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A comprehensive gas chromatography coupled to high resolution mass spectrometry based method for the determination of polybrominated diphenyl ethers and their hydroxylated and methoxylated metabolites in environmental samples

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ABSTRACT

We report here an efficient and comprehensive analytical methodology based on gas chromatography with high resolution mass spectrometry (GC–HRMS) to simultaneously determine PBDEs from mono to deca brominated and hydroxy (OH-) and methoxy (MeO-) PBDE metabolites in environmental samples, particularly, sediment, fish tissue and milk. Among a number of extraction and clean-up methods tested, pressurized liquid extraction followed by gel permeation chromatography and florisil clean-up proved to be simple, robust and optimized so that all target analytes (parent compounds and metabolites) were collected in a single fraction. Extracts were analyzed by GC–HRMS to identify PBDEs. Following, the same extracts were derivatized and re-analyzed by GC–HRMS to determine 11 target and 35 non-target OH- and MeO-PBDEs. Monitoring of the M^+ for MeO-PBDEs and the $[M-CH_2CO]^+$ ions for derivatized OH-PBDEs at 10,000 resolution permitted unequivocal identification of the PBDE metabolites in the environmental matrices examined. The method was validated in terms of accuracy, precision, detection limits and long-term stability. The analytical precision obtained with this method was between 0.3 and 17%, and the limits of quantification were lower than 3.28 pg/g dry weight, 20.5 and 41.4 pg/g lipid weight in sediment, milk and fish, respectively. The method was applied to determine PBDEs and target and non-target metabolites in all three matrices.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are used worldwide as flame retardants and environmental monitoring studies have shown increasing levels of these new-era contaminants in wildlife [1] and in humans around the globe [2]. The cause of concern is based on the accumulating evidence that these compounds could have dioxin-like properties and endocrine disrupting potential [3]. Another issue of concern has been the fate of these contaminants in the environment, i.e. whether these contaminants are transformed or metabolized and whether the degradation products persist and pose health risks to humans and wildlife.

Despite their chemical stability, PBDEs are prone to photochemical degradation [4]. Ultraviolet and solar light cause debromination of deca BDE to tetra- and hexa-brominated compounds [5,6] and reductive debromination is the main photodegradation pathways of tetra- to hexa-brominated PBDEs producing polybromo dibenzofurans [7] or methoxylated brominated dibenzofurans [8]. On the other hand, microorganisms can degrade brominated flame retardants by cleavage of the C–Br bond via action of the enzymes hydrohalidase and dehalogenase and under anaerobic conditions, reductive dehalogenation can produce the substitution of Br by an OH group [9] generating hydroxylated PBDEs (OH-PBDEs) as a result of biotransformation of PBDEs through the CYT P450 mediated oxidation [10].

The presence of OH- and methoxy PBDEs (MeO-PBDEs) in the environment has not been fully explored. MeO-PBDEs were first identified as natural products formed by spongi [11] and algae [12]. More recently OH- and MeO-PBDEs were detected in pike [13], salmon [14], artic cod [15] and carp [16]. Residues of MeO-PBDEs have also been detected in mammals such as seals [17], whales [18] and rats [5]. In these studies, different OH- or MeO-PBDEs have been detected and their concentration varies largely depending on the methods used and type of sample analyzed.

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Several gas chromatographic techniques have been employed for the determination of OH- and MeO-PBDEs although to date the lack of authentic standards and quantification methods have been identified as the main drawback in the identification of PBDE metabolites. Haglund et al. [17] used for the first time gas chromatography coupled to mass spectrometry (GC–MS) and high resolution mass spectrometry (HRMS) at a resolution of 8000 to determine the presence of tribromo- to hexabromoMeO-PBDEs in Baltic biota and since no authentic reference standards were available for MeO-PBDEs, quantification was based on comparison with PBDE standards with the same degree of bromination. In this study, a limit of detection (LOD) of 0.1 ng/g lw was reported and three congeners of MeO-TeBDEs and one MeO-PeBDEs of unknown substitution were identified in herring, seal, salmon muscle and fish oil at concentrations up to 158 ng/g lipid weight (lw). Marsh et al. [14] identified the structures of 9 OH- and 6 MeO-PBDEs substituted with four or five bromines in Baltic sea salmon blood by comparison with synthesized 26 OH- and MeO-PBDE standards on a non-polar CP-Sil8CB and a polar SP-2331 GC column and MS in electron ionization (EI) and negative chemical ionization (NCI) modes providing relative retention times (RRTs) and mass spectral characterization for the derivatized (methylated with diazomethane) OH- and MeO-PBDEs. Following, 7 OH- and 4 MeO-PBDEs were identified by GC–MS in EI and NCI in the phenolic and neutral fractions of algae and mussels from the Baltic sea by comparison of the relative retention times of 26 synthesized reference standards versus BDE 138 [19]. GC–HRMS at a resolving power of 4000 and GC–LRMS in full-scan mode for confirmation was used to identify 7 PBDEs and 5 tri to penta brominated MeO-PBDEs in two fractions and using 1,2,3,4,7,8-hexaCDD as the quantification standard, levels up to 16,390 pg/g lw were detected in fish and guillemot [15]. Using GC–HRMS at 9000 resolving power with the internal standard PCB 153 added to the extracted lipid prior to sulphuric acid treatment and PCB 189 as the injection standard, the temporal trends of 5 PBDEs, 6-MeO-2,2′,4,4′-BDE 47 and 2′-MeO-2,3′,4,4′-BDE 68 were studied in pike, although the recoveries of MeO-BDEs were not determined [13].

The aim of our work was to develop a comprehensive, simple, highly sensitive and robust ultratrace analytical method for the identification and quantification of OH- and MeO-PBDEs and their parent compounds in relevant environmental matrices. Pressurized liquid extraction (PLE) followed by gel permeation chromatography (GPC) and florisil in conjunction with GC/HRMS was used to determine the target analytes. Contra to previous studies where the PBDEs and the OH- and MeO-PBDEs were collected in more than one fraction [14,19], our aim was to elute all the target compounds (parent and metabolized) in a single fraction which could be analyzed by GC–HRMS. This was achieved by optimizing the sample clean-up steps and by derivatizing the final extract by acetylation prior to GC–HRMS analysis. The performance of the overall method was validated in terms of accuracy, precision, detection limits and long-term stability. In addition to the 11 OH- and MeO-PBDEs authentic standards we had available we collected relevant information available from the literature [14] and established relative retention times (RRTs) of an additional 35 OH- and MeO-PBDEs compounds. As such, the methodology we present has the capacity to provide quantitative data for 43 PBDE congeners from mono to deca and some 46 OH- and MeO-PBDEs. The applicability of the method to environmental samples was explored by analyzing selected sediment, fish tissue and milk samples for PBDEs and OH- and MeO-PBDEs. This approach, i.e. simultaneous detection of parent and metabolized compounds in a single analysis, provides unique data which are essential to unravel patterns of OH- and MeO-PBDEs in the environment and to assist us in understanding the biotic and abiotic transformation of PBDEs into OH- and MeO-PBDEs.

2. Experimental

2.1. Chemicals and reagents

The Polybrominated Diphenyl Ether Analytical Standard Solution EO-5099 from Cambridge Isotope Laboratories (CIL), Inc. (MA, USA) contained: 3 monoBDEs (BDE # 1,2 and 3), 7 diBDEs (BDE # 7, 8, 10, 11, 12, 13 and 15), 8 triBDEs (BDE # 17, 25, 28, 30, 32 33, 35 and 37), 6 tetraBDEs (BDE # 47, 49, 66, 71, 75 and 77), 7 pentaBDEs (BDE # 85, 99, 100, 116, 118, 119 and 126), 5 hexaBDEs (BDE # 138, 153, 154, 155 and 166) and 3 heptaBDEs (BDE # 181, 183 and 190) at 100 pg/μL for the mono congeners to 250 pg/μL for the hepta congeners. Octa to deca BDEs were analyzed using the commercial mixture OCBDE (79-8DE from CIL) and native 209 (Wellington Laboratories Inc., Canada) at a concentration of 50,000 pg/μL.

