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RESEARCH PAPER

HPLC–MS/MS methods for the determination of 52 perfluoroalkyl and polyfluoroalkyl substances in aqueous samples

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Abstract Two quantitative methods using high-performance liquid chromatography (HPLC) combined with triple quadrupole tandem mass spectrometry (MS/MS) were developed to determine perfluoroalkyl and polyfluoroalkyl substances (PFASs) in aqueous samples. The first HPLC–MS/MS method was applied to 47 PFASs of 12 different substance classes with acidic characteristics such as perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkane sulfonic acids (PFSAs), as well as precursor substances and biotransformation intermediates (e.g., unsaturated fluorotelomer carboxylic acids). In addition, 25 ^{13}C-, ^{18}O-, and ^{2}H-labeled PFASs were used as internal standards in this method. The second HPLC–MS/MS method was applied to fluorotelomer alcohols (FTOHs) and perfluorooctane sulfonamidoethanols as these compounds have physicochemical properties different from those of the previous ones. Accuracy between 82% and 110% and a standard deviation in the range from 2% to 22% depending on the substances were determined during the evaluation of repeatability and precision. The method quantification limit after solid-phase extraction ranged from 0.3 to 199 ng/L depending on the analyte and matrix. The HPLC–MS/MS methods developed were suitable for the determination of PFASs in aqueous samples (e.g., wastewater treatment plant effluents or influents after solid-phase extraction). These methods will be helpful in monitoring campaigns to evaluate the relevance of precursor substances as indirect sources of perfluorinated substances in the environment. In one exemplary application in an industrial wastewater treatment plant, FTOHs were found to be the major substance class in the influent; in particular, 6:2-FTOH was the predominant compound in the industrial samples and accounted for 74% of the total PFAS concentration. The increase in the concentration of the transformation products of FTOHs in the corresponding effluent, such as fluorotelomer carboxylic acids, unsaturated fluorotelomer carboxylic acids, *n*:3 polyfluorinated saturated carboxylic acids (*n* indicates the number of nonfluorinated carbon atoms), and PFCAs, indicated biotransformation of FTOHs or their derivatives during wastewater treatment. However, only 33 mol% of the total amount of PFASs present in the influent was quantified in the corresponding effluent.

Keywords Perfluoroalkyl and polyfluoroalkyl substances · Wastewater treatment plants · High-performance liquid chromatography–electrospray ionization tandem mass spectrometry · Precursor compounds of perfluoroalkyl acids

Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are a class of anthropogenic chemicals that have been used in industrial and consumer applications for more than six decades [1, 2]. PFASs are classified as micropollutants, and may possess harmful, persistent, and bioaccumulative properties. Among these substances, perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkane sulfonic acids (PFSAs) have been studied most thoroughly, and the toxicological and ecotoxicological profiles of their C_8 homologs *n*-perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) have been compiled to a large extent [2–6]. Aside from a small number of exceptions, the application of PFOS

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was strictly regulated and forbidden by the European Union [7], and PFOA was included in the candidate list of substances of very high concern by the European Chemicals Agency [8]. The frequent use of PFASs in industrial and consumer products consequently led to an increase in the amount of PFCAs and PFSAs in the environment. Thus several studies dealing with the occurrence and exploring the sources of PFCAs and PFSAs in the environment as "direct sources" [9] were initiated. The abiotic transformation of precursor substances to PFCAs in the atmosphere as well as the biotransformation of these compounds by microorganisms were investigated and can be referred to as "indirect sources" [2, 10]. An increase in the concentration of certain PFASs in the effluent of wastewater treatment plants (WWTPs) compared with the corresponding influent was observed in numerous studies and indicated the transformation of precursor substances, which were not included in these studies [11–14]. Whereas individual PFASs have been measured frequently in diverse environmental compartments, only a small selection of whole PFASs were monitored, most notably the group of perfluoroalkyl acids (PFAAs), where all hydrogen atoms of the alkyl chain have been replaced by fluorine.

Several precursor compounds of PFCAs and PFSAs have been identified. These can be classified into a group consisting of fluorotelomer-based compounds, such as fluorotelomer alcohols (FTOHs), and a group containing non-fluorotelomer-based compounds, for instance, perfluoroalkane sulfonamides. Biotransformation of 8:2-FTOH by different microorganisms was investigated in several studies, and demonstrated the formation of PFOA and perfluorohexanoic acid, among other compounds [15–17]. Especially for the volatile FTOHs, abiotic transformation in the atmosphere also results in the formation of PFCAs [18, 19]. Further intermediates that were detected included unsaturated fluorotelomer carboxylic acids (FTUCAs), fluorotelomer carboxylic acids (FTCAs), fluorotelomer aldehydes, and polyfluorinated saturated carboxylic acids (*n*:3-acids). Additionally, biotransformation of other precursor substances, such as polyfluoroalkyl phosphates (PAPs), fluorotelomer methacrylates, and fluorotelomer acrylates, may lead to the cocktail of transformation products described above.

For the simultaneous quantification of multiple relevant precursor substances and PFASs in aqueous samples, the development of an analytical high-pressure liquid chromatography (HPLC)–tandem mass spectrometry (MS/MS) method was deemed necessary. The required analyte spectrum covered 12 different substance classes with 47 target compounds and an additional 25 ^{2}H-, ^{18}O-, and ^{13}C-labeled PFASs, used as internal standards. Fluorotelomer aldehydes, however, were not determined by this method because of their short lifetime [15]. In addition, a second method for the determination of relevant PFASs with a neutral characteristics, namely, FTOHs and perfluorooctane sulfonamidoethanols (FOSEs), by the same HPLC–MS/MS system needed to be developed. The structures, acronyms, and compound classes of the analytes in this study are shown in Table 1.

Experimental

Chemicals and reagents

Several standard mixtures of PFASs and individual substances with the highest purity available were obtained from Neochema (Bodenheim, Germany), Wellington Laboratories (Guelph, Canada), and DuPont (Wilmington, DE, USA). Two compounds were synthesized by the Hochschule Fresenius (Idstein, Germany) [20]. All compounds and labeled standards had a chemical purity of 98% or greater, except for perfluoroundecanoic acid and fluorotelomer ethoxycarboxylates ($FTEO_1Cs$) (purity 96% or greater) and perfluorododecanoic acid, perfluorotridecanoic acid, and perfluorotetradecanoic acid (purity 97% or greater). An overview and detailed information regarding the substances and the ^{13}C-, ^{18}O-, and ^{2}H-labeled internal standards are given in the electronic supplementary material. The spiking solutions were prepared in methanol (MeOH; ultra liquid chromatography–mass spectrometry grade, purity 99.95% or greater, Roth, Karlsruhe, Germany) and stored in glass vials protected from light at -18 °C. SupraSolv® acetone (purity 99.9% or greater), 2-propanol (liquid chromatography–mass spectrometry grade, purity 99.95% or greater), and ammonia (30%) were also obtained from Roth (Karlsruhe, Germany). Ammonium acetate (p.a., purity 99.0% or greater) purchased from Sigma-Aldrich (Buchs, Switzerland) was used for the eluents in the HPLC–MS/MS methods. All aqueous solutions and sample preparations were made with ultrapure water (Direct-Q3 system, Millipore, Milford, MA, USA) unless stated otherwise.

