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# Simultaneous determination of perfluoroalkyl phosphonates, carboxylates, and sulfonates in drinking water

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ABSTRACT

A trace analytical method based on high performance liquid chromatography coupled to quadrupole time-of-flight high resolution mass spectrometry was developed for simultaneous determination of perfluoroalkyl phosphonates (PFPAs, carbon chain lengths C6,8,10), perfluoroalkyl carboxylates (PFCAs, C5–12), and perfluoroalkyl sulfonates (PFSAs, C4,6,8,10) in drinking water (tap water). Analytes were enriched on a mixed mode co-polymeric sorbent (C8 + quaternary amine) using solid phase extraction. Chromatographic separation was achieved on a Zorbax Extend C18 reversed phase column using a mobile phase gradient consisting of water, methanol, and acetonitrile containing 2 mM ammonium acetate and 5 mM 1-methyl piperidine. The mass spectrometer was operated in electrospray negative ion mode. Use of 1-methyl piperidine in the mobile phase resulted in a significant increase in instrument sensitivity for PFPAs through improved chromatographic resolution, background suppression, and increased ionization efficiency. Method detection limits for extraction of 500 mL tap water were in the ranges of 0.095–0.17 ng/L, 0.027–0.17 ng/L, and 0.014–0.052 ng/L for PFPAs, PFCAs, and PFSAs, respectively. Whole method recoveries at a spiking level of 0.5 ng/L to 500 mL HPLC grade water were 40–56%, 56–97%, and 55–77% for PFPAs, PFCAs, and PFSAs, respectively. A matrix effect (signal enhancement) was observed in the detection of PFPAs in tap water extracts, leading to calculated recoveries of 249–297% at a 0.5 ng/L spiking level. This effect resulted in an additional improvement of method sensitivity for PFPAs. To compensate for the matrix effect, PFPAs in tap water were quantified using matrix-matched and extracted calibration standards. The method was successfully applied to the analysis of drinking water collected from six European countries. PFPAs were not detected except for perfluorooctyl phosphonate (PFOPA) at close to the detection limit of 0.095 ng/L in two water samples from Amsterdam, the Netherlands. Highest levels were found for perfluorobutane sulfonate (PFBS, 18.8 ng/L) and perfluorooctanoate (PFOA, 8.6 ng/L) in samples from Amsterdam as well as for perfluorooctane sulfonate (PFOS, 8.8 ng/L) in tap water from Stockholm, Sweden.

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## 1. Introduction

Perfluoroalkyl acids (PFAAs) are a group of anthropogenic compounds with unique physical-chemical properties [1]. They have been used for the last 60 years in many consumer products such as paints, polishes, packaging materials, fire-fighting foams, cookware, lubricants, and stain repellents as well as in industrial processes for the production of fluoropolymers [1]. PFAAs consist of a fully fluorinated carbon chain of typically four to sixteen carbon atoms and an acidic functional group, such as a carboxylic, sulfonic, or phosphonic acid. Due to the extraordinary stability of the carbon–fluorine bond, PFAAs are resistant to degradation, including reaction with acids and bases, and they persist in the environment. During the last decade, these properties have triggered an increasing interest among scientists and regulators seeking to understand environmental processes and implications of PFAAs. This has led to the discovery that some PFAAs are bioaccumulative [2], toxic in animal studies [3,4], and prone to long-range transport [5].

PFAAs have been detected globally in a variety of matrices such as sea water [6], sludge [7], air [8], wildlife [9], and in humans [10]. However, at typical environmental pH values of 5–8, PFAAs predominantly dissociate into their ionic forms and the shorter chain homologues including PFOA and PFOS are expected to partition to water [11,12]. Because classical water purification processes are not efficient in removing short chain PFAAs, these may eventually end up in drinking water [13], which is thus a potential vector for human exposure [14]. Monitoring of tap (or drinking) water has mainly focused on perfluorooctanoate (PFOA), perfluorooctane sul-

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fonate (PFOS), and possibly some other perfluoroalkyl carboxylates (PFCAs) and sulfonates (PFSAs) [15–17]. Screening data for perfluoroalkyl phosphonates (PFPAs) in tap water samples have not been published to our knowledge.

PFPAs are a new class of PFAAs, which contain a functional group with two acidic protons in contrast to PFCAs and PFSAs [18]. PFPAs have been used mostly in the industrial sector as commercial surfactants with favorable wetting and leveling properties. Applications include cleaning products and aqueous coatings, as well as defoaming additives in pesticide production [19]. The total annual production volume of perfluorooctyl phosphonic acid (PFOPA) has been estimated to be 4.5–230 tons between 1998 and 2002 [20]. PFPAs are very strong acids with estimated p*K*a values of the two acid groups in PFOPA of 2.4 and 4.5 [21], resulting in a high migration capability from emission sources into environmental waters [18]. Recently, the environmental occurrence of PFPAs (predominantly PFOPA) was described for the first time in Canadian surface water and waste water treatment plant effluents [18]. The presence of PFPAs in the European environment was confirmed in a study from the Netherlands where 1 ng/L of PFOPA was found in surface water collected from Amsterdam [22]. To better understand the risk associated with potential exposure, the biological fate of PFPAs and perfluoroalkyl phosphinic acids was investigated in rats [23].

