ON-LINE SPE LC/ESI-MS/MS ANALYSIS OF PFAAs. BEHAVIOR IN A DRINKING WATER TREATMENT PLANT THAT COMBINES CONVENTIONAL AND ADVANCED TREATMENTS IN PARALLEL LINES.

Flores C[1], Palacios O[1], Ventura F[2], Martín-Alonso J[3], Caixach J[1]*

[1]Mass Spectrometry Laboratory/Organic Pollutants, IDAEA-CSIC, Jordi Girona 18, 08034 Barcelona, Spain;
[2]Department of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18, 08034 Barcelona, Spain
[3] AGBAR-Aigües de Barcelona, Gral Batet 5-7, 08028 Barcelona, Spain

Introduction

Perfluoroalkyl acids (PFAAs) are a subgroup of polyfluoroalkyl substances (PFASs) widely used in industrial and commercial applications for the past sixty years as water and oil repellents, fire retardants, herbicide and insecticide formulations, cosmetics, greases and lubricants, paints, polishes and adhesives[1]. Two important classes of PFAAs are the perfluoroalkyl carboxylic acids (PFCAs) and the perfluoroalkyl sulfonic acids (PFSAs). Specifically, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS) have received more attention because so far are the PFASs most commonly used, found in the environment and included in international regulations[2]. A number of recent studies have indicated serious health effects associated with PFOA and PFOS in various animal models. They are worldwide distributed, environmentally persistent, bioaccumulative, present in remote regions and potentially toxic and therefore have increasingly attracted global concerns in recent years. Both have also been included in the OSPAR List of Chemicals for Priority Action (revised 2009) and listing of persistent organic pollutants (POPs) in the Stockholm Convention. Different from other typical POPs, PFOA and PFOS have high water solubility, and thus can exist and easily transport in water environments. So far, they have been detected in wastewater, surface water, groundwater and even tap water throughout the world[3]. PFOA and PFOS are listed as chemical contaminants on the Drinking Water Contaminant Candidate List CCL3 considered for future regulation (US EPA, 2009). Recently, PFOS has been included in the Proposal for a directive of the European Parliament and of the Council amending Directives 2000/60/EC and 2008/105/EC in regards to priority substances in the field of water policy (European Commission Proposal, 2012). In this Directive, the environmental quality for the PFOS has been set (0.65 and 0.13 ng/L for the annual average of inland and other surface waters, respectively). On the other hand, a wide range of other PFASs are receiving increasing attention because they occur as alternative to PFOA and PFOS, are intermediates for their production, and by-products or products of biodegradation. Some PFAAs currently in use as substitutes are PFBA, PFPeA, PFHxA, PFHpA, PFNA, PFDA, PFBS and PFHxS, mainly the shorter chain, and fluorinated organic compounds containing phosphorus[3, 4]. However, while most of the studies in the literature focusing on PFOA and PFOS monitoring, few information are devoted to detection of the other PFAAs in treated drinking water and their fate during drinking water treatment.

PFAAs concentrations reported in water cover a range of several orders of magnitude (pg-μg/L in contaminated samples). Usually, lower concentrations are found, requiring enrichment of the sample. Off-line solid phase extraction (SPE) and liquid chromatography tandem mass spectrometry (LC-MS/MS) is the most commonly used method for PFASs analyses due to its robust quantitative analysis and good sensitivity and specificity. In fact, ISO standard method 25101:2009 for determination of PFOS and PFOA for unfiltered samples and EPA standard method 537 for determination of selected perfluorinated alkyl acids in drinking water uses this methodology. Sample treatment using an off-line SPE procedure, is tedious, laborious, time consuming and poorly reproducible at trace levels. However, rapid analytical approaches continue to be required. In contrast, on-line SPE allows a fast and reliable approach to the monitoring of trace pollutants in water proving excellent analytical performance characteristics (reduction of analysis times and sample manipulation, low solvent consumption, low amounts of sample required, excellent precision, accuracy and robustness) and advantages for routine analysis (improvement of the sample throughput and automation of the method). In this context, the main objectives of this work were (i) to develop an on-line SPE LC-MS/MS approach based in a retention and preconcentration chromatographic column which can be reused for the rapid screening of PFAAs in water; and (ii) to assess the occurrence and behavior of 11 PFAAs at the different stages of a drinking water treatment plant

(DWTP) that combines conventional and advanced treatments including superficial (raw water) and groundwater.

Materials and methods

Water samples from a DWTP that combines conventional and advanced treatments were analyzed. Sampling was done in 2013 and 2014. Additionally, groundwater involved in the DWTP process was analyzed. This water comes from wells where it is subjected to stripping and chlorination before blending. The conventional potabilization process consists of dioxychlorination, coagulation, flocculation, settling, sand filtration, groundwater dilution, ozonation, granular activated carbon (GAC) filtration and a final postchlorination step. Since 2010, a new parallel treatment line after sand filtration and raw groundwater dilution was in operation. This advanced treatment included ultrafiltration (UF) followed by UV disinfection, reverse osmosis (RO) and remineralization. Finally, treated waters from the two potabilization lines are blended and subjected to a final postchlorination step.

