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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,

Table 2
Validation data for breast milk and serum samples (*c* = concentration).

Breast milk					Intra-day precision ($n = 6$)		Inter-day precision ($n = 6$)	
Compound	LOQ [μg/l]	LOD [μg/l]	Mean relative recovery [%]		CV [%]		CV %]	
			c [0.15 μg/l]	*c* [1.5 μg/l]	*c* [0.15 μg/l]	*c* [1.5 μg/l]	*c* [0.15 μg/l]	*c* [1.5 μg/l]
PFOA	0.15	0.07	89	73	12	2	17	7
PFOS	0.03	0.01	92	93	4	4	4	5
PFHxS	0.02	0.007	109	106	6	4	6	6

Serum					Intra-day precision ($n = 6$)		Inter-day precision ($n = 6$)	
Compound	LOQ [μg/l]	LOD [μg/l]	Mean relative recovery [%]		CV [%]		CV [%]	
			c [0.4 μg/l]	*c* [2.0 μg/l]	*c* [0.4 μg/l]	*c* [2.0 μg/l]	*c* [0.4 μg/l]	*c* [2.0 μg/l]
PFOA	0.4	0.1	93	95	13	11	15	9
PFNA	0.4	0.1	89	94	12	5	17	8
PFDA	0.4	0.1	84	89	11	10	12	13
PFDoA	0.4	0.1	102	112	22	7	18	7
PFOS	0.1	0.03	92	99	6	2	5	5
PFHxS	0.2	0.07	101	104	6	1	6	5
PFBS	0.4	0.1	87	93	6	9	8	9

vortex mixed and then stored at −20 °C for 1 h. Afterwards the specimen was centrifuged again for 5 min. The supernatant was transferred into a HPLC glass vial together with 350 μl of 2 mM ammonia acetate buffer solution.

2.4. Standard preparation

A stock solution (1 ng/μl) for all analytes (also mass labelled) was prepared in methanol/water (50/50, v/v) and then further diluted with methanol/water (50/50, v/v) to obtain the standard mix working solutions with concentrations of 1, 10 and 100 pg/μl.

2.4.1. Breast milk

Solutions for calibration (0.005, 0.01, 0.03, 0.06, 0.1, 0.2, 0.4, and 0.6 μg/l) were prepared similar as the samples described under Section 2.3. Instead of breast milk 400 μl of 2 mM ammonium acetate buffer instead of breast milk and 270 μl buffer solution (50 mM of ammonium acetate, pH 7.5) instead of lipase and protease respec-