The di-anionic character of PFPAs makes them extremely challenging to analyze. Routine extraction and clean-up methods commonly applied in trace analysis of PFCAs and PFSAs may not be applicable to PFPAs. Furthermore, trace analysis of PFPAs has so far been hampered by methodological challenges, such as poor resolution in high performance liquid chromatography (HPLC) and low detector response in mass spectrometry (MS). In the present study, a highly sensitive analytical method based on HPLC coupled to quadrupole time-of-flight high resolution MS (HPLC/QToF-HRMS) was developed and validated to quantify PFPAs in drinking water. The presented method overcomes the challenges mentioned above and enables the simultaneous determination of PFPAs, PFCAs, and PFSAs with a variety of carbon chain lengths. It was successfully applied to a set of drinking water samples from six European countries.

## 2. Experimental

### 2.1. Chemicals

The abbreviations for the analytical standard compounds including surrogate internal standards (IS) and recovery internal (volume) standards (RIS) are listed in Table 1. All standards were obtained as solutions in methanol. Native PFCAs, PFSAs, and PFPAs as well as the isotopically mass-labeled RIS were purchased from Wellington Laboratories (Guelph, Ontario, Canada), while the mass-labeled PFCAs (carbon chain lengths C6,8-12) and PFSAs (C6,8) used as IS were donated by Wellington. All reference standards were purely linear compounds, apart from native PFOS, which was a mixture of 78.8% of the linear isomer (lin-PFOS) and 21.2% sum of branched isomers (br-PFOS). The following reagents and solvents with the highest purity available were purchased and used as received: 1-methyl piperidine (Merck Eurolab, Stockholm, Sweden), ammonium hydroxide solution (Fluka, Buchs, Switzerland), triethylamine (Fluka, Bornem, Belgium), sodium hydrogen carbonate ($NaHCO_3$, Sigma–Aldrich, St. Louis, MO, USA), formic acid (Fisher Scientific GTF, Gothenburg, Sweden), ammonium acetate (Merck, Darmstadt, Germany), HPLC water Chromanorm grade (VWR International, Stockholm, Sweden), methanol LiChrosolv grade (Merck) and acetonitrile Chromasolv grade (Sigma–Aldrich, Stockholm, Sweden).

### 2.2. Method validation experiments

For method development and validation, 500 mL of HPLC grade water containing 50 mg/L $NaHCO_3$ (mimicking the ionic strength of tap water) were spiked with different concentrations of native PFPAs, PFCAs, and PFSAs. The pH of the water was adjusted to approximately 7 by addition of 2 μL formic acid. The complete method was validated by assessing the instrumental and procedural blank contamination, detection and quantification limits, method linearity, recoveries, reproducibility, and accuracy. Due to the presence of traces of PFCAs in the bottled HPLC grade water, procedural blanks were evaluated by extraction of only 5 mL of this water. The method detection limits (MDLs) and method limits of quantification (MLQs) were calculated from sample chromatograms based on a signal-to-noise ratio of 3 and 10, respectively. For PFOA, which showed a minor procedural blank contamination, the MDL and MLQ were defined as 3 and 10 times the signal of the blank contamination. Whole method linearity for PFCAs, PFSAs, and PFPAs was assessed over a spike concentration range of the individual MLQs up to 20 ng/L water (5 data points). Individual compound recoveries were determined in triplicates from spiked water (500 mL) on two different days. Spike concentrations were 0.5 ng/L as well as 5 ng/L for all individual PFPAs, PFCAs, and PFSAs. Additionally, recoveries for PFPAs were calculated at a 0.5 and 5 ng/L spike concentration in Stockholm tap water, as well as at a 2 ng/L level in European tap water samples (see Section 2.3). The coefficient of variation of the inter-day triplicate determination of recoveries was used as a measure for method reproducibility. The accuracy of the method for PFCAs and PFSAs was evaluated by analysis of a bulk water sample collected from a freshwater canal near Amsterdam in April 2009. This water sample had previously been analyzed in an inter-laboratory comparison study (ILS) with 35 laboratories reporting results for at least one analyte [24]. Accuracy in PFPA analysis was achieved by using matrix-matched and extracted external calibration standards. For each individual tap water sample an aliquot of 500 mL was spiked with 2 ng/L of each PFPA and extracted along with the unspiked sample. The extract of the spiked sample was used as external calibration standard for quantification of PFPAs in the unspiked sample extract.

### 2.3. Tap water samples

Samples of local tap water (2 L of cold water) were collected at the following seven research institutes in six European countries in July, 2010: (1) Stockholm University, Sweden; (2) Institute for Environment and Sustainability, Ispra, Italy; (3) University of Antwerp, Belgium; (4) University of Amsterdam, The Netherlands; (5) VU University, Amsterdam, The Netherlands; (6) Norwegian Institute for Air Research, Tromsø, Norway; (7) Fraunhofer Institute, Schmallenberg, Germany (Fig. S1 in the supplementary data). Samples were collected in polypropylene bottles previously rinsed with methanol and the sampled water itself and sent to Stockholm University. Upon receipt samples were stored in the freezer at −20 °C until analysis in August, 2010.

