Anal Bioanal Chem (2006) 386:2123–2132
DOI 10.1007/s00216-006-0902-7

ORIGINAL PAPER

Method optimization for determination of selected perfluorinated alkylated substances in water samples

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Received: 19 May 2006 / Revised: 15 September 2006 / Accepted: 29 September 2006 / Published online: 1 November 2006

Abstract In recent years perfluorinated alkylated substances (PFAS) have appeared as a new class of global pollutant. Besides being an industrially important group of compounds, PFAS are regarded as highly toxic and extraordinarily persistent chemicals that pervasively contaminate human blood and wildlife throughout the world. They are therefore regarded as PBT (persistent, bioaccumulative, and toxic) chemicals. Two comprehensive methods have been developed for determination of eleven of the most environmentally relevant PFAS (seven perfluoroalkylcarboxylates, two perfluoroalkylsulfonates, and two perfluoroctanesulfonamides) in aqueous samples. The compounds were isolated by liquid–liquid extraction (LLE) and solid-phase extraction (SPE), and identification and quantification of the target analytes were achieved by liquid chromatography–electrospray ionization–tandem mass spectrometry (LC–ESI–MS–MS). With LLE detection limits ranged from 0.26 to 0.62 ng L^{-1} for enrichment of 900-mL water samples; recovery of PFAS with a carbon chain longer than C_7 was excellent (80–93%). With SPE, carboxylates with carbon chains $<C_{10}$ could be extracted efficiently (70–98%) under acidic conditions, and PFOS and PFOSA could be extracted efficiently (81% and 96%, respectively) under basic conditions, resulting in MDLs between 0.25 and 0.64 ng L^{-1}. The LLE method was applied successfully to Austrian wastewater effluent samples.

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Keywords PFAS · PFOS · PFOA · LC–MS–MS · Wastewater

Introduction

Perfluorinated alkylated substances (PFAS), which include perfluorooctane sulfonate (PFOS) and PFOS-related substances, are a diverse class of chemicals characterized by a hydrophobic alkylated chain saturated with fluorine atoms, usually attached to a hydrophilic head. Because of their structures, PFAS have both lipid and water-repellent properties, making them ideal for several commercial uses, mainly those requiring surface-active properties. They are constituents of a wide range of products including fluoropolymers, for example polytetrafluoroethylene (PTFE) or polyvinylidene fluoride (PVDF) (largely fluorinated along the polymer backbone), liquid repellents for paper, packaging, textiles, leather, carpet goods, industrial surfactants, additives, coatings, and firefighting foams [1].

PFAS-related chemicals are focus of international public environmental concern. Recent studies have revealed their extreme persistence in the environment [2], their tendency to bioaccumulate and their biomagnification [2–5], and their potential to adversely affect human health and the environment (toxicity/ecotoxicity) [2, 6–12].

In recent years the number of publications reporting concentrations of PFAS in different environmental aqueous matrices has increased. Thus, PFAS have been detected in surface water [13–22], rainwater [23], sea water [24–27], drinking water [28], and waste water [29–32]; PFOS and PFOA (perfluorooctanoic acid) are the most studied compounds.

Attempts to develop instrumental methods for analysis of perfluorinated alkylated substances were hindered by non-volatility and the absence of chromophores [1] until the end

of the nineteen-nineties, when more sensitive techniques became available. Since then, gas chromatography–mass spectrometry (GC–MS), with prior derivatization, has been used to measure fluorinated carboxylates [33]. More recently, gas chromatography–chemical ionization-mass spectrometry (GC–CI-MS) has been used for analysis of some fluorinated neutral compounds and the applicability of this method to ambient atmospheric monitoring has been described [34]. Perfluorinated sulfonates, for example PFOS, do not form stable, volatile derivatives, however, and, consequently, cannot be easily analyzed by GC–MS. This problem was solved by use of liquid chromatography in combination with mass spectrometry (LC–MS) [14, 17, 24] or tandem mass spectrometry (LC–MS–MS) [10, 13, 15, 16, 18–23, 25–28, 30, 31, 35]. Nowadays, these techniques are by far the most commonly selected for analysis of biota and abiota samples. It is, nevertheless, known that the use of LC–MS for quantitative analysis still requires substantial sample clean up to reduce mass interferences that, usually, coelute with the analytes [1]. The instrumental configuration best adapted to current demanding analytical requirements is LC coupled with tandem mass spectrometry.

Despite these instrumental advances, the most prestigious researchers claim that current analytical methods and tools for quantification of perfluoroalkyl substances are limited or only in their infancy, which restricts further research aimed at understanding their sources and environmental dynamics [36]. To contribute to improvement of this knowledge it is necessary to develop more reliable methods for extraction and determination of these substances in environment compartments.

The objective of our study was to develop a comprehensive and sensitive LC–ESI(–)–MS–MS analytical method for determination of a range of fluorinated alkyl substances in water samples–seven perfluoroalkylcarboxylates (C_6–C_{12}), two perfluoroalkylsulfonates (C_8, C_{10}), and two fluoroalkyl sulfonamides. These substances were chosen as being representative of several commercially, industrially, and domestically used fluorinated alkyl chemicals.

Most publications on PFAS have concentrated on two compounds, PFOS and PFOA, and one extraction technique, solid-phase extraction (SPE) with or without ion pairing or acidification.

To the best of our knowledge, reports of measurements of short and long-chain perfluorinated acids (between C_6 and C_{12}) and polyfluorinated sulfonamides in water are scarce. In one report on these compounds, including fluorotelomer alcohols and fluorotelomer acids [18], analysis involved solid-phase extraction with the recently commercialized weak anion-exchange (WAX) cartridges. Recoveries of long-chain perfluorinated carboxylates were between 50 and 90%; for fluoroalkyl sulfonamides they were between 45 and 65%. Milli-Q water (100 mL) was used throughout for determination of the recovery of the procedure and no recovery data from natural water samples was reported. Because of our earlier experiments [37] and the above mentioned results, one objective of our study was to improve extraction efficiencies, especially for the long-chain PFAS. It was also intended to extend these experiments to real water samples.

