sativus roots, from plants growth under Fe and/or Mo deficiencies

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Introduction

Molecular mechanisms involved in the nutrient homeostasis represent an important research topic for all living organisms. In plants, both Iron (Fe) and Molybdenum (Mo) are essential microelements involved in several key metabolic processes [1,2]. Recently, an interesting link between Mo and Fe metabolism has been highlighted [2]. The aim of this study was to investigate, at proteomic level, the changes occurring in mitochondria ofCucumis sativusroots, growth under Fe and/or Mo deficiencies. In particular, we aimed to identify the proteomic profile related to the investigated conditions, as well as the proteins most affected by Fe and/or Mo nutritional stress.

Methods

Cucumber seedlings were grown in hydroponic medium for 10 days in following different nutrient solutions: 1) +Fe +Mo (control), 2) +Fe -Mo, 3) -Fe +Mo, 4) -Fe -Mo. For each conditions, mitochondria have been purified from roots and their proteins were extracted and analyzed in biological and technical replicates. Specifically, we applied a gel-free approach called MudPIT (Multidimensional Protein Identification Technology) [3]. In addition, label-free quantitative approaches [4], based on spectral count evaluation, were applied for identifying differential expressed proteins.

Results

Preliminary results allowed to identify above 1200 different proteins. For each sample were identified about 3000 redundant peptides corresponding to about 500 distinct proteins. These proteins were predominantly located, according to programs predicting protein targeting, in the mitochondrion or in the plasma membrane, confirming the usefulness of MudPIT for identifying highly hydrophobic proteins [5]. In addition, more than 150 proteins changed their expression by comparing the analyzed conditions; of note, some of them were involved in Mo and/or Fe trafficking and storage.

Conclusions

Our preliminary studies confirmed the usefulness of MudPIT for investigating membrane proteins, which represent an important aspect in the study of nutrient homeostasis in the plant field. In this context, it allowed to identify interesting proteins, among which also membrane proteins, as candidates involved in intracellular Mo and/or Fe trafficking and storage [6].

Novel Aspect

Our results open the way to further investigate the Mo and/or Fe intracellular uptake by applying new computational procedures. In particular, it will allows to shed light on molecular pathways that regulate plant nutrient homeostasis, and therefore to evaluate the analyzed conditions at proteomic systems level.

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WPS22-10 / Breaking the chain and cracking the code: interpreting cyclic peptide fragmentation spectra

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Introduction

Natural and synthetic macrocyclic peptides often exhibit powerful bioactivity, which, along with their versatility, make them desirable targets for future pharmaceutical development. Cyanobacteria are an especially rich source of cyclic peptides and cyclic depsipeptides. The cyanobacterium Prochloron spp produces multiple cyanobactins including trunkamides and patellamides. Recent work1,2 has shown that a diverse range of macrocyclic patellamides can be produced by harnessing the enzymes of related cyanobactin biosynthetic pathways. Mass spectrometry serves as a vital tool in structure confirmation and elucidation of these macrocyclic peptides. However, interpretation of the MSMS of cyclic peptides is non-trivial due to non-specific ring opening during the fragmentation process, and atypical fragmentation patterns compared to the conventional b and y ions seen in a linear peptide. Additionally, cyclic peptide natural products often contain unusual or highly modified amino acids which further complicate the interpretation of the spectra. The patellamides not only contain serine and threonine amino acids which lose water under CID conditions but also heterocycles, such as oxazolines and thiazolines, which are formed in the backbone of the peptide sequence, and further complicate the fragmentation pattern.

Methods

We have compared the fragmentation patterns of a number of structurally related biosynthetically and semi-synthetically prepared patellamides, along with isotopically labelled variants, using accurate mass CID, at a range of collision energies, on an ABSciex 5600 instrument, to elucidate how this class of cyclic peptides fragment. We have also explored ion mobility changes upon cyclization using a Waters Synapt G2S mass spectrometer.

Results

We have elucidated the major CID fragmentations occurring in a panel of patellamide molecules, identifying those signals which act as signature peaks indicative of macrocyclization and heterocyclization as well as those that allow sequence determination.

Conclusions

We have developed a robust method for the structural elucidation of patellamides and can now apply our set of rules to novel patellamide structures.

Novel aspect

Interpretation of the MSMS of simple cyclic peptides containing only natural amino acids remains challenging, although some progress has been made in producing software to allow this to be performed in an automated fashion3. However, such programmes are not applicable to patellamides, where heterocyclizations involving the peptide backbone occur. We are the first group to study a large panel of non-natural patellamides and delineate the major fragmentation events occurring in CID fragmentation.

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WPS22-11 / Characterization of beta-Arrestin2 Protein Interactions in the Wnt Signaling Network by Label-free Quantitative Mass Spectrometry

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Introduction

Wnt ligands trigger the canonical and multiple non-canonical signaling pathways. Despite their variability different Wnt pathways share subsets of proteins including beta Arrestin2 (Arrb2) and it is still unknown which protein interactions confer specific and concerted activity of different signaling cascades.
We have characterized the Arrb2 protein interaction network by one-step co-immunoenrichment and label-free quantitative mass spectrometry.
Our analysis revealed differential recruitment of additional proteins to a core complex of beta-Arrestin2, Dishevelled, and trimeric G-proteins beta and gamma in a signaling context dependent manner.

Methods

Plasmids encoding desired proteins were transiently transfected into HEK293T cells. Flag-tagged Arrb2 was affinity-isolated on anti-Flag beads (Sigma) and eluates were digested with trypsin in-solution and analyzed by nanoflow HPLC-MS/MS (2D-NanoLC, Eksigent; Velos Orbitrap MS, Thermo Fisher Scientific). Protein identification was performed with Mascot V2.2 (Matrixscience) and label-free quantification was performed with Progenesis LCMS V2.6 (Nonlinear Dynamics).

Results

Interestingly, the analysis of eluates from affinity enriched beta-Arrestin2 (Arrb2) alone using different tagging techniques did not lead to detection of interaction partners. We hypothesized that a minimal Arrb2 protein core complex might be required to assemble a functional signaling complex and co-expressed different potential interacting proteins.
Indeed, co-expression of Dishevelled (Dvl) or trimeric G-proteins beta and gamma (Gb/g) with an Arrb2 bait led to successful isolation of protein complexes. Label-free quantitative analysis by LC-MS/MS identified numerous novel as well as known protein interaction partners which were enriched and distinguishable from the unspecific background of hundreds of proteins.
The results supported our hypothesis that Arrb2 forms a core complex with Dvl which is also required for the Wnt/Ca2+ signaling pathway as we have described recently [1].
The analysis of an Arrb2 affinity enrichment experiment from cells co-expressing Dishevelled (Dvl) and the G-proteins beta, gamma (Gb/g) alone identified 36 potential interacting proteins. Seven proteins had been reported to interact with either Arrb2 or Dvl previously. We discovered 7 proteins with a known role in Wnt signaling to interact physically with the Arrb2/Dvl complex. In ongoing validation studies so far 5 novel proteins have been functionally validated in cell culture or in vivo in Xenopus embryos.
In summary, the quantitative mass spectrometric analysis provided new insight into the Wnt signaling cascade and identified several new, functionally relevant interacting proteins.

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Novel Aspect

Characterization of novel beta-Arrestin2 protein complexes in Wnt signaling by LFQ mass spectrometry

WPS22-13 / Toxicity and adaptive responses of the green algae Chlamydomonas reinhardtii exposed to silver as manifested on the transcriptome, proteome and phenotype

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Understanding mechanistic and cellular events underlying a toxicological outcome allows the prediction of impact of environmental stressors to organisms living in different habitats. A systems based approach aids in characterizing molecular events, and thereby the cellular pathways, that have been perturbed. However, mapping just adverse outcomes of a toxicant in an organism falls short of describing the stress response that is mounted to maintain homeostasis on perturbations and may confer resistance to the toxic insult. Silver is a toxicant which is a potential threat to aquatic organisms, at least partly due to the increasing use of silver-based nanomaterials, which release free silver ions. In this study, we have derived a mechanistic understanding of the temporal dynamics and pathways involved in toxicity, detoxification and repair in the unicellular green algae, Chlamydomonas reinhardtii, exposed to silver. Mechanistic understanding was accomplished by studying the effects of silver at the transcriptome, proteome and cellular level of the algae. The initiating event is the binding and translocation across the cell membrane of silver ions by copper transporters. Silver accumulates in the cells very fast and reaches amounts more than 1000 times higher than present in the water. Almost immediately, the cells lose energy and reduce photosynthesis and growth but at the same time mount an enormous, temporal stress response. Of the roughly 15'000 genes and 4'000 proteins that we were able to analyze, more than 1000 responded to combat the stress in an intricate interplay of toxicity and detoxification. An example of toxicity was perturbations in the copper transport system. On the other hand, cells mount a defense reaction to combat oxidative stress and to eliminate silver via efflux transporters. Taken together, regulation of genes and proteins allowed the algae to partly recover, for example, to resume photosynthesis, but not regain full growth or eliminate the silver from the cells. These results reveal a certain resilience of the cells to silver exposure but raise concern about the transfer of silver through algae in the aquatic food chain and the fitness of algae if exposed to silver for extended times.

WPS22-14 / Stressor-induced proteome alterations in zebrafish: a meta-analysis of response patterns characterized by gel-based and gel-free proteomics

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Introduction
Proteomics are applied in ecotoxicology on the premise that the identification of changes in the expression of specific proteins induced by a certain chemical would allow elucidation of molecular mechanisms of action. Unfortunately, most global proteomics techniques, both gel-based and gel-free, do not allow a complete coverage of all proteins present in the sample, especially if no sample pretreatment is applied before analysis. Consequently, only a fraction of those proteins being most abundant in the sample is usually measured. Disturbingly, a few protein groups seem to respond regardless of the tissue analyzed or the nature of the stressor applied.

Methods
We performed a meta-analysis of differential proteomics studies performed in zebrafish exposed to diverse stressors. Differentially expressed proteins identified in each study were extracted and converted to a common identifier. Several curation criteria were applied to avoid multiple counts per study and to ensure the inclusion only of those proteins that were reliably shown to respond to a treatment.

Results
The final dataset included 40 studies, 36 of them done by gel-based and 4 by gel-free proteomics methods. On average, each study reported 24 proteins to be differentially expressed. A list of «top 21» zebrafish proteins repeatedly identified as responding to various stressors was assembled. Hspa8 (heat shock protein 8) was found to be detected most often, namely in 30% of studies. Several other heat shock proteins were also on this list, accompanied by oxidative stress defense and diverse energy metabolism proteins. Additionally, an astonishing variety of cytoskeletal proteins, including actin, myosin and tubulin family members, as well as three keratins, were found to be frequently detected. Similar situation was observed for «Top 25» repeatedly identified zebrafish protein families. On average, around 50% of all proteins that were identified as differentially expressed in different studies belonged to the «Top 25» zebrafish protein families. Furthermore, zebrafish «top list» proteins were similar to those identified in an earlier meta-analysis of mammalian proteomics data.

Conclusions
Similar proteome responses to different stressors are commonly seen in zebrafish as well as in other species. Most often detected groups include cytoskeleton, energy metabolism and stress response proteins, followed by a much scarcer representation of other proteins. Thus, the usefulness of global proteomics for deciphering chemical-specific mechanisms appears rather limited at the moment. Thus, current proteomics methodology needs further development to allow increasing the routinely achieved coverage.

Novel aspect
We suggest that any differential proteomics experiment performed with zebrafish (or any other species) should be interpreted keeping in mind the list of most frequent responders. Careful consideration of the reliability and significance of observed changes is absolutely necessary. Furthermore, our discussion emphasizes the need for a shift in proteomics discovery strategy in ecotoxicology, from a global descriptive approach towards targeted, hypothesis-driven analysis.

WPS22-15 / Revealing the mystery behind the Epithelial-mesenchymal transition (EMT)

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More than 80 % of the cancer cases are carcinomas, formed by the transformation of epithelial cells . Thus, revealing epithelial cell conversions and their network of cell-cell adhesion is a primary interest for scientists. In the early stages of carcinogenesis, the cancer cells are restricted at the primary site by a network of cell-cell adhesion molecules and integrated to the basal lamina (Lobo et al., 2007). Eventually, a primary tumor cell invades the surrounding extracellular matrix; penetrate the basement membrane and intravasate a blood or lymphatic vessel and cancer cell then propagates to a distant organ via the circulation. If the cell survives the journey through the circulation, it attaches to the endothelium and extravasates into the surrounding tissue, where it may in time generate a metastatic tumor (Mathias et al., 2009). Carcinoma cells utilize a normally latent embryonic program, EMT to facilitate the initiation and progression of metastasis. Throughout EMT, epithelial cells lose cell-cell contacts and cell polarity. Cells acquire a mesenchymal appearance with increased

motility and invasiveness with a characteristic down regulation of E-Catherin protein (Guarino et al., 2007).
Studies of embryonic development showed that the mesenchymal cells in the mesoderm gives rise to epithelial organs including kidney and ovary by undergoing a reversible process called MET (Mathias and Simpson, 2009). This could be an evidence for the reversible process of cancer cells during the later stage in metastasis. During tumor metastasis, disseminated cancer cells would seem to require the ability to renew themselves as stem cells and this can suggest a possible link between stem cells and the cells generated by EMT. A recent study illuminated the mechanistic relations between EMT induced cells and cancer stem cells. This showed the importance of phosphorylation events and variation between several kinase expressions is shown by inhibition, and inhibitions showed less effect on mesenchymal and cancer stem cell compared to epithelial cells (Tam et al., 2013). Although, such studies helps us to determine biomarkers for the gene expression, it is still impossible to detect the level of phosphorylation events and regulation at mRNA level.
The aim of the project is to understand protein and phosphopeptide`s regulations during EMT by proteomics and to compare results with mRNA studies (Tam et al., 2013; Taube et al., 2010)(Jechlinger et al., 2009). We investigated whole proteome with quantitative methods to determine epithelial vs. mesenchymal cells protein regulations and their correlation with mRNA studies. We have also performed phosphoproteomics analysis and determined around 5500 proteins, 50000 unique peptides, 13000 unique phosphosites. To our knowledge this dataset will be the first systematically produced phospho-proteome data for EMT transition.
Our results are revealing promising regulations, differentiations in phosphorylation events and their regulations, active kinase motives and their signaling pathways.

WPS22-16 / Revealing the structural and spatio-temporal plasticity of the COP9-Signalosome complex using a combination of mass-spectrometry and cell biology approaches

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Introduction
The COP9 signalosome (CSN) is an evolutionarily conserved protein complex composed of eight subunits (CSN1-CSN8), which is found in all eukaryotes. The major role of the CSN is to regulate the ubiquitin-26S proteasome pathway, by controlling the activity of cullin-RING-ubiquitin ligases. The complex has been shown to be involved in numerous cellular and developmental processes, including gene expression, signal transduction, cell cycle progression, embryogenesis, DNA repair and cancer. Despite the progress made in revealing its biological functions, little is known about the regulatory mechanisms that control the CSN itself. Here, we aimed to expose the subunit diversity of the CSN complex, and study its plasticity in response to UV damage.

Methods
In our studies we used endogenous CSN complexes, which were biochemically purified from blood, or immuno-affinity purified from cells using a FLAG tag. In order to investigate the CSN complex we used various mass spectrometry (MS) methods, including nUPLC-ESI-MS, native MS, proteomic and phosphoproteomic analyses, in combination with cell biology and biochemistry methods.

Results
Initially, we subjected the purified complexes to nUPLC separation on a monolithic column, followed by ESI-MS. We found that most CSN subunits exist in one major form that lacks the first methionine, and are N-terminally acetylated. Using this approach, we were able to identify, in addition to the known CSN7a and CSN7b isoforms, new forms of the CSN2 and CSN8 subunits, which appear to result from alternative translation initiation sites. Structural MS analysis of the CSN showed that the endogenous complex is remarkably stable, even after prolonged incubation with a denaturing agent, and does not tend to exist in equilibrium with smaller subcomplexes. In accordance with our early model of the architecture of the recombinant human CSN, we found that the endogenous subunits are present at unit stoichiometry and that the CSN3, CSN4 and CSN8 subunits occupy peripheral positions. Cellular studies indicated that the complex is localized in the cytoplasm, nucleoplasm, and in chromatin-bound fractions, and upon DNA damage, a transient and dose-dependent recruitment of the complex into the nucleus was seen, accompanied by significant phosphorylation changes.

Conclusions
Taken together, our results suggest that the CSN is a heterogeneous complex which is composed of different subunit variants, and can undergo differential modifications. This specific spatio-temporal composition of the CSN may reflect a means by which the complex can rapidly response to the changing needs of the cell.

Novel Aspect
The endogenous CSN complex is composed of a versatile array of heterogeneous populations, which are regulated in a spatiotemporal manner to accommodate for the changing needs of the cell.

WPS22-17 / Blood Cell Interactions in Atherosclerosis involves the Coordinated Regulation of Multiple Protein Modifications

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Introduction
Atherosclerosis is the most frequent cause of cardiovascular diseases, the leading cause of death globally. Platelet activation as well as platelet-monocyte and monocyte-endothelial cell interactions is believed to contribute to the development of plaques in the early stage of atherosclerosis. To better understand this process, we have established a mixed cell model composed of human platelets, primary HUVEC (human umbilical vein endothelial cell), and THP-1, a monocytic cell line.

Methods
To our model, we applied a number of proteomic methods we have established to investigate changes in global protein expression and secretion, in small GTPase activity, and in glyco- phospho-, and redox sub-proteomes. As stimuli, we used lysophosphatidic acid, LPA (16:0), and thrombin, the strongest platelet activators in atherosclerotic plaques and during haemostasis, respectively. Differential analysis relied on SILAC labeling and mixing of stimulated/non-stimulated samples, using a range of time points between 10 minutes and 24 hours, sub-proteome-specific pre-fractionation, and LC-MS/MS using Thermo LTQ-FT and Orbitrap platforms.

Results
LPA activated platelets in a donor-dependent fashion, but did not significantly increase platelet-monocyte aggregate formation. In contrast, thrombin resulted in homogeneous platelet activation and noticeably increased aggregate formation. Analysis of the signaling pathways in platelets linked these differences to different small GTPase activation profiles and differential release of soluble factors and microparticles. Exposure of THP-1 cells to thrombin- and LPA-induced platelet releasate (PR) resulted in increased monocyte adhesion and migration,

and higher reactive oxygen species (ROS) levels.Comparative glycoproteome analysis revealed the up-regulation of integrin α5 and β1, which form a major adhesion complex. Redox proteomics identified proteins with altered redox state on their cysteine residues, including cytoskeletal and metabolic proteins such as GAPDH.Phosphoproteomics indicated the involvement of RhoA and actin cytoskeleton signaling pathways in this response. Platelet releasates were found to contain soluble P-Selectin and P-Selectin-expressing microparticles, yet blocking P-Selectin binding to PSGL-1 eliminated platelet and microparticle binding to monocytes, but did not alter ROS production, Cys oxidation, and increased monocyte adhesion

Conclusion
Our data established a platelet-monocyte aggregate and P-Selectin-independent pathway to monocyte activation: platelet activation releases factors that signal through NADPH oxidase, ROS production, and cysteine oxidation in monocytes, resulting in up-regulation of membrane proteins that facilitate monocyte adhesion, migration, and extravasation.

Novel aspect
A newly established multi-cell model that enables time-resolved quantitative proteome studies identifies a cell interaction axis that does not require P-Selectin/PSGL-1 engagement.

WPS22-18 / Calculating cell-to-cell metabolic variability using single-cell mass spectrometry

Alfredo Ibanez[1], Florian Buettner[2], Renato Zenobi[1]
[1]ETH Zurich, [2]Helmholtz Zentrum München

Mass spectrometry is one of the state-of-the-art techniques used for imaging the incorporation of a drug into organs, monitoring -omic (systems biology) differences between healthy and stressed cell populations, etc. In –omic applications, mass spectrometry data can be used to create mathematical models to characterize and even predict cell responses. However, multiple studies have demonstrated that processes within cells are rarely identical between different individuals of a population. Thus, it is necessary to "assume" coefficients of variation, while developing predictive mathematical models, for the metabolic intermediates.
In order to experimentally find the coefficient of variations, we need to deliver reproducible results with single cell sensitivity. Microarrays for mass spectrometry (MAMS) is a substrate for matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) that allows us to reach limits-of-detection that are compatible with single-cell metabolite detection. However, the original data processing method used for MAMS-based measurements was inefficient, since it required numerous normalization steps.
Using the know-how associated with the signal processing used in DNA/RNA microarray applications, we can now pool more efficiently data collected from different runs (i.e., analytical and biological replicates). By combining multiple measurements, we are able to perform a multivariable analysis to calculate the cell-to-cell differences in glycolytic intermediates, a biological insight that is not accessible with other techniques.

Novel aspects
The use of an improved MAMS analytical protocol bestowed lower levels of technical variability in our single-cell level mass spectrometry measurements. This allowed us to calculate the variance associated with the glycolytic intermediates at the single-cell level.

WPS24 - Trace Gas Analysis of Breath and Food Flavours

11:00-15:00
Poster Exhibition, Level -1

WPS24-01 / Online measurement of volatiles from a liquid flow by PTR-ToF-MS: The case of coffee extraction.

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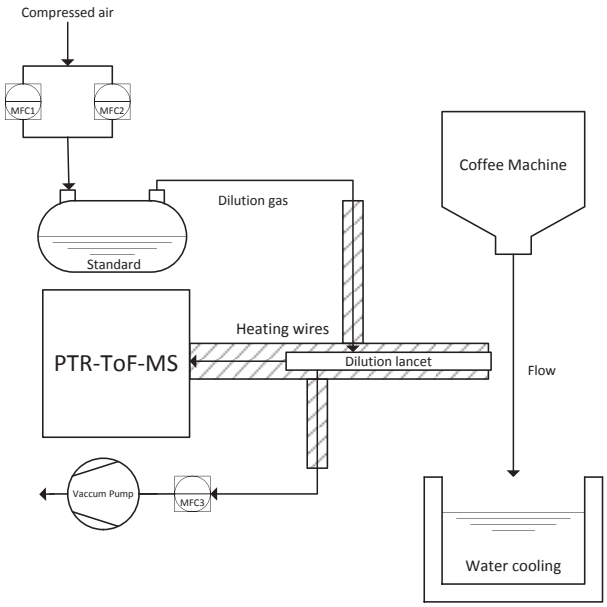
Introduction
The temporal evolution in the formation of volatiles during food processing gives valuable information about the physicochemical changes of the food stuff. Being able to measure them in real-time will also help to optimize and control those processes. In continuous processes, the output is generally a mass flow, and the most common option is to take samples at different time points and analyse them offline. We assumed it is possible to measure directly volatiles from a liquid flow with PTR-ToF-MS, in a similar way that it is done with membrane-introduction mass spectrometry, and that it could be easily done when the liquid flows in an open atmosphere where no membrane is needed. To probe that, we have developed a simple method to analyse VOCs released from a liquid flow, and we selected coffee extraction as an example of liquid flow containing a gradient of concentration.

Methods
Ten different capsules from six different coffee varieties were extracted in a commercial coffee machine according to manufacturer specifications (Delica, Switzerland). A customized inlet with a gas dilution system was placed 0.5 cm from the coffee flow and coupled to a PTR-ToF-MS 8000 (Ionicon Analytik GmbH, Austria). Drift tube was operated at 2.2 mbar, 70°C and 600 V. Mass spectra were recorded in the m/z range 0-205 at 1 spectrum per second. Data processing was done using PTR-TOF DATA Analyzer software v4.171.



Results
Results on coffee extraction showed good reproducibility among the ten replicates and the extraction kinetics observed are in agreement with offline measurements2. We characterized

the time intensity profile of each m/z by three time-dependent parameters: time at maximum intensity (tmax), time elapsed until the intensity drops to half of maximum intensity (t1/2) and the area under the curve at each time point (at). The decay of intensity gave information on how fast the compounds were extracted and the area under de curve represented the total amount of compound extracted at each time point. We used the area under the curve at different time to classify the different coffee capsules. Both hierarchical cluster analysis and Principal Component Analysis showed that all coffees could be differentiated at the end of the extraction time.

Conclusions
The method presented showed good reproducibility and was useful to study the kinetics of coffee espresso extraction. It was also useful to differentiate among different coffees with the data recorded is just few seconds.

Novel Aspect
This is the first time that espresso extraction is monitored online by direct injection mass spectrometry and results indicate that the set up could be easily adapted to measure volatiles from other liquid flows.

1. Müller, M., Mikoviny, T., Jud, W., D'Anna, B. & Wisthaler, A. A new software tool for the analysis of high resolution PTR-TOF mass spectra. Chemom. Intell. Lab. Syst. 127, 158–165 (2013).
2. Mestdagh, F., Davidek, T., Chaumonteuil, M., Folmer, B. & Blank, I. The kinetics of coffee aroma extraction. Food Res. Int. (2014). doi:10.1016/j.foodres.2014.03.011

WPS24-02 / Identification of volatile and semi-volatile compounds in honey by gas chromatography time-of-flight accurate mass spectrometry

Mohammed Moniruzzaman[1], Issac Rodríguez[2], Maria Ramil[2], Rafael Cela[2], Siti Amrah Sulaiman[1], Siew Hua Gan[1]
[1]Universiti Sains Malaysia, [2]University of Santiago de Compostela

Introduction
The performance of gas chromatography (GC) combined with a hybrid quadrupole time-of-flight (QTOF) mass spectrometry (MS) system for the determination of volatile and semi-volatile compounds in honey samples is evaluated.

Methods
Honey sample preparation was conducted using headspace (HS) solid-phase microextraction (SPME). After HS-SPME, the accurate mass capabilities of the above system were evaluated for compounds identification. Accurate scan electron impact (EI) MS spectra allowed discriminating compounds displaying same nominal masses, but having different empirical formulae. Moreover, a narrow mass window was selected (0.005 Da) during extracting the chromatogram on basis of possible quantification ions.

Results
The use of narrow mass window provided highly specific chromatograms for selected ions, avoiding the contribution of interferences to their peak areas. Additional information derived from positive chemical ionization (PCI) MS spectra and ion product scan MS/MS spectra permitted confirming the identity of novel compounds. Overall, 84 compounds, from a total of 89 species, could be identified in 19 honey samples from 3 different geographic areas in the world. The suitability of responses measured for selected ions, corresponding to above species, for authentication purposes is assessed through principal components analysis.

Conclusions
GC-QTOF-MS analysis following HS SPME provides enough sensitivity for the identification of a relevant number of volatile and semi-volatile compounds in honey samples, with mass errors usually remaining below 1 mDa. Identified compounds can be classified in four major classes: benzene derivatives, non-aromatic carboxylic acids and esters, terpenes and nitrogenated compounds. The accurate scan EI-MS spectra are of high usefulness to discriminate between compounds rendering ions with same nominal masses, but having different empirical formula.

Novel Aspect
For first time, the capabilities of hybrid quadrupole time-of-flight (QTOF) mass spectrometry (MS), following gas chromatography (GC) separation, have been evaluated for the characterization of volatile and semi-volatile species, responsible for aroma of honey samples. Accurate, scan spectral features of the above system, together with the possibilities offer by different ionization and detection modes, were employed for the characterization of compounds present in complex chromatograms of honey samples. Two new alkaloids are reported in some of the investigated honey samples.

WPS24-03 / Use of High-Resolution Accurate Mass Spectrometry for studying of the changes of the proanthocyanidins during beer brewing process

Martin Dusek, Jana Olšovská
Research Institute of Brewing and Malting, PLC

Novel Aspects
The method using of High-Resolution Accurate Mass Spectrometer was successfully used for monitoring of bioactive proanthocyanidins during beer brewing process.

Introduction
Proanthocyanidins represent a group of condensed flavan-3-ols, such as procyanidins, prodelphinidins and propelargonidins that can be found in many plants. Hops and barley, as an ingredients used in the brewing industry, represent an important natural sources of these bioactive polyphenols, which can contribute to prevention of several civilization diseases, owing to their antioxidant and radical scavenging activity. Previously study showed that in the hops can be found significant amounts of di-, tri- and tetramer proanthocyanidins consisting of (epi)catechin and (epi)gallocatechin [1]. The intention of this work was to determine the proanthocyanidins composition and describe their changes during whole beer brewing process.

Experimental
An UHPLC-ESI-HR/AM-MS based method for the profiling of proanthocyanidins was developed. Analytes were extracted from samples of wort, hopped wort, green beer and beer using QuEChERS method [2] and separated on the XSelect HSS T3 2.5 µm column. The data were acquired by using a quadrupole-Orbitrap hybrid (Thermo Q-Exactive) mass spectrometer by targeted-MS2 experiment in negative ionization mode.

Results
High-resolution mass spectrometry allowed distinguishes between individual proanthocyanidins composed from different 3-flavon units (catechin, gallocatechin and afzelechin) on the base of exact mass and consequently confirmed these compounds o the base characteristic fragmentation spectra. The profile of the proanthocyanidins was studied in the samples that correspond to whole beer brewing procedure. Therefore, the proanthocyanidins changes could be observed during beer brewing process and also proanthocyanidins coming from hops and barley malt could be distinguished.

Financial support of this project was provided by the Czech Science Foundation No. GAP303/14/10233S.

References
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[2] Anastassiades M. et al., J. AOAC Int., 86 (2), 412 (2003)

WPS24-04 / Fast GC-MS/MS Analysis Of Multicomponent Pesticide Residues (360) In Food Matrix

Hendrik Schulte, Stéphane Moreau, Hans-Ulrich Baier
Shimadzu Europa GmbH

The determination of pesticide residue in food prepared by the well-established QuEChERS method is mainly done by GCMS/MS and LCMS/MS using multiple reaction mechanisms (MRM). The selectivity of tandem MS detectors is often preferred because samples prepared by QuEChERS contain large matrix signals which may interfere with target peaks. To reduce analysis time regarding GCMS the use of narrow bore capillary columns have become a powerful tool. This approach reduces analysis time drastically while mainly maintaining the chromatographic resolution. As the sample capacity for 0.1 mm inner diameter capillary columns is reduced in comparison with standard columns in this work a RTX-5 20m, 0.18 mm, 0.18 mm was used as capacity with respect to matrix is larger compared to the ones with 0.1 mm I.D.. Using the selectivity of the tandem MS a large number of compounds can be measured. Regarding the detector part the system must be able to follow sharp increases of signals as the peak widths at half height (FWHM) in fast GC with narrow bore columns are expected to be down to about 0.5 s. Therefore fast MRM switching modes are needed with no interfering cross talk. Each pesticide was measured with one quantifier and one qualifier transitions to ensure necessary selectivity. The method was adapted to QuEChERS extracts from apple. The run time was below 10 minutes to screen all compounds (360). The limit of quantification (MRM) was below 0.1 ppb. Good reproducibilties of below RSD%<5 were found for nearly components. Linear calibration curves were done between 0.5 ppB and 100 ppB using triphenylphosphate (TPP) as internal standard.

WPS24-05 / Merits of fast, high resolution time-of-flight mass spectrometry for the aroma profiling of cheese samples at different maturity levels

Juergen Wendt[1], Thomas Groeger[2], Ralf Zimmermann[2]
[1]LECO Instrumente GmbH, [2]Joint Mass Spectrometry Centre, University of Rostock and Helmholtz Zentrum München

Introduction
Kars kashkaval is a semi-hard Turkish traditional cheese with distinctive flavour characteristics. The manufacturing process of the cheese is unchanged during decades and it represents a core product in the agricultural economy of the Kars province. The authenticity of this cheese variety is a matter of concern for the region. Therefore the precise characterization of the aroma profile of the cheese is important for the producers. Small volatile compounds containing heteroatoms (N,S,O) are assumed to be the key aroma active compounds and therefore of special interest. Fast and high resolution time-of-flight mass spectrometry offers the ability for a further comprehensive characterization of the aroma profiles, especially with view on the different levels of maturity.

Methods
Recently introduced fast and high resolution time-of-flight mass spectrometry in combination with automated solid phase matrix extraction (SPME) and gas chromatography has been used for the profiling and identification of odor active compound in a time series of different matured cheese samples. The unique characteristics of the multi-reflecting time-of-flight instrument (Folded Flight Path® (FFP) technology) with a resolution up to 50.000, a mass accuracy of about 1ppm and a acquisition rates up to 200 Hz provide a novel tool to determine the exact mass information of fast changing time profiles. Electron and chemical ionization were used for the sample measurements. Build-in kinetic algorithmic data acquisition system allowed a expeditious analysis of high resolution data.

Preliminary Data
Solid phase micro extraction was applied in combination with gas chromatography-time-of-flight mass spectrometry to characterize the volatile organic compounds (VOC) of Kars kashkaval semi-hard Turkish traditional
cheese.
A time series of different matured cheese samples were analyzed and the aromatic profiles were compared to generate a mature depended VOC profile. The recently introduced fast and high resolution time-of-flight mass spectrometer (HRT) facilitated the identification of heteroatomic key compounds and the identification of ambiguous compounds of the aroma profile. Accurate mass information could be used to filter especially for heteroatomic compounds.

Conclusions
Fast and accurate time-of-flight mass spectrometry allows the application of novel visualization tools for the analysis of complex molecular mixtures.Kendrick mass defect information could be integrated with chromatographic data to separate nominal isobars and structural isomers at the same time (chromatographic resolved Kendrick mass defect plot). Discriminating features could be detected by applying state-of-the-art biomarker detection software and identification benefits from speed (spectrum deconvolution) and high mass accuracy (confirmation of molecular ion) of the applied HRT technology.

Novel aspect
Usage of fast, high resolution time-of-flight mass spectrometry for verifing the authenticity of agricultural products

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WPS24-06 / Characterization of Food Products by GC×GC-TOFMS and GC-high resolution TOFMS: A Food "omics" Approach

Lorraine Kay[1], Elizabeth Humston-Fulmer[2], Joe Binkley[2], Jeffrey Patrick[2]
[1]LECO Instruments UK Ltd., [2]LECO Corporation, St. Joseph, MI.

Introduction
Gas chromatography (GC) coupled with mass spectrometry (MS) is an effective tool for characterizing and distinguishing food products. Individual analytes can be isolated from complex food matrices with GC and identified with MS. Information on individual analytes provides food "omics" insight at various stages throughout production, including differentiation of raw materials, process changes, and finished products. As sample complexity increases, additional resolution can be gained with two-dimensional GC (GC×GC) or with high-resolution MS (HR-TOFMS) to isolate additional analytes. These capabilities for investigating food "omics" have implications in quality control, process optimization, and detection of food fraud, among others. Analyses of hops, beer, and edible oils are demonstrated here.

Methods
For each food type, sample preparation was accomplished with head space solid-phase microextraction (HS-SPME). A divinylbenzene/carboxen/polydimethylsiloxane (50/30 µm DVB/CAR/PDMS, Supelco, Bellefonte, PA, USA) fiber was used to

collect and concentrate headspace analytes for GC analysis. The volatile and semi-volatile analytes in these samples were separated and identified with Pegasus 4D GC×GC-TOFMS and/or Pegasus GC-HRT. Visually distinct two-dimensional chromatograms were utilized for fingerprinting and individual analytes were identified through library matching of nominal mass spectra and accurate mass information in high resolution spectra, when available. Data analyses were accomplished with a combination of ChromaTOF software for peak finding and identification, Excel for data compilation, and XLStat for chemometric analysis, including PCA.

Preliminary Data
Sample preparation, chromatographic, and mass spectral methods were determined to analyze a variety of food items, including hops, beer, and edible oils. These methods provided good sample characterization and the ability to distinguish variations in products. HS-SPME was used in each case to pre-concentrate the volatile and semi-volatile analytes for analysis. Process changes were determined by monitoring hops through a simulated boil in the beer brewing process. A collection of analytes were identified that showed distinct time-dependent trends, including a decrease in essential oil levels corresponding to an increase in the duration of the boil. Characterization of the finished product was also accomplished with both fingerprinting and the determination of individual analyte differences. Beer varieties clearly clustered with principle component analysis (PCA). The broad applicability of these approaches is highlighted by applying similar methods to other food products.

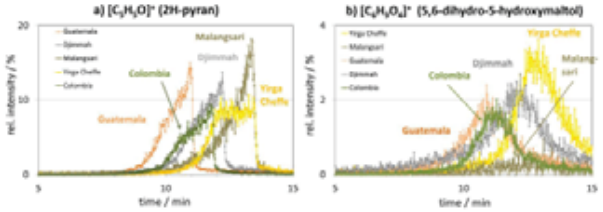
Novel aspect
HS-SPME, GC, and MS methods are presented that effectively characterize and differentiate a variety of food products.

WPS24-07 / On-line analysis with PTR-ToF-MS of coffee roasting reveals different flavour formation for coffee from different origins

Alexia N. Gloess[1], Anita Vietri[1], Flurin Wieland[1], Samo Smrke[1], Barbara Schönbächler[1], Jose A. Sanchez-Lopez[1], Sergio Petrozzi[1], Sandra Bongers[2], Thomas Koziorowski[2], Chahan Yeretzian[1]
[1]ZHAW, [2]PROBAT-Werke von Gimborn Maschinenfabrik GmbH

Introduction
Roasting is the crucial step in coffee processing towards the delightful aroma of a good cup of coffee. It is there, where in different chemical reactions hundreds of volatile organic compounds (VOCs) are formed. But, albeit coffee roasting is performed since hundreds of years, little is known about the formation pathways of these VOCs during roasting. On-line monitoring of coffee roasting with proton-transfer-reaction time-of-flight mass-spectrometry (PTR-ToF-MS) provides a direct insight into these VOC formation dynamics helping to understand how different roasting conditions may affect the aroma profile of a cup of coffee, and why different coffees evolve different aroma profiles, although they are roasted under identical roasting conditions.

Methods
A PTR-ToF-MS (Ionicon, Austria) was coupled to the exhaust gas of a drum roaster (Probatino, PROBAT, Germany). PTR settings: f(inlet): 100ml/min, p(drift): 2.13mbar, U(drift): 600V, T(drift): 80°C. Roasting profile: high temperature short time (HTST) up to low temperature long time (LTLT), medium and dark roast degree. Coffee:Coffea arabica: Colombia, Guatemala (Antigua La Ceiba), Yirga Cheffe (Ethiopia), Djimmah (Ethiopia);Coffea canephora var. robusta:Malangsari (Indonesia). Off-line analysis on coffee brew: HS SPME GC/MS, content of total solids, total polyphenols (Folin Ciocalteu), organic acids (ion chromatography), chlorogenic acids, caffeine (HPLC), titratable acidity, pH.



Results
On-line analysis with PTR-ToF-MS of roasting different coffees applying the same roasting conditions showed significant differences in the VOC formation (Figure 1): The dynamics of VOC formation differed as well as the time when the VOC formation started differed for each of these coffees. The earliest VOC formation was observed for Guatemala coffee, the latest in case of Malangsari and Yirga Cheffe.
Changes in the time-temperature roasting profile changed the release dynamics of the on-line monitored VOCs and were reflected in the characteristics of the cup of coffee brewed from the respectively roasted beans.

Conclusions
On-line analysis with PTR-ToF-MS accompanied by off-line analysis is a powerful tool for gaining a deeper understanding of how a specific profile of a cup of coffee can be generated, based on the choice of coffee and the way of roasting it.

Novel Aspect
Up to date, this is the most comprehensive study on on-line monitoring of VOC formation during coffee roasting, accompanied by off-line analysis of the respective coffee brews. A clear difference in VOC formation was observed when roasting different coffees along the same time-temperature roasting profile.

Figure 1: Time intensity profiles for the medium time-medium temperature roasting profile to a medium roast degree for a) [C5H5O]+and b) [C6H9O4]+.

WPS24-08 / The Combining of an Integrated Microfluidic Device with Collision Cross Section Ion Mobility Screening for the Analysis of Pesticide Residues in Food

Séverine Goscinny[1], Michael McCullagh[2], David Douce[2]
[1]Scientific Institute of Public Health, [2]Waters Corporation

Introduction
Full spectra acquisition and the specificity of accurate mass measurement is well characterised. It is used in combination with time tolerances, isotopic matching, fragment ions/ratios and response thresholds to help reduce false positive and false negative identifications in screening assays. Advances in mass spectrometry have vastly improved sensitivity for full spectral analysis, but further sensitivity enhancements would improve the mass spectral data quality. This is especially important to avoid compromised precursor ion or fragment ion information, and ensure high mass accuracy below the legislation levels. The integrated microfluidic device was interfaced to a Synapt G2-S mass spectrometer operating in ion mobility data acquisition mode, enabling enhanced sensitivity and selectivity to be obtained for the sample acquisitions.

Methods
The assay is based on the analysis of sample extracts, matrix matched calibrants (pear, ginger, leek and mandarin) and quality control samples generated for an EU-RL proficiency test. These

samples were analysed using an integrated microfluidic device containing an analytical channel (150µm x 100mm) along with the ionisation emitter, coupled with ion mobility mass spectrometry.

Results
Initially, ion mobility data was acquired using the integrated microfluidic device, for a series of solvent standard mixtures. These were utilized to generate retention time information and collision cross section (CCS) measurements for the pesticide library of the scientific information system. This subsequently enabled the correct identification of the pesticide residues in the matrix matched samples and proficiency samples. The results were compared to those previously obtained, where analysis was performed using conventional UPLC. Initial results have shown gains in both sensitivity and signal to noise with excellent linearity correlation coefficients being obtained for the matrix matched calibrants (r2≥0.95). Improvements in sensitivity have enabled matrix dilution to be performed and detection of 1pg on column to be obtained. In addition the CCS measurements obtained during the UPLC ion mobility acquisitions, were used to rapidly determine the ionKey/MS retention times of the pesticide solvent standards and identify the residues present in a previous proficiency sample.

Conclusions
IonKey/MS with ion mobility offers some unique advantages for profiling complex matrices.
Spectral clean up and collision cross section measurements provide unique selectivity and added confidence in identifcation.

Novel Aspect
CCS measurement, improved sensitivity and matrix suppression reduction for screening pesticide residues in food using an integrated micro fluidic device

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WPS24-09 / Discovery of Pesticide Protomers Using Routine Ion Mobility Screening

Séverine Goscinny[1], Michael McCullagh[2], Kieran Neeson[2]
[1]Scientific Institute of Public Health, [2]Waters Corporation

Introduction
Criteria to instill confidence in identification include acceptable product ion ratio tolerances and relative intensities of the detected ions, expressed as a percentage of the intensity of the most intense (abundant) ion or product ion, which should correspond to those of the calibration standard at comparable concentrations and measured under the same conditions. Ion ratio performance can vary with instrumentation, matrix and is affected by sample concentration. SANCO/12571/2013 guidance document describes the method validation and analytical quality control requirements to support the validity of data used for checking compliance with maximum residue limits, enforcement actions, or assessment of consumer exposure to pesticides in the EU. Here we use ion mobility mass spectrometry to gain greater understanding of ion ratio variation.

Methods
The assay is based on the analysis of sample extracts and matrix matched calibrants of pear, ginger, leek and mandarin, as well as quality control samples generated for an EU-RL proficiency test. Positive ion electrospray with ion mobility mass spectrometry data acquisition was performed using a Synapt G2-S mass spectrometer.

Results
Empirically isobaric pesticide protomers have been identified and characterised using ion mobility. It has been possible to separate the protomers (ions different only by their protonation site), determine their respective collision cross section and individual protomer fragmentation dissociation pathways. This has allowed unique visibility of product ion formation information, enabling the product ions to be selected that will result in improved product ion ratio reproducibility.For indoxacarb determined to be present in proficiency sample FV-13, two mobility separated species, with CCS values of 136.49 Å² and 147.94 Å² were obtained. The fragmentation spectra generated from the mobility separated protomers of indoxacarb allowed the distinctive different fragments of each respective protomer to be identified, as well as common fragments (m/z 190 and m/z 249). A further example of protomer formation observed, is for fenpyroximate with measured CCS values of 147.15 Å² and 158.33 Å². The protomers of fenpyroximate each respectively produce one of the two most abundant fragment ions.

Conclusions
For the first time using ion mobility, observation, separation and characterization of the protomers of indoxacarb and fenpyroximate have been shown.Ion mobility has enabled a unique insight into the process of ionization and fragmentation. Using ion mobility improved selectivity of fragment ions can be made and in turn, more robust analytical assays can be developed where ion ratios are required.

Novel Aspect
Identification and collision cross section measurement of empirically isobaric pesticide protomers in residue analysis.

WPS24-10 / Determination of vitamin B3 vitamers in milk and milk products by LC-MS SIDA

Kristel Hälvin[1], Allan Vilbaste[2], Toomas Paalme[1], Ildar Nisamedtinov[2]
[1]Tallinn University of Technology, [2]Competence Centre of Food and Fermentation Technologies

Vitamin B3 vitamers, e.g. nicotinamide, nicotinamide riboside (NR), have claimed to have positive effect on health; maintaining and raising NAD+ levels can improve metabolism and prevent age-related functional decline and associated disease (Houtkooper & Auwerx 2012). In the present work we demonstrate a rapid, convenient and cost-efficient method for determination of vitamin B3 vitamers (nicotinic acid, nicotinamide, NR, nicotinamide mononucleotide (NMN) and NAD+) in milk and milk products (ice cream, yogurt, kefir) using LC-MS with stable isotope dilution assay (SIDA). Sample preparation contained protein precipitation with methanol, evaporation of methanol and reconstitution of sample with 0.05M ammonium formate buffer (pH 4.5). To determine NR, NMN and NAD+ pre-prepared vials with homemade 15N labelled yeast hydrolysate and to determine nicotinic acid and nicotinamide pre-prepared vials with commercial stable isotope labelled standards were used. Although, total amount of B3 vitamers in milk and milk products was very similar, their distribution in milk and milk products was totally different. Milk (pasteurized and raw) and ice cream contained mostly simpler forms of vitamin B3 vitamers, pasteurized milk and raw milk contained nicotinamide 80±8 and 75±4 µg/100g and NR 22±1 and 49±0 µg/100g, respectively, ice cream contained besides nicotinamide 35±3 and NR 24±2 µg/100g also nicotinic acid 22±4 µg/100g. Yogurt and kefir contained mostly vitamin B3 coenzyme NAD+ (341±8 and 426±13 µg/100g, respectively). NMN levels in the milk and milk products were minor. Low NAD+ levels (4±0 µg/100g) in pasteurized milk can be explained by NAD+ degradation during pasteurization, but as NAD+ levels were also very low in raw milk (13±2 µg/100g) the cause is rather enzymatic degradation of NAD+ into simpler vitamers by endogenous enzymes. High NAD+ content in yogurt and kefir could be explained by the fermenting microorganisms,

which may have consumed all simple forms of vitamers and produced NAD+ instead. The developed LC-MS-SIDA method with relatively fast and simple sample preparation showed to be a good high throughput method for studying vitamin B3 vitamers distribution in milk and milk products.

References

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WPS24-11 / Proteomic analysis of protein changes in milk products during processing and storage

Thao Le[1], Lotte Larsen[1], Hilton Deeth[2], John Holland[2]
[1]Aarhus University, [2]The University of Queensland

Milk proteins undergo chemical changes such as lactosylation, deamidation, proteolysis and protein cross-linking during processing and storage. These modifications can cause deterioration of their functional (e.g, solubility) and nutritional quality (e.g., digestibility). Proteomic techniques were used to investigate chemical modifications to proteins in different heat-treated milk products during storage (e.g., UHT, pasteurized milk and milk powders). Lactosylation and protein cross-linking were the most significant modifications that were observed on 2-DE gels. Lactosylation were well-separated on 2-DE in vertical stacks of spots and multiple lactosylation sites were detected by MALDI and LC/MS/MS. αS1-casein was found to be dominant in cross-linked protein complexes, however the mechanism of protein cross-linking is still unknown. Further studies are required for characterisation of protein cross-linking in stored milk products and a possible correlation between the formation of cross-links and product functionalities. Generally, the results show that proteomics can be used as a marker of milk quality in commercially milk and milk powders.

WPS24-12 / Application of a Prototype Microfluidic Device with MS for the Screening of Pesticide Residues in Food Analyses

John Chipperfield[1], Michael McCullagh[1], Severine Goscinny[2], David Douce[1], Ramesh Rao[1]
[1]Waters, [2]ISP WIV

Introduction

Full spectra acquisition and the specificity of accurate mass measurement is well characterised. It is used in combination with time tolerances, isotopic matching, fragment ions/ratios and response thresholds to help reduce false positive and false negative identifications in screening assays. Advances in mass spectrometry have vastly improved sensitivity for full spectral analysis, but further sensitivity enhancements would improve the mass spectral data quality. This is important to avoid compromised precursor ion or fragment ion information, and ensure high mass accuracy below the legislation levels. The micro fluidic device was interfaced to a Xevo G2 S QTof mass spectrometer operating in MSE data acquisition mode.

Methods

The assay is based on the analysis of sample extracts, matrix matched calibrants (pear, ginger, leek and mandarin) and quality control samples generated for an EU-RL proficiency test. These samples were analysed using a ceramic microfluidic device (ionKey) containing an analytical channel (150µm x 50mm) along with the ionisation emitter, coupled to a MS. The chromatographic gradient was provided from a nanoUPLC system with all separations occurring on the iKey micro fluidic device. The ionKey/MS device was interfaced with a QTof mass spectrometer operating in MSE data acquisition mode.

Results

Initial MSE data acquired on the microfluidic system was for a series of solvent standard mixtures. These were utilized to generate retention time information within the pesticide library of the scientific information system. This subsequently enabled the correct identification of the pesticide residues in the matrix matched samples and proficiency samples. The results were compared to those previously obtained, where analysis was performed using conventional UPLC. Initial results have shown gains in both sensitivity and signal to noise with no compromise in the linearity correlation coefficients for the matrix matched calibrants ($r2 \geq 0.99$). Improvements in sensitivity have enabled matrix dilution to be performed and routine detection of 1pg on column sample loadings to be obtained.

Conclusions

Significant sensitivity gains are observed due to the improved ionisation efficiency of the microfluidic device. Linearity for the pesticides using matrix matched standards produced correlation coefficients of 0.95 and above. Sensitivity improvements for iKey vs UPLC were shown to have a factor improvement x5 to x45 for 80% of the pesticides when analysed from solvent standards. Sensitivity improvements for iKey vs UPLC were shown to have a factor improvement x5 to x60 for 80% of the pesticides when analysed from matrix matched pesticides.

Novel Aspect

Improved sensitivity and matrix suppression reduction in the screening of pesticide residues in food using a prototype micro fluidic device.

WPS24-13 / Breath analysis for diagnostics of gastro-esophageal reflux disease

Patrik Španěl[1], Kseniya Dryahina[1], Jarmila Turzíková[2], Jiří Votruba[3]
[1]J. Heyrovsky Institute of Physical Chemistry of the ASCR, [2]Paediatric Department, Faculty Hospital Bulovka, Czech Republic, [3]First Clinic of Tuberculosis and Respiratory Diseases, General University Hospital in Prague, Czech Republic

Objective

Experimental study was carried out in order to discover volatile metabolites present in exhaled breath that could be used as biomarkers of Gastroesophageal Reflux Disease, GERD, one of the most common causes of chronic cough.

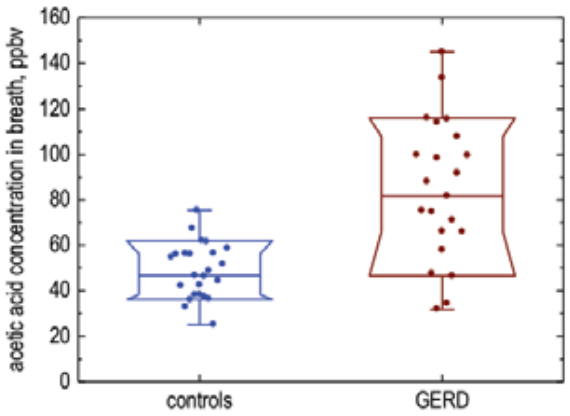


Figure 1. Concentrations of acetic acid in exhaled breath of controls and patients with GERD obtained using SIFT-MS. In addition to the individual data points the box and whisker plots are included to indicate the range, 10th, 25th, 50th, 75th and 90th percentiles.

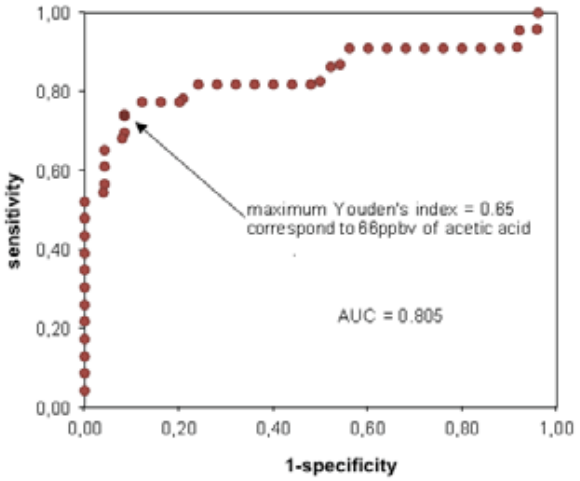


Figure 2. The receiver operating characteristics (ROC) curve plotted for breath concentration of acetic acid as a diagnostic variable for GERD. The area under the curve (AUC) is 0.805 and the acetic acid concentration corresponding to a maximal Youden's index (0.65) is 66 ppbv.

Methods
An in-vitro model based on pork tissue samples exposed to a challenge by artificial gastric fluid was used to identify specific volatile compounds to be chosen as candidates for biomarkers. GC/MS analyses of the headspace of this in vitro model indicated that the only volatile compound significantly increased was acetic acid.
Acetic acid was thus identiied as a potential biomarker and it was quantified in directly exhaled breath of GERD patients and controls using selected ion flow tube mass spectrometry, SIFT-MS. Acetic acid vapour was analysed in real time in single exhalations using the selected NO+ reagent ions injected into a flow tube with helium carrier gas. The count rates of the product ions at m/z 90 and 108 were used to calculate the absolute concetrations of acetic acid in orally exhlaed breath. Additionally, exhaled concentrations of water vapour were analysed using the product ions at m/z 48 and 66 .

Results
End expiratory concentration of acetic acid measured by SIFT-MS in mouth exhaled breath of GERD patients (N=22; median 85 ppbv) was found to be significantly higher than that in breath of a control group (N=24; median 48 ppbv) see Figure 1. The receiver operating characteristics (ROC) curve was plotted for breath concentration of acetic acid as a GERD biomarker. The area under the curve (AUC) was 0.805 and the acetic acid concentration corresponding to a maximal Youden's index (0.65) was 66 ppbv.

Conclusions
Breath acetic acid may be useful for non-invasive diagnostics of GERD and other conditions resulting in the lowering of pH of the lining of the airways.

WPS24-14 / Wine analysis by FastGC proton-transfer-reaction time-of-flight mass spectrometry

Andrea Romano[1], Lukas Fischer[2], Jens Herbig[2], Hugo Campbell-Sills[1], Joana Coulon[3], Patrick Lucas[4], Luca Cappellin[1], Franco Biasioli[1]
[1]Fondazione Edmund Mach, [2]Ionicon Analytik GmbH, [3]BioLaffort, [4]Univ. Bordeaux

Introduction
Proton Transfer Reaction - Mass Spectrometry (PTR-MS) has successfully been applied to a wide variety of food matrices; nevertheless, the reports about the use of PTR-MS in the analysis of alcoholic beverages remain anecdotal. Indeed, due the presence of ethanol in the sample, PTR-MS can only be employed after dilution of the headspace or at the expense of radical changes in the operational conditions.

Methods
A commercial PTR-TOF 8000 instrument was modified in order to accommodate a short (fast) dimethyl-polysiloxane GC capillary column, which was resistively heated. The modified instrument was equipped with a switching mechanism that allowed to choose between direct injection and FastGC PTR-MS mode.

Results
The prototype FastGC system allowed for a rapid (90 seconds) chromatographic separation of the sample headspace prior to PTR-MS analysis. The system was tested on red wine: the FastGC step allowed to rule out the effect of ethanol, eluted from the column during the first 8 seconds, allowing PTR-MS analysis to be carried out without changing the ionization conditions. Eight French red wines were submitted to analysis and could be separated based on the respective grape variety and region of origin.

Conclusion
In comparison to the results obtained by direct injection, FastGC provided additional information, thanks to a less drastic dilution of the sample and due to the chromatographic separation of isomers. This was achieved without increasing duration and complexity of the analysis.

Novel Aspect
This work represents the first reported application of a new PTR-ToF-MS accessory. A novel strategy for the analysis of matrices containing ethanol by PTR-MS is also presented.

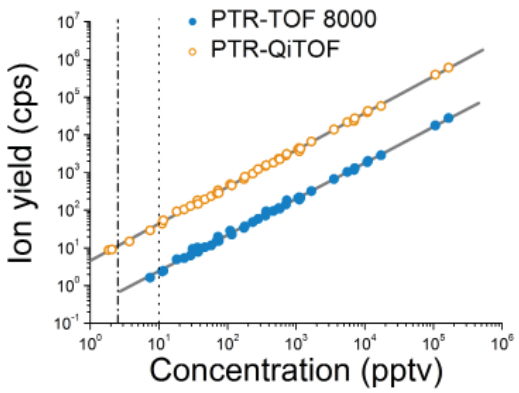
WPS24-15 / The Future of Proton-Transfer-Reaction Time-Of-Flight Mass Spectrometry

Lukas Märk[1], Christian Lindinger[1], Alfons Jordan[1], Eugen Hartungen[1], Gernot Hanel[1], Jens Herbig[1], Simone Jürschik[1], Philipp Sulzer[1], Tilmann D. Märk[2]
[1]IONICON Analytik GmbH., [2]IONICON Analytik GmbH. / Universität Innsbruck

Introduction
The introduction of commercial Time-Of-Flight (TOF) mass analyzers (in 2009) can be considered as one of the most important development in Proton-Transfer-Reaction Mass Spectrometry (PTR-MS). In contrast to Quadrupole mass filter, TOF based PTR-MS instruments typically have a sufficiently high mass resolution to separate many isobaric compounds and always acquire full mass spectra instead of monitoring single (pre-selected) nominal masses. Here we present the latest developments in PTR-TOFMS.

Methods
Established PTR-TOFMS instruments utilize conventional transfer lens systems to guide the ions from the reaction region (drift tube) to the mass spectrometer. We coupled for the very first time a PTR ion source (IONICON Analytik GmbH., AT) via a Quadrupole interface (Qi) to a high resolution TOF mass analyzer (both: Tofwerk AG, CH), hence we call this instrument PTR-QiTOF. This extremely efficient coupling, in combination with a slight increase of the drift tube pressure as well as the drift tube voltage, leads to a gain in sensitivity and a clear improvement of the mass resolution due to favorable injection conditions. On the

other hand we present the data on the newly developed PTR-TOF 1000, which is comparable to conventional Quadrupole based setups in terms of dimensions and weight.



Results

We present comparison data obtained by simultaneously introducing a certified gas standard into a common PTR-TOF 8000 (conventional transfer lens system) and the novel PTR-QiTOF at different concentration levels via a T-piece (see figure for a sensitivity comparison of dichlorobenzene). Both instruments show an increase in sensitivity with increasing m/z and thus have their highest sensitivities at the heaviest molecule under investigation at m/z 147 (dichlorobenzene) with a value of about 210 cps/ppbv for the PTR-TOF 8000 and 4,660 cps/ppbv for the PTR-QiTOF. For the latter this corresponds to a Limit-of-Detection (LoD) of 20 pptv after 100 ms and 750 ppqv after 1 min integration time. Depending on whether the instrument has been tuned for maximum sensitivity or maximum mass resolution we get between 6,900 and 10,400 m/Δm (FWHM) for the PTR-QiTOF at m/z 149.

Utilizing the same gas standard we find that the compact PTR-TOF 1000 performs with a mass resolution of over 1,500 m/Δm and a maximum sensitivity of over 100 cps/ppbv which leads to a LoD below 10 pptv.

Conclusions

We conclude that with the PTR-QiTOF we have developed a PTR-MS instrument with the highest sensitivity and mass resolution ever reported. The PTR-TOF 1000 on the other hand could replace Quadrupole based setups because of its comparable size, while being superior to those because of the advantages of TOF mass spectrometry.

We gratefully acknowledge financial support from the FFG (Wien, AT).

Novel Aspect

We introduce two novel PTR-TOFMS instruments: One with extremely high sensitivity and mass resolution and one with compact dimensions and reduced weight.

WPS24-16 / Modified PTR-MS operating conditions for in vitro and in vivo analysis of wine aroma

Jean-Luc Le Quéré[1], Etienne Sémon[2], Elisabeth Guichard[3], Gaëlle Arvisenet[4]
[1]INRA - SFC , [2]INRA, [3]INRA-CSGA, [4]AgroSup Dijon-CSGA

At PTR-MS standard operating conditions (ensuring excess of H3O+ primary ions, low water clusters formation and limited analytes fragmentation) analysis of alcoholic beverages is an analytical challenge. Ethanol reacts with H3O+ leading to depletion of reagent ions, and to formation of ethanol-related ions and clusters. This results in unstable ionization and to significant fragmentation of analytes. This is a main drawback for quantitative headspace analysis [1] and for in vivo nosespace analysis [2] of alcoholic beverages by PTR-MS. Alternate methods, either based on headspace dilution with N2 [3] or on non-conventional operating conditions using large E/N values in the drift tube [1] were proposed to maintain operational levels of H3O+. All these methods result in competitive ionization between H3O+ and ethanol-related ions, significant fragmentation of analytes and ligand-switching reactions. The purpose of this study was to propose an alternative method based on ethanol chemical ionization.

A PTR-MS (Ionicon ToF 8000) was operated at very low E/N drift tube value (80Td) to study ethanol-water solutions or model wines (10-13% v/v). In these conditions the main reagent ions (C2H5OH)H+ at m/z 47.049 (100%) and (C2H5OH)2H+ at m/z 93.091 (15%) were stable, further ethanol clusters were not detectable and H3O+ at m/z 19.018 did not exceed 2%. Linearity and LOD were checked for ethyl hexanoate. Fragmentations and ligand-switching reactions were checked for a series of 10 volatiles commonly found in wines. Finally the method was applied to the nosespace analysis of model wines flavored at white wines concentrations.

An excellent linear curve (r^2=1) was found for ethyl hexanoate from 0.128 to 128 ppb in hydro-alcoholic solutions and LOD was found at sub-ppb level, both being comparable to results obtained for aqueous solutions. Due to the low E/N drift tube value, fragmentation of the protonated analytes was limited to a few ions of low intensity [i.e. for ethyl octanoate MH+ at m/z 173.154 (100%), McLafferty rearrangement ion at m/z 145.122 (6%)] or to specific fragment ions with no further fragmentation [i.e. for 3-methylbutanol MH+ at m/z 89.096 (25%), MH+-H2O at m/z 71.085 (100%) and isobutyl fragment at m/z 57.070 (<1%)]. Ligand-switching reactions from ethanol clusters were insignificant. Applied to the nosespace analysis of flavored model wines, the method was found particularly suited as the simple fragmentation patterns permitted a precise flavor release study.

Operation of a PTR-ToF-MS at low E/N drift tube value (80Td) to study alcoholic solutions and beverages without dilution resulted in ethanol chemical ionization. Linearity, LOD, simple fragmentation patterns and absence of clusters made the method suitable for direct headspace and nosespace analyses of wines.

[1] Fiches et al., Int. J. Mass Spectrom. 356, 41 (2013)
[2] Déléris et al., Chem. Senses 36, 701 (2011)
[3] Spitaler et al., Int. J. Mass Spectrom. 266, 1 (2007)

WPS24-17 / Analyses of volatile metabolites in breath by a combination of thermal desorption, TD, with selected ion flow tube mass spectrometry, SIFT-MS

Kseniya Dryahina[1], Patrik Španěl[1], Alexandr Nemec[2], Pavel Dřevínek[3]
[1]J. Heyrovsky Institute of Physical Chemistry of the ASCR, [2]The National Institute of Public Health, [3]University Hospital in Motol

Development of sample collection method for off-line GC-MS and SIFT-MS analyses will be presented that has been carried out in order to study breath biomarkers of bacterial infections in patients with cystic fibrosis by quantitative analyses of volatiles in exhaled breath and headspace of sputum and bacterial cultures. Research of non-invasive methods for diagnosis and monitoring of bacterial infection in cystic fibrosis is currently a topic of great interest. Hydrogen cyanide has been identified as a breath biomarker of Pseudomonas aeruginosa (PA) infection. However, some strains of PA do not produce HCN. Thus other volatile breath biomarkers are needed order to improve the sensitivity and specificity. We propose to study trace amounts of volatile compounds in breath of CF patients using selected ion flow tube mass spectrometry (SIFT-MS) and gas chromatography mass spectrometry (GC-MS). SIFT-MS quantification of trace gases at low concentrations (<10 parts-per-billion by volume,

ppbv) is achieved by reactions of ions H3O+, NO+ and O2+ with the molecules of analyte. Sample collection using thermal desorption tubes is used for off-line analyses and for discovery of breath biomarkers of bacterial infections in patients with cystic fibrosis by quantitative analyses of volatiles in exhaled breath and headspace of sputum and bacterial cultures.
Selected ion flow tube mass spectrometry (SIFT-MS) method for absolute quantification of volatile compounds in humid air is based on a combination of the fast flow tube technique, chemical ionisation, and quantitative mass spectrometry. A chosen reagent ion (H3O+, NO+ or O2+) is selected from a microwave discharge by a mass filter and injected into helium carrier gas. The selected reagent ions ionize the trace gases (excluding the major components of air) in a sample introduced via a heated capillary. The ratio of the count rates of the product ions to the reagent ions is used to quantify the analytes. SIFT-MS thus allows precise and simultaneous quantification of multiple trace gases present in humid air samples at very low concentrations (with detection limit in the range of ppbv units or nmol/mol) in real time.
Thermal desorption device was constructed for pre-concentration of VOCs from breath samples for off-line SIFT-MS analysis. This device is based on controlled heating of a glass tube filled with sorbent and can be directly connected to sampling capillary of SIFT-MS without any cryo-focusing stage. Because SIFT-MS allows time resolved recording of concentrations of several VOCs, it is possible to use the information about time and temperature profiles of the desorption rates to separate the compounds.
In conclusion, the initial data have been obtained for selected sorbents that indicate that the propose method is feasible to facilitate accurate and precise SIFT-MS off-line breath analysis.

WPS26 - Metabolomics

11:00-15:00

Poster Exhibition, Level -1

WPS26-01 / Highly Standardised, Fast and Easy Determination of 25-hydroxyvitamin D3/D2 by Supported Liquid Extraction and U/HPLC-MS/MS Analysis

Fabio Polato, Ines Zitturi, Daniele Seppi, Therese Koal
BIOCRATES Life Science AG

Introduction
For the survey of vitamin D synthesis and deficiency a fast and reliable quantitation of 25-hydroxyvitamin D3/D2 is highly demanded. In this work a fast forward U/HPLC-MS/MS workflow was developed to reduce lab costs, labour and analysis time being at the same time highly precise, accurate and robust.

Methods
50 µl of plasma/serum and IS are loaded on a 96 well SLE-plate. The easy, fast and highly effective sample preparation provides clean extracts ready for LC-MS/MS in only 3-steps. For the method also all the reagents (CAL, IS, QC) and disposables required were developed. The validation included sensitivity, selectivity, matrix effects, inter- and intraday accuracy and precision for two major triple quad manufacturers: AB Sciex and Waters.

Results
The developed workflow enables the analysis of 80 samples within 7 hours by baseline separation of 25-hydroxyvitamin D3/D2 in 3 min run time. Moreover the LC-column is suitable for both HPLC and UHPLC instruments. The developed reagents include 4 CAL, 2 QCs, an isotopic labelled IS and a system suitability test-mix. The performance on different triple quads will be compared and proficiency test results discussed.

Conclusions
The new developed workflow can analyse 25-hydroxyvitamin D3/D2 in only 50 µl within a 3 min U/HPLC-MS/MS analysis, reducing lab time and costs, being easily automatable and dependable being traceable to the new NIST Standard SRM 972a.

Novel Aspects
The new developed workflow enables a fast forward analysis of 25-hydroxyvitamin D3/D2 for both HPLC and UHPLC - APCI -Triple Quad MS/MS qualitative and quantitative analysis.

WPS26-02 / Identification of unknown metabolites in bamboo leaf extracts by a non-targeted metabolomics approach using UHPLC-QTOF MS/MS driven by chemometrics tools

Götz Schlotterbeck, Timm Hettich
FHNW

Introduction
Bamboo is an important plant for a large part of the world's population. The use of bamboo is manifold ranging from constructive material, energy source, nutriment, cosmetics to traditional Chinese medicine applications. Bamboo is a rich natural source of promising phytochemicals including flavonoids and other secondary plant metabolites. These metabolites exhibit a broad range of health-promoting effects including anti-inflammatory, anti-oxidant, anti-viral and anti-aging properties. However, there is still a lack of information on the responsible metabolites present in many different bamboo species around the world. Thus, there is a great need for detailed information on the metabolite level of different bamboo species influenced by genus, age and geographical origin. A targeted LC-MS/MS based analysis of major flavonoid composition did not show any correlation regarding anti-oxidative and anti-inflammation properties. Thus, samples of different bamboo leaf extracts of genera Phyllostachys, Fragesia and Sasa as well as young and old leafs were analyzed in a non-targeted metabolic profiling workflow.

Method
Dried bamboo leaves of 25 different species were analyzed by UHPLC-MS/MS with an Agilent QTOF 6540 system.
Statistical data analysis was performed with mass profiler professional (MPP, Agilent).

Results
The investigated bamboo species can be differentiated by principle component analysis of high resolution MS data. In addition, for Phyllostachys edulis young and old leaves significant separating features were identified by the non-targeted metabolic profiling workflow. Non-targeted assessment of the statistical data (with respect to covariance) directed the selection of features for further MS/MS experiments. This results in a focused MS/MS list containing the most differentiating entities. Based on high resolution MS/MS experiments with high mass accuracy (≤ 3ppm) and Product Ion Scan data we structurally identified the most important compounds based on retention time, mass, and fragmentation pattern.

Conclusion
Non-targeted metabolic profiling in combination with integrated software-directed feature selection was found to be extremely helpful for rapid structural assessment of differentiating features between young and old leaves of Phyllostachys edulis. The implementation of this integrated workflow accelerates the tedious process from statistical feature identification to structural characterization of relevant metabolites. Pharmacological studies with the same bamboo extracts exhibit positive effects on the anti-inflammatory and wound-healing assays for young leaves of

Phyllostachys edulis. With the non-targeted metabolite profiling workflow structure identification of the significant metabolites is facilitated and allows an interpretation of pharmacological properties on a molecular level. Our study provides a model workflow for a comprehensive phytochemical assessment combing LC-MS/MS data with pharmacological testing.

WPS26-03 / Metabolic Phenotyping of Bile Acids - Standardized quantitative bile acids analysis in human plasma/serum and mouse plasma on different (U)HPLC-MS/MS platforms.

Hai Pham Tuan, Doreen Kirchberg, Ines Zitturi, Fabio Polato, Daniele Seppi, Therese Koal
BIOCRATES Life Sciences AG

Introduction

Bile acids are considered not only as endogenous markers for liver cell functions, but also as signaling molecules regulating triglycerides, cholesterol and glucose metabolism. Accurate determination of individual bile acids and their conjugates is, therefore, very important in accessing liver damages as well as hepatic and biliary tract diseases. It would provide a powerful tool for applications in precision medicine, toxicology, and clinical biomarker research. We have developed and validated a standardized (U)HPLC-ESI-MSMS assay for the analysis of more than 20 bile acids from only 10 µL human plasma/serum or mouse plasma samples. The bile acid panel consists of cholic acid, deoxycholic acid, chenodeoxycholic acid, ursodeoxycholic acid, hyodeoxycholic acid, alpha-, beta-, omega-muricholic acid and their glycin as well as taurine conjugates.

Method

10 µL sample and 10 µL IS mixture are pippeted onto the paper filter spot suspended in a 96-well filter plate. After a short drying under nitrogen stream, bile acids are extracted with 100 µL methanol. The methanolic extract is filtered through the plate into the 96- deep well receiving plate, under light centrifugation. 60 µL water is added to the extract before injecting into the (U) HPLC-ESI-MSMS for analysis. The analysis runtime for UHPLC and HPLC is 5 and 11 min, respectively. Bile acids detection is performed using MRM in negative ESI mode. 7-points calibration curves are used for quantitation. The assay has been rigorously validated according to the EMA guideline.

Results

Due to the special arrangement of the paper filter spot, proteins which have been precipitated are largely captured by the filter, while allowing the target metabolites to be extracted and filtered through. Only 3 steps are needed to complete the sample preparation. Seven calibrators levels and three quality control levles are used to guarantee the accuracy and precision of the measurements. This new assay in kit format has been validated for different LC-MS/MS platforms from AB Sciex, Waters, and Thermo Scientific. In general an LLOQ of 0.01 to 0.02 µM have been achieved for all target bile acids. Among the tested LC-MS/MS platforms, increasing sensitivity for bile acids analysis can be graded as follows: Xevo TQ MS < TSQ Vantage < 400QTRAP< QTRAP5500.

Conclusion

With the help of the very simple and robust bile acids kit, the analysis of several human plasma/serum samples and mouse plasma samples reveals that the bile acid profile of mice is quite different from that of human. While taurine conjugates of bile acids are prevalent and glycin conjugates are amost absent in mouse plasma, the situation is reversed in human plasma/serum. Moreover, the male/female differences found in mouse plasma is much more profound than that found in human samples.

Novel aspect

High-throughput and standardized bile acid analysis based on LC-MSMS, simple and robust sample preparation, universal for all LC-MS/MS platforms.

WPS26-04 / Primary metabolites ultra Performance HILIC-MS/MS targeted profiling method in IVF culture medium for the assessment of IVF procedure outcome.

Christina Virgiliou[1], Ioannis Sampsonidis[1], Georgiostheodoridis Theodoridis[1], Eleni Gika[2], Katerina Chatzimeletiou[3], Nikolaos Raikos[4]
[1]Department of Chemistry, Aristotle University Thessaloniki, Greece, [2]Department of Chemical Engineering, Aristotle University Thessaloniki, Greece, [3]Section of Reproductive Medicine, First Department of Obstetrics and Gynaecology, Aristotle University Medical School, Papageorgiou General Hospital, Thessaloniki, Greece, [4]Laboratory of Forensic Medicine and Toxicology, Medical School, Aristotle University, Greece

In-vitro fertilisation (IVF) represents a major sector of health industry (estimation of market size €3.7 billion, annually), however success rates remain relativelylow (<30%). The metabolic content of human embryo culture media is expected to correlate with IVF outcome and may provide prognostic markers.High possibilities of miscarriage, multiple births and the high cost of IVF treatment are the mainly areas that need to be improved in IVF. Thus info contained to IVF spent culture medium may aid to the selection of the most viable embryo with the higher developmental potential.Spent culture medium has been mostly assayed for this purposes byNIR, NMR and FT-IR spectroscopy, although technical limitations (sample volume, method sensitivity) hindered the development of robust assays that would help embryologist in decision making.To address this challenge we decided to develop a hydrophilic interaction liquid chromatography (HILIC) MS/MS method for profiling and quantitation of key end- point metabolitesin spent culture medium, Specifically the method provides the quantitative analysis of circa 100 metabolites including carbohydrates, amino -acids, organic acids and amineson an ultrahigh pressure liquid chromatography (UPLC) system with a HILIC Amide Column (ACQUITY UPLC BEH Amide), coupled (ESI)with a triple quadrupole spectrometer. UPLC conditions were optimized in order to reach maximum peak capacity and retention for all hydrophilic metabolites in a single run of 40 minutes. Mobile phase composition and pH were studied in order to improve elution factors, retention, separation and peak shape of all metabolites. Mobile phase adjusted at pH3.0,6.5 and9.0, while the composition differed according to buffer modifier and organic content. Additionally, due to low sample volume availability, different injection volumes (2, 5 and 10µl) were tested so as to achieve low limits of detection and to avoid adverse effects. The developed method was validated for the whole set of compounds and was proven to be reliable, robust, sensitive and displayed excellentlinearity over a wide range of concentrationThe method was applied in the analysis ofmore than 320 real samples of extracellular medium deriving from IVF procedures collected at the Obstetrics and Genecology clinic of PapageorgiouGenaral Hospital, Thessaloniki Greece following a standardised collection procedure. The sample bank consisted of medium from embryos that resulted in pregnancy and delivery and of embryos that failed to implant. The results from the analysis provided information on the metabolicactivityreflecting from embryos with different growth potential. Multivariate statistical analysis principal component analysis (PCA), Partial least squares discriminant analysis (PLS-DA) and advanced visualisation tools were used for data scrutiny to correlate metabolite concentrations/peak areas with embryo quality and implantation potentialThis research has been co-financed by the European Union (European Social Fund- ESF) and Greeknational funds through the Operational Program «Education and Lifelong Learning» of the NationalStrategic Reference Framework (NSRF) Research Funding Program: Thales II.

WPS26-05 / Metabolite profiling study of shikonin's cytotoxic activity in human Huh7 cancer cells.

Helen Gika[1], Angeliki Kyriazou[2], Christina Virgiliou[3], Georgios Mosialos[4], Evgenia Spyrelli[5], Vasilios Papageorgiou[5], Andreana Assimopoulou[5]
[1]Aristotle University of Thessaloniki, [2]School of Medicine, Aristotle University of Thessaloniki, [3]Department of Chemistry, Aristotle University of Thessaloniki, [4]Department of Biology, Aristotle University of Thessaloniki, [5]Department of Chemical Engineering, Aristotle University of Thessaloniki

Shikonin and its enantiomer alkannin, which are naturalproductsmainlyfoundin the roots of several plants of the Boraginaceae family such as Alkanna tinctoria, Lithospermum erythrorhizon etc, have been extensively studied for, among others, their antitumor activityin vitro andin vivo [1,2]. Several mechanisms have been proposed for their antitumor activity [1-3], therefore the investigation of the mechanisms involved is of interest. Metabolic profiling in cells is a promising tool for studying the underlying mechanisms. The holistic approach, which allows identifying changes in the context of the global network of metabolic pathways in a cell, can provide a picture of cell's phenotype. In this study we focused on shikonin's effect in human Huh7 hepatocarcinoma cell line aiming to identify primary metabolites affected and thus approach the mechanism of action.
The initial experiments were focused on the determination of the IC50 of purified shikonin in Huh7 cells at 48 hours. Concentrations of shikonin from 10-8 M to 10-5 M were tested with experiments performed in duplicates and it was found that the IC50 was~5x10-6 M. Studies on the cell growth and viability after treatment with 5x10-6 M shikonin showed that shikonin inhibited cell growth after 24 hours comparing to the control Huh7 cultures. Shikonin-treated cell cultures exhibited gradual accumulation of non-viable cells that started 24 hours after treatment and reached the maximum value of over 50% at 96 hours. Experiments were performed in triplicates and every 24 hours till 96 hours.
MetaboliteprofilingbyUPLC/MS-MSwas performed in cell culture medium and intracellular content in order to obtain information on their metabolite content. A multi-analyte HILIC-MS/MS profiling method capable to determinate 105 primary metabolites was applied both in cell culture medium and intracellular samples of control and shikonin treated (5x10-6 M) cell cultures after 72 hours. Results showed that metabolic profiling can sensitively detect and distinguish changes in metabolite concentrations induced by exposure to treatment with shikonin. It was found that in intracellular content certain aminoacids such as glutamic acid, alanine, proline and aspartic acid but also nicotinamide and inositol were decreased in shikonin treated cell lines, whereas glycose, pyruvate and arginine showed significant increase. In cell culture medium there were other metabolites that showed increased such as choline or descrease such as threonine, ornithine and others. Additionally it was found that thetreatmentproducedareproduciblemetabolic profile. The study demonstrates the potential of metabolomics to improve the knowledge on the response of hepatocarcinoma cell line to shikonin exposureand could be further used as a roadmap for shikonin, alkannin and other cytotoxic compounds on several cancer cells andin vivo experiments.

WPS26-06 / Metabolic pathway driven targeted metabolomics – a «quickstep" from mass spectrometric raw data to biologically relevant conclusions

Andrea Kiehne, Aiko Barsch, Verena Tellström, Heiko Neuweger
Bruker Daltonics GmbH

Biologists often think in terms of biological pathways and try to interpret results derived from Transcriptomics, Proteomics or Metabolomics experiments by making use of these. In addition to the interpretation of known target compounds, scientists in Metabolomics research often also intend to observe significant changes in abundances of as yet unidentified metabolites. Modern full scan high resolution QTOF instruments provide the advantage that both researcher's requests can be answered using the same data set. In this study a profiling for compounds contributing to the differentiation of coffee according to the assigned strength is performed.
The acquired LC-MS data was initially evaluated using a non-targeted workflow. This non-targeted approach enabled differentiation of coffee types based on their assigned flavor intensity and identified trigonelline (N-methyl-nicotinic acid) as one characteristic compound for weak coffee. We postulated that other compounds contained in metabolic pathways involved in generating trigonelline or derived from trigonelline might have higher abundance in weak coffee as well. Using a novel software tool we queried the elemental composition for trigonelline in the KEGG database (http://www.kegg.jp/). This returned several metabolic pathways involved in trigonelline metabolism. The nicotinate metabolic pathway was selected for automatically creating a targeted profiling list containing all metabolites of this pathway map. The initial screening list was extended by several compounds known to be characteristic for coffee.
The software tool also enabled to quickly screen for the presence of the compounds by generating high resolution Extracted Ion Chromatograms (hrEIC) with narrow mass tolerance window for the target compounds. Interactive views allowed for a quick evaluation of the compounds detected within the samples. The tentative identity of the compounds screened for was substantiated by taking into account accurate mass and isotopic pattern information. For further statistical evaluation all data was exported for PCA calculation. This revealed a clustering according to the coffee intensity as expected based on the previous non-targeted profiling results. The targeted profiling revealed chlorogenic acid and quinic acid as further compounds responsible for differentiating weak and strong coffee, respectively. This statistical targeted evaluation guided the purchase of reference standards for the final confirmation of the identity of chlorogenic and quinic acid.
In summary a novel workflow for combined non targeted and pathway driven targeted metabolomics based on the same data high resolution QTOF data files will be presented.

WPS26-07 / A novel high resolution MS/MS Human Metabolite Spectral Library enabling rapid and accurate metabolite identification in human metabolomics studies

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Introduction

Human biofluid metabolomics is being increasingly used for the discovery of metabolite biomarkers of diseases. However, metabolite identification remains to be a major analytical challenge in metabolic profiling. Here, we describe a workflow for rapid and accurate metabolite identification based on the use of a high resolution MS/MS spectral library.

Methods

An MS/MS spectral library of 800 human endogenous metabolites from the Human Metabolome Database (HMDB) was created by running individual standards in a high resolution impact QTOF mass spectrometer (Bruker Daltonics). All spectra were manually curated and possible structures and/or chemical formula of the fragment ions were manually deduced with the assistance of the SmartFormula 3D software. Human urine after filtering was analyzed directly by LC-QTOF-MS and MS/MS.

Results
We used ~800 human endogenous metabolites from HMDB to create a high resolution MS/MS spectral library using a QTOF-MS instrument. For each metabolite, at least 5 different collision energies were used to generate a set of standard MS/MS spectra, which provides flexibility for spectral match in a real sample analysis with different instrumental settings. We found that the high mass accuracy we defined as part of the Standard Operation Procedure (SOP) for adding spectra to the library (<2 ppm) was important for narrowing the match list and improving match specificity.
Preliminary work to apply this library for rapid identification of metabolites present in human urine was performed. Searching over 1500 MS/MS spectra from a urine sample measured by LC-QTOF-MS against the spectral library resulted in matches of many different metabolites to the standard metabolites. We are currently in the process of manually examining these matches in order to confirm their identifications. We will report the specificity and sensitivity of this approach for analyzing urine metabolome.

Conclusion
A novel high resolution MS/MS spectral library for human metabolites enabled for rapid and accurate metabolite identification in a human urine based metabolic profiling case study.

Novel Aspect
A high resolution MS/MS library of 800 human metabolites was created and applied for urine metabolomics.

WPS26-08 / Metabolite alteration in epithelial-mesenchymal transition-induced cells using GCMS-based metabolomics

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SHIMADZU Corp.

Introduction
Metabolomic analysis is one of a platform in the field of systems biology, which focuses on the dynamic changes of small molecules in response to the disturbance of the organism. Recently, non-targeted metabolomics approaches are being widely used for the discovery of new biomarkers and investigation of the carcinogenesis mechanism. Gas chromatography mass spectrometry (GCMS) is highly efficient, sensitive, and reproducible for analysis of low molecular weight metabolites. Moreover, it is quantitative, and its compound identification capabilities are superior to other separation techniques because GCMS instruments obtain mass spectra with reproducible fragmentation patterns, which allow for the creation of public databases.
In this study, we evaluate and compare the metabolite profiles of epithelial-to-mesenchymal transition-phenotypic cells to parental cells using metabolomics approach based on GCMS. Epithelial to mesenchymal transition of cancer cells plays a crucial role in cancer metastasis.

Methods
Human hepatocellular carcinoma HepG2 cells were maintained in DMEM medium (containing 10% FBS, 100 U/mL penicillin, 100 U/mL streptomycin, and 1×N-acetyl glutamine) Epithelial to mesenchymal transition was induced with BMP-9 for 3 days incubation.
Intracellular and extracellular metabolites were extracted with 80% MeOH at -80 ºC. Supernatant was collected and dried with Speedvac. For GCMS analysis, samples were derivatized by the addition of 40 µL methoxyamine hydrochloride in pyridine (for 90 min) followed by 80 µL MSTFA for trimethylsilylation of acidic protons (for 180 min). Sample materials were separated using a GCMS-TQ8030 (Shimadzu, Japan) equipped with a 30 m long, 0.25 mm i.d. Rtx5Sil-MS column with 0.25 mm 5% diphenyl film (Restek). The GC oven temperature program was held at 100ºC for 2 min and increased to 320ºC at 4ºC/min-1.

Results
Metabolites were identified using GCMS Metabolite Database Ver. 2 and NIST. About 300 ions were detected and half of them exhibited significant differences between BMP-9-treated and untreated cells.
PLS-DA S plot can be used to define the differential metabolites for distinguishing the EMT cells from original cells. Alteration of metabolic profiles was observed in a time-dependent manner of BMP-9 stimulus for epithelial-mesenchymal transition.

Conclusion
The different amount of metabolite in metastatic phenomenon is expected to clarify the alteration of metabolic pathway and correlation networks between none-metastatic and metastatic cancer cells. We hypothesize that the hallmark of metabolic network interactions contributes to diagnosis and treatment of metastatic cancer.

WPS26-09 / A Strategy to Determine Metabolite Elemental Compositions using Isotopic Fine Structure Information from High-Resolution Mass Spectrometry

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Introduction
For metabolite identification, libraries or database search strategies using the exact MS and MS/MS pattern of mass spectra of known and available compounds are well-established chemical annotations with MS. However, there are still a tremendous number of unknown metabolites other than commercially available compounds. Therefore, reference-free MS-based metabolite identification is a key requirement in metabolomic studies. We previously demonstrated quantitative relative isotopic area (RIA) measurement of an authentic compound by ultra-high resolution FT-ICR-MS that enabled reference-free determination of elemental composition of metabolites. In the present study, we evaluated the potential effectiveness of the RIA as a constraint for determining elemental composition in metabolomic research. Some challenges regarding further use of the approach are also discussed.

Methods
From the dataset of more than 100,000 known metabolites (<1,000 Da), duplicated elemental compositions were removed, and 20,258 unique elemental compositions (compounds containing CHNOPS) were obtained. Mass spectra with ultrahigh resolution and certain analytical errors were then simulated from each metabolite entry in the datasets, and subjected to the calculation of elemental compositions. The analytical errors included the mass accuracy and the ion intensity. Considering FT-ICR-MS, 0.1 to 1.0 ppm of random error was added to the theoretical m/z of each peak and the relative isotopic peak areas were multiplied by a random error of −25% to +10%. The simulation was performed 100 times using mass spectra generated for each trial.

Results
The simulation indicated that, in combination with reported constraint rules, the RIA led to unambiguous determination of the elemental compositions for more than 90% of the tested metabolites. It was noteworthy that, in positive ion mode, the process could distinguish alkali metal-adduct ions ([M+Na]+ and

[M+K]+). However, a significant degradation of the elemental compositions determination performance was observed when the method was applied to real metabolomic data (mouse liver extracts analyzed by infusion ESI), because of the influence of noise and bias on the RIA. To achieve ideal performance, as indicated in the simulation, we developed an additional method to compensate for bias on the measured ion intensities. The method improved the performance of the calculation, permitting determination of ECs for 72% of the observed peaks. The proposed method is considered a useful starting point for high-throughput identification of metabolites in metabolomic research.

Conclusion
RIA-based constraint rules are complementary to previously reported rules, enabling unambiguous determination of the elemental compositions of metabolites. The proposed method is considered a useful starting point for high-throughput identification of metabolites in metabolomic research.

Novel Aspects
We developed a calculation algorithm for direct determination of elemental compositions of metabolites using ultra-high resolution MS data.

[1] Miura, D. et al. Anal. Chem. (2010) 82, 5887-5891.
[2] Nagao, T. et al. Anal. Chem. Acta (2014) 813, 70–76.

WPS26-10 / Metabolic soft spot identification workflow: Efficient analyses, review, reporting and storage of accurate mass data using Mass-MetaSite and WebMetaBase

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Introduction
In drug discovery identification of metabolic soft spots in molecules is important to support rational design of metabolic stable compounds. A standard approach is the generation of untargeted accurate mass spectrometry data of hepatic microsomal incubations. However, evaluation and structural assignment of complex data is often time-consuming and limits the throughput of compounds. Using the software Mass-MetaSite (MMS) and the server-based application WebMetaBase (WMB) we set up a workflow for in vitro metabolic soft spot identification that enables automated and efficient data analysis, review, reporting, and storage.

Methods
Verapamil, nefazodone, losartan, cisapride, clopidrogel and ticlopidine were incubated with human and rat liver microsomes at 1 µM using a liquid handling system. Aliquots were taken after 0.3, 10 and 30 minutes. The LC-MS/MS system consisted of an Acquity UPLC coupled to an AB Sciex TripleTOF5600+. The data were acquired using TOF/information-dependent acquisition /product ion mode with multiple mass defect filter criteria for phase I metabolism. The raw data files were processed using MMS in batch processing mode and automatically uploaded to the WMB server.

Results
The workflow was defined in WMB and allowed parallel analysis for up to 7 compounds in two matrices with/without NADPH for multiple time points. Sample lists for MS data acquisition and for MMS batch processor were automatically compiled by WMB. The structure based analysis by MMS was accessible via a web browser in WMB. MMS detected and integrated parent related chromatographic peaks in the incubation samples with up to 95% agreement with manual inspection and confirmed literature data. Further, MMS was able to automatically assign and propose chemical structures of metabolites based on the MS/MS fragmentation pattern. Here, common phase I metabolites such as N-demethylation, N-dealkylation and hydroxylation were identified and the proposed structure assignments for the metabolites were in accordance to likely 75% with literature. However, most of the proposals for secondary and tertiary metabolites needed manual inspection and refinement by an expert user. With WMB an efficient review of all chromatographic and mass spectral information (fragmentation analysis) was possible by visualization and matching of metabolites across samples, time points and conditions used in the experiment.

Conclusion
Assay cycle time is a crucial factor to inform medicinal chemistry on metabolic soft spots of high clearance compounds. Basic information - rather than having definitive metabolite ID data - on the major metabolites formed in microsomes is in many cases sufficient. For this purpose the workflow based on MMS and WMB proved to be a powerful tool.

Novel Aspect
A new approach for high-throughput metabolite identification by automatic peak detection and structure assignment in combination with a server application for efficient data review and reporting.

WPS26-11 / Ion-Mobility-Derived Collision Cross Section as an Orthogonal Measure for Metabolomic Phenotyping

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Introduction
Metabolomic phenotyping is a rapidly-evolving analytical approach in life and health sciences. The structural elucidation of the metabolites of interest remains a major analytical challenge in the metabolomic phenotyping. Here, we investigate the use of ion mobility as a tool to aid metabolite identification. Ion mobility allows for the measurement of the rotationally-averaged collision cross-section (CCS), which gives information about the ionic shape of a molecule in the gas phase. The CCS value is a unique physicochemical property of a molecule and can be used as an orthogonal molecular descriptor in addition to retention time and mass-to-charge ratio (m/z) to improve the identification confidence and the reproducibility of analysis.

Methods
Multiple travelling-wave ion mobility mass spectrometers (TW-IM-MS) located in independent laboratories were used to derive CCS information for a variety of low molecular weight metabolites. Chromatographic separation was achieved using an ACQUITY UPLC system based on HILIC (polar metabolites) and reversed phase (lipids) UPLC. CCS values obtained in nitrogen were experimentally determined using polyalanine oligomers as the TW mobility calibrant. Theoretical collisional cross-sections were calculated using Mobcal after energy minimization. Data analysis and processing was performed using novel bioinformatics solutions.

Results
We created a CCS database containing over 300 common metabolites and lipids using TW-IM-MS. CCS measurements

were highly reproducible on instruments located in multiple independent laboratories (RSD <5% for 99%). We also determined the reproducibility of CCS measurements in various biological matrices including urine, plasma, platelets, and red blood cells, using UPLC coupled with TW-IM-MS. The mean RSD was <2% for 97% of the CCS values, compared to 80% of retention times. Finally, as proof of concept, we used UPLC/TW-IM-MS to compare the cellular metabolome of epithelial and mesenchymal cells, an in vitro model used to study cancer development. CCS data were filtered and matched against the CCS database to confirm the identity of key metabolites potentially involved in cancer.

Conclusions

Experimentally-determined and computationally-derived CCS values were used as orthogonal analytical parameters in combination with retention time and accurate mass information to improve identification confidence and reproducibility of analysis.

Novel Aspect

Adding CCS data to searchable databases for metabolomic phenotyping improves the identification confidence and the reproducibility of analysis compared to traditional analytical approaches.

WPS26-12 / Untargeted analysis of reactive aldehydes produced by lipid peroxidation using selective derivatisation and detection by LC/HRMS.

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Diets rich in red or processed meat were recently associated with an elevated risk of colorectal cancer (CRC) in some epidemiological studies. One of the major hypotheses involves dietary heme iron which could promote CRC by favouring lipid peroxidation. In particular, peroxidation of polyunsaturated fatty acids (PUFA) can lead to several decomposition products among which aldehydes and more specifically γ-hydroxy-α,β-unsaturated aldehydes have received attention due to their toxicity. Aldehydes such as 2(E)-4-hydroxynonenal (HNE) and 2(E)-4-hydroxy-hexenal (HHE) formed from respectively ω6- and ω3-PUFA, are known to be cytotoxic and genotoxic and may play a promoting role in CRC development, by selecting precancerous cells.

Those aldehydes are specific of their precursor fatty acids, but they are not fully identified and other lipid peroxidation toxic aldehydes may also be formed in the intestinal lumen and may be linked with CRC. In this context, we present in this work the development of a mass spectrometry based strategy for a non-targeted aldehyde analysis to provide a qualitative and semi-quantitative image of lipid peroxidation in the intestinal lumen.

Due to the volatile and reactive characteristics of aldehydes, an in-situ derivatization was considered. For a specific detection of aldehyde related signals by mass spectrometry, the introduction of a bromine atom was also considered. After preliminary trials, a brominated probe reacting under aqueous buffered, almost neutral conditions (namely 1-[(Aminooxy)methyl]-2-bromobenzene hydrochloride (BBHA)) was selected for derivatisation.

Our method was then applied to the analysis of fecal waters prepared from rats fed four variously prooxidative diets (i.e. different precursor fatty acid profiles with added heme iron). After solid phase extraction of derivatised aldehydes, analyses were carried out by positive electrospray ionization on a LTQ-Orbitrap mass spectrometer. Signals of brominated compounds were filtered and aldehydes were identified by MS/MS and comparison with home-made synthetic standards when available. This allowed a sensitive and selective detection of aldehydes on the basis of the 79Br and 81Br isotopomers of their BBHA derivatives (isotopic pattern, exact mass, mass defect). The method performances (repeatability, sensitivity, selectivity) were then assessed using several synthesized standard aldehyde BBHA derivatives.

Results will be presented showing that (i) several expected derivatised aldehydes were detected (e.g. linear saturated aldehydes, alkenals, hydroxyl and keto-alkenals such as 4-HHE and/or 4-HNE), (ii) the method could be used in a semi-quantitative way and (iii) several other brominated signals, corresponding to potentially unknown aldehydes were also detected. Work is currently under progress for the structural identification of these potentially new aldehydes, which may be linked to CRC promoting role of red meat rich diets.

WPS26-13 / Serum 25-Hydroxyvitamin D Status of Healthy Adults: Results from the Karlsruhe Metabolomics and Nutrition Study (KarMeN)

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Max Rubner-Institut

Introduction

The aim of the KarMeN study was to investigate associations between nutrition, physical activity and the human metabolome. 301 healthy adults were thoroughly characterized (medical history, body composition, clinical parameters, nutrition, physical activity and fitness). Metabolome analysis in plasma and urine was performed by NMR, GCxGC and targeted LC-MS. Food consumption and nutrient intake was estimated by two 24 hour recalls with a time lag of two weeks. However, data about the vitamin D status cannot be obtained by this way, since the majority of 25-hydroxyvitamin D (25OH-D) is synthesized in vivo in the skin exposed to sunlight and only a minor part is ingested via food. Due to the central role of this hormonally active compound in the metabolism (bone mineralization, immune response) it is essential to directly quantify the vitamin D status.

Methods

Accepted parameter for the vitamin D level is 25OH-D2/D3 in serum. The biological significance of isomers is unclear, but there is evidence that the 3-epimers are partially physiologically active. A quantitative UPLC-MRM method for 25OH-D2/D3 and epi-25OH-D3 in serum was established in course of the KarMeN study using a PFP column with a methanol gradient. Sample preparation was performed by protein precipitation plus extraction with hexane, and matrix-matched calibrators and controls were used together with d6-25OH-D3 as internal standard.

Results

With the established method the epimers of 25OH-D can be accurately quantified down to 5 nmol/L. The upper limit of the linear range is 166 nmol/L for 25OH-D3 and 72 nmol/L for the other isomers. Precision of serum controls is 5-7% (batch, day-to-day), the bias is below 3% (accuracy) and the analytical recovery including sample preparation is 72-81%.

The median of 51.1 nmol/L for 25OH-D3 is slightly above the concentration of 50 nmol/L recommended by the German Nutrition Society, 47% are below the cutoff. There is no significant correlation with age or sex. Only 21 samples (7%) showed 25OH-D2 values above the LLOQ, and epi-25OH-D3 was detected in 47 samples (15%) above the LLOQ. In these 47 samples epi-25OH-D3 correlates with 25OH-D3. The expected seasonal influence (sun exposure) for 25OH-D3 follows a sinus trend and can be compensated by a mathematical correction function. This may be important for detection of possible associations between vitamin D status and other metabolites, which otherwise may be masked by the seasonal fluctuation.

Conclusions
The vitamin D status of a thoroughly characterized study population of healthy adults has been accurately quantified. This allows further examination of the metabolomics data for associations with parameters such as nutrition behavior, physical activity and fitness, and other metabolites. The data show that about 50% of the study participants do not reach the recommended vitamin D serum concentration.

Novel Aspect
Quantification of the vitamin D status of a healthy study population in the context of nutrition, physical activity and metabolomics

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WPS26-14 / Negative ion electrospray tandem mass spectrometry of prenylated fungal metabolites from Suillus species (Basidiomycetes)

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Introduction
Meroterpenoid quinones are widespread in various living organisms and play an important role in several life processes. The basidiomycetous genus Suillus (Boletales) is known for the occurrence of a series of prenylated phenols and boviquinones. The fragmentation behaviour of such compounds obtained by negative ion electrospray (ESI) tandem mass spectrometry is discussed. Furthermore, crude extracts from basidiocarps of four different Suillus species were investigated by using UPLC/ESI-MS and direct infusion ESI-FTICR-MS. The obtained mass spectral data were used for a classification of Suillus spp. like S. bovinus (L.) Roussel, S. granulatus (L.) Roussel, S. tridentinus (Bres.) Singerand and S. variegatus (Sw.) Richon & Rozeby a principal component analysis (PCA).

Methods
The UPLC/ negative ion ESI-ion trap mass spectra were recorded on a LCQ Deca XP MAX (Thermo Scientific) coupled with a Waters ACQUITY UPLC system. High-resolution negative ion ESI mass spectra were obtained from a Bruker Apex III FTICR mass spectrometer (Bruker Daltonics, Billerica, USA) equipped with an Infinity™ cell and a 7.0 T superconducting magnet (Bruker, Karlsruhe, Germany). The elemental composition of the fragment ions were approved by a LTQ Orbitrap Velos Pro (Thermo Scientific) measurements under negative ion ESI conditions.

Results
The fragmentation behaviour of the boviquinones is mainly characterized by consecutive losses of isoprene units leading to a benzyl radical anion at m/z 152. In addition to the loss of the whole side chain, the characteristic neutral losses of 68 and 69 amu, respectively, from the oligoprenyl side chain give information with respect to the isoprenoid and aromatic system both for prenylated phenols and benzoquinones. So, the mass spectral behaviour under negative ion electrospray conditions of prenylated benzoquinones and phenols provides useful information for their structural features and allows a characterization and identification of these compounds.

Conclusions
Prenylated benzoquinones and phenols exhibit a characteristic fragmentation pattern under negative ion ESI conditions.
The mass spectral decomposition of both prenylated phenols and boviquinones is mainly characterized by typical successive losses of the isoprene units.
The loss of the isoprenoid side chain of the boviquinones leads to a common radical anion comprising the quinoid moiety.
A UPLC/ESI-MS based PCA analysis clearly showed that the metabolite profiles can be used not only for the identification and classification of such fungi, but also as a sophisticated and powerful tool for chemotaxonomical approaches of fungi.

Novel Aspect
characterization of prenylated fungal metabolites by liquid chromatography / negative ion electrospray tandem mass spectrometry and classification of Suillus species by PCA analysis

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WPS26-15 / Complimentary LC- and GC-Mass Spectrometry Techniques Provide Broader Coverage of the Metabolome

Jean-Baptiste Vincendet, Neil Devenport
AB SCIEX

Introduction
The metabolome is difficult to characterize; there is a wide dynamic range of concentrations of metabolites, which are chemically and structurally diverse with various polarities and sizes and creating a single analytical method for all of these components is challenging. Here we highlight the value added in using both GCMS and LCMS analyses for untargeted metabolomics as an "integrative" workflow by interrogating a well-established rat model for diabetes, obesity and cardiovascular disease effects.

Methods
Using a HPLC system and a high strength silica column (Acquity HSS T3 1.8µm, 2.1 x 100mm @ 60oC), polar metabolites were separated using a 5ul injection volume at a flow rate of 600 µL/min. Full scan TOF MS and IDA MSMS data was acquired on a TripleTOF® 5600+ system (AB SCIEX). Serum from the Zucker rat model was taken from 7-9 week old lean (n = 10), fatty (n = 10) and obese rats (n = 10).

Preliminary Data
Interrogating the raw TIC data, it was observed that there were many lipids changes amongst the three groups of rats (lean, fatty and obese) therefore LipidView software was used to identify the lipid classes and molecular species before undertaking any statistical data analysis. Specifically changes in the glycerolipids, glycerophospholipids, sphingolipids, cholesterol esters and carnitines were detected. Significant changes amongst the bile acids were also detected between all three groups of samples. Large differences in the signal intensities for example of the short chain acyl carnitine 4:0 where there are very low levels in the lean rats compared to the other two groups indicating an increase in the catabolism of BCAAs in diabetes or obesity.
Using LCMS data alone we were only able to generate statistical models using discriminant analyses. However combining the LC-MS and the GCMS data into Expressionist MSX software from Genedata we were able to generate PCA models with clear differences between the samples groups using non-discriminant analysis. We can then start to build a correlation network and group metabolites together with similar profiles not only by analyte group but by sample group and also by analysis type.
From our results we can observe which metabolites were more amenable to GC and LC and where there was an overlap. The overlap gave an extra level of confidence to our biological interpretation and validated our results. We also observed that whilst GCMS addresses primary metabolites, LCMS most readily addressed secondary metabolites and in turn each primary metabolite affects multiple secondary metabolites.

Novel Aspect
Use of Complimentary LC- and GC-Mass Spectrometry Techniques Provide Broader Coverage of the Metabolome.

WPS26-16 / Green or black what's your favorite? Fast biomarker detection and identification in green and black tea using flow injection and FT-ICR mass spectrometry

Matthias Witt, Aiko Barsch
Bruker Daltonics GmbH

Introduction

In this proof of concept study we analysed different mixtures of black and green teas. Possible health benefits have been hypothesised for both types of teas and a deeper understanding of potential health promoting effects as well as an improvement in quality and taste is of high interest in academia as well as food industry. Metabolic profiling based on LC-MS is considered an established method to pinpoint and identify characteristic compounds, although several bottlenecks remain. LC-MS measurements typically take 10-20 minute per sample, and this can become a challenge if large numbers of samples should be analysed – like in food quality control.

Methods

Here a series of tea metabolite extracts was analysed by direct infusion Electrospray Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI-FT-ICR) with ultrahigh resolution resulting in an about 10x decrease in sample analysis time compared to standard LC-MS setups. Statistical analysis was used to determine compounds unique to each sample, black or green tea. Therefore, up- and down regulation of specific compounds can be recognized with this method. The reproducibility of the results was tested with repetitive measurements and analysis of green and black tea mixtures.

Results

The identification of target compounds is a further bottleneck in metabolomics research. Mass spectra acquired by ESI-FT-ICR enable to calculate theoretical molecular formula based on the accurate mass values determined. Because elemental composition calculation can return multiple hits even within 0.5 ppm mass accuracy, ultrahigh resolution mass spectrometry is used to determine isotopic fine structure of the unknown compounds. The isotopic fine structure of each molecular formula candidate is compared against the experimental isotopic fine structure using a novel software algorithm. This enabled to literally "read out" the correct elemental composition for the target compounds. Based on this workflow several characteristic metabolites for black and green tea extracts were identified.

Conclusions

In this proof of concept study flow injection combined with ultrahigh resolving FT-ICR-MS detection enabled a significant increase in sample throughput for profiling of complex metabolite mixtures, here tea extracts, without sacrificing the capability for detection and identification of marker compounds. The MS resolving power of 600.000 at m/z 400 enabled unambiguous molecular formula generation by making use of isotopic fine structure information, a novel capability not accessible before by different MS platforms with lower mass resolution.

Novel Aspect

This workflow addresses three of the major requirements in current metabolomics research: sample throughput, quickly detecting potential biomarkers and compound identification by making use of isotopic fine structure information.

WPS26-17 / Derivatization and Enantioselective Separation of Sugar Metabolites

Roland Wohlgemuth, Bernhard Schönenberger, Rudi Köhling, Paul Rodwell
Sigma-Aldrich

Chiral D-and L-glyceraldehyde phosphates have various key roles in metabolic pathways. We have recently described the preparation of enantiomeric glyceraldehyde 3-phosphates [1]. The analysis of chiral D- and L-glyceraldehyde phosphates is not only important for the determination of its enantiomeric purity, but also for stereochemical characterization of relevant metabolic pathways. Chromatographic separation of the corresponding D- and L-forms of glyceraldehyde 3-phosphate or glyceraldehyde 2-phosphate has not been described so far, despite much effort. In our approach we have chosen to explore the combination of aldehyde derivatization, HPLC-separation on a chiral stationary phase and detection by mass spectrometry. The rationale behind this approach was that the derivatization of a chiral molecule may change its interactions with a chiral stationary phase and may therefore offer new opportunities for the separation of the enantiomers. 4-(Dialkylamino) benzoyl hydrazides, e.g. 4-(diethylaminomethyl)-benzhydrazide, are suitable derivatization reagents for aldehydes and ketones. This simple and short derivatization reaction made glyceraldehyde-3-phosphate detectable with high sensitivity by UV and MS. Additionally the two enantiomers are well separated on a zwitterionic chiral stationary phase. This reagent is also suitable for the derivatization of glyceraldehydes and other sugars or sugar metabolites with carbonyl groups.

[1] D.Gauss, B.Schönenberger, R.Wohlgemuth, Carbohydrate Research, 389, 18-24 (2014).