### 2.4. Sample extraction

Prior to analysis the water samples were thawed and the sample bottles were put into an ultrasonic bath at room temperature for 15 min. The pH was adjusted to 7 with formic acid and two aliquots of 500 mL water were taken from each sample. One aliquot was spiked with the isotopically mass-labeled IS of PFCAs and PFSAs (Table 1) to a concentration of 4 ng/L. The other aliquot was spiked with native PFPAs at 2 ng/L and served as matrix-matched and extracted calibration standard for PFPA analysis (see also Section 2.2). The analytes were enriched on a CUQAX256 solid phase extrac-

**Table 1**
Abbreviations of analytical standard compounds used in this study and selected instrumental parameters for quantification of each compound.

| Compound name | Abbreviation | Counter ion | Quantification mass [*m*/*z*] | Cone voltage [V] | Scan function |
|---|---|---|---|---|---|
| *Native standards* | | | | | |
| Perfluoro-*n*-pentanoate | PFPeA | $H^+$ | 218.98 | 20 | 1 |
| Perfluoro-*n*-hexanoate | PFHxA | $H^+$ | 268.98 | 20 | 1 |
| Perfluoro-*n*-heptanoate | PFHpA | $H^+$ | 318.97 | 20 | 1 |
| Perfluoro-*n*-octanoate | PFOA | $H^+$ | 368.97 | 20 | 1 |
| Perfluoro-*n*-nonanoate | PFNA | $H^+$ | 418.97 | 20 | 1 |
| Perfluoro-*n*-decanoate | PFDA | $H^+$ | 468.96 | 20 | 1 |
| Perfluoro-*n*-undecanoate | PFUnDA | $H^+$ | 518.96 | 20 | 1 |
| Perfluoro-*n*-dodecanoate | PFDoDA | $H^+$ | 568.96 | 20 | 1 |
| Perfluoro-*n*-butane sulfonate | PFBS | $K^+$ | 298.94 | 50 | 3 |
| Perfluro-*n*-hexane sulfonate | PFHxS | $Na^+$ | 398.93 | 50 | 3 |
| Perfluorooctane sulfonate (isomer mixture with 78.8% linear PFOS) | PFOS | $K^+$ | 498.93 | 50 | 3 |
| Perfluoro-*n*-decane sulfonate | PFDS | $Na^+$ | 598.92 | 50 | 3 |
| Perfluoro-*n*-octane sulfonamide | FOSA | – | 497.94 | 50 | 3 |
| Perfluoro-*n*-hexyl phosphonate | PFHxPA | 2 $H^+$ | 398.94 | 50 | 2[a] |
| Perfluoro-*n*-octyl phosphonate | PFOPA | 2 $H^+$ | 498.93 | 50 | 2[a] |
| Perfluoro-*n*-decyl phosphonate | PFDPA | 2 $H^+$ | 598.93 | 50 | 2[a] |
| *Surrogate internal standards (IS)* | | | | | |
| 1,2-$^{13}C_2$-Perfluoro-*n*-hexanoate | MPFHxA | $H^+$ | 269.99 | 20 | 1 |
| $^{13}C_8$-Perfluoro-*n*-octanoate | M8PFOA | $H^+$ | 375.98 | 20 | 1 |
| $^{13}C_9$-Perfluoro-*n*-nonanoate | M9PFNA | $H^+$ | 426.98 | 20 | 1 |
| 1,2,3,4,5,6-$^{13}C_6$-Perfluoro-*n*-decanoate | M6PFDA | $H^+$ | 473.98 | 20 | 1 |
| 1,2,3,4,5,6,7-$^{13}C_7$-Perfluoro-*n*-undecanoate | M7PFUnDA | $H^+$ | 524.97 | 20 | 1 |
| 1,2-$^{13}C_2$-Perfluoro-*n*-dodecanoate | MPFDoDA | $H^+$ | 569.97 | 20 | 1 |
| $^{18}O_2$-Perfluoro-*n*-hexane sulfonate | MPFHxS | $Na^+$ | 402.93 | 50 | 3 |
| $^{13}C_8$-Perfluoro-*n*-octane sulfonate | M8PFOS | $Na^+$ | 506.93 | 50 | 3 |
| *Recovery internal (volume) standards (RIS)* | | | | | |
| 1,2,3,4-$^{13}C_4$-Perfluoro-*n*-octanoate | M4PFOA | $H^+$ | 371.98 | 20 | 1 |
| 1,2,3,4-$^{13}C_4$-Perfluoro-*n*-octane sulfonate | M4PFOS | $Na^+$ | 502.93 | 50 | 3 |

[a] Enhanced Duty Cycle (EDC) at *m*/*z* 499 (PFOPA) was used in function 2.

tion (SPE) cartridge (C8 + quaternary amine, 500 mg–6 mL; United Chemical Technologies, UCT, Bristol, PA, USA). Before loading the sample, the cartridge was rinsed and conditioned with 3 mL each of methanol with 0.1 vol% 1-methyl piperidine (1-MP), methanol, and water. The sample was loaded at 5 mL/min. The cartridge was then rinsed with 1 mL methanol and the analytes were subsequently eluted by gravity with 8 mL of 80:20 methanol:acetonitrile with 2 vol% of 1-MP. The elution solvent was warmed up to 60 °C before application. The extract was evaporated to incipient dryness under nitrogen at 40 °C and the residue was reconstituted in 200 μL of 50:50 water:methanol containing 5 pg/μL of the isotopically mass-labeled RIS M4PFOA and M4PFOS (Table 1).

### 2.5. Instrumental analysis and quantification

Extracts were analyzed by HPLC/HRMS using an Acquity Ultra Performance LC (Waters, Milford, MA, USA) coupled to a QToF Premier HRMS instrument (Micromass, Manchester, UK). Instrumental operation, data acquisition and peak integration were performed with MassLynx v4.1 control software (Waters). The HPLC instrument was equipped with a trapping column (Zorbax Extend C18 50 mm × 2.1 mm, 3.5 μm particles; Agilent Technologies, Santa Clara, CA, USA) installed between the eluent mixer and the injector to trap and delay PFCA contamination from the HPLC system. The target analytes were separated on a Zorbax Extend C18 reverse phase column (150 mm × 1.0 mm, 3.5 μm particles, Agilent Technologies) by injecting 5 μL sample volume at a mobile phase flow rate of 50 μL/min. Separation was achieved at room temperature by gradient elution using a mobile phase A consisting of 95:5 water:methanol and a mobile phase B consisting of 75:20:5 methanol:acetonitrile:water, with 2 mM ammonium acetate and 5 mM 1-MP in both A and B. The gradient profile started with 90% A (hold time 0.3 min) and continued with a linear change to 80% A up to 1 min and to 50% A up to 1.5 min followed by a linear change to 100% B up to 12.5 min and hold until 18 min. Initial conditions were regained at 18.5 min followed by equilibration until 25 min.