The methods developed have been fully validated and, finally, the LLE method was applied to effluents from five municipal wastewater treatment plants (WWTPs) in Austria.

Experimental

Chemicals and standards

All organic solvents used for sample preparation were residue-analysis grade or better. HPLC-grade solvents were used for work related to liquid chromatography.

Eleven perfluorinated compounds were examined in this study. Perfluorohexanoic acid (PFHxA, >97%), perfluoroheptanoic acid (PFHpA, >99%), perfluorooctanoic acid (PFOA, ammonium salt, >98%), perfluorononanoic acid (PFNA, 97%), perfluorodecanoic acid (PFDA, 98%), perfluoroundecanoic acid (PFUnA, 95%) and perfluorododecanoic acid (PFDoA, 95%), perfluorooctane sulfonate (PFOS, potassium salt, >98%) and perfluorodecane sulfonate (PFDS, ammonium salt, 25% (*w*/*w*) solution in water–2-butoxyethanol) were purchased from Sigma–Aldrich (Vienna, Austria). Perfluorooctane sulfonamide (PFOSA, 97%) and *N*-ethylperfluorooctane sulfonamide (*N*-EtPFOSA, 95%) were both supplied by ABCR (Karlsruhe, Germany).

The surrogate standards perfluoro-*N*-[1,2,3,4-$^{13}C_4$]octanoic acid ([$^{13}C_4$]-PFOA, >98%), perfluoro-*N*-[1,2-$^{13}C_2$] decanoic acid ([$^{13}C_2$]-PFDA, >98%), and *N*-ethyl-d5-perfluoro-1-octanesulfonamide (*N*-d5-EtPFOSA, >98%), were purchased from Wellington Laboratories (Ontario, Canada). The internal standard perfluoro-*N*-[1,2-$^{13}C_2$] octanoic acid ([$^{13}C_2$]-PFOA, 98.1%) was supplied by Perkin–Elmer Life and Analytical Sciences (Wellesley, MA, USA).

Sulfuric acid (95–97% for analysis), ammonia solution (25% for analysis), sodium chloride extra pure, sodium hydroxide (99% for analysis), and potassium hydroxide (85.0% for analysis) were supplied by Merck (Vienna, Austria).

A solution (13.13 mg mL^{-1}) in methanol (MeOH) of the ion pair reagent didecyldimethylammonium bromide (Sigma–Aldrich) was used for some experiments.

Stock solutions (10 mg mL^{-1}) of the analytes were prepared in MeOH, in polypropylene (PP) volumetric tubes, and then diluted with MeOH to prepare the standards needed. All stock solutions were stored at 4 °C. The long-term stability of stocks was monitored to guarantee the consistency of standards.

Cleaning and pretreatment of laboratory and sampling containers

To minimize possible contamination, samples were collected and stored in 1-L PP bottles, precleaned by thorough rinsing first with methyl *tert*-butyl ether (MTBE) and then with MeOH. Disposable PP and polyethylene (PE) laboratory-ware were used during sample preparation as a substitute of glassware, to prevent binding of the analytes to the glass surface [21, 36]. All this equipment was also precleaned in the same way.

Sample preparation

Solid phase extraction (SPE)

Before solid-phase extraction, NaCl was dissolved in 500 mL water samples to give a final concentration of 4 g L^{-1}. The samples were then acidified with sulfuric acid to pH 4. All SPE procedures were performed automatically by the AutoTrace Extraction WorkStation (Zymark, Caliper Life Sciences, Rüsselsheim, Germany), using Isolute 1 g C_{18} endcapped cartridges. The cartridges were conditioned with 2×1.5 mL MeOH–ethyl acetate, 1:1 (*v/v*), 2×1.5 mL MeOH, and 2×1.5 mL H_2O adjusted to pH 4 with H_2SO_4. The sample was loaded at a flow rate of 7 mL min^{-1} and the cartridges were rinsed with 1.5 mL H_2O–MeOH, 95:5 (*v/v*), and dried completely under a nitrogen stream for 1 h. The analytes were eluted with 5 mL MeOH–ethyl acetate, 1:1 (*v/v*). Finally, the solvents were reduced in volume by rotary vacuum concentration (75 mbar, 35 °C) and adjusted to an exact volume of 1 mL with MeOH.

Liquid–liquid extraction (LLE)

NaCl was added to water samples (400 and 900 mL), to give a final concentration of 50 g L^{-1}, and the pH was adjusted to 4 with sulfuric acid. After this step the target analytes were extracted once (10 min) with 60 mL and twice (2×10 min) with 30 mL MTBE by LLE (liquid–liquid extraction) directly in the sampling containers. The solvent was removed by rotary vacuum concentration (75 mbar, 35 °C) to a final volume of 1 mL, with previous exchange of the solvent for MeOH.

Liquid chromatography

Liquid chromatography was performed with an Agilent 1100 HPLC comprising a quaternary pump, auto sampler, degasser, and column department. Separations were performed with a 100 mm×2 mm (length×i.d.), 5-μm particle, Luna C_{18} analytical column from Phenomenex HPLC Service (Dr Wagner-Löffler, Breitenfurt, Austria) and a 4 mm×2 mm i.d., 5-μm particle, guard column containing the same packing material. The temperature of the HPLC column was kept constant at 25 °C.