WPS26-18 / LC-MS Analysis of Gluconate dehydratase-catalyzed Formation of KDG

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2-Keto-3-deoxy-sugar acids are key intermediates in central metabolic pathways like the Entner-Doudoroff (ED) type hexose degradation pathways as well as the Dahms and the Weimberg pathways for pentose degradation described from all three domains of life, i.e. Archaea, Bacteria and Eukarya. These metabolites are also constituents of bacterial polysaccharides, lipopolysaccharides and cell wall components. A direct one-step route from the corresponding sugar acids has been developed in the biocatalytic synthesis of 2-keto-3-deoxy-D-gluconate (KDG) from D-gluconate using recombinant gluconate dehydratase [1]. An essential prerequisite thereby was the development of a quantitative and qualitative LC-MS analysis method which could detect simultaneously the product KDG and the starting material D-gluconate. The analysis of the samples during the reaction time were performed on a DionexUltimate 3000 RSLC connected to a Dionex CoronaCAD and Bruker microTOF-Q II via a static flow-splitting device (LC Packings) at a ratio of 1:5 (MS:CoronaCAD). The efficient separation of the polar metabolites gluconic acid and KDG has been achieved by ion-pair chromatography using 5 mM dihexylamine acetate in the aqueous mobile phase at pH 7 (A) and acetonitrile as organic mobile phase (B) on a Supelco Ascentis Express C8 column (4.6 x 100, 2.7 µm) and KDG. The response linearity of gluconate has been analyzed and the curve fit result (R2=0.999) indicates a direct proportional relationship between the peak area (MS) and the concentration in the reaction solution. The CAD chromatograms and the extracted ion chromatograms (EIC) of both gluconic acid and KDG were integrated and the peak areas were plotted against the reaction in

hours. At t=0 h an additional sample of the reaction solution was taken before the enzyme was added and then the reaction was started, which follows the scheme of a simple 1st order reaction.

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WPS26-19 / UFLC-MS based metabolomic profiling reveals oxidative stress related early biological effects induced by ambient air pollutants exposure in general population

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Introduction

Air pollution has caused increasing public concern in the past decades. Extensive epidemiologic studies have focused on and established the association of late-stage end points of morbidity and mortality with air pollution exposure. However, it is worth paying attention to the understanding of the early molecular events in the exposure–disease continuum to provide valuable information for the intervening and redirecting of the outcome. Metabolomics offers great potential to give a global picture of the pathways affected by pollutants exposure and to greatly increase our understanding of their effects on the health of organisms. The aim of this study is to assess the personal internal exposure level of air pollutants and find early metabolomic markers of air pollution exposure in general population.

Methods

A total of 244 participants were divided into two groups. Exposed group included 148 participants from a polluted area subjected to coking industry related air pollution and control group included 96 participants from a national ecological demonstration area. Urine samples of all participants were collected and stored at -80°C until analysis. The urinary concentrations of nine monohydroxy metabolites of polycyclic aromatic hydrocarbons (PAHs) were determined by LC-MS/MS methods for personal exposure assessment. The metabolomic analysis of urine samples was performed on an ultra fast liquid chromatography (UFLC) system hyphenated to a TripleTOF 5600 mass spectrometer operating in positive and negative ion mode.

Results

Personal exposure assessment results showed that exposed participants had a significantly higher PAHs burden compared to controls. The increased exposure of PAHs caused a series of changes of levels of endogenous metabolites among exposed population. Urinary metabolomic analysis identified 18 metabolites that had a significantly different urinary level between the exposed and control group. Most of the metabolites, including 2 amino acids, 3 organic acids, 1 glucuronide conjugate, 11 acylcarnitines were significantly increased in the exposed participants. Uric acid was the only metabolite that significantly decreased in the exposed population. The perturbation of these metabolites reflected the oxidative stress-related early biological effects induced by ambient pollutants exposure, including the depletion of antioxidants, accelerated breakdown of muscle proteins, increased lipid peroxidation, dysfunction of medium-chain acyl-CoA dehydrogenase and the increased detoxification, among the exposed population.

Conclusions

The results revealed the overall early biological effects induced by ambient pollutants exposure in human, supporting oxidative stress as the underlying mechanism found in vitro and in vivo animal models.

Novel aspect

The study unveiled the oxidative stress related early biological effects caused by ambient pollutants exposure and highlighted the potential of metabolomics as a promising tool for environmental health risk assessment.

WPS26-20 / Nontargeted Metabolite Profiling Approach to Investigate the Role of Reactive Oxygen Species and Ethylene in Compatible Plant-Pathogen Interaction

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Introduction

Reactive oxygen species (ROS) signaling is linked with signaling networks in plant-pathogen interaction, activating plant immune response to pathogen (=incompatible response) or plant cell death (=compatible response). A biphasic production of ROS and ethylene is reported in compatible response between tobacco and a pathogen, Phytophthora parasitica var. nicotianae (Ppn). The levels of ethylene and ROS produced are associated with disease severity. ROS and ethylene might influence the susceptibility of plant to pathogen, changing the levels of metabolite related to disease resistance or susceptibility.

Methods

Nontargeted metabolite profiling using UPLC-Q-TOF/MS was applied to investigate the metabolic responses at the first (1h) and second (48 h) phases of ROS production in wild type and ethylene signaling-impaired transgenic tobacco plants (EIN3-AS). Ppn-responsive mass ions were selected using independent sample t-tests and multivariate statistical analysis. The selected mass ions were identified by analysis of their MS/MS fragmentation patterns referring to metabolome databases and relevant literature.

Results

Phenolic amino acids, phenylpropanoids hydroxycinnamic acid amides, linoleic acid, linolenic acid, lysophospholipids, glycoglycerolipids, and oxidized phospholipids were identified as changed by Ppn inoculation. We also compared Ppn-induced metabolic profiles from wild type (WT) and Ein3-AS transgenic plants. Two-way hierarchical clustering analysis revealed that phenylpropanoid-polyamine conjugates and their intermediates were present at lower levels in Ein3-AS transgenic plants during Ppn-interaction than in WT, whereas galactolipid and oxidized free fatty acid levels were higher in Ein3-AS mutant. Application of lyso phosphatidylcholine and phytosphingosine which were increased by Ppn revealed that these molecules may be act as death signal by Ppn penetration, influencing the production of ROS.

Conclusions

Using metabolomics approaches, we could get some information for the understanding of metabolic response related to the action of ROS and ethylene produced during tobacco-Ppn interaction. Lysophosphatidylcholine and phytosphingosine might act as signal molecule related to the ROS production and cell death.

Novel Aspect

A function of ROS and ethylene signaling was revealed by nontargeted metabolite profiling during plant defense response to susceptible pathogen.

WPS26-21 / Integrated metabolomics for urine biomarker discovery of esophageal carcinoma

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Introduction
Metabolomics is the comprehensive analyticalapproach for the study of all lowmolecular metabolites present in a given biological systemof interest via noninvasive analyses of biofluids. In this study, apatients ns lyod sample ofhe ORs and non-ORs. nd non-ORs, metabolomics studyusing urine from esophageal carcinoma (EC) patients (before and after treatment) and healthy controls, was originally carried out by LC-MS in conjunction with multivariate data analysis to discriminate the global urine profiles of EC patientsand healthy controls. To further validatethe reliability of these potential biomarkers, an independentvalidation was performed by using the selectedreaction monitoring (SRM) based targeted approach. The purpose of this study is to establish an integrated metabolomics method to discover urine potential diagnostic and therapeutic biomarkers for EC by LC-MS.

Methods
62 EC patients and 62 healthy volunteers from the Cancer Institute and Hospital of the Chinese Academy of Medical Sciences (Beijing, China) were enrolled in this study. All the patients were diagnosed with histopathology examination. The urine sample was prepared by creatinine value calibrated dilution.Chromatographic separation was performed on a Zorbax Aq-C18column (1.8 μm, 10 cm × 2.1 mm; Agilent, USA), using an Agilent 1200 Series rapid resolution liquid chromatography system. MS experiments were performed on a Q-TOF equipped with ESI sources. Raw LC–MS data files were converted into mzData format using Wiff to mzData utility and directly processed by open-source XCMS package to carry out peak discrimination, filtering and alignment. The data matrices were imported into SIMCA-P for further PCA, PLS-DA and OPLS-DA analysis.

Results
The feasibility of using urine metabolomics for the diagnosis and monitoring therapeutic responses and predictingoutcomesof EC has been evaluated. The LC-MS/MS have been carried out to identify the potential biomarkers that made great contribution to the discrimination. As a result,83 potential diagnostic biomarkers and 43 potential therapeutic biomarkers for EC were screened. Furthermore, 49 metabolites and 26 metabolites were selected by independent validationtest based on LC-MS/MS targeted metabolomics. Some of these metabolites were identified. In addition, in-depth ROC analysis was performedto characterize these putative biomarkers and a potential diagnostic biomarker panel was found.

Conclusion
The present study develops an integrated metabolomics to discoverpotential biomarkers of EC. It is possible that the current biomarkers are not unique to EC but just the result of any malignancy disease. In further, we will apply a larger urine sample sets toverify these conclusions. In addition, we will establish the quantitative methods of the potential biomarkers and elucidate the related pathophysiology of EC.

Novel aspect
This study established a LC-MS-based integrated metabolomics method to discover the potential diagnostic and therapeutic biomarkers of EC in urine.

WPS26-22 / Metabolite Labeling with Fluorinated Alkyl Chloroformate and Concurrent Liquid Liquid Microextraction for Targeted GC-MS and LC-MS Metabolomics

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Introduction
Protic metabolites, the most common targets in current GC-MS based metabolomics, are most commonly subjected to prior oximation-silylation before analysis. In this study, we investigated a novel approach employing fluorinated alkyl chloroformate (FCF) labeling and concurrent liquid liquid microextraction (LLME) for targeted metabolomic analysis.

Methods
We systematically investigated reaction products and analytical properties of more than 200 metabolites involved in central and steroid metabolism. The derivatives of amino acids, organic acids, steroids, sterols and tocopherols with trifluoroethyl chloroformate (TFECF) or heptafluorobutyl chloroformate (HFBCF) were separated on achiral/chiral GC columns and reversed phase (RP) HPLC columns and were detected by EI(PICI)-MS and ESI-MS(/MS), respectively. By means of the obtained EI and ESI spectra, the particular structures were unequivocally assigned. The novel approach was examined in GC-MS analysis of more than 150 metabolites in human urine, in chiral GC-MS analysis of 35 D,L-amino acids and GC-MS of 16 steroids in human serum. Complementarily, N-protic metabolites which were not amenable for robust GC-MS analysis, were analyzed by RP-LC-MS(/MS).

Results
Predictable structures were obtained in most cases (> 82 %). Only a minor part of metabolites provided more distinct reaction products (ca 10 %, typically 2 - 4 entities). Some highly N-protic or non-stable metabolites, poorly chromatographed on capillary GC columns or not at all (about 8 % of the set), were measured by RP-LC-MS(/MS) analysis. The labeling of steroids with FCFs proceeds efficiently under anhydrous conditions and is easily combined with LLME between an isooctane – acetonitrile phase. Majority of the examined FCF labeled metabolites gave well defined, interpretable EI spectra or ESI tandem mass spectra with diagnostic fragment ions.
The developed protocols were applied to GC-MS analysis of 100 morning urines obtained from healthy patients where 108 metabolites relevant to the major human metabolite pathways were clearly detected in a 40 μl sample aliquot. The method was validated with special attention to potential biomarker candidates of metabolic disorders. Amino acids labeled with HFBCF are also amenable to chiral GC-MS profiling. Except D,L-arginine, D,L-cystine (not eluted) and D,L-proline (not separated), 35 D,L enantiomeric amino acid pairs are well separated on a 25 m Chirasil-ValTM capillary column. The new steroid labeling method was successfully applied to GC-MS profiling in 40 woman sera and amniotic fluids, the results are well-comparable with those reported by other authors.

Conclusions
The results suggest that the described metabolite FCF labeling and concurrent LLME have been promising tools fox expanding metabolite coverage in GC-MS and LC-MS based metabolomics and towards the chiral metabolite profiling.

Novel aspect
New, efficient metabolite profiling strategy for targeted non-chiral & chiral MS based metabolomics.

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WPS26-23 / Label-free and standard-free quantitative metabolomics approach by using liquid chromatography mass spectrometry

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Introduction

Quantification of compounds in biological samples plays an indispensable role in metabolomic research. However, obtaining the standard compounds of all metabolites in samples is nearly impossible and the isotope-labeled method has the limitations of time-consuming, complex spectrum and incomplete label. To address such a critical problem, a label-free and standard-free quantitative metabolomics approach has been established preliminarily on account of 47 discriminated metabolites detected previously in plasma of lung cancer.

Methods

EDTA-K2 anticoagulated plasma samples stored at -80 °C were thawed at 4 °C and vortexed on ice before use. Each 50μL aliquot plasma was deprototeinized by adding 150μL dilution (10μL methanol and 140μL acetonitrile with internal standard) stored at 4°C. Then took supernatant after vortexed for 5min at 2500rpm and centrifuged at 10000 rpm at 4°C for 5 min. For the pooled plasma sample that was used to prepare the mixed calibration curves, 100μL dilution was added after separating 100μL supernatant. Then diluted sequentially as before to obtain the 5, 2.5, 1.25, 0.62, 0.31, 0.16, 0.08, 0.04, 0.02, 0.01, 0.005, 0.0025 relative plasma concentration and injected the same volume for LC-MS analysis in MRM scan mode. Thus we can acquire analytes' calibration curves themselves. Then this method has been applied to analyze the plasma samples from 20 lung cancer patients and 20 normal controls. At the same time, the conventional quantitative method has been carried out to evaluate the feasibility of the new method.Acylcarnitines-free plasma spiked with IS and acylcarnitine standards at 12 concentration levels were treated in triplicate to establish calibration curves.

Results

The mixed calibration curves were constructed from the ratios of the peak areas to that of the area of IS against the dilution fold and fitted by linear regression with 1/x weighting. The linearity of 40 analytes were favorable (r>0.99). When analysis the plasma samples of the lung cancer patients and healthy controls using this approach, 21 metabolites have been observed to have significant differences. Compared with the conventional method, the change fold of discriminated metabolites and ability of differentiation detecting were similar.

Conclusions

The label-free and standard-free quantification metabolomics approach can be used to quantify the metabolites relatively even for the unknown metabolites. So, it can be used after untargeted metabolomics method to further validate the potential biomarkers quantitatively and accurately. More important is that it might replace conventional metabolomics method to quantify the whole metabolome of biological samples extensively.

Novel Aspect

The new established metabolomics quantification method get rid of the complexity and the incompleteness by using standard and isotope-labeled compounds.

WPS26-24 / Study of glucose metabolism in several prostate cancer and normal cell lines using 13C metabolic flux analysis and GC-MS

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^{13}C metabolic flux analysis (^{13}C-MFA) is considered as an accurate and reliable method for measuring intracellular metabolic fluxes. Fluxes provide valuable information on cellular physiology that can be applied to engineering metabolic, regulatory pathways improvement of cellular phenotypes and to understand mechanisms of cancer disease. ^{13}C-MFA consists in three principal steps: i) the design and execution of isotopic labeling experiments ii) the measurement of the isotopic labeling by mass spectrometry iii) the estimation of metabolic fluxes.

The developed approach is based on the addition of ^{13}C-labelled glucose (universally labelled or labelled with one ^{13}C atom to a cell culture medium of different prostate cancer cell lines. Then, intracellular metabolites are extracted with MeOH at -80°C, derivatized with N-Methyl-N-tert-butyldimethylsilyltrifluor oacetamide and analyzed by GC-MS to measure their isotopic distribution. Finally multiple linear regression is applied to determine the molar fraction of the different isotopic forms and their isotopic enrichment for the different intracellular metabolites detected (28 compounds including small organic acids, amino acids, and fatty acids).

The validation of the methodology was carried out with the comparison of the results obtained by GC-MS with those obtained by GC-IRMS. Then the methodology was applied to the analysis of six cell lines (androgen dependent and androgen independent) of prostate cancer and one normal prostate cell line. We obtained a significantly different isotopic enrichment in several of the studied metabolites, in the different cell lines. Also differences between androgen-dependent and androgen-independent cell lines were observed indicating a different metabolic behaviour as a function of the degree of tumor progression.

The proposed methodology provides accurate measurement of the isotopic enrichment of glucose metabolites in cancer cell lines allowing the measurement of intracellular metabolic fluxes with improved precision.

WPS26-25 / Detection of betulin and its derivatives from birch

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Introduction

Betulin and lupeol are triterpenes and plant metabolites commonly occurring in the nature, e.g. in birch trees. They are known to have medicinal properties like antimicrobial and anti-inflammatory effects. Betulin and its derivatives have non-polar lupane structure, and they also have low solubility in both polar and non-polar solvents. These qualities complicate their extraction and analysis from birch. Here, betulin, lupeol and other betulin derivatives were screened directly from birch pieces with ambient mass spectrometry (MS) technique desorption atmospheric pressure photoionization (DAPPI). DAPPI allows the analysis of compounds directly from solid surfaces and thus avoids the difficulties involved in the extraction of triterpenes. DAPPI ionization mechanisms involve reactions similar to those in atmospheric pressure photoionization (APPI); e.g. charge exchange and proton transfer reactions, which enable the ionization of both polar and nonpolar compounds.

Methods
MS method optimization was done with direct infusion µAPPI system for betulin, lupeol, betulinic acid, betulonic acid, allobetulin and allobetulone. The authentic birch samples were collected from three cloned birch trees. Samples were taken from four layers of each birch tree trunk and from the three different heights. In DAPPI the birch samples were exposed to hot toluene vapor and nebulizer gas (N2, 180 mL/min), which caused thermal desorption of analytes from the sample surface. The gas-phase analytes were ionized through photon-initiated reactions mediated by the dopant (toluene). The ions were transferred through a transfer capillary into the MS. Data was analysed with multivariate methods.

Results
Studied triterpenes were efficiently ionized in DAPPI and they formed molecular ions or protonated molecules depending on the compound. All four tree trunk layers showed different chemical patterns. Also, the chemical pattern changed according to the height of the tree. Identified compounds were e.g. betulin, lupeol and betulinic acid. Outer layer (bark) of the trunk showed the highest intensity for betulin, but also the lupeol signal was abundant. Both were detected as molecular ions. The betulin intensity decreased when monitoring the inner layers of the tree so that in the fourth layer there was no trace of betulin. The intensity of betulin in the bark decreased with the height of the tree.

Conclusions
Photoionization based detection method suites well for the analysis of betulin and its derivatives. DAPPI was successfully used for chemical fingerprinting of vaporized compounds present in the birch.

Novel Aspect
This is the first time that DAPPI is applied in the analysis of non-polar triterpenes from tree trunk.

WPS26-26 / Enhanced Pharmaceutical Stability Testing Using On-line Electrochemical Reactions Up-front MS

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Introduction
Stability testing of active pharmaceutical ingredients (API) and finished pharmaceutical products (FPP) is of prime importance. It provides evidence of how the quality of such product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. Purposeful degradation experiments using chemical and thermal methods are widely applied to aid understanding the stability and degradation of API and FPP. As a result of stability testing a shelf-life can be established and storage conditions can be recommended.

Method
Many pharmaceutical degradation reactions occur by REDOX mechanisms. With the recent availability of flow-through electrochemical (EC) reaction cells by Antec, a new on-line Electrochemistry/Mass Spectrometry (EC/MS) method is now available, providing the means to identify and quantify degradation product under varied experimental conditions. Furthermore, EC/MS also has utility to study drug-excipient interactions and can be scaled up and coupled to liquid chromatography to separate and isolate degradation products for further study, e.g., NMR.

Results
In this lecture results will be shown using EC/MS and EC/LC/MS/MS to study the electrochemical degradation of active pharmaceutical compounds and excipients. Furthermore, data will be shown on the influence of antioxidants and their stabilizing capacity on the API based on direct EC/MS measurements.

Novel Aspect
Use of an electrochemical reaction cell on-line with MS for faster and better Pharmaceutical Stability Testing

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WPS26-27 / Analysis of Isoprenoid Pathway Metabolites by LC-MS

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Sigma-Aldrich

Introduction
Isoprenoid pathway metabolites such as the isoprenoidphosphates and isoprenoid-pyrophosphates are central metabolites leading to sterols, dolichols, ubiquinones, prenylated natural products and proteins. The development of new methodologies for the preparation as well as the separation and quantitation of pure phosphorylated isoprenoid metabolites is key for detailed investigations of these pathways on a molecular level. It is therefore of much interest to develop new high-performance separation methods which are able to determine the whole range of isoprenoid-(pyro)phosphates.

Methods
The analysis of a whole range of synthetic metabolites of isoprenoid pathways by LC-MS methods will be presented.

Results & Conclusions
New HLPC-MS methods for the simultaneous analysis of the stereoisomeric dimethylallyl- and isopentenyl-(pyro)phosphates respectively have been established using the cyclo-dextrine-based stationary phase Supelco Cyclobond 2000 and a buffer/acetonitrile eluent in HILIC mode. Whereas the separation of these polar single unit isoprenoid-(pyro)-phosphates is based on selective ionic interactions on a cyclodextrin-based stationary phase, the separation of the larger isoprenoid-(pyro) phosphates has been achieved with IPC-UHPLC-MS using ion-pair chromatography on a Supelco Ascentis Express C8 column and dihexylamine acetate. The resolution of this IPC has been improved significantly with Fused Core particles.

Novel Aspect
This IPC method turned out to be very versatile and applicable for a large range of other phosphorylated and polar metabolites.

WPS26-28 / Development of a standard protocol for high-throughput metabolomic fingerprinting of urine using FIA- and Nano-ESI coupled with FT-ICR MS

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Introduction
The direct infusion (DI) coupled to high-resolution mass spectrometer (HRMS) has been developed as an alternative method to increase the sample throughput for large-scaled metabolomic fingerprinting. Compared to traditional liquid chromatography LC-MS, DI-HRMS increases the analysis speed and also simplifies the data pre-treatment process. However, mass spectra were largely complicated with the presence of thousands of peaks. Furthermore, an exhaustive metabolite detection can be prevented by the matrix effect. Therefore, novel experimental design and data pre-treatment process ensuring constant

non-biased results should be sought and developed. Herein, we attempted to develop (i) a standard DI-HRMS protocol, specifically using both the Flow Injection Analysis (FIA) and nano-injection modes adapted to ESI-FT-ICR MS for urinary samples and (ii) in addition, the data pre-treatment to evaluate the experimental approach.

Methods
Urine was prepared at different dilutions and the final sample contained 75% MeOH and 0.1% formic acid. For FIA-ESI-MS, 10 µL of sample was injected at a 10 µL/min flow rate. The isocratic mobile phase composition was the same as the sample. For nano-ESI, 5 µL of sample was injected at 200 nL/min flow rate combined with a 7T FT-ICR (Bruker Solarix) within a resolving power of 260K at m/z 400. Data were acquired from 100 to 600 m/z range in «chromatographic» acquisition mode. The peak lists were exported and annotated using public database and ESI-MS database (CEA-Saclay). Additionally data pre-treatment process and statistical analysis were carried out on R 3.0.3 and SIMCA 13.0, respectively.

Results
Dilution of the complex mixture can be a remedy to decrease the matrix effect responsible for signal suppression/enhancement when such mixtures are introduced into the API source. However, it is quite delicate and a compromise should be found between the concentration and matrix effect to succeed a metabolomic fingerprinting. To this end, urine was diluted with different factors. Due to the large size of datasets, statistical analysis has been employed for the best dilution factor determination. Significant outliers can be observed for dilution factors that are either too small or too big, probably due to the matrix and concentration effect. The optimal dilution factor has been determined as 200-fold for FIA-ESI and 50-fold for nano-ESI. For such optimal dilution factors, standard additions of 4 reference compounds in various concentrations showed good linearity of the signal response. Hence, 80 samples could be analyzed in less than 22 hours in positive and negative ESI without significant signal bias (< 20%). A proof-of-concept study has been conducted to compare the performance of traditional LC-MS with this DI-MS approach.

Conclusions
This established approach could be applied as a protocol for high-throughput metabolomic involving numerous cohorts.

Novel Aspect
Development of DI-MS method for cohort analysis in FT-ICR MS

WPS26-29 / Mass spectrometric investigation of biotransformation pattern of palmatine in human hepatocytes

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Introduction
Palmatine is a protoberberine alkaloid with important biological activity and therapeutic value including treatment of jaundice, hypertension or inflammation [1]. Despite its common use in form of phytopreparation in traditional Chinese herbal medicine, the metabolic transformation of palmatine in human hepatocytes is yet to be disclosed.

Methods
High-performance liquid chromatography coupled to electrospray ionization–mass spectrometry (LC-ESI/MSE) was employed in this study. Suspensions of human hepatocytes in serum-free medium were incubated with 10 µM palmatine for 0, 1 and 2 h, then centrifuged and the cell pellets and media were stored separately at -80 °C. For LC-MS analysis, the cell pellets were washed with phosphate buffered saline, resuspended in methanol containing 5% (v/v) acetic acid, sonicated, centrifuged and the supernatants were analyzed. All samples were chromatographically separated by gradient elution on a reversed-phase cyanopropyl (CNP) column with water/methanol/acetic acidmobile phase (89:10:1, v/v A, methanol B) and detected by a high resolution MS detector. The evaluation of the data was performed using Metabolynx XS software.

Results
A detailed fragmentation study for palmatine molecule was carried out by quadrupole-time-of-flight mass spectrometry. The knowledge of fragmentation pattern and the elemental composition of fragments was applied for elucidation of metabolites' structures together with the software calculations. A total number of ten metabolites was identified in hepatocytes from eight human donors. LC-MS data were evaluated semi-quantitatively according to the previously published procedure [2]. Phase I and phase II biotransformation products of palmatine in human hepatocytes did not exceed 5 % and 2 % of parent compound after 2 h cultivation, respectively. Overall, it can be concluded that palmatine is transformed by human hepatocytes at limited rate.

Conclusions
O-demethylated palmatine and the conjugates, glucuronide and sulfate, were found to be the main biotransformation products in human hepatocytes.

Novel Aspect
The present study gives a more detailed and comprehensive view on the biotransformation of palmatine and adds novel aspects to the limited knowledge of human metabolism of this frequently used bioactive compound.

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WPS26-30 / A sample preparation protocol for metabolomics studies in Leishmania using gas chromatography-mass spectrometry as analytical tool

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Metabolomics is a new approach used to understand changes associated to disturbance at cellular level. The application of mass-spectrometry (MS) for analysis of cellular metabolites has grown dramatically over the last years, and nowadays MS is the single most important detector method in biotechnology. MS combined with separation techniques such as gas chromatography offers tremendous opportunities for analysis of complex biological samples because it enables the determination of a large number of metabolites in a single analysis. Metabolomics analysis represents a great analytical challenge, mainly due to the variation of the physicochemical properties and metabolites concentration in biological samples. Despite of recent developments of sample preparation for biofluids, the extraction process remains the critical step that requires much time in analytical method.Thus, the aim of this work is to develop a careful protocol of sample preparation and extraction of intracellular metabolites in Leishmania infantum for untargeted

metabolomics analysis by GC-MS. Firstly it was studied the best cell preparation for sample collection, investigating some parameters such as: number of washes steps with PBS for total removal of extracellular metabolites, growth phase of parasite, residence time of parasites in culture medium and number of cells used. Thus, after cell collection, the extraction of metabolites was evaluated by checking the best cell lysis (tissuelyzer or ultrasound probe), followed by experimental design based on triangle solvents (11 points assessed) by combination of methanol, water and chloroform (binary and ternary mixtures) for evaluation of the best solvent extractor by response surface methodology. The sample preparation workflow was constructed by evaluation of GC-MS results and consists of: collecting samples of L. infantum from different cultivations, minimizing biological variability, put them in dry ice for metabolic quenching and washing 2 times with cold PBS, sampling 6.107 promastigotes/mL, drying and storing at -80°C until use. The extraction of intracellular metabolites is performed by ultrasound probe operating at 30% for 60 seconds with the mixture extract solvent of methanol, chloroform and water, since it has been proven to be effective partitioning of polar compounds in the aqueous fraction. The results were evaluated according to the best chromatographic profile, as well as the area of extracted peaks of metabolites identified in GC-MS libraries. Overall, metabolomic studies are not concerned with sample preparation, thus, the contribution of this work is to show that different compositions of extractor solvents can lead to different metabolic profiles, which may cause erroneous measurements in metabolome of biological systems. This is the first part of a wide study for leishmaniasis, which is a neglected disease that affects over 12 million people worldwide.

WPS26-31 / Identification of human metabolites in urine with a high-quality metabolomics library combined with UHPLC-SWATH-MS/MS analysis

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Introduction
Metabolomics studies commonly report changes in the occurrence of hundreds of observed metabolites. To understand the biological meaning of these changes, correct assignment of LC/MS peaks to the known metabolites is of course essential, however the identification of small molecules from LC/MS data remains a challenge. It ultimately relies on the comparison with reference standards that are often not easily obtainable for metabolites. The currently available MS/MS libraries are of varying quality and dependent on the instrumentation used for acquisition. Furthermore no information is available for relative MS response factors. We generated a MS metabolomic library for 554 metabolites reported in the human metabolome database (HMDB) taking into account MS response adducts, fragment annotation and retention times and used it for identification of metabolites in urine.

Methods
High resolution TOF MS and MS/MS spectra were acquired on a Triple TOF 5600 mass spectrometer in ESI positive and negative mode by flow injection analysis of each reference compound. The retention factors of the typically polar metabolites were determined by UHPLC with two RP C18 columns of medium and high polarity and a HILIC column with gradients based on different solvents at various pH values. The results were correlated with predictive retention times using a software package from ACD labs. Urine samples were analysed by UHPLC-MS using data independent acquisition (DIA) with SWATH (sequential window acquisition of all theoretical fragment ion spectra) acquisition mode. Metabolite assignments were performed by searching the annotated library and the LC retention time.

Preliminary results
Our MS metabolomic database includes the MS response for the various adducts such as [M+H]+, [M+NH4]+, [M+Na]+, [M+K]+, [M-H]-, [M+Cl]- [M+Na-2H]-, [M+FA-H]-, [M+AA-H]-, dimers and losses of H2O, CO2 and NH3 based on TOF MS data. It also includes the observed MS/MS fragments and their structure assigned using a prototype software tool for fragment structure annotation as well as the retention factors determined by RP and HILIC chromatography. Retention times were predicted based on a linear regression between the retention times for a set of model compounds and their log D values. Accuracy was improved when the model compounds were selected based on structural similarities with the compound of interest. This library provides the basis for identifying and quantitating these metabolites in the LC-MS-SWATH data.

Conclusion
An annotated high resolution library of human metabolites could be generated considering relative MS response factor and LC retention time. This library could be successfully used to perform metabolite identification in urine for LC-SWATH MS data.

Novel aspects
Metabolite library search using SWATH-MS data and LC retention times

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WPS26-32 / Towards a standardized metabolomics MS/MS databank : advantages and limitations

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Introduction
In metabolomics, mass spectrum annotation and the statistic treatments are the principal aims. The metabolite identification by electron impact ionization was currently achieved by using a data bank, owing the strong reproducibility of the mass spectra. By contrast, atmospheric pressure ionization (API) produces various molecular species depending upon experimental conditions. Thus, these ions do not give a lot of reproducible fragment ions, but the product ions obtained by CID can help to identify the molecular structure from the molecular weights obtained from the annotation of the API mass spectrum. The study aims to develop an appropriate protocol to provide standard experimental conditions for CID spectra recorded using different types of mass spectrometers.

Methods
Theoretical studies were made in order to understand and to control the influence of experimental parameters on the CID spectrum patterns using the triple quadrupole and the ion trap instruments. The internal energy deposition on the mono-protonated species was probed using a calculation approach based on RRKM modeling with some thermometer molecules characterized by different degrees-of-freedom (para-iodobenzylpyridinium salt, leucine enkephalin and RLDIFSDF).

Results
A preliminary study in the French Network of Metabolomics (RFMF) demonstrated the need of standardized conditions to reduce differences among the various instrumental platforms available by the laboratory partners. This work leads us to a better understanding and a better control of the relative abundance of fragment ions with an optimization of internal energy deposition on the precursor ions. CID spectra recorded

at low collision energy using various instrumentations are poorly reproducible and comparable. The internal energy deposition, the dissociation rate constant, the kinetic shift, the gas pressure and its nature in the collision cell are the main origin of such reproducibility limitation. Thus, various excitation modes such as (i) resonant CID and PQD, and (ii) non-resonant CID as HCD were investigated and evaluated by using high resolution analysis with Orbitrap-based instruments. CID spectra provided from the PQD and HCD experiments suggest that only the collision voltage amplitude parameter has an influence on the low energy collisional-activation spectra. Therefore, a calibration method based on this collision voltage should provide reproducible dissociation spectra for PQD and HCD modes, which should present high similarity with ion beam CID spectra recorded by using tandem-in-space instruments.

Conclusion
We present a method for building standardized metabolomics databank as well as the advantages and limitations, by presenting a first metabolomics inter-laboratory exercise for comparison of CID spectra using different type of instruments.

Novel Aspect
The development of a method allowing the standardization of the experimental conditions based on theoretical studies

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WPS26-33 / Accurate mass GC-qTOFMS - a novel tool in metabolic flux analysis

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Introduction
In the past two decades 13C based metabolic flux analysis (13C-MFA) of microbial organisms has become a key analytical technology especially in support of metabolic engineering [1,2]. Besides the proof of successful genetic manipulations, flux measurements allow the quantification of intracellular metabolic rates to unravel non-obvious metabolic networks and their regulation. The introduction of stable isotope tracers, like 13C, into an organism, leads to a characteristic pattern of labeled metabolites, which are most frequently measured by NMR or MS based techniques [3]. In this context the principle of orthogonality is essential and can be additionally achieved when employing GC and LC. In the present work, we have developed a GC-qTOFMS based method for accurate analysis of isotopomer distribution of amino acids and organic acids.

Methods
An Agilent 7200 GC-qTOFMS system was used for the analysis of biotechnological samples from a 13C based metabolic flux experiment. Derivatization was performed via trimethylsilylation or propylchloroformation. Both EI and CI were employed for ionization; CID was achieved by using either Ar or N2 as collision gas.

Results
The data obtained by the novel GC-qTOFMS method under different experimental conditions are compared in terms of precision and accuracy. Both derivatization procedures delivered accurate results. However, chloroformation was advantageous concerning derivative stability, reduced sample preparation time and simpler correction algorithms. CI gave the quasi molecular ion enabling selective fragmentation of isotopologues and the unambiguous assignment of isotopomeres to the respective isotopologue.

Conclusion
Due to the information gain when employing CI with consecutive CID, GC-CI-qTOFMS proved to be most valuable for the analysis of tandem mass isotopomer distribution of high abundant primary metabolites, as this approach has its drawbacks concerning sensitivity. Whereas the application of EI is also compatible lower abundant metabolites, this hard ionization technique require sophisticated tailored bioinformatics platforms due to the overlapping of isotopomeres.

Novel Aspects
We present a novel analytical approach based on accurate mass GC-qTOF-MS for the analysis of isotopologue fractions of 13C-MFA experiments from biotechnological applications. We consider the orthogonality to LC-MS or NMR as highly valuable in terms of validation of MFA related analytical data.

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WPS26-34 / Metabolomic study of trans-trans 2, 4-decadienal induced lung lesions in mice by liquid chromatography-mass spectrometry

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Introduction
Recent epidemiological studies have demonstrated that exposure to cooking oil fumes (COF) is strongly associated with female lung adenocarcinoma in China, Hong Kong, Singapore, and Taiwan. COF are a complex mixture of chemicals in which the fatty acids in the cooking oils, especially polyunsaturated fatty acids, decompose readily upon heating or oxidation to yield aldehydes. Among these aldehydes, trans, trans 2,4-decadienal (tt-DDE) is the most abundant and cytotoxic chemical. tt-DDE has been reported to interact with DNA, inducing DNA breaks that cause genetic damage, as well as producing increased oxidative stress and causing genotoxicity in A549 cells. Metabolomics, the global study of metabolites and their concentration changes, interactions and dynamics in complex biological systems, also have become a vital tool in clinic research and diagnosis of human disease. Recently LC-MS has been applied widely in metabolomic studies owing to its high sensitively and reproducibly. The objective was to identify potential disease biomarkers and to examine potential early diagnostic and stratification strategies that would allow tt-DDE exposure in vivo to be followed.

Methods
5 µL of urine or serum was injected and separated on a 2.1×150 mm T3 3 µm column using an Agilent 1200 HPLC system. The mass spectrometric data were collected using a QSTAR Elite operated in positive and negative ionization with needle voltages of 5.5 and 4.2 kV, respectively. Mass calibration of the TOF-MS was performed by infusion of 10 pmolµL−1 of renin substrate tetradecapeptide at a flow rate of 10 µLmin−1 and 400 pgµL−1 of taurocholic acid at a flow rate of 5 µLmin−1 for positive and negative ionization, respectively

Results
In this study, we investigated the mouse urine and serum metabolic profiles after the intratracheal instillation of tt-DDE. First, we performed non-targeted metabolomics analysis using LC-TOF-MS to distinguish between the vehicle and treated groups at different time points. The nontargeted analysis candidate peaks were then used to characterize the groups treated with tt-DDE after selection by principal component analysis. Next,

candidate metabolites were analyzed using LC-MS. The results demonstrated that the amino acid profiles of urine and serum were changed when mice were treated with tt-DDE. Ten amino acids were found to be significantly reduced in the serum of mice treated with tt-DDE at the 8 weeks after tt-DDE was instilled.

Conclusions
LC-MS method has been proved to be powerful and reliable analytic platform for urine and serum metabolites screening with relative high sensitivity. The main conclusion of our study is related to the identification of the metabolic profiling in urine and serum of mice treated to tt-DDE. The results demonstrated that the amino acid profiles of urine and serum were changed when mice were treated with tt-DDE.

Novel Aspect
The objective of this study was to identify any changes in metabolite profiles associated with the development of tt-DDE-induced lung lesions.

WPS26-35 / Analysis of untargeted MS-based metabolomics data: the metaMS package for R

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Introduction
Untargeted MS metabolomics data provide a wealth of data on the presence and abundance of metabolites in biological samples. The extraction of relevant information can be difficult, and many software platforms have been proposed. One of the most popular tools for analysing LCMS and GCMS data is XCMS, written in the R language. An add-on to XCMS, developed specifically in the context of untargeted metabolomics, is metaMS, providing facilities for building in-house databases of chemical standards geared towards specific organisms or groups of metabolites, automatic annotation, and quantification. MetaMS, like XCMS, is publicly available from the Bioconductor repository.

Methods
For LCMS, the main part of the metaMS pipeline is similar to the XCMS pipeline. The additions from metaMS focus on improved annotation using in-house databases, an m/z and intensity-dependent mass accuracy window and an explicit definition of minimal support for annotation. The outcome is a matrix summarizing for all samples the intensities of the aligned peaks. The GCMS pipeline is different, working on so-called pseudospectra rather than individual peaks; here, the output is a relative intensity measure for chemical compounds rather than individual peaks (Wehrens et al., 2014). The compounds may be annotated (when there is a match with the database), or labelled as Unknowns.

Results
A web-based pipeline has been built using the metaMS package, which now is in daily use by the metabolomics platform at FEM. Processing hundreds of samples takes only a couple of hours on a regular four-core linux desktop computer. The generated tables can be immediately be used for subsequent statistical analysis. A common application is Quality Control: a score plot from a Principal Component Analysis can be inspected to see whether the quality control samples do not show a trend with injection order.

Conclusions
Open-source software like metaMS provides ultimate control over data processing, which is of utmost importance when analysing data as complex as GCMS or LCMS data. In addition, the large user base of the underlying XCMS package guarantees rapid adaptation to new developments, timely bug reporting and on-line user feedback. MetaMS provides a top layer over XCMS, specifically geared to untargeted metabolomics.

Novel Aspect
The novel aspects of metaMS are found at several levels: at the most abstract level the functionality of XCMS is extended and geared towards untargeted metabolomics data. At more detailed levels this includes tools for setting up in-house databases, doing annotation in a principled way, and a completely new approach to handle GCMS metabolomics data.

R. Wehrens, G. Weingart and F. Mattivi: J. Chrom. B DOI: 10.1016/j.jchromb.2014.02.051

WPS26-36 / The potential of two-dimensional chromatography in non-targeted metabolome analysis

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Introduction
Non-targeted metabolomic approaches have received more and more attention during the past years. Nowadays, many laboratories rely on the selectivity and resolving power of mass spectrometry based systems to identify the individual metabolites and create metabolic fingerprints via non-targeted analysis. This strategy has clear shortcomings for unstable metabolites and those that occur as multiple isomers. Moreover, peak capacity and sufficient retention of both polar and apolar compounds are the limiting factors in LC-MS based non-targeted analysis. Metabolic fingerprints obtained by such methods might therefore be biased and have to be evaluated carefully.

Methods
In this work, the potential of two-dimensional (2D) chromatography in non-targeted metabolomic approaches with a special focus on isomeric metabolites is explored exemplarily for sugar phosphates. Aiming at the establishment of an online combination in a 2DLC-TOFMS setup, different modes of chromatography including hydrophilic interaction liquid chromatography (HILIC), reversed-phase chromatography (RPLC) as well as ion chromatography techniques were evaluated.

Results
A comparison between data sets generated using conventional non-targeted LC-MS methods and employing a 2DLC-MS approach in non-targeted analysis will show if the application of the latter increases the amount of extractable isomer information from the resulting metabolic fingerprints.

Conclusions
LC-MS based non-targeted analysis in metabolomics requires a comprehensive approach that takes the high chemical variability of intracellular metabolites into account. If left unassessed, a potential methodological bias might result in misleading metabolic fingerprints and wrong biological interpretations. Especially the determination of sugar phosphates and their isomeric forms demands for method optimization even in non-targeted analysis.

Novel aspect
To the authors' knowledge, it is the first time that different modes of chromatography are systematically evaluated in a 2DLC-MS setup in terms of their potential beneficial effects on the

information content of metabolic fingerprints created by non-targeted analysis, with a special focus on isomeric metabolites.

WPS26-37 / HRMS dereplication and MS/MS networking to decipher cryptic metabolite pathways in fungal microorganisms

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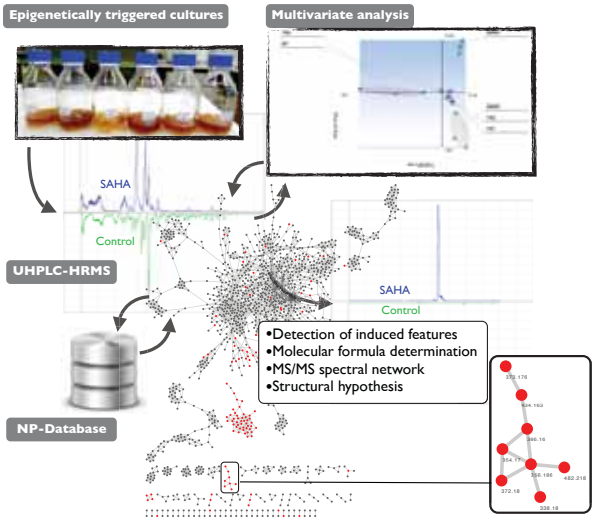
Introduction

The interest of microorganisms as a valuable source of bioactive compounds needs no more justifications. Since the discovery of Flemming's penicillin G from Penicillium notatum at the beginnings of the XXth century to the isolation of the proteasome inhibitor salinosporamide A from Salinospora tropica in 2003, numerous valuable biologically active metabolites have been isolated from microorganisms. Recent insights from the progress in genetics have shed a new light on the biosynthesis of microbial natural products. It is now well known that under classical laboratory culture conditions, microorganisms only express a small proportion of their biosynthetic potential.[1] This phenomenon, known as gene cluster silencing, is very common and has been reported to occur in a vast range of living organisms.[2] Recently, new approaches aiming to address silenced biosynthetic pathways in eukaryotes have appeared. [3,4] One of our research interest is the de novo induction of secondary metabolites stimulated by the co-culture of diverse fungal strains [5,6]. In the present study, de novo inductions mechanisms are studied under a different angle and it is hoped to gain new perspectives and deeper understanding of communication between fungi at the metabolite level.

Methods

In order to explore the hidden biosynthetic potential of filamentous fungi we used small molecule epigenetic modificators (EM) of various classes (HDACi, DNAMTi) on phylogenetically diverse fungal strains. A metabolomic approach implying UHPLC-HRMS analysis, semi-automated dereplication procedures, MS/MS spectral networks generation and multivariate data analysis was set up to detect the induction of novel metabolites and select promising fungal candidates for further scale-up culture.



Results

This workflow allowed us to highlight the production of various secondary metabolites not detected in control conditions. The application of HR-MS/MS networking provided valuable information regarding structures of the induced features. In particular, a family of closely related compounds was induced in one of the treated strain, thus indicating the probable unlocking of a common biosynthetic cluster.

Conclusions

An integrated metabolomics workflow taking advantage of high-resolution MS data acquisition, semi-automated dereplication procedures and spectral networks construction was applied to screen and highlight inductions of secondary metabolites biosynthesis in epigenetically modified fungal strains. Combined with HRMS, MS/MS networking appears as a very informative tool for natural products dereplication.

Novel aspects

Taking advantage of the high resolution provided by Orbitrap mass spectrometer in early natural products dereplication. Generation of spectral MS/MS networks highlighting effects of epigenetic modificators on fungal secondary metabolomes.

WPS26-38 / Identification of two plant sources of red propolis by UHPLC-MS

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Introduction

Red propolis is a resinous substance collected by bees from different plant sources. It exists in the northeastern states of the Brazilian Coast, in Venezuela, Cuba, México and it has also been found in China. The chemical composition of red propolis varies widely and recent studies verified the existence of two types of red propolis: Type A, whose ion marker is m/z 601.35 ($C_{38}H_{49}O_6$), and type B whose ion markers are m/z 255.06 ($C_{15}H_{11}O_4$), 267.06 ($C_{16}H_{11}O_4$), 271.06 ($C_{15}H_{11}O_5$), 271.10 ($C_{16}H_{15}O_4$), 283.06 ($C_{16}H_{11}O_5$), and 301.07 ($C_{16}H_{13}O_6$). The chemical differences can be explained by the geographical origin of the resin and the fact that the weather conditions affect the flora around the hive, which might not be the same in all the places where red propolis has been found.

Materials and methods

SAMPLES

Red colored propolis was collected in hives by different beekeepers in the following Brazilian states: Sergipe; Alagoas; Paraiba; Espirito Santo and Bahia. The sample of Dalbergia ecastophyllum was collected near João Pessoa, state of Paraiba, and a sample of red propolis from Cuba was kindly donated by Dr. Osmany Cuesta-Rubio.

UPLC-ESI(-)-MS

The chromatographic analyses of the ethanolic solutions of the dried propolis extracts (1 mg/mL) were performed on a UPLC Acquity chromatographer coupled with a TQD Acquity mass spectrometer (Micromass-Waters Manchester, England), with an ESI source. A C18 BEH Waters Acquity column (2.1 mm _ 50 mm _ 1.7 lm particle size) was used. Solvent A was mili-Q purified water with 0.1% formic acid and solvent B was methanol. The flow rate was 0.2 mL/min and 5 uL of samples were injected. ESI ionization in the negative ion mode was used under the following conditions: Capillary -3.00 kV, Cone -30 V, Source Temperature 150 ºC, Desolvation Temperature 350 ºC , acquiring data between 100 and 800 m/z.

Results and discussion

It was observed that both types of propolis have several substances in common, including the ion markers. However, their richness is different in each type of propolis: most of type B ion markers were present in type A as well, but in lower concentration. The chromatograms showed two groups of constituents. The first one included ions present in D. ecastophyllum, while the second one included the ion marker of red propolis type A, m/z 601.35. Previous data showed that this substance is not present in D. ecastophyllum, which suggests that the second group of ions observed in the chromatograms belongs to a different and unknown plant source.

Conclusions
At least two plant species are the source of red propolis, being D. ecastophyllum responsible for the main compounds found in red propolis type B.

Novel aspect
At least two plant sources are responsible for the composition of red propolis, being each one of them responsible for the classification in type A and B of the resin. This is the first study of red propolis using UPLC-MS.

WPS26-39 / Localization of Flavonoids Affects Blue Color Expression of Flower Petals

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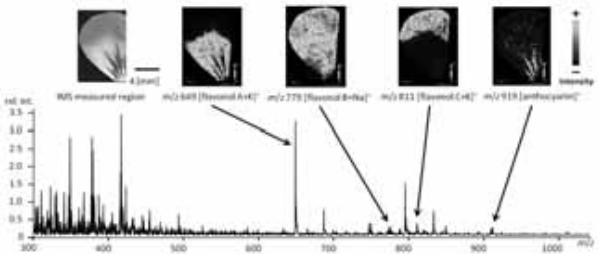
Introduction
Flowers have a large variety of colors, and the causative secondary metabolites are known as carotenoids, chlorophylls, betacyanins, and flavonoids. Flavonoid glycosides are related to various color expression in the yellow, red, violet and blue for petals. We are interested in the blue viola petals because the blue color expression mechanism has not revealed yet. We hypothesized that flavonoid glycoside contents and their localizations are directly related to the blue color expression in the blue viola petals.Although whole viola petals were subjected to LC-MS analysis in the ordinal research approach, the localization information of the substances disappeared. Therefore, we attempted an imaging MS technique for the viola petals to resolve the above problems and describe the color expression mechanism.

Methods, Result
At first, appropriate petal samples for imaging MS measurement could not be prepared due to the rough surface, thickness, and high water content of them. Thus, we developed pre-measurement treatments, target modification, and sample fixation methods on the target. Finally, we obtained MS images (figure) of several flavonols and an anthocyanin, indicating co-localization of the anthocyanin and flavonols is essential for blue color expression in the viola petal.



Conclusion, Novel Aspects
In this study, we could identify some flavonoids related to blue color expression of blue viola flower by using imaging MS techniques.
For petal imaging MS measurements, we attempted an inventive modification on the sample preparation to obtain accurate MS images.

WPS26-40 / Metabolomic Analysis of Gingival Crevicular Fluid Using GC/MS

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Introduction
Periodontal disease is one of the most concerned threats to dental health. But there is no method to appropriately evaluate pathological condition and disease activity. Therefore, new analysis method to diagnose periodontal disease conditions is required. In this study, gingival crevicular fluid was analyzed using gas chromatography/mass spectrometry (GC/MS) and metabolome analysis was performed to identify characteristic metabolites that represent pathological condition.

Methods
Gingival crevicular fluid was collected from 4 patients and 27 healthy volunteers by capillary, and from other 4 patients and 18 healthy volunteers by periopaper. Maximum collectable volume of capillary is 2 μL and that of periopaper that is quick and easy to use is 0.2 μL. Gingival crevicular fluid with pretreatment such as extraction using a solvent mixture (MeOH:H2O:CHCl3=2.5:1:1), condensation and derivatization was analyzed using GC/MS. 6890N (Agilent, USA) was used for GC. Capillary column used in this analysis was a CP-Sil 8 CB for Amines 30 m×0.25 mm (0.25 μm). Miniaturized time-of-flight mass spectrometer "MULTUM-S II" was used for mass analyzer.1) JMS-Q1000GC (JEOL, Japan) was used for cross validation. The data obtained by GC/MS was analyzed by using principal component analysis (PCA), which is a basic method of multiple classification analysis.

Results
The gingival crevicular fluid collected by capillary and periopaper was measured by GC/MS and some metabolites were specified. Both GC/MS indicated a similar tendency. Even at minimum collection quantity of 0.1 μL, metabolites in the gingival crevicular fluid was detectable. The levels of metabolites were significantly changed in patients compared with those in healthy volunteers. The PCA scores plots using 29 peaks show that the patients and the healthy volunteers were clustered in two groups. Intensities of 6 metabolites with higher loading of the PCA scores were obviously different to distinguish the patients and the healthy volunteers. Thus these metabolites are considered as characteristic metabolites that represent pathological condition.

Conclusions
This results suggested that metabolome analysis of gingival crevicular fluid is useful for diagnosis for periodontal disease. The results will aid the new analysis method to diagnose periodontal disease conditions by using characteristic metabolites that represent pathological condition.

Novel Aspect
Metabolome analysis by GC/MS reveal that the levels of metabolites of gingival crevicular fluid were significantly changed in patients compared with those in healthy volunteers. Even at minimum collection quantity of 0.1 μL, metabolites in the gingival crevicular fluid was detectable.

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WPS26-41 / Analyzing Durable Anti-fungal Resistance Processes in Cereals by Metabolomics Using UHPLC-HR-MS

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Introduction

Fungal pathogens cause substantial losses of crop yield every year. Introduction of durable resistance genes in crops is an important strategy to prevent yield loss and to maintain food security. The resistance gene Lr34 of wheat (Triticum aestivum), which is durably conferring resistance to four major fungal pathogens [1, 2], was cloned into barley (Hordeum vulgare). The molecular resistance mechanism of Lr34, which encodes for an ATP-binding cassette transporter [3], is not known yet.

The objective of our research is to identify the molecular nature of the transported molecule(s) and to understand the mechanisms of Lr34-mediated durable resistance.

Methods

An untargeted metabolomics approach [4] based on ultra-high performance liquid chromatography high-resolution mass spectrometry has been developed. Control plants were compared with transgenic lines for non-quantitative pattern recognition by statistical evaluation of LC-MS data.

Results

Different classes of metabolites that accumulate to a different level in control and transgenic lines were detected in barley extracts. Characterization of these differential metabolites using accurate mass and MS/MS fragmentation in combination with in silico fragmentation and spectral databases will be presented.

Conclusions

A workflow for plant metabolomics based on UHPLC-HR-MS was established for crop tissue extracts, and is applied to barley, rice, wheat and sorghum samples.

Novel Aspect

The newly developed metabolomics method enables us to gain valuable insight into the Lr34 resistance mechanism of this highly relevant wheat resistance gene.

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WPS26-42 / Determination of soybean-derived isoflavones in the rumen fluid by HPLC-MS-TOF

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Isoflavones belong to a class of phytoestrogens that exhibit the most potent estrogenic activity. The major isoflavones are aglycones genistein, daidzein and glycitein and are found primarily in leguminoses. Phytoestrogens have received increasing attention for their potentially beneficial effects as estrogen agonists in human health and disease. In order to study quantitative aspects of the isoflavones metabolism in the rumen, it is necessary to quantify accurately their levels in rumen fluid. The objective of the study was to optimise and validate the HPLC-MS-TOF method for the determination of isoflavones genistein, daidzein, glycitein and metabolite equol in bovine rumen fluid. Rumen fluid samples ware centrifuged and supernatant was deproteinized by mixing with trichloracetic acid. After centrifugation, supernatant was incubated with beta-glucuronidase and sulfatase. After incubation, the solution was added to methanol and centrifuged. Alternatively, was tested method without enzymatic hydrolysis, with direct MS-TOF identification of glycosylated isoflavones. HPLC analysis of isoflavones ware performed by an Agilent Technologies 1260 Infinity HPLC instrument equipped with an Agilent Technologies 6224 TOF with ESI ionization on C-18 column by buffered methanol gradient elution. Isoflavones will be identified by standards.

Rumen fluid was obtained from three lactating Holstein cows fitted with rumen cannulas Cows were fed individually twice daily the diet based on maize silage, lucerne hay and supplemental mixture that contained extruded full-fat soya as a source of isoflavones. During the collecting period individual samples of ruminal fluid were taken in five time intervals. Concentration of daidzein in the rumen fluid varied from 92.5 µg/L to 113.2 µg/L with an average value of 102.9 µg/L. Mean concentration of equol, a metabolite of daidzein, in rumen was 42.5 µg/L. The lowest concentration (22.2 µg/L) was found at start time and then raised up to 66.4 µg/L. Average concentration of genistein in rumen fluid was 239,3 µg/L. The lowest concentration (205.7 µg/L) was determined prior feeding. Concentration of genistein in other collection times was relatively stable ranging from 242.7 to 249.0 µg/L. Concentration of glycitein was on average 23.6 µg/L and did not vary greatly among collecting times.

The results of our research include the development of a method that is both fast and highly sensitive in the simultaneous quantitation of isoflavones from rumen fluid.

This work was supported by the Grant 13-34134S (Grant Agency of Czech Republic).

WPS26-43 / Identification of an unexpected de novo metabolite from Acinetobacter baylyi ADP1 : a particular challenge for the HRMS and HR/MSn arsenal

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Introduction

Combination of untargeted metabolomics to whole genome RNAseq transcriptomics can be a useful strategy to tackle functional genomics, enlightening hidden features of the metabolism inaccessible by classical methods. Shifting the carbon source of the soil bacterium Acinetobacter baylyi ADP1 from succinate to quinate triggered a large scale of transcriptional reorganization that led to the production of many unexpected and unidentified metabolites. Our aim is to elucidate their structure, to gain insight in their biosynthesis and function. Herein, for this purpose, the analytical arsenal offered by mass spectrometry (i.e., positive and negative LC/HRMSn modes, H/D exchanges, and «in axis collision» and resonant excitation for ion activation) associated to fragmentation interpretation were used for the de novo structural elucidation of one of this metabolite.

Methods

LC-HRMS as HILIC-LTQ-Orbitrap XL (Thermo Electron, Germany) with a heated HESI source was used in positive and

negative modes. Raw data were processed by XCMS Online (Scripps Center, USA). Metabolites were putatively identified by matching their masses to public KEGG and Metlin databases. For unknown metabolites, structural elucidation was done by using sequential CID-MSn experiments under high resolution conditions (30,000 FWHM at m/z 400) from light samples and on deuterated samples prepared by H/D exchanges with D2O.

Results

Matching the accurate mass of the unknown compounds produced exclusively in quinate-grown cells with databases gave putative identities that were not consistent with both in-source CID and data-dependent MSn experiments. This is why we made the structural elucidation of this de novo metabolite. In-source protonated molecules yield fragment ions consistent with the methylamine loss and CID-MSn experiments generated diagnostic ions. In negative mode, CID-MS3 lead to distonic C6H5NO•- ion similar to that provided from tyrosine-containing peptides. This de novo metabolite seems to be a tyrosine substituted by a benzyl methylamine. The H/D exchange experiments respectively leading to hexa-deuterated and tetra-deuterated species in both the HESI polarities confirm the structure.

Conclusion

To our knowledge, this de novo metabolite has never been described, and its function and biosynthesis remain unknown. We are currently working on two additional unknown compounds exclusively detected in quinate metabolom. Preliminary results suggest that these de novo metabolites are structurally related to the tyrosine derivative, and may belong to a new family of secondary metabolites. Their structure should help to find genes associated with them to eventually discover hidden metabolic pathways.

Novel Aspect

Identification of de novo metabolite involved in a hidden metabolic pathway using LC-HRMSn

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WPS26-44 / Gender-specific metabolic profiling study in patients with myocardial infarction using UPLC/Q-TOF MS

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Myocardial infarction (MI) is the leading causes of death worldwide, and caused by prolonged myocardial ischemia with necrosis of myocytes due to obstruction of blood supply to an area of heart. Many studies have showed gender-related differences in the clinical features of MI, but the reason for the difference between genders remains unclear. In this study, we applied ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC/Q-TOF MS) to identify gender-specific metabolic patterns in aqueous and lipid metabolites of serum from healthy individuals and patients with myocardial infarction. Patients with diagnosed MI (male, n=35; female, n=33), and age- and body mass index-matched healthy individuals (male, n=35; female, n=33) were included in this study. The orthogonal projections to latent structures-discriminant analysis (OPLS-DA) model was generated from metabolic profiling data, and the score plot showed a significant difference between genders in MI. Interestingly, the result of OPLS-DA score plots showed significantly higher separations between control and MI in male than female. Phosphatidylcholine species containing unsaturated fatty acids were significantly different in male between control and MI, whereas phosphatidylethanolamine-plasmalogen species were significantly different in female. This study demonstrates that serum metabolite profiling using UPLC/Q-TOF MS provides specific information on gender variation in MI and could be used to observe gender-specific metabolic patterns between control and MI.

WPS26-45 / GC-qTOFMS for determination of accurate isotopologue ratios and tandem mass isotopomer ratios for metabolic flux analysis of the central carbon metabolism

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Introduction

Metabolomics, i.e. the comprehensive analysis of the metabolome of a biological system, demands for the combination of different analytical techniques. Currently mass spectrometry (MS) and nuclear magnetic resonance spectroscopy are most frequently employed for targeted and untargeted analysis of metabolites, and metabolic flux analysis (MFA). In this context flux assessment via the introduction of isotope labeled compounds, i.e. 13C-based MFA is regarded as the key strategy. For bioinformatic modeling, the quality of analytical data is essential, as the accuracy and uncertainty of measurement of the imported data is directly influencing the validity and informative value of the derived kinetic data. We are presenting a novel GC based separation method for the analysis of accurate isotopologue and tandem mass isotopomer ratios of metabolites from the central carbon metabolism, with a special focus on sugar phosphates, using a new generation GC-qTOFMS system. An inter-comparison with LC-MS/MS derived data was performed in terms of method validation and will be discussed regarding the analytical figures of merit.

Methods

Cellular extracts were analyzed (1) with an Agilent 7200 GC-qTOFMS system after derivatization via just-in-time ethoximation/trimethylsilylation and (2) with a Thermo Vantage LC-MS/MS system applying both reversed phase LC and HILIC separation.

Results

Isotopologue or tandem mass isotopomer distribution of organic acids, amino acids and sugar phosphates were determined in cellular extracts from biotechnological applications. The isotopologue or tandem mass isotopomer ratios measured in natural, non-labeled metabolites were in excellent agreement with the theoretical values. The precision of the ratios from LC-MS/MS and GC-MS/MS were in the same range and mainly influenced by compound specific sensitivity and concentration of the metabolites of interest.

Conclusions

In the context of metabolomics and metabolic flux analysis GC-qTOFMS is a valuable, orthogonal alternative to LC-MS based techniques, which is beneficial for assessment and quality assurance of mass spectrometric data.

Novel Aspect

We are presenting a novel GC-qTOFMS based method including an intercomparison with LC-MS/MS for analysis of isotopologue and tandem mass isotopomer distribution of important metabolites of the central carbon metabolism in cellular extracts from industrial biotechnology.

WPS26-46 / Characterization of the Hepatocellular Metabolome and its Changes upon Primaquine Exposure Using LC/MS

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Introduction

Metabolomics is an expanding research area dealing with the analysis of all cellular metabolites, thus, endogenous as well as exogenous low molecular weight compounds, and their altering concentrations upon system stimuli. Targeted and non-targeted liquid chromatography (LC) mass spectrometry (MS)-based techniques are commonly applied to plasma, serum or urine samples for identification and quantification of up- or down-regulated compounds. However, the potential biomarkers sought-after and discovered by such approaches may differ from those in the target organ (e.g. liver) if remaining instead of being excreted. Hence, a LC/MS method was developed to characterize the metabolome of hepatocyte cells. Its changes upon exposure to primaquine were monitored and a potential link to hepatic drug toxicity was evaluated.

Method

Rat or human hepatocytes were first analyzed without adding stress-inducing factors, later treated with different concentrations of primaquine, an anti-malarial drug. Cell samples were flash-frozen and mechanically lyzed by cryogenic grinding with glass beads. For extraction of metabolites the Bligh & Dyer approach was utilized. LC/MS analysis of the recovered extracts was performed via dual-column separation injecting aqueous fractions on a C18 and organic fractions on a C8 column, both coupled to a TripleTOF 5600 (AB Sciex) with an ESI source. MS/MS data were acquired in SWATH mode ("sequential window acquisition of all theoretical fragment-ion spectra") and periodical TOF autocalibration was applied.

Preliminary Data

Since hepatocytes still contain the various cell organelles comprising exogenous metabolites and endogenous substances they represent a suitable model for studying the hepatic metabolome at an original state. Different cell concentrations were tested and LC/MS conditions optimized to enable the analysis of a majority of cellular metabolites simultaneously. Subsequently, hepatocytes were incubated with primaquine using selected drug concentrations (low µM to upper pM range) and incubation time points. It was intended to maintain the detection of endogenous metabolites and to prevent signal superimposition by primaquine and/or related exogenous metabolites. After quenching metabolic activity with a cold (-70°C) solution of 80% methanol, cell pellets were flashfrozen and disrupted via cryo-lysis. Aqueous and organic phases resulting from the Bligh & Dyer extraction were analyzed via LC/MS. The resulting high resolution (HR) MS2 spectra from acquisition in SWATH mode were evaluated using multivariate statistical tools in combination with database search. Next to commonly known platforms like HMDB and MassBank, an in-house created library was additionally exploited for a more reliable validation of potential hits. Thereby, SWATH provides the advantageous possibility of integrating qualitative and quantitative analysis (QUAL/QUAN). Thus, changes in the hepatocellular metabolome distribution upon primaquine exposure could readily be assessed.

Novel Aspect

Metabolomic changes were considered at cell stage in hepatocytes using a dedicated
workflow based on Bligh & Dyer extraction and LC/HR-MS in SWATH mode.

WPS26-47 / LC-MS of Chiral Hydroxycarboxylic Acids

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Hydroxycarboxylic acids represent interesting optically active building blocks used by nature as metabolites and by industry as synthetic intermediates. The direct determination of their optical purity by enantioselective separation is preferred over the measurements of the optical rotation, as this enables also to distinguish metabolic pathways and to quantify the enantiomers in complex samples. The direct separation on chiral stationary phases of highly polar or charged metabolite enantiomers carrying hydroxy- and carboxy-functional groups and their sensitive detection requires high separation efficiency and MS compatiblity of the separation conditions. LC-MS methods for the separation of the D- and L-enantiomers of a series of chiral 2-hydroxycarboxylic acids and 2-hydroxy-glutaric acids will be presented. The enantioselective separation of D- and L-hydroxyglutarate has important applications in the area of inborn errors of metabolism as well as in cancer cell metabolism. The chiral differentiation and quantification of (S)- and (R)-2-hydroxyglutaric acids is key for characterizing neurometabolic disorders like the 2-hydroxyglutaric acidurias, which cause neurological impairment at young age [1]. The milestone discovery that cancer-associated IDH1 mutations result in a new ability of the enzyme to catalyze the NADPH-dependent reduction of 2-ketoglutarate to the oncometabolite D-2-hydroxyglutarate demonstrates the importance of chiral metabolite analysis [2].

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WPS26-48 / Dereplication of aporphine, oxoaporphine and protoberberine alkaloids from Guatteria australis by ESI IT MS

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Introduction

Brazilian medicinal plants have proved to be a rich source of compounds that might be useful for the development of new pharmaceutical agents. The Annonaceae family, comprising about 135 genera and more than 2500 species, is a large family of tropical and subtropical trees and shrubs. Members of this family are known for their edible fruit and for their medicinal properties. Previous chemical and pharmacological investigations on some species revealed the presence of bioactive compounds exhibiting pharmacological activities such as cytotoxicity against human tumor cell lines, antimicrobial, antiparasitic and antiinflammatory properties. Guatteria australis, popularly known as pindaúva-preta, is a native tree from Brazilian Atlantic Forest. Despite their importance in folk medicine, the number of Guatteria species that have been chemically investigated is still very small. In order to obtain qualitative information about of chemical composition, the dereplication of aporphine, oxoaporphine and protoberberine alkaloids by direct infusion in ESI-IT-MSsystem was applied for alkaloidal fractions of the G. australis.

Methods

Dried and powdered bark and leaves of G. australis were separately and successively extracted with n-hexane followed by

methanol, to yield hexane and methanol bark extracts and leaf extracts. TLC investigations indicated a high concentration of alkaloids in both methanolic extracts. These were subjected to an acid-base extraction to give and the CH2Cl2 alkaloid fraction (0.1 mg/μL) was directly injected into a Bruker-Daltonics Ion-Trap-ESI equipment. The experiment was performed in positive ion mode; flow rate of 5 μL/min. MS and MSn acquisition conditions were: capillary exit –83 V, nebulizer temperature 300ºC, nebulizer 30 psi; drying gas and nebulizing gas (N2) flow rate 8 L/min; HV capillary 3.5 kV; scan range 100-1000 m/z units; CID energy 30%.

Results
Analysis of MS2 spectra showed that the extract contains mainly isoquinoline alkaloids. Substances were differentiated using MS2 fragmentation, whereas mixtures of aporphine, oxoaporphine and protoberberine alkaloids were identified due to the characteristic fragmentation pattern. Using only the fragmentation keys and comparison with literature the alkaloids: palmatine, demethyl-palmatine, liriodenine and lisycamine were identified. Confirmation was further performed by the isolation of these compounds using preparative HPLC.
Conclusions: Direct insertion ESI-IT-MS of extracts of the leaf and bark of G. australis provided a reliable fingerprint of the secondary metabolites in this medicinal plant.

Novel Aspect
The dereplication study of aporphine, oxoaporphine and protoberberine alkaloids by ESI IT MS proved to be a useful tool for the analysis of isoquinoline alkaloids and to identify these substances in complex mixtures without the use of standards.

Financial support: CNPq, CAPES, FAEPEX-UNICAMP and FAPESP

WPS26-49 / UHPLC-MS analysis of damage-induced variation in metabolites in species of medicinal plants: Mikania glomerata Sprenguel and Mikania laevigata Schultz

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UNICAMP

Introduction
The use of medicinal plants and herbal medicine is being increasingly stimulated by Brazilian government policies and cough syrup based on Mikania glomerata is presently furnished by the Brazilian Health System. However, variations in the cultivation conditions of medicinal plants may result in different concentrations of active components, affecting the safety, quality and efficiency expected, but there are few studies regarding the cultivation of the two species of interest: Mikania glomerata e Mikania laevigata. We evaluated how the secondary metabolites were influenced by mechanical damage of leaves, simulating herbivory. As both species are found in the Brazilian Phytotherapic Formulary, and apparently may be used indiscriminately, they were studied in parallel.

Methods
Healthy plants grown under controlled conditions had 10% of their leaf area damaged with forceps. Control (undamaged leaves) and leaves 1, 12, 24, 48 hours and 7 days after damage were collected, lyophilized and extracted with 70% ethanol. Analysis of the extracts using a gradient of acetonitrile and water, C18 column and ESI ionization in both positive and negative ions mode was performed using a Waters Acquity UPLC-MS (TQD) equipment. The chromatograms were compared to detect differences in composition and marker ions were selected, using the Markerlynx software and manually. Significant differences in peak area were determined using ANOVA ($p<0,05$ t test).

Results
For M. glomerata reduction in the area of peaks of disaccharides and caffeoyquinic acids were observed; for M. laevigata the area of peaks of disaccharides decreased but those of caffeoyquinic acids increased. The concentration of coumarin varied between 0.9 and 3.0 ug /200mg leaves for M. glomerata and between 276.9 and 436.5 ug /200mg leaves for M. laevigata, showing a distinct difference between species. Other unidentified ions also varied and further studies are needed for their identification.

Conclusions
Differences in composition between species were greater than those induced by damage, showing that both species should not be used interchangeably. However significant changes in the metabolites were induced by damage which could affect their therapeutic activity.

Novel Aspect
Unsupervised metabolomic study of medicinal plants by UHPLCMS showed distinct differences between species and variation in composition induced by leaf damage.

WPS26-50 / Potential of high resolution mass spectrometry and additional all ion fragmentation mass spectrometry for targeted and untargeted metabolomics

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Introduction
In the last years metabolomic studies have used many different LC-MS methods that address intracellular metabolite pools either in a targeted (quantitative) or untargeted (fingerprinting) way. But a method combining targeted and untargeted approaches to accomplish a comprehensive metabolomic analysis is not yet available. Thus we aimed to develop a comprehensive method based on high resolution mass spectrometry (HRMS) and all ion fragmentation MS (AIFMS) coupled to ion pair reversed phase liquid chromatography. The applicability of this method is demonstrated for four different biological platforms (Escherichia coli, Schefferomyces stipitis, Saccharomyces cerevisiae, Glioblastoma multiforme).

Methods
A standard reversed phase HPLC setup was used for compound separation prior HRMS and AIFMS detection with an ExactiveTM Orbitrap system (Thermo Fisher ScientificTM). A 40 minutes two eluent multi-step gradient of 2-propanol and an aqueous mobile phase including tributylamine as ion pair reagent was applied. Negative ionization of metabolites was carried out via heated electrospray ionization. Data acquisition was conducted via full scan mode and an alternating AIF scan of fragments released from a higher-energy collisional dissociation cell. Both scans can be generated within 750 msec. Appropriate software packages were used for processing of targeted and untargeted data. Biological samples were prepared from cells grown at midexponential phase in mineral medium under anaerobic conditions on glucose as sole carbon source.

Results
HRMS and alternating AIFMS generated enormous data sets (500-800 mbytes/sample) including valuable information for targeted and untargeted metabolomics. Metabolites of central carbon metabolism, amino acids, energy metabolites and redox cofactors were quantified applying the established method. Common chromatographic issues, such as hexose phosphates isomer separation, were addressed over specific AIF fragments.

As HRMS and MS/MS (generated by AIF) information for all metabolites was covered, no SRM scans for metabolites of interest were required. Beside the targeted approach the dataset also enabled untargeted metabolic fingerprinting. Usability of the developed method was successfully approved for all tested biological platforms.

Conclusion and novel aspects
Ion pair HPLC coupled to HRMS using an alternating sequence of full scans and AIF scans is a valuable tool to gain simultaneously comprehensive targeted and untargeted metabolomics data. This new method also demonstrates a broad range of application for diverse biological platforms.

WPS26-51 / UHPLC-HRMS metabolomics as a tool to decipher complex chemotaxonomic relationships in plants: the case of the Gentianaceae

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Introduction:
Metabolomics is emerging as powerful complementary tool to DNA analysis to assess phylogenetic relation within plant species. The Gentianaceae, a cosmopolitan family of over 1700 species and 99 accepted genera, has been taxonomically well studied and several molecular phylogenies at the family level are available. However, representatives of only four tribes (out of six) have been chemically investigated. Furthermore, until recently, phytochemical investigations have focused on single species hindering the uses of metabolomic as a tool to classified taxa.

Method
A rapid protocol based on UHPLC-HRMS allowing metabolomic comparison of related taxa in Gentianaceae was developed. The method was optimised based on a careful evaluation of column geometry and temperature to provide sufficient LC resolution and high throughput. Metabolite profiling was tested using five Chironia and seven Exochaenium species obtained from both herbarium samples of different ages and standardized sample. Additionally, representatives of twenty genera of Gentianaceae were investigated for their chemotaxonomic characteristics. Each of the investigated species had at least three to six biological replicates.

Results
Following ball and mill extraction of only 50 ± 2 mg and sample pre-treatment with solid phase extraction, a rapid UHPLC-HRMS fingerprinting protocol was developed to analyse methanolic extracts. In total more than 700 metabolites profiles were recorded generating about 4000 features that were finally reduced to ca 330 after a filtering and validation procedure. As an example principal component and hierarchical clustering analysis revealed a clear separation within the five Chironia species. Interestingly, Orphium frutescens was clustered together with Chironia congruent with recent finding based on molecular phylogeny that places Orphium within the genus Chironia. Similarly, Exochaenium species were independently clustered. Furthermore, the metabolite profiles suggest the presence of two chemotypes within one single species (Exochaenium baumianum). This result is surprising regarding the biological homogeneity of the species and highlights the usefulness of metabolomics to pinpoint botanical discrepancies.

Conclusions
The developed HRMS based profiling approach allows the distinction of closely related taxa and the metabolomic result were congruent with taxonomical and phylogenetical analyses. Interestingly good correlations were obtained regardless of the sampling method used (i.e. herbarium sample or silicagel stored material).

Novel aspect
Our results have implication (i) for the systematic search of new secondary metabolite and (ii) as a potential chemotaxonomic tool for the rapid identification of closely related taxa with sample amount that are compatible with the high throughput analysis of limited amounts of herbarium sample.

WPS26-52 / A novel approach for acquiring and processing LC-MS metabolomics data

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Introduction
Liquid chromatography coupled with mass spectrometry is routinely used in metabolomics applications to measure the level and variation of metabolites within biofluids. Data generated through these studies may yield insight into fundamental biological processes as well as being used to differentiate disease onset and progression from healthy individuals. LC-MS based metabolomics generates large and complex data sets on highly complex biological fluids. As such improvements in MS performance and data acquisition, analysis and interpretation are required. In this paper we will detail advances in MS performance and data handling, including data processing and advanced multivariate approaches, being described for the analysis of large scale metabolomics datasets.

Methods
Urine from a healthy individual was centrifuged and the supernatant diluted. The urine was divided into control, low dosed (LD) and high dosed (HD) groups. To create a sample set, 11 different drugs were differentially spiked into LD and HD urine, contrasted with blank urine. A reversed phase gradient was applied and MS data acquired in positive ion LC-MS mode on a quadrupole orthogonal acceleration time-of-flight mass spectrometer.

Results
Distinguishing biological variation and metabolic change from analytical interference is key to data processing and analysis. Samples were randomized and measured six times, including QC runs, to ensure statistically valid analysis. LC-MS data were retention time aligned and deconvoluted to produce a feature list. Identified features were compound searched and interrogated with multivariate statistics to provide marker ions of interest. Relative high abundance levels of the standards were reported for LD and HD compared to controls, confirmed by trend plots analysis showing an increase in LD and HD groups compared to control. The standards were identified with an average score of 91 and mass error of 1.2 ppm. Three sample clusters were produced with the standards being the most differentiating features (top 20 based on q value) between groups. Functionality of the software will be demonstrated using biological samples. A comparison of LC-MS data obtained from different MS instrument platforms will be presented

Conclusion
Modern state-of-the-art LC-MS systems coupled with advanced data processing and multivariate statistical tools provides a comprehensive tool for probing the metabolome of biological samples.

WPS26-53 / Quantitative metabolomics using isotope-labeling, differential analysis and RP-LC-HRMS: Investigation of metabolic perturbations in a cellular model of cancer

Michel Wagner, Leanne Ohlund, Tze Chieh Shiao, Amelie Vezina, Borhane Annabi, Rene Roy, Lekha Sleno
UQAM - Department of chemistry

Introduction

One of the main limitations of typical metabolomics workflows is metabolite quantitation, because it relies on using a set of internal standards not optimized for every compound in an untargeted analysis. We propose an alternative strategy based on isotope-labeling, which allows relative quantitation for all metabolites between two sets of samples. Each set of samples is derivatized with a reagent available in two isotopic forms. With RP-LC-MS analysis, the resulting metabolite derivatives co-elute and their signal ratios track metabolite concentration changes in samples. This strategy was used to determine metabolite perturbations occurring with a cellular model for cancer, namely HL60 cells cultured under hypoxic conditions with or without serum present in the culture media.

Methods

HL60 cells were cultured under four conditions (n = 6 for each): normoxia (control) and hypoxia (cancer model), with or without serum. Metabolites were extracted, and labeled with N-benzoyl oxysuccinimide (figure 1), available in 12C6 and 13C6 versions for differential analysis. Derivatized samples were mixed together (12C6/13C6, 1:1) and analyzed by LC-MS, using a C18 column for RP-HPLC and a high resolution QqTOF-MS/MS (AB Sciex 5600 TripleTOF). MS/MS experiments were automatically triggered to support structure elucidation. Automated peak picking was done using a custom differential filtering tool within MetabolitePilotTM. Data were inspected with PeakView®, followed by peak area integration for quantitation using MultiQuantTM.

Quantitative metabolomics using isotope-labeling, differential analysis and RP-LC-HRMS: Investigation of metabolic perturbations in a cellular model of cancer

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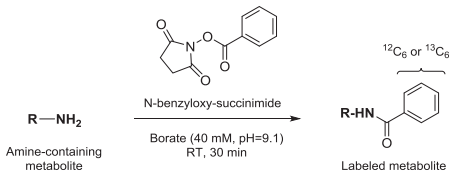
Figure 1. Labeling reaction

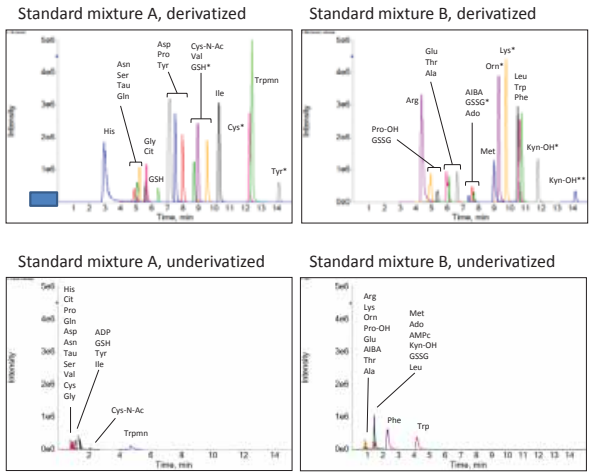


Results

Derivatization of standard mixtures of 34 model metabolites showed that amine- and phenol-containing metabolites compounds are effectively derivatized. The strategy allowed increasing the hydrophobicity of metabolites and analyte signal responses (figure 2).

The workflow used for differential analysis of cell extracts is presented in figure 3. Peak picking resulted in a list of 431 peak pairs (12C6/13C6) that exhibited the same retention time and a m/z difference of 6.0201 u (singly labeled) or 12.0602 u (doubly labeled). Only meaningful signals were used for data processing and redundant signals (e.g. sodium and potassium adducts, dimers, doubly charged species) were systematically removed. This resulted in a list of 301 features, for which peak area ratios (13C6/12C6) were determined and compared across samples (figure 4).

Figure 2. Impact of derivatization on retention time and signal intensity



* : doubly derivatized metabolite. ** : triply derivatized metabolite

Model metabolites:
2-Aminoisobutyric acid (AIBA), N-acetyl-cysteine (NAC), adenosine (Ado), adenosine 5'-diphosphate (ADP), adenosine cyclic 3',5'-monophosphate (AMPc), alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), citrulline (Cit), cysteine (Cys), glutamic acid (Glu), glutamine (Gln), glutathione (GSH), glutathione disulfide (GSSG), glycine (Gly), histidine (His), hydroxykynurenine (Kyn-OH), hydroxyproline (Pro-OH), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), ornithine (Orn), L-phenylalanine (Phe), L-proline (Pro), serine (Ser), taurine (Tau), threonine (Thr), tryptamine (Trpmn), tryptophan (Trp), tyrosine (Tyr) and valine (Val)

Figure 3. workflow used for differential analysis of cell extracts

Sample analysis

1. ***Individual samples****: control/control and cancer/control ($^{12}C_6$/$^{13}C_6$) cell extracts*
2. ***LC-MS/MS analysis***

↓

Data processing

1. ***Peak picking*** *(MetabolitePilot)*
2. ***Pair finding*** *(MetabolitePilot add-in)*
   LC-MS peak pairs with same RT and a 6.02 or 12.04 amu difference
3. ***Export and anotate results in an Excel speadsheet***
   Remove Na+ and K+ adducts, multiply charged species, multimers

↓

Quantitation & statistical analysis

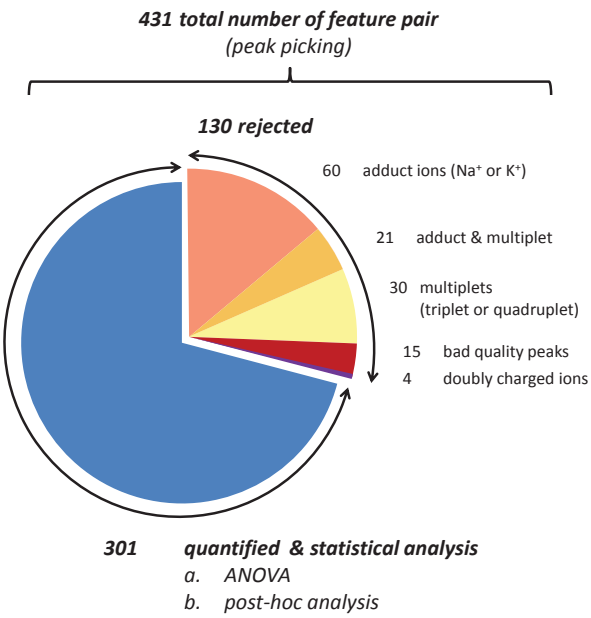
1. ***Integrate peak areas of remaining features*** *(MutliQuant)*
2. ***Determination of discriminating features***
   a. ***ANOVA***
   b. ***Post-hoc tests***

↓

Structure elucidation

1. ***Determination of the underivatized monoisotopic mass of discriminating features***
2. ***Database search*** *(e.g. HMBD)*
3. ***Refine with MS/MS*** *(from IDA experiments)*
4. ***Comparison with reference standards***

Figure 4. Data processing:
Number of feature pairs identified at various steps of the workflow



Conclusions
A novel isotope-labeling strategy was developed and used successfully for differential analysis of cell extracts. Statistical analysis of discriminant features, using ANOVA and post-hoc testing, and structural elucidation are underway to determine the metabolic perturbations involved in the cellular model of cancer.

Novel Aspect
Application of a novel isotope-labeling strategy for untargeted, differential analysis of biological samples, including statistical analysis of data.

WPS26-54 / UPLC/MS method for determination of panel of neurotransmitters in rat cerebrospinal fluid: application to the rat model for tauopathy

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Introduction
Alzheimer's disease (AD) is most common neurodegenerative disease. Currently, there is no cure and early preclinical diagnostic assay available for AD. Therefore much attention is now being directed at the development of novel methods for quantitative determination of AD biomarkers in the cerebrospinal fluid (CSF). Here, wedeveloped and validated the liquid chromatography-tandem mass spectrometry method for determination of 5-hydroxytryptamine (SER), 5-hydroxyindoleacetic acid (5-HIAA), homovanilic acid (HVA), noradrenaline (NADR), adrenaline (ADR), dopamine (DA), glutamic acid (Glu), γ-aminobutyric acid (GABA), 3,4-dihydroxyphenylacetic acid (DOPAC) and histamine (HIS) in cerebrospinal fluid (CSF) from the rat model for human tauopathy.

Methods
Neurotransmitters and metabolites were derivatized with benzoyl chloride and analysed on ultra-performance liquid chromatography (UPLC) on C18 column in combination with tandem mass spectrometry. For optimal separation the gradient elution program was established by use of ammonium formate with formic acid as mobile phase A and acetonitrile as mobile phase B.

Results
The method is very simple, highly sensitive and showed excellent linearity with regression coefficients higher than 0.99. The accuracy was in a range of 93-113% for all analytes. The intra-day precision (n=6), expressed as %RSD, was in the range 2-12% for all analytes. The inter-day precision (n=5 days), expressed as %RSD, was in a range 2-10% for all analytes. The method was subsequently used for analysis of CSF samples from transgenic rat model for tauopathy. We detected significant changes of CSF levels of two important neurotransmitters/metabolites, ADR and 5-HIAA, which correlates with progression of neurodegeneration in our animal model.

Conclusion
In this study, we developed and further validated new UPLC/MS method using a benzoyl chloride as pre-column derivatization reagent for the simultaneous determination of 10 NT and metabolites in rat CSF. The developed method is easy, highly sensitive and requires only small volume of CSF sample. The concentrations determined by the current method are in the same order of magnitude as in the literature. Using this method we analysed neurotransmitters and metabolites in the CSF of transgenic rat model for human tauopathy.

Novelty
Transgenic rats expressing human truncated tau, developed in our laboratory displayed several AD characteristic features which are important in a process of tauopathy. Transgenic rats offer another advantage over other transgenic AD models, such as mice – easy and reproducible collection of CSF from single animal. Therefore we took this advantage and in the current paper we for the first time looked how tau cascade affects several important neurotransmitters and metabolites.

WPS26-55 / Influence of mass spectrometry resolution on metabolite coverage in plasma

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Introduction
Nowadays metabolite identification is one the most crucial step in approach of untargeted metabolomics where important/discriminating compounds are picked up first and afterwards identified. To confidently identify compound, comparison of two orthogonal properties (e.g. retention time, spectra) with commercial standard should be used according to Metabolomics Standards Initiative. However not all compounds are commercially available. Thus most of the compounds are identified as putatively annotated compounds based upon comparison of physicochemical properties, spectral similarity with publicly available libraries. High resolution accurate mass MS and MSn is a very useful tool in metabolite identification, but has its limitations. Higher resolutions requires usually higher scanning times and thus less data points across the peak. The aim of this work is to find what resolution is sufficient for metabolomics analyses and determine number of possible overlapping compounds. This was addressed by in silico calculations and analyzing human plasma by LC-HRMS at different resolutions.

Experimental
In silico calculations based on mzMine database ontaining 41 448 metabolites were run in R software to see which groups of metabolites are experiencing most of these "overlaps". Analyses of the same sample can deliver different number of compounds when using various resolutions. R package Rdisop was used to calculate isotopic patterns and monoisotopic masses from chemical formula of each compound. Afterwards the common

adducts were added to the list ([M+NH4]+, [M+K]+...). These models were calculated for increasing resolutions from 15k up to 3840k in both – positive and negative mode.
To evaluate these models LC-MS of one identical plasma sample were run on Orbitrap Elite (Thermo Fisher Scientific, CA, USA) from 15k up to maximum resolution of the instrument of 480k. The XCMS package in R and Thermo Excalibur 2.2 SP1.48 (Thermo Fisher Scientific, CA, USA) were used for data evaluation.

Results
By filtering metabolites (in silico) from mzMine we get 15 711 unique masses without isobars with range of masses from 70 to 2000 Da. This number stands basically for maximum amount of peaks visible in mass spectrum representing real metabolites. In lower resolution (15k) this number is significantly smaller of approximately 6000 possible peaks. This is caused by "fusion" of several peaks in one. Using in silico calculations, this set showed that from resolution of 240k the number of distinguishable peaks is not rising. In real plasma samples this plateau was observed at 60k – 120k resolutions in LCMS experiment, in both positive and negative. The maximum number of compounds observed in positive mode in mass range 70 – 2000 m/z was 4100 peaks.

Conclusion
Above resolution of 60k there was no significant change in number of detected peaks, suggesting that resolution above 60k is sufficient for metabolomic experiment.

Acknowledgements
Infrastructural part of this project (Institute of Molecular and Translational Medicine) was supported from the Operational program Research and Development for Innovations (project CZ.1.05/2.1.00/01.0030).

WPS26-56 / Molecular insight into the postoperative state of diabetic patients

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The aim of the presented clinical study is to clarify on a molecular level the impact of bariatric surgery treatment on patients who suffer from diabetes mellitus type 2 (T2DM). Along with monitoring of levels of well-known biomarkers of inflammatory processes the other important task has been to identify new potential biomarkers specific for postoperative state of patients with T2DM.
Newly developed methods of analysis taking advantage of combination of high performance liquid chromatography with high-resolution mass spectrometric detection have proven to be an ideal choice for analyses of complex matrices such as urine or blood plasma. The levels of biomarkers of inflammatory processes (aliphatic aldehydes, 8-isoprostane, cysteinyl leukotrienes, o-tyrosine, 3-chlorotyrosine, 3-nitrotyrosine, 8-hydroxyguanosine, 5-(hydroxymethyl)uracil, 8-hydroxy-2'-deoxyguanosin) were significantly elevated speaking of increase as high as 80% in the case of LTB4 and 8-isoprostane. There have been identified structures already associated with diabetes such as L-3-methylhistidine (muscle tissue degradation metabolite) or 2»-Deoxy-N-methyladenosine (insulin excretion signal molecule) as well as novel structures corresponding with the state of the patients, e.g. 2'-deoxy-5'-inosinic acid (hypoxanthine nucleoside, which possibly triggers DNA replication errors), symmetric and asymmetric dimethylarginine (kidneys damage markers), anserine and homocarnosine (endogenic dipeptide antioxidants), acetyl-N-formyl-5-methoxykynurenamine (oxidative stress preventer), 17-octadecynoic acid (inhibitor leukotriene B4 20-hydroxylase and renal CYP450 ω-hydroxylase). Despite the general success of bariatric surgery in the treatment of T2DM the levels of inflammatory processes biomarkers have proven to be significantly elevated even after six months from performed surgery. Some of the novel potential biomarkers exhibit the elevated values as well.
The proper molecular insight into the postoperative changes in metabolic pathways may define a new approach to the treatment of diabetic patients. Deep knowledge of the effects of bariatric surgeries represents the key to the effective and successful treatment making a promise of full life of the patients.

Acknowledgments
This work was financially supported by the EU structural funds – "Operational Programme Prague – Competitiveness" (Grant CZ.2.16/3.1.00/22197), by the Ministry of Education, Youth and Sports Czech Republic in program "National Programme of Sustainability I" – NPU I (LO) (Grant No.: MSMT-34807/2013) and, by the Ministry of Health Czech Republic (grants No. NT 13299).

WPS26-57 / Metabolomic Profiling of Anionic Metabolites in Oral Cancer Cells by Capillary Ion Chromatography HR/AM Mass Spectrometry

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Introduction
Metabolomics approach has obtained increasing attention in oral cancer study. The Cap IC has demonstrated outstanding separation for anionic polar metabolites. Here we couple the capillary ion chromatography (Cap IC) with a benchtop quadrupole Orbitrap mass spectrometer, and compare the separation performance of Cap IC on untargeted metabolic profiling in oral cancer cells and polar metabolites characterization to RPLC and HILIC methods.

Methods
Anionic polar metabolites were used to optimize the MS conditions, Cap IC gradient and settings for minimal sample consumption and high analytical sensitivity.
Three OSCC cell lines, UMSCC1, UMSCC5, cancer stem-like cells (CSC), and according wild-type controls with biological replicates were harvested and counted. Cellular metabolites were extracted. IC was run at 25µL/min using an electrolytic suppressor to convert potassium hydroxide gradient to pure water. HILIC and RPLC methods were run at flow rates from micro flow to high flow.
Differential analysis was performed using Thermo Scientific™ SIEVE 2.1 and metaXCMS online. Components of interest (p-value<0.05, fold change>2) were identified using high resolution accurate mass, MS/MS, and retention time.

Results and Conclusions
Compared to RPLC and HILIC experiments, Cap IC had 100-fold increased analytical sensitivities. The detection limits for 40 standard metabolites ranged from 0.2 to 0.8 nmol/L (0.2 and 3.4 fmol) at S/N of 3. The inter day (n = 5) relative standard deviation (RSD) of retention time and intensity were below 8%.
Cap IC demonstrated outstanding separation and peak shape for anionic polar metabolites. Monophosphates and diphosphates with up to 10 isomeric species for a single m/z were well separated by Cap IC, as compared to a single peak or unsolved peaks in both high- and micro-flow rate RPLC and HILIC methods.
Data from three OSCC cell lines UM1, UM5, CSC were analyzed using SIEVE 2.1 and metaXCMS online. Numbers of feature with significant change (p-value<0.05, fold change>2) are 4597 for UM5 KD vs. WT; 2856 for UM1 KD vs. WT and 3861for cancer stem cells (CSC) vs. control. The common feature number for all three is 218.

Novel Aspect
Cap IC was successfully coupled to HR/AM MS for untargeted metabolic profiling in oral cancer cells and polar metabolites characterization

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WPS26-59 / Large-scale metabolomics & lipidomics to discover biomarkers of healthy aging and personalizing medicine
Thomas Hankemeier
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Introduction
New biomarkers revealing disease mechanisms are urgently needed as yet cardiovascular disease remains a leading cause of mortality, but despite detailed knowledge of its pathogenesis, half of the cardiovascular events occur in persons without known risk factors, and we still cannot predict and individual's disease risk, progression and therapeutic response accurately. Large-scale metabolomics studies are required to discover and validate biomarkers personalized medicine.

Methods
We have developed LC-MS and LC-MS/MS methods for analyzing more than 2000 metabolites and lipids in urine and plasma samples. Extensively used methods are the analysis of lipids with RPLC-qTOFMS, the analysis of biologically relevant lipid mediators with RPLC-MS/MS and of biogenic amines after derivatization with RPLC-MS/MS.
The lipids are quantified via a target list or, alternatively, with an in-house developed algorithm, mzExtract. A range of internal standards and a QC/QA procedure are applied to correct for possible shifts in sample preparation, chromatography and MS detection. For the biogenic amines and lipid mediators, absolute concentrations are reported. For the lipids, only relative responses are reported as reference compounds are not available for most lipids. An algorithm was developed to merge lipid data sets of cohorts measured at different moments in time.

Results
The established metabolomics platforms, data handling and processing protocols delivered robust data for studies with 100's and 1000's of samples; the repeatability and reproducibility expressed at RSD was generally better than 15% if concentrations were enough above the LOQ.
To increase the power for biomarker discovery, the lipid data of three cohorts of each 2500-3000 persons were combined using transfer samples and a novel algorithm. It is demonstrated that combination of LC-MS data sets is possible even when no absolute concentrations have been determined. This study allowed the identification of lipid biomarker for healthy aging. In another example biomarkers for the response to cardiovascular treatment of diabetes patients are shown.

Conclusions
The developed metabolomics methods proved to be robust and suitable for large-scale metabolomics and discovery of explorative biomarkers. The increase in the throughput of metabolomics by innovative sample pretreatment methods such as electroextraction will be discussed.

Novel Aspect
A novel data preprocessing procedure is shown in combination with a QA/QC pipeline. Lipid data of different studies were combined using so-called transfer samples and a novel algorithm. Biomarkers are reported for healthy aging and for cardiovascular treatment of diabetic patients. Finally, new emerging modules for high throughput metabolomics will be discussed.

WPS26-60 / Combining Raman microscopy and LESA-HR mass spectrometry to identify and image metabolites produced by Schizophyllum commune in fungal co-cultures
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Schizophyllum commune, the renowned white-rot fungus produces secondary metabolites in the form of pigments, etc. when accosted by various white-rot fungi in nature. These fungal interactions may be mediated at a distance or by direct contact resulting in physiological responses like cessation of mycelial extension, barrage formation, increased secretion of enzymes, or pigmentation. Such compounds, produced dynamically, are of potential interest as new leads for drug discovery. Because of the complexity of microbial extracts, advanced analytical methods are key for the successful detection and identification of co-culture-induced metabolites.
Here, we employed two analytical techniques, liquid extraction surface analysis (LESA) combined with high-resolution mass spectrometry (HRMS) and Raman mapping, to directly examine and localize the metabolites produced by S. commune upon interaction with another wood-decay fungus Hypholoma fasciculare. LESA is a nanoelectrospray-based, highly reproducible extraction infusion technique for mass spectrometry. Confocal micro-Raman spectroscopy allows for direct imaging combining Raman spectroscopy with light microscopy.
To investigate the interaction between S. commune and H. fasciculare, agar plate based confrontation assays were performed. In self-paired cultures the fungi did not exhibit induction of pigmentation, while coloured substances and discolouration of the medium was observed when S. commune interacted with H. fasciculare. A greenish blue/sometimes black pigment developed at the bottom of the plate within the domain occupied by S. commune at 12 h post contact. The intensity of this pigmentation increased with the duration of contact and was visible in the interaction zones of the fungi. Raman mapping was performed on selected hyphae growing in the zone of interaction by recording the Raman spectra. Based on the spectral information and VCA, it was discovered that the pigment indigo was present in the interaction zone and localized only in S. commune hyphae. LESA-HRMS was performed on the confrontation assay plates directly from the surface of the agar. Analyzing the interaction zone, a mass signal corresponding to the pigments indigo, indirubin and isatin were observed. Indigo was distinguished from the isobaric indirubin by implementing UHPLC-ESI-MS.
Thus, we showed that both techniques are efficient tools to identify compounds which have a relatively low solubility in water and organic solvents, necessitate only low invasion and are label-free applications requiring minimal sample preparation. Therefore, they are well suited for biological applications. The applied techniques open up new avenues for further investigations of fungal interactions with co-occurring organisms to obtain more information on their mechanisms, as targets for drug development, or plant protection agents.

WPS26-61 / Molecular Diversity and Body Distribution of Saponins in the Sea Star Asterias rubens by Mass Spectrometry
Marie Demeyer, Pascal Gerbaux
University of Mons

Introduction
Saponins are natural molecules that the common sea star Asterias rubens produces in the form of steroid glycosides bearing a sulfate group attached on the aglycone part. These molecules reveal a large chemical diversity and different biological activities. The

generalobjective of this project is to link specific saponins with selected biological activities of the starfish Asterias rubens. In this context, we determined the saponin inter-organ variability by mass spectrometry, 5 organs have been selected for the present work.

Methods
Amongst all the available analytical methodologies, mass spectrometry is definitely a first-choice technique for tackling the large diversity of saponins in echinoderm tissues. MALDI-ToF experiments were selected as the primary tool for a rapid screening of the saponin mixtures, whereas LC-MS(MS) techniques were used to achieve chromatographic separation of isomers. Spatial distribution of saponins within the organs of A. rubens has then be probed by MALDI Imaging analysis (University of Lille, Laboratoire de Spectrométrie de Masse Biologique, Fondamentale & Appliquée).

Results
First of all, on the basis of all the collected MSMS data,our analyses demonstrated that the diversity of saponins is higher than previously reported [1]. Secondly, the comparison of the saponin contents from the five body components revealed that each organ is characterized by a specific mixture of saponins and that between animals there are also qualitative and quantitative variability of the saponin contents which could be linked to the sex or to the collecting season [2]. Thirdly, the MALDI Imaging analyses allow to localize saponins on a cross-section of sea star arm with a good resolution. Particularly, these molecules are preferentially localized in the surface layers (i.e., mucus layer) of the body wall and tube feet. This distribution, together with the lack of saponins in the fooprints, suggests that saponins would be involved in defense rather than in locomotion in the sea star Asterias rubens.

Conclusions
The observed high variability of the saponin contents, in terms of organs and animals, unambiguously confirms that saponins probably fulfill several biological functions in A. rubens. The results of the present report will pave the way for our future studies that will be devoted to the clarification of the biological roles of saponins in A. rubens at a molecular level.

References
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Wednesday

WPS26-62 / High Sensitivity Analysis of Metabolites in Serum Using Simultaneous SIM and MRM Modes in a Triple Quadrupole GC/MS/MS

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Introduction
Gas chromatography/ mass spectrometry (GC-MS) is an accepted technique for metabolomics analysis because of the chromatographic separation, reproducible retention times, and sensitive and selective mass detection. However, unambiguous identification can be difficult in the presence of a complex matrix. To improve the mass spectrometric selectivity, Multiple Reaction Monitoring (MRM) mode in GC/MS/MS is used. The MRM mode is a powerful technique which creates product ions from a single precursor ion during collision-induced dissociation (CID). However, some compounds with low CID efficiency produce insufficient product ions for MRM transitions, and the MRM mode is consequently less sensitive than SIM for these compounds. In this study, SIM, MRM, and simultaneous SIM/MRM modes are evaluated for analysis of metabolites in human serum.

Methods
A human serum sample was extracted using 250 uL of methanol / water / chloroform (2.5:1:1) and the supernatant was subjected to methyloximation and trimethylsilylation (TMS) derivatization prior to analysis. The work present here was performed on a GCMS-TQ8030 (Shimadzu, Japan) equipped with a 5%-phenyl-methylpolysiloxane capillary column (30 m x 0.25 mm ID x 1.0 um film) with helium carrier gas. The SIM, MRM and simultaneous SIM/MRM analysis modes (SIM/MRM) in GC/MS/MS were used for analysis of metabolites in human serum and the results compared. The methods provided targeted analysis of 186 metabolites, and were created using the GC/MS Metabolites Database Ver.2 (Shimadzu, Japan).

Preliminary Data
Analytical results from the SIM and MRM modes identified 116 and 146 metabolites, respectively. In SIM mode, interfering matrix co-eluted with some peaks, and these compounds could not be detected. On the other hand, the MRM mode separates masses in two stages, making this technique significantly more selective than SIM. As a result, the compounds, which could not be measured by SIM, were easily detected using the MRM mode. However, the results using MRM produced signal intensity approximately 10 times lower than SIM for selected compounds, e.g. S-Benzyl Cysteine-4TMS, because of poor CID efficiency, even when these compounds were easily detected by SIM. To solve this problem, the compounds which had insufficient sensitivity in MRM mode were measured by SIM, and the remaining compounds were measured by MRM in a single analysis using simultaneous SIM/MRM. Comparison of the results from MRM and SIM/MRM analyses, revealed detection of 146 and 156 metabolites, respectively. Six replicate analyses using the SIM/MRM method produced relative standard deviation (RSD) of less than 20% for 126 compounds, confirming presence of the targeted metabolites. The MRM mode by itself confirmed fewer metabolites, 116, with RSD less than 20% because ten compounds had poor CID efficiency and were not detected using the MRM mode alone.
The results demonstrate that simultaneous SIM and MRM analysis modes, SIM/MRM, improves the sensitivity and reproducibility for analysis of metabolites in human serum compared to MRM alone, and expands the utility of triple quadrupole GC/MS/MS for this type of analysis.

WPS26-63 / Myth Busters: The Truth About Metabolomics &Gas Chromatography-High Resolution Time-of-Flight Mass Spectrometry

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Introduction
In recent years, there has been a significant increase in the number of publications associated with metabolomics. Gas chromatography-time of flight mass spectrometry is still the “Gold Standard” for metabolomic profiling of blood, plasma, urine, and plant extracts. While the best approach for comprehensive profiling of samples is a combination of analytical techniques, gas chromatography-high resolution time-of-flight mass spectrometry offers desirable analytical characteristics such as robust data acquisition, speed, effective peak deconvolution, high quality spectral data for database comparisons and excellent mass accuracy values (< 1 ppm) for confident formula determinations. In addition, high resolution mass spectrometry reduces interferences and is not limited to monitoring specific ions during spectral acquisitions but rather provides a profile from a single injection.

Methods
A combination of EI-HRT and CI-HRT (Reagent Gas = 5% Ammonia in Methane) data was collected to obtain profiles of polar and nonpolar compounds with molecular weights approaching 1000 Da. Sample components were derivatized using an optimized two-step procedure: 1) Treatment with methoxylamine hydrochloride and 2) MSTFA. Compounds were separated using an Rxi-5MS column and detected with a high resolution time-of-flight mass spectrometer operating at a resolution of 25,000 (m/z =219). System performance was monitored using internal standards (e.g., octafluoronaphthalene and fatty acid methyl esters). After untargeted data processing using the automated peak find capabilities of the ChromaTOF HRT software, formula and spectral similarity searches using commercially available libraries facilitated confident identification of analytes in complex samples.

Preliminary Data
The workflow resulted in the identification of hundreds of compounds in plasma and plant extracts. Compound characterization was facilitated through effective peak deconvolution of data that was then matched to well-established databases. Compounds identified included acids, diacids, amino acids, sugars, fatty acids and sterols. Mass accuracy values of 1ppm or better were obtained for fragment, molecular, quasi-molecular ions and adducts. The methodology was particularly useful for the identification of difficult to characterize monosaccharides where characterization via retention indices and database searches were inconclusive.

Novel Aspect
A robust workflow using complementary ionization techniques and high resolution mass spectrometry is applied to metabolomic profiling

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WPS26-64 / Sensitivity improvement in negative mode electrospray ionization mass spectrometry using 2-(2-methoxyethoxy)ethanol (2-MEE) for non-targeted metabolomics

Wendelin Koch[1], Sara Forcisi[1], Rainer Lehmann[2], Philippe Schmitt-Kopplin[1]
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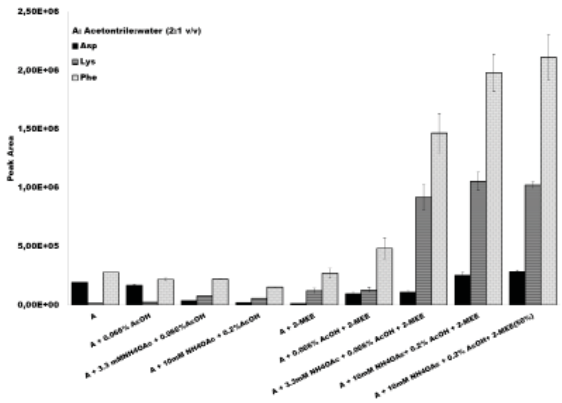
Introduction
In order to obtain robust, valid and efficient separations in liquid chromatography (LC) pH control of mobile phase needs to be assured. Nowadays in reversed phase (RP) analyses pH control is mostly achieved by acidification with formic or trifluoroacetic acid, especially when coupling to mass spectrometry (MS) via electrospray ionization (ESI) is applied. In the expanding field of Hydrophilic liquid chromatography (HILIC), application of volatile buffers such as ammonia acetate (NH4OAc) is far more frequent compared to RP. While increasing HILIC efficiency on the one hand NH4OAc is known to concomitantly lead to ion suppression in (ESI-MS) detection on the other hand. In literature it was shown that post-column infusion of 2-(2-methoxyethoxy) ethanol (2-MEE) is capable to compensate this negative side effect of NH4OAc buffered solvents in negative ESI mode. The gaining importance of HILIC separation methodology in research and pharmaceutical applications encouraged us to a deeper evaluation of the effects of 2-MEE on negative ESI.

Methods
A systematic setup of direct infusion and HILIC post-column infusion experiments was established in order to investigate the beneficial effects of 2-MEE. To elucidate the 2-MEE dopant mechanism, metabolite standards were analyzed. In order to confirm the obtained results, a urine sample was analyzed with and without post-column infusion of 2-MEE in a non-targeted HILIC-MS metabolomics approach. In addition, biological samples were spiked with stable isotope labeled standards and analyzed with post-column infusion HILIC-MS to evaluate the impact of matrix effects on 2-MEE post-column infusion systems.



Results
In this work 2-MEE does not only show a compensating effect for ESI suppression derived from NH4OAc application. The use of NH4OAc in combination with post-column infusion of 2-MEE, outperforms the analysis without any modifier. Besides an analyte structure specific response concerning signal intensities was revealed when 2-MEE is applied. 2-MEE especially improves the ESI response for small and polar molecules of low acidity. Signal intensities of stable isotope labeled amino acids spiked into biological matrices increases up to 50-fold (i.e. D5-L-glutamic acid). The ESI dopant effect of 2-MEE is supposedly caused by the formation of smaller ESI droplets and stripping of positive charge from ESI droplets due to evaporation of acetic acid anions.