The mass spectrometer was calibrated with a solution of sodium formate, which formed cluster ions in the *m*/*z* range 75–1000. Electrospray ionization in negative ion mode was employed, and data were acquired in full scan mode (*m*/*z* 75–780) utilizing three parallel scan functions for PFCAs, PFPAs, and PFSAs. Enhanced Duty Cycle (EDC) at a centre mass of *m*/*z* 499 (PFOPA) was used in the function for PFPAs. The following optimized parameters were applied: capillary voltage, 3.0 kV; sample cone voltage, 20 V for PFCAs and 50 V for PFSAs and PFPAs; collision energy, 5 eV; source temperature, 100 °C; desolvation temperature, 400 °C; nitrogen desolvation gas flow, 800 L/h. The applied cone voltages and scan functions for all reference standards and analytes are given in Table 1. For on-line mass scale correction the LockSpray utility was used infusing a 0.1 ng/μL solution of sulfadimethoxine in methanol. Quantification was done using extracted mass chromatograms from full scan recording with a *m*/*z* window of 0.05 u. The quantification *m*/*z* values for all target compounds are listed in Table 1. Quantification *m*/*z* values for PFSAs and PFPAs of the same carbon chain length were identical; however, the respective compounds were baseline separated in the HPLC system. Only the linear isomer was quantified for all compounds except for PFOA and PFOS, which additionally showed quantifiable concentrations of branched isomers in the water sample extracts. For these two compounds the linear isomer (lin) and sum of branched isomers (br) were quantified separately. Lin-PFOS and br-PFOS were quantified using the relative response factors (relative to the IS) of lin-PFOS and br-PFOS, respectively, obtained from the calibration standard (mixture of 78.8% lin-PFOS and 21.2% br-PFOS). The relative response factor of lin-PFOA (purely linear reference standard) was applied for quantification of both lin-PFOA and br-PFOA. Concentrations of PFCAs and PFSAs in the water samples were calculated using the internal standard method employing nine isotopically mass-labeled PFCAs and PFSAs as IS (Table 1). Concentrations of PFPAs were calculated using an external matrix-matched and extracted reference standard for each sample (see Sections 2.2 and 2.4).

## 3. Results and discussion

### 3.1. Elimination of blank contamination

Procedural and instrumental blank contamination is a major challenge in most of the laboratories performing trace analysis of PFAAs. Possible sources of instrumental and procedural blank contamination as well as techniques for reducing the contamination have been described [25,26]. In the present study, a trapping column was installed between the mobile phase mixing chamber and injector in the HPLC instrument to reduce the instrumental background contamination. Fluoropolymer parts of the instrument were not exchanged. After installing the trapping column, background signals of PFCAs, PFSAs, or PFPAs were not observed in solvent blank injections. Procedural blank contamination was reduced by avoiding the use of fluoropolymer materials in the lab during sample preparation and extraction and by rigorously rinsing all equipment with methanol before use. Very low levels of procedural blank contamination, leading to a slightly elevated MDL and MLQ for PFOA, were occasionally observed (see Section 3.5). However, the blank contamination was negligible compared to quantified PFOA concentrations in tap water samples.

### 3.2. Optimization of the instrumental method

In the present study QToF-HRMS was chosen as detector for the following three reasons. (1) It is very selective in single stage MS due to the high mass resolution (here operated at a resolution of 10,000 fwhm), and thus the inherent ion intensity loss in multiple stage MS can be avoided [27]. Mass accuracy data of the HRMS instrument for all analytes in a tap water extract are given in Table S1 in the supplementary data. (2) The high acquisition speed of the QToF instrument allows for data acquisition in full scan mode resulting in chromatograms that contain information on co-extracted matrix constituents. (3) Response factors of different structural isomers of PFAAs vary much less in single stage MS compared to tandem MS [28–30], making the quantification of an unknown pattern of branched isomers of a given PFAA more reliable.

PFPAs suffer from a relatively low detector response in MS compared to PFOS [22]. Enhanced Duty Cycle (EDC) was therefore used to increase the sensitivity for PFPAs. With selection of $m/z$ 499 (the pseudomolecular ion of PFOPA) as target mass for EDC, an increase in sensitivity was obtained not only for PFOPA (+350%) but also for PFHxPA (+338%) and PFDPA (+43%). In addition, the chromatographic separation and detector response of PFPAs was optimized. In optimization of the chromatographic conditions four distinct columns (Ace3 C18, Advanced Chromatography Technologies, Aberdeen, Scotland; Eclipse plus C18 and Zorbax Extend C18, Agilent; Acquity UPLC BEH C18, Waters) were tested and mobile phases containing methanol, acetonitrile, and water at pH values between 3 and 11 (using formic acid and ammonium hydroxide, respectively) in the presence of 2 mM ammonium acetate were used. This did not give satisfactory results for PFPAs. However, addition of 1-MP as an ion-pairing agent to the mobile phase, resulting in a pH between 10 and 11, considerably improved both the chromatographic resolution and the instrumental response of PFPAs, and the baseline noise in the chromatogram was suppressed (Figs. 1 and 2). The Zorbax Extend C18 HPLC column was chosen because it is specially designed for applications at high pHs and excellent peak shapes for PFPAs were obtained. Additionally, also the PFCAs and PFSAs showed a distinctive sensitivity increase in the presence of 1-MP, which was especially pronounced for short chain compounds (Fig. 2).

