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Analysis of perfluorinated phosponic acids and perfluorooctane sulfonic acid in water, sludge and sediment by LC–MS/MS

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ABSTRACT

Residues of perfluorinated phosphonic acids (PFPAs) and perfluorooctane sulfonic acid (PFOS) were investigated in various Dutch surface waters, sludge and sediments. For this purpose, a liquid chromatographic (LC) method was optimized by testing several columns with different mobile phases. Atmospheric pressure chemical ionization (APCI) was chosen for the LC tandem mass spectrometry (MS/MS) analysis. An ion-pair reagent was added to the injection solvent to improve peak shape. Different solvents were studied for the extraction from solid samples. For clean-up and pre-concentration, weak anion-exchange solid-phase extraction cartridges were used. Water samples were extracted using the same cartridges. The method was used for screening PFPAs in the Dutch aquatic environment. PFPAs were not observed in sediment or sludge samples. PFOPA was found at 1 ng L^{-1} in one surface water sample. PFOS was found at levels between 0.07 ng g^{-1} and 48 ng g^{-1} (dry weight) in sediments and sewage sludge samples. PFOS concentrations in surface water ranged from 3.3 ng L^{-1} to 25.4 ng L^{-1}.

1. Introduction

Since the 1950s fluorochemicals have been produced and, due to their favourable surface tension lowering properties, since the early 1980s their use has increased in a variety of industrial and commercial applications [1,2]. Perfluorinated alkylated substances (PFASs) were used as polymerization aid for the production of fluorinated polymers, for metal plating, in the photographic, semi-conductor and aviation industry (hydraulic fluids), in fire fighting foams and as fat and water repellents for textiles, paper and leather [3]. PFASs consist of a hydrophobic alkyl chain of varying length (typically C4 to C16) and a hydrophilic end group. The hydrophilic part defines the type of PFASs. The carboxylic acids and the sulfonates, especially perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), are the main ones studied. Other functional groups, such as alcohol, amide or N-substituted sulfonamides, have received less attention. Due to their persistence and other properties the main manufacturing companies have decided to reduce their emission or phase them out [4,5].

Recently, a new class of PFASs has emerged with a phosphonic acid as hydrophilic group; the perfluorinated phosphonic acids (PFPAs). PFPAs are used as anti-foaming agents in the textile industry, in pesticides and lubricants (registered use in Sweden) [6]. The structures and some properties of the PFPAs are shown in Table 1, together with linear PFOS. Their similarity with perfluoroalkylsulfonic acids (PFSA) suggests that their behavior, in terms of degradation and migration may be similar. The strength of the fluorine-carbon bond (approx. 466 kJ mol^{-1}) and the high electronegativity of fluorine atoms [7] make most PFAS persistent in the environment [8] and resistant to hydrolysis, photolysis, microbial degradation, metabolism by vertebrates, relatively high temperatures and X-ray and nuclear radiation [3]. PFPAs have no known precursor compounds. Physicochemical properties have been estimated and are shown in Table 1, pKa_1 values vary from 2.1 to 3.4 and pKa_2 values from 4.4 to 5.6. This means that at environmentally relevant pH values in surface water (pH = 6–8), PFPAs occur to a large degree in their dianionic state. Due to their high log *P* values calculated by sparc on-line calculator software [9] PFPAs are expected to accumulate in soils and sediments. It should be noted that this holds for the neutral, fully protonated species (i.e. at very acidic conditions).

PFPAs have chain lengths of 6–10 carbon atoms, which are completely fluorinated. Perfluorooctyl phosphonic acid (PFOPA, 8 carbon atoms) was listed by the US-EPA as a high production volume chemical in 2007 with an annual production between 4500 and 230 000 kg [10]. The information on production, import and application of PFPAs in Europe is scarce. In Denmark between 1 and 5 tones of PFPAs were registered in the Danish Product Register

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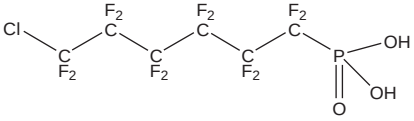
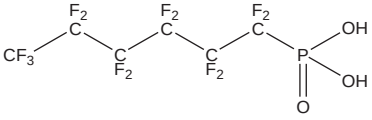
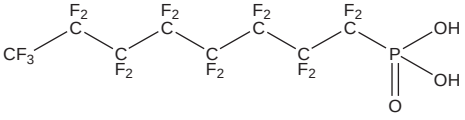
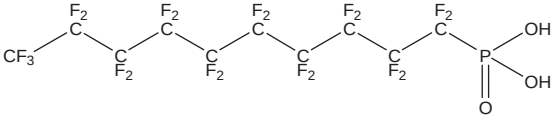
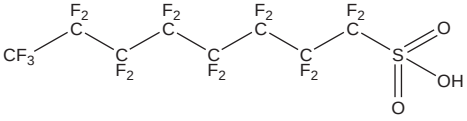
Table 1
Structures and estimated properties of perfluoroalkylphosphonic acids (PFPAs) and perfluorooctanesulfonic acid (PFOS).

Name	Acronym	Structure	Log *P*	pKa_1	pKa_2
1-Chloro-perfluorohexyl phosphonic acid	Cl-PFHxPA[a]		4.01[b]	2.1[c]	4.5[c]
Perfluorohexyl phosphonic acid	PFHxPA		3.55[b]	2.1[c]	4.4[c]
Perfluorooctyl phosphonic acid	PFOPA		5.85[b]	2.4[c]	4.5[c]
Perfluorodecyl phosphonic acid	PFDPA		8.27[b]	3.4[c]	5.6[c]
Perfluorooctyl sulfonic acid	PFOS		5.5[b]	0.1[b]	n.a.

