Research Article

Online Solid-Phase Extraction LC-MS/MS: A Rapid and Valid Method for the Determination of Perfluorinated Compounds at Sub ng·L^{-1} Level in Natural Water

Salvatore Barreca (iD)**, Maddalena Busetto, Matteo Vitelli, Luisa Colzani, Laura Clerici, and Pierluisa Dellavedova**

Agenzia Regionale per la Protezione dell'Ambiente della Lombardia (ARPA Lombardia), U.O.C. Laboratorio di Milano, Sede laboratoristica di Monza e Brianza via Solferino N° 16, 20900 Monza, MB, Italy

Correspondence should be addressed to Salvatore Barreca; salvatore.barreca@unipa.it

In this research paper, we report a method able to detect perfluorinated compound at pg·L^{-1} levels in superficial and underground water samples. An online solid phase extraction HPLC-MS/MS method was developed for the analysis of 12 perfluoroalkyl acids (PFASs). The method is based on injection of 5000 μL of water sample in SPE online WAX column followed by chromatography separation and mass spectrometry determination. SPE online elution was performed by water + 0.05% NH$_4$OH and methanol + 0.05% NH$_4$OH, while separation of target compounds was achieved within 10 min by Gemini chromatographic column operating from 1 to 12 pH range and using a mixture of water-methanol + 0.05% NH$_4$OH. Sub ng·L^{-1} method detection limits (from 0.2 to 5 ng·L^{-1}), linearity (from 0.2 to 250 ng·L^{-1}), accuracy (from 80 to 120%), and precision (RSD less than 15% at LOQs levels) were achieved. The method is capable of measuring PFAS at trace levels, but above all it can reach the limit of 200 pg·L^{-1} required by European regulation for PFOS determination in surface and underground waters. The method was validated for quantitative analysis of PFASs in real water samples.

1. Introduction

In the last decades, a wide variety of different pollutants have been found in several environment samples [1–3]. The research community has made significant efforts in order to develop analytical techniques capable of detecting these pollutants at trace levels [4–6].

The term "perfluorinated compounds" (PFCs) is referred to a class of organic substances in which all the hydrogens of the hydrocarbon backbones are substituted with fluorine atoms [7].

PFCs have been synthesized and commercialized since the 1960s and are used in numerous industrial and consumer products especially for intermediates and/or additives in the synthesis of certain fluorine compounds [8]. Such compounds are widely used in various domestic and industrial products including adhesives, polishes, paints, waxes, textiles stain/water/grease repellents in carpets and clothing, or in cooking utensils such as nonstick coatings [9–11].

PFC's extensive uses may depend on whether the fluorine-carbon bonds are extremely stable, and it should imply very high thermal and chemical stability [7]. Due to these properties, PFCs are classified as persistent pollutants, and some of these substances bioaccumulate in the environment. In addition, former research suggests that impact of exposure from consumer products is usually lower than impact of exposure in contaminated drinking water or contaminated food, such as fish [12, 13].

Although several PFAs such as perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonate (PFHxS), and perfluorononanoic acid (PFNA) have

been more widely studied, research has not yet determined the health effects and mechanisms resulting from human exposure to PFAs at levels typically found in water and food [14, 15].

Generally, PFAs accumulate in the human body, and their levels decrease slowly over time.

Some studies in humans have shown that certain PFAs may have an impact on the developing fetus and child and decrease fertility, interfere with the natural hormones of the body, increase cholesterol, affect the immune system, and increase cancer risk [16, 17].

Further studies are required to determine whether the same effects are caused by the same mechanism of action.

In the last ten years, the scientific and the health communities have focused interest in perfluorinated compounds by investigating in particular perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). The most common PFCs have been identified at considerable levels in various environmental matrices, predominantly in aqueous environment [18, 19]. Indeed, these compounds may present a potential risk to humans via drinking water and to wildlife via receiving waters.

More recently, detection of PFAs relevant levels in the area of Veneto, Italy, have highlighted the necessity of undertaking studies evaluating the concentration of perfluorinated compounds in different water matrices.

In this respect, several Environmental Protection Agency of Italy (ARPA) are endeavoring to develop and validate methods able to assess PFAs concentrations in environmental water samples.

Generally, the analysis of PFAs at trace levels in different environmental matrices represents a challenge in analytical chemistry. Indeed, quantification of PFAs at concentration of $ng \cdot L^{-1}$ or $pg \cdot L^{-1}$ in drinking water requires extraction techniques and cleanup procedures to provide enrich the sample and reduce matrix interferences. In this respect, the most effective analytical technique for the determination of perfluorinated alkyl acids (PFAs) in drinking water consists of extraction procedures by offline solid-phase extraction (SPE) cartridge containing polystyrene divinylbenzene followed by elution in methanol, concentration to dryness with nitrogen, injection and analysis using liquid chromatography/tandem mass spectrometry (LC/MS/MS) [20–22].

Unfortunately, due to several challenges such as matrix effects and cross-contamination, classic analytical techniques cannot detect PFAs at levels below $10 \, ng \cdot L^{-1}$, especially for PFOS and PFAs at long chain [23, 24]. For this reason, it is necessary to develop and validate an analytical procedure capable of improving detection limits for all PFAs.

