SIMULTANEOUS EXTRACTION OF SELECTED PERFLUORINATED COMPOUNDS AND POLYFLUOROALKYL PHOSPHATE SURFACTANTS IN HUMAN MILK

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Introduction

Over the last decade, perfluorinated compounds (PFCs), particularly perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), have received worldwide attention, mainly due to their widespread occurrence, biopersistence and toxicity. PFCs have been used for many years in a variety of industrial and commercial applications [1]. Following the voluntary phasing out of the production of perfluorooctanesulfonyl fluoride (POSF, $C_8F_{17}SO_2F$)-based materials in 2002 by the primary manufacturer, a decrease in concentrations of PFOS and PFOA was observed in the general population in North America [2, 3]. At the same time, however, an increase in serum levels of longer-chain (*i.e.,* C_9 to C_{11}) perfluorinated carboxylic acids (PFCAs) has been observed, suggesting continued PFCA exposure. Recent studies have demonstrated that the biotransformation of polyfluoroalkyl phosphate surfactants (PAPS) may be a source of perfluorinated carboxylic acids [4-6]. These fluorotelomer-based products are used on food contact paper to impart oil/grease resistance and as such, were shown to be able to migrate into food [7, 8]. These chemicals, therefore, should be monitored in both biological and environmental matrices, in order to determine the most suitable matrices to assess human exposure to PAPS and to generate exposure data for the purposes of health risk assessment. In this study, selected PFCs and four diPAP congeners (4:2, 6:2, 8:2, and 10:2 diPAP) were simultaneously extracted from human milk.

Material and Methods

Milk samples

Human milk samples were collected from healthy donors in the Kingston region of Ontario (Canada) in 2003-2004. A pooled milk sample was purchased from Lee BioSolutions (St. Louis, MO, USA). NIST (Gaithersburg, MD, USA) SRM 1954 Organic Contaminants in Fortified Human Milk was used as a QC sample for PFC analysis.

Standards and reagents

Perfluorinated compounds (PFHxA, PFHpA, PFOA, PFNA, PFDA, PFBS, PFHxS and PFOS) and labelled internal standards were purchased from Wellington Laboratories (Guelph, ON, Canada). Ammonium acetate (ACS grade) was purchased from BDH (Toronto, ON, Canada). Omnisolv acetonitrile and methyl-tert-butyl ether (MTBE) were obtained from EMD Chemicals (Gibbstown, NJ, USA). Tetrabutylammonium bisulfate (99%) and sodium carbonate (99%) were purchased from Fluka (Oakville, ON, Canada). Sodium bicarbonate (ACS grade) was supplied by Fisher Scientific Company (Fair Lawn, NJ, USA). DiPAPS (Table 1, x =y) were synthesized at the University of Toronto by Professor Mabury's group.

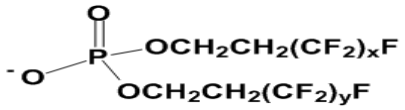
Sample extraction

To a 15-mL polypropylene tube containing 1 mL of milk, 10 µL of a 1 µg/mL mixture of internal standard, containing ^{13}C-labelled PFCs, was added and gently mixed. 1 mL of 0.5 M tetrabutylammonium bisulfate solution (adjusted to pH 10) was added with gentle mixing of the spiked milk, followed by 2 mL carbonate/bicarbonate buffer addition, and the repeated, gentle solution mixing. 5 mL of MTBE was added to the spiked milk and the mixture was vortexed. The organic and aqueous phases were separated by centrifugation at 3500 rpm for 10 min. The organic phase was collected and the MTBE solvent was evaporated to just dryness under nitrogen at 30°C. Extracts were reconstituted in 220 µL of acetonitrile. 40 µL of the extract was transferred into a glass autosampler vial and an additional 160 µL of water was added. This extract was used for the determination of PFCs. The remaining sample extract was used for the determination of diPAPS. This extract was subsequently divided into aliquots and used to generate a matrix-matched calibration curve unique for each sample analyzed.