The OH- and MeO-PBDE were: 2′-MeO-2,4,4′-BDE 28, 4′-MeO-2,2′,4-BDE 17, 4′-OH-2,2′,4-BDE 17, 2′-MeO-2,4,4′,6-BDE 75, 6-MeO-2,2′,4,4′-BDE 47, 2′-MeO-2,4,4′,5-BDE 74, 6′-MeO-2,3′,4,4′-BDE 66, 2′-OH-2,4,4′,6-BDE 75, 6-OH 2,2′,4,4′-BDE 47, 2′-OH 2,4,4′,5-BDE 74 and 6′-OH-2,3′,4,4′-BDE 66. These were synthesized and characterized by Nikiforov et al. [20]. Stock solutions were prepared from the authentic standards at 890–3110 μg/mL in nonane and working solutions at 500 pg/μL in hexane. PBDEs, OH- and MeO-PBDEs standard solutions were used to establish calibration curves and to spike samples for method development.

The surrogate spiking solution EO-5100 (^{13}C labeled BDE) containing ^{13}C-BDE 3, ^{13}C-BDE 15 ^{13}C-BDE 28, ^{13}C-BDE 47, ^{13}C-BDE 99, ^{13}C-BDE 100, ^{13}C-BDE 118, ^{13}C-BDE 126, ^{13}C-BDE 153 and ^{13}C-BDE 183 at 50 to 250 pg/μL from CIL and was used to quantify mono to octa PBDEs and OH- and MeO-PBDEs. The solution EO-5003 from CIL containing ^{13}C-BDE 209 was used to quantify nona and deca BDEs. EO5101 ^{13}C-BDE 77 from CIL was used as the performance standard. At the time this study was performed, ^{13}C-OH- or MeO-PBDEs were not commercially available. Solvents were pesticide residue grade (Caledon Laboratories Ltd., Georgetown, Ontario, Canada). Pyridine was anhydrous 99.5% from Caledon and acetic anhydride was 99% from Aldrich. Biobeads S-X3 was from Bio-Rad Laboratories Ltd., Mississauga, Ontario, Canada. Sodium sulphate (Mallinckrodt Baker, Inc., Paris, Kentucky) was baked overnight at 475 °C and cooled to room temperature in a dessicating chamber. 100–200 mesh silica (Mallinckrodt Baker, Inc., Paris, Kentucky) and alumina (ICN Biomedicals, Eschwege, Germany) were activated overnight at 200 °C and cooled at room temperature in a desiccating chamber.

2.2. Samples studied

The study comprised the analysis of three matrices (sediments, fish and milk) which were used first in spiking experiments to evaluate method performance and second to determine the suitability of the method to analyze real environmental samples. River sediment collected in year 2000 from Beaver Creek, northwest from the town of Golden (62°21′N, 140°52′W) was used in the spiking experiments (39.3% moisture) and from the Kootenay River in Genelle, British Columbia (49°12′N, 117°40′W) for determination of target analytes (44.3% moisture). Sediments were collected from the top 2–3 cm at 2 m of the shoreline. For fish samples, whole Pacific herring homogenate (68.4% moisture and 12.8% lipid) was used in spiking experiments and another herring sample (83.1% moisture and 3.9% lipid) and whole trout (84.8% moisture and 8.6% lipid) were used for determining target analytes. Fish were bought from the local supermarket. Finally, commercial powdered milk used in the spiking experiments was bought from the supermarket and breast milk analyzed to determine target compounds (4.7% lipid) corresponded to a 33-year-old mother giving birth to the third child. This milk sample was collected with an electric milk extraction pump

machine, carefully washed to avoid external contamination, and was immediately dispensed in precleaned glass vials and frozen at −18 °C until analysis.

2.3. Sample extraction

Method performance was evaluated first using standard mixtures and then with the three environmental matrices: sediment, fish and mothers' milk. For method development, samples (triplicates) were spiked with 5000 pg of OH- and MeO-PBDEs and 5000 pg of the surrogate standard solution, giving a concentration of 5–10 ng/g. Method performance for PBDEs was tested only using ^{13}C-PBDEs in unspiked and spiked samples since the analytical protocol for PBDEs is well established [21–23].

Extraction using sodium sulphate was first tested with standards and was performed as follows: the standard mixture was mixed with 100 g of sodium sulphate. The mixture was transferred to an extraction column with rinses of CH_2Cl_2/hexane (1:1, v/v) at approximately 5 mL/min. The extract was reduced by rotary evaporation and dissolved in hexane.

The ASE 200 (DIONEX, USA) was used for the PLE experiments. Five to 10 g of the sample (wet weight) were homogenized with hydromatrix at a proportion of 2/1 (w/w) and were placed in an extraction cell. Extraction solvents tested were (i) hexane–acetone (4:1, v/v); (ii) hexane–acetone (1:1, v/v); (iii) hexane:CH_2Cl_2 (1:1, v/v); (iv) hexane:CH_2Cl_2 (1:2, v/v) and (v) methanol:CH_2Cl_2 (1:1, v/v). The system pressure was set at 1500 psi and the temperature at 100 °C (100% flush volume) with a heat-up time of 6 min. Three cycles of extraction were performed during 5 min in static mode and the purge time was set at 90 s. Extracts were reduced by rotary evaporation to approximately 0.5 mL and afterwards were purified as described below. A parallel PLE extraction was performed to extract the lipids from milk and fish samples, and % lipid values were determined gravimetrically.

2.4. Sample clean-up and derivatization

Gel permeation chromatography (GPC), acidic–basic silica, alumina and florisil chromatography were tested individually using spiking experiments without the presence of matrix. Specific protocols are given in supplementary information 1.

Resulting extracts obtained with the different clean-up steps were reduced by rotary evaporation to 500 μL of toluene. At this stage, 100 μL of performance standard ^{13}C-BDE 77 was added giving a total amount of 1200 pg. Extracts were first analyzed by GC–HRMS to determine PBDE concentrations. Once the extracts were analyzed for PBDEs, they were then quantitatively transferred with toluene, from microvials into centrifuge tubes and were dissolved in 500 μL of toluene. 100 μL of pyridine and acetic anhydride were added to the sample, which was vortexed for 2 min and heated at 60 °C for 30 min. After derivatization, 700 μL of Milli-RX 20 processed water (15 MΩ-cm 50 ppb total organic carbon (Millipore, Canada), Ltd., Nepean, Ontario), which had been washed twice with toluene, was added to pull out the reaction by-products and left over reagents. The sample was vortexed and back extracted into another centrifuge tube using three hexane washes. The extracted sample was passed through a Pasteur pipette filled with hydromatrix to remove any water. The sample was then nitrogen evaporated to 100 μL, transferred to a microvial, nitrogen evaporated to almost dryness and reconstituted in 100 μL of CH_2Cl_2. These extracts were analyzed again by GC–HRMS for OH- and MeO-PBDEs.

2.5. GC–LRMS analysis

A Voyager single quadrupole mass spectrometer (Thermo Finnigan, USA) equipped with Trace GC gas chromatograph and a CTC A200S autosampler (CTC Analytics, Zurich, Switzerland) was used. An HP-5 (30 m × 0.25 mm ID, 0.25 μm film thickness) containing 5% phenyl methyl siloxane capillary column was used with helium as the carrier gas at 1 mL/min. The temperature program was from 110 °C (1 min) to 220 °C (1 min) at 18 °C/min, then from 220 °C to 240 °C (2 min) at 8 °C/min, and to 300 °C (10 min) at 8 °C/min. One microliter of sample was injected using the splitless injection mode with a splitless time of 1 min. The injector, interface and source temperatures were set at 260, 250 and 250 °C, respectively. The LRMS was operated in the positive electron impact ionization mode at 70 eV and acquisition was performed in full-scan over a mass range from 85 to 550 Da. The GC–LRMS was used to set up and optimize the chromatographic conditions and to obtain full-scan mass spectral information of the OH- and MeO-PBDEs. It was also used to check the long-term stability of target OH- and MeO-PBDEs compounds in the derivatized extracts.

2.6. GC–HRMS analysis

GC–HRMS was performed using a VG-AutoSpec-S (Micromass, Manchester, UK) triple sector mass spectrometer equipped with a Hewlett Packard model 5890 series II gas chromatogram (Agilent, Palo Alto, CA, USA) and a CTC A200S autosampler (CTC Analytics, Zurich, Switzerland). The GC was operated in the splitless injection mode and the splitless injector purge valve was activated at 2 min after a sample injection of 1 μL. The analysis of PBDEs using GC–HRMS is described elsewhere [21,22]. Briefly, PBDE analysis is run on DB5 HT 17 m × 0.25 mm ID × 0.1 μm film and the GC program: 100 °C (1 min), 2 °C/min to 140 °C, 4 °C/min to 220 °C, 8 °C/min to 330 °C (1.2 min). The injector, interface and source temperatures were set at 300 °C, 260 °C and 300 °C, with constant pressure 90 kPa. Octa to deca BDEs were analyzed on a DB5 5 m × 0.25 mm ID × 0.1 μm film thickness and the GC program: 110 °C, 10 °C/min to 300 °C, with a pressure program from 110 kPa, 150 kPa/min to 95 kPa, 120 kPa/min to 35 kPa (hold time 18.3 min).