[a] Internal standard used for chemical analysis.
[b] http://sparc.chem.uga.edu/sparc/ September 2009 release w4.5.1529-s4.5.1529.
[c] Reference and handling guide: perfluoroalkyl compounds. www.well-labs.com.

in 2006 [11]. D'Eon et al. have been the first in demonstrating the presence of PFPAs in surface and waste water treatment plant effluent in Canada [12]. They found levels in waste water ranged from 330 to 6500 pg L^{-1} and from 26 to 3400 pg L^{-1} in surface water with PFOPA as the predominant PFPA. Howard and Muir listed PFOPA as a priority substance because of its high predicted bioconcentration factor of 19 510 for the mono-substituted compound along with a long atmospheric oxidation half life [13]. There are no reports known on the presence of PFPAs in the Dutch environment. In this work we aim to (i) develop an extraction and clean-up technique for sediment, sludge and surface water, (ii) to develop liquid chromatography (LC)–tandem mass spectrometry (MS/MS) method for PFPAs and (iii) to carry out a survey on the presence of PFPAs in sludge, sediments and surface water from the Netherlands. PFOS was also analyzed as a PFAS reference compound.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals were analytical reagent-grade. Perfluorohexyl phosphonic acid (PFHxPA), PFOPA, perfluorodecyl phosphonic acid (PFDPA) and PFOS were purchased from Wellington Laboratories (Guelph, ON, Canada). The internal standards, 1-chloro-perfluorohexyl phosphonic acid, $^{13}C_8$-perfluorooctyl sulfonate ($^{13}C_8$-PFOS), $^{13}C_4$-perfluorooctyl sulfonate ($^{13}C_4$-PFOS) and ^{18}O-perfluorohexyl sulfonate (^{18}O-PFHxS), were also purchased from Wellington Laboratories. LC/MS grade methanol, acetonitrile and water were obtained from Merck (Darmstadt, Germany). Ammonium acetate (>98%), and ammonium hydroxide (25%) were provided by Fluka (Steinheim, Germany) and acetic acid (98%) was obtained from Sigma–Aldrich (Steinheim, Germany) as well as dichloromethane (PESTANAL®, >99.8), ethyl acetate (PESTANAL®), tetrabutylammonium hydrogensulfate (TBA) (97%), methyl *tert*-butyl ether (99.8%), potassium carbonate (99%) and tetrahydrofuran (THF). Stock standard solutions (1 μg mL^{-1}) were individually prepared by weight in methanol and stored in a polypropylene (PP) vial. Intermediate solutions were prepared weekly from the stock standard solution by appropriate dilution in methanol:water (1:1, v/v). Calibration standard solutions ranging from 0.1 to 100 ng mL^{-1} were prepared. Mobile phases were filtered through 0.22 μm nylon filters obtained from Varian (Palo Alto, CA, USA) and samples were filtered using 0.2 μm hydrophilic polypropylene membrane GHP syringe filters obtained from Waters (Mildford, MA, USA).

2.2. Sample treatment

Sediment, sludge and surface water samples were collected from The Netherlands. In order to estimate extraction efficiencies of different solvents and methods for the solid samples, a sediment sample from the river Western Scheldt was spiked with PFPAs at approx. 40 ng g^{-1} dry weight (dw). Water samples were passed through an SPE cartridge as explained below.

The sediments and sludge samples were freeze-dried with a Lyph.lock 1L (Labconco, Kansas city, MO, USA). It took approx. 24 h for sediments and approx. a week for sludge samples. One gram of freeze-dried sediment sample was weighed into a 15 mL PP tube and 50 μL of 100 ng mL^{-1} of Cl-PFHxPA and $^{13}C_4$-PFOS were added. Finally, 10 mL extraction solvent was added (THF:water, ethyl acetate, methanol, methanol:water at pH 9, or tetrabutylammonium, see Table 4). The sample was shaken for 1 h in an SM-30 shaker (Edmund Bühler GmbH, Hechingen, Germany) and then centrifuged during 15 min at 4500 rpm in an SW9 centrifuge (Firlabo, Meyzieu, France). The supernatant was evaporated under a stream of nitrogen (5.0 grade, purity >99.99%) until the water layer was left over. Extraction efficiency was also tested with pressurized liquid extraction (PLE, ASE 350, Dionex). Two and a half gram of freeze-dried sample were weighed in a 22 mL cell and the extraction was performed with 3 cycles at 100 °C. The solvents used for extraction by PLE were methanol, ethyl acetate, and dichloromethane (Table 4). The solvent was evaporated in the same way as explained

Table 2
Conditions and transitions used in mass spectrometry experiments for PFPAs and PFOS.

	Transitions	Fragmentor		Collision energy (eV)
		ESI	APCI	
ClPFHxPA	m/z 415 → 79	175	150	45
PFHxPA	m/z 399 → 79	175	150	45
PFOPA	m/z 499 → 79	200	175	45
PFDPA	m/z 599 → 79	225	175	50
PFOS	m/z 499 → 80	200	200	45
	m/z 499 → 99			45
$^{13}C_8$-PFOS	m/z 507 → 80	200	200	45
	m/z 507 → 99			45
$^{13}C_4$-PFOS	m/z 503 → 79	200	200	45
	m/z 503 → 79			45
^{18}O-PFHxS	m/z 401 → 82	175	175	45
	m/z 401 → 81			45

before, and reconstituted with 10 mL water. After evaporation, the entire aqueous phase was loaded on an SPE cartridge.

A mixed mode of weak anion exchange (WAX) Oasis cartridges from Waters (6 mL, 150 mg, Mildford, MA, USA) were used according to D'eon et al. [12] (for PFPAs) and Ballesteros-Gomez et al. [14] (for PFSAs and perfluorinated carboxylic acids (PFCAs)). The cartridges were first conditioned with 4 mL of methanol with 0.1% ammonia, 4 mL of pure methanol and 4 mL of water. Subsequently, the sample was loaded and the cartridge was washed with 4 mL 25 mM ammonium acetate at pH 4 and 8 mL of THF:methanol (75:25, v/v). Finally, the compounds were eluted with 10 mL methanol with 0.1% (v/v) ammonia which again were evaporated to dryness under pure nitrogen and then reconstituted in 0.25 mL of 25 mM TBA (methanol:water 1:1, v/v). The extract was filtered through a 0.22 μm GHP filter and 5 μL was injected in the LC–MS/MS.

The water samples were homogenized with a stir bar and after adding the internal standards and conditioning the cartridges, 500 mL was passed through the SPE cartridge (Oasis WAX) and treated as mentioned above.

