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The determination of perfluoroalkyl substances, brominated flame retardants and their metabolites in human breast milk and infant formula

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ABSTRACT

In the present study, a novel analytical approach for the simultaneous determination of 18 perfluoroalkyl substances (PFASs) and 11 brominated flame retardants (BFRs) including their hydroxylated metabolites and brominated phenols has been developed and validated for breast milk and infant formula. The sample preparation procedure based on extraction using acetonitrile and subsequent purification by dispersive solid-phase extraction (d-SPE) employing C18 sorbent is rapid, simple and high-throughput. Ultra-high performance liquid chromatography (UHPLC) interfaced with a tandem mass spectrometry (MS/MS) was employed for the identification/quantification of these compounds. The method recoveries of target compounds for both matrices ranged from 80% to 117% with relative standard deviations lower than 28% and quantification limits in the range of 3–200 pg/mL for milk and 5–450 pg/g for infant formula. Within the pilot study, the new method was used for the analysis of PFASs and BFRs in 50 human breast milks and six infant formulas. In the breast milk samples the total contents of PFASs and BFRs were in the range of 38–279 and 45–16,200 pg/mL, respectively. The most abundant PFASs detected in all tested breast milk samples were perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS), the latter contaminant was present not only as a linear form but also as a branched isomers. The incidence of BFRs was lower, the only representatives of this group, tetrabromobiphenol A (TBBPA) and α-hexabromocyclododecane (α-HBCD), were detected in less than 30% of breast milk samples. None of the infant formulas contained BFRs, traces of either PFOS, PFOA or PFNA were found in three samples.

1. Introduction

A wide range of halogenated contaminants are present in the human environment. While most of the early monitoring studies focused on various chlorinated persistent organic pollutants (POPs), in recent decades, toxicological concerns emerged on the ubiquitous occurrence of fluorine and bromine containing compounds that may also accumulate in food chains. The high volume production of perfluoroalkyl substances (PFASs) and brominated flame retardants (BFRs), the latter represented mainly by polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDs) and tetrabromobisphenol A (TBBPA), have led to their widespread distribution in the environment. As regards to PFASs, due to their unique characteristics such as chemical inertness, stability, hydrophobicity and lipophobicity, they are used in a variety of industrial and consumer applications while BFRs are used to reduce the flammability of treated materials [1,2].

Non-occupational human exposure to PFASs and BFRs, that may occur through a variety of pathways including inhalation of contaminated dust particles [3,4] or food [5,6]/drinking water ingestion [7], has been clearly documented by findings of these chemicals and their (bio)transformation products in human tissues and fluids including plasma and breast milk [8–10]. The latter matrix is a widely used bioindicator that can be used to assess the body burden of these environmental pollutants especially with regard to its importance as the first food for the newborn.

Recently, the European Food Safety Authority (EFSA) has outlined European Union (EU) framework and respective activities of the Panel on Contaminants in the Food Chain (CONTAM Panel) in the field of BFRs. Six Scientific Opinions on the main classes of these contaminants completed between October 2010 and October 2012 have been presented [11]. It is worthy to note that the very recent Scientific Opinion is also concerned with brominated phenols and their derivatives. With the exception of 2,4,6-tribromophenol (2,4,6-TBP), the data for risk assessment are lacking. In

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general, the requirement for the continuation of BFRs surveillance and the need to fill in the gaps in occurrence data on emerging/novel BFRs has been emphasized by EFSA [12]. Similarly, in the last few years, the CONTAM Panel has paid a great deal of attention to public health concerns for a wide range of PFASs entering human food chain [13]. The occurrence of PFASs in various food commodities and estimation of dietary exposure has been recently reviewed by EFSA within a comprehensive scientific report [14]. Nevertheless, the CONTAM Panel has acknowledged the limitation in information available on other PFASs and recommended further monitoring of food contamination. The use of analytical methods with improved sensitivity are needed to increase the proportion of quantified results and thereby the reliability of exposure assessments that has been highlighted.

In our study, we attempted to respond to the EFSA requirement and to implement a highly sensitive method for selected representatives of both the above discussed POP groups. Since the only data on exposure to halogenated POPs in the Czech Republic is available for organochlorine pesticides (OCPs) [15], polychlorinated biphenyls (PCBs) [16], polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) [17] and PBDEs [18], we decided to provide a complementary information on two other groups of halogenated POPS. For this reason a set of breast milk samples for PFASs and polar BFRs represented by brominated phenols and hydroxylated derivatives of PBDEs (OH-PBDEs) was examined for the first time in the Czech Republic. Considering the fact that some newborns have to be fed by infant formula, we also included this matrix in our experiments as another potential exposure source to fluorine/bromine containing POPs.