Conclusion
Especially in targeted and non-targeted metabolomics studies maximum detection sensitivity is essential. HILIC separation techniques generally address highly polar and smaller metabolite molecules. Here the application of NH4OAc buffered eluents and post-column infusion of 2-MEE can offer a valuable improvement.

Novel Aspects
The dopant mechanism of 2-MEE was systematically investigated and an analyte structure dependent behavior has been revealed. An extended dopant mechanism is suggested compared to previously published ones.

WPS26-65 / Integrated analytical platform including automated Bligh and Dyer extraction and dual-column UHPLC-MS/MS separations for metabolomic analyses of cells extracts

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Introduction
Sample preparation in metabolomic studies require generally a manual Bligh-Dyer extraction. This step is cumbersome but necessary to separate the aqueous fraction containing polar endogenous metabolites from the organic fraction containing apolar compounds.
We propose to integrate an automated Bligh-Dyer extraction on a robotic system including a dual-column UHPLC-MS/MS platform for the metabolomic analysis of cells extracts of Chlamydomonas reinhardtii. The aqueous fraction is split and analysed on a C18 column with two mobile phase pH values, alternately with the lipidic fraction analysed on a C8 column.

Method
The Bligh-Dyer extraction was performed with a PAL RTC system (CTC Analytics) equipped with a vortex mixer, a centrifuge and two injection ports. Two quaternary low-pressure Nexera LC30AD UHPLC pumps (Shimadzu) were used for the dual-column separation. A TripleTOF® 5600 (AB SCIEX) was operated in positive or negative ESI with TOF autocalibration. Each aqueous diluted fraction was analysed on a 100x2.1mm XBridge BEH C18 XP column (Waters) alternately with a pH3 and a pH8 mobile phase. Organic fractions were evaporated, reconstituted and injected onto a 150x2.1mm XBridge BEH C8 XP column. MS/MS data were acquired in SWATHTM mode.

Results
1mL of algae was mixed with 1mL of cold methanolic solution to quench the metabolic activity. Cells were then pelleted, flash-frozen and disrupted with cryogenic grinding. A solution of water-methanol-chloroform (0.8/2/1,v/v) was added and the supernatant was transferred in a glass vial. The sample preparation platform was used to perform the Bligh-Dyer extraction by adding 225µL of water and 225µL of chloroform. After vortex-mixing and centrifugation, 0.5mL from the upper aqueous phase was aspirated and transferred in a fresh vial. An aliquot was diluted 5-fold to reduce the organic sample solvent concentration and 25µL was injected onto the C18 column running with an acidic mobile phase gradient of 20min. In parallel, 0.25mL of the lower organic phase were aspirated and transferred into a glass vial for evaporation under a gentle nitrogen flow provided by a dedicated tool of the RTC platform. After evaporation and reconstitution, 5µL were injected onto the C8 column and analyzed on the second UHPLC system during the washing and conditioning steps of the first column with an alkaline mobile phase. Then, a second aliquot of the aqueous fraction was diluted and separated on the C18 UHPLC system during the reconditioning of the C8 column. High resolution MS2 spectra were acquired in a SWATHTM mode and subsequently searched against annotated high resolution spectral libraries.

Conclusions
Overlapping the sample preparation with chromatographic separation could increase the analytical throughput and showed similar performance but improved repeatability than the manual approach.

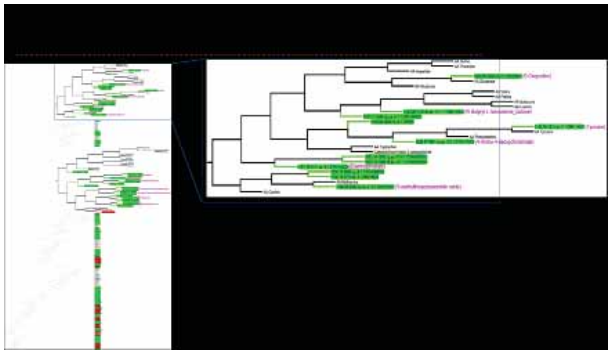
Novel aspects
Automated Bligh-Dyer extraction integrated in a sample preparation workflow with a dual-column UHPLC-MS/MS platform.

WPS26-66 / A combined metaXCMS and automated fragmentation trees alignment approach for rapid characterization of differentially induced metabolites.

Amol Fatangare[1], Kerstin Scheubert[2], Marco Kai[1], Sebastian Böcker[2], Aleš Svatoš[1]
[1]Mass spectrometry research group, Max Planck institute for chemical ecology, Jena., [2]Chair for Bioinformatics, Friedrich Schiller University, Jena.

Introduction
The qualitative and quantitative analysis of all metabolites represents the metabolite profile of a plant at a given instant. In addition to factors like stage and state of a plant, biotic stresses e.g. leaf herbivory or bacterial infection could significantly alter the metabolite profile of a plant. In metabolomics, characterization of such metabolites induced due to particular biotic stress is still a challenging task. High-throughput analysis of metabolites using modern LCMS techniques can be combined with metaXCMS and automated fragmentation trees alignment programs for rapid characterization of such metabolites.

Methods
Arabidopsis thaliana Col-0 plants (25 days old, early flowering stage) were used for all experiments. Plants were subjected to S. littoralis leaf herbivory or P. syringe infection. Plants were cut into above and below ground parts and extracted in methanol:water (80:20, v:v). Extracts were analyzed using UPLC (C-18 RP column) coupled to Orbitrap XL mass spectrometer. Mass spectra were acquired at various fragmentation energies. Analysis of differentially up/down regulated mass features was performed using metaXCMS. Extracted mass features are categorized into related chemical classes using the automated fragmentation tree alignment approach.



Results and Discussion
UPLC-MS data were analyzed using MetaXCMS which identified total of 6560 differentially induced mass features (fold change > 2; P value >0.05). We acquired MS/MS data using online data-dependent settings in which total of 873 differentially induced mass features were fragmented. Molecular formula and corresponding fragmentation tree was computed for each mass feature. We assigned hypothetical identifications to some mass features based on their presence in AraCyc or KEGG. These mass features were then clustered together into chemical classes using automated comparison of computed fragmentation trees.
Biotic and abiotic stresses induce various defense pathways and thus whole new ensemble of metabolites in each stress. It is highly likely that metabolites involved in same pathway depict similar up/down differential induction. They might share chemical similarity if they arise from the same precursor molecule. We propose that such clusters comprise of compound arising from same or similar pathways and/or belong to the same chemical class. The hypothetical identification and clustering of these masses will shed light on underlying stress induced metabolic pathways.

Conclusion
Unknown metabolite mass features could be putatively identified and clustered together according to their chemical class using a combined metaXCMS and automated fragmentation trees alignment approach. High throughput analysis of differentially induced metabolites will pave way for rapid characterization of metabolites induced in various stress responses.

Novel aspects
An automated high-throughput approach to classify differentially induced metabolites and help unraveling underlying metabolic pathways.

WPS26-67 / Chamomile characterization combining ambient ionization & LC-ESI MS/MS high resolution data for a novel metabolomics approach

Elizabeth Crawford, Jaromír Hradecký, Eliška Humlová, Jana Hajšlová
Institute of Chemical Technology Prague

Introduction

Characterization of natural products and dietary supplements is a growing task for product manufacturers to protect their product brand and regulatory bodies as counterfeiters trend toward economic adulteration of high end herbal products. Other common counterfeiting of herbal products is adulteration with active pharmaceutical ingredients for more pronounced and faster impact of the product on the patient. The focus of this work deals with characterization of chamomile herbal products using complementary ambient ionization and traditional LC separation with electrospray ionization for a combined characterization approach to identify the important marker compounds that are biologically active.

Methods

A Direct Analysis in Real Time (DART) ambient ionization source was coupled to a high resolution accurate mass (HRAM) qTOF mass spectrometer with independent data acquisition (IDA) MS/MS for MS profiling and was combined with the classical MS analytical approach employing LC separation (C18 solid phase) with electrospray ionization HRAM MS/MS. For both approaches the 40 chamomile samples, ranging from loose crushed herb, packaged teas to dried blossoms were extracted using 500 g of homogenized crushed sample wetted with 1 mL of distilled water (MilliQ) for approximately 30 minutes and then extracted with 5 mL of HPLC grade methanol. The methanolic extracts were then analyzed by both DART (5 µL/sample analysis) and LC-ESI (2 µL injection onto column) HRAM MS/MS methods in both the positive and negative ionization modes.

Results

Biologically active marker compounds were positively identified in the German chamomile samples including α-bisabolol, α-bisabolol oxide B, bisabolol oxide A and spathulenol. The profiles and ionization efficiencies between DART ambient ionization where there is no separation of analytes prior to ionization and the LC-ESI ionization approach complement one another as DART ionization promotes ionization of more non-polar analytes and ESI covers a range of polarities favoring more polar analytes. A Roman chamomile sample was compared against the set of German chamomile samples and using unsupervised principle component analysis it was clearly separated from the German type. A larger sample set of Roman chamomile needs to be acquired for true identification of its markers.

Novel aspect

HRAM MS combined with different ionization techniques improves classification power based on complementary marker ions.

WPS27 - Small Molecules – Data Acquisition and Analysis

11:00-15:00

Poster Exhibition, Level -1

WPS27-01 / The porous size effect in functionalized porous silicon surfaces by desorption electrospray ionization mass spectrometry analysis

Nicolas Schwab[1], Moriam Ora[2], Alessandra Tata[2], Marcos Eberlin[3], Sylvie Morin[2], Demian Ifa[2]
[1]Departament of Chemisttry - York University/ Thomson Lab - Unicamp, [2]Departament of Chemistry/York University, [3]ThoMSon Mass Spectrometry Lab/Unicamp

Introduction

In desorption electrospray ionization mass spectrometry (DESI-MS), in addition to low gas and solvent flow rates, the type of surface help avoid the 'splashing of solvent' or 'washing effect' where samples are promptly removed from the surface by the spray. These effects operate on smooth surfaces and generally result in unstable signals as the spray moves over the spot. The aim of this work is to compare the performance of different porous silicon surfaces (PSi) for small molecules analysis with regard to the sensitivity and stability of the signal observed in DESI-MS.

Methods

Silicon crystalline were first etched in hydrofluoric acid and anhydrous ethanol mixture galvanostatically under different conditions to produce and control the porous sizes. To avoid the fast oxidation of the freshly etched H-terminated porous silicon substrates were then reacted thermally with 1-decene reagents in Schlenk tubes under argon in order to produce a chemically stable Si–C bond creating a purely hydrophobic surface. Aliquots of the sample solutions in different concentrations were deposited on porous silicon surfaces and after dried, the spots were scanned by DESI for the comparisons. The mass spectrometer (LTQ Finnigan, Thermo Co.) was operated in the multiple reaction monitoring (MRM) mode and the ion current was used for data analysis to find the lowest concentration detected (LOD) for each analyte. The signal stability was also investigated.

Tabel 1: Lowest concentrations detected for representative compounds on different porous surfaces.

Compound	Polarity	CID	Precursor → Product (m/z)	LOD (ng/mL)	
				pSi (10-50nm)	pSi (< 10nm)
Cocaine	+	14	304 $[M+H]^+$ → 182	1	1
Diazepam	+	22	285 $[M+H]^+$ → 257	10	10
Oxycodone	+	30	316 $[M+H]^+$ → 298	10	1
Propranolol	+	25	260 $[M+H]^+$ → 183	10	1
MRFA (peptide)	+	25	524 $[M+H]^+$ → 288	100	100
Chloramphenicol	-	27	321 $[M+H]^-$ → 194	10	10
Taurocholic acid	-	27	514 $[M+H]^-$ → 353	100	100

Preliminary Results

The table 1 show the LOD for seven ordinary compounds on distinct surfaces. Four different concentrations of each compound, ranging from 1 ng/mL to 1 µg/mL were deposited in a straight line and the DESI spray scanned across the dried spots. Generally, the LOD for microporous (porous smaller than 10 nm) show the best results. New experiments are being performed with bigger porous size to evaluate the LOD for these surfaces.

Signal stability was investigated by DESI imaging on dried spots using 5ul of propranolol (m/z 260) at 1 µg/mL. This procedure was repeated several times for the same dried spot to observe the signal intensities and the imaging also provide information about size and distribution of the analyte spoted due the cristalinization process over the surface in different porous sizes.

Conclusion

The functionalized PSi surfaces provides a good alternative for

DESI-MS, increasing the sensitivity and improving stability of the signal. This is possible because the weak sample-surface interactions due the hyprophobic characteristic favoring desorption of the analyte. The porosity size of the surface may provide more surface area for interaction between the dried analyte and the thin solvent film created by the DESI spray, resulting in more effective dissolution of the analyte in the spray solvent.

Novel Aspect
Functionalized porous silicon surfaces for DESI-MS to enhanced the sensitivity and stable signal for small molecules

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WPS27-02 / Quantitative analysis of bromate in non-alcoholic beer using ultra performance liquid chromatography-electrospray ionization mass spectrometry

Ibrahim Alsohaimi[1], Mohammad Khan Khan[1], Zeid Alothman[1], Nasser Alqahtani[1], Mu Naushad[1], Mohammad Algamdi[2], Ahmed Alomary[1]
[1]King Saud University, [2]King Saud University; King Abdulaziz City for Science and Technology

Bromate (BrO3−) is a possible human carcinogen which is formed asa bromide (Br−) containing source waters ozone disinfection by-product. Thus, BrO3− is subject to assess for its threat to humans. In the present study, an ultra performance liquid chromatography-electrospray ionization mass spectrometry method has been developed for the quantitative analysis of BrO3− in non-alcoholic beer. The chromatographic separation of both transitions 81BrO3− and 79BrO3− (isotope contributions of 79Br and 81Br) was carried out and achieved in <1 min, with superior peak symmetry. The good quality parameters were obtained such as linearity (R2>0.9999), precision (repeatability and reproducibility in terms of relative standard deviation) <1% at 1.0 µg/mL standard and <3% in the analysis of sample (moussy classic) and sensitivity with low limit of detection (LOD, 81BrO3−, 0.03 ng/mL and 79BrO3− 0.03 ng/mL) and limit of quantification (LOQ, 81BrO3−, 0.1 ng/mL and 79BrO3− 0.1 ng/mL) in standard. Nevertheless, LOD and LOQ in sample (moussy classic)were obtained only for transition 81BrO3−, 0.05 ng/mL and 0.16 ng/mL, respectively. For transition 79BrO3− the obtained peak was below limit of detection. A total of 39 non-alcoholic beer samples of different flavors were analyzed, the 81BrO3− level in classic samples was found between 1.20 µg/mL and 3.73 µg/mL, however, in other flavored samples the concentration levels reached up to 14.04 µg/mL with excellent recovery rates (96-99%). In most of the analyzed samples, transition 81BrO3− was only found, nevertheless, transition 79BrO3− was either <LOD or completely diminished from the obtained sample chromatogram. This is one of the main findings from the present study and the mechanism of this cause is still unknown. The obtained BrO3− levels in such products were so high and beyond recommendation limits (10-25 ng/mL) established by different international agencies in drinking water. The overall high throughput (including absence of matrix effects and minimal sample preparation without the loss of the target analyte) offered by proposed method can be a benefit for this type of analysis.

WPS27-03 / Comparison of metabolite formation for CYP specific substrates in human and rat lung S9 using single or pooled incubations.

Anna Abrahamsson, Anna-Pia Palmgren
AstraZeneca

In drug development it is necessary to understand the routes of metabolism using human and pre-clinical animal species in vitro systems to ensure effective planning of drug safety testing studies. For projects in which inhalation is the intended route of drug administration, it is imperative to investigate the metabolism using lung tissue in vitro (typically using lung S9). To ensure the metabolic competence of the CYPs (Cytochrome P450 enzymes) and other relevant drug metabolizing enzymes expressed in the lung tissue, incubations with CYP-selective substrates are performed, ensuring that relevant pulmonary CYPs are studied (Olsson et al., 2011). Pooling of substrates in a single control incubation is desirable to reduce the complexity and cost of experiments.
Selected substrates for CYP1B1, 2B6, 2D6, 2J2, 3A4 and FMO were incubated in lung S9 from human and rat both as single incubations and as pooled incubations. Analysis was performed using a UPLC-Q-TOF high resolution LC-MS system with positive and negative ionization. Data was processed for metabolite profiling automatically with MetaboLynx software and manually by extracting of masses.
Comparison between human and rat lung S9 showed a difference in capacity of the metabolism of the different substrates, although the same metabolites were formed in the different species. Some decrease in amount of the metabolites formed when using a pool of substrates compared to single incubations could be seen. Nevertheless all metabolites formed in the single incubations were also formed in the pooled incubation. This indicates that a pool of substrates from selected CYPs can be used when controling the metabolism capacity in human and rat lung S9.
Olsson B, Bondesson E, Borgström L, Edsbäcker S, Eirefelt S, Ekelund K, Gustavsson L, Hegelund-Myrbäck T, Pulmonary drug metabolism, clearance and absorption in Controlled Pulmonary Drug Delivery ed. H.D.C Smyth and A.J. Hickey, Springer, 2011, Chapter 2, 21-50.

WPS27-04 / A new UHPLC-MS method to evaluate S/G ratio in lignin

Joao Benhur Mokochinski, Giovana A. Bataglion, Marcos N. Eberlin, Paulo Mazzafera, Alexandra C.H.F Sawaya
UNICAMP

Introduction
Lignin is the second major component of plants and is of great commercial importance in the pulp and paper industry and ethanol production. The proportion of coumaryl, coniferyl and sinapyl alcohols (monolignols H, G and S respectively) is directly linked to the release processes in pulp delignification. Nitrobenzene oxidation of the biomass followed by GC-MS analysis is the most widely employed method for this purpose; however it is a laborious process and uses toxic chemicals. Alternatively, we developed a new method based on alkali hydrolysis of monolignols, which is faster and cleaner and allows quantification of S/G ratio.

Methods
The monomers of lignin were hydrolyzed using NaOH 4.0 mol/L for 24 hours at 95 °C. After cooling, samples were acidified with HCl 6.0 mol/L and extraction was carried out using ethyl acetate. Solvent was evaporated under a N2 flow and samples were resuspended in water prior analysis. Samples were quantified in a UPLC- ESI-TQD-MS (Waters). Chromatographic separation was carried out using a C8 column and a gradient of water/acetonitrile as mobile phase. MS conditions (capillary and cone) were optimized according each analyte. Ions were identified by the comparison of their m/z, retention time and ESI(-)MS/MS dissociation patterns with pure standards. The analytical method developed was validating in terms of specificity, linearity, range, accuracy, precision, robustness, LOD and LOQ. Samples of E. globulus and E. grandis were employed in triplicate to verify the effectiveness of the method.

Results
For each monolignol (H, G and S) only one hydrolysis product is formed, 28 Da less than its precursor, which were identified by FT-ICR-MS as4-hydroxy benzaldehyde, vanillin and syringaldehyde, respectively. Different from other methods, the procedure proposed here does not have the inconvenience of formation of various products from the same alcohol precursor, indicating that these products may be considered as markers of monolignols for qualitative and quantitative purposes. Data were acquired in SIR mode for 4-hydroxy benzaldehyde, vanillin and syringaldehyde in samples of E. globulus and E. grandis. Based on peak area, S/G ratio was calculated. The results are in agreement with other previously reported results. For E. globulus S/G ratio is 4.56 and the content of H, G, S is 5.97, 102.22, 466.09 µg/g, respectively. For E. grandis S/G ratio is 3.53 and the content of H, G, S is 12.28, 78.91, 278.93 µg/g, respectively.

Conclusions
Alkali hydrolysis of monolignols is a new alternative to evaluate S/G ratio. The reaction products of H, G and S are 4-hydroxy benzaldehyde, vanillin and syringaldehyde, respectively.

Novel Aspect
Based on procedure described, we propose a new method to evaluate the S/G ratio in samples containing lignin. Hydrolysis reaction, liquid extraction and a UHPLC-MS analytical method were developed and validated in order to quantify monolignols.

WPS27-05 / Towards a high-throughput workflow via the Critical Assessment of Small Molecule Identification (CASMI) 2013 using MetFrag, MetFusion and MOLGEN-MS/MS

Emma Schymanski[1], Michael Gerlich[2], Christoph Ruttkies[2], Juliane Hollender[1], Steffen Neumann[2]
[1]Eawag, Swiss Federal Institute of Aquatic Science and Technology, [2]IPB

Introduction
The Critical Assessment of Small Molecule Identification (CASMI) contest ran for the second successive year in 2013, to compare structure elucidation methods using a common dataset provided by the organisers. The 2013 challenges comprised of generally high resolution tandem mass spectra with various acquisition techniques and fragmentation information for 16 compounds (known to the organisers but not the participants), together with some biochemical background information. A joint team from Eawag, Switzerland and the IPB, Germany participated in CASMI 2013 with an automatic workflow-style entry for small molecule identification.

Methods
MOLGEN-MS/MS was used for Category 1 (molecular formula calculation), while MetFrag and MetFusion were used for Category 2 (structure identification), retrieving candidates from the compound databases KEGG, PubChem and ChemSpider and joining these lists pre-submission using InChI Key matching. MetFusion additionally queried MassBank. Some pre-processing was performed, akin to strategies already used at Eawag.

Results
The Category 2 results were impressive considering the database size and automated regime used and were the (joint) winner in 6 of the 16 challenges (all compounds correctly ranked in 1st place, including challenges 11 and 14 with over 2000 possible candidates) and outright winner of one further challenge. The joining of the three database results meant that the correct entry was present in all submissions despite the fact that 7, 1 and 1 of the correct entries were missing in KEGG, ChemSpider and PubChem, respectively. The visualisations developed for results interpretation (cluster plots, maximum common substructures, similarity plots) assisted greatly in determining whether the spectrum contained sufficient information to identify the candidates reliably, e.g. by showing whether all highly-scoring candidates were structurally related and clustered together (e.g. Challenge 14-16), whether distinct clusters with dissimilar, high-scoring structures were formed (e.g. Challenge 2) or whether all candidates were very similar and thus difficult to separate with a purely automated approach (Challenge 12).

Conclusions
The results for CASMI 2013 with a high throughput identification workflow on the basis of MetFrag and MetFusion were very good considering the large compound databases used. As none of the three compound databases used contained all 16 challenges, the joining of results from several databases is certainly advantageous. The strategies presented will be implemented in the workflow developed for the EU project SOLUTIONS.

Novel Aspects
Data analysis and structure elucidation with various acquisition techniques using joint compound database queries and in silico fragmentation strategies combined with open mass spectral database queries.

WPS27-06 / Incorporation of a modified Waters APGC system into an open access environment for the rapid, automated analysis of small organic molecules.

Peter Stokes, David Parker, Jackie Mosely
University of Durham

Introduction
ASAP (atmospheric pressure solids analysis probe) has been successfully introduced into laboratories as it is an excellent tool for the analysis of small (typically < 1000 u) organic molecules. It has been used successfully in the analysis of both polar and non-polar samples as is capable of analysing both solid and liquid samples. The technique is limited however, in that it is labour intensive and is not easily automated. In this presentation we show how the issue of automation has been overcome using an APGC (atmospheric pressure gas chromatography) in a "loop injection mode" and how it has been incorporated into an open access environment providing both rapid (~ 2 mins.) and accurate mass measurements.

Methods
Liquid samples were injected into a 1 metre piece of fused silica guard column using a standard split/splitless GC inlet, oven and autosampler. A novel timing delay circuit was developed and integrated into the system to control acquisition start times and a bespoke heating block/calibrant delivery system was used to transport either the calibration or reference compound into the ionisation source.

Results
The modified system has allowed the successful analysis of those samples normally submitted for ASAP in a fully automated manner. The analysis is rapid and a typical analysis can be carried out in less than 2 minutes and the results obtained are directly comparable with those obtained by ASAP. Mass accuracy is excellent and results are typically within ± 5ppm of the theoretical molecular mass.

Conclusions
An existing APGC system has been modified which is suitable for the automatic analysis of samples normally analysed by ASAP. The APGC instrument incorporates the use of a novel reference compound and delivery system which allows accurate mass

measurements to be performed with results typically within 5 ppm of the theoretical molecular mass. This and other modifications described have allowed the technique to be incorporated into the existing suite of open access experiments in an open access laboratory.

Novel aspect
The use of APGC –ToF MS in "loop injection mode" automated for the automated analysis of organic compounds normally analysed by ASAP.

WPS27-07 / Solvent effects on electrospray ionization

Jaanus Liigand[1], Anneli Kruve[1], Ivo Leito[1], Marion Girod[2], Rodolphe Antoine[3]
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Introduction
Electrospray ionization mass-spectrometry (ESI/MS) is an increasingly used analytical technique. Although ESI/MS is applied for a diverse range of analyte and sample types and despite numerous studies, the mechanism of ESI is still not fully understood. The ionization efficiency (IE) dependence on different variables has been studied, but there is no comparative study of solvent effects on ionization efficiency. This work aims to systematically study how solvent composition (organic phase content and pH) influence ionization efficiency.

Methods
The electrospray ionization efficiency scales of ten compounds in seven different solvent compositions were measured. Compounds under study have different extent of protonation in the solvent and different hydrophobity. The studied solvent compositions had three different organic phase (acetonitrile) concentration (80%, 50% and 20%) and three different pH values (2.68, 5.0, and 9.75).

Results
The obtained results show that the higher organic phase percentage results in higher ionization efficiencies and lower differentiating power of ionization efficiencies.
Ionization efficiency was also found to be pH dependant when the ionization degree of analyte changes in the studied pH range. The IE of analytes that are fully ionized in the solution does not depend on solvent pH. The results show that ionization efficiency depends on the combination of hydrophobity and basicity, because the IE of analytes with higher hydrophobity are less influenced by the solvent pH.
The obtained results show that hydrophobicity is very important in the ESI process. The phthalates that have ionization degree nearly zero under the used experimental conditions show high ionization efficiencies even in highly basic conditions.

Conclusions
Depending on molecular characteristics there are some solvent effects.
Firstly the ionization efficiency is found to be influenced by the changes of organic phase content in the solvent.
Secondly the weak bases with changing ionization degree in the solvent pH range show also significant changes in IE.
Finally, very weak bases with high hydrophobicity have moderate or high ionization efficiencies in spite of negligible degree of ionization in the used solvents.

Novel Aspects
Comparative study of solvent effects (organic phase percentage and pH) on ESI ionization efficiency was carried out.

WPS27-08 / Analysis of crude oil mixtures by Atmospheric pressure photoionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

Matthias Witt
Bruker Daltonics GmbH

Analytical methods are needed to better utilize heavy oils. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) is a very powerful technique to study oil on the molecular level. This technique is a well-established method in petroleomics since more than a decade. Routinely a resolving power of more than 800.000 can be achieved by FT-ICR MS with mass accuracies in the ppb range. However, even with this technique not all compounds in oils can be detected due to ionization effects and isomers can't be separated. Not only the chemical composition of oil can be extracted from ultrahigh resolution mass spectra, the mass spectrum is also very specific for the analysed oil. Therefore, the mass spectrum is a fingerprint for the analysed oil. Oils can not only be classified by statistical methods by FT-ICR MS, also mixtures of oils can be identified and also quantified based on the chemical composition based on their compound classes.

Methods:
Two crude oils were mixed in different ratios and analysed by Atmospheric Pressure Photoionization (APPI) FT-ICR mass spectrometry on the molecular level. The relative abundances of the compound classes of the oil mixtures were calculated and correlation plots were generated. Repetitive measurements were performed to check the reproducibility of the results and to calculate errors based a) sample preparation and b) mass detection. The correlation was based on the calculated compound classes detected in the APPI mass spectra.

Results:
The correlation of the detected compound classes based on the mixing ratios have shown linear trends with very good regression factors. The mixing ratios of crude oils were calculated based on the detected compound classes in good agreement with the known ratio. It has to be taken into account that a sample preparation error exists beside the error of reproducibility of the mass measurement using repetitive measurements. The reproducibility of the abundant compound classes have shown a good relative reproducibility based on compound class calculation. The spectra of the mixed crude oils were also analysed by the statistical method Principle component analysis (PCA). The samples with different mixing ratios were well separated in the scoring plot of PCA.

Conclusions:
Mixing ratios of two crude oils even with small differences can be measured by APPI FT-ICR mass spectrometry. The calculation is based on the accurate detection of compound classes. Due to the very high complexity of crude oils and crude oil mixtures especially in APPI mass spectra, ultrahigh mass resolution achieved by FT-ICR mass spectrometry is needed to detect all compound classes accurately.

WPS27-09 / Establishing A New Standard in Triple Quadrupole Detection Limits

Michael Ugarov, Anabel Fandino, Michael Flanagan, Na Pi, Lester Taylor, Laszlo Toelgyesi, Thomas Glauner
Agilent Technologies

Introduction
The use of an instrument detection limit (IDL) based on relative standard deviation (RSD) of a series of replicates is a most accurate measure of "ion efficiency" and analytical utility. This

approach eliminates typical ambiguities related to different ways in which S/N ratio can be defined.
Several recent improvements to a high-end triple quadrupole (QQQ) mass spectrometer have resulted in higher analytical performance. Enhanced sensitivity gives enhanced peak area response and improved peak area precision, which ultimately lead to lower IDL for both check-out analytes as well as samples from food safety and environmental applications.

Methods
An Agilent triple quadrupole has been updated with a new collision cell, new ion detector operating at dynode accelerating voltages of up to 20kV as well as a Q1 pre-filter featuring new geometry and tune.
Extracts of black tea obtained using QuEChERS and dispersive SPE were diluted 1:20 with acetonitrile and then spiked with several pesticides presenting high risk relevance in the concentration range of 0.2 ppt to 100 ppb.
Steroids spiked in neat solvents were directly injected into the mass spectrometer using an injection volume of 20µL. Chromatography was performed under gradient conditions using 2.1x100mm columns in both applications.

Results
The use of the new high voltage acceleration detector design resulted in expected gains in sensitivity for large m/z ions as well as an appreciable increase in detection efficiency of negative ions across a broad mass range. Testing of new instrument pre-filter optics has demonstrated a significant increase in ion transmission in various isolation modes (up to a factor of 3).
The enhanced instrument sensitivity directly translated into improved area RSD of the signal response and the ability to achieve IDL down to 10 attogram of verapamil on column. This result can be readily used to deduce the ultimate ion efficiency of the mass analyzer which corresponds to about 1 ion detected for each 500 analyte molecules in the injection volume. The “ion transfer function” can be subsequently reconstructed indicating ion generation and transmission efficiency along the instrument ion path.
Similarly, a larger number of pesticides can achieve low IDL in the 1:20 diluted black tea extracts (for example, area RSD of only 7% for 1fg bentazon). Additionally, the detection of estrone and equilin in neat solvents (area RSD of 9% and 15% at1ppt level, respectively) is shown as an example of improved sensitivity relevant to streamlining sample preparation in drinking water analysis.

Conclusions
The RSD of signal response is shown to be a better approach to determining quantitation limits compared to S/N measurements. For the analysis of very low sample amounts in QQQ LC/MS the peak area RSD is a direct indication of the number of ions that reach the detector.

Novel Aspect
New ultralow QQQ MS instrument detection limits linked to data analysis based on ion statistics

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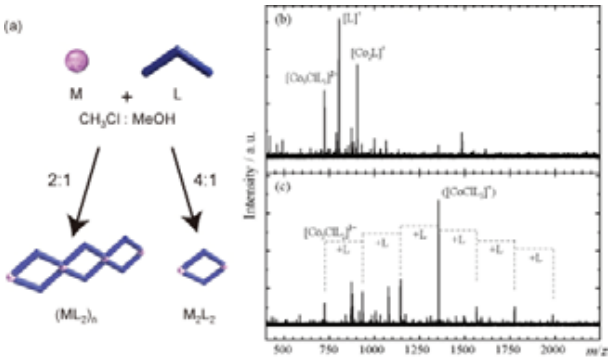
WPS27-10 / Continuous Complexation of CoCl2 and Admantane-Based Ligands observer by CSI-MS
Kazuaki Ohara, Masahide Tominaga, Isao Azumaya, Kentaro Yamagurhi
Tokushima Bunri University

Novel Aspect
CSI-MS can detect pre-organized structure of discrete and polymeric coordination complex.

Introduction
Conventional mass spectrometry (MS) using electron ionization, fast atom bombardment, and electro-spray ionization cannot detect large coordination complexes due to the dissociation of the weak coordination bonds during ionization.We have been developed cold-spray ionization MS (CSI-MS) which can detect coordination complexes without dissociation to give the numbers of ligands and metals as well as ions based on the solvent adducts attached. In our previous work, we clearly observed oligomeric coordination species of coordination polymers (CPs) in the crystallization solution by CSI-MS. We believe that CSI-MS is suitable for the detection of a complex constructed by the coordination bonds, because it has a large dynamic range and high sensitivity even if the complex has very low solubility. In this work, we demonstrated the construction of two crystal structures: a discrete cyclic complex with tetrahedral geometry and a polymeric 1D complex bearing cyclic units with octahedral geometry, depending on the methanol concentration in a methanol–chloroform mixture, using the same ligand as that used in metal-ligand stoichiometry (Fig.a). The crystallization solution was investigated by CSI-MS, UV-vis, and NMR spectroscopy in order to discuss how solvent differences affect the formation of a discrete complex and a continuous metal complex.



Experimental and Results
A bidentate organic ligand is intrinsically capable of forming a bridge or a ring with metals. Owing to its nature, ligand L can form diverse supramolecular architectures in the crystals. The ligand was prepared from a copper(I)-catalyzed reaction between imidazole and 1,3-bis(4-iodophenyl)adamantane with a yield of 50%. In a methanol–chloroform mixture (1:4 and 1:2), CoCl2 ·6H2O and L at 1:1 ratio were stirred for 5 min. After vapor diffusion of tetrahydrofuran into the solution for 1 day at room temperature, blue (Fig.b) and colorless (Fig.c) crystals were obtained from 1:4 and 1:2 MeOH/CHCl3, and single-crystal X-ray analysis found them to be a discrete cyclic complex and a polymeric 1D complex, respectively. CSI-MS can reveal two structures, discrete and continuous metal complexes in solution. In the methanol-rich solution, several molecular ion peaks were clearly observed and assigned to the coordination complexes without coordination bond dissociation. On the other hand, ion peaks corresponding to low-molecular-weight complex were observed in the methanol-poor solution.

Conclusion
We succeeded to synthesize two different coordination structures, a discrete cyclic complex and a polymeric 1D complex by diffusing method. Crystallization solutions were subjected to CSI-MS and some ion peaks were clearly observed as continuous ligand adducts in polymeric structure condition, though an ion peak of cobalts and lignads complex was observed in a cyclic dimer condition. We hope that CSI-MS is a promising analytical method for use in coordination chemistry to determine a large oligomeric complex.
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WPS27-12 / iElement: New UHRM Signal Handling Approach for More Accurate Elemental Composition Determination

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Academia Sinica

Introduction

The field of metabolomics has become increasingly important in the functional genomics. Until now, the large scale identification of metabolites is still challenge. The key step for the metabolite identification is on the determination of accurate elemental composition. The accuracy for the determination of metabolite elemental composition is based on the mass accuracy and isotopic ratio. An accurate elemental composition determination can largely reduce the number of possible chemical structures need to be further validated. To date, the current development of ultra-high resolution MS (UHR-MS) can facilitate the separation and molecular weight determination of the metabolites. Unfortunately, most of UHR-MS such as FT-ICR and Orbitrap may produce inaccurate isotopic pattern because of the ion decay effect during the mass measurement.

Methods

There are several issues needed to be considered for the determination of accurate isotopic pattern. First, the spectrum noise should not interfere the isotopic envelope. Second, the signal should stable to obtain accurate peak intensity. Third, the mass discrimination due to ion decay must be compensated. In the considering of above issues, we have developed the a new software based on the software "UniQua" to have three new functions. First, the adaptive background subtraction function can improve the noise removal in different m/z range. Second, the use of two-dimensional spectra smoothing can improve signal stability of individual mass spectrum. Third, the isotopic pattern error cause by ion-decay effect can be corrected using polyfit regression.

Results

In this study, a sample of complex lipid mixture was analyzed by LC-OT-MS (Orbitrap Elite). With conventional signal processing approach, the average isotopic pattern error observed by Orbitrap operated in 120K resolution was ~10%. With noise removal, spectra smoothing and isotopic pattern correction by iElement, the isotopic pattern error can be reduced to ~1%. With the sub-ppm mass accuracy and ~1% isotopic accuracy, the UniQua can reduce the possible lipid compound candidates for each MS peak from 21 to 1. This reduction can significant reduce to effort for unknown metabolite or compound identification/quantitation and highlight the application of UHR-MS in metabolomics application.

Conclusions

With the combination of appopriate signal processing apporach the S/N and the precision of the isotopic signals and pattern can be improved. The low accuracy of the isotopic pattern due to the ion-decay effect can be effectively corrected by the polyfit regression. This approach was successfully to improve the elemental composition and mass accuracy of the UHR-MS.

Novel Aspect

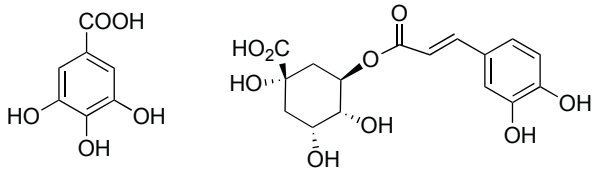
A new approach to reach sub-ppm mass accuracy and < 1% RIA error for specific elemental composition determination.

WPS27-13 / Solvent free analysis of biphenyl-hydroxyacids by APCI- Direct Insertion Probe-High Resolution Mass Spectrometry (APCI-DIP-TOF-HRMS)

Noemí Cabello, Sofía Arnal, Vanessa Martínez, Joan Gallardo-Donaire, Xuequiang Wang, Ruben Martin
ICIQ (Institute of Chemical Research of Catalonia)

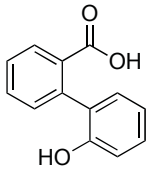
Introduction

Aromatic hydroxy acids are an important family of compounds with antioxidant and anti-inflammatory properties (Fig. 1). Among these, the analysis of compounds containing the 2'hydroxy-1,1'-biphenyl-2 carboxylic acid moiety present a challenge for Mass Spectrometry due to their low stability in solution (Fig. 2). Herein we present a rapid, solvent free method for the analysis of these substances using APCI (DIP)-MS in positive mode. Moreover, a new mixture of commercially available compounds has been prepared and employed to perform direct calibration of the APCI source, affording accurate mass results of the peaks of interest.



Gallic acid Chlorogenic acid

Fig.1



2'-hydroxy-[1,1'-biphenyl]-2-carboxylic acid

Fig.2

Methods

The Maxis Impact mass spectrometer (QqTOF, Bruker Daltonik) was calibrated in APCI+ mode with a new mixture of commercially available compounds in optimized conditions. Hydroxyacids were analyzed as neat solids, by using the DIP (Bruker Daltonik), and the accurate mass of the (M-OH)+ fragment was employed to characterize them.

Typical source conditions employed:

Gas: Nitrogen. Capillary: 4500v, corona: 4000nA, nebulizer: 2.0 bar, dry gas: 3.0 L/min, APCI heater: 350 ºC, dry heater: 250 ºC.

Results

The first ionization mode that we tried for the analysis of several hydroxyacids was, as usual for carboxylic acids, negative ESI. As expected, in several cases (M-H)- was observed. However, for several biphenyl-hydroxyacids (Fig. 2) we were not able to detect clear peaks related to the compound of interest. MALDI and LDI ionization modes also failed to yield clean spectra. Due to the low stability of these acids in solution, we tried to analyze them by DIP, with a solvent free procedure. Good results were obtained in positive mode: in general, (M-OH)+ was the base peak of the spectrum, and mass measurement accuracy was better than 5 ppm, useful to identify the compounds of interest.

Conclusions
This solvent-free APCI-DIP(+)-MS method was applied to the analysis of unstable compounds that could not be detected previously by ESI. In addition, direct calibration of APCI source with a new mixture of compounds afforded these results including accurate mass values (external calibration, accuracy better than 5 ppm). This method can also be applied to compounds that are insoluble in common Mass Spectrometry solvents.

Novel aspects
A new mixture of compounds was developed for direct calibration in APCI mode (to obtain accurate mass of molecules ranging from 135 to 600 m/z).
A new, rapid, solvent free APCI-DIP method was developed for analysis of insoluble compounds and molecules that are unstable in solution.

References
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WPS27-15 / Detection and structural characterization of reactive metabolites using liquid chromatography coupled with high resolution mass spectrometry.
Tommaso Miraval, Thomas Pfeifer, Ali Selimi, Carmela Gnerre
Actelion Pharmaceuticals Ltd.

The formation of covalent adducts between reactive metabolites and nucleophiles can be used to gain information on the nature and amount of reactive metabolites. Traditional approaches use e.g. reduced glutathione (GSH) as trapping agent followed by precursor ion scans of 272Da (in negative ion mode) or a more traditional neutral loss scan experiment for 129 Da (pyrroglutamic acid) in positive ion mode with triple quadrupole mass spectrometers. A high resolution mass spectrometer (Orbitrap™) was used to detect and characterize reactive metabolites. Human liver microsomal incubations were utilized as in vitro test system. A specific isotopic pattern was generated with an equimolar mixture of isotopically labeled and unlabeled glutathione. The MetWorks ™ software package was used to perform background subtraction using a control file and automatic isotope pattern recognition for accurate mass data. None of the trapped reactive metabolites discovered were losing pyroglutamic acid (129 Da) using different fragmentation techniques. The isotopic search using labeled glutathione showed to be a useful tool to detect and characterize reactive metabolites. Furthermore glutathione trapped metabolites must be always screened using single and multiply charged ions.

WPS27-16 / Maximizing Efficiency in UHPLC-MS/MS Method Development for multi component analysis
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Shimadzu Europa GmbH

Introduction
With the development of high sensitive and ultra-fast LC-MS/MS instruments, the triple quadrupole technology has found its way into a huge area of applications. Nowadays, it is the method of choice for trace level analysis and identification of various compounds.
The steadily increasing number of applications in different fields like pharmaceutical, environmental, food, forensic and clinical analysis demand fast and efficient development of new LCMSMS methods. The basis for stable generation of high quality data is a well optimized chromatographic separation. Fully automated optimization of the UHPLC/HPLC method in combination with automated MS optimization for MRM and Interface parameters are the perfect platform for the generation of new triple quad MS methods. Here, we report a fast procedure for LCMSMS method optimization for multi component analysis.

Methods
Choosing the best UHPLC/HPLC column and composition of eluents are often the most important but time-consuming steps during method development. We used Shimadzu´s Method Scouting System in combination with the ACE Excel Method Scouting Column Kit in order to elucidate the best UHPLC/HPLC parameters for the analysis of different compounds.
The Method Scouting System allows the combination of up to 6 HPLC columns with up to 16 different eluent combinations, resulting in the investigation of up to 96 different combinations, which requires only a fraction of the time required by traditional approaches. The MS control software LabSolutions offers the possibility to select MS parameters like precursor ion selection, collision energy and fragment optimization for MRM via flow injection analysis in an automated way while the Interface Setting Support Software is a useful tool to find the most suitable Interface settings.

Results
Traditional method development in UHPLC is extremely time consuming. The combination of automated UHPLC and MS method development allows the development of complete LCMSMS methods within two days. In this study we show an automated method scouting procedure including the search for optimum column and mobile phase. The final UHPLC method validation is also software assisted. The combination with the fully automated MRM-optimization and the use of the Interface Setting Support Software allows a fast development of a final method for the analysis of clinical drugs.

Conclusion
Here we show the successful establishment of an automated generated method for the separation, identification and quantification of a mixture of drugs. All steps combined lead to generate a fully automated final method for the analysis of multi components.

Novel aspect
Combination of fast automated UHPLC and MS method development on Shimadzu's LCMS 8050 Triple Quad Mass analyser.

WPS27-17 / A Rapid LC-hrMS Method for Metabolite Identification Simultaneously to Metabolic Stability Assessment on Microsomes at an Early Screening Stage
Didier Bressac, Emmanuel Hardillier, Olivier Lacombe
Inventiva

Introduction
Metabolic stability assessment on liver microsomes is one of the first earlyADME assay in drug discovery. The aim is to evaluate the intrinsic hepatic clearance of compounds in very short timelines after their synthesis. Structure-Activity Relationship based on metabolic stability has been a powerful tool for medicinal chemists to optimize drug candidates relating to their pharmacokinetic properties. With the leverage of recent chromatography and mass spectrometer technologies, bioanalytical conditions combining a rapid LC method with hrMS detection have been set-up. This will allow the simultaneous study of the metabolic clearance and the metabolite identification for an in-depth drug optimization and for an interspecies comparison.

Method
For the metabolic stability assessment, compounds are incubated at 0.5 µM with human and mouse liver microsomal proteins and NADPH-generating system. After each incubation time, the reaction is stopped by adding a quenching solution with internal standard. The samples are injected into a LC-hrMS (Exactive Plus, ThermoFisher) with a generic method in full scan detection mode. QuickCalc® software (Gubbs Inc) is used for quantifying tested compounds based on an automatic search of the molecular ions within the full scan chromatogram. The remaining parent compound is automatically determined. A metabolite identification reprocess with MetWorks® software (ThermoFisher) allows the metabolites data extraction. The present work was focused on Midazolam, Diclofenac and Amitriptyline as examples in human and mouse species.

Results
A first processing with QuickCalc® was used for the microsomal stability assessment of tested compounds. A second reprocessing with MetWorks® software allowed rapid metabolite identification. Midazolam was slightly more metabolized in mouse than in human, but with similar main metabolites: oxidations and di-oxidation. Diclofenac was highly metabolized in human and slightly in mouse. The proportion of oxidized derivatives could explain this interspecies discrepancy. Amitriptyline was more metabolized in mouse than in human. This interspecies discrepancy could be explained by the proportion of desmethylated and oxidized derivatives.

Conclusion
A generic and fast method has been set-up for helping medicinal chemists to optimize the drug candidates based on their microsomal stability and additional metabolic pathway comprehension. The relatively low incubation concentration doesn't allow to precise the correct position of metabolism, but provides helpful information on the metabolite class and potentially on the interspecies metabolism pathway. Specific metabolite identification study will bring structural information.

Novel Aspect
A LC-MS system with high resolution analyzer is a very adapted tool to combine during the first earlyADME studies the parent compound metabolic stability with metabolite identification using a simple, generic and fast analytical method and process.

WPS27-18 / Routine Targeted Quantitation and Identification of Pesticide Residues using Triple Quadrupole LC-MS/MS and Advanced Scheduling of MRM Transitions

Ashley Sage, Jianru Stahl-Zeng, Harald Moeller, Jean-Pierre Lebreton
AB SCIEX

Introduction
Recent regulations on food analysis require the screening for pesticides using confirmatory techniques, such as GC-MS(/MS) and LC-MS/MS. With more than 1000 pesticides of more than 100 compound classes there is a demand for powerful and rapid analytical methods, which can detect very low concentrations in a variety of food matrices. Here we present a high-throughput routine LC-MS/MS method that combines screening with identification based on Multiple Reaction Monitoring (MRM) and full scan MS/MS data.

Experimental
Fruit and vegetable samples from local supermarkets were extracted using a QuEChERS procedure and injected into LC-MS/MS after dilution to minimize possible matrix effects. LC separation was performed using Phenomenex Kinetex (50 x 2.1 mm) column and a gradient of water and methanol and ammonium formate buffer with a total run time of less than 20 min. Detection was performed on an AB SCIEX triple quadrupole mass spectrometer using Electrospray Ionization.

Results & Discussion
Targeted pesticides were quantified and identified using the Scheduled MRM™ pro algorithm. This new algorithm allows setting of flexible detection windows for each target compound, dynamically extends the detection window if needed, and triggers qualifier MRM transitions when the quantifier is present: resulting in enhanced selectivity, sensitivity, accuracy, and reproducibility. The MRM ratio was used for pesticide identification and is automatically calculated in MultiQuant™ software.
The method provided sufficient sensitivity, accuracy and reproducibility to quantify and identify all targets at a concentration of 10µg/kg or below.

WPS27-19 / Characterisation of small pharmaceutical molecules by electron-transfer dissociation

Andy Ball[1], Jackie Mosely[1], Anthony Bristow[2], Martin Sims[2], Mike Morris[3]
[1]Durham University, [2]AstraZeneca, [3]Waters

Introduction
Collision-induced dissociation (CID) is a widely used technique for characterising pharmaceutical molecules. In some cases, such as for the pharmaceutical compound cediranib, only limited information can be obtained from CID and so alternative methods are being sought. Any new technique would need to be fast, efficient and sensitive, and ideally compatible with liquid chromatography mass spectrometry (LC-MS). Electron-based techniques such as electron-capture dissociation (ECD) and electron-induced dissociation (EID) of doubly and singly charged precursor ions respectively, have been shown to generate unique and diagnostic product ions in comparison with collision-induced dissociation (CID). However, both of these electron-based techniques are limited to Fourier transform-ion cyclotron resonance (FT-ICR) instruments.
An alternative fragmentation method is electron-transfer dissociation (ETD). ETD is known to generate similar results to ECD for highly multiply charged peptides and proteins, and has the advantage that it can be used on any mass spectrometer, typically following electrospray ionisation (ESI). In this report, ETD has been compared to CID for the characterisation of a selection of small pharmaceutical molecules including cediranib [M+2H] 2+.

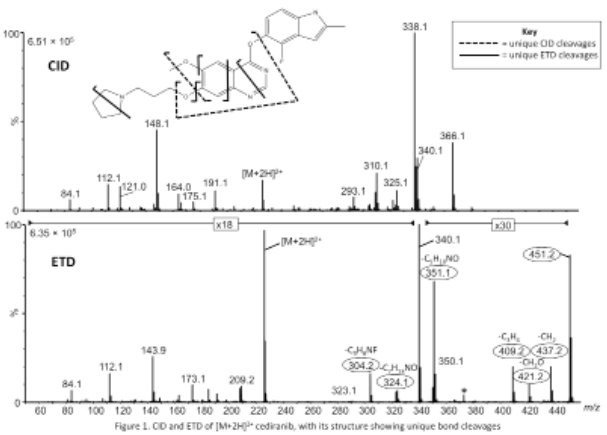

Figure 1. CID and ETD of [M+2H]2+ cediranib, with its structure showing unique bond cleavages
*peak relates to noise and can be disregarded

Methods
A selection of small molecules was provided by AstraZeneca. Compounds were made into solution at 0.1 mg/mL with CH3CN:H2O. Direct infusion electrospray was used to generate positive ions. A Synapt G2-S (Waters corp.) was

used to perform CID (5-20 eV) and ETD, the ETD reagent was 1,4-dicyanobenzene.

Results
Figure 1 shows the CID and ETD spectra for [M+2H]2+ cediranib, highlighting the number of unique peaks present following each technique and the bond cleavages for some unique product ions . Cross-ring cleavage is only observed following ETD. ECD (data not shown) is largely comparable to CID with respect to m/z observed, although relative peak intensities differ. ETD shows significantly more peaks at higher m/z, which are unique to this technique (circled). Some of these product ions relate to small neutral losses, while others are the result of bond cleavages, or combinations of bond cleavages, unique to ETD pertaining to the central region of the ion.

Conclusions
ETD gives diagnostic, unique and complementary information to other dissociation techniques for the analysis of small, doubly charged ions when compared to CID and ECD. The unique peaks at a higher m/z range are caused by charge reduction from [M+2H]2+ à [M+H]+ followed by dissociation. Charge reduction is only observed following ETD, explaining why these product ions are unique to this technique.

Novel Aspect
The use of ETD for small pharmaceutical ions.

WPS27-20 / A combination of quantitative structure-property relationship and machine learning to predict MALDI efficiency of metabolites

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Kyushu university

Introduction
MALDI method has been used for the analysis of a wide range of small biological compounds. Although the fundamental mechanisms of the ionization process has been studied extensively, there is no decisive rationale to determine optimal matrices for given compounds. In the present study, we developed a prediction model of the ionization profile using a quantitative structure-property relationship (QSPR) approach and machine learning.

Methods
The ionizability and ionization efficiency of 200 metabolite standard compounds in MALDI-TOF-MS were examined with 9-aminoacridine (9-AA) to cover a wide range of structural diversity of metabolites. The MDL molfiles (files containing information about the constituent atoms and their connectivity and coordinates of molecules) of the compounds were acquired from the PubChem. The molecular descriptors, which include 905 1-2D and 3D type and 10 types of fingerprinting descriptors, were calculated by the PaDEL-Descriptor. We employed Random Forest, one of machine learning methods, to construct QSPR models in classification and regression modelling. The limit of detection (LOD) is used as the response variable. LOD values and categorical values (ionizedornot ionized) were used in the regression and classification models respectively.

Results
Out of 200 compounds examined, 104 compounds were detected as deprotonated peaks, and the LOD values ranged from 0.0025 to 100 ppm. The descriptors generated by the PaDEL-Descriptor were used to construct a Random Forest QSPR model for the LOD values. First, we constructed a QSPR model to classify ionizability (ionized or not ionized) as a classifier. The overall accuracy of the prediction using the model was 86.0 %, and there were no significant biases with regard to the estimation error and the metabolic class. Besides, the respective types of descriptors (1-2, 3D types and fingerprints) were applied to construct Random Forest prediction models. As the result, 3D model exhibited the highest performance (91.0 %) followed by 2D model (88.5 %). Secondly, we constructed a regression model using the LOD values, indicating ionization efficiency. In Global and 3D models, predictive performance reached p=0.77, and 2D model achieved the best performance p = 0.78. An analysis of the descriptors contributing to such model construction suggested that the proton affinity is a major determinant of the ionization, whereas some substructures hinder efficient ionization.

Conclusions
The relevant descriptors found in this study can be interpreted as the structural preference specific to 9- AA and/or negative mode MALDI-MS analysis. The QSPR approach should also be applicable for other MALDI matrices to characterize the structural properties of target compounds for preferred ionization.

Novel Aspect
This study employed a combination of systematic analysis of the ionization profile in 9-AA-MALDI-MS and machine learning method to model ionization profile and achieved high predictive performance.

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WPS27-21 / High-speed MRM quantification for multiple metabolites in biological samples using parallel UHPLC-MS/MS system with fast electrospray polarity switching

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[1]Life Sciences Mass Spectrometry, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, [2]Shimadzu Corporation, Manchester

Introduction
In targeted metabolomics, the objective is to quantify many metabolites representative of biological pathways in relatively large cohorts of samples using different LC conditions. Depending on the lipophilicity range of the compounds gradient elution is almost mandatory. With electrospray ionization and depending on the mobile phase conditions, certain analytes need to be detected in the positive mode while others display best signals in the negative mode. The consequence is that a single sample needs to be analyzed several times which affect the sample throughput. Reducing the analysis cycle time without compromising chromatographic performance can be achieved by integrating parallel UHPLC with column switching (for sample clean-up or preconcentration) and acquiring MRM data with a high data speed. In the present work the quantitative performance of ultrafast MRM acquisition (1-5 msecs of dwell time) with fast polarity switching (5-15 msecs) was investigated.

Methods
Parallel UHPLC system was constructed with units of Nexera series (Shimadzu). Two quaternary low-pressure-gradient LC-30AD UHPLC pumps and switching valves to inject samples onto analytical columns (C18) or trapping columns were included. Analytes representative of different classes of endogenous and exogenous metabolites were separated by gradient elution and detected by a triple quadrupole MS (LCMS-8040/50, Shimadzu) operated with high-speed electrospray polarity switching and short MRM dwell times less than 5 msecs.

Results
The UHPLC-MS/MS system used in the present work is based on two low-pressure UHPLC gradient pumps and column switching valves. Each UHPLC pump can handle four different solvents and several gradient combinations can be considered in parallel or serial mode. The present system can be operated in three modes: i) parallel UHPLC with two identical gradients, ii) parallel UHPLC with different gradients and iii) column-switching with parallel UHPLC or two different trapping columns chemistry (C8, C18) and one UHPLC gradient. In endogenous metabolites analysis, representative analytes including fatty acids, bile acids, steroids, amino acids metabolism, nucleotides, vitamins and xenobiotics were monitored. With the possibility of windows MRM acquisition, a very short dwell time was not found to be too critical. However, fast polarity switching did significantly contribute to increasing the number of analytes that could be detected in a single analysis.

Conclusions
A single UHPLC-MS platform allowing various parallel and column-switching setups could be applied for the quantification of endogenous metabolites in biological matrices.

Novel aspect
Novel approaches to increase throughput for metabolomic UHPLC-MS/MS analysis based on parallel UHPLC, column-switching and fast MS polarity switching.

WPS27-22 / Direct analysis of complex impregnation products by thermal solid phase extraction GCMS and low temperature plasma ionization MS

Asger W. Nørgaard, Per Axel Clausen, Peder Wolkoff
The National Research Centre for the Working Environment

Introduction
Commercial impregnation spray products, for e.g. waterproofing or inducement of "easy to clean" effects, are complex mixtures of film-forming compounds, solvent, and in some cases a propellant. Several cases of pulmonary injury have been associated with use of impregnation spray products; thus, identification of the causative compounds is crucial. Detailed chemical analysis is often required, because data about the formulation of the products are seldom sufficient for exposure assessment. Here, we present a two-method approach for characterization of such products:
1. Thermal solid phase extraction (TSPE) GCMS; impregnation products are injected onto a clean Tenax TA adsorbent tube. Polar solvents (water, alcohols) are subsequently removed by purging with helium at ambient temperature. VOCs and SVOCs with boiling points up to ca. 400 °C are subsequently released by thermal desorption; larger and non-volatile species (e.g. particulates and polymers) are retained by the adsorbent.
2. Low temperature plasma ionization (LTP) MS; a cold helium plasma is utilized for direct analysis of impregnation products deposited on Teflon or filter paper.
Both methods facilitate analysis of raw products without prior sample preparation and minimal risk of instrument contamination.

Methods
TSPE-GCMS: 1 mL of raw product was injected into a stainless steel tube containing Tenax TA adsorbent. The tube was, after purging with He, analyzed by thermal desorption and GC-MS using EI, CI and CID for identification of eluting compounds.
LTP-MS: 5 mL of raw product was applied to filter paper or Teflon and placed in front of the inlet of a Bruker micrOTOF-Q mass spectrometer. An in-house built LTP probe was used for ionization. Experiments were carried out in both negative and positive ion mode and CID were applied for identification of film-forming compounds.

Preliminary results
The methodology was tested on a water-based product for impregnation of wood. The TSPE-GCMS analysis showed mainly glycol ethers and methylsiloxanes. In addition, a series of compounds with masses of 426, 526, 626 and 726 Da (confirmed by CI) were observed at eluting temperatures below 150 °C that indicates s a perfluorinated polymer. This was confirmed by LTP-MS; several of the observed ions showed neutral losses of HF and C2F4 in CID. Further, a large series of ions (m/z ca. 500-1400) with an internal spacing of 44 suggested presence of poly ethylene glycol.

Preliminary conclusions
A combination of TSPE-GCMS and LTP-MS informed about the content of VOCs, SVOCs as well as polymeric material in a water-based impregnation product. The applied methodology is simple and proved effective for the analysis of impregnation products. The two-method approach may easily be applied to other types of consumer and industrial products.

Novel aspects
Use of novel methodology for direct analysis of complex impregnation products.

WPS27-23 / Mechanism studies of Ullmann-type coupling reactions: ESI-MS Detection of Intermediates by Using an Ionically-Tagged Ligant

Antonion Cesar de Amorim Borges, Jones Limberger, Alessandra Pazini, Jairton Dupont
UFRGS

Introduction
Most copper-catalyzed coupling reactions involve aryl halides as the electrophile partner with nucleophiles, leading to C(aryl)-N or C(aryl)-O bond formation (Ullmann Reactions). These reactions are important and useful knowledgement for the Organic Synthesis Sciences. With respect to the reaction mechanism, discussions are focused over: (1) How is proceeding the activation of the organic halide? There are two proposals (a) oxidative addition/ reductive elimination (CuI →CuIII), (b) radical processes (CuI – CuII). (2) Formation of the specie Cu-Nu (here, Cu-phenoxide). Discussion is related to wether this formation occurs if before or after the halide activation.

Methods
We report here in, the employement of an ESI(+)-MS and MS/MS q-Tof technique aiming to detect reaction intermediates in the Ullmann condensations reactions, for this purpose , we used an ionically-tagged ligant labeled L = [C16H34N4S2]2+, as the ligand (Di-cathionic specie) and ((CF3SO2)2)N- noted (N(Tf)2-) as counter ion. The system used was the reaction between 4-t-buthylphenol and 4'-bromoacetophenone catalyzed by CuI/L . The Cs2CO3 was the choosen base, toluene was used as solvent of the reaction and condensation proceeded for four hours at the temperature of 100ºC.

Results
We can fortunatly report that the following most intenses ratio mass charge (m/z) signs were detected at: in positive ion mode (m/z); 133, 243, 339, 621, 981, 1263 and in negative mode(m/z): 149, 279 and 693. Among these signs, the one at m/z 339, in the positive mode, can be attributed to the L-Cu-phenoxyde with iodide as counter ion noted as the following ion notation [C26H41N4S2IOCu]2+; we should mention that the isopic model is in agreement with the experiment spectrum. Moreover in the Ms/Ms spectrum of the m/z 340 it was observed the related free ligant (L). The last ones signs at positive mode are possibly clustters built from the the ligant L and the N(Tf)2-. m/z 133 can

be attributed to a fragment of the ligant. In the negative mode, m/z 149, 279 are due to [C10H13O]- and [N(Tf)2]-respectively and m/z 693 is not yet attributed to a structure.

Conclusions

ESI-MS technique was able to detect important ion specie, from wich can be infered that the formation of the Cu-phenoxyde precceds the aryl bromide activation, helpping scientists better to understand Ullmann Reactions mechanism.

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WPS27-24 / We have the analyte – but where is the dross? A systematic approach to investigate the matrix removal during sample preparation

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In quantitative analyses, the main criterion for selecting a sample preparation method is high recovery of the desired analyte, or, in the case of the metabolomics community, to obtain high recoveries for as many analytes as possible. The focus, however, is never on what is removed during sample preparation. A better understanding of which compounds are removed during the sample preparation process can help to avoid interfering matrix components and signal suppression in mass spectrometry. This is especially valid for complex biological matrices like serum and plasma. Therefore, we performed a systematic approach, which follows the recovery of the analyte during the sample preparation and determines the efficiency of the matrix removal at the same time. Several sample preparation strategies like liquid-liquid extraction, solid-phase extraction (cation-exchange, reversed phased, hybrid), and protein precipitation were applied to plasma samples. The recovery of the lipophilic antidepressant amitriptyline and three of its metabolites was determined by reversed-phase HPLC/HRMS. The efficiency of the matrix removal was evaluated by an untargeted metabolomics-based HILIC HPLC/HRMS approach. The recovery of the analytes varied between 20% and 120%, with lowest recovery obtained with liquid-liquid extraction and highest recovery obtained with protein precipitation. The metabolomics data showed substantial differences between the various sample preparation strategies and also distinctive behaviors of individual compound groups were observed. Polar compounds, which are still present in the sample after protein precipitation and some SPE cleanup, may not interfere with the detection of the analytes in this case. But compound groups like phospholipids that were still present after liquid-liquid extraction and precipitation, could impact on the detection of the analyte.

The selection of the sample preparation method depends on the special requirements of the application. Analyte recovery, clean-up, costs and time consumption are important factors that have to be taken into account. A better insight of what really happens with the matrix during clean up can facilitate this decision. The combination of quantitative analysis and untargeted metabolomics is an innovative tool to get better insight into these processes.

WPS27-26 / Identification and Quantitation of Designer Drugs in Urine by LC-MS/MS

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[1]AB SCIEX, [2]LGC Standards

Introduction

Recently, trends in the drugs of abuse field suggest novel compounds, similar in structure to current drugs of abuse, are rapidly appearing on the market in an attempt to evade controlled substance laws. These “designer drugs” or “legal highs” have caused concern due to their unknown quantity in terms of potency, side effects, health consequences and potential for abuse.

A method is presented here using a simple dilute and shoot sample preparation, using the QTRAP 3200 to quantify and identify a number of these compounds in urine, specifically ketamine, norketamine, dehydronorketamine, 3-methoxyeticyclidine, 3-methoxyphencyclidine and methoxetamine, generating library searchable spectra for high confidence identification.

Methods

The sample preparation consists of a simple pre-treatment step using dilution of urine samples (1:5 in aqueous mobile phase), followed by direct injection at a volume of 20ul. HPLC methodology consists of a water/methanol/buffer mobile phase with a short gradient on a Kinetex 50x2.1 PFP column. Data was generated using a Multiple Reaction Monitoring (MRM) method with two transitions per compound and an MRM triggered Enhanced Product Ion (EPI) method to not only obtain quantitative results but additionally allow confirmation of the analytes using library searchable MS/MS spectra from the same injection.

Results

Using this method it was possible to analyse the compounds ketamine, norketamine, dehydronorketamine, 3-methoxyeticyclidine, 3-methoxyphencyclidine and methoxetamine with an injection - injection time of 8 minutes.

Sensitivity for the compounds is shown to be between 1 and 5ng/ml in urine. This equates to1 to 5pg on column sensitivity for these compounds.

Quantitative performance has been demonstrated and shows accuracies within 20 % of nominal at the LOQ and %CV of 20 %, also at the LOQ and within 15 % for the higher concentrations. Mean linearity (r2) has been shown for all compounds to be > 0.993 across at least three orders of magnitude.

Additional verification parameters including matrix effects have been investigated.

Conclusion

An LC-MS/MS method for the analysis of 6 designer drugs was achieved utilizing a designer drug library for high confidence identification.

Novel Aspect

LC-MS/MS method for designer drugs with unambigious identification of LIT based MS/MS spectra and easy sample preparation

WPS27-27 / Outgas analysis for zeolites by DIP-GC-HR-TOFMS and Complemental Interpretation by NMR and FE-SEM

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Introduction
Zeolites are well known absorbents and catalysts used in industry and medicine. Their structure, composition and ion-exchange properties is rather different between different types. We therfore analysed differences between three different zeolites properties by using Direct Inlet Probe (DIP) HR-TOFMS, Solid state Nuclear Magnetic Resonance (NMR) and Scanning Electron Microscope (SEM).
DIP- HR-TOFMS is suitable for analysis of outgases from solid material.HR-TOFMS can moreover, perform separation of molecules that have very small mass differences, i.e.CO, N2 and C2H4. On the other hand, Solid-state NMR has been rapidly developing in recent years due to technical achievement that provides higher spinning rate of Magic-Angle Spinning (MAS). If the material is analysed by DIP- HR-TOFMS and NMR, SEM may provide additional data on the surface structure by secondary and backscattered electrons. SEM is thus widely used to obtain image of material shapes and structure.

Methods
We analysed 4 zeolity types comprising one synthetic zeolite (Zeolite A, Panaceo, Austria), natural clinoptololite (Panceo, Austria) and two treated clinoptilolites (patented PMA-technology tretament, Panaceo, Austria) . For DIP- HR-TOFMS we applied 50 °C to 500 °C (16 °C/min). For the interpretation of outgases, we compared ±1 mDa width extracted mass chromatograms. Si SS-NMR measurements were done by 500MHz spectrometer at 5 kHz MAS frequency with 8 mm HX MAS probe. SEM images were obtained by FE-SEM at several magnification ratios.

Results and discussion
From SEM observation major differences were visible between synthetic and natural zeolites. Synthetic zeolite contains relatively big and cubic crystals while natural non-treated and treated zeolites have almost similar shapes like flakes. A difference between natural zeolites seems sharpness of the edge of crystals. Treated samples have looser edges. Si SS-NMR results confirmed differences in Si between synthetic and natural zeolites related tointernal chemical structure around Si. Internal structure between natural zeolites has a high digree of simialrity. DIP-MS showed several outgases by narrow range mass chromatography. Each outgas was separated from background ions by higher resolving power. Differences were again observed between synthetic and natural zeolites. Interstingly, H2O and CO2 show different behavior in each sample.
Alltogether, the results suggests differences of affinity, surface area and micropore size.

Conclusion
We observed differences between analysed zeolites samples by each of used instrumentation. We can consider them from different aspects depending on the instrument. It would be a good solution to find reasonable physical and chemical differences between the samples.

Novel Aspect
Mass spectrometry methods in combination with SEM analyses proved extremely useful for differentiation between natural and synthetic zeolites. Differences were visible from outgas analyses by DIP HR-TOFMS, SEM images and NMR peak shapes of Si. Obrained data suggests substantial surface and internal chemical structure differences as well as differences in microporous material structure.

WPS27-28 / Ultrarapid auxin metabolite profiling for high-throughput Arabidopsis mutant screening

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Introduction
The phytohormone auxin (Indole-3-acetic acid; IAA) has a fundamental role in plant growth and development, acting as a signal molecule in several developmental processes. Crucial for its action is the formation of local auxin gradients, which are resulting from the interplay between auxin transport, biosynthesis, degradation and conjugation. When studying pathways of auxin metabolism, it is crucial to combine data obtained from genetic investigations with the identification and quantification of individual metabolites. In such cases, a high-throughput metabolite profiling method for rapid mutant screening would be a very valuable tool.

Methods
Homogenization and extraction with sodium-phosphate buffer (pH 7.0) was done in one microcentrifuge tube and accelerated by crushing the plant material in a vibration mill. The extracts from minute amounts of fresh plant material were immediately purified using an in-tip microSPE (micro Solid-Phase Extraction). A fast chromatography technique, the ultra-high performance liquid chromatography (1290 Infinity, Agilent Technologies) was coupled to triple quadrupole mass spectrometer (6490 Triple Quad LC/MS, Agilent Technologies) equipped with an electrospray interface (ESI). The mass spectrometric conditions were optimized for each analyte and quantification was obtained by selected reaction monitoring (SRM) of precursors and the appropriate product ions.

Results
We are presenting a new high-throughput method for simultaneous screening of IAA and its key precursors and metabolites in minute amounts (<10mg fresh weight) of Arabidopsis thaliana tissues. For the isolation of IAA metabolites from plant extracts, a simple one-step purification protocol based on in tip microSPE was utilized. Combining two types of reversed phase sorbents, we achieved a more than 80% extraction recovery of all analyzed compounds. We then merged this in tip microSPE technique with fast liquid chromatography during the final mass spectrometry step to facilitate the rapid analysis of a large number of samples in very short time (5 min), and we applied the method on a large collection of Arabidopsis mutant lines which were isolated based on their leaf phenotypes. Finally, multivariate data analysis was used to evaluate the large data set generated, in order to identify mutants that were altered in their metabolite profile.

Conclusions
Together with genetic screening, the new high-throughput auxin metabolite profiling approach will provide new insights into the pathways and regulation of auxin metabolism in Arabidopsis thaliana.

Novel Aspect
A new sensitive and selective high-throughput profiling method for simultaneous screening of IAA and its precursors and degradation products in Arabidopsis mutant lines.

WPS27-29 / Development of Software for Identifying Fungal Species with PLS Analysis of SPME GC/MS and IMS Data of Microbial Volatile Organic Compounds

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Introduction
Fungal contaminations were found on the mural paintings of the Takamatsuzuka and Kitora Tumuli in Japan.1,2 The ability to detect and control these contaminations at an early growth stage is essential in preserving such cultural properties. An ion mobility spectrometer (IMS) is suitable for on-site measurements because of its portability. However, no software had been designed to detect and identify fungal species from IMS spectra. In this paper, a software for identifying fungal species was constructed with Partial Least Squares (PLS) analysis of SPME GC/MS and IMS data of microbial volatile organic compounds (MVOCs) in order to monitor MVOCs emitted from fungi at such cultural sites.

Methods
The MVOC database named «MVOC Finder» was constructed using the GC/MS, LC/MS and IMS data, which includes information of fungal species, strains, genes, environmental conditions and spectral measurement conditions.3,4 All data is loaded in Mass++ format.5 In order to analyze fungal species from GC/MS and IMS data of MVOCs, the PLS analysis was applied in the «MVOC Finder» program.6

Results
MVOCs emitted from soil-derived fungi (A. fumigatus; A. nidulans; F. solani and P. paneum) in various growth stages were analyzed using IMS, GC/MS and LC/MS spectrometers.3,4 Because each fungal strain emitted a characteristic group of sesquiterpenes before spore reproduction, sesquiterpenes are useful in identifying both fungal species and their reproduction periods. The emitted ketones, aldehydes and alcohols increased linearly with spore number. Therefore, the ketone 3-octanone is suited to be an indicator of fungal amounts.3,4 By PLS plots of GC/MS and IMS data of an unknown fungi sample, the fungal species could be identified using «MVOC Finder». Furthermore, fungal species were also identified using fungal type prediction scores calculated by principal component analysis. MVOC Finder also has the ability to predict an IMS drift time from molecular geometry of ions and IMS measurement conditions.6 The PLS plot is displayed with fungal type prediction scores.

Conclusions
The fungal database «MVOC Finder» was constructed using both GC/MS and IMS data of volatile fungal metabolites. The fungal species could be determined by the software.

Novel Aspect
A new software for identifying fungal species was developed with PLS analysis of SPME GC/MS and IMS data of MVOCs for conservation technology of cultural properties

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WPS27-30 / Deiodination of iodinated aromatic compounds with electrospray ionization mass spectrometry

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Introduction
Dehalogenation of iodinated X-ray contrast media (ICM) has been reported using electrochemical and bioelectrochemical systems. Correspondingly, dehalogenation of aromatic halogens has also been reported in mass spectrometry (MS) using different ionization techniques like CI, thermospray, FAB and FAB-LSIMS. The aim of the present work was to study deiodination of iodinated aromatic compounds in MS with electrospray ionization.

Method
The iodinated aromatic compounds were characterized by liquid chromatography/tandem mass spectrometry (LC-MS/MS) using a QTof-micro MS instrument and electrospray ionization (ESI) in both positive and negative ion mode. The effect of mobile phase additives like formic acid, acetic acid, trifluoroacetic acid, ammonium formate and ammonium acetate on the negative and positive ESI mass spectra of the iodinated aromatic compounds was studied.

Results
Formic acid and ammonium formate induced deiodination of the iodinated aromatic compounds with ESI-MS. Neither acetic acid, trifluoroacetic acid nor ammonium acetate induced the deiodination reaction. The effect was most pronounced with negative ESI where the HI product of the deiodination reaction easily adhered to the aromatic compounds giving rise to HI adducts in the mass spectra. The deiodination reaction was shown to take place in the ESI capillary, since the extent of the reaction was largely dependent on the capillary voltage. The calculated heat of reaction for deiodination of the iodinated aromatic compounds was significantly exothermic for formic acid. This was not the case for acetic acid and trifluoroacetic acid.

Conclusion
Care should be taken when using formic acid as a mobile phase additive in LC-MS analyses of iodinated aromatic compounds, since the interpretation of the mass spectra might be influenced by potential dehalogenation reactions taking place in the ESI capillary.

WPS27-31 / Mass spectrometric studies of free radiolyzed amino acids and in analogous meteoritic matrix

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The discovery of amino acids in meteorites was the beginning of numerous studies aimed to explain how they formed, how they survived and rationalise their presence in enantiomeric excess. It is thought that the evolution of life on Earth was influenced by amino acids (in enantiomeric excess) arrived with meteorites. Considering the fact that amino acids are supposed to be buried at a depth of >20 m, the main energy source against their preservation derives from the presence of radioactive elements in the meteorites. It is believed that during the life of Solar System (4,6x109 years), the total radiation produced by radionuclide decay is almost 14 MGy. These radiations can destroy the amino acids, creating new products and changing the initial chiral signature of molecules. We are studying the behavior of amino acids subjected to radiations to understand how oxygen can influence amino acids reactivity towards radiations and

how they behave if included in analogous meteoritic matrix or silica. The amino acids (proteinogenic and non proteinogenic) are γ irradiated in a 60Co source at a total dose of 3.2 MGy, in order to reproduce the actions of radionuclide decay for 1.05x109 years. The chemical processes induced by γ ray are investigated with different methodologies: Differential Scanning Calorimetry (DSC), Optical Rotatory Dispersion (ORD), FT-IR spectroscopy in Attenuated Total Reflectance (ATR) and ESI Mass Spectrometry (ion trap). The possibility to operate a MSn analysis is essential to elucidate the structure of radiation products, allowing us to make assumptions regarding the reactivity of solid amino acids subjected to γ irradiation. Moreover, it is possible to couple the mass spectrometry with an HPLC instrument, equipped with a teicoplanine based chiral column, to investigate the radioracemization process of amino acids. We perform the qualitative analysis as well as the quantitative analysis, estimating the amount of radiation products and determining the exact amount of D-enantiomer formed by radiations. From the comparison between the data derived from irradiation carried out with or without oxygen we deduce that oxygen inhibits, at some extent, the radioracemization process. In addition, its presence doesn't change the chemical nature of radiation product, but their relative amounts is generally modified. Thanks to the collision induced dissociation (CID) technique, we are able to isolate and fragment the new radiation products. For each amino acid and for each radiation product we are able to make a scheme of dissociation that lead us to rationalise the structure. This study is also important to provide information concerning the grafting process of amino acids on inorganic support. The amino acids are submitted to irradiation in solid state, a novel approach respect to the majority of study, which are conducted in solution.

WPS27-32 / The importance of matrix effect investigations in human biological matrices for accurate and sensitive quantification of polyphenols with LC-ESI/MS/MS

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Novel aspects

Simultaneous, sensitive and accurate quantification of polyphenols in human biological matrices with LC-ESI/MS/MS focused on matrix effects

Abstract

The application of LC-ESI/MS/MS analysis has expanded in bioanalytical and pharmaceutical research. Residual co-eluting matrix components, e.g. endogenous phospholipids, salts and mobile phase modifiers affect ESI-ionization of the target analytes. This can contribute to matrix effects (ME), resulting either ion suppression or ion enhancement. Aside from diverse matrices such as plasma and serum a high individual variability of different sources of e.g. plasma from the same species needs to be considered. Current FDA and EMA guidance documentation require that ME have to be evaluated in different lots of matrix as a part of quantitative LC/MS/MS method development and validation. There exist few main strategies to overcome ME like an adequate sample preparation, adjustments in the chromatography or calibration.

We compared more than 30 different sample preparation techniques like protein precipitation, liquid-liquid extraction and solid-phase extraction in human plasma for quantification selected polyphenols at trace levels and evaluated the sample preparation recovery, quantitative ME and process efficiency. Besides the post-column infusion method for qualitative determination of ME, we used the post-extraction spike method which assesses quantitative ME by comparing the signal response of an analyte spiked postextracted into blank matrix to the response present in neat mobile phase.

Moreover, we focused on studying and quantifying ME with the optimized sample clean-up in six different lots of plasma and in human pooled plasma, which is commonly used for calibration. Target analytes-free blank matrix for calibration might not always be available. We also investigated the influence of the total analyt concentration in human pooled and individual plasma on ME. Furthermore, we present a comparison of the accuracy and thus the performance of the quantification with a structural and a stable isotope labelled (SIL-) internal standard (IS) carried out using the example of an analyt with the use of spiked individual plasma samples. It is generally believed that SIL-IS yield better assay performance, but their use is rather expensive and for many compounds SIL-IS are not commercially available. By the selection of an appropriate sample clean-up and the associated resulting compensated ME the accuracy of our quantification with structural IS in different lots of plasma can compete with SIL-IS.

Our investigations emphasize the importance of evaluating ME during development and validation of analytical methods in complex biological fluids such as plasma or serum. ME might also impact the sensitivity of an analytical method. If utilized for pharmacokinetic or clinical studies it is essential to examine the relative ME due to the individual variability of the matrix samples, which can compromise accuracy and yield to uncorrected quantification results.

WPS27-33 / Matrix effect correction in drug analysis for a LC-TOF platform using post column infusion

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Matrix effect is nowadays one of the main concerns for any analyst working in quantitative LC-MS analysis. This phenomenon can influence method sensitivity and, what is more troublesome, the reliability of the obtained results. Since matrix effect can hugely vary from one sample to another depending on the amount and the nature of compounds present in the matrix, the accuracy, repeatability and robustness of the method can be severely affected.

Post-column infusion has been traditionally used for qualitative matrix effect evaluation but it has rarely been considered as an alternative to isotopically labelled internal standards from a quantitative point of view1. Although the internal standard approach is widely accepted it has some drawbacks such as the lack of availability of some analogues and more importantly, the retention time dependence which requires one internal standard per analyte in multi-component analysis. In order to avoid the latter issue we propose an alternative approach based on the post-column infusion of the internal standards (matrix effect markers). Presuming that the standards and the analytes suffer from the same matrix effect, its impact can be compensated over the complete chromatographic run range.

Here, we applied the post-column infusion approach to a generic UPLC-TOF platform developed for combined quantitative drug analysis and metabolomic profiling. We used 8 different markers for post-column infusion and evaluated the approach for 20 drugs with very different physicochemical properties covering a wide chromatographic range. Several urine samples from different volunteers (age, BMI, sex were different) were equally spiked with the drugs and analysed in order to study the absolute and relative matrix effect, the precision and the robustness of the method. Furthermore, calibration curves for each analyte were built and different QC samples analysed to check the applicability

of this approach for quantitative analysis.
The matrix effect profiles of the markers showed to be very different agreeing with the compound dependent matrix effect hypothesis, therefore, the most suitable marker had to be chosen for each analyte. After that, corrected chromatograms generated using analytes and markers signals were used for data analysis. The approach proposed here improved the results in terms of relative and absolute matrix effect dramatically. Furthermore, dynamic range was enhanced, accuracy of the analysis increased and method robustness improved.
In this work matrix effect compensation using post-column infusion of different compounds has been systematically applied for the first time. This is a very promising step towards a matrix effect free analytical platform which can make LC-MS analysis even more successful, adding to its intrinsic high sensitivity and selectivity a higher reliability.

1Stahnke et al. Anal. Chem 81 (2009) 2185-2192.

WPS27-34 / Improving resolving power for complex reactive and instable gas mixtures by combining tunable synchrotron radiation with advanced mass spectrometric techniques

Arnas Lucassen
Sandia National Labs

WPS27-35 / A Gas Chromatography High Resolution Time-of-Flight Mass Spectrometry Method to Characterize and Semi-Quantify Constituents in Aerosol Fractions

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Philip Morris

Introduction
The use of fuels from renewable sources is an important strategy in reducing carbon dioxide emissions. However, combustion of those substances may also cause new risks and problems. For example, biomass, and thus the potential fuel, may contain elements like nitrogen and oxygen, not present in conventional hydrocarbon fuels. Oxygen functions in the fuel are prone to produce harmful pollutants including acetaldehyde and formaldehyde. Therefore, understanding of the combustion chemistry of model substances containing fuel-nitrogen is crucial to take precautions against pollutant release. Flame sampling molecular-beam mass spectrometry has proven to be a method which can provide vital information on flame chemistry.
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Method
Gas is sampled from variety of combustion and similar reactive gas systems, for instance laminar premixed flames, jet stirred reactors, or counter flow flames. A molecular-beam setup is used preserving the molecule and radical composition at the sampling position. Time-of-flight (TOF) mass spectrometry is well suited for simultaneous and quantitative detection of all relevant combustion intermediates. Complex fuel structures, especially N- and O-containing fuels, can result in a rich mixture of different flame species which presents a challenge for the analysis. Ambiguities in species assignment can be overcome by combining a state of the art orthogonal reflectron time of flight mass spectrometerwith tunable Vacuum ultra violet synchrotron radiation. Identification and quantification are performed by using reference ionization cross sections.

Results
A number of isomer pairs of prototypic hydrocarbon and bio derived fuels are investigated. 2-methylbutanol and 3-methylbutanol, 2-methylheptane and 3-methylheptane, 2-methylhexane and n-heptane. Several key intermediate species, as for instance ketene and propene, could now be quantified unambiguously, which could previously only be separated by combining data from several instruments. Furthermore several species, as for instance 1,2 ethendiol,are observed for the first time which were obstructed in the previous combination of instruments. In the low temperature oxidation of dimethylether small contributions of the critical intermediate formic acid could be separated in presence of large quantities of the fuel using peak separation routines.

Conclusions
Orthogonal time of flight mass spectrometry could be successfully used as a powerful tool to reduce ambiguities in the analysis of key intermediates in complex reactive gas systems.

Novel Aspect
For the first time a mass spectrometer able to separate quasi isobaric species of different elemental compositions is combined with isomer selective tunable single photon ionization.

WPS27-35 / A Gas Chromatography High Resolution Time-of-Flight Mass Spectrometry Method to Characterize and Semi-Quantify Constituents in Aerosol Fractions

Introduction
In order to develop and assess potentially reduced risk tobacco products, it is essential to reliably characterize the chemical composition of the aerosols generated by these products and to compare it against the chemical composition of smoke generated by combustible cigarettes.

Methods
Gas chromatography with high resolution time-of-flight mass spectrometry (GC-HR-TOF-MS) combined with state-of-the-art computational methods was used to identify aerosol/smoke constituents and to generate semi-quantification data using isotopic dilution.

Results
A total of 500 reference standards (including isotopically labeled internal standards) were analyzed by GC-HR-TOF-MS equipped with a DB-5-MS GC column to monitor apolar and polar compounds, and by headspace (HS)-GC-HR-TOF-MS using a DB-624 GC column to monitor volatile and semi-volatile constituents. The EI accurate mass spectra of these reference standards were registered in an in-house database.
In order to strengthen the confidence level for compound identification, RapidMiner and Dragon software were used to develop a model for the prediction of retention times (RT) based upon the structural properties of the reference standards. Furthermore, these predicted RT were compared with those generated by ACD/ChromGenius software. Consistent results were obtained by both methods when compared to experimentally determined RT values (r2>0.9300). These predictive models were then applied to a wide selection of compounds (approximately 6000) known to be present in tobacco smoke. This approach, together with accurate mass measurement, enabled the identification of smoke constituents that were not present in the original set of reference standards.
Semi-quantification was performed using internal standards and sample dilution, hence avoiding time-consuming calibration curve preparation and any problems associated with reference standard stability. After the selection of characteristic quantifier and qualifier ions, this novel approach demonstrated good linearity for the monitored compounds and appropriate working ranges for quantification were defined.

Conclusions
The combination of RT prediction models together with high mass accuracy enabled the identification of several hundred smoke

constituents in addition to those used as reference standards. Semi-quantification data were compared with available targeted reference analytical methods and revealed a very good match for the monitored aerosol constituents.

NovelAspect
Implementation of RT prediction models and EI high mass accuracy data to increase the confidence level for compound identification.

WPS28 - Biomolecular Conformation in the Gas-Phase and in Solution

11:00-15:00
Poster Exhibition, Level -1

WPS28-01 / Porbing Structural Dynamics of Intrinsically Disordered Proteins in Heterogeneous Systems from Solution to the Gas Phase

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Introducion
α-Synuclein (α-Syn) and human islet amyloid polypeptide (hIAPP) are intrinsically disordered proteins (IDPs), which are known to be associated with pathogenesis of degenerative diseases, such as Parkinson's disease and type II diabetes, respectively. Due to its structural heterogeneity and fluctuation, it is challenging to investigate the conformations of an IDP using conventional tools for structural biology. We report detection of structural changes of α-Syn and hIAPP under heterogeneous complex systems using electrospray ionization ion mobility mass spectrometry (ESI-IM-MS).

Methods
CD spectroscopy, synchrotron small-angle X-ray scattering (SAXS), and ESI-IM-MS were used for structural transition study of α-Syn and hIAPP from solution to the gas phase. MD simulations were performed based on the experimental data. Interactions of α-Syn in large unilamellar vesicles were investigated using hydrogen deuterium exchange mass spectrometry. ECD fragmentation was used to deduce the metal binding sites of hIAPP.

Results
The helix formation of α-Syn in solution was analyzed using solution SAXS and ESI-IM-MS. The secondary structural characteristics of α-Syn in solution were preserved in the gas phase suggestingpotential utility of ESI-IM-MS for the study of the structural dynamics of IDPs. Then, ESI-IM-MS is applied to investigate structural transitions of α-Syn to helix via association with large unilamellar vesicles as model lipid membrane systems. Combined with HDX-MS experiments, it is found that α-Syn forms helix by adsorption onto the anionic phospholipid vesicles via electrostatic interactions between the N-terminal region of the protein and the anionic head groups of the lipids. α-Syn also associates with zwitterionic lipid vesicles and forms helical structures via only hydrophobic interactions.
Structural change of hIAPP by metal association was also observed in IM-MS spectra suggesting potential utility of ESI-IM-MS for the study of the structural dynamics of IDPs. For example, Zn2+ and Cu2+ bound hIAPP showed compact structures while only extended structure was observed from other divalent metallated (Mg2+and Ca2+) hIAPP. The ECD analysis suggests that Zn2+ and Cu2+ are bound to the central region of the peptide holding the peptide to be b-turn structure in the gas phase.However, magnesium ion associates with the C-terminal region of hIAPP yielding extended structures by charge-charge repulsions with protonated N-terminal region.

Conclusions
We demonstrated that the structural characteristics of IDPs in solution can be preserved in the gas phase providing the potential utility of MS for the investigation of the structural dynamics of IDPs.We also defined the plausible intermolecular interacting regions of IDPs in heterogeneous systems using MS techniques.

Novel Aspect
Solution SAXS and IM-MS experiments provide overall picture of structural transition of IDPs along with structural relevance of desolvated structures of IDP to the solution phase structures. In addition, potential utility of mass spectrometry to study equilibrium dynamics and intermolecular interactions of IDPs in heterogeneous systems is demonstrated.

WPS28-02 / Further development of decomposition method of charge-state distributions of biopolymer ions produced by electrospray ionization of solutions

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Initially our decomposition method of charge state distributions of biopolymer ions was designed to estimate probabilities to keep charges by ionogenic groups of the biomolecule without distinction of different charge carriers. The analysis of the mass spectra produced by electrospray ionization (ESI) of several peptides and proteins carried out on this basis results in some conclusions for their ion structure and behaviour inside the ion source. It is quite natural to try to specify charge carriers in our processing of electrospray mass spectra of bioions. The aim of the work is getting information about retention of such charge carriers as proton, Na+, K+ and so on by individual specific sites of the biomolecule. Solution of reverse problem by decomposition of summary distribution of ion intensities for all possible combinations of possible charge carriers attached to the biomolecule is the way to do it.
Generalization of our method for the case of the multidimensional charge state distributions corresponding retaining by biomolecule ions of various charge carriers and corresponding program are described. Hopefully, such approach will allow to get more substantial information concerning structure and transformations of bioions in solutions and under electrospray conditions that may be important for various biological applications.
Testing of the proposed method carried out for numerous simulated data has shown higher accuracy of obtained results. Higher accuracy was demonstrated and for particular case of the single type of charge carriers in comparison with earlier implemented method. A number of calculations for published experimental mass spectra of polyprotonated cytochrome C molecules was made. The results, apparently, specify their both native and denaturated structure changes at different compositions of the solution and they are correlated with the published data obtained by other methods.
A new method and the program for decomposition of multidimensional charge-state distributions of biopolymer ions were developed. Effective operation of the software was demonstrated for multidimensional simulated data and experimental one-dimensional data. Hopefully, decomposition of experimental charge-state distributions for at least two types of charge carriers will allow to get reliable structural information about bioions in solution.
The possibility to characterize individual sites in the biomolecule

by their ability to retain different charge carries such as proton, ions of alkali metals and so on is a new feature of the approach.
The work is carried out within the framework of the Program of 9 Basic researches of Presidium of the Russian Academy of Science.

WPS28-03 / Acid-induced Expansion of Lysozyme Structure during Electrospray Ionization

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Acid-induced Expansion of Lysozyme Structure during Electrospray Ionization
Jong Wha Lee and Hugh I. Kim*

Introduction
Electrospray ionization mass spectrometry (ESI-MS) combined with ion mobility spectrometry (IMS) has become a powerful tool in studying structure of biomolecules. However, the extent to which MS studies can provide insight into solution-phase protein structures is still an area of debate. Acids are frequently used in protein denaturation experiments and to generate calibrant ions for travelling wave (TW) IMS. However, their influence on protein conformation during ESI has not been studied thoroughly yet. In this study, the structure of protein lysozyme (Lyz) in different acid solutions was investigated using MS techniques and other various solution-phase techniques, and the effect of acids to Lyz conformation during ESI is discussed.

Method
A Waters Synapt G2 HDMS instrument was used for ESI-TWIMS-MS experiments. Effects of different parameters on the experimental results were tested and soft ionization conditions were used. Small-angle X-ray scattering (SAXS), circular dichroism (CD) spectroscopy and tryptophan fluorescence spectroscopy was used to probe solution-phase structures and/or stabilities of Lyz in different acid solutions.

Results
Lyz has been discussed to maintain its native structure at pH 2.2, and our solution-phase experimental results also indicate minor differences in the overall structure regardless of the acid used. However, a bimodal charge state distribution is observed for Lyz ions from HCl solution. Moreover, a large fraction of highly extended conformation is found for Lyz ions from formic acid solution at +6 charge state, whereas Lyz ions from HCl and acetic acid solution exhibit a narrow mobility distribution with collision cross section (CCS) values similar to theoretical CCS of Lyz crystal structure. This more extended conformation is also observed for Lyz in organic solvent/formic acid solution where Lyz is in its acid-state (A-state), but is absent for Lyz in organic solvent/HCl or acetic acid. It is inferred that formic acid destabilizes Lyz, and further expands its structure during the ESI process.

Conclusion
Different acids can destabilize Lyz to different extent and yield different conformations during ESI. This study shows that a specific acid can destabilize protein Lyz, forming a different, highly extended structure during ESI.

Novel aspect
Not much attention has been paid to the effect of acids on protein conformation during ESI, because acids have been regarded to be passive agents, serving its sole role as a pH control. However, in this study, we show that proteins from solutions of the same pH can take different conformations as a result of structural transition during ESI. We believe that insights provided in this study on how protein structure undergoes structural transitions during ESI would hold great significance to MS-based studies on protein structures.

WPS28-04 / Characterisation of immunoassay antibody-antigen interactions: ion mobility mass spectrometry as a potential tool

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LGC

Novel Aspect
Use of IMS-MS for antibody-antigen interaction studies and its potential as a complementary technique for the characterisation of the measurand of immunoassay antibodies.

Introduction
Immunoassays are widely used in routine clinical laboratories to inform diagnostic and therapeutic decisions, yet cross reactivity can lead to inaccuracy and imprecision of measurements. To improve accuracy and precision, reference measurement procedures are required. A fundamental component of a reference method is an understanding of what is being measured, i.e. the measurand, which for proteins includes an understanding of the tertiary structure. However, variations in protein tertiary structure, due to translation and biochemical modifications have made full definition of protein measurands challenging. Developments in mass spectrometry-based methods has allowed for measurements to help facilitate the definition of the measurand. Ion mobility spectrometry-mass spectrometry (IMS-MS) is one such tool though its potential in this area has yet to be fully explored. This potential is here explored using human growth hormone (rhGH) as a model system.

Method
Native IMS-MS experiments have been performed using travelling wave ion mobility spectrometry (TWIMS). Three rhGH reference standard materials and two commercially available monoclonal antibodies (MAb) known to bind to rhGH via two distinct epitopes were analysed by nano-ESI-TWIMS. MAb-rhGH binding was investigated by conducting competitive binding experiments followed by nano-ESI-TWIMS. Collisional cross-section (CCS) measurements were determined using calibration performed as described in Smith et al1.

Results
IMS-MS analysis of the intact rhGH standards revealed three distinct monomeric forms of each standard: a compact monomer, a further extended conformer and a third transitional state-like conformer of which the average CCS fell between the two other distinct forms. The conditions required to perform accurate measurements and minimize CCS measurement uncertainty were assessed. The bias of each antibody showed towards the three monomeric conformers of rhGH was observed. Any discrepancies in calculated average CCSs between the two hGH-antibody binding complexes could be observed, highlighting the potential of IMS-MS as a discriminatory tool between antibodies.

Conclusions
These results demonstrate the potential for IMS-MS to distinguish the variety of conformers present in protein reference standards and discriminate between immunoassay antibodies known to bind to different epitopes. In particular IMS-MS can be used to highlight the specific conformations an antibody interacts with, data not obtainable from conventional MS approaches, to further understand the specific measurand of each immunoassay approach.

1. Smith, D.P.; et al; Eur. J. Mass Spectrom. 2009, 15, 113−130.

WPS28-05 / Complexes of nucleic acid bases with polyethylene glycol oligomers: from solution to the gas phase

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Introduction

Information on intermolecular interactions of biomolecules with organic polyethers and their associates formation is necessary for elaboration of pegylated nanomaterials for drug delivery. Correlations between conformations of the associates in the initial solution and in the gas phase are of interest for mass spectrometry fundamentals. Here we report the results of our study of elementary steps of noncovalent interactions between nucleic acid nitrogen bases cytosine (Cyt), methyl-cytosine, and adenine (Ade) with oligomers (Mn) of polyethylene glycol PEG-400 in solution and in the gas phase.

Methods

Electrospray ionization (ESI) mass spectrometry was applied to detect noncovalent complexes formed in the system containing a mixture of bases and the polyether dissolved in methanol. Molecular dynamics (MD) simulation was used to determine conformations of the complexes in the gas phase and to model disintegration of a liquid methanol droplet containing a base-oligomer cluster.

Results

ESI mass spectrometry revealed formation of stable complexes of protonated nitrogen bases with PEG oligomers, recorded in the ESI mass spectra as a set of abundant clusters Mn•Cyt•H+ or Mn•Ade•H+ (where the degree of polymerization n ranges from 3 to 17). MD simulation has shown that a self-assembly of nanoclusters from the individual protonated bases and Mn oligomers took place. In the gas-phase structures the polymeric chains adopted quasi-cyclic or quasi-helical conformations winding over the organic cation. To mimic the process of disintegration of a sprayed droplet under ESI, nanodroplets composed of 3000 methanol molecules and the preformed Mn•Cyt•H+ complex (n=4, 8) were allowed to expand into a low pressure (10-2 Pa) medium. It was demonstrated, that the base-oligomer clusters did not decompose during the methanol solvent evaporation and the droplet disintegration; the steps leading to stable gas-phase conformation followed the desolvation of the complex.

Conclusions

PEG oligomers form complexes with protonated Cyt and Ade nitrogen bases; the complexes are stabilized by winding of the polymeric chain around the organic cation. MD simulation has demonstrated that the gas-phase clusters recorded in the ESI mass spectra reflect adequately noncovalent complexes present in the initial solution.

Novel Aspect

The process of preformed nocovalent nitrogen base-PEG oligomer clusters release to the gas phase on disintegration of methanol droplet under ESI conditions is described by MD simulation for the first time.

WPS28-06 / Simultaneous protein N- and C-termini identification using tandem mass spectrometry, isotope labelling and database searches.

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DuPont

In 2009, Xiang et al[1]. published a neat application to identify N- and C-termini, for proteins isolated in an electrophoresis gel, by MALDI-TOF MS. The basic principles of this work have been extended to the use of chromatographic separation with tandem mass spectrometric detection and identification by database searches.

In addition, the present application demonstrates the complete sample preparation made on a spin filter in contrast to the in-gel protocol of Xiang et al. The sample preparation includes reduction and alkylation, protein guanidination, acetylation of the protein N-terminal, protein digestion in 40% 18O-water. After digestion the N-termini of the peptides are labelled with a 1:1 mixture of PIC and PIC-d5 (PhenylIsoCyanate).

The digested and PIC-labelled sample is analysed by reversed phase nanoLC with high-resolution MS detection (Thermo LTQ Orbitrap MS). In each MS scan cycle, a full-scan high-resolution mass spectrum and two data dependent low-resolution MS/MS spectra were acquired in each scan cycle.

The acquired LC/MS run was subjected to a MASCOT search. Fixed modifications were Lys-guanidination and carbamidomethylation. Variable modifications were acetylation at any N-terminal, 18O-labeling at any C-terminal, PIC at any N-terminal and PIC-d5 at any N-terminal.

The MASCOT search results demonstrate the feasibility of using spin filters for the sample derivatization and digestion having protein samples in solution. The Mascot Search Result gives a list of identified peptides including the variable modifications; acetyl, PIC, PIC-d5 and 18O-label.

N-terminal peptides are characterised by doublet basic isotopic peaks at Mo (monoisotopic) and Mo+2; 16O-/18O-label at the carboxylic end. C-terminal peptides are characterised by doublet basic isotopic peaks at Mo and Mo+5; PIC/PIC-d5 labelled. Finally, the internal peptides are characterised as quartet basic isotopic peaks at Mo, Mo+2, Mo+5 and Mo+7; the combination of PIC/PIC-d5 at the amino end and 16O-/18O-label at the carboxylic end.

The isotopic mass pattern reveals the type of peptide; NTP, CTP or ITP. For a single protein sample, ideally a single NTP and a single CTP should be in the protein digest, while all other peptides are ITPs. If the protein(s) exists in the databases, MASCOT should easily be able to identify the NTP and CTP.

Xiang et al. used MALDI-TOF MS and accordingly revealed the isotopic peptide patterns in MS mode, i.e. no peptide sequence information could be obtained. Database searches were limited to PMF searches of single protein samples.

The present method has the potential of identifying N- and C-termini of mixtures of proteins due to the information in the acquired MS/MS spectra in combination with database searches.

[1]Bosong Xiang, Xiaolong Yang, Theodore Thannhauser, Rapid Commun. Mass Spectrom. 2009; 23: 2102–2106

WPS28-07 / An Improved HDX MS System for online Digestion, Separation and Data Analysis

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Introduction

This study reports recent improvements in hydrogen deuterium exchange mass spectrometry (HDX MS) for protein higher order structure analysis. These improvements are gained from 1) on-

line digestion using a novel immobilized pepsin column that can sustain high pressure digestion; 2) targeted ETD for single amino acid resolution; and 3) H/D uptake data including intact protein, ETD data are processed and annotated using DynamX 3.0 software automatically.

Methods
Pepsin was immobilized onto high-pressure resistant Ethylene Bridge Hybrid (BEH) particles and packed into a 2.1x30 mm UPLC column. This column was placed on a temperature-controlled chamber specifically designed for HDX MS analysis. A pressure regulator was placed before the waste line in the trap valve to provide high pressure. Phosphorylase b, cytochrome C, and IgGs were digested online under a pressure range above 8000 psi at 0, 10, or 25 °C, respectively. Peptides were separated on a 1x100 mm C18 column, and the eluent was directed into an ESI-QTOF SYNAPT G2-S mass spectrometer. All data were processed by DynamX 3.0.

Results
Data from online digestion using pepsin immobilized BEH column confirmed that the chemical immobilization process was properly carried out and enzymatic activity of pepsin was not affected by the immobilization chemistry. The online digestion of phosB was robust and reproducible, with and without pressurization. An increased number of overlapping peptic peptides were generated from pressurized digestion resulting in improved coverage and redundancy score. Increasing the digestion temperature resulted in higher sequence coverage of cytoC from 63% at 0 °C to 100% at 25 °C. The rate of back-exchange from deuterated proteins was measured for both BEH pepsin column and commercially available pepsin columns and comparable back-exchange rates were obtained. An enhanced chromatographic resolution was achieved for protein digests separated in less than 10 min at 0 °C. Increasing spatial resolution in order to locate the deuterium is a long-standing goal of HDX MS. There are two ways to achieved it: generating more peptic peptides that are shorter in length and highly overlapped; or choosing ETD fragmentation. In this study, the spatial resolution was significantly improved by applying high pressure digestion and ETD. DynamX 3.0 improves the speed and consistency of deuterium uptake measurement for the peptides generated using the HDX platform and has the capability to process deuterated data in intact protein level and peptides with ETD fragmentation.

Conclusions
Data generated from this study demonstrated that the spatial resolution could be significantly improved by either increasing the digestion efficiency of the pepsin column operating under higher pressure or performing targeted ETD.

Novel Aspect
Improved HDX MS system and new processing capabilities in a HDX MS software

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WPS28-08 / Differentiation of topoisomeric peptides by ion mobility - mass spectrometry

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Introduction
Lasso peptides are bioactive peptides produced by bacteria that present a mechanically interlocked structure where the C-terminal tail of the peptide is threaded through and trapped within an N-terminal lactam macrocycle. The structural characterization of lasso structures and the differentiation from the unthreaded topoisomeric peptides (named branched cyclic) can be carried out by NMR, but relatively large amount of pure samples are needed and the method cannot be applied to mixtures.
Ion mobility mass spectrometry comes out as an obvious choice for the differentiation of lasso peptides from their biologically inactive branched cyclic topoisomers, as these peptides are expected to produce different gas phase conformations. These peptides can also be used as a test to investigate their gas phase conformations compared to their conformations in solution.

Methods
Experiments were carried out in a hybrid quadrupole ion-mobility time of flight mass spectrometer (Waters, Synapt G2) equipped with an ESI source and operated in the positive ion mode. Data was collected on a set of five class II lasso peptides and their synthetic cyclic branched topoisomers. Denaturing solvent conditions with and without sulfolane as supercharging agent were used.

Results
The lower charged multiprotonated topoisomers displayed similar drift times, indicating that the branched cyclic peptide was folded in the gas phase into a conformation as compact as the lasso peptide. By contrast, high charge states, obtained in the presence of the supercharging agent sulfolane, permitted to clearly discriminate lasso and branched cyclic topoisomers. Sulfolane was shown not to affect ion mobility results. Comparison of the lasso and branched cyclic peptides for all charge states including the higher charge states obtained with sulfolane yielded three trends that allowed differentiation of the two topologies: low change in collision cross section with increasing charge state of multiprotonated peptides, low intensity of highly charged protonated molecules, even with the supercharging agent and narrow ion mobility peak widths.

Conclusions
Although these three trends were observed with variable proportions in the set of class II lasso peptides tested, they could constitute a fast method to discriminate mechanically interlocked topologies for uncharacterized lasso peptides.

Novel Aspect
Ion mobility of mechanically interlocked peptides from denaturing solutions in combination with a supercharging agent.

WPS28-09 / Chemical Cross-linking and Mass Spectrometry - Tools for Characterization of Conformational Changes in Proteins

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Introduction
Some proteins and enzymes require presence of their specific ligand, cofactor or prosthetic group for their activity. Binding of this specific molecule cause conformational changes which permit to perform their function. In some occasions the identification of conformational changes is difficult. Chemical cross-linking coupled with mass spectrometry provides complex tool for searching and low resolution visualization of these changes. The aim of our project was study of conformational changes induced by binding of ligand to protein molecule. For this purpose were used calcium-binding protein calmodulin whose structures with and without ligand were described by both X-Ray and NMR spectroscopy

Methods
Protein samples modified via homobifunctional cross-linking reagents (DSG, DSS and their deuterated forms) in presence/absence of cofactor were analyzed using reverse-phase chromatography coupled to FT-ICR mass spectrometer. The acquired distance constraints between modified amino acids allowed us to complete molecular models of proteins for each state (with and without ligand). Homology modeling followed by a short steepest descent minimization was performed by using the Modeller 9.12 and Yasara-Structure 14.1 package. Formed lysine-lysine cross-links were quantified using mMass 5.5. program that determined ratio of peptides cross-lined by isotope unlabeled reagents in the presence of calcium ions and peptides cross-linked by isotope labeled reagents in calcium free conditions.

Results
The cross-linking experiments with calmodulin revealed 7 intramolecular distance constraints with DSG and 5 intramolecular distance constraints with DSS. Quantification of obtained cross-links revealed that the ratio between unlabeled and labeled cross-links varies case to case. However all cross-links can be divided into three groups. First group contains cross-links whose ratio is close to 1:1 and therefore calcium ions do not have any effect on their formation. Second group consists of cross-links whose ratio (1:9) is shifted to the favor of peptides cross-linked in calcium free condition whereas third group is composed of cross-links which are mainly formed in the presence of calcium ions (ratio 9:1). Based on these data were generated homology models that describe calmodulin structure in solution in presence or absence of calcium ions. Both molecular models nicely correspond to the structures revealed by techniques with atomic resolution thereby our method for determination of conformational changes was validated.

Conclusions
Presented results show that chemical cross-linking in combination with mass spectrometry could be a new tool for characterization and quantification of structural changes in proteins in which it is not possible to do it by using techniques with atomic resolution.

Novel aspect
Chemical cross-linking and mass spectrometry with high resolution allow quantification of structural changes in proteins.

WPS28-10 / Immobilization of aspartic protease nepenthesin-1 for protein digestion in hydrogen/deuterium exchange mass spectrometry

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Introduction
Combination of hydrogen/deuterium exchange and mass spectrometry (HDX-MS) is widely used technique to probe protein dynamics and structure in solution. In the classical (bottom-up) HDX-MS setup studied proteins are enzymatically digested (usually by porcine pepsin) into peptides whose H/D exchange rate is then followed by mass spectrometry. Recently, a group of aspartic proteases nepenthesins produced by carnivorous pitcher plants of the genus Nepenthes has gained an increased attention for use in HDX-MS due to their unique cleavage preferences [1].