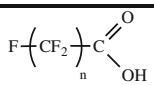
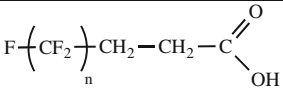
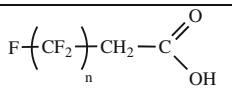
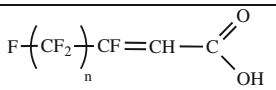
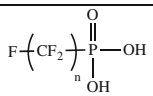
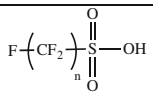
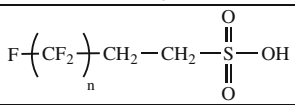
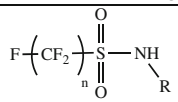
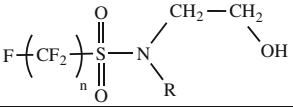
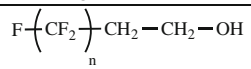
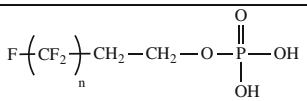
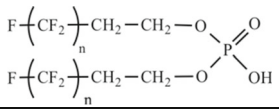
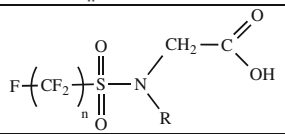
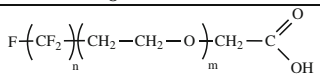
HPLC–MS/MS parameters

The inclusion of all analytes in only one HPLC–MS/MS experiment was impossible because of the different HPLC parameters needed, such as the flow rate and solvent composition of the injected samples.

The first HPLC–MS/MS method was applied for the analytes and isotopically labeled internal standards with acidic characteristics. The substances were measured as the deprotonated molecule following negative electrospray ionization (ESI). The temperature of the turbo heater gas (nitrogen 5.0) was set to 600 °C in this method. Because of the acidic characteristics of the analytes, this method is named "'HPLC–MS/MS-a."

A second HPLC–MS/MS method, namely, "'HPLC–MS/MS-n," where *n* refers to the neutral characteristic of the substances for which this method was used, allows the analysis of

Table 1 Structures, acronyms, and homologs of the analytes used in this study

Chemical Structures	Acronyms (class)	Compound class	Acronyms (compound)	Homologs
	PFCA	Perfluoroalkyl carboxylic acid	PF*X*A	n=3-13
	n:3 Acid	n:3 saturated acid	n:3-acid	n=3-7
	n:2-FTCA	n:2-Fluorotelomer acid	n:2-FTCA	n=6,8,10
	FTUCA	Unsaturated fluorotelomer acid	n:2-FTUCA	n=6,8,10
	PFPA	Perfluoroalkyl phosphonic acid	PF*X*PA	n=6, 8, 10
	PFSA	Perfluoroalkane sulfonic acid	PF*X*S	n=4, 6, 7, 8, 10
	FTS	Fluorotelomer sulfonic acid	n:2-FTS	n=4, 6, 8
	FASA	Perfluoroalkane sulfonamide	N-Me/N-Et*FX*SA	n=8; R=H, Me, Et
	FASE	Perfluoroalkane sulfonamido-ethanol	N-Me/N-Et*FX*SE	n=8 R=Me, Et
	FTOH	Fluorotelomer alcohol	n:2-FTOH	n=6, 8, 10
	mono-PAP	Monoalkylated fluorotelomer phosphate	n:2-PAP	n=6, 8
	di-PAP	Dialkylated fluorotelomer phosphate	n:2-diPAP	n=6, 8
	FASAA	Perfluoroalkane sulfonamido acetic acid	F*X*SAA	n=8 R=H, Me, Et
	FTEOC	Fluorotelomer ethoxycarboxylate	n:2-FTEO$_m$C	n=6, 8 m=1

Adapted with permission from [21]

Et ethyl, *Me* methyl, *X* represented the number of C of the alkyl-chain, *B* butyl, *Pe* pentyl, *Hx* hexyl, *Hp* heptyl, *O* octyl, *N* nonyl, *D* decyl, *UnD* undecyl, *DoD* dodecyl, *TrD* tridecyl, *TeD* tetradecyl

volatile 6:2-FTOH, 8:2-FTOH, 10:2-FTOH,*N*-MeFOSE, and *N*-EtFOSE. In negative ESI, acetate adducts are formed, which are not stable at high temperature. Therefore, the temperature of the turbo heater was reduced to 150 °C.

An HPLC system (series 200, PerkinElmer Norwalk, CT, USA) with a reversed-phase C_{18} column (MZ-Aqua Perfect C_{18}, 50 mm × 2.1 mm, 5 μm MZ Analysentechnik, Mainz, Germany) and a precolumn (MZ-Aqua Perfect C_{18}, 10 mm ×

2.1 mm, 5 μm, MZ Analysentechnik, Mainz, Germany) connected to a QTrap 3200 (Applied Biosystem, Foster City, CA, USA, software Analyst®, version 1.5.1 and MultiQuant®, version 3.0) hybrid triple-quadrupole linear ion trap tandem mass spectrometer equipped with an ESI source in negative ion mode (V = -4.5 kV) was used in this study. The mobile phases in both methods were 5:95 (v/v) MeOH–H_2O with 5 mM ammonium acetate (solvent A) and 95:5 (v/v) MeOH–H_2O with 5 mM ammonium acetate (solvent B).

The gradient of the HPLC–MS/MS-a method started at 100% solvent A at a flow rate of 300 μL/min for 0.5 min, changed to 65% solvent B in 2 min, changed to 100% solvent B in 10 min, and was maintained at that level for 5 min. At the end, the gradient was returned to the original conditions and the system was equilibrated for 10 min before the next run. The total run time was 27 min and the injection volume was 50 μL.

Because of the large number of multiple reaction monitoring (MRM) transitions with this method (in total 123), the application of scheduled MRM (sMRM) mode was crucial. A window of 90 s for each transition was used during the analysis, except for the "mixed" diPAPs (6:2/8:2-diPAP and 8:2/10:2-diPAP). No reference material was available for these compounds to determine the retention time (t_R). Therefore, these analytes were measured continuously. A summary of the MS/MS data, including the corresponding internal standard, molar mass, MRM transition for the qualifier and quantifier, and t_R of all the compounds, is given in Table 2. A second MRM transition could not be observed for all the compounds during method development.

The concentration of the isotopically labeled compounds in the internal standard mixture used in this method ranged from 0.1 to 1 ng/μL (see the electronic supplementary material). The gradient for the HPLC–MS/MS-n method started at 95% solvent A at a flow rate of 400 μL/min for 1.5 min, changed to 100% solvent B in 4 min, and remained at that level for 3 min. At the end, the gradient was returned to the original conditions and the system was equilibrated for 9 min before the next run. The total run time was 17 min. The injection volume for this method was 20 μL and the mass spectrometer was operated in MRM mode. The concentration of isotopically labeled M-6:2-FTOH and M-8:2-FTOH used in the HPLC–MS/MS-n method was 5 ng/μL in the internal standard mixture. A summary of the MS parameters, molar mass, and t_R is given in Table 3.