Although the current trend in food contaminants control is to integrate various contaminants groups into a single procedure, the review of the available scientific literature did not reveal a procedure that was applied for analysis of BFRs and PFASs in breast milk/infant formula. In some studies, both groups of contaminants were monitored [19–21] by using two alternative methods for each compound class. As regards BFRs, gas chromatography coupled to mass spectrometry (GC–MS) is the method of choice for the most often monitored representatives-PBDEs [22]. This approach can also be employed for the determination of other widely used BFRs, nevertheless, some limitations need to be taken into consideration: the separation of HBCD isomers is hardly feasible by common GC capillaries [23], and in the case of rather more polar TBBPA, derivatization of the hydroxyl group is needed prior to injection [24]. Similarly, brominated phenols or OH-PBDEs are not amenable to GC–MS analysis without derivatization [25]. As a result, high performance liquid chromatography (HPLC) based methods represent a more convenient option. Several methods have been recently published describing the use of this approach for examination of polar BFRs, their metabolites [26], HBCD isomers or TBBPA in body fluids [27,28]. Regarding PFASs, the other group of contaminants involved in our study, HPLC–MS(/MS) is also the key technique used for their quantification in biotic matrices [9].

The objectives of our study were: (i) to implement an solution for the simultaneous analysis of PFASs and BFRs including several metabolites amenable to LC–MS analysis in milk and infant formulas and (ii) to apply a new method for the examination of breast milk and infant formula samples.

2. Materials and method

2.1. Samples collection

The samples of human breast milk were obtained from 50 Czech women living in the Olomouc region (located in the north-east part of the Czech Republic) from April to August 2010 thanks to co-operation with the Gyneacological-maternity Clinic, Faculty Hospital in Olomouc. The age of participating mothers ranged from 20 to 43 years (mean and median age was 30 years). To acquire information that could be relevant to the estimation of contamination pathways, patients completed a questionnaire about their age, body weight, current area of residence (rural/urban), number of children (primapara/multipara), occupation, and dietary habits. Approximately 50 mL of each breast milk sample was collected by hand expressing into a pre-cleaned glass bottle and samples were stored at −20 °C until analysis. The lipid content of the human breast milk samples was determined gravimetrically (results ranging from 0.5–4.9%, mean 2.4%); for this purpose the liquid–liquid extraction (LLE) with hexane and diethyl ether followed by filtration of organic phase through anhydrous sodium sulfate was used [18].

In addition, 6 different types of infant formula from the Czech retail market were examined in this study: (i) one powdered infant and two toddler milk formulas, and (ii) one special formula for babies with lactose intolerance, one formula for premature babies and one soya based formula for babies with non-milk diets.

(i) Milk formulas were supplied in 800 g paper packages and the content of proteins, fats and carbohydrates were in the range of 9.3–10.4, 2.9–3.1 and 55.8–59.1 g in 100 g of formula, respectively.
(ii) Special formulas were obtained in 400 g tin packaging with the composition in proteins, fat and carbohydrates in the range of 10.8–12.8, 10.9–27.3 and 46.1–56 g in 100 g of formula, respectively.

2.2. Standards and chemicals

The individual standards of PFASs and HBCD isomers as well as isotopically labeled internal standards of PFASs and HBCD isomers were purchased from the Wellington Laboratories (Guelph, ON, Canada). PFOS standard supplied by Wellington Laboratories contained 78.8% linear (L-PFOS) and 21.2% branched isomers (Br-PFOS), thus separate quantification of L- and Br-PFOS was possible. Individual standards of OH-PBDEs: 6-hydroxy-2,2′,4,4′-tetrabromodiphenylether (6-OH-BDE-47), 2′-hydroxy-2,3′,4,5′-tetrabromodiphenylether (2′-OH-BDE-68), 4′-hydroxy-2,2′,4,5′-tetrabromodiphenylether (4′-OH-BDE-49), 6-hydroxy-2,2′,4,4′,5-pentabromodiphenylether (6-OH-BDE-99), and brominated phenols: 2,4-dibromophenol (2,4-DBP), 2,4,6-TBP, pentabromophenol (PBP), were purchased from AccuStandard (New Haven, CT, USA). The standard of TBBPA was obtained from Cambridge Isotope Laboratories (Andover, MA, USA). The purity of individual standards was at least 98%. Working standard mixtures of all analytes were prepared in methanol (MeOH) and stored in the refrigerator (5 °C); PFASs and OH-PBDEs were at concentrations 0.25; 0.5; 1; 5; 10; 50 and 100 ng/mL, the concentrations of HBCD isomers, TBBPA and brominated phenols were five times higher. Calibration was prepared by mixing 30 μL of particular working standard mixture with 270 μL of blank matrix extract prepared as described below (without addition of isotopically labeled internal standards) to obtain matrix-matched standards corresponding to the relevant concentration levels: 0.025; 0.05; 0.1; 0.5; 1; 5 and 10 ng/mL for PFASs and OH-PBDEs, and 0.125; 0.25; 0.5; 2.5; 5; 25 and 50 ng/mL for HBCD isomers, TBBPA and brominated phenols.