PFASs analyzed in this study were supplied from Wellington Laboratories Inc. (Canada), except perfluoropropinoic acid (PFPrA): i) perfluorobutanoic (PFBA), perfluoropentanoic (PFPeA), perfluorohexanoic (PFHxA), perfluoroheptanoic (PFHpA), perfluorooctanoic (PFOA), perfluorononanoic (PFNA), perfluorodecanoic (PFDA) acids; ii) perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS), perfluorooctanesulfonate (PFOS); iii) [$^{13}C_4$]-perfluorooctanoic acid ($^{13}C_4$-PFOA), [$^{13}C_2$]- perfluorodecanoic acid ($^{13}C_2$-PFDA), ion [$^{18}O_2$]-perfluorohexanesulfonate ($^{18}O_2$-PFHxS) and ion [$^{13}C_4$]-perfluorooctanesulfonate ($^{13}C_4$-PFOS). PFPrA was purchased from Aldrich (Steinheim, Germany).

Analytes were isolated by on-line SPE. Mass-labeled internal standards ($^{13}C_4$-PFOA, $^{13}C_2$-PFDA, $^{18}O_2$-PFHxS and $^{13}C_4$-PFOS) were added prior to analysis for internal standard quantification. 1.5 mL of unfiltered water samples was spiked with an internal standard mixture in methanol to obtain a final concentration of 50 ng/L (7.5 µL at 10 µg/L) and then an aliquot of 1 mL was directly processed using Thermo Electron's EQuan environmental quantification system. The system consists of a two Surveyor LC and MS pumps with a preconcentration column, an analytical column, a PAL autosampler (CTC Analytics, Zwingen, Switzerland) and one switching device unit. The entire system was connected to a triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source and was controlled via Xcalibur software, version 2.1. To optimize the enrichment of target compounds different SPE sorbents were tested. The optimum SPE column for most of the compounds was a combination of mixed-mode Strata-X cartridge (2.0×20 mm, 25 µm particle size from Phenomenex, Torrance, CA, USA) plus Hypersil GOLD C_{18} column (2.1×20 mm, 12 µm particle size from Thermo Fisher Scientific, Franklin, MA). After enrichment, the analytes are transferred to the analytical column for their separation by switching the MS valve into loading mode. The chromatographic separation was performed by linear gradient elution on a reversed-phase C_{18} Hypersil Gold analytical column (50 x 2.1 mm, 1.9 µm, Thermo Fisher Scientific, San Jose, CA, USA) preceded by an C_{18} XBridge guard column (10 x 2.1 mm, 2.5 µm, Waters, Mildford, MA, USA). The mobile phase was composed of Milli Q water as solvent A and methanol as solvent B at a flow rate of 200 µL/min. Briefly, the whole procedure consisted first with, loading the sample into the enrichment column using ultrapure water. After the enrichment step, the analytes were transferred to the analytical column for separation. The last step was the equilibration in which the initial conditions were set for the next run while the MS connector changes to waste. After separation, detection was carried out by LC coupled to ESI tandem mass spectrometry (LC/ESI-MS/MS) with a triple quadrupole instrument (TSQ Quantum, Thermo Fisher Scientific, San Jose, CA, USA). The analyses were carried out in negative ion electrospray and selected reaction monitoring (SRM) modes. Two transitions for each compound were registered, one for quantification and one for confirmation. A relation between transitions was calculated. The spray voltage was chosen at 3.5 kV and the tube lens voltage and collision energy were optimized for each *m/z* and for each transition, respectively. The ion transfer tube temperature was set at 350 ºC. Nitrogen was used as a sheath gas, ion sweep gas an auxiliary gas at flow rates of 30 psi, 0 and 5 a.u. (arbitrary units), respectively. The argon gas collision-induced dissociation was used with a pressure of 1.5 mtorr.

For identification purposes the following criteria were accomplished: i) the ratio of the chromatographic retention time of the analyte to that of the internal standard, shall correspond to that of the calibration solution at a tolerance of ±2.5%; ii) two m/z transitions were confirmed for each analyte; iii) the ratio between the two transitions in the sample compared to ratio in the calibration curve should be in agreement to [calibration curve average ± maximum permitted tolerances for relative ion intensities as proposed in European Commission

2002/657/EC]. The quantification of analytes was performed by internal calibration curve: the plot ratio of the most intensive transition peak area divided with the internal standard area against the ratio of concentrations.