Methods
Recombinant nepenthesin-1 (rNep-1) from N. gracilis was produced by heterologous expression in E. coli and refolded in vitro by several dialysis steps [2]. The mature active form of rNep-1 was obtained after acidification which led to the cleavage of the propeptide chain. Enzymatic characterization of rNep-1 was done by spectrophotometric assays. rNep-1 was also immobilized on a POROS resin and tested under HDX-MS compatible conditions [3]. The digestion efficiency of the immobilized rNep-1 and its cleavage specificity were tested on several model proteins and compared to the commonly used porcine pepsin.

Results and Conclusions
We present a convenient protocol yielding tens of miligrams of the active rNep-1 per liter of the production culture. Despite the lacking glycosylation the biochemical and enzymatic properties of rNep-1 closely match to those decribed for the protease isolated from the pitcher fluid. Interestingly, the recombinant protein exhibits activity and stability over a wide range of pH and temperature. However, some instability under low pH leading to auto-digestion and the loss of activity at high temperatures have been observed which could be attributed to the missing glycosylation. Similarly to the natural enzyme, the recombinant protease is also susceptible to denaturing and reducing agents. This sensitivity has been overcome by immobilization of the rembinant enzyme. Immobilized rNep-1 has been successfully used for online digestion in HDX-MS workflow. Regarding the cleavage specificity the rNep-1 showed typical pepsin-like cleavages but additionally it also cleaved efficiently after all the three basic amino acids (Arg, Lys, and His), which would increase the sequence coverage and spatial resolution of the HDX-MS approach.

[1] Rey M. et al.: Nepenthesin from monkey cups for hydrogen/deuterium exchange mass spectrometry. Mol Cell Proteomics. 2013;12(2):464-72.
[2] Kadek A. et al.: Expression and characterization of plant aspartic protease nepenthesin-1 from Nepenthes gracilis. Protein Expr Purif. 2014; 95:121-8.
[3] Kadek A. et al.: Aspartic protease nepenthesin-1 as a tool for digestion in hydrogen/deuterium exchange mass spectrometry. Anal Chem. 2014 Apr 8. [Epub ahead of print].

Novel Aspect
Recombinant immobilized nepenthesin-1 for protein digestion in HDX-MS workflow.

Acknowledgements
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WPS28-11 / Structural analyses of gas phase molecules using different drift gases in a high resolution ion mobility time-of-flight mass spectrometer

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Introduction
Ion mobility-mass spectrometry (IM-MS) has become a valuable tool in structural studies of biological compounds. Uniform-field drift tube ion mobility instruments can be used to directly calculate collision cross section (CCS) values for gas-phase ions which provide insights into the structure of the molecules studied. This study will include the CCS calculations for selected peptides, carbohydrates, small molecules, lipids and small proteins and the comparison of some experimental CCS values with the theoretical CCS calculations. This study will also discuss the use of different drift gases for improving mobility separation and selectivity in complex sample analyses.

Methods
Both HPLC and direct infusion methods were employed with an ESI source to introduce gas phase ions into the IM-QTOF system.

The instrument used in this study was a modified high resolution IM-QTOF instrument which allows the use of different gases in the drift cell. This instrument is equipped with flow regulators to automatically maintain the correct pressure in the drift tube irrespective of the drift gas used. Molecular dynamics simulations and theoretical CCS calculations were used to interpret the structures for the molecules studied.

Results

The IM-QTOF instrument used in this study consists of a low pressure drift tube ion mobility device coupled to a high resolution quadrupole time-of-flight mass spectrometer. This instrument can maintain proper pressures throughout the instrument when using different drift gases without any modifications to the vacuum system. The ion mobility resolving power for this instrument is 60-85. Currently, helium, nitrogen, argon, nitrous oxide and carbon dioxide drift gasses have been used in this system. Absolute CCS measurements for species in different drift gases are compared with previously reported CCS values and are structurally interpreted using computational approaches. The arrival time information for each ion was directly converted to CCS values using the Mason-Schamp equation. Empirical CCS calculations were performed using custom software that automates the CCS data processing workflow. The preliminary CCS data obtained using this instrument for different classes of biomolecules in nitrogen and helium drift gases agreed well with the literature data. The utility of different drift gases to enhance selectivity in complex sample analyses will be addressed.

Conclusions

Structural analysis of molecules in gas phase can be achieved using ion mobility mass spectrometry techniques. In this study we have calculated CCS values for different classes of molecules using different drift gases and compared some of those results with theoretical calculations.

Novel Aspect

Comprehensive evaluation of the use of different drift gases and CCS calculations in a low-pressure uniform-field drift tube instrument.

WPS28-12 / Real-time native MS to monitor the effect of point mutations, inhibitor or tRNA binding on Tgt subunit exchange and dimer stability

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Introduction

The bacterial tRNA modifying enzyme, tRNA-guanine transglycosylase (Tgt), constitutes a target for new antibiotics against Shigella bacteria. As Tgt is only active in its homodimeric form, a way to address selectivity is to design inhibitors affecting the dimer interface. Time-resolved native mass spectrometry (MS) has been used to study the dynamics of Tgt subunit exchange on wtTgt in presence of different inhibitors as well as in presence of tRNA. Effects of Tgt point mutations that destabilize the dimer interface on the kinetics of subunit exchange are also described.
Time-resolved native MS can be proposed as a complementary biophysical technique for real-time monitoring of protein dynamics, bringing additional information for both Tgt catalytic process characterization and design of new inhibitors.

Method

Native mass spectrometry experiments were performed on a Synapt G2 HDMS (Waters) coupled to an automated chip-based nanoelectrospray device (Triversa Nanomate, Advion Biosciences). All experiments were performed in 1M ammonium acetate buffer at pH 7.5. Instrumental settings were tuned so that ions could be transferred intact and without extensive activation in the gas phase while keeping a sufficient ion desolvation and transmission. For kinetic measurements, proteins bearing a N-terminal Streptavidine tag were mixed with cleaved Tgt. Quantifications were realized based on most intense charge states areas for kinetic measurement and on peaks intensities for protein/ligand complexes ratio determination.

Preliminary data

First, native MS experiments and subsequent MS-based homodimer quantitation performed at different Tgt concentrations allowed to conclude that mutations E498Q and Y258F drastically impact Tgt homodimerization, which was consistent with in silico simulation data. Native MS was thus prone to confirm hot spots for dimer stability. Similarly, the potential of newly designed inhibitors for destabilizing Tgt dimer interface was screened by native MS.
Further real-time native MS experiments were next conducted in order to address dynamics of Tgt subunit exchange for the different Tgt mutants. For this, formation of a heterodimeric complex resulting from monomer subunit exchange between cleaved Tgt and strep-tag Tgt was monitored at different time points over 24h by native MS. Associated rate constants were deduced from native MS spectra based on peak areas. Such kinetics measurements were also performed with several inhibitors or in presence of tRNA. While inhibitors were shown to accelerate monomer exchange, presence of tRNA rather seemed to stabilize Tgt homodimer.
Altogether our time-resolved native MS results combined to X-ray and in silico simulation data allowed to conclude that dynamics of Tgt subunit exchange is strongly affected by inhibitors or point mutations aimed at targeting the dimer interface, while tRNA conversely stabilizes Tgt dimers.
Our results illustrate how native MS and real-time monitoring of protein dynamics can provide strong support for rational design of new lead molecules targeting protein/protein interfaces.

WPS28-13 / Behavior of the disordered tail regions of the histone H2A/H2B dimer

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Introduction

The histone H2A/H2B dimer, a component of nucleosome core particle, has three intrinsically disordered tail regions. We have characterized gas-phase behaviors of these disordered regions attached to the folded core part. In the previous study combined ion mobility mass spectrometry (IM-MS) and molecular dynamics simulation, two populations (Figure 1), Group I (small) and Group II (large), in the collision cross-section (CCS) by IM-MS were recognized for the histone multimers, which was estimated to be due to varied behaviors of the tail regions. To investigate the factors causing the conformational diversity in detail, we have prepared the histone mutant dimers, in which most parts of N- and/or C-terminal tail regions were deleted, as well as wild-type, and analyzed them by IM-MS and hydrogen/deuterium exchange MS (HDX-MS).

Methods

Human histone monomers H2A, H2B and these tail-truncated mutants (deltaN-H2A, deltaNC-H2A and deltaN-H2B) (Figure 2) were overexpressed in E. coli. and purified. Each histone dimer was prepared by refolding the recombinant monomers. The dimers were subjected to IM-MS and HDX-MS experiments (Synapt G2).

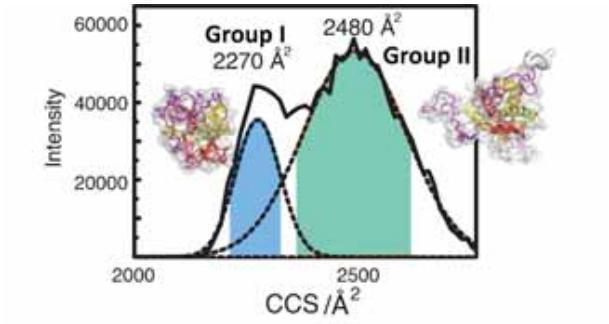

Figure 1.CCS distribution of wild-type H2A/H2B dimer

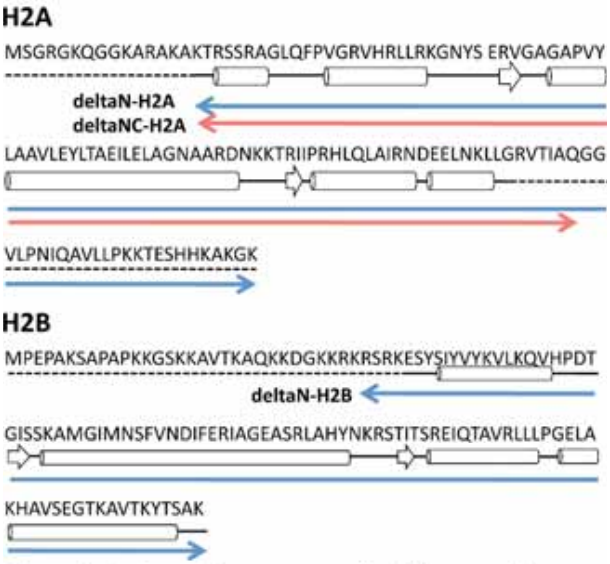

Figure 2. Amino acid sequences of wild-type and mutant histone proteins

Results

In order to investigate the effect of the tail regions on the conformational variety of the histone H2A/H2B dimer, we prepared three histone mutants; deltaN-H2A, deltaNC-H2A and deltaN-H2B. Then three histone dimers, wild-type H2A/H2B dimer, deltaN-H2A/deltaN-H2B dimer and deltaNC-H2A/deltaN-H2B dimer, were prepared with corresponding monomers. Using ESI-IM-MS, the CCS values of the observed ions of the dimers were analyzed. The relative ratio of Group II (large) in the CCS distribution of the dimer got smaller as the tail length was reduced, and the deltaNC-H2A/deltaN-H2B dimer presented slight evidence of Group II. Thus it was confirmed that the gas-phase conformational variety was derived from the multiple behaviors of the tail regions, and the dimer with long tails could generate two populations in the CCS values evidently. Stability of these dimers in the gas phase is now under study using ESI-IM-MS. Next, to examine the structural stability of H2A/H2B dimer in solution, HDX-MS experiments were performed for the histone dimers of wild-type and deletion mutants. It was revealed that the deltaNC-H2A/deltaN-H2B dimer was rather unstable compared with others. This suggests that the tail regions are necessary to retain the folded structure of the H2A/H2B dimer in solution. The difference and similarity between the structures in the gas- and solution-phases will be discussed.

Conclusions

In the IM-MS analysis of the H2A/H2B dimers with various tail lengths, it was identified that the long tail regions contributed to generation of the conformers with diverse CCS values. The HDX-MS and IM-MS experiments suggested the importance of the tail regions to stabilize the dimer structure not only in solution but also in the gas phase.

Novel Aspect

A specific behavior of disordered regions of the histone dimer in the gas phase was characterized, and importance of the disordered tails was verified by IM-MS and HDX-MS.

WPS28-14 / Active Control of Protein Conformation on Surfaces by Hyperthermal Ion-Surface Interaction

Stephan Rauschenbach[1], Gordon Rinke[1], Ludger Harnau[2], Alyanzan Albarghash[1], Matthias Pauly[1], Klaus Kern[1]

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Introduction

The physical, chemical, and biological properties of macromolecules like proteins strongly depend on their conformation. The degrees of freedom of their chemical bonds inflate a huge conformational space, of which however only a small fraction is accessible in thermal equilibrium. The conformation of a protein can be influenced by the chosen solvent, or in electrospray ionization, by the charge state and mode of transfer into the gas phase.

Methods

We show that soft-landing electrospray ion beam deposition (ES-IBD) of unfolded proteins on a surface allows to choose their conformation from of a wide range spanning from fully extended to completely compact, evidenced on the single molecule level by scanning tunneling microscopy (STM) at sub-nanometer resolution. Our experiments are supported by molecular dynamics simulations of the landing process.

Results

We image proteins at the surface as extended strands or as compact patches for high and low charge states, respectively (see Fig. a). Upon increasing the deposition energy, we find that the high charge state proteins adopt more compact conformations.

Conclusions

Our results demonstrate that the final conformation of the protein on the surface is reached through a mechanical deformation of the gas phase conformation during the ion-surface-collision. This process can be actively steered by controlling either the charge state to change the stiffness or the deposition energy in the hyperthermal energy regime to have a more intense collision. Therewith a new dimension to the processing of macromolecular materials is added to the existing coating technology, with the potential to reach otherwise inaccessible conformational states.

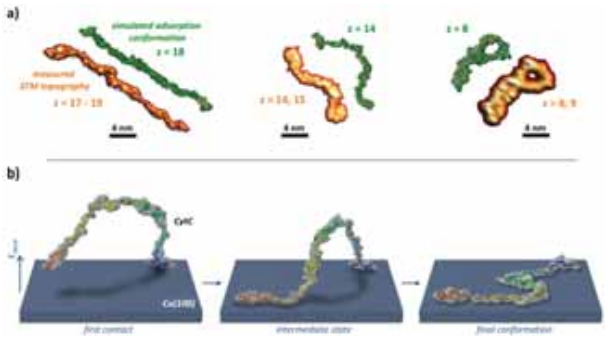

Figure: a) Selected proteins immobilized on a Cu(100) surface imaged by STM in ultrahigh vacuum. Different charge states have a strong effect on the conformation. b) Molecular dynamics simulations of the landing process, showing the deformation of the protein conformation.

Novel Aspect
We show that both, gas phase conformation and ion surface collision are important factors for the structure formation of large molecules on the surface. Our methodology offers two novel modes of active conformation control, beam energy and charge state, which are easily accessible in a preparative mass spectrometry environment.

WPS28-15 / Controlled Reduction of Disulfide Bonds in Biopharmaceuticals Using an Electrochemical Reactor Cell online with LC/MS

Agnieszka Kraj, Hendrik-Jan Brouwer, Nico Reinhoud, Jean-Pierre Chervet
Antec, Zoeterwoude, The Netherlands

Introduction
Reduction of disulfide bonds in peptides and proteins prior to MS analysis is done for several reasons. It is one of the steps in the determination of the disulfide bonds positions which is important for understanding the folding processes of a protein. Also disulfide bonds can hindrance MS identification and their reduction is necessary for efficient sequence analysis of proteins; or prior to HDX exchange procedures. In this lecture, an alternative, electrochemical (EC) method for the efficient and fast reduction of disulfide bonds in peptides and proteins combined online with separation and mass spectrometric detection is presented. The method utilizes an alternating potential pulse and does not require any reducing chemicals, which is an advantage over traditional methods.

Methods
To demonstrate the performance of the EC reactor cell in an online LC/EC/MS platform, somatostatin, insulin, α-lactalbumin, and lysozyme were used as model compounds. The disulfide bonds were reduced in the EC reactor cell placed between the column and electrospray source of a mass spectrometer (HCT Plus (Bruker, USA). Reduction was performed in the µ-PrepCell (Antec, The Netherlands) consisting of a titanium-based working electrode, a titanium auxiliary electrode and a Pd/H2 reference electrode. The separation was performed using reversed phase chromatography.

Results
We present reduction of the disulfide bonds using the EC reactor cell that is placed between column and electrospray ionization source (ESI).
Insulin with 3 disulfide bridges was used as model. Reduction of somatostatin with one disulfide bond (1638 Da), α-lactalbumin with four bonds (14178 Da) and lysozyme with four bonds (14 313 Da) will be shown to demonstrate the efficiency of electrochemical disulfide bond reduction The reduction efficiency is controlled by parameters such as flow rate, mobile phase composition (formic acid and acetonitrile content) and the square-wave pulse settings. Based on the example of α-lactalbumin and lysozyme, the controlled reduction of disulfide bonds will be shown. By changing of the applied potential the extent of disulfide bond cleavage is controlled. More negative potentials result in a shift of the charge state distribution indicating increased disulfide bond cleavage and unfolding of the protein.
The effects of different experimental parameters are tested and the optimized protocol for the electrochemical reduction of disulfide bonds in online LC/EC/MS platform is presented. Furthermore, we show data on long term stability and repeatability.

Conclusions
The electrochemical cell can be positioned pre- and post-column resulting in a fully automated platform for fast characterization of disulfide bonds in protein/peptide based biopharmaceuticals.

Novel Aspect
Controlled and sequential reduction of disulfide bonds in proteins using an electrochemical reactor cell online with LC/MS.

WPS29 - Ambient Ionization and Miniaturization

11:00-15:00
Poster Exhibition, Level -1

WPS29-01 / Functionalized porous silicon surfaces as DESI-MS substrates for small molecules analysis

Nicolas Schwab[1], Moriam O. Ore[2], Alessandra Tata[2], Marcos Eberlin[3], Sylvie Morin[2], Demian Ifa[2]
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Introduction
In desorption electrospray ionization mass spectrometry (DESI-MS), in addition to low gas and solvent flow rates, the type of surface help avoid the 'splashing of solvent' or 'washing effect' where samples are promptly removed from the surface by the spray. These effects operate on smooth surfaces and generally result in unstable signals as the spray moves over the spot. The aim of this work is to compare the performance of different porous silicon surfaces (PSi) for small molecules analysis with regard to the sensitivity and stability of the signal observed in DESI-MS.

Methods
Silicon crystalline were first etched in hydrofluoric acid and anhydrous ethanol mixture galvanostatically under different conditions to produce and control the porous sizes. To avoid the fast oxidation of the freshly etched H-terminated porous silicon substrates were then reacted thermally with 1-decene reagents in Schlenk tubes under argon in order to produce a chemically stable Si–C bond creating a purely hydrophobic surface. Aliquots of the sample solutions in different concentrations were deposited on porous silicon surfaces and after dried, the spots were scanned by DESI for the comparisons. The mass spectrometer (LTQ Finnigan, Thermo Co.) was operated in the multiple reaction monitoring (MRM) mode and the ion current was used for data analysis to find the lowest concentration detected (LOD) for each analyte. The signal stability was also investigated.

Tabel 1: Lowest concentrations detected for representative compounds on different porous surfaces.

Compound	Polarity	CID	Precursor → Product (m/z)	LOD (ng/mL)	
				pSi (10-50nm)	pSi (< 10nm)
Cocaine	+	14	304 $[M+H]^+$ → 182	1	1
Diazepam	+	22	285 $[M+H]^+$ → 257	10	10
Oxycodone	+	30	316 $[M+H]^+$ → 298	10	1
Propranolol	+	25	260 $[M+H]^+$ → 183	10	1
MRFA (peptide)	+	25	524 $[M+H]^+$ → 288	100	100
Chloramphenicol	-	27	321 $[M+H]^-$ → 194	10	10
Taurocholic acid	-	27	514 $[M+H]^-$ → 353	100	100

Preliminary Results
The table 1 (attached) show the LOD for seven ordinary compounds on distinct surfaces. Four different concentrations of each compound, ranging from 1 ng/mL to 1 µg/mL were deposited in a straight line and the DESI spray scanned across the dried spots. Generally, the LOD for microporous (porous smaller than 10 nm) show the best results. New experiments are being performed with bigger porous size to evaluate the LOD for these surfaces.
Signal stability was investigated by DESI imaging on dried spots using 5ul of propranolol (m/z 260) at 1 µg/mL. This procedure was repeated several times for the same dried spot to observe the

signal intensities and the imaging also provide information about size and distribution of the analyte spoted due the cristalinization process over the surface in different porous sizes.

Conclusion
The functionalized PSi surfaces provides a good alternative for DESI-MS, increasing the sensitivity and improving stability of the signal. This is possible because the weak sample-surface interactions due the hyprophobic characteristic favoring desorption of the analyte. The porosity size of the surface may provide more surface area for interaction between the dried analyte and the thin solvent film created by the DESI spray, resulting in more effective dissolution of the analyte in the spray solvent.

Novel Aspect
Functionalized porous silicon surfaces for DESI-MS to enhanced the sensitivity and stable signal for small molecules

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WPS29-02 / Investigation of Programmable Temperature Vaporisation as a Sample Introduction Method for Ambient Ionisation MS

Bryan McCullough, David Bell, Camilla Liscio, Christopher Hopley
LGC

Novel Aspect
Use of a PTV inlet in ambient MS allowing temperature discrimination in sample introduction yields better quality MS data and improved robustness.

Introduction
Fuels are often transported in pipelines which transport more than one fuel type and are therefore subject to some degree of cross-contamination when the fuel type is changed. Potentially the most serious type of cross-contamination is contamination of jet fuels with fatty acid methyl esters (FAMEs) from biodiesels as their presence in this fuel may lead to engine problems. To counter this, a limit of 5 ppm total FAME content in jet fuel has been set. Lab-based tests for FAME content are now fairly routine but, given the potential seriousness of this contamination, an at-site testing solution would be desirable; with this in mind we have investigated the potential for analysis of FAME content in jet fuel using corona discharge ionisation (CDI) based ambient MS techniques with a view to combining them with emerging transportable mass spectrometers for at-site analysis.

Methods
The source of a Micromass Ultima QqQ MS was modified such that it could be used for ambient ionisation via a hole in the "goldfish bowl" source housing. This set-up was used to analyse a series of jet fuels spiked with different levels of FAME.
This source was then further modified to include an ATAS OPTIC II programmable temperature vaporisation (PTV) inlet the output of which was positioned near the heated nebuliser of the APCI source thus allowing temperature discrimination in the sample introduction step. Analysis of the jet fuel samples was then carried out with and without a solvent flow through the heated nebuliser in order to allow comparison between PTV-CDI and PTV-extractive APCI.

Results
ASAP-MS was able to detect FAMEs in jet fuel with good sensitivity, however, the spectra generated are extremely noisy due to the complex sample matrix and often only a few scans in duration due to the rapid nature of the desorption thus making accurate quantitation very difficult. In addition the source region was rapidly contaminated with fuel leading to a drop-off in sensitivity with each sample analysed.

The use of the PTV inlet allows far greater control of sample introduction as the lower boiling components of a sample (e.g. jet fuel) can be analysed separately to the higher boiling components (e.g. FAMEs) or even sent to waste. Using the PTV-CDI set-up with and without solvent flow demonstrates the clear gains which can be obtained by temperature discrimination in sample introduction giving better sensitivity and robustness than that observed for ASAP-MS.

Conclusions
The inclusion of a PTV inlet allows temperature discrimination in sample introduction for ambient mass spectrometry giving the user greater control over their experiment and greatly increasing data quality. The combination of this technique with transportable MS technology may prove very powerful for point-of-use analysis of fuels.

WPS29-03 / On-line monitoring of continuous flow chemical synthesis using a portable, small footprint mass spectrometer

Tony Bristow[1], Andrew Ray[1], Anne O'Kearney-McMullan[1], Louise Lim[2], Bryan McCullough[3], Alessio Zammataro[4]
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Introduction
For on-line monitoring of chemical reactions mass spectrometry (MS) can provide data to (i) determine the fate of starting materials and reagents, (ii) confirm the presence of the desired product, (iii) identify intermediates and impurities, (iv) determine steady state conditions and (v) speed up process optimisation. Recent developments in portable mass spectrometers further enable this coupling, as they can be easily positioned with the reaction system to be studied. A major issue for this combination is the transfer of a sample that is representative of the reaction and also compatible with the mass spectrometer. This is particularly challenging as high concentrations of reagents and products can be encountered in organic synthesis.

Methods
Continuous on-line monitoring by MS was applied to one of the stages of an industrial chemical process under development at AstraZeneca; a Hofmann rearrangement reaction. A FlowStart Evo B-400 flow reactor was coupled to a Microsaic 4000 MiD® mass spectrometer. Due to its size, the mass spectrometer could be placed next to the reactor within the fume hood, housing the reactor. From the reaction mixture emerging from the flow reactor, small aliquots were continuously transferred using a mass rate attenuator (MRA) into a sampling make-up flow provided by a high pressure Knauer pump. The make-up flow is then directed to the mass spectrometer. This process dilutes the output of the reactor and also makes it compatible to electrospray ionisation and mass analysis. The consumption of reactants and the formation of products and impurities were monitored continuously over a range of reaction temperatures. The MS data was used to determine optimum conditions for reaction completion. A key additional aspect of this study was the evaluation of the effect that different make-up flow compositions and dilution factors had on the resulting MS data.

Results
The Microsaic 4000 MiD® portable mass spectrometer was successfully coupled to FlowStart Evo flow reactor to monitor the Hofmann rearrangement reaction on-line. The progress of the reaction as a function of the reaction temperature was established and the optimum reaction conditions were identified. Impurity formation during the reaction was also detected and characterised. The composition of the make-up flow and the dilution factor used to sample aliquots of reaction mixture clearly influenced the on-

line MS data. Variation in ionisation efficiency of the components monitored directly was identified as an important factor as issues of detector saturation were encountered. This influences the selection of a dilution factor and the ions monitored to provide the required process understanding

Conclusions
On-line reaction monitoring using mass spectrometry provided chemical process understanding and allowed reaction optimisation to be established in real time.

Novel Aspect
Overall evaluation and optimisation of the key parameters of this experimental approach to on-line MS reaction monitoring in an industrial environment. The study also describes the limiting factors of such an approach which must be considered and overcome.

WPS29-04 / Substantial Release of Silicones from Household Items and Baby Articles Analyzed by Direct Analysis in Real Time-Mass Spectrometry

Jürgen Gross
Heidelberg University

Introduction
The release of low molecular weight silicone oligomers from articles of daily use such as flexible silicone baking molds, beakers, watch bands, pacifiers, and teething rings has been analyzed by direct analysis in real time (DART) [1-3] combined with a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer. The substantial release of silicones at elevated temperatures within seconds indicates a potential health hazard from daily use of such silicone items. Thus, a substantial dose of silicones may be taken up by humans, in particular during elongated exposure under extracting conditions as in case of pacifiers or teething rings or when used at elevated temperature as is the case with baking molds. Here, the use of DART-MS is demonstrated as a rapid screening technique for articles of daily use to assess their tendency to release silicones.

Methods
Experiments were performed using a Apex-Qe FT-ICR instrument (Bruker Daltonik). A DART-SVP ionization source (IonSense) was mounted at an angle of 45° relative to the axis of the ceramics tube of the VapurInterface. Items were positioned halfway between helium exit and capillary entrance and directly exposed to the ionizing gas. Ions were accumulated for 1.0 s prior to ICR mass analysis. Ions were excited and detected using standard setting from previous DART work [4-6]. Broadband spectra were acquired with 1 M data points. Per magnitude spectrum, 16 transients were accumulated. Initial positive-ion mode mass calibration was established in DART mode using silicone oil [4] or ionic liquids [6].

Results
Semiquantitative analyses of silicone release have been performed by DART- FT-ICR-MS. By defining a standard temperature and analysis time, which is applied for any item subjected to analysis it was possible to rank the items by their silicone releasing tendency. After screening the DART gas temperature in the range of 150–350 °C, a standard temperature was defined and applied for all analyses. Furthermore, silicone ionic compositions were verified by accurate mass measurement based on an independent external mass calibration (mass accuracy ≈ 2 ppm up to m/z 1200) [4, 5].

Conclusions
DART-MS is very sensitive for the analysis of silicones and can be used to analyze silicone release from articles of daily use. Thus, it should be useful for similar applications, e.g., to breast implants or food after having been in contact with silicone rubber.

Novel Aspect
DART-FT-ICR-MS reveals substantial release of silicones from articles of daily use and indicates potential health hazard from such items.

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WPS29-05 / Towards an Add-on Secondary Electrospray Ionizer for pre-existing API-MS and for high sensitivity analysis of volatiles

César Barrios-Collado[1], Renato Zenobi[2], Guillermo Vidal-de-Miguel[1]
[1]ETH Zurich, Department of Chemistry and Applied Biosciences; SEADM. S. L.; Valladolid University, Energy and Fluid Mechanics Engineering Dep., [2]ETH Zurich, Department of Chemistry and Applied Biosciences

Introduction
Despite the interest in the detection of volatiles by mass spectrometry, currently only two dedicated instruments for the task (SIFT & PTR) exist. Notably, no commercial alternative exists for owners of a pre-existing MS with an atmospheric pressure ionization source to turn their instruments into a vapor analyzer.
Secondary ElectroSpray Ionization (SESI) in tandem with Atmospheric Pressure ionization Mass Spectrometry (API MS) has already shown sensitivities below the ppt level for polar vapors. It has been successfully used in different applications requiring the analysis of ambient vapors, with very promising results. In the security field of explosives detection, in which an extraordinary level of sensitivity and selectivity is required, an improved version of this technique (named Low Flow SESI) enabled, in tandem with a Differential Mobility Analyzer, the detection of explosives in cargo containers at the sub-ppq level.
In a collaborative project, we have joined efforts to further develop an Add-on Low Flow SESI platform to bring these improvements to the general MS user. Here, we present the first results of this development.

Methods
The new architecture utilizes the optimized electrostatic and fluid dynamic configuration of the LF-SESI, which enables high efficiency ionization, and is further improved to enable a fast coupling or disassembly to the API-MS, to operate safely at high temperature, and to be compliant with safety and work place regulations. The new ionizer will be coupled to AB Sciex, Thermo, and Waters API-MS instruments. It is composed of three main parts: (i) an interface, which is specific for each type of MS, (ii) a core, which incorporates the ionization chamber, and (iii) an external module, which incorporates the flow and temperature controllers. In the first stage of this development we have evaluated the expected ionization efficiency, and the transmission of ions to the MS.

Results
The mechanical limitations imposed by the MS are compatible

with high transmission as long as the LF-SESI electrodes are sharp enough to avoid stagnation regions. The expected result of this development is a vapor ionizer which, due to its versatility, will allow the user to take full advantage of the high sensitivity of LF-SESI, and the fast evolving performances of the MS developped by the main vendors. The new ionizer, if compared with other vapor analyzers, which incorporate a built-in MS, will drastically reduce the acquisition costs for users who already own an API-MS (including the most commonly used LC-MS platforms).

Conclusions
According to our compatibility and transmission studies, the Add-on LF-SESI configuration for general MS is viable.

Novel Aspect
The new Add-on architecture enables high efficiency ionization and high ion transmission, and brings LF-SESI to the general MS user.

WPS29-06 / A comparison of ion mobility spectrometry and direct ionisation mass spectrometry for the detection of trace explosives on hand swabs.
Christopher Hopley, Bryan McCullough, Camilla Liscio, David Bell
LGC

Novel Aspect
A performance comparison of direct ionization mass spectrometry to ion mobility spectrometry for the analysis of trace explosives on swabs with hand sweat matrix.

Introduction
Ion mobility spectrometry is the technique of choice for explosives screening in the security environment, such as transport and entertainment venues, due to the rapid analysis and simplicity of operation. However there are acknowledged drawbacks with the technology, including issues with matrix suppression, non-detection of novel explosives and poor linearity. Direct ionization coupled to transportable mass spectrometry could address some of these drawbacks due to better ionization sources, better linearity and more efficient ionization less prone to matrix effects. The recent advances in lower price miniaturized mass spectrometers together with the rapid development of alternative direct ionization sources, could enable the wider adoption of mass spectrometry technology, for the development of rapid semi-quantitative screening in the security screening environment Here an ASAP (atmospheric solid analysis probe) direct ionization source coupled to a mass spectrometer was applied to the direct analysis of swabs, which are the sampling system of choice for security screening. The results were compared with the results obtained by using two commercially available IMS instruments.

Methods
Explosive solutions prepared in methanol were spiked onto commercial cotton (Smiths) and fiberglass (GE) swabs. The solvent was allowed to evaporate prior to analysis and swabs were wiped over the palms of the hand prior to spiking with the explosives to simulate matrix sampling conditions (hand sweat). The spiked swabs, both with and without matrix were then analysed on two ion mobility spectrometers, a Smiths ion scan DT500 and a GEItemiser 3. In addition, an ASAP direct ionization source coupled to a Waters QuattroUltima QqQ was modified to enable the direct analysis of swabs in order to generate comparison data.

Results
A discussion on the relative merits of each of the techniques will be presented. The performance of the ASAP direct ionization source coupled to a Waters Quattro Ultima QqQ will be compared with the performance of the ion mobility devices and limits of detection and linearity will be shown for a range of explosives. In addition, the effects of the presence/absence of the matrix (hand sweat), for the detection of a range of explosives will be discussed.

Conclusions
The data obtained shows similar performance for the ASAP-MS system to the traditional devices, dependant onoperation mode of the mass spectrometer and source conditions. It is felt that further refinement of the swabdesorption interface coupled to the MS would improve the usefulness of the technique. However the data presented demonstrates the potential of the ASAP-MS technology for security screening.

WPS29-07 / On-site detection of ecstasy tablets by portable mass spectrometer
Hiroyuki Inoue[1], Yukiko Nakazono[1], Yuko Iwata[1], Masuyoshi Yamada[2], Akihito Kaneko[3], Hidetoshi Morokuma[3], Shun Kumano[2], Yuichiro Hashimoto[2], Fumiyo Kasuya[4]
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Introduction
Drug abuse is a serious social problem all over the world. For rapid screening of illicit drugs, an about 11-kg portable mass spectrometer was developed. The system consists of pulse introduction of sample vapor, dielectric barrier discharge ionization (DBDI), and miniature linear ion trap mass spectrometry. To minimize vacuum pumps, the sample gas is introduced into the mass spectrometer for only short time (20−50 ms per second). The DBDI source can efficiently ionize the sample molecules as [M+H]+. For reliable identification of target ions from the other chemical noises without chromatographic separation, tandem mass spectrometry is applied. In this study, we show applicability of the portable mass spectrometer to rapid screening of ecstasy tablets.

Methods
About 0.2−0.4 mg of powdered samples of ecstasy tablets were attached on the top of the rod-like sampling probe. After the probe was set on a heater and heated, the sample was vaporized and the gas was introduced into the DBDI source through a pulse valve and an introduction tube. Then the ions were sent to the linear ion trap mass spectrometer. After the ions were trapped and the pulse valve was closed, the pressure inside ion trap chamber was decreased to less than 0.1 Pa. Then the ions in the trap were scanned and detected.

Results
Direct analysis of powdered samples of ecstasy tablets containing 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), and methamphetamine (MA) showed full mass spectra with ions at m/z 194, 180, and 150, corresponding to the protonated molecules of MDMA, MDA, and MA, respectively. Collision-induced dissociation of protonated molecules gave characteristic product ion spectra. Lower limit of detection of the compounds in tablets was less than 1 mg. 4-Methoxymethcathinone, which is controlled as “designated substances” in Japan since May 2011, is one of the isomers of MDMA. The system could differentiate between the two compounds by comparing their product ion spectra.

Conclusions
We developed the portable mass spectrometer for rapid screening of ecstasy tablets. The time for screening one sample was less than 5 min, and it can be driven by a battery. The system would

be useful for rapid on-site drug screening.
This work was supported in part by "R&D Program for Implementation of Anti-Crime and Anti-Terrorism Technologies for a Safe and Secure Society", Funds for Integrated Promotion of Social System Reform and Research and Development of the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Novel Aspect
The portable mass spectrometer enables direct analysis of illicit drugs in ecstasy tablets.

WPS29-08 / Thermal Desorption/Electrospray+Atmospheric Pressure Chemical Ionization/Mass Spectrometry for Simultaneously Detecting Polar and Nonpolar Compounds in Complica

Minzong Huang, Siou-Sian Jhang, Jentaie Shiea
National Sun-Yat Sen University

Introduction
Ambient mass spectrometry (AMS) has been used to characterize chemical compounds under ambient conditions without sample pretreatment. Based on the differences on ionization mechanisms, the technique can be classified as: electrospray ionization (ESI) based AMS or atmospheric pressure chemical ionization (APCI) based AMS. ESI-based AMS is useful for characterizing polar and large compounds, while APCI-based AMS - including corona discharge and plasma ionization- is useful for characterizing small and less polar compounds. A combination of both ionization methods will extend the detective mass range as well as polarity. In this study, we combine a thermal desorption sampling unit with a ESI/APCI ionization source so that both polar and nonpolar chemical compounds in complicated mixtures are detected simultaneously.

Methods
The ambient mass spectrometry consisted of: (1) sampling probe - for taking the sample in solution or on solid; (2) heated oven - for thermally desorbing analytes from the sampling probe; and (3) ESI/APCI source - for post-ionizing the desorbed analyte via interactions with the reactive species in ESI/APCI plume. The reactive species were generated by electrospraying an acidic methanol solution and discharging nitrogen gas under high electric field. The ESI/APCI source can be operated at ESI-only, APCI-only, and ESI+APCI mode, respectively. The analyte ions were detected by an ion trap mass analyzer attached to the ion source.

Preliminary Data
The TD/ESI+APCI/MS was used to detect both polar and nonpolar chemical compounds in various samples. A solution containing lidocaine, carbazole, and ferrocene standards was analyzed, where protonated lidocaine and carbazole (MH+) ions were detected in ESI-only mode, protonated lidocaine (MH+) ions and carbazole and ferrocene (MH+) radical ions were detected in APCI-only mode, and protonated lidocaine and carbazole ions and carbazole and ferrocene radicals were detected in the ESI+ACPI mode. The experimental results indicated that the dual ionization source incorporated the features of ESI and APCI and is useful for simultaneously characterizing both polar and nonpolar chemical compounds. The technique was applied to study the composition of the samples with very complicated components such as crude oils, heavy oils, and plant extracts.

Novel Aspect
Simultaneous detection of polar and nonpolar compounds in complicated mixtures was achieved by thermal desorption/ESI+APCI/mass spectrometry.

WPS29-09 / Atmospheric pressure vacuum ultraviolet ionization via microplasma ionization sources

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Georgia Institute of Technology

Microhollow cathode discharges (MHCDs) have been recently used to photoionize neutrals desorbed from solid samples at atmospheric pressure via the generation of vacuum ultraviolet (VUV) photons.1 These photons ionize analytes with minimum fragmentation. MHCDs operating in a windowless mode have also been shown to protonate analytes via chemical ionization pathways.2 These devices are compact, robust, require minimal gas flow, and operate at lower power than other ionization techniques, giving MHCDs an advantage when comparing cost and portability. We expand on the applications of MHCDs by demonstrating their use as ionization sources for direct analysis and laser desorption surface imaging experiments.These spatially-resolved mass-specific imaging experiments use a programmable translation stage, a Nd:YAG laser for desorption, and a differentially pumped atmospheric pressure sampling triple quadrupole mass spectrometer.

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WPS29-11 / Mechanistic understanding on factors determining ionization efficiencies of (+) APPI-MS

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Kyungpook National University

Introduction
Gas phase basicity (GPB) has been considered as an important factor governing production of protonated ion with (+) mode atmospheric pressure photo ionization (APPI). However, analyses of standard compounds in this study show that a significant portion of basic compounds with high gas phase basicity produce mostly molecular ions. Therefore, it is clear that a factor other than GPB is needed to explain the tendency to generate protonated ions by (+) APPI. In this study the mechanisms for the generation of molecular and protonated ions for basic PAH compounds were thoroughly investigated by combination of quantum mechanical calculation and MS analysis.

Methods
Standard compounds and HPLC grade toluene were purchased from Sigma-Aldrich (MO, USA) and used without further purification.Each standard sample was dissolved in toluene to final concentration of 1μM~10μM. The solutions were analyzed by positive mode APPI Q Exactive ion trap mass spectrometer (Thermo Scientific). All the solutions were directly injected to the APPI source at a flow rate 40 μl/min and the APPI source temperature 200~400 °C. Other operating mass spectrometric parameters were: sheath, aux and sweep gas flow rate (arb) = 10, 5 and 0 respectively. Quantum mechanical calculations were carried out using GAMESS software.

Results and Conclusions

Fourteen PAH compounds with GPB values higher than 200 kcal/mol were selected, dissolved into 100% toluene and analyzed with (+) mode APPI MS. For an example, N-methylaniline and benzylamine have gas phase basicity more than 210 kcal/mol. However, it was observed that benzylamine dominantly produces protonated ion but N-methylaniline produce molecular ions. Therefore, these results strongly suggest that a parameter other than gas phase basicity is needed to understand protonation behavior of molecules in (+) mode APPI. To find a better parameter, quantum mechanical calculation has been performed. In our previous study, it was proposed that protonation mainly occurs through two step reactions. The first step is either composed of charge exchange reaction with toluene radical ions or direct photo ionization. The second step is composed of hydrogen transfer reaction (A•+ + C6H5CH3 à H+(A) + (C6H5CH2)•). The reaction enthalpy (ΔH) values of the hydrogen transfer reactions of fourteen compounds were calculated and compared with experimental results. It was observed that compounds with negative ΔH values produced mostly protonated ions and ones with positive ΔH values produced molecular ions. Therefore, it is concluded that ΔH of hydrogen transfer reaction is a good parameter to predict protonation behavior of molecules in (+) APPI MS analyses.

Novel aspect (19 out of Limit 20 words)
The reaction enthalpy of hydrogen transfer reaction correlates well with protonation behavior of compounds in (+) APPI MS analysis.

WPS29-12 / Alternative ionization methodologies for the broadening of intra-operative applications of rapid evaporative ionization mass spectrometry

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Introduction
The intra-operative application of REIMS for real time tissue identification has recently been demonstrated as a powerful new tool providing additional information for the surgeon, reducing the amount of healthy tissue taken and increasing the assurance that all cancerous cells have been excised.
Whilst highly successful for some cancer types, other applications are hampered by the local environment and surrounding tissue type. To have a technique that can be used for all cancer types a greater control over ionisation is required, the work presented here will describe these advancements.

Method
Developmental work was carried out on food grade meat whilst proof of concept and validation was carried out on human tissues collected and used in strict accordance with ethical guidelines. All experiments were carried out on a Waters Xevo G2S Q-ToF instrument.
The aerosol from the cutting procedure is drawn up a PTFE tube and introduced orthogonally to the inlet capillary of the mass spectrometer using a Venturi pump.
Modifications to the inlet interface to increase the ion yields and biochemical species obtained include cold and heated collision surfaces, in-plume electrospray post-ionisation and the introduction of solvents to the nitrogen gas of the Venturi pump.

Results
Due to the underlying ionization mechanism, REIMS from tissue typically yields high intensity signal for phospholipids in negative ion mode. Charge segregation on the aerosolised evaporate droplets leads to predominantly negative ions which provides a characteristic fingerprint of phospholipid ions for a given tissue.
In cases where the electrosurgery is being carried out in very moist conditions (colorectal for example) or when the tissue surrounding the tumour is not conducive to the formation of negative ion phospholipid peaks, additions to the interface are required to either increase the ion yield or to bias the ionization towards new biochemical groups.
In the case of breast cancer, normal breast tissue, due to high tri and di glycerides levels, does not produce negatively charged ions and thus the tissue cannot be characterised. Electrospray post-ionisation of the aerosol was carried out with formic acid added between the Venturi pump and the MS inlet. This addition created a range of phospholipid and TG and DG in the positive ion mode not seen within the normal experiment. When coupled to a heated collision surface many orders of magnitude extra ion intensity is achievable making otherwise difficult to identify tissues easily characterised.
Within the negative ion mode, the heated collision surface yields a highly reproducible strong signal from a range of tissues. We demonstrate that the addition of solvents to the gas flow can alter the ionisaiton occurring within the interface and open up the ability to detect additional groups of biochemicals to further aid in the characterisation of tissues.

Novel Aspects
Modified inlet interfaces for iKnife applications increase ion yield and accessible biochemical information through creating different ionisation pathways increasing the range of surgical procedures the technique can be applied to.

WPS29-13 / Direct-infusion and paper spray ionisation mass spectrometry for high-throughput screening of rapid oak extracts

Ross Farrell[1], Richard Wilson[2], David Nichols[2], Michael Breadmore[1], Robert Shellie[1]
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Introduction
Wines are normally matured in contact with oak wood to improve sensory quality through the positive contribution of oak extracts to wine aroma, taste, colour and stability. The long extraction procedures and sample preparation requirements for traditional analysis of complex oak extracts present challenging problems. The development of high-throughput screening methods that allow direct analysis of samples with minimal sample preparation are of great interest due to their potential for improving quality control in the wine industry.

Method
In this preliminary study we combined rapid ultrasonic extractions of milled oak samples with alternative mass spectrometry (MS) approaches. Methanol extractions of milled oak from two separate French oak staves were carried out over two time periods of five and fifteen minutes. Extractions were conducted in duplicate.
Extracts were characterised using traditional liquid chromatography-mass spectrometry (LC-MS) with a high-resolution Orbitrap mass spectrometer. Oak extracts were also analysed using two different direct mass spectrometry approaches with no sample preparation step, namely direct-infusion electrospray ionisation (DI-ESI) and paper spray ionisation mass spectrometry. Significant components of the different extracts were identified via the combination of tandem mass spectrometry and high-resolution mass spectrometry data. All MS data were collected in negative ionisation mode. Principal components analysis (PCA) was performed to visualise and evaluate the data.

Results
The primary components of the rapid oak extracts identified by LC-MS were also observed using the direct MS methods, although matrix effects are evident. The major components of the

rapid oak extracts were identified as hydrolyzable ellagitannins and ellagic acid. Triterpenes and lignols such as lyoniresinol were also tentatively identified. Differences between staves and between the two extraction periods were observed.

Conclusions
Rapid ultrasonic oak extracts taking less than fifteen minutes were found to contain compounds known to be important taste-active components of oak aged wines. Characteristic spectra were obtained using both DI-ESI MS and Paper spray MS methods indicating their potential for high-throughput screening of oak extracts.

Novel Aspect
There are few studies concerning ultrasonic extraction of oak compounds and this is the first study coupling ultrasonic oak extractions to DI-ESI and Paper spray ionisation MS methods.

WPS29-14 / Halo-shaped flowing atmospheric pressure afterglow for ambient desorption/ionization mass spectrometry
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WPS44 - Very Large Biomolecules and Structural Biology

11:00-15:00
Poster Exhibition, Level -1

WPS44-01 / In vivo catabolism of the fusion protein Tetranectin-Apolipoprotein A1 in rabbit
Manfred Zell[1], Christophe Husser[1], Gregor Jordan[2], Axel Paehler[1], Roland Staack[2], Wolfgang Richter[1], Manfred Zell[1]
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Introduction
Tetranectin-Apolipoprotein A1 (TN-ApoA1) is a fusion protein of 32.8 kDa composed of the Apolipoprotein A1 (ApoA1) and endogenous Tetranectin (TN) protein. Lipidated TN-ApoA1 was intravenously administered to rabbits and its plasma concentration determined by an ELISA assay to establish pharmacokinetic (PK) profiles. Since there were hints of the occurrence of active metabolite, an LC-MS method was developed to investigate this issue in further detail.

Methods
TN-ApoA1 and its catabolites were immunoextracted from rabbit plasma using a biotinylated monoclonal capture antibody (mcAb) coupled to streptavidin coated magnetic beads. After incubation of the beads in 50 µl of plasma, the captured analytes were released from the isolated beads. The immuno-extraction eluate was analyzed on a monolithic HPLC column(1.0 mm i.d. x 50 mm) to isolate the protein. The intact protein (top-down) was analyzed by LC-QTof MS or after digestion with proteinases (bottom-up). Peptide analysis of digests (bottom-up) were conducted utilizing a column-switching capillary HPLC system (NanoAcquity, Waters) using Xbridge BEH130 (0.3 mm i.d. x 10 cm, 3.5 µm) as analytical capillary (AC) and a shorter capillary of the same type as trapping column. The eluents were 0.1% formic acid and acetonitrile. Peptide separation on AC was performed by raising the gradient from 3% acetonitrile to 40% in 90 min at a flow rate of 10 µl/min. The effluent of AC was directed to an electrospray ion source of the QTof MS (Synapt G2, Waters). MSE spectra were acquired in the mass range m/z 50 to 2000 in 0.5 sec using collision energy ramping from 25 to 45V.

Results
Immunoextraction was employed to isolate active metabolites since the mcAb used was directed to the epitope in the fusion region of TN-ApoA1. Intact protein analysis using deconvolution of the mass spectra revealed the occurrence of a major metabolite with a loss of 168.1 Da. Apparently AP was cleaved off the N-terminus of TN-ApoA1. This catabolic metabolite M1 was the main carrier of the pharmacological response showing a terminal half-life being much longer than that of administered TN-ApoA1. The cleavage of AP was further confirmed by peptide mapping and accurate mass determination of peptides following protein digestion with LysC. In contrast to trypsin, LysC produced a more abundant signature peptide comprising the N-terminus of TN-ApoA1 which demonstrated the rapid AP cleavage in rabbit after administration of TN-ApoA1. Further minor catabolic metabolites could also be identified where bigger peptide portions from the C- and N-terminus were cleaved off.

Conclusions
Complete PK profiles of catabolic metabolites could be established from ELISA concentration data using the ratios of deconvoluted mass peaks from TN-ApoA1 and its metabolites as determined by intact protein mass measurements using mass spectrometry.

Novel aspect
To derive PK profiles of metabolites of therapeutic proteins from ELISA concentration data by combination with mass spectrometric peak ratios.

WPS44-02 / Ion-mobility mass spectrometry analysis of the conformational conversion of amyloid aggregation
Mei-Chun Tseng[1], Chia-Sui Sun[2], Chun-Hua Hsu[3], Gerard, Chun-Hao Lin[4], Cindy Y.-H. Wang[2], Joseph Jen-Tse Huang[5], Yet-Ran Chen[6]
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Introduction
Ion mobility spectrometry is able to separate complex mixtures of ions based on their shape and/or charge, yielding structural information complementary to molecular mass measurements. The technique of IMS relies on separating ion's characteristic collision cross-section (CCS) according to their mobility through a drift-tube filled with a buffer gas and electric filed. Using this approach, IMS-MS has benefited greatly from analyses in which transient intermediate species are lowly populated and short lived, such as in amyloid fibril formation. In different amyloid disorders, fibrils accumulate in specific regions of the body causing the variety of symptoms associated with individual diseases. TAR DNA-binding protein (TDP-43) has been recognized as a histopathological marker of Amyotrophic Lateral Schelerosis, Alzheimer's and Parkinson's diseases. While literature has shown that the mutated C-terminal peptide fragments in TDP-43 plays an important role in proteinopathy, and the elucidation of their morphology of amyloid aggregation remained limited. In this study, we have revealed the pathways of amyloid formation of various TDP-43 mutated peptides by ion mobility-mass spectrometry (IM-MS).

Methods
To characterize the impacts of mutations on the amyloid formation,

various TDP-43 C-terminus peptides, including the wild type (D1), pathological mutations (G294V), sporadic mutations (G294A) and de novo single and triple mutants (GGG308PPP) were synthesized. After incubation in physiological condition (50mM ammonia acetate, pH=7, 37°C) for 10 days, all peptides showed pronounced oligomerization under the electrospray ion-mobility mass spectrometry (ESI-IM-MS) conditions employed at Day 0.

Results
For D1 peptide, the signals of larger oligomer, such as trimer and tetramer, are diminished significantly after 5 days, suggesting elevated presence of amyloid fiber during this phase. In contrast, accelerated aggregation can be observed in pathological mutants (G294V and G294A) compared with D1 after the third day. However, the replacement of proline in TDP-43 C fragment (GGG308PPP) is able to disrupt the aggregation characteristics and stabilize the oligmer structure with the signals maintenance after 10 days.

Conclusions
Through comparison of the CCSs of species, ESI-IM-MS revealed differences in the distribution of oligomers formed under each of the mutant peptides; whereas oligomers up to tetramer in size are populated during the formation of fibrils. The G294V and G294A are β-amyloid and the aggregation propensity accelerates while comparing to D1. In addition, the ESI-IM-MS analysis showed that GGG308PPP remained random-coiled and it was extremely low in aggregation ability, which may imply the importance of this region in amyloidogenesis process.

Novel Aspect
By using IMS-MS, the dynamics of oligomer population for several mutated TDP-43 peptides have been detected to study the aggregation mechanism and pathway of fibrils formation.

WPS44-04 / Ion-mobility mass spectrometry analysis reveals quaternary structural and conformational changes for thermal-induced activation of thermophilic SNR

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Introduction
Sulfonucleotide reductase (SNR) is an enzyme that catalyzes the first committed step in the biosynthesis of reduced sulfur compounds and required for bacterial survival and virulence. In addition, mammals do not possess the sulfate reduction pathway, which makes SNRs being a promising target for drug development against human pathogens. The thermophilic SNR fromSulfolobus solfataricus(SsoSNR) is a tetramer in solution determined by analytical gel filtration chromatography. The crystal structure of SsoSNR was solved recently and showed that the tetramer is made of a dimer of dimer. Unexpectedly, the C-terminal tail containing the active residue was restricted on the dimeric interface of two dimers. Since the optimal temperature for enzymatic activity of SsoSNR is around 348 K, the raising question is how the quaternary structural and conformational changes occurred corresponding to elevated temperature.
The ion mobility mass spectrometry (IM-MS) is an emerging technology to study the dynamics of protein conformations and structure changes base on their mass-to-charge ratio and collisional cross-sections through a buffer gas. This technology can act as a tool to separate complex mixtures, to resolve ions that may be indistinguishable by mass spectrometry alone, or to determine structural information (for example rotationally averaged cross-sectional area), complementary to more traditional structural approaches. In this study, IM-MS was used to gain insights into the quaternary structural change and conformational dynamics of the SNR enzyme with thermal-induced activation property.

Methods
To characterize the impacts of temperature-dependent oligomer dissociation correlated to thermal-induced activation, recombinant SsoSNR were produced and purified. After incubation in physiological condition at various temperature for 10 min, all samples was monitored the oligomerization states and collisional cross section using electrospray ion-mobility mass spectrometry (ESI-IM-MS).

Results
For SsoSNR heat-treated at 298 K, the result suggests the protein samples are mostly in a tetrameric form. In contrast, the signals of tetramer are diminished significantly for SsoSNR heat-treated at more than 338 K, suggesting dissociation of SsoSNR at high temperature. However, the signals of dimer are present, showing elevated presence of stable dimer form of SsoSNR.

Conclusions
Through comparison of the CCSs of SsoSNR at different temperature, IM-MS revealed differences in the distribution of tetrameric and dimeric forms, which are correlated to the inactivated and activated conformations of SsoSNR, respectively. Our results show the oligomerization state of SsoSNR could be dissociated from tetramer to dimer in a temperature-dependent event, and corresponding to the thermal-induced catalytic mechanism. IM-MS approach combing with other structure-based studies of SNR would extend the knowledge of the role of C-terminal tail in the catalytic mechanism of SNR.

Novel Aspect
By using IM-MS, the different populations of SsoSNR structures at different temperatures have been detected to characterize the thermal-induced activation and catalytic mechanism.

WPS44-05 / There goes the neighbourhood – Mapping protein proximity in highly complex samples, using chemical cross-linking, LC-MS and novel bioinformatics.

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Introduction
A number of small nucleic proteins have been shown to be directly linked to the chronic autoimmune disease "Lupus erythematosus", and purification has suggested that these proteins assemble into large complexes of yet uncharacterised composition and structure. Chemical crosslinking combined with LC-MS is commonly used to derive information on protein-protein interaction between a few key proteins, but the method is rarely used on complexes of this size and complexity. This is partly due to the exponentially increasing search-space, which makes the data-search very time consuming and prone to false positives.
Using MS, and a newly developed search algorithm designed for handling very complex datasets, we have investigated the protein neighbourhood of complexes related to Lupus erythematosus, in samples purified from human nuclei.

Methods
Nucleic protein extract from healthy individuals was obtained from Statens Serum Institute, and the proteins were cross-linked with BS3. Cross-linked samples were analysed with LC-MS in CID and HCD modes. The datasets were analysed for cross-links with in-house developed software.

Results
We have reliably identified a number of cross-linked proteins

from the purified samples, and crosslinks between them show a distinct proximity pattern between several key proteins.

Conclusions
Traditionally, cross-link searches are carried out with pure protein samples and against defined search spaces to cut down on both time and FDR. Here we show that even complex samples with an undefined search space can be cross-linked and analysed with confidence using LC-MS and novel algorithms specialised for this purpose.

Novel aspects
The sample presented is the most complex cross-linked complex reported so far. In order to identify the identify components in the complex we have had to search for cross-links throughout the entire human proteome. We have made this possible by implementing a new search algorithm. We have used only common methods, readily available materials and free software to accomplish this.

WPS44-06 / A New Cross-Linker targeting Asp/Glu residues

Mariana Fioramonte, Fabio Gozzo
UNICAMP

Chemical cross-linking is an attractive technique to study protein and protein complex structures due to its attractive features, like low sample consumption and short analysis time. The distance constrains obtained by the identification of cross-linked peptide by mass spectrometry can be used to construct protein models that fits those constrains. The interaction region of several protein complexes have been mapped by this technique and the use of distance constrains can be extended to determine the folding of a protein. Such task would be possible if the number of cross-linking species is increased. Currently, one of the limitations of this technique is the specificity of cross-linkers related to amino acid side chain. The usual cross-linkers are reactive towards amino groups present in lysine and N-terminal residues. Lysines are relatively common residues in proteins however the number of Glu and Asp residues combined are double the number of lysines. Moreover, some proteins are poor in Lys residues. In this work we show a potential methodology for Glu and/or Asp cross-linking, which could provide additional distance constrains when combined with conventional Ly-Lys cross-links.
Standard proteins, like hemoglobin and myoglobin were diluted in phosphate buffer and mixed with the cross-linker for periods between 30 minutes and 2 hours. The reaction products were then digested and injected into a UPLC system coupled to a Synapt mass spectrometer (Waters). The MS/MS spectra were acquired using data dependent acquisition.
The standard proteins presented the formation of both cross-link and dead end speies, showing that the new cross-linker is able to cross-link acid residues (Asp/Glu) in proteins. The proteins presented a number of cross-links compatible with the number of acidic residues in the protein surface. Overall the formation of these extra cross-links in different regions of proteins (compared to lysine cross-links) provides an additional set of distance constrains that can be used to model both protein and protein complexes.

WPS44-07 / Insights into the interaction between Human Hsp90 C-terminal and Tom70 by chemical-cross-linking and HDX coupled to mass spectrometry

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Biological processes are essential for cell's, normally presented on a higher organization level that are mediated and controlled by protein-protein interactions.Studies of the function on many proteins are closely related to the analysis of the three-dimensional structure and identification of interaction partners. Chemical-crosslinking and mass spectrometry is an alternative technique for obtain the interaction region between proteins. In cross-linking two residues are covalently connected and the identification of these cross-links are performed after proteolytic digestion of the cross-linked complex. The location of cross-links imposes a distance constrain on respective residues allowing the study of protein interaction.Another method to obtain structural information is hydrogen/deuterium exchange (HDX) coupled to MS. Conformational aspects or dynamics of protein is measure by rate deuterium exchange over time and can be perturbed by different factors, as examples protein interaction, denaturation and pH. The translocase of the mitochondrial outer membrane (TOM) is responsible for polypeptide translocation across the outer membrane and inside the organelle. The Tom70 is a chaperone dependent activity, which have key role in this mechanism. The chaperone 90kDa or Hsp90 protect the preprotein aggregation and target the preproteins to the Tom70.
The complex of proteins (Human Hsp90 C-terminal and Tom70) was incubated with DSS (1:50 ratio) for 2 h. Then, the cross-linked protein was digested by trypsin for 16 h (37 ºC). Peptides analyses were performed by LC-MS/ MS using a Synapt G1-S HDMS and Q-Exactive. Cross-linked peptides were identified by using Crux-for-Xlinks and p-Link software followed by manual validation. The H/D exchange reaction was perfomed by a dilution of the Hsp90 and protein complex in D2O buffer. Quenched samples were immediately injected into a nano-Acquity UPLC System with HDX Technology coupled to Synapt G1-S HDMS and analyzed by DynamX.
The structural model for Hsp90 C-terminal and Tom70 interaction were generated based on experimental data from combined cross-linking and HDX experiments. The H/D exchange results showed dynamics changes in C-terminal of Hsp90 upon Tom70 binding. These models are in full agreement with the biological data obtained so far for these proteins and complexes.

WPS44-08 / Cross-linking as a key experimental data in Stanniocalcin-1 structural modeling

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The Stanniocalcin-1 (STC1) protein is an endocrine glycoprotein hormone of approximately 27 kDa and composed of 247 amino acids. Discovered firstly in glans of bone fishe and late identified in human as a homodimeric specie by differential expression of mRNA related to cellular immortalization, being also involved in many physiological, pathological and development process, including carcinogenesis, pregnancy, lactation, angiogenesis, organogenesis, cerebral ischemia, and others [1]. Recently research in patients with Acute Lymphoblastic Leukemia (ALL) has shown large increase in the expression of the STC1 gene in leukemic cells, reinforcing its potential role in cancer [2].
Previous analysis, such as circular dichroism and SAXS, show that STC1 is a protein with a well-defined tertiary structure but the absence of sequence homology with proteins in PDB makes the homology modeling of STC1 very difficult. I-TASSER [3] and QUARK [4] are one of the best examples of a computational tool for structural prediction of a protein which combines various techniques such as threading, ab initio modeling and atomic-level structure refinement approaches and allows one to use some

experimental data in order to improve the refinement of possible structures.
In this work it was used the chemical cross-linking agent disuccinimidyl suberate which to obtain a list of distance constrains and generate models in I-TASSER and QUARK that obeys all these conditions showing the usefulness of the integrative approach.

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WPS44-09 / Native MS and Ion Mobility MS (IM-MS) for Antibody Drug Conjugate Characterization

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Introduction
Antibody drug conjugates (ADCs) are biomolecules composed of a cytotoxic drug linked covalently to an antibody (mAb). ADCs are next generation of empowered mAbs foreseen to treat cancer by combining the selectivity of mAb for tumor-associated antigen with the potency of cytotoxic molecules. A major issue underlying ADCs' development is to improve their analytical characterization. Compared to "unconjugated" mAbs, ADCs have increased level of complexity as the heterogeneity of conjugation (number and distribution of drug on the mAb) cumulates with the initial variability of the biomolecule. Here we highlight the benefits of high resolution native MS and native ion mobility MS for the determination of ADCs' average DAR (drug to antibody ratio) and drug load distribution.

Method
Native mass spectrometry experiments were performed on a high resolution Orbitrap Exactive Plus EMR mass spectrometer (Thermo Fisher Scientific). Native travelling wave ion mobility mass spectrometry (TWIMS) experiments were achieved on a Synapt G2 HDMS (Waters) instrument. Both instruments were coupled to an automated chip-based nanoelectrospray device (Triversa Nanomate, Advion Biosciences).
All experiments were performed in a 150 mM ammonium acetate buffer at pH 7.4. For both native MS and IM-MS, instrumental settings were tuned to transfer intact ions without extensive activation in the gas phase while keeping sufficient ion desolvation and transmission.

Preliminary data:
The analytical potential of native mass spectrometry (MS) and native travelling wave ion mobility MS (IM-MS) is compared to hydrophobic interaction chromatography (HIC), known as the analytical gold standard for ADC characterization.
The utmost interest of native MS for the characterization of cysteine-linked ADCs is illustrated with Brentuximab vedotin (AdcetrisTM). High resolution native MS provided accurate mass measurements (<10 ppm) of intact ADCs together with average DAR and DAR distribution. The main advantage of using native MS for exact mass measurements of ADCs with interchain Cys linked drugs, lies in the ability to detect noncovalent associations of light and heavy chains which cannot be analyzed by classical LC-MS methods.
Native travelling wave ion mobility MS (IM-MS) was next used for the first time to characterize Brentuximab vedotin. In these experiments, mAb heterogeneity due to drug distribution was uniquely evidenced by differences in drift times. Collisional cross sections were measured for each payload species attesting slight conformational changes induced by drug conjugation. Finally, a semi-quantitative interpretation of IM-MS data allowed the average DAR and DAR distribution to be directly extrapolated.
Both native MS and IM-MS experiments were in agreement with results obtained from HIC. Interestingly, HIC fractions were collected and analyzed by native MS and IM-MS, allowing HIC limitations to be circumvented and HIC results to be specified.
Our results illustrate how native MS and IM-MS can rapidly assess ADC structural heterogeneity and how easily these methods were implemented into MS workflows for in-depth ADC characterization.

WPS44-11 / Native Mass Spectrometry of Reconstituted Human Nucleosome Core Particle

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Introduction
The minimum structural unit of chromatin is the nucleosome core particle (NCP, ~201 kDa) composed of ~146 bp DNA wrapped around a histone octamer containing two H2A/H2B dimers and one (H3/H4)2 tetramer. In each histone protein, N-terminal tail parts are highly modified such as acetylation, methylation, etc., and responsible for regulation of transcription initiation. To exactly identify each modification effect on the structure and function, it is preferable to characterize singly modified NCP and analyze its biophysical and biological characteristics. In the present study, human NCP with no modification was prepared in vitro with four recombinant histone proteins (H2A, H2B, H3, and H4) and 146 base-pair DNA, and reconstituted products were analyzed by native mass spectrometry in addition to native- and SDS-PAGE.

Methods
NCP was reconstituted with recombinant human histone proteins and 146 bp DNA which were prepared in house. Mass spectra for the reconstitution products were acquired by Tri-wave SYNAPT G2 HDMS (Waters) with a nanoESI source. The samples in ammonium acetate solutions (pH 6.8) were deposited in gold-coated nanoESI spray tips manufactured in house, and placed in the nanoESI source. For ESI ion mobility mass spectrometry (IM-MS), ions generated by nanoESI were injected into the Tri-wave ion mobility cell, in which the Tri-wave mobility separation was operated at a wave velocity of 800 m/s with a wave height of 40 V.

Results
In native- and SDS-PAGE, no distinct difference was recognized for the fractionated samples of the reconstituted NCP. In contrast, native mass spectrometry suggested that the reconstituted product consisted of two types of nucleosomes, i.e., canonical NCP and hexasome NCP (one H2A/H2B dimer is depleted from the canonical NCP). Although the canonical NCP was 28 kDa larger than the hexasome NCP, the peaks of multiply charged ions of the canonical and hexasome NCPs substantially overlapped. In the two-dimensional contour plots obtained by IM-MS analysis, these two NCPs could be separately observed. In addition, the hexasome NCP was found to be more unstable than the canonical NCP when prepared in a high concentration of ammonium acetate; remarkable peaks of dissociated DNA were observed in the mass spectrum of the hexasome NCP.
The evidence of the hexasome NCP in the reconstituted sample solution would suggest that the hexasome NCP could be an

intermediate of assembly and disassembly processes of NCP not only in vitro but also in vivo.

Conclusion
Conclusive evidence of the hexasome NCP was obtained using native mass spectrometry. This might support the hypothesis that NCP assembly and disassembly processes in vivo are executed via an intermediate of the hexasome NCP.

Novel Aspect
First observation of the hexasome NCP by nanoESI-MS: evidence of the hexasome as a possible intermediate of the NCP assembly process.

WPS44-12 / Chemical cross-linking and MALDI-MS for the characterization of intact protein complexes

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Understanding the way how proteins interact with each other to form transient or stable protein complexes is a key point in structural biology [1]. Electrospray ionization mass spectrometry (ESI-MS) has shown a high potential for characterizing intact assemblies, especially for determining the complex stoichiometry or monitoring dynamic changes [2]. However, finding conditions compatible with both complex stability and ESI-MS might be very challenging. Thanks to its tolerance to concentrated salt over ESI-MS, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) can also be used after coupling with chemical cross-linking; the protein complex is thus covalently stabilized, which allows more flexibility regarding the sample preparation [3].
In this project, we combined chemical cross-linking with MALDI-MS and ESI-MS to determine the binding stoichiometry of a protein complex involved in transcription processes. Glutaraldehyde was first used; but in order to get more information on the interacting area, cross-linking with isotopically labeled bis[sulfosuccinimidyl] suberate (BS3) d0-d4 was performed. After incubation, the cross-linking reaction was quenched and submitted to tryptic proteolysis, digested peptides were then analysed by nanoLC-MS/MS. Intra- and intercross-linked peptides were matched by two search engines dedicated to identification of cross-links: pLink [4] and XQuest [5]. Afterwards, the identification was validated manually.
The heterotetrameric stoichiometry was unambiguously resolved, the intercross-linked peptides were also interpreted by pLink and XQuest with relevance. Therefore, five protein-protein interaction sites were identified. These MS data are consistent with the structure prediction obtained by molecular modeling, and are important clues to refine the one obtained by electron microscopy and molecular biology. Hence, the combination of MS and biological analysis techniques can help us to gather a relevant 3D structure of the complex.

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WPS44-13 / Mass Spectrometric Estimation of the Flexibility of Intact Proteins

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Introduction
One of the recent interests in protein science is the concept of flexibility relating to several functions such as post-translational modifications and interactions. The flexibility of intact proteins is estimated by several measures such as B-factor and turn preference factor with X-ray crystallography, protection factor with NMR. Although MALDI and ESI have outstanding abilities allowing the rapid and high sensitive identification of proteins, it is difficult to have the kind of information about higher structures and flexibility that can be determined by X-ray and NMR. Here, we report that discontinuous intense peaks observed in the MALDI-ISD spectra of intact proteins give flexible amino acid residues such as Asp, Asn, Gly and Cys [1], and that hydrogen-radical accessibility in the MALDI-ISD experiments can be adopted as a novel factor for estimating the flexibility of intact proteins.

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Methods
MALDI-ISD spectra were acquired on a time-of-flight mass spectrometer (TOF MS) AXIMA-CFR (Shimadzu, Kyoto, Japan) equipped with a nitrogen laser (337 nm wavelength, 10 Hz pulse rate, 4 ns pulse width). The ions generated by MALDI were accelerated using 20 kV with delayed extraction. The analyzer was operated in linear mode. 5-Amino-1-naphthol (5,1-ANL) was used as a matrix. The proteins used were BSA, myoglobin and thioredoxin.

Results
The ISD spectra of BSA, myoglobin and thioredoxin here gave discontinuous intense fragment peaks originating from the one-side preferential cleavage at N-Ca bonds of Xxx-Asn, Xxx-Asp, Xxx-Cys and Gly-Xxx residues. It is of importance to recognize that in protein science Asp, Asn, Cys and Gly residues preferred in flexible turn structures and tend to destroy the helix structures. Therefore, the results obtained suggest that the protein molecules embedded on matrix crystals are partially maintain their helix structure, although it is difficult to prove the presence of secondary structures under MALDI conditions.

Conclusions
It had been believed so far that any MS methods are in a disadvantageous position for obtaining information about higher order structures and flexibility of intact proteins even when ESI and MALDI were used. Here we reported that the discontinuous intense ISD fragment peaks observed originated from the N-Ca bond cleavage of Xxx-Asp/Asn/Cys and Gly-Xxx residues lying in helix/sheet-free regions that were determined by X-ray crystallography. Asp, Asn and Gly residues estimated with MALDI-ISD were common to all the measures such as the B-factor, the turn preference, the protection and the fluorescence decay factors. Further, Asp, Asn, Gly and Cys residues with MALDI-ISD were common to those estimated by turn preference factor with X-ray.

Novel Aspect
MALDI-ISD experiments can be adopted as a novel method for estimating the flexibility of intact proteins.

WPS44-14 / Investigating the familial Parkinson's disease's mutations in DJ-1 and their affect on the 20S proteasome

Almog Spector, Alina Zhuravlev, Oren Moscovitz, Michal Sharon
Weizmann Institute

Introduction

Parkinson's disease (PD) is a degenerative disorder of the central nervous system. The symptoms of Parkinson's disease result from the death of dopamine-generating cells in the substantia nigra that are critical for regulating motor function. The cause of this cell death is unknown, however, potential mediators including oxidative stress and failure of the proteasome were proposed. Few genes associated with the disease were found, one of them is DJ-1. It was found that early onset, familial PD patients have a high prevalence of certain mutations in the DJ-1 gene. Six point mutations were found- L166P, L10P, M26I, E64D, A104T, D149A and one deletion mutation P158Δ, but their effects on the progression of the disease are still unknown. We recently discovered that DJ-1 physically binds the 20S proteasome and inhibits its catalytic activity. My research aims at investigating link between the familial PD DJ-1 mutations and their effect on DJ-1's ability to inhibit 20S proteolysis.

Methods

Native mass spectrometry (MS) analyses were performed on a QTOF instrument modified for high mass measurements under conditions that allow maintaining non-covalent complexes intact within the mass spectrometer. Specifically, MS, tandem MS (MS/MS) and ion mobility mass spectrometry (MS-IMS) measurements were utilized to investigate the stability, oligomeric state and compactness of each of the DJ-1 mutations. Moreover, the interactions between these mutational variants and the 20S proteasome were characterized.

Results

The different mutations were found to have altered conformations, for example, unlike DJ-1WT which is activeas a dimer, MS analysis indicated that DJ- L10P is less susceptive of forming dimers. The broad distribution of charge states of DJ-1L166P suggest that this mutant has a relatively open conformation, emphasizing that this mutation changes the folding state of the protein, and possibly turns it to a non-active protein.

Conclusions

The altered conformations of the different mutations, like the lack of dimerization or unfolded state suggest that the protein has impaired activity and possibly impaired binding and inhibition of the 20S proteasome. Their interaction with the 20S proteasome should be tested in order to see if they have a different effect on the binding or the inhibition of the 20S proteasome, than the wild type form.

Novel aspects

Our results will not only be able to shed light on the functional properties of DJ-1, but also to provide a functional link between the pathogenic DJ-1 mutants and the 20S proteasome.

PS00-01 / Francis William Aston: Postcards from Switzerland
Kevin Downard
University of Sydney

ThPS32 - Ion Mobility MS

11:00-15:00
Poster Exhibition, Level -1

ThPS32-01 / Comparative study of APCI and MPI/REMPI in atmospheric pressure IMS

Marvin Ihlenborg, Jürgen Grotemeyer
CAU Kiel

Introduction
The home build IMS used in the working group has a 3H-source for APCI measurements. To extant the range of analytes to substances with low proton affinity (PA) e.g. toluene or xylene the APCI source should be changed to a MPI/REMPI source.
Here the advantage from a MPI/REMPI source in comparison to an APCI source under almost the same experimental conditions will be shown. Hence benzaldehyde and anisole have a high PA and a good absorption in the UV range this components will be compared first with this three methods. Furthermore the range to molecules with low PA like toluene, p-xylene or mesitylene will be extended.

Method
A home build IMS is used for measurements with different ionization techniques. A 3H-source is used for APCI, a Nd:YAG laser (Surelite I, Continuum, 4. harmonic) for MPI and a MOPO (MOPO 730, Spectra-Physics) pumped by a Nd:YAG laser (Pro 230, Spectra-Physics, 3. harmonic) for REMPI. The ions are detected by a Faraday cup with an analogue amplifier (Dräger Safety, Dräger IMS 5000) coupled to a storage oscilloscope (Waverunner 6051, LeCroy). The applied voltages over the electrodes are produced by a power supply (Dräger Safety, Dräger IMS 5000 for APCI and FUG, HCN14-3 500 for MPI/REMPI respectively).
A system consisting of 3 massflowcontroller (MKS Instrument, Typ MF1) is used to provide the gases (N2 or synthetic air) for the drift tube and the analyte transport. In APCI a syringe pump is used additionally.

Results
By changing the ionization technique with otherwise almost constant conditions the ion yield is dramatically changed in intensity. Furthermore species with low PA are ionized as well.
While benzaldehyde and anisole show a shift in the RIP by the use of APCI, the same is not observed for toluene, xylene or mesitylene. Therefore latter ones are not ionized under APCI conditions due to their low PA.
A change to MPI conditions shows a significant ion signal for each substance. Using the same drift voltages an increase of the signal level of approximately the tenfold is observed with a high broadening of the resulting signal. Since an external power supply can be used for MPI measurements the drift voltage is increased to result in smaller and higher signals.
Due to the change from MPI to REMPI lower laser intensities can be used to get the same signal level under same conditions. REMPI also leads to an additional separation capability due to different resonance wavelength of the used analytes.

Conclusion
It was shown that a change in the ionization method from APCI to MPI/REMPI leads to a rise in the ion yield by at least the sixtyfold in terms of the signal level depending on the used wavelength and laser intensity. When REMPI was used also an additional separation can be obtained.
Furthermore species with low PA like toluene or xylene are observed in high yields.

Novel Aspect
Quantitative comparison of the ion yields obtained by an APCI-, MPI- and REMPI-process under atmospheric pressure conditions in an IMS.

ThPS32-02 / Method of duty cycle enhancement for orthogonal accelerator TOF MS with axial symmetric mass analyser, connected with drift tube IMS

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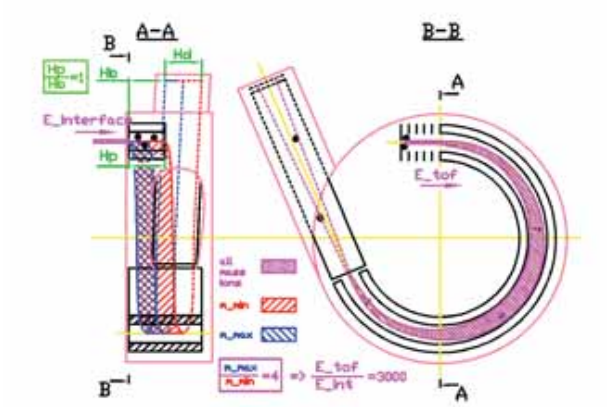
LTD Linantek, Moscow, Russia

On the basis of recently made ion mobility mass spectrometer [1,2], including a drift tube ion mobility spectrometer and an orthogonal acceleration electrostatic sector time-of-flight mass analyzer, a method of duty cycle enhancement was developed for orthogonal accelerator.
Axial symmetric mass analyser allows increasing ratio of push region height of orthogonal accelerator and height of mass analyser to 100%, because detector and push region are divided by space. It allows reaching 100% duty cycle for heavy ions in oaTOF MS, that is impossible in case of reflectron mass analyser, as reported by Guilhaus [3]. But it saves mass discrimination of duty cycle that leads to significant losses of middle-weighted and light ions.
New method is described that allowes achieving 100% duty cycle for ions of any mass. At first, it demands to refuse usual TOF mass spectra timing, confined by consecutive orthogonal accelerator push impulses. And define it by TOF of the fastest and the slowest ions of interested mass. At s econd, to exclude intersection of spectra, difference between these times must be smaller then period of push pulses. After that substituting significant electrical and geometrical parameters of analysers into TOF expression, some algebra and scaling law gives condition on ion's kinetic energies in orthogonal directions dependent of interested mass range:

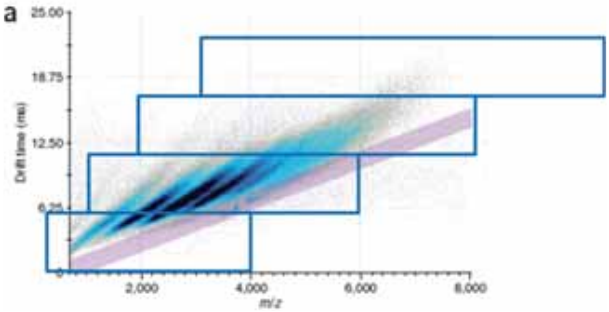
$$\frac{U_1}{U_p} \geq \left[\frac{1}{l_p}\left(\sqrt{\frac{m_{max}}{m_{min}}} - 1\right)\frac{l_j}{\sqrt{a_j}}\right]^2$$

, where U1 is ion's energy in mass analyzer, Up is ion's energy in orthogonal accelerator, lp is height, mmax and mmin is the heaviest and the lightest ions mass, lj and αj is parameters of analyzer j-region – length and ratio of ion's energy to U1, respectively.
In the case of developed TOF MS it's showen that only small ratio of masses can be realized, like 4-5. But mass-mobility correlation, K~m2/3, in fact, divide mass-mobility spectra for numerous mass spectra with rather small ratio of masses, like 4-5, that was empirically proofed. This all allows to reach 100% duty cycle of oaTOF MS. Limits of method's application are discussed. Among them are simultaneous existence of different ion's charge states, dissociation in interface.
Novel aspects: use of mass-mobility correlation for duty cycle enhancement for orthogonal accelerator IMS/TOF MS.



Trace of ion trajectories in oaTOF MS with 100% duty cycle for all mass ions in case of ratio of interested masses equal to 4:

Example of division of mobility-mass spectrum into regions with ratio of masses equal to 4:



Spectrum from:
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ThPS32-03 / Resolution Enhancement in a Multiplexed, High-Pressure Drift Tube IMS-MS

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Tofwerk AG

Introduction
Ion mobility spectrometry coupled to mass spectrometry (IMS-MS) is increasingly widespread, but challenges remain in satisfactorily separating many isomeric and isobaric compounds, especially in complex mixtures. We report on resolution improvements in a short (20 cm) IMS by operation above atmospheric pressure. A multiplexed ion gating method allows application of novel post-processing algorithms which further increase resolution, while improving sensitivity. Resolutions above 200 are demonstrated, and a fingerprinting example shown.

Methods
The Tofwerk IMS-TOF systems includes an ESI source, a 10cm desolvation tube, a 20cm drift tube (both made from resistive glass, field approx. 400 V/cm) and a Tofwerk HTOF TOF-MS. Measurements were carried out in both positive and negative ion modesat IMS pressures between 1 and 1.4 atm nitrogen. Samples were introduced either directly by syringe pump or using an Ultimate3000 LC system (Thermo Fisher Scientific).

Results
In the Tofwerk gas-tight drift cell, and up to 1.4 bar absolute pressure, mobility resolution increased sub-linearly with pressure. Amaximum resolution increase of approximately 10% was demonstrated. Larger resolution improvements were achieved by postprocessing of the Hadamard multiplexed data. Using operations in the multiplexed domain, the effective transformed resolution can be increased to or above the classical diffusion limit. At the same time, Hadamard artifacts due to modulation defects and/or noise are suppressed or eliminated. Mobility resolutions beyond 200 are demonstrated. The 50% Hadamard duty cycle improves ion transmission over 200 times and S/N ratios 10 times compared to conventional pulsed mode, at maximum resolution. The high pressure multiplexed IMS-TOF is well suited for fingerprinting of complex natural products, as it almost doubles the number of detected features.

Conclusions
Super-atmospheric pressure and multiplexing lead to improved resolution and sensitivity in a drift-tube IMS. Using a fast scanning TOF MS as detector guarantees that IM resolution is preserved and allows compound identification in complex samples thanks to high mass accuracy.

Novel Aspect
First bench-top multiplexed high-pressure drift-tube IMS-MS with ion mobility resolution >200.

ThPS32-04 / Analysis of Ions Produced By Laser-Desorption Ionization In Air and Liquid – A Theoretical and Experimental Study

Yi-Sheng Wang, Yi-Hong Cai, Yin-Hung Lai
Academia Sinica

Introduction
The rapid diagnosis of ions in ambient and liquid environments is important for the miniaturization of analytical devices and is an emerging field of research. Although ion mobility spectrometry facilitates the measurement in low-pressure conditions, the analysis under elevated pressure environments or liquid is still highly challenging. In this work, the method for ion separation and detection in ambient conditions and liquid is studied theoretically and experimentally. The results of predicted ion motion were compared with experimental results to determine the suitable theoretical model of ion migration. The objective of this work is to design a new type of sample analyzers with compact size, fast response, and high sensitivity.

Methods
The migration of ions in air and liquid was analyzed theoretically by various models. Electrohydrodynamical simulation was conducted with a commercial finite-element method package with a spatial resolution of roughly 1 micron. The experimental results were obtained with a laser desorption ionization (LDI) device, which was workable in both air and liquid environments. The typical liquids included toluene and hexane due to their low electric conductivity. The ions were accelerated by an electric field of 5,000 – 15,000 V/cm across roughly 3.5 mm, and the signal were collected by a detection plate. The signal were amplified with a commercial charge amplifier and recorded. The samples included metals, graphite, C60, CsI, and typical MALDI samples.

Results
The predicted result suggested that the drag force played a critical role in the migration of ions in the studied environments. Although both electric and magnetic fields imposed forces to the ions, the electric force was considerably higher than the magnetic force for the control of ion motion. When conducting the experiment in air, intense signal was observed at roughly 15 – 25 µs after laser irradiation on various samples, and the peak width was a few µs. The result was predicted with Cunningham-Knudsen-Weber-Millikan equation, which showed a superior accuracy than the original Stoke's equation. Comparing the speed of ions in air, the predicted speed of ions in toluene was roughly 8000 times slower. The result observed in toluene was also compared to the prediction to determine the suitable ion mobility model.

Conclusions
Intense signal of LDI-generated ions was obtained in ambient condition. The result was predicted reasonably using Cummingham-Kundsen-Weber-Millikan equation. The ion mobility in liquid reduced by roughly 3 – 4 orders of magnitude. The prediction suggested that separation in liquid provides superior resolving power than that in ambient conditions.

Novel Aspect
The diagnosis of LDI-ions in air and liquid was studied theoretically and experimentally.

ThPS32-05 / Coupling of ion mobility and mass spectrometry as a new alternative for the analysis of pharmaceutical diastereomers

Laurence Queguiner[1], Virginie Domalain[2], David Speybrouck[1], Marie Hubert-Roux[2], Eric Arnoult[1], Carlos Afonso[2], Jérôme Guillemont[1]
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Tuberculosis still ranks as one of the most deadly infectious diseases in the world. A new structural class of antimycobacterials, the diarylquinolines, has been synthesized and is being highly effective against bothMycobacterium tuberculosisand multidrug-resistant tuberculosis. As these molecules contain two chiral centers, stereomers are therefore obtained. A conformational analysis of the stereomers has already been carried out by tandem mass spectrometry, NMR and molecular modelling. (Chem Biol Drug Des, 2006, 68: 77-84) In order to obtain new and complementary information, we propose to perform analyses by traveling wave ion mobility - mass spectrometry (TWIM-MS). This coupling with a new dimension of separation permits to obtain information about the conformation of ions in gas phase.

Methods
ESI-TWIM/MS experiments were performed on a commercial hybrid ion-mobility-mass spectrometer (Synapt G2 HDMS, Waters). Analyses have been carried out on protonated molecules and also on cationic adducts with alkali metals. In order to improve the precision of the drift time measurements, Gaussian fit of the IMS data was performed with the Origin pro software (v9.1). Experimental CCS have been determined after the calibration of IMS cell with polyalanines. These experimental values have been compared to theoretical CCS calculated with the Mobcal software.

Results
After optimization of IMS conditions, several cases have been observed depending on the structure of the molecule. Indeed, for some molecules, the drift time difference (DTd) for the [M+H]+ ion between diastereomers is high (≈0.10 ms) whereas for others this DTd is medium (≈0.06 ms) or small (≈0.03 ms). A systematic investigation with different metal ions has been carried out in order to increase the DTd between the two diastereomers. This study showed that the addition of lithium allows in some cases an increase of DTd. In most cases, the diastereomers differentiation has been evidenced by two different ways: 1) the differentiation is obtained with a calibration curve plotted from standard solutions with known proportion of the diastereomers thanks to the very high accuracy and reproducibility of the drift time measurement. 2) peak shoulders are evidenced when the drift time difference is higher than 0.15 ms which allows to obtain the direct proportion of each isomers with a peak fitting algorithm. Finally, these results are in agreement with CCS calculated from theoretical structures which were obtained by DFT calculation.

Conclusions
IM-MS allows to obtain information about conformation in gas phase of pharmaceutical diastereomers of the diarylquinoline chemical family. This fast method enables the differentiation of diastereomers by the measurement of their drift time and permits to determine the values of collision cross section.

Novel Aspect
This is the first analysis of diastereomers of diarylquinolines by IM-MS.

ThPS32-06 / A New High Resolution Temperature Regulated Ion Mobility Mass Spectrometer

Jakub Ujma[1], Perdita Barran[1], Kevin Giles[2], Micheal Morris[2]
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Introduction
The rapidly expanding field of ion mobility mass spectrometry correlates experimental ion mobility with the three dimensional ion conformation. This technique is versatile and allows measurements on ions ranging from small organic molecules to intact viruses. Currently the "golden standard" approach is to correlate the experimental collision cross section with a simulated collision cross section. The majority of commercial and homebuilt instruments allow for IMS measurements at room temperature, wherein vacuoconformers may interconvert impeding direct comparison with simulated data. Furthermore, with the increasing size of the ions studied, the reliability of this "measure, simulate and compare" approach diminishes as it is possible to find multiple matching structures which may be inherently different although produce similar collision cross sections.
Here, we present a new high resolution IMS-MS instrument with the additional capability of performing IMS experiments on the ions with altered temperatures, hence inducing or preventing conformational changes in the ion of interest. By cooling the ion we may slow down conformational changes and effectively "freeze" multiple conformations. Moreover, the lower drift gas temperature offers improvements in resolution, as thermal diffusion effects are surpassed. On the other hand, heating enables the study of thermal denaturisation and refolding. Rapid cooling of hot ions may allow us to access unusual conformations which could be found via replica exchange MD protocols. Finally, this instrument enables us to enter the relatively small field of correlating temperature induced changes in the experimental collision cross sections with simulated structures optimised at different temperatures.

Results
The presented work will feature the design and preliminary results obtained with the new temperature regulated drift cell (80-550K, 50 cm) interfaced with a commercial mass spectrometer - Micromass QToF2. The new type of drift electrode assembly has been designed to minimise problems related to the differential thermal expansion of the insulating and conducting materials. The applied drift fields are between 2-10 Vcm-1 under 3-10 Torr pressure of helium. The high pressure ion trap and the

ion funnel have been designed for the effective ion bunching and high transmission in the specified temperature range. The primary use of the instrument will be to study large protein ions and non-covalently bound clusters; therefore an emphasis was put on minimising the effects of the ion injection energy and of collisional heating. The resolving power has been measured and compared with the theoretical value.

Novel Aspect
New high resolution, temperature regulated ion mobility device coupled with the commercial quadrupole – time of flight mass spectrometer.

ThPS32-07 / Monitoring of active ingredients release from different delivery systems by GC-IMS
Andrea Amantonico, Laurent Wunsche
Firmenich SA

Delivery systems include different technologies extensively applied in the design of drugs, cosmetics, food and beverages. Encapsulation, for example, is an effective method to preserve active ingredients from evaporation and degradation and to achieve their controlled release. For these reasons, flavors and fragrance industry is constantly developing innovative encapsulating systems and is extending the range of application of this technology.
The development of novel delivery systems needs to be paired by analytical methods which are able to assess the performances of these technologies. Therefore, it is crucial to obtain quantitative information on the release of encapsulated active compounds. In flavors and fragrances domain this is generally done using GC-MS (e.g. via SPME). This technique, which remains the workhorse in the field, can be time consuming and requires relatively complex instrumentation. In this work, we present an alternative fast GC method coupled to ion mobility spectrometry (IMS) detection. Since the IMS produces itself a separation, GC-IMS can profit of a two-dimensional orthogonal separation. This allowed to reduce the chromatographic time to just a few minutes even if a simple detection system (no high vacuum needed) with very small footprint was used. A commercial GC-IMS (Flavourspec®) equipped with a short multi capillary GC column and with a tritium ion source (300 MBq) was employed during the work.
Since the ultimate objective was to find a fast method to assess volatile ingredients release, no particular sample preparation was performed. All the samples were simply diluted in water and, after a short equilibration period, between 50-500µL of the static headspace was directly injected. As first step, the quantitative capabilities of the IMS detector were assessed by building the calibration curve for typical perfumery ingredients (e.g. benzaldehyde). LOD below 1ppm (in the liquid phase) and linear range over 2 orders of magnitude were readily achieved. The same tests were conducted spiking the ingredient in food substrates to evaluate the influence of the matrix on the response. Finally the release of encapsulated blends of ingredients was detected from complex substrates (e.g. bouillon cube). GC-IMS showed that encapsulated ingredients are more efficiently released in the final application.
IMS offers the possibility to easily and rapidly add another dimension to almost any separation technique. This is particularly advantageous when fast screening methods are required. By monitoring the quantities of selected component in the headspace, GC-IMS showed to be a simple and sensitive tool to evaluate the release of ingredients from delivery systems even in complex matrices.
GC-IMS was employed as novel tool for the fast assessment of delivery systems performances. Semi-quantitative headspace analysis could be obtained using a simpler, cheaper, and lower maintenance instrument respect to GC-MS.

ThPS32-08 / Ion Mobility-Mass Spectrometry of Linear alcohol ethoxylates
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Introduction
Linear alcohol ethoxylates (LAEs) represent an important tenside group. Surfactants are widely used as cleaning, dispersing, emulsifying, foaming and anti-foaming agents. They often contain oligomeric mixtures which enlarges requirements on applied analytical methods. Mass spectrometry especially in combination with chromatographic methods is widely used [1]. Hyphenation of ion mobility-mass spectrometry has been proven to be useful in analysis of complex mixtures including tensides [2 - 3]. This work demonstrates capability of ion-mobility mass spectrometry in characterization of LAEs, shows influence of experimental parameters on arrival time distributions.

Methods
Ion mobility-mass spectrometric experiments were carried out using a SYNAPT G2-S spectrometer (Waters, Manchester, UK) using direct infusion or flow injection analysis with positive electrospray ionization. All standards were obtained from Sigma-Aldrich. Solvents were of HPLC grade, other chemicals were of analytical grades.

Results
Traveling-wave ion mobility-mass spectrometry (TWIMS) provided sufficient discrimination of different ion series of LAE and eliminated interferences from matrix components. It made data evaluation more straightforward. Nevertheless, acquired data can be influenced by some effects changing arrival time distribution. In the case of LAEs, oxyethylene chain conformers or LAE adducts with metal cations can play a role. To evaluate the extent of such effects, ion mobility of LAEs was followed under different instrumental parameters. Wave height, wave velocity, trap CE, transfer CE, trap bias were systematically changed. Comparison of compounds with various oxyethylene and alkyl chain length revealed that besides diffusion, ions (conformers) of individual LAE with different collision cross section caused ion mobility peak broadening.

Conclusion
Ion mobility-mass spectrometry has been proven an efficient analytical tool to characterize LAEs. Under evaluated conditions, oxyethylene chain conformers noticeably increased peak widths of analytes.

Novel aspects
Evaluation of influence of TWIMS instrumental parameters on arrival time distribution of LAEs.

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ThPS32-09 / Ion mobility spectrometry of foldamers

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Introduction

Foldamers are synthetic organic molecules that have the capability to fold into specific secondary, tertiary, or quaternary structures [1]. They are inspired by biopolymers, but the theoretically infinite chemical diversity of their constituting monomers is opening novel avenues for the design of biomimetic artificial folds. Because folding and self-assembly is the key to foldamer function, it is important to go beyond the simple mass confirmation of the synthetic products, and characterize their folding using ion mobility spectrometry and collision cross section determination. Here, we present ion mobility characterization of aromatic oligoamide foldamers with masses up to 16 kDa, forming single helices or multi-helix structures.

Methods

Quinoline-derived oligoamide foldamers, linear or branched, were synthesized and the samples were electrosprayed from dichloromethane solutions doped with a few percents of methanol. Experiments were carried out with an Agilent 6560 ESI-IMS-Q-TOF, equipped with a drift tube ion mobility cell operated in nitrogen or helium. Experimental collision cross sections were obtained by ramping voltages on the drift tube. Theoretical cross sections in helium were calculated using the EHSS2k method [2] with modified parameters [3].

Results

Charge state distributions were obtained by protonation of the oligoamide foldamers, with on average one proton for eight monomeric units (m/z = 2000). For the largest foldamers, ion mobility separation greatly improved the MS sensitivity by separating the multiply charged foldamers from the singly and doubly charged chemical background. For linear foldamers, the collision cross section (CCS) distribution is monomodal and narrow. The CCS increases when the charge state increases, indicating some expansion of the helical structure due to Coulomb repulsion at the highest charges. We then compared the collision cross sections of linear and branched foldamers, consisting of two helices covalently linked by their middle. Branched foldamers showed bimodal CCS distributions, indicating a mixture of two conformations: one with the helices side-by-side like a «H», and one with the helices crossed like a «X». For the largest foldamers (starting at 8 kDa, 32 monomeric units), the CCS of H and X branched structures were both significantly lower than the CCS of a linear foldamer of same mass, which adopts a straight conformation like a «I».

Conclusions

These results highlight the high potential of ion mobility spectrometry to characterize the folding equilibria and pathways of large synthetic organic polymers, directly from dilute solutions.

Novel Aspect

First ion mobility spectrometry study of the conformation of synthetic organic foldamers, which are as large as small proteins

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ThPS32-10 / The Dispersion Characteristics of Lipids in High-Field Asymmetric Waveform Ion Mobility Spectrometry

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MRC-HNR

Introduction

High field asymmetric waveform ion mobility spectrometry (FAIMS) is a chip based adaptation of conventional ion mobility (IM). The FAIMS system utilises a differential compensation field to focus ions through into the detector. The chip-based system allows for higher field strengths to be applied such that ion separation can be achieved using a smaller instrument design. This enables compatibility of the FAIMS system with different mass spectrometry setups/instruments. FAIMS separation is determined by several factors, including ion mass, collisional cross section, charge state and charge position. We studied the effect of differential compensation fields on the mobility of lipids from different classes to determine the utility of FAIMS for separation lipids of different classes.

Methods

Lipid standards including; fatty acids, phospholipids, glycerolipids and cholesteryl esters (n=8, 18, 10, 1 respectively) were used at different concentrations (100nm to 1000µm). Each of these standards, individually as well as in combinations, and plasma extracts were directly infused using chip based nanospray (Advion TriVersa NanoMate) into the chip based FAIMS system (prototype from Owlstone, Cambridge UK) and spectra were obtained using a bench top Orbitrap (Thermo Exactive). A two dimensional approach was used with regards to the FAIMS data acquisition. The differential separation (compensation field, starting on 150Td going to 300Td) was cycled over several values for the aspiration mechanism (dispersion field, starting on -1Td and finishing on +4.25Td). We plotted the data in 3 dimensions, which allowed the behaviour of the ions to be assessed as a result of the two main varying parameters in FAIMS.

Results

The FAIMS system was able to separate ions of different lipid classes depending on the location of the charge. Therefore isobaric lipids (e.g. PCs v PEs) could be dispersed aiding in the identification of the ions in question. In addition FAIMS assisted in the discrimination of ions that were either authentic or an isobaric fragmentation ions. The localisation of the charge affected the behaviour in the asymmetric field, therefore concentration dependent ionisations mechanisms introduced an extra level of complexity to mixtures of lipids such biological samples. Further works is necessary to test if the addition of electrolytes (such as Li+ or Na+) can lead to uniform ionisation and therefore a more focussed dispersion in FAIMS.

Conclusions

The FAIMS system allows post ion source separation of isobaric lipids. This separation is dependent on charge localisation and ionisation mechanism.

Novel Aspect

Isobaric lipid separation, with regards to class and authentic / fragmentation ion mass matching. Ion-adduct proportionality, for the determination of charge position and adduct formation.

ThPS32-11 / Pressure-tunable, UV- and IR-laser based ion mobility spectrometer for the determination of ion mobilities and the investigation of laser ionization mechanisms

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University of Potsdam

Introduction

Ion mobility (IM) spectrometers are used as inexpensive detectors for explosives, chemical warfare agents and industrial chemicals. They are also increasingly employed in combination with MS, providing complementary structural information in addition to the ion mass. Commercial detectors are usually operated at 1 bar, while drift tubes used in MS are held at 1-10 mbar. The large majority of available data on mobilities and ionization mechanisms has therefore been recorded at either of those pressure regimes.

In the current work, an IM spectrometer is presented that can be operated over a wide range of pressures. Two laser based ionization sources have been implemented, REMPI by pulsed UV lasers and MALDI by pulsed IR lasers. The aim of the study was the measurement of ion mobilities at different pressures and in various drift gases, and the investigation of the influence of the pressure on ionization mechanisms. In support of the latter, additional REMPI experiments were carried out in a ToF-MS (REMPI in vacuum) and in a novel laser ionization cell in front of an API-MS (REMPI at atmospheric pressure).

Methods

The IM spectrometer was developed in house. The lasers used are a pulsed UV laser (NL204-FH, Ekspla) for REMPI and an OPO (IR Opolette, Opotek) for IR-MALDI. The laser ionization cell was also developed in house and was installed in front of an API-MS (LTQ XL, Thermo). The REMPI-ToF-MS (RFT10, Kaesdorf) was fitted with an effusive inlet.

Results

The newly developed IM spectrometer can be operated at pressures between 10-1000 mbar. Resolutions above 80 could be achieved, facilitating precise mobility determinations. The mobilities of aromatic radical cations formed by REMPI and singly charged peptide ions and peptide cluster ions formed by IR-MALDI are reported for various drift gases with different polarizabilities. Mobilities recorded in different drift gases were correlated to the polarizabilities of the drift gases and the interaction energies between ions and drift gas molecules obtained by quantum chemical calculations.

The influence of the pressure on the composition of the ionization products obtained by REMPI of selected phenothiazine derivatives (neuroleptics) was also investigated. While the primary ions found in the REMPI-ToF-MS are mainly fragment ions and radical cations, the atmospheric pressure ionization chamber in front of the API-MS yielded secondary ions such as protonated molecules as well.

Conclusions

Measurements carried out with a newly developed IM spectrometer provided precise ion mobilities in a number of drift gases over a wide pressure range. The results could be correlated to the polarizabilities of the drift gases and interaction energies of ions and drift gas molecules.

Novel Aspect

For the first time, a laser-based IM spectrometer was developed that facilitates the determination of mobilities over a wide pressure range (10-1000 mbar), bridging the gap between drift tubes operated at 10 mbar and 1 bar.

ThPS32-12 / HPLC-ESI ion mobility spectrometry: Characterization and applications

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Introduction

Ion mobility (IM) spectrometry is a well established technique for the detection of gaseous substances such as explosives, drugs and industrial chemicals. However, polar, nonvolatile substances in liquids cannot be investigated by currently available commercial IM spectrometers. These compounds are preferably ionized by electrospray ionization (ESI) which was introduced into IM spectrometry by the Hill group. This group was also the first to demonstrate the application of an ESI-IM spectrometer as an HPLC detector. However, these studies were limited to flow rates below 20 µl/min and required LC flow-splitting. The current work established ESI-IM spectrometry for flow rates between 50-1500 µl/min.

Methods

A home-build ESI-IM spectrometer was characterized as a stand-alone device and as an HPLC detector. The ESI source is orthogonally aligned to the drift tube. The HPLC setup consists of an HPLC pump (P6.1L, KNAUER) and either an RP column (Hypersil Gold, Thermo) or a HILIC column (Eurospher II, KNAUER).

Results

The ESI-IM spectrometer was characterized with regard to HPLC-compatibility (flow rate, solvent composition) and analytical parameters, such as limits of detection (LOD) and linear ranges, were established. The sensitive detection of substances via ESI-IM spectrometry at flow rates up to 1.5 ml/min could be demonstrated for the first time. All substances investigated in this work could be detected sensitively over a wide range of solvent compositions of water/methanol and water/acetonitrile mixtures (up to 90 % water), showing the gradient compatibility of the device. LOD in the negative ESI mode are in the low micromolar range (TNT: 2.5 µM), LOD in the positive ESI mode are in the nanomolar range (promazine: 50 nM).

The separation capability of the standalone IM spectrometer on the millisecond time scale was demonstrated for different mixtures of low complexity (neuroleptics, surfactants, isomers). More complex mixtures could be investigated by multidimensional separation in the dimensions retention time and drift time. The reduced resolution of fast HPLC separations could be compensated by the separation in the IM spectrometer. Furthermore, the IM spectrometer allows the identification of ions by their characteristic ion mobilities.

Conclusions

An ESI-IM spectrometer can be used as an HPLC detector for various polar substances including neuroleptics, pesticides and amino acids. The operation at high flow rates up to 1.5 ml/min and a wide range of solvent compositions could be demonstrated along with LOD down to 50 nM. Mixtures of lower complexity could be separated in the millisecond range in a stand-alone spectrometer. The separation of more complex mixtures benefits from the multidimensional separation in the retention time and drift time domains.

Novel Aspect

For the first time, the application of an ESI-IM spectrometer as an HPLC detector at flow rates between 50-1500 µl/min is demonstrated.

ThPS32-13 / Direct Sample Ionization and Separation Using a High Kinetic Energy Ion Mobility Spectrometer (HiKE-IMS)

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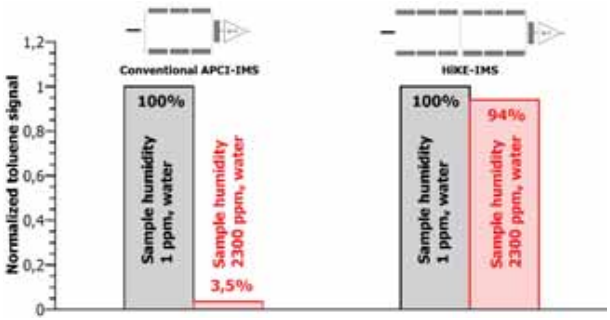
Ion mobility spectrometers (IMS) separate different ion species by studying their motion through a neutral buffer gas under the influence of an electric field. Being a gas phase separation at atmospheric or reduced pressure using chemical ionization, ion mobility spectrometry offers fast and extremely sensitive analysis with medium resolution. Due to these advantages, IMS have gained widespread use as both sensitive standalone detection systems and millisecond-scale pre-separation for mass spectrometers. Furthermore, we have been able to increase the resolution of compact atmospheric pressure IMS from typical values of 20-60 to above 180, vastly improving their separation capabilities. However, while being extremely sensitive, the chemical ionization causes non-linearities and matrix effects from humidity in the sample or substances with higher proton affinities. These problems typically necessitate an additional pre-separation, e.g. by gas chromatography, or membrane inlets for water removal before the ionization, contradicting the advantage of fast IMS analysis. In order to mitigate these problems, we have added an additional reaction tube, similar to those known from PTR-MS or SIFT-MS, between the ion source and the ion shutter. This modification can easily be integrated into the setup, as the required reaction tube is a stacked-ring design similar to typical IMS drift tubes. By controlling the pressure and the reduced electric field strength inside this reaction tube, we are able to influence the chemical ionization and thus suppress the mentioned effects. For example, increasing the reduced electric field strength and therefore the kinetic energy of the reactant ions reduces water cluster size and thereby facilitates ionization. Such a high kinetic energy ion mobility spectrometer (HiKE-IMS) exhibits significantly less dependence on high proton affine interferents and sample humidity compared to standard IMS systems as shown in Fig 1. for the detection of toluene (10 ppm in air). Therefore, additional slow pre-separation techniques when measuring complex mixtures or humid samples are no longer necessary. Hence, a HiKE-IMS can directly and quickly ionize and separate complex samples, either for use as a stand-alone device or to provide pre-separated ions for further mass spectrometry analysis.



ThPS32-14 / "Secondary effects" changing arrival time distribution in ion mobility-mass spectrometry of tyramine-based hyaluronan derivatives

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Introduction

Sodium hyaluronate or hyaluronic acid (HA) belongs to naturally occurring linear polysaccharides. [1] Mass spectrometric (MS) analysis of polysaccharides is challenging due to stereochemistry of monomeric units, variability of their linkage and eventual polysaccharide chain branching. Ion mobility (IM) can separate ions according to their shapes and IM-MS has been recognized to be very useful in carbohydrate analysis. [2] This work aims to study ion mobility separation of positional isomers - tyramine based HA.

Methods

Experiments were carried out using a Synapt G2-S ion mobility mass spectrometer (Waters, Manchester, UK) equipped with electrospray. Hyaluronan oligosaccharides modified by tyramine were provided by Contipro Pharma. Methanol, acetonitrile (LC-MS Chromasolv grade) and other chemicals were purchased from Sigma Aldrich (Czech Republic). Purified water was obtained using a Milli-Q Reference Water Purification System (Merck Millipore, Czech Republic).

Results

Sufficient ion mobility separation was achieved for tyramine positional isomers of HA oligosaccharides with six units in negative-ion mode. Detailed evaluation of fragmentation spectra identified isomers corresponding to individual mobility peaks. One of the isomers provided a characteristic fragment, they also differed in intensity ratios of some product ions but the differences were not conclusive without ion mobility separation.
Interestingly, one positional isomer provided two ion mobility peaks if analyzed as sodiated molecules. Peak broadening was observed for protonated molecules but sodium cation probably stabilized at least two separable structures.