Background elimination

The prevention of contamination during the sample preparation, storage, and investigation is an important quality aspect in analysis of PFASs. All laboratory devices and vessels used were rinsed three times with MeOH before use. During all preparations and storage, contact of the spiking solution or sample with polyfluorinated or perfluorinated materials such as polytetrafluoroethylene was avoided to prevent contamination. An analytical HPLC column (MZ-Aqua Perfect, C_{18}, 50 mm × 2.1 mm, 5 μm) was used as a trapping column and was installed between the mobile phase mixing chamber and the injector of the HPLC system to reduce background contamination.

Calibration of the HPLC–MS/MS-a and HPLC–MS/MS-n methods

Two series of standards with ten concentrations, ranging from 0.05 to 48 ng/mL, were prepared in MeOH–H_2O (1:1; v:v) and were measured with the HPLC–MS/MS-a method in sMRM mode. Two series of standards with eight concentrations in the range from 1 to 500 ng/mL were prepared in MeOH for the calibration of the HPLC–MS/MS-n method. The first series of standards were measured in duplicate and the second series were measured in triplicate on two different days. The instrumental detection limit was determined by a signal-to-noise ratio of 3, and the instrumental quantification limit was determined by a signal-to-noise ratio of 9. Only concentrations with accuracy between 70% and 130% of the nominal concentration were used as the lowest concentration in the calibration curve. The ratio of the response (area) of MRM transitions (M_1/M_2) with an acceptance criterion of ±30% was an additional quality parameter for the concentration. Weighting by 1/x was used for the calibrations to assign a higher priority to the lower concentrations in both methods.

Repeatability and precision

Six individual standards in the concentration range from 5 to 25 ng/mL were used for the evaluation of the repeatability and precision of the HPLC–MS/MS-a method. The standards were prepared in MeOH–H_2O (1:1; v:v) and analyzed with the HPLC–MS/MS-a method. Six standards, prepared in pure MeOH and with a concentration of 50 ng/mL (FTOHs and FOSEs), were measured with the HPLC–MS/MS-n method to asses the repeatability and precision for this method [21].

Solid-phase extraction method

Effluent water samples from a municipal WWTP were fortified with all the target analytes for the two HPLC–MS/MS methods. An aliquot of 200 g of the water sample was filtered with a sucking filtration setup with Whatman GF/F glass micro filters (0.7-μm pore size, 4.7-cm diameter, Sigma-Aldrich, Buchs, Switzerland) and collected in a 500-mL high-density polypropylene (HDPE) bottle with a narrow neck. The aliquot was spiked with all the internal standards for the two HPLC–MS/MS methods (10 μL of the HPLC–MS/MS-a internal standard mixture and 10 μL of the HPLC–MS/MS-n internal standard mixture respectively), the bottle was closed with a screw cap, and the solutions were intensively mixed with a

Table 2 Summary of the data for the high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS)-a method (see the text for details)

Compound	Molar mass (g/mol)	MRM transition (*m/z*, $[M-H]^-$)		Internal standard	t_R (min)
		Quantifier	Qualifier		
PFBA	214	213 → 169	-	MPFBA	3.9
PFPeA	264	263 → 219	-	MPFPeA	4.3
PFHxA	314	313 → 269	313 → 119	MPFHxA	4.6
PFHpA	364	363 → 319	363 → 169	MPFHpA	5.0
PFOA	414	413 → 369	413 → 169	MPFOA	5.4
PFNA	464	463 → 419	463 → 169	MPFNA	6.0
PFDA	514	513 → 469	513 → 269	MPFDA	6.9
PFUnDa	564	563 → 519	563 → 319	MPFUnDa	7.7
PFDoDa	614	613 → 569	613 → 219	MPFDoDa	8.5
PFTrDa[a]	664	663 → 619	663 → 169	MPFDoDa	9.1
PFTeDa[a]	714	713 → 669	713 → 169	MPFDoDa	9.8
6:2-FTCA	378	377 → 243	377 → 63	M-6:2-FTCA	5.1
8:2-FTCA	478	477 → 63	477 → 393	M-8:2-FTCA	6.4
10:2-FTCA	578	577 → 63	577 → 493	M-10:2-FTCA	8.0
6:2-FTUCA	358	357 → 293	-	M-6:2-FTUCA	5.1
8:2-FTUCA	458	457 → 393	-	M-8:2-FTUCA	6.3
10:2-FTUCA	558	557 → 493	-	M-10:2-FTUCA	8.0
PFHxPA	400	399 → 79	-	M-(Cl)PFHxPA	4.3
PFOPA[a]	500	499 → 79	-	M-(Cl)PFHxPA	5.0
PFDPA[a]	600	599 → 79	-	M-(Cl)PFHxPA	6.2
3:3-Acid[a]	242	241 → 117	241 → 177	MPFHxA	4.3
4:3-Acid[a]	292	291 → 167	291 → 187	MPFHxA	4.7
5:3-Acid[a]	342	341 → 217	341 → 237	MPFHxA	5.1
6:3-Acid[a]	392	391 → 267	391 → 287	MPFOA	5.6
7:3-Acid[a]	442	441 → 317	441 → 337	MPFOA	6.2
PFBS[a]	300	299 → 99	299 → 80	MPFHxA	4.3
PFHxS	400	399 → 80	399 → 99	MPFHxS	5.0
PFHpS[a]	450	449 → 80	449 → 99	MPFOA	5.4
PFOS	500	499 → 80	499 → 99	MPFOS	6.0
PFDS[a]	600	599 → 80	599 → 99	MPFUnDa	7.6
4:2-FTS[a]	328	327 → 81	327 → 307	M-6:2-FTS	4.6
6:2-FTS	428	427 → 81	-	M-6:2-FTS	5.4
8:2-FTS[a]	528	527 → 81	-	M-6:2-FTS	6.8
FOSAA[a]	557	556 → 498	556 → 78	M-*N*-MeFOSAA	6.7
N-MeFOSAA	571	570 → 169	570 → 219	M-*N*-MeFOSAA	7.3
N-EtFOSAA	585	584 → 419	584 → 169	M-*N*-EtFOSAA	7.8
6:2-$FTEO_1C$[a]	422	421 → 75	421 → 255	MPFOA	5.6
8:2-$FTEO_1C$[a]	522	521 → 75	521 → 355	MPFDA	7.2
6:2-PAP[a]	444	443 → 79	443 → 97	M-8:2-PAP	5.8
8:2-PAP	544	543 → 97	543 → 79	M-8:2-PAP	6.9
6:2-DiPAP[A]	790	789 → 97	789 → 79	M-8:2-diPAP	9.4
8:2-DiPAP	990	989 → 97	989 → 79	M-8:2-diPAP	11
FOSA[a]	499	498 → 78	-	M-*N*-MeFOSA	6.7
N-MeFOSA	513	512 → 169	512 → 219	M-*N*-MeFOSA	7.3
N-EtFOSA	527	526 → 219	527 → 219	M-*N*-EtFOSA	7.8
6:2/8:2-diPAP	890	889 → 97	889 → 79	-	-
8:2/10:2-diPAP	1090	1089 → 97	1098 → 79	-	-

