DETERMINATION OF PERFLUORINATED COMPOUNDS IN FRENCH WATER SAMPLES USING LC-MS/MS

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Introduction

Perfluorinated compounds (PFCs), especially perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), described by some scientist as the "PCBs of the twenty-first century", are a class of emerging environmental contaminants. These compounds present the particularity to repel both water and oil, and exhibit very high chemical and thermal stability. These properties have made PFCs widely used in many commercial applications including surfactants, lubricants, paper and textile coatings, polishes, food packaging, and fire-retarding foams. Most of these compounds are present in the environment as a result of human manufacture and use. It has been established that significant amounts of these compounds were detected in human blood and in the liver of many species, and it appears that a potential risk could exist in terms of developmental and other adverse effects associated with such human exposure.

The aquatic environment appears to be an important route from which PFCs are released in the environment. Thus, their concentrations in water have to be monitored, in order to determine their occurrence as a first step of risk assessment.

Materials and Methods

Standard compounds were obtained from Wellington Laboratories (Guelph, Canada). Solvents were Picograde® quality and provided by LGC Promochem (Wesel, Germany). Fourteen compounds are analysed : perfluorobutane sulfonate (PFBS) ; perfluorohexane sulfonate (PFHxS) ; perfluoroheptane sulfonate (PFHpS) ; perfluorooctane sulfonate (PFOS) ; perfluorodecane sulfonate (PFDS) ; perfluorobutanoic acid (PFBA) ; perfluoropentanoic acid (PFPA) ; perfluorohexanoic acid (PFHxA) ; perfluoroheptanoic acid (PFHpA) ; perfluorooctanoic acid (PFOA) ; perfluorononanoic acid (PFNA) ; perfluorodecanoic acid (PFDA) ; perfluoroundecanoic acid (PFUnA) ; perfluorododecanoic acid (PFDoA) ; perfluorotridecanoic acid (PFTrDA) ; perfluorotetradecanoic acid (PFTeDA).

The LC separation was achieved on a Gemini C18 3µm (50 x 2,0 mm, Phenomenex), coupled to an Agilent pump HP1200. The elution solvents were ammonium acetate in water (20 mM) (A) and methanol (B). The compounds were separated with the following gradient: A/B 50/50 for 0.5 min, from 50/50 to 0/100 in 6.5 min, 0/100 for 5 min, from 0/100 to 50/50 in 3 min, 50/50 for 5 min. The gradient was linear and the flow rate was set at 0.6 mL/min. The injected volume was 50 µL. The detection was performed on a Agilent HP6410 triple quadrupole analyser (Palo Alto, CA, USA) operated in negative electrospray ionization (ESI-) and in selected reaction monitoring acquisition mode (SRM). Nitrogen was used as nebulisation and desolvatation gas, at 40 psi and 6 L/min, respectively. Potential applied onto the capillary was 4.0 kV. Cone potential and collision energy were optimized for each molecule. In the collision cell, nitrogen was used as a collision gas.

Sample preparation procedure

Sample aliquot of 100 ml was spiked with 1 ng of $^{13}C_4$-perfluorooctane sulfonate and $^{13}C_4$ perfluorooctanoic acid. Sample was filtered and loaded onto an Oasis HLB SPE cartridge (Waters). Column was washed with 5 ml of purified water and perfluorinated compounds were eluted with 10 ml of a methanol/ammonium hydroxide mixture (99.9/0.1; *v/v*). The extract was evaporated under a gentle stream of nitrogen before being reconstituted with 200 µl of a methanol/water mixture (50/50, *v/v*).

Results and discussion

Performances
Performances of the method are described in Table 1.

Table 1: Performances of the analytical method, in terms of coefficient correlation (R^2), limit of detection (LOD), recovery, repeatability and trueness.