The mobile phase was a gradient prepared from water containing 10 mmol L^{-1} ammonium acetate (component A) and MeOH (component B). The gradient was 10% B for 2 min, change to 98% B in 8 min, hold for 6.5 min, and, finally, change to 10% B in 0.5 min; the analysis time was 23 min. The injection volume was 10 μL and the LC flow rate was 0.25 mL min^{-1}.

Mass spectrometry

Mass spectrometry was performed with a Quattro Ultima triple-quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with a Z-spray electrospray interface operating in negative-ion mode. Argon (>99.999% purity grade, Messer, Gumpoldskirchen, Austria) was used as collision gas and nitrogen as drying and nebulizing gas (provided by means of a Whatman, Air Liquid, Austria Model 75-72 nitrogen generator).

The instrument was operated in multiple reaction-monitoring (MRM) mode for quantification of each compound. Analyte identification was verified by comparison of chromatographic retention time, by mass spectral daughter characterization, and by selecting additional ion pair transitions.

The capillary potential was 3.0 kV and the cone potential was between 10 and 60 V, depending on the compound of interest. The temperatures of the source block and desolvation capillary were 120 and 300 °C, respectively. The flow rates of the nebulizer and desolvation gases were approximately 90 and 500 L h^{-1}, respectively. The collision gas was set between 9 and 43 eV, depending on the analyzed compound.

Retention times, monitored transitions for quantification and confirmation of the investigated PFAS, and the nature of the daughter ions are listed in Table 1.

Sampling of wastewater

Five effluent spot-samples from municipal Austrian wastewater treatment plants (WWTPs) were collected in 1-L narrow-mouth PP bottles in November 2005. The samples were stored frozen (−20 °C) without any further treatment until analysis.

Results and discussion

Solid-phase extraction (SPE)

The technique most extensively used for extraction of PFAS from environmental water samples is, undoubtedly, solid-phase extraction (SPE). Bearing this in mind, this

Table 1 HPLC–tandem MS data and ions monitored for perfluorochemical analysis

Compound[a]	Molecular formula	t_R (min)	Parent ion (*m/z*)	Daughter ion (*m/z*)	Daughter ion	Collision energy (eV)	Cone potential (V)
PFHxA	$C_5F_{11}CO_2H$	12.6	313.20	269.30	$[M-COOH]^-$	9	20
PFHpA	$C_6F_{13}CO_2H$	13.6	363.20	319.30	$[M-COOH]^-$	10	25
PFOA	$C_7F_{15}CO_2H$	14.0	413.20	369.20	$[M-COOH]^-$	10	25
PFNA	$C_8F_{17}CO_2H$	14.3	463.20	419.20	$[M-COOH]^-$	9	25
PFDA	$C_9F_{19}CO_2H$	14.5	513.10	469.20	$[M-COOH]^-$	9	25
PFUnA	$C_{10}F_{21}CO_2H$	14.8	563.10	519.20	$[M-COOH]^-$	11	25
PFDoA	$C_{11}F_{23}CO_2H$	15.0	613.10	569.20	$[M-COOH]^-$	11	25
PFOS	$C_8F_{17}SO_3^-$	14.2	499.15	130.25[b]	$[CF_2SO_3]^-$	43	10
				230.25[c]	$[C_3F_6SO_3]^-$	37	10
PFDS	$C_{10}F_{21}SO_3^-$	14.6	599.15	230.20[b]	$[C_3F_6SO_3]^-$	40	10
				280.25[c]	$[C_4F_8SO_3]^-$		
				99.25[c]	$[FSO_3]^-$		
PFOSA	$C_8F_{17}SO_2NH_2$	14.8	498.20	78.30	$[SO_2N]^-$	30	60
N-EtPFOSA	$C_8F_{17}SO_2NHCH_2CH_3$	15.5	526.15	169.25[b]	$[C_3F_7]^-$	27	10
				219.25[c]	$[C_4F_7]^-$	24	10
[$^{13}C_2$]-PFOA	$^{13}C_2C_6HF_{15}O_2$	14.0	415.10	370.17	$[M-^{13}COOH]^-$	10	15
[$^{13}C_4$]-PFOA	$^{13}C_4C_4HF_{15}O_2$	14.0	417.07	372.22	$[M-^{13}COOH]^-$	10	15
[$^{13}C_2$]-PFDA	$^{13}C_2C_8HF_{19}O_2$	14.5	515.01	470.11	$[M-^{13}COOH]^-$	10	15
N-d5-EtPFOSA	$C_{10}D_5HF_{17}NO_2S$	15.5	531.02	169.14	$[C_3F_7]^-$	30	30

[a] Acronym
[b] Used for quantification
[c] Used for confirmation

study began with comprehensive optimization of the conditions that could affect the efficiency of the extraction.

First, several SPE adsorbents were tested—alkyl-bonded silica-based adsorbents C_{18} and CH, polystyrene–divinylbenzene–based adsorbents ENV+ and Isolute 101, and the copolymer poly(divinylbenzene-co-*N*-vinylpyrrolidone)-based adsorbent Oasis HLB. Because all the cartridges resulted in similar recoveries of all the compounds the C_{18} material was selected for the rest of the study, basically because of its low cost.

Subsequently, critical factors affecting the extraction process were studied and optimized step by step by use of 500-mL tap-water samples spiked with 500 ng of the target analytes. The conditions studied for sample pretreatment were sample pH (between 4 and 11), sample volume, and use of different additives, for example NaCl, dodecyldimethylammonium bromide (ion pair reagent), and MeOH. In the elution step, different volumes of MeOH and the mixtures MeOH–ethyl acetate, 1:1 (*v/v*), and MeOH–ammonia, 99:1 (*v/v*), were tested. The optimized conditions are reported in the section "Solid phase extraction (SPE)".