LC-MS/MS

The chromatographic separation of extracts was performed using a Finnigan Surveyor Plus HPLC System (Thermo Electron Corporation, San Jose, CA). PFC separation was achieved by using two Discovery HS C_{18} (7.5 cm x 2.1mm, 3 μm) columns where one was used as a pre-column followed by another used as the analytical column. An opti-guard C_{18} column (10 mm x 1 mm, 3 μm) was installed between the pump and the analytical column, in order to trap and delay PFCs originating from the solvents and the pumping system. DiPAPS were separated on a single Kinetex column (5 cm x 4.6 mm, 2.6 μm). The mobile phase consisted of 20% acetonitrile in 2 mM ammonium acetate (A) and 90% acetonitrile in 2 mM ammonium acetate (B). For the separation of PFCs and PAPS, the column temperatures were maintained at 30 and 50°C, respectively. The gradient elution started with 100% A for 1 min, followed by an 8 min linear gradient to 100% B, then 3 min hold at 100% B, and returned back to 100% A in 4 min, at a flow rate of 200 μL/min. The system was equilibrated for 4 min at the initial conditions before the next injection. Sample injection volumes were 10 and 20 μL for PFCs and PAPS, respectively. Mass spectrometric experiments were performed using a Thermo Finnigan TSQ Quantum Ultra EMR triple quadrupole mass spectrometer (Thermo Electron Corporation, San Jose, CA). The samples were analyzed in negative ion electrospray ionization, using selective reaction monitoring mode. The following SRM transitions (*m/z*) were monitored for PFC and diPAP analysis: PFHxA (312.9 → 268.9), ^{13}C-PFHxA (315.0 → 270.0), PFHpA (362.8 → 318.8), PFOA (412.9->368.9), ^{13}C-PFOA (416.9 → 372), PFNA (462.9 → 419), ^{13}C-PFNA (468 → 423), PFDA (513 → 469), ^{13}C-PFDA (515 → 470), PFBS (298.7 → 80.4), PFHxS (398.9 → 80.4), PFOS (498.9 → 80.4) and ^{13}C-PFOS (502.9 → 80.4), 4:2 diPAP (589 → 343), 6:2 diPAP (789 → 443), 8:2 diPAP (989 → 543), 10:2 diPAP (1189 → 643). Quantitation of polyfluoroalkyl phosphate surfactants was performed by matrix-matched calibration. For both PFCs and PAPS, isomers were not determined separately.

Table 1. Structures, congeners, and acronyms of PAPS

Structure	Congeners	Acronym
	8 congeners x = 4, 6, 8, or 10, y = x or x+2	If y = x, x:2 diPAP If y = x + 2, x:2/y:2 diPAP

Results and Discussion

Perfluorinated Compounds

There are a few published studies on the levels of perfluorinated compounds in human milk, which have revealed that PFCs tend to be present at much lower levels in human milk than in serum. Of the PFCs detected in human milk, PFOS and PFOA were the most prevalent [9-16]. In this study, PFOA was detected in 85% of 13 individual human milk samples, in the pooled milk sample and in NIST SRM 1954. Other PFCs were not detected at a concentration above the method detection limit. Compared to the determination of PFCs in human serum, it has been demonstrated that the analysis of these compounds in human milk is more challenging [17]. In NIST SRM 1954, the values for PFOA and PFOS were 0.08 and 0.10 ng/mL, while consensus values for PFOA and PFOS were 0.13±0.04 and 0.16±0.03 ng/g, respectively [9]. The distribution of PFOA in this study is presented in Figure 1. The current extraction method was developed for participation in the "Worldwide Proficiency Testing on PFCs in Human Milk 2009/2010" [17]. Table 2 shows how values obtained with this method compared to the average and median values obtained from all the participants in this proficiency testing. Overall, the results obtained in the above study demonstrated that the extraction method developed here is suitable for the determination of PFCs in human milk.

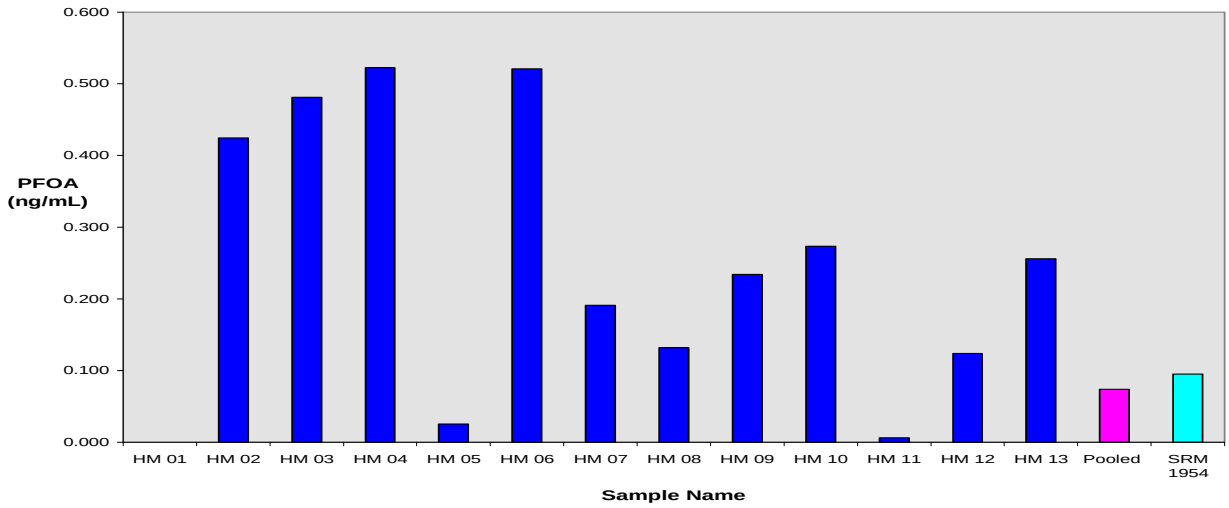


Figure 1. Distribution of PFOA (ng/mL) in individual and pooled milk samples, and in NIST SRM 1954.

