A MULTI-CHEMICAL METHOD FOR DETERMINATION OF PERFLUOROALKYL CARBOXYLATES, SULFONATES AND PHOSPHONATES IN FOOD CAULDRONS

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Introduction

Perfluoroalkyl carboxylates (PFCAs), sulfonates (PFSAs), and phosphonates (PFPAs), together referred to as perfluoroalkyl acids (PFAAs), are used in a wide range of products and industrial applications, due to their inertness and exceptional surface tension lowering potential. As a consequence, some PFAAs have been found ubiquitously in humans and wildlife. Nevertheless, exposure pathways are still not well understood. Dietary intake was suggested to be one of the major routes of human exposure to perfluorooctanoate and perfluorooctane sulfonate[1]. PFCAs and PFSAs have been detected in food from various countries[2, 3]. However, nothing is known so far on the occurrence of PFPAs in food or on possible human exposure to this emerging class of contaminants. More reliable and sensitive analytical methods are needed to accurately quantify the low levels of PFAAs occurring in food, which is a prerequisite for tracking the sources of food contamination and quantifying the relative importance of diet to the total human exposure. In this study a multi-chemical method based on ultra-performance liquid chromatography coupled to quadrupole time-of-flight high resolution mass spectrometry (UPLC/qToF-HRMS) was developed and validated for the determination of PFCAs (C4-12), PFSAs (C4,6,8,10) and PFPAs (C6,8,10) in food cauldrons.

Materials and methods

Chemicals

All standards were purchased from Wellington Laboratories. Native compounds were obtained as solutions of the acids of PFCAs (perfluorobutanoic acid (PFBA, C4), perfluoropentanoic acid (PFPeA, C5), perfluorohexanoic acid (PFHxA, C6), perfluoroheptanoic acid (PFHpA, C7), perfluorooctanoic acid (PFOA, C8), perfluorononanoic acid (PFNA, C9), perfluorodecanoic acid (PFDA, C10), perfluoroundecanoic acid (PFUnDA, C11), perfluorododecanoic acid (PFDoDA, C12)), salts of PFSAs (perfluorobutane sulfonate (PFBS, C4), perfluorohexane sulfonate (PFHxS, C6), perfluorooctane sulfonate (PFOS, C8), perfluorodecane sulfonate (PFDS, C10)) and di-protonic acids of PFPAs (perfluorohexane phosphonic acid (PFHxPA, C6), perfluorooctane phosphonic acid (PFOPA, C8), perfluorodecane phosphonic acid (PFDPA, C10), perfluorinated chloro-hexane phosphonic acid (Cl-PFHxPA)). A solution of mass-labeled compounds contained PFCAs (C4,C6-C12) and PFSAs (C6,C8). Acronyms for mass-labeled compounds contain the letter M in front of the acronym of the corresponding native compound. Other chemicals, reagents and solvents including 1-methyl piperidine (1-MP), formic acid, ammonium acetate, HPLC grade water, methanol and acetonitrile were purchased with the highest commercial purity and used as received.

Sample matrix

As a sample matrix for method development and validation, a mixture of ten different commercial glass jars of baby food was used. This composite baby food matrix was found to be free from detectable levels of PFAAs.

Extraction

An aliquot of 5 g homogenized baby food was placed in a 50 mL polypropylene (PP) tube and spiked with mass-labeled PFCAs, PFSAs and Cl-PFHxPA. Extraction was performed with 6 mL acetonitrile:water (90:10) by vortex-mixing for 1 min followed by ultra-sonication for 15 min. After centrifugation for 5 min at 3000 rpm, the supernatant was transferred to a 14 mL PP tube. The extraction was repeated with 5 mL acetonitrile and the combined supernatants were concentrated to 3 mL by N_2 evaporation prior to solid phase extraction (SPE) clean-up. A CUQAX256 SPE cartridge (C8 + quaternary amine, Clean-Screen, THC, 500 mg – 6 mL; United Chemical Technologies, UCT, Bristol, PA, USA) was rinsed and conditioned with 3 mL each of methanol (containing 0.1% 1-MP) and pure methanol followed by 1 mL water. The sample was loaded and the cartridge was then rinsed with 2 mL of 2 % formic acid in methanol:MTBE (95:5) followed by 1 mL pure methanol. The

analytes were subsequently eluted by gravity with 8 mL of methanol:acetonitrile (60:40) containing 2 % of 1-MP. The extract was evaporated to incipient dryness under nitrogen at 40 ºC and the residues were reconstituted with 200 µL of 50:50 methanol:water containing the $^{13}C_4$-mass-labeled PFOA and PFOS as recovery internal standards. The extract was kept in the refrigerator overnight to precipitate remaining particles. At the day of analysis, the extract was micro-centrifuged at 10,000 rpm for 10 min and the clear solution was transferred to a PP autoinjector vial for LC/MS analysis.