Conclusions

Ion mobility –mass spectrometry can well characterize modified hyaluronan oligomers. Peak splitting for metal cation adduct that is not related to positional isomers or collision energy influenced arrival time distribution as "secondary effects" to primary effect of positional isomers.

Novel Aspect

Ion mobility-mass spectrometry was successfully applied in analysis of modified hyaluronic oligomers.

Acknowledgement: The Czech Science Foundation (P206/12/1150), the Operational Program Education for Competitiveness – European Social Fund (project CZ.1.07/2.3.00/30.0004 of the Ministry of Education, Youth and Sports of the Czech Republic).

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ThPS32-15 / Differential mobility spectrometry of endogenous peptides: Modifiers effect on ion mobility and selectivity

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Introduction

Qualitative and quantitative analysis of peptides often follows a generic workflow where peptides are separated by liquid chromatography, ionized by electrospray and detected by mass spectrometry (LC/MS and LC/MS/MS). Differential ion mobility (DMS), a gas phase separation technology, can be implemented between the ion source and the entrance of the mass spectrometer and brings an additional orthogonal separation step for peptides with the same m/z. Thus, DMS gives the possibility to improve

selectivity of the analytical workflow by filtering the interferences, if the mobility of the compounds differs. More recently, it has been described that the addition of modifier (organic solvents such as MeOH or 2-propanol), increases the differences in ion mobility and thus can improves separation power of DMS. The goal of the present work is to evaluate modifiers effect on peptide ions separation at different charge states (from z=2 to z=7) and also to evaluate their effect on selectivity.

Methods
A set of seven endogenous peptides (related to obesity disorder) ranging from 1265 Da to 4193 Da were used for this study. Evaluation of peptides separation was done by direct infusion of peptide mixture into the DMS-MS instrument. DMS spectra were recorded with a fixed separation voltage (SV) and compensation voltage (CoV) were scan by step of 0.2V. Organic solvents were used as modifiers and their effects on separation were screened. The evaluation on modifiers selectivity effects was performed on a LC-DMS-MS platform. DMS was operated as a filter with a fixed SV and fixed CoV specific to each peptide ion species. The set of peptide was spiked in biological matrix and then analyzed by LC-DMS-SIM/SIM (single ion monitoring) and LC-DMS-SRM (selected reaction monitoring). Peptides ions and interferences were monitored across the LC run and plotted for each modifiers employed.

Results
For the evaluation of peptides separation, 2-propanol as modifier has shown the best separation performance with CoV amplitude of 10.64 V between the largest and the smallest peptides, where the CoV amplitude was about 7.6 V without modifier. Additionally, it was observed that CoV values were in positive region for modifiers with lower proton affinity (PA) such as methanol with PA of 761 kJ/mol and CoV ranging from 3.3 V to 7.7 V. For the acetone, with a PA of 823 kJ/mol the CoV ranged from -11.7 V to -2.1 V. For the evaluation of selectivity improvement, the 2-propanol has shown the best interferences removal capacity. For the LC-DMS-SIM/SIM, with 2-propanol as modifier, interferences peaks were reduced by a factor 10 approximately and for the LC-DMS-SRM experiment the remaining inferences were completely removed.

Conclusions
The present work demonstrate the capability of the DMS, with the presence of organic modifier, to separate intact peptides ranging from 1265 Da to 4193 Da and also the ability to filter interferences when SV and CoV are fixed specifically for the targeted peptides.

Novel aspect
Application of DMS for the analysis of endogenous peptides and evaluation of modifiers effect on the DMS separation selectivity

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ThPS32-16 / Formation of isomeric ions in collision-induced dissociation process probed by energy-resolved ion mobility tandem mass spectrometry (ER-IMS/MS2)

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Introduction
Traveling-wave ion mobility spectrometry (TWIMS) coupled with tandem mass spectrometry (MS2) enabled separation and characterization of isomeric ions generated in gas-phase processes. We've proposed energy-resolved ion mobility tandem mass spectrometry (ER-IMS/MS2) as a tool for probing behavior of organic ions in collision-induced dissociation (CID) process and examined fragmentation and isomerization of various organic molecules simultaneously [1]. Isomerization may happen in parallel with or prior to fragmentation and ER-IMS/MS2 provided us indirect evidence that a cationized macrocyclic compound isomerized prior to give product ions [2]. To further confirm the putative intermediate in the fragmentation process, we've investigated simplified model systems by using ER-IMS/MS2.

Methods
Cationized molecules of synthetic 20-membered macrocyclic ethers were analyzed by a Synapt G2 (Waters, UK) tandem mass spectrometer equipped with an ultra-performance liquid chromatograph and an electrospray ion source. Alkaline metal salts were added post-column to assist formation of cationized molecules. Mass selected cationized molecules were subjected to energetic collisions with Ar at the 1st collision cell (CC1) followed by IMS and time-of-flight (TOF) mass analysis. Collision energy (CE) at CC1 was gradually increased and IM spectrum at each CE were inspected after a series of acquisitions (ER-IMS/MS2 Type-I experiments). Alternatively, ions of interest were partially activated at CC1 (fixed CE) and let through TWIMS device and then re-activated at the 2nd collision cell (CC2) with varied CE prior to TOF mass analysis (Type-II experiments).

Results
All the analyzed precursor ions from macrocyclic ethers showed CE-dependent mobility change (Type-I experiments). Two distinct ion species were observed in IMS, i.e., the original precursor ions and another species that only appears after substantial activation. The latter showed longer arrival time and suggested to have an extended structure compared to the original precursor ions. Energy-resolved CID experiments on those early arriving species (original precursor ion) and late arriving species (Type-II experiments) have shown that the latter requires lower CC2 CE to give fragment ions. Examination of the experimental data together with molecular modeling results, the late arriving species were suggested to be an intermediate to give fragment ions from those cationized macrocyclic ethers.

Conclusions
Energy-resolved ion mobility tandem mass spectrometry (ER-IMS/MS2) was shown to be a useful tool for interrogating isomerization of organic ions induced by collisional activation.

Novel Aspect
Isomeric ions formed in collision-induced dissociation process were detected and characterized by energy-resolved ion mobility tandem mass spectrometry (ER-IMS/MS2).

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ThPS32-17 / Solving Selectivity Challenges in Qualitative and Quantitative Analysis of Drugs and Metabolites

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AB SCIEX

Introduction
Accurate metabolite identification and quantification are important for drug discovery and development studies. These studies can be challenging due to the limited amount of sample available from clinical or preclinical studies as well as the lack of authentic metabolite standard. There is a tradeoff between separation and acquisition time, typically, and method development for separation of isobaric metabolites and minimization of co-eluting contaminants with short run time can be time-consuming. Ion mobility technology delivers a new dimension of selectivity and separation for metabolite identification in complex matrices, separation of isobaric metabolites and accurate quantification.

In this presentation, we are demonstrating the key benefits of using differential mobility spectrometry for LC-MS analysis of model compounds and their metabolites in biological matrices.

Methods
Sample: Mixture of isobaric metabolites, norverapamil and p-o-desmethyl verapamil were prepared in water, spiked in rat plasma and bile at different concentration ranges (0.1 to 1000 ng/mL).
Chromatography: A Shimadzu Prominence HPLC system equipped with a Kinetex C18 column (2.1X50mm, 2.6µm) was used. The run consisted of a 3.5 minute 0.1% formic acid in water / 0.1% formic acid in acetonitrile gradient (0.5-95%) at a flow rate of 400 µL/min.
Differential Mobility Separation (DMS) and acquisition method: A prototype differential ion mobility spectrometry (DMS) device interfaced with an accurate mass instrument was used for all experiments (TOFMS, product ion scan, IDA, SWATH). Isopropanol was used as a chemical modifier. All data was acquired in positive ESI mode.

Preliminary Results/Abstract
1) We developed a high throughput DMS optimization procedure on a novel instrument using limited amount of samples in the absence of analyte standards. This approach can be applied for analysis of any biological sample.
2) We could obtain qualitative and quantitative data of the co-eluting isobaric metabolites in a single acquisition run without time-consuming optimization of a separation method.
3) We successfully decreased interference of co-eluting contaminants which resulted in lower background, higher signal to noise ratio and cleaner MS and MS/MS spectra in complex biological samples.

Novel Aspect
Separation of isobaric metabolites in biological samples using differential mobility spectrometry technique without the need for long LC gradient

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ThPS32-18 / Rapid quantitation of Substance P in plasma using Differential Mobility Spectrometry and Microflow chromatography

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AB SCIEX

Introduction
Substance P is an 11 amino acid neuropeptide that is known to modulate neural responses primarily associated with pain perception Recent studies have shown that this peptide also plays a significant role in regulating the immune system and its increased production is part of the pathology of several autoimmune/inflammatory disorders including Inflammatory Bowel Disease and Rheumatoid Arthritis.Consequently there is significant interest in methods that enable detection of Substance P at physiologically relevant concentrations .Here we describe a fast and robust method to detect and quantitate Substance P in protein precipitated plasma. We demonstrate that Subfemtomole limits of quantiation (LOQs) are obtained by combining traditional Multiple-Reaction Monitoring with microflow chromatography and Differential mobility Spectrometry (DMS).

Methods
Substance P was spiked in to a crashed plasma matrix at physiologically relevant concentrations. The resulting mixture was separated on a 1x50mm C18 Column using a Microflow LC. The eluting peptides were analyzed by MRM on a DMS enabled triple quadrupole mass spectrometer. LOQs were evaluated with and without DMS.

Preliminary Results/Abstract
One of the main challenges of peptide quantitation using multiple-reaction monitoring in plasma and other complex matrices has been interference from isobaric, non-target transitions. This has resulted in a significant burden on the chromatographic separation of targets from interfering species, leading to longer run times and significant method development. While ion mobility has previously been explored as a means to improve the selectivity of these assays, it does not always result in an improvement in LOQ. We have developed a rapid method that combines the sensitivity of microflow liquid chromatography with the selectivity of DMS. Quantitation of substance P under fast microflow chromatography was limited by isobaric interference that resulted in poor LOQs. However, by combining microflow with DMS we observed a greater than 10 fold improvement in the LOQ, enabling accurate quantitation at low pg/ml concentrations in under 3 minutes.

Novel Aspect
This work represents a new strategy for the rapid quantitation of Substance P in plasma.

ThPS32-19 / High Resolution IMS-MS and UHPLC-HRMS for the analysis of natural products and complex natural extracts

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Introduction
The efficient analytical profiling of molecules present in plant or fungal extracts represents a key element in natural product research. Commonly these complex extracts are characterized by highly chemodiverse secondary metabolites and by the presence of many multiple isomers. HPLC and UHPLC coupled with mass spectrometry are the most frequent methods used to analyze these types of samples but these techniques sometimes fail to resolve multiple isomers especially when used in a high throughput mode. In such cases, Ion Mobility Spectrometry (IMS) is an attractive alternative for the efficient separation of mixtures containing isomers, without increasing cycle time and with minimal added experimental complexity. In this study, the potential of High Resolution IMS-MS has been evaluated and compared to UHPLC-MS, for the analysis of closely related isomeric flavonoids. Moreover the applicability of IMS-MS has been demonstrated for the fingerprinting of complex mixtures, such as fungal co-culture and mono-culture extracts.

Methods
IMS-MS analyses were performed on a Tofwerk IMS-TOF equipped with an ESI source. Measurements were carried out in both Positive and Negative Ion mode at an applied ESI potential of 2kV and 1.7 kV, respectively. Desolvation and drift tube were kept at atmospheric pressure and 150°C with nitrogen as the buffer gas. Ion mobility separation was carried out at a field strength of ca. 400V/cm. UHPLC-TOF-MS analyses were performed in both PI and NI on a Waters Acquity UPLC system coupled to a Waters Micromass-LCT Premier Time-of-Flight mass spectrometer, equipped with an ESI interface.

Results
UHPLC-MS and IMS-MS were investigated for the rapid analysis of closely related isomeric flavonoids and their glycosides. On a time scale of a few minutes, the flavonoid aglycones were all separated by ion mobility, but not by UHPLC. The glycosides were better resolved by IMS-MS, but not completely separated by both methods. The ion mobility resolving power was routinely >150, indicating that the system provides sufficient resolution for separation of isomeric natural products even in complex samples.

Furthermore, HPLC-IMS-MS and UHPLC-MS were evaluated for the analysis of complex fungal co-culture and mono-culture extracts.

Conclusions
This study demonstrates that isomeric flavonoids can be better resolved by IMS-MS compared to UPLC-MS. Moreover, a significant number of compounds were detected by direct analysis of the fungal extracts using IMS-MS and were not detected by UHPLC-MS or MS only. These results suggest that high resolution IMS is well suited for the separation of isomeric natural compounds (even in high-throughput metabolomics studies) and appears as an attractive alternative to established UHPLC-MS methods.

Novel aspect
High Resolution IMS-MS utilized for the analysis of isomeric natural compounds and for the fingerprinting of complex natural extracts.

ThPS32-20 / Separation of Lipid Classes, Subclasses and Isobaric/Isomeric Lipids Using a Novel FAIMS Device

Julie Horner, Michael Belford, David Peake, Satendra Prasad, Tina Settineri
Thermo Fisher Scientific

Introduction
Lipids play a key role in cell, tissue and organ physiology and with diseases such as cancer and diabetes which involve disruption of their metabolic enzymes and pathways. While the fields of genomics and proteomics have experienced widespread study and generation of information, lipidomics has not enjoyed similar advancement. This is due largely to the complexity of lipids and to the lack of tools for their analysis. In this work we describe a novel method for separation of lipid classes, subclasses, isomers and isobars which employs high Field Asymmetric waveform Ion Mobility Spectrometry (FAIMS). This method may be used with or without prior separation by liquid chromatography.

Method
Modification of three key properties of a standard FAIMS device were investigated and deployed resulting in significant improvement in analytical performance including sensitivity, speed and cost. First, a novel ESI-FAIMS interface was developed to rectify poor ion sampling in FAIMS and achieve significant improvement in sensitivity. Second, gas velocity through the FAIMS device was increased resulting in reduced residence time and therefore increased speed of analysis. Third, electrode geometry was optimized leading to simultaneous improvements in sensitivity and speed.

Preliminary Data
A standard device was used to collect ESI-FAIMS Full Scan HR/AM Mass Spectra on an Orbitrap Elite hybrid mass spectrometer (Thermo Fisher Scientific, San Jose, CA) for a crude rat retina lipid extract prepared using a monophasic Svennerholm method. The standard method furnished separation of lipid classes (gangliosides from the crude extract) at compensation voltages ranging from 28 V-38 V and separation of subclasses (GT1, GD1, GD3) within this compensation voltage range. A modified prototype device utilizing an elliptical, progressively curved inlet orifice of the outer electrode has been used to demonstrate increased sensitivity and speed of separation of small molecule active pharmaceutical ingredients (APIs) and for improved-S/N small-molecule and peptide quantitation using 100% Nitrogen (Helium-free) FAIMS gas. This prototype device will be used to separate lipid mixtures for qualitative identification of class and subclass components.

Novel Aspect
Optimized prototype FAIMS device for qualitative analysis of lipid mixtures.

ThPS32-21 / Using Ion Mobility Mass Spectrometry to Identify Multiple Protonation Sites and Different Fragmentation Patterns For The Fluoroquinolone Class of Antibiotics

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Waters Corporation

Introduction
Fluoroquinolones are a class of antimicrobial agents which have been administered to livestock for different purposes, (a) prevention and control of infections and (b) growth promotion. Due to concerns regarding the spread of resistant microorganisms in the human population, the USA FDA introduced a ban on the use of enrofloxacin and ciprofloxacin in livestock production in 2005. Use of antibiotic growth promoting agents in animal husbandry has been forbidden in the EU since 2006. Here we report, use of High Definition Mass Spectrometry (HDMS) as a powerful tool for method development to support, unequivocal identification of fluoroquinolone antibiotic residues.

Methods
The assay is based on the analysis of porcine sample extracts and a mixture of veterinary drug solvent standards, containing fluoroquinolones, tetracyclines and macrolides. Positive ion electrospray with HDMSE data acquisition was performed using a Synapt G2-S HDMS mass spectrometer.

Results
Ultra performance liquid chromatography (UPLC) HDMSE data was initially acquired for a series of porcine extracts, solvent and standard mixtures of fluoroquinolones, tetracyclines and macrolides. These were utilized to generate mobility separated single component MSE spectra for the [M+H]+ species, hence precursor ion, fragment ions and drift time were determined. The enhanced analytical performance facilitated the detection of antibiotics in a selection of sample types requiring only a simple and generic extract preparation step. HDMS can not only provide additional peak capacity but also new insights into the molecular characteristics of the analytes during the method development process. The data presented here shows the detection and elucidation of multiple sites of protonation within a single compound. The presence of the multiple sites of protonation that were observed in this study, may account for variations seen in proficiency test results for these compounds.

Conclusions
The elucidation of different sites of intra-molecular protonated species has been shown using ion mobility MS and have been determined from the different single component fragmentation spectra. The HDMSE observations have the potential to explain the differences sometimes observed in inter-laboratory studies where participants report results obtained from monitoring specific MRM transitions. Ion mobility can be used as an investigative tool to fully understand the impact of the parameters employed in an analytical assay and on the results that can be obtained. Using this information, improved experimental designs can be employed to ensure more reliable and reproducible results.

Novel Aspect
Ion mobility separation and structural characterization of isobaric species formed from the multiple sites of protonation for fluoroquinolone veterinary drugs.

ThPS32-22 / Using the Routine Separation Dimension and Identification Criteria of UPLC Ion Mobility to Enhance Specificity in Profiling Complex Samples

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Introduction

The combined peak capacity of UPLC/ion mobility and collision cross section measurements (CCS) can be used to produce routine unequivocal identification of marker flavonoid isomers in complex mixtures such as herbal tea products that are used as functional foods or phytomedicines. Several Passiflora (Passifloraceae) species known to have sedative properties are utilized as phytomedicines. The species contain flavonoids, mainly C-glycosylflavones (apigenin and luteolin derivatives) which frequently occur as isomeric forms. Flavonoids are one of the largest and most wide spread classes of compounds and possess diverse pharmacological and biological properties.

Methods

Hydroethanolic extracts of P.incarnata, P.edulis, P.caerulea and P.alata were analysed using ultra performance liquid chromatography. Negative ion electrospray with ion mobility HDMSE data acquisition was performed using a Synapt G2-S mass spectrometer.

Results

Collision cross sections (CCS), accurate mass, fragment ions and retention time have been used to profile the hydroethanolic extracts of Passiflora speices grown in Brazil. This approach offers a unique selectivity in profiling complex mixtures. Results obtained clearly show the benefits of using the collision cross section measurements and the combined peak capacity of UPLC with ion mobility. The enhanced peak capacity enabled more information to be extracted from fragmentation studies and the individual fragmentation spectra have been obtained for co-eluting flavonoid isomers. Characteristic assignment for 6-C and 8-C flavonoid glycosides isomers (vitexin and isovitexin) (orientin and isoorientin) were obtained from analysis of the extracts.The four Passiflora extracts were analysed and screened against the flavonoid CCS library, to identify the 6-C and 8-C flavonoid glycosides isomers. CCS measurements for marker glycoside pairs vitexin and isovitexin (188.8 Å2/195.5Å2) and orientin and isoorientin (187.7 Å2/198.1 Å2) have been determined. In the case of isoorientin/orientin (which co-eluted chromatographically and had the same fragment ions), ion mobility resolution enabled unique fragment ion ratios to be observed. For the first time unique collision cross section measurements and corresponding isomer fragmentation spectra have been obtained for these compounds.

Conclusions

UPLC ion mobility mass spectrometry can be used to profile complex samples and identify marker flavonoid compounds. Vitexin, isovitexin, isoorientin and orientin C-glycosyl flavonoids have been determined to be present in the four Passiflora species and have been distinguished using ion mobility mass spectrometry and collision cross section measurement.

Novel Aspect

Using ion mobility mass spectrometry, individual collision cross section measurements and unique fragmentation spectra were obtained for flavonoid isomers that chromatographically co-elute.

ThPS32-23 / Collision Cross Section a powerful parameter for the identification of pesticides in food

Séverine Goscinny[1], Michael McCullagh[2], Kieran Neeson[2]
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Introduction

Recent advances in mass spectrometry (MS) and separations technology have a potential impact on complex mixture analysis such as enhanced MS ion transmission and increased peak capacity with UPLC. Such enhancements in TOF hardware technology provide improved precision and accuracy in the data generated, which has enabled more intelligent data processing software packages to be created and utilised. Nonetheless, it is a challenge to rapidly and efficiently identify targeted compounds present in the sample with a large number of co-extracted matrix components. We will illustrate how ion mobility added to conventional MS screening enhances the selectivity of the overall method.

Methods

The assay is based on the analysis of different mandarin samples as it is known to be one of the most complex commodity in the fruit and vegetables group. Extracts of an organic sample was used as matrix blank and for matrix matched calibrants, as well as quality a control sample generated for an EU-RL proficiency test (FV-13 2011).
UPLC HDMSE data was initially acquired for a series of solvent standard mixtures. These were utilized to generate mobility separated single component MSE spectra for the [M+H]+ or adducted species. The data generated was processed using prototype software, estimated CCS values, precursor ion, fragment ions and retention time values were determined for the pesticide standards. These were entered into the scientific library. Subsequently non targeted data sets were acquired for the mandarin matrix.

Results

The poster will demonstrate how CCS of the pesticide standards can be utilized as a confirmatory parameter to increase confidence in identification.The use of CCS as a newly integrated parameter for screening, offers the potential to reduce the initial specificity of applied traditional screening parameters. The results will show how CCS values have been utilized to efficiently reduce false identifications and avoid false negatives in the EU-RL proficiency test sample analysed. To ease manual interrogation of the data, especially for difficult identification cases, we also took advantage of the mobility spectral clean-up to support the final decision.

Conclusions

Retention time, precursor ion and fragment mass accuracy are traditionally used for screening parameters, the results obtained indicate that CCS can be used as a complimentary identification point. CCS values provide added confidence which permits less stringent screening parameters to be used reducing the chance of false negatives to occur.

Novel Aspect

CCS as a new identification point for screening assay.

ThPS32-24 / Elucidation of unexpected reaction pathways during synthesis of strained PAH macrocycles by ion mobility mass spectrometry

Wen Zhang, Martin Quernheim, Hans Joachim Räder, Klaus Müllen
Max Planck Institute for Polymer research

ThPS32-25 / Direct visualization of intact protein ion beam focusing transmitted by an ion funnel using a position-sensitive detector at elevated pressure

Tiffany Porta, Shane R. Ellis, Ron M.A. Heeren
FOM Institute AMOLF

Introduction

An active pixel detector is used to investigate the ion beam focusing properties of an ion funnel (IF) guide, used for efficient transfer of ions from an AP ion source into a mass analyzer. The detector is capable for direct charge detection after the IF and recording the position of ions hitting its surface. The detector is operated at elevated pressure and without signal pre-amplification, reducing electronic noise usually generated by MCP detectors. By means of deflecting electrodes inserted in-between the IF exit and the detector, we demonstrate the potential of the detector for parallel detection of ions with different mobility in a single experiment. This may provide a mean to increase the throughput of ion mobility-based measurements.

Methods

Experiments were performed on a simple device containing: a heated capillary, to introduce ions generated by ESI; a 2 stage vacuum chamber hosting the ion funnel (PNNL) and the detector (Timepix), held at 3 and 0.95 mbar, respectively. A grid was inserted in-between the IF and the detector to monitor the transmitted ion current. Optimized conditions were: inlet capillary voltage: 310 V; IF entrance and exit electrode: 300 and 50 V, respectively; conductance limit: 30V. Proteins standard solutions were prepared in 50%ACN/2%HCOOH (denaturing conditions) and 25 mM ammonium acetate:10%ACN, pH=7 (native conditions).

Results

Our experimental results show the ability of the IF-Timepix combination to efficiently transmit and detect intact proteins (apomyoglobin, 12kDa; BSA, 66 kDa and IgG, 150kDa) and protein complexes (holo-myoglobin) down to a concentration of 1 µM without signal pre-amplification. Ion images illustrate the effect of the potentials applied at the conductance limit (which determines the gas composition in the detector chamber) and the grid, greatly influencing the ion beam focusing. Experiments conducted without the grid illustrate the charge state distribution after focusing by the IF. Results for BSA display a ring-shape distribution, with more intense signal at the outer region. This can be expected with an IF ion guide due to charge repulsion in the center of the focused ion beam. The addition of a deflecting potential perpendicular to the ion trajectory show that ions are directly mobility separated in a single experiment, without the need of a scanned potential.

Conclusions

A position-sensitive detector provides insight in ion beam focusing properties of an ion funnel. This approach is exploited for direct differential ion mobility experiments. Enough current is generated and transmitted to activate detector pixels without pre-amplification at elevated pressures. This allow the separation and detection of various ion species driven by the IF down to the µM range, including intact proteins and complexes.

Novel Aspect

Free electronic-noise and direct charge detection at elevated pressure using an active pixel and position-sensitive detector, capable of parallel detection of mobility separated ions in a single experiment.

ThPS32-26 / Multiplexed IM-QTOF analysis of complex proteomics and metabolomics samples using a real-time dual filtering technique

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Agilent Technologies

Introduction

The benefit of applying Hadamard transformation multiplexing to an ion mobility mass spectrometry experiment (IM-MS) is to significantly increase the ion mobility duty cycle and also improve the dynamic range of the instrument. One of the main challenges is to efficiently deal with removing noise due to fluctuations in the ion beam intensity and to execute the de-multiplexing step in real-time for handling high-throughput IM-MS applications. The challenges are exacerbated with high density data such as those obtained from complex proteomics or metabolomics samples.

Methods

The instrument used in this study consist of a uniform-field ion mobility drift cell interfaced to a quadrupole time-of-flight mass spectrometer. Multiplexing is accomplished by applying a pseudo random sequence (PRS) generated gating pulse to the ion funnel trap. De-multiplexing and filtering are accomplished with a new two filter approach of high computational efficiency to handle noise in the multiplexed ion signal. The first filter is a column-type filter applied before the de-multiplexing step. The second filter is a pattern matching filter used in a post-processing step following de-multiplexing of the input data. Both of these filters are implemented in firmware and therefore capable of handling complex samples in real-time.

Results

We have observed significant improvements in sensitivity for both simple and complex biological samples using multiplexed ion mobility experiments with an LC IM-QTOF system. These improvements led to enhanced protein sample coverage for complex samples such as Ecoli protein digest. Four-dimensional feature extraction is used to analyze de-multiplexed data. Results using transformation multiplexing algorithms with a pseudo random sequence (PRS of 4 and 5-bit) producing 8 and 16 pulses will be presented. Preliminary data for Ecoli and BSA protein digests show factor of 8 and 16 improvements in signal intensity for 4-bit and 5-bit experiments respectively compared to single pulse experiments while preserving low level signal. A multicore implementation further increases the performance of our system to handle high density multiplexed ion mobility data.

Conclusions

We have demonstrated the successful implementation of multiplexing experiments on complex biological samples using a new de-multiplexing and filtering algorithm that can be used in real-time data processing.

Novel Aspect

Real-time de-multiplexing and processing of high density data using a dual filtering technique for complex biological sample analyses.

ThPS32-27 / Characterisation of metabolites by utilising Collision Cross Section measurements in conjunction with an integrated microfluidic device

John Chipperfield[1], David Douce[1], Richard Gallagher[2], Christine Pattison[2], Kathryn Pickup[2], Kristin Samuelsson[2], Mike McCullagh[1]
[1]Waters, [2]AstraZeneca

Introduction

Full characterisation and identification of drug metabolites is a

key requirement in all drug development programs. It is often difficult to definitively identify phase II drug metabolites formed by conjugation reactions as often the conjugate moiety simply falls off under MS/MS experiments with no evidence of the point of biotransformation. The measurement of the collision cross section (CCS) of molecules, allows a metabolism scientist unique additional information to potentially deduce the exact nature of the metabolites. Previous work confirmed the advantage in using the microfluidic tile, with additional metabolites being identified from a small sample volume. Here a larger sample was utilized to help in the structural elucidation of these metabolites with the aid of the ion mobility function.

Methods
Metabolites of midazolam were produced from incubations in rat and human hepatocytes. Midazolam was also dosed to chimeric mouse strains and whole blood was extracted. The assay is based on the direct analysis of the extracts produced by protein precipitation and subsequent analysis using a microfluidic LCMS interface. Chromatographic gradient was provided from a nano Acquity system with all separations occurring on the integrated microfluidic device. All experiments were performed on a Waters Synapt G2-Si Q-tof mass spectrometer operating in HDMSE data acquisition mode. Detected metabolites of interest were subject to separation by ion mobility and the CCS determined from the calibrated system for all the isomeric forms detected.

Preliminary Data
CCS values have been measured for midazolam, 1-OH midazolam and 4-OH midazolam from solvent standards. From these experiments, a library of known midazolam metabolites containing both the retention time information from the microfluidic device and the CCS value was used to identify these expected metabolites within the hepatocyte and whole blood samples. The increased sensitivity from the integrated microfluidic device has allowed the tentative identification of a number of additional metabolites, and the use of ion mobility resolution to clean up the precursor and fragmentation spectra, which has allowed clarification of these metabolites. Midazolam is known to produce many phase II metabolites which are formed either by glucuronidation of the 1-OH or 4-OH midazolam or by direct N-glucuronidation, of which there are three possible sites for this to occur. We are currently using the ion mobility capability to definitively characterise the possible N-glucuronide secondary metabolites. In addition, similar experiments have been used to investigate the O-glucuronides and confirmation will be completed when an authentic reference standard becomes available.

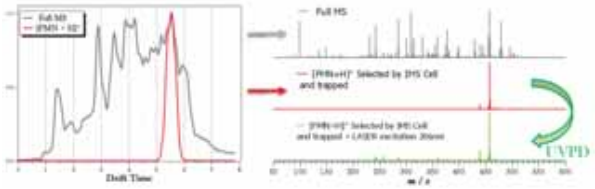
Novel Aspect
Integrated microfluidic device. Combined nanoflow analytical column and source sprayer. Collision Cross Section measurements. Characterization of isobaric metabolites.

ThPS32-28 / Photodissociation of trapped ions selected by drift-time separation. IMS-UVPD-MS

Bruno Bellina[1], Jeff Brown[2], Mike Morris[2], Isabelle Compagnon[3], Perdita Barran[1]
[1]University of Manchester, [2]Waters, [3]University Claude Bernard LYON1

Introduction
Photodissociation induced by laser excitation of selected ions is a powerful analysis method for proteomics or gas-phase spectroscopy. By adding an IMS cell to this approach we bring a new dimension allowing us to perform photodissociation of conformer selected ions as well as cross sections measurements of species generated by laser excitation.
Here we describe the first results obtained on an ion mobility enabled Q-ToF research platform coupled with a UV laser. The photodissociation has been performed on ions selected through m/z in a quadrupole and/or drift-time in an IMS cell.

Methods
The Waters Synapt G2S ion mobility enabled Q-ToF has been modified to allow the injection of a laser beam through the entire setup. We have machined a lens into the pusher plate of the TOF head. In this configuration the ion beam is collinear with the optical way all along the trap cell, the IMS cell and the transfer cell. The ions are generated by a nano-spray source and detected by a Q-ToF. Waters programs have been developed to provide IMS selection and trapping in the transfer cell.



Results
We have trapped ions post mobility cell (in the third part of the tri-wave assembly). We can trap them for several seconds with the application of electrostatic trapping. UVPD experiments have been achieved with a 349 nm kHz UV laser and with 266 nm YAG laser. We observed photo-fragments on different molecules such as peptides, dyes or entire proteins. We also achieved electron photo-detachment along with sequential loss of CO2 following irradiation of the 12.4 kDa protein Cyctochrome C in negative ionisation mode. We also present photodissociation of the Flavin mononucleotide [FMN+H]+ isolated by quadrupole versus Drift-time selected from the full MS. Also UVPD and CID have been performed on this drift-time selected ion.
This is the first time (to our knowledge) that photo-dissociation has been demonstrated in a Q-ToF instrument and shows the feasibility of this approach.

Conclusion
We have trapped and photo-dissociated ions selected in function of their drift-time through an IMS cell. These first results show the feasibility and the vast potentiality of implementing photo-excitation to an ion mobility mass spectrometer. This system enables various approaches on many systems such as isobaric molecules, protein conformations and spectroscopic/FRET studies.

Novel aspect
Photodissociation on drift time selected ions.
Drift-time measurements of ions generated with laser excitation.

ThPS33 - Data Analysis – General
11:00-15:00
Poster Exhibition, Level -1

ThPS33-01 / Adaptive noise smoothing of the mass spectra

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Ryazan State Radio Engineering University

Initial preconditions for the research are problems with relatively low quality of the mass spectra recorded by special purpose instruments, such as mass spectrometers for spacecraftwith limited vehicle-borne resources. The development of such mass spectrometers highlights the task to reduce the weight

and dimensions of the device, its power consumption. This task becomes particularly relevant at the present stage of the space technology development under the transition to the format of the micro-and nano-satellites weighing less than 1 kg.
The purpose of these researches was development of mathematical methods to increase the limit of detection of sample components with minimal distortion of the major mass spectral peaks. The detection limit can be characterized by a minimum concentration of components reliably recorded. Reliability of the components registration with a low concentration is smaller if the noise level is higher.
Ways to increase the signal/noise ratio are based on the suppression of the noise component of the recorded signal. Essentially, there are two known noise elimination methods – smoothing by means of thenormalization windows in the reading field (mass, in this case) and filtering in the frequency domain.
Smoothing of signals is expressed mathematically by the convolution. In this paper we proposed and tested the method of smoothing of noises by the use of normalization windows with adjustable (locally adaptable) width. Theprinciple of the proposed method is an algorithm that is used to smooth the signal by the convolution operation with any of its standard windows, where in each i-th point of a signal width of the window is controlled by the condition of coincidence of total intensity included in the vicinity of the point and a certain reference intensity threshold.
Another approach to the suppression of the signal noise component is expressed mathematically by multiplying the Fourier transform of the original signal and the amplitude characteristic of the filter and the restoration of the smoothed signal by means of an inverse Fourier transform. The most effective methods of filtering, including Wiener, are based on the utilization of experimental information about the noise. For this purpose the portion of the signal containing only the noise component is allocated, Fourier transform of the noise is determined, unbiased sample estimates of the spectral density of the entire signal and noise are computed and the amplitude characteristic of the filter is constructed. In this paper, the method of calculating of the unbiased sample estimate of the mass spectrum through the using of two widths of smoothing Tukey lag window is proposed in order to construct a Wiener filter.
Experimental studies have shown that the proposed methods in contrast to the commonly used ones, allow reducing the noise component of the signal without noticeable distortions of intense peaks (Fig.1).

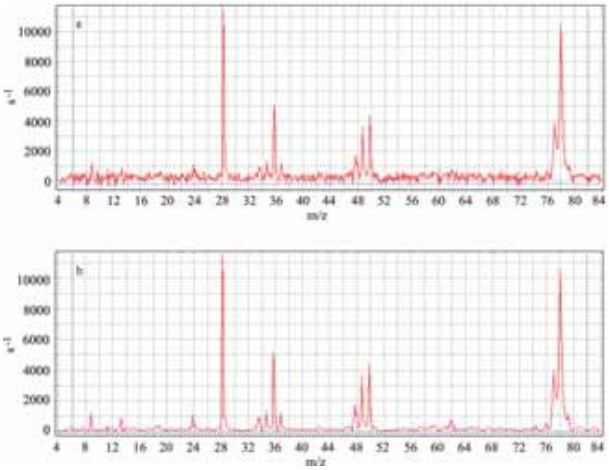

Fig.1. Mass spectrum of benzene: a – with high level of the noise, b –smoothed by window with adjustable width.

ThPS33-02 / A new calibration method of vacuum mass spectrometer

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[1]*Company Science and Technology on Vacuum & Cryogenics Technology and Physics Laboratory*

The vacuum mass spectrometer is a kind of vacuum gauge for partial pressures measurement. For many vacuum applications, the partial pressures of certain component species of a gas are of more interest than the total pressure of the gas in a system. If precise measurement of the partial pressures is necessary, the calibration of mass spectrometer must be made. However, the sensitivity to single gas in a mixture has been found to change as other gas pressure was varied. Therefore, for a specific measurement object, the specific standard sample mixture must be used to calibrate mass spectrometers. For this purpose, a calibration apparatus of mass spectrometers was developed. The apparatus not only prepares the standard sample mixture but also keeps the gases proportion constant in the condition of molecular flow.
The apparatus consists of pumping system, gases supply system, standard sample chamber, inlet system, calibration chamber, etc.. The pumping system could be used for obtaining good vacuum in the inlet chamber, standard sample chamber and pipeline to ensure the purity of prepared gases mixture. The gases supply system can provide various high-purity gases filled into the standard sample chamber. The pressures of different gases filled into the standard sample chamber can be measured by the capacitance diaphragm gauges, which will determine the gases proportion. After preparing the standard sample mixture, the mixture is sampled by the small vessel, and then expanded into the inlet chamber. The mixture pressures in the inlet chamber are greatly reduced into molecular flow region for the orifice 1 because the inlet chamber is much larger than the small vessel. When calibrating a mass spectrometer installed on the calibration chamber, the mixture in the inlet chamber is led through the orifice 1 into the calibration chamber and pumped continuously through the orifice 2 between the calibration chamber and the pumping chamber. The dynamic equilibrium pressures will be generated in the calibration chamber. During calibration period, the mixture pressures nearly maintain constant because the orifice 1 is small enough and the inlet chamber is large enough. Moreover, two orifices were designed to be molecular flow conductances for passing mixture from them, which makes the gases proportion in the calibration chamber as same as that in the inlet chamber, and also as same as that in the standard sample chamber. This implies that the gases proportion is not different from that preparing originally. If volumes of the small vessel and the inlet chamber, and conductances of the orifices are known, the partial pressures in the calibration chamber can be calculated theoretically. By this method, the sensitivity and linearity of the mass spectrometer would be calibrated according to its actual requirements.

Novel aspects
A new calibration method of vacuum mass spectrometer

ThPS33-03 / The relationship between electron-ionization mass spectra and conformation of (substituted phenyl)ferrocenes

Yutaka Okada
Ritsumeikan University

Introduction
In this paper, electron-ionization mass spectra of these (substituted phenyl)ferrocenes were measured. The fragmentation was discussed in the view of their conformation.
Methods
The mass spectra were measured using a Shimadzu QP-5050A by introducing the sample through the GC. The electron energy used to ionize the sample was 70eV.

Results
1. (p-substituted phenyl)ferrocenes showed less fragmentation than o-derivatives. No cleavage of the Fe-Cp bond possessing no substituents was found, while that of the Fe-Cp bond, which has phenyl group, was observed.
2. (o-substituted phenyl)ferrocenes showed intense fragment ions. In particular, cleavage of the Fe-Cp bond possessing no substituents was found.
3. For the (o-chlorophenyl)ferrocene, cleavage of the Fe-Cp bond possessing a phenyl group was found due to the interaction between the high electronegative chlorine atom and positive charge on the iron atom.
For the (o-methoxy-, hydroxyl- and acetoxylphenyl)ferrocenes, a stable fragment ion of m/z=212 was observed due to the formation of the 6-membered chelate ring containing positive charge on the iron atom.
For the [o-(methoxycarbonyl)- and acetylphenyl]ferrocenes, fragment ions that the unsubstituted Cp ring eliminates were observed as large peaks. Furthermore, no elimination of the (methoxycarbonyl)- or acetyl group was observed in contrast to the p-isomers. This is because a 7-membered chelate ring is formed by an interaction between the carbonyl oxygen and positive charge on the iron atom.

Conclusions
Based on the mass spectra of the (p-substitutedphenyl)ferrocenes, cleavage of the Fe-Cp bond, which has a phenyl group, mainly proceeds. However, for the o-derivatives, cleavage of the Fe-Cp bond possessing no substituents was mainly observed. This result was due to the interaction between the electronegative atom in their substituents and the positive charge on the iron atom.

Novel Aspect
Electron-ionization mass spectra of (substituted phenyl) ferrocenes were related to the conformations.

ThPS33-04 / Analytical application of alkylation for the study of amino acids

Nino Todua, Anzor Mikaia
National Institute of Standards and Technology

Introduction
Alkylation is widely applied to amino acids and their condensation products of natural origin prior to their analysis by mass However systematic study of per-alkylated amino acids and identification of characteristic ions useful for structure elucidation have not been carried out. In the present study EI spectra of partially and completely alkyl substituted amino acids are reported and diagnostically important ions presented.

Methods
Commercial Glycine, L-Alanine, beta-Alanine, L-Phenylalanine, L-Valine, L-Leucine, L-Isoleucine, L-Threonine, L-Serine, L-Threonine, L-Methionine, L-Lysine, L-Histidine, L-Tyrosine, L-Proline, and L-Tryptophan were objects of the study. Alkylation of these amino acids has been accomplished with the use of commercial derivatization reagents: methyl, trideuteromethyl, ethyl, n.-propyl, isopropyl, 1,1-dimethyl-propyl, n.-butyl, sec.-butyl, tert.-butyl, 2-methylbutyl, n.-pentyl, isopentyl and neopentyl iodides in presence of NaOH. The EI mass spectra of resulting alkyl/polyalkyl amino acids were recorded on GC-MS and GC-MS-MS instruments with quadrupole analyzers.

Preliminary data
Fragmentation of alkylated amino acids under EI conditions proceeds according to the known mass spectrometry rules, and most of resulting ions can be explained easily. Only nitrilium cation [Alkyl-N+≡CH]+ has not been earlier considered to be characteristic for these molecules; peaks of these ions are prominent in the spectra of mono- and dialkyl-amino acids when the amino group is connected to 2-C-atom. The interesting differences in localization of a charge can be useful in data analysis: (a) while alkylated Tryptophans and Histidines mostly localize charges on "aromatic" moieties, the ions containing "non-aromatic" parts of a molecule are the most intensive peaks in the spectra of alkylated Tyrosines; (b) mass spectra of alkyl derivatives of "Lysine-type" amino acids indicate localization of charge on Nepsilon part of the molecule. Emphasis will also be made on specific types of fragmentation of Threonine, Serine and Methionine.

Novel aspect
Fragmentation pathways of alkylated amino acids are defined, moieties localizing the charge in each amino acid are identified, diagnostically important ions for structure elucidation are established, and the results are systematized.

ThPS33-05 / Development of ultralow energy (1-10 eV) ion scattering spectrometry coupled with RAIRS and TPD for the investigation of molecular solids

Rabin Rajan J Methikkalam[1], Soumabha Bag[2], Radha Gobinda Bhuin[2], Thalappil Pradeep[2], Luke Kephart[3], Jeff Walker[3], Kevin Kuchta[3], Dave Martin[3], Jian Wei[3]
[1]Indian Institute of Technology, Madras, [2]DST Unit of Nanoscience (DST UNS), Department of Chemistry, Indian Institute of Technology Madras, India, [3]Extrel CMS, LLC, Pittsburgh, USA

Introduction
Extremely surface specific information, limited to the first atomic layer of molecular surfaces, is essential to understand the chemistry and physics in upper atmospheric and interstellar environments. Ultra-low energy ion scattering in the 1-10 eV window with mass selected ions can reveal extremely surface specific information which when coupled with reflection absorption infrared (RAIR) and temperature programmed desorption (TPD) spectroscopies, almost complete information of the molecular species at surfaces could be derived. These experiments have to be performed at cryogenic temperatures and at ultra-high vacuum conditions without the possibility of collisions of neutrals and background deposition in view of the poor ion intensities and consequent need for longer exposure times. Here we combine a highly optimized low energy ion optical system designed for such studies coupled with RAIR and TPD and its initial characterization.

Experimental Section
Instrument consists of three ultra-high vacuum chambers namely, ionization chamber, octopole chamber and ion scattering chamber. Ion scattering chamber is fitted with a Ruthenium single crystal at the tip of a closed cycled Helium cryostat, which is used as the substrate to deposit various molecular solids by vapour deposition. Ions for the low energy ion scattering setup are produced in the ionization chamber by electron impact ionization on the gases fed into the chamber. The ions are given a desire kinetic energy, mass selected by a quadrupole mass analyser, bended 90 degrees by an ion bender again mass filtered by the octopole and are then made to collide on the ruthenium substrate where the molecular solids which to be studied were pre-deposited at desired temperature. The scattered ions are analysed by another quadrupole mass spectrometer fixed at the other end, which give a resultant spectrum of the scattered ions mass verses its intensity. Together with low energy ion scattering setup, the scattering chamber is fitted with a TPD and RAIRS.

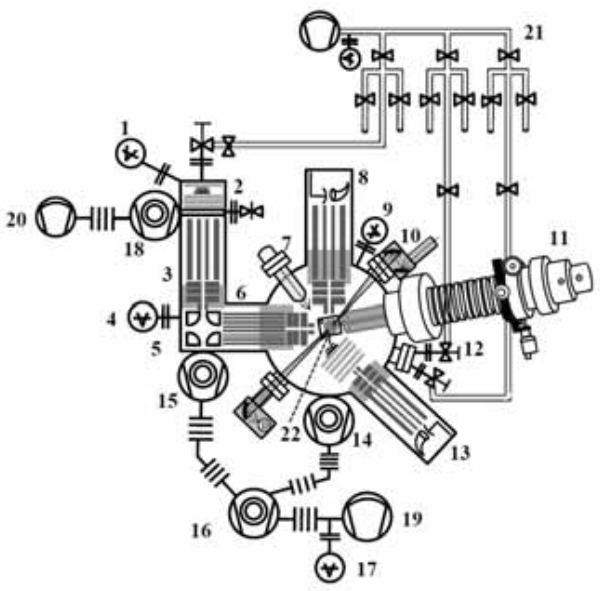

Figure 1: Schematic of the instrument. Various parts of the instrument are: 1. ionization chamber B-A gauge, 2. ionization chamber, 3. quadrupole mass filter (Q1), 4. octupole chamber cold cathode gauge, 5. ion bender, 6. Octupole chamber, 7. low energy alkali ion gun (Cs+/Li+), 8. quadrupole mass analyzer (Q3), 9. scattering chamber B-A gauge, 10. RAIRS setup with KBr view port and external MCT detector (D), 11. sample manipulator fitted with closed cycle He-cryostat, 12. scattering chamber leak valves, 13. TPD probe fitted with ionizer and quadrupole mass analyzer and detector, 14. Scattering chamber turbomolecular pump (685 l/s), 15. octupole chamber turbo molecular pump (260 l/s), 16. Backing turbo molecular pump (260 l/s), 17. Pirani gauge, 18. ionization chamber turbomolecular pump (80 l/s), 19. Diaphragm pump (9.6 m3/h), 20. diaphragm pump (3.8 m3/h), 21. gas manifold arrangement with shutoff valve, gas line, pirani gauge, and diaphragm pump, and 22. Ru(0001) single crystal. Standard vacuum symbols are used (wherever necessary) for clarity.

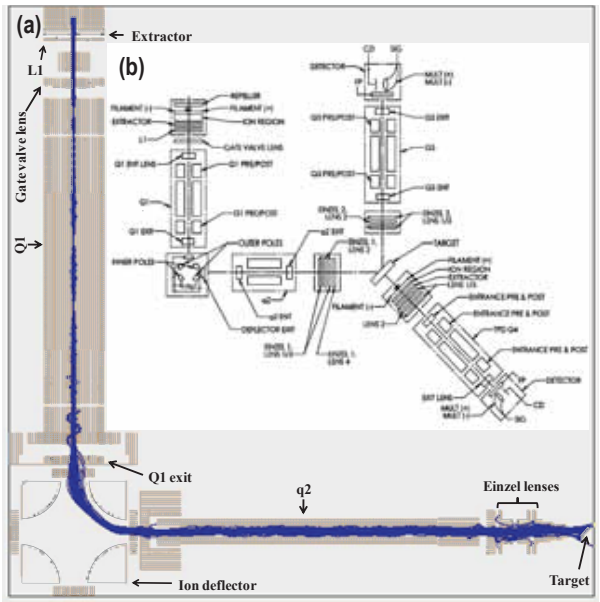

Figure 2: (a) SimIon simulation of the ion trajectory of 1 eV Ar+ (m/z 40), colliding on the target. (b) A schematic of the instrument with a description of the components. Appropriate labels are shown on the calculated trajectory so as to relate (a) and (b).

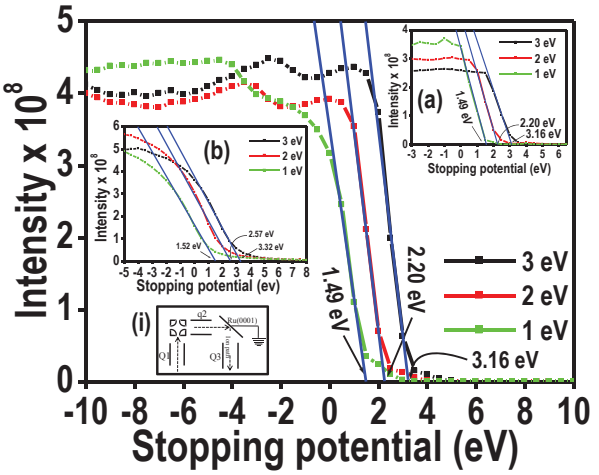

Figure 3: Plot of Ar+ stopping potential data at quadrupole 1 (Q1). Data corresponding to octupole 2 (q2) and quadrupole 3 (Q3) are in (a) and (b). The experimental scheme is shown in inset.

Results

The instrument's performance with low energy ion scattering was tested with SimIon simulations (fig 2), for 1 eV Ar+ ion collision on the target and stopping potential experiment (fig 3) with various quadrupoles. Ion scattering measurement of C6D6+ ion on Ru(0001) substrate at 10 K in the energy range 1-6 eV and Ar+ ion chemical sputtering at 30 eV on D2O solid deposited at 100 K (fig 4) was also done. The performance of TPD was checked using a thickness dependent measurement of Argon gas deposited at 10 K (fig 5) which was giving a linear fit with increase in monolayers. The characteristic C-Cl stretching frequency for CCl4 (fig 6) was obtained for a thickness of 100 ML (~30 nm) confirms RAIRS sensitivity and performance.

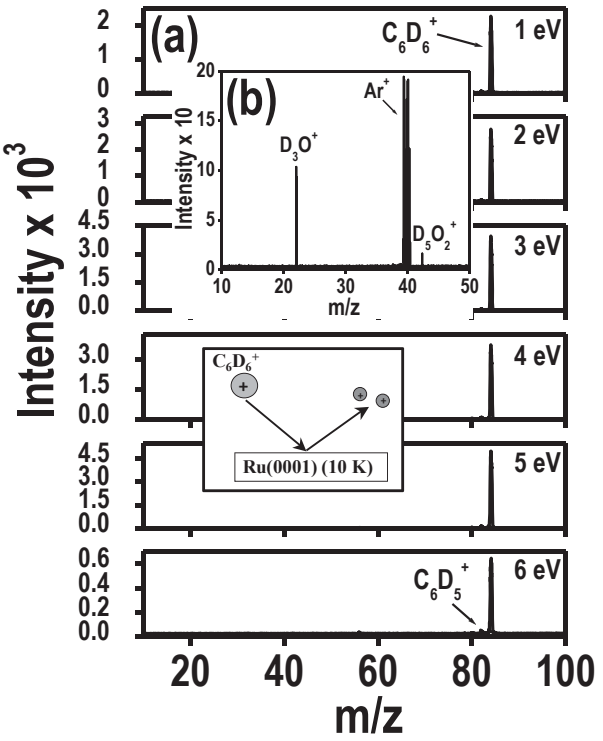

Figure 4: (a) Results of 1–6 eV C6D6+ scattering on Ru(0001) at 10 K. (b) Chemical sputtering spectrum due to 50 eV Ar+ on amorphous ice at 100 K. Bottom inset shows a schematic of the ultralow energy ion scattering experiment. The ejection of D3O+ is due to the proton-transfer reaction upon collision of Ar+ ions (2D2O→D3O+ + OD−).

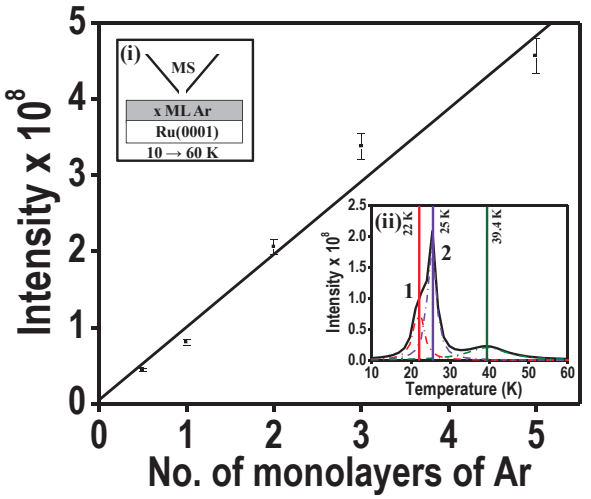

Figure 5: Increase in intensity with respect to number of monolayers of Ar; 0.5, 1, 2, 3, 5 ML during the TPD experiment. Intensity refers to that of the m/z 40 peak. Inset (i) shows a schematic representation of the TPD experiment (MS stands for mass spectrometer). Inset (ii) shows the TPD spectrum of 5 ML Ar.

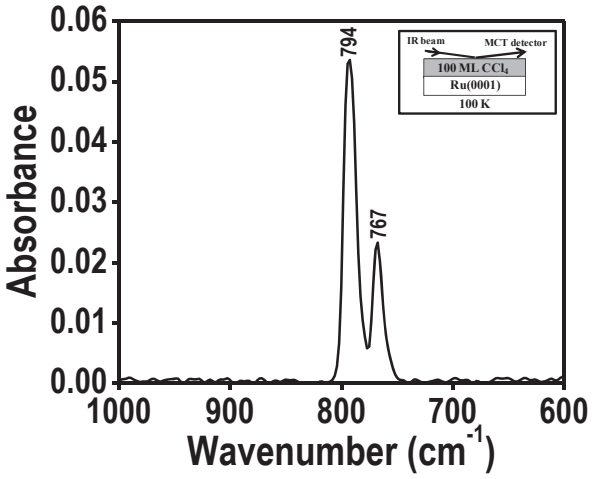

Figure 6: RAIR spectrum of 100 ML CCl4 deposited on Ru(0001) at 100 K.

Conclusion

A custom-built instrument with low energy ion scattering coupled with TPD and RAIRS was built and its performance was tested.

Novel aspect

The instrument can study molecular solids at low temperature (8 K) and the simultaneous probing of such solids with low energy ion scattering setup, TPD-MS and RAIRS can unravel structure, reactivity, kinetics and thermodynamics of molecular materials.

ThPS33-06 / Determination of mesoridazine by liquid chromatography–tandem mass spectrometry and its application to pharmacokinetic study in rats

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[1]Korea Research Institute of Chemical Technology (KRICT), [2]College of Pharmacy, Chungnam National University

The object of the present study was to develop and validate an assay method of mesoridazine in rat plasma using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Plasma samples from rats were prepared by simple protein precipitation and injected onto the LC-MS/MS system for quantification. Mesoridazine and chlorpromazine as an internal standard (IS) were separated by a reversed phase C18 column. A mobile phase was composed of 10 mM ammonium formate in water and acetonitrile (ACN) (v/v) by a linear gradient system, increasing the percentage of ACN from 2% at 0.4 min to 98% at 2.5 min with 4 min total run time. The ion transitions monitored in positive-ion mode [M+H]+ of multiple-reaction monitoring (MRM) were m/z 387>126 for mesoridazine and m/z 319>86 for IS. The detector response was specific and linear for mesoridazine at concentrations within the range 0.001 – 4 µg/ml and the correlation coefficient (R2) was greater than 0.999 and the signal-to-noise ratios for the samples were ≥10. The intra- and inter-day precision and accuracy of the method were determined to be within the acceptance criteria for assay validation guidelines. The matrix effects were approximately 101 and 99.5% from rat plasma for mesoridazine and chlorpromazine, respectively. Mesoridazine was stable under various processing and/or handling conditions. Mesoridazine concentrations were readily measured in rat plasma samples after intravenous and oral administration. This assay method can be practically useful to the pharmacokinetic and/or toxicokinetic studies of mesoridazine.

ThPS33-07 / A Workflow for Nontarget Screening of Transformation Products formed in Biological Wastewater Treatment using Multivariate Analysis

Jennifer Schollee[1], Emma Schymanski[1], Heinz Singer[1], Richard Ottermanns[2], Juliane Hollender[1]

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Introduction

Micropollutant transformation products (TPs) pose a risk to the environment and may be formed during wastewater (WW) treatment but the formation of TPs during these processes has not been thoroughly investigated. The goal of this project was to use liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) to detect unknown TPs along the WW treatment train. A workflow with nontarget screening and multivariate analysis was assembled to select peaks of possible TPs. A linkage analysis between influent and effluent peaks was developed based on LC-HRMS data with the goal of faster identification of TPs.

Methods

Composite samples (48-hr) were collected from the influent and effluent of a WW treatment plant in Switzerland. Samples were enriched with an off-line solid-phase extraction (SPE) cartridge containing reverse phase, ionic exchange, and activated carbon layers. A subset of samples was used for method validation and was spiked with known parent and TP compounds. Samples were measured with LC-HRMS using electrospray ionisation (ESI). Data processing of the validation set was done with Thermo QuanBrower. Nontarget screening and linkage analysis was done with R statistical software. The workflow for selection of peaks characteristic of the influent or effluent included principle component analysis (PCA), hierarchical cluster analysis, and indicator species analysis.

Results

PCA scores for the validation set showed that influent and effluent samples were separated in the first PC and loadings indicated parent compounds were associated with influent samples and TPs with effluent samples. For nontarget screening, PC1 explained 60% of the variance and separated influent and effluent samples. As 19457 features were present, peak selection was difficult and additional statistical methods were included,

resulting in 4663 influent peaks and 2202 effluent peaks. From these lists, a linkage analysis resulted in a possible 1689 links, spread across 7 transformation reactions. Further confirmation of these transformations is currently carried out on LC-HRMS data (i.e.,exact mass, retention time, isotope and/or adducts, fragments).

Conclusions
A workflow was developed combining multiple lines of evidence which facilitated the selection of peaks characteristic of influent and effluent samples, representing possible parent compounds and possible TPs, respectively. A linkage analysis included known information about the peaks and possible transformations for fast prioritization of TPs for faster structure elucidation and identification.

Novel Aspect
Multivariate methods were applied to WW treatment steps to identify possible parent and TPs and linkages between these groups were considered. This approach combines current methods of nontarget screening with a novel method for linking parent and TP in order to incorporate as much information as possible for quicker identification of TPs.

ThPS33-08 / The choice for centroid or profile data in high-resolution MS quantification: more than a detail?
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Pharmacokinetics, Dynamics & Metabolism, Janssen R&D, Beerse, Belgium

Introduction
With the increased sensitivity and dynamic range of the newest generation high-resolution MS (HRMS) instrumentation (both Time of Flight and Orbitrap based systems), HRMS has gained more and more interest in quantitative analysis in different fields of pharmaceutical research. The users need to adapt to different work flows compared to those applied for triple quad analyses typically used for quantification.
Understanding high resolution data acquisition and processing is a very important part of good quantitative analysis. Here we discuss the impact of processing continuum/profile data versus centroid data on quantification with HRMS.

Methods
A midazolam standard was analysed by LC/HRMS on a Synapt G2-S QTof (Waters) and a Q-Exactive (Thermo). Data were acquired in profile/continuum mode and processed with XCalibur 2.2 (Thermo) or Masslynx 4.1 (Waters). The Masslynx data were compared with the same data after conversion to centroid data.

Results
The effect of different minimum extraction windows (MEW) and use of the theoretical or measured accurate mass on the resulting extracted ion chromatograms (XICs) has been explored. The application of a narrower MEW and the choice between a theoretical and measured accurate mass had a clear effect on the intensity of the extracted peak in the Masslynx profile data, while there was no effect on intensity for the centroid data and the XCalibur profile data. Therefore, narrower MEWs can be used for the latter data resulting in increased selectivity and enhanced signal-to-noise.

Conclusions
The choice between centroid and profile data can have a significant impact on sensitivity and precision in quantification with high-resolution MS. It is important to understand how profile data are handled by different vendor softwares.

ThPS33-09 / Development and applications of proton transfer reaction-mass spectrometry for homeland security: trace detection of explosives
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[1]University of Birmingham, [2]KORE Technology

Introduction
To overcome problems of recovery times and to quantify limits of detection for the screening of explosives using proton transfer reaction mass spectrometry (PTR-MS), KORE Technology Ltd. has developed a thermal desorption unit (TDU)(see design in figure 1). This TDU directly couples to a short heated inlet system leading to the reaction region of the PTR-TOF-MS and makes use of polytetrafluoroethylene (PTFE) circular swabs mounted on card (see photograph 1). When inserted into the TDU, a high force concentric annular "anvil", with a diameter slightly less than the swab, compressed the PTFE to plastically deform and convert it into a gas tight circular seal around the rim. Immediately heated laboratory air (140ºC) rapidly heated and flowed through the PTFE carrying thermally desorbed explosive material into the drift tube where reactions with H3O+ via proton transfer reactions occurred, which may be nondissociative leading to MH+ as the product ion (where M is the explosive) or dissociative leading to [M+H-A]+ as a product ion (where A is a neutral eliminated from the protonated parent). Given that the thermal desorption of an explosive material from a swab is rapid then for a short period of time (seconds to tens of seconds depending on the explosive) a relatively high concentration pulse of the explosive material entered the drift tube. In this work we illustrate the potential of this system for the detection of the most common explosives.

Methods
For the developemnt of this project a KORE PTR-ToF-MS and a KORE TDU was attached to the front inlet of the PTR-ToF-MS. Single component standards for the explosives used in this study were purchased from AccuStandard Inc. and from Dr. Ehrenstorfer GmbH.

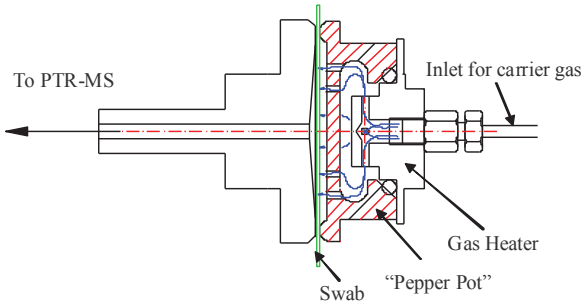

Figure 1. Schematic cross-section of the thermal desorption unit. The laboratory air is heated as it travels through the heating block to insure that it reaches the same temperature as the temperature-controlled block. This heated air is then dispersed across the surface area of the swab via a series of equally spaced holes (the pepper pot) and directed towards the swab. The passage of the air heats the swab resulting in thermal desorption of material placed on it, which is carried through by the carrier gas to the drift tube reactor

Photograph 1. Circular PTFE swab mounted onto card (real scale).

Explosive	Product Ion and nominal *m/z*	E/N (Td)	Linear range (ng)	r^2	LOD (ng)	Repeatability (RSD%) (n=5)	Reproducibility (RSD%) (n=5)
EGDN	$EGDNH^+$, 153 NO_2^+, 46	110	0.5-300	0.9982 0.9910	4.4 ± 0.5 7.2±0.6	13.8 9.2	19.0 16.1
1,3-DNB	$DNBH^+$, 169	170	0.063-25	0.9981	0.13 ± 0.02	2.9	7.7
3,4-DNT	$DNTH^+$, 183	140	0.1-25	0.9982	0.07 ± 0.01	5.0	3.7
HMTD	$HMTDH^+$, 209	90	1-500	0.9996	0.74 ± 0.08	3.8	5.2
TNB	$TNBH^+$, 214	210	0.063-25	0.9980	0.14 ± 0.02	3.9	6.5
RDX	$([RDX\text{-}HONO)H]^+$, 176 $CH_2NO_2^+$, 75 NO_2^+, 46	110	5-1000	0.8938 0.9974 0.9993	36 ± 6 6 ± 2 14.9 ± 0.8	0.5 2.8 3.1	17.7 23.1 7.1
NG	NGH^+, 228 NO_2^+, 46	80	1-500	0.9763 0.9849	2.0 ± 0.2 12 ± 2	9.6 6.6	10.8 6.5
TNT	$TNTH^+$, 228	180	0.25-50	0.9974	0.15 ± 0.01	1.2	2.9
PETN	$PETNH^+$, 317 NO_2^+, 46	110	1-500	0.9996 0.9953	0.6 ± 0.1 14 ± 1	2.0 12.4	3.9 16.4

Table 1. Results for the different explosives. Table shows followed product ions and nominal m/z values, as the optimun E/N value for achieving the best sensitivity for each of the explosives. Linear range means the range measured and the regression cofficient (r2) is also presented. The precision of the method was evaluated by determination of the repeatability and reproducibility in terms of relative standard deviation (RSD). Repeatability determinations involved measurements of 5 replicates consecutively while reproducibility determinations were 5 replicates over 5 different days.

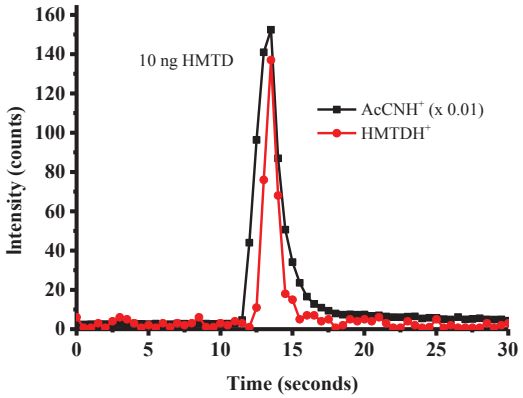

Figure 2. The ion intensities of HMTDH+ and AcCNH+ as a function of time for a deposit of 10 ng of HMTD in 1 µl of acetonitrile on a swab.

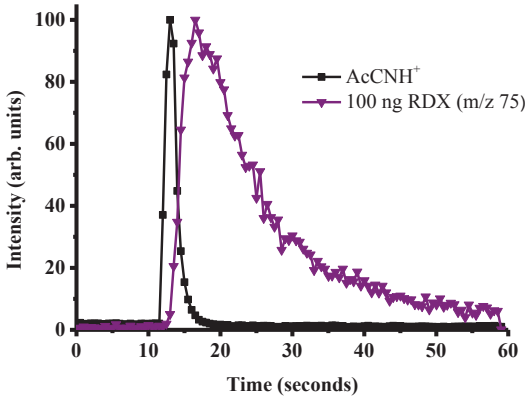

Figure 3. Plot of ion intensities for protonated acetonitrile and m/z 75, both set to a height of 100 for a swab onto which 100 ng of RDX in a 1 µl droplet of acetonitrile had been placed.

Results

Table 1 shows the calculated limits of detection (LOD) for the explosives investigated in this study presented in order of increasing molar mass. Note that for RDX no protonated parent ion was observed. Instead two fragment ions were used in the determination of the LODs. Figure 2 provides the response time for HMTD, which resulted in the shortest response time. Figure 3 presents the results for the notoriously sticky explosive, RDX. But even for this explosive, the complete cycle time of sampling, detecting and clearing is only about 60 seconds.

Conclusions

We have demonstrated the use of a TDU for the high sensitive and high selectivity detection of several common explosives. The calculated limits of detection vary are in the nanograms region. Memory effects have been shown to be negligible for all explosives with the exception of RDX. However, even for RDX, recovery times are not severe.

Novel Aspect

The novel aspect presented in this work is the development and use of a thermal desorption unit (TDU) specifically designed for PTR-MS. The results presented here have shown that the TDU/PTR-MS system is capable of not only high sensitivity (sub ng. levels) but also high selectivity.

Acknowledgements

This work and the funding of RGM were supported through the PIMMS Initial Training Network.

ThPS33-10 / Structure characterization of intact monoclonal antibody using Orbitrap Tribrid mass spectrometer

Terry Zhang, David Horn, Jenny Chen, Vlad Zebrouskov, Zhiqi Hao
ThermoFisher

Introduction

Monoclonal antibodies (mAbs) are increasingly developed and utilized for the detection and treatment of diseases. The quality of the antibodies is crucial for both safety and efficacy. Due to the heterogeneity of mAb products, thorough characterization is very challenging. Among the analytical tools used for the analysis of therapeutic mAb, mass spectrometry (MS), especially high resolution and accurate mass MS, has become more and more important in providing accurate information on various protein properties, such as intact molecular mass, glycosylation form distribution, amino acid sequence, post-translational modifications, minor impurities and high order structure. In this study, new methods coupling capillary LC with new generation Orbitrap mass spectrometer were developed and experiment parameters optimized for structure characterization of intact mAb.

Experimental

Monocolonal antibody was diluted with 0.1% FA in water for intact protein analysis. Reduced monocolonal antibody were prepared in the 6 M Gunidin, 100mM DTT, heated at 60°C for 30min for top-down analysis. The protein solutions were directly injected and analyzed by using capillary LC coupled with a Orbitrap Fusion Tribid high resolution mass spectrometer. Experimental parameters for both ion source and mass spectrometer were optimized. The MS full spectra for intact mAb were analyzed using Protein Deconvolution. The top-down ETD and HCD spectra were analyzed using ProSightPC 2.0.

Results

For intact mAb molecular mass measurement, various parameters including ion transfer tube temperature, in-source activation energy, s-lens values, AGC target and others have been optimized. With the optimized experimental condition, mAb was accurately measured with low ppm mass error. Different glycoforms were also accurately identified.

For further characterization of this mAb, the sample was reduced using DTT and light and heavy chains were completely separated on a Pepswift column on a 20min gradient method. The molecular mass of both light and heavy chain was measured low ppm mass accuracy. ETD, HCD fragmentation approaches were employed for top-down analysis. More than 40% sequence coverage was obtained for light chain from ETD or HCD experiments.

Combining ETD and HCD fragments together, about 70% for light chain and 20% for heavy chain backbone sequence coverage were achieved. Result from the topdown approach not only characterized variable regions of both heavy and light chains, but also identified and located modifications. Using the top down method developed in this study, comprehensive structural information can be obtained for intact mAb

Novel aspect
Characterization of intact mAb using capillary LC coupled with Orbitrap Tribid mass spectrometer.

ThPS33-11 / Recent developments of ChemCalc (www.chemcalc.org): an online tool for mass spectrometrists
Luc Patiny, Laure Menin, Michaël Zasso
EPFL

Introduction
Following the appearance of cloud computing accessible from the web browser, we may wonder how this novelty can be applied to the analysis of scientific information and if in a couple of years the only application that will be installed on your computer is a navigator.
In this context ChemCalc (i.e. www.chemcalc.org) allows to quickly and efficiently calculate isotopic distribution, calculate fragmentation and find possible molecular formula from a monoisotopic mass.
The algorithms of those core functions have been fine tuned to pave the way to a general tool allowing to solve specific questions encountered daily in research laboratories.

Results
3 core functions have been developed:
1. Isotopic distribution can be calculated using ChemCalc by specifying the FWHM or the resolution. This will apply a gaussian that will yield to a spectrum that match perfectly experimental results. It is possible not only to specify the charge and to create mixture of molecules but also to specify the isotopic enrichment of a specific elements and to use common group abbreviations. For example molecular formula may be entered like «HAla10OHH+. HAla10OHH2++» (i.e. a mixture of 2 decapeptide single and doubly protonated).
2. Research in the synthesis of peptide has led to many non-natural amino acids as well as unconventional side chain modifications and complexation with organometallic molecules. Despite those modifications, the mass analysis of such peptides may yield to usual fragmentation (a,b,c and x,y,z ions) but most of the tools are unable to predict the corresponding fragments masses. Using simple regular expression we are able to virtually split the string representing the sequence in all its fragments. For example «HAlaLys(H+)GlySer(H-1Ph)ProGlyOH» describes a peptide sequence with a protonated lysine side chain and a replacement of the alcohol hydrogen of the serine by a phenyl group.
3. The analysis of complex mixtures using high resolution and precision mass spectrometers requires the development of algorithms that allow to determine possible molecular formula for a specific monoisotopic mass. On ChemCalc it is possible not only to specify the molecular formula range but also to specify uncleavable groups of atoms (e.g. {CH2CH2O}0-10Ala0-10, i.e. allowing 0 to 10 copies of the PEG like chain and between 0 and 10 alanine). It is even possible to find all possible amino acids composition of a peptide for a monoisotopic mass up to 1000.

Innovations and conclusions
Real life research problems are often not easy to solve using commercial softwares. We will show innovative applications of the ChemCalc tools to help the mass spectrometrist to analyze data directly from a web browser. Based on a simple scripting language it is possible to automatically match predicted spectra with experimental data and generate interactive reports that can be shared between colleagues. Real case examples involving peptide complexes, isotopic enrichment and GC/MS will be shown as example.

ThPS33-12 / Structure of arene-linked dinuclear ruthenium(II) organometallics–peptide complexes
Laure Menin, laure Menin, Benjamin S. Murray, Luc Patiny, Yury O. Tsybin, Paul Dyson
EPFL

Introduction
Revealing the characteristics of drug binding to peptides provides insights on their mode of action. Tandem mass spectrometry (MS/MS), particularly electron transfer dissociation (ETD) and also Ion Mobility (IM) MS have shown tremendous utility in determining drug binding sites and probing conformational changes on drug binding. Ruthenium(II)-based organometallic compounds are actively researched for their promising anticancer properties. Here, a series of dinuclear analogues of the RAPTA series of organometallic ruthenium(II) compounds was investigated with ETD MS/MS and IM-MS upon binding to a model peptide, a fragment of the amyloid b-protein. The conformation of the RAPTA units relative to each other is controlled by the stereochemical conformation of a 1,2-diphenylethylenediamine (DPEN) linker molecule, leading to isomeric compounds with open or more closed conformations.

Methods
To probe the binding sites of the dinuclear Ru(II) ligands to a peptide, ETD MS/MS was performed on an hybrid linear ion trap (LTQ) Orbitrap Elite FT mass spectrometer. To assist in locating all putative binding sites of the organometallics-peptide adducts, an in-house script based on http://www.chemcalc.org was developed to match the experimental and theoretical MS/MS data. Through simultaneous variation of the elemental composition of the ligands including theoretical loss(es) of fragile moieties during fragmentation and consideration of a number of proton and charge additions or defects, theoretical fragments were simulated alongside scores based on the full isotopic distribution similarities. The data were supplemented by IM-MS experiments to probe conformational differences between the organometallics-peptide adducts.

Results
ESI-FTMS mass spectra exhibited 1:1 mononuclear (L1) and dinuclear (L2) metal complex:amyloid b-protein fragment peptide adducts with the chlorido ligands lost at each Ru, implying that each metal of the mono or dinuclear compounds is coordinated to one or more amino acid residues in the 16-mer peptidesequence. It is likely that crosslinked species are formed by each metal bound to a different residue.
Analysis of the ETD mass spectra of such complexes is challenging, especially due to the occurrence of internal fragmentation and loss of fragile ligands moieties in particular those coordinated to Ru, in addiction to common backbone C and Z ions. The script developed for this purpose and available on chemical.org has been of great help to interpret such complex data and tentatively assign all the ions observed to deduce the binding sites.
In the IM-MS experiments, we observed a variety of arrival time distributions (ATDs) for the different complexes. In particular, the dinuclear complexes exhibit two peaks in the ATDs profile and this split distribution may be a reflection of the metallation of the peptide at the three His positions, as determined by ETD analysis, yielding isomeric adducts of similar but distinguishable drift time.

ThPS33-14 / An AMDIS-based GC/MS Procedure for the Rapid Characterization of Algal Liquefaction Products and Process Optimization

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[1]King's College London, [2]Imperial College London

Introduction
The pyrolysis or supercritical fluid treatment of biomass gives a vast array of organic molecules. In industrial research it is important to characterize such product slates quickly. The OH functionality is widely distributed in organic oxygenates [1]. Methodologies are being developed to evaluate the composition of processed biomass by NMR and conventional and novel MS methods (GC/MS, ESI, MALDI). A GC/MS procedure based on AMDIS software is illustrated by application to a study of the removal of acidic OH groups from products derived from the esterification of algae with methanol.

Methods
Mixtures of freeze-dried algae powder (Nannochloropsis gaditana) were processed in supercritical methanol for several minutes. Dichloromethane extracts were analyzed by GC/MS following trimethylsilylation). Components in the resulting profiles were analyzed by the Automated Mass Spectral Deconvolution and Identification System (AMDIS [2], NIST [3]).
AMDIS libraries were created from representative profiles of liquefaction products (derivatized and underivatized) and are undergoing continual development and refinement.
Quantification used both the AMDIS batch job report [4,5] and the instrument manufacturer's software (Chemstation).
The resulting data arrays were then available for analysis and process optimization/modelling.

Results
The current TMS library has > 150 unknown (no good NIST library match) mass spectra which are available for further study incl. comparison with other databases.
The AMDIS quantitation procedure yields similar results to those obtained with the conventional retention time routine (Chemstation).

Conclusions
A simple GC/MS procedure with trimethylsilylation is described for the rapid characterization of algal liquefaction products.
The AMDIS-based procedure has the advantage of being applicable to data files from most manufacturers and incorporates convenient application of retention indices as well as times.
The analytical procedure is undergoing validation.
Library development and refinement is ongoing.
Complementary electrospray , MALDI and NMR procedures are also being developed.

Novel Aspects
Quantitative application of AMDIS software

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ThPS33-15 / Cerebrospinal Fluid Proteome of Patients Diagnosed with Alzheimer Disease: Focus on Data Analysis

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Uppsala University

Introduction
Mass Spectrometry-Based proteomics is a powerful technique for identifying and quantifying proteins. Particularly important is the label-free shotgun approach in which the proteins are digested into peptides and subsequently analyzed by tandem mass spectrometry, allowing for identification and quantification of thousands of proteins in unlimited number of samples. Considering the complexity and amount of data being produced, having an accurate and high performance down-stream data analysis platform has become especially important. Numerous commercial and open source software tools have been developed in the last decade which makes the selection of tools difficult especially for the non-technical users. The aim of this study was to compare two well-known commercial software tools (Sieve by Thermo, DecyderMS by GE Healthcare) to an open source platform (OpenMS) in terms of reproducibility and performance using data on cerebrospinal fluid (CSF) from patients diagnosed with Alzheimer's disease and age-matched healthy controls.

Methods
Seven highly abundant proteins were depleted from each sample by immunoaffinity chromatography. Samples were then digested using LysC-trypsin mixture and desalted on a C18 solid phase extraction column. Each sample was run in duplicate using a 7 T hybrid LTQ FT mass spectrometer (ThermoFisher Scientific, Germany). The data was exported and for each software, we adjusted the parameters to quantify the peaks and performed the identification using MASCOT (Sieve and DeCyderMS) and for OpenMS we used a combination of X! Tandem and OMSSA. Multiple factors were considered for the comparison such as user interface, advanced controlling/viewer, reproducibility, identification, speed, and limitation in the number of samples.

Results
Our results showed that in a majority of the cases there is a negligible difference between the three platforms when it comes to reproducibility. We also showed that there is a great overlap between the two identification approaches.

Conclusions
In conclusion, the Sieve software performed slightly better than OpenMS and DecyderMS by showing higher reproducibility. However, the open source solution showed higher identification power by identifying and mapping more proteins and peptides compared to the commercial counterparts.

Novel aspect
This study compares reproducibility and identification performance of an open source solution to the commercial counterparts using multiple factors, thus assisting non-technical users for selecting the platform which suits their needs.

ThPS33-17 / New methods of data analysis for FTMS with improved analytical performance

Anton N. Kozhinov, Konstantin O. Nagornov, Konstantin O. Zhurov, Yury O. Tsybin
Ecole Polytechnique Fédérale de Lausanne

Although Fourier transform mass spectrometry (FTMS) was significantly revolutionized in the past, new developments in its instrumentation and data analysis methods are vital for further progress in FTMS-based applications. Over the past five years,

numeric programming with the Python programming language armed with gold-standard C/Fortran libraries became a widely recognized and efficient approach for software development in science and engineering. Here, we demonstrate the utility of such approach for FTMS and present signal processing methods with increased resolution performance and mass calibration methods with increased mass accuracy.
Experiments were conducted on Q Exactive Orbitrap, Orbitrap Elite, and 10 T LTQ ICR FTMS (Thermo Scientific). Built-in data acquisition systems with advanced software interface (Thermo Scientific) or an external data acquisition system (National Instruments) were employed. Data analysis methods were implemented using the functionality of the pyFTMS framework, which was developed in-house with Python and standard software packages for scientific computing. Specifically, the NumPy, SciPy, and matplotlib extensions were used for fundamental numeric programming and visualization, the FFTW library for the discrete Fourier transform, the MINUIT library for least-squares fitting (LSF) of transients, and a custom library for the filter-diagonalization method (FDM).
Based on LSF, we present a method tailored for increased scan rate in FTMS-based quantitative proteomics with 10-plex TMT reagents. We also propose a double phase correction method, which is tailored for regular m/z distributions and provides a 2-fold gain in resolution performance compared to absorption-mode FT, thus reducing the required detection period to one isotopic beat in the analysis of proteins. Next, a prototype method designed for analysis of wide bandwidth (multiple harmonics) transient signals and providing increased resolution performance compared to the conventional FTMS is presented and evaluated for peptides and proteins. We also discuss the signal-to-noise ratio limit to FDM resolution performance and present a sub-method for representing probability density distributions of the FDM's amplitude and frequency values. Additionally, a method for advanced mass calibration in the analysis of petroleomic samples with Orbitrap FTMS is presented, reducing root-mean-square mass errors to the level of about 350 ppb. Finally, we describe a mass calibration method for an ICR cell operating in the unperturbed cyclotron frequency regime, routinely overcoming the current 1 ppm mass accuracy barrier in FT-ICR MS.
Thus, the presented methods provide increased resolution performance and mass measurement accuracy while numeric programming with Python enables successful implementation of these methods.
Novel aspect: Taking advantage of Python's extensions for scientific computing, we implemented novel methods for FTMS with improved analytical performance.

ThPS35 - Elemental and isotopics, MS, ICP-MS General, Cultural Heritage and Archeology

11:00-15:00

Poster Exhibition, Level -1

ThPS35-01 / Methods to improve analytical characteristics of monopole mass spectrometers

Michael Dubkov, Michael Burobin, Vladimir Ivanov, Igor Kharlanov
Ryazan State Radio Engineering University

Monopole mass analyzers are notable for the simple electrode system design and low power consumption. It allows using them while developing compact analytical devices to control and analyze substances in a gaseous state.
The main disadvantage of a monopole mass analyzer causing decrease of its resolution is a tighten front of a mass peak on the side of heavy masses. It is known some ways to improve the characteristics of a monopole mass analyzer relating to ion sorting time increment.
The most obvious method is extension of the electrode system. But such approach leads to the growth of overall dimensions of a monopole mass analyzer which limits its usage as a part of mobile analytical equipment.
The next method included decrease of energy of ions inputting in a mass analyzer looks more attractive. But presence of the adequately extended entranceperiphery region, electric field of which differs from the field in the center of the analyzer, constrains the usage of the mentioned method substantially.
Our studies show that there are optimal ion transit times of the entranceperiphery region of a mass analyzer expressed in periods of radio-frequency voltage enclosed to electrodes by which the transmittance of an analyzer is maximal. In order to realize such conditions of ion input it is supposed to use the frequency deployment of a mass spectrum and a special construction of the electrode system in the areas of input and output of ion flux providing quadratic distribution of a potential in the diametral plane of an analyzer and linear distribution in its longitudinal direction. This proposed ion-optical system allowsconsiderably decreasing the periphery region extent in the longitudinal direction and therefore reducing the energy of input ions not causing losses in this area, and qualitatively improvingthe distribution of a potential in it.
Another prospective method to increase the ion sorting time consists in creation of the decelerating electric field directed along the analyzer axis. The mentioned effect is constructively realized by the inclination of a rod electrode relative to the longitudinal axis, then the distance between a rod electrode and a corner electrode in the symmetry plane of a monopole analyzer is less at its input that at its output. Usage of this method allows increasing the resolution of a monopole mass analyzer in 1.5 times, and its sensitivity, sweep generator construction and weight-size parameters are not changed.

ThPS35-02 / Development of a Certified Reference Material (KRISS CRM 114-01-001) for the Determination of Hazardous Elements in Cosmetics

Sook Heun Kim, Young Ran Lim, Euijin Hwang, Yong-Hyoen Yim
Korea Research Institute of Standards and Science

Accurate measurement of the hazardous elements in cosmetics is significantly important in response to the cosmetic safety issue. A cosmetic certified reference material (CRM) in a power form was developed for the validation of analytical methods and quality control. For the certification of trace elements (Cd, Hg, Pb) in cosmetic samples, a method employing the sample preparation using microwave (MW)-assisted acid digestion combined with the analysis using double isotope dilution inductively coupled plasma-mass spectrometry (ID-ICP/MS) was optimized and validated.
The candidate cosmetic powder material was prepared by spiking and homogenizing toxic elements, including Cd, Hg and Pb, in commercially available baby powder. For complete dissolution of the sample matrix and sufficient chemical equilibration between the spiked enriched isotopes and the analytes in the sample, microwave-assisted acid digestion was carried out in a closed vessel using a mixture of nitric acid (HNO3), hydrofluoric acid (HF) and hydrogen peroxide (H2O2). For complete recovery and equilibrium of the analytes from the baby powder, the major component of which is talc, use of HF in the digestion mixture is inevitable. But the presence of HF in sample digest requires extreme precautions in sample handing and use of ICP/MS sample introduction parts made of special non-corrosive materials. To overcome this problem, an analytical method was developed to quantitatively recover the analytes from sample digest by evaporation of acid mixture and subsequent

re-dissolution using nitric acid. Although the majority of talc materials were precipitated and filtered out, the analytes were recovered quantitatively by taking advantage of isotope dilution calibration method. The method was successfully applied to the ID-ICP/MS analysis of non-volatile elements, Cd and Pb, and validated by comparing with the result obtained by direct ID-ICP/MS analysis of the HF-containing sample digest. In the case of Hg, however, the content was determined only with direct analysis of the HF-containing sample digest to prevent the loss of volatile Hg during evaporation process. The inherent problem of memory effect in the ICP/MS analysis of Hg was resolved by employing a fast flushing sample introduction system. The performance of ID-ICP/MS method with fast flushing sample introduction system was evaluated by analyzing the Hg isotopic standard solution, IRMM 639. The analysis methods optimized and validated here were applied to the certification of baby powder CRM of Korea Research Institute of Standards and Science (KRISS). The certified values were obtained for Cd, Hg and Pb with less than 2.5 %, 0.6 % and 5 % combined standard uncertainty, respectively. Proper use of the developed CRM for quality control and method validation in testing laboratories and cosmetic industries is expected to significantly improve reliability and quality of the testing results for hazardous elements in cosmetics, thereby, contribute to the cosmetic safety.

ThPS35-03 / Simple cobalamin speciation using TLC-DLTV ICP MS

Antonín Bednařík[1], Iva Tomalová[1], Tomáš Vaculovič[2], Viktor Kanický[2], Jan Preisler[2]
[1]Masaryk University, [2]Masaryk University/CEITEC

Introduction
Sample introduction to inductively coupled plasma (ICP) by means of a diode laser thermal vaporization (DLTV) is a simple and low-cost technique of dried droplet elemental analysis. The diode laser scans across the surface of a substrate, induces its pyrolysis and the generated aerosol is carried to the ICP. A typical substrate is a common filter paper covered with an absorber, a regular black ink. The technique provides low pg limits of detection for many metals.
Here we present a coupling of thin-layer chromatography (TLC) separation to the sensitive DLTV ICP MS detection on the example of cobalamins, cobalt bearing complex molecules known as vitamins B12.
The efficiency of vaporization for different substrates, species and different sample preparation procedures will be discussed.

Methods
Rectangles (2.6 × 1.2 mm) were printed using a common black ink onto filter paper and aluminum-cellulose TLC plates. Solutions of Co^{2+} and four different cobalamins were deposited on the preprinted rectangles of both substrates as 200-nL droplets by a micropipette.
The TLC plates were eluted with 1-butanol:ethanol:water = 10:3:7 (v/v/v) containing 0.5 % (v/v) of concentrated NH_3 solution under the red light. The sample deposition was done using 2 µL TLC capillary. After drying, the centers of separation channels were overprinted with 1.5-mm wide black ink strips by a commercial ink-jet printer. The plates were cut into the stripes, each stripe with one separation channel.
The TLC stripes and sets of pipette deposited samples were inserted into a simple tubular DLTV cell and were scanned by the 1.2 W 808 nm continuous-wave diode laser. The generated aerosol was carried to the ICP MS.

Results
The DLTV parameters were optimized for the new substrate, the aluminum-cellulose plates. The individual species showed different DLTV ICP MS response. The cobalamins exhibited considerably higher response compared to the cobalt ion Co^{2+}. The signal intensity increased with the affinity of the species to the substrate.
The TLC-DLTV ICP MS coupling provided ~3 orders of magnitude lower detection limits for separated cobalamins compared to the visual detection.

Conclusions
The simple speciation of cobalamins using DLTV ICP MS for detection of TLC-separated cobalamins is demonstrated. This technique presents a sensitive and price competitive alternative to HPLC coupled to ICP MS.

Novel Aspect
Original DLTV sample introduction technique to ICP MS was employed for simple and sensitive detection of TLC separations.

Acknowledgement
We acknowledge the grants CZ.1.05/1.1.00/02.0068 and Grant No. GAP206/12/0538.

ThPS35-04 / Investigation of Sulfur Allotropes with Thermal Analysis – Single Photon Ionization Mass Spectrometry

Janos Varga[1], Michael Fischer[1], Sebastian Wohlfahrt[1], Mohammad R. Saraji-Bozorgzad[2], Georg Matuschek[1], Thomas Denner[3], Armin Reller[4], Ralf Zimmermann[1]
[1]Helmholtz Zentrum München, [2]Photonion GmbH, [3]Netzsch-Gerätebau GmbH, [4]University of Augsburg Chair of Resource Strategy

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ThPS35-05 / Determination of elemental impurities in active pharmaceuticals by single ICP-MS run after prior stabilisation of osmium

Denis Besic[1], Irena Raič[1], Ernest Meštrović[1], Sanda Rončević[2]
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Introduction
A commercially available simultaneous thermal analyzer (STA) – skimmer mass spectrometer system was modified: the quadrupole mass analyzer was replaced with a single photon ionization time of flight mass spectrometer (SPI-TOFMS). The application of SPI allows a soft ionization eliminating fragment peaks which make mass spectra interpretation difficult. The applicability of the newly developed system was tested on the investigation of elemental sulfur clusters.

Methods
STA refers to the application of thermogravimetry (TG) and differential scanning calorimetry (DSC)/differential thermal analysis (DTA) to a (single) sample at the same time. The coupling in the skimmer system between STA and MS takes place directly in the furnace of the STA and it enables a wall contact free sampling which is necessary for the investigation of low volatile species such as inorganic clusters of the 5 and 6 main groups of the periodic table. The first pressure reduction step is arranged directly above the sample container. The orifice is designed to work as a divergent nozzle. The conically distended aluminium oxide ceramic aperture is arranged in the forming jet expansion behind the nozzle. Vacuum ultraviolet photons for SPI are generated by a deuterium lamp. It is possible to operate SPI and electron ionization (EI) in a rapidly alternating modus. The advantages of a fast modern time of flight mass analyzer are increased time resolution and sensitivity [1].

Results
In this study the applicability of the system was tested on inorganic clusters. Sulfur vapor exhibits a complex molecular composition, it consists of all molecules with 2-8 atoms in temperature and pressure dependent equilibria.

Conclusions
The developed system enables a new approach for the investigation of sulfur and other inorganic elemental clusters.

Novel Aspect
In this study for the first time a successful skimmer hyphenation of simultaneous thermal analyzer and photo ionization time-of-flight mass spectrometer was implemented and successfully tested in the field of inorganic chemistry. The determination of the relative photoionization cross sections will be the next step. It allows an exact quantitation of the different allotropes in the gas phase which is poorly investigated in the literature so far.

[1] Saraji-Bozorgzad et al. J Therm Anal Calorim (2011) 105:691-697 DOI 10.1007/s10973-011-1383-2

ThPS35-05 / Determination of elemental impurities in active pharmaceuticals by single ICP-MS run after prior stabilisation of osmium

The aim of this study is to develop method for the analysis of elemental impurities (Li, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Ru, Rh, Pd, Ag, Cd, Sn, Sb, Ba, Os, Ir, Pt, Au, Hg, Tl and Pb) in active pharmaceutical substances. Pharmaceutical active substance is considered to be a pharmaceutically active ingredient (API) or excipient. Elements which are covered by this method are typically used as process catalysts or reagents during the synthesis of pharmaceuticals but also we can find them in manufacturing equipment and piping, cleaning solvents and packaging. Their use can lead to residues in the final pharmaceutical active ingredients, and thus in the final product - a drug. Analysis of elemental impurities is performed at every stage of production process of API, including the analysis of intermediates and starting materials. Development of analytical method had included combination of elements listed in recommandations such as US pharmacopoeia <232>, European pharmacopoeia (Ch 5.20) and ICH guideline (Q3D). Analysis of these elements was carried out by inductively coupled plasma - mass spectrometry (ICP-MS) and taken in a single run, which is the biggest benefit of the development of such method. Sample preparation procedure is the most critical point in analysis and detection of elemental impurities, because it is not considered to be only destruction of solids, but also conversion of substance suitable and stable for introduction into modern ICP instrumentation.Specific chemical properties of some elements such as formation of volatile products during preparation step usually affect the final result. One of the most challenging tasks is determination of osmium in the complex matrices. Regarding the fact that osmium produces volatile species in oxidising and acidic conditions, there is a need for stabilisation of osmium during the analysis in order to prevent the loss of these fractions and to prevent the overestimation due to vapour enrichment in spray chamber. Osmium stabilisation was carried out by addition of very low concentrations of thiourea. The efficiency of applied method was tested on set of API's. A satisfactory recoveries for all analysed elements (70 – 150%) were obtained in a single ICP-MS run.

ThPS35-06 / Coupling of single HPLC separation run to ESI, MALDI and SALD ICP MS for metallothionein characterization

Kateřina Jägerová[1], Iva Tomalová[2], Kristýna Dlabková[2], Ondřej Polanský[2], Viktor Kanický[3], Jan Preisler[3]
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Introduction
Metalloproteins account for nearly a half of all proteins in the nature; the metal-binding sites are often responsible for catalysis in important biological processes.

Recently, we introduced the off-line coupling of capillary electrophoresis (CE) to both matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) and substrate-assisted laser desorption inductively coupled plasma (SALD ICP) MS. Here we demonstrate the coupling of high performance liquid chromatography (HPLC) to three complementary detection systems, MALDI MS, electrospray ionization (ESI) MS and SALD ICP MS.

Methods
For the separation of MT isoforms (MT1a, MT2d, MT2e), an effluent coming out of the HPLC column was split. One part of the flow 200 µL.min-1 was acidified and on-line introduced to ESI MS for detection of protein-metal complexes or protein apoforms. Second part 5 µL.min-1 was mixed with MALDI matrix and deposited on MALDI plastic targets (Prespotted AnchorChip 96). Subsequently, off-line MALDI and SALD ICP MS detection was performed from the same target.

Results
Gradient separation on C-18 column was optimized and performed with mobile phases (A) 5 mM amonium acetate at pH 7.5 and (B) 5 mM amonium acetate at pH 7.5 with 60% MeOH (v/v) at the flow rate 0.2mL.min-1. The solution acidity was regulated for ESI detection by added flow of 99 % formic acid: methanol (30:70, v/v).
ESI MS detection provided information about apoforms at pH < 2 and about protein-metal complexes with the highest signal observed at pH 3.0 - 3.5. For MALDI MS, the LC effluent was mixed with CHC matrix (50% MeOH (v/v), 1% TFA) and deposited on the target; the uniform crystal layer was formed on every deposited spot allowing the automatic analysis. Only MT apoforms were observed at MALDI MS.
Detection limits of MTs were one to two orders of magnitude lower for ESI compared to MALDI MS, which can be partially attributed to the flow ratio after the splitter.
The supplementary SALD ICP MS is valuable for quantitative analysis of the MTs and for verification of the stability of protein-metal complexes during HPLC separation.

Conclusions
Mutlidetection platform comprising of ESI, MALDI and SALD ICP MS was used for analysis of MTs. Commercially available MALDI targets made of conductive plastic was employed for the determination of MTs by means of MALDI and SALD ICP MS. Off-line MALDI MS was found to deliver comparable results to on-line ESI MS detection of protein apoforms.

Novel aspect
New analytical methodology for characterization of MTs based on HPLC separation coupled with on-line ESI MS and off-line MALDI MS and ICP MS detections from conductive polymer targets.

Acknowledgement
We acknowledge the grants GAP206/12/0538, CZ.1.05/1.1.00/02.0068 and CZ.1.07/2.3.00/30.009.

ThPS35-08 / Electrospray Ionisation Mass Spectrometry for the Complexation of Palladium with Thiourea, Benzoylthiourea and N,N-diethyl N′-benzoylthourea

Suresh Kumar Aggarwal[1], Pranaw Kumar[2], Jaison P. George[2], Alamelu D.[2]
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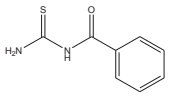
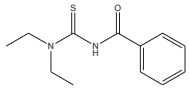
Introduction
Increase in the demand of Platinum Group of Elements (PGEs) has necessitated the development of various extractants and analytical methods for their selective extraction and determination, respectively.Due to soft nature of PGEs, sulfur based donor ligands such as thiourea and its aryl derivatives are commonly used for their selective complexation. Electrospray Ionization ESI-MS being a soft ionization technique, is useful for speciation studies of metal complexes and was used for comparing the complexation of Pd with thiourea (TU), benzoylthiourea (BTU) and N,N-diethyl N′-benzoylthiourea (DEBT).

Methods
A micrOTOFQ-II (Bruker Daltonics) mass spectrometer with electrospray ion source and quadruple-time-of-flight analyzer was used. Samples were introduced using a syringe pump at a rate of 4 µL/min. Analyses were performed in positive ion mode with 1000 V plate potential and capillary at 4500 V. Nitrogen was used as both auxiliary gas and sheath gas whereas high purity argon was used as a collision gas for MS/MS. TU (Merck) and BTU (Alfa Aesar) were available commercially whereas DEBT was synthesized. PdCl2 (M) and ligand (L) solutions were mixed on volume basis to obtain the required L to M ratios. Methanol (LC-MS grade, Fluka) was used as a mobile phase.

Table 1. Different species of Pd with TU, BTU and DEBT in ESI-MS (positive ion mode)

Ligand	Species	m/z	% Abundance
Thiourea (TU) 	$[Pd(TU)_2]^+$	256.9	53.0
	$[Pd(TU)_2(OH)]^+$	276.9	4.5
	$[Pd(TU)_3]^+$	332.9	96.4
	$[Pd(TU)_3Cl]^+$	370.8	100*
Benzoylthiourea (BTU) 	$[Pd(BTU)(TU)]^+$	360.9	50.8
	$[Pd(BTU)_2]^+$	464.9	86.6
	$[Pd(BTU)_2Cl]^+$	502.9	98.4
	$[Pd(BTU)_3]^+$	645.0	14.9
	$[Pd(BTU)_3Cl]^+$	682.9	100*
N,N-diethyl N′-benzoylthiourea (DEBT) 	$[Pd(DEBT)Cl(H_2O)]^+$	359.0	3.1
	$[Pd(DEBT)Cl]^+$	378.9	10.8
	$[Pd(DEBT)OH]^+$	396.9	16.7
	$[Pd(DEBT)_2]^+$	577.1	100*
	$[Pd(DEBT)_2Cl]^+$	615.1	4.6
	$[Pd(DEBT)_3]^+$	813.2	1.4
	$[Pd(DEBT)_3Cl]^+$	851.2	< 0.1

** Base peak in the mass spectrum*

Results
Table 1 shows various major species of Pd complexes with the three ligands viz. TU, BTU and DEBT. With thiourea, Pd(TU)3 and Pd(TU)3Cl were the most abundant species. With benzoylthiourea, the maximum abundance species obtained were [Pd(BTU)2Cl]+ and [Pd(BTU)3Cl]+. In the case of DEBT, [Pd(DEBT)2]+ was observed as the most intense peak . As given in Table, with BTU and TU, Pd forms ML2,ML3 as well as ML2Cl and ML3Clcomplexes. In case of DEBT, the peak corresponding to the ML3Cl was negligibly small (< 0.1%) and the most intense peak was of ML2 species. These observations suggest that DEBT is having a strong bidentate behavior in contrast to TU and BTU. Collision induced dissociation experiments were carried out to compare the stabilities of Pd complexes with the three ligands. The effect of collision cell energy (2 eV to 15 eV) was monitored for similar types of species viz. [Pd(TU)2]+, [Pd(BTU)2]+ and [Pd(DEBT)2]+. In case of Pd(DEBT)2, the percentage decrease in intensity was lower than those in Pd(BTU)2 and Pd(TU)2 showing that Pd(DEBT)2 is more stable compared to Pd(BTU)2 and Pd(TU)2 resulting from the bidentate nature of DEBT.

Conclusions
ESI-MS studies were carried out to compare the behavior of Pd complexes with thiourea and two of its derivatives viz. BTU and DEBT. Preferential bidentate coordination mode of DEBT compared to BTU and TU was observed. The Pd (DEBT)2 complex was found to be more stable compared to Pd(TU)2 and Pd(BTU)2 under the conditions employed.

Novel Aspect
The difference in the complexation behaviour of Pd and the stabilities of DEBT complex as compared with TU and BTU complexes were brought out by ESI-MS.

ThPS35-09 / Investigation of Uranyl-Hydroxycarboxylic Acid Complexes by Electrospray Ionization Mass Spectrometry

Suresh Kumar Aggarwal[1], Jaison P. G.[2], Pranaw Kumar[2], Vijay M. Telmore[3], Alamelu D.[3]
[1]Bhabha Atomic Resaerch Centre, [2]FCD, BARC, [3]FCD,BARC

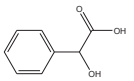
Introduction
Hydroxycarboxylic acids such as α-hydroxyisobutyric acid (HIBA), mandelic acid are used for liquid chromatographic separation of lanthanides and actinides and also as model compounds of humic substances to understand the migration of actinides in environment. Electrospray ionization mass spectrometry was used to investigate species formed between uranyl and the two ligands. The different monopositive and the dipositive monomeric and dimeric species were identified and intensities were compared. The relative binding strength amongst the species was compared by CID.

Experimental
Measurements were performed using micrOTOFQ-II (Bruker Daltonics) MS equipped with ESI and Q-ToF. Positive ion mode with experimental conditions: capillary potential: -4500 V; dry gas temperature: 180 oC; sheath gas flow: 4L/min and nebulizer gas pressure: 0.3 bar were employed. Uranyl nitrate hexahydrate, mandelic acid, HIBA, Methanol and Milli-Q water were used in the studies. Solutions of uranyl nitrate with mandelic acid and HIBA (M:L v/v ratio 1:10) prepared in 1:1 methanol-water mixture containing 10-5 M NaClO4 were directly infused into the ESI-MS system at a flow rate of 3µL/min.

Results and Discussion
Table 1 presents the comparison of different species in positive ion mode. The different species can be categorized into ML1, ML2 and ML3. In ML1 type, the species observed were ML1, ML1(H2O), ML1(CH3OH), ML1(H2O)(CH3OH), ML1(H2O)2 and ML1(CH3OH)2. No species of ML1 type containing NO3- ion was noted. The abundances of ML1 type species containing water as a ligand were higher than those containing methanol. In case of ML2 type, species obtained were ML2 and ML2(H2O). There was no peak corresponding to ML2(CH3OH) in the case of both the ligands and this observation further supports the

preference of uranyl ion for water over methanol. In contrast to mandelic acid, HIBA showed the peak at m/z 540.1 corresponding to ML2(NO3). The base peak for the ligands was of ML3 species corresponding to m/z 581.1 and m/z 725.1 for HIBA and mandelic acid, respectively. It was concluded that ML3 species must be formed by the protonation of two ligands and deprotonation of one ligand. In all the singly charged monomeric species, maximum number of ligands around the uranyl ion was 3 with no significant difference between the two ligands. Amongst the dimeric species, (UO2(L2)2)2+ was the maximum abundant species for HIBA and (UO2L(NO3)(H2O)(CH3OH))2+ for mandelic acid. The maximum number of the ligands associated with the metal for doubly charged dimeric species was 3 and 4 for HIBA and mandelic acid, respectively. In the case of mandelic acid [UO2 L3 (H2O)]22+ type species was also present. In most of the dimeric species, nitrate ion was found to be present.

Table 1: Diiferent species of Uranyl with HIBA and mandelic acid

Species	Uranium –mandelic acid 	Uranium –HIBA 
	m/z	*m/z*
Singly charged monomeric species		
$[UO_2 L]^+$ (M-H)	421.08 (16.6)*	373.1 (9.7)*
$[UO_2 L (H_2O)]^+$ (M-H)	439.09 (23.3)	391.09 (11.0)
$[UO_2 L(CH_3OH)]^+$ (M-H)	453.1 (19.3)	405.1 (14.2)
$[UO_2L(CH_3OH)(H_2O)]^+$ (M-H)	471.1 (38.8)	423.1 (22.2)
$[UO_2 L(H_2O)_2]^+$ (M-H)	457.1 (41.8)	409.1 (16.0)
$[UO_2 L(CH_3OH)_2]^+$ (M-H)	485.1 (1)	437.1 (1)
$[UO_2 L(NO_3)]^+$	484.08 (NA)	436.07 (NA)
$[UO_2 L_2]^+$ (M-H)	573.1 (52.3)	477.1 (58.9)
$UO_2 L_2 (H_2O)]^+$ (M-H)	591.1 (71.9)	495.1 (70.7)
$UO_2 L_2 (CH_3OH)]^+$ (M-H)	606.1 (NA)	509.1 (NA)
$[UO_2 L_2(NO_3)]^+$	636.1 (NA)	540.1 (4.9)
$[UO_2 L_3]^+$ (M-H)	725.1 (100) BASE PEAK	581.1 (100) BASE PEAK
Doubly charged dimeric species		
$[UO_2 L (NO_3) (H_2O)]_2^{2+}$	1004.1 (NA)	908.1 (NA)
$[UO_2 L (NO_3) (CH_3OH)]_2^{2+}$	516.1 (2.3)	468.1 (3.0)
$[UO_2 L_2]_2^{2+}$ (M-H)	573.6 (9.0)	477.6 (2.8)
$[UO_2 L_2]_2^{2+}$ (M-H)	953.2 (3.9)	1145.2 (12.3)
$[UO_2 L (NO_3)(H_2O)(CH_3OH)]_2^{2+}$ (M-H)	1067.2 (7.8)	971.2 (NA)
$[UO_2 L (NO_3)(H_2O)(CH_3OH)]_2^{2+}$	534.1 (20.2)	486.1 (NA)
$[UO_2 L_2 (NO_3)(H_2O)]_2^{2+}$	654.1 (3.6)	558.1(NA)
$[UO_2 L_2 (NO_3)(CH_3OH)]_2^{2+}$	668.1 (1.5)	571.1 (NA)
$[UO_2 L_3 (H_2O)]_2^{2+}$ (+5 O.S)	744.1 (11.9)	600.1(NA)

NA: Not Available (less than 1%)
***Denotes % intensity with respect to the base peak**

Conclusions
ESI-MS allowed identification of different species of UO22+-mandelate and UO22+-HIBA in methanol-water medium. The preference for water was found in uranyl-ligand species. Doubly charged dimeric species with the 4 ligands was found only in mandelic acid system.

Novel Aspect
Different species of uranyl with HIBA and mandelic acid were identified and intensities were compared.

ThPS35-10 / A Comparison of Groundwater δ18O and Speleothem-derived water δ18O isotopes.

Lewis Adler[1], Pauline, C Treble[2], Karina Meredith[2], Jon Hellstrom[3], Andy Baker[1]
[1]University of New South Wales, [2]Institute for Environmental Research, ANSTO, [3]University of Melborne

Introduction
Groundwater δ18O records have the potential to provide an important long-term record of Australia's past climate, but many uncertainties exist when using groundwater archives and they are therefore considered low-resolution records. In contrast, speleothems (cave formations) are much higher-resolution records that can be used to obtain terrestrial paleoclimate records, specifically they are used for climate reconstruction from their oxygen and carbon isotopes (δ18O and δ13C). In this study we compare measured δ18O values from both groundwater and speleothem records for the first time from the South West of Western Australia (SW WA).

Methods
Groundwater samples from SW WA were analysed for δ18O by IRMS and radiocarbon (14CDIC) by AMS. A MAT253 IRMS in conjunction with a Kiel IV carbonate device was used to obtain δ18O from a speleothem deposited in a cave in the same region. The speleothem was dated using U-Th methods.

Results
Groundwater δ18O varied between -3 and -5 per mil for groundwater dated between 0 and 40,000 years before present. The speleothem U-Th dates covered the same time period, and had δ18O of calcite between -5.0 and -2.5 per mil. These values were corrected for the temperature dependent fractionation of water-calcite.