Adapted with permission from [21]

MRM multiple reaction monitoring, t_R retention time

[a] Compounds for which no isotopically labeled internal standard was available. Existing internal standards with a similar retention time (t_R) and/or structure are assigned to these analytes. No second MRM transition was determined for PFBA, PFPeA, FTUCAs, PFPAs, 6:2-FTS, 8:2-FTS, and FOSA

Table 3 Summary of the data for the HPLC–MS/MS-n method (see the text for details)

Substance	Molar mass (g/mol)	MRM transition (m/z, $[M + Ac]^-$)	Internal standard	t_R (min)
6:2-FTOH	364	423 → 59	M-6:2-FTOH	8.7
8:2-FTOH	464	523 → 59	M-8:2-FTOH	9.9
10:2-FTOH[a]	564	623 → 59	M-8:2-FTOH	10.7
N-MeFOSE[a]	557	616 → 59	M-8:2-FTOH	10.0
N-EtFOSE[a]	571	630 → 59	M-8:2-FTOH	10.3

Adapted with permission from [21]

Ac acetate, *MRM* multiple reaction monitoring, t_R retention time

[a] Compounds for which no isotopically labeled internal standard was available. Existing internal standards with a similar t_R are assigned to these analytes

vortex mixer. Following conditioning of an Oasis WAX® sold-phase extraction (SPE) cartridge (60 mg, 3 cm^3, Waters, Milford, MA, United States) with 2 mL MeOH containing 0.1% NH_3, 2 × 2 mL MeOH, and 6 mL H_2O, the sample was passed through the cartridge with the aid of a membrane pump at a flow rate of approximately one drop per second and cleaned with 3 mL H_2O–MeOH (80:20; v:v). Afterward, the cartridge was dried for 10 min by a gentle stream of nitrogen. In the first elution step, the target compounds for the HPLC–MS/MS-n method (FTOHs, *N*-MeFOSE, and *N*-EtFOSE) were eluted with 2 × 1 mL MeOH, and the solvent was reduced to a final volume of 500 μL by application of a nitrogen stream. The sample was filtered with a regenerated cellulose syringe filter with pore size of 0.45 μm (Schleicher & Schuell, Dassel, Germany) to remove all solids, transferred into an HPLC injection vial (500 μL, polypropylene), and analyzed with the HPLC–MS/MS-n method to determine the FTOHs and FOSEs. The same eluate was also measured by the HPLC–MS/MS-a method to determine perfluoro-1-octanesulfonamide (FOSA) and the derivatives (*N*-MeFOSA and *N*-EtFOSA). In the second elution step, the target compounds with an acidic characteristic were eluted with 2 × 1 mL MeOH containing 1% NH_3 into a glass vial. The eluate was vaporized at 50 °C by a gentle nitrogen stream, and the resulting residue was redissolved in 250 μL MeOH. After vortex mixing for 1 min, the eluate was added to 250 μL H_2O, mixed, and filtered with a syringe filter. Following transfer into an HPLC injection vial (polypropylene), the sample was measured with the HPLC–MS/MS-a method. Blanks (H_2O), which contained only the internal standards, were prepared and analyzed simultaneously.

For the determination of percent recoveries, 200-mL aliquots of selected samples were spiked before SPE with target analytes as follows: 50 μL of spiking solution containing analytes for the HPLC–MS/MS-a method at a concentration of 0.1 ng/μL and 10 μL spiking solution containing analytes for the HPLC–MS/MS-n method at a concentration of 5 ng/μL. Afterward, the samples were filtered and fortified with the internal standard mixtures used in the two HPLC–MS/MS methods as described earlier.

Sampling sites

All aqueous samples were collected in 1-L HDPE bottles, because no potential absorption of higher molecular weight compounds to the HDPE bottle wall occurs [22]. The samples were stored at -18 °C in the dark until analysis. One effluent water sample from a municipal WWTP in Germany and one 24-h composite influent sample and the corresponding 24-h composite effluent sample of an industrial WWTP in Europe were collected and used in this study.

Because of the extraordinarily high concentration of PFASs in the industrial samples, no enrichment via SPE was necessary. An aliquot of each sample was spiked with the internal standards used for the HPLC–MS/MS-a method, mixed with MeOH (1:1; v:v), filtered with a syringe filter, and analyzed with the HPLC–MS/MS-a method. The samples were prepared and analyzed in duplicate. One influent sample and the corresponding effluent sample of an industrial WWTP were spiked with the internal standards M-6:2-FTOH and M-8:2-FTOH, mixed with MeOH (1:1; v:v), filtered, and measured immediately after the sample preparation with the HPLC–MS/MS-n method. The influent samples, which were analyzed with the HPLC–MS/MS-n method, were prepared in triplicate and the corresponding effluent samples were prepared in duplicate.

Results and discussion

Method development

The gradient profile of a published HPLC method [23] was optimized so as to determine a high number of target analytes within an acceptable run time of 27 min. A consequence of this optimization was the improvement of the peak shape of PFCAs, in particular the short-chain *n*-perfluorobutanoic acid (PFBA) and *n*-perfluoropentanoic acid. The problem with the peak shape of PFBA during the chromatographic separation

is already known from the literature [24–26]. The first 18 min of the two gradient profiles and the corresponding chromatograms of selected PFCAs (C_4–C_{10}) are shown in Fig. 1. The chromatogram of a 12 ng/mL standard, analyzed with the gradient profile (Fig. 1a) from [23] is compared with the chromatogram of a 10 ng/mL standard measured with the newly developed gradient profile (Fig. 1b). The chromatogram shows only one MRM transition for each of the selected PFCAs. The other target analytes and the internal standards used in this method are not included in Fig. 1. The PFBA signal of the previous gradient profile (Fig. 1a, 4.2–7.3 min) is magnified tenfold to assess the differences in the peak shape clearly.

The peak shape for the short-chain PFCAs (C_4–C_7) and for PFOA, *n*-perfluorononanoic acid, and *n*-perfluorodecanoic acid was improved significantly by the application of the new gradient. A comparison of the width at half peak height of the previous HPLC method and the optimized method as well as the tailing factors, which were determined at 5% peak height, is given in Table 4.

