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“One-shot” analysis of polybrominated diphenyl ethers and their hydroxylated and methoxylated analogs in human breast milk and serum using gas chromatography-tandem mass spectrometry

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Abstract

The presence of polybrominated diphenyl ethers (PBDEs) and their hydroxylated (OH-BDE) and methoxylated (MeO-BDE) analogs in humans is an area of high interest to public health due to their neurotoxic and endocrine disrupting effects. Consequently, there is a rise involving the investigation of these three classes of compounds together in order to understand their bioaccumulation patterns in environmental matrices and in humans. Analysis of PBDEs, OH-BDEs, and MeO-BDEs using liquid chromatography-mass spectrometry (LC-MS) can be accomplished simultaneously, but detection limits for PBDEs and MeO-BDEs in LC-MS is insufficient for trace level quantification. Therefore, fractionation steps of the phenolic (OH-BDEs) and neutral (PBDEs and MeO-BDEs) compounds during sample preparation are typically performed so different detection techniques can be used to achieve the needed sensitivities. However, this approach involves multiple injections, ultimately increasing analysis time. In this study, an analytical method was developed for a “one-shot” analysis of 12 PBDEs, 12 OH-BDEs, and 13 MeO-BDEs using gas chromatography tandem mass spectrometry (GC-MS/MS). This overall method includes simultaneous extraction of all analytes via pressurized liquid extraction followed by lipid removal steps to reduce matrix interferences. The OH-BDEs were derivatized using N-(t-butyldimethylsilyl)-N-methyltrifluoroacetamide, producing OH-TMDS derivatives that can be analyzed with PBDEs and MeO-BDEs by GC-MS/MS in “one shot” within a 25-min run. The overall recoveries were generally greater than 65%, and the limits of detection ranged from 2–

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14 pg in both breast milk and serum. The applicability of the method was successfully validated on four paired human breast milk and serum samples. The mean concentrations of total PBDEs, OH-BDEs, and MeO-BDEs in breast milk were 59, 2.2, and 0.57 ng g⁻¹ lipid, respectively. In serum, the mean total concentrations were 79, 38, and 0.96 ng g⁻¹ lipid, respectively, exhibiting different distribution profiles from levels detected in the breast milk. This GC-MS/MS method will prove useful and cost-effective in large-scale studies needed to further understand the partitioning behavior and ultimately the adverse health effects of these important classes of brominated diphenyl ethers in humans.

Keywords

Polybrominated diphenyl ethers; Hydroxylated polybrominated diphenyl ethers; Methoxylated polybrominated diphenyl ethers; Breast Milk; Serum; Gas chromatography tandem mass spectrometry

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are additive flame retardants that are mixed with, or coated onto consumer products to prolong flame dispersion. Because these compounds are not chemically bound to the polymer and have low vapor pressures, they are leached into the surroundings more readily [1]. Consequently, PBDEs are ubiquitous in the environment and have been found to bioaccumulate in humans [2]. Furthermore, the hydroxylated (OH-BDE) and methoxylated (MeO-BDE) analogs of PBDEs have become a concern to environmental chemists and toxicologists because of their retention in higher trophic level organisms such as in fish, birds, seals, polar bears, sharks, and humans [3–8]. The OH-BDEs and MeO-BDEs are not commercially produced, but are biotransformation products of PBDEs and are also known to be naturally occurring in the environment [9]. These three classes of brominated diphenyl ethers (BDEs) have also been linked to the development of neurological disorders, and are considered endocrine disrupting chemicals with effects related to developmental delays, disruptions of neurotransmitter release, and cytotoxicity [10–12]. Due to their lipophilic nature, BDEs have all been detected in human blood (maternal and fetal), breast milk, and umbilical cord tissue, and their levels vary depending on age, diet, occupation, and geographical location [13–17]. Therefore, understanding their accumulation patterns can ultimately provide information on their sources and metabolism in humans.

Previously established methods for analyzing PBDEs in human samples often include extraction with a non-polar solvent, sample clean-up, and separation by gas chromatography (GC) or liquid chromatography (LC) [1]. However, inclusion of OH-BDEs and MeO-BDEs necessitates that the sample treatment and instrumental analysis be modified in order to account for these classes of compounds. While simultaneous determination of PBDEs, OH-BDEs, and MeO-BDEs is theoretically possible using liquid chromatography-tandem mass spectrometry (LC-MS/MS), limits of detection (LOD) for PBDEs and MeO-BDEs are much higher than OHBDEs due to their poor ionization efficiencies using APCI [8]. Therefore, to achieve the necessary detection limits, PBDEs, OH-BDEs, and MeO-BDEs have been

analyzed separately through fractionation using an acidified silica column and then analyzing the OH-BDEs by LC-MS/MS, and the PBDEs and MeO-BDEs by GC/MS [18]. Another common technique used to separate the neutral (PBDEs and MeO-BDEs) and phenolic (OH-BDEs) analytes involves partitioning with potassium hydroxide [13, 14, 17, 19–22]. However, an added concern of this method is that small amounts of PBDEs can be detected in the phenolic fractions resulting from incomplete separation, which may lead to erroneous results [22]. Gel-permeation chromatography is also used to isolate target analytes from interfering lipids and as well as be able to fractionate compounds based on polarity and size; however this approach is laborious and not environmentally friendly because of the high organic solvent requirement [6, 23].