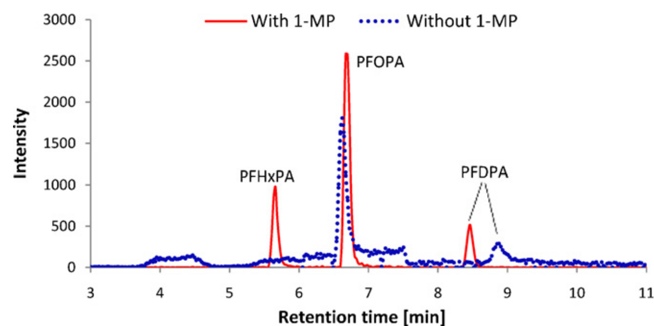


**Fig. 1.** Extracted HPLC/HRMS mass chromatograms of PFPAs (25 pg on column) with and without 1-MP in the mobile phase. A shorter retention time was observed for PFDPA in the presence of 1-MP.

### 3.3. Use of 1-methyl piperidine in HPLC/MS

In the present study, the use of 1-MP in the mobile phase was the key to highly sensitive analysis of PFPAs. The increase in sensitivity was based on (i) better chromatographic resolution resulting in sharper peaks, (ii) suppressed baseline noise (Fig. 1), and (iii) better detector response (Fig. 2). A drawback of 1-MP in the eluent is the resulting high pH, which may lead to depletion of the stationary phase of a silica-based column. However, earlier studies demonstrated that the solubility of silica supports at a pH around 11 is surprisingly low in certain buffers based on organic amines such as 1-MP, triethyl amine, and pyrrolidine [31]. In the present study 1-MP and triethyl amine were tested, both showing similar results. 1-MP was finally chosen because it has been reported that methanol-modified buffers at pH 11 made with 1-MP showed better column stability compared to buffers made with triethyl amine or potassium phosphate [32]. In HPLC 1-MP presumably acts in two ways. As an ion-pairing agent it masks the negative charges of the phosphonate group leading to an increase in the retention of PFPAs on a C18 stationary phase through hydrophobic interaction. Furthermore, the (protonated) amine group of 1-MP may sorb to negative charges on the silica surface, thus shielding the remaining active sites of the silica.

A high pH value of the HPLC mobile phase generally favors the formation of negatively charged ions in MS detection, leading to better sensitivity for acidic analytes. The superior effect of 1-MP on the MS response for the PFAAs (compared to e.g. ammonium hydroxide at the same pH) is probably attributable to the low escaping force, or fugacity, of the (protonated) 1-MP in the shrinking electrospray droplets. The ammonium ion would be depleted from the droplets through volatilization of ammonia, therewith driving the deprotonation of ammonium even further. In contrast, the concentration of 1-MP in the shrinking droplet remains unaltered, or even increases. Hence, the pH increasing effect of 1-MP likely remains up to the point when the droplets burst due to electrostatic repulsion.

### 3.4. Optimization of extraction

In the initial phase of method development, 500 mL of deionized Milli-Q water from a water purification unit (Millipore AB, Solna, Sweden) were spiked with different concentrations of native PFPAs, PFCAs, and PFSAs. However, compared to tap water samples, lower recoveries were achieved for the extraction of the target compounds from Milli-Q water due to the absence of ions. Additionally, considerable levels of PFCAs were found to be present in the Milli-Q water. Therefore, final method optimization and validation was performed with aliquots of 500 mL HPLC grade water to which 50 mg/L $NaHCO_3$ were added in order to mimic the ionic

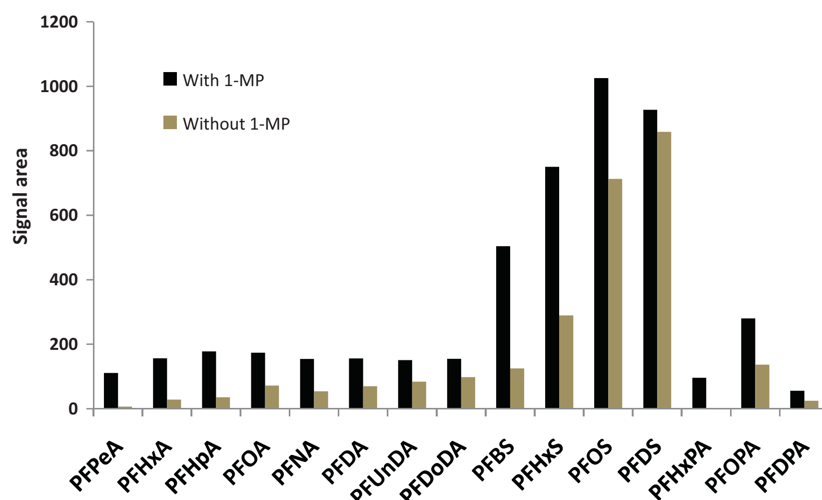


**Fig. 2.** Response enhancement of PFCAs, PFSAs, and PFPAs using 1-MP in the mobile phase. A total amount of 25 pg of each compound was injected on column.

