[insert journal title]. The final authenticated version is available online at: http://dx.doi.org/[10.1007/s00216-020-02434-w)]

Applicability of mixed-mode chromatography for the simultaneous analysis of C_1-C_{18} perfluoroalkylated substances

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Acknowledgments

This work was supported by the Water Challenges for a Changing World Joint Program Initiative (Water JPI) Pilot Call (ref. WATERJPI2013 – PROMOTE, the Spanish Ministry of Economy and Competitiveness/Spanish Agencia Estatal de Investigación (refs. JPIW2013-117 and CTM2017-84763-C3-2-R), the Galician Council of Culture, Education and Universities (ref. ED431C2017/36) and FEDER/EDRF funding. Special thanks go to Dr. Leo Yeung (former member of University of Toronto and currently at Örebro University) and Prof. Thomas Knepper and Dr. Daniel Zahn (Hochschule Fresenius) for providing us standards of perfluoroethane sulfonic acid, perfluoropropane sulfonic acid and perfluoromethane sulfonic acid, respectively.

Abstract

A new analytical method for the determination of 22 perfluoroalkylated (carboxylic and sulfonic) acids in water samples is presented. The method's objective was to achieve the simultaneous quantification of compounds with different chain length (from C_1 to C_{18}). To this end, 500 mL of water were extracted with Oasis WAX solid-phase extraction cartridges and eluted with 3 mL of 5% ammonia in methanol. After evaporation to dryness, extracts were reconstituted in methanol:ultrapure water (1:1) and analyzed by mixed-mode liquid chromatography-tandem mass spectrometry (MMLC-MS/MS) using a weak anion exchange/reversed-phase column. The method provided good results, with limits of quantification lower than 1 ng/L in river water for most of compounds, except the two perfluorocarboxylic acids with the longest alkyl chain (>C_{14}) and trifluoroacetic acid, for which a blank contamination problem was observed. The method proved good trueness and precision in both ultrapure and river water (R ≥ 81%, RSD ≤15%). After validation, the method was applied to the analysis of nine water samples where 9 perfluoroalkylated acids were quantified. Seven of them were ultrashort- (C_1-C_4) and short-chain (C_4-C_8) perfluoroalkylated acids, pointing out the importance of developing methods capable to target such substances for further monitoring.

Keywords: Perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkyl sulfonic acids (PFSAs), persistent and mobile organic contaminants (PMOCs), water samples, solid-phase extraction.

Introduction

Awareness on perfluoroalkylated acids (PFAAs) and other fluorinated substances has substantially raised in the last 2 decades. Although this class of organic compounds contains several chemical species, the most frequently studied groups are perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs). These compounds are used in several industrial applications due to their physical and chemical properties and stability, such as manufacturing of fire-fighting products, coatings, lubricants, etc. Thus, the global emission of PFCAs, has been estimated as thousands of tons worldwide [1]. The main reason for the increasing concern on these compounds is that some of them, specially long-chain (C_8-C_{18}) PFAAs, are nowadays known to be very stable in the natural environment (resistant to degradation), present high mobility (can be easily transported for long distances) and because of their potential bioaccumulation in the food chain and long half-lives in humans [2]. For those reasons, perfluorooctanoic acid (PFOA) and its salts were included in 2017 in the candidate list of regulatory substances in the EU (Annex XVII to Regulation (EC) No 1907/2006). Also, perfluorooctanesulfonic acid (PFOS) was added to the persistent organic pollutants (POPs) list at the Stockholm Convention on Persistent Organic Pollutants in 2009 (Part A of Annex I to Regulation (EC) No 850/2004) and also included in the Directive 2013/39/EU as regards priority substances in the field of water policy in 2013 [3]. Besides them, perfluorohexane sulfonic acid (PFHxS) and PFCAs from C_9 to C_{14} are included in the candidate list of substances of very high concern (SVHC) under REACH regulation, and should, therefore, be progressively replaced by less dangerous substances.

The presence of PFAAs in the environment, wildlife and even human fluids and tissues has been reported worldwide [4], which lead to limit the production and emission of some of

the most widely used PFAAs. Due to this limitation on the use of long-chain PFAAs, the industry has searched for alternative substances, such as short- (C_4-C_8) and ultrashort- (C_1-C_3) chain PFAAs, which exhibit similar persistence and lower bioaccumulative potential than long-chain PFAAs but with lower occurrence and toxicological data available [5-7].

Solid-phase extraction (SPE), using mixed-mode weak anion-exchangers, is the preferred sample extraction procedure [8-10] for this class of analytes. Although recoveries using for example Oasis Hydrophilic-Lipophilic Balance (HLB) are also acceptable for long-chain PFAAs [11, 12], for short-chain compounds the use of ionic exchangers is mandatory [13]. Early analytical determination of PFCAs was carried out by gas chromatography (GC) [14]. However, the GC analysis involves a previous derivatization step due to the high polarity of them, cluttering the procedure, on the other hand, the GC determination of PFSAs is quite difficult, because the derivatives are highly unstable. Thus, high-performance liquid chromatography (HPLC) coupled to tandem mass spectrometry (MS/MS) is currently the most preferred and extensively employed analytical technology for PFAAs quantitation. Although high resolution analyzers have been employed, such as quadrupole time-of-flight mass spectrometry (QTOF-MS), providing a high resolving power, selectivity and mass accuracy necessary for the discovery of novel PFAAs [15, 16], the highest sensitivity is still provided by triple quadrupole mass spectrometers (QqQ) [17]. The main analytical challenge in ultrashort- and short-chain PFAAs analysis is chromatographic separation. Common reversed-phase LC (RPLC) based methods used as routine for the analysis of long-chain PFAAs, fail for these compounds with high polarity, which elute early and exhibit poor peak shape [18, 19]. To improve the separation, alternative mechanisms have been considered, such as hydrophilic interaction liquid chromatography (HILIC) [20], ion-exchange HPLC [13] and supercritical fluid chromatography (SFC) [19, 21]. The main limitation in these

cases is the inability to jointly analyze ultrashort-chain PFAAs and the longer chain congeners, so that two different methodologies are normally employed when they all need to be analyzed [13, 19].

Mixed-mode liquid chromatography (MMLC) has been previously applied for the analysis of perfluoromethane sulfonic acid (PFMS) [22] providing good results, while being capable of determining other less polar analytes. Thus, in the present study we investigate the suitability of a new method based on mixed-mode SPE and MMLC, aiming at analyzing at the same time 22 ultrashort-, short- and long-chain PFCAs and PFSAs in water samples.

Experimental

Reagents and materials

Detailed supplier information is provided in Electronic Supplementary Material, Table S1. Most of analytes' standards and isotopically labeled analogs employed as internal standards (IS) were supplied by Wellington Laboratories (Ontario, CA) as mixtures of 2 μg/mL in methanol (MeOH). Five analytes (short-chain PFAAs) were obtained as individual standards from Sigma-Aldrich (San Luis, Mi, USA), Kanto Corporation (Portland, OR, USA) and Carbolution (Saarbrücken, Germany), and prepared as a mixture of 2 μg/mL in methanol (MeOH). Diluted working solutions (500 ng/mL) containing all the analytes or all the IS were prepared in MeOH and stored in the dark at -20°C until use.