In the past, PBDEs have been analyzed by GC with an electron capture detector (ECD) because of its inherently high sensitivity for halogenated compounds [24]. However, GC-ECD is less selective than GC-MS because the former relies solely on retention times, and co-eluting compounds cannot be distinguished from each other. Therefore, GC with electron capture negative ionization mass spectrometry (ECNI-MS), high resolution mass spectrometry (HRMS), and triple quadrupole mass spectrometry (QqQ-MS) have gained popularity because of their enhanced selectivity. While ECNI-MS is very sensitive, it generally monitors for the signal of the negative ions of the bromine isotopes m/z 79 and 81. Consequently, structural isomers of BDEs are difficult to differentiate, and isotopically-labeled standards (^{13}C -BDEs) are not useful for quantification. On the other hand, the use of electron ionization (EI) coupled to HRMS or QqQ can take advantage of isotopically-labeled compounds for accurate quantification based on isotope dilution. The latter technique also has the additional advantage of identifying co-eluting BDEs based on the characteristic mass spectral fragmentation obtained from the collision cell [25].

The objective of this study is to develop an efficient extraction, clean-up, and detection method for the simultaneous analysis of 12 PBDEs, 12 OH-BDEs, and 13 MeO-BDEs using gas chromatography with tandem mass spectrometry (GC-MS/MS). Derivatization via silylation with N-(t-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) was chosen based on the high analytical responses and distinct fragmentation patterns observed in the derivatives formed [26]. A programmable temperature vaporizer (PTV) injector was optimized to avoid thermal degradation and analyte discrimination of BDEs due to their range of vapor pressures, molecular weights, and degrees of bromination [27]. The GC-MS/MS was operated under selected reaction monitoring (SRM) mode to achieve high selectivity and signal-to-noise ratios. Four paired breast milk and serum samples were used for method validation to demonstrate the applicability of the method in analyzing human samples for trace levels of PBDEs, OH-BDEs, and MeO-BDEs. The novelty of this “one-shot” GC-MS/MS method lies in its ability to simultaneously quantify the three classes of BDEs, with high specificity and sensitivity, without the need for the tedious fractionation that lends to analyte losses. The method can prove useful in epidemiological studies requiring large number of samples for analysis, such as in determining the partitioning behavior of the three classes of BDEs in complex biological samples.

2. Materials and methods

2.1. Chemicals and reagents

Chromatographic silica gel (60 Å, 40–63 µm) was purchased from Sorbent Technologies (Norcross, GA). The derivatization agent, MTBSTFA, was obtained from Sigma Aldrich (St. Louis, MO) and analytical standards including all individual PBDEs, OH-BDEs, and MeO-BDEs were purchased from Accustandard (New Haven, CT). A surrogate mix containing nine stable isotope-labeled ^{13}C -PBDEs (^{13}C -PBDE-3, -7, -15, -28, -47, -99, -153, -154, -183) and the internal standard solution of ^{13}C -PBDE-77 were obtained from Wellington Laboratories, Inc. (Guelph, ON, Canada). For method development, the sheep serum was obtained from Quad Five (Ryegate, MT), and human breast milk was donated from lactating mothers in Buffalo, NY. For the analysis of paired breast milk and serum specimens analyzed, volunteers from the Mothers' Milk Bank in Austin, Texas each donated breast milk and blood serum which were collected in chemically clean containers. More detail on these paired samples have been previously published [28].

2.2. Sample preparation

2.2.1. Extraction—Sheep serum and donated human breast milk were used for all method development and samples were stored at -40°C until extraction. Five grams of homogenized breast milk and one gram of serum were each spiked with a ^{13}C -labeled surrogate mixture as shown in the schematic diagram of the method in Fig. 1. Isopropanol was added to the milk (5 mL) and serum (1 mL) to denature proteins, and then samples were mixed with 7 g of pre-washed HydromatrixTM (Agilent Technologies, Santa Clara, CA). The extraction procedure was briefly modified based off a previously published method using pressurized liquid extraction with an accelerated solvent extractor (Dionex, Sunnyvale, CA) with 50/50 hexanes:dichloromethane (hex:DCM) at a temperature of 100°C , pressure of 1500 psi, 5 min cycle time performed 3 times, flush volume of 150%, and purge time of 100 seconds, resulting in an extraction volume of 120 mL [29]. Then, lipid content for breast milk was determined gravimetrically by evaporating the extracts down to dryness. Due to the small sample volume of serum, lipid content was determined by calculating total triglyceride and cholesterol levels enzymatically, presented previously by Schechter et al. [28].