High performance liquid chromatography (HPLC) grade MeOH was supplied by Merck (Darmstadt, Germany). Acetonitrile (MeCN), anhydrous magnesium sulfate and HPLC grade ammonium acetate (99.99%) were obtained from Sigma-Aldrich (Taufkirchen, Germany). Water purified by a Milli-Q® Integral system (no PFASs containing polymers), supplied by Merck (Darmstadt, Germany), was used throughout the study. Sodium chloride was supplied by Lach-Ner

(Neratovice, Czech Republic). Formic acid (85%) was purchased from Penta (Chrudim, Czech Republic) and Bondesil C18 sorbent (40 μm) was supplied by Varian (Harbor City, CA, USA). Polypropylene (PP) centrifuge tube filters (nylon, pore size 0.22 μm) were supplied by Sigma-Aldrich.

2.3. Extraction and instrumental analysis

An amount of 15 mL of human breast milk sample was transferred into a 50 mL PP centrifuge tube and the isotopically labeled internal standards, 360 μL formic acid, and 15 mL MeCN were added and the mixture was vigorously shaken by hand for 1 min. In the case of infant formula samples, 5 g of powdered sample was weighed and 15 mL of 18 MΩ MilliQ water was added and mixed by shaking for 1 min.

The following steps of sample preparation were identical for both matrices. 6 g anhydrous $MgSO_4$ and 1.5 g NaCl were added and the tube was immediately shaken again for 1 min. The tube was then centrifuged (Hettich, Germany) for 5 min at 10,000 rpm. An amount of 12 mL of the upper organic layer of supernatant was transferred to the new centrifuge tube containing 180 mg C18 sorbent and 1.8 g anhydrous $MgSO_4$. The tube was shaken again for 1 min and centrifuged for 5 min at 10,000 rpm. Subsequently, 8 mL of a purified extract was evaporated near to dryness and the residues were dissolved in 0.5 mL MeOH. The reconstituted extract was filtered through a 0.22 μm nylon centrifuge tube filter and transferred into the vial for the LC–MS/MS analysis.

The UHPLC analyses of PFASs and BFRs were performed using an Acquity Ultra-Performance LC system (Waters, USA) equipped with PEEK tubing and a 10 μL sample loop. Analytes were separated on an Acquity UPLC HSS T3 analytical column (100 mm × 2.1 mm i.d., 1.8 μm particle size, Waters, USA) maintained at 40 °C. The column isolator (50 mm × 2.1 mm i.d., Waters, USA) was inserted between the mixer and the sample valve to separate background contaminates from the sample to be analyzed. The mobile phase consisted of (A) 5 mM ammonium acetate in Milli-Q water and (B) MeOH. The elution gradient conditions for the LC mobile phase were as follows: 10–50% B over 0.5 min, then 50–100% B over 7.5 min followed by an isocratic hold at 100% B for 4 min. The total run time for each injection was 15 min. The flow rate began at 0.3 mL/min and the sample volume injected was 5 μL. The UHPLC system was coupled to a triple quadrupole mass spectrometer Xevo TQ-S (Waters, USA) with negative electrospray ionization (ESI–) and operated in multiple reaction monitoring (MRM) mode. The retention times and quantitative and qualitative MRM transitions of target analytes are listed in the Table S1 in the Supplementary Data.

2.4. Quality assurance/quality control

To demonstrate the applicability of the implemented analytical method, validation experiments on milk and infant formula were performed. Together with each batch of samples, a procedural blank (i.e. sample prepared in a common way, but without the use of test matrix) was prepared to document the absence of background contamination. Recoveries, repeatabilities expressed as relative standard deviations (RSDs), and limits of quantification (LOQs) were established on the data obtained by analyzing 6 spiked blank cow milk (3.5% fat content) and 6 blank powdered milk infant formula samples. For recovery testing, the spike concentrations for milk were 30 pg/mL for PFASs and OH-PBDEs and 300 pg/mL for BFRs (namely HBCDs, TBBPA and brominated phenols). Powdered milk infant formula was spiked at a concentration of 60 pg/g for PFASs and OH-PBDEs, 1000 pg/g for HBCDs, TBBPA and brominated phenols. The LOQs were estimated as the lowest matrix matched calibration standard which provided a signal-to-noise ratio (S/N) higher than 10 and the second MS/MS transition (if available) had to provide a $S/N > 3$. Weighted linear regression ($1/x$) was used and regression coefficient (R^2) was calculated for the calibration curve from the LOQ up to the highest calibration point. Finally, the whole optimized procedure was validated by employing isotopically labeled surrogates.