2.3. LC–MS/MS

Chromatographic separation was performed on a 1200SL LC system (Agilent, Santa Clara, CA, USA) equipped with a binary pump, an autosampler and a column oven. Four analytical columns were tested, a Symmetry C18 (50 × 2.1 mm, 5 μm; Waters, Mildford, MA, USA), a ZORBAX Extended C18 (50 × 2.1 mm, 5 μm), a Fluorosep-RP Octyl (150 × 2.1 mm, 5 μm; ES-Industries, West Berlin, NJ, USA) and a ZORBAX Rapid Resolution High Throughput (30 × 2.1 mm, 1.8 μm) from Agilent. Gradient elution was performed to separate the PFPAs and PFOS. Solvent A was acetonitrile and solvent B was 2 mM ammonium acetate. The gradient elution started with a 0.5 min isocratic step at 10% of solvent A, followed by a linear gradient of solvent A up to 100% in 19.5 min, followed by an isocratic step of 2 min. The mobile phase flow rate was 300 μL min^{-1}, the column temperature was maintained at 20 °C and the injection volume was 5 μL.

Mass spectrometry was performed using a 6410 triple quadrupole (Agilent) equipped with an electrospray (ESI) and an atmospheric pressure chemical ionization (APCI) as ionization sources. Working conditions for ESI were as follows: the gas flow was 6 a.u. (arbitrary units) with a temperature of 325 °C, and a nebulizer of 25 psi. The capillary voltage was 2500 V. The scan time was fixed to 200 ms for all the analytes and 20 ms for the IS. For the APCI source, the gas flow was 8 a.u., at the same temperature and capillary voltage. The nebulizer pressure was 50 psi and the vaporizer temperature was set at 250 °C. The corona was set to 1 μA. Table 2 shows the fragmentor values and the collision energies for each transition for the two sources. PFOS was monitored as a reference due to the similarities in fragmentation, where only the hydrophilic fragment can be obtained at high collision energies. It was also analyzed because it is one of the most abundant PFASs in the environment [15–18]. High purity nitrogen (>98%) supplied by a nitrogen generator NitroflowLab provided by Parker (Cleveland, OH, USA) was used for the ESI and APCI sources. Data acquisition was performed in MS/MS using the only transition observed for the PFPAs that yields to the product ion m/z 79 corresponding to the fragment PO_3^- and the product ions m/z 80 and m/z 99 for PFOS.

3. Results and discussion

3.1. Liquid chromatography–tandem mass spectrometry

Initially, the Symmetry C18 column with a 10–100% gradient of methanol using 2 mM ammonium acetate in the aqueous phase was used according to Ballesteros-Gomez et al. [14]. PFOS was used as a retention time reference. Wide peaks and tailing were observed for the PFPAs, whereas PFOS and isomers showed narrow Gaussian peaks (data not shown). Some authors like Holm et al. [19] and Ahrens et al. [20] used an ammonium acetate buffer in both aqueous and organic phases for the analysis of PFCAs and PFSAs and D'eon et al. for the analysis of PFPAs [12]. Therefore, 2 mM and 10 mM ammonium acetate buffers were tested in the aqueous phase only, and in both phases. When working with the 2 mM buffer concentration in both phases, more retention for PFPAs and PFOS was obtained than with buffer in the aqueous phase only. The same effect was observed when increasing the buffer concentration. The peak shape was not influenced by the salt amount in the mobile phase but there was a loss of sensitivity due to broader peaks.

The pH of the mobile phase can play an important role for these compounds due to the possible formation of double charged ions. As shown in Table 1, the pKa_2 values are estimated at 4.5–5 (www.well-labs.com) and the supplier recommends elevating the pH of the eluent to 9 for optimal chromatography [21]. By increasing the pH, it is possible to obtain the dianionic form of PFPAs, which will influence the retention behavior. The pH of the aqueous phase was studied between 6 and 10.5 using 2 mM ammonium acetate and adjusting the pH with ammonia. For this purpose a C18-extended column was used, which allows working at pHs above 9. When increasing the pH, the PFPAs are less retained but the peak width of e.g. PFOPA increased (see Fig. 1). Therefore, 2 mM ammonium acetate, which provides a pH of 6.2, was used as optimal for further determinations.

The injection solvent was another important parameter to study. The supplier recommends the use of methanol:water (75:25, v/v) at pH 9 to obtain a good peak shape and to avoid adherence

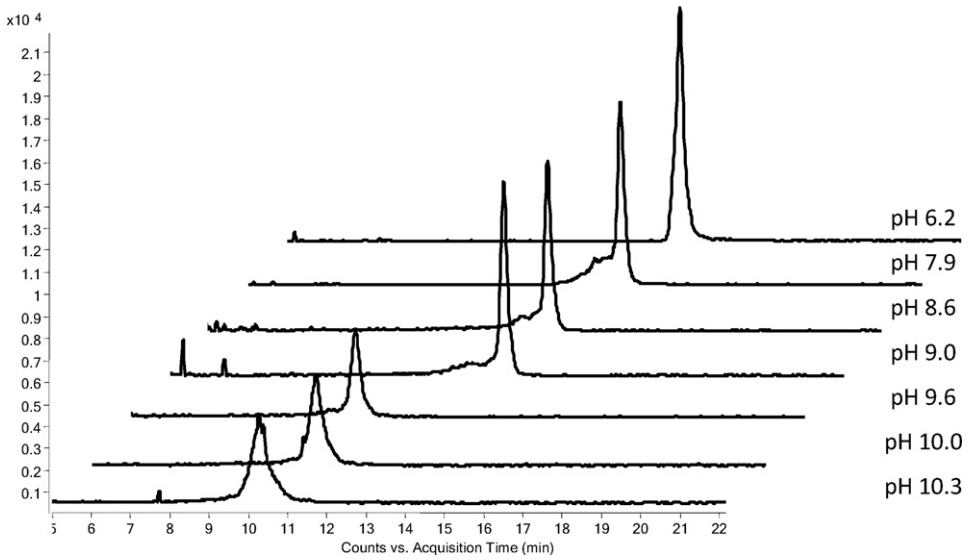


Fig. 1. LC–MS/MS chromatograms of a 100 μg mL^{-1} PFOPA (*m/z* 499 → 79) standard in MeOH:water at pH 9 (75:25) at several pHs.