2.2.2. Lipid clean-up—The extracts were cleaned-up with a liquid-liquid extraction (2×) using 2 mL concentrated sulfuric acid and 10 mL hexane. The organic layers were combined and evaporated down to 0.75 mL. This extract was then passed through an activated silica column consisting of a self-packed glass cartridge with 1.2 g pre-baked anhydrous sodium sulfate, and 3.2 g acidified silica (44% sulfuric acid), held by a layer of glass wool at the bottom of the cartridge. The PBDEs, OH-BDEs, and MeO-BDEs were eluted with 10 mL 50/50 hex:DCM. Samples were quantitatively transferred to 2 mL amber vials and derivatized by adding 500 µL of acetonitrile and 50 µL of MTBSTFA, followed by a 30-min incubation in an oven at 80°C . The derivatized extracts were then evaporated to dryness and reconstituted with isooctane in 50 µL inserts. Finally, 20 ng mL⁻¹ of ^{13}C -PBDE-77 was spiked into each sample as an internal standard for GC-MS/MS analysis.

2.3. GC-MS/MS analysis

Sample analysis was performed on a Trace GC Ultra coupled to a TSQ Quantum XLS triple quadrupole mass spectrometer (Thermo Fisher Scientific, West Palm Beach, FL). Separation was achieved on a 30-m DB-5HT capillary column, with a 0.25-mm inner diameter, and a 0.10- μ m film thickness (Agilent Technologies, Santa Clara, CA.). The oven temperature program was as follow: initial temperature of 120° C was held for 2 min, ramped at 23° C min⁻¹ to 250° C, followed by a second ramp at 3° C min⁻¹ to 275° C, and then finally increased at a rate of 33° C min⁻¹ to a final temperature of 330° C which was held for 5 min. The flow rate was held at 1.2 mL min⁻¹ with helium (99.99% purity) as the carrier gas. For the inlet, the PTV was set at an initial temperature of 89° C and was increased at a rate of 7.5° C min⁻¹ for 0.2 min. The analytes were then transferred to the column at a temperature of 330° C for 0.1 min. The split flow was 60 mL min⁻¹ with a splitless time of 1.13 min.

The GC-MS/MS was initially operated under the full scan mode at 60 eV, scanning from m/z of 100–1000 to determine the retention times and the m/z values of the most abundant ions for each analyte. To optimize for fragmentation ions, product scans were then conducted from 10–50 eV to obtain the collision energies that provided the most abundant signal of the characteristic fragment ion. Collision energies were chosen based on a 5–10% relative intensity remaining for the base peak. Once the m/z values for the transitions were identified, a GC-MS/MS method based on SRM mode was created for the analysis of all PBDEs, derivatized OH-BDEs (OH-TMDS-BDEs), and MeO-BDEs within a 25-min run time. The observed retention times, m/z values of ions monitored, collision energies, recoveries, and method LODs (mLOD) for all analytes in the breast milk and serum matrices are presented in Table 1.

2.4. Quality control

Isotope dilution was used for the quantification of PBDEs using ¹³C-PBDEs, and a 7-point external calibration curve from 0.75–50 ng mL⁻¹ was used to quantify OH-TMDS-BDEs and MeO-BDEs. Method detection limits ranged from 2–14 pg and were determined based on the standard deviation of the lowest spiked concentration (2.5 ng mL⁻¹) in both serum and milk matrices, along with a signal to noise ratio greater than 3. For positive identification, the retention time of an analyte was set to match that of the standard within ± 0.1 min; and isotopic ratios of the monitored m/z had to be within $\pm 15\%$ of the expected ratios of the standard. Intra-day precision of this method proved reproducible with relative standard deviations (RSD) of <8% for all PBDEs, OH-BDEs, and MeO-BDEs in both breast milk and serum. A calibration curve using a concentration range of 0.625 to 360 ng mL⁻¹ showed good linearity resulting in $R^2 > 0.995$ for all analytes. The extraction and clean-up steps were performed in a positive pressure laboratory to limit atmospheric contamination. All glassware were pre-washed with Alconox®, rinsed with deionized water, and then submerged in a 2% nitric acid bath overnight for removal of potential contaminants adsorbed on the glass. Reported concentrations were background-subtracted by subtracting the endogenous levels in unspiked samples.

