





...ability of a multi-enzyme ...  
 ...the methylation ...  
 ...using a four step ...  
 ...characterization ...  
 ...the biological ...  
 ...elements were used ...  
 ...of reacting biomolecules ...  
 ...examples design.

## **HUMAN URINE BY HPLC- STANDARD**

**1. M. Anastasia 2**

*Studia di Milan*

...of free pyridinoline (Pyd),  
 ...Gal-Pyd) and glucosyl-  
 ...age-34±7). For the first  
 ...all these compounds, the  
 ...the coefficients of variation for  
 ...around 2.3 pmol injected,  
 ...and 96.4±20.1 pmol/  
 ...from limit. The addition of the  
 ...unequivocal quantification  
 ...of all selected analytes is

work are four proteins. It shows which display a low level of interspecific genetic diversity. Among all the study are, the characterization of the protein expression profile and the identification of proteins differentially expressed in the four B. clausi strains during the stationary phase of growth. Few protein spots show statistically significant expression changes, and, among this group of proteins, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) show a significant decrease in the stationary phase only two out of four strains. The comparative proteomic data correlate with the enzyme activity and the expression level of the mRNA of ADH and ALDH. Acknowledgement: Grant from FAR-MUR, DM 23154.

## **BETA-AGONISTS IN LIVER - VALIDATION METHOD**

**FRANCESCA DI GAUDIO**

Department DIBIMEL, Medical Biochemistry Section, Faculty of Medicine, University of Palermo, Italy

Administration of beta-agonists through feeds in meat-producing livestock has been illegally introduced in EU in order to improve muscle tissue to fat tissue ratios. Only few methods have been validate for Clenbuterol like anabolic drugs determination in bovine liver using stringent criteria imposed by Decision 657/2002/EC. The aim of this work was to validate a method following the EU guidelines, using LCMS QqQ after cleanup procedure. 5,00 g of tissue were homogenized, added with IR, extracted and digested with  $\beta$ -glucuronidase/arylsulfatase and cleaned up. The validation procedure was carried out on a LCMS QqQ. Several MS/MS spectra, at increasing collision energy, were recorded. Some of the fragments in the MS/MS spectra can be explained by a common fragmentation pathway that imply competitive losses of water and alkene. Identification and quantification was performed using Clenbuterol D9 as IS, specific SRM transition and a calibration curve in certified matrix. The validation following the Decision is challenging for retrieval of certified matrices and isotopically Labeled Standards and a large number of samples necessary in order to optimize parameters.



## A TWO-DIMENSIONAL ELECTROPHORESIS AND MASS SPECTROMETRY PROTEIN ANALYSIS OF FOUR BACILLUS CLAUSII STRAINS

A. Abbrescia<sup>1</sup>, A. Gaballo<sup>1</sup>, A. Gnoni<sup>2</sup>, R. Lippolis<sup>1</sup>, L. L. Palese<sup>2</sup>, D. Pane<sup>1</sup>, M. S. Patemoster<sup>2</sup>, S. Papa<sup>1,2</sup>, A. M. Sardanello<sup>2</sup>

<sup>1</sup> Ist. of Bioenerg. and Biomembr., CNR, Bari, Italy; <sup>2</sup> Dept. of Med. Biochemistry, Biophysics and Physiology, University of Bari, Italy

The alkaliphilic *Bacillus* species constitute a large, heterogeneous group of microorganisms which have relevant applications and commercial interest. Probiotic species have been proved to contribute in preventing and treating various gastrointestinal disorders by improving the hosts intestinal microbial balance. The focus of this work are four probiotics *B. clausii* strains which display a low level of intraspecific genome diversity. Aims of this study are, the characterization of the protein expression profile and the identification of proteins differentially expressed in the four *B. clausii* strains during the stationary phase of growth. The proteomic data show statistically significant expression changes, and, among this group of proteins, several dehydrogenases (ADH) and aldehyde dehydrogenase (ALDH) show a significant decrease in the stationary phase (day 10) of four strains. The comparative proteomic data correlate with the enzyme activity and the expression levels of the mRNA of ADH and ALDH. Acknowledgement: Grant from FAR-MIUR; DM 23154

## BETA-AGONISTS IN LIVER - VALIDATION METHOD

FRANCESCA DI GIACOMO

Department DIBIMEL, Medical Biochemistry, Section of Experimental Medicine, University of Bari, Italy

Administration of beta-agonists through feeds in meat-producing animals and other species introduced in EU in order to improve muscle tissue to fat tissue ratios. Only few methods involving clenbuterol like anabolic drugs determination in bovine liver using stringent chromatographic and mass spectrometric procedures. The aim of this work was to validate a method following the EU guidelines using LCMS-MS after cleanup procedure. 5,00 g of tissue were homogenized, added with IR, extracted and digested with 8-g. carboxypeptidase Y, sulfatase and cleaned up. The validation procedure was carried out on a LCMS QqQ. Several MS/MS spectra, at increasing collision energy, were recorded. Some of the fragments in the MS/MS spectra can be explained by a common fragmentation pathway that imply competitive losses of water and alkene. Identification and quantification was performed using Clenbuterol D9 as IS, specific SRM transition and a calibration curve in certified matrix. The validation following the Decision is challenging for retrieval of certified matrices and isotopically Labeled Standards and a large number of samples necessary in order to optimize parameters.