The analysis of OH- and MeO-PBDEs was done using a DB-5 30 m × 0.25 mm ID × 0.25 μm film thickness from J&W Scientific (Folsom, CA) with the following program: 80 °C (2 min), 10 °C/min to 300 °C (10 min) with a constant pressure 135 kPa. Injector, interface and source temperatures were set at 260 °C, 260 °C, and 300 °C.

The HRMS was operated under positive electron ionization conditions with the filament in the trap stabilization mode at 600 μA, and an electron energy of 35 eV. The instrument was operated at a resolving power of 10,000 and data were acquired in the selected ion monitoring mode (SIM) monitoring the molecular peak $[M]^+$ for mono, di, and BDE 77 and the $[M-2Br]^+$ for the rest of the PBDEs. The ions monitored for the OH- and MeO-PBDEs are presented in Table 1.

2.7. Quality assurance quality control

Instrumental limits of detection (IDLs) for HRMS were calculated by a signal-to-noise ratio of three using the lowest concentration standard and the same criteria was used to determine the limits of detection (LOD) of the method from spiked matrices. Each analytical sequence included quality control standards and blanks to monitor background levels, possible carryover between samples and to monitor and adjust retention time variations. Procedural blanks contained 2′-MeO-2,4,4′,5-BDE 74 and 6-MeO-2,2′,4,4′-BDE 47 and PBDE 47, 99, 100 and 183 below the LOD.

For PBDEs, calibration curves were determined at 5, 20, 50, 100, 300 and 500 pg/μL with surrogate and internal standard at 50–250 pg/μL and were linear over this concentration range. For OH- and MeO-PBDE, the calibration curves were prepared using standards with concentration of 12, 25, 50, 100 and 250 pg/μL and surrogate and internal standards at a concentration of 30–70 pg/μL.

Table 1
OH-, MeO-PBDEs, surrogate standards and performance standard (in italics) included in the analytical method, retention times, relative response factors (RRF), relative standard deviation and instrument detection limits (IDL, pg) and ions monitored for OH- and MeO-PBDEs obtained under GC–HRMS conditions. The RRFs were established relative to ^{13}C-BDE 47 for tri-brominated OH- and MeO-BDEs and ^{13}C-BDE 100 to quantify tetra-brominated OH- and MeO-BDEs and the IDLs (S/N = 3) were obtained from monitoring *m/z* 1. The 50 pg/μL derivatized standard solution was used to perform these experiments.

Compound	Formula	Rt (min)	RRF	RSD	IDL (pg)	GC–HRMS ions monitored	
						m/z 1	*m/z* 2
2′-MeO-2,4,4′-BDE 28	$C_{13}H_9O_2Br_3$	20.54	1.22	5	0.02	435.8133 $[M+2]^+$	437.8113 $[M+4]^+$
4′-MeO-2,2′,4-BDE 17	$C_{13}H_9O_2Br_3$	20.95	1.05	5	0.03	435.8133 $[M+2]^+$	437.8113 $[M+4]^+$
2′-MeO-2,4,4′,6-BDE 75	$C_{13}H_8O_2Br_4$	22.10	0.95	5	0.04	513.7237 $[M+1]^+$	515.7217 $[M+3]^+$
6-MeO-2,2′,4,4′-BDE 47	$C_{13}H_8O_2Br_4$	22.38	0.85	6	0.04	513.7237 $[M+1]^+$	515.7217 $[M+3]^+$
2′-MeO-2,4,4′,5-BDE 74	$C_{13}H_8O_2Br_4$	22.57	0.27	6	0.13	513.7237 $[M+1]^+$	515.7217 $[M+3]^+$
6′-MeO-2,3′,4,4′-BDE 66	$C_{13}H_8O_2Br_4$	22.90	0.80	3	0.04	513.7237 $[M+1]^+$	515.7217 $[M+3]^+$
4′-OH-2,2′,4-BDE 17	$C_{12}H_7O_2Br_3$	21.93	1.24	7	0.06	421.7976 $[M-CH_2CO+2]^+$	423.7956 $[M-CH_2CO+4]^+$
2′-OH-2,4,4′,6-BDE 75	$C_{12}H_6O_2Br_4$	22.45	0.89	5	0.26	501.7061 $[M-CH_2CO+3]^+$	503.7041 $[M-CH_2CO+5]^+$
6-OH-2,2′,4,4′-BDE 47	$C_{12}H_6O_2Br_4$	22.71	0.59	6	0.39	501.7061 $[M-CH_2CO+3]^+$	503.7041 $[M-CH_2CO+5]^+$
2′-OH-2,4,4′,5-BDE 74	$C_{12}H_6O_2Br_4$	22.96	0.73	8	0.29	501.7061 $[M-CH_2CO+3]^+$	503.7041 $[M-CH_2CO+5]^+$
6′-OH-2,3′,4,4′-BDE 66	$C_{12}H_6O_2Br_4$	23.11	0.71	6	0.33	501.7061 $[M-CH_2CO+3]^+$	503.7041 $[M-CH_2CO+5]^+$
^{13}C-2,2′,4,4′-BDE 47	$^{13}C_{12}H_6OBr_4$	21.24	n.c.	n.c.	n.c.	495.7534 $[M+2]^+$	497.7514 $[M+4]^+$
^{13}C-2,2′,4,4′,6-BDE 100	$^{13}C_{12}H_5OBr_5$	22.88	n.c.	n.c.	n.c.	415.8273 $[M-2Br+2]^+$	417.8252 $[M-2Br+4]^+$
^{13}C-2,2′,4,4′,5-BDE 99	$^{13}C_{12}H_5OBr_5$	23.31	n.c.	n.c.	n.c.	415.8273 $[M-2Br+2]^+$	417.8252 $[M-2Br+4]^+$
^{13}C-3,3′,4,4′-BDE 77	*$^{13}C_{12}H_6OBr_4$*	*22.02*	n.c.	n.c.	n.c.	*495.7534 $[M+2]^+$*	*497.7514 $[M+4]^+$*

n.c.: not calculated because they are performance standards.

Calibration standards for OH- and MeO-PBDEs were first dissolved in toluene or hexane but those resulted in nonlinear calibration curves over that concentration range which was attributed to poor solvation of these compounds in the two solvents. Using CH_2Cl_2 as solvent, good linear calibration curves were obtained (typically, $R^2 > 0.990$) over the concentration range indicated above for all compounds (Table 1).

3. Results and discussion

3.1. Mass spectra of OH- and MeO-PBDEs

Individual OH- and MeO-PBDEs were analyzed on the GC–LRMS instrument to establish retention times and mass spectral features. The mass spectra of 4′-OH-2,2′,4-BDE 17, 2-MeO-2,4,5′-BDE 28 and 4′-MeO-2,2′,4-BDE 17 are shown in Fig. 1. The bromine isotopic pattern was observed in all cases and permitted to establish the MS patterns. All MeO-PBDEs with three and four Br substituents had the molecular ion as base peak. Fragmentation differed according to the MeO substitution of the diphenyl ether ring. MeO-PBDEs with the methoxy group in the ortho position to the diphenyl ether bond presented $[M-BrCH_3]^+$ as an intense characteristic ion with 80–90% abundance (see Fig. 1). The presence of $[M-CH_3]^+$ was characteristic of MeO-PBDEs with the methoxy group in the para position to the diphenyl ether bond. The $[M-2Br]^+$ ion of all MeO-BDEs had abundances between 40 and 60% of the base peak. The fragmentation patterns observed with our standards is in accordance with previously published information [14,17]. The $[M-CH_2COBr_2]^+$ was the base peak in the mass spectra of all derivatized OH-PBDEs and the $[M-CH_2CO]^+$ ion had abundances between 70 and 90% to that of the base peak. Other fragment ions characteristic of OH- and MeO-PBDEs were *m/z* 233 $[C_6H_3Br_2]^+$ and *m/z* 154 $[C_6H_3Br]^+$ (Fig. 1C). A characteristic ion of OH-PBDEs was at *m/z* 187 $[C_6H_4O_2Br]^+$ corresponding to a cleavage of the diphenyl ether bond while for MeO-PBDEs it was at *m/z* 201 $[C_7H_6O_2Br]^+$. Another characteristic ion of MeO-PBDEs was at *m/z* 261 $[M-CH_3Br_2]^+$.