Compounds	R^2	LOD ($ng.L^{-1}$)	Recovery (%)	Repeatability* (%)	Accuracy (%)
PFBA	0,9950	0.09	94	17.9	9.8
PFPA	0,9975	0.15	100	13.2	11.5
PFHxA	0,9962	0.10	102	11.3	1.3
PFHpA	0,9969	0.12	98	7.2	3.6
PFOA	0,9979	0.04	99	4.4	5.0
PFNA	0,9992	0.03	91	6.4	1.0
PFDA	0,9927	0.01	83	8.2	5.1
PFUnA	0,9962	0.03	74	9.0	27.1
PFDoA	0,9983	0.01	67	15.6	3.1
PFTrDA	0,9902	0.05	55	19.0	13.5
PFTeDA	0,9877	0.02	75	32.0	13.0
PFBS	0,9941	0.06	101	9.2	0.8
PFHxS	0,9995	0.03	98	5.6	21.7
PFHpS	0,9995	0.03	90	4.9	5.9
PFOS	0,9982	0.04	81	5.1	1.4
PFDS	0,9994	0.01	76	10.1	19.3

* : Repeatabilty (%) = RSD / average

Repeatability has been assessed on the basis of 20 samples fortified at 20 $ng.L^{-1}$ and analysed according to the previously described method. Globally, the observed signal variability appeared below 15% which was found fully satisfying. However, higher values have been found for long-chain and short-chain, due to the quantification of these compounds with the imperfectly adapted internal standards PFOA $^{13}C_4$ or PFOS $^{13}C_4$, compounds which present significantly different physical-chemical properties. LODs were estimated on the basis of the signal observed for the lowest point of the calibration curve and subsequent evaluation of concentration inducing a signal to noise ratio of 3. These values ranged from 0.01 to 0.15 $ng.L^{-1}$, which appeared fully compatible with the expected concentration levels in water, and to allow determination of the contamination pattern in an efficient manner even at low-concentration level. The linearity was assessed on seven calibration levels for each analyte over the respective range of 1 to 100 ng-L-1. Coefficient of determination (R^2) were found better than 0.99 for all the analytes, except PFTeDA (R2 = 0.9877). Recoveries was calculated based on comparison between a sample spiked at the beginning of the extraction and the same sample spiked just before injection. They were found globally between 80% and 102%. However, lower values were obtained for long-chain compounds (55 – 75 %), probably due to an ion suppression phenomenon caused by co-elutions of interferences at the end of the gradient. Accuracy was evaluated based on comparison between amount spiked and amount calculated with the calibration curve. It varied from 0.8 to 27.1%.

Quality control of external contamination
A major problem associated to the analysis of perfluorinated compounds is the risk of external contamination in PFOA due to the presence of fluoropolymers in the analytical system. These types of fluoropolymers have to be avoided in order to increase limits of detection of the method, and quantify accurately the PFCs, even at $ng.L^{-1}$ level. As described in Figure 1, no significant contamination was observed with the applied method and the equipment used for LC-MS/MS measurement.