LCMS-grade MeOH, formic acid, acetic acid and ammonia solution in ultrapure water (25%) were supplied by Scharlab (Barcelona, Spain). LCMS-grade acetonitrile (ACN) and ammonia (NH_3) in MeOH (7N) were supplied by Fisher Scientific (Hampton, NH, USA) and Acros Organics (Geel, Belgium), respectively. Ultrapure water was obtained in the laboratory

by purifying demineralized water in a Milli-Q Gradient A-10 system (Merck-Millipore, Bedford, MA, USA).

Sampling and sample treatment

Water samples were collected at different locations (see Electronic Supplementary Material Table S2) in Galicia (NW of Spain). They were vacuum-filtered through 0.7 μm glass microfiber and 0.45 μm low protein binding membrane filters. Then, 500 mL of water were spiked with 2 ng of IS and solid-phase extracted onto mixed mode reversed-phase-weak anion exchange cartridges (Oasis WAX-150 mg, Waters). Prior sample loading, the cartridges were consecutively conditioned with 5 mL of MeOH containing 2% of formic acid and 5 mL of ultrapure water. Subsequently, samples were passed through the cartridges using a vacuum pump, and after sample loading, cartridges were washed with 10 mL of ultrapure water and dried under a nitrogen stream (99.999%) for 30 min. Analytes were recovered with 3 mL of 5% NH_3 in MeOH. Eluates were evaporated to dryness under a nitrogen stream and redissolved in 100 μL of MeOH:ultrapure water (1:1) for analysis.

UHPLC-MS/MS analysis

10 μL of extract (or standard) were injected into a Waters Acquity UPLC® H class system (Milford, MA, USA) equipped with a sample manager, a binary solvent pump and a column oven. Chromatographic separation was carried out on an Acclaim ™ mixed-mode WAX-1 120 Å column (50 × 3 mm I.D., particle size 3 μm) from Thermo (Waltham, MA, USA) kept at 40°C. Mobile phases consisted of (A) ultrapure water, (B) acetonitrile and (C) 1 M aqueous ammonium acetate at pH 5.5. The concentration of C was maintained constant at 4 % during the separation. The elution gradient was as follows: 0 min (45% B), 10 min (90% B), 13 min (90% B), 13.05 min (45% B), 16 min (45% B).

During the chromatographic optimization, another mixed-mode column, an Acclaim™ Trinity™ P1 column (50 × 2.1 mm I.D., particle size 3 μm) was used and the separation compared with that obtained with the WAX column under the same gradient conditions.

A triple quadruple mass spectrometer Xevo TQD (Waters Corp., Milford, MA, USA) equipped with an electrospray ionization (ESI) source, working in negative mode, was used. Nitrogen and argon were used for ionization and collision induced dissociation, respectively. Ionization parameters were as follows: 3 kV (capillary voltage), 150°C (source temperature), 400°C (desolvation temperature), 900 L/h (desolvation gas-N_2 flow) and 50 L/h (cone gas-N_2 flow). Collision energy (CE) and cone voltage (CV) values were adjusted individually for every compound. One (IS) or two (analytes) ion transitions per compound were recorded in the Selected Reaction Monitoring (SRM) mode. For 4 analytes (TFA, PFPrA, PFBA and PFPeA) only one transition could be registered. Selected transitions, together with their corresponding CE and CV values, retention times (RT) and the labeled compound used as IS for each analyte, are shown in Electronic Supplementary Material, Table S3.

Method validation

The method was evaluated in terms of linearity, instrumental repeatability, instrumental and whole method limits of quantification (IQLs and MQLs), trueness and precision. Analytes were quantified using the isotopic labeled analogs as IS. In those (six) cases where no labeled analog was available, the labeled compound providing the best results in terms of trueness was selected (see Electronic Supplementary Material, Table S3).

Calibration curves were prepared in MeOH:ultrapure water (1:1) between 0.5 and 500 ng/mL for all the analytes. The IS level was 20 ng/mL in all cases. IQLs were calculated as the concentration of a standard providing a signal-to-noise ratio (S/N) of 10. Instrumental

repeatability was assessed as the relative standard deviation (%RSD) of six consecutive injections of two different standards (containing either 5 or 50 ng/mL of all analytes and 20 ng/mL of IS).

Trueness and precision of the whole method were estimated from recovery experiments performed in ultrapure and river water spiked with 10 ng/L of all the analytes and 4 ng/L of all IS. Samples were also analyzed without analyte addition in order to correct for their native content. MQLs were assessed from measured concentrations in river water samples containing (or spiked with) low concentrations of all analytes, downscaling the levels for which the signal-to-noise ratio is 10. For estimation of TFA MQL, 10 replicates of the procedural blank were done and the MQL calculated following the Eurachem guide [23] recommendations. Trueness and precision for this compound were evaluated separately at higher spiking level (100 ng/L).

Results and discussion

Chromatographic separation

The chromatographic behavior of the analytes has been tested in two MMLC columns. The selected mixed-mode columns were the Acclaim Trinity P1 (hereafter Trinity), which provides at the same time strong cation exchange (SCX), WAX and RP functionalities, and the Acclaim WAX-1 (hereafter WAX), which only contains WAX and RP functionalities. The Trinity column was firstly tested since it provided good results for TFMS according to our previous experience [22, 24]. Fig. 1 shows the chromatograms obtained with both columns for the five ultrashort-chain PFAAs. The chromatograms for the remaining compounds are provided in the Electronic Supplementary Material, Fig.S1. For all of them,

both peak shape and width were similar using the WAX and Trinity columns, but the WAX column provided more retention than the Trinity.

The WAX column was selected as it provides more retention of the analytes and it will not retain basic species, thus, possible basic interferences present in the matrix would elute in the void volume and consequently less matrix effect is expected.

A limitation when using MMLC columns is their durability when compared with RP columns, as retention times become less stable with time, especially when injecting complex matrices. Thus, the injection of daily quality standards to control retention time stability is mandatory (a maximum variability of 10% for $^{13}C_4$PFBA retention time was stablished).

Solid-phase extraction

Our previous experience with long-chain PFAAs [11] and specially, literature for ultrashort-chain PFAAs [13] led us to select SPE mixed-mode cartridges with WAX functionality that should provide good recovery for all PFAAs. Two different types of WAX SPE cartridges were tested, Oasis WAX and Strata-X-AW. Fig. 2 shows the recovery obtained using both cartridges when 500 mL of ultrapure water spiked at 10 ng/L (20 ng/L IS) are extracted. TFA was evaluated separately at a higher concentration (100 ng/L). Both cartridges provided similar results for ultrashort- and short-chain PFAAs, in agreement with published methods [25]. However, those compounds containing more than ten atoms of carbon in the alkyl chain (lower polarity) presented better recoveries with Oasis WAX cartridges.