The results showed recoveries were exceptionally good (>89%) with low standard deviations (<6%) for perfluorinated carboxylates with a C-chain below 9 (Table 2, Recovery method A). It is clearly apparent, however, that recoveries deteriorated with longer carbon chains. Recovery was also low (approx. 50%) for PFOS.

For some of the analytes it was possible to enhance recovery by changing the pH of the sample to 11, without addition of salt, and by eluting with 5 mL MeOH (Table 2, Recovery method B). The increase was notable for PFOS (49 to 81%) and for PFNA and PFDA (70 to 96% and 34 to

Table 2 Recoveries obtained by use of two different SPE methods

Compound	Method A[a] recovery (%±SD%)	Method B[b] recovery (%±SD%)
PFHxA	96 (6)	8 (1)
PFHpA	98 (4)	50 (2)
PFOA	89 (6)	102 (0.4)
PFNA	70 (7)	96 (1)
PFDA	34 (7)	69 (10)
PFUnA	29 (5)	47 (9)
PFDoA	23 (3)	30 (6)
PFOS	49 (6)	81 (2)
PFDS	17 (2)	26 (2)
PFOSA	–[c]	96 (3)
N-EtPFOSA	11 (2)	57 (12)

[a] SPE conditions for Method A (n=8, on three non-consecutive days): sample volume 500 mL spiked with the target analytes at a concentration of 1 μg L^{-1}; NaCl concentration 4 g L^{-1}; pH 4; elution solvent 5 mL MeOH–ethyl acetate, 1:1 (*v/v*)
[b] SPE conditions for Method B (n=3): sample volume 500 mL spiked with the target analytes at a concentration of 1 μg L^{-1}; NaCl concentration 0 g L^{-1}; pH 11; elution solvent 5 mL MeOH
[c] PFOSA was not available when this study was performed

69%). It is likely that adsorption of the compounds by the PP containers is reduced under basic conditions. The extraction efficiency was, nevertheless, low for C_6 and C_7 perfluorinated carboxylates—because of their strongly acidic behavior, they are probably not retained by the C_{18} SPE material. Recoveries of analytes with longer carbon chains (>10) were enhanced, but were still unsatisfactory (<50%).

With regard to the other factors studied, extraction efficiencies were not altered significantly and PFAS with longer carbon chains could not be extracted quantitatively. As has been reported by Taniyasu et al. [18], irreversible adsorption of the analytes by the PP containers, and by the tubes of the SPE extraction system in our work, could be the reason for the low recoveries obtained. Although Taniyasu et al. [18] used only 100 mL samples, they recovered compounds between 45 and 90%. These results are similar to those obtained in this study with the difference of 500-mL samples (Table 2).

Liquid—liquid extraction (LLE)

The poor recoveries of PFAS with long carbon chains led to the use of a different method of extraction — liquid–liquid extraction (LLE). Factors that can affect extraction efficiency were again investigated. Extraction solvent and sample pH and the ionic strength, altered by addition of NaCl to the sample, were tested. The optimized conditions are reported in the section "Liquid–liquid extraction (LLE)".

In the first experiments 400 mL tap water, without pH adjustment, spiked with 500 ng of the target analytes, was used to test different extraction solvents. Figure 1 shows the results obtained from use of three organic solvents — *n*-hexane, MTBE, and trichloromethane (TCM). Although short-chain carboxylates could not be extracted, MTBE was the best extraction solvent.

In the next step 400-mL samples of tap water containing PFAS at two different concentrations (50 and 500 ng) were adjusted to pH 4 and NaCl was added to enhance the efficiency of extraction of short-chain carboxylates, because of their acidic behavior. Excellent recoveries (between 73 and 100%) were achieved except for PFHxA (64%) (Fig. 2).

It was, however, necessary to increase the amount of sample to approximately 1 L to achieve low detection limits. In this way, similar recoveries of the target compounds were obtained except for PFHxA and, partly, for PFHpA (Fig. 2). Excellent results are obtained by use of this method, especially for PFAS with long carbon chains and the perfluorinated sulfonamides.

The method described above was subsequently applied to five wastewater samples, effluents from municipal WWTPs in Austria. Samples (900 mL) were analyzed at least three times, both unmodified and spiked at two concentrations, 50 and 500 ng, to enable subsequent calculation of the efficiency of extraction of the target analytes. Recoveries obtained for tap water and wastewater samples are compared in Fig. 3. The results were very similar, showing the robustness of the extraction method.

It was possible to optimize a LLE method for quantitative extraction of perfluorinated acids with carbon chains longer than C_6 and the perfluorinated sulfonamides, in contrast with the SPE methods mainly described in the literature. Another advantage of LLE over SPE is that water samples with a high suspended matter content can easily be extracted without prior filtration and, therefore, the overall concentration of the selected PFAS in water (aqueous and particulate fraction) can be determined. If only the content of the aqueous phase is required, the samples can be