strength of tap water. During the optimization of sample extraction, two different SPE cartridges with mixed mode co-polymeric sorbents, CSTHCM506 (C8 + primary amine, 500 mg–6 mL; UCT) and CUQAX256 (C8 + quaternary amine, 500 mg–6 mL; UCT) were tested, as well as two ion exchange cartridges, CUPSA156 (N-2 aminoethyl, 500 mg–6 mL; UCT) and Oasis WAX (weak anion exchange, 150 mg–6 mL; Waters). An aliquot of 500 mL water sample spiked with PFPAs, PFCAs, and PFSAs was applied to the SPE cartridges. The HPLC water as well as the tap water samples (typical pH around 8) were adjusted with formic acid to pH 7, as this resulted in better recoveries for most analytes compared to acidic or basic conditions. Various solvents, solvent mixtures and reagents consisting of methanol, acetonitrile, water, ammonia, ammonium acetate, formic acid, and 1-MP were tested for the washing and elution steps. Good recoveries for PFCAs and PFSAs were obtained with all tested columns; however, only the CUQAX256 column was found to also enrich the PFPAs. The Oasis WAX cartridge used by D'eon et al. [18] and by de Boer et al. [22] did not result in satisfactory recoveries for PFPAs in the present study. The quaternary amine function of the CUQAX256 sorbent may be responsible for the efficient retention of the phosphonates as well as the short chain PFCAs and PFSAs An elevated temperature of 60 °C of the elution solvent and addition of 1-MP helped in quantitatively recovering the PFPAs as well as the PFCAs and PFSAs from the CUQAX256 cartridge. Also perfluorobutanoate (C4 PFCA) was successfully trapped on the CUQAX256 column (data not shown). This compound was omitted from the complete method due to poor chromatographic resolution in some sample extracts.

### 3.5. Method validation

Achieved method detection limits (MDLs) and method limits of quantification (MLQs) are summarized in Table 2. MDLs were low, ranging between 0.014 and 0.17 ng/L for the different analytes including PFPAs. The reported MDLs directly reflect the sample concentration factor, injection volume and sensitivity of the instrument. Sample concentration factor and injection volume could easily be increased if lower MDLs were desirable. The MDL of PFOA was slightly elevated due to occasional procedural blank contamination (see Sections 2.2 and 3.1). The MLQs were in the sub ng/L range for all target compounds. Whole method linearity was evaluated with spiked water samples at five different concentrations between the individual MLQs and 20 ng/L. Excellent $r^2$ values of at least 0.99 (except for PFDPA with 0.98) were obtained (Table 2). This showed that extraction and sample processing recoveries were not concentration dependent.

Inter-day averages of whole method recoveries ($n$ = 3) and coefficients of variation (CVs) for all analytes spiked at 0.5 ng/L and at 5 ng/L to 500 mL HPLC grade water are given in Table 3. Recoveries for PFCAs and PFSAs were good (≥60%) except for the long chain compounds. Recoveries of 45–60% for PFUnDA, PFDoDA, and PFDS were considered satisfactory given the low water solubility of these compounds, which promotes the tendency to partition to boundary layers or to adsorb to vessel walls [30]. The recoveries of the mass-labeled IS of PFCAs and PFSAs spiked to the European tap water samples (results not shown) agreed well with the values for the native compounds determined for spiked HPLC water. The CVs showed good precision of the method except for the lower

**Table 2**
Method detection limit (MDL) and method limit of quantification (MLQ) as well as method linearity ($r^2$ values) for PFCAs, PFSAs, and PFPAs over the spike concentration range of the individual MLQs up to 20 ng/L in water (5 data points).

| | PFPeA | PFHxA | PFHpA | lin-PFOA | br-PFOA | PFNA | PFDA | PFUnDA | PFDoDA |
|---|---|---|---|---|---|---|---|---|---|
| MDL [ng/L] | 0.17 | 0.12 | 0.035 | 0.091 | 0.089 | 0.040 | 0.027 | 0.13 | 0.14 |
| MLQ [ng/L] | 0.53 | 0.38 | 0.12 | 0.30 | 0.30 | 0.13 | 0.090 | 0.43 | 0.45 |
| Linear regression | 0.995 | 0.994 | 0.999 | 1.000 | na | 0.999 | 1.000 | 1.000 | 0.991 |

| | PFBS | PFHxS | lin-PFOS | br-PFOS | PFDS | PFHxPA | PFOPA | PFDPA |
|---|---|---|---|---|---|---|---|---|
| MDL [ng/L] | 0.028 | 0.048 | 0.024 | 0.052 | 0.014 | 0.17 | 0.095 | 0.16 |
| MLQ [ng/L] | 0.092 | 0.16 | 0.080 | 0.17 | 0.045 | 0.52 | 0.32 | 0.51 |
| Linear regression | 0.995 | 0.999 | 1.000 | na | 0.996 | 0.989 | 0.997 | 0.982 |

na, not analyzed.