Different research groups have endeavored to develop innovative online SPE techniques to detect PFASs in water samples.

Within this context, several methods employ formic acid or other substances to improve LODs, but at ultratrace level analysis, the risk of cross-contaminations caused by external additions such as acid or other substances represent a critical point.

Recently, Dasu et al. have reported an ultrasensitive method for the analysis of perfluorinated alkyl acids in drinking water using an SPE online column coupled with high-performance liquid chromatography-tandem mass spectrometry. This method uses water and methanol containing 0.1% of NH_4OH [25] as mobile phases. Unfortunately, eluents at pH value above 8 are not totally compatible with classic C18 or polymeric columns employed in chromatographic separations. To the best of our knowledge, the best LOQ value for PFOS obtained by using SPE online technique values were reported at $1 \, ng \cdot L^{-1}$ [25]. The research centre at ARPA Lombardia pioneers the monitoring of activities on superficial water samples on the Italian territory. It has recently developed an analytical method based on solid phase extraction online coupled with HPLC/MS-MS to detect PFCs concentration in natural water samples by using a C18 column with pH working range from 1 to 12.

2. Experimental

2.1. Chemicals and Materials.
Native standard solutions of the individual analytes in methanol were purchased from Wellington Laboratories. ^{13}C mass-labeled methanolic solutions of each analyte were used as internal standards at $25 \, ng \cdot L^{-1}$, while ^{13}C M3PFBA, ^{13}C MPFDA, ^{13}C MPFOA, and ^{13}C MPFOS were used as surrogate standards in order to check percentage recovery by final concentration of surrogate standard solution at $25 \, ng \cdot L^{-1}$. All mass-labeled standards were purchased from Wellington Laboratories.

Stock solutions were prepared in methanol and were stored at $-18°C$ in amber glassware. Methanol HPLC grade was purchased from Sigma-Aldrich. High purity water (Milly-Q water grade) was prepared using a Millipore Milli-Q purification system. All standard solutions were purchased at concentration of $2000 \, mg \cdot L^{-1}$. Mass-labeled solution and calibration solutions were prepared by serial dilution of stock solutions in tap water (S. Anna). For the preconcentration and cleanup experiments, an Oasis WAX 150 mg/6 mL was used for SPE online, relying upon the good recoveries reported in existing literature by using Oasis WAX-SPE cartridges including short-chain (C4–C6) compounds.

Two LC Column Poroshell 120 EC-C18 3×50 mm, $2.7 \, \mu$m, were used to eluent cleanup. NH_4OH concentrate solution was purchased from Sigma-Aldrich.

A Gemini C18 $3 \, \mu$m, 100×3 mm, made Phenomenex operating a pH value ranged 1–12 was used for chromatographic separation column. Samples were collected in a propylene bottle of 0.250 mL and refrigerated at a temperature of $4°C$ until analyses.

2.2. Solvent Cleanup.
Methanol and Milli-Q water were precleaned by connecting separate online scrubber columns for each mobile phase. In this context, two columns were used. The first one was positioned before injection valve to clean up the eluent used for SPE online, and the second one was used to clean up the eluent employed in chromatographic separation.

2.3. Liquid Chromatograph and Mass Spectrometric Instruments.

Analysis were performed using high-performance liquid chromatograph (HPLC) consisting of binary and quaternary pump Nexera LC pump, a DGU-20A 5R degassing unit, a SIL-30AC autosampler equipped with a 5000 μL loop, a CTO/20AC thermostated column compartment, and a CBM-20A module controller.

The Nexera LC system was coupled with a 6500 QTrap mass spectrometer (Sciex), equipped with a Turbo V® interface by an ESI probe. Elution conditions and mass spectrometry optimal parameters were reported in Tables 1–3, respectively. About SPE online column, load processes occur from 0.8 to 3.5 min, while elution in analytical column occurs from 3.5 to 10 min.

In order to minimize MS source contamination, the first 3.5 min and the last 3.5 min of the chromatographic run have been diverted to waste by means of a two-position six-port valve installed before the mass spectrometer.

Analysis was performed by multiple reaction monitoring (MRM) in negative ionization mode by using m/z, declustering potential, and collision energy as reported in Table 4.

Moreover, analytes were identified comparing both their retention times (RT) with the RT of the internal standards and by qualification ions.

3. Results and Discussion

Sample preparation and analytical operating conditions play an important role to ensure accuracy, precision, and repeatability recovery and yield the finest possible LOD.

Optimization of analytical separation was carried out by direct injection of standard solutions with a 100 μL loop, while the separation gradient elution for SPE online were investigated by injection of 2000 and 5000 μL, respectively. The best results, in terms of S/N and standard area value, were obtained by injection of 5000 μL. In detail, for standard at 0.2 ng·L^{-1}, the S/N for 2000 and 5000 μL was 2.0 and 7.5, respectively.

Moreover, laboratory has valuated different SPE online columns as reported in Section 2.1. Best results were obtained by using an Oasis WAX SPE online.