Instrumental analysis

Samples were analyzed by LC/MS using an Acquity Ultra Performance LC (UPLC; Waters, Milford, MA) with a trapping column BEH C18 reversed phase (50×2.1 mm, 1.7 µm particles) installed between the eluent mixer and the injector to trap the C4-C9 PFCA contaminants accumulating from the LC system. The analytes were separated on a BEH C18 reversed phase column (50×2.1 mm, 1.7 µm particles). Separation was achieved by gradient elution using a mobile phase A consisting of 95 % water and 5 % acetonitrile, and a mobile phase B consisting of 75 % methanol, 20 % acetonitrile and 5 % water, with 2 mM ammonium acetate and 5 mM 1-MP in both A and B. The injection volume was 5 µL, column oven temperature was 40 ºC and the flow rate was 70 µL/min. The gradient profile started with 90 % A (hold time 0.3 min) and continued with a linear change to 80 % A up to 1 min and to 50 % A up to 1.5 min followed by a linear change to 75 % B up to 12 min, hold until 15.5 min, linear change to 100 % B to 16 min and hold until 21 min. Initial conditions were regained at 21.2 min followed by equilibration until 26 min.

The mass spectrometer was a QToF Premier HRMS instrument (Micromass, Manchester, England). Electrospray ionization in negative ion mode was employed, and data acquisition was performed in full scan mode utilizing three parallel scan functions. Enhanced Duty Cycle was used in one of these scan functions to analyze PFPAs. The following optimized parameters were applied: Capillary voltage, 3.0 kV; sample cone voltage, 20 V for PFCAs and 50 V for PFSAs and PFPAs; collision energy, 5 eV; source temperature, 100 ºC; desolvation temperature, 400 ºC; nitrogen desolvation gas flow, 800 L/h. Quantification was performed using extracted mass chromatograms from full scan recording with a m/z window of 0.05 u.

Results and discussion

In an initial phase of method development, the chromatographic separation and ionization efficiency for PFPAs was optimized. Due to the di-anionic character and high water solubility of the phosphonate moiety, obtaining a good chromatographic resolution was very challenging. Adding 1-MP as ion pairing agent to the mobile phase resulted in a significant increase of detector response and improved chromatographic resolution for PFPAs. However, extra care has to be taken when choosing the LC column due to the high pH obtained by employing 1-MP in the mobile phase. The BEH C18 column is specially designed for applications at high pH values. Applying this column resulted in excellent peak shape for PFPAs at a relatively low column temperature of 40 ºC, which was chosen in order to minimize degradation of the column performance. Furthermore, this method also resulted in superior separation and sensitivity for detection of PFCAs and PFSAs compared to separation using a mobile phase without 1-MP. Representative chromatograms of PFCAs, PFSAs and PFPAs spiked to a baby food sample are shown in Figure 1.

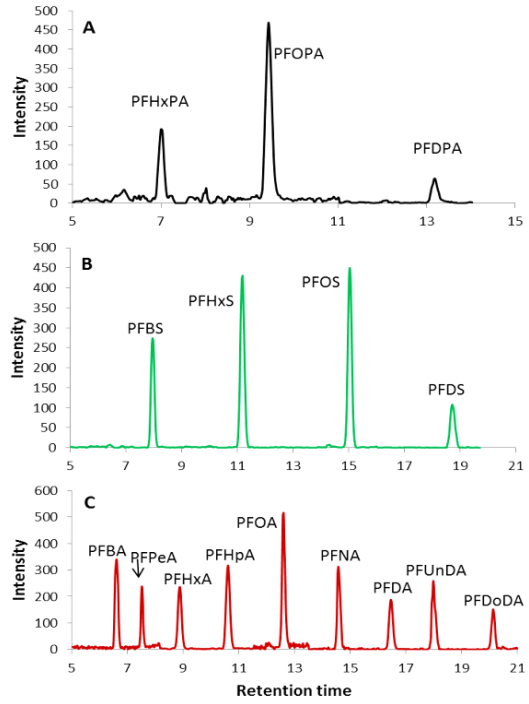

Figure 1: Extracted mass chromatograms of (A) perfluoroalkyl phosphonates (PFPAs), (B) perfluoroalkyl sulfonates (PFSAs) and (C) perfluoroalkyl carboxylates (PFCAs) spiked at 0.1 ng/g to a baby food sample.