The correlation coefficient (*r*) of the calibration curves was 0.99 or greater, except for 8:2-FTEO$_1$C, 3:3-acid, 8:2-FTCA, and 10:2-FTCA, which showed *r* between 0.97 and 0.98. Because of the lack of a certified reference, the mixed diPAPs (6:2/8:2-diPAP and 8:2/10:2-diPAP) could be analyzed only qualitatively. The MRM transitions of these compounds were determined on the basis of the MRM parameters of the diPAPs. The mixed diPAPs were measured in MRM mode and not in sMRM mode, because of the unknown retention time. The individual standards, used for the evaluation of the repeatability and precision of the HPLC–MS/MS-a method, showed accuracy between 82% and 110% and coefficients of variation (CV) in the range from 2% to 22%. Because of the different sensitivities, the instrumental quantification limits of the analytes differed by a factor of 100. As a consequence, three concentrations (5 ng/mL, 10 ng/mL, and 25 ng/mL) of standards were used for the evaluation. The accuracy of the six individual standards (50 ng/mL) for the evaluation of the HPLC–MS/MS-n method was between 89% and 105% and the CV ranged from 6% to 8%. The method detection limit (MDL) and the method quantification limit (MQL) to analyze water samples without enrichment and the accuracy and CV of the validation parameters for the HPLC–MS/MS methods are shown in Table 5. The instrumental detection limit and instrumental quantification limit can be estimated from the MDL and MQL by division by 2.

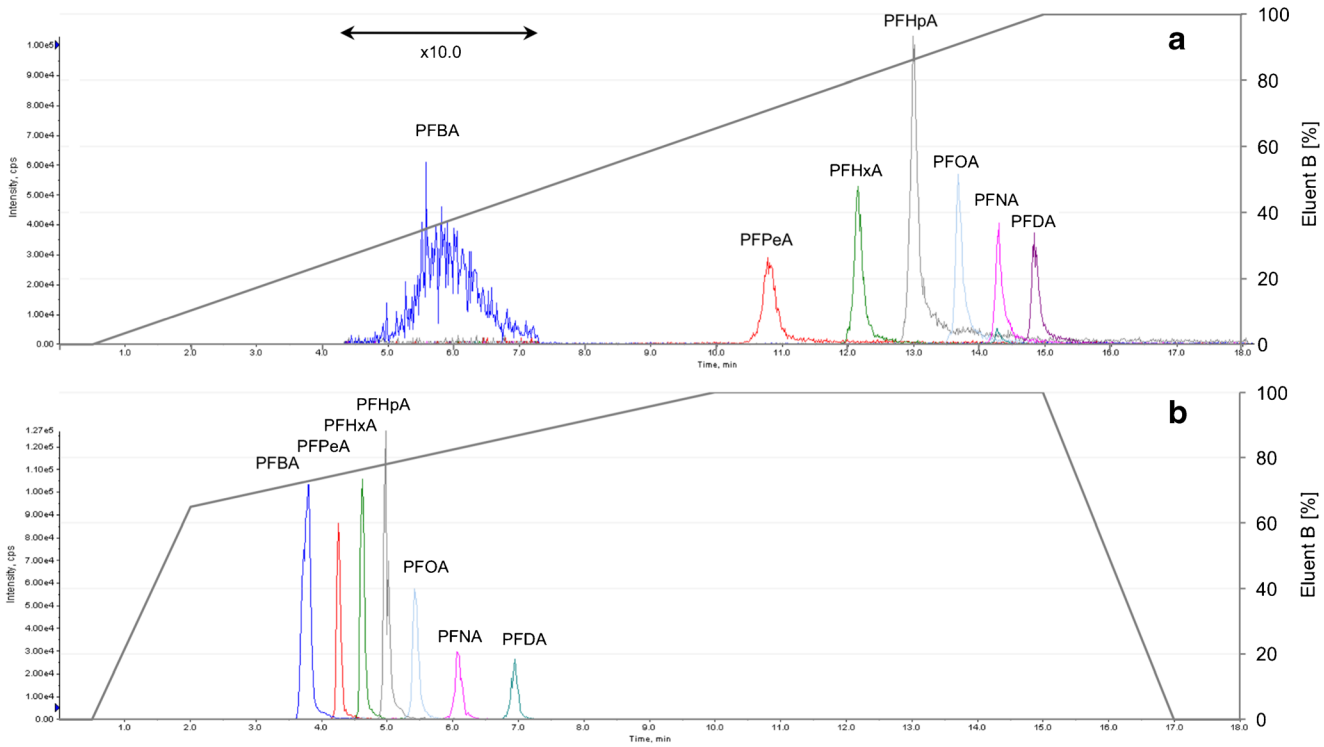


Fig. 1 Extracted ion chromatograms of perfluoroalkyl carboxylic acids (C_4–C_{10}) obtained with a previous gradient profile (**a**) compared with the newly developed gradient profile (**b**). The *n*-perfluorobutanoic acid (*PFBA*) signal obtained with the previous gradient profile (**a**, 4.2–7.3 min) is magnified tenfold. *PFDA* *n*-perfluorodecanoic acid, *PFHpA* *n*-perfluoroheptanoic acid, *PFHxA* *n*-perfluorohexanoic acid, *PFNA* n-perfluorononanoic acid, *PFOA* *n*-perfluorooctanoic acid, *PFPeA* *n*-perfluoropentanoic acid. (Adapted with permission from [21])

Table 4 Comparison of the width at half peak height ($W_{0.5}$) and the tailing factor (*TF*; at 5% peak height) of a previous method and the improved method

Analyte	Method adapted from [23]		Optimized method	
	$W_{0.5}$	TF	$W_{0.5}$	TF
PFBA	0.874	2.22	0.145	0.96
PFPeA	0.208	1.69	0.083	1.11
PFHxA	0.141	1.45	0.088	1.20
PFHpA	0.132	2.25	0.105	1.37
PFOA	0.121	1.99	0.112	1.26
PFNA	0.119	2.03	0.120	1.18
PFDA	0.138	2.58	0.123	1.02

Solid-phase extraction

The elution from the solid phase was done in two steps as described "Experimental." Following the first elution step with MeOH, the FTOHs and FOSEs as well as the internal standards (M-6:2-FTOH and M-8:2-FTOH) were quantified by the HPLC–MS/MS-n method. Reanalysis of the same eluate by the HPLC–MS/MS-a method allowed the determination of FOSA, *N*-MeFOSA, and *N*-EtFOSA, together with corresponding internal standard (M-*N*-MeFOSA) for these analytes. The recovery rate of the compounds in the HPLC–MS/MS-n method ranged from 86% to 121%. The absolute recoveries without consideration of the internal standards are given

Table 5 Method detection limit (*MDL*) and method quantification limit (*MQL*) of the HPLC–MS/MS methods for the determination of perfluoroalkyl and polyfluoroalkyl substances in water samples by direct injection. Accuracy and coefficient of variation (*CV*) of individual standards investigated for the determination of repeatability and precision; $n = 6$