**Table 3**
Individual inter-day average recoveries ($n = 3$) and coefficient of variation (CV) for PFCAs, PFSAs, and PFPAs spiked at 0.5 ng/L and at 5 ng/L to 500 mL HPLC grade water.

| | PFPeA | PFHxA | PFHpA | PFOA | PFNA | PFDA | PFUnDA | PFDoDA |
|---|---|---|---|---|---|---|---|---|
| Recovery 0.5 ng/L [%] | 72 | 96 | 87 | 97 | 84 | 64 | 60 | 56 |
| CV [%] | 13 | 15 | 14 | 12 | 2 | 3 | 9 | 5 |
| Recovery 5 ng/L [%] | 84 | 86 | 89 | 82 | 86 | 64 | 52 | 45 |
| CV [%] | 7 | 8 | 10 | 14 | 10 | 8 | 8 | 15 |

| | PFBS | PFHxS | PFOS | PFDS | PFHxPA | PFOPA | PFDPA |
|---|---|---|---|---|---|---|---|
| Recovery 0.5 ng/L [%] | 77 | 64 | 60 | 55 | 53 | 56 | 40 |
| CV [%] | 17 | 8 | 5 | 25 | 5 | 18 | 43 |
| Recovery 5 ng/L [%] | 75 | 70 | 63 | 50 | 57 | 65 | 33 |
| CV [%] | 14 | 10 | 13 | 15 | 20 | 21 | 1 |

spiking level of PFDS, again due to the challenges with long chain PFAAs described here above. PFPAs spiked to HPLC grade water showed recoveries of 33–65%. However, these values were not representative for PFPA extraction from tap water. Calculated whole method recoveries of PFPAs from spiked tap water samples were almost consequently above 100% and up to 300% (Table 4). This is most probably due to a matrix effect from co-extracted compounds, enhancing the ionization of PFPAs in electrospray ionization in negative ion mode. It is not known why this effect was observed for all PFPAs but not for the other PFAAs. The effect seemed to be slightly more pronounced at the 0.5 ng/L spiking level compared to 5 ng/L. However, the whole method linearity was good even for PFPAs (Table 2), confirming that the matrix effect was independent of the PFPA concentration within the investigated concentration range. To compensate for this effect, matrix-matched and extracted external standards for each of the European tap water samples were used for quantification in the present study. The average recoveries and CVs of these spiked and extracted samples are given in Table 4. They show that the ionization enhancement effect is commonly present in tap water extracts with a similar magnitude in all samples. Since the present study targeted a first screening of PFPAs in European tap water, and only very low levels below the MLQ for PFOPA were found in two samples (see Section 3.6), the matrix effect was not further investigated or eliminated. On the contrary, the effect was exploited to achieve very low MDLs for PFPAs.

Accuracy of results for PFPAs was secured by using matrix-matched external calibration standards as described in Sections 2.2 and 2.4, and here above. This procedure is time consuming and for future work the use of isotopically mass-labeled PFPAs as IS to mitigate matrix effects is recommended. However, such standards are currently not available. Accuracy of results of the present method for PFCAs and PFSAs was investigated by analyzing a bulk water sample that had previously been used in an ILS [24]. Results are given in Table S2 in the supplementary data. The concentrations obtained with the present method are in agreement with median values from the ILS (relative deviations of 3–83%, Table S2), given the large relative standard deviations of the results reported in the ILS (35–160%, Table S2) and thus the high uncertainty of current methods for trace level analysis of PFAAs in water samples [30].

**Table 4**
Recoveries of PFPAs spiked to 500 mL Stockholm tap water at two different concentrations (single analysis) and average recoveries and coefficient of variation (CV) from the European tap water samples.

| | PFHxPA | PFOPA | PFDPA |
|---|---|---|---|
| *Stockholm tap water* | | | |
| Recovery at 0.5 ng/L [%] | 292 | 297 | 249 |
| Recovery at 5 ng/L [%] | 202 | 234 | 181 |
| *European tap water samples* | | | |
| Average recovery ($n = 7$) at 2 ng/L [%] | 285 | 225 | 152 |
| CV [%] | 32 | 33 | 27 |

### 3.6. European tap water samples

The present method was successfully applied to analyze PFAAs in tap water samples from seven research institutes situated in six European countries (Fig. S1 in the supplementary data). Extracted mass chromatograms of PFSAs, PFCAs, and spiked PFPAs (0.5 ng/L) from the tap water sample from Stockholm University, Sweden, are shown in Fig. 3. The results for all samples are summarized in Table 5 and in Fig. S2 in the supplementary data. PFPAs were not detected in any tap water sample apart from PFOPA in the two samples collected in Amsterdam. The PFOPA concentrations in the Amsterdam samples were close to the MDL of 0.095 ng/L. PFOPA has also been determined to be approximately 1 ng/L in a surface water sample from the same region [22]. Amsterdam municipality uses surface water for the production of drinking water. These results could therefore indicate that the drinking water purification may not be able to efficiently remove PFOPA. The present study together with the results reported by de Boer et al. [22] showed for the first

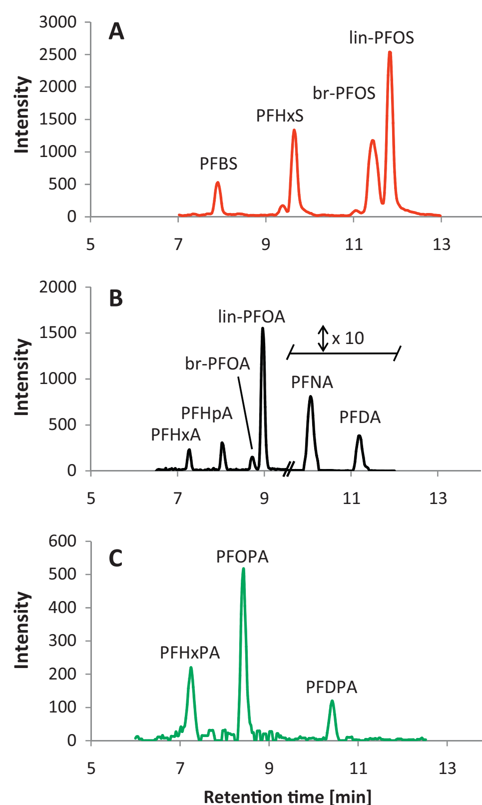


**Fig. 3.** Extracted HPLC/HRMS mass chromatograms of (A) PFSAs, (B) PFCAs, and (C) spiked (0.5 ng/L) PFPAs in a tap water sample from Stockholm University, Sweden. The signals of PFNA and PFDA in (B) are enlarged by a factor of 10 for better visibility. For quantified concentrations of PFCAs and PFSAs see Table 5.

