Determination of per- and polyfluorinated alkyl compounds using liquid chromatography tandem mass spectrometry in water samples

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Abstract
Recently per- and polyfluorinated alkyl compounds (PFAS) were discovered as new emerging persistent organic pollutants. They have been found in air, biota tissue, solid and aqueous matrices around the globe. A method has been developed for the determination of 41 PFAS (one fluorotelomer sulfonate, seven perfluorosulfonates, three perfluorosulfinates, seventeen perfluorocarboxylic acids, three fluorotelomer acids, three unsaturated telomer acids, four perfluorosulfonamides and three perfluorosulfonamidoethanols) in water samples. In addition 18 mass-labelled internal standards (IS) from the same group of substances were introduced. Two litres of water sample are filtered with glass fibre filter (GFF) and extracted by solid phase extraction (SPE) with Oasis WAX-cartridges from Waters. Extraction of particulate matter (> 1.2 µm) is carried out with sonication. All extracts are analysed using high performance liquid chromatography-electrospray ionisation-tandem mass spectrometry. The method detection limits for the water and particle phase were in the range of tens to hundreds ppq. Except for compounds which have no appropriate mass-labelled IS the recoveries for the particle phase range from 65 to 128% using sonication and for the filtrate they range from 80 to 121% using SPE.

Introduction
Per- and polyfluorinated alkyl compounds (PFAS) are widely used in a lots of consumer products such as polymerization aids, stain repellents on carpets, textiles, leather, and paper products. PFAS are persistent in the environment and they have been found in water, wildlife and human tissues around the globe.[1] The longer chained compounds are known to bioaccumulate and toxic effects in biota like neuroendocrine effects and peroxisome proliferation were examined.[2,3]

The perfluorinated acids have high water solubilities, low pK_a values and are therefore dissociated at environmentally relevant pH values. PFAS were detected in precipitation, surface, ocean, and tap water.[4-7] Detected concentrations are around some hundreds of pg L^{-1} to a few tens of ng L^{-1}, depending on location and compound. Perfluorooctanoic acid (PFOA) and Perfluorooctanesulfonate (PFOS) are the predominant substance in most cases. They can be found mostly in water or bound to particles, sediments and soil.[8] The perfluorosulfonamides are neutral compounds and consequently not as water-soluble as the acids and also more volatile. It is assumed that they are transformed to perfluorinated sulfonic acids (PFSAs) in the atmosphere, while fluorotelomer alcohols (FTOHs) are degraded over the intermediates fluorotelomer acids (FTCAs), unsaturated telomer acids (FTUCAs) to perfluorocarboxylic acids (PFCAs).[9,10]

The aim of this study was to develop a sensitive and compound-specific method for the analysis of 41 compounds and the corresponding 18 deuterated and ^{13}C-labelled substances as internal standards (IS) (6:2 fluorotelomer sulfonate (6:2 FTS), PFSAs, perfluorosulfinates (PFSiAs), PFCAs, FTCAs, FTUCAs, perfluorosulfonamides, and sulfonamidoethanols). Because the production was already and will be shifted towards the short-chained compounds, it was necessary to develop a method where short and long-chained compounds are analysed together. Some PFAS were found to bind to a high extent to the particulate matter and therefore the water and particle phase are extracted separately when water samples are analysed.

Materials and Methods
In this study 41 poly- and perfluorinated substances and 18 ISs are analysed. Perfluoropropionic acid (PFPrA, 98%) was purchased from Fluorochem, Perfluorohexanoic acid (PFHxA, chemical purity 97%), and potassium salts of perfluorobutanesulfonate (PFBS, 97%), Perfluorohexanesulfonate (PFHxS, 98%) and PFOS (98%) were supplied by Fluka, 1H,1H,2H,2H-Perfluorooctanesulfonic acid (6:2 FTS, 98%), perfluorobutanoic acid (PFBA, 99%), perfluoroundecanoic acid (PFUnA, 96%), perfluorooctane sulfonamide (FOSA, 97%) and n-ethylperfluorooctane sulfonamide (EtFOSA, 95%) were purchased from ABCR, perfluoropentanoic acid (PFPA,