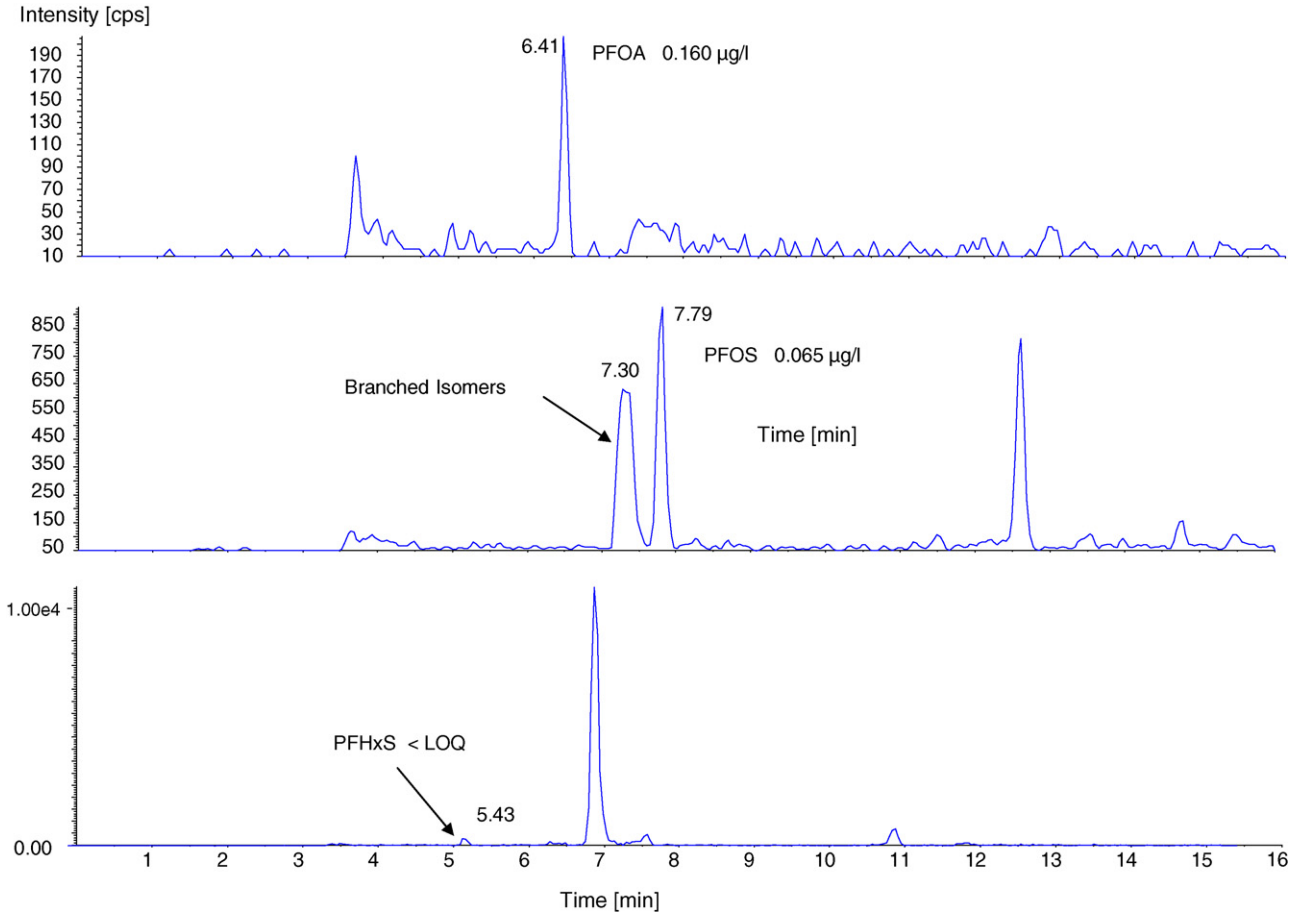

Fig. 1. Separations of PFOS, PFOA, and PFHxS contained in real breast milk samples are shown.