Results and discussion

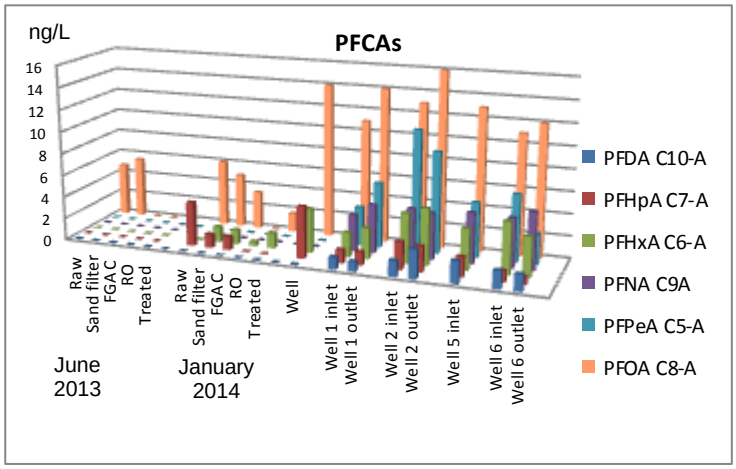
To optimize the enrichment of target compounds different SPE sorbents were tested: C_{18} and modified C_{18} (Hypersil GOLD and aQ columns, 2.1×20 mm, 12 µm particle size from Thermo Fisher Scientific, Franklin, MA); porous graphitic carbon (Hypercarb column, 2.1×20 mm, 7 µm particle size from Thermo Fisher Scientific, Franklin, MA); polymeric (Strata-X cartridge, 2.0×20 mm, 25 µm particle size from Phenomenex, Torrance, CA, USA); and different combined in series between C_{18}, modified C_{18} and polymeric columns. The best enrichment rates for most of the compounds were achieved using mixed-mode Strata-X plus Hypersil GOLD C_{18} columns. In order to avoid the possible carry over between samples, the extraction column was cleaned with methanol and water after every injection. The total run time was 25 min. One of the main advantages of this system in comparison to the common online-SPE cartridges is that the enrichment column can be used for more than 1000 samples, being an important reduction in the analysis expenses. Additionally, LC method for on-line trace enrichment carried out by means of column switching technique is very simple and easily configured. The quality parameters achieved using the on-line enrichment procedure are reported in the table. The method was linear in the working range (LOQ-500 ng/L) with regression coefficients (R^2) greater than 0.9974 and residuals below 19%. Good recoveries were obtained, between 79 and 119%. Initially, PFPrA and PFBA were enclosed in the study. For PFPrA the recovery was too low, below 30%. On the other hand, PFBA exhibited good recovery (75%) but matrix effect was detected in the samples. To compensate this matrix effect in future analyses we will add $^{13}C_4$-PFBA for reliable quantification of PFBA. Therefore, PFPrA and PFBA results are not presented in the present work. The intraday precision at two concentration levels (low-medium and high concentrations) also was satisfactory with RSDs always lower than 15%. The limit of quantification (LOQ) was considered as the lowest validated level and the limit of detection (LOD) was estimated for a signal-to-noise (S/N) ratio equal to 3 from the chromatograms of the samples spiked at the lowest level (LOQ of each analyte). LOQs were between 0.5 and 5.0 ng/L and LODs ranged from 0.1 to 3.0 ng/L.

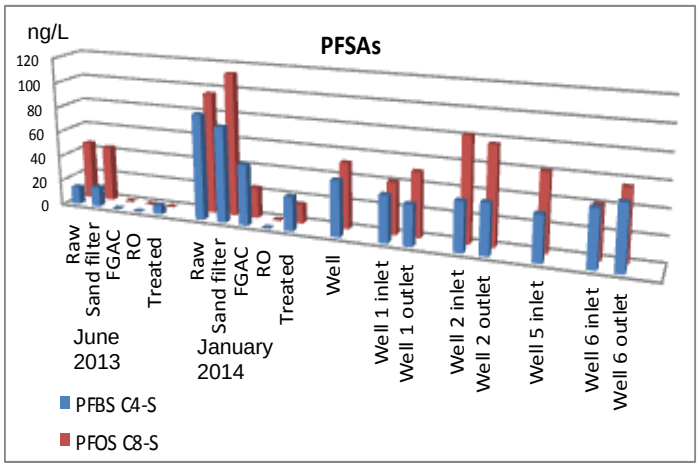
Analyte	Linearity			Recovery (%)	%RSD (n=3)		(ng/L)	
	Range (ng/L)	R^2	Residuals (%)	(n=3, SD)	5 ng/L	200 ng/L	LOD	LOQ
PFPeA (C_5-A)	LOQ-500	0.9986	<10	80 (7)	13	4	3.0	5.0
PFHxA (C_6-A)	LOQ-500	0.9981	<15	79 (11)	8	2	0.1	0.5
PFHpA (C_7-A)	LOQ-500	0.9987	<6	109 (1)	6	4	0.2	0.5
PFOA (C_8-A)	LOQ-500	0.9995	<19	100 (1)	15	2	0.1	1.0
PFNA (C_9-A)	LOQ-500	0.9959	<17	109 (3)	6	4	0.3	1.0
PFDA (C_{10}-A)	LOQ-500	0.9991	<10	119 (5)	8	1	0.4	1.0
PFBS (C_4-S)	LOQ-500	0.9974	<18	89 (10)	12	8	1.1	5.0
PFHxS (C_6-S)	LOQ-500	0.9991	<11	93 (4)	9	3	1.0	5.0
PFOS (C_8-S)	LOQ-500	0.9999	<13	108 (9)	5	5	1.0	5.0