For the optimization of the extraction method two different SPE cartridges with mix mode co-polymeric sorbents, CSTHCM506 (C8 + primary amine, 500 mg – 6 mL) and CUQAX256 (C8 + quaternary amine, 500 mg – 6 mL) (Both obtained from UCT) were evaluated. Good recoveries for PFPAs, PFSAs and PFCAs were only obtained with the CUQAX256 cartridge. The quaternary amine functions in the sorbent may be responsible for the efficient retention of all tested PFAAs. A positive matrix effect, probably due to ionization enhancement, was observed for PFPAs in food extracts (Figure 2). The reason for this matrix effect is so far unidentified.

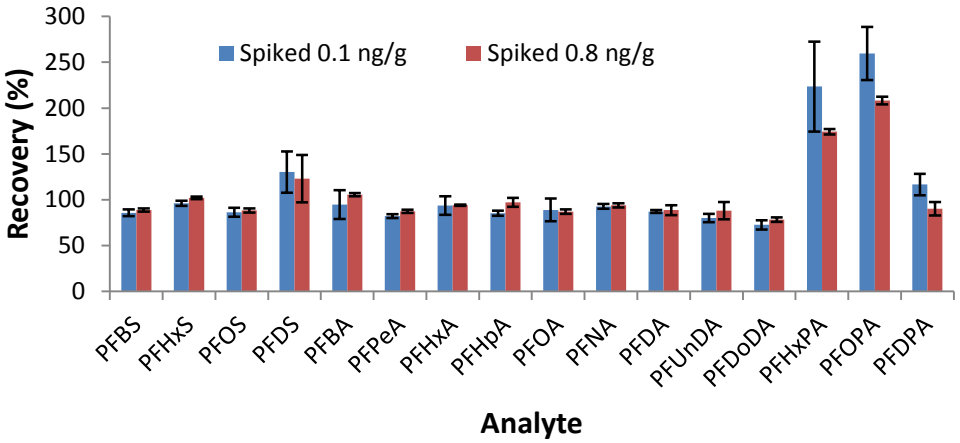

Figure 2: Inter-day method recovery (n = 3) of PFAAs at two different concentrations spiked to a baby food sample.

The complete method was validated by assessing the detection limits, linearity, blank contamination, recovery, repeatability, reproducibility and robustness. The method detection limits (MDLs) and method limits of quantification (MLQs) were determined with native PFPAs and with mass-labeled PFCAs and PFSAs spiked to baby food, in order to avoid biases due to occasional procedural blank contamination at the MDL levels. They are summarized in Table 1. The whole method linearity for PFCAs, PFSAs and PFPAs was evaluated over a range of spike concentrations of 0.05 – 1.2 ng/g to baby food (6 data points including a blank). Excellent r^2 values >0.996 were obtained for all analytes. Average method recoveries (n = 3 at three different days) at a spiking level of 0.1 ng/g were 73-109 %, 86-130 % and 117-260 % for PFCAs, PFSAs and PFPAs, respectively, and at a spiking level of 0.8 ng/g the corresponding recoveries were 78-97 %, 88-123 % and 90-208 % (Figure 2). All other validation parameters (repeatability, reproducibility and robustness) showed excellent performance of the analytical method. The method will be applied to a food cauldron, baby food and diet composite samples.

Table 1: Method detection limits (MDLs) and method limits of quantification (MLQs) determined with mass-labeled PFCAs, PFSAs and native PFPAs.

Perfluoroalkyl carboxylates (PFCAs)							
	MPFBA	MPFHxA	MPFOA	MPFNA	MPFDA	MPFUnDA	MPFDoDA
MDL (pg/g)	12	3	1.8	3.6	2.4	2.4	9
MLQ (pg/g)	40	10	6	12	8	8	30
Perfluoroalkyl sulfonates (PFSAs) and phosphonates (PFPAs)							
	MPFHxS	MPFOS		PFHxPA	PFOPA	PFDPA	
MDL (pg/g)	2.5	1.8		17	5.5	6	
MLQ (pg/g)	8.3	6		57	18	20	

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