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Sample preparation method for the speciation of polybrominated diphenyl ethers and their methoxylated and hydroxylated analogues in diverse environmental matrices

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ABSTRACT

A reliable analytical method was developed here for the simultaneous separation, identification and quantification of ten polybrominated diphenyl ethers (PBDEs), nine methoxylated PBDEs (MeO-PBDEs) and ten hydroxylated PBDEs (OH-PBDEs) in various environmental matrices, including water, soil, sediment, plant, mollusk and fish. PBDEs and MeO-PBDEs were measured by gas chromatography coupled with mass spectrometry (GC/MS) and liquid chromatography coupled with electrospray ionization (negative)-tandem quadrupole mass spectrometry (LC/ESI(−)-MS/MS) for the separation and determination of OH-PBDEs without prior derivatization. After preliminary sample cleaning using acid silica gel, water-impregnated silica column separation of PBDEs, MeO-PBDEs and OH-PBDEs was proved to be rapid, simple, and efficient. For phenolic analytes, the method detection limits (MDLs) were 3.2–11.6 pg/L in water sample and 2.8–18.4 pg/g dry weight in solid samples. For neutral compounds, MDLs were 48.8–150.3 pg/L in water sample and 46.5–170.8 pg/g dry weight in solid samples. The method was validated using six kinds of environmental samples spiked with all analytes at three concentration levels (0.3 ng, 2 ng and 5 ng, respectively) for recovery (71–113%) and repeatability determination (4–12%RSD).

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are widely distributed in the environment as flame retardants. As structural analogues to PBDEs, hydroxylated polybrominated diphenyl ethers (OH-PBDEs) and methoxylated polybrominated diphenyl ethers (MeO-PBDEs) have been found in water samples [1], red algae [2] as well as marine animals [3–7], and even in human blood and breast milk [8,9]. These compounds are persistent and lipophilic and can be bio-accumulated via the food chain [10]. Both types of analogues present health risks such as thyroid disruptions and neurotoxic effects [11–14]. MeO-PBDEs are often identified as natural products [15,16]. However, some reports indicated that MeO-PBDEs may be formed via methylation of PBDEs or OH-PBDEs [4]. OH-PBDEs are known of natural origin [17,18] and the products of PBDEs metabolism [19–23]. Recently, Wan et al. [24] found the demethylation of MeO-PBDEs could be a source of OH-PBDEs. The unclear biological process and greater toxicities relative to PBDEs have attracted increasing interests in environmental fate and toxicology of OH-PBDEs and MeO-PBDEs in biota and abiotic environment.

The analysis of PBDEs and MeO-PBDEs is mainly based on GC–MS or GC–ECD [5,25–28]. Although some works used LC–MS/MS methods for the analysis of PBDEs and MeO-PBDEs [29–31], the high quantification limits made it unsuitable for the detection of trace PBDEs and MeO-PBDEs in environmental samples. OH-PBDEs can be determined by GC/MS as their MeO-analogues via a methylated derivatization using diazomethane [10,25,26,32], but the toxicity and easy explosive of diazomethane make the analysis difficult to handle. Therefore, atmospheric pressure chemical ionization (APCI)-LC/MS/MS [29,33] and ionspray ionization (ISP)-LC/MS/MS [34] methods have been developed for the analysis of OH-PBDEs without the time consuming step of derivatization. However, the selectivity and sensitivity of LC–MS/MS methods need to be further improved to make it more efficient in OH-PBDEs analysis. Phenolic compounds were usually separated from neutral compounds by partitioning with potassium hydroxide after extraction. Thus the mentioned methods are always time-consuming and cause the loss of analytes [10,25,26] Kato et al. [29] proposed a single cleanup procedure using gel permeation chromatography (GPC) for simultaneous collection of

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OH-PBDEs and MeO-PBDEs but limited to only some types of matrices such as biosamples.