The HRMS method was set up using mass spectral information obtained from the LRMS experiments. Molecular ions were chosen for the HRMS analysis to enhance selectivity and sensitivity. There was no problem of isobaric peaks overlapping and all target OH- and MeO-PBDEs could be identified by retention time at each characteristic ion. The exact masses of ions monitored being M^+ for MeO-PBDE and $[M-CH_2CO]^+$ for OH-PBDE, etc. are listed in Table 1 and since the OH-derivatives were acetylated, the mass of the derivatized OH-PBDEs did not match those of the MeO-PBDE homologues. Our unique method feature of acetylation of the OH-metabolites solves the problem of analyte coelution which occurs when OH-PBDEs are methylated and thus have the same mass as MeO-PBDEs. With this approach both OH- and MeO-PBDEs are collected in a single fraction and determined simultaneously in a single GC–MS analysis. During the ionization process of the OH-PBDEs the acetyl group is lost, resulting in the $[M-CH_2CO]^{+\bullet}$ as the base peak, i.e., its *m/z* is equivalent to that of the *m/z* of the corresponding OH-PBDE but it is not the same ion structurally as there is no H on the O for $[M-CH_2CO]^{+\cdot}$ but rather an extra H on the ring. $[M-CH_2CO]^{+\bullet}$ are characteristic ions for aryl acetate spectra due to a hydrogen rearrangement through a six-membered-ring intermediate [24]. For OH-tetra PBDEs, the *m/z* 503 $[M+5]^+$ was used instead of *m/z* 499 $[M+1]^+$ as a trace of coeluting ^{13}C-BDE 100 was observed at the latter *m/z*. Acquisition was kept in a single MS function for the analysis of both OH- and MeO-PBDEs, so that any non-target compounds could also be identified within the entire time range monitored. Identification criteria is given in supplementary information 2.

The ion chromatogram of a solution containing all the target analytes obtained by using the DB5 column is shown in Fig. 2. Based on data from Marsh et al. [14] who provide the most comprehensive list of OH- and MeO-PBDEs and their chromatographic profiles, and data available from our work we used linear regression analysis and established relative retention times (RRTs) for 46 OH- and MeO-PBDEs for the DB5 column (Table 2). We incorporated the RRTs of the compounds that we did not have standards for into our analytical procedure and this enabled us to monitor quantitatively 11 OH- and MeO-PBDEs and qualitatively and semi-quantitatively an additional 35 compounds. The GC–HRMS response factors we used for the compounds for which we did not have standards were the average RRF of the homologues for which we had standards. For non-target OH- and MeO-PBDEs, the closely eluting isobaric peaks were separated given the excellent resolution and acquisition rate of the GC–HRMS method developed.

3.2. Quantification

PBDEs, OH- and MeO-PBDEs were quantified by the isotope dilution method using ^{13}C standards which give the same response as native compounds under GC–HRMS (Table 1). The mean relative response factors (RRFs) were calculated from calibration solutions,

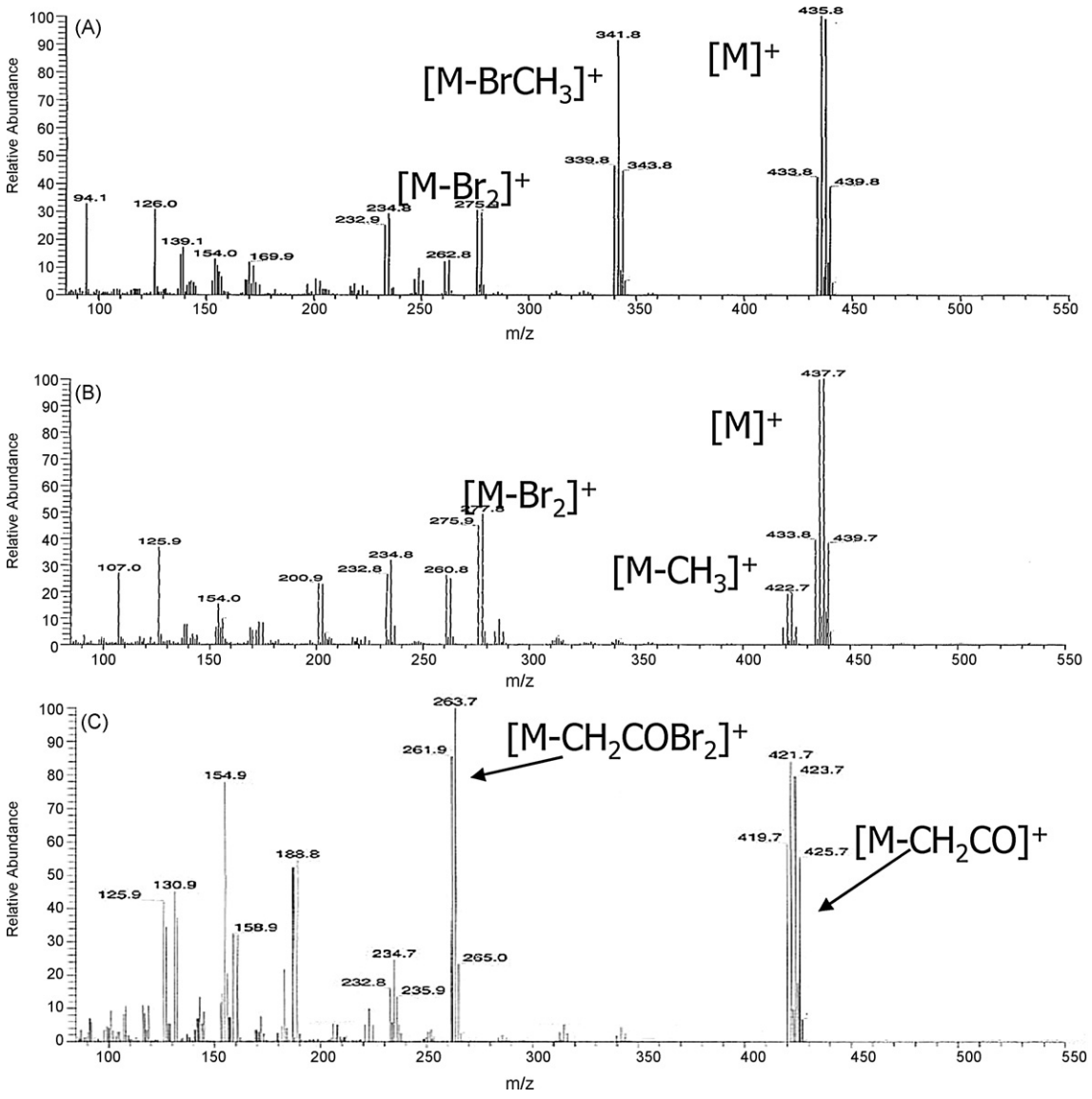

Fig. 1. GC–LRMS mass spectra of 2′-MeO-2,4,4′-BDE 28 (A), 4′-MeO-2,2′,4-BDE 17 (B) and 4′-OH-2,2′,4-BDE 17 (C). The concentration of the solution to obtain these spectra was of 250 pg/μL.

using the base peak or the sum of the areas of both isotope peaks monitored. For all analyses, ^{13}C-BDE 77 was used as the performance standard. PBDEs were quantified using ^{13}C-BDE 15, 28, 47, 100, 99, 118, 153, 183 and 209. Details on deciding which surrogate standards to use for analyte quantification is given in supplementary information 3. Since ^{13}C-OH- and MeO-PBDEs were not available at the time this study was performed, we used ^{13}C-PBDEs to quantify OH- and MeO-PBDEs. ^{13}C-BDE 47 was used to quantify tri-brominated OH- and MeO-BDEs and ^{13}C-BDE 100 to quantify tetra-brominated OH- and MeO-BDEs. These combinations resulted in the best overall long-term RRF stability tested over many injections. The RRF used for quantification method and the %RSD obtained over a concentration range from 2.5 to 100 pg/μL are reported in Table 1. The RRF values ranged between 0.27 and 1.24 with %RSD from 3 to 8, which are similar to PBDEs RRFs which range between 0.3 and 1.2. Thus, despite the lack of ^{13}C-OH-PBDEs as surrogate standards, it is demonstrated that OH- and MeO-PBDEs can be precisely quantified using the ^{13}C-PBDEs homologues. Also in Table 1 we present the IDLs available with the GC–HRMS technique. The advantage of using GC–HRMS for the analysis of these compounds is evident from the ultra-low and consistent IDLs obtained, 0.02–0.39 pg. IDLs were higher for OH-PBDEs than for MeO-PBDEs.