In the first step of the optimization process, the influence of eluent pH on elution procedures of PFAs from SPE online using Oasis WAX cartridge was jointly investigated. Best conditions were obtained by using NH$_4$OH 0.05% in water-methanol eluent. Unfortunately, the most C 18 and polyvinyl column employed in HPLC operate a pH range 2–8 and mobile phase used to eluate total PFAS (C-4/C-12) from Oasis WAX cartridge has a pH value above 8. Within this context, to overcome such challenges, a Gemini C18 column able to operate at pH near 12 was used.

A representative chromatogram for PFASs by using developed analysis conditions is represented in Figure 1.

An acceptable chromatographic separation was achieved for 12 PFAs investigation, while best ionization responses were obtained for perfluoroalkyl acid compounds.

Calibration solutions were run at the beginning of each analytical session to ensure instrument response. The percent

TABLE 1: Elution conditions employed in SPE online column.

Time (min)	Total flow (mL·min^{-1})	% eluent A1 water 0.5% of NH$_4$OH	% eluent B1 methanol 0.5% of NH$_4$OH	Valve
0.0	0.8	50	50	A: exhaust
3.5	0.8	50	50	B: mass instrument
6.5	0.8	0	100	B: mass instrument
8.0	0.8	0	100	B: mass instrument
8.5	0.8	0	100	B: mass instrument
8.6	0.8	50	50	B: mass instrument
9.5	0.8	50	50	B: mass instrument
10	0.8	50	50	B: mass instrument
12	End			A: exhaust

TABLE 2: Elution conditions employed in Gemini HPLC Column.

Time (min)	Flow (mL·min^{-1})	% eluent A Milli-Q water loading	% eluent B water/methanol 50 : 50 w/w water	Valve
0.0	1.6	100	0	A: exhaust
3.5	1.6	100	0	B: column
6.5	1.6	100	0	B: column
8.0	1.6	100	0	B: column
8.5	1.6	0	100	A: discharge
8.6	1.6	0	100	A: discharge
9.5	1.6	0	100	A: discharge
10.0	1.6	100	0	A: exhaust
12.0	End			

TABLE 3: Parameter used in ESI-MS/MS.

Source	Unit	Value
Curtain gas (CUR)	psi	25
Collision gas	—	High
Ion spray voltage (IS)	V	−4500
Temperature TEM	°C	450
Ion source gas (GS1)	psi	45
Ion source gas (GS2)	psi	55
Entrance potential (EP)	V	−10
Collision exit potential (CXP)	V	−15

relative standard deviation between different replicate levels of calibration curves ranged from 0.5% to 10%.

Prior to analysis and every ten samples, blank solutions were analyzed, and for each analyte, the obtained signal was

Table 4: m/z, declustering potential and collision energy used.