3. Results and discussion

3.1. Sample clean-up

To develop a robust analytical method to detect trace levels of PBDEs, OH-BDEs, and MeO-BDEs in breast milk and serum, appropriate clean-up steps were investigated. Concentrated sulfuric acid and an additional activated silica adsorption column were used to denature proteins and effectively remove interfering lipids that could cause ion suppression in GC-MS/MS analysis. Percent recoveries were generally higher than 65% for tri- to hexa-BDEs, but were much lower (<15%) for mono- and di-BDEs in breast milk and serum (Table 1). To determine the cause of analyte loss, hexane was spiked with 25 ng mL⁻¹ of PBDE-10, 2'-OH-BDE-03, 2'-OH-BDE-07, 3'-OH-BDE-07, 2'-MeO-BDE-07, and 3'-MeO-BDE-07, and individual recoveries were determined either after treatment with concentrated sulfuric acid (18M) or after passing through an activated silica column. Data presented in Fig. S1 indicates that the poor recoveries of lower brominated analytes can be attributed mainly to the analyte losses during treatment with 18M sulfuric acid, even though other factors such as evaporation and elution steps could contribute to analyte loss as well. However, treatment with 18M sulfuric acid is necessary to remove proteins in the samples and reduce the high fat content in breast milk causing matrix effects. Samples that were not properly cleaned-up caused significant decrease in GC-MS/MS signals, as observed from a series of injections that showed loss of sensitivity over time. This is the first study that provides an explanation for the loss of lower brominated BDE congeners that is commonly observed. Since treatment with 18M sulfuric acid in combination with the activated silica column was essential for an effective clean-up, the lower BDE congeners were not quantified in this study. Many studies have not been able to detect lower BDE congeners in biological samples, most likely because of their degradation during sulfuric acid clean-up.

3.2. Instrumental performance

The derivatization step converting OH-BDEs into OH-TDMS-BDEs did not affect the amounts and composition of MeO-BDEs and PBDEs, as depicted in Fig. 1. The selective derivatization of OH-BDEs in the presence of PBDEs and MeO-BDEs is key to the successful development of a “one-shot” GC-MS/MS method that can quantify all PBDEs, OH-TDMS-BDEs, and MeO-BDEs in one run without the need to separate the phenolic BDEs from the neutrals prior to derivatization, which was done in previous methods [13, 14, 17, 20, 30–32].

3.2.1. PTV optimization—The main advantage of using a PTV in splitless mode is the ability to have controlled transfer of analytes from the inlet to the column to achieve lower detection limits and avoid analyte discrimination [27]. In this study, five parameters were investigated: transfer temperature, rate of increase in transfer temperature, evaporation rate, split flow, and splitless time. A transfer temperature of 330° C was chosen because temperatures higher than 330° C resulted in signal reduction due to thermal degradation of analytes in the inlet liner. This coincided with maximum responses at a transfer temperature rate of 7.5° C min⁻¹. No apparent difference was observed with changes in evaporation rate. However, the rate of solvent evaporation should be equivalent to the rate of analyte transfer to prevent flooding onto the column, that may lead to poorly resolved peaks [33]. Therefore,

an evaporation rate of $7.5^{\circ}\text{C sec}^{-1}$ was chosen along with a split flow of 60 mL min^{-1} to efficiently trap analytes and to release the solvent through the solvent vent. A splitless time of 1.13 min, defined as the duration of the whole injection step, was long enough to ensure quantitative transfer of all analytes onto the column. All PTV parameters were optimized on a 3- μL injection volume and are presented in Fig. S2.

3.2.2. Mass spectra fragmentation—The mass spectral patterns of BDEs were characterized using the isotopic bromine signature of ^{79}Br and ^{81}Br . The base peak monitored for di- through penta-substituted PBDEs was the molecular ion $[\text{M}]^{+}$, while for any hexa- and hepta-substituted PBDEs the base peak was $[\text{M}-2\text{Br}]^{+}$. The fragmentation of PBDEs occurred with a common loss of two bromines except PBDE-138 and PBDE-154, which resulted in the loss of $[\text{M}-\text{COBr}]^{+}$. For MeO-BDEs, the base peak was $[\text{M}]^{+}$ for the para- and ortho- MeO-BDE position, and $[\text{M}-2\text{Br}]^{+}$ for the meta-MeO-BDE position. The fragmentation patterns of MeO-BDEs in the samples were consistent with the generalized fragmentation patterns reported by Athanasiadou et al [25] for the different positional isomers of MeO-BDEs in electron ionization mode.

For the OH-BDE derivatives using MTBSTFA, the base peak was the loss of the t-butyl moiety $[\text{M}-\text{C}(\text{CH}_3)_3]^{+}$. Mass spectra of the OH-BDE derivatives can be seen in Fig. S3. Fragmentation of the ortho-substituted OH-TMDS-BDEs was monitored via cleavage of the ether bond, corresponding to the loss of the opposing phenyl ring and its subsequent bromines from the attached silyl group (Fig. S3a). Furthermore, fragmentation of both meta- and para-substituted OH-TMDS-BDEs shared common losses of an odd number of bromines, as depicted in Figs. S3b and S3c for 5-OH-TMDS-BDE-47, and 4'-OH-TMDS-BDE-49, respectively.