The aim of this work was to develop a highly sensitive and effective analytical method for simultaneous identification and quantification of ten PBDE congeners, nine MeO-PBDE congeners and ten OH-PBDE congeners in diverse environmental matrices using GC/MS and LC/ESI-MS/MS. A faster and simpler clean-up and pre-separation method for various environmental samples was developed. Water, soil, sediment, plant, mollusk and fish samples were employed to explore the applicability of the method to various environmental matrices. The performance of the overall method was validated in terms of linearity, recovery, repeatability, and sensitivity.

2. Experimental

2.1. Chemicals and reagents

Nine stock standards of methoxylated polybrominated diphenyl ethers, 4-MeO-BDE-42 (10 μg/mL), 4′-MeO-BDE-49 (10 μg/mL), 3-MeO-BDE-47 (50 μg/mL), 5-MeO-BDE-47 (50 μg/mL), 6-MeO-BDE-47 (10 μg/mL), 2′-MeO-BDE-68 (10 μg/mL), 6-MeO-BDE-85 (10 μg/mL), 5′-MeO-BDE-99 (10 μg/mL), 6′-MeO-BDE-99 (10 μg/mL), ten hydroxylated polybrominated diphenyl ethers, 3′-OH-BDE-28 (50 μg/mL), 4-OH-BDE-42 (10 μg/mL), 4′-OH-BDE-49 (10 μg/mL), 3-OH-BDE-47 (50 μg/mL), 5-OH-BDE-47 (50 μg/mL), 6-OH-BDE-47 (10 μg/mL), 2′-OH-BDE-68 (10 μg/mL), 6-OH-BDE-85 (10 μg/mL), 5′-OH-BDE-99 (10 μg/mL), 6′-OH-BDE-99 (10 μg/mL) and ten standards of polybrominated diphenyl ethers, BDE-28 (50 μg/mL), BDE-47 (50 μg/mL), BDE-66 (50 μg/mL), BDE-68 (50 μg/mL), BDE-85 (50 μg/mL), BDE-99 (50 μg/mL), BDE-138 (50 μg/mL), BDE-153 (50 μg/mL), BDE-154 (50 μg/mL) and BDE-183 (50 μg/mL) were purchased from AccuStandard (New Haven, CT, USA). Recovery surrogate standards, ^{13}C-6′-MeO-BDE100, ^{13}C-6′-OH-BDE100 and ^{13}C-BDE-99 and injection internal standards, ^{13}C-6-MeO-BDE47, ^{13}C-6-OH-BDE47 and ^{13}C-BDE-47 at 50 μg/mL were purchased from Wellington (Guelph, ON, Canada). Individual PBDEs and MeO-PBDEs working solutions were prepared at 500 ng/mL in hexane and OH-PBDEs working solutions were prepared at 500 ng/mL in acetonitrile.

Methyl tert-butyl ether (MTBE), methanol and acetonitrile were HPLC grade, hexane and dichloromethane (DCM) were pesticide grade and all purchased from J.T. Baker (Phillipsburg, NJ, USA) and Honeywell Burdick & Jackson (Seelze, Germany). Deionized water (18.2 MΩ) was obtained from ultrapure water purification system (Barnstead International, Dubuque, USA).

Silica gel (0.063–0.100 mm) (Merck, Darmstadt, Germany) and Florisil (0.150–0.250 mm) (Sigma–Aldrich, St Louis, USA) were prebaked at 140 °C for 7 h. Acid silica was prepared by 56 g activated silica with 44 g concentrated H_2SO_4. Water-impregnated silica was prepared by 95 g activated silica with 5 g deionized water.