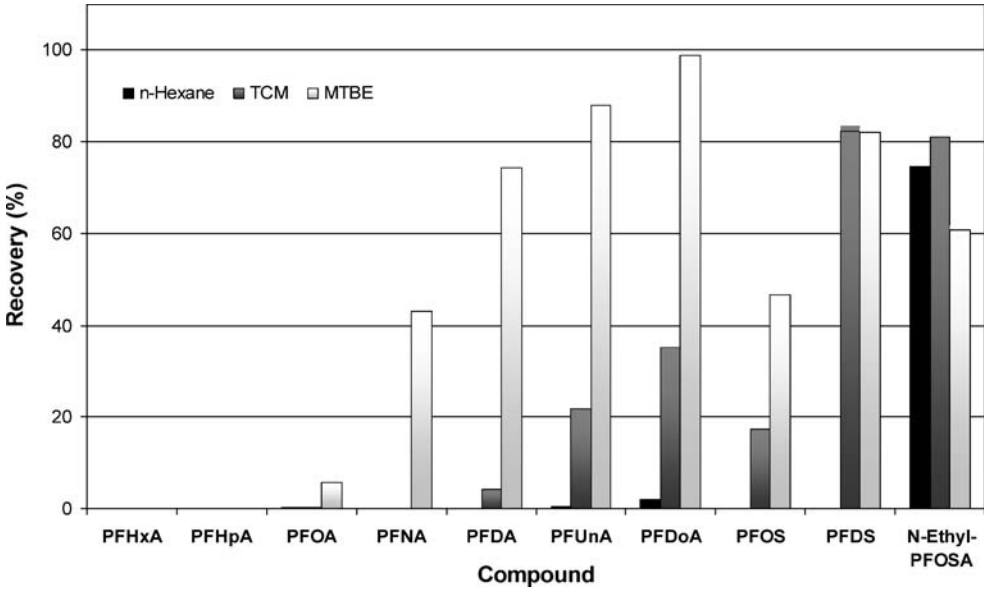


Fig. 1 LLE recoveries obtained with different solvents in analysis of 400-mL tap water samples spiked with 500 ng of the target compounds (PFOSA was not available when this study was performed)

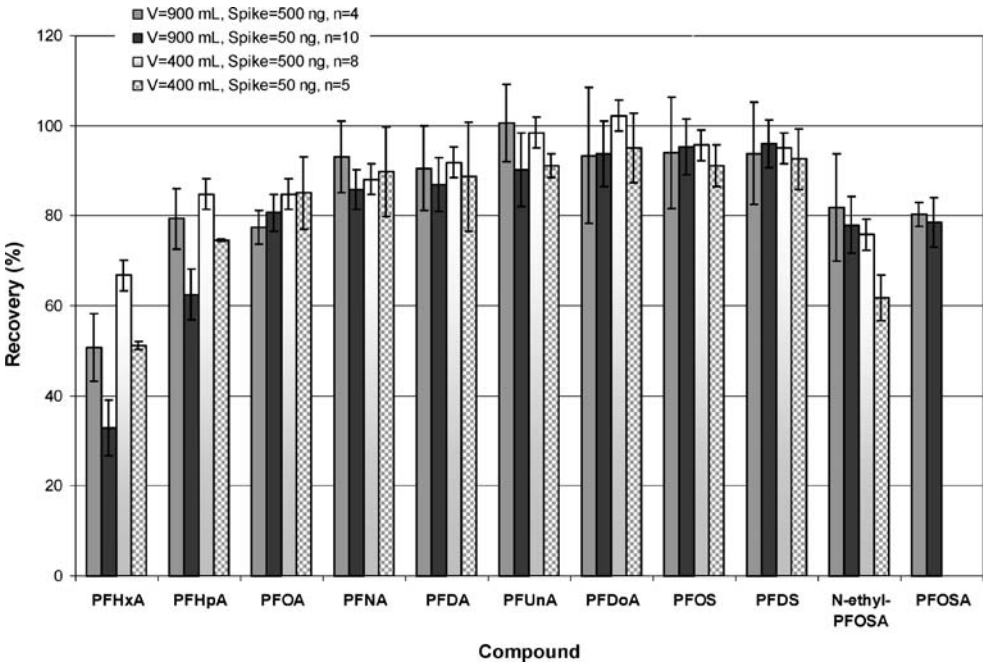


Fig. 2 LLE recoveries of perfluorinated alkylated compounds from spiked tap water samples under different conditions—two concentration levels (50 and 500 ng absolute) and two sample volumes (400 and 900 mL) (PFOSA was not available when this study was performed)

filtered, as described in several SPE applications [14, 17]. The only drawback of LLE was that recovery of PFHxA, especially, was low — approximately 40%.

Validation of the analytical method

The LLE method was validated for linearity, limits of detection and quantification, procedural blanks, surrogate recoveries, and matrix effects.

Complete regression data, including slope, intercept, and precision of the curves (RSD%), are listed in Table 3. To verify the linear range, a Mandel fitting test (P=99%) was also performed [38]. Linear calibration plots were obtained over a concentration range of two or three orders of magnitude, depending on the compound.

Basic validation for standard solutions was performed by use of the software package SQS 98 [39], in accordance with the German standard method DIN 32645 [40]. Instrumental detection and quantification limits (IDLs, IQLs) are summarized in Table 4. To calculate the method detection and quantification limits (MDLs, MQLs), the basic validation values were multiplied by the enrichment factors and by the recoveries of the analytes. Mean recoveries (n=44) for each analyte were evaluated by extraction of samples with and without matrix spiked at two different concentrations (55 and 555 ng L^{-1}).