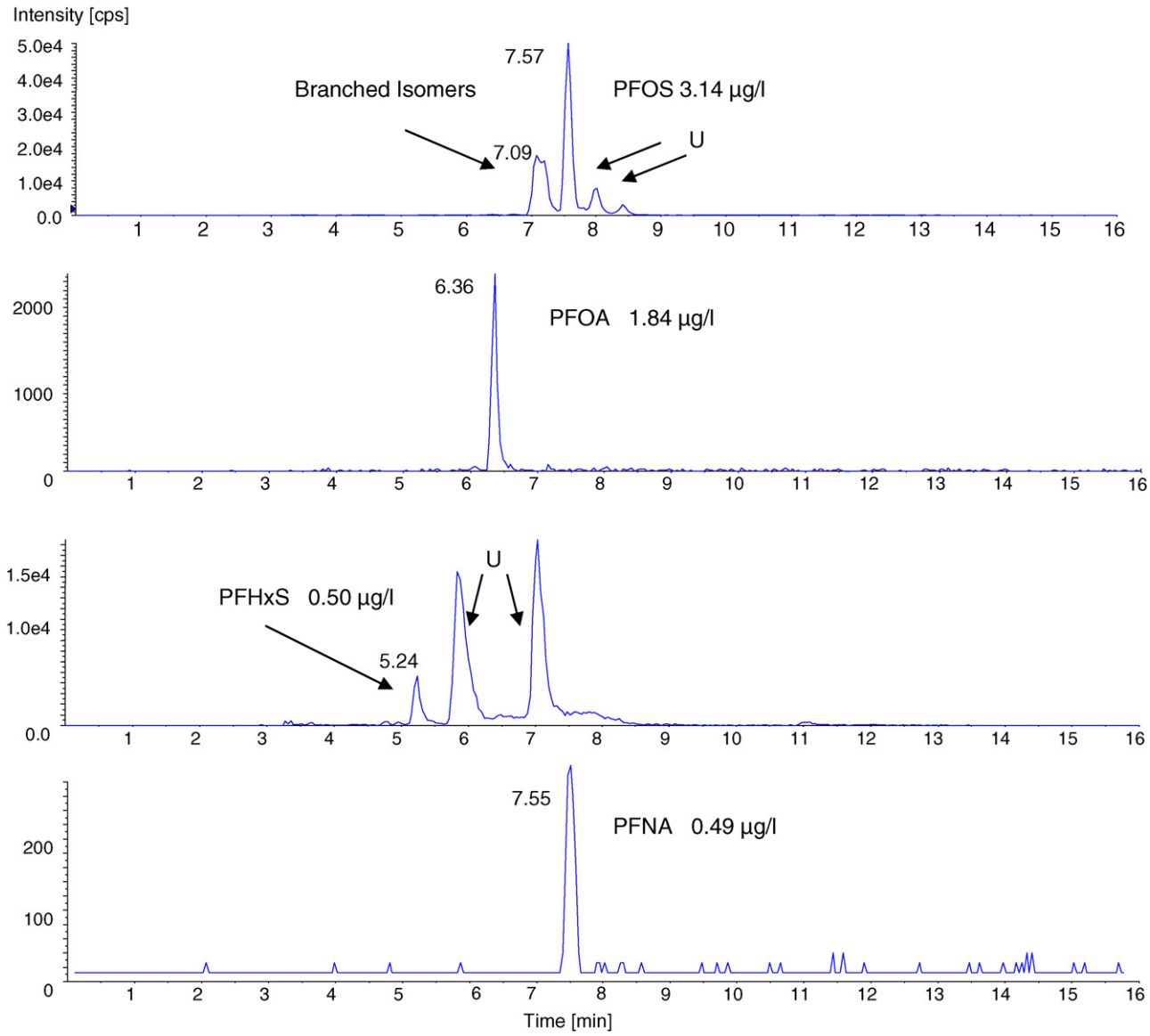

Fig. 2. Separations of PFOS, PFOA, PFHxS, and PFNA contained in real serum samples are displayed. The signals marked with U are unknown compounds (details see text).

tively. After adding of 14 µl internal standard mix and a definite volume of one of the standard mix working solutions (concentrations of analytes were 1, 10, and 100 pg/µl respectively) to each calibration standard methanol was added to achieve a total volume of 1 ml. So the ratio aqueous to organic in the calibration solutions is identical to breast milk samples. The steps "incubation" and "filtration" were omitted.

2.4.2. Human serum

Solutions for calibration (0.02, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, and 10 µg/l) were prepared as described under 2.3 but with 200 µl 2 mM ammonium acetate buffer instead of human serum. After adding of 14 µl internal standard mix and a definite volume of one of the standard mix working solutions (concentrations of analytes were 0.01, 0.1, and 1 ng/µl respectively) to each calibration standard methanol/water (50/50, v/v) was added to achieve a total volume of 1 ml. The calibration solutions were not further centrifuged or frozen.

2.4.3. Quantitation

An internal standard method was used for quantitation based on isotope labelled standards. Only for PFBS no isotope labelled standard was available and we used MPFOS as internal standard for quantitation. For calibration the ratio of the peak area of standard to internal standard was plotted versus the concentration by Analyst 1.42 software with no regression weighting.

2.5. Liquid chromatography

The online extraction LC–MS/MS system was already described [16]. Meanwhile some changes have been done. The auto sampler introduced the sample (200 µl) into the system and pump 1 (Ultimate 3000 micro, Dionex, Idstein, Germany) carried the mobile phase (100% 2 mM ammonium acetate buffer solution, A) at 1 ml/min to load the sample onto the trap column (Oasis® HLB, 25 µm, 2.1 mm × 20 mm, Waters, Eschborn, Germany). After 2 min the sample loading and elimination of matrix components were completed and then the 10-port valve (Dionex, Idstein, Germany) switched into the elution position. Pump 2 (Ultimate 3000 micro, Dionex, Idstein, Germany) flushes the trapped analytes back from the trap column onto an analytical column (ReproSil-Pur-ODS-3, 5 µm, 150 mm × 2 mm, Dr. Maisch, Ammerbuch, Germany, with Security Guard Cartridge C 18 AQ, Phenomenex, Aschaffenburg, Germany) with a flow rate of 400 µl/min and a gradient of 62%