2.2. Sampling and sample preparation

Six different environmental matrices were selected for spiking experiments to evaluate method performance. Water and sediment samples were collected from Gaobeidian Lake, Beijing in 2010. Surface water samples were collected using 4 L amber glass bottles and immediately transferred into the laboratory. Sediment samples were collected by a grab sampler. Agricultural soil and leaves of cushaw were obtained in Zhejiang Province, China in 2010. Mollusk and fish samples, *Mytilus edulis* and *Pseudosciaena polyactis* were collected from the Bohai Sea and the Donghai Sea, China in 2009, respectively. *Mya arenaria* was collected from Tianjin, China in 2009. All the solid samples were stored at −20 °C until analysis.

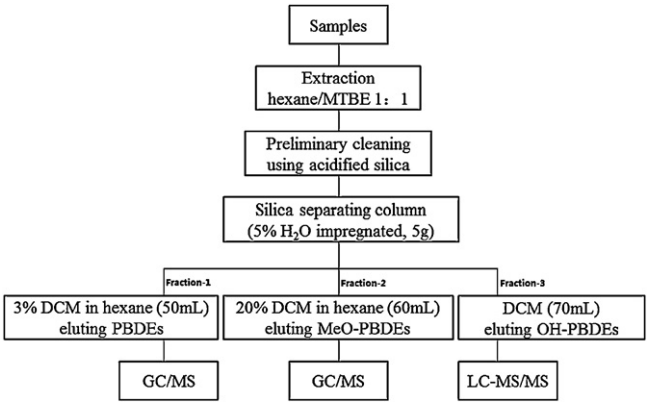

Fig. 1. Optimized extraction and cleanup procedure of environmental samples for the simultaneous separation of PBDEs, MeO-PBDEs and OH-PBDEs.

Before analysis, solid samples were freeze-dried, ground and sieved through a stainless steel 75-mesh (0.5 mm) sieve. The mollusk samples were mixtures of the soft tissues from several mussels. 500 mL lake water and 1.0 g solid samples were spiked with three recovery surrogate standards (5 ng for each) and mixture of ten PBDEs, nine MeO-PBDEs and ten OH-PBDEs (three spiking levels: 0.3 ng, 2 ng and 5 ng for each of the analytes). The pretreatment procedure is illustrated in Fig. 1. Lake water sample was extracted twice with 25 mL hexane/MTBE (1:1; v/v) after the addition of 2 mL 2-propanol. The extracts were combined and concentrated. The solid samples were extracted with 5 mL hexane/MTBE (1:1; v/v) in an ultrasonic bath for 20 min after the addition of 2 mL 2-propanol. The samples were centrifuged at 3000 rpm for 10 min and the solvent was transferred to clean vials. This extraction procedure was repeated three times and the extracts were combined and dried under gentle flow of nitrogen gas. The dried residues were then dissolved in 20 mL of DCM and cleaned by acid silica gel. For sediment sample, sulfur was removed by 2 g activated copper powder before adding acid silica gel. The acid silica gel was removed through an anhydrous Na_2SO_4 column (4 g). DCM (40 mL) was used as eluent to ensure all the compounds were desorbed from acid silica gel. The collected extracts were concentrated using a rotary evaporator and re-dissolved with 1 mL of hexane. Then, the extract was cleaned by a multi-layered column consisting, from bottom to top with 5 g of silica deactivated with 5% water (w/w) and 1 g of Na_2SO_4 for fractionation of PBDEs, MeO-PBDE and OH-PBDEs. After prewashing with 30 mL of hexane, the extract was loaded to the column. 50 mL 3% DCM in hexane was used to elute PBDEs firstly. Secondly, 20% DCM in hexane (60 mL) was applied to elute MeO-PBDEs from the column and finally, DCM (70 mL) was used to elute OH-PBDEs. Fractions of PBDEs and MeO-PBDEs were concentrated to 100 μL and added internal standards (^{13}C-BDE-47 and ^{13}C-6-MeO-BDE47) prior to instrument analysis. Fraction of OH-PBDEs was evaporated to dryness and solvent exchanged with 100 μL acetonitrile. ^{13}C-6-OH-BDE47 was added prior to LC–MS/MS analysis.