The stability of the derivatized standards was measured over a period of 7 days by performing successive injections of the same extract maintained at room temperature. Fig. 3 shows the response of each compound measured with LRMS. Data was normalized to ^{13}C-BDE 77 to eliminate injection errors and changes in absolute instrument response that vary from day to day. The derivatized standard solution at 50 pg/μL was stable for 7 days (standard deviation from 0.3% to 1.1%, $n = 18$), keeping the standards at room temperature (20 °C). Thus unlike other derivatization agents employed for the derivatization of OH- and MeO-PBDEs, the use of pyridine and acetic anhydride ensured the stability of OH- and MeO-PBDEs over at least one week without losses in sensitivity.

3.3. Method performance

In our efforts in developing a comprehensive analytical technique of isolating and quantifying PBDEs, OH- and MeO-PBDEs at trace levels in diverse matrices (sediment, milk and whole fish),

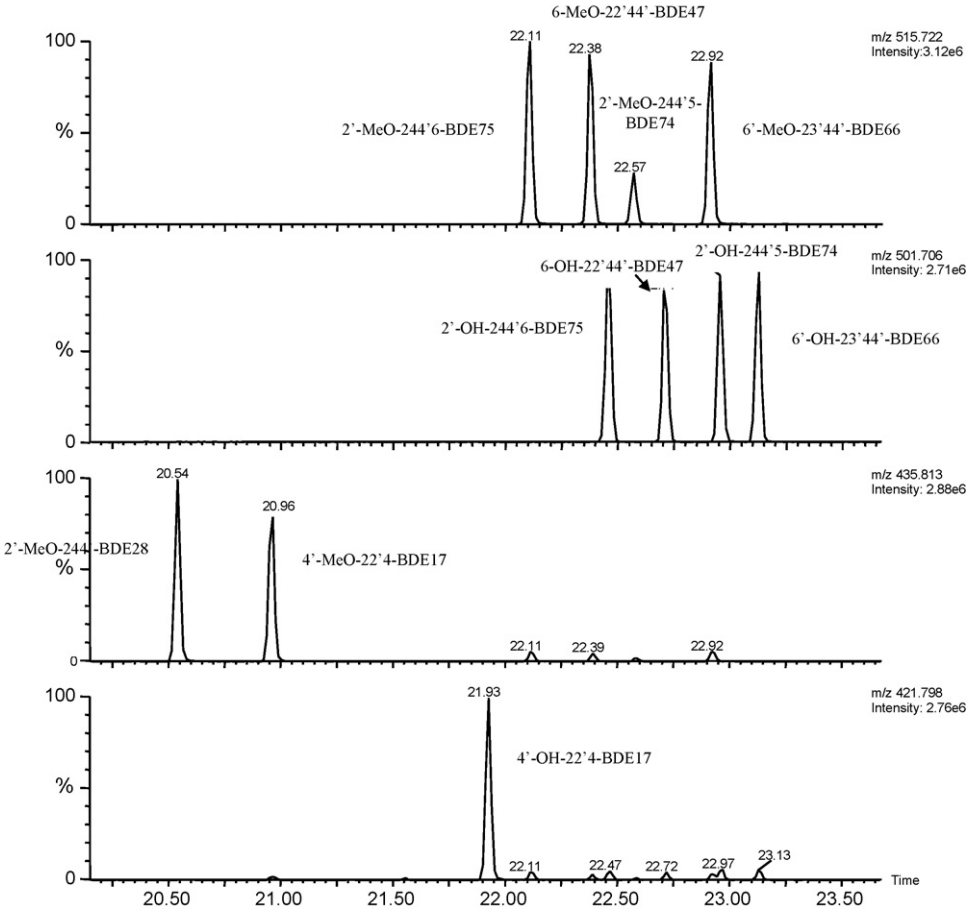

Fig. 2. GC–HRMS ion chromatogram for OH and MeO-PBDEs studied using a DB-5 column. The concentration of the solution to obtain these chromatogram was of 22–27 pg/μL.

several extraction and clean-up procedures were explored. First extraction and clean-up procedures were tested using standard mixtures containing ^{13}C-PBDEs and OH- and MeO-PBDEs. Among the extraction methods tested, pulverizing the sample with sodium sulphate followed with gravimetric extraction did not work as the OH-PBDEs were not recovered presumably due to adsorption onto sodium sulphate. PLE was selected as an alternative and hydromatrix was used as the filler instead of sodium sulphate. With the selection of proper solvents and extraction temperature and pressure, PLE provided high extraction efficiency for all the target analytes. Other inherent advantages of PLE are automation, making the methods highly reproducible and enhancing sample throughput and ease of use. Using PLE, extraction with methanol:CH_2Cl_2 (1:1, v/v) was not efficient in extracting OH-PBDEs, but all other solvents hexane–acetone (4:1, v/v), hexane–acetone (1:1, v/v), hexane:CH_2Cl_2 (1:1, v/v) and hexane:CH_2Cl_2 (1:2, v/v) permitted

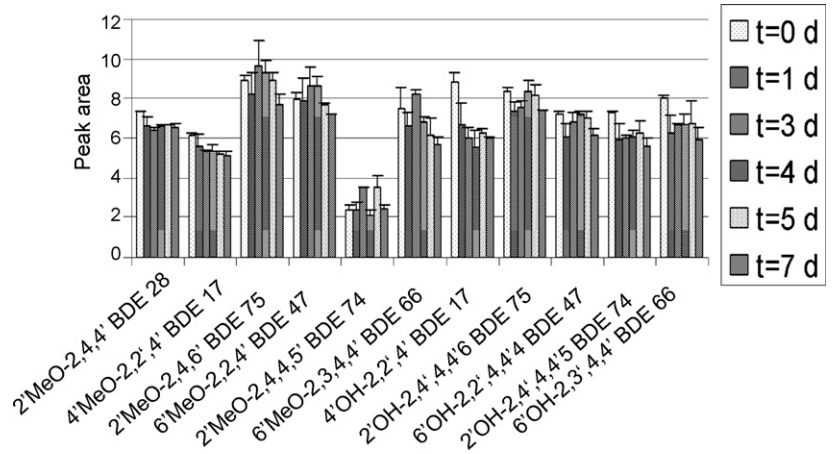

Fig. 3. Stability of MeO-PBDE and derivatized OH-PBDE ($n = 3$) during 7 days. Data is obtained from GC–LRMS and scan mode acquisition mode using the molecular ion for quantification. Peak areas are normalized to ^{13}C-BDE 77 and error bars represent the standard deviation ($n = 3$). These experiments were performed using a standard solution of 50 pg/μL.