Assessment of blank contamination

As one of the main problems reported in the literature associated with analysis of PFAAs is background contamination [8, 26], instrumental blanks were performed by injection of MeOH:ultrapure water (1:1). Instrumental contamination was discarded since none of the target compounds were observed in the instrumental blanks. Procedural blanks were carried out, eluting directly the cartridge after conditioning and IS addition, without sample loading. Also, samples of ultrapure water were submitted to the entire protocol. Fig. 3 shows the chromatograms for TFA in an instrumental blank, procedural blank and an ultrapure water sample, where it can be observed that this compound was detected in both the procedural blank and the ultrapure water sample. Thus, the source of TFA contamination in procedural blanks was studied. The elution solvent was injected (before and after a concentration step) and TFA was not detected. A deep rinse with LC-MS quality MeOH and ACN of every plastic material used in the protocol was made, also an additional cleaning step (5 mL MeOH containing 5% of NH_3) was included in the cartridge conditioning. None of the efforts managed to completely eliminate the plastic material contamination with TFA. However, the repeatability of the signal in procedural blanks was appropriate (RSD 8%, n=10), the MQLs for this compound were then estimated using the Eurachem guidelines [23]. This problem led to an increase in the MQL for this compound compared with the obtained IQLs (Table 1). MQLs in the same order were reported for TFA by other authors [19, 27], who quantified TFA by direct injection. In that cases, they do not report blank contamination problems [27] and when observed, they performed a blank subtraction [19]. Given the fact that TFA can be considered ubiquitous and has been reported in drinking water after several oxidation processes at high levels (ca. 50 µg/L) [28] and that the ultrapure water obtained at the laboratory (see Figure 3) contains ca. 110 ng/L of TFA, we consider the MQL still valid to detect TFA in many samples.

Method performance

Firstly, the performance of the LC–MS/MS method was evaluated in terms of precision, linearity, and instrumental LODs and LOQs (Table 1). Linearity was satisfactory with determination coefficients (R^2) higher than 0.9972. Moreover, a Durbin-Watson statistic test provided a p-value greater than 0.05 for all compounds, which indicates no significant correlation in the residuals at the 95% confidence level. Precision, in terms of RSD, was evaluated at two concentration levels, 5 and 50 ng/mL, providing values below 12 and 10 %, respectively. IQLs were calculated and ranged from 0.01 to 0.56 ng/mL. These values are similar or even 10 times lower (in some cases, such as PFES or PFOS) than those obtained by SFC [21] or using other ion exchange columns [13].

After optimization of the sample preparation protocol, the performance of the entire method was assessed. Trueness, precision and MQLs are shown in Table 2. Trueness was acceptable with recovery values ranging between 81 and 115 % in both ultrapure and river water, except for the most lipophilic compounds, PFHdA, PFOdA, PFDeS. Moreover, RSD was below 15% for all compounds but PFHdA, PFOdA, PFDeS. Thus, although the instrumental methodology performed well for these compounds, the extraction method does not meet the quality criteria for them. The significance of this limitation is relatively low since the partition coefficients (log D, pH 7.4) are higher than 7 in case of both carboxylic acids and 4.5 for PFDeS, and thus, it seems unlikely to find these compounds dissolved in the water samples water phase and their presence may be more relevant in suspended particulate matter.

The MQLs were lower than 1 ng/L for most of compounds, except PFHdA, PFOdA and TFA. These values are comparable to those reported in the literature for short and long-

chain PFAAs in surface water samples [8, 10]. Furthermore, in the case of PFOS, the MQL is 0.4 ng/L, fulfilling the requirements of the European existing legislation on PFOS in inland surface water which sets the annual average (AA) and the maximum allowable concentration (MAC) environmental quality standard (EQS) in 0.65 ng/L and 36 μg/L, respectively [3]. For ultrashort-chain PFAAs, the highest MQL was obtained for TFA (63.5 ng/L), due to the contamination problem reported in the previous section. The remaining ultrashort-chain compounds presented MQLs below 0.6 ng/L, similar to those reported in other studies [13, 21, 25] where the MQL for these 4 compounds ranged between 0.1 and 4 ng/L. Yet, the main advantage of the method reported in this work when compared with the literature [13, 19] is its ability of determine all studied PFAAs, from 1 to 18 carbon atoms, in one single chromatographic run and without the requirement of any special equipment, beyond the chromatographic column.

Occurrence in river water

The concentrations of the analytes detected in the samples are shown in Table 3 (see sample location in Electronic Supplementary Material, Table S2). TFA, PFMS and PFBA were found in all samples. PFMS, reported in 2016 for the first time in drinking water [20], was detected at levels higher than 5 ng/L, while TFA and PFBA levels ranged between 66-262 and 1.8-174 ng/L, respectively. The levels of PFBA were higher in drinking water than in surface water, and even higher than those found by other authors in highly polluted river water [29], this suggests that this compound may originate in the water supply treatment or tubing. Within the other ultrashort- and short-chain PFAAs, PFBS and PFPrA were found in 7 and 3 samples, respectively, while PFHxA and PFHxS appeared only in 1 sample at levels near their MQL. PFOA and PFOS were the only long-chain PFASs found in this sampling

set being detected only in river water, at levels ranging between 1.2-5 and 1.3-1.6 ng/L, respectively. In the case of PFOS, these levels are lower than the maximum allowable concentration set by EU authorities as environmental quality standard in inland waters (36 μg/L), but higher than the annual average value (0.6 ng/L), thus a monitoring campaign along the year should be performed.

Conclusions

A new method based on MMLC was developed and validated for the quantification of PFAAs including ultrashort-, short- and long-chain compounds in water samples. The chromatographic method was capable of determining a total of 22 PFAAs (C_1-C_{18}) with one single chromatographic run. However, the three most lipophilic analytes did not perform well during SPE with Oasis WAX in river water due to its lower solubility and lack of isotopically labelled internal standards. The methodology was applied to the analysis of 9 river and drinking water samples where 9 PFAAs were found in at least one sample. Among them, 7 were ultra-short and short-chain PFAAs. The long-chain compounds found were PFOA and PFOS. These findings point out the relevance of the most hydrophilic chemicals in the aqueous environment, where further monitoring is required.