2.3. GC/MS analysis

PBDEs and MeO-PBDEs analysis was performed on an Agilent Model 6890 gas chromatograph (GC) coupled with a 5973C mass spectrometer (MS) detector (Agilent Technologies, Palo Alto, CA). Samples were injected by a 7683B Series Injector into a DB-5MS column (J & W Scientific, Folsom, CA, 30 m, 0.25 mm i.d., 0.1 μm film thickness) with splitless mode (280 °C). Helium was used as carrier gas at a constant flow of 1.0 mL min^{-1}. The oven program started at 90 °C, increased at 15 °C min^{-1} to 220 °C, then at 1 °C min^{-1} to 260 °C. The post run was set at 300 °C, held for 3 min. The total run

Table 1
List of target PBDEs and MeO-BDEs and their retention times (t_R) and SIM ions obtained with GC/MS on DB-5 MS column.

Compounds	Formula	GC t_R (min)	GC/MS ions monitored	
			m/z 1	m/z 2
BDE-28	$C_{12}H_7OBr_3$	10.637	245.8	247.8
BDE-47	$C_{12}H_6OBr_4$	13.671	325.8	327.8
BDE-66	$C_{12}H_6OBr_4$	14.354	325.8	327.8
BDE-68	$C_{12}H_6OBr_4$	13.114	325.8	327.8
BDE-85	$C_{12}H_5OBr_5$	21.660	405.8	407.8
BDE-99	$C_{12}H_5OBr_5$	18.817	405.8	407.8
BDE-138	$C_{12}H_4OBr_6$	30.514	485.8	487.8
BDE-153	$C_{12}H_4OBr_6$	26.413	485.8	487.8
BDE-154	$C_{12}H_4OBr_6$	23.327	485.8	487.8
BDE-183	$C_{12}H_3OBr_7$	35.955	565.8	567.8
2′-MeO-BDE68	$C_{13}H_8O_2Br_4$	15.483	515.7	517.8
6-MeO-BDE47	$C_{13}H_8O_2Br_4$	16.139	515.7	517.8
3-MeO-BDE47	$C_{13}H_8O_2Br_4$	17.143	515.7	517.8
5-MeO-BDE47	$C_{13}H_8O_2Br_4$	17.534	515.7	517.8
4′-MeO-BDE49	$C_{13}H_8O_2Br_4$	17.855	515.7	517.8
4-MeO-BDE42	$C_{13}H_8O_2Br_4$	20.421	515.7	517.8
6′-MeO-BDE99	$C_{13}H_7O_2Br_5$	21.902	595.7	597.8
5′-MeO-BDE99	$C_{13}H_7O_2Br_5$	24.360	595.7	597.8
6-MeO-BDE85	$C_{13}H_7O_2Br_5$	25.715	595.7	597.8

time was 51.67 min. Quantitative determination by GC–MS (EI) was in the selected ion monitoring (SIM) mode. Quantification ions were $[M]^+$ or $[M-2Br]^+$ and identity confirmation ions were $[M+2]^+$ or $[(M+2)-2Br]^+$. The ions monitored for PBDEs and MeO-PBDEs are presented in Table 1.

2.4. LC–MS/MS analysis

Analysis of OH-PBDEs was carried out using an Agilent 1290 Series LC system coupled with an Agilent 6460 Triple Quadrupole MS/MS system (Agilent Technologies, Palo Alto, CA) using a C18 column (100 mm × 2.1 mm, 2.2 μm particle size, Dionex, USA). The mobile phase consisting of acetonitrile (Solvent A) and water (Solvent B) was used with a gradient elution of A:B from 55:45 to 75:25 in 20 min at a flow rate of 0.38 mL min^{-1}. The column was equilibrated for 5 min between runs. The column temperature was set at 40 °C and the volume injected onto the column was 10 μL.