98%), perfluorododecanoic acid (PFDoA, 96%), perfluorotetradecanoic acid (PFTA, 96%), perfluorohexadecanoic acid (PFHxDA, 95%) and perfluorooctadecanoic acid (PFODA, 97%) from Alfa Aesar. Perfluoroheptanoic acid (PFHpA, 98%), PFOA (95%), 3,7-dimethylperfluorooctanoic acid (Me_2PFOA, 97%), PFNA (97%) and PFDA (97%) were obtained from Lancaster and n-methylperfluorobutane sulfonamide (MeFBSA), n-methylperfluorobutane sulfonamidoethanol (MeFBSE), n-methylperfluorooctane sufonamide (MeFOSA), n-methylperfluorooctane sulfonamidoethanol (MeFOSE) and n-ethylperfluorooctane sulfonamidoethanol (EtFOSE) from 3M. Sodium perfluorohexanesulfinate (PFHxSi, 98%), sodium perfluorooctanesulfinate (PFOSi, 98%), sodium perfluorodecanesulfinate (PFDSi, 98%), and Perfluorodecanesulfonic acid (PFDS, 98%), 2-Perfluorohexyl ethanoic acid (6:2 FTCA, 98%), 2-Perfluorooctyl ethanoic acid (8:2 FTCA, 98%), 2-Perfluorodecyl ethanoic acid (10:2 FTCA, 98%), 2-Perfluorohexyl-[1,2-$^{13}C_2$]ethanoic acid (6:2 FTCA [M+2], 98%), 2-Perfluorooctyl-[1,2-$^{13}C_2$]ethanoic acid (8:2 FTCA [M+2], 98%), Perfluorodecyl-[1,2-$^{13}C_2$]ethanoic acid (10:2 FTCA [M+2], 98%), 2H-Perfluoro-2-octenoic acid (6:2 FTUCA, 98%), 2H-Perfluoro-2-decanoic acid (8:2 FTUCA, 98%), 2H-Perfluoro-2-dodecanoic acid (10:2 FTUCA, 98%), 2H-Perfluoro-[1,2-$^{13}C_2$]-2-octenoic acid (6:2 FTUCA [M+2], 98%), 2H-Perfluoro-[1,2-$^{13}C_2$]-2-decanoic acid (8:2 FTUCA [M+2], 98%), 2H-Perfluoro-[1,2-$^{13}C_2$]-2-dodecanoic acid (10:2 FTUCA [M+2], 98%), perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid ($^{13}C_4$-PFBA, 98%), perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid ($^{13}C_2$-PFHxA, 98%), perfluoro-n-(1,2,3,4-^{13}C)-octanoic acid ($^{13}C_4$-PFOA, 98%), perfluoro-n-(1,2,3,4-^{13}C)-nonanoic acid ($^{13}C_4$-PFNA, 98%), perfluoro-n-(1,2,3,4-^{13}C)-decanoic acid ($^{13}C_4$-PFDA, 98%), perfluoro-n-(1,2-^{13}C)-dodecanoic acid ($^{13}C_2$-PFDoA, 98%), sodiumperfluoro-1-(1,2,3,4-^{13}C)-octanesulfonate ($^{13}C_4$-PFOS, 98%), sodiumperfluoro-1-(1,2,3,4-^{13}C)-octanesulfinate ($^{13}C_4$-PFOSi, 90%), n-methyl-d_3-perfluoro-1-octanesulfonamide (d_3-MeFOSA, 98%), n-ethyl-d_5-perfluoro-1-octanesulfonamide (d_5-EtFOSA, 98%), 2-(n-deuteriomethylperfluoro-1-octane-sulfonamido)-1,1,2,2,-tetradeuterioethanol (d_7-MeFOSE, 98%), 2-(n-deuterioethylperfluoro-1-octane-sulfonamido)-1,1,2,2,-tetradeuterioethanol (d_9-EtFOSE, 98%) were purchased from Wellington Laboratories. The injection standard (InjS) 2,4 dichlorophenol $^{13}C_6$ was obtained from Dr. Ehrenstorfer GmbH. Methanol (SupraSolv), acetonitrile (LiChrosolv), ammonium hydroxide (25% for analysis), formic acid (98-100% suprapur) and ammonium acetate were purchased from Merck.

The water samples are collected with one or two litre brown glass bottles. The glass bottles are dropped under water by a sampler and are opened at approximately one to three meter below the surface. Alternatively the water samples are taken from a ship intake system. The samples are filtered through a glass fibre filter (GFF) (Whatman, ø 47 mm) at the laboratory the same day; the water phase is stored at 4°C and the GFF at -18 °C.