3. Results and discussion

3.1. Chromatographic separation of PFASs and BFRs

In the first phase of our study, the UHPLC conditions had to be optimized with regard to OH-PBDEs separation. As far as UHPLC system consisted of common C18 column with 5 mM ammonium acetate and MeOH mobile phase, isomers of PBDE metabolites (2′-OH-BDE-68 and 6-OH-BDE-47) were totally co-eluted. Due to the missing characteristic fragments, a spectral resolution was not possible. The separation of these isomers was achieved only when mobile phase selectivity was changed, i.e. MeOH was replaced by MeCN, see Fig. 1 [29]; however, an undesirable decrease in signal sensitivity for other BFRs (TBBPA by 80% and PBP by 90%) was detected. The adverse drop of PFASs signal was also found (FOSAs by 50%, PFCAs and PFSAs by 10%). To obtain as low as possible detection limits for most of major target analytes (see Table 1), 5 mM aqueous ammonium acetate and MeOH was selected as a primary mobile phase, supposing OH-PBDEs signal was detected, sample re-analysis in the second UHPLC system enabling separation of 2′-OH-BDE-68 and 6-OH-BDE-47 might be performed.

3.2. Method for determination of PFASs and BFRs in milk and infant formula

For the development of the new analytical approach for the determination of PFASs and BFRs in milk and infant formula, we took an inspiration from the QuEChERS (“Quick, Easy, Cheap, Effective, Rugged and Safe”) procedure. This method was originally developed for the pesticide residues analysis [30], but has been

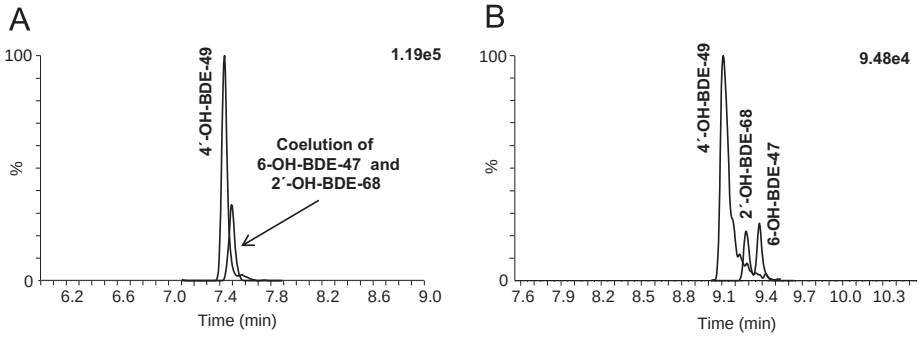

Fig. 1. Chromatograms of OH-PBDEs in matrix matched calibration standard (milk) at 0.5 ng/mL recorded with (A) the mobile phase consisted of 5 mM aqueous ammonium acetate/MeOH and (B) 5 mM aqueous ammonium acetate/MeCN.

Table 1
Recoveries, repeatabilities and LOQs achieved during validation on spiked cow milk (3.5% fat content) and milk infant formula.

Analyte	Milk ($n=6$)				Milk infant formula ($n=6$)			
	Spike PFASs and OH-PBDEs: 30 pg/mL				Spike PFASs and OH-PBDEs: 60 pg/g			
	Spike BFRs[a]: 300 pg/mL				Spike BFRs[a]: 1000 pg/g			
	Recovery (%)	RSD (%)	LOQ[b] (pg/mL)	LOQ[c] (pg/mL)	Recovery (%)	RSD (%)	LOQ[b] (pg/g)	LOQ[c] (pg/g)
PFBA	92	19	20	20	98	23	50	50
PFPeA	91	10	20	20	115	13	20	20
PFHxA	97	4	6	6	107	4	9	9
PFHpA	95	1	6	6	96	3	9	9
PFOA	104	12	6	6	111	4	9	9
PFNA	94	12	6	6	113	3	9	9
PFDA	97	7	6	6	111	4	9	9
PFUdA	99	1	6	6	117	4	9	9
PFDoA	90	3	6	6	110	1	9	9
PFTrDA	101	10	6	6	105	3	9	9
PFTeDA	98	5	6	6	117	3	9	9
PFBS	99	3	6	6	93	4	9	9
PFHxS	101	2	6	6	98	3	5	5
Br-PFOS	110	11	10	10	115	3	20	20
L-PFOS	98	9	5	5	108	3	7	7
PFDS	92	13	6	6	96	2	9	9
PFOSA	101	5	3	10	105	2	5	20
N-EtFOSA	94	5	6	20	104	3	9	20
N-MeFOSA	106	4	6	20	94	5	5	20
6-OH-BDE-47	87	5	n.s.	3	101	9	n.s.	5
4′-OH-BDE-49	94	6	3	3	105	6	5	5
2′-OH-BDE-68	87	5	n.s.	3	95	7	n.s.	5
6′-OH-BDE-99	80	5	3	3	82	10	5	5
2,4-DBP	89	5	200	200	101	6	450	450
2,4,6-TBP	94	4	30	30	99	6	50	50
PBP	82	4	30	100	89	10	50	120
α-HBCD	111	7	30	30	83	11	90	90
β-HBCD	108	5	30	30	81	7	50	50
γ-HBCD	111	4	6	6	84	16	90	90
TBBPA	103	6	60	150	94	7	50	150