3.3. Analysis of BDEs in human samples

3.3.1. Method validation through PBDE comparison—To demonstrate the applicability of the “one-shot” method in biological samples, four paired human breast milk and serum samples were analyzed. The PBDE concentrations in these paired samples have been previously determined using GC-HRMS, and results have been published [28]. Therefore, these samples were useful in validating the accuracy of this developed GC-MS/MS method. A comparison of breast milk and serum concentrations for the 6 PBDE congeners (PBDE-28, -47, -99, -100, -153, and -154) most commonly detected in humans determined by GC-HRMS and GC-MS/MS shows good correlation ($R^2=0.75$; slope=0.91) in Fig. 2. However, the previous study did not analyze for OH-BDEs and MeO-BDEs in these breast milk and serum samples, and therefore no comparison can be made for these compounds. Nevertheless, it is reasonable to infer based on the results of the spiked samples, and the high correlation between PBDE concentrations determined by the two methods, that the determination of OH-BDEs and MeO-BDEs using this “one-shot” GC-MS/MS method is reliable.

3.3.2. Evaluation of PBDEs, OH-BDEs and MeO-BDEs in breast milk and serum—A total of 9 PBDEs were detected in the breast milk and serum samples; and the concentrations are represented in Table 2. PBDE-47 and PBDE-99 were the most abundant

congeners detected in both matrices, with concentrations that are 44% ($\pm 13\%$) and 25% ($\pm 9\%$) relative to the other PBDE congeners, respectively. This accumulation reflects the high presence of PBDE-47 and PBDE-99 in the commercial penta- and octa-PBDE mixtures that were used in consumer goods [34]. Total levels of PBDEs in the paired breast milk and serum samples ranged from 16.6–112 and 16.5–146 ng g⁻¹ lipid, respectively.

Results from these analyses were consistent with previous studies that indicated a dominance of tetra- and penta-brominated OH-BDEs and MeO-BDEs in biological samples [17, 19, 35]. The relative abundances of OH-BDEs were highly variable within individual serum subjects and were detected at 10–100 fold greater levels in serum than in breast milk (Table 2). This pattern of OH-BDE accumulation is very similar to the observed behavior of hydroxylated polychlorinated biphenyls [14]. Note that 5-OH-BDE-47 and 6-OH-BDE-47 had the highest detection frequencies in all samples (88%), and 5'-OH-BDE-99 was detected at the highest concentration (20.7 ng/g lipid). Interestingly, these findings mirror the results from *in vitro* metabolism studies on PBDE-47 and PBDE-99 in humans [36, 37].

Total MeO-BDE concentrations were much lower than total OH-BDEs, ranging from none detected to 1.99 ng g⁻¹ lipid in breast milk, and none detected to 2.29 ng g⁻¹ lipid in serum. Notably, 2'-MeO-BDE-68, one of the MeO-BDE congeners that has been postulated to be naturally occurring in the environment [5, 7], was the most frequently detected (63%) in the four paired samples, along with 2'-MeO-BDE-28. Sample chromatograms of OH-BDEs and MeO-BDEs detected in the breast milk and serum subject #2 are presented in Figure S-4a and S-4b, respectively.

We summarized the levels of BDEs reported in literature (Table 3), which do not appear to vary amongst men and women, but vary widely based on geographical location. In paired samples, PBDE levels were either similar to or lower than OH-BDEs, specifically in paired blood/cord blood samples (No MeO-BDEs were analyzed) [15, 38]. Since prenatal exposure to BDEs is of major concern, the concentrations of these compounds in blood/cord blood samples can provide insight on the transfer of BDEs from the mother to the infant during pregnancy [15, 17]. One study from Hong Kong, China, reported elevated levels of MeO-BDEs in blood plasma (ng g⁻¹ lipid), which suggested that a seafood-rich diet was the main source of these compounds [19]. All other studies examining MeO-BDEs in either breast milk or serum reported lower levels (pg g⁻¹ lipid) [13, 35, 39]. However, further studies with a larger number of human subjects are necessary to investigate cytochrome P-450 mediated oxidative metabolism and dietary factors which may contribute to the large inter-individual variability in the retention of PBDEs, OH-BDEs, and MeO-BDEs in serum, and the partitioning of these agents into breast milk [36, 37, 40].

4. Conclusion

In this study, we developed an efficient method that can simultaneously analyze PBDEs, OH-BDEs, and MeO-BDEs in a “one-shot” GC-MS/MS run. This “one-shot” GC-MS/MS method offers several advantages over existing methods for PBDEs, OH-BDEs and MeO-BDEs such as: (1) simultaneous extraction and clean-up steps that effectively minimize matrix effects, (2) ability to detect PBDEs, OH-BDEs and MeO-BDEs simultaneously in

one GC-MS/MS run without the need for multiple injections, ultimately decreasing analysis time, and (3) high efficiency separation by GC coupled with the selectivity offered by MS/MS detection allowing improved LOD and specificity. While the four paired breast milk and serum subjects are limited in sample number, this preliminary data provided insight on the partitioning of PBDEs, OH-BDEs, and MeO-BDEs in humans, which is still not fully understood. The mean concentrations of total PBDEs, OH-BDEs, and MeO-BDEs in breast milk were 59, 2.2, and 0.57 ng g⁻¹ lipid, respectively. In serum, the mean total concentrations were 79, 38, and 0.96 ng g⁻¹ lipid, respectively, exhibiting different distribution profiles from levels detected in the breast milk. The developed “one-shot” GC-MS/MS method described in this paper provides an efficient and cost-effective analytical approach that will be used for further investigations with in large-scale studies to understand the fate, accumulation patterns, and potential health effects of these compounds in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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1. A simultaneous sample preparation method was developed for the “one-shot” analysis of PBDEs, OH-BDEs, and MeO-BDEs in human breast milk and serum.
2. Method positively correlated with previously published literature on PBDE concentrations in the same paired samples, with $r^2 = 0.75$.
3. Findings provide insight on the different partitioning behavior of PBDEs, OH-BDEs, and MeO-BDEs in humans.