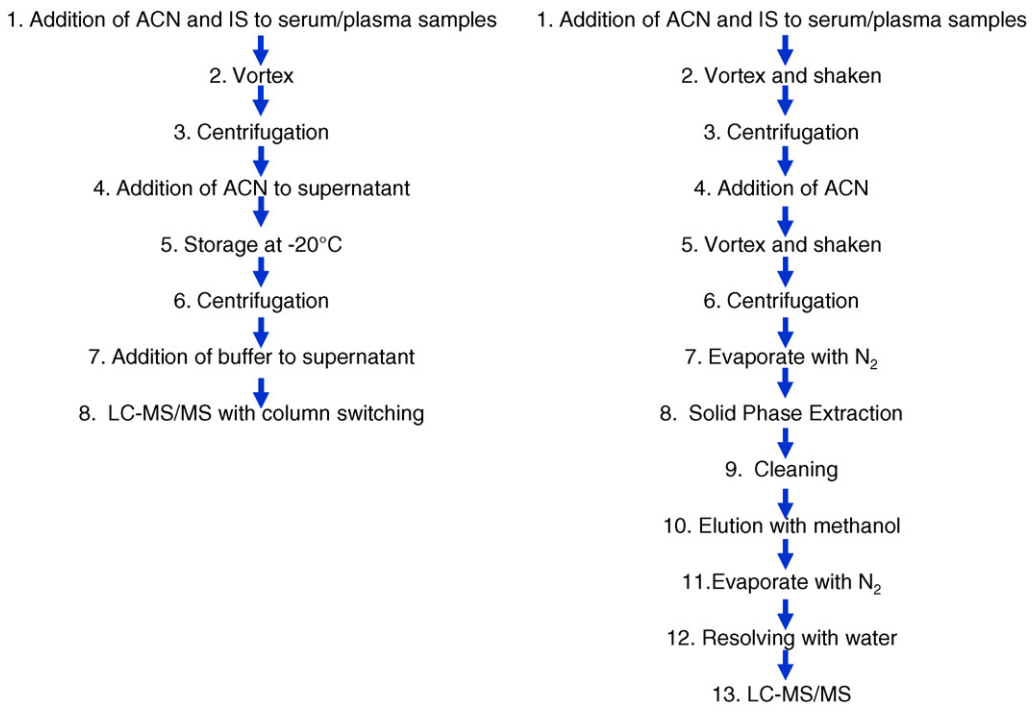

Fig. 3. Comparison of the presented column switching method described in this study to a common method used to analyse PFAA in serum according to [27].

methanol (B) and 38% 2 mM ammonium acetate buffer (A; adjusted to pH 5 with acetic acid). Elution is performed with an increasing gradient to 90% B in 11 min and held for 2 min. Within 1 min the gradient decreased to 62% and held at 62% for 2 min to equilibrate. After 16 min the valve switched back into the first position. The column oven temperature was determined at 35 °C.

As already mentioned a second column (ReproSil C-18AQ, 5 μm, 33 mm × 3 mm, Dr. Maisch, Ammerbuch, Germany) similar to the trap column was integrated between loading pump and injector, respectively trap column to prevent contamination of the injector and trap column system mainly by PFOA. Teflon® may contain small amounts of PFOA and therefore contamination resulting from parts of the HPLC pump or degasser may occur. This additional column trapped such contaminations and while the system was equilibrating for the next analytical run this additional column was washed with 100% of MeOH for 2 min into waste.

2.6. Mass spectrometry

All quantitations were performed using a triple-stage quadrupole mass spectrometer (API 3200 QTRAP™ Applied Biosystems, Darmstadt, Germany) equipped with TurboIonSpray® interface.

Following settings were used for recording spectral data:

Polarity: negative; CAD gas: medium (N_2); Curtain gas: 20 (N_2); Nebuliser gas 1: 55 (N_2); Turbo ion gas 2: 65 (N_2); Ion spray voltage: −4500 V; Source temperature: 600 °C; Interface heater: on; Dwell time: 75 ms; Entrance potential offset: −10 V (for more details see Table 1).