Analyte	Q1 precursor ion [M-h]⁻ (m/z)	Q3 product ion (m/z)	Declustering potential (DP)	Q2 collision energy (CE)
PFBA-1 perfluorobutanoic acid	213	169.1	−10	−14
PFPeA-1 perfluoropentanoic acid	263	219	−10	−12
PFBS-1 perfluorobutane sulfonic acid	299	80	−40	−70
PFBS-2 perfluorobutane sulfonic acid	299	98.8	−40	−40
PFHxA-1 perfluorohexanoic acid	313	269	−10	−13
PFHxA-2 perfluorohexanoic acid	313	119	−10	−30
PFHpA-1 perfluoroheptanoic acid	363	319	−10	−15
PFHpA-2 perfluoroheptanoic acid	363	169	−10	−20
PFHxS-1 perfluorohexansulfonic acid	399	79.9	−40	−100
PFHxS-2 perfluorohexansulfonic acid	399	98.9	−40	−76
PFOA-1 perfluorooctanoic acid	413	369	−10	−15
PFOA-2 perfluorooctanoic acid	41o3	169	−10	−20
PFNA-1 perfluorononanoic acid	463	419	−10	−15
PFNA-2 perfluorononanoic acid	463	168.9	−10	−25
PFOS-1 perfluorooctanesulfonic acid	499	80	−60	−120
PFOS-2 perfluorooctanesulfonic acid	499	99	−60	−100
PFDA-1 perfluorodecanoic acid	513	469.1	−10	−15
PFDA-2 perfluorodecanoic acid	513	218.9	−10	−22
PFUnDA-1 perfluoroundecanoic acid	563	519	−10	−15
PFUnDA-2 perfluoroundecanoic acid	563	269	−60	−140
PFDoDA-1 perfluorododecanoic acid	613	569	−10	−15
PFDoDA-2 perfluorododecanoic acid	613	269	−10	−20
MPFBA-labeled perfluorobutanoic acid	217	171.9	−10	−14
M_5PFPeA-labeled perfluoropentanoic acid	268	233	−10	−12
M_3PFBS-labeled perfluorobutane sulfonic acid	302	99	−40	−70
M_5PFHxA-labeled perfluorohexanoic acid	318	273	−10	−13
M_4PFHpA-labeled perfluoroheptanoic acid	367	322	−10	−20
M_3PFHxS-labeled perfluorohexansulfonic acid	402	99	−40	−100
M_8PFOA-labeled perfluorooctanoic acid	421	376	−10	−14
M_8PFOS-labeled perfluorooctanesulfonic acid	507	99	−60	−100
M_9PFNA-labeled perfluorononanoic acid	472	427	−10	−15
M_6PFDA-labeled perfluorodecanoic acid	519	474	−10	−15
M_7PFUdA-labeled perfluoroundecanoic acid	570	525	−10	−15
MPFDoDA-labeled perfluorododecanoic acid	615	570	−10	−15
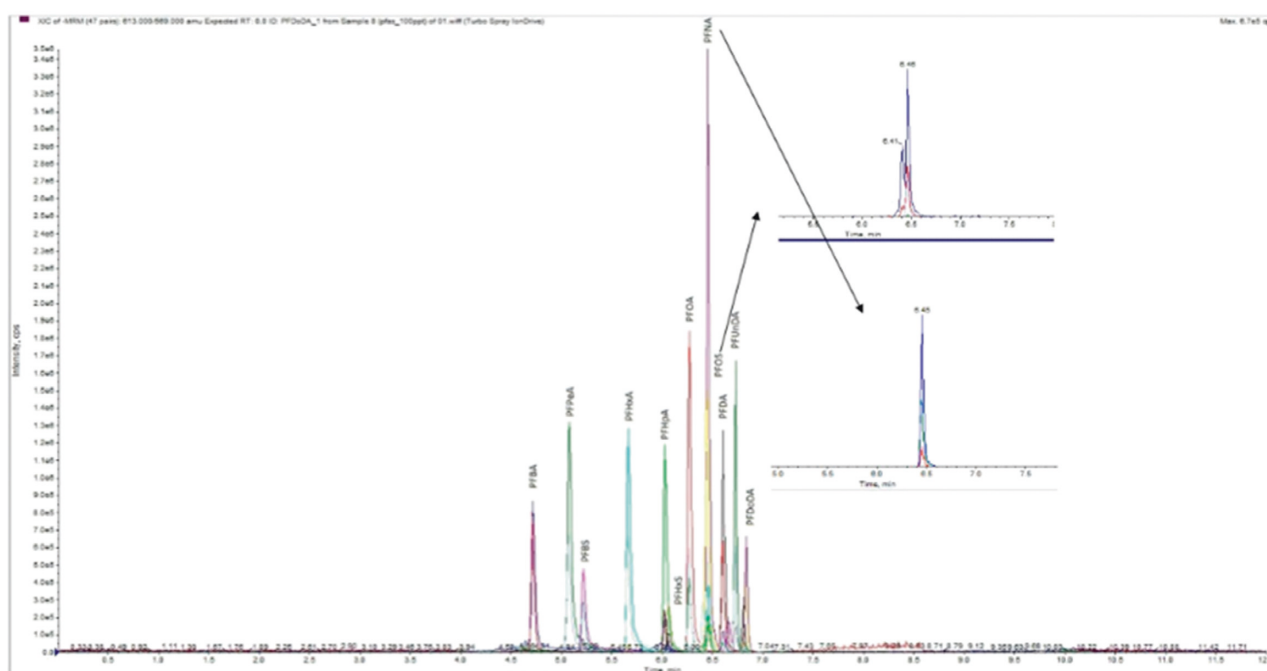
Figure 1: Chromatogram of PFAs solutions at 50 ng·L⁻¹.

<1/2 LOQ. According to ISO 17025 criteria, an independent solution was used to check standards used in calibration curves.

Moreover, to check possible instrumental drift, low and middle level calibration solutions were run after every ten sample analyses to verify the previously established calibration curve. Bias of each quality control ranged from ±20 to 30% for low and middle levels, respectively.

The method was validated in terms of detection limits, accuracy, and precision. Analyses were conducted by using real superficial water samples.

The limits of quantification (LOQs) were estimated by 10-time standard deviation calculated at first level of calibration curve prepared in real superficial water sample.

Table 5 summarizes the results obtained for these performance parameters. In all analyses, analyte recovery was checked by mass-labeled standard. Accuracy and repeatability were calculated on data set of six analyses conducted on water-spiked samples.

All data obtained were subject to different statistical tests. Normal distribution was evaluated by using the Shapiro–Wilk test, while outlier data were checked by the Dixon test.

Linearity ranges were obtained from 0.2–250 to 5–250 ng·L^{-1} depending on the different analytes considered.

PFBA shows a linear range from 5 to 250 ng·L^{-1}. Due to its short alkyl chain, chemical-physical properties of PFBA are very different compared to other PFASs compounds. In this regard, PFBA is adsorbed less than other PFASs in SPE online, and in this context, the first level corresponding to 5 ng·L^{-1}. In any cases, by comparing this method with other direct injection or SPE methods, a good detection limit for PFBA was achieved. For the most PFASs, good correlation can be obtained in calibration curve ranged from 1 to 250 ng·L^{-1}.