The filtrate is extracted by solid phase extraction (SPE), while the particulate matter (> 1.2 µm) was analysed by sonication. The water phase and the GFF are spiked with 50 µL IS (c = 0.2 µg mL^{-1}) separately. The SPE of the water phase is done with WAX cartridges (Waters, 150 mg, 6 cc, 30 µ). They are preconditioned with 5 mL methanol and Millipore water. A one or two litres sample is extracted with approximately 4 drops sec^{-1} and the cartridges are washed with 5 mL 0.1% formic acid in Millipore water. The cartridges are dried for 40 min. The elution is divided into two parts; before the first elution 1 mL methanol is added to the vial as a keeper, then the sulfonamides are eluted by 14 mL acetonitrile; thereafter the acids are eluted by 5 mL 0.1% ammonium hydroxide in methanol. Both eluates are reduced to 200 µL under a nitrogen stream. The extraction of the GFF is done via sonication by placing the GFF into a glass fibre thimble in a beaker. Methanol (100 mL) is added and sonicated for one hour. This is done twice and the two fractions are combined, evaporated, filtered and reduced to 200 µL. As an injection standard (InjS) are added 20 ng absolute of 2,4 dichlorophenol $^{13}C_6$ to the final extracts. Finally the extracts are analysed with high performance liquid chromatography-electrospray ionisation-tandem mass spectrometry (HPLC-ESI-MS/MS).

A Synergi Hydro RP 80A column (Phenomenex, 150 x 2 mm, 4 micron) plus a suitable guard column Synergi 2 µ Hydro RP Mercury (Phenomenex, 20 x 2 mm, 2 micron) for the HPLC-system (Agilent Technologies) are used. Water [A] and methanol [B] are employed as mobile phases, both with 10 mM ammonium acetate as an ionisation aid. The acquisition time of the final method is set to 40 min plus 7 min equilibration. The gradient starts with 30% solvent B. After this, it is increased to 70% over 3 min, continuously increase to 90% over 26 min and then to 100% over 2 min, which is hold over 7 min. During the last 2 min it is decreased to 30% B. The flow is set to 200 µL min^{-1}, 10 µL of the sample is injected and the needle is rinsed in methanol prior to

injection. An oven is applied to keep the temperature constant, which is set to 30 °C. Some modifications to the HPLC system are done due to instrumental blank contamination. All Teflon-containing tubes are replaced and the filters for the mobile phase solvents are exchanged with stainless steel filters. The degaser is excluded from the system and helium is pumped into the mobile phase before every sample batch in order to minimise the amount of gas getting onto the column. A cartridge (Gemini 5 µ C18 110A Mercury, 20 x 2 mm by Phenomenex) is installed behind the pump to trap all contaminants resulting from the mobile phase. Barrier septums from Supelco make of silica and aluminium are used.

An API 3000 triple-quadrupole mass spectrometer (Applied Biosystems/MDS SCIEX) with an ESI interface is used. All compounds are analysed in negative ionisation mode and after optimisation the instrument is operated in multiple reaction monitoring (MRM) mode with a dwell time of 15 msec. Nebulizer, curtain and collision gas are set to 14, 8 and 6 L min^{-1}, respectively, the ion spray voltage is set to - 4500 V and the temperature of the source block is adjusted to 300 °C. Perfluorinated pentane-, heptane- and nonanesulfonate (PFPS, PFHpS, PFNS) and perfluorinated tridecanoic, pentadecanoic and heptadecanoic acid (PFTrDA, PFPDA, PFHpDA) are not available as standards. Therefore they are integrated into the method taking the MS/MS parameters of the compound having one carbon atom less in the carbon chain.

Results and Discussion

The determination of PFAS in water is complicated due to potential blank contamination during sample preparation and instrumental analysis. Instrumental blanks showed no contamination after all above mentioned modifications were applied to the HPLC system. Blank contamination for the SPE of the water phase were only found for PFPA and PFOA with a maximum of 44.1 pg absolute; the contamination using sonication ranged from the lower instrument detection limit (IDL) to 134.6 pg absolute (PFOA) (Tab. 1).

The IDLs were determined in two different ways. First with the use of the calibration method of the German DIN 32645; IDLs vary between 3 pg (PFHxA) and 68 pg absolute (MeFBSA) (results are not shown). Furthermore the IDLs were calculated from the chromatogram with a signal to noise ratio (S/N) of 3; the IDLs ranged from 0.05 pg (PFOS) to 1.35 pg (PFBA) (Tab. 1). Method detection limits (MDLs) were calculated for substances that were found in real samples using the S/N of 3. They varyed between 2.3 to 298.5 pg L^{-1} (Tab. 1).

Table 1: Typical IDLs (S/N = 3), MDLs (S/N = 3), blanks (n = 3), IS-corrected recovery rates (n = 3) and Minimum and Maximum concentrations of the water (n = 6) and particle phase (n = 3) that were found in the river Elbe at Tesperhude/Geesthacht, Germany.