Fig. 1. Schematic diagram showing each step of the sample preparation procedure for the simultaneous detection of PBDEs, OH-BDEs, and MeO-BDEs in breast milk and serum.

Fig. 2.

PBDE concentrations in paired milk and serum subjects determined using the “one-shot” method developed in this study compared with the values obtained for the same samples using a previously reported method employing GC-HRMS [28]. The PBDE congeners represented here are PBDE -28, -47, -99, -100, -153, and -154. The equation of the line is $y = 0.91x + 2.67$ with an R^2 of 0.75 showing good agreement between the two methods.

GC-MS/MS parameters of PBDEs, OH-BDEs, and MeO-BDEs and their recoveries (n=2) and method limits of detection in breast milk and serum

Table 1

Analytes	Retention Time (min)	Base Peak (m/z)	Q1, Q2 (m/z)	Collision Energy (eV)	Recovery (%)		mLODs (pg)	
					Breast Milk	Serum	Breast Milk	Serum
PBDEs								
PBDE-10	6.98	328	168, 147	18	8 ± 2	2 ± 3	n.d	n.d
PBDE-7	7.23	328	168, 147	15	103 ± 4	100 ± 9	5.3	4.2
PBDE-15	7.53	328	168, 142	18	82 ± 2	75 ± 15	3.3	3.1
¹³ C-PBDE-15	7.53	340	180, 151	25	n/a	n/a	n/a	n/a
PBDE-28	8.26	406	248, 246	18	103 ± 1	102 ± 1	7.5	3.2
¹³ C-PBDE-28	8.26	258	150, 178	25	n/a	n/a	n/a	n/a
PBDE-49	9.90	486	326, 328	15	86 ± 3	82 ± 5	3.8	4.9
PBDE-47	10.21	486	326, 328	18	84 ± 2	85 ± 0	6.0	4.0
¹³ C-PBDE-47	10.21	338	230, 228	28	n/a	n/a	n/a	n/a
¹³ C-PBDE-77 (IS)	10.89	498	338, 388	25	n/a	n/a	n/a	n/a
PBDE-100	11.93	564	404, 406	20	86 ± 3	91 ± 2	8.2	7.0
PBDE-99	12.52	564	404, 406	18	91 ± 3	91 ± 1	10.0	11.8
¹³ C-PBDE-99	12.52	416	308, 306	30	n/a	n/a	n/a	n/a
PBDE-85	13.81	564	404, 406	20	92 ± 2	72 ± 6	6.6	9.0
PBDE-154	14.59	484	375, 324	33	82 ± 4	82 ± 4	4.1	10.9
¹³ C-PBDE-154	14.59	496	335, 388	38	n/a	n/a	n/a	n/a
PBDE-153	15.82	484	326, 324	38	77 ± 3	86 ± 4	8.4	12.2
¹³ C-PBDE-153	15.82	496	387, 386	33	n/a	n/a	n/a	n/a
PBDE-138	17.78	484	377, 324	35	88 ± 9	96 ± 6	5.9	8.0
PBDE-183	20.51	564	457, 484	30	77 ± 2	85 ± 5	13.6	9.6
¹³ C-PBDE-183	20.51	576	468, 418	38	n/a	n/a	n/a	n/a
OH-BDEs								
2'-OH-BDE-3	8.22	321	166, 227	15	0 ± 0	1 ± 0	n.d	n.d
2'-OH-BDE-7	9.37	401	166, 320	20	1 ± 0	8 ± 2	n.d	n.d
3'-OH-BDE-7	9.90	401	225, 320	15	0 ± 0	0 ± 0	n.d	n.d
3'-OH-BDE-28	12.07	479	148, 399	23	28 ± 0	86 ± 2	12.7	3.8