Conflict of interest

The authors declare no conflict of interest

References

1. Armitage JM, MacLeod M, Cousins IT. Comparative Assessment of the Global Fate and Transport Pathways of Long-Chain Perfluorocarboxylic Acids (PFCAs) and

Perfluorocarboxylates (PFCs) Emitted from Direct Sources.Environ Sci Technol. 2009;43(15):5830-6.
2. Lindstrom AB, Strynar MJ, Libelo EL. Polyfluorinated Compounds: Past, Present, and Future.Environ Sci Technol. 2011;45(19):7954-61.
3. Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy (1).56.
4. Wang Z, DeWitt JC, Higgins CP, Cousins IT. A Never-Ending Story of Per- and Polyfluoroalkyl Substances (PFASs)?Environ Sci Technol. 2017;51(5):2508-18.
5. Brendel S, Fetter É, Staude C, Vierke L, Biegel-Engler A. Short-chain perfluoroalkyl acids: environmental concerns and a regulatory strategy under REACH.Environ Sci Eu. 2018;30(1):9.
6. Wang Z, Cousins IT, Scheringer M, Hungerbuehler K. Hazard assessment of fluorinated alternatives to long-chain perfluoroalkyl acids (PFAAs) and their precursors: Status quo, ongoing challenges and possible solutions.Environ Int. 2015;75:172-9.
7. Ateia M, Maroli A, Tharayil N, Karanfil T. The overlooked short- and ultrashort-chain poly- and perfluorinated substances: A review.Chemosphere. 2019;220:866-82.
8. Gremmel C, Frömel T, Knepper TP. HPLC–MS/MS methods for the determination of 52 perfluoroalkyl and polyfluoroalkyl substances in aqueous samples.Anal Bioanal Chem. 2017;409(6):1643-55.
9. Boone JS, Guan B, Vigo C, Boone T, Byrne C, Ferrario J. A method for the analysis of perfluorinated compounds in environmental and drinking waters and the determination of their lowest concentration minimal reporting levels.J Chromatogr A. 2014;1345:68-77.
10. Coggan TL, Anumol T, Pyke J, Shimeta J, Clarke BO. A single analytical method for the determination of 53 legacy and emerging per- and polyfluoroalkyl substances (PFAS) in aqueous matrices.Anal Bioanal Chem. 2019;411(16):3507-20.
11. Villaverde-de-Sáa E, Fernández-López M, Rodil R, Quintana JB, Racamonde I, Cela R. Solid-phase extraction of perfluoroalkylated compounds from sea water.J Sep Sci. 2015;38(11):1942-50.
12. So MK, Taniyasu S, Yamashita N, Giesy JP, Zheng J, Fang Z, et al. Perfluorinated Compounds in Coastal Waters of Hong Kong, South China, and Korea.Environ Sci Technol. 2004;38(15):4056-63.
13. Taniyasu S, Kannan K, Yeung LWY, Kwok KY, Lam PKS, Yamashita N. Analysis of trifluoroacetic acid and other short-chain perfluorinated acids (C2–C4) in precipitation by liquid chromatography–tandem mass spectrometry: Comparison to patterns of long-chain perfluorinated acids (C5–C18).Anal Chim Acta. 2008;619(2):221-30.
14. Shafique U, Schulze S, Slawik C, Kunz S, Paschke A, Schüürmann G. Gas chromatographic determination of perfluorocarboxylic acids in aqueous samples – A tutorial review.Anal Chim Acta. 2017;949:8-22.
15. Barzen-Hanson KA, Field JA. Discovery and Implications of C2 and C3 Perfluoroalkyl Sulfonates in Aqueous Film-Forming Foams and Groundwater.Environ Sci Technol Letters. 2015;2(4):95-9.
16. Picó Y, Farré M, Barceló D. Quantitative profiling of perfluoroalkyl substances by ultrahigh-performance liquid chromatography and hybrid quadrupole time-of-flight mass spectrometry.Anal Bioanal Chem. 2015;407(15):4247-59.
17. Trojanowicz M, Koc M. Recent developments in methods for analysis of perfluorinated persistent pollutants.Microchim Acta. 2013;180(11):957-71.

18. Ruan T, Jiang G. Analytical methodology for identification of novel per- and polyfluoroalkyl substances in the environment.TRAC Trends Anal Chem. 2017;95:122-31.
19. Björnsdotter MK, Yeung LWY, Kärrman A, Jogsten IE. Ultra-Short-Chain Perfluoroalkyl Acids Including Trifluoromethane Sulfonic Acid in Water Connected to Known and Suspected Point Sources in Sweden.Environ Sci Technol. 2019;53(19):11093-101.
20. Zahn D, Frömel T, Knepper TP. Halogenated methanesulfonic acids: A new class of organic micropollutants in the water cycle.Water Res. 2016;101:292-9.
21. Yeung LWY, Stadey C, Mabury SA. Simultaneous analysis of perfluoroalkyl and polyfluoroalkyl substances including ultrashort-chain C2 and C3 compounds in rain and river water samples by ultra performance convergence chromatography.J Chromatogr A. 2017;1522:78-85.
22. Montes R, Rodil R, Cela R, Quintana JB. Determination of Persistent and Mobile Organic Contaminants (PMOCs) in Water by Mixed-Mode Liquid Chromatography–Tandem Mass Spectrometry.Anal Chem. 2019;91(8):5176-83.
23. Magnusson B, Örnemark U. Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics. 2nd edition. 2014.
24. Montes R, Aguirre J, Vidal X, Rodil R, Cela R, Quintana JB. Screening for Polar Chemicals in Water by Trifunctional Mixed-Mode Liquid Chromatography–High Resolution Mass Spectrometry.Environ Sci Technol. 2017;51(11):6250-9.
25. Janda J, Nödler K, Brauch H-J, Zwiener C, Lange FT. Robust trace analysis of polar (C2-C8) perfluorinated carboxylic acids by liquid chromatography-tandem mass spectrometry: method development and application to surface water, groundwater and drinking water.Environ Sci Pollut R. 2019;26(8):7326-36.
26. Yamashita N, Kannan K, Taniyasu S, Horii Y, Okazawa T, Petrick G, et al. Analysis of Perfluorinated Acids at Parts-Per-Quadrillion Levels in Seawater Using Liquid Chromatography-Tandem Mass Spectrometry.Environ Sci Technol. 2004;38(21):5522-8.
27. Seitz W, Schulz W, Winzenbacher R. Advantage of liquid chromatography with high-resolution mass spectrometry for the detection of the small and polar molecules trifluoroacetic acid and sulfamic acid.J Sep Sci. 2018;41(24):4437-48.
28. Scheurer M, Nödler K, Freeling F, Janda J, Happel O, Riegel M, et al. Small, mobile, persistent: Trifluoroacetate in the water cycle – Overlooked sources, pathways, and consequences for drinking water supply.Water Res. 2017;126:460-71.
29. Zhao P, Xia X, Dong J, Xia N, Jiang X, Li Y, et al. Short- and long-chain perfluoroalkyl substances in the water, suspended particulate matter, and surface sediment of a turbid river.Sci Total Environ. 2016;568:57-65.

Tables:

Table 1: Instrumental figures of merit.

