A MODIFIED METHOD FOR DETERMINATION OF PERFLUOROALKYCARBOXYLIC ACIDS AND PERFLUOROALKYL SULFONIC ACIDS IN HUMAN MILK BY ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY AND TANDEM MASS SPECTROMETRY

Liu JY, Li JG, Zhou PP, Zhao YF, Wu YN
National institute of nutrition and food safety, 29 Nanwei road, Beijing 100050, China

Indroduction

During the last decade a worldwide contamination of perfluorinated compounds (PFCs) have been found at considerable levels in various biota samples like liver[1], tissue[2], especially human blood and serum[3,4,5]. Human milk might be another perfect biomarker to assess the extent of human exposure and the intake of PFCs for infant by lactation. However, data on PFC in human milk is limited as the result of low contamination levels and complicated matrix. In this study, a robust and sensitive method has been developed to overcome the problems for analyzing the trace level of PFCs in breast milk samples.

Materials and Methods

Chemicals and Materials: standard solution of native perfluoroalkylcarboxylic acids (PFCAs) and standard solution of native perfluoroalkylsulfonates (PFSAs) were purchased from Wellington Laboratories (Guelph, Canada) with purities ≥98%, including perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUndA) and perfluorobutanesufonate (PFBS), perfluorohexanesulfonate (PFHxS), perfluoroheptanesulfonate (PFHpS), perfluorooctanesulfonate (PFOS), respectively. $^{13}C_4$-PFOA and $^{13}C_4$- PFOS (≥98%, Wellington Laboratories, Guelph, Canada) were internal standards. Methanol of HPLC grade was purchased from J.T.Baker (Phillipsburg, USA). Ammonium acetate and formic acid of HPLC grade were purchased from Dikma Pure (Richmond hill, USA). Weak anion exchange SPE cartridges (Oasis® Wax, 60 mg, 3 ml, 30 μm) were provided by Waters (Milford, Ireland).

Sampling: Human milk samples were donated by mothers in lactation from rural areas of china and collected in pre-washed polypropylene container. The samples were stored in refrigerators at -20℃ until analysis.

Sample preparation: 2ml human milk with internal standards were added 9 ml 2% formic acid in water followed by sonication for 15 min and centrifugation at 9,384 ×g and 0℃ for 25 min . The supernatant was transferred to the preconditioned SPE cartridge. PFCs were eluted with 2 ml 9% NH_4OH in methanol, after washing the column with 1 ml 2% formic acid in water and 1ml 2% formic acid solution/methanol (v/v; 1/1). SPE eluate was evaporated under a gentle stream of nitrogen, then methanol/water (v/v; 1/1) was added to a final volume of 200 ul.

UPLC-ESI-MS/MS: Analytes were separated and quantified using an ultra-performance liquid chromatography

and tandem mass spectrometry (UPLC-MS/MS). Chromatographic separation was achieved by an ACQUITY UPLC BEH C18 column (2.1mm i.d.×50mm length, 1.7um; Waters, USA). For eliminating of the contaminant from LC system, two pre-Columns were installed tandem between post-pump and pre-injector. The gradient was performed with methanol and 2mM ammonia acetate in water with flow rate of 0.4 mL/min, increasing methanol from 20 to 100% within 5.1 minutes.

Mass spectrometry was performed by electron spray ionization (ESI) in the negative ion mode and multiple reaction monitoring. MS/MS transitions are displayed in table 1.

Results and Discussion

The developed method was proved to be a robust and sensitive means to analyze eleven compounds. LOD of instrument, MDL and recoveries are displayed in table 1. Recoveries were investigated by a real human milk sample spiked with two different concentrations (1ng/ml and 10ng/mL).

Table 1: The performance of developed method

Compound	Mass transition	LOD (pg)	MDL (pg/ml)	Recovery (%)		RSD (%) n=5
				High level (10ng/ml)	Low level (1ng/ml)	
PFBS	299→80, 299→99	1	2.84	175	155	0.13
PFHxS	399→80, 399→99	0.025	0.69	89	123	0.08
PFHpS	449→80, 449→99	0.05	3.77	85	126	0.11
PFOS	499→80, 499→99	0.025	6.39	99	97	0.11
PFPeA	263→219	0.2	5.50	129	127	0.07
PFHxA	313→269	0.2	2.91	135	123	0.09
PFHpA	363→319	0.1	2.98	93	89	0.08
PFOA	413→369	0.05	14.15	112	115	0.14
PFNA	463→419	0.1	5.46	93	98	0.10
PFDA	513→469	0.1	1.44	128	114	0.07
PFUndA	563→519	0.1	1.30	70	57	0.06

One of the major problems associated with trace level analysis of perfluorinated acids is background contamination. To eliminate the background contamination of PFOA, PFNA and PFUnDA from the liquid chromatography system, two Van Guard^{TM} Pre-Columns were installed tandem between post-pump and pre-injector to separate the analytical peaks and contamination peaks. Separation effect is displayed in figure 1. Two pre-columns could postpone the contamination peak for almost two peak widths that the analytical peak and contaminating peak were totally separated. In figure 2, the chromatogram of some PFCs in the fortified human milk sample (1ng/ml) are shown.

