ANALYSIS OF PERFLUOROALKYL PRECURSORS IN FISH SAMPLES: CHALLENGE AND FIRST APPLICATION

Pollono C[1,2], Veyrand B[1,2], Barbarossa A[3], Durand S[1,2], Marchand P[1,2], Antignac JP[1,4], Pagliuca G[3], Le Bizec B[1,2]

[1]ONIRIS, USC 2013 LABERCA, Nantes, F-44307, France; [2]Université Nantes Angers Le Mans, France; [3]University of Bologna, Department of Veterinary Public Health and Animal Pathology, Via Tolara di Sopra, 50 I 40064 Ozzano Emilia, BO, Italy; [4]INRA, Nantes, F-44307, France;

Introduction

Perfluorinated compounds (PFCs) are synthetic chemical substances produced and used to exploit their particular physico-chemical properties through anti-sticking material or surfactant related products. PFCs are then used in many applications, and consumers from industrialised countries are today in contact with these chemicals in their daily life, through a high number of manufactured products. In parallel, as many other chemicals of entropic origin, PFCs may be released into the environment at each step of their living cycle, and retrieved in various components of the food chain. Food exposure, especially through particular vectors of chemical exposure such as fish, represents a main route of exposure to PFCs for consumers. In addition, first scientific pieces of evidence relying PFCs to reproductive troubles in human were recently delivered. For these reasons, increasing attention is being given to the molecules, and European Union issued in 2010 a Commission Recommendation on the monitoring of these contaminants[1]. In this document it's required to focus not only on the most commonly considered perfluorinated compounds, namely perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), but also on precursors forms including perfluorooctane sulfonamidoethanols (FOSEs), fluorotelomer alcohols (FTOHs), or polyfuoroalkyl phosphate surfactants (PAPS)[1,2,3]. These compounds are as well largely employed in different applications, such as surface treatment formulations for paper and textile products, to give oil and water repellency[3,4].

Regarding FOSEs, two example of compounds of interest are N-methyl heptadecafluorooctane sulfonamidoethanol (N-MeFOSE) and N-ethyl heptadecafluorooctane sulfonamidoethanol (N-EtFOSE). These molecules are very volatile, so they can easily be transferred to the atmosphere from wastewater treatment plants (WWTP)[4]. FTOHs are also volatile substances, and the various compounds belonging to this group are differing from the length of the fluorinated carbons chain. PAPS are as well precursors of PFOA, *via* metabolism of the fluorotelomer alcohol which they release as a first step of degradation[3]. This family includes various compounds, which differs mainly for the number of polyfluoroalkylated chains (MonoPAPS, DiPAPS and TriPAPS); secondly, they can differ for the length of these chains (for example 6:2 MonoPAPS, 8:2 MonoPAPS,…).

In this general context, the objective of the present work was to develop an efficient analytical strategy for isolating (extract and purify) FOSEs and FTOHs from fish samples (notably 6:2 FTOH, 8:2 FTOH and 10:2 FTOH), and then identifying and quantifying these analytes by gas chromatography coupled to tandem mass spectrometry (GC-MS/MS). A similar analytical development was also initiated for PAPS.

Materials and methods

Chemicals

Methanol, dichloromethane and acetone picograde were provided by Promochem (Wesel, Germany), ammonium acetate by Merck (Darmstadt, Germany), glacial acetic acid by SDS (Peypin, France), Silica gel by Sigma-aldrich (St Louis, USA) and EnviCarb phase by Supelco (Bellefonte, USA). Standard reference solutions were purchased from wellington laboratories (Southgate, Canada) and prepared at 10 ng/µL in methanol. Working solutions were prepared at concentrations ranging from 0.01 ng/µL to 1 ng/µL and stored at 4°C.

Sample preparation procedure

One gram of freeze-dried fish sample was spiked with 5 ng of ^{2}H-labelled internal standard (d7 NMeFOSE). After adding 15 mL of methanol, samples were agitated through a horizontal shaker for 15 min and then centrifuged for 15 min at 4000 rpm. The methanolic extract was transferred to a tube containing 800 mg of EnviCarb phase, previously activated with 1 mL of glacial acetic acid. After agitation (1 min) and centrifugation

(5 min, 4000 rpm), methanol was transferred into another tube and evaporated under a gentle stream of nitrogen to around 500 µL. Then, 3mL of dichloromethane were added and the extract was loaded onto a column containing hydrated silica gel (1,5%), previously activated with dichloromethane. After washing with dichloromethane, analytes were eluted with acetone. Acetone was evaporated under a gentle stream of nitrogen at 35°C to 200 µL, transferred to 1.5 mL microtube and centrifuged at 12000 rpm for 45 min. Finally, 150µL were transferred in a polypropylene GC vial.

