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Simultaneous quantitation of perfluoroalkyl acids in human serum and breast milk using on-line sample preparation by HPLC column switching coupled to ESI-MS/MS☆

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ABSTRACT

A high throughput analytical method using a column switching high-performance liquid chromatography combined with isotope dilution tandem mass spectrometry (column switching-HPLC–MS/MS) was developed to simultaneously quantitate the concentrations of 7 perfluoroalkyl acids (PFAAs) in serum and 3 PFAAs in breast milk samples. The sample preparation includes addition of the isotope-labelled internal standard solution to breast milk and serum, enzymatic hydrolysis and filtration of milk samples, precipitation of proteins and analysis by column switching-HPLC–MS/MS. The limits of quantitation ranged from 0.1 to 0.4 μg/l for serum and 0.02 to 0.15 μg/l for breast milk samples. The method accuracies ranged between 73.2% and 100.2% for the different analytes at two concentrations in PFAAs spiked samples. The validity of the method was confirmed by analysing 20 serum and 20 breast milk samples.

1. Introduction

Perfluoroalkyl acids (PFAAs) are a large group of chemicals that consist of a lipophilic carbon chain typically 4–14 in length and a hydrophilic functional moiety (primarily carboxylate, sulfonate, or phosphonate). PFAAs have specific physico-chemical characteristics such as chemical and thermal stability or surface-active properties. Therefore a lot of applications like impregnation of carpets, textiles, and leather, coatings in paper, cardboards, food packing materials or the use in fire-fighting foams are described for PFAAs [1,2].

The toxicity of PFAAs have recently been summarised by Lau et al. in two reviews [1,2]. The acute toxicity of PFAAs especially shown for perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) is low in humans and rodents. In chronic feeding studies with rodents and non-human primates, the liver was the primary target organ and after exposure of Sprague–Dawley rats to PFOS and PFOA within a 2-years bioassay hepatocellular adenomas were reported [3,4]. Furthermore different signs of developmental and reproductive toxicity in rodents have been observed for PFAAs [2].

Due to their environmental persistence and their discussed bioaccumulative potential several PFAAs are widespread in nearly all areas of ecosystems [5]. Especially for PFOS and PFOA a lot of data are published and reviewed in several publications since both compounds were produced in highest amounts in the past [1,6,7]. Additionally, only for some PFAAs stable isotope labelled standards were available, which permit a validated quantitation of these compounds in most different samples.

Recently an inter laboratory study for quantitation of PFAAs in human serum samples were published [8]. Mainly PFOS and PFOA were analysed by 15 different laboratories. If all data were compared for PFOA relative standard deviations (%RSD) between 47% and 89% were observed for three different serum samples containing PFOA in the range of 0.6–10.2 μg/l. Better results were obtained for PFOS. For other PFAAs %RSD up to 133% were obtained. All laboratories used LC–MS instruments with electrospray ionisation including triple quad, ion trap, single quad, or TOF mass analysators. Sample preparation varies between ion pair extraction, acetonitrile precipitation and solid phase extraction. Only one laboratory used an online SPE-HPLC system. This description of methods will be also true for the most published biomonitoring data describing concentrations of PFOS and PFOA in blood summarised previously in Fromme et al. and Lau et al. [1,6].

In contrast to serum samples for breast milk samples only few data are available and an inter laboratory study for breast milk was not performed so far. To our knowledge only 5 studies describe levels for PFAAs in breast milk samples (n = 12–267) [9–17]. PFOS

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Table 1
MS/MS-transitions, declustering potential, collision entrance potential, and collision energy used (offset values).

Analyte		Transition (*m*/*z*)	DP (V)	CEP (V)	CE (V)
PFOA	Quantifier	412.8 → 168.8	−15.0	−20.0	−26.0
	Qualifier	412.8 → 368.9	−15.0	−20.0	−12.0
MPFOA		417.0 → 169.0	−15.0	−20.0	−26.0
		417.0 → 372.0	−15.0	−20.0	−12.0
PFNA	Quantifier	462.9 → 169.0	−15.0	−18.3	−26.0
	Qualifier	462.9 → 419.1	−15.0	−18.3	−16.0
MPFNA		468 → 169.0	−15.0	−18.5	−26.0
		468 → 412.5	−15.0	−18.5	−16.0
PFDA	Quantifier	512.9 → 218.9	−15.0	−20.1	−24.0
	Qualifier	512.9 → 468.9	−15.0	−20.1	−16.0
MPFDA		515.0 → 219.0	−15.0	−20.2	−24.0
		515.0 → 470.0	−15.0	−20.2	−16.0
PFDoA	Quantifier	613.0 → 168.9	−20.0	−23.7	−38.0
	Qualifier	613.0 → 569.0	−20.0	−23.7	−18.0
MPFDoA		615.0 → 169.1	−20.0	−23.8	−38.0
		615.0 → 570.0	−20.0	−23.8	−18.0
PFHxS	Quantifier	398.8 → 79.7	−60.0	−16.2	−72.0
	Qualifier	398.8 → 99.0	−60.0	−16.0	−48.0
MPFHxS		402.7 → 84.1	−60.0	−16.2	−72.0
		402.7 → 103.1	−60.0	−16.2	−48.0
PFOS	Quantifier	499.0 → 79.9	−70.0	−19.6	−90.0
	Qualifier	499.0 → 98.9	−70.0	−19.6	−68.0
MPFOS		503.0 → 79.9	−70.0	−19.8	−90.0
		503.0 → 98.9	−70.0	−19.8	−68.0
PFBS	Quantifier	298.8 → 79.9	−40.0	−12.4	−56.0
	Qualifier	298.8 → 98.7	−40.0	−12.4	−40.0

and PFOA was determined in all studies, perfluorohexane sulfonate (PFHxS) in 4 studies but Bernsmann and Fürst found PFHxS only in 1 of 203 samples [9]. Only one laboratory used a column switching unit for sample preparation [16]. All other laboratories used a solid phase extraction method to extract PFAAs similar to methods described for serum samples. Bernsmann and Fürst did an enzymatic hydrolysis to digest fat and protein components in milk prior to analysis [9].