to the LC–MS system [21]. Initially, methanol:water (1:1, v/v) was compared with the mixture proposed by the supplier. No difference was observed in sensitivity or peak shape for PFHxPA when using pH 9, whereas peak shape was clearly better for PFOPA and PFDPA obtaining narrower peaks (Fig. 2B). Some authors have used the ion-air reagent directly in the injection solvent [22]. The use of TBA as an ion-pair reagent at a concentration of 25 mM was tested because Marín et al. [22] used this for the determination of ethephon, of which the polar group is also a phosphonate. TBA in the injection solvent provided a better signal for the PFPAs, especially for the PFHxPA, of which the signal was 8-fold higher than without TBA (Fig. 2C). Furthermore, PFHxPA eluted several minutes later, meaning that addition of TBA decreases the polarity of the analyte. The addition of TBA in the injection solvent improved the sensitivity of PFOPA ca. 4-fold, while for PFDPA a 2-fold loss in sensitivity was observed, due to ion suppression caused by the ion-pair reagent. Furthermore, as PFPAs stick to metal [21], all connections were changed for PEEK tubing. The unique metal part which could not be changed was the injection needle. To prevent cross contamination, washes with methanol:water (75:25, v/v) at pH 9 were performed after every injection to avoid background contamination.

Once the mobile phase and the injection solvent were optimized, different columns were tested. The Symmetry C18 column provides wider peaks than the extended C18 column although they

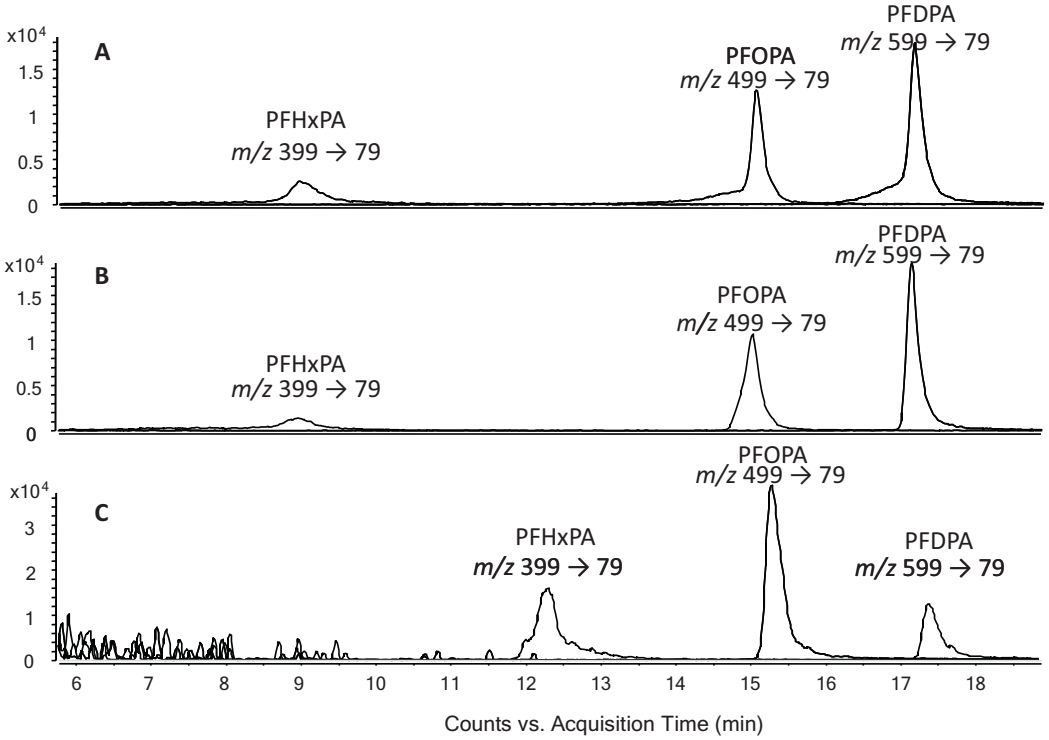


Fig. 2. LC–MS/MS chromatograms of a 100 μg mL^{-1} PFPAs standards with different injection solvents: (A) MeOH:water (1:1); (B) MeOH:water at pH 9 (75:25); (C) MeOH:water (1:1) with TBA 25 mM.

Table 3
Instrumental and method quality parameters for PFPAs and PFOS.

Parameter	PFHxPA	PFOPA	PFDPA	PFOS
Instrumental LOD (ng mL^{-1})	0.45	0.5	1.5	0.05
Instrumental LOQ (ng mL^{-1})	1.5	1.5	5	0.15
Run-to-run (% RSD $n=6$)				
Low level[a]	4.2%	4.6%	3.2%	3.5%
High level[b]	3.4%	2.3%	2.2%	2.4%
Method LOD/ng L^{-1} (water samples)	1	1	2	0.05
Method LOD/ng g^{-1} (sediment samples)	0.25	0.25	0.5	0.01
Run-to-run variation (% RSD $n=6$, level 25 ng g^{-1})	2.6%	4.5%	8.9%	3.9%

[a] Low level: 10 ng mL^{-1}.
[b] High level: 50 ng mL^{-1}.

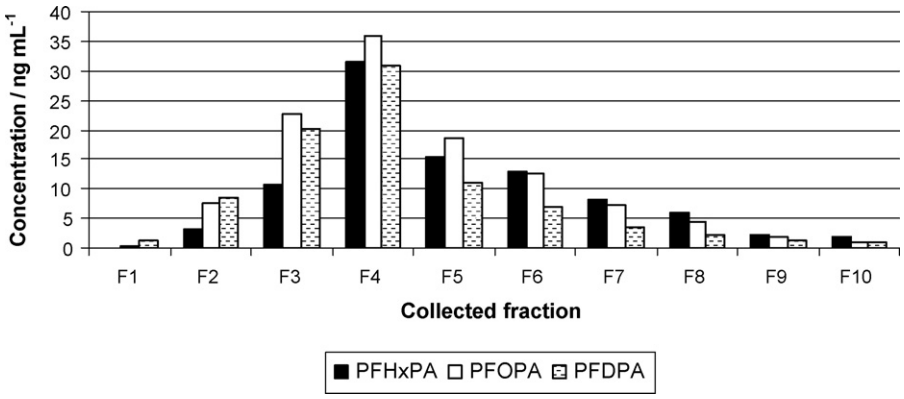


Fig. 3. Oasis WAX SPE recoveries of PFPAs from a standard of 100 μg mL^{-1} in terms of concentration for every fraction of 1 mL.