Analyte	IDL [pg]	MDL [pg L^{-1}]		Blank [pg]		Recovery [%]		Concentration [ng L^{-1}]	
		Water phase	Particle phase	Water Phase	Particle phase	Water phase	Particle phase	Water phase	Particle phase
PFBS	0.30	60.2	n.d.	n.d.	n.d.	116	72	1.70-2.20	n.d.
PFPS	n.a.	32.0	n.d.	n.d.	n.d.	n.a.	n.a.	(0.33-0.56)	n.d.
PFHxS	0.15	50.0	n.d.	n.d.	n.d.	99	90	0.73-0.96	<0.01-(0.03)
PFOS	0.05	34.3	21.3	n.d.	n.d.	96	79	3.22-4.40	0.62-0.71
PFBA	1.35	298.5	18.3	n.d.	n.d.	117	98	0.88-1.98	0.10-0.70
PFPA	0.33	83.1	n.d.	<IDL-44.1	<IDL-101.7	92	102	2.15-2.63	0.14-0.34
PFHxA	0.30	74.6	3.5	n.d.	<IDL-49.3	94	96	3.46-3.91	0.03-0.06
PFHpA	0.14	30.2	n.d.	n.d.	n.d.	99	103	0.97-1.10	<0.01-0.03
PFOA	0.09	43.6	3.0	<IDL-33.6	31.1-134.6	80	100	4.78-5.16	(0.01)-0.11
PFNA	0.12	19.4	2.3	n.d.	<IDL-6.7	95	94	0.72-0.85	0.05-0.07
PFDA	0.18	29.6	4.4	n.d.	<IDL-15.2	95	103	0.61-0.71	0.12-0.15
PFUnA	0.19	6.7	4.3	n.d.	n.d.	104	106	<0.01-(0.04)	<0.01-0.05
FOSA	0.25	11.4	n.d.	n.d.	n.d.	56	77	(0.08)-0.33	n.d.
MeFBSE	0.70	53.2	n.d.	n.d.	n.d.	106	75	<0.05-(0.19)	n.d.

n.a., not available n.d., not detected (), below the lowest calibration point

For the extraction of the water phase WAX cartridges were used, because they are suitable for the detection of the neutral compounds as well as the long- and short-chained perfluorinated acids. A breakthrough test showed that everything was kept on the first cartridge. The IS-corrected recoveries of the PFSAs, PFSiAs, PFCAs (C_3 to C_{14}), FTCAs, FTUCAs, persulfonamidoethanols and two perfluorosulfonamides (MeFOSA, EtFOSA) are quite good (80 to 121%); only 6:2 FTS (64%) and the not IS-corrected compounds FOSA (56%), MeFBSA (161%), PFHxDA (132%) and PFOcDA (199%) showed higher or lower recoveries, respectively. Furthermore the IS, which were used for the correction of the analytes, showed recoveries of 41 (10:2 FTCA) to 120% ($^{13}C_4$-PFOS), except for $^{13}C_4$-PFBA, d_3-MeFOSA, d_5-EtFOSA, for which they were 181, 30 and 30%, respectively. The result sof the extraction of the particle phase are similar; the recoveries ranged from 65 (PFPrA) to 111% (MeFOSE), except for the not IS-corrected compounds PFTA (156%), PFHxDA (185%) and PFOcDA (215%). Recoveries for the IS ranged from 88 ($^{13}C_2$-PFHxA) to 118% (8:2 FTUCA [M+2]), only the FTCA had lower recoveries (45 to 60%). Therefore the compounds without appropriate mass-labelled IS were corrected by a spiked Millipore recovery sample that was analysed within the same sample batch, although this would not correct for any matrix effects.

In order to evaluate the instrumental precision, within-day precision was tested by tenfold injection of the same calibration solution at 25 ng mL^{-1}. It ranged from 2.8% (PFDoA) to 7.4% (PFPrA) relative standard deviation (RSD). Between-day precision at 25 ng mL^{-1} was shown to be between 6.2% (PFNA) and 19.1% (6:2 FTCA) RSD.

Concentrations from the river Elbe at the location Tesperhude/Geesthacht, Germany are in the low ng L^{-1} range (Tab. 1). The highest concentrations in the water phase were found for PFOA, while in the particulate matter PFOS was the predominant compound. The distribution of the analysed concentrations between particle and water phase was calculated for each of the samples. The mean percentages in the particulate matter were relatively high for PFBA (15.3 %), PFOS (14.6 %) and PFDA (11.4 %).

PFAS in the water as well as the particle phase can be analysed with the developed method. This could be used to determine the distribution of the substances between water and particles in future analyses. For further work more IS are needed for the quantification. With their help an over- or underestimation of environmental concentrations could be minimised. Because of the high concentration factor (one or two litre sample concentrated up to 200 µL extract) and the sensitive HPLC-MS/MS analysis a level of tens to hundreds parts per quadrillion (ppq) can be quantified in environmental water samples.

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