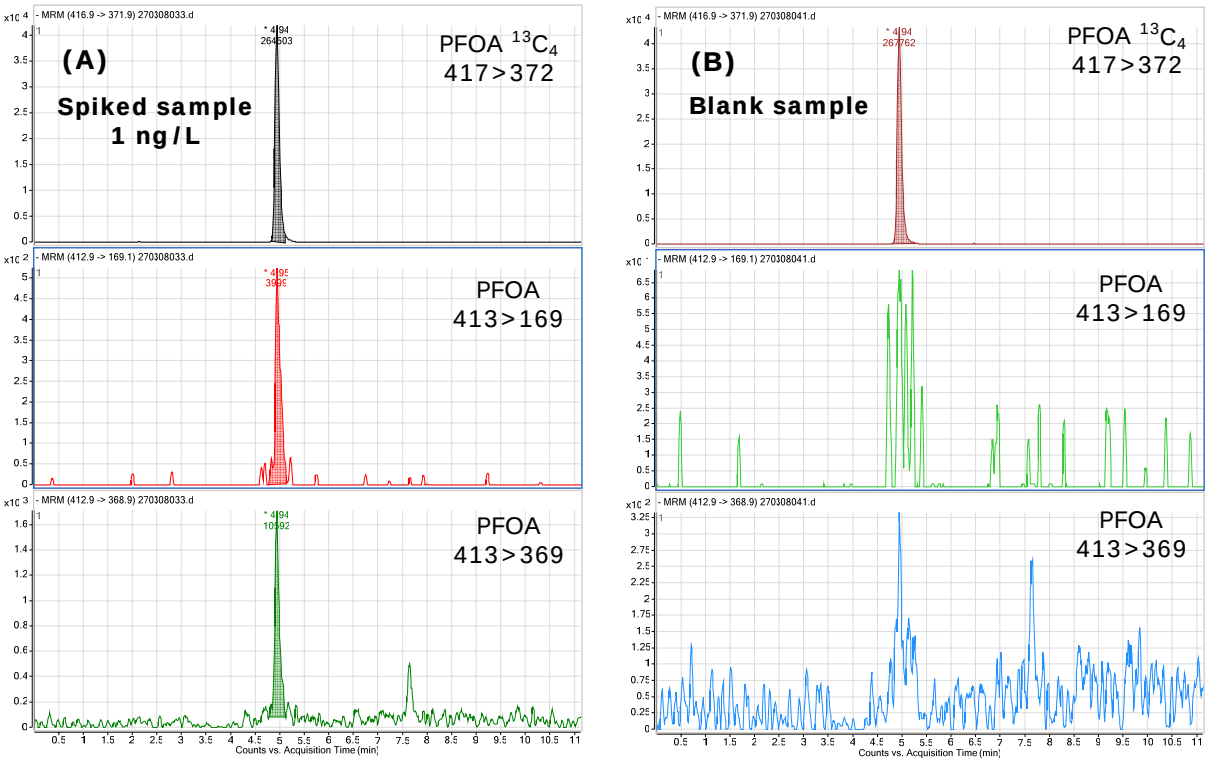

Figure 1 : Diagnostic ion chromatograms of the internal standard (IS) PFOA $^{13}C_4$ (417>372) and PFOA (413>169 and 413>369) obtained for (a) a spiked sample at 1 ng/L and (b) a blank sample.

Analysis of river water

The developed method was applied to 48 water samples collected from various French rivers. Significant concentrations have been found for several compounds.

Taken as a whole, and in accordance with other studies, PFOS was the main detected compound. It was detected in 24 samples at a concentration ranging from 0.5 to 540 ng.L^{-1}. It was also observed that when PFOS was not detected (LOD = 0.2 ng.L^{-1}), the other perfluorosulfonates were not detected either. These results seam to confirm the relevance of measuring systematically PFOS for assessing the global occurrence of PFC in water.

Concerning the other perfluorosulfonates, PFHxS seems to be the second more important analyte, representing in the present case around 50% of the PFOS level. PFBS and PFHpS were detected only in few samples, which presented the highest level of PFOS. PFDS was not detected in any sample.

Regarding perfluorocarboxylic acids, PFOA was the main detected compound (detected in 21 out of 48 samples, at concentration level between 1 and 228 ng.L^{-1}). No significant correlation was observed between the concentration levels of PFOS and PFOA. In particular samples, PFOA has been found at high concentration levels (20-200 ng.L^{-1}) while no traces of PFOS were detected. Concerning other acids, PFHxA is the second more important. Other compounds were detected only in few samples, which presented the highest levels of PFOA.

Examples of typical diagnostic ion chromatograms obtained for various water samples are shown in Figure 2. Concentrations of PFOS in these 4 water samples were found in-between 108 and 3.5 ng.L^{-1}. Chromatogram (D) represents the analysis of a water sample without any detected concentration of PFOS. The first peak represents the branched-PFOS which is present in significant amount. Quantification of this isomer was not evaluated due to the commercial non-availability of the corresponding reference substances.