2.7. Precision

To determine the intraday and inter day precision a pooled breast milk sample was spiked at two different concentrations. For each concentration we analysed a set of six samples on two different days and a set of six samples without spiking. These samples were handled as described under Section 2.3. Recoveries and coefficient of variation were specified. Recovery rates were calculated as the quotient of spiked concentration to measured concentration multiplied with 100; in case of any content in the non-spiked samples, the "measured concentration" has been corrected for this value.

Instead of human serum we used pooled bovine serum for the determination of precision (see Table 2).

2.8. Limit of detection and limit of quantitation

Limits of quantitation were defined as a signal-to-noise ratio of ten for the quantifier ion trace and the limit of detection were defined as one third of the limit of quantitation. In cases where the qualifier ion trace has a lower magnitude as the corresponding quantifier, the limit of detection was defined as a signal-to-noise ratio of three to five for the qualifier.

Because there was no PFOS-free human serum the LOQ was estimated (S/N ratio 90 for 0.3 μg/l).

3. Results and discussion

Based on an existing method for enzymatic hydrolysis of proteins and lipids of breast milk by Bernsmann and Fürst a robust and reliable method was developed to quantify PFAAs in breast milk [9]. After enzymatic hydrolysis and centrifugation a more homogenised solution was formed in contrast to a method using only a precipitation step with acetonitrile which results for some samples in a layer separation as previously published by Völkel et al. [16]. In addition to moderate modification of the chromatographical parameters these changes resulted in slightly lower LOQ (0.03 for PFOS and 0.15 μg/l for PFOA; Table 2). Robustness of the method became also better since coefficients of variation were lower if the same matrix samples were run in doublet and the deterioration of the trap and analytical columns regarding the chromatographical parameters were reduced compared to the method published previously [16].

Table 3
Concentrations of 7 PFAAs in serum samples ($n = 20$).

	PFOS [μg/l]	PFOA [μg/l]	PFHxS [μg/l]	PFNA [μg/l]	PFDA [μg/l]	PFDoA [μg/l]	PFBS [μg/l]]
Range	0.78–9.43	0.65–3.87	<LOQ–3.03	<LOQ–4.37	<LOQ–0.55	<LOD	<LOD
Median	2.96	1.78	0.56	0.54	0.20	n.d.	n.d.

n.d.: not determined.

Furthermore the existing method for PFOA and PFOS was extended to 5 further PFAAs. As described by Bernsmann and Fürst only PFOS, PFOA, and PFHxS was found in breast milk samples with given LOQ. Therefore validation parameters for breast milk samples were optimised only for these PFAAs as described in Table 2.

Calibration curves were linear within the given concentration range with correlation coefficients >0.99 for PFOS, PFOA, and PFHxS.

Fig. 1 shows two typical separations of a breast milk samples containing PFOS and PFOA respectively PFOS and PFHxS. PFOS was quantitated in 20 real breast milk samples with concentrations from <LOQ (0.03 μg/l) to 0.195 μg/l (1 sample <LOQ; median: 0.049 μg/l) and were comparable to results previously published by several studies summarised in Fromme et al. [6]. Only for 3 samples (15%) PFOA concentrations above the LOQ of 0.15 μg/l were obtained. Again these data were comparable to already published data summarised in Fromme et al. [6]. PFHxS was only detected in 1 sample above LOQ of 0.02 μg/l and corresponded to the observation of Bernsmann and Fürst [9]. Validation data and comparability to previously published data suggested that the presented method based on a simple sample work up and a column switching unit is appropriate to be used for quantitation of PFAAs in breast milk samples.

In contrast to breast milk samples quantitation of PFAAs in serum samples is easier to perform due to at least 2 reasons. First, concentrations of PFAAs in serum samples are about 20–60-fold higher (as shown for PFOS with median of 0.05 μg/l in breast milk and 2.96 μg/l in serum) and second the matrix serum is easier to handle. A previously published work shows typically totally different LOQs in milk and serum with 0.6 in breast milk and 0.05 μg/l in serum although two solid phase extractions steps were applied for sample work up of milk samples and equal sensitive mass spectrometers were used [17]. In comparison to milk serum contains lower contents of lipids and therefore the precipitation of proteins with acetonitrile is completely sufficient as sample work up step.