Conclusions
Using both groundwater and speleothem derived measurements of δ18O is a novel method for identifying disequilibrium processes in speleothem deposition, and understanding groundwater recharge processes in a drought affected region.

Novel Aspect
This is the first such comparison of groundwater and speleothem-derived water isotopes in a drought affected region.

ThPS35-11 / Ultra-trace analysis of plutonium isotopes by thermal ionization mass spectrometry with a continuous heating technique without chemical separation

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Thermal ionization mass spectrometry (TIMS) with a continuous heating technique is well known as an effective method for measuring the isotope ratios in trace amounts of uranium. In this study, its analytical performances were investigated using a standard plutonium solution (SRM947) and mixed particulate sample containing plutonium (SRM947) and uranium (U500). In order to examine the analytical performance of the plutonium solution sample, the influence of the heating rate of evaporation filament on precision and accuracy of the isotope ratios was studied using plutonium solution samples at the fg level. Changing the heating rate of the evaporation filament on samples ranging from 0.1 fg to 1000 fg revealed that no influence of heating rate on the precision and accuracy of the isotope ratios occurred over a heating rate range of 100 to 250 mA/min, although the average intensity of 239Pu increased by 2.5 times at 250 mA/min compared to the heating rate of 100 mA/min. All of the isotope ratios on plutonium (SRM947), 238Pu/239Pu, 240Pu/239Pu, 241Pu/239Pu and 242Pu/239Pu, were measured down to sample amounts of 70 fg. The ratio of 240Pu/239Pu was measured down to the sample amount of 0.1 fg, which corresponds to the PuO2 particle with a diameter of 0.2 μm. Moreover, the signal of 239Pu could be detected for a sample amount of 0.01 fg, the detection limit of 239Pu of 0.006 fg estimated by the 3-sigma criterium. In the mixture samples of plutonium and uranium, 238Pu and 238U were clearly distinguished owing to the difference in evaporation temperature between 238Pu and 238U. In addition, 241Pu and 241Am formed by the decay of 241Pu were able to be discriminated due to the difference of the evaporation temperature. As a result, the ratios of 238Pu/239Pu and 241Pu/239Pu as well as 240Pu/239Pu and 242Pu/239Pu in the plutonium sample were successfully measured by TIMS with a continuous heating technique without any chemical separations. The ratios of 238Pu/239Pu and 241Pu/239Pu without using a chemical separation have not been reported in the conventional method such as TIMS with a total evaporation. It is expected that TIMS with a continuous heating technique could be a promising

method for measuring the isotope ratios in various fields such as nuclear forensics and safeguards.

ThPS35-12 / Size distribution of Sulfur, Vandium and Nickel in four crude oils complete distillation series using GPC ICP HR MS

Alain Desprez[1], Brice Bouyssière[1], Carine Arnaudguilhem[1], Gabriel Krier[2], Lionel Vernex-Loset[2], Pierre Giusti[3]
[1]IPREM/LCABIE, [2]LCP-A2MC, [3]Total Refining and Chemicals

Sulfur and metals such as vanadium and nickel are present in crude oils in concentrations going up to 10% for sulfur and hundreds of parts-per-million for metals. The study of those three elements is of great interest because the information given by their total concentrations and molecular size distributions can help when choosing the catalysts properties used during the conversion of heavy fractions (atmospheric residue, vacuum residue) into transportation fuels.
The complete distillation series of four crude oils of different geographical origins and having different physico-chemical properties were determined using Gel Permeation Chromatography Inductively Coupled Plasma High Resolution Mass Spectrometry (GPC ICP HR MS). The size distribution of sulfur, vanadium and nickel containing compounds within the samples were determined, compared and correlated with other properties such as viscosity, Total Acid Number or total sulfur, vanadium and nickel concentrations.
A trimodal distribution was obtained for vanadium and nickel in the crude oils, Total Atmospheric Residues and Vacuum Residues whereas sulfur exhibited either a monomodal or a bimodal distribution. This bimodal behavior was attributed to trapped sulfur compounds in a higher boiling point fraction and a correlation was found between the viscosity of the crude oil and the proportion of trapped sulfur compounds. A correlation between the sulfur fractions retention times and the temperature cuts of the distillation was also evidenced. The thermic treatment applied for the distillation increases the aggregation of low and medium molecular weight compounds of vanadium and nickel into higher molecular weight aggregates between the crude oil on the one hand and the atmospheric residue and vacuum residue on the other hand. This phenomenon is more pronounced when the crude oil has high total sulfur content.
This study improves our understanding of the sulfur, vanadium and nickel mobility within the refinery in terms of size distribution, boiling temperature and aggregation. Monitoring and trying to control those parameters will allow the improvement of the optimization of hydrotreatment and hydrocracking processes.

ThPS35-13 / Occurrence and Impact of Doubly Charged Alkaline Earth Argon Ions [MAr]2+ in ICPMS.

Bodo Hattendorf[1], Bianca Gusmini[1], Ladina Dorta[2], R. Sam Houk[3], Markus Reiher[4], Detlef Günther[1]
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Doubly charged diatomic ions containing argon in mass spectra from an inductively coupled plasma mass spectrometer were recently detected using a highly sensitive magnetic sector instrument (NU Plasma HR). Their presence was previously unexpected as only polyatomic ions with high binding energies were expected to survive the high temperature, high density atmospheric ICP ion source. Quantum mechanical calculations indicate that such species can be formed via association of a doubly charged elemental ion to a neutral argon atom and their bond energy can reach values of up to 0.3 eV. Doubly charged atomic ions are present in the ICP at varying degree for elements with low 2nd ionization energy, particularly alkaline earth or rare earth elements amongst others. In accordance with their higher bond energies, the absolute intensities of the corresponding doubly charged polyatomic ions [MAr]2+ are about 10 times higher than for the corresponding singly charged [MAr]+ ions, even though the latter are more than 10 times more abundant in the ICP source. However, due to the fact that [MAr]2+/M+ signal ratios are in the 10-5 range, relative to the corresponding elemental ions, their influence on quantification should remain small. Significant impact can, however, occur in isotope ratio analyses, when aiming at accuracy in the 0.1 permil range. Significant bias can be introduced in Sr isotope ratio measurements where 86Sr+, 87Sr+and 88Sr+ cannot be resolved from [132Ba40Ar]2+, [134Ba40Ar]2+ and [136Ba40Ar]2+ respectively, or 63Cu+ and 64Zn+, which cannot be distinguished from [86Sr40Ar]2+ and [88Sr40Ar]2+. The contribution form [MgAr]2+ isotopes to the sulfur isotopes would however only be significant for extremely high Mg/S concentration ratios. [CaAr]2+ ions may affect Ca isotope ratio measurements, especially when their abundance is variable between samples, and, to a much lesser degree, potassium.

ThPS35-14 / Development of Simplified Nanoparticle Quantification Protocols

Yong-Hyeon Yim[1], Jung-No Yoon[2], Sook Heun Kim[1], Myungsub Han[1], Jongwon Kim[2]
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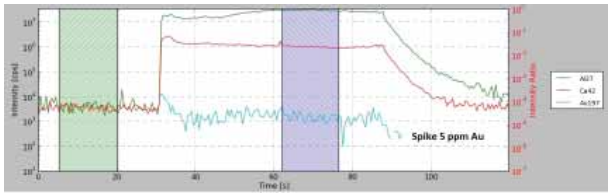
Increasing productions and applications of engineered nanoparticles (NPs) in consumer and medical products demand reliable analytical tools for more accurate characterization of NPs. The elemental composition and concentrations are the most fundamental and important information for characterization of NPs, which is complementary to the information on particle size and particle number concentration. ICP/MS and ICP/OES are usually used as quantitative tools to determine the concentration and composition of elements in NPs. Typically the NP samples are digested in an acid before the analysis to transform analytes in solid particles or their suspensions into simplest ionic forms in solution. Acid digestion of NPs, however, involves use of hazardous chemicals and complex dissolution procedures especially for intractable NPs, such as silica and TiO2. In the present study, simplified sample preparation methods for the ICP/MS and ICP/OES analysis of aqueous NP suspensions were developed and validated by comparing with the rigorous results obtained from isotope dilution (ID) ICP/MS or characteristic values of the reference materials.

Commercial aqueous suspensions of Ag or Au NPs were used as test samples for the development of simplified sample preparation protocols for NP samples. For Ag NPs, total content of Ag in the NP suspension was accurately determined by ID ICP/MS and used as the reference value. In the case of the suspension of Au NPs obtained from NIST, the total Au content of the information value given in the report of investigation was used as the reference value. Two different simplified sample preparation methods, using simple agitation with addition of acid and direct infusion of diluted NP suspensions, were tested for Ag and Au NPs.

ICP/MS as well as ICP/OES results from the simple agitation method were well-matched with the reference values both for Ag and Au NP suspensions. It was also validated for large polymeric capping molecules by using NPs capped with polyvinylpyrrolidone. It was demonstrated that the major elements of NP suspensions investigated here can be determined by the simple agitation method with less than 2 % of measurement biases, provided the acid content of the calibration standards is closely matched with those in the sample solution. In the case of easily dissolved NPs, simple agitation in acid turned out to

be enough to dissolve constituting elements from NPs and the residual organic molecules in diluted sample solutions didn't affect the analysis. Direct infusion of diluted NP suspensions was also tested for Ag and Au nanoparticles. As far as the acid content of the standard solutions is close to that of diluted NP sample solutions by extensive dilutions, the results from direct infusion were matched with the reference values within 5 % of measurement biases. Due to extremely small sizes of NPs investigated here, neither the transportation efficiency through the ICP sample introduction system nor the atomization/ionization efficiency in the Ar plasma seem to be changed noticeably from those of standard solutions.

As result reproducibility, LOD's, deviation from alternatively measured values were evaluated for LA-ICP-MS bulk analysis of powder geological samples without matrix-matching calibration standards.



ThPS35-15 / Current evaluation of powder geological samples elemental analysis using laser-ablation inductively coupled plasma mass-spectrometry

Maxim Blokhin[1], Daniel Tabersky[2], Vladimir Molchanov[1], Evgeny Medvedev[1], Detlef Günther[2]
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Laser-ablation inductively coupled mass-spectrometry (LA-ICP-MS) has been widely used for elemental measurements of geological samples and other solids for many years. Its particular interest in geology has been usually focused on spatial resolution trace-element determinations of different mineral phases or analysis of fluid inclusions. The bulk elemental analysis of geological materials with LA-ICP-MS is not so spread because it meets several serious restrictions e.g. homogeneity of samples and absence of matrix-matching standards.

In the current research we evaluated the possibility to utilize LA-ICP-MS for quantitative analysis of unknown geological specimens trying to develop the most appropriate technique of calibration ensuring reproducible and precise determination with minimum sample preparation procedure.

In our experiment we used several geological samples with various matrixes presented by Far East Geological Institute, Vladivostok, Russia. The samples were fine grinded and dried at 105 °C up to the constant weight. Each sample was divided into two representative portions. The first one was analyzed in FEGI using total dissolution technique with LiBO2 fusion and ICP-MS/AES determination of elements (trace and petrogenic) in wide range. To prevent loss of some valuable elements (e.g. gold) during sample preparation the neutron activation method was employed for quantitative analysis as well. The second part of the samples was studied in Swiss Federal Institute of Technology, Zürich, Switzerland. The powders were measured by LA-ICP-MS with two types of lasers: 193 nm ArF-eximer laser (Lambda Physik, Germany) compared with 213 nm Nd:YAG solid-state laser (CETAC Tech., USA). The lasers were hyphenated to Q-ICP-MS Elan 6100 (Perkin Elmer Corp., USA). Sample preparation technique included just obtaining of pressed pellets by steel tools with a pressure of 1 GPa.

The measured elements involved rare-earth and noble (gold) metals which are of great interest for geologists. It has already been demonstrated before that there is a possibility to use a homogeneously distributed element like Ca as an internal standard to determine REE within different mineral phases versus glass standards. In our work we applied as internal standards the petrogenic elements (Ca, Si, Al, Mn) those had been previously measured by ICP-AES.

The matrix unification had to be performed because glasses NIST 610 and 612 were used as calibration standards against powder samples. It was decided to prepare solution-doped powder pellets spiked with 1, 5 and 20 ppm of gold to check this element LOD's and achieved homogeneity (Fig.1). By the way gold is considered to be a very dispersive element in nature. For example, the same rather challenging calibration strategy was also shown for the analysis of pure copper and zinc by fs-LA-ICP-MS.

ThPS35-16 / ICP-TOFMS based on iCAP Qc ion source and interface

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Introduction

Multi-element detection of short transient signals is a challenge of growing interest in inductively coupled plasma mass spectrometry (ICP-MS). Signals can be as short as 0.5 ms e.g. in single nanoparticles (NPs) analysis. Another example with great analytical potential is imaging of biological tissues using laser ablation (LA) in combination with ICP-MS where the maximum speed of the scanning laser determines the total analysis time. The analysis time per laser spot is ultimately limited by the washout time of the LA-cell. LA-cells with washout times <50 ms have been described which consequently demand for fast acquisition of multi-element signals in ICP-MS. ICP with a time of flight mass spectrometer (TOFMS) is the ideal instrumentation to detect short transient signals if the following can be provided:

- time resolution better than duration of transient signals: always getting optimum S/N ratio.
- linear signal response: reliable quantification and isotope ratio determination.
- sensitivity and detection limits comparable to quadrupole based MS: necessary sample consumption must not exceed state of the art ICP-MS requirements for market acceptance.

Methods

A medium resolution TOFMS with 4000 Th/Th mass resolving power has been coupled to the iCAP Qc ICP ion source and interface from Thermo Scientific. The instrument was equipped with a conventional pneumatic nebulizer as sample introduction. Multi-element standard solutions were measured to investigate instrument performance.

Results

The instrument performance has been evaluated with regard to sensitivity, dynamic signal range and isotope precision. The results will be discussed.

Conclusion

The demonstrated performance of the ICP-TOF shows clear advantages over scanning mass spectrometers for applications like imaging, single particle analysis, fluid inclusion analysis, where full mass spectra are reqtw uired on a millisecond time scale. The non-scanning TOF technology allows to reach the limits of detection (LD) of quadrupole based ICP-MS instruments for all masses on much shorter time scale, depending of the mass range of interest. Furthermore, due to the simultaneous measurement of isotopes, isotope ratio precision is significantly improved compared to scanning instruments. The resolution of the mass analyzer allows to separate interferences on the same nominal mass, improving the detection of e.g. Phosphorus, Sulfur and Iron.

Novel Aspects
LDs comparable to quadrupole instruments in single ion monitoring mode were demonstrated for the first time.

ThPS36 - Advanced MS in Food and Nutrition

11:00-15:00
Poster Exhibition, Level -1

ThPS36-01 / Simultaneous analysis of flonicamid and its metabolites in agricultural products by liquid chromatography tandem mass spectrometry

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Simultaneous analysis of flonicamid and its metabolites in agricultural products by liquid chromatography - tandem mass spectrometry
Flonicamid which is insecticide decompose to TFNG and TFNA and remains in the agricultural products. The definition of residue of flonicamid is different between Korea and other countries. In Korea, the residue of flonicamid is the concentration of flonicamid only, but in other countries such as USA and Japan, the residues of flonicamid is expressed as the sum of flonicamid and its metabolites. Therefore this study was performed to develop the simultaneous analytical method of flonicamid, TFNG and TFNA in a sweet pepper, a strawberry, an apple and a pear using EU official QuEChERS method with HPLC-MS/MS.
Sample in this study is pesticide-free organic agricultural products. Grinded samples are kept in the refrigerator under –70°C. Sample, which was confirmed to be pesticides-free, was used as control.
In order to extract pesticide in the sample, acetonitrile 10 mL was added in the sample 10 g and then homogenized for 10 minutes. By adding MgSO44 g, NaCl 1 g, trisodium citrate dihydrate 1 g and disodium hydrogencitrate sesquihydrate 0.5 g, coextractants and interference materials were removed and cleaned up. After filtering with 0.2 μmsyringe filter, sample was injected into LC-MS/MS to confirm the residue of flonicamid and its metabolites.
The recoveries of flonicamid, TFNG and TFNA was in the range of 69.54 % ~ 117.67 % at the level of 0.1 mg/kg and 82.29 ~ 103.58 % at the level of 1 mg/kg. The linearity was above 0.99. LOD and LOQ of TFNA were 0.006 ~ 0.012 mg/kg and 0.018 ~ 0.036 mg/kg, respectively. LOD and LOQ of TFNG were 0.003 ~ 0.012 mg/kg and 0.009 ~ 0.036 mg/kg, respectively. LOD and LOQ of flonicamid were 0.003 ~ 0.006 mg/kg and 0.009 ~ 0.018 mg/kg, respectively.

ThPS36-02 / Phenolic Compounds from the Leaves of Vitis labrusca and Vitis vinifera L. as a Source of Waste Byproducts: Validation of LC Method and Antichemotactic Activity

Roger Dresch, Maria Dresch, Aline Guerreiro, Renata Biegelmeyer, Maribete Holzschuh, Douglas Rambo, Amélia Henriques
Faculdade de Farmácia - UFRGS

The cultivation of Vitis (Vitaceae) grape varieties is one of the most important economic activities in agribusiness in southern Brazil. Vitis varieties are rich in polyphenolic compounds with several pharmacological and biological activities, such as antioxidant action. In this context, this study analyzed qualitatively and quantitatively the anthocyans and flavonoids found in the leaves of grape varieties Vitis vinifera and Vitis labrusca. For this purpose, vine leaf extracts were prepared and the chemical profile of each was characterized by LC/MS-MS. Two high performance liquid chromatography-validated methods were performed using UV/VIS-LC-DAD detector to quantify phenolic compounds. The main anthocyanins isolated from vine leaves were cyanidin-3-O-glucoside and peonidin-3-O-glucoside. The flavonoids identified were rutin, quercetin-3-O-galactoside, quercetin-3-O-glucoside, and quercetin-3-O-glucuronide, which was the predominant compound. The Waters X-Terra® RP18 column allowed the effective separation of quercetin-3-O-glucuronide from the other flavonoids for the first time, besides the partial separation of quercetin-3-O-galactoside from quercetin-3-O-glucoside. Furthermore, another phenolic compound was confirmed by MS spectrometry, using direct infusion, as being trans-caftaric acid. The present study also investigates the in vitro antichemotactic activity of grape crude extracts, fractions, and isolated compounds. It was demonstrated that almost all fractions and isolated compounds showed increased antichemotactic effect in response to LPS with a more pronounced values of IC50 for anthocyanins fraction, rutin, quercetin-3-O-galactoside, and trans-caftaric acid (0.9, 1.6, 3.7, and 5.1 ng/mL, respectively). The results obtained are promising and pointed out the great potential for the anti-inflammatory action of the varieties of Vitis analyzed. Is important to take into consideration that the effects observed are associated with a wide range of compounds displaying synergistic effect. Furthermore, we highlight the applicability of wasted raw materials derived from extensive crops as materials with great potential for industrial applications resulting in increased value of these byproducts.

ThPS36-03 / A residual feature of gibberellin in pears using the LC/MS/MS determination

Soon-Kil Cho[1], Ji-Mi Cho[1], Yang-Mo Jeong[1], Jeong-Heui Choi[2], Jae-Han Shim[2]
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An oriental pear (Pyrus pyrifolia cv. Niitaka) has better excellent and storage qualities than those of the Western and Chinese pears, and the variety of a pear occupying 80% domestic cultivation area is Singo. However, the pithiness of Singo Pears leads to deterioration, which is a big problem during selection process of pears due to its sorting difficulty in appearance. Therefore, the treatment and residues of gibberellin should be verified from field-pears in advance. The present study investigated residual changes of gibberellin in production stage after application of its liniment, and checked the application and existence of gibberellin at the final pear production stage. Gibberellin (2.5% gibberellic acid and 0.2% gibberellin A4+7) was applied to a peduncle at 25 mg/peduncle after 40 days (May 29) of the flowering season. Weekly weight changes and residues in peduncles and fruits were determined from Jun 4 to October 15 after the application. Subsample was placed into a 50-mL tube, to which acetonitrile 10 mL and QuEChERS EN Pouch were added. The mixture was then shaken and centrifuged for 5 min at 3200 rpm. One mL supernatant was filtered and 15 μL was injected into LC/MS/MS to detect residues of gibberellin. Recovery was tested at 0.02 and 0.2 mg/kg and the mean values were 92.7±4.4 and 95.0±0.5%, respectively. Limits of detection and quantification were 0.005 and 0.015 mg/kg, respectively. The average weight of the control fruit was 8.6 g at Jun 4 and 500.8 g at October 15, and it had increased by 58.2 times, whereas in the treatment, it was 10.1 g at Jun 4 and 706.5 g at October 15 and had increased to 70.0 times, which meant that the growth rate of the treatment was 1.2 times higher than those of the control. Gibberellin residues in the applied peduncles were detected until harvest, and they were also in the control during certain period, which is likely

to be associated with physiologic changes of pear. Nothing of gibberellin was identified in fruits of the control and treatment. Therefore, a peduncle should be detected to establish the fact of use of gibberellin.

ThPS36-04 / Confirmatory LC/MS/MS methods for monitoring of feedingstuffs contamination by authorized coccidiostats at carry-over level

Marilena Muscarella[1], Antonio Armentano[1], Pasquale Gallo[2], Cinzia Civitareale[3], Maurizio Fiori[3], Paolo Stacchini[3]
[1]Istituto Zooprofilattico Sper. Puglia e Basilicata, [2]Istituto Zooprofilattico Sper. del Mezzogiorno, [3]Istituto Superiore di Sanità – Roma

Coccidiostats have antiprotozoal actions and play an important role in the prevention of coccidiosis in animal production, particularly in intensive animal rearing. The usual feedingstuffs production practices lead inevitably to carry-over between consecutive production batches, so cross-contamination of the batch following the production of a medicated/additivate feed is practically unavoidable. Recent Commission Regulation (EU) N. 574/2011 of 16 June 2011 established that the carry-over is unavailable during feeds production and to minimize the potential hazard adopted a pragmatic solution setting maximum acceptable levels of contamination for additives coccidiostats in non-target feed.
To ensure effective control of carry-over, official laboratories have to develop and validate analytical methodologies able to detect these drugs at very low concentration.
Two optimized and validated LC/MS/MS methods were used to analyse the positive samples obtained by a microbiological kit (Premi®Test) for the detection of ionophores since the Italian National Surveillance Program namely Piano Nazionale Alimentazione Animale (PNAA) focus a particular attention on this class of coccidiostats.
The two methods employed different mass analyzers: a method has a triple quadrupole in the experimental set-up, the other method an ion trap.
Over a total of 100 feed samples addressed to the "Istituto Zooprofilattico della Puglia e della Basilicata" collected from different farms of Apulia region 8 samples were found with a concentration of ionophores higher than the legal limits for carry-over. To confirm these results, these 8 samples were analysed used the two different confirmatory LC/MS/MS methods. Three samples were confirmed to be non-complaints (the 3% of the total number of samples). The quantification results obtained with the two confirmatory methods considering the uncertainties are comparable. Monensin and Narasin were the only two molecules detected. The present work demonstrated how the combined use of fast and reliable screening methods and LC/MS/MS confirmatory methods, which require more expensive experimental set-up, is essential to obtained reliable results with considerably reduced costs and times.

ThPS36-05 / Accurate determination of adulterants in dietary supplements using shotgun high-resolution tandem mass spectrometry

Nathalie Martins-Froment[1], Catherine Claparols[1], Véronique Gilard[2], Stéphane Balayssac[2], Rabab Hachem[2], Myriam Malet-Martino[2]
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Introduction
Sales of dietary supplements (DS) increase each year, particularly in developed countries, where they remain considered by many consumers and physicians as harmless because of their natural origin or presumed safe composition. Nowadays, the growing trend consisting in the intentional adulteration of DS with synthetic drugs represents an alarming emerging risk to public health. DS for treating sexual dysfunction are among the most sold and moreover affected by adulteration. Indeed, manufacturers add synthetic actives like phosphodiesterase type 5 (PDE-5) inhibitors (sildenafil, tadalafil, vardenafil) to the natural matrix in order to intensify the pharmacological effect. Furthermore, in an attempt to evade regulatory inspection, unscrupulous manufacturers use not only approved active pharmaceutical ingredients but also unapproved analogs in which minor chemical modifications were brought to the parent structure. This fact enhances the need to propose a performing analytical procedure for detecting these multiple adulterations.

Methods
MS analysis: DS were dissolved in CH3CN:H2O (80:20 v/v) and analyzed after direct infusion using a Waters XEVO G2 QTOF mass spectrometer. Positive and negative ionization modes were performed.
NMR analysis: DS were dissolved in CD3CN:D2O (80:20 v/v). 1H NMR experiments were performed on a Bruker Avance 500 spectrometer equipped with a 5 mm cryoprobe at 298K.

Results
135 DS were analyzed using both 1H NMR and MS. NMR was used for a rapid screening of these preparations and for the detection of adulterants mainly based on the characteristic signals of their aromatic protons. Then HR MS/MS with direct infusion was performed as an orthogonal analytical method for the determination of their accurate molecular masses and elucidation of their structures from the analysis of their fragmentations. Because the chemical structures of some PDE-5 analogs are very similar, HR-MS is not sufficient for the non-ambiguous determination of some analogs, and the exact structure can be only assigned after analysis of the MS/MS fragmentation pattern. Among the DS analyzed, 65% were adulterated. Adulterants were either registered PDE-5 inhibitors (sildenafil, tadalafil, vardenafil) or unregistered analogs. Formulations containing analogs alone or as mixtures represented 55% of the adulterated formulations.

Conclusions
The problematic of adulteration with synthetic compounds is an increasing phenomenon and all possible analytical tools should be used in order to ensure the quality and safety of commercialized products available for consumers. This study combining 1H NMR profiling and HR-MS/MS analysis allowed the detection of 3 PDE-5 inhibitors and 13 of their analogs in the DS analyzed.

Novel Aspect
Despite the chemical complexity of DS, the shotgun MS/MS analysis allows a rapid and accurate determination of contaminants.

ThPS36-06 / Development of ID-LC-MS/MS for the Accurate Determination of Ochratoxin and Application to the Certification of Fermented Soybean Paste Reference Material

Byungjoo Kim, Seonghee Ahn
Korea Research Institute of Standards and Science

Introduction
Ochratoxin A (OTA) is a naturally occurring mycotoxin produced by several species of general Aspergillus and Penicillium. OTA is known to cause teratogenic, embryotoxic, genotoxic, neurotoxic, immunosuppressive, carcinogenic effect on human and animals. OTA occurs in a large variety of foods, i.e., cereals, beans, spices, dried fruits, grapes and cocoa. In Korea, it has been reported that

Doenjang, which is traditional fermented soybean paste, might contain OTA as the bad fungi can grow if the fermentation condition is not well controlled. Therefore, the maximum limit of OTA in fermented soybean paste has been set 20 µg/ kg in Korea. Though several analytical methods are widely used for the screening of OTA in food samples, there is lack of measures to ensure quality and comparability of the analytical results of laboratories. In this study, the isotope dilution-liquid chromatography tandem mass spectrometry (ID-LC-MS/MS) was developed as a reference method for the accurate determination of OTA in various food samples by using 13C20-OTA as an internal standard material.

Methods
Sample was extracted with water/methanol (40/60, v/v) solution and further cleaned up with an immunoaffinity cartridge. A C18 LC column was used with isocratic elution with 15 mM ammonium formate (pH 3 by adding formic acid) in water/methanol (40/60, v/v). The MS analysis was performed in positive ion mode with monitoring CID channels at m/z 404 → 239 for OTA and m/z 424 → 250 for the isotope analogue

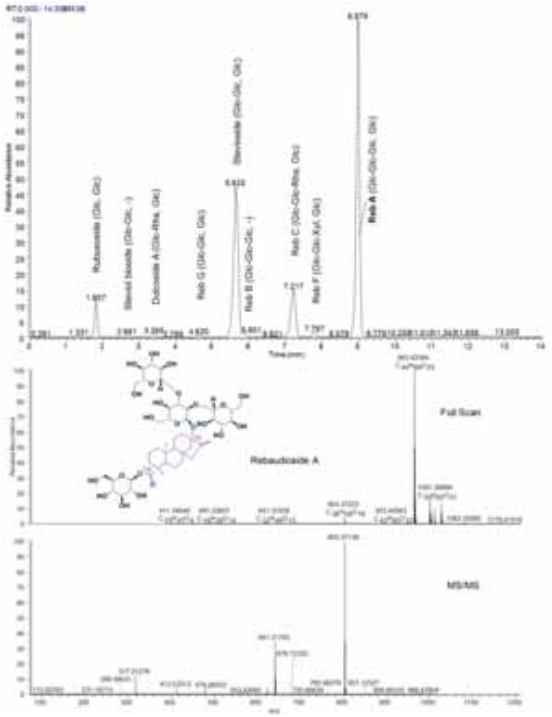
Results
For proper retention and separation of OTA with a C18 LC column, pH of the mobile phase was tested. As pKa of OTA is at 4.4 and 7.3, it is expected that OTA is in a neutral form when pH is below 4. Upon our test, OTA was well retained with showing good peak shape when pH of the aqueous phase was 3.With the optimized experimental parameters, this method showed less than 2 % of repeatability and reproducibility, and overall relative measurement uncertainty was estimated to be 2 % for samples of higher than 1 µg/ kg. This method applied to the certification of reference material of fermented soybean paste, which was recently developed in this laboratory.

Conclusion
It was proved that the ID-LC-MS/MS method has a higher-order metrological quality as a reference method and successfully applied for the certification of a reference material.

Novel Aspects
A ID-LC-MS/MS method has been developed as a as a reference method for the accurate analysis of OTA in various food samples and applied to the certification of reference method, which will be used by testing laboratories to ensure quality and comparability of their analytical results.

ThPS36-07 / Analysis of Stevia Extracts by Ultra Performance Liquid Chromatography coupled with High-Resolution Quadrupole-Orbitrap Mass Spectrometry (UPLC®-HRMS).
Eric Frerot, Nicolas Jeckelmann
FIRMENICH S.A.

Stevia (Stevia rebaudiana) extract is a natural sweetener found in many foods. The sweet taste principles in stevia are diterpene glycosides called steviol glycosides or rebaudiosides. The diterpene (steviol) core possesses two sites of glycosylation: the hydroxyl in position 13 and the carboxyl 19. More than 20 steviol glycosides were described which differ by the nature, the position and the number of the sugar units. These different steviol glycosides do not possess the same sweet taste potency and quality therefore making it very important to know the composition of stevia extracts.
Steviol glycosides were poorly separated by reversed-phase HPLC. Hydrophilic interaction liquid chromatography (HILIC) using a BEH Amide UPLC column (1.7 µm particles) gave a very good separation. A quadrupole-Orbitrap® high-resolution mass spectrometer was used with the negative electrospray ionization. The full scan (FS) mode was first used to identify the major compounds. Then the targeted single ion monitoring (t-SIM) mode also showed the minor isomers while the targeted MS/MS (t-MS2) mode was used to record their MS/MS spectra.
A commercial stevia extract was analyzed by HILIC-UPLC-HRMS. In particular, MS/MS spectra of various known rebaudiosides were examined to understand their fragmentation behavior. This allowed identifying minor steviol glycosides.
UPLC-HRMS in HILIC mode was found very powerful for the analysis of stevia extracts. The excellent chromatographic resolution combined with the accurate mass capabilities of the Orbitrap allowed for the rapid identification of many steviol glycosides. The advanced features of the quadrupole-Orbitrap MS could distinguish subtle structural variations among this family of rather high molecular mass products.
This work represents a good illustration of the possibilities offered by a modern LC/MS instrument in the analysis of food products.



ThPS36-08 / Method Validation for Determination of polycyclic aromatic hydrocarbons in food by GC-MS
Joongoo Lee, Jung Hyuck Suh, Su Yeon Kim, Jung Sik Moon, Hae Jung Yoon
Ministry of Food and Drug Safety

Polycyclic aromatic hydrocarbons, PAHs, produced naturally in food as a result of pyrolytic processes, particularly incomplete combustion of organic matter are genotoxic and carcinogenic. Benoz(a)pyrene, one of PAHs, has been a marker for the occurrence of them in food, but EFSA recently concluded that four specific substances of PAHs would be the most suitable indicators of PAHs in food. Many methods for determination of PAHs are proposed for food, such as HPLC-FLD, GC-MS and GPC. However it need to develop a method for determining 4 PAHs for food. To make a simple and efficient method, Saponification followed by Liquid-liquid extraction, Solid-phase extraction for purification and gas chromatography-mass spectrometry(GC-MS) for detection of 4 PAHs are used. Two isotope labelled PAHs are used as internal standards. A method validation is also carried out for testing of selectivity, linearity, detection Limit, recovery and uncertainty.
To identify 4 PAHs, 228, 229, 226 m/z ions for Benz(a)Anthracene and Chrysene, 252, 253, 250 m/z ions for benzo(a)pyrene and

benzo(b)Fluoranthene, 240, 241, 236 m/z ions for Chrysene-d12, 264, 265, 260 m/z ions for benzo(a)pyrene-d12 are monitored by SIM mode of GC-MS and Qualification was based on the ratio of quantitative ion and 2 qualitative ions. Isolation of 4 PAHs is confirmed by testing it on various blank types for Selectivity. 6 standards at different concentrations are tested and calculated appropriatly regression coefficient for linearity. detection limit was assumed from sample standard deviation. Recovery was also calculated at 3 different concentrations. Uncertainty was calculated according to GUM(Guide to the Expression of Uncertainty of Measurement)
GC-MS chromatogram showed good isolation among 4PAHs, internal standard and nearby interferences such as benzo(e)pyrene, and Benzo(k)fluoranthene. Ranges of calibration r-squared was 0.999~1. LOD and LOQ were 0.02~0.13µg/kg and 0.06~0.44µg/kg. Recovery was 70.8% ~ 114.8% in 2µg/kg, and 10µg/kg.
The Method developed in this study were suitable for analyzing 4 PAHs in food stuff compare with others for doing PAHs. It is needed to Monitor 4 PAHs on food stuff and to assess the risk of 4 PAHs to people by food intake.

ThPS36-09 / Surveillance of chloramphenicol residues in milk,eggs and Chicken meat by LCMSMS

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Chloramphenicol has been banned for use in all food-producing animals by the European Union (EU), and Most of the developed countries.. The EU recently set a minimum required performance limit (mrpl) for chloramphenicol determination at 0.3 µg/kg (ppb) in all foods of animal origin. The growing food safety concerns call for intensive surveillance of chloramphenicol in food products. The objective of the study was to assess whether milk, eggs and chicken meat produced by the livestock farmers in TamilNadu state of India were contaminated with chloramphenicol residues. Liquid chromatography/mass spectrometry (LC/MSMS) method was employed for the determination of chloramphenicol (CAP) residues in milk, eggs, chicken muscle and liver, and kidney. CAP was extracted from the samples with acetonitrile and defatted with hexane. The acetonitrile extracts were then evaporated, and residues reconstituted in 10mM ammonium acetate--acetonitrile mobile phase and injected into the LC system, and detection was by a triple quadrupole mass spectrometer operated in selected reaction monitoring (SRM) mode.
The method studied was sensitive enough to detect and quantify 0.050 ug/kg (ppb) chloramphenicol for screening purposes, much lower than the Minimum Required Performance Limit (MRPL) of 0.3 µg/kg imposed by European Commission's regulation.The study revealed that most of the samples were in compliance with MRL and growing awareness amongst farmers to avoid banned antibiotic CAP

ThPS36-10 / Structural analyses of marine polyether toxins by means of high-energy CID MS/MS using MALDI SpiralTOF-TOF tandem MS system

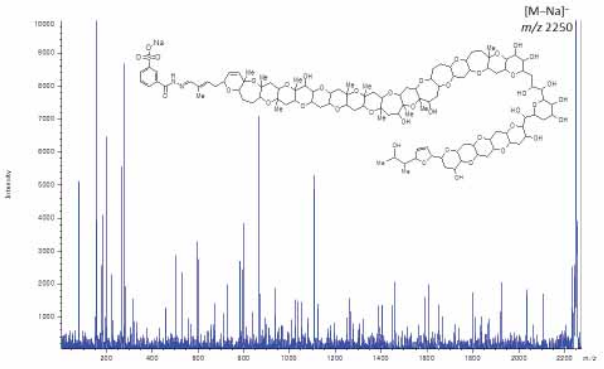
Yoshiyuki Itoh[1], Masahiro Hashimoto[1], Yoshihisa Ueda[1], Akihiko Kusai[1], Jun Tamura[1], Junich Osuga[2], Yuka Hamamoto[3], Masatoshi Yamazaki[3], Masayuki Satake[3]
[1]JEOL Ltd., [2]JEOL Europe SAS, [3]Department of Chemistry, School of Science, The University of Tokyo

Introduction

The ladder-frame polyether is the structure unique to marine natural products. It is necessary to determine the chemical structure and biosynthesis to understand their strong bioactivities. In late 1990's, structures of many marine polyether toxins had been determined using 4-sector tandem mass spectrometers. In 2010, we released a unique, new MALDI-SpiralTOF-TOF tandem MS utilizing a spiral ion trajectory TOFMS as the 1st MS and a reflectron as the 2nd MS. The MALDI-SpiralTOF-TOF system has features common to those of 4-sector tandem MS; monoisotopic precursor selectivity and high-energy collision induced dissociation (HE-CID). In this study, we analyzed two kinds of marine polyether toxins, brevisulcenal-F (KBT-F, from karenia brevisulcata toxin) and yessotoxin (YTX) using the MALDI-SpiralTOF-TOF system.
[Structural analysis of KBT-F]
A red tide of K. brevisulcata in New Zealand, in 1998 was extremely toxic to fish and marine invertebrates and also caused respiratory distress in harbor bystanders. A novel marine toxin, KBT-F was isolated from K. brevisulcata.
[Identification of the origins of oxygen atoms in YTX]
YTX is a ladder-frame polyether produced by a marine dinoflagellate P. reticulatum. Biosynthetic studies on polyethers have been limited to 13C-incorporation patterns to their carbon backbones. We prepared 18O labeled YTX with two different oxygen sources and investigated the origins of the oxygen atoms in YTX.

Methods

[Preparation of KBT-F benzene sulfonate derivative]
KBT-F was extracted and purified from mature cultures of K. brevisulcata. KBT-F benzene sulfonate derivative was prepared by reacting purified KBT-F with 3-(hydrazinecarbonyl)benzene sulfonate in 80% pyridine.
[Preparation of 18O2 labeled yessotoxin]
P. reticulatum was cultured (a) under the atmosphere of N2-18O2 (4:1) in a sealed flask with 2.5 mM sodium bicarbonate and (b) under an atmosphere of N2-O2 (4:1) with 2.5 mM sodium [18O2] acetate. YTX was extracted and purified from each culture.
[Mass spectrometric analyses]
Negative-ion product ion mass spectra of the KBT-F derivative and 2 kinds of 18O-labeled YTX were acquired using JMS-S3000 (JEOL, Ltd., Tokyo, Japan.)



Product ion mass spectra of the KBT-F benzene sulfonate derivative.

Results

[KBT-F]
Product ion mass spectrum of the KBT-F derivative showed fragmentations at the characteristic sites of ether rings. This information complemented the NMR analyses and led to elucidation of the complete structure of KBT-F.
[YTX]
We identified origins of all oxygen atoms in two kind of 18O-labeled YTX. Selection of monoisotopic precursor ions followed by HE-CID was essential to complete the study.

Conclusions
We solved the complete structure for KBT-F, and fully identified origins of all oxygen atoms in YTX. The new MALDI-SpiralTOF-TOF tandem MS provided valuable structural information comparable to that from a 4-sector tandem MS.

Novel Aspect
Complete structural analysis and identification of biosynthesis pathways of ladder-frame polyether marine toxins using MALDI-TOF-TOF tandem MS.

ThPS36-11 / Liquid Chromatography-Mass Spectrometry and Chemometric Techniques for the Authentication of Natural Extracts using the Compositional Profiles of Polyphenols
Oscar Nuñez[1], Lídia Puigventós[2], Meritxell Navarro[2], Élida Alechaga[2], Javier Saurina[2], Santiago Hernández-Cassou[2], Lluis Puignou[2]
[1]University of Barcelona, [2]Department of Analytical Chemistry, University of Barcelona

Recently there has been increasing interest in certain compounds that are present in foods and that are beneficial to human health. For the American cranberry (Vaccinium macrocarpon), its health benefits have been linked to high content of various types of polyphenols as flavonols, flavan-3-ols, oligomeric and polymeric tannins (proanthocyanidins, PAC), anthocyanins and phenolic acids. Moreover, the consumption of cranberry derived products has been related to preventive effects on urinary tract infections due to the ability of A-type PAC oligomers to inhibit adhesion of Escherichia coli bacteria responsible for these infections [1]. Therefore, some dietary supplements containing cranberry extracts are being developed nowadays. Lately, it has been suspected that some of the products sold as cranberry derivatives come actually from other fruits like grapes or blueberries, which do not contain the polyphenols that inhibit the adhesion of bacteria to the urinary tract tissues. Analytical methods for the characterization and authentication of the natural extracts are essential to protect the consumers, to prevent inaccurate declaration of fruit products, as well as to avoid unfair competition.
An LC-ESI-MS(/MS) method in a triple quadrupole instrument was used for the determination of phenolic compounds in fruit-based products. Several fruits (cranberry, blueberry, grape), fruit-based raisins, juices, as well as commercial pharmaceutical products such as natural extracts, powder capsules, syrup, and sachets, were analyzed using a simple sample extraction procedure involving sonication in an acetone/water/HCl solution. Separation was carried out on a C18 column under gradient elution using 0.1% formic acid aqueous solution and methanol [2]. Data acquisition was performed in full-scan (m/z 50 to 500) for polyphenolic profile characterization and in selected reaction monitoring for polyphenolic quantification. Principal component analysis (PCA) was used in order to explore the connection between polyphenolic profiles of products and their fruit of origin, thus achieving discrimination and characterization of these products to prevent misuses. Accurate mass measurements in a LTQ-Orbitrap mass spectrometer were also performed to identify and confirm the presence of discriminant polyphenols in the commercial natural extracts.
The results showed that LC-ESI-MS(/MS) polyphenolic profiles were useful for reliable discrimination between fruit-based products via PCA analysis. Samples were characterized regarding polyphenolic content and classified according to type of fruit, allowing the identification of a believed cranberry-based natural extract as a fraud.
[1] I. Tarascou, J. Mazauric, E. Meudec, J Souquet, D. Cunningham, S. Nojeim, V. Cheynier, and H. Fulcrand. Food Chem., 128 (2011) 802
[2] M. Navarro, O, Núñez, J. Saurina, S. Hernández-Cassou, and L. Puignou. J. Agric. Food Chem., 62 (2014) 1038

Novel Aspect:
Easy characterization of natural extracts by means of the LC-ESI-MS(/MS) polyphenolic profiles after a simple sample extraction procedure.

ThPS36-12 / Enhanced reduction of matrix effects using LC-MS/MS with online extraction for the rapid quantitation of antibiotics in milk
Louis Maljers[1], Helen Sun[1], Yann Hebert[2]
[1]Bruker Daltonics Inc., [2]Bruker Daltonique S.A.

Introduction:
Matrix effect is one of the major issues of LC-MS analysis when handling complicated samples. For example, milk is a very complicated matrix, composed of various carbohydrates, proteins, etc. Testing milk for antibiotics is a routine LC-MS/MS analysis, which typically involves off-line sample clean-up steps to minimize matrix interference. The clean-up steps are usually labor-intensive, tedious, and time consuming. A quantitative method was developed to quantify 8 antibiotics in milk matrix using an LC-MS/MS system with an integrated online extraction option. The milk matrix clean-up was all fulfilled online using an innovative UHPLC integrated with a binary pump and one loading pump. The analytical method used short run liquid chromatography (LC) coupled with mass spectrometry (MS) and using electrospray ionization (ESI) as an ionization technique.

Methods:
Organic milk was purchased from a local organic store. Proteins were precipitated and removed from the blank milk. The supernatant was collected as the blank milk matrix. 7 cephalosporin and penicillin G antibiotics were spiked in the milk matrix to prepare quantitative curve solutions. Online extraction was set up on the Bruker EVOQ LC-MS/MS OLE system to clean up the milk matrix prior to LC-MS/MS analysis. The MRM transitions of each antibiotic were optimized as well as the chromatography conditions. 2% ACN in water with 0.1% formic acid was used to clean up the matrix at 1mL/min. The entire analysis time including matrix clean-up is 15 min, including a 3-min online clean-up and a 12-min LC run.

Results:
The spiked milk matrix samples were directly injected to the LC-MS/MS system without online clean-up. The results were compared with the analysis of the same solutions with online clean-up. Without online clean-up, some antibiotics, such as cefsulodin, exhibit high matrix effects, for example, ~ 50%, versus < 5% (with online clean-up). The integrated UHPLC-OLE system was able to minimize the matrix effect by washing the loading column at a high flow rate (1 mL/min) to remove minerals, sugars, and other interfering compounds in milk. During elution, the UHPLC gradient back-flushed the loading column to elute analytes to the analytical column for separation, followed by MS/MS quantitative analysis. The turn-around time for each sample was 15 min, including 3-min clean-up time and 12-min analysis time. The online sample preparation is under complete software control and allows setting up timed events in one run, such as clean-up, elution, and column conditioning. The calibration range of this method is from 0.02 – 100 ng/mL. The linear response for each antibiotic is above 0.996, demonstrating excellent linearity. Reproducibility of the online-cleanup LC-MS/MS method was also evaluated. At 0.5 ng/mL level, the peak area RSD% of all 8 antibiotics is less than 9%, and 4 of them even have a RSD% < 4%.

Novel Aspect:
A rapid LC-MS/MS quantitative assay using a novel system with a completely integrated UHPLC pump with online sample clean-up capabilities.

ThPS36-13 / Determination of protein adducts originating from 1-methoxy-3-indolylmethyl glucosinolate using isotope-dilution UPLC-ESI-MS/MS

Wolfram Engst, Gitte Barknowitz, Mareike Bernau, Hansruedi Glatt
German Institute of Human Nutrition Potsdam- Rehbrücke

1-Methoxy-3-indolylmethyl (1-MIM) glucosinolate (GLS) occurring in cabbage, broccoli, and other cruciferous plants is a potent mutagen requiring metabolic activation by myrosinase present in plant cells and intestinal bacteria (1). Humans are exposed to high amounts of 1-MIM glucosinolate by consumption of Brassica vegetables.The nutrition-related exposure of humans to 1-MIMglucosinolate is usually muchhigher compared to that of known foodborne genotoxicants such as benzo[a]pyrene and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP).
We previously reported that reactive metabolites of 1-MIM GLS form adducts with the DNA in vitro and in vivo (2). In the present study we investigated whether analogous reactions also occur in proteins. The aim was to generate a human biomarker which can be used for screening the exposure to electrophilic metabolites of 1-MIM GLS .
We found two major adducts after in vitro incubations of individual amino acids, serum albumin or hemoglobin with 1-MIM GLS in the presence of myrosinase. These adducts were identified as histidine (His) adducts (τN-(1-MIM)-His and πN-(1MIM)-His) by MS-MS fragmentation pattern and NMR data.Consequently, we developed and validated an isotope-dilution UPLC-MS/MS method for the specific detection and quantification of these adducts. For this purpose isotope-labeled internal standards of τN- and πN-(1-MIM)-[15N3]His were synthesized. Hemoglobin and serum albumin samples were enzymatically hydrolyzed with Pronase E adding isotope-labeled standards of τN- and πN-(1-MIM)-[15N3]His. The adducts were isolated from unmodified amino acids and digestion mixture components by solid phase extraction or butanol extraction and analysed by UPLC-ESI-MS/MS within 10 minutes. MRM data were acquired in the positive ion mode using two mass transitions for each adduct. The analyses were conducted with an ACQUITY UPLC (Waters) connected to a Xevo TM TQ MS (Waters).
By means of this new analytical method we were able to identify and absolutely quantify τN- and πN-(1-MIM)-His adducts in samples resulting from in vitro and in vivo experiment. The dose dependence as well as the time course of adduct formation can be demonstrated in mice after oral treatment with 1-MIM GLS and Brassica-rich diet. Moreover, 1-MIM His adducts are also detectable in serum albumin and hemoglobin from humans, even without intervention with Brassica-rich diet.
In summary the developed MS method is a specific and sensitive procedure suited for the detection of histidine adductsoriginating from 1-methoxy-3-indolylmethyl glucosinolate. In order to evaluate whether this protein adducts can be used as biomarker of human exposure to the genotoxicant 1-MIM glucosinolate further studies with more subjects are necessary.
[1] Glatt et al., 2011, Chem. Biol. Interact. 192, 81-86
[2] Schumacher et al., 2012, Anal. Chem. 84, 6256-62621

Novel aspects:
Development and application of UPLC-ESI-MS/MS method for quantification of protein adducts originated from metabolites of the brassica constituent 1-methoxy-3-indolylmethyl glucosinolate

ThPS36-14 / Quantification of Micropollutants in Ground Water with LCMS-8050 - Qualifier and Quantifier ions with different Polarity Modes

Udo Burger, Pascal Looser
Shimadzu Schweiz GmbH

Quanti cation of Micropollutants in Ground Water with LCMS-8050- Quali er and Quanti er ions with different Polarity Modes

Udo Burger, Pascal Looser1
1Shimadzu Schweiz GmbH, Basel, Switzerland

Introduction
Micropollutants, like pesticides, pharmaceuticals, personal care products, chemicals, hormones, flame retardants and disinfection by-products have been monitored in surface water for many years. Tandem mass spectrometry (LC-MS/MS) has emerged as the gold standard in trace analysis of such polar and medium polar contaminants. Due to very low environmental concentrations, several multi-residue applications with solid-phase extraction (SPE) or large-volume injection (LVI) have been reported.

Methods
With direct aqueous injection-liquid chromatography/tandem mass spectrometry (DAI-LC/MS/MS), it has been demonstrated that a broad dynamic range of quantification and a low maximum residue limit (MRL 0.1 µg/L), as required by European Directive 2006/118/EC for individual pesticides and degradation products in ground water, is possible.

Results
Using triple quadrupole mass spectrometer LCMS-8050, a method for quantification of acesulfame, amidotrizoic acid, benzotriazole, carbamazepine, diclofenac, mecoprop, sulfamethoxazole, and tolyltriazole was developed. A LOD of 1 ng/L or lower was achieved. With a polarity switching time of 5 ms, signal behaviour of diclofenac in positive and negative mode was not influenced.

Conclusions
Direct aqueous injection-liquid chromatography/tandem mass spectrometry (DAI-LC/MS/MS) with LCMS-8050 allows quantification of the illustrated analytes with a LOD of 1 ng/L using different polarity modes in one method.

Novel Aspects
With very fast polarity switching it is possible to identify and quantify analytes using qualifier and quantifier ions from different polarity modes, without loss of information and sensitivity.

ThPS36-15 / Screening and Quantitation of About 250 Pesticides in Fruit Juices with Positive/Negative Switching LC/MS/MS

Zicheng Yang[1], Louis Maljers[1], Yann Hebert[2]
[1]Bruker Daltonics Inc., [2]Bruker Daltonique S.A.

Introduction:
Liquid chromatography coupled with tandem mass spectrometry operated in multiple reaction monitoring (MRM) mode with electrospray ionization (ESI) is widely used for polar, semi-volatile, and thermally labile pesticides in food testing. Many contract labs currently perform multi-residue analysis of pesticides using separate positive and negative methods due to instrument limitations especially for methods with hundreds of MRM transitions. This requires twice the sample and twice the analysis time. Recently, the Bruker EVOQ Elite LC-triple quadrupole system has been introduced to the market; thereby providing fast positive/negative switching allowing for simultaneous determination of positive and negative co-eluting compounds numbering in the hundreds.

Method:
The measurements were conducted by dilute-and-shoot without sample enrichment. The fruit juices were centrifuged and diluted 10-fold before injection. An YMC-Pack ODS-AQ, 3 µm, 150 mm x 3 mm (I.D.) column with mobile phases (A) 5 mM ammonium fluoride in water, and (B) methanol were used. The total run time was 18 minutes including re-equilibration.

Preliminary Results:
A study using the EVOQ analyzed about 250 pesticides in apple juice, cranberry juice, grape juice, orange juice and V8 vegetable juice using only one method with positive negative switching for about 500 MRM transitions. The preliminary results showed that both positive and negative co-eluting peaks have R2 >0.99 with linear range 0.1 to 100 µg/L.

Conclusion:
The Elite LC-triple quadrupole system was able to detect and quantify over 250 pesticides in one analysis using both positive and negative ionization modes in the analysis.

Novel Aspect:
Fast polarity switching for 500 MRM transitions for pesticide analysis in fruit juices

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ThPS36-16 / Comparison of Ionization Techniques for the Analysis of Trace-Level Pyrethroid Insecticides by GC/MS/MS

Ed George[1], Yann Hebert[2], Muntean Felician[1]
[1]Bruker Daltonics Inc., [2]Bruker Daltonique S.A.

Introduction:
Pyrethrins are natural insecticides derived from chrysanthemum flowers, and were used as the basis to create a class of synthetic pyrethroids. These pyrethroids are stable, toxic, and are more effective against a broader range of pests. They make their way into the environment through agricultural use and domestic control of mosquitos, and tend to persist in soils and sediments. Run-off from residential sources contributes to surface water and waste water treatment plants. State agencies such as the California Department of Food and Agriculture and others have implemented monitoring programs requiring low to sub-part-per-billion reporting limits in soil and water samples.

Method:
Tandem GC/MS is an ideal technique to analyze for the synthetic pyrethroids, because it can discriminate effectively against matrix and provide trace level detection. There have been several publications that have evaluated both ion trap and triple quadrupole instruments with either electron or chemical ionization. In many cases, relatively large sample pre-concentration and/or large volume programmed temperature vaporization (PTV) injections have been used to reach the required detection limits.

Preliminary Results:
The Bruker SCION triple quadrupole mass spectrometer has a unique axial ion source and lens free design, resulting in robust operation and excellent sensitivity for the pyrethroid insecticides. In this work, electron and chemical ionization techniques are optimized and compared in terms of calibration range, method detection limits, and precision. A standard hot splitless injection is used which reduces instrument matrix load and is far simpler than PTV. Discrimination against matrix components resulting from surface and waste water extracts will also be evaluated.

Conclusions:
The GC-triple quadrupole system successfully screened for pyrethrins in surface water and waste water extracts.

Novel Aspect:
Evaluation of matrix components in surface and waste water extracts for pyrethroid analysis using tandem GC/MS.

ThPS36-17 / Ultra High Performance LC-QTOF workflow for Multi-Residue Pesticide Screening using Diagnostic Ion Enhanced Confirmation Criteria

Peter Brechlin, Matthias Szesny
Bruker Daltonics GmbH

Introduction
In recent years, the improvements in sensitivity, dynamic range and resolution of accurate mass LC-MS instruments have extended their application to multi-residue screening workflows. A major advantage of this technology is the ability to screen for a potential unlimited number of analytes per run. However, manual visual inspection of thousands of extracted ion chromatograms per sample necessitates that this step is replaced by sophisticated screening software with automatic cross referencing to accurate mass databases for the reporting of positive findings.
We describe the application of a pesticide accurate mass database and associated screening and quantitation software on a high performance LC-QToF to screen for pesticide residues in tomato at levels below the current EU maximum residue level.

Methods
264 pesticides covering several compound classes were screened in this study. Tomato samples were extracted using a QuECHERS protocol and the extract was spiked at 0.5, 2.0 and 10 ppb levels. 10µl of the spiked extracts were injected onto a binary gradient, reverse phase UHPLC system using a 15 minute separation. Detection was carried out in ESI(+) mode on the impact HD LC-QToF MS using alternating TOF MS and broad band Collision Induced Dissociation (bbCID) modes to generate diagnostic ions (i.e. isotopes, adducts and fragments) for confirmation of pesticide residues. Data processing, mining and reporting was facilitated using a new quan/ qual screening software package that employs use of diagnostic ions within the workflow to reduce false positive findings.

Preliminary Data
Screening results for spiked tomato samples:
Using the generic QuECHERS protocol, all 264 pesticides were detected at the 10 ppb concentration level using the UHR-QToF system. At 2 ppb, 95.3% of the pesticides could be detected and at 0.5 ppb, 82.9% were detected. Quantitation of positive findings using the same screening data file was carried out thus allowing a simultaneous quan/qual workflow to be realized. Good linearity was observed for matrix matched calibration curves for all pesticides and limits of quantitation were as low as 50fg on column for the pesticide fluoaxstrobin.

Conclusion
The combination of True Isotopic Pattern (TIP) and fragmentation ions on a Ultra-High Performance LC-QTOF system has enabled an enhanced confirmation criteria for pesticide residue detection to be established. This in turn enables unprecedented levels of false positive detection rates of pesticide residues at the current European Union MRL in complex samples at mass resolution 20,000 to 30,000 FWHM. The successful application of a novel screening and quantitation software tool to simplify data processing is key to the facile deployment of this high resolution, accurate mass screening workflow.

Novel Aspect
Ultra-High Performance LC-QTOF system using a diagnostic ion enhanced confirmation concept reduces false positive findings of pesticide residues at the permitted European MRL

ThPS36-18 / Identification of low molecular weight thiols in plants by fluorescence derivatization and LC-MS/MS

Marta Fabrega Prats[1], Anna Rita Trentin[1], Antonio Masi[1], Stefano Dall'Acqua[2]
[1]University of Padua dept. DAFNAE, [2]University of Padua/ dept. Pharmaceutical and Pharmacological Scienze

Thiols are reduced sulphur molecules that occur both in plants and animals with relevant roles, as for example the modulation of oxidative stress and participation to enzymatic reactions.
Low molecular weight (LMW) thiolsare key biological molecules due to the intrinsic reactivity of their nucleophilic -SH group. They can modify the redox state of sensitive molecules by participating to reversible redox reactions. Moreover, they can conjugate or make complexes with xenobiotics and toxic compounds, and deactivate them; they can post-translationally modify regulatory enzymes and control metabolism. They may have important implications in food quality and safety, as well as in human health. A limited number of LMW thiols are described in literature, but ahuge amount of unknown thiols exists in plants. Their identification represents a major challenge, since they are at very low concentration in plant tissues.
In order to discover new un-identified thiols, as starting approach, extracts from plants were derivatized with SBD-F (ammonium 7-fluoro 2,1,3-benzooxadiazole-4-sulfonate) and analyzed by HPLC-fluorescence and LC-MS/MS in negative mode using an ion trap (Varian 500 MS), to obtain their fragmentation pattern. Known LMW thiols such as cysteine, homocysteine, glutathione, cysteamine, g-glutamylcysteine, N-acetylcysteine and cysteynilglycine were used as reference compounds. Also high resolution measurements were obtained on a Xevo G-2 Q-TOF mass spectrometer (Waters).
We found that after SBD-F derivatization, thiols can be easily recognized in fragmentation spectra due to the presence of SBD-S fragment (m/z 231), the fluorophore with attached sulphur. Therefore we used this signal as a marker to confirm the presence of thiol groups in unknown molecules. In this way we identified and further confirmed by Q-TOF analysis, the presence of thio-glucose and glutathione containing derivatives in Brassicaceae. Furthermore, extracts from different plant species showed distinctive thiol composition, indicating that such compoundsare species-specific. In maize leaves, we observed a light dependence of some of these unknown-LMW thiols, suggesting that they could be related to photosynthesis. Given their importance in plant metabolism, and due to the potential health benefits, LMW thiols deserve more attention from the scientific community.

ThPS36-19 / Screening Of Toxic Natural Substances in Herbal Products by Liquid Chromatography-Coupled Quadrupole Tandem Mass Spectrometry

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Plant herbs containing toxic natural substances are commonly used as effective ingredients in herbal products such as traditional medicines, health supplements etc. Due to the toxicity of the natural substances, many adverse drug reaction cases have been reported. As a result, we see the need to develop a method using liquid chromatography-coupled quadrupole tandem mass spectrometry for screening the toxic natural substances commonly occurring in herbal products. The instrument used in this study was AB Sciex QTRAP® 5500 mass spectrometer equipped with an Agilent 1290 liquid chromatography system. Multiple reaction monitoring (MRM) was used for the first cut screening and enhanced production ion (EPI) was used for the confirmation. Over 20 different natural substances such as aconite alkaloids, bufotenine, colcnicum alkaloids, digitalis glycosides, solanaceous alkaloids, tetrahydropalmatine etc, were successfully analyzed using this method. This method was then applied as a rapid and sensitive technique with limit of detection being less than 50 parts per billion for the screening of toxic natural substances in herbal products.

ThPS36-20 / Determination of Collision Cross-Section and Analysis of Isomeric Vitamin K1 Using Electrospray Ion Mobility Time-of-Flight Mass Spectrometry

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Introduction
Vitamin K1 is a kind of nutrients that the human body needs for post-translational modification of certain proteins required for blood coagulation, and in metabolic pathways in bone and other tissue. In general, all-trans-vitamin K1 plays an active role in physiological reactions and cis- one has no activity. Therefore, determination of all-trans- and cis- proportion in food or medicine will be beneficial to nutritional analysis. On account of separating isomeric vitamin K1 using HPLC or LC-MS/MS is complex, the potential for ion mobility mass spectrometry (IM-MS) to analyze vitamin K1 isomers rapidly without chromatography was investigated using SYNAPT G2.

Methods
Vitamin K1 was diluted to 20 µg/mL in hexane which was used as working solution. For minimizing isomerization and degradation, vitamin K1 was stored in tinfoil covered vials no matter of storage (-20°C) or experimental environment. IM-MS was carried out using a Synapt G2. Positive ion electrospray parameters were set as shown below: capillary voltage, 3.5 kV; sampling cone voltage, 25.0 V; extraction cone voltage, 4.0 V; ion source temperature, 90 °C; cone gas flow rate, 50 L/h; desolvation gas flow rate, 500 L/h; trap collision energy, 6.0 V; transfer collision energy, 4.0 V. Ion mobility separation conditions included as follows: trap gas flow rate, 5.0 mL/min; ion mobility gas flow rate, 20.0 mL/min (~0.51 mBar); wave velocity, 300 m/s; and wave height, ramping from 8.0 to 13.0 V. For CCS calculation, digested peptides of BSA were used for CCS calibration, and CCScalc 1.0 software was used for experimental CCS determination. Theoretical CCS value was obtained from PDB database.

Results
The sample was prepared and infused into the electrospray ion source at 2 µL/min along with 50 µL/min acetonitrile as a makeup solvent. The molecular ions of m/z 451.4 was detected and selected. Two drift time of 2.22 ms and 3.94 ms represented for all-trans- and cis- isomers respectively. Seven peptides digested from BSA protein were selected for calibration curve establishment. According to the calculate methods reported from previous published articles, CCS of two isomers were calculated, which were 172.6 and 264.1 Å2 for all-trans- and cis-isomer respectively. In recent, a series of infant formulas were selected and then all-trans-vitamin K1 component will be analyzed.

Conclusions:
Vitamin K1 isomers separation was demonstrated using IM-MS. Experimental CCS values calculated from drift time and mathematical models showed that it is a reliable analytical method. A single analysis only need few minutes besides the sample preparation procedures, hence, it will provide an effective and efficient analysis method for all-trans-vitamin K1 determination in the domains of food and medicine safety control.

Novel Aspect
1. A rapid and straightforward analytical approach for vitamin K1 isomers was established based on ion mobility technology;

2. IM-MS performed a stable and high-efficient analysis traits compared with LC or other relative separation methods.

ThPS36-21 / Residue patterns of flonicamid and its metabolites in paprika cultivated using manually pulled trolley sprayer

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Flonicamid is increasingly being used for aphid control of several agricultural commodities in developed countries such as EU, US and Japan. Maximum residue limit for flonicamid is included together with its metabolites such as TFNG, TFNA and TFNA-AM. Although flonicamid, a systemic pesticide, has a high controlling efficacy and residual activity, the studies on field residue pattern of the pesticide are insufficient. To investigate residue patterns of flonicamid and its metabolites (TFNG, TFNA) in paprika during cultivation, we sprayed the pesticide (SETIS, a.i. 10%) diluted 3,000 fold using a high pressure pump (INTERPUMP WS104, Italy) and manually pulled trolley sprayer equipped with fourteen fan type nozzles of one orifice. The spray conditions such as pump pressure, flow rate and travelling speed were 1,960 kPa, 8 L/min and 0.48 m/s, respectively. The pesticide residues were extracted by buffered EN15662 based QuEChERS method, and anlayzed using LC-MS/MS. The method quantitative limit of flonicamid, TFNG and TFNA were determined as 0.002, 0.005 and 0.01 mg/kg, respectively. The recoveries of the residues at two fortification levels (0.05, 0.2 mg/kg) were 75.2%~98.2%. Initial concentration of flonicamid right after pesticide spray was 0.028 mg/kg, then detected 0.033, 0.020, 0.020 and 0.003 mg/kg after 10, 20, 30 and 40 days of pesticide application, respectively. The residual concentration of TFNG was 0.005 mg/kg after 10 days, then steadily increased 0.028, 0.068 and 0.075 mg/kg after 20, 30 and 40 days, respectively. Total flonicamid residues including its metabolites (TFNG and TFNA) during this study increased from 0.022 mg/kg (5 days) to 0.103 mg/kg (30 days), then lasted up to 60 days at the level of 0.067 mg/kg. Our study shows that total flonicamid in paprika sprayed by manually pulled trolley sprayer were increased over 30 days after application, probably due to translocation of the pesticide and its metabolites within plants. This study suggests that flonicamid and its metabolites would be highly accumulated in paprika fruits by spraying the pesticide on whole plants.

ThPS36-22 / GC-MS/MS and LC-MS/MS

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Pesticide residue analysis on food samples is essential for the protection of human health but also to guarantee international trade and to comply with regulatory controls. More than 1000 substances of different chemical groups that are active against pests - triazoles, strobilourines, carbamates, pyrethrins, organophosphates, sulfonylureas, triazines and others - are currently used worldwide. EU adopted Regulation -396/2005 for the maximum permissible pesticide residues in food, the majority of which ranges over the concentration range 0.01-1 mg / kg. For the control of residues of these categories of plant protection substances in different types of food commodities there is the need to develop and validate large scale multi-residue methods based on general extraction procedures in combination with hyphenated instrumental analysis techniques such as - gas and liquid chromatography with mass spectrometry [1].
This work shows the development and validation of multi-residue methods for the detection and quantification of pesticides -parent compounds and transformation products -from different chemical classes, in various food matrices, usinggeneral extraction procedures such as QuEChERS in combination with LC -MS/MS and GC-MS/MS techniques fulfilling the performance criteria described in the European Union guidelines (SANCO Doc. No 12571/2013 [2]).
During LC -MS/MS and GC-MS/MS methods development, the influence of selected per case analytical operating parameters on pesticide response was thoroughly investigatedin order to achieve the highest possible analytical sensitivity, accuracy and detectability in the complex food matrices. A LC-MS/MS method using QqQ-MS technology has been developed for the simultaneous analysis of 140 compounds based on SRM acquisition data. Additionally a GC-MS/MS method using Ion-Trap MSn technology has been applied for the determination of 60 GC-amenable compounds. More than 2500 pesticide/food commodity combinations have been validated using both LC- and GC-MS/MS methods, with limits of quantification of 0.01mg/Kg in most of the cases. Recoveries in the range 70-120% with relative standard deviations less than 20-25% have been achieved. Both methods have been successfully applied for the monitoring of multiclass pesticide residues in more than 1500 food samples enhancing the capacity and the productivity of the laboratory. Results of our monitoring program are included in Annual Report of European Food Safety Authority (EFSA).

References

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ThPS36-23 / Demonstration of Two-Dimensional Liquid Chromatography for the Elimination of Matrix Effects in the Food Analysis

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Introduction

One of the current issues in the food analyses from a variety of food sources is to overcome the matrix effects which affect the quantification in LC/MS experiments suppressing the MS signal of the analytes. A conventional liquid chromatography (1D LC) has shown the limitation in separating target compounds from the matrix due to its relatively low resolving power requiring a long gradient time to improve the problem. Hence, a two-dimensional liquid chromatography (2D LC) has been come up with the alternative. Here, we present the demonstration of our 2D LC system as the first step of building LC system to eliminate the matrix effects. The performance of our 2D LC system was first demonstrated with C18 stationary phases using a chicken sample with fluoroquinolones that causes severe matrix effects.

Method

Fluoroquinolones standards (norfloxacin, ciprofloxacin, enrofloxacin) were mixed with one gram of chicken breast milled finely with a grinder. The fluoroquinolones were extracted from the mixture using consecutive acetonitrile and n-hexane liquid-liquid extraction methods and then the extract was cleaned up using a SupelMIPTM SPE-Fluoroquinolons cartridge and an Oasis MAX cartridge.
2D LC system was constructed based on the Waters I-class LC system. Three six-ports valves (sample injection valve, #1, #2) and two binary pumps (α-, β-pump) were used for the configuration of the system and multiple reaction monitoring (MRM) method was

used for MS analyses. Briefly, the sample was loaded and then separated in the first dimension column (C18) connected to #1 with mobile phase delivered by a α-pump. The eluate containing one of the fluoroquinolones was diluted with mobile phase from a β-pump through a tee connected to #1, #2 and second dimension column (C18) to stably isolate onto the second dimension column which is connected to #2. Elution times for all fluoroquinolones from the first dimension column were identified by pre-testing of the LC/MS experiment during which all the eluates were flowed to MS instrument via #2. The #1 was switched to waste while undesired compounds were eluted. LC/MS experiment of the isolated compound was performed by the gradient of mobile phases delivered by a β-pump. The result was compared with the one of 1D LC/MS of the same sample.

Results
The 2D LC system showed highly reproducible sample analyses in terms of peak shapes and intensities of the analytes in the analysis of fluoroquinolones from chicken sample.

Conclusions
We implemented the demonstration of performance of our 2D LC system as a first step to eliminate matrix effects in the analyses of a variety of food sources. We are looking for the best combination of the stationary phases for successful analyses of the fluoroquinolones using meat sample and expecting to have the result that the matrix effects are eliminated through our 2D LC system before IMSC.

Novel Aspect
Our 2D LC system allows the operation of both 1D and 2D LC without any modification of the system for the effective LC/MS experiments with showing highly reproducible sample analyses.

ThPS36-24 / Development of a rapid method for the analysis of anabolic steroids and stilbenes in bovine muscle using liquid chromatography tandem mass spectrometry

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Introduction
Over the years time-consuming procedures based on GC-MS with derivatisation were used in order to achieve the aim of monitoring for the use of illegal growth promoters in meat samples. More recent the LC-MS/MS procedures without derivatisation step have been developed. But the sample preparation for these methods is complicated because it involves two different solid phase extraction steps.
The aim of this study was the development of a rapid and sensitive method for the analysis of different growth promoters in meat using LC-MS/MS.

Method
Muscle samples (5.0g) were weighed into a 50 ml centrifuge tubes. Samples were fortified with mix of internal standards at a level corresponding to 10 µg kg−1. Then 6 mL of sodium acetate buffer was added and the samples were homogenized with a WiseTis homogenizer for about 1 min. The mixture was extracted with 15 ml TBME (10 min rotating and centrifuged at 3000 rpm). The organic layer was evaporated to dryness and the residue was redissolved in 1.0 mL of methanol/water (80/20, v/v). The mixture was washed with 3 ml of hexane for defattening. Then the hexane layer was decanted and the sample was transferred to a vial and analyzed by LC-MS/MS.
A binary solvent delivery system (Eksigent UltraLC-100, Eksigent, USA) and a hybrid quadrupole mass spectrometer (QTRAP 5500, AB SCIEX, Toronto, Canada) were used. Separation of analytes was carried out on a Pursuit 3 C18 column (150 mm×2.1 mm, Agilent, USA). Samples were injected twice: once in positive polarity mode for triamcinolone acetonide, chlormadinone acetate, betamethasone, dexamethasone, methylboldenone, methyltestosterone, β-testosterone, medroxyprogesterone acetate, megestrol acetate, progesterone, medroxyprogesterone and once in negative one for diethylstilbestrol, dienestrol, hexestrol, prednisolone, methylprednisolone.

Results
The method was validated in the range of 0.5 – 30 µg kg-1 for all analytes.The validation experiment was based on full factorial design for two factors and consisted of 4 runs. The specificity of the method was demonstrated as no interfering peaks were observed at the retention time of analytes in a variety of blanks. The recovery, corrected by matrix-matched calibration and by the use of internal standards, lies in the range of 75 – 110 % for all analytes. The combined uncertainty falls below 40% for all analytes. The method proved to be robust with regard to the two factors: operator and storage time for extracts after sample preparation.

Novel aspects
A rapid and sensitive LC-ESI-MS/MS method for the determination of stilbenes, gestagens, corticosteroids and androgens in bovine muscle has been developed and validated. The sample preparation of the developed method includes liquid-liquid extraction with TBME and defattening with hexane thus making it more suitable for routine analysis in comparison with previously published methods.

ThPS36-25 / Quick and sensitive analysis of multiclass veterinary drug residues in animal products using a benchtop Orbitrap mass spectrometry system

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Introduction
Usually the analysis of veterinary drugs in animal products is a time consuming process with respect to sample preparation as well as mass spectrometric analysis. The quantitative analysis of 45 multi-class veterinary drug residues from meat products and waste usually involves not only different extraction methods either with SPE or LLE extraction, but also multiple sample injections. A new method, utilizing ultra fast chromatography and a bench top Orbitrap mass spectrometer is described in this study. The advantage to this approach is a short overall analysis time and to have a robust method to meet future regulation requirements, offering all options for additional targeted and non-targeted screening.

Method
10 uL injections of extracted meat and urine containing many veterinary drugs were injected onto C18 reverse phase column. All compounds of interest were eluted using one standardized fast gradient elution profile. A bech top quadrupole Orbitrap mass spectrometer with a heated electrospray source (HESI) was used to analyze the compounds of interest in positive ionization mode. All method development, data acquisition, data processing and reporting were done using one customized software application.

Preliminary Data
45 multi-class veterinary drug residues (Abamectin, Amoxicillin, Ampicillin, Benzylpenicillin (G), Cefalexine, Cefalonium,

Cefapririm, Cefoperazon, Cefquinom, Chlortetracyclin, Ciprofloxacin, Cloxacillin, Danofloxacin, Dapson, Difloxacine, Dimetridazole, Doramectine, Doxycycline, Enrofloxacine, Eprinomectine, Erythromycine, Flumequin, Ipronidazol-OH, Ivermectin, Marbofloxacine, Metronidazole, Metronidazole-OH, Moxidectin, Nafcilline, Oxacilline, Phenoxymethylpenicillin (V), Ronidazol, Sarafloxacine, Sulfadiazine, Sulfadimethoxine, Sulfadimidine (Sulfamethazine), Sulfadoxine, Sulfamethoxazole, Sulfamethoxypyridazine, Sulfathiazole, Tetracycline, Thiamphenicol, Tilmicosine, Trimethoprime, Tylosin) from meat products and waste were analyzed in one standardized chromatographic and mass spectrometric method. For quantification, standard curves with eight calibration points were prepared covering the range 100 pg/mL (ppt) to1 µg/mL (ppm). Quasi molecular ions were monitored for quantitation, while additionally up to five fragment ions were monitored for qualification, achieving linear calibration curves over the ranges described above. In this assay, a quick and robust sample preparation method is combined with one short generic analysis method for all compound classes. Together with a very short method development time this new approach stands for high productivity and robustness at the same time. In addition, we could show the potential of this method for additional successful targeted and non targeted screening approaches inside the same data processing software using the same data set.

Novel Aspect
Reduced development and analysis time for multiclass veterinary drug residues in accordance to EU guidelines using HRAM MS detection

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ThPS36-26 / Eliminating matrix effects during multi-residue pesticide analysis by extensive dilution using the high-sensitive 6495 triple quadrupole MS

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Introduction
Pesticides testing in food is the most important analysis in food safety laboratories. Most pesticides are analyzed with multi-residue methods containing hundreds of compounds for screening and quantitation. Matrix effect is a major concern as it hampers accurate quantitation. Sample dilution is one approach to minimize matrix effects but requires the use of highly sensitive analytical instruments as maximum residue limit (MDL) levels stipulated by the EU need to be undercut. In this work we evaluate a newly developed high sensitivity triple quadrupole LC/MS for multi-residue pesticide analysis in extensively diluted food samples.

Method
Extraction was performed using QuEChERS. >250 pesticides with high risk relevance were selected. Extracts were spiked with pesticides at 0.005, 0.05, 0.5, 5 and 10 µg/kg. Samples were diluted 1:10, 1:20, 1:50 and 1:100 with acetonitrile and injected into the LC/MS/MS system. Chromatography was performed under gradient conditions using water and methanol containing ammonium formate. Detection was performed using triple quadrupole LC/MS operated in dynamic MRM mode with polarity switching. Two transitions are monitored per compound.

Preliminary Data
A method for the analysis of more than 250 pesticides has been developed and applied to the analysis of different food commodities including e.g. tomato, orange, and black tea. For matrices with significant matrix effects, samples were diluted up to 100-fold. The sensitivity of the method was characterized by the method detection limit (MDL) which is based on the precision of analysis at low concentrations. The newly developed ion optics and detector allowed the quantitation of majority of the pesticides at 10% of MRL levels even with 50 to 100-fold dilution. Matrix effects after 50-100x dilution could be neglected. With the improved sensitivity and precision, accurate quantitation was achieved even with such high dilution factors. Correlation coefficients (R2) for calibration curves were higher than 0.99, and area RSD [%] were less than 20%. Accuracy for the diluted samples was within 70 to 120%, which is in good agreement with the guidance document SANCO/12571/2013. > 50 pesticides of different chemical classes with high risk relevance, i.e. listed as high in "Check your scope" ranking, were selected for this evaluation covering the full polarity range and including pesticides classified as difficult to analyze due to their low proton affinities, thermal lability or susceptibility to matrix effects.
The efficient UHPLC separation combined with the highly sensitive QQQ MS detection resulted in minimized matrix effects by diluting the sample extracts up to 100-fold. Even with the extensive sample dilution, LLOQs are achieved at or below the MRLs for most of the pesticides. Using sample dilution, a lower amount of matrix was introduced into the LC/MS system leading to improved assay robustness.

Novel aspect
High sensitivity MS detection allowed extensive sample dilution in a multi-residue pesticide analysis while still confidently achieving the MRLs

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ThPS36-27 / Investigation of nicotine contents of chamomile samples by HPLC/TOF-MS method

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Three different reasons may lead to nicotine content in plants:

i biosynthetic processes in the plants,
ii pesticide residues,
iii the treatment of the plants.

An ordinary nicotine level for example is 2-5 µg/kg in Solenaceae.
HPLC-MS system: Waters 1525 pump, 717 autosampler, Agilent 6210 TOF-MS
Eluent:10 mM ammonium acetate–methanol–acetonitrile (15:32:53)
Column: Kinetex C18 100x4,6 2,6µm. Flow rate:0,6 ml/min.
Sample preparation: 1 g sample was extracted with 0.1 N ammonium solutions, then it was extracted into chloroform. After drying it was soluted in acetonitrile.
The nicotine content of chamomile samples was 1-3 mg/kg. It is several times higher than the limit value.
This fact indicates that chamomile consumption increases the total daily amount of nicotine intake.
In the future we will be investigating the effects of different chamomile species and the effect of different treatment methods.

ThPS36-28 / Determination of 8 Estrogens in Milk by the 1290 UHPLC and Highly Sensitive 6495 Triple Quadrupole Mass Spectrometer

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Introduction
Human exposure to estrogens through the consumption of milk and dairy products is of increasing concern. The estrogens in milk impact child development and cause cancers. Routine determination of estrogens in milk or dairy products is required by many governmental agencies. Estrogens, which are hard to

ionize in MS, pose significant analytical challenge as maximum residue level (MRL) is set low. We demonstrate that a highly sensitive 6495 triple quadrupole MS allows the measurement of 8 estrogens at 10x below MRL without the necessity of enrichment or derivatization.

Method
Eight estrogens include estriol, estradiol (17-α-estradiol and 17-β-estradiol), estrone, dienestrol, ethynyl estradiol, diethylstilbestrol, and hexestrol. The sample preparation employs simple QuEChERS kits for animal tissue. The analytes spike levels were 0.1, 0.2, 0.5, 1, 2, 5, 10µg/kg. After dispersive SPE, 4mL of supernatant was dried under nitrogen and reconstituted with 2 mL of 50% methanol water. Chromatography was performed under gradient conditions using water containing NH4F and acetonitrile. Detection was performed using a newly designed 6495 triple quadrupole MS operated in dynamic MRM and negative mode.

Results
The sensitivity of the instrument is characterized by the method detection limit (MDL) which is calculated based on the precision at low concentrations. The MDLs for 8 estrogens ranged from 1.2 to 3.7ppt. The LLOQs ranged from 5 to10ppt. The linear calibration range for estrogens in milk was from 0.1µg/kg to10µg/kg. The LOQs for estrogens in milk were much less than the lowest spike concentration at 0.1µg/kg. The milk blank contained small amount of estrone, but its quantity was far less than the 0.1 µg/kg spiked sample. The correlation coefficients (R2) from 0.1µg/kg to10µg/kg at 7 levels were above 0.997. The QuEChERS recovery and reproducibility were evaluated at 0.2µg/kg, 1µg/kg and 5µg/kg spike levels with 6 replicates per level. The recoveries ranged from 83 to 106%. Reproducibility was characterized as RSDs of 6 replicates for each analyte at three spike levels. RSDs were excellent for all 8 estrogens as single digit, except for hexestrol and diethylstilbestrol at 5µg/kg, which are 13, and 15 respectively.

Conclusion
The high-sensitivity 6495 triple quadrupole MS is a potential tool for estrogens measurement in food matrices where MRLs are low. The advanced instrumentation allows the use of simpler sample preparation procedures as significant enrichment or derivatization is unnecessary. The impurities and observed matrix effect were minimal for the milk blank. This method has potential applications for the measurement of estrogens in other similar matrices, such as milk powder.

Novel Aspect
High sensitivity MS detection allowed direct measurement of estrogens in milk without enrichment or derivatization while still achieving the MRLs.

ThPS36-29 / Multi-residue method for the determination of 262 pesticides in crops by gas chromatography- mass spectrometry

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Gyeongin MFDS

Introduction
The Gas chromatography-mass spectrometry(GC-MS/MS) multi-residue method for the analysis of 262 pesticides in vegetable, fruits and cereals has been developed. The monitoring of pesticide residues in food is nowadays a priority objective in order to get extensive evaluation of food quality and to avoid possible risks to human health. The pesticides included in this study belong to different chemical families of insecticides, herbicides, fungicides and so on. GC-MS is rapidly becoming an accepted technique in pesticide residue analysis for regulatory monitoring purposes. This survey was aimed to establish for determination of multi-residue method for 262 pesticides in commercial agricultural products by GC-MS/MS.

Method
Briefly, the homogenized samples were mixed with 1% acetic acid in acetonitrile. The mixture was vigorously shaken, then added sodium chloride and repeated the shaking process, and cleanup the resulting extract with dispersive solid-phase extraction using primary-secondary amine and MgSO4. After a mixing and centrifugation step, the extracts were transferred to vials for analysis by GC-MS/MS. Pesticides were confirmed by their retention time and multiple reaction monitoring of two fragment ions by GC-MS/MS.

Results & Conclusions
Recoveries, precision, linear dynamic ranges, and limit of quantitation (LOQ) in the analytical method were validated in different matrixes. Calibration curves were linear for most of the compounds studied. The method was demonstrated by the analysis of extracts from crops, spiked at three concentration levels for each pesticide. The recoveries obtained at fortified levels of 0.05-0.5 mg/kg were 60-130 % for all pesticides, with relative standard deviations (RSDs) of £20 %, with a few exceptions. The proposed method was applied successfully for the residue determination of the 262 pesticides in crops.

Novel Aspect
A GC-MS/MS multi-residue method for the simultaneous target analysis of 262 pesticides in various crops.

ThPS36-31 / Vitamin B complex detection in infant formula by LC/MS/MS

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AB SCIEX

Introduction
Vitamin B complex is a group of water-soluble vitamins that play important roles in cell metabolism, absence of individual vitamins in a diet can lead to several conditions including depression and high blood pressure so they are often added to foods especially infant formula. Vitamin B is a complex mixture of different compounds each structural different. Traditionally individual methods have been used to screen for each vitamin B so one method that is capable to screen for several vitamin B compounds in a single analysis would be beneficial.

Experimental
Here we present some new data acquired by LC/MS/MS with a screening method which contains all the major forms of Vitamin B. The required detection limits vary greatly between each vitamin B and range from low parts per billion to low parts for million levels.

Results & Discussion
The method has therefore been developed to detect all the vitamins in the required ranges and has meant that some transitions have had to be detuned to maintain their linear response and enable one simple extraction for all. The LC/MS/MS uses reverse phase chromatography and positive mode electrospray ionisation and meet the requirements of all the limits of detection. The mass spectrometry methods utilises Scheduled MRM™ and a small particle size HPLC column. NIST reference material was extracted and then simply diluted and analysed by LC/MS/MS to show the applicability of this method to routine sample analysis.

ThPS36-32 / HPTLC combined with ambient mass spectrometry: Current trends in food & natural product analysis

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Introduction

Regulation of dietary supplements and rapid screening for their quality and potential adulteration, in particular with regards to regulated pharmaceuticals, including stimulants, has become an area of interest for regulatory bodies, as well as the manufacturers who need to protect their brand from counterfeiters. Ambient mass spectrometry (MS) offers a high level of sensitivity and specificity combined with high resolution MS with the advantage of extremely rapid sample screening. Combining high performance thin layer chromatography (HPTLC) separation with direct MS analysis allows the analyst to more concretely identify compounds from complex mixtures and matrices. With direct monitoring of herbal supplements, manufacturers and regulatory bodies are able to quickly pinpoint counterfeits and alert the consumer public.

Methods

A Direct Analysis in Real Time (DART) ambient ionization source was coupled to a high resolution accurate mass (HRAM) Orbitrap mass spectrometer for MS screening with all ion fragmentation (AIF). High performance thin layer chromatography (HPTLC) silica gel glass backed plates were prepared from polar (methanol) and non-polar (hexane) extracts of several marketed herbal supplements suspected to contain regulated active pharmaceutical ingredients. The HPTLC plates were developed (EtOAc:MeOH:H2O eluent for polar extracts and toluene eluent for non-polar extracts) and then cut using a CAMAG smartCUT plate cutter to bisect the developed spots. The cut HPTLC plates were mounted onto the DART source sampling stage via a customized holder and directly analyzed as the bisected spots passed directly into the DART ionization region.

Results

A number of marketed herbal supplements were screened using this direct HPTLC ambient MS method and were found to contain several stimulants including dimethylethanolamine, caffeine, synephrine and hydroxyamphetamine. The TLC separation adds a layer of confirmation based on Rf values from standards and permits clean direct MS spectra. The plate scanning is automated yielding MS chromatographic timing that can be compared against standards. An overview of publications and current trends on the topic of combining HPTLC with ambient mass spectrometry will also be shown.

Novel aspect

Direct TLC high resolution ambient-MS analysis for rapid mass spectral characterization of dietary supplements.

ThPS36-34 / Multi-residue screening method of 47 veterinary drugs in fishery products by ultra performance liquid chromatography–tandem mass spectrometry

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Introduction

Antibiotics are the most important drugs used in veterinary medicine to treat mainly bacterial diseases. They are also often administered as growth-promoting substances. Their use in food producing animals may result in antibiotics residues in edible tissues, which are monitored to protect human and animal health, support the enforcement of regulations, provide toxicological assessment data and resolve international trade issues.

For monitoring and enforcement purposes, multi-residue methods, i.e. methods capable of analyzing residues of multiple compounds, are favored by regulatory and other food testing laboratories because of their time and cost-effectiveness.

We developed a multi-component quantitative screening method using a ultra-high-performance liquid chromatography coupled with tandem mass spectrometer (UHPLC–MS/MS). The screened veterinary drugs belonged to amphenicols, β-lactams, lincosamides, macrolides, quinolones, sulfonamides and tetracyclines.

Methods

The drugs were extracted from eel, flatfish, and shrimp muscle samples with acetonitrile–water (4:1, v/v) containing 2 mM ammonium formate, and the extracts were applied to the dispersive solid phase extraction and n-hexane clean-up procedure. Reverse-phase LC separation was accomplished on a C18 column and gradient elution with a mobile phase consisting of water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid.

The separated compounds were detected with a tandem quadrupole mass spectrometer operating with an electrospray ion source (ESI) in positive and negative switching mode by applying a time scheduled multiple reaction monitoring of 2 or 3 transitions. The calibrations were performed in sample matrix and the interference effect of sample matrix on the ionization was effectively eliminated.

Results

The confirmation according to Commission Decision 2002/657/EC with the retention time was successful in all compounds. Good linear relationship (R2>0.98) was observed within the concentration range of 6 calibration points (0.25, 0.5, 1.0, 2.0 and 4.0 times VL including blank). Average recoveries at three fortification levels from 0.5, 1.0 and 2.0 times VL were 85.1~106.2 % in three muscle matrices. The percent relative standard deviation for the described method was less than 15.0 % over the range of concentrations studied. The limits of quantitation(LOQs) were ranged from 1/10~1/1 000 of VLs.

Conclusions

The multi-residue method was developed for screening of veterinary drugs in a single analytical run. The method comprising a simple sample preparation and the measurement by UHPLC–MS/MS allowed simultaneously the screening of 47 compounds belonging to 10 groups of veterinary drugs in eel, flatfish, and shrimp muscle. The results of this study was validated according to the EU Commission Decision 2002/657/EEC for a quantitative screening method. All parameters have acceptable values and are in agreement with the criteria of Commission Decision 2002/657/EC.

In short, we believe the proposed method can be successfully used in screening studies for veterinary drugs in fisheries.

ThPS36-35 / Proton-Transfer-Reaction Mass Spectrometry for the study of the aromatic impact of yogurt starter cultures

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Introduction

Proton-Transfer-Reaction Time-of-Flight Mass Spectrometry (PTR-ToF-MS) presents several characteristics of interest for food research and innovation, including a “soft” ionization by means of a pure beam of hydronium ions, coupled to the high time and mass resolution guaranteed by the ToF mass analyzer. In reason of these characteristics, PTR-ToF-MS allows to monitor,

in non-invasive fashion, also the changes in volatile profile during microbial fermentation of food matrices. Lactic Acid Bacteria (LAB) play a key role in the production of fermented foods/beverages and human health.

Methods
In the present work a matrix constituted by heat-treated (30 minutes at 80°C) low-fat milk was inoculated with different yogurt starter cultures (Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus). Micro-lactic acid fermentations of milk were carried out and monitored in automated fashion by means of a multifunctional autosampler. In a typical experimental setup, this allowed to follow five different experiments, with a time resolution of three sampling points/hour and minimal need for manual operation.

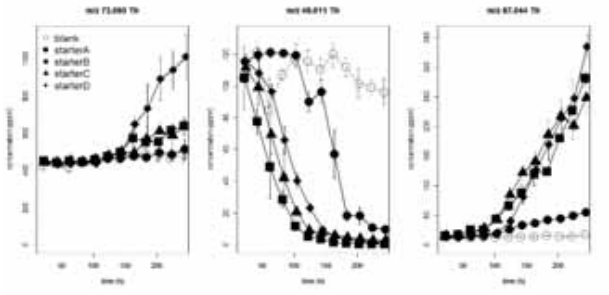

Figure 1 - Time evolution for three selected mass peaks. Tentative identifications: m/z 73.065 Th = 2-butanone, m/z 49.011 Th = methane-thiol, m/z 87.044 = diacetyl.

Results
The experimental design allowed to monitor the kinetics of production and depletion of volatile compounds of major importance in the manufacturing of fermented dairy product both from technological and sensory point of view. Important differencesin volatile compound profile among starter cultures were observed.

Conclusion
PTR-ToF-MS, along with the autosampler/micro-fermentation apparatus and tailored software packages for data analysis, constitute an integrated platform for the rapid and online monitoring of food fermentations and, more generally, of food processes.

Novel Aspect
This work provides an example of the applicability of PTR-ToF-MS as a valuable tool for the real-time monitoring of micro-lactic acid fermentations and for the rapid screening of LAB strains of industrial interest.

ThPS36-36 / MSE strategy for characterization of phenolic compounds

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Euskal Herriko Unibertsitatea (UPV/EHU)

Introduction
Previous studies on the characterization and identification of phenolic compounds in fruit and plant extracts have shown that high performance liquid chromatography coupled with diode array detection and mass spectrometry (HPLC-DAD-MSn) is a powerful tool for structure elucidation. However, with the emergence of UHPLC-coupled ToF or Q-ToF instruments, it is possible to develop a highly attractive analytical method with very high resolution and accurate mass measurements of the precursor and fragment ions.

Methods
An ultrahigh performance liquid chromatography 18 min method was developed using an Acquity UPLC BEH C18 (1.7 mm, 2.1 x 100 mm) column. Flow rate was 0.35 mL/min, column temperature was set at 40 ºC and injection volume was 5 mL. All MS data acquisitions were performed on a quadrupole-time of flight analyzer equipped with an electrospray ionization source operating in both positive and negative modes. Leucine-enkephalin solution was used as the lock mass. To perform MSE mode analysis, two discrete and independent interleaved acquisition functions were created. The first function collects low energy or unfragmented data while the second function collects high energy or fragmented data. MS1 and MS2 product ion mode analyses were performed as well to compare the fragmentation data.

Results
The spectral data for phenolic compounds obtained using this acquisition mode are comparable to those obtained by conventional LC-MS/MS as exemplified in this work.
Application of this analytical strategy has been exemplified with apple products (fruit, pomace and juice). 52 phenolic compounds of five different classes were readily characterized in apple extracts in both positive and negative ionization modes. Among the phenolic compounds identified these samples, 2 dihydrochalcones and 3 flavonols have been tentatively identified for the first time in apple products.
MSE spectral data acquisition overcomes chromatographic co-elution problems, performing simultaneous collection of fragmented and unfragmented ions from precursor ions, which allows resolving complex spectra from mixtures of precursor ions in an unsupervised way and eases their interpretation.

Conclusions
The usefulness of MSE as an untargeted methodology for identification of phenolic compounds in complex fruit and plant samples in just one injection has been clearly demonstrated. MSE can differentiate product ions from a precursor ion collecting them in a single analytical run, obtaining similar information to MS/MS.

Novel Aspect
A new, rapid and selective ultrahigh performance liquid chromatography with diode array detection coupled to electrospray ionization and quadrupole time-of-flight mass spectrometry (UHPLC-DAD-ESI-Q-ToF-MS) strategy using automatic and simultaneous acquisition of exact mass at high and low collision energy, MSE has been developed for the study of phenolic profiles in complex fruit and plant samples.

ThPS36-37 / Analysis of 200+ Pesticides in a Short LC Run Using Non-Timed SRMs on Triple Quadrupole Mass Spectrometer

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Thermo Fisher Scientific

Introduction
Increasing food safety concerns and the growing agricultural trade have resulted in stringent pesticide regulations globally. To comply with strict food safety standards, fast screening and quantitative methods for large numbers of pesticides are becoming important. Tandem quadrupole mass spectrometry offers highly specific and selective detection. However, it is also limited by intra-scan delays and dwell times required to get the maximum sensitivity and reproducibility. Therefore timed-SRMs are needed for analyzing large numbers of analytes. This poster describes a method for rapid analysis of 200+ pesticides in food samples using a triple quadrupole mass spectrometer without the need for setting up a specific time window for each analyte.

Methods
A Thermo Scientific Hypersil GOLDTM column (100 x 3 mm, 3mm) was utilized for the separation of all analytes. The mobile phases were 0.1% formic acid in water and 0.1% formic acid in acetonitrile, respectively. Detection was performed using a Thermo Scientific TSQ Endura™ triple stage quadrupole mass spectrometer. The food sample was extracted with organic solvent using a QuEChERS method.

Results
A multi-residue method was developed for screening and quantitation of 200+ pesticides/500SRMs in peach matrix using a short LC run (10 minutes). One or two ion ratios were used to confirm each analyte. Calibration curves from 0.1 to 100 ppb were generated for the pesticides analyzed. This instrument is able to perform at 500 SRM/sec. data acquisition rate, which allow us to eliminate the need to set up a specific time window for each compound. 200+ pesticides were monitored throughout the entire LC run. The method development was significantly simplified. Furthermore, the method was developed using software with built-in workflows for streamlining method development and routine analysis. The experiment results will be discussed in detail.

Conclusions
The ultra fast data acquisition rate of 500 SRM/s allowed us to improve productivity by simplifying method development, especially for large compound lists and shortening LC run time. The non-timed SRM method can also accommodate shifting retention times in different matrices.

Novel Aspect
Analysis of 200+ pesticides/500SRM using non-Timed SRMs in short LC run (10 min) on triple quadrupole mass spectrometer

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ThPS36-38 / The structural identification of cereal-based arabinoxylooligosaccharides by ESI-MSn

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University of Helsinki

Multiple-stage mass spectrometry (MSn) is a useful tool for structural analysis of oligosaccharides. In MSn analysis the structural characterization is based on fragmentation pattern of an ionized analyte.

In present study MSn method was used for structural analysis of isomeric arabinoxylooligosaccharides (AXOS) isolated from wheat and rye arabinoxylan hydrolysate. Arabinoxylan is common plant cell wall polysaccharide. In cereal grains arabinoxylan is structured by β(1→4)-linked xylopyranosyl residue backbone which is usually mono- or disubstituted by α(1→2)- and α(1→3)-linked arabinofuranosyl residues, but also β-D-Xylp-(1→2)-α-L-Araf-(1→3)- substituents and (Me-4-O)-α-D-GlcA-(1→2)-substituents has been found.
The AXOS were enzymatically hydrolyzed and isolated from wheat and rye arabinoxylan and analyzed with HPAEC-PAD, MALDI-TOF-MS and NMR as reported earlier [1,2]. At first stage of present study the underivatized AXOS of known structures were analyzed with positive and negative ESI-IT-MSn as sodium and chloride adduct ions. At next stage two sample derivatization techniques, reduction and permethylation, were tested.
Preliminary results show that by using negative ionization and chloride adduct formation the MSn analysis can be used for identification of underivatized AXOS. Also permethylated oligosaccharides produced characteristic fragment ions from different isomers.

Novel Aspect
The MSn method for identification of arabinoxylooligosaccharides by using negative ionization and ammonium chloride adduct formation is introduced.

[1] Rantanen et al. 2007 Carboh Polym 68, 350-359
[2] Pastell et al. 2008 Carboh Res 343, 3049-3057

ThPS36-39 / Fast SRM Transition Speeds for High Sensitivity, High Capacity and Selective Multi-Residue Pesticide GC-MS/MS Analysis

Paul Silcock, David Steiniger, Cristian Cojocariu, Jason Cole
Thermo Fisher Scientific

Introduction
The increased accessibility of high selectivity GC-MS instrumentation has enabled more generic sample preparation approaches in pesticide residue testing. Taking a generic approach allows the possibility to consolidate a large number of pesticides and matrices into extensive multi-analyte methods when using GC triple quadrupole MS. However, these high capacity methodologies often lead to a compromise in instrument sensitivity due to lower dwell times for individual SRMs. Many users compensate for this by decreasing quadrupole resolution, an approach which increases the risk of interference, especially in more complex matrices. This work address the need for high sensitivity, selectivity and method capacity using GC-MS/MS with faster collision cell technology uniquely combined with smart software tools.

Method
Thermo Scientific GC-MS/MS was used at unit mass resolution in conjunction with TraceFinder EFS software to construct a multi-analyte MRM method for a large number of pesticide residues. This method was then used to measure 10 ppb spikes in food extracts generated using the popular QuEChERS technique. The low level quantitative performance was determined for in measured matrices at both high and low transition speeds. Statistically calculated LODs for this method were compared along with a comparison between a timed-SRM and the classical segmented approach. These comparisons were made, and will be discussed, with respect to the levels required by the EU for pesticide residues.

Results
Data obtained using low dwell times (500 µs) for SRM transitions produced good RSDs and LODs suitable for reporting data with respect to EU MRLs. Good linearity over the calibrated was observed with also quantified compounds at >0.99 r2 .

Conclusions
Faster transition speeds using fast collision cell designs enables sub-millisecond dwell times that maintain sensitivity and precision at low regulated concentrations of pesticides. This is possible without the need to open up Q1 or Q3 beyond unit mass resolution maintaining the selectivity within the method. This cability allows more compounds to be added to a method or more transitions being added per compound to ensure confirmation in a wider variety of matrices.

Novel Aspect
Higher performance SRM using new fast collision cell technology for pesticides at the EU MRL levels, at lower dwell times, without the need to open quadrupole resolution to compensate for sensitivity losses.

ThPS36-40 / Enhanced aroma profiling by GC-TOF MS with variable-energy electron ionisation

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Aroma profiles, such as those for wine, contain a wide variety of components at a range of concentrations. Detection and identification of important keynote compounds with a low odour threshold and compounds responsible for off-odours is a challenging prospect.

Gas chromatography coupled with time-of-flight mass spectrometry (GC-TOF MS) is an ideal choice for such analyses. Fast acquisition speeds, full-range spectra and low detection limits allow trace components, including adulterants, to be identified even within the most challenging of matrices. Novel data-mining software for the pairwise comparison of complex chromatograms is described, allowing such minor differences to be readily distinguished.

An additional complication in the analysis of aroma profiles is the differentiation of isomeric compounds, such as the monoterpenes. Select-eV, a revolutionary variable-energy electron ionisation technology, has been developed to solve this problem by enabling fast and simple switching between hard and soft electron ionisation with no inherent loss in sensitivity. Select-eV provides enhanced molecular ions and reduced fragmentation to aid speciation of challenging compounds.

This poster describes the combination of GC-TOFMS with Select-eV ionisation and novel data mining tools for rapid and reliable aroma profiling.

ThPS36-41 / GC-MS volatile profiles of ground spice from Hungary extracted by HS-SPME compared with distillation techniques

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Corvinus University of Budapest

Four different SPME fibres (Polyacrylate, PDMS, Carboxen-PDMS, PDMS-DVB) and two distillation techniques (common steam distillation and Likens-Nickerson SDE) were applied to extract the volatile constituents of a special quality sweet, ground red pepper sample. Obviously, the preparation methods produced substantially different scent aromagrams recorded with GC-MS. Each procedure showed individual characters. The two distillation methods were proved to be the most effective ones considering both the number and the sum of the peak intensities expressed in the undecanol-1 internal standard (ISTD). Extracted and identified compounds were methyl esters of open chain acids, terpenes, sesquiterpenes, carotenoid derivatives and the benzene relative components were the most intense. Among the SPME fibres, PDMS-DVB showed the highest general sensitivity in the chemical classes. Among the fruity and sweet nuance bearing "lacton" and "furans" in the high smell activity categories Carboxen-PDMS seems acceptably good. The PDMS fibre is not really proper for red paprika scent analysis and Polyacrylate practically can not be used at all. Comparison of the SPME and distillation results permits to recognize and circumscribe what artefacts, if any,were induced by the distillation steps of the sample preparation procedure.

ThPS36-43 / Influence of selenium species in aquaculture feeds on the selenium status of farmed rainbow trout

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Introduction

The important development of aquaculture in the last decades has induced a significant rise in the use of aquaculture feeds. Due to the finite availability of fishmeal and fish oil, these traditional major ingredients of fish diets can no longer meet the growing demand. There is therefore an increasing interest in the development of a sustainable aquaculture using feeds based on plant products[1]. In order to match requirements of fish, a supplementation of these alternative feeds is often necessary. This is particularly true for micronutrients such as selenium (Se) which is essential to several metabolic functions like cellular redox status regulation[2]. Assessment of the efficiency of such supplementation is most often performed by measuring total concentration of selenium, but it is nowadays known that a more accurate evaluation of the metabolism is obtained by processing speciation of selenium[3]. Consequently, the aim of this work was to evaluate the inputs of selenium speciation in fish nutrition studies and the added value in studying metabolic utilization of selenium depending on the chemical form of Se brought through supplementation in fish diets.

Methods

Two feeding trials were performed in an experimental fish farm comparing different feeds and supplementations, as well as farming conditions. Total selenium determination and selenium speciation were carried out in both feeds and fish. Total selenium was measured after acid digestion by using inductively coupled plasma mass spectrometry (ICP MS). Selenium speciation was performed by the use of several extractions followed by liquid chromatography coupled to ICP MS measurements.

Results

Quantification of total selenium in feeds and fish tissues brought out significant differences. Selenoamino acids determination was able to differentiate forms of dietary supplementation and allowed for comparison of the use of selenium. Selenium metabolites were found in different tissue and levels were compared.

Conclusions

Speciation of selenium in such feeding trials allows for accurate comparison of different supplementations through metabolism evaluation. In particular, selenocysteine determination reflects the actual use of selenium and its expression through selenoproteins.

Novel Aspect

Selenium speciation takes one step further the results of a feeding trial and clearly brings new informations in comparison to the classic determination of total selenium usually performed.

[1] A. G. J. Tacon and M. Metian, Aquaculture, 2008, vol. 285, pp 146–158.
[2] Y. Ogra and Y. Anan, Biological and Pharmaceutical Bulletin, 2012, vol. 35, pp 1863–1869.
[3] K. Bierla, M. Dernovics, V. Vacchina, J. Szpunar, G. Bertin, and R. Lobinski, Analytical and bioanalytical chemistry, 2008, vol. 390, pp 1789–1798.

ThPS36-44 / Application of HPLC-ESI(+)-CID-MS/MS in MRM mode to determine the evolution profile of pyranoanthocyanins in red wine from Rioja.

Zuriñe Rasines Perea, Blanca Gallo Hermosa, Noelia Prieto Perea, Luis Ángel Berrueta Simal
Euskal Herriko Unibertsitatea (UPV/EHU)

Introduction

The use of new soft ionization techniques (ESI, APCI) combined with mass spectrometry (MS) or tandem mass spectrometry (MS/MS) has been useful in the characterization of the anthocyanin derivative pigments during wine maturation and ageing.

Methods

Before carrying out the anthocyanin derivative pigments assay, a solid phase extraction (SPE) was performed. The conditions for the sample analysis were:

The solvents used in the chromatographic separation for the quantification were TFA:H2O (0.5:99.5 v/v) as mobile phase A and acetonitrile as mobile phase B.

The column used was a Phenomenex Onyx Monolithic C18 (100 x 3.0 mm) and the elution gradient used was: 0 0.29 min, isocratic, 12% B; 0.29 4.29 min, lineal gradient, 12 15% B; 4.29 9.17 min, lineal gradient, 15 25% B; 9.17 12.72 min, lineal gradient, 25 40% B; 12.72 13.17 min, isocratic, 40% B; 13.17 13.63 min, lineal gradient, 40 100% B; 13.63 21.63 min, isocratic, 100% B,.

The column temperature was 30ºC and the automatic injector was thermostated at 4 ºC. The injection volume was 50 µL and the flow was

0.3 mL/min.

The five tested derivative pigments formed by anthocyanins with pyruvic acid, acetaldehyde and acetoacetic acid are compiled in Table 1.

Table 1. Retention times achieved by HPLC-DAD-ESI(+)-CID-MS/MS, λmax and the m/z relationship for molecular ion and fragment ion selected for each derivative pigment formed with pyruvic acid, acetaldehyde and acetoacetic acid.

Compound	t_R (min)	$[M]^+$ (m/z)	Fragment ion (m/z)	λ_{max} (nm)
Mv-3-glc-acetaldehyde	11.63	517	355	499*
Mv-3-(6-p-cmam)-glc-acetaldehyde	15.01	663	355	509
Mv-3-glc-vinylmethyl	12.78	531	369	480*
Mv-3-glc-pyruvic	10.68	561	399	510
Mv-3-(6-p-cmam)-glc-pyruvic	13.98	603	399	513

*Approximately values due to the small concentrations of the compounds.

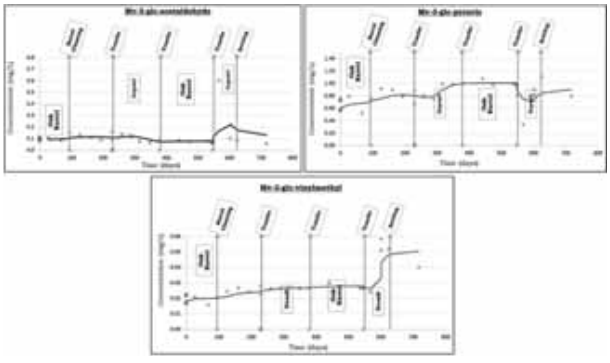

Figure 1. Evolution profile for Mv-3-glc-acetaldehyde, Mv-3-glc-pyruvic acid and Mv-3-glc-vinylmethyl during the ageing in oak barrel of a 2009 Reserva wine from Rioja.

A wine qualified as "D.O.Ca. Rioja Reserva" from 2009 was analysed during its ageing period in oak barrel using the methodology described above.