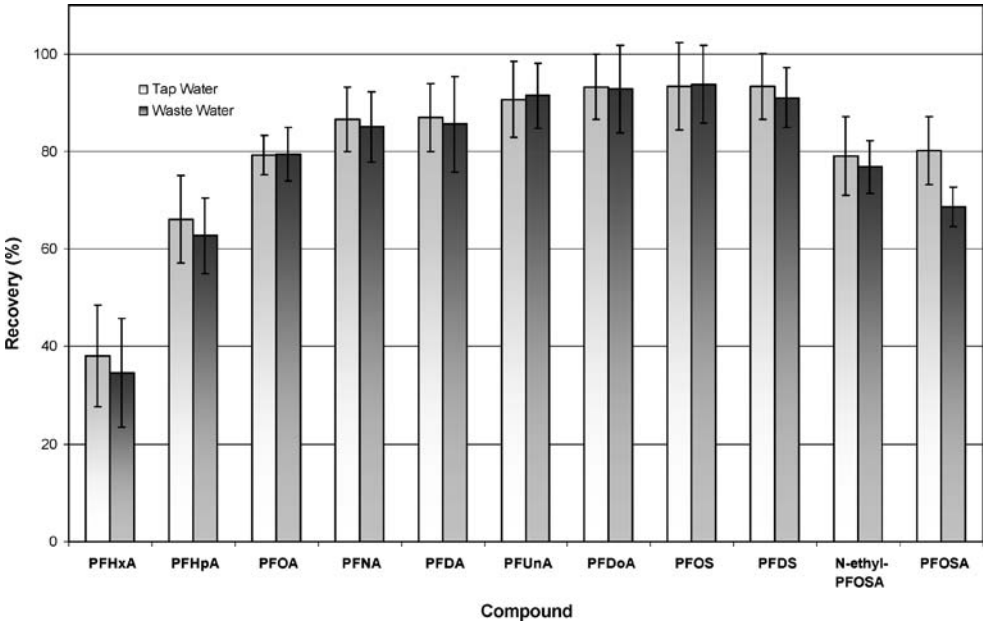


Fig. 3 LLE recoveries of perfluorinated alkylated compounds from tap water (n=14) and from effluent wastewater samples (n=10) spiked at two concentrations (55 and 555 ng L^{-1})

Table 3 Performance characteristics of the LC–ESI–MS–MS method

Compound	Linear range (ng L^{-1})	Correlation coefficient (r)	RSD (%)	$y = bx + a$			
				b	±	a	±
PFHxA	0–150	0.99968	4.8	0.00349	0.0000699	0.000482	0.00104
PFHpA	0–150	0.99949	6.1	0.00693	0.000176	0.000337	0.00157
PFOA	0–100	0.99991	2.6	0.0119	0.000134	0.00287	0.00134
PFNA	0–100	0.99951	6.0	0.0112	0.000296	0.00823	0.00578
PFDA	0–100	0.99971	4.6	0.0131	0.000264	0.00706	0.00533
PFUnA	0–20	0.99868	6.4	0.0134	0.000646	0.000560	0.00469
PFDoA	0–150	0.99985	3.3	0.0157	0.000213	0.000177	0.00350
PFOS	0–50	0.99927	5.4	0.000322	0.0000134	0.0000386	0.000233
PFDS	0–150	0.99904	7.4	0.000706	0.0000290	−0.000439	0.00147
PFOSA	0–150	0.99962	4.9	0.00198	0.0000483	0.000668	0.00103
N-EtPFOSA	0–50	0.99963	4.1	0.00186	0.0000509	−0.000396	0.000851

To verify the limits for real samples (e.g. effects of ion suppression), signal-to-noise ratios for the analytes in extracts of wastewater effluents in which concentrations were close to the calculated MQLs were determined. They were always ten or more. The high sensitivity of the method for water samples is illustrated by the low MDLs and MQLs (Table 4). MDL values ranged from 0.26 to 0.62 ng L^{-1} and MQL values from 0.94 to 2.3 ng L^{-1}, except for PFHxA (4.4 and 16 ng L^{-1}, respectively). Values for PFHxA were enhanced substantially, to 0.64 and 2.3 ng L^{-1}, when SPE was used.

A major problem associated with trace-level analysis of PFAS is background contamination of the analytical blanks [18, 36]. After several improvements of the analytical and instrumental procedures, for example thorough rinsing of the material and avoidance of the use of fluoropolymers in laboratory instrumentation, no background and blank levels of perfluoroalkylsulfonates and perfluoroctanesulfonamides were detected. For perfluoroalkylcarboxylates the signal to noise background was below 3 for all instrumental and procedural blanks.

Table 4 Limits of detection and quantification

Compound	LC–MS–MS		Water samples	
	Instrumental detection limit (IDL) (ng mL^{-1})[a]	Instrumental quantification limit (IQL) (ng mL^{-1})[a]	Method detection limit (MDL) (ng L^{-1})	Method quantification limit (MQL) (ng L^{-1})
PFHxA	0.49	1.7	4.4[b]	16[c]
PFHpA	0.21	0.76	0.48	1.7
PFOA	0.20	0.71	0.33	1.2
PFNA	0.17	0.63	0.26	0.95
PFDA	0.21	0.75	0.30	1.1
PFUnA	0.19	0.70	0.27	1.0
PFDoA	0.19	0.69	0.26	0.95
PFOS	0.19	0.69	0.26	0.94
PFDS	0.28	1.1	0.39	1.5
PFOSA	0.21	0.77	0.35	1.3
N-EtPFOSA	0.33	1.2	0.62	2.3

[a] Calculated by use of the software package SQS 98, based on DIN Standard 32 645
[b] MDL=0.64 ng L^{-1} when SPE is used
[c] MQL=2.3 ng L^{-1} when SPE is used