Mass spectrometric detection was completed using ESI source in the negative ion multiple-reaction monitoring (MRM) mode. An Agilent Jet Stream was coupled to ESI source. Nitrogen was used as the curtain, nebulizer gas and collision gas. The flow was optimized at 5 L/min at a constant temperature of 350 °C and the sheath gas flow was optimized at 11 L/min at a constant temperature of 400 °C. The capillary and nozzle voltages were held at 3500 V and 500 V, respectively. Nebulizer pressure and Delta electron multiplier voltage (EMV) was set as 50 psi and 800 V, respectively. A dwell time of 20 ms was used for each of the MRM transitions. The formula, LC retention time, and the MRM transitions of all analyzed OH-PBDEs are summarized in Table 2.

2.5. Quality assurance and quality control

Seven-point standard calibration curves were prepared using standards with concentration of 2, 5, 10, 25, 50, 100 and 200 ng/mL and surrogate and internal standards at constant concentration of 50 ng/mL. The equipment detection limits (EDLs) for all the investigated compounds by GC/MS and LC–MS/MS were estimated based on a signal-to-noise ratio (S/N) of 3 using the lowest concentration standard. The same criteria were used to determine the method detection limits (MDLs) from spiked matrices. Repeatability was evaluated by intra- and inter-assay variation. Intra-assay variation was assessed by five consecutive injections of a blank soil sample spiked at 5 ng, and inter-assay variation was determined by measuring the same spiking sample each day for five consecutive days. All targets could be identified by retention time at each characteristic ion. The calibration of working curves was done daily to monitor possible carryover effect between runs and adjust retention time variations. No memory effects were observed between consecutive runs of the spiking concentration, and the target compounds were under MDL in solvents.

3. Results and discussion

3.1. Optimization of sample preparation conditions

The formula and GC retention time of target PBDEs and MeO-PBDEs are listed in Table 1.

Sample preparation was designed to fractionate PBDEs, MeO-PBDEs and OH-PBDEs within a single separation column. In our experiment, different clean-up procedures were explored. Several chromatographic columns were tested for clean-up procedure including Florisil, acid silica gel and neutral silica gel. The proportions of hexane and DCM were also optimized as eluent. The results showed co-elution existed among MeO-PBDE congeners and OH-PBDE congeners on acid silica gel column no matter how much H_2SO_4 was used to acidify the silica gel. The recovery of 3-MeO-BDE-47 was only 27%, and co-eluted by 3-OH-BDE-47 (recovery 25%) and 5-OH-BDE-47 (recovery 31%) when using 80% hexane/DCM as eluent. For Florisil column, PBDEs and MeO-PBDEs could be eluted separately while OH-PBDEs were strongly adsorbed even though Florisil was deactivated by water (1.2–5%). Almost all the OH-PBDEs had low recoveries (<50%) except 3′-OH-BDE28 (~90%). Activated neutral silica showed similar performance to Florisil when using DCM as eluent. However, good separating property and eluting capability could be obtained when silica was impregnated with 5% water. Finally, 5% water impregnated silica column was chosen as separating column and the optimized loading amount was 5 g. The mixture of hexane and DCM was optimized from 100% hexane to 100% DCM and the eluent was established as 50 mL of 3% DCM/hexane for PBDEs, 60 mL of 20% DCM/hexane for MeO-PBDEs and 70 mL of DCM for OH-PBDEs. In order to remove abundant impurities in the environmental samples, GPC and acid silica gel (44% H_2SO_4 acidified) were tested for the preliminary purification procedure. Although both methods showed good clean-up performance, acid silica gel was chosen finally because less solvent and less time was needed. For sediment sample, activated copper powder was used for elimination of sulfur-containing compounds. Although all target PBDEs and MeO-PBDEs can be separated by a DB-5 MS column and determined simultaneously by GC/MS, the fractionation of PBDEs and MeO-PBDEs on water-impregnated silica column could decrease the interferences when more PBDEs and MeO-PBDEs species were analyzed. In addition, solid-phase extraction (SPE) method was tried and C18 and HLB columns (Waters, Canada) were tested. Although less solvent was needed by SPE method, lower recoveries and worse separation effect were obtained.