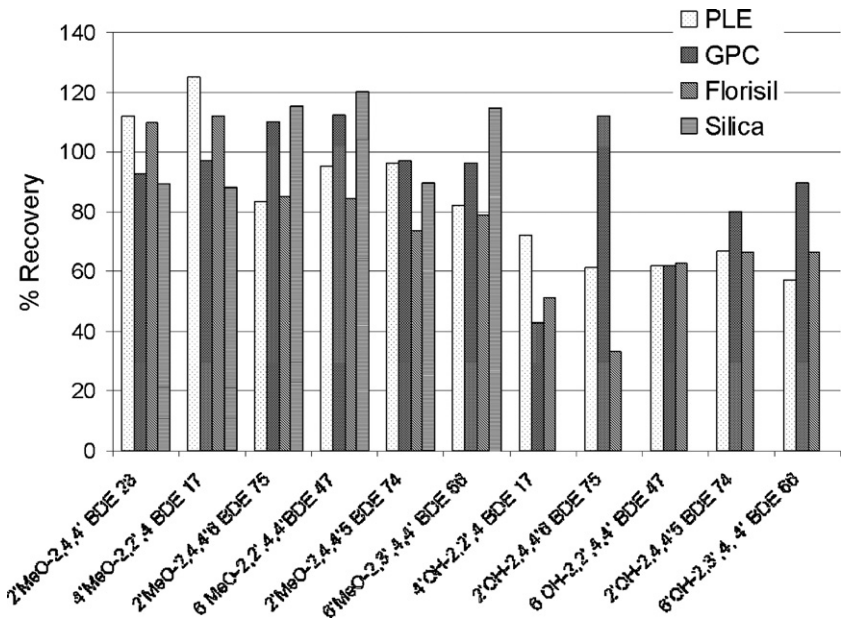

Fig. 4. Percentage of recovery of OH- and MeO-PBDE standards using PLE, GPC, florisil and silica chromatography with no matrix included.

efficient recovery of OH-, MeO-PBDEs and the surrogate standards. However, best recoveries of OH-PBDEs were obtained with hexane–acetone (4:1, v/v), but since surrogate standards were better recovered in hexane:CH_2Cl_2 (1:1, v/v), this solvent system was chosen to provide highest sensitivity and accuracy for all PBDEs and OH- and MeO-PBDEs. The target analyte recoveries obtained using PLE with hexane:CH_2Cl_2 (1:1, v/v) are shown in Fig. 4. Fig. 4 also provides recovery data using GPC, florisil and silica chromatography. Specific optimization parameters of each analytical step is given in supplementary information 4.

Considering all extraction and clean-up steps tested using standard mixtures, the most efficient protocol was PLE extraction followed by GPC and florisil clean-up. The recoveries ($n = 3$) and LOD for OH- and MeO-PBDEs and ^{13}C-PBDEs from spiked river sediments, milk and fish are summarized in Table 3. In these matrices, ^{13}C-PBDEs were recovered from 83% to 112% (Table 3). Although the recoveries of individual PBDEs were not studied, the good extraction efficiency of ^{13}C-PBDEs proves that native PBDEs will recover with similar efficiency [21,22]. Regarding the LOD of PBDEs, the method achieved values of 40 pg/g dw and 113 pg/g lw for monoBDEs in sediment and fish, respectively and for the rest of the congeners from 0.7 to 8.3 pg/g dw and 3.4 to 39 pg/g lw. In milk, LOD were between 12.5 and 197 pg/g lw (BDE 209). For most MeO-PBDEs, good recoveries were obtained, demonstrating no matrix effects in the PLE and florisil clean-up protocol for all matrices studied. 2′-MeO-2,4,4′-BDE 28 and 2′-MeO-2,4,4′,5-BDE 74 presented lowest recovery in sediment and fish, respectively. Recoveries for OH-PBDEs were slightly lower than for MeO-PBDEs (Fig. 4). The use of ^{13}C-PBDEs surrogate standards corrects for analyte losses during sample preparation and controls possible matrix effects, especially for PBDEs. However, the solvent used during the GC–HRMS analysis of PBDEs before the derivatization step could have an impact on the solvation of OH-BDEs, producing a risk of adsorption of OH-PBDEs to the walls of microvials especially at low concentrations. This adsorption cause looses of especially OH-PBDEs. The standard deviation of three replicates varied from 0.3 to 17 (Table 3), showing higher values for whole fish than for sediment or milk. The method is also very sensitive – by extracting 5–10 g of samples on a wet weight basis, LOD were between 0.61 and 3.28 pg/g dw for sediments and from 3.59 to 41.4 pg/g lw for milk and fish (Table 3), low enough to determine these compounds in those environmental matrices. A flowchart of the overall analytical procedure proposed is given in Fig. 5. Following PLE extraction, GPC and florisil clean-up, the extracts are analyzed underivatized by GC–HRMS for mono to decabromo diphenyl ethers. This same extract is thereafter derivatized by acetylation, and OH- and MeO-BDE are analyzed in a second GC–HRMS analysis. Recovery values of ^{13}C-PBDEs are checked for consistency in the spiking level of 5–10 ng/g.

Table 2
List of targeted OH- and MeO-BDEs and their RRTs (relative to BDE 47) obtained with GC–HRMS on the DB-5 column. Bolded congeners identify those for which authentic standards were available.

OH-BDEs	RRT	MeO-BDEs	RRT
6′-OH-BDE 17	0.983	6′-MeO-BDE 17	0.948
4′-OH-BDE 30	0.984	4′-MeO-BDE 30	0.950
2′-OH-BDE 28	0.996	**2′-MeO-BDE 28**	**0.966**
3′-OH-BDE 28	1.010	3′-MeO-BDE 28	0.986
4′-OH-BDE 17	**1.032**	**4′-MeO-BDE 17**	**0.986**
6′-OH-BDE 49	1.039	6′-MeO-BDE 49	1.024
2′-OH-BDE 68	1.050	2′-MeO-BDE 68	1.038
2′-OH-BDE 75	**1.057**	**2′-MeO-BDE 75**	**1.041**
6-OH-BDE 47	**1.069**	**6-MeO-BDE 47**	**1.054**
4′-OH-BDE 69	1.064	4′-MeO-BDE 69	1.058
3-OH-BDE 47	1.074	**2′-MeO-BDE 74**	**1.063**
2′-OH-BDE 66	1.075	3-MeO-BDE 47	1.072
5′-OH-BDE 47	1.078	2′-MeO-BDE 66	1.073
4′-OH-BDE 49	1.080	5′-MeO-BDE 47	1.077
2′-OH-BDE 74	**1.081**	**6′-MeO-BDE 66**	**1.079**
6′-OH-BDE 66	**1.089**	4′-MeO-BDE 49	1.079
4′-OH-BDE 121	1.109	4′-MeO-BDE 121	1.118
4-OH-BDE 42	1.111	4-MeO-BDE 42	1.121
6-OH-BDE 90	1.122	6-MeO-BDE 90	1.136
6-OH-BDE 99	1.125	6-MeO-BDE 99	1.140
4-OH-BDE 90	1.150	4-MeO-BDE 90	1.173
2-OH-BDE 123	1.156	2-MeO-BDE 123	1.181
6-OH-BDE 85	1.167	6-MeO-BDE 85	1.197

3.4. Applicability of the method using environmental samples

The method was tested in a preliminary study to prove its applicability in unspiked sediment, breast milk and fish (trout and herring). Using the developed approach, levels of both target

Table 3
Method performance: spike recovery and standard deviation (%R ± standard deviation, n = 3) and LOD (S/N = 3, using spiked samples at 5–10 ng/g) for all the target analytes using three different matrices (sediment, powdered milk and fish).

Compounds	Sediment (pg/g dw)		Milk (pg/g lw)		Fish (pg/g lw)	
	%R ± SD	LOD	%R ± SD	LOD	%R ± SD	LOD
2′-MeO-2,4,4′-BDE 28	35 ± 1	1.04	100 ± 1	4.74	102 ± 3	21.9
4′-MeO-2,2′,4-BDE 17	99 ± 4	1.17	102 ± 3	5.26	99 ± 1	26.5
2′-MeO-2,4,4′,6-BDE 75	89 ± 2	0.61	89 ± 3	3.59	92 ± 4	11.5
6-MeO-2,2′,4,4′-BDE 47	88 ± 2	0.68	94 ± 1	4.66	106 ± 3	12.8
2′-MeO-2,4,4′,5-BDE 74	94 ± 3	2.26	73 ± 11	14.3	37 ± 4	41.4
6′-MeO-2,3′,4,4′-BDE 66	92 ± 2	0.77	95 ± 3	4.64	80 ± 17	14.5
4′-OH-2,2′,4-BDE 17	67 ± 15	1.73	33 ± 5	3.82	26 ± 9	31.4
2′-OH-2,4,4′,6-BDE 75	43 ± 7	2.13	71 ± 7	12.9	49 ± 4	24.0
6-OH-2,2′,4,4′-BDE 47	69 ± 3	3.28	94 ± 7	20.5	89 ± 8	34.9
2′-OH-2,4,4′,5-BDE 74	74 ± 3	2.45	77 ± 6	18.2	55 ± 6	26.5
6′-OH-2,3′,4,4′-BDE 66	33 ± 4	2.71	57 ± 12	17.3	23 ± 4	29.2
^{13}C-2,2′,4,4′-BDE 47	88 ± 6	–	94 ± 14	–	83 ± 7	–
^{13}C-2,2′,4,4′,6-BDE 100	98 ± 10	–	98 ± 12	–	96 ± 17	–
^{13}C-2,2′,4,4′,5-BDE 99	105 ± 11	–	103 ± 11	–	101 ± 15	–

(–) Not relevant.