Table 2. Method performance data obtained from a pooled human milk sample (Pool G) in the PT PFC Human Milk 2009/2010

PFC	This method (ng/mL)	Average (ng/mL) (PT PFC 2010)	Median (ng/mL) (PT PFC 2010)	SD (ng/mL) (PT PFC 2010)	%RSD (PT PFC 2010)	Nb. of Labs
PFHxA	0.107	0.230	0.183	0.230	100%	4
PFHpA	0.002	0.007	0.007	0.006	78%	5
PFOA	0.178	0.082	0.063	0.058	71%	18
PFNA	0.034	0.020	0.021	0.011	55%	6
PFDA	0.0004	0.003	0.003	0.002	75%	3
PFHxS	0.026	0.015	0.006	0.014	90%	7
PFOS	0.026	0.055	0.026	0.021	38%	20

Polyfluoroalkyl phosphate surfactants

The analysis of PAPS is challenging since the labelled internal standards are not commercially available. SRM 1954 was shown not to contain detectable levels of PAPS and was used to calculate recoveries by spiking it with native PAP at 1.25 ng/mL. Recoveries were acceptable for 6:2 diPAP (58.5±28%) and 10:2 diPAP (90.4±16.4%) while very poor recovery was obtained for 4:2-diPAP (12±7.6%) and 8:2 diPAP was not recovered at this spiking level. The instrument detection limit (IDL) was 0.01 ng/mL for 4:2 diPAP, 6:2 diPAP and 10:2 diPAP. The IDL for 8:2 diPAP was determined at 0.2 ng/L. Figure 2 shows SRM mass chromatograms of a human milk sample containing 4:2, 6:2 and 10:2-diPAPS at a concentrations of 0.14, 0.04 and 0.10 ng/mL, respectively, while 8:2 was not detected. 46% of samples contained 4:2 diPAP with a concentration range of 0.01-0.13 ng/mL, 6:2 diPAP was detected in only one sample at 0.04 ng/mL, and the 10:2 diPAP was quantified in 92% of milk samples, with concentration range of 0.01-0.17 ng/mL. 10:2 diPAP was also found in the commercially available pooled milk sample at 0.33 ng/mL. Other congeners were not detected in any of the samples. No correlation was established between PFCAs and PAPS levels in this small sample size. However, this study suggests that PFCs and PAPS can be simultaneously extracted from human milk samples. To the best of our knowledge, this is the first work reporting the presence of PAPS in human milk; nevertheless, additional work is still required to increase the extraction efficiency for 4:2 PAP and 8:2 diPAP.

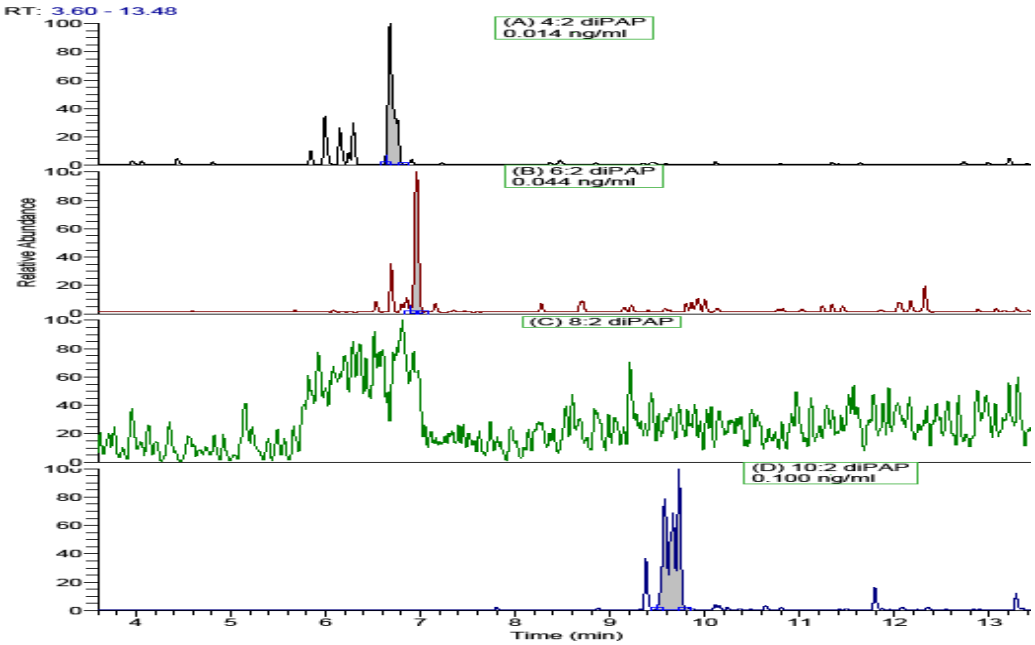


Figure 2. Mass chromatograms for the SRM transitions at (A) *m/z* 589 → 343; (B) *m/z* 789 → 443; (C) *m/z* 989 → 543; and (D) *m/z* 1189 → 643 of a human milk sample.

Acknowledgments

The authors wish to thank Dr. Jiping Zhu (Health Canada) for donating individual milk samples.

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