PERFLUORINATED CONTAMINANTS IN FISH: SOLUTION FOR ULTRA TRACE ANALYSIS

Hajslova J[1], Lacina O[1], Hradkova P[1], Poustka J[1], Rosmus J[1], Pulkrabova J[1]

[1] Department of Food Chemistry and Analysis, Institute of Chemical Technology, Prague, Technická 3, 166 28 Prague 6, Czech Republic
[2] State Veterinary Institute Prague, Sidlistni 136/24, 165 03 Prague 6, Czech Republic
E-mail address: jana.hajslova@vscht.cz

Introduction

Perfluorinated compounds (PFCs), a wide group of anthropogenic substances, are widely used in industrial and consumer applications including stain-resistant coatings for fabrics and carpets, oil-resistant coatings for paper products approved for food contact, fire fighting foams, mining and oil well surfactants, floor polishes and insecticide formulations. Mainly their ability to repel water and oil has become crucial to their widespread industrial use.[1]

In last decade, PFCs have become "emerging" food and environmental contaminants, since they have been found in various types of both abiotic and biotic matrices including human tissues and fluids. PFCs are ranked among persistent organic pollutants with bioaccumulative potential. The molecule consists of hydrophilic functional group and hydrophobic perfluorinated carbon chain.[2]

In May 2009, PFOS and its salts were together with other 8 new halogenated persistent organic pollutants (POPs) included in the list of Stockholm convention on purpose to limit production, usage and emission release of mentioned chemicals to the environment. [3]

In February 2008, EFSA´s CONTAM Panel adopted a Scientific opinion on PFOS, PFOA and their salts. Moreover, with regards to associated dietary intake and since only limited information are available on their occurrence in food and environment, EFSA recommended that further data on PFCs levels in food and in humans would be desirable, particularly with a respect to the human exposure assessment. [4]Therefore, an additional monitoring focused not only on PFOS and PFOA but also on other PFCs is needed.

Subsequently, in March 2010, Commission Recommendation 2010/161/EU recommends the Member States to monitor the presence of following PFCs: PFOS and PFOA and, if possible, their precursors such as perfluorooctane sulphonamide (PFOSA), N-ethyl perfluorooctane sulfonamidoethanol (NEtFOSE) and 8:2 fluorotelomer alcohol in food and if possible, include compounds similar to PFOS and PFOA but with different chain length (C4–C15) and polyfluoroalkyl phosphate surfactants (PAPS) such as 8:2 diPAPS and 8:2 monoPAPS in order to estimate the relevance of their presence in food [5].

Up to date, limits of detection /quantification in analysis of PFCs in food or biota were typically in the range of 0.1 to 1 μg/kg wet weight. This is by far not low enough for reliable quantification of a suite of PFCs in different food items, which is a prerequisite for exposure assessment and source appointment. Typical levels of PFCs in common food items are expected to be in the low ng/kg wet weight or even pg/kg wet weight range.

The presented study, conducted within PERFOOD EU project, introduces a novel, highly sensitive LC-MS/MS method with detection limits that are, thanks to effective clean-up and highly sensitive MS detection, about two orders of magnitude lower than what can be achieved today. The benefits resulting from improved detection capability are demonstrated on analyses of real life samples of canned fish. Not only abundant PFCs but also ultra-trace representatives of these "emerging" contaminants could be reliably identified and quantified.

Materials and methods

All standards and ^{13}C labelled standards of perfluorinated compounds were purchased from Wellington Laboratories (Canada). Methanol (HPLC grade) was from Merck (Germany), acetonitrile (HPLC grade), magnesium sulfate, ammonium acetate (for LC-MS), ENVIcarb and activated charcoal were from Sigma (Germany). Bondesil C18 sorbent was from Varian (Germany).

Charcoal method

Homogenized sample (fish muscle) was weighted (2 g) into Ultra Turrax Tube Drive (IKA, Germany) added ^{13}C labelled isotopic standards and extracted with 6 mL of methanol for 2 min. Whole content of extraction tube was transferred to 50 mL polypropylene centrifugation tube and 340 mg of activated charcoal (170 mg per 1 g of sample) was added. The tube was shaken centrifuged (Hettich, Germany). The aliquot of final extract is filtered and transferred into vial. The matrix equivalent in extract is 0.33 g/mL.

"New method"

Into polypropylene centrifugation tube (50 mL) was weighted thoroughly homogenized sample and PFC free water and ^{13}C labelled internal standards were added. The tube was shaken and than 15 mL of acetonitrile was added and mixed. After addition of $MgSO_4$ and NaCl was sample mixed again and centrifuged. Aliquot of upper layer was transferred into new 50 mL tube with pre-weighted C18 and ENVIcarb sorbents. Aliquot of purified extract was evaporated on rotary evaporator near to dryness, reconstituted in methanol and transferred into vial. The matrix equivalent in extract is 8 g/mL.

UHPLC–MS/MS analysis

The UHPLC analyses were performed using Acquity Ultra-Performance LC system (Waters, USA) equipped with an Acquity UPLC HSS T3 separation column (100×2.1 mm I.D., 1.8 µm, Waters, USA) maintained at 40°C. The mobile phase consisted of 0.005 M ammonium acetate (A) and methanol (B). Sample injection volume 5 µL was used in all experiments and the sample temperature was maintained at 10°C.