have the same length and particle size (5 cm, 5 μm), probably due to a different end capping of the columns. No results were obtained for the Fluorosep-RP Octyl column, because the baseline for the PFOPA transition (m/z 499 → 79) was too high and raised with the progressing gradient (for unknown reasons). Finally, a gradient was performed with a rapid resolution high throughput column (Zorbax, 30 × 2.1 mm) providing narrower peaks because of the smaller particle size (1.8 μm). Methanol was replaced by acetonitrile to decrease the backpressure of the system. With acetonitrile narrower peaks and less tailing PFPA peaks were observed than with methanol, whereas no difference in peak shape was observed for PFOS. The rapid resolution column with acetonitrile as modifier was selected as the optimum column.

Initially, the source used was the ESI because PFCAs and PFSAs are mainly analyzed by ESI. When comparing the ionization with ESI between the PFPAs and PFOS by flow injection analysis (FIA) a substantial difference in ionization efficiency was found. PFOS was 10-fold more sensitive than PFPAs. When the APCI source was tested, the sensitivity of PFDPA increased 2-fold, while for PFHxPA and PFOPA a 3-fold improvement was observed. PFOS remained 10-fold more sensitive than PFPAs due to a more efficient ionization. PFCAs, such as PFOA, were poorly ionized at the optimum PFPAs ionization conditions and were therefore excluded from further study. Finally, the LC–MS/MS system was evaluated for sensitivity and run-to-run precision. The instrumental detection limit (iLOD) for PFHxPA and PFOPA was 0.5 ng mL^{-1} and 2-fold higher for PFDPA (1 ng mL^{-1}). The iLOD of PFOS is an order of magnitude lower than that of PFOPA (0.05 ng mL^{-1}). The instrumental limits of quantification (iLOQ) based on 10 times the signal-to-noise ratio were 1.5 ng mL^{-1} for PFHxPA and PFOPA, 5 ng mL^{-1} for PFDPA and 0.15 ng mL^{-1} for PFOS. The RSD values ($n=6$) for all compounds were lower than 5% at 10 and 50 ng mL^{-1} (Table 3).

3.2. Sample clean-up and concentration

The technique most extensively used for extraction of PFCAs and PFSAs from aqueous environmental samples is SPE. Oasis®HLB

Table 4
PFPAs recoveries using different solvents and extraction methods for Western Scheldt sediment spiked at a concentration of 40 ng g^{-1} dry weight.

Solvent	Extraction	SPE	Recovery		
			PFHxPA	PFOPA	PFDPA
DCM	PLE	Yes	n.r.	n.r.	n.r.
Ethyl acetate	PLE	Yes	n.r.	n.r.	n.r.
Methanol	PLE	Yes	70%	76%	240%
Ethyl acetate	Shaker	Yes	n.r.	n.r.	n.r.
Acetone	Shaker	Yes	n.r.	n.r.	n.r.
TBA/MTBE	Shaker	Yes	12%	14%	20%
TBA/MTBE	Shaker	No	20%	23%	35%
THF	Shaker	Yes	10%	13%	5%
THF:H_2O 75:25	Shaker	Yes	35%	84%	94%
THF:H_2O 50:50	Shaker	Yes	32%	75%	92%
THF:H_2O 25:75	Shaker	Yes	75%	82%	85%
MeOH:H_2O 75:25 (pH 9)	Shaker	Yes	40%	67%	54%

n.r.: no recovery.

and Oasis-WAX phases are often used in the analysis of PFASs in water [2,14,15,23–29]. D'eon et al. developed a method employing Oasis-WAX cartridges for the extraction of the PFPAs [12]. In this study the same cartridges were used, but according to Ballesteros-Gomez et al. [14], two washing steps were performed with 4 mL ammonium acetate 25 mM adjusted at pH 4 and 8 mL of tetrahydrofuran:methanol (75:25, v/v). Furthermore the elution of the cartridge was carried out with methanol with 0.1% (v/v) of ammonia instead of using methyl-*tert*-butyl ether:methanol with 1% (v/v) ammonia (90:10, v/v). For this study, 10 mL of water with each PFPA in a concentration of 100 ng mL^{-1} was loaded onto the WAX cartridges. To test the elution pattern, 10 mL of methanol was collected in 1 mL fractions and injected individually into the LC–MS/MS (n = 3). Recoveries of target fluorinated compounds spiked into WAX cartridges were 92%, 112% and 87% for PFHxPA, PFOPA and PFDPA, respectively, with respective RSD values of 3.5%, 1.1% and 8.7%. As can be observed in Fig. 3, the highest amount of PFPAs eluted in the fourth fraction and 80% of the compounds eluted between the second and the seventh fraction. Moreover the concentration of the PFPAs in the last milliliter of the elution step was around the iLOQ of the method. The use of a WAX method serves to pre-concentrate and clean the water samples and the extracts. A concentration factor of 2000 was obtained after reconstituting the loaded 500 mL to 250 μL.

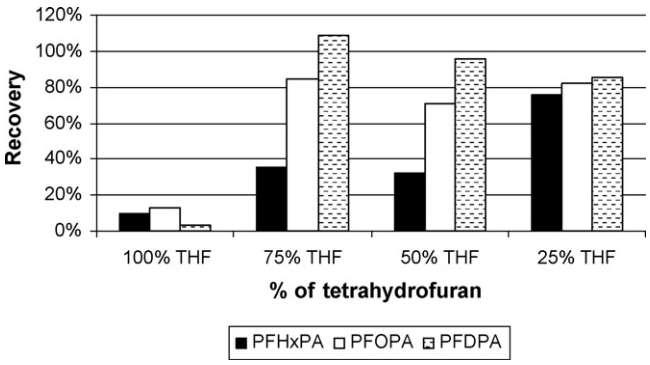


Fig. 4. Recoveries of the whole extraction method for PFPAs form sediment samples at different percentage of THF:water as extraction solvent.