[a] Brominated phenols, TBBPA and HBCDs.
[b] The mobile phase consisted of 5 mM ammonium acetate (A) and MeOH (B) was used.
[c] The mobile phase consisted of 5 mM ammonium acetate (A) and MeCN (B) was used n.s. - not specified, because of coelution of 6-OH-BDE-47 and 2′-OH-BDE-68.

already employed also for the analysis of veterinary drugs [31] or mycotoxins [32]. The QuEChERS method is based on a sample extraction with a mixture of MeCN/H_2O and subsequent solvents partition induced by added inorganic salts, NaCl and $MgSO_4$. Crude MeCN extracts can be purified by a dispersive solid-phase extraction (d-SPE) employing e.g. C18 silica, PSA and/or EnviCarb sorbent, depending on the type of co-isolated matrix components. For our purpose C18 sorbent was used for the removal of co-extracted fat and other lipophilic compounds from MeCN extract [33]. To assess the efficiency of this clean-up step, matrix effects (signal suppression/enhancement, SSE) for individual analytes were determined (comparison of a matrix matched calibration slope with the solvent calibration slope in the LOQ range). Matrix effects for most of the PFASs and all brominated phenols were within 30% of ion signal enhancement, except for N-MeFOSA and N-EtFOSA with 10% ion signal suppression. In the case of other BFRs ion signal suppression was observed for OH-PBDEs and TBBPA at 20% and for HBCD isomers at 50%. To overcome these matrix effects, matrix matched calibration was used for the quantification of each analyte.

3.3. Method validation

The validation experiments for milk (Table 1) were performed with spike concentrations of 30 pg/mL for PFASs and 300 pg/mL for BFRs. BFRs were spiked higher, because of their lower instrumental sensitivity compared to PFASs. The recoveries of PFASs were 90–110% with RSD $<19\%$, and for BFRs recoveries ranged from 80–111% with RSD $<7\%$. LOQs of PFASs ranged from 3 to 20 pg/mL, which are slightly lower or comparable with other recent published studies in the range of 5–25 pg/mL for PFOS and PFOA [9,20,21,34–36]. The higher LOQs of short chain PFCAs, PFBA and PFPeA (both 20 pg/mL) were due to frequently observed matrix interferences. LOQs of BFRs were in the range of 3–60 pg/mL, except 2,4-DBP with an LOQ 200 pg/mL. The detection sensitivity of this compound is approximately 10 times lower in comparison to 2,4,6-TBP and PBP (both LOQ 30 pg/mL). In regards to our LOQs of α, β and γ-HBCD (30, 30 and 6 pg/mL; 1, 1 and 0.2 ng/g lw, respectively) and TBBPA (60 pg/mL; 2 ng/g lw), values are also comparable to recent published studies. Shi et al. reported LOQs for α, β, γ-HBCD 50, 30 and 20 pg/g, respectively and 20 pg/g for TBBPA [37]; Kakimoto et al. published LOQs for isomers of HBCD 0.1, 0.1 and 0.2 ng/g lw [38].

The validation experiments of the method for infant formula were performed by at spike concentration of 60 pg/g for PFASs and OH-PBDEs and 1000 pg/g for brominated phenols, HBCDs and TBBPA. Recoveries of PFASs and BFRs were in the range of 93–117% and 81–101%, respectively. RSDs for all target analytes were lower than 23%. The achieved LOQs for PFASs and BFRs were in the range of 5–50 pg/g and 50–450 pg/g, respectively. LOQs for 2,4-DBP (450 pg/g), PFBA (50 pg/g) and PFPeA (20 pg/g) were higher due to the above mentioned reasons. This method was

also verified for special formulas with higher contents of proteins, fats and carbohydrates than milk formula. Recoveries and RSDs were comparable for both matrices. An example chromatogram including all of the target analytes involved in this study is shown in Fig. 2.

Within our experiments, all target analytes fulfill the linearity in the calibration range mentioned above with R^2 higher than 0.99.