UHPLC system was connected to hybrid quadrupole-linear ion trap mass spectrometer QTRAP 5500 (AB SCIEX, Canada) equipped by Turbo W ion source operated in negative mode. The needle voltage was -4500 V, the pressure of both, Gas 1 Gas 2 was 55 psi and temperature of drying gas was 600°C. The analytes were acquired in sMRM (scheduled multiple reaction monitoring) mode which automatically optimized dwell times and simplify development of multi-analytes LC–MS/MS method. Parameters of sMRM were following: detection window 60 s, scan time 0.55 s, pause between masses 3 ms. Analyst software 1.5 was used for data acquisition and processing.

Results and discussion

In response to Commission Recommendation from March 17, 2010 on the monitoring of perfluoroalkylated substances in food (2010/161/EU), we were searching for a fast analytical method enabling to generate reliable results with recovery rates in the 70–120 % and with limits of quantification of 1 µg/kg wet weight.[5] Of several published methods [6], LC–MS procedure based on a methanol extraction and activated charcoal clean-up was tested in the 1st phase of our study. We managed to meet the required performance characteristics by this approach for all target compounds control of which is required by EFSA.

The method was found suitable for fast and simple analysis in fish which is one of the major sources of PFCs in human diet. However, in real life samples, we were able to quantify only most abundant PFCs such as PFOS, PFNA and/or PFDA, while limits of quantification (LOQs) for PFOSA and PFOA occurring in fish at ultra trace levels, were not low enough (although the AB SCIEX 5500 QTRAP instrument is extremely sensitive in negative ESI mode). Our effort to lower LOQs for these compounds by increased injection of sample equivalent was not feasible due to precipitation of sample components left in extract during concentration step.

Due to this limitation, it was impossible to obtain data needed for a dietary exposure assessment, decision to develop a new sample preparation strategy, thus allows to quantify ultra-trace concentration of a wide range of FOSAs, PFCAs and PFSAs, was taken. As described in experimental, in this new sample processing procedure a transfer of target analytes to organic layer from aqueous sample homogenate (induced by added salts) occurs in the first step.

For removing of polar matrix co-extracts from a crude extract, ENVIcarb and C18 silica sorbent is used. Thanks to the effective removal of matrix co-extracts, pre-concentration of purified extract is possible. In Figure 1 the comparison of these two procedures is presented. While in case of a "charcoal" method, equivalent of injected matrix was (only) 1.7 µg, the new procedure allowed in our experiments injection 40 µg and it would be possible even to increase it, the chemical noise was negligible.