Analytes	Retention Time (min)	Base Peak (m/z)	Q1, Q2 (m/z)	Collision Energy (eV)	Recovery (%)		mLODs (pg)	
					Breast Milk	Serum	Breast Milk	Serum
PBDEs								
4'-OH-BDE-17	12.98	479	400, 228	13	3 ± 0	6 ± 1	n.d	n.d
6-OH-BDE-47	13.25	559	324, 478	25	89 ± 3	87 ± 5	2.1	6.2
2'-OH-BDE-68	14.28	559	324, 326	23	90 ± 5	79 ± 2	8.5	6.7
5-OH-BDE-47	14.67	559	318, 138	23	104 ± 2	84 ± 1	4.5	3.4
4'-OH-BDE-49	15.94	559	318, 227	23	83 ± 3	84 ± 6	7.6	6.3
6'-OH-BDE-99	16.01	639	324, 326	25	86 ± 1	82 ± 3	3.8	7.9
3-OH-BDE-47	17.24	559	318, 399	25	87 ± 5	77 ± 8	6.1	6.8
4-OH-BDE-42	18.06	559	318, 226	20	80 ± 5	86 ± 4	7.3	2.9
6-OH-BDE-85	18.56	639	404, 402	25	81 ± 3	80 ± 7	4.0	3.8
5'-OH-BDE-99	18.56	639	398, 139	25	94 ± 1	87 ± 2	9.6	6.2
6-OH-BDE-82	18.72	639	404, 402	25	84 ± 2	79 ± 1	6.8	5.7
4-OH-BDE-90	22.60	639	398, 227	25	89 ± 2	84 ± 6	5.5	8.0
MeO-BDEs								
2'-MeO-BDE-07	8.08	358	264, 262	20	2 ± 0	18 ± 4	n.d	n.d
3'-MeO-BDE-07	8.30	358	198, 183	15	1 ± 0	4 ± 0	n.d	n.d
2'-MeO-BDE-28	9.66	436	342, 340	20	74 ± 4	77 ± 0	5.7	5.7
4'-MeO-BDE-17	9.98	436	261, 233	25	71 ± 4	81 ± 1	5.0	5.4
3'-MeO-BDE-28	9.98	436	276, 278	13	48 ± 9	64 ± 3	5.0	5.4
2'-MeO-BDE-68	10.99	516	422, 420	20	87 ± 2	102 ± 3	2.7	10.3
6-MeO-BDE-47	11.36	516	422, 420	23	89 ± 1	98 ± 9	8.8	7.3
3-MeO-BDE-47	11.79	516	356, 358	15	91 ± 4	103 ± 3	2.5	6.3
5-MeO-BDE-47	11.94	516	356, 341	15	85 ± 4	100 ± 5	2.0	4.5
4'-MeO-BDE-49	12.05	516	341, 501	23	90 ± 2	97 ± 4	5.8	5.9
4-MeO-BDE-42	13.23	516	501, 356	20	84 ± 3	90 ± 4	8.0	7.6
5'-MeO-BDE-100	13.63	436	393, 421	25	82 ± 1	74 ± 3	10.3	5.1
6'-MeO-BDE-99	13.87	596	436, 500	20	91 ± 1	84 ± 2	11.6	12.3
3-MeO-BDE-100	14.00	596	421, 393	15	87 ± 5	86 ± 8	8.2	6.7
4-MeO-BDE-90	14.89	596	581, 421	15	85 ± 1	85 ± 3	10.1	6.7

IS: internal standard
n/a: not applicable

n.d.: not determined due to loss of analytes in sulfuric acid treatment during clean-up step (Figure S-1)

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Table 2

Concentrations in ng g⁻¹ lipid of PBDEs, OH-BDEs, and MeO-BDEs detected in the four paired breast milk and serum subjects.

	Sample #1		Sample #2		Sample #3		Sample #4	
	Breast Milk	Serum	Breast Milk	Serum	Breast Milk	Serum	Breast Milk	Serum
PBDE-28	2.16	ND	1.31	ND	0.554	ND	2.20	1.95
PBDE-49	0.520	<LOD	0.190	<LOD	0.367	1.67	0.415	1.85
PBDE-47	57.2	43.1	19.6	22.2	9.42	2.22	32.6	58.4
PBDE-85	1.84	1.91	0.444	1.30	0.201	0.990	0.564	1.30
PBDE-99	23.5	34.4	6.61	19.2	2.89	6.72	7.53	25.3
PBDE-100	13.1	16.8	3.12	2.06	1.34	2.46	4.93	19.1
PBDE-153	12.0	10.2	2.71	<LOD	1.77	2.42	23.0	38.5
PBDE-154	1.32	1.37	0.234	ND	0.091	ND	0.249	ND
PBDE-183	0.186	ND	0.082	ND	ND	ND	0.202	ND
<i>ΣPBDEs</i>	<i>112</i>	<i>108</i>	<i>34.3</i>	<i>44.8</i>	<i>16.6</i>	<i>16.5</i>	<i>71.7</i>	<i>146</i>
3'-OH-BDE-28	ND	ND	ND	ND	ND	ND	<LOD	ND
3-OH-BDE-47	3.01	ND	ND	7.46	ND	ND	0.393	11.7
5-OH-BDE-47	0.171	7.53	0.181	0.562	ND	7.42	0.481	9.15
6-OH-BDE-47	0.090	4.68	0.084	4.16	ND	4.37	0.408	5.72
4-OH-BDE-42	ND	ND	0.186	10.2	ND	ND	0.442	12.6
4'-OH-BDE-49	ND	ND	ND	5.15	ND	0.668	0.285	7.93
2'-OH-BDE-68	ND	ND	0.011	<LOD	ND	ND	0.180	<LOD
6-OH-BDE-82	0.124	ND	0.14	ND	ND	ND	0.300	9.14
6-OH-BDE-85	0.116	ND	0.141	ND	0.821	7.63	ND	ND
5'-OH-BDE-99	0.311	ND	ND	17.2	ND	20.7	0.718	ND
6'-OH-BDE-99	ND	ND	ND	ND	ND	ND	0.201	ND
<i>ΣOH-BDEs</i>	<i>3.81</i>	<i>12.2</i>	<i>0.743</i>	<i>44.7</i>	<i>0.821</i>	<i>40.8</i>	<i>3.41</i>	<i>56.2</i>
2'-MeO-BDE-28	ND	ND	0.059	0.214	ND	0.224	0.360	0.397
3'-MeO-BDE-28	ND	ND	ND	<LOD	ND	ND	0.344	<LOD
3-MeO-BDE-47	ND	ND	ND	ND	ND	ND	0.163	0.325