For PFOS, one of the most important analytes monitored, linearity of method was evaluated at low levels over concentration range from 0.2 to 250 ng·L^{-1} finding a linear response (correlation coefficients in Table 5). Generally, in the literature, detection limit for PFOS, by direct injection or SPE online, ranged from 5 to 10 ng·L^{-1}, and only few cases reported LOD at the level of 1 ng·L^{-1} [25, 26]. In this context, quantification limits of 0.2 ng·L^{-1} without sample preparations can be considered an interesting analytical result.

Calibration curves for PFOS in the range from 0.2 to 250 ng·L^{-1} for quantitative and qualitative ions (499 m/z and 80 m/z resp.) are reported in Figure 2.

Moreover, accuracy was evaluated by performing recovery tests in terms of percentage recovery by using spiked samples (tap and superficial water) fortified at various levels (from 10 to 50 ng·L^{-1}), and the results are shown in Figures 3(a)–3(c) for low-, middle-, and high-level (10, 25, and 50 ng·L^{-1}) concentrations, respectively.

For all analyzed samples, analytical recovery, determined by surrogate standard, ranged from 80 to 120%.

For all PFASs, recovery ranged from 80 to 120% in good agreement with accuracy required by the regulations on the determination of PFASs in water which is a satisfactory result.

To determine repeatability of method, different experiments were conducted in replicated analysis at low level, middle levels, and high levels using spiked-water samples with native standard solution at different concentrations. At LOQ levels, RSD were less than 15% (Table 5), while at other levels, RSD were less than 10%.

Moreover, in order to verify the robustness of the analytical method, the matrix effect was investigated by comparing calibration curves for each compound by using tap and superficial water samples. Three replicates for each level were used to obtain calibration curves, and robustness was studied by ME (matrix effect) index evaluated according to the following equation:

$$ME\% = \frac{m(\text{sample})}{m(\text{standard})} * 100 - 100, \tag{1}$$

where $m(\text{sample})$ is the slope of the calibration curve obtained in matrix and $m(\text{standard})$ is the slope of the calibration curve in solvent; accordingly, ME values <0 indicate ion suppression, whereas ME values >0 indicate ion enhancement.

The ME index calculated for each analyte in tap water and real superficial water is reported in Table 6.

As reported in the literature [27], ME index value in the range of ±20 are considered to have a negligible influence on the performance of an analytical method. For the proposal method, for all analytes, ME index is less than ±20. In this regard, the method developed can be considered robust.

4. Conclusions

This paper reports the development, validation, and application of a new SPE online HPLC-MS/MS method able to detect perfluorinated compound in water samples.

The proposed method provides an easy, rapid, and without samples preconcentration application to analyze PFASs. The proposed method is accurate and precise, shows lower limits of quantification and presents a good linearity at the range of pg·L^{-1}-ng·L^{-1} levels.

The method can measure PFASs at the trace levels in water samples and attain most of the adopted quality standards except for European EQS for PFOS (0.65 ng/L) which is considered a challenging limit for every LC-MS analytical method. Furthermore, compared with other SPE online analytical methods, the new analytical method achieves a new real limit for PFOS (200 pg·L^{-1}). Moreover, working under pH conditions above 10, it is better to use a column operating at pH range between 1 and 12.

Finally, a matrix effect depending on superficial water was not detected.

The optimized method was successfully applied to the quantitation of PFASs in natural water samples, and the value of method was successfully tested by using spiked samples.

TABLE 5: Limits of quantification (LOQs), linearity range, R2, accuracy, and repeatability parameters evaluated at LOQ.