With a first method almost identical to the breast milk method previously published [16] more than 300 serum samples were analysed for concentrations of PFOS and PFOA. The results (data not shown) were comparable to serum or plasma samples published elsewhere for example summarised in Fromme et al. [6].

In addition a round robin test initiated from "Institute and Out-Patient Clinic for Occupational, Social and Environmental Medicine of the University Erlangen-Nuremberg: external quality assessment scheme" (http://www.g-equas.de) was successfully completed with the method presented here.

In the actual method described here 5 additional PFAAs were included and separation was optimised for all analytes. Table 2 shows the validation data of 7 PFAAs in serum samples. The means of relative recoveries ranged from 84 to 101% for the lower concentration and 89 to 112% for the higher concentration, depending on the PFAAs. Only for PFDoA intra-day precision with 22% of $N = 6$ samples was greater than 13%. Although for PFBS no isotope labelled standard was available and therefore MPFOS was used as internal standard the precision and recovery data fit quite well to all other PFAAs.

Fig. 2 shows a separation of a typical serum sample with PFOS, PFOA, PFHxS, and, PFNA as PFAAs which are typically observed in serum samples. In contrast to milk (Fig. 1) in the chromatogram of PFOS two additional signals were observed marked with U for unknown. For both signals only the transition m/z 479.0–79.9 was observed and not the qualifier m/z 479.0–98.9. Therefore at the moment we are not sure whether these signals correspond for isomers of PFOS or impurities. Both are possible since Arsenault et al. describe more than 10 isomers and on the other hand Benskin et al. describe that isomers of taurodeoxycholic acid mimic the occurrence of PFOS isomers due to an identical MS–MS-transition of this bile acid [25,26]. In further studies with single isomers of PFOS we hope to elucidate the chemical structure of these signals. The same is true for the additional signals observed in the chromatogram of PFHxS.

Again, the levels observed for PFOA (range: 0.65–3.87 μg/l) and PFOS (range: 0.78–9.43 μg/l) in 20 serum samples were comparable to data published elsewhere. This was also true for the 5 additional analysed PFAAs. Moreover, the concentrations presented in Table 3 were comparable to data summarised in Fromme et al. [6].

As already mentioned the "old" method really comparable to the previously published breast milk method [16] was applied to some other matrixes such as eggs, liver, fish (meat), or water. Some results were published in conference abstracts summarised in Fromme et al. [6]. Therefore we suggested that both methods, old one for PFOS and PFOA and the presented one here, may be adapted to other matrices after performing an appropriated validation of sample work up.

All these data show that the presented method using a minimum of sample work up and a column switching unit to get the opportunity to inject up to 200 μl of the sample is applicable to serum samples. Fig. 3 illustrates the advantage of the method presented here in comparison to a previously published method [27] which uses the traditional sample work up including solid phase extraction which seems to be the most common method to quantitate PFAAs in different matrices as previously reviewed [28].

Nevertheless not all PFAAs which may occur in serum or breast milk could be analysed with this method. For PFBA or shorter carbon chains containing PFAAs the trapping efficiency together with separation onto the analytical column became worse since the elution of such short chain PFAAs from trap columns with a solvent mix of 10% MeOH and 90% of buffer resulted in elution of these PFAAs from the analytical column without any chromatographical separation. The results were poor LODs and poor validation data and therefore these PFAAs were not included in the presented method.

4. Conclusion

We developed a robust and reliable method to determine three, respectively seven PFAAs in human breast milk respectively serum. Determination of PFAAs in trace level concentrations in breast milk is possible after enzymatic hydrolysis of lipids and proteins and application of an on-line sample preparation by HPLC column switching coupled to ESI-MS/MS. In case of serum samples only a protein precipitation step is necessary to get sufficient clean samples usable for the column switching unit.

The main characteristics of both methods are the high reproducibility, reliability, robustness, high sensitivity and high selectivity due to application of MS/MS techniques such as multiple reaction monitoring.

The developed methods are sensitive enough to determine PFAAs especially PFOS, PFOA, and PFHxS in native human breast

milk samples and up to 7 PFAAs in native human serum samples in the Bavarian general population. It is assumed that the present on-line sample preparation by HPLC column switching coupled to ESI-MS/MS can be extended to other matrixes (egg, meat, water, liver, etc.) if homogenates of these samples are easily transformed to sufficient clean specimens usable for the column switching unit.

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