	Sample #1		Sample #2		Sample #3		Sample #4	
	Breast Milk	Serum	Breast Milk	Serum	Breast Milk	Serum	Breast Milk	Serum
5-MeO-BDE-47	ND	ND	ND	0.230	ND	ND	0.310	0.415
6-MeO-BDE-47	ND	<LOD	ND	0.174	ND	<LOD	0.148	0.176
4-MeO-BDE-42	ND	ND	ND	0.162	ND	ND	0.214	0.416
4'-MeO-BDE-49	0.062	ND	ND	0.301	ND	ND	0.166	ND
2'-MeO-BDE-68	0.048	ND	0.066	0.240	ND	ND	0.284	0.560
<i>ΣMeO-BDEs</i>	<i>0.110</i>	<i>0</i>	<i>0.125</i>	<i>1.32</i>	<i>0</i>	<i>0.224</i>	<i>1.99</i>	<i>2.29</i>

ND: not detected

<LOD: Below method limits of detection

Table 3

Summary showing the ranges of concentrations in ng g⁻¹ lipid of PBDEs, OH-BDEs, and MeO-BDEs in humans reported in literature.

PBDEs	OH-BDEs	MeO-BDEs	Matrix	Cohort	Location	Reference
0.17–29	0.07–11	ND	Blood	Maternal	Indiana, USA	Qiu et al. (2009)
0.10–550	0.03–250	ND	Blood	Fetal	Indiana, USA	
0.14–91	2.5–140 ^a	NA	Blood	Maternal	Ohio, USA	Chen et al. (2013) ^b
0.41–96	2.5–230 ^a	NA	Cord blood	Maternal	Ohio, USA	
0.99–58.4	0.56–20.7	0.16–0.56	Blood	Maternal	Texas, USA	This study (2015) ^b
0.082–57.2	0.011–3.01	0.048–0.36	Breast Milk	Maternal	Texas, USA	
0.16–290	0.11–32	NA	Pooled blood	Children	Managua, Nicaragua	Athanasiadou et al. (2008)
0.023–1,100	0.021–0.89	0.012–15	Breast milk	Maternal	Barcelona, Spain	Lacorte et al. (2009)
NA	NA	1.1–3.0	Breast milk	Maternal	Bizerte, Tunisia	Hassine et al. (2015)
0.09–2.0	ND ^c	0.25 ^d	Breast milk	Maternal	Okinawa, Japan	Fujii et al. (2014)
<LOQ–0.003	0.17 ^c	ND ^d	Blood	Maternal	Okinawa, Japan	
11–95 ^e	2.5–51 ^e	NA	Blood	Maternal	Kashiwa, Japan	Kawashiro et al. (2008) ^b
17–670 ^e	NA	NA	Breast milk	Maternal	Kashiwa, Japan	
0.57–15 ^e	0.83–11 ^e	NA	Cord blood	Maternal	Kashiwa, Japan	
0.47–11 ^e	1.6–19 ^e	NA	Umbilical cord	Maternal	Kashiwa, Japan	
2.0–2,800 ^e	1.2–60 ^e	0.40–6.1 ^e	Blood	E-waste workers	Bangalore, India	Eguchi et al. (2012)
1.4–2,700 ^e	0.67–120 ^e	0.54–18 ^e	Blood	Coastal area residents	Chidambaram, India	
0.11–18	0.012–0.22	0.50–47	Blood	Female	Hong Kong, China	Wang et al. (2012)
0.40–16	0.062–0.30	0.40–16	Blood	Male	Hong Kong, China	
0.19–230	7.5–360	ND	Blood	E-waste workers	Shantou, China	Ren et al. (2010)

ND: not detected, NA: not analyzed

^e Units reported in pg g^{-1} wet weight

^d Only 6-MeO-BDE-47 was targeted

^c Only 6-OH-BDE-47 was targeted

^b Study involves paired samples

^a Units reported in pg mL^{-1}