Name	**Acronym**	**Linearity (R2)**	**Repeatability (RSD, n=6)**		**IQL (ng/mL)**
		0.5-500 ng/mL	5 ng/mL	50 ng/mL	
Trifluoroacetic acid	**TFA**	0.9989	11%	9%	0.56
Perfluoropropanoic acid	**PFPrA**	0.9995	7%	4%	0.17
Perfluoro-n-butanoic acid	**PFBA**	1.0000	6%	8%	0.08
Perfluoro-n-pentanoic acid	**PFPeA**	0.9999	8%	9%	0.08
Perfluoro-n-hexanoic acid	**PFHxA**	0.9995	5%	2%	0.02
Perfluoro-n-heptanoic acid	**PFHpA**	0.9992	3%	6%	0.03
Perfluoro-n-octanoic acid	**PFOA**	0.9998	10%	9%	0.03
Perfluoro-n-nonanoic acid	**PFNA**	0.9999	8%	9%	0.05
Perfluoro-n-decanoic acid	**PFDeA**	0.9999	10%	5%	0.04
Perfluoro-n-undecanoic acid	**PFUnA**	0.9996	7%	6%	0.02
Perfluoro-n-dodecanoic acid	**PFDoA**	0.9993	10%	6%	0.05
Perfluoro-n-tridecanoic acid	**PFTriA**	0.9997	7%	10%	0.05
Perfluoro-n-tetradecanoic acid	**PFTeA**	0.9998	6%	8%	0.06
Perfluoro-n-hexadecanoic acid	**PFHdA**	0.9997	5%	8%	0.07
Perfluoro-n-octadecanoic acid	**PFOdA**	0.9972	8%	9%	0.07
Perfluoromethane sulfonic acid	**PFMS**	0.9994	5%	10%	0.02
Perfluoroethane sulfonic acid	**PFES**	0.9986	5%	2%	0.02
Perfluoropropane sulfonic acid	**PFPrS**	0.9978	8%	3%	0.06
Perfluorobutane sulfonic acid	**PFBS**	0.9985	7%	3%	0.05
Perfluorohexane sulfonic acid	**PFHxS**	0.9996	5%	2%	0.01
Perfluorooctane sulfonic acid	**PFOS**	1.0000	10%	2%	0.01
Perfluorodecane sulfonic acid	**PFDeS**	0.9994	12%	2%	0.02

Table 2: Percentages of recovery (%R), relative standard deviations (%RSD) and method quantification limits (MQL) of the SPE-MMLC-MS/MS analytical method.

	Recovery % (RSD, n=4)		**MQL (ng/L)**
Analyte	Ultrapure water	River water	River water
TFA[(1)]	92(12)	85(8)	63.5
PFPrA	102 (7)	93 (11)	0.5
PFBA	115 (9)	99 (15)	0.7
PFPeA	94 (7)	96 (12)	0.6
PFHxA	117 (6)	87 (9)	0.6
PFHpA	99 (7)	105 (13)	0.5
PFOA	103 (6)	90 (14)	0.5
PFNA	98 (7)	94 (15)	0.3
PFDeA	92 (8)	95 (10)	0.5
PFUnA	103 (8)	92 (13)	0.4
PFDoA	103 (12)	89 (12)	1.0
PFTriA	82 (15)	85 (15)	0.4
PFTeA	82 (13)	81 (14)	0.5
PFHdA	103 (12)	34 (30)	3.4
PFOdA	115 (15)	11 (35)	1.7
PFMS	95 (9)	114 (13)	0.1
PFES	103 (8)	90 (12)	0.5
PFPrS	104 (12)	92 (14)	0.6
PFBS	101 (11)	86 (13)	0.2
PFHxS	104 (12)	87 (13)	0.7
PFOS	96 (10)	96 (15)	0.4
PFDeS	75 (26)	58 (23)	0.2

[(1)] Recovery and RSD evaluated at 100 ng/L, MQL calculated from procedural blanks.

Table 3: Concentrations (ng/L) of the analytes that were detected in river water samples (n = 3). N.B.: those analytes which are not presented were not detected in any of the samples.

	Conc. ± SD (ng/L)								
Analyte	**SW 1**	**SW 2**	**SW 3**	**SW 4**	**SW 5**	**SW 6**	**DW 1**	**DW 2**	**DW 3**
TFA	71 ± 6	81 ± 5	262 ± 15	101 ± 11	230 ± 15	113 ± 9	66 ± 13	77 ± 13	79 ± 8
PFMS	5.1 ± 0.8	5.8 ± 0.2	9.4 ± 1.2	15 ± 0.4	52 ± 3	26 ± 2	5.2 ± 0.2	7 ± 1	7.9 ± 0.2
PFPrA	3.2 ± 0.3	5.4 ± 0.1	nd	nd	nd	3.3 ± 0.2	nd	nd	nd
PFBA	1.8 ± 0.1	3.0 ± 0.1	7 ± 1	3.6 ± 0.1	22 ± 1	9.8 ± 0.5	51 ± 1	47 ± 3	174 ± 9
PFBS	0.68 ± 0.06	0.32 ±0.02	0.25 ± 0.02	nd	0.65 ± 0.03	0.31 ± 0.01	0.29 ± 0.01	0.28 ± 0.02	nd
PFHxA	0.81 ± 0.12	nd	nd	nd	nd	nd	nd	nd	nd
PFHxS	nd	nd	nd	nd	0.8 ± 0.1	nd	nd	nd	nd
PFOA	1.2 ± 0.1	2.3 ± 0.3	nd	nd	5.0 ± 0.4	nd	nd	nd	nd
PFOS	nd	nd	nd	nd	1.6 ± 0.2	1.3 ± 0.1	nd	nd	nd

nd. Not detected

Caption to figures

Figures:

Fig.1: Chromatograms of a standard (10 ng/mL) of ultrashort-chain PFAAs separation on the mixed-mode columns: WAX (A) and Trinity (B). Peak identification: 1: TFA, 2: PFMS, 3: PFPrA, 4: PFES, 5: PFPrS.

Fig. 2: SPE extraction efficiency (relative recovery, %) obtained with the two studied cartridges (spike level: 10 ng/L, *except TFA: 100 ng/L).

Fig. 3: Extracted-ion chromatogram of TFA in an instrumental blank (red line), a procedural blank (green line) and an ultrapure water sample (black line).