3.4. Concentrations of PFASs and BFRs in human breast milk samples

The results obtained by the analysis of 50 breast milk samples are summarized in Table 2. From the 18 target PFASs, only 5 analytes were detected above the LOQ, specifically PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoro-1-hexanesulfonate (PFHxS) and PFOS. The total concentration of PFASs in human breast milk ranged from 38 to 279 pg/mL (mean 115, median 105 pg/mL). The most abundant substances were PFOA and PFOS, which were detected in 100% of the examined samples. Concentrations were in the range of 12–128 pg/mL (mean 50, median 44 pg/mL) and 7–114 pg/mL (mean 33, median 30 pg/mL) for PFOA and L-PFOS, respectively. Other detected analytes were PFNA found in 48% of milk samples in the range of $<$6–15 pg/mL, followed by PFDA and PFHxS found in less than 10% of samples with concentrations ranging from $<$6 to 22 pg/mL. In the case of PFOS, its isomers, L-PFOS and Br-PFOS, were determined separately. Br-PFOS were quantified in 72% of the samples in the concentration range of $<$10–63 pg/mL (mean 20, median 17 pg/mL). The chromatogram of L-PFOS and Br-PFOS in a real milk sample is shown in Fig. 3. Origination of branched isomer occurs during electrochemical fluorination (ECF), process used for PFOS synthesis [2]. The resulting technical mixture typically contains 21–35% of Br-PFOS and 65–79% of L-PFOS [39]. The relative contribution of L-PFOS in the samples positive also for Br-PFOS was in the range of 31–88%. It was slightly wider compared to the technical PFOS mixture. Several studies [40–42] published L/Br-PFOS relative contributions in human serum/plasma ranging from 50% to 70% for L-PFOS, which also did not match the ratio in the technical mixture. The recent studies [43,44] reported isomer specific rates of PFOS-precursor (PFOSA) biotransformation, which may explain the common

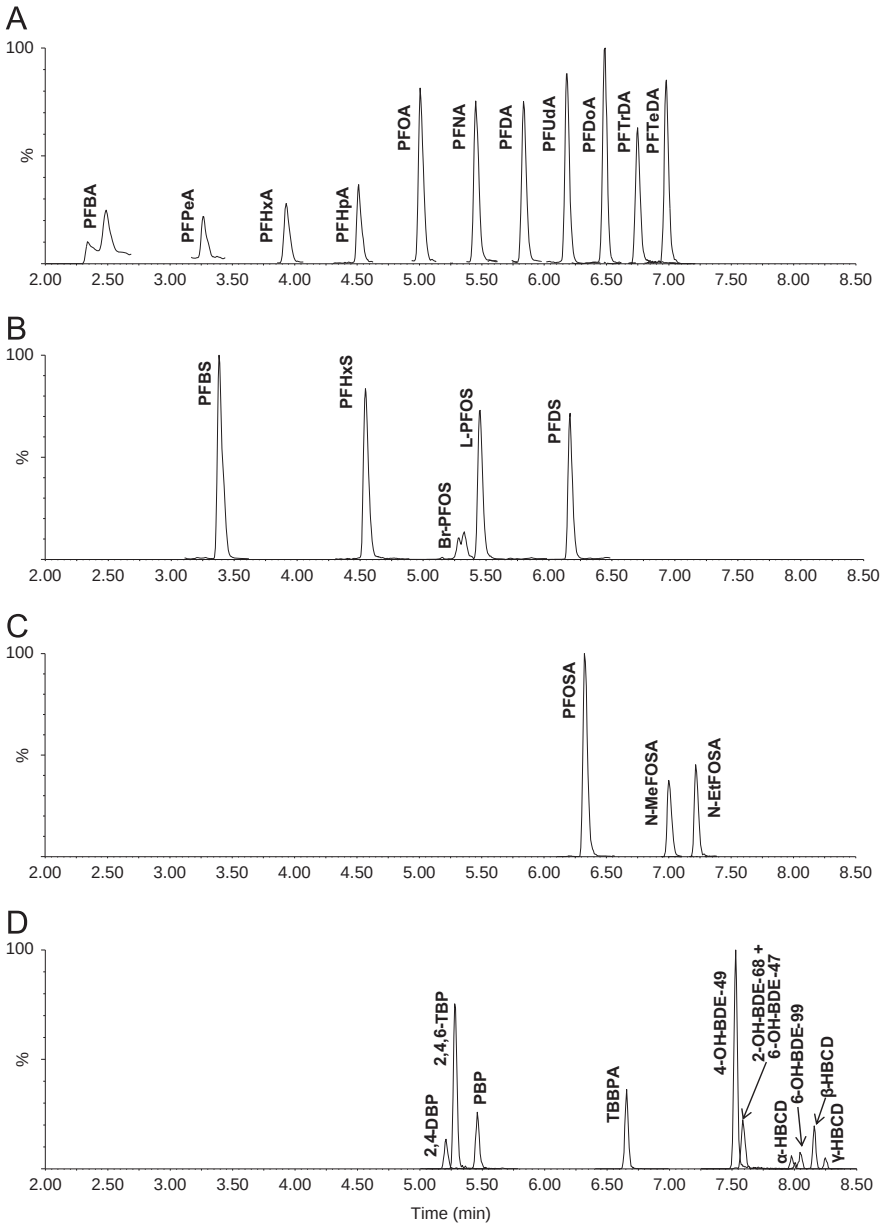

Fig. 2. Chromatograms of (A) PFCAs, (B) PFSAs, (C) non-ionic FOSAs and (D) BFRs in matrix match calibration standard (milk) at concentration 0.5 ng/mL.