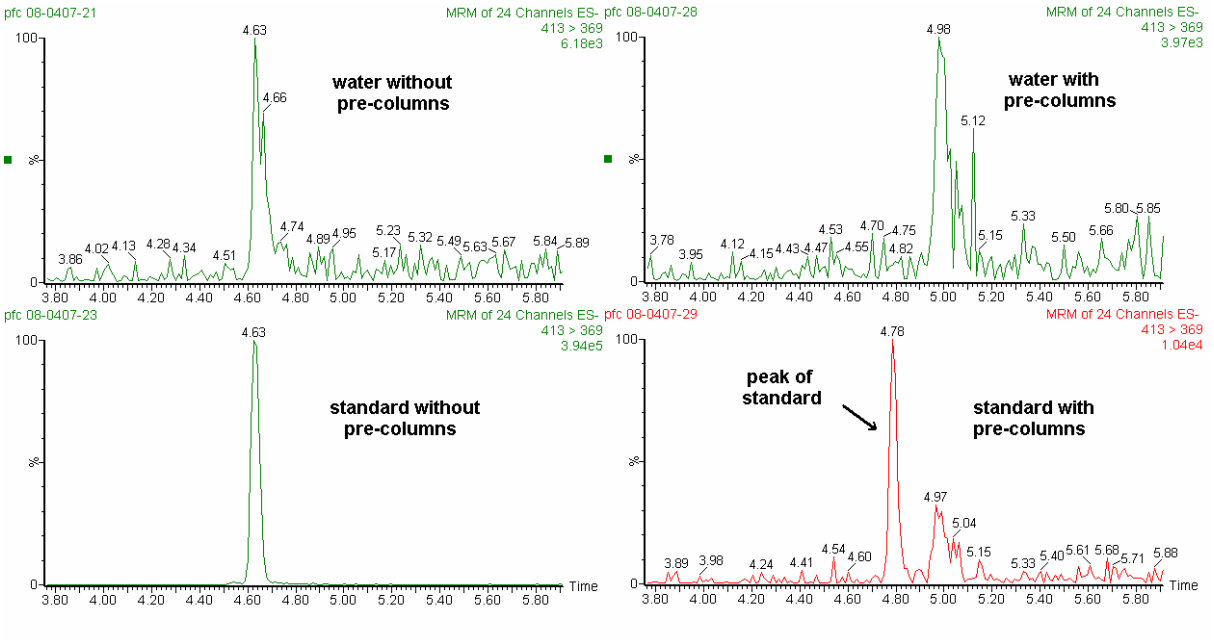

Fig.1 Instrumental contaminating peaks of PFOA with and without pre-columns

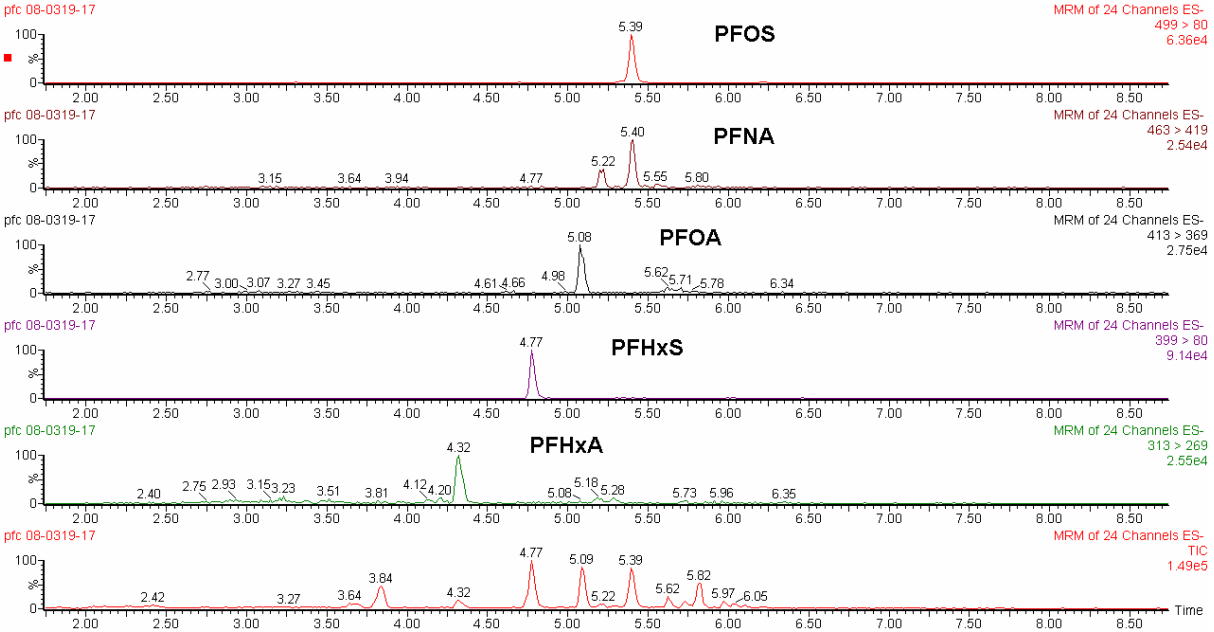

Fig.2 The chromatogram of some PFCs in the fortified human milk sample (1ng/ml)

Acknowledgements

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