3.3. Extraction of sediment and sewage sludge

Sludge and sediment samples were freeze-dried prior to extraction. Methanol, at basic or acidic conditions, has been used by several authors to extract the analytes [30,31]. Other authors used MTBE with TBA as ion-pair reagent [2] and sometimes PLE has been used [32]. In the present study several solvents and methods have been tested for the extraction of PFPAs from sediments and sludge

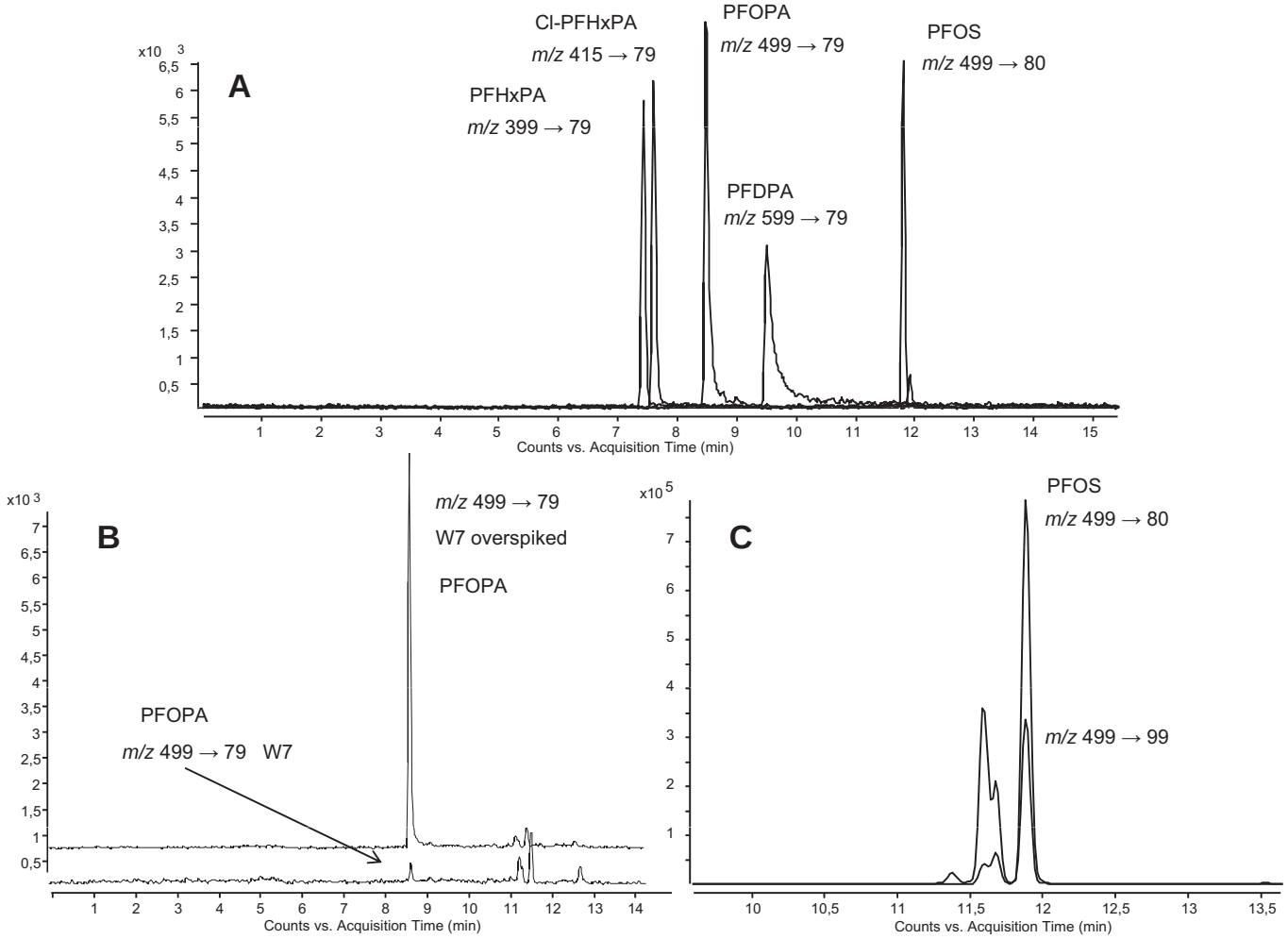


Fig. 5. LC–MS/MS chromatograms of: (A) 10 ng mL^{-1} standard; (B) PFOPA trace of water sample from IJmuiden (W7) and overspiked at 10 ng mL^{-1}; (C) PFOS transitions of water sample from Twente Kanaal (25.4 ng L^{-1}).

Table 5
Concentrations of PFOS in sediment, sewage sludge and water.

Sample	Location	Sampling period	PFOS concentration ± SD (dry weight)
Ss1	Eindhoven	July 2009	35 ± 1.5 ng g^{-1}
Ss2	Kralingseveer	August 2009	36 ± 2.4 ng g^{-1}
Ss3	Bath	July 2009	43 ± 2.7 ng g^{-1}
Ss4	Amsterdam	July 2009	48 ± 1.8 ng g^{-1}
Sd5[a]	Elbe	July 2009	0.75 ± 0.12 ng g^{-1}
Sd6[a]	Seine	July 2009	1.5 ± 0.25 ng g^{-1}
Sd7[a]	Ems	July 2009	1.9 ± 0.31 ng g^{-1}
Sd8	Middelplaat, Western Scheldt	September 2008	0.07 ± 0.01 ng g^{-1}
W1	IJ, Amsterdam	December 2009	9.2 ± 0.76 ng L^{-1}
W2	Deventer	December 2009	7.1 ± 0.57 ng L^{-1}
W3	Twente Canal	December 2009	5.6 ± 0.69 ng L^{-1}
W4	Hopestein	December 2009	6.3 ± 0.42 ng L^{-1}
W5	Nieuwe Waterweg km 1017.5	December 2009	6.7 ± 0.96 ng L^{-1}
W6	Zwarte Water, Genemuiden	December 2009	3.3 ± 0.71 ng L^{-1}
W7	IJmuiden	December 2009	12 ± 0.68 ng L^{-1}
W8	Twente Kanaal, Almelo	December 2009	25 ± 0.94 ng L^{-1}
W9	Kampen	December 2009	6.7 ± 1.2 ng L^{-1}
W10	Vuren	December 2009	12 ± 0.39 ng L^{-1}

[a] These are not Dutch samples: S5 and S7 are from Germany and S6 from France. Ss = sewage sludge, Sd = sediment, W = water. SD: standard deviation.

by weighing 1 g of freeze dried sample and spiking a PFPAs mixture at a concentration of 40 ng g^{-1} and leaving it to incubate overnight. Furthermore for each method an unspiked sample, a blank and a standard were extracted.