Table 2
Concentrations of detected PFASs and BFRs in human breast milk ($n=50$)[a,b].

	Analyte	Samples > LOQ (%)	LOQ	Mean	Median	Minimum	Maximum
pg/mL	PFOA	100	6	50	44	12	128
	L-PFOS	100	5	33	30	7	114
	Br-PFOS	72	10	20	17	< 10	63
	PFNA	48	6			< 6	15
	PFDA	10	6			< 6	12
	PFHxS	8	6			< 6	22
	α-HBCD	28	30			< 30	1660
	TBBPA	30	60			< 60	16,200
ng/g lw	α-HBCD	28	1			< 1	76
	TBBPA	30	2			< 2	688

[a] Mean and median values were calculated when more than 70% samples were positively detected in concentrations above the LOQ.
[b] For results below LOQ one-half the LOQ value was used.

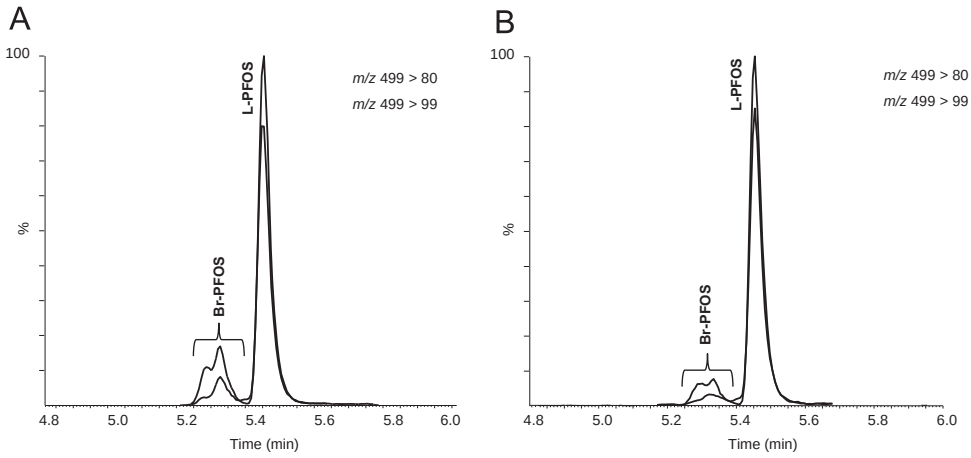

Fig. 3. Chromatograms of (A) Br- and L-PFOS in matrix matched calibration standard ($c=1$ ng/mL) and (B) human breast milk sample. The concentrations of Br- and L-PFOS in sample were 16 pg/mL and 114 pg/mL, respectively.

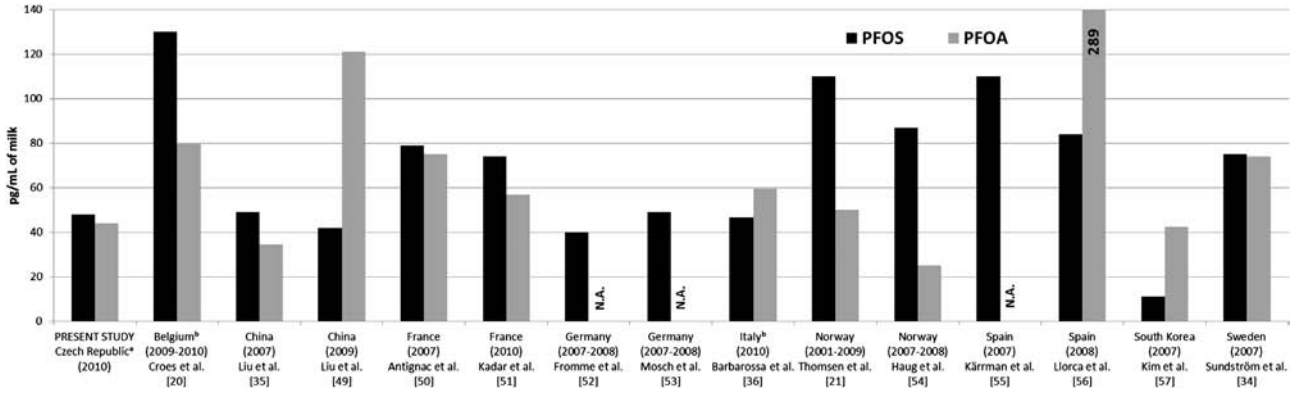

Fig. 4. Median concentrations of PFOS and PFOA (pg/mL) in human breast milk samples from various countries according to years of sampling (in the brackets), N.A. – not available. [a]Median of PFOS is expressed based on the sum of L- and Br-PFOS (pg/mL). [b]Mean of PFOS and PFOA (pg/mL).

observation of enriched PFOS isomers in human sera. However, the PFOSA concentrations in food are often minimal or undetected [45,46]. It is assumed, that the main route of exposure to PFOS isomers is through diet [47] or dust [48]. When we searched data from similar studies concerned with PFASs in other countries, mainly PFOS and PFOA were studied. Fig. 4 gives an overview on PFOS and PFOA concentrations in human milk samples collected since the year 2007. Our data are comparable with other recent reported results [20,21,34–36,49–57].