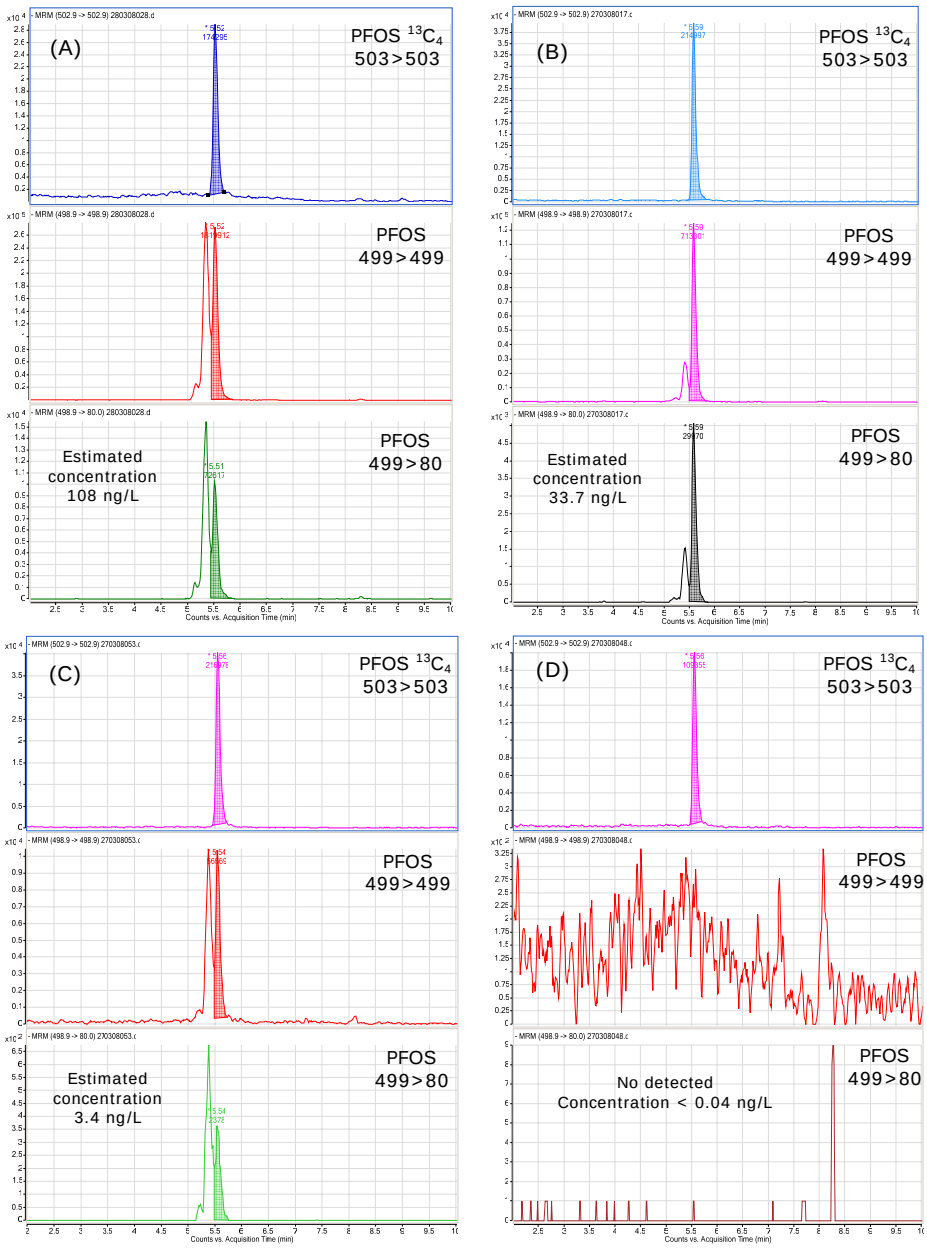

Figure 2 : Diagnostic ion chromatograms of PFOS $^{13}C_4$ (503>503) and PFOS (499>499 and 499>80) obtained for 4 river samples in which PFOS was quantified at a concentration estimated to (a) 108 ng/L, (b) 33.7 ng/L and (c) 3.4 ng/L. Chromatograms (d) correspond to a no-detected sample, the limit of detection being evaluated at 0.04 ng/L.

Conclusion

The described method was proven to be fit-for-purpose in terms of sensitivity and specificity for measuring perfluorinated compounds in water. It was applied to the analysis of 48 water samples which allowed to generate the first exposure data and contamination pattern in French rivers. It was found that PFOS represents the major contributor to the PFC global pollution, followed by PFOA. These 2 compounds were detected in around 50% of the analysed samples. PFHxA and PFHxS were also detected in most of the samples, but to a minor extend. The other PFCs were detected only in few samples, which presented the highest global contamination level.