Compound	LOQ ($ng \cdot L^{-1}$)	Linear range ($ng \cdot L^{-1}$)	R^2	Accuracy (%)	Repeatability CV%
PFBA	5	5–200	0.999	101.1	10.7
PFPeA	1	1–250	0.999	91.7	10.6
PFBS	1	1–250	0.999	95.6	11.5
PFHxA	1	1–250	0.999	97.4	8.28
PFHpA	1	1–250	0.999	97.2	10.4
PFHxS	1	1–250	0.999	88.94	9.7
PFOA	1	1–250	0.999	99.9	11.5
PFNA	1	1–250	0.999	93.0	13.8
PFOS	0.2	0.2–250	0.999	85.0	12.4
PFDA	5	5–250	0.999	81.0	12.1
PFUnDA	5	5–250	0.999	107.2	11.3
PFDoDA	5	5–250	0.999	107.4	9.5
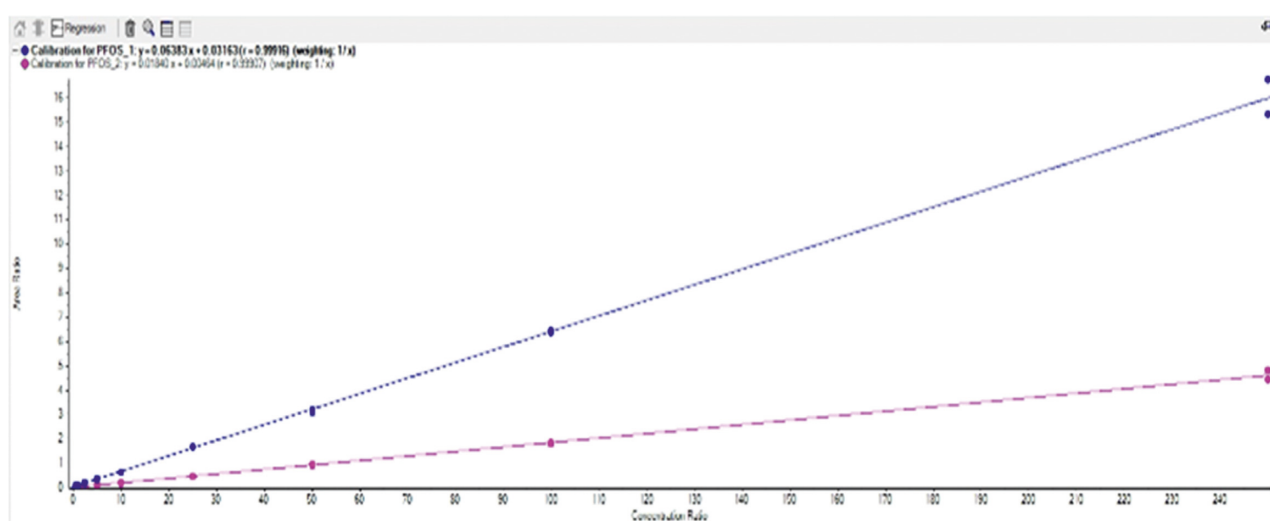
FIGURE 2: PFOS calibration curve in the range from 0.2 to 250 $ng \cdot L^{-1}$. Dotted blue line represents m/z transition $499 \rightarrow 80$; dotted red line represents m/z transition $499 \rightarrow 99$.
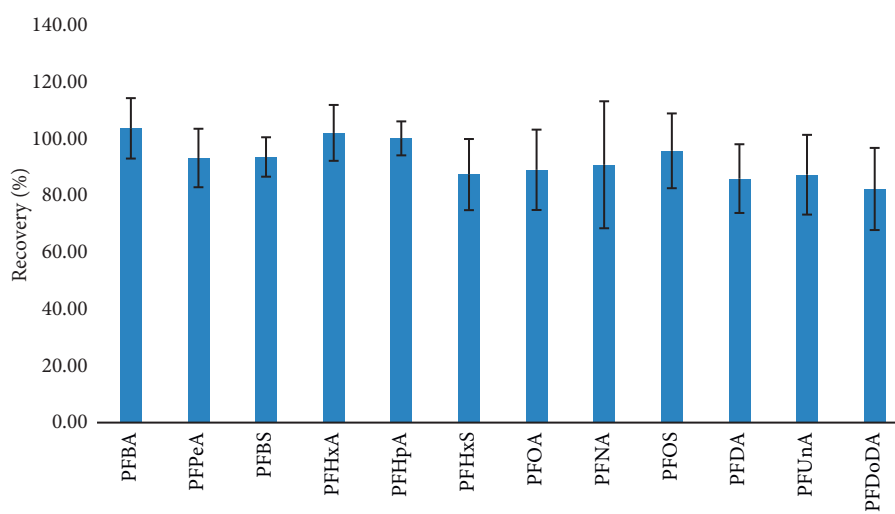
(a)

FIGURE 3: Continued.
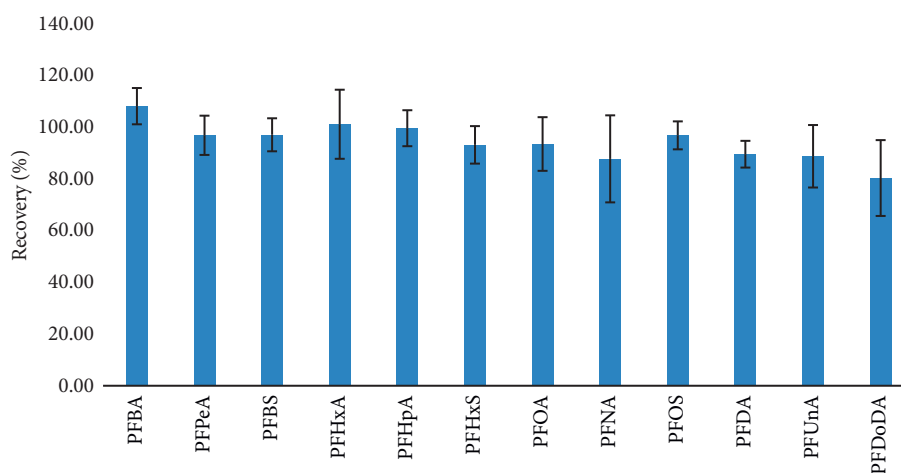
(b)
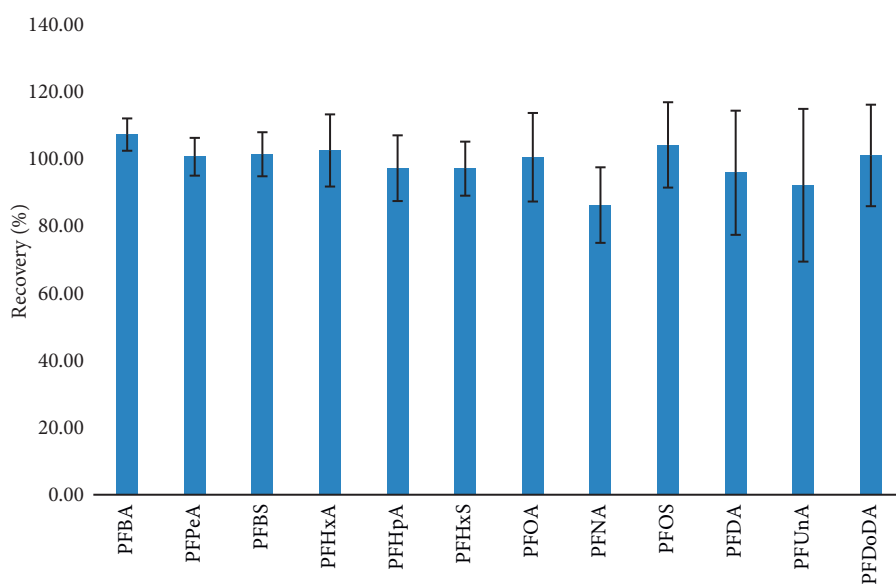
(c)