Analyte	MDL (ng/mL)	MQL (ng/mL)	CV (%)	Accuracy (%)	Analyte	MDL (ng/mL)	MQL (ng/mL)	CV (%)	Accuracy (%)
PFBA[a]	0.1	0.2	4	102	5:3-Acid[a]	0.2	1.0	10	97
PFPeA[a]	0.2	1.0	10	96	6:3-Acid[b]	1.0	10	8	82
PFHxA[a]	0.1	0.2	8	110	7:3-Acid[b]	1.0	10	6	83
PFHpA[a]	0.2	1.0	6	102	PFBS[a]	0.1	0.2	6	107
PFOA[a]	0.1	0.2	5	99	PFHxS[a]	0.1	0.5	7	103
PFNA[a]	1.0	2.0	11	98	PFHpS[a]	0.1	1.0	10	90
PFDA[a]	0.5	1.0	9	100	PFOS[a]	0.1	1.0	4	101
PFUnDa[a]	0.5	2.0	2	90	PFDS[a]	0.5	1.0	12	85
PFDoDa[a]	1.0	2.0	11	96	4:2-FTS[a]	0.5	2.0	5	109
PFTrDa[a]	0.5	1.0	8	98	6:2-FTS[a]	0.2	1.0	9	101
PFTeDa[a]	0.5	1.0	12	98	8:2-FTS[a]	0.5	1.0	15	96
6:2-FTCA[b]	2.0	10	22	97	FOSA[a]	0.1	0.2	8	110
8:2-FTCA[c]	10	20	2	99	*N*-MeFOSA[b]	1.0	10	8	96
10:2-FTCA[c]	10	20	6	110	*N*-EtFOSA[a]	0.5	2.0	5	100
6:2-FTUCA[a]	0.1	0.5	9	97	FOSAA[a]	0.5	2.0	13	103
8:2-FTUCA[a]	0.1	2.0	5	104	*N*-MeFOSAA[b]	2.0	10	6	100
10:2-FTUCA[b]	1.0	10	8	105	*N*-EtFOSAA[b]	1.0	10	7	96
PFHxPA[a]	0.5	2.0	7	109	6:2-FTEO$_1$C[b]	0.5	10	4	88
PFOPA[b]	2.0	10	8	101	8:2-FTEO$_1$C[c]	10	20	9	94
PFDPA[c]	10	20	2	94	6:2-PAP[b]	2.0	10	11	104
3:3-Acid[c]	10	20	8	96	8:2-PAP[b]	2.0	10	7	106
4:3-Acid[a]	0.5	2.0	12	93	6:2-diPAP[b]	0.5	10	12	103
6:2-FTOH[d,e]	5.0	10	8	98	8:2-diPAP[a]	0.1	1.0	8	103
8:2-FTOH[d,e]	2.0	10	7	93	*N*-MeFOSE[d,e]	<2	10	7	102
10:2-FTOH[d,e]	2.0	10	6	89	N-EtFOSE[d,e]	< 2	10	6	105

The instrumental detection limit and the instrumental quantification limit can be calculated from the MDL and MQL by division by 2

[a] Concentration of 5 ng/mL

[b] Concentration of 10 ng/mL

[c] Concentration of 25 ng/mL

[d] Concentration of 50 ng/mL

[e] Measured with the HPLC–MS/MS-n method

in the electronic supplementary material. Because of the use of the two isotopically labeled internal standards, the matrix effects were compensated for 6:2-FTOH and 8:2-FTOH, and consequently excellent recovery rates were achieved for these two substances. Since 10:2-FTOH showed a retention time different from that of the corresponding internal standard M-8:2-FTOH, a lower recovery rate and a higher standard deviation were calculated. The recovery rate of the SPE method for the two HPLC–MS/MS methods is shown in Fig. 2. The absolute recoveries of the compounds and internal standards are given in the electronic supplementary material.

The remaining analytes in the solid phase were eluted in a second elution step with MeOH with 1% NH_3 and consisted of all the other analytes determined with the HPLC–MS/MS-a method except for FOSA, *N*-Me-FOSA, and *N*-EtFOSA. Because of the high MQL of 8:2-FTCA, 10:2-FTCA, *n*-perfluorodecanoic acid, 3:3-acid, and 8:2-$FTEO_1C$, the recovery rates of these analytes can be considered as only semi-quantitative. The recovery rates of the PFCAs ranged from 70% to 149%, except for *n*-perfluorotetradecanoic acid (53%), in the spiked water sample. The recovery rates of FTCAs, FTUCAs, $FTEO_1Cs$, FOSAs, perfluoro-1-octanesulfonamidoacetic acids (FOSAAs), PAPs, PFSAs, 1*H*,1*H*,2*H*,2*H*-perfluorooctane sulfonate (6:2-FTS), 1*H*,1*H*,2*H*,2*H*,-perfluorodecane sulfonate (8:2-FTS), perfluorohexylphosphonic acid, and 8:2-diPAP ranged from 72% to 145%. The recovery rates of sodium perfluoro-1-heptanesulfonate, perfluorooctylphosphonic acid, and perfluorodecylphosphonic acid were extraordinarily high, ranging from 115% to approximately 240% (Fig. 2). This could be due to the coextracted compounds that enhance the ionization of PFPAs during the negative ESI process. This phenomenon was observed in a previous study where selected PFASs in drinking water (tap water) were determined [27] and can be even more pronounced by the matrix effect of the effluent samples. The matrix effect can be compensated for only by a structurally identical isotopically labeled internal standard. Different absorption properties of the corresponding internal standard and the analyte might lead to too high or too low recoveries if the structure is not equivalent. In this study, only perfluorohexylphosphonic acid had such an internal standard. Compared with the reference standard, the peak area of the corresponding internal standard M-8:2-diPAP decreased significantly, and thus an extraordinary high recovery for 6:2-diPAP was observed. Because of the very high value, the recovery rate of 6:2-diPAP (490%) is excluded from Fig. 2. Several interferences, such as the sulfite radical anion at *m*/*z* 80, which is a common fragment of sulfonates, disturbed the quantifier MRM transition of perfluorobutane sulfonic acid (PFBS) (*m*/*z* 299 → 80) [28]. Therefore, the more selective MRM transition *m*/*z* 299 → 99, which represents FSO_3^-, was used as the quantifier transition as well as for the recovery evaluation of PFBS.

The recovery rates for 1*H*,1*H*,2*H*,2*H*-perfluorohexane sulfonate and *n*:3-acids ranged from 12% to 104%.