PLE with ethyl acetate, dichloromethane and methanol and a shaking extraction with acetone and ethyl acetate were tested. No PFPAs were recovered when using dichloromethane, acetone or ethyl acetate. The very poor extraction of PFPAs could be due to matrix effects or to the inefficacy of the solvent to extract these compounds from the sediment. The addition of labeled PFOS and PFHxS as internal standards after the extraction procedure was done to assess the possible occurrence of matrix effects (i.e. ESI suppression or enhancement). Because this experiment showed that no matrix effects were present, it was concluded that acetone, dichloromethane and ethyl acetate cannot extract PFPAs from sediments under the tested conditions. Methanol with ASE provided recoveries around 70% for PFHxPA and PFOPA but recoveries higher than 200% for PFDPA. This high recovery was attributed to matrix effects (i.e. ESI). The method suggested by Hansen et al. using TBA as ion pair reagent with an extraction with MTBE gave recoveries between 20% and 25%, when performed without SPE (because TBA may prevent the anionic group from interacting with the WAX adsorbent).

Mixtures of solvents such as methanol:water at pH 9 and tetrahydrofuran:water have also been tested. Ballesteros-Gomez et al. [14] were the first in using THF:water for the extraction of biological samples because of its Hildebrand solubility parameter (δ_T), which provides a measure of the overall intermolecular forces resulting from the additive effect of dispersion (δ_d), dipole–dipole (δ_p) and hydrogen bonding (δ_h) forces ($\delta_T^2 = \delta_d^2 + \delta_p^2 + \delta_h^2$). When the sample is extracted with 100% THF, the recoveries are lower than 12% for all PFPAs. As can be seen in Fig. 4, when 75% or 50% of THF is used for the extraction, recoveries around 80% are obtained for PFHxPA and PFDPA but recoveries under 40% are obtained for the PFHxPA. When the percentage of THF decreases to 25%, the recovery for PFHxPA is doubled (78%) and the recoveries for the other two are still around 80%. Therefore, THF:water, 25:75, v/v was used to study the recovery of the entire procedure. A freeze-dried sediment sample (Western Scheldt), which was previously found to be free of PFPAs, was fortified with the native compounds and analyzed using the optimized procedure (25:75%, v/v THF:water, Oasis WAX sample clean-up and LC–APCI-MS/MS analysis and quantification). The recoveries thus obtained were 75% ± 2.6% for PFHxPA, 82% ± 4.5% for PFOPA and 85% ± 8.9% for PFDPA (n = 6).

3.4. Survey of PFPAs in Dutch environmental samples

The proposed methods (for sediment: THF–water (25:75%) extraction and Oasis WAX clean-up; for water: extraction and clean-up using Oasis-WAX) were used to analyze ten surface water samples, four sewage sludge samples from The Netherlands and four sediment samples from Dutch river basins. The aim was to get a first impression on the presence of PFPAs in the Dutch environment. Therefore, a selection of surface water samples was sampled from different origins, as well as sediment and sewage sludge. None of the samples, either water, sediment or sludge, were found to be contaminated with PFPAs. Only one water sample, W7 from IJmuiden (near Amsterdam), showed a small PFOPA trace (m/z 499 → 79) at a concentration around the LOD (approx. 1 ng L^{-1}). Due to the lack of a confirmation transition, no confirmation could be performed. To ensure the presence of the PFOPA, the sample extract was spiked, demonstrating the presence of a small amount of PFOPA in the sample. Fig. 5A shows a chromatogram of a standard and B shows both chromatograms of the spiked and unspiked W7 sample. There are no known sources of PFPOA contamination in the Netherlands, but in a recent study by Ullah et al. [33], a trace of PFOPA was found in drinking water sourced in Amsterdam indicating that there may be a local PFOPA contamination source. Nevertheless, our results indicate the PFPAs are not major pollutants in the Dutch environment, although more research is needed to confirm this.

Concentrations of PFOS in sediment samples, shown in Table 5, were 0.07–1.9 ng g^{-1} dry weight. The highest concentrations were found in sewage sludge (35–48 ng g^{-1}). These concentrations were similar to the concentrations found by Loganathan et al. [18]. PFOS was also detected in water in low ng L^{-1} concentrations. These results are consistent with the literature [15–18,34]. Fig. 5C shows the quantification and the confirmation transitions of the W8 sample from the Twente Canal. As can be seen in this figure, more peaks are observed due to the presence of branched PFOS isomers. All values were quantified using a standard that only contained linear PFOS, meaning that the actual PFOS concentrations may be slightly deviating due to different MS response factors for the branched and linear isomers.

4. Conclusions

Several LC columns, a perfluorooctyl column and a conventional C18 column have been tested to obtain the best peak shape for

and optimum injection solvent (mobile phase, pH 9 and ion-pair). The optimum LC–MS/MS method for the determination of PFPAs and PFOS in environmental samples included a ZORBAX Rapid Resolution High Throughput column (30 × 2.1 mm, 1.8 μm). Gradient elution using acetonitrile:2 mM ammonium acetate as mobile phase at a flow rate of 300 μL min^{-1} was used. APCI was used as ionization technique.