The method presented here enables the quantitation of 7 PFAAs in serum and 3 PFAAs in breast milk samples. It is based on a column switching technique as described elsewhere [18–20]. Especially for PFOA, PFOS and PFOSA (Perfluorooctanesulfonylamide) two column switching methods are already published using an single quadrupole or an ion trap mass spectrometer for detection of these PFAAs in blood samples [21,22]. In contrast to these methods of Holm et al. and Inoue et al. a second column was integrated between loading pump and injector, respectively trap column to prevent contamination of the trap column as already described by Kärrman et al. for a conventional LC–MS/MS system [11]. The combination of both column switching and this additional column enables a considerably lower LOQ in contrast to the methods of Holm et al. and Inoue et al. and therefore only 200 μl of serum and 400 μl of breast milk were used for quantitation.

2. Experimental

2.1. Chemicals

Methanol (LiChrosolv) and acetonitrile (LC–MS grade) were purchased from Merck KGaA (Darmstadt, Germany); water (Rotisolv HPLC Gradient Grade) was purchased from Roth (Karlsruhe, Germany) and ammonium acetate p.a. was from Riedel-de-Haën (Hannover, Germany).

Perfluorobutanesulfonic acid tetrabutylammonium salt (PFBS), potassium perfluorooctanesulfonate (PFOS), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA) and perfluorododecanoic acid (PFDoA) were from Sigma–Aldrich (Taufkirchen, Germany). Perfluorooctanoic acid (PFOA), sodium perfluorohexanesulfonate (PFHxS) and the corresponding isotope labelled internal standards sodium perfluoro1-hexane [18O2] sulfonate (MPFHxS), sodium perfluoro-1-[1,2,3,4-13C4] octanesulfonate (MPFOS), perfluoro-n-[1,2,3,4-13C4] octanoic acid (MPFOA), perfluoro-n-[1,2,3,4,5-13C5] nonanoic acid (MPFNA), perfluoro-n-[1,2-13C2] decanoic acid (MPFDA) and perfluoro-n-[1,2-13C2] dodecanoic acid (MPFDoA) were from Wellington Laboratories (Ontario, Canada).

Lipase Type VII (Enzyme Commission number: 3.1.1.3; EC: 232-619-9) and Protease Type XIV (Mix of different enzymes; EC: 232-909-5) were purchased from Sigma–Aldrich (Taufkirchen, Germany).

2.2. Sample collection

Breast milk samples were collected (period of collection from November 2007 to April 2008) within the surveillance program called “Bavarian Monitoring of Breast Milk” (BAMBI) as previously described [23,24].

Serum samples were collected within a feasibility study (Mother Infant Biomonitoring Study, MIBS) in the years 2007 and 2008 to determine the exposure in pregnancy and during childhood. All serum samples used for testing the method with real samples were obtained from women before childbirth.

Samples were stored at −20 °C before analysis. All participants completed a detailed questionnaire about age, weight, height, and possible exposure through occupational contact, dietary habits, smoking habits, living area, etc.

The ethic committee of the Bavarian Chamber of Physicians approved the studies and written informed consent of all participants was obtained in case of infants by the parent.

2.3. Sample preparation

2.3.1. Breast milk

Sample preparation was performed referring to an already published method [9] with some modifications.

After thawing 400 μl of the breast milk was fortified with 14 μl internal standard mix (containing 0.2 ng of MPFOS and MPFHxS as well as 1 ng MPFOA). The specimen was vortex mixed and 135 μl of the protease and 135 μl of the lipase mixture (each 10 mg/ml in 50 mM ammonium acetate, adjusted to pH 7.5 with ammonia) were added. It was again mixed thoroughly. 10 μl of a solution containing 2.5% of ammonia was added to adjust the pH to about 7.5. Subsequently the sample was incubated over night at 37 °C in a thermo mixer.

After incubation the sample was centrifuged at 20,800 × *g* for 5 min. Then 400 μl of the supernatant and 176 μl methanol were mixed and centrifuged for another 5 min. 500 μl of this supernatant were transferred into the reservoir of a Microcon® Centrifugal Filter Device (YM-10; Millipore, USA), centrifuged at 14,000 × *g* for 45 min and 200 μl of the obtained filtrate was injected.

2.3.2. Human serum

In this case human serum was also thawed. 200 μl of serum, 14 μl internal standard solution (containing 0.2 ng of MPFOS and MPFHxS, 1 ng MPFOA as well as 2 ng MPFNA, MPFDA, and MPFDoA) and 36 μl of methanol/water (50/50, v/v) were mixed and 200 μl acetonitrile – for protein precipitation – were added. Then it was vortex mixed and centrifuged (20,800 × *g* for 5 min). The supernatant and another 200 μl of acetonitrile were put together,