From 11 target BFRs, only TBBPA and α-HBCD were identified and quantified in the human breast milk samples. TBBPA was found in 30% of samples at a wide concentration range, < 60–16,200 pg/mL (< 2 to 688 ng/g lw). As the number of published studies related to TBBPA concentration levels in human breast milk is extremely limited, a robust comparison of our results with other available data is hardly achievable. Nevertheless, the maximum concentration of TBBPA was approximately 100 times higher compared to those previously published studies [37,58–60].

α-HBCD was detected in 28% of milk samples at concentrations ranging from < 30 to 1660 pg/mL (< 1 to 76 ng/g lw). In the technical mixture γ-HBCD is the most abundant isomer (> 70%), but in the majority of biota samples including our results the

α-HBCD dominated. Some reports have suggested that β- and γ-HBCD can be more extensively metabolized in organisms than α-HBCD [61]. Nevertheless, a few published studies showed the dominance of γ-HBCD in milk samples, which may be related to a recent exposure to a technical mixture [62,63]. In any case, there is a high variability between reported concentrations of the sum of the three isomers (Σ HBCDs) previously published and recently summarized [64]; the mean concentrations are varied from 0.09 to 27 ng/g lw, compared with our results, these data are lower.

Similar to other studies dealing with the occurrence of halogenated pollutants in human milk, the correlation between the age of mother and other factors (e.g. number of children, body weight, occupation and dietary habits) was assessed, but no such trend was found for any of the target compounds. Also the correlation between detected analytes representing both target groups was investigated, nevertheless no relationship was observed.

3.5. PFASs and BFRs in infant formula

No significant contamination was found in examined infant formula samples. In a three samples, the analytes exceeding the LOQ were PFOS, PFOA and PFNA, and the highest contamination in one sample was 19, 10 and 11 pg/g, respectively. BFRs were not detected in investigated samples of infant formula.

Currently, there is no information available regarding the occurrence of BFRs in this type of samples and also limited data exists on PFASs occurrence in infant formula. Although a few positive samples were found in our study, infant formula, under certain conditions, can be also important source of exposure to PFASs and, therefore should be controlled. As documented by a recent study, several long chain PFCAs were detected in milk infant formula made in Japan and China [65]. PFOA, PFNA and PFDA were detected and the mean concentrations ranged between 10 and 28 pg/mL for both countries. In a former study [66] PFOS and PFHxS were found in concentrations also comparable with our study. Within a German study, no PFASs were detected in infant formula samples above the LOQs which ranged from 10 to 50 pg/mL [52]. Finally, in the Spanish study [56], six PFASs were found in milk infant formula and cereals baby food samples, represented by PFOA, perfluoro-7-methyl perfluorooctanoic acid (i,p-PFNA), PFDA, PFOS and perfluorodecanesulfonate (PFDS) in the concentration range of 55–1290 pg/g. The authors assumed that the presence of PFASs in samples could be associated with possible migration/contamination from packaging and production processes. This was supported by the fact that the profile of PFASs present in these products differed from that in most human breast milk analyzed within this Spanish study in which only PFOS, PFOA and i,p-PFNA were detected.

4. Conclusions

Within this study, the new analytical approach for the simultaneous determination of 18 PFASs and 11 BFRs including their metabolites in milk and infant formula has been developed and validated. The sample preparation procedure is based on extraction with acetonitrile and subsequent purification of a crude organic extract (obtained by partition induced by added inorganic salts) with d-SPE using a C18 sorbent. Good performance characteristics were achieved for all target analytes in both matrices, the method recoveries ranged from 80 to 117% with the relative standard deviations lower than 23% for all analytes and the quantification limits were in range from 3–200 pg/mL for milk and 5–450 pg/g for infant formula. The benefits of the method are a rapid and simple sample extraction (ten samples/hour) with a minimal solvent consumption and practically no need of expensive laboratory equipment.

This novel method was successfully applied within the pilot survey for examination of PFASs and BFRs in 50 human breast milks from Olomouc region in the Czech Republic. The results clearly indicated the ubiquitous occurrence of PFASs and some of BFRs in the general Czech population, with PFOS and PFOA being the most abundant contaminants. Subsequently, investigation of six commercial powdered milk formula samples showed no significant contamination, only traces above LOQs were found in few samples.

This is the very first study enabling simultaneous analysis of PFASs and BFRs including several metabolites in human milk and infant formulas, which has never been published before.

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Appendix A. supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.08.040.

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