Both water and sediment/sludge needed a pre-concentration and clean-up step. Oasis WAX cartridges showed recoveries over 90% for PFPAs and PFOS. 0.5 L was passed through a WAX cartridge, eluted, evaporated and reconstituted in methanol:water (1:1, v/v) with 25 mM tetrabutylammonium (TBA). Sediment samples were extracted with a mixture of THF:water (75:25%) and then followed the same procedure as the water samples. Before injecting 5 μL in the LC–MS/MS extracts were filtered through 0.22 μm GHP filters. The method showed LOD values at ng L^{-1} level and provided good linearity and precision (RSD < 20%). PFOPA was observed in only one surface water sample around LOD level. PFOS was found at low ng g^{-1} levels in sediment and sludge and at low ng L^{-1} levels in surface water. This suggests that PFPAs are not major contaminants in the Dutch environment, but more research is needed to confirm this.

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References

[1] E. Kissa, Fluorinated Surfactants, Marcel Dekker, New York, NY, USA, 2002.
[2] K.J. Hansen, L.A. Clemen, M.E. Ellefson, H.O. Johnson, Environ. Sci. Technol. 35 (2001) 766.
[3] OECD Hazard assessment of perfluorooctane sulfonate (PFOS) and its salts. Report ENV/JM/RD (2002)17/FINAL, 2002.
[4] 3M. Phase-out plan for POSF-based products. U.S. EPA Docket ID OPPT-2002-0043. Specialty Materials Markets Group, St. Paul, MN, USA, 2000.
[5] E.I. du Pont de Nemours and Company. DuPont global PFOA strategy Comprehensive source reduction. U.S. EPA public docket AR226-1914. U.S. Environmental Protection Agency, Washington, DC, 2005.
[6] Perfluorinated substances and their uses in Sweden, Report Nr 7/06, ISSN: 0284-1185.
[7] G. Lewandowski, E. Meissner, E. Milchert, J. Hazard. Mater. A 136 (2006) 385.
[8] J.P. Giesy, K. Kannan, Environ. Sci. Technol. 36 (2002) 146A.
[9] http://sparc.chem.uga.edu/sparc/ Last update. September 2009 release w4.5.1529-s4.5.1529.
[10] P.H. Howard, W. Meylan, EPA Great Lakes study for identification of PBTs to develop analytical methods: selection of additional PBTs—Interim Report. EPA Contract EP-W-04-019. U.S. Environmental Protection Agency, Syracus, NY, 2007.
[11] R. Bossi, Survey and Environmental/health Assessment of Fluorinated Substances in Impregnated Consumer Products and Impregnating Agents, vol. 99, Allan Astrup Jensen & Pia Brunn Poulsen FORCE Technology, 2008.
[12] J. D'eon, P.W. Crozier, V.I. Furdui, E.J. Reiner, E.L. Libelo, S.A. Mabury, Environ. Toxicol. Chem. 28 (2009) 2101.
[13] P.H. Howard, D.C.G. Muir, Environ. Sci. Technol. 44 (2010) 2277.
[14] A. Ballesteros-Gomez, S. Rubio, S.P.J. van Leeuwen, J. Chromatogr. A 1217 (2010) 5913.
[15] S. Taniyasu, K. Kannan, M.K. So, A. Gulkowska, E. Sinclair, T. Okazawa, N. Yamashita, J. Chromatogr. A 1093 (2005) 89.
[16] P. Rostkowski, N. Yamashita, M.K. So, S. Taniyasu, P.K.S. Lam, J. Falandysz, K.T. Lee, S.K. Kim, J.S. Khim, S.H. Im, J.L. Newsted, P.D. Jones, K. Kannan, J.P. Giesy, Environ. Toxicol. Chem. 25 (2006) 2374.
[17] S. Takagi, F. Adachi, K. Miyano, Y. Koizumi, H. Tanaka, M. Mimura, I. Watanabe, S. Tanabe, K. Kannan, Chemosphere 72 (2008) 1409.
[18] B.G. Loganathan, K.S. Sajwan, E. Sinclair, K.S. Kumar, K. Kannan, Water Res. 41 (2007) 4611.
[19] A. Holm, S.R. Wilson, P. Molander, E. Lundanes, T. Greibrokk, J. Sep. Sci. 27 (2004) 1071.
[20] L. Ahrens, S. Felizeter, R. Sturm, Z. Xie, R. Ebinghaus, Mar. Pollut. Bull. 58 (2009) 1326.
[21] Reference and handling guide: perfluoroalkyl compounds. www.well-labs.com.
[22] J.M. Marín, O.J. Pozo, J. Beltrán, F. Hernández, Rapid Commun. Mass Spectrom. 20 (2006) 419.
[23] N. Yamashita, K. Kannan, S. Taniyasu, Y. Horii, T. Okazawa, G. Petrick, T. Gamo, Environ. Sci. Technol. 38 (2004) 4056.
[24] K.J. Hansen, H.O. Johnson, J.S. Eldridge, J.L. Butenhoff, L.A. Dick, Environ. Sci. Technol. 36 (2002) 1681.
[25] C.A. Moody, J.W. Martin, W.C. Kwan, D.C.G. Muir, S.A. Mabury, Environ. Sci. Technol. 36 (2002) 545.
[26] S. Taniyasu, K. Kannan, Y. Horii, N. Hanari, N. Yamashita, Environ. Sci. Technol. 37 (2003) 2634.
[27] M. Llorca, M. Farré, Y. Picó, D. Barceló, J. Chromatogr. A 1216 (2009) 7195.
[28] S. Chu, R.J. Letcher, J. Chromatogr. A 1215 (2008) 92.
[29] H. Yoo, J.W. Washington, T.M. Jenkins, E.L. Libelo, J. Chromatogr. A 1216 (2009) 7831.
[30] C.R. Powley, S.W. George, T.W. Ryan, R.C. Buck, Anal. Chem. 77 (2005) 6353.
[31] C.P. Higgins, J.A. Field, C.S. Criddle, R.G. Luthy, Environ. Sci. Technol. 39 (2005) 3946.
[32] H.F. Schroder, J. Chromatogr. A 1020 (2003) 131.
[33] S. Ullah, T. Alsberg, U. Berger, J. Chromatogr. A 1210 (2011) 6388.
[34] C. González-Barreiro, E. Martínez-Carballo, A. Sitka, S. Scharf, O. Gans, Anal. Bioanal. Chem. 386 (2006) 2123.