Fig.1

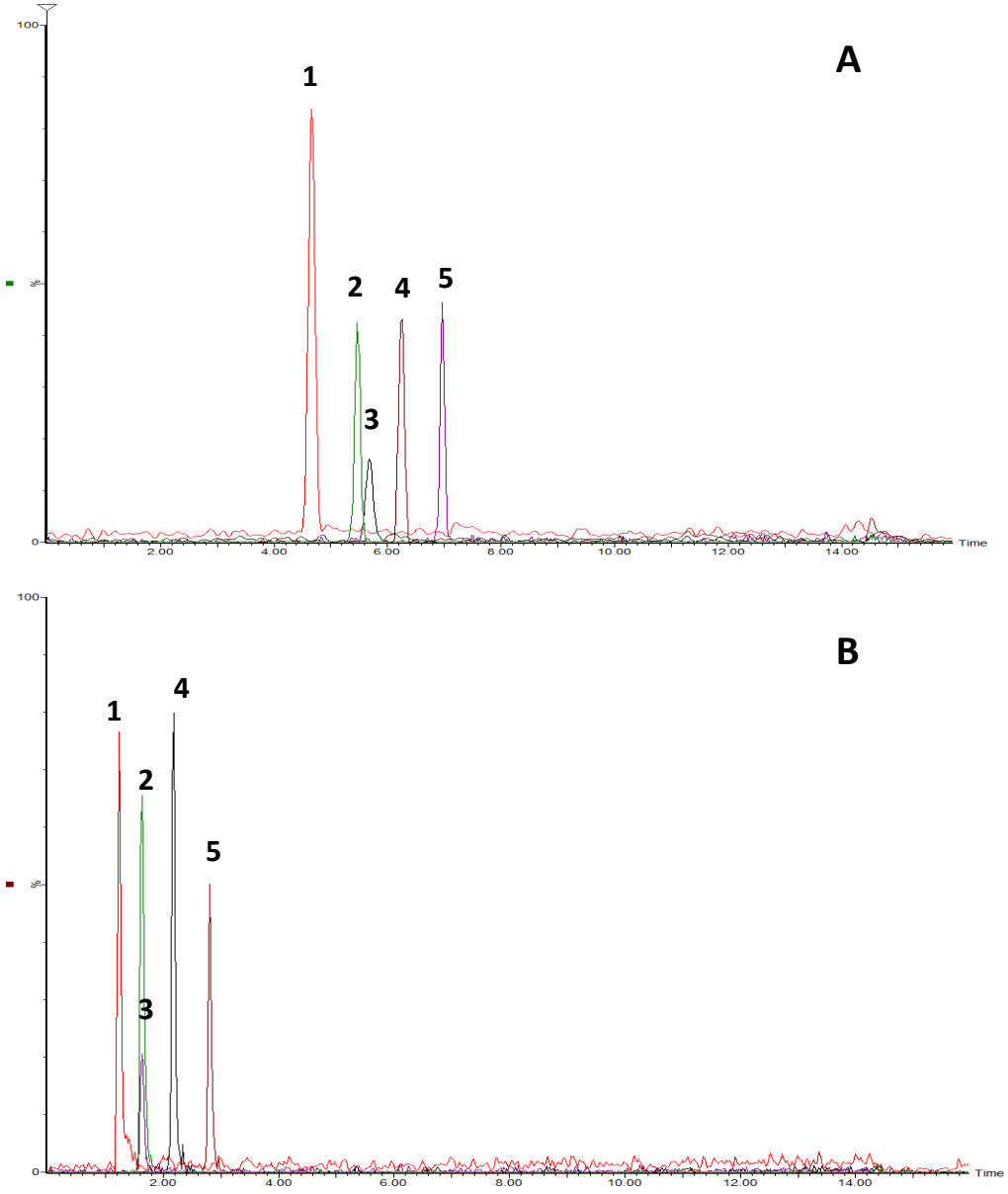
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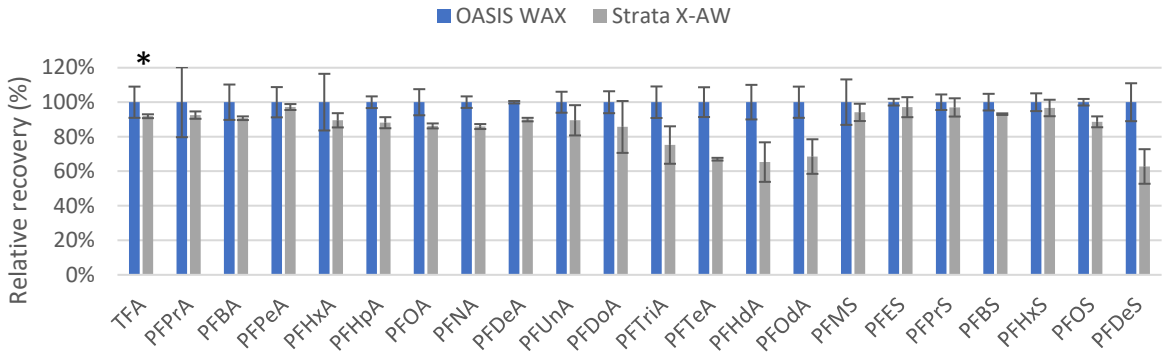
Fig. 2


OASIS WAX
Strata X-AW
Relative recovery (%)
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100%
80%
60%
40%
20%
0%
*
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PFPeA
PFHxA
PFHpA
PFOA
PFNA
PFDeA
PFUnA
PFDoA
PFTriA
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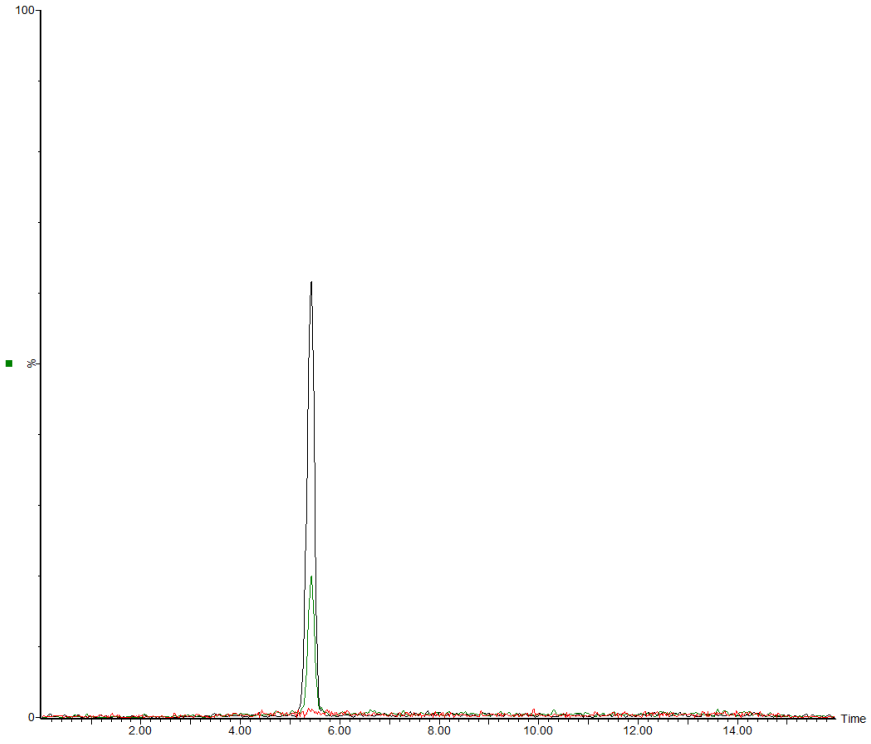
Fig. 3



Supplementary material to:

Applicability of mixed-mode chromatography for the simultaneous analysis of C_1-C_{18} perfluoroalkylated substances

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Table S1: PFCAs and PFSAs considered in the study. Chemical formulae, acronyms and standards supplier information.