Results and Discussion

Results for Mv-3-glc-acetaldehyde (Vitisin B), Mv-3-glc-pyruvic acid (Vitisin A) and Mv-3-glc-vinylmethyl (Figure 1)show the greater stability of pyranoanthocyanins during the ageing step in spite of some technological process carried out during the process, which could modify the oxygen level of the medium.

Figure 1. Evolution profile for Mv-3-glc-acetaldehyde, Mv-3-glc-pyruvic acid and Mv-3-glc-vinylmethyl during the ageing in oak barrel of a 2009 Reserva wine from Rioja.

Novel Aspect

A new, rapid and selective high performance liquid chromatography with diode array detection coupled to electrospray ionization and triple quadrupole mass spectrometry (HPLC-DAD-ESI-MS/MS) has been developed for the study of pyranoanthocyanins evolution profiles in red wines from Rioja.

Acknowledgements.

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ThPS36-45 / Dispersion of olive oil in aqueous fine bubbles

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Introduction

Aqueous fine bubbles are known as a media of dispersion for oils and fats, although the dispersion mechanism is not well discussed. In this study, dispersion of olive oil in aqueous fine bubbles are investigated.

Methods

Aqueous fine bubbles are produced by GALF system (IDEC Co. Ltd.). Olive oils are dispersed in aqueous fine bubbles by stirring. The undispersed part, surfaced on water, and the dispersed part are investigated by GC/MS (Shimadzu QP-2010 and Thermo Polaris Q).

Results

Fatty acids like oleic acid were dispersed in aqueous fine bubbles produced with tap water, however they were not dispersed in aqueous fine bubbles produced with distilled water. Undispersed organic compounds were found and characterized by MS.

Conclusions

Dispersion properties are related to the zeta potential of aqueous fine bubbles and were controlled by the electrical conductivity of original water.

Novel Aspect

Dependence of functional group of compounds on dispersion in aqueous fine bubbles was discussed.

ThPS36-46 / Flavonoid profiling of meat products treated with selected plant extracts using HPLC-MS/MS

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Introduction

As UV-VIS spectra of flavonoids have limitations (sensitivity, time consuming nature…) in quantitative and qualitative analysis, therefore tandem mass spectrometry have increasingly been used for their analysis because of the possibility of analysing a large number of analytes in a short period of time. Some flavonoids has

antimicrobial activity, others, especially anthocyanins has natural red colour at certain pH range. Also, some of these compounds are known to protect colonic cells against damaging effects of cancer causing agents that may be formed in the large intestine after meat consumption. Hence plant extracts rich in flavonoids has a potential to use as additive in processed meat to create a healthier product. The aim of the present study is to establish a quick method to evaluate plant extracts based on their flavonoid content.

Methods
Analyses were performed by a Jasco HPLC system using a reversed-phase C18 column (Phenomenex Kinetex, 100 x 2.1 mm; 1.7 mm). The mobile phase consisted of 0.1% formic acid in water and acetonitrile, carrying out a gradient elution. The LC system was connected to a Waters Quattro Ultima triple quadrupole MS instrument, equipped with an ESI interface; parameters for analysis were set using both negative and positive ion modes. The ESI source were optimized by flow injection analysis using quercetin, quercetin-3-O-rutinoside, (-)-epicatechin, (-)-epigallocatehin gallate, apigenin-7-O-glucoside, taxifolin, isorhamnetin, luteolin-glucoside, apigenin. Different MRM methods allowed us to assign the identity of many flavonoids. Quantitative method development was considered the differences in concentration of analyte of interest, the nature of the matrix and the stability of compounds in different solvents.

Results
Results of qualitative and quantitative differences on commercial plant extracts will be presented. The analytes were identified by comparing the MS/MS data with the respective literature data when commercial standards were not available, using positive and negative ion modes together. The chromatographic run time was under 30 minutes. The method has good sensitivity (~10-100 µg/L), depending on the compound ~3 orders linear range, and good intraday/interday repeatability.

Conclusions
The developed method were used to select, among several plant extracts the most suited one as food ingredient, for the content of flavonoids using LC-MS/MS analysis.

Novel Aspect
Present study is a part of a project aimed to develop innovative meat products in which the food additive nitrite has been partially replaced by natural compounds originating from fruits and vegetables. In a number of meat products, carefully selected combinations of natural antioxidants and other biologically active compounds will be added during meat processing. The flavonoid content of plant extracts were evaluated before and after the addition to the meat products.

ThPS36-47 / The Analysis of Horsemeat for the Banned Drug Phenylbutazone

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Food and Environment Research Agency

The use of phenylbutazone is permitted for use on sport horses and horses kept as companion animals not destined for the food chain. There is no maximum residue limit (MRL) or any other action limits set for horses that have received phenylbutazone, so they are not permitted to enter the food chain. Low level residues have been detected during routine monitoring of horse meat from abattoirs for some years. The increased exposure of the UK public to horsemeat via the unwitting consumption of illegally adulterated beef products led to an increase in the frequency of testing quickly followed by a product release scheme for which all results needed to be reported within 48 hours. As phenylbutazone has no MRL set for horse, the very presence of a residue is enough to assign a sample as non-compliant. Modern instruments are extremely sensitive and so detection limits tend to be sub parts per billion concentrations. An analytical method based upon LC-MS/MS was quickly developed, validated and accredited to ISO17025. The performance of that method and results from the surveillance of horse meat in the UK will be presented.

ThPS36-48 / A comprehensive approach of Cognac 'crus' typicity by GC-O and GC-MS analysis of extracts and PTR-MS direct analysis of samples headspace

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[1]INRA - SFC , [2]INRA and CR Pernod-Ricard, [3]CR Pernod-Ricard

Introduction
Cognac is a blend of white wine distillates, ageing in oak barrels. According to Cognac experts, spirits originating from different 'crus' present specific sensory characteristics. These characteristics are already perceived in freshly distilled spirits and should partially found their origin in their related wines, for which areas of production together with viticultural and oenological expertise are relevant parameters. The objective of this study was to identify in freshly distilled spirits originating from different crus the key molecular features of the alleged specific characteristics. For this a targeted approach combining identification by GC-MS of aroma components selected after gas chromatography-olfactometry (GC-O) analyses of chosen extracts and a non-targeted procedure using headspace PTR-MS analyses of a set of samples were used and compared.

Methods
Six freshly distilled spirits selected in four Cognac crus were extracted and submitted to GC-O using the detection frequency method with duplicate injections. The odour events generated by a panel of 8 assessors were grouped into 94 olfactive areas (OAs). Correspondence and discriminant (PLS-DA) analyses were performed on the complete set of data. The OAs were then associated with compounds identified using GC-MS. Besides, the headspaces of 36 spirits originated from the four crus were analyzed in duplicates by PTR-ToF-MS (Ionicon ToF 8000) after dilution of the spirit in water (1% v/v, resulting in an ethanol content of ca. 0.7%). Multivariate analyses (PCA and PLS-DA) were performed on the data.

Results
Multivariate analyses conducted on the complete set of GC-O data allowed to distinguish the samples and to identify their characteristic OAs using GC-MS. Esters and alcohols were the two main chemical classes represented. No 'cru'-specific compound was found but differences were clearly perceived in GC-O, so that spirit typicity should be due to an equilibrated balance between molecules concentrations rather than expression of exclusive aromatic elements. Statistical results showed that 32 OAs exhibited no real changes between samples, and thus may represent the background of the overall spirit aroma. The 62 remaining OAs appear to be more relevant to discriminate spirits according to their crus. Without-'a priori' analyses of the PTR-MS data revealed a hundred significant peaks. Multivariate analyses allowed to distinguish the samples and to identify the peaks responsible for this differentiation, among which long chain aliphatic esters played a prominent role. Comparison of the two data sets revealed similarities but also differing features.

Conclusion
Combined GC-O and GC-MS analyses of freshly distilled Cognac extracts revealed key features of crus typicity. Non-targeted direct headspace analyses of Cognac samples by PTR-MS displayed another pattern, illustrating the different sampling conditions.

ThPS36-49 / New methods for assessing quality of milk powder using particle size analysis and NIR technique

Hyeo Joong Kim, Chulyoung Kim, Dong Gil Baek, Kyeong Heo, Young Min Ahn, Jae Hwan Lee, Jae Hun Sim
Korea Yakult

Noble aspect

Particle size analysis and near-infrared(NIR) technique to present new indicators of milk powder quality assessment

Introduction

Liquid precipitation in the manufacture of yogurt products are the leading causes attributable to the quality of the milk product. International and manufacturer specification to determine the quality of powdered milk are heat stability test, contents of non–protein nitrogen, moisture, protein and lactose. Heat stability test are not consistent depending on users, it seems to require more scientific techniques. In addition, rapid lot test of incorporation of non-protein nitrogen and other adulterants are required. Key indicator of heat applied powdered milk quality is protein aggregation its size. Especially mixed milk powder is more sensitive to heat because of whey protein. The size of significant proteins are distributed from sub-micro meters to hundreds so that corresponding particle size analyzer was applied.

Methods

1. Particle size analysis

Analysis of the particle size distribution of milk powder graded after heat resistance test was accomplished by the particle size analyzer of Malvern.

Various sampling conditions like agitation, ultrasonic, post-ultrasonic were applied to particle size analysis of milk powder protein. To validate particle sizes, other instruments like Bio-SEM, TEM were used.

2. Near-infrared analysis

Dairy guard milk analyzer of Perkin-Elmer Corporation was used to measure protein, moisture, fat, ash, lactose and melamine as the specification test of milk powder. Incorporation of non-protein nitrogen and other adulterants were analyzed.

Results and Conclusions

Size distribution of milk powder protein after heat resistance test ranged from sub micro meter to hundreds and two size region(less than 1um or less than 50um) are correlated with the quality of milk powder. Size distribution of milk protein before and after the ultrasonic were significantly correlated with quality grades.

By near-infrared analysis, basic specification, non-protein nitrogen and other adulterants were quantified to verify the quality of milk powder.

Two methods above can be used as new indicators of milk powder quality control.

ThPS37 - Hyphenated Techniques – Applications

11:00-15:00

Poster Exhibition, Level -1

ThPS37-01 / Monitoring of coccidiostat residues in eggs according to the EU legislation using liquid chromatography tandem mass spectrometry

Anneli Niemi, Seija Berg
Finnish Food Safety Authority Evira

Coccidiostats cover a various range of pharmaceutical substances used in the treatment of an infectious disease called coccidiosis caused by a protozoan parasite. Several coccidiostats are licensed for use in the EU as feed additives for treating infection in poultry, but are not authorized for laying hens. The occurence of carry-over of feed additives in non-target feed or improper use on farm may result in residues of coccidiostats in eggs.

In this study we developed and validated a multiresidue method for the confirmation of ten coccidiostats in eggs. These include the ionophores as lasalocid (LAS), monensin (MON), narasin (NAR), salinomycin (SAL) and maduramicin (MAD). The chemical coccidiostats are robenidine (ROB), diclazuril (DIC), nicarbazin (DNC), halofuginone (HFG) and clopidol (CLO).

For the sample preparation 12 eggs were broken and mixed, and then 5 g of homogenized egg mixture was weighed into a centrifuge tube. The sample was extracted with acetonitrile and cleaned up on a silica based solid phase extraction column (Bond Elut, 500 mg, 3 ml, Agilent). The eluate was evaporated to dryness and the residue was dissolved into a solution of acetonitrile and 0.01 M ammonium formate (250 µl, 95/5, v/v).

Chromatographic separation of the analytes was performed on a Kinetex C 18 (2.6 µm, 75 mm x 3.0 mm, Phenomenex) protected with a C-18 guard column. Chromatographic solvents used for a gradient elution were 0.01 M ammonium formate (A), methanol (B) and acetonitrile (C); the both organic solvents contained formic acid (0.1 %). The mass spectral analysis was achieved on a Micromass Quattro Micro triple quadrupole mass spectrometer (Waters) using both a positive and a negative ion MS/MS mode. Two diagnostic fragmentation ions were monitored for each analyte.

Coccidiostats were validated according to the permitted limits set by the European Commission. The limits for each compound in egg were LAS (150 µg/kg), MON (2 µg/kg), NAR (2 µg/kg), SAL (3 µg/kg), MAD (2 µg/kg), ROB (25 µg/kg), DIC (2 µg/kg), DNC (100 µg/kg) and HFG (6 µg/kg), which were used for the determination of a decision limit (CC alfa) and a detection capability (CC beta). The permitted limit was not listed for CLO. Ionophores and chemical coccidiostats differ greatly in their chemical structures and properties, so LC-MS/MS combined with a simplified sample preparation provide their simultaneous determination. Furthermore, by monitoring two product ions, LC-MS/MS is specific enough to confirm the presence of coccidiostat residues in eggs as required by the EU legislation

ThPS37-02 / On-line trapping LC-MS for the determination of DEHP metabolites, PFOS and PFOA in breast milk and cord plasma samples from European birth cohorts

Marja Lamoree[1], Jacco Koekkoek[1], Tomáš Trnovec[2], Greet Schoeters[3], Margot Van de Bor[4], Merete Eggesbø[5], Juliette Legler[1]
[1]Institute for Environmental Studies, VU University, [2]Slovenska Zdravotnicka Univerzita v Bratislave, [3]Flemish Institute for Technological Research VITO, [4]Institute of Health Sciences, VU University, [5]Norwegian Institute of Public Health

Introduction
The incidence of childhood obesity has reached epidemic proportions globally. Early life exposure to environmental contaminants has been implicated in altering developmental programming, resulting in possible higher susceptibility to obesity. The OBELIX (OBesogenic Endocrine disrupting chemicals: LInking prenatal eXposure to the development of obesity later in life) project examined the hypothesis that prenatal exposure to endocrine disrupting compounds (EDCs) in food plays a role in the development of obesity later in life. In this presentation, method development, validation and analytical results of the quantitative trace level analysis of DEHP metabolites, PFOS and PFOA in cord plasma and breast milk samples from different European birth cohorts will be discussed.

Methods
Samples were analyzed at the Institute for Environmental Studies (IVM) at the VU University in the Netherlands, which is accredited by the Dutch accreditation council (ISO17025), and participates in inter-laboratory proficiency testing. Cohorts involved in the study are FLEHS II, HUMIS, Michalovce and Linc, with either cord plasma, breast milk, or both. For the determination of DEHP metabolites, a deglucuronidation step was included in the sample treatment and extraction. The extracts were injected onto a RAM (restricted access material) phase cartridge. After trapping and cleanup, the analytes were eluted from the cartridge and transferred to the analytical column using a gradient. PFOS and PFOA were analyzed using a C8-column trapping column in an on-line system with the analytical column. After loading and enrichment, PFOS and PFOA were eluted from the trapping column and separated on the analytical column using gradient elution. The samples were analyzed on a triple quadrupole mass spectrometer with an electrospray interface (ESI) operating in the negative ion mode

Results
In the presentation, the levels of PFOS, PFOA and selected secondary DEHP metabolites in cord plasma and breast milk will be compared with those obtained in other cohort studies. Other topics to be addressed are e.g. the influence of contamination of the samples and remaining enzymatic activity on the suitability of specific metabolites for exposure assessment.

Conclusions
The methods developed showed to be capable of the reliable analysis of extremely low levels of DEHP metabolites, PFOS and PFOA, supporting further epidemiological studies into the relation of prenatal exposure to these endocrine disrupting compounds and the development of obesity later in life.

Novel aspects
Especially for secondary DEHP metabolites in cord plasma, data are extremely scarce, emphasizing the uniqueness of our study.

ThPS37-03 / Liquid chromatography with substrate-assisted laser desorption/ionization mass spectrometry for determination of sterols

Blanka Vrbková[1], Jan Preisler[1], Vendula Roblová[1], Edward S. Yeung[2]
[1]Masaryk University, [2]Iowa State University

Introduction
Sterols in food are usually analyzed by gas chromatography (GC) with electron impact ionization mass spectrometry (MS) or liquid chromatography (LC) with atmospheric pressure chemical ionization MS. Here we propose use of surface-assisted laser desorption/ionization (SALDI) MS in off-line combination with LC for analysis of sterols.

Methods
The chromatographic separation, fraction collection and subsequent SALDI MS of sterols were optimized using three model sterols, cholesterol, stigmasterol and β-sitosterol. For quantification, 6-ketocholestanol was used as the internal standard. Initially, reverse phase LC was optimized with a UV absorbance detector. A splitter (1:73) was inserted prior to effluent deposition on the target. Samples for SALDI MS were prepared using the overlayer sample spotting with colloidal suspension of silver serving as the SALDI matrix. The ionization and detection of sterols was performed using silver ion cationization with a time-of-flight mass spectrometer operated in the positive reflector mode. The method was applied to analysis of three commercial oil samples after simple sample preparation consisting of alkaline saponification followed by extraction of the unsaponificable fraction with diethyl ether.

Results
Separation of the model sterols on a C18 column using methanol-water gradient was achieved in less than 10 minutes. Optimized SALDI MS of cholesterol, stigmasterol and β-sitosterol standards with silver nanoparticles used as the matrix provided limits of detection 12, 6 and 11 fmol, respectively. Off-line SALDI MS was then applied for mass determination/identification and quantification of the separated sterols. Because of the incorporated splitter, the effective limits of detection of the RPLC–SALDI MS analysis were 4, 3 and 4 pmol (or 0.08, 0.06 and 0.08 μg/mL) for cholesterol, stigmasterol and β-sitosterol, respectively. Quantification of sterols in olive, linseed and sunflower oil samples was demonstrated using 6-ketocholestanol (KE) as the internal standard.

Conclusions
A new analytical method based on the off-line combination of liquid separation and desorption mass spectrometry for the determination of nonpolar compounds has been developed. The application of an unconventional matrix, colloidal silver, was essential for efficient ionization of nonpolar sterols via silver cationization. The method represents a viable alternative to established on-line GC–MS and LC–MS methods for the analysis of nonpolar compounds.

Novel Aspect
Off-line LC - SALDI MS with silver nanoparticle matrix for analysis of sterols.

Acknowledgement
We acknowledge the grants GAP206/12/0538 and CZ.1.05/1.1.00/02.0068.

ThPS37-04 / Detailed analysis of lignin cleavage products from electrochemical degradation by high resolution mass spectrometry

Tobias Dier, Verlaine Fossog, Rolf Hempelmann, Dietrich Volmer
Universität des Saarlandes

The limited availability of fossil fuels in the future has prompted intense investigations of renewable energy sources. Lignin is one of the most abundant natural resource and is promising because of its polyaromatic structure. Lignin degradation, however, is very challenging because of its high chemical resistance. In addition to biochemical degradation, electrochemical cleavage, in particular using protic ionic liquids, was shown to give a wide range of lignin cleavage products by oxidative and reductive pathways. GC-MS has been successfully applied to characterize the thermally stable fraction of the degradation products, but the method only covers a very small segment of the entire complement of products. In this study, a comprehensive scheme for characterization both low and high molecular weight as well as polar and non-polar degradation products of lignin has been developed, including liquid chromatography in combination with a variety of ionization techniques such as ESI, APCI and APPI for complementary data to GC-MS analyses.
Lignin (5% w/w) was dissolved in triethylammonium methanesulfonate and electrochemically degraded. After several extraction and evaporation steps, the resulting solid was dissolved in methanol and fractionated based on HPLC-UV analysis. Every fraction was analysed using QqLIT (QTRAP 2000, AB Sciex, Concord, Ontario) and a FTICR (Solarix 7T, Bruker, Bremen, Germany) mass spectrometers.
Direct injection of analyte solutions revealed several interesting m/z series of ions depending on the ionization technique used. While ESI in positive ion mode exhibited the most abundant masses in the range between m/z 200 and 800, negative ionization gave the majority of ions between m/z 50 to 200. HPLC fractionation of analyte solutions was necessary to reduce the complexity of the samples and avoid ion suppression effects, followed by detailed mass spectral interrogation of the individual fractions. These analyses revealed significant differences between the ionization techniques used: while APPI generated ions for a wide range of polar and non-polar compounds, ESI ionized far fewer products; that is, in negative ionization mode mainly hydrophilic compounds were seen while positive ionization gave ions mainly for lipophilic compounds. APCI ionized a similar number of compounds as compared to ESI in the methanol fraction of the crude degradation mixture. The aromatic nature of most lignin degradation products made ionization by APPI a very promising ionization technique. Importantly, APPI also formed ions for products which were detected by GC-MS, thus making APPI the most universal technique. HRMS analyses additionally enhanced characterization of the products and interpretation of APPI mass spectra. HRMS also allowed the development of intelligent mass defect filters, for prediction of oxidative or reductive pathways during electrochemical degradation.

ThPS37-05 / Rapid characterization of crude oils by thermogravimetry coupled to fast modulated gas chromatography-single photon ionization time-of-flight mass spectrometry

Sebastian Wohlfahrt[1], Michael Fischer[1], Janos Varga[1], Mohammad-Reza Saraji-Bozorgzad[2], Georg Matuschek[1], Thomas Denner[3], Ralf Zimmermann[1]
[1]Helmholtz Zentrum München, [2]Photonion GmbH, [3]Netzsch-Gerätebau GmbH

Introduction

The on-line combination of thermogravimetry with mass spectrometry (TG-MS) is commonly used for evolved gas analysis (EGA). In most cases, electron ionization (EI) with standard kinetic electron energy of 70 eV is applied. Thus, fragment peaks dominate EI mass spectra due to the high excess energy. Single photon ionization (SPI) circumvents this issue. TG-SPI-MS measurements of different crude oils using an electron-beam pumped rare gas excimer lamp (EBEL) as vacuum ultra-violet (VUV) light source have been conducted in the past [1]. In this study, a comprehensive fast gas chromatographic separation step was implemented prior to MS detection to achieve a TG-GCxSPI-MS system. Crude oil samples have been investigated to reveal major differences and advantages compared to direct TG-SPI-MS.

Methods

The samples were heated in a thermobalance from 40 °C to 1000 °C (10 Kmin-1) in N2 atmosphere. Connection of the thermobalance to the GC was realized by use of a heated transfer line. The GC oven was equipped with a dual-stage liquid CO2 modulator for GC×GC measurements. One modulation cycle took 30 s. 3 m mid-polar BPX50 GC column was used for fast separation. For photoionization, VUV light with a center wavelength of 126 nm (9.8 eV) was generated by a laboratory-built EBEL light source. The orthogonal-accelerated TOF was maintained at an acquisition rate of 10 Hz.

Results

Two-dimensional plots with m/z signals over TG temperature depict a high similarity between direct TG-SPI-MS and TG-GCxSPI-MS data for very heavy Turkish crude oil (see Fig. 1, left hand side). Averaged mass spectra over a temperature range from 40-400 °C for both techniques exhibit that the highest signals originate from three major homologous series. These signals have been ascribed to specific compound classes by Geissler et al.: alkanes, cycloalkanes and alkylated benzenes [1]. The introduction of a gas chromatographic separation allows the distinction of isobaric compounds. Multiple signals are detectable for a single m/z value in two-dimensional plots with retention time over TG temperature (Fig. 1, right hand side). The specific contribution of isobaric compounds to one mass signal is determined for alkanes and naphthalenes as well as for alkylated benzenes and one currently unknown compound class.

Conclusions

Introduction of a fast, rapidly modulated GC separation in comparison with solely TG-SPI-MS enables strongly enhanced detection especially with such highly complex organic matrices as crude oil. In contrast to former TG-SPI-MS measurements, separation and identification of overlying substances is possible because of different GC retention times. The specific contribution of isobaric compounds to one mass signal is determined for alkanes, naphthalenes, alkylated benzenes, and other compounds.

Novel Aspect

The soft photoionization favors the formation of molecular ions. In contrast to GCxGC, the whole thermal information of the sample is conserved.

[1] Geissler, R. et al. J Therm Anal Calorim 96 (2009) 813-820.

ThPS37-06 / Off-line CE–MALDI/SALD-ICP MS coupling: What can we acquire from a single separation run?

Iva Tomalová[1], Ondřej Polanský[2], Pavla Foltynová[2], Viktor Kanický[3], Jan Preisler[3]

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Introduction

Comprehensive characterization of the metal-protein or metal-metabolite complexes is becoming increasingly important; it has been estimated that metalloproteins encompass about one third of all proteins.

Here, off-line coupling of a single separation run to both matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) and substrate-assisted laser desorption inductively coupled plasma (SALD ICP) MS is presented as an alternative to the more common on-line coupling employing electrospray ionization (ESI) MS and/or nebulizer ICP MS. Particular attention is focused on metal-protein complex detection by MALDI MS and metal quantitation by SALD ICP MS.

Methods

CE-MS coupling is accomplished via a liquid junction interface and a sub-atmospheric deposition chamber where the CE effluent is collected on Au-coated polyethylene terephthalate glycol sample target. The separation record is then covered with aliquots of MALDI matrix and consecutively analyzed by MALDI MS and SALD-ICP MS. As a model analyte, rabbit liver metallothionein isoform mixture was used.

Results

Single run of CE coupled to MALDI MS and SALD-ICP MS provides highly complementary information about present biomolecules and metals. Moreover, SALD-ICP MS allows reliable metal quantitation, which was performed using a calibration curve approach. Both protein apoforms and protein-metal complexes can be detected using MALDI MS, depending on the applied MALDI matrix and the pH of the matrix solution. MALDI mass spectra of metal-protein complexes are compared with corresponding ESI mass spectra.

Conclusions

Off-line coupling of CE to MALDI MS and SALD ICP MS provides comprehensive characterization of analyte without repetitive separation runs.

Novel aspect:

Acquisition of MALDI and ICP mass spectra from separation record from single separation CE run; focus on metal-protein detection and metal quantitation

Acknowledgement:

We acknowledge the grants GAP206/12/0538, CZ.1.05/1.1.00/02.0068 and CZ.1.07/2.3.00/30.009.

ThPS37-07 / GC-MS for Identification of Volatile Organophosphates in Thermal Aged LiPF6-Based Electrolyte

Waldemar Weber, Vadim Kraft, Martin Grützke, Martin Winter, Sascha Nowak

MEET Universität Münster

LiPF6 based electrolytes constitute the main part of the currently used in lithium-ion batteries in which organic carbonates have been proven as solvents. Currently, an implementation of LiPF6 based batteries in a broad scale is problematic due to potential safety and technical problems. Further optimization is crucial for industrial applications such as power sources for electric vehicles and intermediate storage of renewable energies. A well known phenomenon of LiPF6/organic carbonate based batteries is their pronounced thermal instability at elevated temperatures. The equilibrium of LiPF6 with solid LiF and gaseous PF5 is considered as mainly responsible for a variety of adverse reactions. PF5 is a strong Lewis acid and reacts with solution species/impurities such alcohols, organic carbonates and preferably with water under formation of HF and POF3, whereas the following reactions of POF3 generate a number of phosphate derivatives, organic species and HF.

The focus of our current investigations is the thermal aging process of a commercial LiPF6 based electrolyte regarding the formation of volatile phosphorus containing degradation products. Aging products were analyzed with GC-EI-MS, performed by support of chemical ionization combining spectra in positive and negative modes. A part of the discovered compounds belongs to the group of fluorophosphates (phosphorofluoridates) and as such are interesting due to their potential toxicity, which is well known for relative derivatives, e.g diisopropyl fluorophosphate. Another group of identified compounds belongs to the group of trialkyl phosphates. These compounds may provide a positive impact of thermal and electrochemical performance of Li-based batteries as repeatedly described in the literature. Thus, the knowledge about their formation during battery aging may be of importance for optimization of additive content.

ThPS37-08 / IC-ESI-MS/MS for Investigation of the Organophosphates in LiPF6-Based Electrolyte

Vadim Kraft, Waldemar Weber, Martin Grützke, Martin Winter, Sascha Nowak

University of Münster, MEET

Since the commercial introduction of lithium ion batteries in the early 1990s, LiPF6 is the most common commercially available conducting salt. In contrast to notable advantages of this salt such as high conductivity and great solubility in different organic solvents the cycle life of the LiPF6-based batteries is limited due to the thermal instability. One of the thermal degradation products, POF3, reacts with water, alcohols or organic carbonates to produce different inorganic and organic phosphates or potentially toxic fluorinated organophosphates. The investigation of the decomposition products is therefore important for the understanding of reaction mechanisms of the decomposition as well as with regard to toxicological aspects.

Since many of the formed analytes are of ionic nature, the ion exchange chromatography is suitable method for the investigation of the decomposition process. For identification of the unknown analytes the conductivity detection is not sufficient, so other detection methods are required. Thus a method based on the hyphenation of ion chromatography (IC) and electrospray ionization mass spectrometry (ESI-MS) for the separation and determination of thermal decomposition products of LiPF6-based electrolytes was chosen.

The used electrolyte, LP50 (BASF) consisted of 1 mol/l LiPF6 dissolved in ethylene carbonate/ethyl methyl carbonate (50/50 wt%). To simulate thermal decomposition the electrolytes were stored at 95 °C in completely sealed aluminum vials and measured after defined time. For the separation method development the ion chromatographic columns with different capacities and stationary phases were applied and compared. Besides the hydrolysis products of lithium hexafluorophosphate several new organophosphates were identified, which structure was elucidated in series of fragmentation experiments with IC-ESI-MS/MS. The gain of knowledge about decomposition products should be helpful by improvement of the batteries cycle life and prevention of the toxic product formation.

ThPS37-09 / Using molecularly-imprinted polymers for compound-specific isotope analysis of polar organic micropollutants

Rani Bakkour, Thomas B. Hofstetter
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Compound-specific isotope analysis (CSIA) is a promising tool for assessing sources and transformation processes of polar organic micropollutants such as pesticides, pharmaceuticals and consumer chemicals in aquatic environments. There are, however, two major challenges: (1) Polar organic micropollutants occur at very low levels and, as a consequence, large amounts of water are required to achieve analyte enrichment with factors exceeding 50'000, inevitably leading to large interferences from the aqueous matrix. (2) The polarity of these micropollutants impedes the use of typical non-polar sorbates for solid-phase enrichment without further cleanup steps. In view of these challenges, the use of molecularly imprinted polymers (MIP) is a promising approach to produce tailor-made materials for highly selective retention of polar organic micropollutants with reduced matrix interferences. In this work, we explore the use of MIP to selectively retain benzotriazoles, a popular complexing agent used in dishwashing detergents and important representative of polar aquatic micropollutants.
Polymers were synthesized in the presence of enzotriazole or similar analogous molecule as a template, which leaves cavities in the polymer matrix with a very high affinity to the template and closely related structures including our main target analyte, 1H-benzotrizole. After extraction of the template, specific recognition of substituted benzotriazoles and other compounds is tested in chromatographic columns packed with either the imprinted polymer (MIP) or the non-imprinted polymer (NIP). Retention factors of the MIP and NIP are compared for different synthetic procedures and imprinting factors (IF), thereof, are calculated as indicators of selectivity (i.e. IF > 1 is selective).
Imprinting of benzotriazole molecule with imprinting factors larger than 1.0 was successfully achieved. The synthesized polymers could not only retain the imprinted template, but also its structural analogues such as 5-methyl benzotriazole and 5,6-dimethyl benzotriazole. However, benzotriazoles with substituents at the 1 position nitrogen in the triazole ring (e.g. 1-methyl benzotriazole) have shown imprinting factor close to 1.0 . No selectivity was observed either for compounds like naphthalene or benzothiazole, while, slight selectivity was measured for some s-chloro triazines. No isotopic fractionation for 13C/12C was measured during the process of loading, washing and eluting of 1H-benzotriazole on the imprinted polymers. These initial results show that MIP can be a promising tool as a pre-cleanup step before CSIA.
This approach will enable us to enrich and clean large amounts of aqueous samples while minimizing interferences from organic matter and other organic pollutants in the sample matrix and thus offer new perspectives for CSIA of polar organic micropollutants.

ThPS37-10 / Highly sensitive detection of typical fluoroquinolones in milk by FESI-CE coupled with electrostatic spray ionization MS

Yan Deng[1], Yan Deng[2], Natalia Gasilova[1], Liang Qiao[1], Xin-Xiang Zhang[3], Hubert Girault[1]
[1]EPFL, [2]EPFL; Peking University, [3]Peking University

Fluoroquinolones (FQs) are a group of synthetic antibiotics with a broad activity spectrum against mycoplasma, gram-positive and gram-negative bacteria. Due to the extensive use of FQs in farming and veterinary science, there is a constant need in the analytical methods able to efficiently monitor their residues in food products of animal origin, regulated by the European Union Council Regulation no. 2377/90. Herein, field-enhanced sample injection (FESI) for sample stacking prior the CZE separation was developed inside a bubble cell capillary for highly sensitive detection of five typical FQs in bovine milk. The effect of BGE composition, injection parameters and water plug length on the FESI-CE with UV detection was investigated and optimized. Under the optimized conditions, described FESI-CE-UV analysis of FQs provides a dynamic linear concentration range from 2.5 to 75 ng/mL and LODs varying from 0.5 to 1.4 ng/mL. These LOD values are much lower (from 430 to 1200 times) than those obtained by a conventional CE in a standard capillary without bubble cell. The developed method was applied for the analysis of FQs in bovine milk from a local supermarket. Sample recovery values from 45.2% to 70.2 % for different FQs, as well as LODs from 1.0 to 2.9 µg/Kg, were achieved. Moreover, the proposed FESI-CE was coupled with recently introduced electrostatic spray ionization MS (ESTASI-MS) via an iontophoretic fraction collection interface for qualitative FQs identification.

ThPS37-11 / Structure Determination of Aging Products in Lithium-Ion Battery Electrolytes with Gas Chromatography using Chemical Ionization Mass Spectrometry

Martin Grützke, Waldemar Weber, Martin Winter, Sascha Nowak
University of Münster, MEET Battery Research Center, Institute of Physical Chemistry

Lithium-ion batteries (LIBs) are the fundament of modern consumer electronics and portable devices. Furthermore, these energy storage systems are the most promising technology for electric and hybrid electric vehicles.[1,2] Unfortunately, the lifetime of LIBs is limited due to electrochemical, thermal and calendric aging. Research about aging phenomena has been done in the last years but mechanisms are yet not fully understood.[3-6] We focus our investigations on the electrolyte aging.
The compositions and decomposition products of two different sample types were analyzed with gas chromatography hyphenated to mass spectrometry (GC MS). On the one hand, a LIB electrolyte of ethyl methyl carbonate (EMC) and ethylene carbonate (EC), mixed 1:1 by weight with 1 mol/L LiPF6 was stored in a gas tight aluminum vial and thermally aged for 21 days at 95 °C. On the other hand, commercial 18650 cells of unknown composition were electrochemically aged at different temperatures. The electrolyte was obtained by extracting the cells with supercritical carbon dioxide.
The unknown electrolyte (18650 cells) consists of dimethyl carbonate, EMC and EC as solvents which was determined with comparative electron ionization (EI) spectra and retention times of standards. The organic carbonate solvents were quantified by external calibration with a flame ionization detector. Both samples (thermally and electrochemically aged electrolyte) contained a huge number of volatile organic decomposition products which could be separated with GC. The identification of every compound was neither successful by comparative EI spectra, nor by interpreting them. The following example will demonstrate the difficulties in the exact structure determination: four compounds with slightly different retention times yielded very similar EI spectra. Thus, the ion source was changed to chemical ionization with ammonia which resulted in the same molecular mass of 192 for all four compounds (Fig. 1).

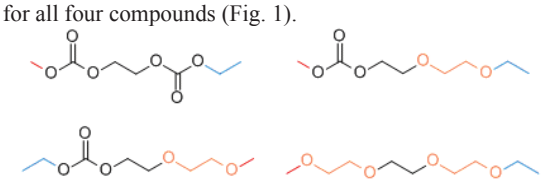

***Figure 1**: A demonstration of the difficult structure determination of four LIB electrolyte aging products with the same molecular mass of 192.*

However, on the basis of intensity ratios of (M+H)+ and (M+NH4)+ as significant structure indicators in combination with

fragments of the further performed negative chemical ionization experiments, clear identification for all detected aging compounds could be achieved. Furthermore, generation and further reaction of each compound was monitored by relative quantification of the samples measured every day with an internal standard during the 21 days of thermal aging.
We kindly thank the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety for funding of the project LithoRec II (project grant number: 16EM1025) and also the project partners for support and cooperation.

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ThPS37-12 / Target and untarget analysis of water soluble compounds in urban aerosols using an innovative coupling of IC-HRMS.

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Introduction
Primary emissions such as biomass burning (BB), chemical and photo chemical reactions introduce polar organiccompounds into the atmosphere. These molecules, such as levoglucosan (Lg) the most specific tracer of BB, are often difficult to characterize using reverse phase chromatography.
In this work it is proposed the innovative coupling between ion chromatography (IC) and HRMS for the first time aimed at the determination of Lg and its isomers and the chemical characterization of water soluble anionic organic compounds in the atmospheric aerosol.

Method
The analytical method was developed coupling a Thermo Scientific™ Dionex™ ICS-5000 system configured with a pump module, a sodium hydroxide Eluent Generator module, a 2-mm ASRS® 300 Suppressor , AS-AP Autosampler , and a Orbitrap XL FTMS (Thermo Fisher Scientific, Bremen, Germany). Chromatogaphic separation was perfomed using a a Dionex CarbopacTM PA10 analytical column (250x2mm) equipped with a Dionex CarbopacTM PA10 guard column (50x2mm).
The IC was connected to an atmospheric pressure chemical ionization source with negative polarity. The Orbitrap mass analyzer was used with a scan range of m/z 60-600 and a nominal resolving power setting of 100,000 (FWHM, at m/z 400; 0.5 s scan cycle time) using palmitic acid at m/z 255.2330 as the lock mass.

Results
The instrumental method was developed with the aim of obtaining an efficient separation between Lg and its isomers: mannosan and galactosan, which are specific BB tracers in the atmosphere. The dynamic linear range, detection and quantification limits were evaluated for each analyzed compound. The internal standard method was used to quantify anhydrosugars in airborne particulate matter using 13C6 Lg.
In order to evaluate the impact of BB on urban aerosols of Belgradecollected during fall and winter seasons, the instrumental method was applied to the quantitative determination of these compounds in an acqueous extract. Moreover one of the advantages of full scan HRMS is that the data can be mined at a later time without any need for re-injecting the samples, obtaining an untarget analysis with the aim of better understanding the chemical composition of urban aerosols.

Conclusion
We obtained the set up of a instrumental method characterized with good instrumental detection limits (Lg 3.6 ng mL-1, mannosan 4.7 ng mL-1 , galactosan 9.9 ng mL-1) for the target determination of anhydrosugars. The seasonal trend of these compounds and the untarget characterization in urban aerosols of Belgrade were obtained .

Novel aspects
To our knowledge for the first time an Orbitrap MS has been coupled to an IC with the aim of quantitatively determining anhydrosugars and at the same time qualitatively perform chemical characterization of atmospheric aerosols.

Acknowledgments
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ThPS37-13 / Historical amphetamines are still here. Development of a reliable turbulent flow LC-MS/MS assay for following intoxication admitted in intensive care unit

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Introduction
Amphetamine-type stimulants were developed as synthetic alternatives to ephedra. Currently illicit, the desired effect for subjects abusing these drugs is euphoria. Recently many studies reported new «designer psychostimulants» which aim to mimic the Amphetamines subjective effects, such as cathinone. However, in the past year in our laboratory, MDMA and its metabolites MDA were found in more than more than 90% of urine drug testing for amphetamines, confirming that historical amphetamines are still here and widely used. This rise creates a need for a reliable confirmatory assay. An on-line, automated turbulent flow sample preparation coupled to the sensitivity and selectivity of tandem mass spectrometry (MS/MS) detection was applied to the analysis of amphetamines (Amphetamine-A, Methamphetamine-MA, Methylenedioxyamphetamine-MDA, Methylenedioxyethylamphetamine-MDEA and methylenedioxymethylamphetamine-MDMA) in human plasma to support intoxication and evaluate toxicokinetic assessments.

Methods
Plasma samples (200 µL) were vortex mixed with deuterated internal standard solution (200 µL containing deuterated analogs, 100 ng/mL in methanol). After centrifugation, 20 µL supernatant were injected directly onto a 50×0.5-mm Cyclone MAX TurboFlow column. Analytes were focused onto a 100×4 mm (3.5 µm) Supelco HSF5 analytical column and eluted with an acidified methanol/water isocratic mixture. Analytes were monitored in selected reaction monitoring mode (positive ESI) at unit resolution. Total analysis time was 12 min.

Results
The method was validated in accordance with the European Medicine Agency guidelines. The standard curves, which ranged from 5 to 1000 ng/mL for all analytes, were fitted to a 1/x2 weighted linear regression model. Calibration was linear ($R2 > 0.99$). The within- and between-day precision was below

11.3% and the accuracy ranged from -13.5% to +17.5% for all analytes. Ion suppression was observed but compensated by using deuterated internal standards. No carryover was detected and the analytes were stable at room temperature, at 4°C for 24h, and three freeze-thaw cycles. For the target analytes studied, the LC-MS/MS analysis was as precise, accurate, and specific as the reference GC-MS method previously used. No derivatization steps were needed and sample volume was 10 folds less. The practicability of the assay has been demonstrated by the analysis of MDMA plasma samples from patients during involuntary intoxication.

Conclusions
An automated LC–MS/MS method has been developed for quantitation of amphetamines in human plasma using turbulent flow chromatography and detection by mass spectrometry (TFC-LC-MS/MS). This method is suitable and is routinely used for the analysis of toxicological samples and is applied for intoxication monitoring in patients admitted in Intensive Care unit. This LC-MS/MS method is robust reliable, and suitable for use as a confirmation assay in the simultaneous urine drug testing and quantification of historical amphetamines.

Novel Aspect
Use of Turbulent flow chromatography for Amphetamine abuse

ThPS37-14 / Input of bonded fluoro(pentafluorophenyl) used as normal phase for determination of Cocaine and Opiates drugs of abuse in plasma

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Introduction
Opiates and cocaine are well-known drugs of abuse and several LC-MS assays are described for their quantitation in biological samples. Most of them include a reversed phase chromatography as separation prior to tandem mass spectrometry detection. In this work an on-line turboflow sample preparation coupled to tandem mass spectrometry (MS/MS) detection was applied to the analysis of cocaine and its main metabolites (methylecgonine-MEC, benzoylecgonine-BZE), opiates (morphine, codeine, dihydrocodéine-DHC and 6 MAM) in plasma to support intoxication and evaluate toxicokinetic assessments. We wil emphasize on original association of mixed mode trapping column coupled to a bonded fluoro(pentafluorophenyl) analytical column.

Methods
Plasma samples (200 µL) were vortex mixed with deuterated internal standard solution (200 µL containing deuterated analogs, 100 ng/mL in methanol). After centrifugation, 20 µL supernatant were injected directly onto a 50 × 0.5-mm Cyclone MAX TurboFlow column. Analytes were focused onto a 100 × 4 mm (3.5 µm) Supelco HSF5 analytical column and eluted with an acidified methanol/water isocratic mixture. HSF5 bonded fluoro(pentafluorophenyl) columns provide separations that are different from C18 columns. Our choice was driven to improve LC-MS detection by using higher % organic mobile phase. To optimize retention time, different buffer concentrations were tested to modify selectivity at high % organic. Analytes were monitored in selected reaction monitoring mode (positive ESI) at unit resolution.

Results
Under certain mobile phase conditions HSF5 can exhibit both reversed- and normal-phase behavior resulting in tedious method development along with extensive re-equilibration time. To avoid these issues, isocratic condition at high organic (20 mM bicarbonate buffer pH 3/methanol 10/90, v/v)) was chosen for analytical separation. Thus, sensitivity is improved and around 10 min re-equilibration time is avoided. Under these conditions, analytes elution exhibited normal phase behavior, with 2.6, 3.1, 3.8 min retention time for BZE (cLogP : -1.51), MEC (cLogP : -1.26) and Cocaïne (cLogP : 1.32) respectively.Sample throughput being important, an online turbulent flow sample preparation was chosen using a mixed mode MAX trapping column. The complete assay allowed us to achieve quantitation with total analysis time of 9 min. It was validated according to international guidelines, was linear in a dynamic range of 2.5-1000 ng/mL. The within- and between-day precision was below 15.8% and the accuracy ranged from -7.9 to +17.6% for all analytes.

Conclusions
This original turbulent flow LC-MS/MS assay method provided the requisite selectivity, sensitivity, accuracy and precision to assess toxicological concentration and pharmacokinetics of cocaine and its major metabolites as well as opiates in patients admitted in Intensive care unit. Bonded fluoro(pentafluorophenyl) chromatography could be an ideal complimentary to reversed-phase for multidrugs analyses.

Novel Aspect
Bonded fluoro(pentafluorophenyl) chromatography for drug of abuse analysis

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ThPS37-15 / FIA-HRMS/MS for the analisis of recreational drugs and legal highs

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The recreational use of psychoactive substances is one of the main causes for concern in our society. Its use may cause irreversible health damage not only because of the main active components in the drug but also due to the presence of adulterants [1]. Moreover, the introduction of new «legal» substances (Legal Highs), which are sold as «bath salts» or «plant food», has also raised concern due to the misinformation showed in its packaging, where the actual composition is not indicated. These new products often contain synthetic drugs such as synthetic cathinones [2] and β-cannabinoids [3]. Although some Legal Highs have been banned, constantly new compounds are being introduced in the black marked and distributed by internet, outdating most of the available analytical methods for their control.
Governmental institutions such as the European Monitoring Centre for Drugs and Drug Addiction (EMCCDA) have started to control and to inform about the dangers of psychoactive substances and adulterants and frauds in recreational drug marketing. However, there is a need for fast and selective non-targeted analytical methods to identify new psychoactive substances constantly introduced in the market.
In this work a method based on Flow Injection Analysis coupled to High Resolution Mass Spectrometry (FIA-HRMS) is proposed for the analysis of recreational drugs and Legal Highs. Samples of recreational drugs provided by the Spanish NGO Cruz Roja and samples of Legal Highs bought from internet websites were dissolved in methanol and injected in a 1:1 mixture of methanol: 0.1% aqueous formic acid used as carrier flow. Electrospray ionization and a Q-Exactive mass spectrometer working in full scan/data dependent MS2 acquisition mode in both positive and negative mode were used. A custom-made database containing over 600 substances, including psychoactive compounds and common adulterants, was used for identification, based on the accurate mass and the isotopic pattern of the ions observed in

full scan mode and the main product ions observed in the product ion scan (MS/MS). Confirmation and quantitation was performed when standards were available. Levamisol was detected in one cocaine sample and several cocaine frauds were identified. Synthetic cathinones (butylone, flefedrone) were detected in some of the Legal Highs and one sample also contained L-DOPA, which is commonly used for the treatment of Parkinson's disease.

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ThPS37-16 / Ion exchange chromatography with non-volatile buffers hyphenated through on-line liquid-liquid extraction to electrospray MSfor the analysis of lipoproteins

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Introduction

Serum lipoproteins have been and still are intensively studied. The lipoprotein concentration (such as HDL and LDL) are among the most common biochemical parameters analysed in the clinic laboratories. Lipidomics is orthogonal and aims to capture information on fatty acids or intact lipids. In almost all methods the first step is the dissociation of the lipids from their original organisation lipoprotein particles and therefore loosing relevant clinical information on the origin and metabolic fate of the lipids analysed. It is possible to separate lipoproteins based on their size and anionic state. This is approach is limited to high salt buffer solutions and therefore considered as incompatible with electrospray based MS. We aimed developed a new method that hyphenates ion exchange chromatography with electrospray MS. This became possible through on-line liquid-liquid extraction (CLL-MS) for which we recently showed that it is compatible to size exclusion chromatography.

Methods

Human plasma was diluted tenfold in a PBS buffer and injected on TSK-GEL DEAE-NPR column (4.6 mm ID x 35 mm, 2.5 µm) column with a gradient of 50 mM Tris-HCl +1 mM ethylenediamine tetraacetic acid, pH 7.5, (at 500 µl min-1) with an increasing concentration of Na-perchlorate (max 500 mM) over a 15 min gradient to achieve the separation of lipoproteins. The eluent was lead to a FLLEX module (Syrris, Royston UK), which also received CHCl3/MeOH (3:1) at (at 500 µl min-1). Using a 150 µl loop the two phases were mixed and subsequently separated using a teflon membrane in an especially designed pressurised glass flow chamber. This yielded both the aqueous phase and the organic phase. The organic phase was mixed with a flow of isopropanol/methanol 7.5 mM NH4Ac (at 150 µl min-1) and introduced to a standard electrospray source. Spectra were obtained using a LTQ Orbitrap Velos (Thermo) or an Expression CMS (Advion).

Results

Ion exchange chromatography has been commonly applied to separate lipoproteins and is usually combined with photo spectroscopic detection to determine the lipoprotein levels (Hirowatari et al., J Lipid Res. 2003.44:1404–12). Nobody has tried to combine analysis with mass spectrometry based analysis of the lipoprotein particles. Through the on-line liquid-liquid extraction method it becomes possible to obtained detailed mass spectra of the lipids in the different lipoprotein fractions. We were able to separate the lipoprotein particles bases using the ion exchange chromatography and directly determine their lipid composition by class using low resolution mass spectrometry or by individual lipid using higher resolution mass spectrometry.

Conclusions

Through the use of on-line liquid-liquid extraction it is possible to hyphenate ion exchange chromatography with electrospray MS, independent of the concentration of non-volatile salts. This can be applied in the analysis of the lipid composition of lipoprotein particles.

Novel aspects

First on-line hyphenation of non-volatile salt based ion exchange chromatography with electrospray mass spectrometry and applied to lipoprotein analysis.

ThPS37-17 / Comparison of different quantification approaches to deal with matrix effects in LC-ESI-MS/MS based determinations of mycotoxins in selected spices

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Introduction

Mycotoxins presence in food have to be controlled due to the health problems that can originate. Thus, maximum levels allowed in food have been established by the European Union in several food commodities.
This work reports a comparative study of different quantitation strategies and their capability to account for matrix effect in UHPLC-ESI-MS/MS: the standard addition method and isotope dilution method with different ways of calculation. To this end, in a first approach, the matrix effect has been evaluated by both monitoring the signal of a compound during the entire chromatogram and by classical post-extraction addition.

Methods

The study was focused in nine mycotoxins (see Table 1) and two matrices: nutmeg and curcuma. Homogenized samples were extracted with a mixture of acetonitrile:water acidified with formic acid and injected in the UHPLC-ESI-MS/MS.
A monitoring substance added to the mobile phase allowed matrix effect assessment via permanent infusion methodology.
Suitable pre and post-extraction addition allowed the quantification by means of standard addition method, by calibration curve, by Isotope Pattern Deconvolution (IPD), and Internal Calibration (ICAL).

Table 1. Matrix effect and recoveriesa in percentage of mycotoxins in nutmeg using different calibration approaches.

		Calibration Curve					Internal Standard Single-point calculations	
			Single Standard Addition					
Compound name	Matrix Effect	Standard Addition	L1	L2	L3	Internal Standard	ICAL	IPD
Fumonisin B_1	111 (5)	95 (**25**)	115 (**30**)	89 (12)	102 (**29**)	90 (9)	108 (20)	99 (20)
Fumonisin B_2	79 (10)	90 (**23**)	94 (13)	92 (12)	93 (21)	104 (7)	**125** (9)	120 (9)
Fumonisin B_3	85 (11)	113 (**25**)	108 (**23**)	105 (12)	118 (**30**)	105 (13)	**125** (10)	111 (10)
Aflatoxin B_1	29 (16)	102 (14)	115 (**46**)	99 (**29**)	103 (20)	95 (13)	94 (16)	100 (17)
Deoxynivalenol	46 (11)	92 (17)	103 (**28**)	92 (9)	95 (15)	92 (9)	104 (12)	97 (12)
HT-2	99 (13)	94 (3)	**128** (**32**)	95 (18)	102 (5)	103 (6)	98 (10)	107 (9)
Ochratoxin A[b]	-	-	-	-	-	-	-	-
T-2	67 (15)	100 (**25**)	**142** (**37**)	101 (10)	112 (**32**)	96 (10)	111 (5)	99 (5)
ZEA	27 (16)	100 (4)	**175** (**29**)	112 (17)	110 (4)	112 (11)	112 (12)	114 (13)

[a]Figures in bold: recoveries outside range 70-120% or RSD > 20%.[b]The chromatographic peak was overlapped by an isobaric interference. ICAL: Isotopic Internal Calibration, IPD: Isotope Pattern Deconvolution.

Results

Permanent infusion of a monitoring compound provided a qualitative way of evaluating the matrix effects although it was not adequate for quantitative purposes.

Recoveries between 70 and 120% were obtained for most post-extraction addition method and the matrix effect was accounted for. High recoveries were obtained with IPD and ICAL calculations for some mycotoxins. As an example Table 1 shows the results for nutmeg samples.

Conclusions

Most analytes were affected by high signal suppression in the studied matrices.

Recoveries and the precision (RSD below 20%) calculated by standard addition method (both for multiple or single addition at higher concentration levels) and calibration curve with internal standard where satisfactory. Thus, the matrix effect is correctly compensated.

This study has also demonstrated that single-point calibration approaches (IPD and ICAL) provides similar results, in terms of accuracy and precision, to the values obtained with the whole calibration curve. Nevertheless, recoveries for single-point calculations with isotope labelled internal standards lead occasionally to unacceptable high recoveries.

Novel Aspects

For the first time an exhaustive study of the extent of matrix effects and applicability of four mathematical methodologies for their correction in LC-ESI-MS/MS mycotoxins analysis in challenging matrices has been developed.

Permanent infusion of a monitoring compound provided a qualitative way of evaluating the matrix effects at each retention time but it was not adequate as a quantitative approach to correct for the matrix effect.

Although the four quantification methods assayed correctly accounts for matrix effect, the single standard addition methods can be the best choice as it reduces considerably the total analysis time.

ThPS37-18 / Buffer salt effects in off-line coupling of capillary electrophoresis and mass spectrometry

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Introduction

The separation mechanisms used in CE differ from those in LC, but problems in quantitative analysis similar to those found in LC-MS (difficulties concerning reproducibility and accuracy in the analysis of compounds present in complex samples at low concentrations – matrix effects) can also be expected in CE-MS. This is because the components of the electrolytes used in CE-MS have an ionic character and are present in large excess over the analyte(s) in the solution sprayed through the ESI capillary. It is recommended that the electrolytes used in CE-MS be volatile, have low ionic strength, be clean and provide sufficient ionization of the target analyte. The way how to achieve sufficient sensitivity and reproducibility of CE-ESI-MS separations is to increase the sensitivity of the CE-ESI-MS analysis through development of the on-line or off-line preconcentration methods, for example preparative isotachophoresis (pITP). In this work, the impact of buffer salts/matrix effects on the signal in direct injection MS with an electrospray interface following pITP fractionation of the sample was studied.

Methods

All DI-ESI-MS experiments were performed on a LCMS-IT-TOF analyzer (Shimadzu). The data in MS experiments were acquired within a range of 100- 1500 m/z in both positive (+4.5 kV) and negative (-3.5 kV) ionization modes. The MS data were evaluated using LCMS Solution ver.3.5 (Shimadzu). A modified isotachophoretic analyzer ZKI-001 (Villa-Labeco) in the column-coupling configuration of the separation unit was used for the pITP experiments. The electrolyte buffers frequently used in CE (beta-alanine, acetate, histidine, phosphate, ammediol, and borate) with a constant ionic strength of 10 mmol/L were studied. Buserelin and cetirizine served as a model analytes.

Results

A range of buffers frequently used in CE analyses (pH 3–10) was prepared containing 10, 50, and 90% v/v of ACN, respectively. The sets of calibration solutions of cetirizine (an antihistaminic drug with an amphiprotic character) within a 0.05–2.0 mg/L concentration range were prepared in different buffers. The greatest enhancements in the MS signal (in terms of change in the slope of the calibration line) were obtained for the beta-alanine buffer (pH 3.5) in positive ionization and for the borate buffer (pH 9.2) in negative ionization, respectively. The procedure was successfully applied to the analysis of buserelin (a peptidic drug). The slope of the calibration line for solutions containing the beta-alanine buffer with 50% of ACN was 4 times higher than for water or urine, respectively.

Conclusions

This study clearly demonstrates that the buffer salt/matrix effects in an off- line combination of pITP and DI-ESI-MS can also play a positive role, as they can enhance the signal in MS. A similar influence of the above effects can also be presumed in the CE techniques combined on-line with ESI-MS.

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Novel aspects

The study of buffer salts/matrix effects in off-line coupling of capillary electrophoresis and mass spectrometry.

ThPS37-19 / Compositional analysis of heavy crude oil samples using size exclusion chromatography in combination with ultrahigh resolution mass spectrometry

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Introduction

Heavy crude oil samples and heavy components of crude oils such as asphaltenes are very problematic components of crude oil since they provide a challenge for oil refining processes and transportation. The exploration of their structure is difficult to study because of their complexity, polycondensed aromatic construction and limited solubility. These complex samples have caused a diverse discussion in the literature since even simple data such as the molecular weight was shown to be interpreted differently. Especially the use of size exclusion chromatography (SEC) has been discussed controversially since it apparently does not represent the original molecular weight but the solvents used are causing the formation of aggregates with higher molecular weight. To understand these processes we have studied the separation of heavy crude oil components by SEC and ultrahigh resolution MS.

Methods

For the separation of heavy crude oil components a combination of different SEC columns has been used. Here, both a 100 and 1000 Å were combined to achieve optimized separation conditions. As mobile phase different combinations of solvents were compared involving chloroform, toluene and THF.

Mass spectrometric analysis was perform using a research type high field Orbitrap mass spectrometer that allows higher resolving power. Ionization was performed by using both APPI and APCI.

Results
Usually, the direct coupling of LC with MS is a routine method but when analyzing crude oil samples this endeavor becomes much more complicated especially due to the large number of different compounds present. Here, the sensitivity is of great importance to be able to detect compounds that are present only in minor amounts. For the observation of changes that occur during the SEC separation a direct coupling system was developed.
The analysis of highly aromatic components of crude oil by size exclusion chromatography is not yielding the separation of different compounds or compound classes into a number of chromatographic peaks. Here the separation yields one broad bump with a retention time width of up to 4 minutes. But when comparing the MS spectra along the retention time it is obvious that compounds with different m/z ranges retard from the chromatographic column at different times. At the beginning larger compounds are retarding earlier, while smaller ones are being chromatographically separated later.

Conclusion
The combination of different solvent systems for SEC for the separation of highly aromatic compounds was studied and the mass spectrometric results were compared. With these studies no aggregate formation was found and the molecular weight for high aromatic compounds such as asphaltenes was determined after SEC separation by MS and not by SEC standards.

Novel aspects
Complex crude oil samples were analyzed by size exclusion chromatography and ultrahigh resolution mass spectrometry

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ThPS37-21 / Routine high throughput quantitative analysis with increased sensitivity using micro flow LC-MS/MS.
Remco van Soest, Subodh Nimkar, Leo Wang, Eike Loge
AB SCIEX

The interest in micro-LC/MS for routine high sensitivity quantitation using 0.2 to 1 mm ID columns, operated at 2 – 100 uL/min flow rates, is growing rapidly. The development of robust microLC pumps with high precision and accuracy, optimized flow path components, micro-columns and ESI interfaces, allow for exploiting the long known but untapped potential of microLC. We will discuss the various engineering considerations that affect micro-LC-MS/MS performance, and present comparisons of quantitation performance in different sample matrices, solvent composition of sample, and workflows such as direct injection and online SPE.
Data will be presented on the ultra-sensitive analysis of the type 2 diabetes drug Exenatide in plasma using online SPE that employs a 0.5 x 5 mm C18 trap cartridge coupled to a 0.5 x 50 mm C18 analytical column. 50 uL of sample was loaded onto the trap cartridge at a flow rate of 50 uL/min. A fast gradient of 5-90% Acetonitrile in Water with 0.1% formic acid at 40 uL/min was used for separation, while detection was performed using MRM on a triple quadrupole mass spectrometer. This approach resulted in a LLOQ better than 5 pg/mL with precision < 7%, accuracy of 91-106% and a dynamic range of over 3 orders of magnitude.
Our data shows that microLC/MS can address the need for increased sensitivity and speed for bioanalytical assays, while maintaining the same level of quantitation performance of conventional LC/MS.

ThPS37-22 / Utilising open access UPC2-MS within a Chemistry environment
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Introduction
An array of open access mass spectrometry and separation science solutions (HPLC-MS (ESI/APCI), GC-MS (EI/CI) and now UPC2-MS (ESI/APCI) are provided within Chemistry at the University of Southampton via a web-based sample introduction and data viewing platform RemoteAnalyzer®. This facility supports more than 150 researchers working on projects that cover a wide range of chemical space e.g. small organic molecules and natural products, organometallics, supramolecular complexes, large proteins and oligonucleotides.
The enhanced capability of the UPC2-MS provides a chromatography solution for the introduction of normal phase compatible analytes and separation of mixtures that would typically require normal phase solvents (e.g. lipids, petrochemicals).

Methods
A Waters Acquity UPC2 system coupled to a triple quadrupole (TQD) Mass Spectrometer (with the addition of an APPI source) has been integrated into the multi-user environment to expand capability and capacity. A specific make-up pump is essential to provide a flow of solvent to the MS source to aid ionisation. The provision of multiple column ovens enables an initial column/co-solvent screen of up to six columns and four different co-solvents for MS specialists and trained power users. This preliminary screen determines which methods are developed and subsequently made available to the open access user.

Results
Successful UPC2-MS assays have been developed for a number of applications within Chemistry e.g.

- Utilising a BEH C18 column and methanol modifier to detect and quantify the conversion of cellulose to hydroxymethylfurfural (HMF), a precursor to second generation biofuel has enabled the Raja research group to monitor slight changes to the design of their novel microporous aluminophosphate catalysts.
- Fluorosugars do not retain using conventional reversed phase HPLC. Working in conjunction with the Linclau research group a UPC2-MS method has been developed to improve the retention and separation of these molecules.
- Single nucleotide bases are often difficult to separate and then ionise by mass spectrometry. Utilising UPC2 separation with positive and negative ion electrospray ionisation provides a rapid screening method for the Watts research group.
- Molecular wires synthesised by the Whitby research group are now analysed routinely using UPC2-MS utilising both APCI and APPI.

Conclusions
The integration of convergence chromatography in addition to liquid chromatography and gas chromatography has proved to be essential to deliver a comprehensive, modern and fast separation science and mass spectrometry service to address the wide range of Chemistry research currently being undertaken at the University of Southampton.

Novel Aspect
The utilisation of UPC2-MS in an open access environment.

ThPS37-23 / The routine sequencing method for one peptide immobilized on a gel-type single bead prepared by the solid-phase synthesis focusing on drug discovery

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Introduction

Peptide libraries constructed using the concept of «one peptide immobilized on a single bead» is a powerful method for discovery of interacting molecules [Lam KS. et, al., Nature 354: 8282, 1991], in particular no preliminary information regarding ligands. While gel-type polymer beads as supports allow assembly in organic solvents and assays in aqueous media, library construction is performed on polyethylene glycol (PEG) based resins. Sophisticated encoding technologies such as DNA or small functional groups followed by deconvolution [Still WC. Acc. Chem. Res. 29, 155, 1996], although the simplest method is the Edman degradation [Liu R. and Lam KS. Analytical Biochemistry 295, 9, 2001], although this method requires several 10 picomole samples and N-terminal amino group should not be blocked, additionally the Edman cycle is time consuming and costly. To overcome these restrictions we have attempted sequencing of selected single bead by MS. However, the strong signals caused by PEG disturb identification of peptide peaks.

Methods

Construction of high quality libraries of cyclic peptides immobilized on TentaGel-S (90 µm) by the split and combine method [Ito H. et. al., Int. J. Pep. Res. Ther., 12, 275, 2006]. Hence 19 natural and 5 non-proteinogenic amino acids and two D-cysteines (enzyme resistance in bioassays) at the both termini were incorporated, thus ca. 200 millions of octa-peptide beads has been prepared.Since peptides immobilized on beads must be liberated before MS analyses, thus partial hydrolysis has been carried out. A novel nano-flow column packed with 2 micron non-porous ODS (designated Presto FF-C18) was developed, of which dimension was 0.2 (id.) X 150 mm. The eluate was analyzed by a MALDI-TOF-MS/MS or a LTQ Orbitrap.

Results

Since complete hydrolysis gave no-sequence information, partial hydrolysis gave several degraded peptide fragments with PEG derived from solid-supports. Optimal hydrolysis conditions have been established (IMSC2012, Kyoto). The hydrolyzate gave abundant PEG-derived peaks which were much easier ionized than peptides. Removal of PEG derivatives was carried out by different packed materials including TiO2, although full length sequence could not deconvoluted. Finally we have found that the nano-flow column packed with non-porous ODS was the most suitable to reveal sequence of peptide immobilized on a single bead of the TentaGel®.

Conclusions

The optimal condition of partial acid hydrolysis and non-porous ODS for isolation of peptide fragments were developed. In the case partial sequence is given, the syntheses of second library can help the complete sequence.

Novel Aspect

A high throughput sequence determination of the peptide immobilized on a single gel-type bead screened by bioassays has been established without labeling. Peptides can be used as a protein mimetic and this method is useful to discover the interacted proteins.

ThPS37-24 / Validated LC-MS/MS method for the determination of enantiomeric purity of S-Amlodipine in human plasma : Application to pharmacokinetic study.

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Introduction

Amlodipine, is a chiral calcium antagonist, currently on the market and in therapeutic use as a racemate and S-form enantiomer. The pharmacokinetic properties of S-amlodipine were studied using racemic amlodipine and single S-enantiomer administration to Korean male volunteers. A sensitive and selective liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-MS/MS) method was developed and validated for the determination of S-Amlodipine in human plasma using S-Amlodipine-d4 as an internal standard.

Method

Plasma levels of the drug were determined using chiral liquid chromatography coupled with tandem mass spectrometry following liquid-liquid extraction. The total run time was 21min and the elution of S-Amlodipine and S-Amlodipine-d4 occurred at 15.5 and 15.4 min, respectively. This was achieved with a mobile phase consisting of 20mM Ammonium acetate pH4.5 –2-propanol (gradient elution) at a flow rate of 1 mL/min on a Chiralpak α-acid glycoprotein (AGP) column.

Result

A linear response function was established for the range of concentrations 0.2–15 ng/mL (r >0.998). The intra- and inter-day precision values met the acceptance criteria. S-Amlodipine stable in a set of stability studies, viz. bench-top, auto-sampler, freeze–thaw cycles and long-term. The current assay was successfully. Geometric mean Cmax values for S-Amlodipine after administration of Amlodipine 10 mg and S-Amlodipine 5mg were 2.77, 2.51 ng/mL, respectively; the corresponding geometric mean AUC values were 93.13, 83.91 ng · h/mL.

Conclusion

This method was used to perform a comparative study of the pharmacokinetics of amlodipine 10mg and S-Amlodipine 5mg. The results revealed that the pharmacokinetic profile of S-amlodipine was comparable to that following the administration of the racemic mixture.

Novel aspects

A stereospecific,high-throughput, simple and highly sensitive LC-MS/MS method has been developed for the estimation of S-Amlodipine. The application of the assay to a clinical study confirmed the utility of the assay.

ThPS37-25 / Lipid residue analysis by derivitisation and comprehensive Gas Chromatography – Time of Flight Mass Spectrometry as applied to South African Iron Age pottery

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Introduction

In order to understand the use of different earthenware pots within an Iron Age culture, lipid analysis is required. The aim is to learn more about the diet and eating habits of this culture. We report, for the first time, a method for the analysis of fatty acid methyl esters (FAMEs) by comprehensive gas chromatography-time of flight mass spectrometry (GCxGC-TOFMS) for application to

southern African Iron Age pottery sherds.

Method
Pottery sherds were obtained from the Mutumba site in the Limpopo province of South Africa where an iron age culture existed around 1300 AD. Lipids were solvent extracted from pulverised pottery sherds followed by trans-methylation of the extract. The product mixture containing the FAMEs was analysed by GCxGC-TOFMS.

Results
The superior resolving power of GCxGC-TOFMS allowed separation of the FAMEs from matrix interferences. In an attempt to identify food residues, indicators for the origin of lipid methyl esters were obtained from ratios of Hexadecanoic acid methyl ester (C16:0)(Methyl Palimitate):Octadecanoic acid methyl ester (C18:0)(Methyl Stearate). This P/S ratio may be used as a guide to distinguish between fat of a ruminant or of a non-ruminant origin [1].

Conclusion
GCxGC-TOFMS is ideally suited for the trace analysis of lipid residues so as to obtain indicators for the origin of lipids from southern African Iron Age pot sherds and to determine the original function of individual vessels. For conclusive archaeological information on the original contents of the earthenware vessels, a more extensive sampling campaign needs to be launched with proper site control samples (e.g. soil surrounding the discovered sherd) and meticulous care to prevent post-sampling lipid contamination.

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Archaeometry 49 (4) 729-747.

Novel aspect
GCxGC-TOFMS analysis of South African Iron Age earthenware

ThPS37-26 / Application of novel "nMS2"technique towards characterisation of "Bio-Active" molecules of Andrographis paniculata extracts.

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Introduction
Triple quad MSMS technique has become a routine tool in all quantification applications, whereas ion-trap technique is routinely employed towards structure elucidation of unknown molecule at trace level concentrations. The simultaneous multiple collision energy mode, herein nomenclatured as "nMS2" mode is becoming popular towards the structural elucidation of unknown molecules similar to that of ion-trap technique. One of the salient features of this techniques is the fragmented ions can always be correlated to the parent ion, recorded under each collision energy and thus structural elucidation of unknown molecule becomes straight forward. This paper reports such an approach towards the structural elucidation of bio-active molecules of Andrographis paniculata, an traditional herb known for its (Bio-activity) anti-hyperglyceminc, anti-viral, anti-inflammatory, immunostimulatory and anti-oxident properties.

Methods
The dried leaves of Andrographis paniculata were powdered and one gram of this powder was extracted with water and other common organic solvents. The extracts were further charcoal treated to remove pigments from leaves. The aqueous extract with water was directly taken for analysis and the organic extracts were evaporated to remove the organic solvents. The residual organic extracts was dissolved in acetonitrile/methanol system and simultaneous multiple collision energies were applied to record MSMS spectra under tandem mass spectrometric approach.

Results
The non-aqueous extracts of Andrographis paniculata are found to contain the bio-active molecules. Thus, the leaf extracts of dichloromethane and ethanol showed the major constituents are andrographolides-bicyclic diterpenoid lactone of molecular weight 351Da and its subsequent dehydrated compounds of molecular weight of 332Da, 314Da. The LCMS analysis also shows the presence of flavone aglycones, thus demonstrating the power of "nMS2" approach towards the structural elucidation of these molecules.

Conclusion
The bioactive molecules such as andrographolide and the flavone aglycones of Andrographis paniculata were characterised through tandem mass spectrometry.

Novel Aspect
Characterisation of bio-active molecules of andrographis paniculata through "nMS2" technique.

ThPS37-27 / FAFOSS : Fast Automated Food Safety Screening

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Introduction
Current methods for measuring contaminants in foodstuffs tend to be slow and costly and often involve an initial screen by immunoassay followed up, where necessary, by analysis using conventional gas or liquid chromatography mass spectrometry (GC-MS and/or LC-MS). The initial testing is expensive and the follow up testing is often characterised by high cost and delay.
In this project, methods for fast and robust parallel measurements of food samples for important contaminants have been developed . This has been achieved by using high throughput laboratory robots to carry out unattended sample preparation for high performance thin layer chromatography.
The use of the CAMAG TLC-MS interface couple to an Advion Expression MS has also been investigated for the quantitative analysis of samples that were indicated as «positive» in the initial HPTLC screen. The use of TLC-MS removes the need for the additional sampling/sample processing and analysis by LC-MS as is currently required.

Experimental
Rhodamine B (in children's sweets) , Patulin (in Apple Juice) and Sudan dyes have been initally studied. Separation was achieved on a silica TLC plate using a mobile phase of toluene: ethyl acetate: formic acid and intial detection was by flourescent imaging.
In order to achieve parallel analysis software protocols for automated solid phase extraction and spotting of the TLC plate were developed for the Gilson 215 liquid handling robot. Plates were developed examined by fluoresence and TLC-MS.

Results
Patulin was readily detectable at the of EU legal limit (50ug/L) using both TLC with flourescent imaging and TLC-MS. Rhodamine B also exhibited excellent sensitivity. Issues with the TLC-MS detection of Sudan 1 when UV254 indicator plates were

used were encountered, these were overcome by the use of an alternative plate.

Conclusions
The proposed technique should prove invaluable in emergency situations when large numbers of samples need to be screened to determine which samples are contaminated and which product batches should be withdrawn. The proposed new methodologies combine ideas in simple sample preparation, parallel sample processing and quantitative analysis directly from TLC plates for the first time.

Novel Aspect
Use of conventional laboratory robots for automated parallel sample processing in SPE-TLC-MS.

ThPS37-28 / Investigation of Solid Phase Micro Extraction in Bioanalysis

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Introduction
There has been a growing trend in bioanalysis toward the utilization of micro extraction techniques for sample isolation and transportation. Not only is there an interest in cost reduction associated with sampling, but also ethical advantages and improving sample retention using microsampling techniques. Major focus has been on utilizing dried blood spot (DBS) media as an inexpensive alternative to terminal blood draws. Primary media used for DBS consists of either cellulose or glass fiber material, with each having different absorption and surface activity characteristics. Though DBS does offer benefits towards cost reduction, shipping and sample storage, it is not without limitation specifically with respect to blood hematocrit levels. There have been numerous citations with regards to quantitation irregularities due to variation in blood spot size derived from blood hematocrit levels. Other alternatives techniques to DBS, such as micro capillary extraction, or solid phase micro extraction may be impacted to a lesser degree by variant hematocrit levels. The purpose of this study is to explore the utility of solid phase micro extraction (SPME) as an alternative sampling device to DBS media cards. Bio-SPME, as described as functionalized particles bound to a core fiber substrate, enabling direct micro sampling of biological matrices without the need for additional sample treatment. Because analytes are extracted partially by diffusion using Bio-SPME, this technique offers the ability to concentrate analytes onto the functionalized particles while also providing sample cleanup. Analytes are concentrated onto the functionalized particles via direct immersion into biological fluids, followed by solvent desorption for analysis.

Methods
The key aspect of this study is a evaluation of the Bio-SPME sampling technique for the isolation of target analytes from human bloods samples. The simple set of model compounds is used to explore extraction efficiencies, detection limits, binding issues, and also hematocrit impact. Blood samples with ranging hematocrit levels are extracted using both techniques for comparison of analyte detection and overall sample cleanliness. Commercially available noncellulose-based media was utilized for the DBS experiments, while silica based particles modified with C18 stationary phases embedded onto a fiber core were utilized for the Bio-SPME experiments. Parameters such as extraction conditions, desorption solvent optimization and desorption times are detailed.

Results
Results demonstrate the capability of analyzing sub ng/mL concentration levels of carbamazepine in whole blood using both techniques. Whole blood samples extracted using the Bio-SPME technique exhibited increased analyte response, while demonstrating significant reduction in detected endogenous matrix as compared to DBS. Key comparison between DBS and Bio-SPME with regards to hematocrit impact is detailed.

Novel Aspect
Micro sampling, simultaneous sample clean-up/ analyte concentration onto fiber,

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ThPS37-29 / Development of Optimized Multiclass Clean-up Methods for LC-MS/MS Analysis of Mycotoxins in Multiple Food/ Feed Matrices Incorporating a Novel SPE Column

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Introduction
Mycotoxins are a group of toxic and often carcinogenic or genotoxic metabolites produced by several strains of fungi found on food crops worldwide. Mycotoxins have great potential to cause harm to humans, crops and farmed animals. As a result, a wide range of food and feedstuff substrates require testing for mycotoxin contamination. The diversity of analyte structure and food substrate generates a significant analytical challenge. Traditionally, mycotoxins have been analyzed using multiple methods, each optimized for a single mycotoxin or group of closely related toxins. Multi-analyte approaches are being made increasingly possible by the adoption of liquid chromatography-tandem mass spectrometry (LC-MS/MS) based analyses. Due to the selective nature of LC-MS/MS, highly selective sample preparation techniques are often no longer essential to meet the residue limits required. However, appropriate sample preparation remains necessary in order to minimize matrix effects and maximize assay robustness.

Methods
Mycotoxin extraction was compared using several organic solvents, organic:aqueous ratios and solvent modifiers. Matrix-specific catch-and-release SPE methods were developed for a novel SPE resin using selective modulation of: liquid extraction, resin equilibration, sample loading, wash and elution conditions. Mycotoxins were analyzed using one of two HPLC-MS/MS platforms dependent on the matrix extracted: a Waters 2795 and Quattro Ultima Pt or Shimadzu Nexera LC-30 and AB Sciex Triple Quad 5500. Analyte monitoring involved polarity-switched ion acquisition using electrospray ionization in the MRM or sMRM mode (where appropriate) for 2 or 3 transitions per analyte. Transitions were chosen from the most intense product ions (selected using full scan, SIR and precursor ion monitoring).

Results
We are able to demonstrate robust and reproducible extraction of multiple mycotoxins: aflatoxins, ochratoxin A, type 1 and 2 trichothecenes, ergot alkaloids, fumonisin B1 and zearalenone from: grain (wheat, maize, barley); nuts (Brazil nut, peanut); animal feed (soya and compound feedstuff); apple juice; ground chili; infant foodstuffs (formula and cereals). We demonstrate sample preparation methods that give quantitative assays for multiple mycotoxins from a variety of matrices over three orders of magnitude linear range with correlation coefficients >0.990. LOQ will be demonstrated at or below current EU MRL for the relevant matrix/mycotoxin combination with S/N >10:1 and reproducibility below specified limits. In the majority of cases recoveries were between 70% and 110%, exceptions will be presented.

Conclusions
We developed simple catch-and-release SPE methods for the selective extraction of multiple classes of mycotoxins from a variety of matrices. We are able to demonstrate robust and reproducible extraction of multiple mycotoxins.

Novel aspect
The development of multi-class SPE clean-up of mycotoxins from multiple matrices using a novel resin.

Novel Aspect
Isolation of natural products and simultaneous UV APCI/MS detection under flash-chromatography normal phase conditions. Analytical to flash chromatography method transfer. Optimization of splitting condition for a simultaneous UV,MS detection

References
[1] Davy Guillarme, Dao T.T. Nguyen, Serge Rudaz, Jean-Luc Veuthey, Eur. J. Pharma.Biopharma. 2008, 68, 430

ThPS37-31 / Methodological development for the establishment of efficient normal phase MS directed purification of natural products at the preparative scale

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Introduction
The improvements of analytical techniques and methodological tools play an important role for the characterization and isolation of bioactive secondary metabolites in natural product research. Reversed phase liquid chromatography MS (RP-LC-MS) is widely used for the metabolite profiling of complex natural extracts and start to be more and more used for targeted MS isolation of biomarkers. Normal phase chromatography (NP-LC) is well suited for the purification of apolar secondary metabolites offering also some advantages compared to RP like low operating pressures and cheapest stationary phases. The potential of NP-LC-APCI-MS for metabolite purification at preparative scale using generic separation methods has been investigated on medium pressure preparative chromatography system (PuriFlash® - MS).

Methods
A mixture of representative apolar natural product standards was chosen and analyzed under normal phase conditions. All parameters were carefully optimized for both separation and detection (gradient system, split rate, flow rate, temperature, Inj. Volume, column length, ionization source parameters). A special care was taken to find ionization and splitting conditions and that provide good detection and preclude source contamination. A linear gradient was applied at the analytical scale to enhance the separation of the molecules considered. The analytical gradient was then geometrically transferred to flash chromatography following a gradient transfer method after the calibration of the chromatographic systems [1].

Results
An efficient, fast and generic approach for the MS targeted isolation of apolar natural products was obtained. MS detection in complement to UV enabled the monitoring of NPs with weak chromophores and the selectivity of MS was of great helps for a precise collection of partially coeluting compounds. APCI-MS detection with optimized splitting and post-column elution of appropriate solvent was found robust and well-suited for this purification.

Conclusion
The analytical to flash level LC method transfer represent an innovative strategy for a rational isolation of specific biomarkers or bio-active compounds based on metabolite profiling results. The MS-triggered fractionation in addition to standard UV detection is a powerful tool to precisely orient the isolation. This rational approach can be widely used for direct purifications and isolation of synthetic and natural mixtures. Separation performed at preparative scale allows to purify tens to hundreds mg of compounds for further structural identification of bioactivity characterization studies.

ThPS37-32 / Characterisation of the polar fraction of diesel using accurate mass spectrometry: the effects of sample preparation, ionisation methods and pre-separation

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Introduction
The trace polar species present in diesel contribute significantly to the physical properties of the fuel. In order to study these species, selective ionisation together with accurate mass detection was performed.

Method
Four reference diesels and nine commercial diesels were analysed. Diluted fuel samples were analysed directly using electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) followed by high resolution accurate mass detection using a quadrupole time-of-flight mass analyser (qTOFMS). Methanol extracts of the fuels were also prepared and analysed directly. The effect of pre-separation on accuracy of mass measurement was investigated by performing ultra performance liquid chromatography (UPLC) and ion mobility spectrometry (IMS). In order to interpret the large amount of data obtained from these techniques, Kendrick mass defect plots were used to identify homologous classes present in each sample. Multivariate statistical techniques were used to investigate the relationship between the chemical class composition and physical properties of the different fuels.

Results
The mass spectra obtained for the diluted and extracted fuels differed, which indicates that ionisation efficiency is influenced by the presence of aliphatic hydrocarbons, which make up the bulk of the fuel. Furthermore compound class distributions indicated that hetero-atom containing species were ionised more efficiently by ESI, whilst aromatic and highly unsaturated hydrocarbons were ionised more efficiently by APCI. Complementary information was thus obtained from these techniques.
Compound class separation (based on polarity) was obtained to a certain extent with UPLC using hydrophilic interaction chromatography (HILIC). Results showed that the ionisation of some species was suppressed during direct infusion due to the presence of other compound classes. Complementary information regarding adduct formation was obtained from positive and negative ion mass spectra.

Conclusions
Results showed that the use of methanol extracts of diesel fuels and chromatographic pre-separation are important for the investigation of diesel fuels when using selective ionisation combined with accurate mass detection. Kendrick mass defect plots were a useful tool for the interpretation and comparison of complex mass spectral data.

Novel aspect
To our knowledge this is the first account of the use of hydrophilic interaction chromatography to obtain compound class separation

of diesel fuels. It was also shown how this pre-separation could enhance ionisation of certain species. The effect of sample preparation and different ionisation techniques were also studied in detail with regards to the analysis of diesel fuels in order to investigate the relationship between chemical composition and physical properties.

ThPS37-33 / UHPLC-UV-MSE analysis for the characterization of carotenoids and chlorophylls in Synechococcus sp. PCC 7002 cyanobacterium

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Introduction

Synechococcus sp. PCC 7002 is a fast growing unicellular marine cyanobacterium, which is found in both freshwater and marine water environments. It is an ideal metabolic engineering platform for carbon-neutral biotechnology because they directly convert CO2 to a range of valuable products, such as biofuels and bioactive carotenoids and chlorophylls. Synechococcus is capable of photoheterotrophic, chemoheterotrophic, photoautotrophic, and even nitrogen fixation, and thanks to the knowledge related to its genome and metabolism, its metabolic pathways can be readily manipulated towards the production of those valuable products. Carotenoids and chlorophylls are used as natural additive in the food (food colorants), pharmaceutical and cosmetic industries, and are known to have an important role as antioxidants.

Methods

Fried-dried cianobacterium material (0.05g) was sonicated with 70% acetone in water (containing NaF 0.2% (w/v) during 20 min. The extract was filtered through a 45mm PTFE filter, prior to injection into the UHPLC system (Waters ACQUITY UPLCTM) coupled to PDA detector and a quadrupole time of flight (Q-ToF) mass spectrometer (Waters SYNAPTTM G2 HDMS), equipped with an electrospray ionization (ESI) operating in positive mode. Analyses were performed on a reverse phase C18 column (Waters Acquity UPLC HSS T3, 1.7 µm, 2.1 mm × 100 mm), as described by Fu et al. (2012). UV-visible spectra were recorded in the range 250–750 nm. Carotenoids and chlorophylls were monitored at 450 nm. Data was acquired in MSE mode from m/z 50-1200, creating two discrete and independent interleaved acquisition functions to collect: i) low energy or unfragmented data, and ii) high energy or fragmented data.

Results

The profile of carotenoids and chlorophylls produced by Synechococcus sp. PCC 7002 during standard growth conditions was characterized by ultra high performance liquid chromatography coupled with diode array detection and mass spectrometry (UHPLC-DAD-ESI-Q-ToF/MSE). A single run allowed isomers separation, UV detection, accurate mass measurements, and MS/MS spectra for structural elucidation. New pigments not previously reported in Synechococcus sp. PCC 7002 were identified and confirmed by combining information coming from unfragmented and fragmented data and using key diagnostic fragmentations.

Conclusions

MSE as an untargeted methodology has been demonstrated to be a useful tool for the identification of carotenoids and chlorophylls in cianobacteria in a single run.

Novel Aspect

A rapid and selective UHPLC-DAD-ESI-Q-ToF-MS strategy using automatic and simultaneous acquisition of exact mass at high and low collision energy, MSE, is used for the fist time to study the profiles of carotenoids and chlorophylls in Synechococcus sp. PCC 7002 cianobacterium.

Fu, W.; Magnúsdóttir, M.; Brynjólfson, S.; Palsson, B. Ø.; Paglia, G., Analytical and Bioanalytical Chemistry, 404 (2012) 3145-3154.

ThPS37-34 / Isolation and Characterization by HRMS of a new compound in Red Wine

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Chromatographic separation of red wines revealed, besides a series of flavan-3 ols, the recurrent presence of an undefined compound in Bordeaux red wine. The present work reports on the different steps of the highlight of this new glycoconjugate from its isolation, characterization by Mass spectrometry, NMR, and chemical synthesis to its quantification in red wines all along the winemaking process of different varieties (Cabernet-Sauvignon and Merlot) and "terroir" (Medoc, Saint Emilion, Côtes de Bourg, Côtes de Bordeaux, Graves, Pauillac) in order to estimate whether this compound may be used as a terroir, variety or wine process tag.

The MS fragmentation pattern of this compound exhibits an interesting daughter molecular ion that evidences the presence of an hexoside moiety in the compound (molecular weight, 162). The structural identification has required the purification of the compound and its analysis by both High-Resolution Mass Spectrometry and NMR.

Quantification of this compound has then been developed directly on wine by using HPLC, the calibration curve performed with the synthesized compound give rise to the quantification with a good accuracy. The results obtained revealed the following trends:

- The concentration decreases all along the winemaking process.
- A significant difference is observed between the two varieties tested.
- No significant difference is observed from a Bordeaux area to another.
- The Pauillac wine exhibits a higher concentration compared to all the other wines tested.

ThPS37-35 / Development of an LC-MS-based purification strategy for the Mass Spectrometry targeted isolation of bioactive compounds

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Introduction

In natural product (NP) research, the targeted purification of bioactive molecules from complex extracts is of prime importance. In this respect, Mass Spectrometry (MS) detection is a key tool enabling the monitoring of specific features for precise fractionation.

Methods

To improve the isolation process efficiency of active natural products, an LC-MS-based purification strategy was developed.

First, the chromatographic separation of the crude extract was optimized by application of a linear gradient at the analytical scale in HPLC-UV-MS. An at-line microfractionation, followed by an agar overlay bioautography test using a Candida albicans hypersusceptible strain, was also performed to identify the bioactive fractions [1]. Then, the gradient was geometrically transferred from analytical to preparative scale using gradient transfer rules based on the calibration of both chromatographic systems [2]. Finally, an MS-triggered isolation of the localized antifungal compounds was realized with a Flash chromatographic system coupled, via splitter, to a single quadrupole mass spectrometer.

Results
The above developed isolation strategy was applied for the MS-direct purification of the antifungal compound Diplophyllolide A from the Chinese liverwort Chiloscyphus Polyanthos (L.) Cord. The bioactive molecule was directly isolated in large amount, without any problem of MS saturation, thanks to the optimization of the MS splitting geometry.

Conclusions
This rational LC-MS-based methodology has high potential not only for the rapid purification and identification of bioactive NPs that lack of UV chromophore, but also for the isolation of biomarkers identified by UHPLC-MS metabolomics.

Novel Aspect
The use of a single quadrupole mass spectrometer coupled to a Flash/Prep LC system (PuriFlash® – MS) appears as a promising tool for the precise MS-direct isolation of natural products from crude complex extracts.

References
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[2] Davy Guillarme, Dao T.T. Nguyen, Serge Rudaz, Jean-Luc Veuthey, Eur. J. Pharma. Biopharma. 2008, 68, 430.

ThPS37-36 / Kinetic Capillary Electrophoresis Coupled On-line with Mass Spectrometry to Study Conformational Dynamics of DNA G-Quadruplex in Solution

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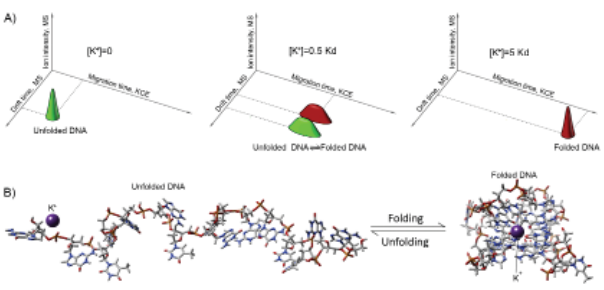
G-quadruplex forming DNA/RNA sequences play an important role in the regulation of biological functions and development of new anticancer and anti-aging drugs. In this work, we couple on-line Kinetic Capillary Electrophoresis with Mass Spectrometry (KCE-MS) to study conformational dynamics of DNA G-quadruplexes in solution. We show that peak's shift and its widening in KCE can be used for measuring rate and equilibrium constants for DNA-metal affinity interactions and G-quadruplex



formation; and ion mobility mass spectrometry (IM-MS) provides information about relative sizes, absolute molecular masses and stoichiometry of DNA complexes. KCE-MS separates a thrombin binding aptamer d[GGTTGGTGTGGTTGG] from mutated sequences based on affinity to potassium, and reveals the apparent equilibrium folding constant (KF ~ 150 μM), folding rate constant (kon ~ 1.70×103 s-1M-1), unfolding rate constant (koff ~ 0.25 s-1), half-life time of the G-quadruplex (t1/2 ~ 2.8 s), and relaxation time (τ ~ 3.9 ms at physiological 150 mM [K+]).

ThPS37-37 / Capillary Electrophoresis On-line Hyphenated with Mass Spectrometry Used for Analysis of Vitamins B in Pharmaceuticals

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Introduction
Vitamins B are organic compounds, essential for human organism, which are present in a lot of dietary supplements and pharmaceuticals. It is necessary to have a powerful analytical method to control the content of these vitamins especially in case of the dietary supplements where the differences between the declared and real contents of the active substances can fluctuate as a result of the absence of an effective analytical method for the post-production quality control of such products.

Methods
The capillary electrophoretic analyzer, Agilent 7100 Capillary Electrophoresis System, was used in this work. CZE analyses were carried out in the cationic regime of the separation.
A mass spectrometer, Agilent 6410 Series Triple Quadrupole, was equipped with an electrospray ionization source (ESI). CE-MS coupling was carried out using a sheath liquid coaxial interface (Agilent). The sheath liquid was delivered by a pump, Agilent 1260 Infinity, and flowed through a splitter set at a ratio of 1:100 into the sprayer.

Results
Vitamins B have very different chemical structures so it is quite difficult to find an optimum separation conditions for their simultaneous analysis. The best compromise between resolution, analysis time, reproducibility, and detection sensitivity was obtained
with BGE composed of 50 mM formic acid with pH 2.05. The optimized method was validated. LOQs were at sub microg/mL concentration levels. RSDs of migration times were less than 0.7% and RSDs of peak areas were less than 4.5 %. The average recoveries were in the range of 96.33-102.7 %, for biotin and folic acid slightly below 95%. The optimized and validated method was applied to identify and determine content of vitamins B in various commercial pharmaceuticals such as injections, tablets, and effervescent tablets. The determined values obtained with the proposed method were consistent and in a good accordance with the declared ones.

Conclusions.
This work demonstrates analytical potential of the hyphenated CZE-ESI-MS/MS method for the analysis of vitamins B in pharmaceutical samples. The validation data and overall analysis time indicate fast, efficient and highly reliable separation process useful for its use in the pharmaceutical analysis such as quality drug control.

Acknowledgements
This work was supported by the projects VEGA 1/0664/12, KEGA 031UK-4/2012,
APVV-0550-11, FaF UK/5/2014, FaF UK/3/2014 and carried out in the Toxicological and Antidoping Center (TAC) of Faculty of Pharmacy, Comenius University.

Novel Aspect
The proposed CZE-ESI-MS/MS method prevents any detection interferences in the real matrices (each vitamin was characterized by two independent signals), offers superior sensitivity (the highest signal-to-noise ratio for given analytes) and specificity for the determination of vitamins B (including vitamins that do not contain UV sensitive chromophores, such as pantothenic acid and biotin) in comparison with UV detection.

ThPS37-38 / High performance time-of-flight mass spectrometry for comprehensive petroleum analysis
Juergen Wendt[1], Clécio F. Klitzke[2], David Alsonso[2], Joe Binkley[2], Jeffrey Patrick[2]
[1]LECO Instrumente GmbH, [2]LECO Corporation, St. Joseph, MI.

ThPS37-39 / Utilizating Hydrogen Carrier Gas for High Throughput Gas Chromatography - High Resolution Time of Flight Mass Spectrometry (GC-HRT): Application Compendium
Juergen Wendt[1], Joe Binkley[2], David Alonso[2], Charles Lyle[2]
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Introduction
Petroleum is the most complex matrix in nature, constituted by many thousands of compounds. Full knowledge of composition of crude oil is the key in the rational use of reserves, optimization of production, refining process, reduction of byproducts, quality control of derivatives and many other crucial aspects of petrochemical field. Advances in the performance of time-of-flight mass spectrometry (TOF MS) such as high resolution, high mass accuracy and high acquisition rate, provides an alternative for most routine studies of volatile and semi-volatile compounds of crude oil and derivatives with GC-TOF MS. In this work we investigate the composition of crude oils and derivatives through a petroleomic software by GC-HRTOF MS, complemented by isomeric composition provided by GCxGC-TOF MS.

Methods
Crude oil samples and derivatives were diluted in CHCl2 and injected into the gas chromatograph's split/splitless inlet with helium carrier gas. The analysis were performed in both EI and CI (CH4) modes with a GC-HRTOF MS (resolving power of > 25,000 and mass accuracy of < 1 ppm), and in EI with a GCxGC-TOF MS. The spectral data of GC-HRTOF MS were exported as txt files and processed using a petroleomic software for classification (HC, S, O, N class) and plots of carbon number versus double bonds (DBE).

Preliminary data and Conclusions
Preliminary results show that GC-HRTOF MS can be useful for petroleomic studies with evaluation of class distribution and composition of the homologous series (carbon number versus DBE plots). The hydrocarbons and aromatics are the most abundant class, and heteroatomic class in abundance greater than 1% can be analyzed. Chemical ionization provides additional information of class composition showing the relative distribution of hydrocarbons and (N, O, S)- heterocyclic compounds. Members of homologous series are better distinguished and compared using CI in conjunction with EI. These results show that petroleomic software complements the traditional workflow analysis. GCxGC-TOF MS complements the results of GC-HRTOF MS with a comprehensive and in depth analysis of isomeric composition showing 3 to 4 times more peaks.

Novel aspect
GC-HRTOF MS and GCxGC-TOF MS are useful for a non-target comprehensive characterization of petroleum and distillates through a petroleomic approach.

ThPS37-39 / Utilizating Hydrogen Carrier Gas for High Throughput Gas Chromatography - High Resolution Time of Flight Mass Spectrometry (GC-HRT): Application Compendium

Introduction
Both the costs associated with a dwindling helium supply and the desire for higher sample throughput have fueled the desire to develop fast gas chromatography methods using hydrogen as a carrier gas. This poster will demonstrate the ability to utilize hydrogen carrier gas on a high resolution GC-HRT instrument. Methods achieving fast separations for multiple application markets including, but not limited to, metabolomics, forensics, and specialty chemicals. Derivatized NIST Human Plasma was used to develop a high speed GC-HRT method for the metabolomics application market. Various drugs of abuse including synthetic cannabinoids, cathinones, psychotropic substances such as psilocin and psilocybin were used to demonstrate capabilities in the forensic market. Polymer additives were used to represent the specialty chemical market.

Methods
Fast GC-HRT methods were developed using hydrogen carrier gas and relatively short, narrow bore GC columns (10m x 0.18mm x 0.2 micron film). Methods were developed for rapid separation of representative metabolomic, forensic, and specialty chemical markets.
NIST plasma extracts were derivatized prior to GC-HRT analysis using an optimized two-step procedure: 1) Treatment with methoxylamine hydrochloride and 2) MSTFA. Drugs of abuse were dissolved in organic solvents and analyzed both underivatized and derivatized.
Polymer additives samples were prepared by extracting 1 gram of ground polymer material with 10 mL of dichloromethane. Extracts were filtered prior to analysis.

Preliminary Data and Conclusions
Fast GC-HRT methods of approximately 12 minutes were achieved for complex NIST human plasma extracts.
Fast GC-HRT methods allowing sub 4 min analyses for drugs of abuse were developed.
Polymer additives screening methods by GC-HRT were achieved in less than 8 minutes.

Novel aspect:
Fast Separation methods using hydrogen carrier gas were developed on a high resolution GC-TOFMS system.

ThPS37-40 / Endogenous metabolite separation by multimode gradient HPLC and detection by MS
Adrian Ammann, Marc Suter
Eawag, Swiss Federal Institute of Aquatic Science and Technology

In metabolomics, multimode separation strategies have rarely been exploited. Preferentially, a single separation mechanism is applied, like reversed-phase (RP), HILIC or ion exchange. One of these chromatographic modus is usually optimized for best separation of certain target metabolites. One single mode chromatography, however, cannot handle all metabolites. For instance, using RP chromatography, lipophilics can easily be separated, while hydrophilic compounds elute at the front. The opposite is true for a HILIC-based separation which elutes lipophilic compounds at the front. Furthermore, RP does not separate ionic compounds, unless they have a large lipophilic part, under HILIC conditions, retention times for ionic compounds are difficult to predict and ion exchange cannot separate uncharged hydrophilic or hydrophobic metabolites. Since a metabolome comprises compounds of all chemical classes (hydrophobics, non-ionic hydrophilics, anions and cations), a single mode

chromatography cannot separate nor a partially separate a metabolome. However, minimizing co-elution is required preventing ion suppression in an electrospray ion source and achieving a reliable detection and identification of the highest number of metabolites in a short runtime chromatogram.
We developed a hyphenated method combining all the above mentioned separation modes in a single run HPLC. Commercially available multi-mode columns (C18, WAX, SCX) were used to perform HILIC, RP, anion exchange and cation exchange chromatography coupled to a triple quadruple MS (Vantage and QExactive). Flows, eluents and gradients were optimized to spread as many compound as possible over the entire runtime. Dozens of endogenous metabolites representing diverse compound classes were separated and serve as base data set to predict retention times of other metabolites in a database.

ThPS37-41 / Identification and Relative Quantification of Anions in Battery Samples with the course of time using Ion chromatography and High Resolution Mass Spectrometer

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Thermofisher Korea

Purpose

A Lithium-Ion battery(LIB) is a member of a family of rechargeable battery types in which lithium ions move from the negative electrode to the positive electrode during discharge and back when charging. LIB use an intercalated lithium compound as the electrode material, compared to the metallic lithium used in non-rechargeable lithium battery. One of the ion which is used in LIBs is LiPF6, and we indentified and quantified degradable amounts of the anions, PF6-, with the course of time using Ion Chromatography and High Resolution Mass spectrometry. Normally speaking, the anions were analyzed by Ion chromatography technology for quantification, so we used the system for separation and relative quantification for the samples prepared as time-course. Moreover, for Identification, we used High Resolution MS (Orbitrap) with Electro Spray Ionization (ESI).

Methods

Ion chromatography system was ICS-3000, dual pressure pump. Suppressed conductivity, ASRS 300 Anion self-regenerating suppressor and CRD 200 Carbonate removal device were equipped for detection. For ionization efficiency, the instrument was coupled to another pump system to flow acetonitrile solvent. MS system was Q Exactive and Ion source was ESI negative mode. Xcalibur software was used for acquiring raw data, and Qualbrowser was used for reviewing the raw data.

ThPS37-42 / The combination of "fast" triple-quad MS with flow-modulated comprehensive 2D gas chromatography for the untargeted and targeted analysis of complex mixtures

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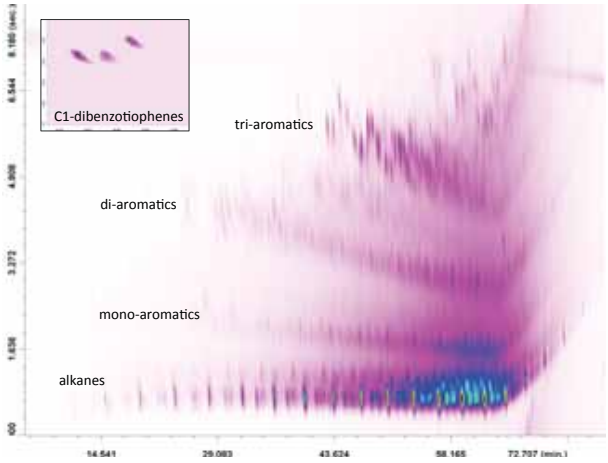
Introduction

Comprehensive 2D GC (GC×GC) is a multidimensional method, and is the most powerful GC approach today-available. With respect to 1D GC, GC×GC is characterized by: 1) increased selectivity and peak capacity; 2) enhanced sensitivity; 3) increased identification power, due to the formation of ordered 2D-chromatogram patterns relative to homologous compounds (i.e., mono-aromatics).
The scope of the present research was to evaluate a novel triple quadrupole (QqQ) MS under challenging GC conditions, specifically those generated by flow-modulated (FM) GC×GC. The QqQ MS system was capable of operation under high-speed conditions, in the simultaneous full-scan and multiple-reaction-monitoring (MRM) modes. An FM GC×GC-MSMS method was developed for the simultaneous untargeted analysis of heavy gas oil (HGO) constituents, and the MRM determination of targeted ones, namely S compounds.

Methods

All HGO FM GC×GC-QqQ MS applications were carried out on a system consisting of two independent GC2010 gas chromatographs (GC1 and GC2), and a TQ-8030 triple quadrupole mass spectrometer (Shimadzu). Data were acquired using the GCMSsolution software v. 4.0 (Shimadzu). Bidimensional chromatograms were generated by using the ChromSquare v. 2.0 software (Shimadzu).
The primary column was a low-polarity 20 m × 0.18 mm ID × 0.18 µm df SLB-5ms, while the second was a medium-polarity 10 m × 0.32 mm ID × 0.20 µm df SPB-50.



Results

Chromatographic conditions were first optimized in the full-scan mode (see Figure). As can be observed in the TIC GC×GC-QqQ MS chromatogram, the HGO constituents are distributed on the 2D plane in highly-organized patterns. Specifically, analytes elute from the medium-polarity secondary column (a non-polar column was used in the first dimension) on the basis of increasing polarity (alkanes, mono-aromatics, di-aromatics, etc.). After optimizing the GC×GC method, MRM transitions were finely tuned using a blank sample, spiked with one representative standard compound for each of the twenty S classes (e.g., tiophenes, C1-tiophenes, benzotiophenes, C1-benzotiophenes, etc.). An MRM bidimensional expansion, relative to the determination of C1-dibenzotiophenes, is illustrated in the inset located in the figure. In the final developed method, each target compound was characterized by a specific location on the 2D plane, a full spectrum, and an MRM trace, with the latter exploited for quantification.

Conclusions

The work has demonstrated two factors: 1) the QqQ MS was capable of operation under "extreme" GC conditions; 2) the combination of QqQ MS with a high-resolution GC method had sense, inasmuch that untargeted data were also generated and, moreover, the second-dimension was useful for the separation of matrix interferences from the target analytes.

Novel aspects

- One of the very few reports of GC×GC-QqQ MS.
- The potential future usefulness of such a combination is highlighted.

ThPS37-43 / Qualitative and quantitative application of UPC2-MS to biofuel screening.

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Introduction

UPC2-MS has been used to study a number of biodiesel (BD) applications, e.g. (1) FAME in AVTUR, (2) optimisation of catalytic synthesis of G2 BD and (3) a screen for steryl glucosides (SGs).

(1) G1 BD (FAME) provides a renewable alternative to petrodiesel but comes with a number of additional challenges to the fuel industry, e.g. purity, stability and compatibility. The latter is an issue in relation to contamination of AVTUR through use of common pipelines. AVTUR must be B0, i.e. no BD, where B0 <5ppm total FAME. Presently the international reference method (IP 585) is a 60 min GC-MS method for rapeseed methyl ester (RME). To address recent industry demands to include short chain methyl esters, alternative 60 min GC-MS and 2 min UPC2-ESI/MS methods have been developed.

(2) Working with the Raja group UPC2-MS has been used to reaction monitor the selective, one-step conversion of sugars to hydroxymethylfurfural (HMF), the precursor to G2 biofuel. Multiple glucose isomerisation catalytic reactions are screened using a combinatorial approach, a reaction sampled and monitored over short time intervals to build an accurate kinetic profile, allowing the determination of rates, enthalpic and entropic factors that aid catalyst design.

(3) Sedimentation has been detected in BD, this being linked to the presence of SGs. After transesterification, acylated SGs (present in plants and vegetable oils) can be converted into SGs, meaning that the level of SG is higher in BD than in the original feedstock. These insoluble solids are thought to cause filter blockages interrupting the flow of fuel towards the engine. Hence the need to monitor levels of SGs in biodiesel and petrodiesel fuels.

Method

Samples are screened using 6 different sub-2 µm particle size UPC2 columns (BEH, 2-EP, fluorophenyl, HSS C18 SB, amide, and cyano) with co-solvents (MeOH, MeCN, IPA and MeOH 25 mM NH4OAc). Chromatographic and API conditions were then optimised for each of the different analytes.

Results

(1) The above method provided the linear dynamic range for the total FAMEs in the range of 0.50-10 ppm (R2 >0.99) and LODs for RME C18:1, C18:2, C18:3 FAME <1 ppm, total time 2 min.
(2) Quantitative separation of the stock sugars (glucose and fructose; ESI) and the reaction product (HMF; APCI), total analysis time 3 min. (BEH column), utilising the ESCI source.
(3) HSS C18 SB column (MeOH modifier) afforded the optimal chromatography conditions for the steryl glucosides with APCI-MS giving sub 5 ppm LODs.

Conclusions

UPC2-MS has been used to solve a number of problems related to the introduction of BD in the fuel chain. The UPC2-MS method is 30 times quicker than the GC-MS ASTM reference method and affords analysis of the lower chain length methyl esters. SGs can also be detected (<5ppm) and a routine assay is used for the optimisation and reaction monitoring of the catalytic synthesis of HMF.

Novel Aspect

Use of UPC2-MS for analysis of biofuels.

ThPS37-44 / ESI mass spectrometry investigation of the chiral recognition of amino acid esters by lariat ethers

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Crown ethers have proven themselves as the synthetic receptors capable of selectively bind and transport organic and inorganic ions and molecules. The ability of the crown ethers for guest binding can be increased substantially when a flexible terminal fragment containing the additional electron donor centers appears in their structure (so called lariat ethers). An expressed ability to a diastereomeric discrimination is an important factor for consideration, for example, when creating a chiral stationary phase for high performance liquid chromatography. For the chiral recognition of the enantiomers of biologically active compounds the mass spectrometry based approaches were successfully used. The aim of this work was to identify the differences in the ability of lariat ethers 1-4 (Fig. 1) to the chiral recognition in the gas phase relative to the amino acid ester hydrochlorides, depending on the size of the cavity of the crown ether and the position of methoxy group within the aromatic ring of the terminal substituent by ESI mass spectrometry. The method allows registering in the gas phase the diastereomeric complexes between the studied crowns and hydrochlorides of alanine, phenylglycine and phenylalanine methyl esters.

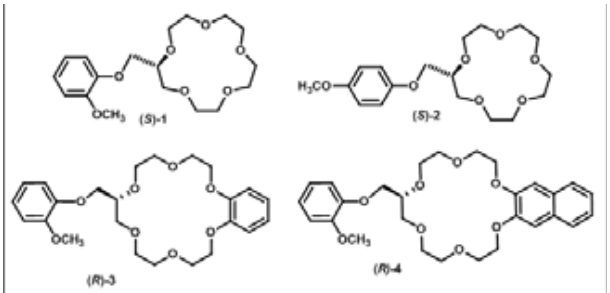

Fig. 1. **The structures and designations of crown ethers used in this work**

Two different approaches were used. The results obtained by isotopically labeled guest-molecules technique appears to be comparable to the results of a method based on the use of achiral amine (benzylamine and cyclohexylamine) displacer. In general, (S)-crowns preferentially bind L-enantiomers of amino acid esters and vice-versa. The best values of discrimination shows chiral crown (S)-1, especially in comparison with crown (S)-2. This fact proves the different nature of ortho- and para-methoxyphenoxymethyl substituents involvement in the processes of complexation with ammonium guests in the gas phase. The marked lariat-effect is characteristic to the crowns, in which the methoxy group is in ortho-position of the aromatic ring. Increasing the size of the cavity and appearing of a flat aromatic moiety in the crown-ether molecules 3 and 4 facilitates the formation of complexes with chiral guests, simultaneously weakening the enantioselectivity. For such a host molecules a moderate chiral recognition was revealed only for methylphenylalaninate as a guest.
We demonstrated in this work for the first time that the lariat-effect influences the chiral recognition.