Application to environmental water samples

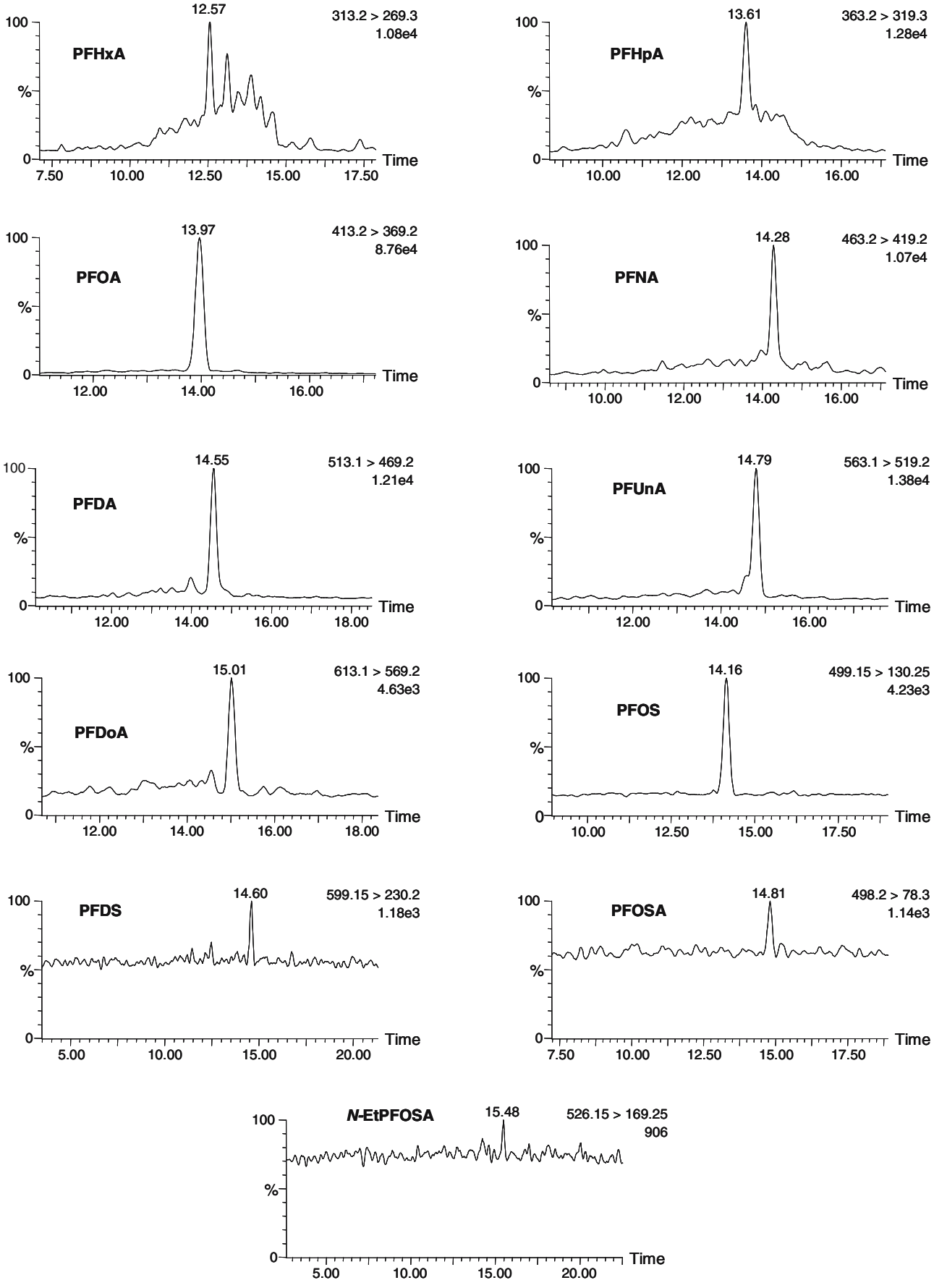
Laboratory reagent blanks (extracted and prepared as for samples) and instrumental blanks were analyzed with each batch of samples to check and correct for possible contamination and interferences.

Three labeled compounds, [$^{13}C_4$]-PFOA, [$^{13}C_2$]-PFDA, and *N*-d5-EtPFOSA, were chosen as surrogates (compounds that would behave similarly to PFAS in the analytical method). Recoveries were tested with the target analytes to evaluate their suitability (Table 5). [$^{13}C_4$]-PFOA was selected for PFOA; [$^{13}C_2$]-PFDA for PFNA, PFDA, PFUnA, PFDoA, PFOS and PFDS; and *N*-ethyl-d5-PFOSA for *N*-ethyl- PFOSA and PFOSA. PFHxA and PFHpA were not surrogate-corrected because recovery of [$^{13}C_4$]-PFOA is not related to recoveries of these compounds. PFHxA and PFHpA were, however, corrected by measurement of recoveries for a spiked sample analyzed within each sample set.

Before LC–MS–MS analysis an internal standard ([$^{13}C_2$]-PFOA) was added to the extracts to compensate

Table 5 Recoveries of the surrogates from tap water (n=14) and wastewater (n=5)

Compound	Tap water		Effluent wastewater	
	Recovery (%)	SD (%)	Recovery (%)	SD (%)
[$^{13}C_4$]-PFOA	78	4	83	0.8
[$^{13}C_2$]-PFDA	85	8	86	8
N-d5-EtPFO SA	77	7	77	9


PFHxA
12.57
313.2 > 269.3
1.08e4
PFHpA
13.61
363.2 > 319.3
1.28e4
PFOA
13.97
413.2 > 369.2
8.76e4
PFNA
14.28
463.2 > 419.2
1.07e4
PFDA
14.55
513.1 > 469.2
1.21e4
PFUnA
14.79
563.1 > 519.2
1.38e4
PFDoA
15.01
613.1 > 569.2
4.63e3
PFOS
14.16
499.15 > 130.25
4.23e3
PFDS
14.60
599.15 > 230.2
1.18e3
PFOSA
14.81
498.2 > 78.3
1.14e3
N-EtPFOSA
15.48
526.15 > 169.25
906
100
%
0
Time

◀ **Fig. 4** LC–MS–MS chromatograms obtained from perfluorinated alkyl compounds extracted from effluent collected from WWTP 2

for possible matrix effects on the extent of ionization. To assess the suitability of this compound as internal standard, comprehensive evaluation of matrix effects, by the approach of Weiss et al. [41], was performed by means of a series of standard addition experiments (known amounts of each target analyte and the ISTD were added to the final effluent extracts). The study showed that suppression was, at worst, not more than 20% for some compounds. The matrix effects were well compensated by the internal standard response because all compounds are eluted between 12.6 and 15.5 min, with no variation of the composition of the mobile phase at this stage (98% MeOH).