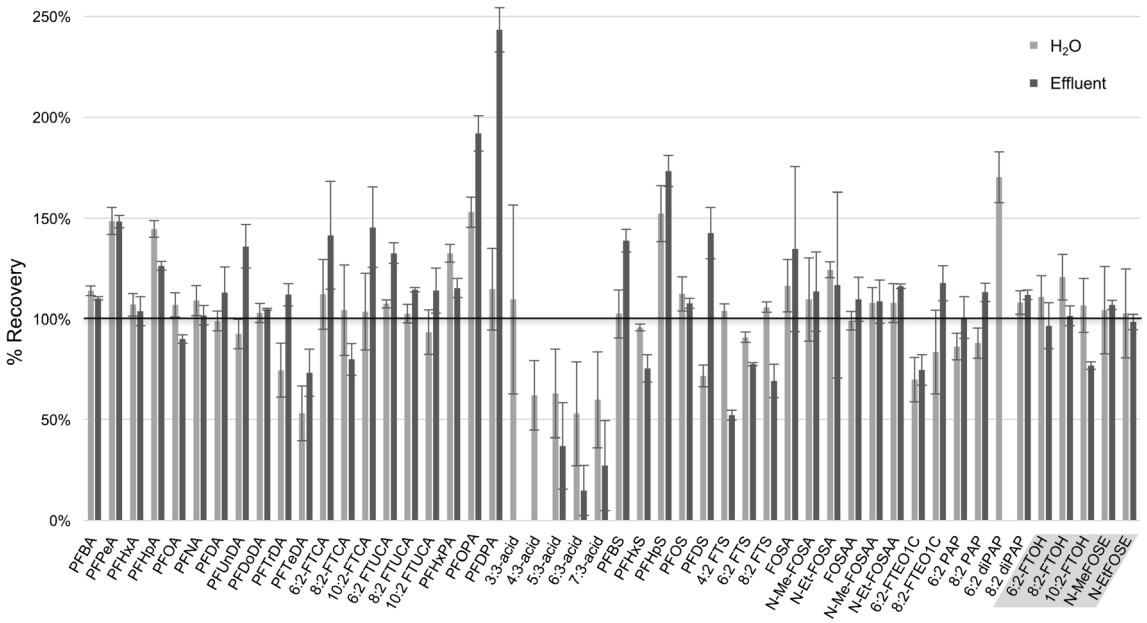


Fig. 2 Recovery rate of spiked water samples (ultrapure water and effluent from a municipal wastewater treatment plant) after solid-phase extraction; compared with a standard [10 ng/mL for the analytes measured with the high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS)-a method (see the text for details), 50 ng/mL for the analytes measured with the HPLC–MS/MS-n method (see the text for details), names *shaded in gray*]. *Error bars* indicate the standard deviation ($n = 3$)

Table 6 MDL and MQL of the sold-phase extraction method and the enrichment from different matrices

Analyte	Water sample		Effluent sample		Analyte	Water sample		Effluent sample	
	MDL (ng/L)	MQL (ng/L)	MDL (ng/L)	MQL (ng/L)		MDL (ng/L)	MQL (ng/L)	MDL (ng/L)	MQL (ng/L)
PFBA	0.2	0.4	2.5	5.0	5:3-Acid	2.2	4.3	6.3	13
PFPeA	0.3	0.6	2.3	4.7	6:3-Acid	1.7	3.3	2.6	5.3
PFHxA	0.4	2.2	1.3	6.3	7:3-Acid	1.7	3.3	2.6	5.3
PFHpA	0.6	3.2	1.2	6.2	PFBS	0.2	0.4	0.6	1.3
PFOA	0.2	1.7	0.3	2.6	PFHxS	0.1	1.3	0.1	1.4
PFNA	0.2	1.9	0.3	3.5	PFHpS	0.3	1.7	0.5	2.6
PFDA	0.3	1.7	0.9	4.5	PFOS	0.3	1.3	0.4	2.2
PFUnDa	1.7	3.4	6.4	12	PFDS	0.3	1.7	1.3	6.4
PFDoDa	2.6	5.2	5.8	11	4:2-FTS	0.3	1.4	0.3	1.3
PFTrDa	2.6	5.2	5.8	11	6:2-FTS	0.1	0.3	0.1	0.3
PFTeDa	2.6	5.2	5.8	11	8:2-FTS	1.4	2.9	1.3	2.6
6:2-FTCA	10	51	22	110	FOSA	0.9	4.4	5.5	28
8:2-FTCA	42	84	72	143	*N*-MeFOSA	4.4	8.8	28	55
10:2-FTCA	51	102	100	199	*N*-EtFOSA	3.5	7.1	32	63
6:2-FTUCA	0.6	2.9	2.0	9.8	FOSAA	1.8	3.5	4.8	9.6
8:2-FTUCA	0.5	2.7	1.4	6.9	*N*-MeFOSAA	1.8	3.5	4.8	9.6
10:2-FTUCA	3.3	6.6	7.4	14.8	*N*-EtFOSAA	1.7	3.3	5.1	10.2
PFHxPA	0.1	0.3	0.1	0.3	6:2-FTEO$_1$C	8.3	17	13	26
PFOPA	0.3	1.3	0.3	1.3	8:2-FTEO$_1$C	3.4	17	8.9	45
PFDPA	1.3	2.6	1.3	2.6	6:2-PAP	2.6	13	3.1	16
3:3-Acid	22	43	63	126	8:2-PAP	2.6	13	3.1	16
4:3-Acid	2.2	4.3	6.3	12.5	6:2-diPAP	1.2	5.9	5.0	25
6:2-FTOH[a]	6.6	20	4.7	14	8:2-diPAP	1.2	5.9	5.0	25
8:2-FTOH[a]	5.5	10.6	3.7	7.1	*N*-MeFOSE[a]	5.5	10.6	3.7	7.1
10:2-FTOH[a]	5.5	10.6	3.7	7.1	*N*-EtFOSE[a]	5.5	10.6	3.7	7.1

[a] Measured with the HPLC–MS/MS-n method

Not all matrix effects could be compensated for by the corresponding internal standards because of the different properties compared with the corresponding analytes. In addition, the matrix of the enriched ultrapure water samples and the enriched effluent samples was very different. Therefore, the MDL and the MQL for the two spiked matrices were estimated by the following formula:

$$\text{Method limit} = F \times \text{instrumental limit}/\text{recovery of internal standard},$$

where F is the enrichment factor.

The recovery rate of the internal standard was determined for each individual isotopically labeled compound in each sample. Therefore, the average of the internal standard peak areas was divided by the average of the internal standard peak areas of the solvent standards, which were measured in the same sample set. The MDL and MQL of the SPE method are given in Table 6. The absolute recoveries of the internal standard are given in the electronic supplementary material.

Several HPLC–MS/MS methods for determination of PFASs in aqueous samples have been reported in the literature. The comparison of these methods, regarding selectivity and sensitivity, is very complicated because of the numbers of analytes, the SPE material used, the sample and injection volume, the number of internal standards, etc. The method reported by Ahrens et al. [29] showed lower MQLs for the PFCAs, compared with the MQLs in this study, in the range from 0.01 to 0.63 ng/L, for the PFSAs a similar MQL range from 0.2 to 1.7 ng/L, and for the only fluorotelomer sulfonic acid analyzed (6:2-FTS) a higher MQL (0.7 ng/L). Kim et al. [30] reported MDLs in the same range as the

Table 7 Summary of perfluoroalkyl and polyfluoroalkyl substances determined in the influent and the corresponding effluent of an industrial wastewater treatment plant by the HPLC–MS/MS-a method