ThPS37-45 / Automated LC-LC-MS/MS system for comprehensive analysis of small RNAs in functional RNP complexes

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Introduction

Non-coding small RNAs are currently the subjects of intense research because of their important roles in various biological processes. In general, those RNAs function as a part of ribonucleoprotein (RNP) complexes. We have recently developed a method for direct RNA analysis using nanoflow LC–MS/MS coupled with the genome-oriented database search engine Ariadne (Taoka et al., Nucleic Acids Res. 2009; 37: e140; Nakayama et al, Nucleic Acids Res. 2009; 37: e47). We report here our attempts to develop a fully automated LC-LC-MS/MS system that serves as an analytical platform for comprehensive identification and chemical analysis of RNA components in functional RNP complexes.

Methods

The system consisted of two independent LC assemblies connected through a liquid handling robot, a high-resolution Orbitrap mass spectrometer equipped with an ESI interface, and a data processing system with Ariadne software. A sample RNA mixture isolated from a cellular RNP complex is first separated by 1st reversed phase LC (Yamauchi et al, J. Chromatogr. A, 1312, 87-92, 2013) into several dozen of fractions, which were then added with aliquots of RNase T1. After 1 h at 37oC, the resulting oligonucleotide mixtures were subsequently injected into 2nd nanoflow reversed phase LC connected to MS via an ESI interface and the resulting tandem MS data are processed for characterization of RNAs. All operations were automated by synchronizing with a computer program.

Results

We validated the performance of our automated LC-LC-MS/MS system by analyzing the box H/ACA small nucleolar RNAs (snoRNAs) in the budding yeast S. cerevisiae, which contains 29 snoRNAs that guide the formation of 44 pseudourines (Ψs) in ribosomal RNAs (Torchet et al, RNA 11, 928-938, 2005). The RNP complex was isolated from yeast cells by the affinity purification using a tagged Gar1 protein as bait, and the RNA components extracted from the purified complex were analyzed by the automated system. After extensive analysis of the resulting tandem MS data by Ariadne, we could finally identify all the known snoRNAs of this Gar1 associated RNP complex, except for two called “snR9 and snR161”, and found one previously unknown component “snRX1”. We also applied this system to the analysis of the box H/ACA RNP in the fission yeast S. pombe and identified 18 known and 20 previously unknown snoRNAs. The subsequent genetic and biochemical analyses showed that at least 17 of the 20 snoRNAs found in this study participated in the pseudouridylation of particular sites of the 18S or 25S rRNA and thereby the novel functional cofactors of the box H/ACA snoRNP complex.

Conclusion

The automated nanoflow LC-LC-MS/MS system reported here was found useful for characterization of RNAs in the RNP complexes. Because the method allows comprehensive, unbiased identification of RNAs and analysis of their chemical structures solely utilizing the genome information, it should be most suitable for use in the discovery-based studies of functional cellular RNP complexes and their metabolites.

Novel aspect

This is the first report describing the fully automated MS-based analytical platform of small RNAs.

ThPS37-46 / Chiral Discrimination of Amino Acids Based on Cyclofrunctans (CFs) in Mass Spectrometry

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Zhejiang University

Introduction

Cyclofrunctans (CFs), the novel component obtained from enzymatic conversion of insulin, is a family of cyclic oligosaccharides composed of β-(2-1)–linked fructofuranoseunits. Different to the cavity structure of cyclodextrin, the crystal data shows that CFs has 18-crown-6 skeleton and neighboring fructofuranose units.[1] Using functionalized CFs as the stationary phase, amines, acids, and alcohols has been enantiomeric separated successfully.[2] We now reported a three component (CFs, amino acid AA, benzoboric acid) in-situ reaction protocol [3] for determining the chirality of AA via mass spectrometry.

Methods

In the initial step, the 1:1 stoichiometry of CFs and AA complex was confirmed by their mass-to-charge ratio. The native CFs shows low selectivity towards the chirality of AA in both full scan mass spectra and tandem mass spectra. According to their mass behaviors, CFs-AA complex was deduced to be rim complex via formation hydrogen bonding and ion-dipole forces. However, when benzoboric acid (B) was introduced into the system, the chirality effect could be observed from the relative peak intensities of uncapped complex ion [CFs+AA]+ and capped complex ion [CFs+B+AA]+.

Results

.The representative spectra for chiral recognition His were shown in Fig. 1. Chiral recognition ability Rchiral was defined as Rchiral = RL/RD = .The detection results were summarized in Table 1. As shown, the Rchiral value was stable and not change with concentration and collision energy. CFs provided large chiral discrimination for compounds with hydrophobic chains Phe (3.56), Trp (2.53), Val (2.56), and His (2.24) than hydrophilic side chains Ser (1.49) and Glu (1.41). And CFs provided larger chiral discriminations for compounds with aromatic side chains.

Conclusion

In summary, a three component in-situ reaction mass spectrometric method has been proposed to recognize the chirality of amino acids using CFs. The relative peak intensity of the capped and uncapped complex ions provides the chiral recognition properties straightforwardly.The present methodology is practically useful for further research on developing CFs to be selective transporters.

Novel Aspect

1. As an uncultivated host, this is the first report for determination the chirality of amino acids using native CFs in mass spectrometry.
2. The technique could apply to various hosts such as cyclodextrins for detecting chiral recognition behavior. Applicable guests are not limited to amino acids as well.

Acknowledgements
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ThPS37-47 / Rapid Phosphopeptide Analysis by Microchip Electrophoresis-Electrospray Ionization Mass Spectrometry

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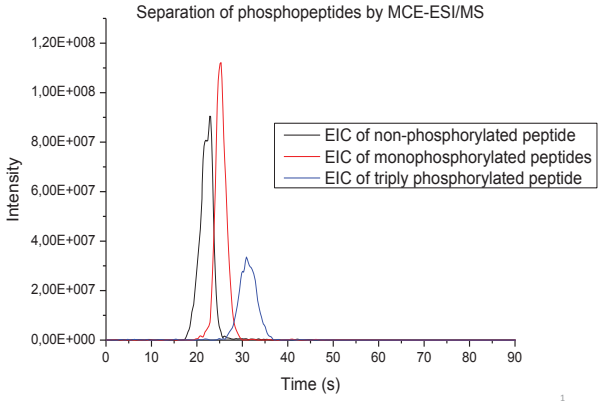
Introduction

Protein phosphorylation is an important post-translational modification that has an effect on protein activity and degradation, as well as cell signaling and apoptosis.1 It is of high importance to determine the different phosphorylation sites (phosphorylated amino acids) in order to understand their effects on the biological processes. Although a variety of analytical techniques for phosphopeptide separation has been described, a clear need for faster and more selective separation methods exist. Separation of peptide isomers with different phosphorylation sites remains challenging. The aim of the study was to develop microchip-based rapid screening methods for phosphopeptide separation.

Methods

The miniaturized analysis system was based on monolithically integrated microchip (capillary) electrophoresis-electrospray ionization (MCE-ESI) on an SU-8 microchip.2 For the method development, two monophosphorylated peptide isomers (TRDIpYETDYYRK, TRDIYETDpYYRK), a triply phosphorylated peptide (TRDIpYETDpYpYRK), and a non-phosphorylated peptide, all having the same amino acid sequence, were used. The peptides were analyzed both in their native form and as conjugates of 9-fluorenylmethyl chloroformate (Fmoc-Cl, 5-fold excess relative to free amino residues).



Results

Using the microchip setup, separation of monophosphorylated peptides from non-phosphorylated (Rs=0.42) and triply phosphorylated peptide (Rs=0.56) was easily achieved within about 30s (see Figure). The repeatability of the migration times was between 2.7 and 4.5% RSD (n=5). The plate numbers were between 1.9×104 and 4.55×104 per meter. However, separation of monophosphorylated peptide isomers (native form) from each other was not achieved regardless of rigorous optimization. Thus, possibility to improve their separation via rapid and selective derivatization by Fmoc-Cl was studied. Our tentative results suggest that phosphorylation may direct Fmoc conjugation. For instance, the monophosphorylated peptide isomers, TRDIpYETDYYRK and TRDIYETDpYYRK, formed conjugates with two and one Fmoc groups, respectively, which facilitates separation of the isomers both electrophoretically and based on m/z. Optimization of the separation conditions for Fmoc-peptides is under way.

Conclusions

The miniaturized system permits fast and effective separation of phosphopeptides with low sample and solvent consumption. Differently phosphorylated peptides were easily separated according to the number of phosphorylated amino acids with the developed MCE method. Our current work focuses on improving the selectivity of the method by derivatization so that the monophosphopeptide isomers could also be separated from each other.

Novel Aspect

Rapid separation of phosphopeptides by microchip (capillary) electrophoresis-electrospray ionization mass spectrometry.

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ThPS37-48 / Improved Sequence Coverage for PMF using a Droplet Interface for Nano-LC MALDI-MS

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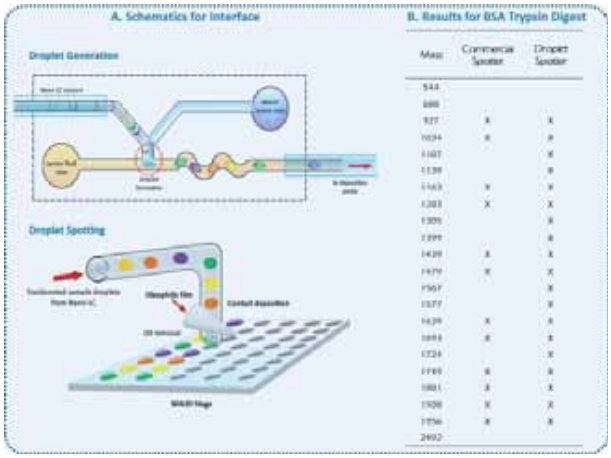
Introduction

Mass spectrometry (MS) and Liquid chromatography (LC) are among the most powerful tools in analytical science. The complexity of biological samples often necessitates these techniques to be coupled for multi-dimensional analysis to aid peptide mass fingerprinting (PMF). Whilst research and development has focused on improving separation and ionisation efficiencies, sample transfer between separation techniques represents a key bottleneck in protein identification. Here we demonstrate the successful application of droplet based microfluidics to sample transfer between LC and MALDI MS. The droplets facilitate fractionation of LC eluent a few centimetres after it leaves the LC column preserving resolution of the upstream separation.

Methods

The device employs a film to remove oil at the point of interface and consists of two fundamental parts: a droplet generation structure that fractionates effluent from an LC separation into droplets and a microprobe that transfers the sample to a "second dimension", here MALDI MS, concurrently removing the oil (Fig. A). The device mixes the sample with matrix prior to droplet formation, allowing the droplets to be delivered to the target in a MALDI-ready format.



Results

The performance of the interface device was compared to a commercially available automated spotter using several standards. One sample, Trypsin digested BSA, generated a profile with a large number of peaks when separated using Nano-LC. The molecular weight information for 22 of these peaks was provided by the manufacturer. Following MALDI analyses,

samples spotted using the droplet interface showed 19 peaks with their elution order preserved while the commercial spotter showed 12 (Fig. B). In fact, for all samples analysed, the droplet interface device performed better, seeing over 86% of all peptides present compared to ~ 56% observed by the automated spotter. Analysis using Mascot saw the protein identified as Bovine Albumin with a protein score of 179 for the droplet interface. Furthermore this was the only protein with a score above 61 and the sequence coverage was 34%. Similarly Bovine Albumin was also identified using the results from the commercial spotter with a protein score of 115 with sequence coverage of 23%. However, a Human Kinase was also identified as a potential hit with a score of 63.

Conclusion

Employing a droplet interface ensures that the resolution gained by LC separation is transferred unchanged downstream for MS analysis, improving sequence coverage and resulting in greater confidence in proteins identified. The next steps will include analysis of mixtures of protein digest to demonstrate the interfaces ability to deal with extremely complex samples.

Novel Aspect

There are several novel features of this technology, most significant of all is the ability to address single droplets and transfer them downstream for analysis while removing the carrier phase (oil) which can be detrimental to the analytical process.

ThPS37-49 / High-resolution peptidomic and transcriptomic profiling of spider venom: a rapid and comprehensive method for assessment of complex venom composition.

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Spider venoms are complex fluids, constituted of hundreds of different components, mainly peptides and proteins but also small molecules such as acylpolyamines. Nowadays 44540 spider species, distributed in 112 families are described (Platnick, The World Spider Catalog, Version 14.5). Among these only a small number of species have been studied so far in term of venom components and proteome investigation. A major limitation in the study of spider venom is accessing sufficient amount of raw material to conduct an in-depth proteomic analysis using traditional methodology.

A spider from the Heteropoda genus never investigated so far was chosen as a model for this study. The aim of our approach was to achieve a complete venom profile, combining UHPLC-QTOF-MS/MS analysis with venom gland transcriptome, obtained from a single run exploiting NextGen sequencing technology. This avoids the need for conventional techniques such as Edman degradation and de novo MS/MS sequencing, which are expensive and extremely sample and time consuming.

Peptidomic analysis on desalted and reduced venom was performed on a Waters Acquity UPLC with an Acquity C18 column, coupled to a Synapt G2 QTOF with an electrospray ionization source system. Chromatographic conditions were optimized in order to obtain the best resolution peptide profiling according to previously validated protocols [1]: column length, temperature, flow rate and gradient time were refined to obtain the best peptide separation efficiency. MS/MS acquisitions were performed in both survey and MSe modes, to generate a complete venom peptidomic map. Transcriptomic analysis using a cDNA library derived from venom gland mRNA was carried out and sequenced using the Ion Torrent Personal Genome Machine pipeline. MS/MS resulting masses were matched to sequences deduced from transcriptome analysis in order to automatically identify all peptide sequences expressed in the venom.

Exploiting the advances in the field of chromatography and mass spectrometry combined to NextGen RNA-Seq sequencing technology allowed the best achievable elucidation of a complex matrix such as venom, and a high resolution peptidomic profile could be obtained with a significant gain in terms of time and raw material consumption.

1 Eugster PJ, Biass D, Guillarme D, Favreau P, Stöcklin R, Wolfender J-L 2012. Peak capacity optimisation for high resolution peptide profiling in complex mixtures by liquid chromatography coupled to time-of-flight mass spectrometry: Application to the Conus consors cone snail venom. J Chromatogr A 1259: 187-199.

ThPS37-50 / Top-down venomics - High resolution mass spectrometry as a fast and accurate tool for the profiling of snake venoms

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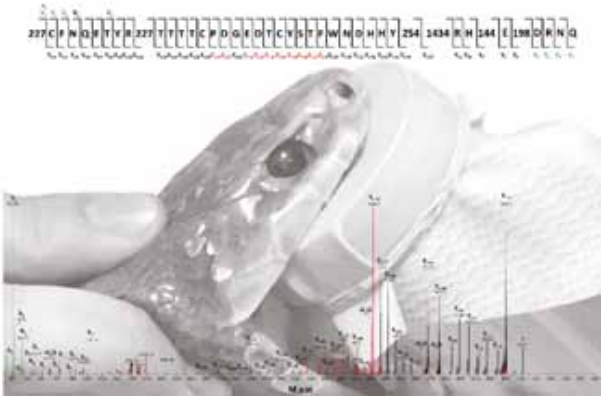
Introduction

Snake venoms are complex mixtures of bioactive peptides and proteins. A deep understanding of the composition of venoms is of high importance not only for exploring their enormous potential as sources of pharmacological novelty, but also to fight the dire consequences of snakebite envenoming. In the last decade several bottom-up proteomic strategies to explore venom proteomes have been developed. Drawbacks of these approaches are the co-elution (HPLC), low mass resolution (SDS-PAGE), or impaired quantification ability (2DE) of venom components. On the other hand, shotgun proteomics of whole venom or IDA protocols involving electrophoretic bands containing mixture of proteins may result in difficulties assigning all toxin isoforms.

Experimental and Results

To overcome these problems, we developed an LC-MS approach for the rapid characterization of snake venoms, which combines mass profiling of native and reduced venom components and online top-down MS/MS using CID and HCD. Combining HCD and CID spectra of various charge states we were able to sequence most of the toxins de novo and identify them by database search of the sequence tags.



Conclusion and Novelty

Here, we present results on the profiling and relative quantification capabilities of top-down MS for elapid snake toxins. This is the first time, top-down mass spectrometry has been applied for the in deep characterization of snake toxins.

ThPS37-51 / On-chip spyhole mass spectrometry for droplets-based microfluidics: Studying β-lactoglobulin interaction with liposoluble vitamins

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LEPA, EPFL

Water-in-oil droplets generation, handling and analysis using microfluidic devices have a wide range of applications in various scientific fields, what requires the integration of different detection methods into such devices. Mass spectrometry (MS) is an attractive alternative to the traditional fluorescence and electrochemical detection methods, owing to its sensitivity and analyte mass spectrum as a clear readout. However, the coupling of droplet-based microfluidics with MS still remains challenging due to the interference of the oil phase with electrospray formation. New ways of combining MS with droplet microfluidics are needed.

Herein, a novel on-chip approach for coupling droplet-based microfluidics with MS using the recently introduced Electrostatic-Spray Ionization (ESTASI)1 method is presented2. For this purpose the polyimide microchip fabricated by laser ablation was designed with a spyhole through which the water droplets were ionized when passing through the microchannel underneath. The ionization of the water-in-oil droplets was realized by the contactless application of high voltage square pulses with an electrode placed under the microchip and aligned with the spyhole and MS inlet. Meanwhile, the oil continued flowing to the microchip outlet to be adsorbed by a swab stick avoiding any contamination to the ion source.

After optimization the presented system was used for the performance of biphasic reaction between β-lactoglobulin and liposoluble vitamins, like vitamin E, A, D and K. It was discovered that in the case of vitamin E, vitamin E acetate and vitamin K, β-lactoglobulin not only bounds with the liposoluble vitamin, but also oxidizes it. The vitamins oxidation was proved by the performance of MS/MS analysis of their oxidation products, while reduction of the protein was proved by the tagging of its reduced cysteine residues with benzoquinone.

Proposed on-chip coupling of droplets microfluidics with MS provides the performance of dilution free droplets ionization without the additional oil removal step insuring sensitive MS detection by the contactless HV application. Due to the ESTASI mechanism, droplets containing only pure water or a basic pH solution can also be efficiently ionized without any sheath-flow of organic solvent and acidic pH. A variety of biphasic reactions in droplets can be performed and analyzed via ESTASI MS, as it was demonstrated for β-lactoglobulin and liposoluble vitamins, revealing new information about these protein-ligand interactions.

1. Qiao, L.; Sartor, R.; Gasilova, N.; Lu, Y.; Tobolkina, E.; Liu, B.; Girault, H.H. Anal. Chem. 2012, 84, 7422–7430.
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ThPS37-52 / Hyphenation of effect-directed enzymatic reactions to mass spectrometry

Therese Burkhardt, Johanna Grassmann, Christine Kaufmann, Thomas Letzel
TU Muenchen

The entirety of biocatalytic reactions is performed by enzymes. Especially in the field of environment and food/nutrition enzymes play an essential role. To determine their kinetic parameters or regulation (e. g. inhibition) conventional assays make use of photometrical, electrochemical or radiometric techniques.

Nowadays, these enzymatic reactions can also be designed in a manner to enable the direct coupling to a mass spectrometer. This hyphenation allows for a simultaneous detection of substrate depletion as well as product formation and moreover potential intermediates. Further advantages are the efficient screening of complex mixtures as well as the simultaneous analysis of different functional assays (multiplex enzyme reactions). So far, the direct hyphenation of multiple enzymatic and functional reactions to mass spectrometry has been realized.

A few enzymatic assays that investigate functional and regulatory characteristics are deeply presented with their advantages and information density.

Using this direct MS coupling insight into the mechanism of intestinal alkaline phosphatase (iAP), an important participant in intestinal pH regulation and inflammatory processes, has been gained [1].

Furthermore, an enzyme relevant in the field of food and nutrition is Xanthine oxidase, which can be used to characterize and identify antioxidants.

Finally, environmental enzyme classes, that have been established yet, are e.g. Chitinases and Cytochrome P450 (CYP) and will be presented. It is supposed that Cytochrome P450 plays an important role in the biotransformation of emerging trace organic chemicals (TOrC) in managed aquifer recharge systems (MAR) [2].

Concluding, various relevant enzymatic reactions, that have been established mass spectrometric compatible, will be presented showing the versatile application and the advantages of this technique in various fields. Robustness and method establishment will be shown as well as kinetic studies of products and intermediates, thus a novel view on effect-directed enzymatic reactions can be given.

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ThPS37-53 / Exploring the effect of DMSO as a mobile phase additive for improving protein identification and SWATH acquisition by NanoLC/MS/MS

Remco van Soest, Jenny Albanese, Mark Yang, Christie Hunter, Eike Loge
AB SCIEX

NanoLC/MS/MS is the current method of choice for high sensitivity identification of proteins, e.g. for the discovery of biomarkers in plasma or other biological fluids. It has recently been demonstrated that solvents like DMSO can increase electrospray ionization efficiency and improve sensitivity. In this paper we will discuss optimization of the concentration of DMSO, the effect on chromatography, and the improvements on proteomics applications.

A nanoLC-MS set-up using chip based columns was used, with DMSO either added post-column, or added directly to the mobile phase. For adding DMSO post-column a nanoLC chip column was developed with an integrated post-column Tee. The effect of adding DMSO was studied by looking at peptide sensitivity and the overall number of peptides/proteins identified in a complex mixture, using a QqTOF type mass spectrometer.

Using post-column addition of DMSO, the optimal % DMSO after addition was found to be 5%, consistent with previous observations in the literature. An increase in peak area for 13 Beta-galactosidase tryptic peptides was observed that varied from 1.2 to 2.6 x, with a median of 1.9 x. For a Yeast digest, a significant increase in peptides and proteins identified was seen (30 and 20% respectively). Next, adding 5% DMSO directly to the mobile phase was explored. A slight effect on retention times,

and minimal impact on peak widths was observed The effect on sensitivity as a function of peptide properties and the effect on protein identification is currently under investigation.
The addition of DMSO promises to improve the number of proteins identified in complex samples using nanoLC/MS/MS, benefiting biomarker discovery research. Next steps will be to characterize the impact of this workflow on SWATH acquisition results.

ThPS37-54 / Glass separation chips with sharp, monolithicically integrated electrospray emitter for rapid bioanalysis

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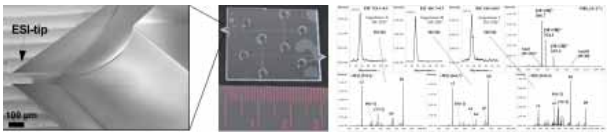
University of Helsinki

Introduction

Microchip electrophoresis (MCE)-electrospray ionization mass spectrometry (ESI/MS) is a very promising technology for modern bioanalysis. Glass provides ideal materials properties for MCE, but fabrication of a sharp emitter tip out of glass using parallel processing approaches (i.e., lithography, etching) has long remained a challenge. We have recently resolved this technological keystone by developing a new microfabrication protocol for standard glass wafers which yields monolithically integrated glass MCE-ESI chips [1]. In this work, we demonstrate the high feasibility of the glass MCE-ESI chip for various bioanalytical applications.

Methods

The MCE-ESI chips were composed of two through-wafer etched, 500-µm-thick borosilicate glass wafers that were thermally bonded together. Through-wafer etching was required to form the inlets and the emitter tip, while shallower etching was used for patterning of the microchannels [1]. For MCE-ESI/MS analysis, the chips were coupled to an iontrap mass spectrometer in positive ionization mode and actuated by electrokinetic flow.



Results

Our microfabrication approach exploits the isotropic etching profile of glass in order to create three-dimensionally sharp tips (Fig. 1). Instead, most previous glass MCE-ESI devices are created by sequential micromachining or manual processing of one emitter at a time [2,3], which may result in large chip-to-chip variation in their performance. Here, rapid separation (<1 min/cycle) of three angiotensin peptides was shown with excellent run-to-run repeatability (3.0% RSD, n=4, migration time) (Fig. 1). Feasibility of glass MCE-ESI devices for intact protein analysis was also demonstrated with selected protein standards of varying size and hydrophobicity. As a result, on-line monitoring of the short-lived pre-steady state hydrolysis products of α-chymotrypsin (i.e., enzyme-product complexes) was achieved. Monitoring of such complexes with conventional instruments is severely compromised because of their poor control over (reaction) time resolution. Last, monitoring of an antiepileptic drug, topiramate, in mouse serum following off-chip liquid-liquid extraction was demonstrated with good linearity (R2=0.9998, 1.6-50 µg/mL) at clinically relevant concentrations.

Conclusions

The results evidence that our microfabrication approach is feasible for mass production of glass MCE-ESI chips for multifaceted bioanalysis. Together with the possibility to use largely varying solvent conditions and ESI voltages as low as 1.4 kV [1], the wafer-scale processing of multiple separation chips simultaneously is likely to race with commercial nanospray in various proteomic and metabolomic applications.

Novel aspect

New glass separation chip design for bioanalysis

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ThPS37-55 / UHPLC-UV-MSE analysis for the characterization of carotenoids and chlorophylls in Scenedesmus obliquus microalgae

Rosa Maria Alonso Salces[1], Beatriz Abad[2], Mauro Do Nascimento[1], Leonardo Curatti[1], Sandra Rosa Fuselli[3], Luis Angel Berrueta Simal[2], Blanca Gallo Hermosa[2]
[1]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Investigaciones en Biodiversidad y Biotecnología (INBIOTEC), [2]Departamento de Química Analítica, Facultad de Ciencia y Tecnología, Universidad del País Vasco/Euskal Herriko Unibertsitatea (UPV/EHU), [3]Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata

Introduction

Carotenoids and chlorophylls are valuable bioactive compounds that can be extracted from microalgal biomass. They are used not only as a natural food colouring agent but also as additive in pharmaceutical and cosmetic products, and have antioxidant as well as antimutagenic properties. Scenedesmus obliquus is a freshwater microalga that can grow in industrial wastewaters of different origins showing good adaptation ability, being a very versatile microalga as raw material for biofuels production and as a source of natural bioactive compounds, such as carotenoids and chlorophylls.

Methods

Fried-dried microalgal material (0.05g) was sonicated with 70% acetone in water (containing NaF 0.2% (w/v) during 20 min. The extract was filtered through a 45mm PTFE filter, prior to injection into the UHPLC system (Waters ACQUITY UPLCTM) coupled to PDA detector and a quadrupole time of flight (Q-ToF) mass spectrometer (Waters SYNAPTTM G2 HDMS), equipped with an electrospray ionization (ESI) operating in positive mode. Analyses were performed on a reverse phase C18 column (Waters Acquity UPLC HSS T3 1.7 µm, 2.1 mm × 100 mm), as described by Fu et al. (2012). UV-visible spectra were recorded in the range 250–500 nm. Carotenoids and chlorophylls were monitored at 450 nm. Data was acquired in MSE mode from m/z 50-1200, creating two discrete and independent interleaved acquisition functions to collect: i) low energy or unfragmented data, and ii) high energy or fragmented data.

Results

The profile of carotenoids and chlorophylls produced by S. obliquus during standard growth conditions was characterized by ultra high performance liquid chromatography coupled with diode array detection and mass spectrometry (UHPLC-DAD-Q-ToF/MSE). A single run allowed isomers separation, UV detection, accurate mass measurements, and MS/MS spectra for structural elucidation. New pigments not previously reported in S. obliquus were identified and confirmed by combining information coming from unfragmented and fragmented data and using key diagnostic fragmentations.

Conclusions
MSE as an untargeted methodology has been demonstrated to be a useful tool for the identification of carotenoids and chlorophylls in microalga in a single run.

Novel Aspect
A rapid and selective ultrahigh performance liquid chromatography with diode array detection coupled to electrospray ionization and quadrupole time-of-flight mass spectrometry (UHPLC-DAD-ESI-Q-ToF-MS) strategy using automatic and simultaneous acquisition of exact mass at high and low collision energy, MSE is used for the fist time to study the profiles of carotenoids and chlorophylls in S. obliquusmicroalgae.

Fu, W.; Magnúsdóttir, M.; Brynjólfson, S.; Palsson, B. Ø.; Paglia, G., Analytical and Bioanalytical Chemistry, 404 (2012) 3145-3154.

ThPS38 - Non-Covalent Interactions

11:00-15:00
Poster Exhibition, Level -1

ThPS38-01 / Study by Electrospray Ionization Mass Spectrometry of the Complexation of Histone Deacetilase Inhibitors With Divalent Metal Ions

Gastón Siless, Gabriela Cabrera
DQO-UMYMFOR, FCEN, Universidad de Buenos Aires

Introduction
Histone deacetylase inhibitors (HDACIs) are a class of anti-cancer drugs which act as epigenetic modifiers. The HDAC family of enzymes consists of 18 known members divided into four classes, three of which are metalloenzymes that require a divalent metal ion for substrate binding and catalysis. Different classes of HDACIs are known, some of which are hydroxamic acids that chelate active Zn2+ in a bidentate manner. Since the mode of action of these compounds is related to its binding capacity of Zn2+, and they are known to chelate other metals such as Co, Cu or Ni, the goal of this study was the analysis by Electrospray of the complexes obtained from HDACIs in the presence of Zn2+ and other metals and compare their differences.

Methods
Mass spectrometric analyses were performed using a Bruker micrOTOF-Q IImass spectrometer equipped with ESI.
The analyte solutions of the HDACIs vorinostat (suberoylanilide hydroxamic acid, SAHA), octyl hydroxamic acid (OHA) and sodium valproate (VPA), at a concentration of 10 mM, were prepared using methanol. The metal ion stock solutions (10 mM) were prepared from the metal salts of Zn (II), Ca (II), Co (II), Ni (II) and Cu (II) in the form of chlorides or sulphate (Cu).

Results
Characteristic spectra were obtained in all the cases (the three inhibitors with the five metals). [M + Me -H]+ and [2M + Me -H]+ were the most abundant metal adducts observed where Me is Zn (II), Co (II) and Ni (II) and M is SAHA or OHA.
The spectra of the HDACIs with Ca2+ showed deprotonated adducts with up to 3 ligands and other dicharged species with 3 to 5 ligands. The base peak was different for the three inhibitors.
The spectra in the presence of Cu2+ showed more ions, including those mentioned above, [3M + 2Cu -3H]+ and [M + Cu(I)]+ .
VPA generally showed much more complex spectra, with a great number of species, including adducts with water, chlorine and up to 5 ligands.
All the ions were characterized by their accurate molecular weight, their characteristic isotopic profiles and MS/MS.

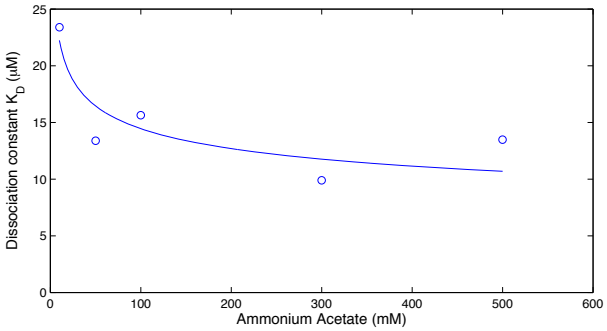
Conclusions
Although some generalizations could be made on the type of adducts observed in the MS spectra of SAHA, OHA or VPA in the presence of different metal cations, the spectra were in general characteristic, with the presence of some particular adduct or with a different relative abundance among the formed species. Although the studied HDACIs have the same type of bioactivity, they exhibit a clearly different behavior in the presence of different metals which may help explain their dissimilar enzymatic specificity. The activity of HDACIs has been previously related to the formation of bidentate chelates, but in the present study other species are also formed in high relative abundance.

Novel Aspect
Metal complexation of HDACIs allows understanding of how these compounds bind to metals and may contribute to explain the different selectivity in their activity.

ThPS38-02 / Effect of Buffer Concentration on Protein-Ligand Binding Affinities Measured by Native ESI-MS

Agni Faviola Mika Gavriilidou, Basri Gülbakan, Renato Zenobi
ETH Zurich, Department of Chemistry and Applied Biosciences, Switzerland

The discovery and characterization of protein-ligand interactions is crucial for the understanding of biochemical reactions and pathways as well as for a subsequent design of new therapeutics for treatment of different human diseases and infections, thus it is of great importance to study the effect of buffer concentration on the protein- ligand affinities.
A powerful and increasingly utilized tool for the investigation of noncovalent interactions is native electrospray ionization mass spectrometry (ESI-MS). The most frequent buffer choice for studying non-covalent protein-ligand interactions by native ESI-MS analysis is ammonium acetate. The buffer concentration used in native ESI- MS titration measurements is generally in the low to high mM range. Studies have shown that by varying the buffer concentration the relative abundances of the protein-ligand complex to the free protein can vary (Benkestock et al. 2004; Kapur et al. 2001). However, no systematic studies of the effect of the buffer concentration on the apparent affinity of the ligand to a receptor have been performed.



A well studied system (lysozyme-NaG3) was chosen in order to show to which extent the concentration of ammonium acetate buffer used for nanoESI-MS affects the value of the dissociation constant (KD). For this study five different ammonium acetate buffer concentrations were used (10 mM, 50 mM, 100 mM, 300 mM and 500 mM). It is observed that the KD is decreasing with increasing the ammonium acetate concentration (Figure 1).
As a general trend, it is observed that by increasing the ammonium

acetate concentration the KD values are decreasing. The affinity of NaG3 to lysozyme is altered by a factor of up to 50% with increasing the buffer concentration.
To date it has not been shown to which extent the buffer concentration affects the value of the dissociation constant (KD). Different model systems (protein/aptamers-ligands) will be tested in order to investigate this effect.

ThPS38-03 / MALDI-MS quantitative analysis of organometallic complexes: application to anti-cancer drug candidates.

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Organometallic compounds currently used in chemotherapy are platinum(II) compounds, the cisplatin being the first member of these Pt-based anti-cancer agent[1]. Its anti-tumoral activity goes along with terrible side effects, like neurotoxicity, nephrotoxicity, ototoxicity... Furthermore, some cancers showed innate or developed resistance towards cisplatin, making the treatment inefficient. In order to find alternative drugs with improved efficiency and acceptable toxicity, research was directed at development of new metal-based drugs. Part of this research is based on the Platinum Group Metals (PGM) which includes Palladium, Ruthenium, Osmium and Iridium. Our study concerns two anti-cancer drug candidates[2],[3]: one Ruthenium based compound 'RDC11' and one Osmium based compound 'ODC9'. Both complexes have shown promising cytotoxic activity in vitro against cancer cell lines. Here, the behaviour of RDC11 and ODC9 in human body was evaluated using mass spectrometry, and especially their stability in vivo.
In this study, a MALDI-MS quantification protocol was developed to monitor the evolution of RDC11 and ODC9 in biological fluids (plasma, cellular extracts). Although such organometallic complexes are usually characterized by ESI-MS, in our case MALDI-MS was preferred because of its higher tolerance towards sample nature.
Calibration curves were built in biological fluid by measurement of area ratio of analyte towards structural analogous references. The kinetic study of ODC9 showed a stability for at least 4 days in human plasma. According to preliminary HPLC data, RDC11 is no more detected after 4 hour-incubation in plasma. A comparative stability study by MALDI-MS was realized to support or disconfirm such results.
In conclusion, we developed a viable and fast MALDI-MS protocol for the quantitative analysis of inorganic anti-cancer drug candidates in biological fluids without sample pretreatment.

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ThPS38-04 / Characterization of a Cisplatin-DNA-Antibody and its Corresponding Antibody-Antigen-Complexes by Mass Spectrometry under Native Conditions

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Since antibodies have been increasingly used in the diagnostic and therapeutic medicine, new analytical tools for their detection and characterization are getting more and more important. In recent years, mass spectrometry, especially in combination with soft ionization techniques like electrospray ionization, has been employed for the detection of high masses. This allows the analysis of precise molecular masses of large native proteins like antibodies without any fragmentation. In order to understand structural aspects of native non-covalent complexes, the analysis of a whole antibody-antigen-complex by mass spectrometry has been investigated and first results are presented here.
We analyzed the rat antibody R-C18 specific for Cisplatin induced DNA adducts that was developed by Thomale et al. [1] The detection of this antibody in its native state was successfully accomplished by using a modified nano-ESI time of flight mass spectrometer. [2] We were also able to identify up to 8 fine structures, probably referring to PTMs like N-glycosylation and C-terminal lysines. The mass of the unmodified antibody for the most intensive signal was determined to 146.0 kDa. CID-based experiments resulted in three main fragments with a mass of 132.6 kDa, 122.3 kDa and 119.5 kDa.
With regards to analyses of antibody-antigen-complexes, we used 30 and 50 base pair long synthetic oligonucleotides to produce the specific platinated antigens. The sequences of these oligomers were chosen that only one of the single strands contains two adjacent guanines to yield the supposed binding pattern G*G*-cisPt(NH3)2 after incubation with Cisplatin. This resulted in unmodified and platinated single and double stranded DNA-oligomers, which we were able to detect by mass spectrometry.
The whole antibody-antigen-complex containing these synthetic platinated oligomers could be successfully analyzed in positive ionization mode. Surprisingly, the antibody does not only bind platinated synthetic double strand, but also the corresponding platinated single strand DNAs. That means that the binding pattern of this special antibody might either not be restricted to Cisplatin modified DNA double strands, or the until now assumed binding pattern of G*G* intrastrand Cisplatin adducts is not correct.
Dissociation of the complex in MS/MS mode was successfully performed by applying collision energy of 150 eV. As a result, we could detect signals for the free intact antibody, the above mentioned specific antibody fragments, while we did not detect any specific signal of the DNA part of the complex. This suggests a high stability of the complex and also of the antibody itself in contrast to the oligomers, which easily undergo fragmentation.
In conclusion we have shown the successful detection and specific fragmentation of the antibody and its antibody-antigen-complexes under native conditions via nano-ESI-High-Mass-Q-ToF-MS and MS/MS. In the future we are aiming to determine the precise epitope-binding structure of DNA antigens and their diagnostically relevant antibodies.

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ThPS38-05 / Native nano-ESI mass spectrometry studies of aptamer-ligand complexes

Basri Gulbakan, Basri Gulbakan, Konstantin Barylyuk, Petra Schneider, Gisbert Schneider, Renato Zenobi
ETH Zurich

Introduction

The study of the non-covalent interactions of biomolecules with various ligands is an area of great importance. In recent years, aptamers has attracted great interest owing to their high affinity and specificity. Therefore development of direct and label-free methods to study aptamer-ligand interactions will be of great value. In this study, we demonstrate the use of native nano ESI-MS to investigate different aspects of aptamer-ligand interactions.

Methods

Adenosine(ABA) and L-argininamide-binding (LBA) aptamers were obtained from Eurogentec. Aptamers and their ligands were dissolved in 50 mM ammonium acetate (AmAc) buffer (pH 7.4). NanoESI-MS analyses were performed with a hybrid Q-TOF mass spectrometer (Synapt G2-S, Waters, UK) in negative ion mode. Samples were infused with gold coated nanoESI emitters. The capillary voltage was set to 0.8 kV. The source temperature was kept at 25 ˚C. Instrumental conditions were adjusted to detect the non-covalent complexes without dissociation.

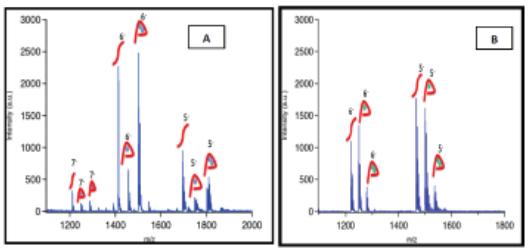

Figure 1. Native nano-ESI mass spectra of adenosine- binding aptamer / 5-NH_2-5`-deoxyadenosine complexes (A) LABA / L-argininamide complexes (B).

Preliminary results

Nano ESI-MS experiments were conducted with ABA and 8 different structural isomers of adenosine: namely adenosine (1), inosine (II), 2`-deoxyadenosine (III), 3`-deoxyadenosine (IV), 5`-deoxyadenosine(V), 2-NH2-2`deoxyadenosine (VI), 3-NH2-3`-deoxyadenosine (VII), and 5-NH2 -5`-deoxyadeosine (VIII). Nano ESI-MS results revealed that binding order of different ligands with the ABA is VIII> VII >1> VI> IV=III=II. This trend is in agreement with the original SELEX study of ABA, in which deletion of the hydroxyl groups resulted in the loss of binding. In the case of ligands (VIII), (VII), and (VI), addition of an amino group on the sugar ring restored the binding. ABA was titrated with (VIII) ligand in 50 mM AmAc buffer under native-like conditions (Figure 1). ABA has two binding sites and both (1:1) and (1:2) complexes are observed. Our results show that the binding occurs cooperatively. To extract the Kd value and to account for the role of non-specific ESI processes, we used a scrambled sequence and developed a binding model and fitted the data accordingly. Our results showed that K1= 82 uM and K2 = 16 uM. Native nano ESI-MS of (LABA) revealed two binding sites. One binding site (1:1 complex) was tighter than the other one (1:2 complex). To investigate the ligand specificity, L-phenylalaninamide (P), L-tyrosinamide (T), and L-argininamide (LA) ligands were mixed with LABA and measured by native ESI-MS. The binding strength followed the trend of A>T>P. LABA was titrated with (LA) in 50 mM AmAc buffer. (Figure 1) We have found the Kd values of K1= 129 uM and K2 = 741 uM. To compare our results, isothermal titration calorimetry experiments were performed in 50 mM AmAc. We found Kd=6.8 uM and Kd=27 uM for (ABA) / (VIII) and (LABA) / (LA) complexes, respectively.

Novel aspect

The first comprehensive native mass spectrometry study on aptamer-ligand interactions revealing the stoichiometry of complexes, ligand specificity, binding affinity, and cooperativity of binding.

ThPS38-06 / Interaction of phospholipid pulmonary surfactants with nanoparticles studied in liquid media by LC MS and MALDI TOF MS

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Introduction

Inhaled nanoparticles (NPs) may be deposited in the lungs, where pulmonary surfactant (PS) is present at the air-liquid interface. NPs may interact with PS and inactivate the surfactant function causing reduced lung function. PS consists of proteins and phospholipids with detergent-like properties, which reduce the surface tension and prevent collapse of alveoli at the end of expiration.

This study aims to investigate the mechanism of interaction between the NPs and PS by studying the ability of the NP to adsorb PS on their surface. To carry out this, we developed an in vitro protocol for different NPs and a phospholipid using organic solvent, which is needed to dissolve phospholipids. Dipalmitoyl-phosphatidyl-choline (DPPC) was selected as model PS. It constitutes about 50% of the phospholipids which constitute about 90 % of the total mass of the PS.

MALDI-TOF-MS was used to measure DPPC directly on precipitated NPs and in solution. LC-MS was used to more quantitatively and reliably measure DPPC in solution, in order to validate the results obtained by MALDI-TOF-MS.

Method

Samples for MALDI-TOF -MS analysis were premixed with matrix. 1 mL of mixture was spotted on to the target plate and dried under a stream of warm air to ensure the homogenous co-crystallization by rapid evaporation. All MALDI spectra were acquired manually in the positive ion mode using Bruker Autoflex III.

LC-MS (Agilent 1200 HPLC–Bruker micrOTOF-Q): Injection of 5 µL sample on a C18 column at a flow of 0.3 mL/min, column temperature at 60°C, and isocratic elution with 20% of A and 80% of B for 10 min. Mobile phase A and B consisted of 0.1% formic acid and 0.1 mmol ammonium acetate in MilliQ water and in 2-propanol, respectively. Data acquisition was in the positive ion mode.

Results

The analysis of supernatants from the interaction study with both LC-MS and MALDI-TOF-MS showed a linear correlation (R2 = 0.99) of the DPPC measured in the supernatant with the initial added DPPC. A similar tendency was observed for the precipitates. We used the theory for gas-particle partitioning in air to explain this linear correlation by directly translating the theory into a thermodynamic particle-liquid partition coefficient.

Conclusion

These preliminary results show that a small amount (8-11%) of DPPC was adsorbed on the surface of NPs by simple physical adsorption.

Novel Aspect

We have developed a protocol for studying the interaction of DPPC with nanoparticles using MALDI-TOF-MS and LC-MS and a theoretical framework describing the results.

ThPS38-07 / Characterization of calcium phosphate salt catalysts by MALDI-TOF mass spectrometry

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CNRS/ICSN

Introduction

Calcium phosphate salts have proved to be catalysts of choice for the electrophile α-bromination of enecarbamates in a highly enantioselective and diastereoselective manner. These complexes have been little studied and described in the literature. A 31Phosphorus NMR study has shown that these complexes exist in various forms. Crystals of these compounds could not be obtained for X-ray diffraction analysis. Thus, a study by mass spectrometry was essential to study their chemical structures. As these compounds are only soluble in volatile organic solvents (tetrahydrofuran, dichloromethane, etc....), electrospray is not well suited for their ionization. To the contrary, MALDI-TOF mass spectrometry offers the possibility to detect more easily compounds prepared in such solvents.

Methods

Two families of MALDI-TOF matrices, an aprotic matrix which contains no electron-donating group, as trans-2-[3 - (4-tert-butylphenyl)-2-methylpropenylidene] malononitrile (DCTB) or 7,7,8,8-tetracyanoquinodimethane-p-quinodimethane (TCNQ), and a matrix proton donor, as 2,5-dihydroxybenzoic acid (DHB) or 1,8,9-anthracenetriol (Dithranol, DIT), were firstly tested. The DCTB proved to be the best choice of matrix for studying this type of complexes, in terms of mass resolution or signal-to-noise ratio. Then, sample preparation was optimized by selecting the solvent (THF for samples and matrix), the ratio between matrix and sample concentration (103), and the sample deposition method (dried droplet). We finally obtained a sensitivity of a few tens of femtomoles deposited on the plate.

Results

The calcium phosphate salt catalysts have chiral groups more or less congested on the BINOL 3 and 3' positions. Results obtained by MALDI-TOF mass spectrometry show that different forms of complexes exist depending on the size of these congested groups. As an example, a phosphoric acid with very bulky groups (triisopropyl) exists as a complex [2 Ligands + Calcium]. By contrast, catalysts bearing less congested groups exist in the form of one species [3 Ligands + 2 Calcium]. Therefore, MALDI-TOF can be considered as an efficient alternative method to determine the chemical structures of calcium phosphate salt catalysts when X-ray diffraction cannot be employed.

Novel aspect

Alternative analytical tool for the characterization of calcium phosphate salt catalysts

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ThPS38-08 / High Resolution Native Mass Spectrometry allows rapid characterization of therapeutic monoclonal antibodies: from PTM to antibody-drug conjugates analysis.

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Introduction

Antibody-based therapeutics are complex molecules, and, as such, put quite some challenge on analytical tools to characterize them in detail. In this context, mass spectrometry (MS) is gaining importance in the analysis of mAbs.
We developed and applied native MS methodologies that represent excellent tools for the rapid and detailed characterization of therapeutic mAbs. In particular, here we describe the qualitative and quantitative analysis of complex mAbs mixtures, the analysis of mAbs glycosylation profiles and the analysis of antibody-drug conjugates (ADCs).

Methods

Purified mAbs samples, buffer exchanged into ammonium acetate, were directly injected into a LCT (Waters) or Orbitrap Exactive Plus EMRTM (ThermoFischer Scientific). Data analysis was done using either MassLynx (Waters) or Excalibur and Protein Deconvolution (ThermoFisher Scientific). When complete deglycosylation or, alternatively, specific glycan cleavage was required, antibodies were incubate overnight at 37 °C, prior to buffer exchange, with specific enzymes such as PNGaseF, β1,4 galactosydase or neuraminidase.

Results

By using native mass spectrometry, we can simultaneously identify and quantify complex mixtures of (bispecific) monoclonal antibodies co-expressed in a single cell platform. To validate our quantitative data, the same antibody mixtures were analyzed by cation exchange chromatography.
We recently pioneered native MS using an Orbitrap mass analyzer, resulting in significant improvements in performance compared to the time-of-flight (TOF) instruments typically used for such measurements. This new instrumentation not only allowed us to further extend the mixture complexity, but also to focus our interest into PTM analysis.
By using the new Orbitrap Exactive Plus EMR instrument we developed a new method that allows confident glycosylation analysis directly at the intact protein level. The improved resolution enabled by using the EMR instrument results in baseline separation and accurate mass determination of all different glycosylation variants decorating mAbs. To validate glycan identification, we adopted a slightly different approach that uses enzymes specific for the cleavage of particular glycan residues.
We finally show how native MS can play also an important role in the analysis of ADCs, wherein the new EMR instrument allows unprecedented detail in their molecular characterization.

Conclusions

Our achievements show how native MS can now be used for a number of applications ranging from characterization of mAb mixtures to PTMs analysis. We are confident that biotechnology and biopharmaceutical companies can benefit from the ease and speed of this technique.

Novel Aspect

Qualitative and quantitative characterization of mAb mixtures can now be obtained in a single step. In addition, with the introduction of the new EMR instrument, detailed glycosylation analysis is now possible at the intact mAb level.

ThPS38-09 / The application of mass spectrometry to identification of the epitope in human cystatin C – monoclonal antibody HCC3 complex.

Monika Rafalik, Martyna Prądzińska, Aleksandra Kołodziejczyk, Aneta Szymańska, Sylwia Rodziewicz-Motowidło, Paulina Czaplewska
University of Gdansk

Introduction

Human cystatin C (hCC) is a protein (120 amino acid, 13.3 kDa), found in all human physiological fluids, which main function is inhibiton of cysteine proteases. HCC belongs to amyloidogenic proteins that can dimerize and precipitate in tissues, leading to amyloid diseases. In the case of hCC it is hereditary cystatin C amyloid angiopathy (HCCAA), in which deposits of hCC L68Q mutant are formed in brain arteries [1,2]. It was proved, that interactions with antibodies might suppress hCC dimerization

and may constitute a basis for immunotherapy. HCC3 is one of anti-hCC antibodies, which reduce the in vitro formation of cystatin C dimers by 60% [3].

Methods
In the experiments, affinity chromatography and the "epitope excision" and "extraction" methods based on proteolysis of antigen-antibody complexes and further MS studies were used [4]. Trypsin, AspN and pronase were used as proteolytic enzymes.

Results
The initially found epitope was assigned to fragments from the region 45-65 and 93-118 of human cystatin C. It was also shown that the synthesized fragments: hCC 43-72 and hCC 93-120 bind to HCC3 antibody and, on the other hand, interact with each other.

Conclusions
The obtained results suggest that the epitope is discontinuous. In order to confirm and narrow down the epitope sequence we are going to use mass spectrometry combined with hydrogen-deuterium exchange.

Novel aspect
Identification of binding site(s) in hCC-HCC3 complex may allow for a search of effective drugs for HCCAA immunotherapy.

Acknowledgements:
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[4] U. Reineke, M. Schutkowski. (2009) Mol. Biol. 524, 87-101

ThPS38-10 / A high-resolution quantitative BN-MS approach for comprehensive analysis of protein complexes

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Introduction
Blue native (BN) gel electrophoresis is a powerful method for size-based separation of solubilized protein complexes [Schägger & Pfeiffer (2000), EMBO J 19(8):1777-83]. Combined with liquid chromatography-tandem mass spectrometry (LC-MS/MS) and, more recently, clustering algorithms, it allows for unbiased identification of protein complexes and new subunits as demonstrated for a number of model systems like mitochondrial membranes [Fandiño et al. (2005), J Mass Spectrom 40(9):1223-31; Wessels et al. (2009), Proteomics 9(17):4221-8; Remmerie et al. (2011), J Proteomics 74(8):1201-17; Sessler et al. (2012), Amino Acids 43(3):1119-29; Heide et al. (2012), Cell Metab 16(4):538-49]. However, current BN-MS approaches do neither make use of the full resolution nor the quantification potential offered by BN separation and mass spectrometry.

Methods
Here we present a new method combining cryo-microtome slicing of BN gels (to obtain large series of well-defined gel slices with an adjustable step size from 0.1 to 0.5 mm) and quantitative high-resolution LC-MS/MS analysis of in-gel digested proteins. To extract reliable protein profiles for clustering of protein populations from a large number of highly complex MS datasets, we developed a stringent workflow involving m/z, retention time and intensity calibration, elimination of MS assignment errors, protein profile building, population fitting and clustering as well as size calibration with marker complexes.

Results
Application of this approach to a mitochondrial reference sample demonstrated a high degree of comprehensiveness and accuracy, effectively resolving complexes differing in size by less than 10%. Using the well-studied mitochondrial respiratory chain complexes as a benchmark, more than 90% of the described (>100) subunits and assembly factors of complexes I-V could be detected and their profiles assigned to a total of more than 20 distinct (super)complex populations. Detailed inspection revealed as yet unresolved subpopulations in particular for cytochrome C oxidase (complex IV) and a novel supercomplex-specific protein subunit for complex III-complex IV assemblies.

Conclusions
In summary, the combination of native gel separation, high resolution sampling and advanced data processing proved as a powerful approach for comprehensive and de-novo identification and quantification of protein complexes.

Novel Aspect
The state-of-the-art BN-MS technique was significantly improved concerning cluster resolution, complex (component) coverage and supercomplex characterization.

ThPS38-11 / Mass spectrometry as a powerful tool in antigen-antibody studies.

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Introduction
Amyloidoses are a group of diseases in which the main role is played by abnormal folding of peptides and extracellular proteins. Amyloid deposits are also formed by ubiquitous protease inhibitor, human cystatin C (hCC) and its amyloidogenic mutant L68Q [1]. Monospecific antibodies could be one of potential weapons able to stop pathological aggregation. Immunotherapy has been successfully used for the treatment of some diseases, such as breast cancer, leukemia, asthma, arthritis and Crohn's disease [2]. In the case of hCC aggregation it was proved that catalytic amounts of antibodies added at conditions promoting formation of dimers can significantly suppress dimerization of both wild type cystatin C (wt-hCC) and its highly amyloidogenic mutant L68Q [3].

Methods
For epitope identification the epitope excision/extraction methods based on proteolysis of antigen-antibody complexes and further MS studies and affinity chromatography were used [4].
In antibody sequencing the bottom-up analysis was the method of choice. The peptide mixture was analyzed by liquid chromatography – tandem MS analysis (LC-MS/MS) or by MALDI MS. The antibody identification was finally made by

comparing the experimental mass spectrometric data (MS and MS/MS spectra of peptides) with the spectra of a theoretical digest of the mAb. Unidentified peptides were sequenced by mass spectrometric de-novo sequencing.

Results
By the combination of different methods we are able to present here the epitope sequence located in C-terminal part of human cystatin C and primary structure for light and heavy chain of mAb Cyst13 with the sequence of CDR regions.

Conclusions
In our laboratory studies are being conducted on identification of epitopes in the cystatin C sequence for selected antibodies. In this case with the use of mass spectrometry we could fully characterize binding sites for protein complex, namely between human cystatin C and monoclonal antibody Cyst13.

Novel aspect
Determination of epitopes of hCC, which bind antibodies is essential for planning potential immunotherapies, designing of inhibitors of the dimerization/oligomerization processes of cystatin C and also for understanding molecular mechanisms of toxic oligomers formation.

Acknowledgements:
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[4] U. Reineke, M. Schutkowski. (2009) Mol. Biol. 524, 87-101

ThPS38-12 / Association behavior of Ser-Ser dipeptide: magic clusters and metal ion binding

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Self-assembly of amino acids is in the focus of intensive interest since years. A unique self-directed oligomerisation of amino acid serine (Ser) was observed previously using electrospray ionization mass spectrometry. Serine shows a strong preference to form protonated octamer clusters. It was demonstrated that this association strongly depends on the enantiomeric composition: the octamer complex is characteristic in optically pure solution only. Since amino acids are the building blocks of macromolecules, their spontaneous, chiroselective aggregation could represent the initial step for the evolution of life on Earth.
In this work, our aim was the investigation of complex formation ability of seryl-serine dipeptides (H2N-Ser-Ser-COOH), focused on the possible formation of "magic" clusters. To have an insight into the chiral preference during the complex formation, we have synthesized an enantiomerically pure peptide with two L-Ser amino acids (L-Ser-L-Ser, SS), as well as its diastereomer pairs: L-Ser-D-Ser (Ss) and D-Ser-L-Ser (sS) peptides.
Complex formation was studied by electrospray ionization mass spectrometry using a Bruker Esquire 3000+ ion trap mass spectrometer. Ser-Ser dipeptides show unusual self aggregation both in the positive and in the negative mass spectra, especially in the presence of metal ions (e.g. K+). Under the conditions applied, L-Ser-L-Ser dipeptide forms intensive protonated or cationated clusters up to 1700 m/z. In the positive electrospray ionization mass spectrum, the most characteristic ions are the dimer [2SS+H]+, the trimer cluster incorporating one potassium ion [3SS+K]+, the tetramer cluster incorporating again one potassium ion [4SS+K]+, as well as diverse multiply charged, larger clusters. In the negative electrospray ionization mass spectrum, the most characteristic oligomer is the tetramer cluster incorporating again one potassium ion [4SS+K-2H]-. In the presence of KCl salt, the most characteristic clusters are [2SS+3K-2H]+, [3SS+4K-3H]+, [4SS+4K-3H]+, [4SS+5K-4H]+, with a moderate preference to form [4SS+4K-3H]+ type cluster ion. Interestingly, we did not observed significant difference between the diastereomer peptide pairs.
Our results show that Ser-Ser dipeptides have a slight preference to form tetramer type cluster ions, which demonstrates the first time, that small peptides can also associate in a selective manner, similarly to amino acids.

Acknowledgements
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ThPS38-13 / Identification of epitopes and characteristic of anti cystatin C antibodies.

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Introduction
Human cystatin C (hCC) is a protein which belongs to the cysteine proteinase inhibitors and is used as a marker for glomerular filtration rate (GFR). Mutation of the gene encoding cystatin C, resulting in single amino acid substitution (L68Q) leads to the amyloid angiopathy (amyloid C cystatin hereditary angiopathy HCCA) associated with the aggregation process of hCC. Grubb and coworkers demonstrated that even catalytic amount of monoclonal antibody (mAb) is able to inhibit the dimerization of hCC [1]. There are several commercially available anti-hCC mAb, which were also shown to influence this process to varying degrees. In this project we present the use of mass spectrometry for A) epitope identification for commercial clone Cyst10, which does not affect the dimerization process, and B) characteristic of isolated naturally occurring antibodies (NAbs) interacting with hCC isolated from serum of healthy people.

Methods
The epitope extraction/excision approach and affinity experiments combined with mass spectrometry technique (MALDI-TOF and ESI TripleTOF) were used for epitope identification for commercial clone Cyst10 and autoantibodies. For the isolation of NAbs affinity chromatography was used. Immobilized hCC has been used as a ligand to bind anti-hCC NAbs from commercially available immunoglobulin G fraction of serum of healthy people (IgG, Aldrich, I2511, I4506). The presence of anti-hCC NAbs was checked by SDS-PAGE electrophoresis, and its concentration was measured in a NanoQuant (Tekan).

Results
The identification of epitope for Cyst10 after epitope excision/extraction experiments revealed two fragments of hCC in eluates, one located in the middle part of hCC and second from C-terminal fragment. This suggest the presence of discontinuous epitope for clone Cyst10. In the case of NAbs, analysis of the isolated fraction using 2D gel electrophoresis revealed the mixture of antibodies. Affinity chromatography between isolated NAbs and synthetic fragments of hCC showed that mixture of anti-hCC NAbs interact with several different fragments of hCC.

Conclusion
Clone Cyst10 is not able to inhibit dimerization of hCC, and its discontinuous epitope is placed in fragments hCC(43-72) and hCC(93-120). With the application of simple affinity chromatography and the use of human cystatin C attached to solid matrix we were able to isolate autoantibodies from commercial IgG serum fraction. Isolated anti-hCC NAbs are mixture of different autoantibodies recognizing some fragments of human cystatin C.

Novel Aspect
The determination of epitopes for commercial and isolated NAbs directed against human cystatin C is important for understanding inhibition process of amyloidogenic molecules and what is more important for planning immunotherapy and designing diagnostic assays.

Acknowledgements
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ThPS38-14 / Mass spectrometry technique in studies of interactions of human cystatin C with antibodies

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Introduction
Generation of amyloid deposits by human cystatin C (hCC), and more precisely by its amyloidogenic variant L68Q, is associated with hereditary cystatin C amyloid angiopathy (HCCAA). The number of molecules proposed as potential inhibitors of hCC dimerization and oligomerization is limited. It was reported that addition of monoclonal antibodies can suppress dimerization of hCC and its L68Q variant. Clone Cyst28 out of twelve of the tested antibodies against hCC raises the biggest hope for dimerization suppression. Identification of an epitope may lead to the design of improved and more specific immunogens and to obtain specific antibodies for future immunotherapy. Therefore, immunization of rabbits by peptide fragments of hCC, which have been identified as epitopes for some of monoclonal anti-hCC antibodies, could give the hope to discover ligands, which would effectively trigger immunological response, which - in turn - would lead to production of antibodies against cystatin C and its toxic type L68Q.

Methods
Identification of hCC epitopes recognized by Cyst28 and antibodies isolated from rabbits was based on the methodology of “epitope excision” and “epitope extraction”. Interactions of antibodies with peptide fragments of hCC was analyzed using affinity chromatography and indirect ELISA system.

Results
The results of rabbits’ immunization revealed that polyclonal antibodies against hCC from a rabbits’ serum were obtained. Identification of epitopes for Cyst28 and rabbit antibodies was performed using several proteolytic enzymes (trypsin, Asp-N, pronase) and digest analysis with the use of high-resolution mass spectrometry. Analysis of interactions of mono- and polyclonal antibodies with the whole molecule of hCC and its fragments was performed by affinity chromatography and ELISA. In order to check whether the antibodies from rabbits are able to suppress the dimerization process of hCC, the wild type protein was incubated with the addition of rabbit antibodies under conditions of forced dimerization. Progress of dimerization process was analyzed using gel filtration technique.

Conclusions
The epitope for monoclonal antibody Cyst28 is nonlinear and is located in the central region and C-terminal part of the protein. The non-linearity of the identified epitopes recognized by rabbit antibodies in connection with their location in various parts of the hCC means that these antibodies are relatively polyspecific in relation to the protein. Two kinds of the antibodies obtained from rabbits did not suppress the hCC dimerization process. They show some affinity to the hCC molecule, but, the most likely, not to the parts crucial for the initiation of the dimerization process.

Novel Aspect
Determination of epitopes of hCC is essential for planning potential immunotherapies and designing inhibitors of the dimerization process.

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ThPS38-16 / Mapping the protein-protein interactions employing the combination of photo cross-linking protein nanoprobe with high resolution mass spectrometry

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Introduction
In this work, is shown that photo-initiated cross-linking of protein nanoprobe (an alternative approach to chemical cross-linking overcoming the limitations/restrictions directed by the employed chemical cross-linker) in combination with mass spectrometry (MS) is suitable to determine the topology of protein complexes. Photo-activation of the nanoprobe - photo-labile methionine analog with diazirine functional group (pMet) is incorporated in the protein sequence - forms a highly reactive carbene biradical that can covalently bind to any amino acid in close proximity.
First, we evaluated the methodology using the well-characterized homodimeric 14-3-3 protein (a highly conserved regulatory protein found in all eucaryotic cells). Later, we applied it to study the interaction within the mammalian microsomal biotransformation system, analyzing the cross-links between cytochrome P450 (P450) with nanoprobe based on one of its redox partners, photo-labile cytochrome b5 (pcytb5).

Methods
The protein of interest, photo-labile nanoprobe, was expressed in E. coli BL21 GOLD in methionine-deficient mineral medium supplemented with pMet. The nanoprobe production was optimized to obtain maximal yield of partially incorporated pMet during recombinant expression. The purified nanoprobe was reconstituted with its interaction partner in functional system in vitro, UV-irradiated, and newly formed covalent complexes were separated by SDS-PAGE. After in-gel digestion with protease, a peptide mixture was analyzed by reversed phase liquid chromatography on-line coupled to the high accuracy MS (LC-ESI-FTICR). For each matched cross-linked peptide pair the tandem mass spectrum (MS/MS) was acquired to identify amino-acids involved in covalent cross-link.

Results and Conclusions
In the first experimental model, 14-3-3 homodimer, the Gln8-Met78 and Met1-Gln77 were determined within contact in agreement with a known structure. The second applied photo-labile nanoprobe (wild-type pcytb5 and all its single-pMet mutants, containing only one Met residue in the sequence) produced cross-links with P450 in the reconstituted lipid vesicles (detected on SDS-PAGE). The high accuracy LC-MS of binary complex allowed identification of contacts between trans-membrane peptides of C-terminal hydrophobic anchor of pcytb5 and N-terminal region of P450. Existence of such contact was, to our knowledge, not reported in the literature so far. The MS/MS analysis for particular amino acid residues determination involved in the interaction is in progress.

Novel Aspect
The construction of nanoprobe (with incorporated photo-labile pMet in protein sequence), cross-linking by UV-irradiation and identification of protein-protein contacts by MS has been successfully tested at both systems (14-3-3 homodimer or cytochromes P450-cytb5).

Acknowledgments
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ThPS38-17 / Peptide folding on metal surfaces in UHV
Sabine Abb[1], Gordon Rinke[1], Girjesh Dubey[1], Ludger Harnau[2], Klaus Kern[3]
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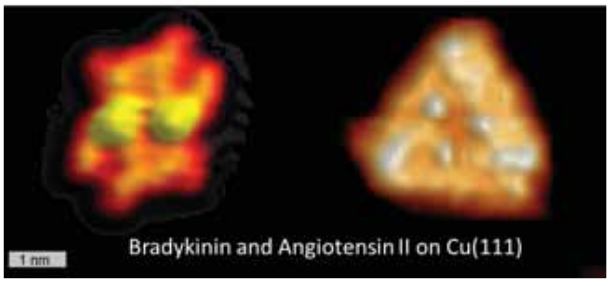
Introduction:
Biomolecules, such as peptides, show specific functionality upon folding into unique structures. Their conformation is steered by non-covalent interactions, such as van der Waals interaction, hydrogen bonding and polar interactions as well as by metal coordination. The mechanisms of the highly complex interplay between these interactions at the atomic level are, however, not yet fully understood. Investigating two peptides, namely bradykinin and angiotensin II, with high resolution imaging, we explore the influence of the different interactions at the single molecule level.

Methods:
A new approach to investigate the structure of peptides and peptide complexes in vacuo is possible with our home-built electrospray ion-beam deposition/scanning tunneling microscopy (ES-IBD/STM) instrument which employs ESI as particle source for intact deposition of large non-volatile molecules. The ion beam composition is monitored with a TOF-MS and controlled by quadrupoles enabling subsequent STM characterization with amino acid resolution. With the support of molecular dynamics (MD) and density functional theory (DFT) calculations, peptide structures are obtained from the STM images.



Results:
In STM, we observe well-defined structures on the surface formed by intact molecules. Upon changing the substrate-peptide interactions by deposition onto different surfaces, we find a variety of non-covalently bound peptide assemblies ranging from individual peptides, to peptide dimers, up to hierarchically ordered assemblies of bradykinin dimers for increasingly weaker substrate-peptide interactions. For angiotensin II, we also observe peptide assemblies, dependent on the strength of the substrate-peptide interaction. However, due to different intramolecular interactions and stereochemical aspect, we observe structures of individual peptides, trimers and highly ordered long-range honeycomb networks with weaker substrate-peptide interactions.

Conclusion:
By comparison of the two peptides, we can extract common behaviour of peptide folding at surfaces in low dielectric environments (vacuum). In principle, assemblies are formed in such a way that the reactive ends and polar residues of the peptide are folded to the inside of the oligomer assembly. With the stereochemical effects of the peptide residues, which can hinder the interfolding of polar groups, the number of peptides in an oligomeric assembly can be influenced. In the end, stable assemblies have no functional group for strong interactions accessible on the outside. Nonetheless, a hierarchical order of these oligomers due to weak, non-polar interactions can be observed on weakly interacting surfaces.

Novel Aspect:
Preparative mass spectrometry based on ESI enables the amino-acid resolved imaging of peptides. We demonstrate a new path to investigate conformational changes of peptides, complementary to gas phase investigations such as ion mobility spectrometry.

ThPS38-18 / Three-body fragment ion in Positive and Negative ESI for Location of Non-covalent Binding Sites in DNA/Peptide complexes
Jean-Claude Tabet[1], Bessem Brahim[2], Sandra Alves[2], jean-claude Tabet[2]
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Introduction
Non-covalent interactions (NCI) are determining features of the three-dimensional structures adopted by proteins and nucleic acids in living organisms. NCI are involved in most of biological processes in which biomolecules bind specifically but transiently to one another and thus determine both specificity and selectivity of a biological activity. Since the beginning of desorption/ionization methods, gas-phase (GP) studies of biological non-covalent complexes (NCX) represented a promising analytical approach to probe directly such intermolecular interactions. Here, GP nucleic acid-peptide complex species were investigated using tandem mass spectrometry.

Methods
Gas-phase fragmentation of single stranded DNA-peptide non-covalent complexes (NCX) was investigated in both positive and negative electrospray ionization (ESI) modes using a LTQ-Orbitrap XL instrument. Collision induced dissociation (CID) experiments performed in positive ion mode into the LTQ analyzer have confirmed the trend previously observed in negative ion mode (Alves et al., J. Mass Spectrom., 2007, 42, 1613-1622) i.e., a higher stability for NCX containing most basic peptidic residues (i.e., R > K) and most acidic nucleotide units (i.e., Thy units), certainly incoming from the existence of gas phase salt bridge interactions (SBI).

Results
Independently of the ion polarity, stable NCX precursor ions were found to dissociate preferentially through covalent bond cleavages of the oligonucleotide and the resulting DNA fragment ions still linked to the peptide, were systematically mostly composed of Thy units. Additionally, the loss of an internal nucleic fragment producing "three-body" non-covalent fragment ions were also observed under collisions and investigated in both ion polarities. The identical fragmentation patterns (regardless to the relative fragment ion abundance) observed in both polarities has shown a commune location of SBI certainly preserved from solution. Nevertheless, most abundant non-covalent fragments ions (and particularly three-body ones) are observed from multiply protonated NCX.

Novel Aspect
The production of "three-body" non-covalent fragment ions observed independently of the ion polarity was used to gain insight into charge induced fragmentation pathways of DNA/peptide complexes.

ThPS38-19 / Mapping of protein-protein interactions in 14-3-3zeta homodimer: Combination of photo cross-linking protein nanoprobe with mass spectrometry.

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Introduction
14-3-3 is a family of highly conserved regulatory proteins found in all eukaryotic cells. Due to binding interactions with hundreds of structurally and functionally diverse partners the 14-3-3 proteins play crucial role in the regulation of many cellular processes including signaling pathways, apoptosis, tumor suppression. Previous studies of 14-3-3 complexes revealed that the dimeric nature of these proteins is very important for their multifunctional character.

Methods
Photo-initiated cross-linking technique in combination with high-resolution and tandem mass spectrometry was applied to map the contact surface regions involved in the homodimerization process of human 14-3-3zeta protein. Presented approach is based on the incorporation of photo-labile amino acid analog with diazirine functional group (photo-Met or photo-Leu) in the sequence of studied protein during its recombinant expression. The highly reactive carbene radical formed from photo-labile diazirine group after UV-light exposure is able to attack any residue in close vicinity to form a new covalent bond that can be identified by MS analysis.

Results
Two recombinant photo-labile proteins 14-3-3zeta wild type (WT) and S58D mutant were successfully prepared and partial incorporation of photo-labile amino acid analog was confirmed by MS. Native structure of both proteins - homodimer formation in case of 14-3-3zeta WT and monomeric character of its S58D mutant was proved by native electrophoresis. In agreement with the previous result, the dimeric photo-14-3-3zeta WT and monomeric photo-14-3-3zeta S58D were detected by SDS-PAGE after photolysis in solution. The cross-linked products were trypsinised and obtained mixtures of peptides were analysed by MS. The high accuracy MS data revealed the list of cross-linked peptide pairs. The tandem mass spectra, acquired for each m/z signal from the mentioned list, helped to identify amino acids involved in covalent cross-link between two molecules of 14-3-3zeta WT (e.g. Gln8-Met78).

Conclusions
The novel methodological approach of photo-initiated cross-linking protein nanoprobe with mass spectrometry extends the number of residues within homodimer interface of 14-3-3zeta. These findings are in accordance with previously published data obtained by chemical cross-linking with EDC, H/D exchange mass spectrometry or X-ray crystallography (PDB 3DHR).

Novel Aspect
The obtained results with human 14-3-3zeta isoform demonstrate the successful application of used technique to map protein-protein interactions. Moreover, this methodology enables acquisition of protein structural data in the native conditions and offers applications for elucidation of functional mechanism.

Acknowledgments
Charles University (GAUK903413, UNCE204025/2012) and Grant Agency of the Czech Republic (P207/12/0627).

ThPS38-20 / 1c2p-REMPI of non-covalent anisole complexes

Heinke V. Thurn, Jürgen Grotemeyer
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Introduction
1c2p-REMPI-spectra of non-covalent anisole complexes were measured. They are important for understanding and explaining supramolecular organization. If anisole and other small aromates are forming dimers, there are different electronic transitions as for the monomers [1]. The 0-0-transistion of the complex is red shifted compared to both monomers. Phenol dimers have two 0-0-transitions, for one working as a proton donor and the other as acceptor [1]. In contrast to them the anisole complexes have one 0-0-transition, which indicates another stabilization interaction.
In this work complexation partners of anisole were varied and their effects on the change of the electronic structure of the complex and their stabilization interactions were investigated.

Methods
All measurements were done with a molecular beam experiment at a modified ReTOF (Bruker). Substances were used without further purification in a ratio of ingredients 1:1 (vol : vol). The expanding gas mixture is produced by an argon flow (3-4 bar) above the samples. One probe reservoir can be heated up to 40°C. The radiation used for ionization is generated by a frequency doubled dye laser (Scanmate, Lambda Physics) operating on Coumarine 153, pumped at 355 nm by a pulsed Nd:YAG laser (SpitLight, InnoLas). Spectra were recorded by a home built software controlling an oszilloscope (LSA1000Series, LeCroy) and by a two-stage MCP [2].

Results
There are homologous complexes of toluene and anisole up to 3 molecules detectable. Heterogeneous complexes are detected in a ratio 1:1. As for anisole-phenol complexes, anisole-toluene complexes have one 0-0-transition which is red-shifted compared to the monomers. While the complexation partners are varied the shift of 0-0-transition are analyzed. At frequencies above 0-0-transition spectra show complex band structure which can be correlated on the basis of DFT-calculation.

Conclusions
REMPI spectra of different anisole complexes were recorded. Their 0-0-transitions are red shifted compared to the monomers and up to 0.3 nm red shifted compared to the 0-0-transition of the anisole-dimer.

Novel Aspects
1c2p-REMPI-Spectra of anisole complexes with varying partners.

Literature
[1] G. Pietraperzia, V. Barone, The Journal of Physical Chemistry, 115 (2011).
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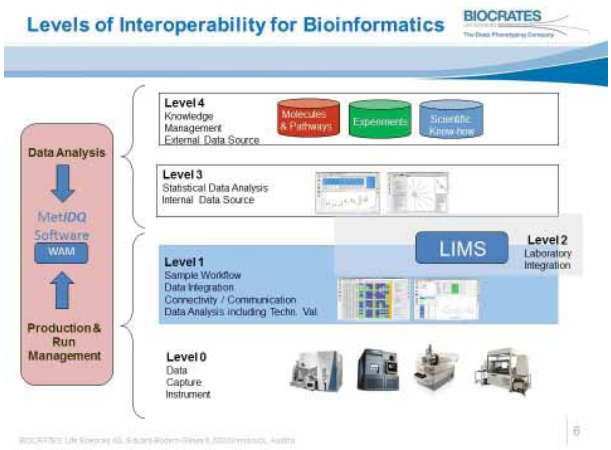
ThPS39 - Informatic tools for MS

11:00-15:00
Poster Exhibition, Level -1

ThPS39-01 / Mass spectrometry based metabolomics work area and data management software «From sample to metabolic pathways»

Bernd Haas, Martin Buratti, Nicole Huber, Hannes Pedevilla
Biocrates Life Sciences AG

The software highly automates the whole FIA-MS/MS and LC-MS/MS mass spectrometry process from project and sample administration to work-list and plate generation, technical validation and statistical analysis to standardize metabolomics project data processing.An optionally integrated dynamic pathway graphics package provides the feature to combine the concentrations of metabolites into the pathways involved (retrieved from common biobases) providing graphical interpretation for more intuitive understanding of biochemical pathway information in the affected metabolic pathways.
Statistically relevant up and down regulated quantitative metabolomics data of sample cohorts (e.g. disease, control) can be easily visualized together with appropriate metabolite pathways identifying well known and additionally new combinations of metabolic signatures. Various export formats provide further data analysis with other tools e.g. MetaboAnalyst.
The graphical font-end is designed as a java swing rich client that connects to an oracle database back-end where all data is available in a well-structured database schema. The software system can be configured to setup a single user system, a workgroup or a cloud solution. Result data from Thermo, Waters, Agilent or AB Sciex Instruments is converted to mzXML. Identification, quantification, and validation is high automated and performed based on the MRMs and method parameters available in the database. The concentration of every data point is stored in µM inside the database to make results comparable across instrument platforms, materials and projects. Pathway visualisation database backend is based on the open source database scheme www.biowarehouse.org.
Because of the harmonization of ms FIA raw data and processed LC data from different MS suppliers to quality controlled concentrations the software suite is a valuable tool for standardization i.e. for ring trials to compare instrument performance. For lipids an integrated isotope correction algorithm (H,C,N,O,S) improves concentration quality significantly. The database backend makes project data online available over many years in a well-structured format and is a valuable source for data mining algorithms. The rapid prototyping software development approach was the ideal solution to deal with complex user requirements to find the right balance between automation for diagnostics environments and flexibility for research environments. Database schema version management is essential to keep databases compatible and handle upgrade procedures. Archiving ms raw data (mzML) inside the database support further automated data analysis with future algorithms. The user acceptance increases if many export formats to other tools like MetaboAnalyst, R, SPSS, XLSX, CSV, XML for further data analysis are supported. By providing a method import feature it is easy to import new metabolite and their MRMs as well as other quantitation parameters to the database from mass spectrometry softwares like Analyst®, MultiQuant™, Xcalibur™, MassLynx™ etc..



ThPS39-02 / Mass Spectrometry software tool enabling full characterization of biopharmaceutical molecules

Joe Shambaugh, Jean Mercier
Genedata AG

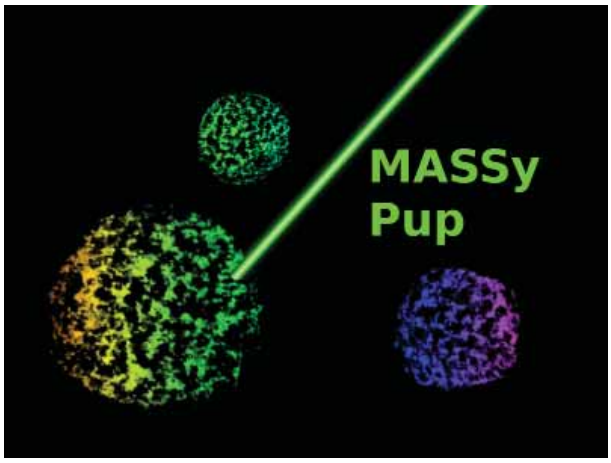
Characterization of biopharmaceutical molecules has been advanced by improved mass spectrometry technologies over the past several years. Analysis of intact proteins and peptide mapping have been accelerated through automated data processing tools. However, some of the more complex analyses such as identification of glycopeptides, and confirmation of disulphide bonds have been more difficult to automate due to the computational complexity required. Here we demonstrate new capabilities within the Genedata Expressionist for Mass Spectrometry software that employ complex in-silico calculations to enable automated, high throughput characterization of glycopeptides as well as disulphide bonds. In this approach, identification of glycopeptides is based on comparison of experimentally measured masses against the theoretical fragmentation of the contents of Glycome DB. Similarly, de novo identification of disulphide bonds can be accomplished by comparing measured data with theoretical masses. Together with the existing suite of tools for mass spectrometric analysis, full characterization of biopharmaceutical molecules is now available in a single software platform.

ThPS39-03 / MASSyPup: A Linux Live DVD for the analysis of mass spectrometry data

Robert Winkler
CINVESTAV Unidad Irapuato

Introduction
In mass spectrometry (MS) related research, the real work starts after the raw data have been collected. MS laboratories generate large amounts of data in a rapid pace. Researchers, who want to squeeze out the maximum of information from their raw data, cannot rely on the automatized outcome of service lab pipelines, but they will carry away their crude data and analyze them off-site. Plenty of excellent free software is available, but incompatible libraries and versions complicate the installation of programs and frustrate scientists. Therefore, we decided to develop a Live DVD for the immediate use of MS data analysis software.

Methods
Based on Puppy Linux, a minimalist Linux live system, we created a collection of mass spectrometry software, which is pre-configured for immediate use [1]. The software was selected to permit data conversion and viewing, protein analysis/ proteomics, metabolite analysis/ metabolomics and statistical analysis. Many popular packages and pipelines, such as ProteoWizard, mmass, Mzmine, ESIprot, XCMS, R and the Trans-Proteomic-Pipeline have been included. The GNU compiler collection and various standard libraries facilitate the installation and development of MS related software. The Live DVD *.iso is available from http://www.bioprocess.org/massypup.



Results
The MASSyPup live DVD was tested on more than 20 computers for teaching courses, MS data analysis and software development. On computers with sufficient memory, the system runs entirely in the RAM, which results in extremely fast program start-up and execution. Lately, we included hardware drivers, to use the system for the control of a sampling/ imaging prototype [2]. Currently, we work on a toolbox called SpiderMass for the automated generation of custom databases and identification of metabolites. Time-critical applications of SpiderMass are compiled on MASSyPup for easier software distribution and best possible performance. Altogether, MASSyPup greatly facilitates our daily tasks due to its speed, stability and functionality. According to our website statistics, we estimate that several hundred DVDs are in use in the MS community.

Conclusions
MASSyPup greatly simplifies the analysis of MS data by providing a standardized working environment. The DVD/USB can be used for off-site MS data analysis, for teaching or as a development platform.

Novel Aspect
Installation-free use of MS data analysis software.

References
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[2] Maldonado-Torres M, López-Hernández JF, Jiménez-Sandoval P, Winkler R. "Plug and Play" assembly of a low-temperature plasma ionization mass spectrometry imaging (LTP-MSI) system. J Proteomics 2014.

ThPS39-04 / A spatially-aware peak picking method for MALDI-imaging data from TOF and FTICR mass analyzers
Dennis Trede[1], Jan Hendrik Kobarg[2], Lena Hauberg-Lotte[2], Michaela Aichler[3], Michael Becker[4], Janina Oetjen[5], Andrew Palmer[5], Stefan Schiffler[1], Judith Berger[6], Stefan Heldmann[6], Peter Maass[5], Axel Walch[3], Theodore Alexandrov[5]
[1]SCiLS GmbH, [2]Steinbeis Innovation Center SCiLS Research, [3]Helmholtz Zentrum München, [4]Bruker Daltonics GmbH, [5]University of Bremen, [6]Fraunhofer MEVIS

Introduction
MALDI-imaging is a spatially-resolved mass spectrometric technique which can obtain the spatial distribution of hundreds of molecules in a thin tissue section. Manual analysis is time-consuming since it requires visual examination of all m/z-images. We introduce a novel method that automatically detects structured m/z-values without specifying a region of interest and without manual visual examination.

Methods
The new approach automatically selects spatially structured m/z-images by ranking all m/z-images by their level of spatial structure. The ranking is based on our original measure of spatial chaos. We illustrate the idea of the measure of spatial chaos by applying it to MALDI-imaging data sets acquired with different types of mass analyzers. We apply it to a 2D TOF data set of a rat brain section, to a 3D TOF data set of a mouse heart after myocardial infarction, and to a 2D FTICR data set of a rat brain section.

Results
The application of our novel spatially-aware peak picking method to MALDI-TOF data shows that the algorithm can be used to automatically discover m/z-values corresponding to structured images in two and in three spatial dimensions.
For FTICR data one typically faces the problem of having too many peak candidates. We show how the number of peaks can be reasonably reduced with the structure detection approach by restricting it to those peaks showing spatial structure in the corresponding m/z-image.

Conclusions
Selecting structured m/z-images after visual examination is the well-accepted approach of manual analysis and it is a part of everyday work. Our parameter-free and unsupervised method supports the imaging mass spectrometrist at this task. The method also complements spectrum-wise peak picking. Our method is sensitive event to small peaks because it does not depend on peak intensity, but only on the measure of spatial chaos of the corresponding m/z-image.

Novel Aspect
The proposed measure of spatial chaos automatically and unsupervisedly detects structured m/z-images and thus complements spectrum-wise peak picking.

ThPS39-05 / Comparison of protein extraction buffers in formalin-fixed paraffin-embedded tissue
Valérie Broeckx[1], Evelyne Maes[1], Kurt Boonen[1], Xavier Sagaert[2], Hans Prenen[2], Bart Landuyt[1], Liliane Schoofs[1]
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Formalin-fixed paraffin-embedded (FFPE) tissues are an unexploited resource and an alternative way to obtain large cohorts of samples, as these tissue blocks are routinely prepared for pathological analysis and stored in hospitals worldwide. In addition, these tissues are linked with pathological, clinical and outcome information, making them a valuable sample type for

biomarker research. Unfortunately, proteome analysis of FFPE tissue is impeded by intra- and intermolecular protein cross-links. These cross-links hamper the extraction of full-length proteins, and unknown/unexpected protein modifications challenge mass spectrometry-based proteomic research. In recent years, several research groups successfully identified FFPE-extracted proteins, based on different extraction protocols. In this study, we compare several of these protein extraction buffers by performing a gel enhanced liquid chromatography (GeLC) proteomics analysis both on murine and human FFPE tissues. Peaks studio software (version 7, Bioinformatics solutions Inc., Waterloo, ON, Canada) was used to analyze the MS/MS spectra. Our data show that a GeLC-MS/MS workflow, combined with the software's de novo sequencing of peptides and detection of frequently occurring posttranslational modifications, enables the identification of hundreds of successfully extracted proteins. Moreover, differences in the protein extraction efficiency of multiple extraction buffers were clearly observed.

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ThPS39-06 / Towards demultiplexing of SWATH spectra for peptide identification: similarity analysis of fragment elution profiles

Aivett Bilbao[1], Ying Zhang[2], Dario Bottinelli[2], Bandar Alghanem[2], Frédéric Nikitin[3], Jeremy Luban[4], Caterina Strambio De Castillia[4], Markus Mueller[3], Frédérique Lisacek[3], Emmanuel Varesio[2], Gérard Hopfgartner[2]
[1]LSMS/SIB, [2]LSMS, [3]SIB, [4]UMASS

Introduction
Data-independent acquisition (DIA) offers several advantages over data-dependent acquisition (DDA) schemes (e.g. no bias toward high abundance peptides and reproducibility for identification and better sensitivity and accuracy for quantification) for complex protein digests analyzed by LC-MS/MS. In SWATH acquisition, a resolving Q1 isolation window (e.g. 20-30u) is stepped repeatedly across an m/z range (typically 500-1500). High-resolution LC-MS maps of multiplexed fragment-ion chromatographic profiles are generated from all peaks. SWATH data is mainly processed based on MS information obtained from previous DDA/SRM analyses for quantification and peptide identification. The ultimate aim of this work is to develop an effective processing strategy to exploit SWATH data without using any prior MS peptide knowledge. We studied the elution profile similarity of fragments from co-eluting peptides and compared several similarity measures and smoothing algorithms which maximises the discrimination of multiplexed fragments from different precursors.

Methods
The first sample investigated was a mixture of 12 standard proteins. The second sample consisted of peptides derived from the digestion of total cell extracts of primary human dendritic cells (hDC) generated from peripheral blood monocytes. Protein extracts were digested with trypsin and analyzed with a 2D+ NanoLC-Ultra system (Eksigent) hyphenated to a TripleTOF 5600 mass spectrometer (AB Sciex). Data have been acquired in both positive SWATH and DDA modes. Each LC-MS product map, corresponding to each SWATH window, is independently analyzed by a 3D peak detection algorithm. Detected peaks (features) are described by their elution profile, m/z, retention time (RT) and charge. RT and fragment m/z values from DDA high confidence peptide identifications are used to annotate features detected in the SWATH data. For each peptide, a similarity matrix is computed comparing the elution profiles from all features within the same RT window: intra-peptide-fragments, co-eluting inter-peptide-fragments and co-eluting non-annotated features.

Results
Five similarity functions (Pearson correlation, Cosine, eJaccard, Spearman's rank correlation and Euclidean) and pre-smoothing of elution profiles (moving average, Gaussian and Savitzky-Golay filters) are compared. Preliminary results from the hDC sample show that Cosine combined with Gaussian smoothing provides the best intra-peptide-fragments similarity (70% peptides having the 3rd best similarity value greater than 0.95 compared to 23% without smoothing), but more than 60% peptides can have inter-peptide-fragments similarity values greater than its 3rd best intra-peptide-fragments similarity.

Conclusions
While for simple peptide mixture spectra demultiplexing was possible, elution profile similarity was not sufficient for demultiplexing SWATH data obtained from the analysis of a biological relevant sample. We are working to find complementary strategies and combine them in a more sophisticated demultiplexing algorithm.

Novel Aspect
Extensive analysis of SWATH data towards the development of a data processing strategy for peptide identification.

ThPS39-07 / Do You See What I See ?

Malcolm Clench, Laura Cole, Arul Selvan, Heath Reed, Chris Wright
Sheffield Hallam University

Introduction
Images of the human body (or its parts) in-vivo are produced in order to reveal, diagnose, or examine disease. Imaging of removed organs and tissues is performed for medical purposes, as a part of pathology. A large number of in-vivo imaging modalities exist including radiography, magnetic resonance imaging and utlrasound and for removed tissues the standard procedure employed is immunohistochemistry, increasingly supported by mass spectrometry. Issues facing imaging technologies include the dissemination and communication of the wealth of information contained in multi-modal imaging data sets to not only medical professionals but also to patients and a lay audience. In this project these issues have been addressed these by examining, (a) improvements to medical image viewing software to support multimodal imaging and (b) design Issues concerning the communication of medical images to different audiences

Methods
Lipid and on tissue tryptic digest images were performed on mouse fibrosarcoma tumours. Tumours were embedded in gelatin blocks for MRI, frozen and then cryosectioned for MALDI MSI. T2 weighted Gradient Echo (3NEX) and XBone (4NEX) were acquired from the blocks. Hierarchical Clustering-based Segmentation (HCS) was used to perform 'up-sampling' of the lower resolution MRI data in order to highlight potential diseased areas.

Results
MALDI-MS images of ions arising from tissue haemorrhaging and phospholipid oxidation were produced from the MS imaging data set. Tissue abnormality in a medical image is usually related to a dissimilar part of an otherwise homogeneous image, the dissimilarity may be subtle or strong depending on the modality

and the tissue type. Hence a dissimilarity highlighting process that yields a hierarchy of segmentation output is useful. A method employing Hierarchical Clustering-based Segmentation (HCS) has been developed. To identify the part of the MR image slice which correlated with the MS image, HCS was applied to the MR data. HCS highlighted major regions which were unclear in the original MR data. The graphic communication mode of these data sets is seen as crucial to bridging understanding gaps for both lay and professional readers.
Design concepts for ways to present multimodal imaging data also need to consider how the imaging information can be conveyed in a number of ways and at a number of levels. In the initial proposed model, a 'plane' is assigned to each imaging model. The planes are intended to ground the three dimensional nature of the sample within a tangible environment and provide an interface by which aspects of each mode (e.g., for MSI, peptide intensity) can be selected for a given slice through the sample

Conclusions
Images are a powerful method for the display of complex information. Presentation of multi-modal imaging data sets in a medical context so that patients, imaging professionals and clinicians can each «see» the important information is a complex issue requiring input from each group and design expertise.

Novel Aspect
Design and software issues in the presentation of multi-modal imaging data sets are explored using «real» data from MALDI-MSI, MRI and conventional microscopy.

ThPS39-08 / Expanding the current performance of precursor ion-based protein quantification using complementary fragment ions

Vladimir Gorshkov, Thiago Verano-Braga, Fedor Kryuchkov, Frank Kjeldsen
University of Southern Denmark

Introduction
One of the recent trends in shotgun proteomics is to obtain complete or near complete quantitative and qualitative proteome coverage in relatively short period of time (hours) in one experiment [1, 2]. This however inevitably leads to significantly increased complexity of the peptide sample. Despite significant progress in the sensitivity and speed achieved by MS instrumentation recently, the problem of precursor ion co-isolation is still one of the most severe limits for the shotgun approach and it gets even worse in case of single-shot proteome analysis. In this contribution we show how complementarity of fragment ions can be beneficial in deconvolution of co-isolated peptides in mixture spectra and allow for assessment of quantitative information in MS1 scans. Overall this should expand the current achievements reported in quantitative proteome coverage.

Methods
An algorithm to identify and extract complementary ion pairs from fragment spectra for further processing was written in C# and implemented as a node for Thermo Proteome Discoverer. The complete explanation of the algorithm is published earlier [3]. Preliminary data was obtained on LOVO cells lysate, as described in ref 3. The data analysis was performed using Thermo Proteome Discoverer with Mascot as the search engine.

Results
Pre-processing of the spectra requires virtually no time compared to the typical analysis time (order of minutes). On average 1.5 – 1.8 additional spectra with more than 3 complementary fragment pairs were extracted per input spectrum. Increase of 17 – 28% in peptide identifications number (based only on PSMs with FDR < 0.01 by target-decoy approach) was observed, while about 80% of additional peptide features can be quantified using precursor ion area. In total 15-20% increase of the number of quantified peptides was observed with the reported pre-processing method. Similar effect can be observed on protein level as well.
Further development includes analysis of triplicates of neural stem cells digested, dimethyl-labeled and analyzed by Orbitrap Fusion (Thermo Scientific) using CID fragmentation and various isolation windows (1, 2 and 4 Da) by the method similar to the one used for the LOVO cells.

Conclusions
The described pre-processing method is capable to facilitate the problem of co-isolated precursor ions significantly increasing the quantifiable portion of proteome. The result can be obtained at little time cost and doesn't require any additional experiments. The developed node is freely available to the public and can be easily combined with any other pre-processing and post-processing tools.

Novel Aspect
Extraction of complementary fragment ions was used to expand the number of quantifiable PSMs in parent ion quantification methods.

References
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ThPS39-09 / Clustering-based ion chromatogram extraction and peak-picking for high-resolution LC-MS data

Martin Loos, Matthias Ruff, Heinz Singer, Juliane Hollender
Eawag, Swiss Federal Institute of Aquatic Science and Technology

Introduction
A first step of data analysis in liquid chromatography-mass spectrometry (LC-MS) comprises the detection of distinct measurement signals of individual known and unknown compounds. Commonly referred to as the extraction of ion chromatograms (EICs) and peak picking, a large variety of algorithms have been proposed for this task. While facing noisy and complex sample matrices, these algorithms are often based on brute data binning, narrowly defined peak shapes or a false assumption of data homogeneity over the full mass and retention time range. Other approaches are not applicable to data already preprocessed during acquisition, causing false negative detections from misleading signal-to-noise estimates. Moreover, the extraction of mass chromatograms can be hampered by the interference of unresolved masses even at high resolutions.

Methods
A novel algorithm for EIC and peak detection is introduced, involving four consecutive processing steps. First, an agglomerative clustering is used to partition the measurements into separable subsets, enhancing the performance of the subsequent processing. Namely, an intensity-ranked unsupervised clustering is performed within each of these partitions in a second step. At the addition of each new measurement to an existing cluster, the mass and the retention time tolerances are gradually restricted and adapted, respectively. With mass estimates thereby decreased towards their expected precision, cluster attributable to a common ion are then hierarchically merged, resulting in individual EICs. In a third step, a recursive peak detection is run for each EIC. To refrain from a particular model of peak shape, the optimal peak width is chosen from a penalized sum of intensity changes in the

neighborhood of a candidate peak apex. In a last step, peaks are filtered by a set of criteria applicable to preprocessed data. We evaluated the algorithm using 220 standard compounds for a temporal sequence of >100 environmental samples.

Results
The algorithm is made publicly available (R-package enviPick) and provides a convenient user interface and interactive data viewer to guide its parameterization. At the required sensitivity, the high specificity of enviPick discriminates against false positive findings, outperforming other standard methodologies.

Conclusion
A fast two-fold clustering strategy for the extraction of mass chromatograms and peaks from centroided high-resolution LC-MS data is presented. Taking advantage of the autocorrelation in the intensity ranks of LC-MS measurements, the algorithm prioritizes the robust processing of high-intensity signals.

Novel aspects
enviPick does not assume a predefined peak shape and adapts to the heterogeneity characteristic of LC-MS data, even at high noise levels. Moreover, the algorithm accounts for the possible occurrence of chemical baselines and isobaric compounds and resolves for mass chromatograms interfering at a given resolution.

ThPS39-10 / Probing the structure of human protein disulfide isomerase by chemical cross-linking combined with LC-MS

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Introduction
We have analyzed native PDI purified from human placenta by chemical cross-linking followed by LC-MS. In addition to PDI the sample contained soluble calnexin and ERp72. Extensive cross-linking was observed within the PDI molecule, both intra- and inter-domain, as well as between the different components in the mixture.

Methods
PDI was obtained from purification of human placenta. The samples were then cross-linked with the BS3 and BS2G cross-linkers. The samples were reduced with dithiothreitol and alkylated with iodoacetamide. Digestion of the protein was performed with 2% trypsin, and the resulting peptides were separated by strong cation exchange chromatography. Mass spectrometry was carried out on an LTQ-Orbitrap XL in HCD and CID modes.
The in-house developed "MassAI" search engine was used for protein and cross-link identification. Initially, MassAI was used to identify the main proteins in the sample, by searching against the Homo sapiens part of the UNIProt database. Based on the proteins identified, a limited protein database was generated for cross-link searching.

Results
A total of 45 runs were performed and over 220.000 scans were recorded. These yielded more than 300 unique cross-links for the key proteins.
The distance constraints derived from the cross-link data were in agreement with the established X-ray structure of the monomeric PDI. The dimer, as presented in the existing crystal model however, does not seem to be prevalent in solution, as modelling on the observed cross-links revealed new models of dimeric PDI. The observed inter-protein cross-links further confirmed the existence of a peptide binding area on calnexin that binds strongly both PDI and ERp72.

Conclusion
The present work demonstrates the use of chemical cross-linking and mass spectrometry for the determination of a solution structure of natural human PDI, and its interaction with the chaperones ERp72 and calnexin. The data further shows that the dimeric structure of PDI may be more diverse than indicated by present models.

Novel aspect
Models of protein structure are commonly carried out by x-ray crystallography, where the proteins are not in solution. We have demonstrated that in-solution cross-linking can complement crystal structures to highlight dynamics not otherwise observable.

ThPS39-11 / A "Universal" data-dependent mass spectrometry method that eliminates time-consuming method optimization for achieving maximal identifications from each sample

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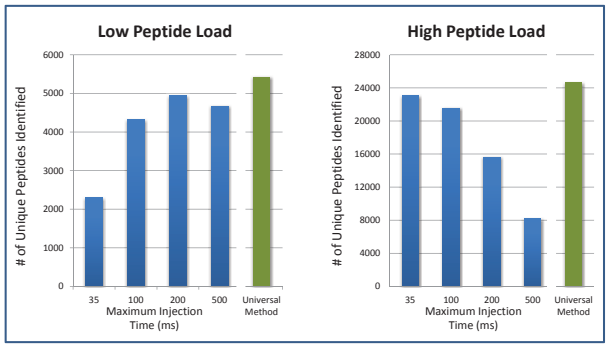
Thermo Fisher Scientific

Introduction
Achieving the maximum identifications from different peptide samples requires optimization of MS methods. Optimizations are time/sample intensive to determine the best balance of scan rate and number of ions per spectrum. This is particularly true when accurate sample concentration, complexity, and dynamic range are unknown, often the case following fractionation/enrichment. Unfortunately, optimizations are often not performed due to sample/time restraints. Here we present a universal method which adjusts parameters "on-the-fly" according to spectral complexity/intensity, eliminating the requirement for optimization.

Methods
Here, we analyzed various samples including HeLa digests and immunoprecipitations. Analysis was performed on an Orbitrap Fusion MS. The resulting LC-MS/MS data were searched using Proteome Discoverer, matches were filtered to 1% FDR. Each sample was analyzed with varying ion targets and maximum injection times to determine optimal parameters.
Novel instrument control software, now implemented on Orbitrap Fusion MS, was used to develop a Universal Method which makes "on-the-fly" decisions about length of injection time per precursor based on the ion flux, complexity of full scan and available cycle time without user input.



Results
Maximum identifications are obtained by reaching a balance between scan rate and quality of spectra. At high peptide load, Orbitrap Fusion achieves maximal identifications using 35 ms maximum injection time and 1e4 ion target. At lower peptide loads, however, maximal identifications are achieved using upwards of 500 ms maximum injection time. Depending on the sample load, complexity, and dynamic range, optimal values change dramatically. A single Universal Method achieved

maximal identifications in all sample types (see attached figure for a subset of results).

Conclusions
Our results show that it is possible to achieve maximal peptide identifications from samples with unknown concentrations without method optimization and lengthy reanalysis, thereby, increasing the throughput of the instrument while simultaneously improving the quality of the data acquired.

Novel Aspect
Development of a universal method that self-optimizes "on-the-fly" to generate maximal identifications for various sample concentrations.

ThPS39-12 / Prediction of peptide fragment ion intensity: a priori partitioning reconsidered

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Introduction
The recent algorithm MS2PIP is the most accurate predictor of observed intensities of peptide fragment ions in collision-induced dissociation (CID). The basic assumption and design choice of the algorithm is that models based on coherent training data are more accurate. MS2PIP therefore segments the training (and test) data into partitions for each combination of peptide length, fragment ion length, and ion type and charge. For each partition a separate random forest (RF) model is trained.
The machine learning literature, however, contains a large volume of experiments demonstrating that with the right algorithm, additional data that is correlated with the main target can often be helpful to a learner. This is found in transfer learning, in multi-label classification, and in relational learning.

Methods
We revisit the basic assumption behind MS2PIP and devise an encoding for all its features, and some additional features, in a way that permits the training of a monolithic RF on all available data irrespective of lengths. Features are computed on demand, avoiding the need to store the training data matrix in memory and allowing a larger number of examples and features. Our system is implemented in C++ and we name it I2P (InSPECtor Intensity Predictor). Heavy use of templates allows a maximum amount of work to be performed at compile-time: the binary code is fully specialized in the type of the data it has to handle and the tasks it has to perform. The predictive model itself can be exported to C++ and compiled, generating a very fast predictor that does not need to load model data files.

Results
Experiments indicate that the predictions of I2P are much better for rare ions and those of MS2PIP are better for the most common ions (short single-charged b and y ions). An ensemble that predicts the mean (in log-space) of the output of both systems is superior to either system on its own.

Conclusions
The intense partitioning of MS2PIP, while advantageous for abundant ions, is harmful for less abundant ions, which collectively make up a large proportion of all cases. I2P mitigates this problem because the predictions of rare classes can benefit from tree structures based on many more cases. The same property makes I2P also more robust against scarcity of training data.
A simple ensemble provides the best overall predictions.

Novel aspect
I2P is a novel system for predicting fragmentation ion intensities with state-of-the-art performance that is more suitable for modeling new fragmentation mechanisms for which fewer historical experiments are available.

[1] Sven Degroeve and Lennart Martens. MS2PIP: a tool for MS/MS peak intensity prediction. Bioinformatics 29 (24), 2013.

ThPS39-13 / Toward a comprehensive bottom-up and top-down analysis of a reference monoclonal antibody

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Introduction
Therapeutic proteins now command a major share of new medicines, and monoclonal antibodies (mAbs) are the most common in this class because they can be readily engineered for a wide range of disease targets. However, careful characterization is required due to their inherent variability. Mass spectrometry is a leading tool for this characterization because of its ability to analyze all kinds of modifications as well as verify the primary sequence. In this study, high resolution mass spectrometric data of the NIST mAb interim reference material (RM 8670, lot 3F1b) was analyzed by new bioinformatics tools to identify and quantify modifications, including oxidation, glycation, glycosylation, and sequence variants.

Methods
Heavy and light chain components were measured by both bottom-up and top-down (sometimes called middle-down) approaches by a high resolution Thermo Fisher Orbitrap Elite mass spectrometer employing CID, HCD and ETD fragmentation modes. Bottom-up digestion was by trypsin, chymotrypsin and glu-C. Intact ~ 25 kDa parts of the reference mAb, light chain and IdeS-digested heavy chain components Fd' and scFc, were analyzed in the top-down assay. The data was analyzed by a combination of the search engine Byonic™ and new inspection software Byologic® that combines and compares MS1 and MS2 data streams and performs label-free quantification by taking the ratio of extracted ion chromatograms (XICs) of the modified to unmodified peptide. In addition, new peptide mapping software, Byomap™, was employed to quantify and annotate the peptide map.

Results
Bottom-up analysis led to site-specific identification and quantification of sequence variants at low concentrations (all below 0.2 %), glycations (below 1%), and a wide range of oxidations (ranging from 0.1 to 6 %). Intact glycopeptides/glycoproteins (both bottom-up and top-down) were measured. The bottom-up approach led to the relative quantification of six different glycosylated forms of the peptides K.TKPREEQYnSTYR.V and R.EEQYnSTYR.V, with FA2 (G0F) and FA2G1 (G1F) being by far the most common. The top-down analysis by Byonic provided annotation of fragments spanning the entire sequences, with ETD, at least initially, providing the greatest degree of fragment coverage. Oxidation sites and truncations were observed in the top-down analysis.

Conclusions
High resolution mass spectrometry of the NIST reference mAb provides a wealth of data, with tens of thousands of scans over numerous LC-MS/MS acquisitions. The bioinformatic software Byonic, Byologic and Byomap were applied to this data to efficiently elucidate and quantify a wide range of modified forms.

Novel Aspect
A reference mAb was extensively analyzed by high resolution mass spectrometry and new bioinformatics tools. Modifications were identified and quantified at low concentrations.

ThPS39-14 / Twin-Ion Metabolite Extraction: Combining stable isotopic labels and LCMS data mining software for non-targeted identification of drug metabolites

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Introduction
Despite their promise and the patients they cure, many widely used medications produce unwelcome side effects that can range from mild discomfort to life threatening toxicity. In some cases these side effects can be linked to metabolism and, accordingly, industry and regulatory bodies alike now place a great emphasis on the identification and characterization of drug metabolites for investigational compounds. In support of these fundamental goals, we are currently devising strategies for identifying metabolite signals from complicated high resolution LCMS data sets.

Methods
Employing the so-called "twin-ion" method, whereby a 1:1 mixture of a drug and a stable isotope labelled derivative is administered to a subject, any metabolites formed will retain the diagnostic ion signature that can be profiled by LCMS. This provides a handle for computational algorithms to differentiate between ions of drug metabolites and endogenous compounds in complex biological samples such as blood plasma.

Results
An algorithm has been developed for the detection of drug metabolites displaying the twin-ion signature. Essentially raw instrument data collected in profile mode is weighted by correlation with a hypothetical 3D twin-ion signature then assigned a confidence score. The weighted data is then plotted as a heat map from which high scoring regions of data can be readily visualised and investigated by the analyst.
The ability of this approach to identify metabolites of paracetamol and 13C6 paracetamol in rat blood plasma was evaluated. Upon manual investigation of the LCMS data, 10 metabolites (Shown in figure 1) ranging in intensity from ~106 to ~103 counts were found. Applying our shape fitting algorithm, every one of these 10 was subsequently identified and differentiated from background signals and instrumental noise.

Conclusion
A new computational approach to scan LCMS data and automatically detect twin-ion signatures has been developed. Using this method, drug metabolites can be rapidly detected, from complex datasets, in a non-targeted manner without resorting to radioactive materials.

Novel Aspect
To fully capitalise on non-targeted metabolite identification methods, raw instrument outputs should be analysed with as little data reduction as possible to preserve the signal integrity of low abundance metabolites. In our experience, the computationally simple centroid data format fails to represent very low abundance signals accurately enough for precise computational identification. Our approach, by contrast, is the first algorithm that is capable of extracting twin-ion signatures from raw profile data generated by high resolution mass spectrometers opening up the potential for greatly enhanced precision and sensitivity.

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