The method described above was applied to several wastewater effluents collected from different Austrian locations.

Before extraction, and without previous filtration, all samples were spiked with 50 μL of a 1 μg mL^{-1} mixed solution of the surrogates [$^{13}C_4$]-PFOC, [$^{13}C_2$]-PFDC, and *N*-d5-EtPFOSA in MeOH. Before injection, 10 μL of a 10 μg mL^{-1} solution in MeOH of the internal standard [$^{13}C_2$]-PFOC was added to 1 mL sample extracts and standards. The target analytes were quantified by internal standard calibration.

Figure 4 shows the chromatogram obtained from an extract from a treated effluent in which all the analytes were detected (WWTP 2).

Five perfluoroalkylcarboxylates (PFHxA, PFHpA, PFOA, PFNA, PFDA) and one perfluoroalkylsulfonate (PFOS) were detected in all wastewater samples at concentrations ranging from <0.95 ng L^{-1} (PFNA) to 21 ng L^{-1} (PFOA) (Table 6). This agrees with previous investigations of fluorinated alkyl substances in wastewater treatment [30, 31]. Boulanger et al. [30] analyzed one effluent from a WWTP and detected 26 ng L^{-1} PFOS and 22 ng L^{-1} PFOA, the PFAS present at the highest concentrations. Schultz et al. [31] investigated ten WWTPs and measured effluent concentrations up to 130 ng L^{-1} for PFOS. (PFHxA, PFHpA, PFOA, and PFOS were the substances found most frequently.)

The relative high concentrations of short-chain compounds (C_6–C_8) in treated wastewaters are possibly because of biodegradation of potential precursors entering WWTPs [31]. It is, for example, known that fluorotelomer alcohols can be degraded to PFOA and other perfluorocarboxylates. PFOS can also be formed during biodegradation of sulfonamidoacetate compounds or sulfonamido alcohols such as perfluorooctanesulfonamidoacetate (PFOS-AA), its *N*-ethyl and *N*-methyl derivatives, and *N*-methyl and *N*-ethyl perfluorooctane sulfonamidoethanol (*N*-MePFOSE, *N*-EtPFOSE).

Table 6 Concentration of PFAS in effluent wastewater samples

Compound	Concentration (ng L^{-1})[a]				
	WWTP 1	WWTP 2	WWTP 3	WWTP 4	WWTP 5
PFHxA	17	16	8.1	16	6.0
PFHpA	4.1	4.0	3.8	4.6	2.5
PFOA	14	16	11	21	10
PFNA	1.6	1.6	1.3	1.9	<0.95[b]
PFDA	1.2	<1.1	<1.1	1.8	<1.1
PFUnA	n.d.[c]	1.2	<0.98	n.d.	n.d.
PFDoA	n.d.	<0.95	<0.95	n.d.	n.d.
PFOS	9.3	20	6.4	7.7	4.5
PFDS	1.8	1.7	<1.5	n.d.	n.d.
PFOSA	<1.3	<1.3	1.4	<1.3	n.d.
N-EtPFOSA	n.d.	<2.3	<2.3	n.d.	n.d.

[a] Concentrations are all corrected for recovery using the individual sample recoveries of the surrogates
[b] Detected but not above the MQL
[c] Not detected

Conclusions

Two methods of extraction have been optimized for analysis of eleven perfluorinated alkylated substances (PFAS) by LC–ESI–MS–MS.

Two SPE methods were used. Under acidic conditions (pH 4) perfluoroalkylcarboxylates with carbon chains <10 were extracted quantitatively, resulting in MDLs between 0.25 and 0.64 ng L^{-1}. Under basic conditions (pH 11) PFOA, PFNA, PFDA, PFOS, and PFOSA could be extracted and low detection limits could be achieved (between 0.20 and 0.47 ng L^{-1}). SPE could not, however, be used to extract PFAS with longer carbon chains.

Recoveries of the analytes by LLE were reasonable, particularly for perfluoroalkylcarboxylates with carbon chains ≥8, perfluoroalkylsulfonates, and fluoroalkyl sulfonamides (80–93%). Detection limits for sample volumes of 900 mL were quite low (0.26–0.62 ng L^{-1}) except for PFHxA (4.4 ng L^{-1}). Application of this technique to wastewater samples proved its robustness (analysis of neutral and acidic compounds was possible) and enabled study of the occurrence and fate of these compounds in the treatment processes used in WWTPs.

LLE has two important advantages over the SPE technique commonly used for environmental water samples:

1. Because of the surface-active nature of PFAS they tend to be adsorbed by small particles and will be partly found in the suspended matter of water samples (especially waste water with a high particulate matter content). By using LLE it is possible to extract samples with or without prior filtration and hence it is possible

to determine concentrations in the aqueous fraction or the whole sample.
2. Probable losses because of adsorption on the sample vessels are minimized, because extraction is performed directly in the sampling containers.

These investigations have shown that this LLE method coupled with liquid chromatography–tandem mass spectrometry is suitable for sensitive analysis of many PFAS, particularly those rarely studied in abiota samples.

Acknowledgements This research was performed in the laboratories of the Umweltbundesamt-Austria and was based in the project "Substance Survey". C. González-Barreiro acknowledges the Spanish Ministerio de Educación y Ciencia for her Postdoctoral grant (supported by the Secretaría de Estado de Educación y Universidades and Fondo Social Europeo).

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