FIGURE 3: (a) Percentage recovery for all analytes at $10 \, ng \cdot L^{-1}$. (b). Percentage recovery for all analytes at $25 \, ng \cdot L^{-1}$. (c). Percentage recovery for all analytes at $50 \, ng \cdot L^{-1}$.

TABLE 6: Matrix effect index for all analytes.

Analyte	ME index
PFBA	−1.4
PFPeA	4.5
PFBS	1.7
PFHxA	−2.2
PFHpA	−0.67
PFHxS	5.1
PFOA	−0,20
PFNA	−1.7
PFOS	0.08
PFDA	−0.64
PFUnDA	−2.4
PFDoDA	−6.8

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

The findings and conclusions of this article are solely the responsibility of the authors and do not represent the official views of ARPA Lombardia.

Conflicts of Interest

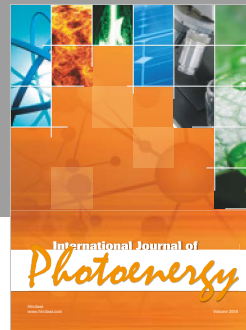The authors declare that they have no conflicts of interest.

Acknowledgments

References

[1] R. Kallenborn, *Perfluorinated Alkylated Substances (PFAS) in the Nordic Environment*, Nordic Council of Ministers, Copenhagen, Denmark, 2004.

[2] S. Barreca, A. Mazzola, S. Orecchio, and N. Tuzzolino, "Polychlorinated biphenyls in sediments from Sicilian coastal area (Scoglitti) using automated soxhlet, GC-MS, and principal component analysis," *Polycyclic Aromatic Compounds*, vol. 34, no. 3, pp. 237–262, 2014.

[3] E. Magi, M. Di Carro, C. Mirasole, and B. Benedetti, "Combining passive sampling and tandem mass spectrometry for the determination of pharmaceuticals and other emerging pollutants in drinking water," *Microchemical Journal*, vol. 136, pp. 56–60, 2018.

[4] V. Paiano, E. Fattore, A. Carra, C. Generoso, R. Fanelli, and R. Bagnati, "Liquid chromatography-tandem mass spectrometry analysis of perfluorooctane sulfonate and perfluorooctanoic acid in fish fillet samples," *Journal of Analytical Methods in Chemistry*, vol. 2012, Article ID 719010, 5 pages, 2012.

[5] S. Barreca, S. Orecchio, and A. Pace, "Photochemical sample treatment for extracts clean up in PCB analysis from sediments," *Talanta*, vol. 103, pp. 349–354, 2013.

[6] R. Loos, B. M. Gawlik, G. Locoro, E. Rimaviciute, S. Contini, and G. Bidoglio, "EU-wide survey of polar organic persistent pollutants in European river waters," *Environmental Pollution*, vol. 157, no. 2, pp. 561–568, 2009.

[7] T. Stahl, D. Mattern, and H. Brunn, "Toxicology of perfluorinated compounds," *Environmental Sciences Europe*, vol. 23, p. 38, 2011.

[8] M. Fricke, "U. Lahl Risikobewertung von perfluortensiden als beitrag zur aktuellen diskussion zum REACH-Dossier der EU-Kommission," *Umweltwissenschaften und Schadstoff-Forschung*, vol. 17, no. 1, pp. 36–49, 2005.

[9] H. Fromme, M. Schlumer, J. Ungewiss, and E. Roscher, "Materialien zur Umweltmedizin-Umweltmedizinische Bedeutung perfluorierter Kohlenwasserstoffe (PFC)," in *Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (Hrsg.), Band 16 der Schriftenreihe*, Bavarian State Office for Health and Food Safety, Bavaria, Germany, 2006.

[10] J. M. O'Brien, A. C. Carew, S. Chu, R. J. Letcher, and S. W. Kennedy, "Perfluorooctane sulfonate (PFOS) toxicity in domestic chicken (Gallus gallus domesticus) embryos in the absence of effects on peroxisome proliferator activated receptor alpha (PPARα)-regulated genes," *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, vol. 149, no. 4, pp. 524–530, 2009.