GC-MS/MS measurement

The GC separation was achieved on a Varian capillary column (CP-WAX 57CB, 25m, 0.25mm I.D, 0.20µm film thickness). Detection was performed on an Agilent 7000 triple quadrupole instrument. A volume of 2 µL was injected in pulsed splitless mode (225°C). Helium was used as carrier gas at 1 mL/min. The oven temperature gradient started at 60°C (held for 4 min), then increased to 70°C (5°C/min) and then to 200°C (15°C/min, held for 6 min). Detection was performed in positive chemical ionization (CI+) using the multiple reaction monitoring (MRM) acquisition mode. Methane was used as reagent gas and the source temperature was set at 200°C. A N_2/He gas mixture was used as collision gas (N_2 flow = 1.5 mL/min and He flow = 2.25 mL/min). Collision energy varied from 1 to 30 V, as described in Table 1.

Table1: Diagnostic signals (MRM transitions) used for detecting sulfonamido-ethanols and fluorotelomers with associated collision energies (eV) after positive chemical ionization mode.

Compound	Diagnostic signal	Collision Energy (eV)
NMeFOSE	557.7 > 557.7	1
	557.7 > 540.0	10
	557.7 > 476.0	30
NEtFOSE	571.7 > 571.7	1
	571.7 > 554.0	10
	571.7 > 462.0	30
$^{2}H_{7}$ NMeFOSE	564.7 > 564.7	1
	564.7 > 547.0	10
	564.7 > 483.0	30
6:2 FTOH	364.6 > 364.6	1
	364.6 > 327	10
8:2 FTOH	464.6 > 464.6	1
	464.6 > 427	10
10:2 FTOH	564.6 > 564.6	1
	564.6 > 527	10
$^{13}C_{2}$ $^{2}H_{2}$ 8:2FTOH	468.6 > 468.6	1
	468.6 > 431	10

Results and discussion

In a first step, some experiments were conducted using LC-MS/MS as measurement technique for FOSEs. However, unsatisfactory results were immediately observed by this way. In particular, only acetate adducts were observed, and the specificity and sensitivity finally obtained were not good enough. In comparison, GC-MS/MS permitted to obtain good results: all compounds were detected in full scan mode, and specific fragments have been observed after fragmentation in the collision cell. Two diagnostic signals (MRM transitions) with good specificity have been determined for all target compounds. An illustration of the chromatic separation obtained in GC-(CI+)-MS/MS is illustrated in Figure 1.

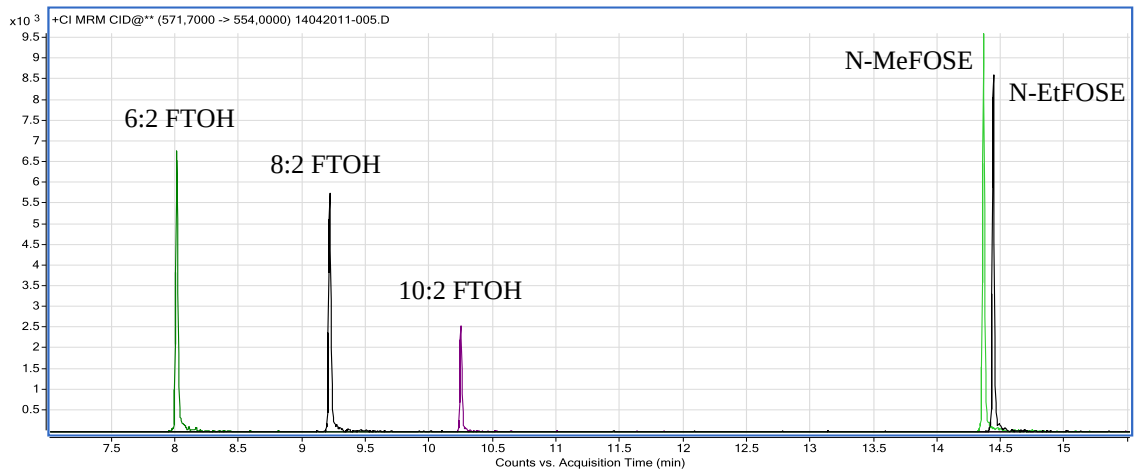

Figure 1: typical diagnostic ion chromatogram obtained in GC-(CI+)-MS/MS for the five perfluoroalkyl precursor compounds analysed (0.02 ng injected on-column).

Regarding the extraction and purification procedure, a particular critical point was identified for FOSEs and FTOHs which is the evaporation steps, due to the high volatility of these compounds. After various tests, this issue has been solved for FOSEs by decreasing samples temperature during evaporation from 45 to 35°C, paying attention to use a more gentle nitrogen flow and reducing samples volume to 200 µL instead of evaporating them to dryness. However, even after this optimization it was not possible to detect properly FTOHs. In order to assess the linearity and limit of detection (LOD) of the method, a six points matrix-matched calibration curve has been prepared (from 0 to 50 µg/kg of dry matter), giving satisfactory results: as described in Figure 1, linearity for both of the compounds were found satisfactory, with coefficient of determination (R^2) higher than 0.99 for both tested FOSEs compounds.