Table S2: Sample location (GSM coordinates).

Table S3: Instrumental parameters and transitions (precursor/product) used for quantification and confirmation, deuterated compound used as surrogate or internal standard (IS) and retention time (RT) for every analyte.

Fig. S1: Chromatograms of a standard (10 ng/mL) of short- and long-chain PFAAs separation using mixed-mode columns A) Trinity and B) WAX. Peak identification: 1: PFBA, 2: PFPeA, 3: PFBS, 4: PFHxA, 5: PFHpA, 6: PFHxS, 7: PFOA, 8: PFNA, 9: PFOS, 10: PFDeA, 11: PFDeS, 12: PFUnA, 13: PFDoA, 14: PFTriA, 15: PFTeA, 16: PFHdA, 17: PFOdA

Table S1

Name	Acronym	Formula	Supplier	Concentration
Analytes				
Perfluoroethanoic acid	TFA	C2HF3O2	Sigma Aldrich	99%
Perfluoropropanoic acid	PFPrA	C3HF5O2	Sigma Aldrich	97%
Perfluoro-n-butanoic acid	PFBA	C4HF7O2	Wellington Laboratories	2 µg/mL
Perfluoro-n-pentanoic acid	PFPeA	C5HF9O2		2 µg/mL
Perfluoro-n-hexanoic acid	PFHxA	C6HF11O2		2 µg/mL
Perfluoro-n-heptanoic acid	PFHpA	C7HF13O2		2 µg/mL
Perfluoro-n-octanoic acid	PFOA	C8HF15O2		2 µg/mL
Perfluoro-n-nonanoic acid	PFNA	C9HF17O2		2 µg/mL
Perfluoro-n-decanoic acid	PFDeA	C10HF19O2		2 µg/mL
Perfluoro-n-undecanoic acid	PFUnA	C11HF21O2		2 µg/mL
Perfluoro-n-dodecanoic acid	PFDoA	C12HF23O2		2 µg/mL
Perfluoro-n-tridecanoic acid	PFTriA	C13HF25O2		2 µg/mL
Perfluoro-n-tetradecanoic acid	PFTeA	C14HF27O2		2 µg/mL
Perfluoro-n-hexadecanoic acid	PFHdA	C16HF31O2		2 µg/mL
Perfluoro-n-octandecanoic acid	PFOdA	C18HF35O2		2 µg/mL
Perfluoromethane sulfonic acid	PFMS	CHF3O3S	Carbolution	98%
Perfluoroethane sulfonic acid	PFES	C2HF5O3S	Kanto Corporation	95%
Perfluoropropane sulfonic acid	PFPrS	C3HF7O3S	Kanto Corporation	95%
Perfluorobutane sulfonic acid	PFBS	C4HF9O3S	Wellington Laboratories	2 µg/mL
Perfluorohexane sulfonic acid	PFHxS	C6HF13O3S		2 µg/mL
Perfluorooctane sulfonic acid	PFOS	C8HF17O3S		2 µg/mL
Perfluorodecane sulfonic acid	PFDeS	C10HF21O3S		2 µg/mL
Internal standards				
Perfluoro-n-(1,2,3,4-$^{13}C_4$)butanoic acid	$^{13}C_4$PFBA	C4HF7O2	Wellington Laboratories	2 µg/mL
Perfluoro-n-(1,2-$^{13}C_2$)hexanoic acid	$^{13}C_2$PFHxA	C6HF11O2		2 µg/mL
Perfluoro-n-(1,2,3,4-$^{13}C_4$)octanoic acid	$^{13}C_4$PFOA	C8HF15O2		2 µg/mL
Perfluoro-n-(1,2,3,4,5-$^{13}C_5$)nonanoic acid	$^{13}C_5$PFNA	C9HF17O2		2 µg/mL
Perfluoro-n-(1,2-$^{13}C_2$)decanoic acid	$^{13}C_2$PFDeA	C10HF19O2		2 µg/mL
Perfluoro-n-(1,2-$^{13}C_2$)undecanoic acid	$^{13}C_2$PFUnA	C11HF21O2		2 µg/mL
Perfluoro-n-(1,2-$^{13}C_2$)dodecanoic acid	$^{13}C_2$PFDoA	C12HF23O2		2 µg/mL
Sodium perfluoro-1-hexane[$^{18}O_2$]sulfonate	$^{18}O_2$PFHxS	C6HF13O3S		2 µg/mL
Sodium perfluoro-1-[1,2,3,4 $^{13}C_4$]octanesulfonate	$^{13}C_4$PFOS	C8HF17O3S		2 µg/mL

Table S2.

Sample Code	Location (GSM coordinates)	Description
SW 1	42°51'26.1'' N 8°38'43.9'' W	River water
SW 2	42°51'40.6'' N 8°39'24.0'' W	River water
SW 3	42°54'18.8"N 8°41'40.9"W	River water
SW 4	42°36'33.5"N 7°44'35.5"W	River water
SW 5	43°10'18.8"N 8°26'59.0"W	River water connected to landfill leachate
SW 6	43°13'40.8"N 8°19'10.2"W	River water used for water facilities, before treatment
DW 1	42°52'28.0"N 8°33'38.9"W	Drinking water
DW 2	42°51'28.9"N 8°39'11.6"W	Drinking water
DW 3	42°36'31.1"N 7°46'04.7"W	Drinking water