Analyte	Influent A (μg/L)	Influent B (μg/L)	Average (μg/L)	Effluent A (μg/L)	Effluent B (μg/L)	Average (μg/L)
PFBA	22.9	22.0	22.5	11.8	11.4	11.6
PFPeA	20.4	18.7	19.6	11.3	11.0	11.1
PFHxA	5.96	5.50	5.73	51.5	47.1	49.3
PFOA	3.00	3.20	3.10	3.27	3.08	3.18
PFNA	<MQL	<MQL	-	<MQL	<MQL	-
PFDA	1.06	1.06	1.06	0.55	<MQL	0.4[a]
PFUnDa	1.86	2.34	2.1	ND	ND	-
PFTrDa	1.32	1.58	1.45	ND	ND	-
6:2-FTCA	<MQL	<MQL	-	8.62	6.78	7.7
6:2-FTUCA	0.94	1.02	0.98	8.91	8.92	8.92
8:2-FTUCA	<MQL	<MQL	-	1.40	1.33	1.37
4:3-Acid	ND	<MQL	-	<MQL	<MQL	-
5:3-Acid	4.48	4.88	4.68	7.27	7.43	7.35
6:3-Acid	<MQL	<MQL	-	n.d.	<MQL	-
7:3-Acid	<MQL	<MQL	-	<MQL	<MQL	-
PFBS	0.54	0.64	0.59	ND	ND	-
6:2-FTS	ND	ND	-	<MQL	<MQL	-
6:2-$FTEO_1C$	ND	<MQL	-	ND	<MQL	-

Adapted with permission from [21]. Because of high instrumental background levels, the results for PFHpA were excluded from the table

ND not detected

[a] The average and CV were calculated by use of the half the MQL (0.5 ng/mL) of PFDA

MQLs of Ahrens et al. and this study (0.4–1.6 ng/L for PFCAs, 0.2–0.7 ng/L or PFSAs, and 0.3–0.4 ng/L for FOSAAs). In 2014, the lowest MQLs were reported by Boone et al. [22], where concentrations of PFCAs in the range from 0.03 to 0.6 ng/L and concentrations of PFSAs in the range from 0.03 to 0.1 ng/L were quantified in surface water samples. All these methods covered only two or four substance classes and mainly PFCAs. To the best of our knowledge, the simultaneous determination of numerous PFASs from 12 substance classes has not been reported in the literature before.

PFAS concentrations in municipal and industrial WWTP samples

Only four PFASs of all the target analytes were detected in the municipal WWTP effluents investigated: namely, sodium perfluoro-1-hexanesulfonate (4.7 ng/L), 8:2-FTS (1 ng/L), PFOA (3 ng/L), and 6:2-FTS (4.7 ng/L) (data not shown). In contrast to the municipal WWTP, high concentrations of PFASs up to approximately 700 μg/L were determined in the industrial WWTP influent. Also the number of target analytes detected in the industrial

Table 8 Summary of perfluoroalkyl and polyfluoroalkyl substances determined in the influent of an industrial wastewater treatment plant by the HPLC–MS/MS-n method and direct measurement

Analyte	Influent A (μg/L)	Influent B (μg/L)	Influent C (μg/L)	Average (μg/L)	CV (%)
6:2-FTOH	458	489	514	487	6
8:2-FTOH	85.9	79.4	88.1	84.4	5
10:2-FTOH	48	37.5	46.0	43.8	13
N-MeFOSE	ND	ND	ND	-	-
N-EtFOSE	ND	ND	ND	-	-

Adapted with permission from [21]. No analytes that were the target of this method were detected in the corresponding effluent

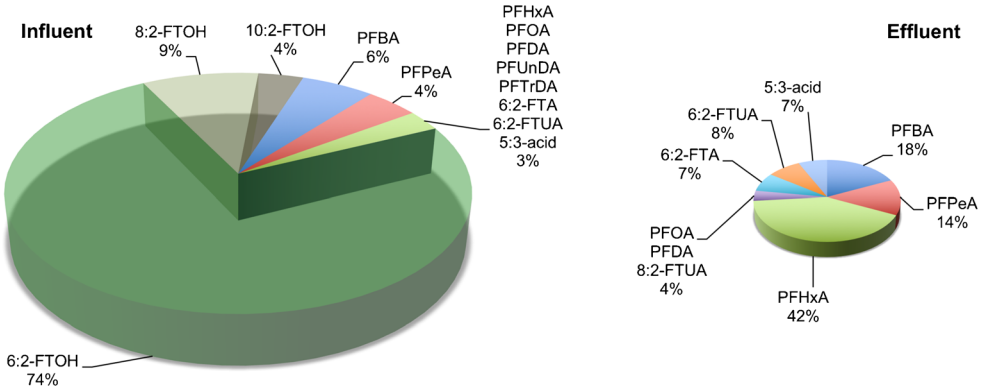


Fig. 3 Comparison of perfluoroalkyl and polyfluoroalkyl substances determined in the influent and the corresponding effluent of an industrial wastewater treatment plant (calculated in mole percent) analyzed with the HPLC–MS/MS-a/HPLC–M/MS-n method via direct measurement. Only 33 mol% of the total amount of perfluoroalkyl and polyfluoroalkyl substances was quantified in the corresponding effluent

samples was considerably higher. A summary of the PFASs detected in the influent of an industrial WWTP and the corresponding effluent is given in Tables 7 and 8. The influent samples were prepared in duplicate and the effluent samples were prepared in triplicate. In contrast to the high FTOH influent concentrations, no target analytes for the HPLC–MS/MS-n method were detected in the corresponding effluent.

For better comparison of the PFAS concentrations in the influent and the concentrations in the corresponding effluent, the results were calculated in mole percent. The dominant substance class in the industrial influent analyzed was FTOH, accounting for 87 mol% of the total PFASs. Only 33 mol% of the total amount of PFASs determined in the influent samples was quantified in the corresponding effluent (see Fig. 3). No FTOHs were detected in the effluent. However, an increase in the amount of perfluorohexanoic acid, PFOA, 6:2-FTCA, 6:2-FTUCA, 8:2-FTUCA, and 5:3-acid, which are transformation products of FTOHs [15], was observed in the corresponding effluent, suggesting the FTOHs had undergone at least partial biotransformation. It can be assumed that a large portion of the FTOHs evaporated and were released into the atmosphere during the wastewater treatment process, especially during the stripping procedure, as demonstrated in the literature [31, 32].

Conclusion

Two HPLC–MS/MS methods were developed and validated for the determination of 52 PFASs (47 analytes with the HPLC–MS/MS-a method and five analytes with the HPLC–MS/MS-n method) in aqueous samples. The wide variety of analytes can provide comprehensive data regarding the PFAS burden in the aqueous environment. Coverage of 12 different substance classes of PFASs, including precursor substances, transformation intermediates, and nondegradable PFAAs, is the main advantage compared with other reported methods dealing with the analysis of PFASs. It was shown that the SPE method used in this study is well suited for the enrichment of PFASs, allowing determination down to the sub-nanogram per liter range. Because of the different physicochemical properties and the high number of analytes, the application of several internal standards was important to compensate for matrix effects for individual analytes as efficiently as possible. The HPLC–MS/MS methods were used for the determination of PFASs in the effluent water of a municipal WWTP as well as in the influent and in the corresponding effluent of an industrial WWTP. Several precursor substances, mainly FTOHs, and biotransformation intermediates were detected in the industrial WWTP samples. The high number of PFASs detected demonstrated that there is a compelling necessity to increase the analyte spectrum so as to assess the relevance of different precursor compounds as sources of perfluorinated substances in the environment via WWTPs.

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Compliance with ethical standards This article does not contain any studies with human or animal subjects performed by any of the authors.

Conflict of interest The authors declare that they have no conflict of interest.

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