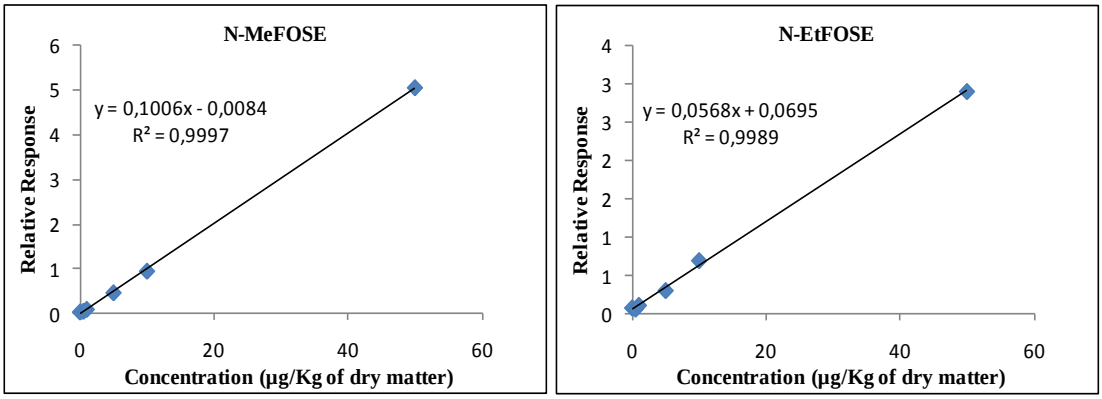

Figure 2: Calibration curves obtained for N-EtFOSE and N-MeFOSE spiked in a fish sample from 0 to 50 µg/kg of dry matter.

Limits of detection of the method have been evaluated (signal-to-noise ratio of 3) at 0.030 µg/kg and 0.040 µg/kg of wet matter for N-EtFOSE and N-MeFOSE respectively. An example of diagnostic chromatograms obtained for a fish sample spiked with N-MeFOSE and N-EtFOSE at 0.25 µg/kg is given in figure 3, permitting to evaluate both the sensitivity at this concentration level as well as the signal specificity.

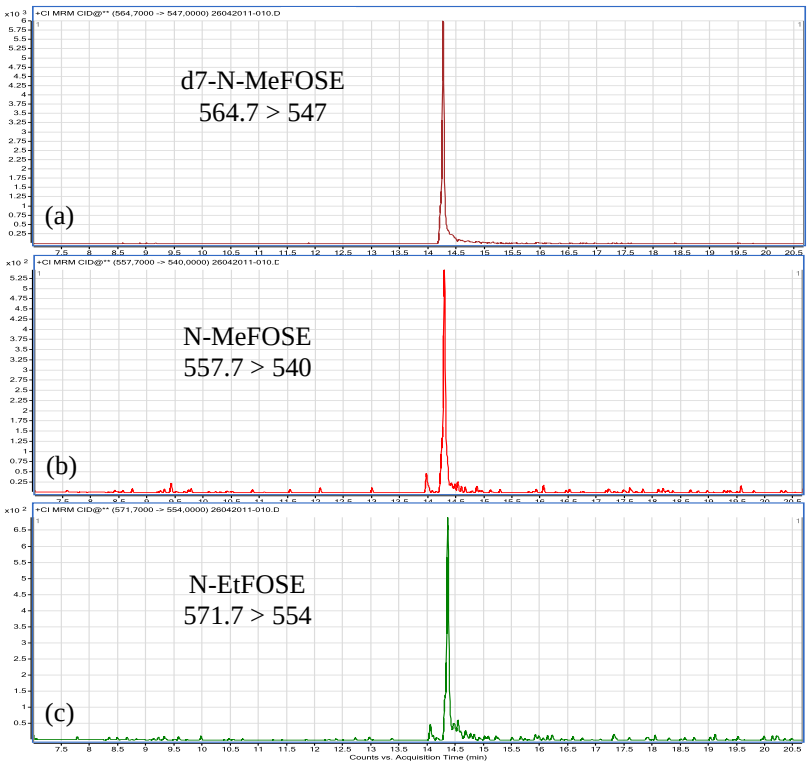

Figure 3: typical diagnostic ion chromatograms obtained for (a) d7-N-MeFOSE, (b) N-MeFOSE and (c) N-EtFOSE in a fish samples spiked at 0.25 µg/kg of wet matter (for d7-N-MeFOSE was used as internal standard at 2.5 µg/kg)

Absolute extraction recoveries have been calculated and estimated to around 40% for both compounds. The developed method is now on track to be applied to a large number of fish samples (n=50), in the scope of (1) evaluate also the repeatability and the reproducibility of the proposed methodology and (2) determine the occurrence of these compounds in various fish samples.

References

1. Commission recommendation of 17 march 2010 on the monitoring of perfluoroalkylated substances in food (2010); O*fficial Journal of European Union.* 2010/161/UE
2. Annika Jahnke, Sandra Huber, Christian Temme, Henrik Kylin, Urs Berger (2007) *Journal of Chromatography A*, 1164:1-9
3. Jessica C. D'Eon and Scott A. Mabury (2007); *Environ. Sci. Technol.* 41:4799-4805
4. Kurt R., Rhoads., Elisabeth M.-L. Janssen, Richard G. Luthy and Craig S. Criddle (2008); *Environ. Sci. Technol.* 42:2873-2878