PBDEs and OH- and MeO-PBDEs and non-target OH- and MeO-PBDEs are provided. For the latter, the RRT reported in Table 2 together with the exact mass and retention time information was used to determine eight non-target OH- and MeO-PBDEs (Table 5). These compounds were tentatively quantified using target OH- and MeO-BDEs of similar bromination level. Their concentration in these samples ranged between 0.61 and 650 pg/g. Furthermore, another 31 non-targets were also observed but could not be identified by RRT and the generic family name is given. All OH- and MeO-PBDEs observed contained three to five bromine substitutions (Tables 4 and 5).

Up to 31 PBDEs, from di to deca brominated, were detected with levels increasing in the order milk < sediment < herring < trout (Table 4). BDE 47, BDE 99, and BDE 100 were the major congeners detected as reported earlier in environmental matrices [13–15,17]. The specific PBDE pattern changed according to the matrix studied. BDE 209 was the most abundant congener in sediment whereas tetra and penta PBDEs were detected in higher concentrations in fish while breast milk showed only trace levels. OH- and MeO-PBDEs were found in highest concentration in fish. Overall, the metabolite/PBDE ratio varied from 0.1 to 0.01, suggesting PBDE degradation is not important in these matrices or either these compounds are not stable.

Sediment contained 24 PBDEs at levels between 0.90 and 1249 pg/g dw whereas 2 OH- and 17 MeO-PBDEs substituted at any of the meta, ortho or para position were identified at 0.61–43.7 pg/g dw (Tables 4 and 5). Among the main PBDEs detected, the concentration decreased in the order BDE 209 > BDE 47 > BDE 99 > BDE 100 > BDE 49 > BDE 17. The total PBDEs concentration in Columbia river sediment is comparable to superficial sediment cores from the Canadian Artic, where BDEs 47, 99 and 100 were detected at levels of 250 pg/g dw [26] but lower than sediment cores from Lakes Michigan and Erie, which contained total PBDE from 12,000 to 320,000 pg/g dw [27]. The differential PBDEs patterns are specific from area to area and depend on PBDE sources and environmental conditions which might influence degradation processes. The presence of OH- and MeO-PBDEs is attributed to soil microorganisms. It has been shown that microor-

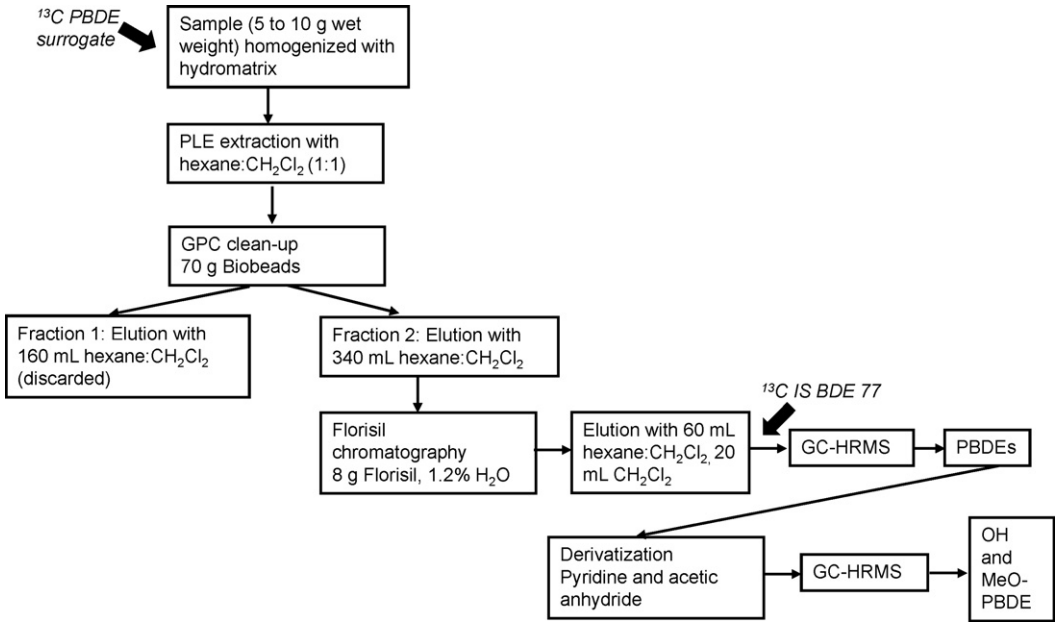

Fig. 5. Extraction and clean-up method used to simultaneously extract and analyze PBDEs, OH- and MeO-PBDEs.

Table 4
Levels of target OH-, MeO-PBDEs and PBDEs (mean ± standard deviation, $n = 2$ for OH- and MeO-PBDEs and $n = 3$ for PBDEs) present in the environmental matrices studied.

Compounds	Sediment (pg/g dw)	Milk (pg/g lw)	Trout (pg/g lw)	Herring (pg/g lw)
Target OH- and MeO-PBDEs				
2′-MeO-2,4,4′-BDE 28	3.89 ± 2	35.9 ± 8	5.74 ± 5	11.8 ± 1
4′-MeO-2,2′,4-BDE 17	0.61 ± 0.5	11.8 ± 5	n.d.	n.d.
2′-MeO-2,4,4′,6-BDE 75		23.1 ± 7	n.d.	n.d.
6-MeO-2,2′,4,4′-BDE 47	43.7 ± 16	n.d.	317 ± 50	368 ± 104
2′-MeO-2,4,4′,5-BDE 74	n.d.	n.d.	n.d.	n.d.
6′-MeO-2,3′,4,4′-BDE 66	5.58 ± 2	n.d.	23.5 ± 1	12.3 ± 1
4′-OH-2,2′,4-BDE 17	n.d.	n.d.	37.8 ± 15	n.d.
2′-OH-2,4,4′,6-BDE 75	n.d.	n.d.	n.d.	n.d.
6-OH-2,2′,4,4′-BDE 47	n.d.	n.d.	n.d.	n.d.
2′-OH-2,4,4′,5-BDE 74	n.d.	n.d.	n.d.	n.d.
6′-OH-2,3′,4,4′-BDE 66	n.d.	n.d.	n.d.	n.d.
PBDEs				
BDE 7	24.8 ± 4	n.d.	n.d	n.d.
BDE 8/11	13.9 ± 1	n.d.	17.7 ± 5	n.d.
BDE 13	0.9 ± 0.2	n.d.	14.7 ± 2.5	n.d.
BDE 15	4.4 ± 1	n.d.	300 ± 66	19.7 ± 4.6
BDE 17	86.1 ± 19	n.d.	202 ± 35	16.5 ± 5
BDE 25	4.5 ± 1	n.d.	63.2 ± 11	15.6 ± 2
BDE 28/33	21.1 ± 2	n.d.	2458 ± 657	2347 ± 60
BDE 75	n.d.	n.d.	553 ± 139	14.2 ± 3
BDE 49	117 ± 3	n.d.	7363 ± 2315	1148 ± 470
BDE 71	8.3 ± 1	n.d.	1663 ± 443	n.d.
BDE 47	580 ± 22	165 ± 14	60011 ± 18213	4924 ± 1817
BDE 66	22.7 ± 1	n.d.	1989 ± 641	156 ± 59
BDE 77	n.d.	n.d.	91.6 ± 31	n.d.
BDE 100	148 ± 7	n.d.	12457 ± 3631	729 ± 247
BDE 119	n.d.	n.d.	485 ± 102	n.d.
BDE 99	568 ± 41	142 ± 30	1477 ± 4527	1208 ± 379
BDE 85	30.8 ± 6	n.d.	n.d.	21.7 ± 8
BDE 101[a]	15.2 ± 1	n.d.	3029 ± 93	66.0 ± 17
BDE 118	n.d.	n.d.	670 ± 190	9.6 ± 3
BDE 155	4.7 ± 0.4	n.d.	1179 ± 317	17.1 ± 6
BDE 154	56.1 ± 6	n.d.	18941 ± 5538	134 ± 52
BDE 153	60.1 ± 7	82.6 ± 27	13696 ± 4204	112 ± 30
BDE 140[a]	2.4 ± 1	n.d.	93.3 ± 30	n.d.
BDE 138/166	11.1 ± 6	n.d.	n.d.	n.d.
BDE 183	17.1 ± 2	204 ± 40	955 ± 287	31.4 ± 2
BDE 181	n.d.	n.d	9.6 ± 3	n.d.
BDE 207	26.7 ± 10	n.d.	56.2 ± 20	48.5 ± 16
BDE 206	49.4 ± 23	n.d.	38.8 ± 8	14.9 ± 6
BDE 209	1249 ± 287	n.d.	712 ± 279	616 ± 214

n.d.: not detected.