Table S3

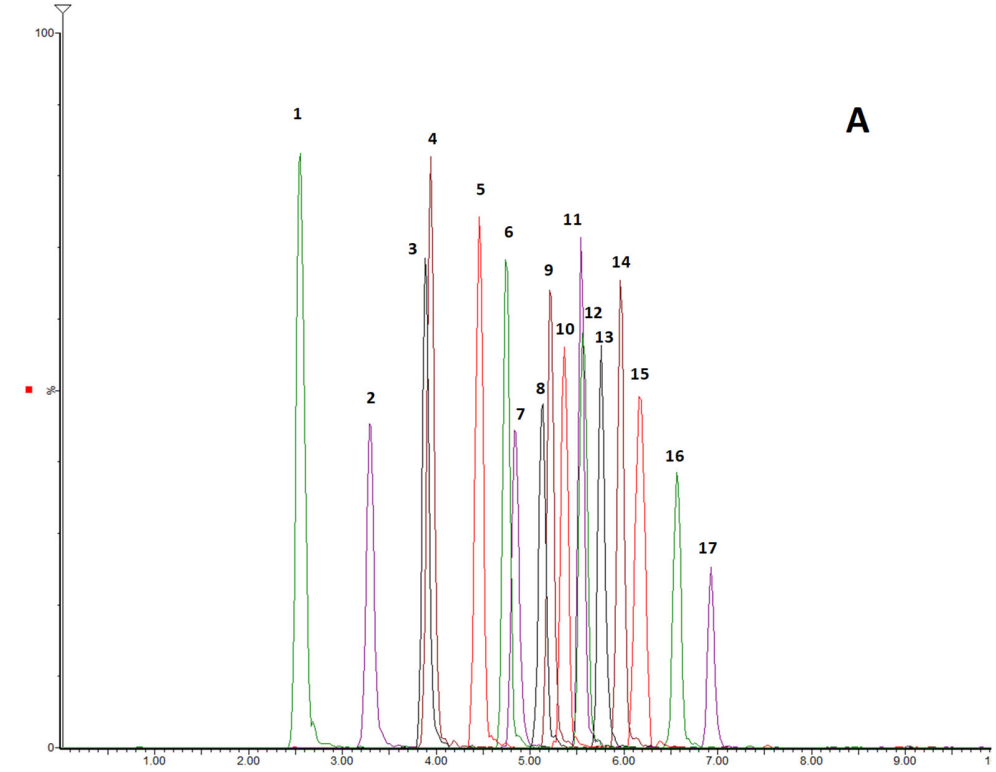
	Precursor	**Product**	**Cone Voltage**	**Collision energy**	**Internal standard**	**Retention time**
Analytes	m/z	m/z	(V)	(V)		(min)
TFA	**113**	**69**	**22**	**8**	$^{13}C_4$PFBA	5.30
PFPrA	**163**	**119**	**18**	**12**	$^{13}C_4$PFBA	6.30
PFBA	**213**	**169**	**20**	**12**	$^{13}C_4$PFBA	7.16
PFPeA	**263**	**219**	**20**	**10**	$^{13}C_4$PFBA	8.02
PFHxA	**313**	**269**	**18**	**10**	$^{13}C_2$PFHxA	8.63
	313	119	18	28		
PFHpA	**363**	**319**	**20**	**12**	$^{13}C_2$PFHxA	9.05
	363	119	20	20		
PFOA	**413**	**369**	**20**	**14**	$^{13}C_4$PFOA	9.32
	413	169	20	20		
PFNA	**463**	**419**	**22**	**14**	$^{13}C_5$PFNA	9.50
	463	219	22	22		
PFDeA	**513**	**469**	**22**	**14**	$^{13}C_2$PFDeA	9.61
	513	269	22	24		
PFUnA	**563**	**519**	**22**	**14**	$^{13}C_2$PFUnA	9.72
	563	169	22	32		
PFDoA	**613**	**569**	**24**	**16**	$^{13}C_2$PFDoA	9.86
	613	169	24	36		
PFTriA	**663**	**619**	**24**	**16**	$^{13}C_2$PFDoA	9.99
	663	169	24	40		
PFTeA	**713**	**669**	**24**	**16**	$^{13}C_2$PFDoA	10.12
	713	169	24	44		
PFHdA	**813**	**769**	**24**	**18**	$^{13}C_2$PFDoA	10.41
	813	169	24	52		
PFOdA	**913**	**869**	**24**	**18**	$^{13}C_2$PFDoA	10.7
	913	169	24	60		

Table S3 cont.

	Precursor	**Product**	**Cone Voltage**	**Collision energy**	**Internal standard**	**Retention time**
Analytes	m/z	m/z	(V)	(V)		(min)
PFMS	**149**	**80**	**46**	**20**	$^{13}C_4$PFBA	5.90
	149	99	46	18		
PFES	**199**	**80**	**50**	**24**	$^{18}O_2$PFHxS	6.70
	199	99	50	20		
PFPrS	**249**	**80**	**54**	**28**	$^{18}O_2$PFHxS	7.38
	249	99	54	22		
PFBS	**299**	**80**	**56**	**36**	$^{18}O_2$PFHxS	7.93
	299	99	56	30		
PFHxS	**399**	**80**	**67**	**42**	$^{18}O_2$PFHxS	8.57
	399	99	67	35		
PFOS	**499**	**80**	**78**	**50**	$^{13}C_4$PFOS	8.84
	499	99	78	40		
PFDeS	**599**	**80**	**85**	**60**	$^{13}C_4$PFOS	9.04
	599	99	85	37		
Internal standards						
$^{13}C_4$PFBA	217	172	20	10		7.16
$^{13}C_2$PFHxA	315	270	18	10		8.63
$^{13}C_4$PFOA	417	372	20	14		9.32
$^{13}C_5$PFNA	468	423	22	14		9.5
$^{13}C_2$PFDeA	515	470	22	14		9.61
$^{13}C_2$PFUnA	565	520	22	14		9.72
$^{13}C_2$PFDoA	615	570	24	16		9.86
$^{18}O_2$PFHxS	403	84	67	42		8.57
$^{13}C_4$PFOS	503	80	78	50		8.84

Quantification transition marked in bolds.

Fig. S1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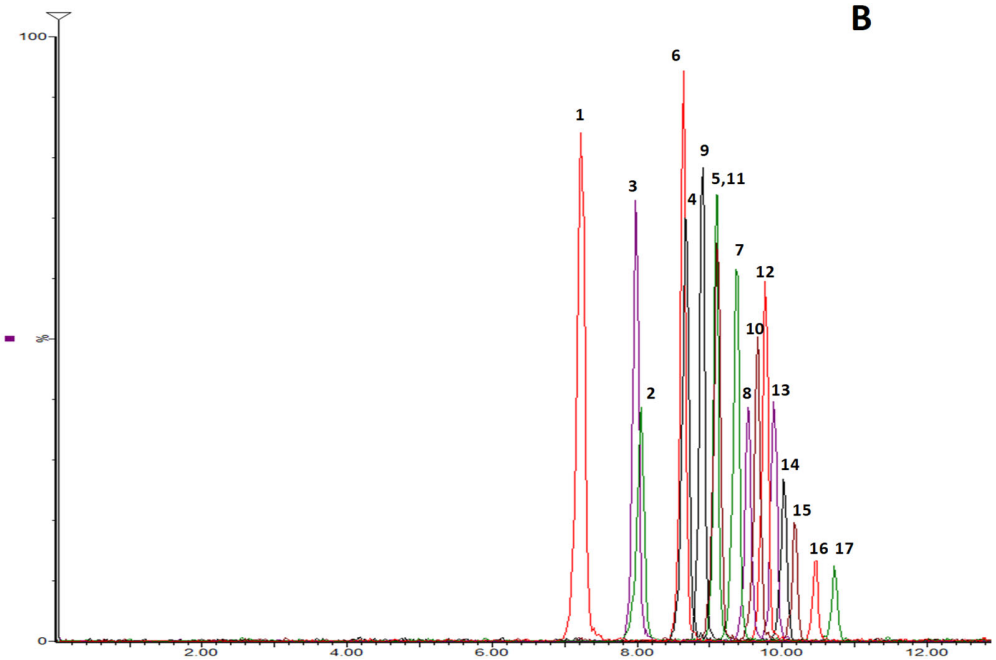
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