**Table 5**
Concentrations of PFAAs in seven tap water samples collected from six European cities. For PFOA and PFOS the percentage of the linear isomer and sum of branched isomers (% lin/br) is additionally given.

| Sampling location | Concentrations of PFCAs [ng/L] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | PFPeA | PFHxA | PFHpA | PFOA (% lin/br) | PFNA | PFDA | PFUnDA | PFDoDA |
| SU[a] | nd[h] | 2.86 | 1.09 | 6.18 (92/8) | 0.433 | 0.506 | nd | <MLQ |
| IES[b] | <MLQ[i] | 2.10 | 1.19 | 4.92 (90/10) | 0.522 | 0.612 | nd | <MLQ |
| UA[c] | 1.39 | 3.00 | 0.996 | 2.70 (100/0) | 0.339 | 0.182 | nd | <MLQ |
| UvA[d] | 0.734 | 3.06 | 1.47 | 8.56 (80/20) | <MLQ | <MLQ | nd | <MLQ |
| VU[e] | 2.69 | 5.15 | 1.91 | 5.66 (65/35) | <MLQ | <MLQ | nd | <MLQ |
| NILU[f] | <MLQ | 0.806 | 0.434 | 2.20 (100/0) | <MLQ | 0.094 | <MLQ | <MLQ |
| FI[g] | <MLQ | <MLQ | <MLQ | 0.302 (100/0) | <MLQ | <MLQ | nd | <MLQ |

| Sampling location | Concentrations of PFSAs and PFPAs [ng/L] | | | | | | |
|---|---|---|---|---|---|---|---|
| | PFBS | PFHxS | PFOS (% lin/br) | PFDS | PFHxPA | PFOPA | PFDPA |
| SU[a] | 0.955 | 2.50 | 8.81 (68/32) | <MLQ | nd | nd | nd |
| IES[b] | 0.502 | 1.15 | 6.92 (74/26) | <MLQ | nd | nd | nd |
| UA[c] | 2.94 | 0.909 | 2.71 (62/38) | 0.074 | nd | nd | nd |
| UvA[d] | 7.61 | 0.556 | 0.861 (62/38) | 0.045 | nd | <MLQ | nd |
| VU[e] | 18.8 | 1.34 | 0.397 (58/42) | <MLQ | nd | <MLQ | nd |
| NILU[f] | <MLQ | <MLQ | 0.573 (70/30) | 0.195 | nd | nd | nd |
| FI[g] | 0.092 | <MLQ | 0.847 (71/29) | <MLQ | nd | nd | nd |

[a] SU: Stockholm University, Sweden.
[b] IES: Institute for Environment and Sustainability, Ispra, Italy.
[c] UA: University of Antwerp, Belgium.
[d] UvA: University of Amsterdam, The Netherlands.
[e] VU: VU University, Amsterdam, The Netherlands.
[f] NILU: Norwegian Institute for Air Research, Tromsø, Norway.
[g] FI: Fraunhofer Institute, Schmallenberg, Germany.
[h] nd: not detected (for method detection limits see Table 2).
[i] <MLQ: detected but below the method limit of quantification (Table 2).

time the presence of PFOPA in the European aquatic environment at concentrations similar to those in surface waters in Canada [18]. Additionally, this very low level of PFOPA was detected only in the Dutch samples in the present study, indicating that PFPA contamination of European drinking water is currently not of concern.

Among the PFCAs and PFSAs highest levels were found for PFBS (18.8 ng/L) in the sample from VU University, Amsterdam, for PFOA (8.56 ng/L) in University of Amsterdam water, and for PFOS (8.81 ng/L) in tap water from Stockholm University (Table 5 and Fig. S2). The highest level of PFBS in Amsterdam was in accordance with the concentration found in tap water collected from the same area [13]. Also the second sample from Amsterdam in the present study showed an elevated level of PFBS (7.61 ng/L). This may be indicative of a current PFBS source close to Amsterdam, of the increasing use of this chemical after the phase out of the production of PFOS related compounds by the 3 M company in 2002, and of the high water solubility of short chain PFAAs. In general, PFOA and PFHxA were the dominant contaminants in the analyzed tap water samples, with concentrations above 2 ng/L in all but one and all but two samples, respectively. These two compounds have also been found to be widely distributed in European rivers [33]. In addition to the compounds shown in Table 5 perfluorooctane sulfonamide was also analyzed in the tap water, but only found at a quantifiable concentration of 0.73 ng/L in the sample from Ispra, Italy. The concentrations determined in the present study for PFCAs and PFSAs in the Italian sample are in good agreement with earlier monitoring data from six tap water samples originating from the same region [16].

## 4. Conclusions

This is the first study to describe the use of 1-methyl piperidine in HPLC/QToF-HRMS analysis of PFAAs resulting in significantly better chromatographic resolution (especially for PFPAs) and increased detector response for all PFAAs due to improved ionization efficiency. The developed method was successfully applied to analyze a suite of 15 PFAAs (among them PFPAs for the first time) in European tap water samples. Contamination of European drinking water with PFPAs seems currently not to be a major problem. On the other hand, PFBS levels of up to 19 ng/L in tap water raise concern for the ongoing use of this PFOS substitute.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.07.005.

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