[a] BDE 101 and 140 are not included in the standard EO5099. BDE 101 has a predicted RT [25] and an average RRF calculated from the other pentaBDE congeners present in EO5099. BDE 140 was present in CIL EO4980 which was used to establish a ratio RRF of BDE 140 with BDE 153.

ganisms from a contaminated soil produced complete reductive dehalogenation of brominated biphenyls more rapidly than for polychloro biphenyls (PCBs), with meta and para positions removed first [28]. Whereas reductive debromination of BDE 209 with zerovalent iron produced debromination and no oxidation products [29], the formation of MeO-PBDEs could be induced from hydroxylated compounds via O-methylation from soil bacteria [30].

In breast milk, four PBDEs were found at levels between 82.6 and 204 pg/g lw and 2′-MeO-2,4,4′-BDE 28, 4′-MeO-2,2′,4-BDE 17 and 2′-MeO-2,4,4′,6-BDE 75 were identified (Table 4). Previous studies indicate the prevalence of BDEs 47, 99, 100, 153 and 154 in breast milk at concentrations ranging from 17 to 128,000 pg/g lw, depending on the sampled area [2]. Recently OH- and MeO-PBDEs have been identified in breast milk although formation of OH- and MeO-metabolites are not reported as a main degradation route [31].

Trout had higher PBDE levels than herring and it contained many more non-target metabolites leading to a higher total metabolite content than the herring (Tables 4 and 5). MeO-PBDEs were detected in trout and herring at levels similar to guillemot from the Baltic, Atlantic and Artic environments [15]. In contrast to our study, MeO-PBDEs were not identified in blood from Detroit river fish, although ∑PBDEs were found at 24–11,475 pg/g ww, depending on the species, and 9 OH-PBDEs were identified at levels ranging from 0.9 to 170 pg/g ww, with 6-OH-BDE 47 detected at highest concentrations [32]. To evaluate whether debromination could be an elimination route in fish we estimated the ratio among BDE homologues. In trout, we observed ∑hepta BDEs/∑nona BDEs of 13, ∑hexa BDEs/hepta BDEs of 26 and ∑tetra BDEs/∑pentaBDEs of 2 and in herring ∑pentaBDEs/hexa BDEs of 8 and ∑tetra BDEs/pentaBDEs of 3, which differs completely from the ratios of formulated PBDEs, suggesting debromination in freshwater fish as reported by Stapleton in a mesocosm experiment that demonstrated debromination of BDE 209 to penta–octa-BDEs [33]. However, none of the samples included in this study contained octa-BDEs. Other studies report high concentrations of BDEs 47, 66, 75, 119 and 183 in exposed salmon, due to debromination preferentially from the meta position to the diphenyl ether bond [34]. When considering PBDEs/OH- and MeO-PBDE ratio, values were always higher than 1, suggesting that CYP enzyme mediated metabolism is not an important route of PBDE degradation in fish [9,10]. Nonetheless, given the relevant levels of MeO-PBDE along with PBDEs, their accumulation potential in fish [35] and the fact that metabolism of PBDEs may produce more potent pseudoestrogens [36], effort should be given to determine the

Table 5
Tentatively identified non-target OH- and MeO-PBDEs in sediment and fish using a DB5 column, retention time, relative retention time and concentration (mean ± standard deviation, $n = 2$). Milk samples were not analyzed for non-target samples.

Compound	*m/z*	Rt (min)	RRT	pg/g dw
Sediment				
MeO-triBDE	435.8133	20.04	0.927	1.16 ± 1
MeO-triBDE	435.8133	20.32	0.940	17.8 ± 9
MeO-triBDE	435.8133	20.38	0.943	2.12 ± 1
MeO-triBDE	435.8133	20.44	0.945	1.03 ± 1
MeO-triBDE	435.8133	20.53	0.950	4.70 ± 2
MeO-triBDE	435.8133	20.72	0.958	2.14 ± 1
MeO-triBDE	435.8133	20.79	0.962	5.50 ± 2
MeO-triBDE	435.8133	21.49	0.994	9.71 ± 5
6′-MeO-BDE 49	513.7237	22.14	1.024	2.59 ± 2
2′-MeO-BDE 68	513.7237	22.45	1.038	18.9 ± 8
3-MeO-BDE 47	513.7237	23.18	1.072	6.16 ± 3
MeO-tetraBDE	513.7237	23.24	1.075	11.9 ± 5
MeO-pentaBDE	593.6323	25.76	1.192	12.5 ± 5
OH-triBDE	421.7976	20.14	0.932	4.83 ± 3
OH-triBDE	421.7976	22.07	1.021	4.53 ± 3
Trout				
MeO-triBDE	435.8133	21.21	0.982	11.3 ± 1
MeO-triBDE	435.8133	21.56	0.998	11.9 ± 2
MeO-triBDE	435.8133	21.9	1.013	12.3 ± 3
MeO-triBDE	435.8133	22.48	1.040	136 ± 38
MeO-triBDE	435.8133	22.79	1.055	62.4 ± 29
MeO-triBDE	435.8133	23.1	1.069	41.7 ± 22
6′-MeO-BDE 49	513.7237	22.14	1.024	10.9 ± 4
2′-MeO-BDE 68	513.7237	22.44	1.038	149 ± 45
5′-MeO-BDE 47	513.7237	23.24	1.075	19.5 ± 6
6-MeO-BDE 90	593.6323	24.49	1.133	24.2 ± 6
OH-triBDE	421.7976	20.14	0.932	299 ± 27
OH-triBDE	421.7976	22.05	1.020	250 ± 132
OH-triBDE	421.7976	22.10	1.023	204 ± 65
OH-triBDE	421.7976	22.25	1.029	59.3 ± 12
OH-triBDE	421.7976	22.54	1.043	97.3 ± 143
OH-triBDE	421.7976	22.73	1.052	46.8 ± 14
OH-triBDE	421.7976	22.82	1.056	209 ± 76
4′-OH-BDE 49	421.7976	23.34	1.080	109 ± 12
OH-tetraBDE	501.7061	22.32	1.033	18 ± 11
OH-tetraBDE	501.7061	22.56	1.044	13.4 ± 7
2′-OH-BDE 68	501.7061	22.69	1.050	9.65 ± 7
OH-pentaBDE	579.6166	24.09	1.115	13.9 ± 1
OH-pentaBDE	579.6166	24.42	1.130	17.2 ± 4
Herring				
MeO-triBDE	435.8133	20.35	0.942	16.8 ± 14
4′-MeO-BDE 30	435.8133	20.52	0.950	22.9 ± 19
MeO-triBDE	435.8133	20.76	0.961	11.7 ± 8
6′-MeO-BDE 49	513.7237	22.13	1.025	75.1 ± 58
2′-MeO-BDE 68	513.7237	22.44	1.039	118 ± 86
6-MeO-BDE 90	513.7237	24.48	1.133	14.9 ± 9

metabolism routes of PBDEs in fish and evaluate their potential effects.

4. Conclusions

From numerous studies performed to date, it is clear that PBDEs are ubiquitous environmental contaminants although their fate in the environment is still not understood basically because there is a lack of precise and accurate analytical methods to determine a broad range of PBDEs along with OH- and MeO-PBDEs. Monitoring of PBDEs should be continued using any of the analytical methods described in the literature, but at the same time it is important to measure those OH- and MeO-derivatives which could be PBDE degradation products that might have a deleterious effect towards the environment or to humans. In this study, we have demonstrated that OH- and MeO-PBDEs can be present in environmental samples although at concentrations lower than those found for PBDEs. However, the relevance of these compounds in the environment has to be elucidated. For that reason, the proposed method will be useful to resolve some environmental problems related to the presence and degradation of PBDEs in the environment, e.g. determine whether PBDE degradation or metabolism is important, establish those matrices where OH- and MeO-PBDEs are formed and explore if these compounds persist and are bioavailable to finally elucidate the fate of PBDEs in the environment. So far, quantitative data for congener specific OH- and MeO-PBDEs is limited.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2009.11.024.

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