[11] F. Xiao, T. R. Halbach, M. F. Simcik, and J. S. Gulliver, "Input characterization of perfluoroalkyl substances in wastewater treatment plants: source discrimination by exploratory data analysis," *Water Research*, vol. 46, no. 9, pp. 3101–3109, 2012.

[12] J. Hölzer, O. Midasch, K. Rauchfuss et al., "Biomonitoring of perfluorinated compounds in children and adults exposed to perfluorooctanoate-contaminated drinking water," *Environmental Health Perspectives*, vol. 116, no. 5, pp. 651–657, 2008.

[13] S. P. J. Van Leeuwen, C. P. Swart, I. Van der Veen, and J. De Boer, "Significant improvements in the analysis of perfluorinated compounds in water and fish: results from an interlaboratory method evaluation study," *Journal of Chromatography A*, vol. 1216, no. 3, pp. 401–409, 2009.

[14] S. T. Wolf and W. K. Reagen, "Method and validation for the analysis of perfluorinated compounds in water by pre-sampling isotope dilution-direct injection-LC/MS/MS," *Analytical Methods*, vol. 5, no. 10, pp. 2444–2454, 2013.

[15] A. K. Rosenmai, C. Taxvig, T. Svingen et al., "Fluorinated alkyl substances and technical mixtures used in food paper-packaging exhibit endocrine-related activity in vitro," *Andrology*, vol. 4, no. 4, pp. 662–672, 2016.

[16] A. P. Starling, J. L. Adgate, R. F. Hamman et al., "Perfluoroalkyl substances during pregnancy and offspring weight and adiposity at birth: examining mediation by maternal fasting glucose in the healthy start study," *Environmental Health Perspectives*, vol. 125, no. 6, 2017.

[17] G. W. Olsen, D. C. Mair, C. C. Lange et al., "Per-and polyfluoroalkyl substances (PFAS) in American Red Cross adult blood donors, 2000–2015," *Environmental Research*, vol. 157, pp. 87–95, 2017.

[18] S. P. Van Leeuwen, A. Kärrman, B. Van Bavel, J. De Boer, and G. Lindström, "Struggle for quality in determination of perfluorinated contaminants in environmental and human samples," *Environmental Science & Technology*, vol. 40, no. 24, pp. 7854–7860, 2006.

[19] J. Thompson, G. Eaglesham, and J. Mueller, "Concentrations of PFOS, PFOA and other perfluorinated alkyl acids in Australian drinking water," *Chemosphere*, vol. 83, no. 10, pp. 1320–1325, 2011.

[20] X. Zhao, Y. Cai, F. Wu, Y. Pan, H. Liao, and B. Xu, "Determination of perfluorinated compounds in environmental water samples by high-performance liquid chromatography-electrospray tandem mass spectrometry using surfactant-coated Fe_3O_4 magnetic nanoparticles as adsorbents," *Microchemical Journal*, vol. 98, no. 2, pp. 207–214, 2011.

[21] S. Valsecchi, S. Polesello, M. Mazzoni, M. Rusconi, and M. M. Petrovic, "On-line sample extraction and purification for the LC–MS determination of emerging contaminants in environmental samples," *Trends in Environmental Analytical Chemistry*, vol. 8, pp. 27–37, 2015.

[22] K. Bielicka-Daszkiewicz, "Extraction techniques based on solid state and connected with liquid chromatography," *Journal of Liquid Chromatography & Related Technologies*, vol. 39, no. 10, pp. 477–487, 2016.

[23] J. L. Wilkinson, P. S. Hooda, J. Swinden, J. Barker, and S. Barton, "Spatial distribution of organic contaminants in three rivers of Southern England bound to suspended particulate material and dissolved in water," *Science of the Total Environment*, vol. 593-594, pp. 487–497, 2017.

[24] P. Zhao, X. Xia, J. Dong et al., "Short-and long-chain perfluoroalkyl substances in the water, suspended particulate matter, and surface sediment of a turbid river," *Science of the Total Environment*, vol. 568, pp. 57–65, 2016.

[25] K. Dasu, S. F. Nakayama, M. Yoshikane, M. A. Mills, J. M. Wright, and S. Ehrlich, "An ultra-sensitive method for the analysis of perfluorinated alkyl acids in drinking water using a column switching high performance liquid chromatography tandem mass spectrometry," *Journal of Chromatography A*, vol. 1494, pp. 46–54, 2017.

[26] N. P. H. Lien, S. Fujii, S. Tanaka et al., "Perfluorinated substances in tap water of Japan and several countries and their relationship to surface water contamination," *Environmental Engineering Research*, vol. 43, pp. 611–618, 2006.

[27] L. Ciofi, L. Renai, D. Rossini et al., "Applicability of the direct injection liquid chromatographic tandem mass spectrometric analytical approach to the sub-ng L^{-1} determination of perfluoro-alkyl acids in waste, surface, ground and drinking water samples," *Talanta*, vol. 176, pp. 412–421, 2018.