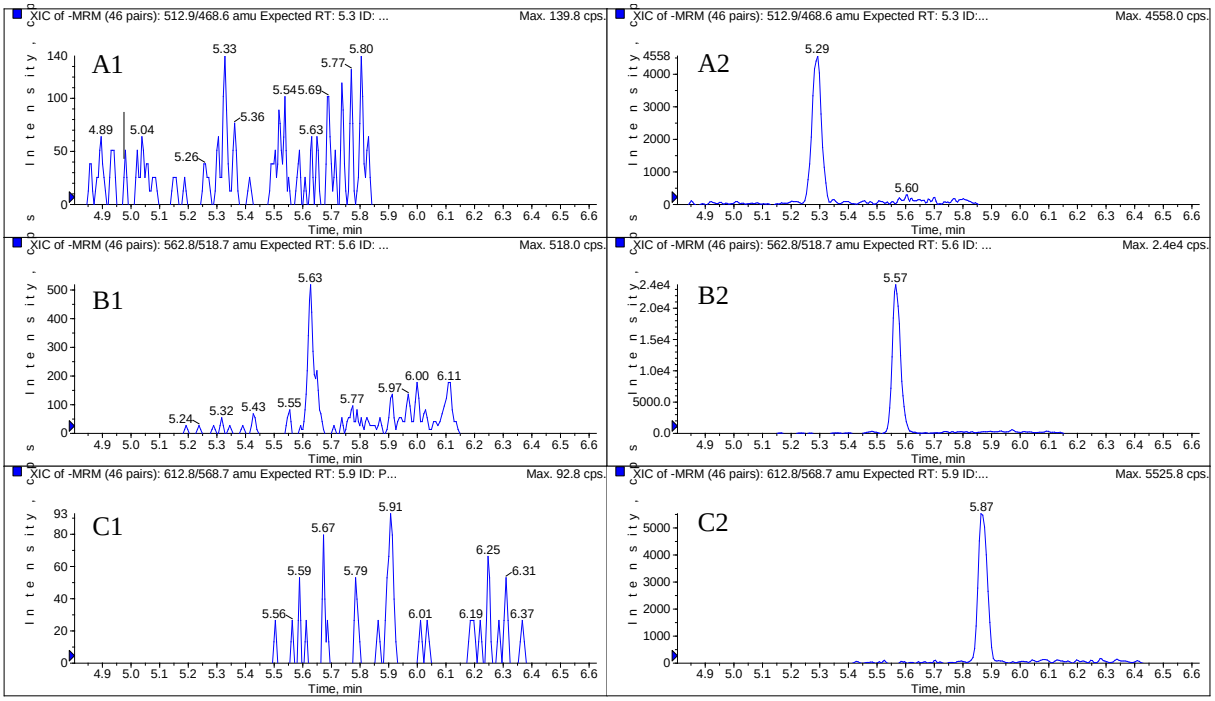

Figure 1. Comparison of "charcoal" and "new" method sensitivity: LC-MS/MS analysis of canned mackerel. PFDA was not detect in "charcoal" extract (A1), thanks to more sensitive "new" method was found 0.04 µg/kg (A2). In the same sample was PFUdA found at 0.1 µg/kg (B1 – charcoal, B2 – new method) and PFDoA at concentration 0.07 µg/kg (C1 – charcoal, C2 – new method)

In Table 1 the performance characteristics of both analytical approaches is shown. While sample throughput rather dropped, significantly lower LOQs, approx 20 times, were achieved (the trueness results for major PFCs was comparable for both procedures). Due to high sensitivity of the new method, broader spectrum of PFCs could be identified/ quantified in canned fish samples: in addition to PFOS, PFNA and PFDA, also FOSA, PFOA, PFUdA, PFDoA and PFTrDA were found in most samples, see Figure 2.

Thanks to implementation of new, powerful analytical procedure, convenient collection of comprehensive data sets required for dietary exposure assessment will be enabled in PERFOOD and other related project.

Table 1. Performance characteristics of two methods obtained within validation study (n=6). LC-MS/MS conditions were identical; the difference was in sample preparation procedure

Analytes	Charcoal method			New method		
	Spike 1000 ng/kg		LOQ	Spike 66 ng/kg		LOQ
	Recovery (%)	RSD (%)	ng/kg wet weight	Recovery (%)	RSD (%)	ng/kg wet weight
C_4–C_{14} PFCAs (11)	87–100	3–7	75–150	60–103	5–12	3–12
C_4–C_{10} PFSAs (4)	72–81	3–5	75	85–103	3–11	3
C_8 PFOSAs (3)	85–100	4–16	30–150	92	2	1–12
Samples prepared per hour	18			6		

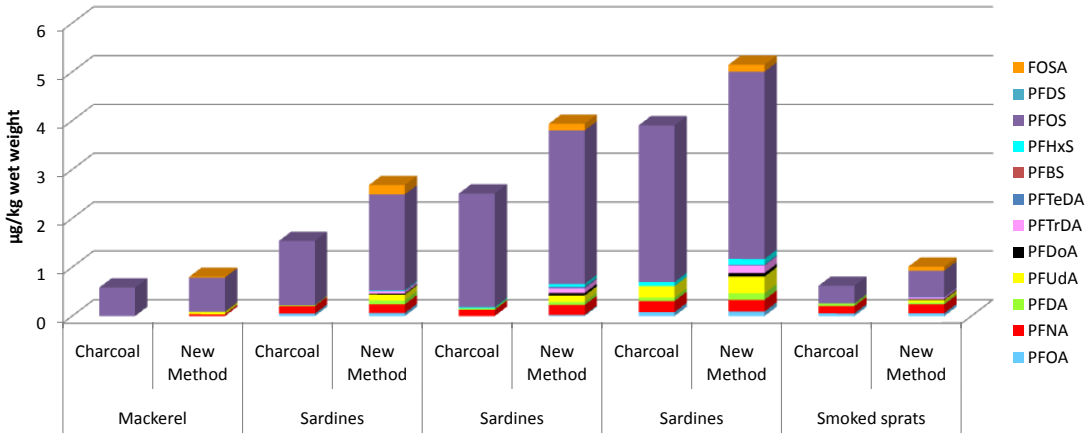

Figure 2. Comparison of PFCs determined in canned fish samples by two alternative methods (µg/kg wet weight)

Acknowledgements
This study was funded by: (*i*) the Research Support Fund of the National Training Fund within the project EMERCON (Identification and quantification of emerging organic contaminants in the Czech aquatic ecosystem and food market supply) with focus on perfluorinated alkylated compounds (PFCs), no. A/CZ0046/2/0026, and (*ii*) the FP7 EU project PERFOOD (PERFluorinated Organics in Our Diet), no. 227525.

References:

[1] Kärrman A, van Bavel B, Järnberg U, Hardell L, Lindström G (2006); Chemosphere 64: 1582-1591.
[2] Jahnke A, Berger U (2009); J Chromatogr A. 1216: 410-421.
[3] Stockholm Convention on Persistent Organic Pollutants (POPs) http://chm.pops.int/Convention/ThePOPs/tabid/673/language/en-US/Default.aspx
[4] CONTAM, EFSA Panel on Contaminants in the Food Chain. Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. The EFSA Journal. 2008:1-131
[5] Commission recommendation of 17 March 2010 on the monitoring of perfluoroalkylated substances in food. (2010/161/EU)
[6] Van Leeuwen SPJ, de Boer J (2007); J Chromatogr A. 1153: 172-185.