PFASs have become ubiquitous in the environment. Therefore, special care was taken to prevent contamination from sampling and laboratory material and instrumental parts. To minimize background contamination throughout the procedure, all known sources of contamination, including accessible polytetrafluoroethylene (PTFE) and other fluoropolymer materials from the instruments and apparatus, were removed. Blank samples and blank spikes were used as quality controls. Additionally, a trapping column was installed after LC pump and before injection valve and a by-pass of degasser was done for MS pump. The trapping column improved LOQ in an order of magnitude for PFHxA and PFOA analytes.

Water samples from a DWTP along to different treatments in two sampling (June 2013 and January 2014) and groundwater involved in this DWTP were analyzed. This study is at an early stage and is expected to continue next sampling. The results obtained to date are shown in the diagrams and described below. PFPeA, PFNA and PFDA were not analyzed in June 2013 samples. PFHxS and PFPeA were not detected in any sample. Regarding DWTP samples, overall levels of analytes were higher in January 2014 than in June 2013 with significant differences of concentrations at the intake of the DWTP (raw water). This behavior observed for all compounds was more significant in the more frequently found compounds, PFOS and PFBS, for which concentrations were higher, 96 and 84 ng/L in January 2014 and 46 and 14 ng/L in June 2013, respectively. Focusing on June 2013 samples, PFOS, PFBS and PFOA were detected in raw water (46, 14 and 4.8 ng/L, respectively) and only PFBS

was not been completely removed until final treated water (7.0 ng/L). PFHxA, PFHpA and PFHxS were not detected in any sample. Dioxychlorination and sand filtration have not produced any effect on the concentration of the compounds under study and sorption onto GAC and RO have produced a 100% removal. On the other hand, in January 2014 samples, the RO practically completely removed all PFAAs under study. Meanwhile, the GAC filter had an irregular performance with removal of 79% for PFOS, around 40% for PFOA, PFDA and PFBS and <18% for other analytes. This behavior is consistent with other authors who described RO as an effective treatment to remove PFAAs >C_5 in the potabilization process and GAC filters as useful treatment for removing PFASs from drinking water, although the efficiency of GAC is compromised in the presence of natural organic matter and frequent carbon reactivation may be necessary[4, 5]. Also, it has been demonstrated that longer chain PFASs will sorb better onto GAC compared to the shorter chain compounds. PFASs such as PFBA and PFBS may pass through or reach breakthrough very quickly. In summary, for DWTP samples, PFOS and PFBS were the predominant PFAAs detected with PFOA and PFHpA in a lesser extent. The other PFAAs were quantified at concentration levels below 1.8 ng/L. Regarding groundwater, all wells have a similar profile as the type of PFASs detected and the concentration range. As expected, the stripping and chlorination does not produce any reduction in the initial levels of PFAAs in groundwater. The more frequently found compounds were PFSAs, PFOS (71-82 ng/L) and PFBS (36-51 ng/L). The predominant PFCAs were PFOA with concentrations in the range between 11 and 16 ng/L. The others PFCAs were quantified a concentration below 9.2 ng/L.





It should be remarked that all samples analyzed (raw, treated, final treated and groundwater) presented at least one of the compounds at quantifiable concentrations.

Finally, it should be noted that taking as a reference the more restrictive individual guidance levels, i.e. 40 ng/L (preliminary health-based guideline value, New Jersey) and 200 ng/L (Provisional Health Advisory, US EPA) for PFOA and PFOS, respectively, all treated drinking samples may not pose an immediate health risk to consumers.

References:

1. Giesy JP, Kannan K. (2002); *Environ. Sci. Technol.* 36: 146A–52A
2. Kissa E. (2001); 2nd ed. New York: Marcel Dekker
3. Lindstrom AB, Strynar MJ, Libelo EL. (2011); Environ. Sci. Technol. 45: 7954–61
4. Rahman MF, Peldszus S, Anderson WB. (2014); *Water Res.* 50: 318–40
5. Flores C, Ventura F, Martin-Alonso J, Caixach J. (2013); *Sci. Total Environ.* 461-462: 618–26