Comparing with the methods reported in the previous papers [25,26] in which partitioning with potassium hydroxide and subsequent acidification and back extraction of the aqueous fraction was always used to separate the neutral and phenolic analogues, a single 5% water impregnated silica column was used instead in this paper for the simultaneous separation of three analogues, PBDEs, MeO-PBDEs and OH-PBDEs. Derivatization of OH-PBDEs was unnecessary so that the entire preparation was simplified.

3.2. Optimization of LC–MS/MS conditions

Five HPLC chromatographic columns were tested to resolve the coelution of isomeric species, including ZORBAX C18 column

Table 2
Characterization of target OH-PBDEs and MRM parameters used for qualitative and quantitative determination.

Compounds	Formula	LC t_R (min)	$[M-H]^-$	Product ions	MRM transition (m/z)	Frag (V)	CE (V)
3′-OH-BDE28	$C_{12}H_7Br_3O_2$	8.058	420.7	$[C_{12}H_6Br_2O_2]^-$	420.7 → 340.6[a]	−160	−15
				$[C_6H_3Br_2O]^-$	420.7 → 80.8[b]	−160	−32
				$[Br]^-$			
4-OH-BDE42	$C_{12}H_6Br_4O_2$	9.669	500.7	$[C_6H_2Br_2O_2]^-$	500.7 → 265.6[a]	−135	−22
				$[Br]^-$	500.7 → 80.8 [b]	−135	−40
3-OH-BDE47	$C_{12}H_6Br_4O_2$	10.445	500.7	$[C_{12}H_5Br_3O_2]^-$	500.7 → 418.6[a]	−135	−38
				$[C_{12}H_5Br_2O_2]^-$	500.7 → 80.8 [b]	−135	−40
				$[C_6H_3Br_2O]^-$			
				$[Br]^-$			
4′-OH-BDE49	$C_{12}H_6Br_4O_2$	10.966	500.7	$[C_6H_2Br_2O_2]^-$	500.7 → 265.6[a]	−135	−25
				$[Br]^-$	500.7 → 80.8 [b]	−135	−40
5-OH-BDE47	$C_{12}H_6Br_4O_2$	11.207	500.7	$[C_{12}H_5Br_3O_2]^-$	500.7 → 418.6[a]	−100	−20
				$[C_{12}H_5Br_2O_2]^-$	500.7 → 80.8 [b]	−100	−32
				$[C_6H_3Br_2O]^-$			
				$[Br]^-$			
6-OH-BDE47	$C_{12}H_6Br_4O_2$	12.002	500.7	$[Br]^-$	500.7 → 80.8[a]	−100	−8
					500.7 → 78.7 [b]	−100	−30
2′-OH-BDE68	$C_{12}H_6Br_4O_2$	12.838	500.7	$[Br]^-$	500.7 → 80.8[a]	−100	−8
					500.7 → 78.7[b]	−100	−30
6-OH-BDE85	$C_{12}H_5Br_5O_2$	14.158	578.6	$[Br]^-$	578.6 → 80.8[a]	−100	−12
					578.6 → 78.7[b]	−100	−27
5′-OH-BDE99	$C_{12}H_5Br_5O_2$	15.258	578.6	$[C_{12}H_4Br_4O_2]^-$	578.6 → 498.2[a]	−135	−20
				$[Br]^-$	578.6 → 78.7[b]	−135	−42
6′-OH-BDE99	$C_{12}H_5Br_5O_2$	16.166	578.6	$[Br]^-$	578.6 → 80.8[a]	−120	−35
					578.6 → 78.7[b]	−120	−25

[a] MRM transition used for quantification.
[b] MRM transition used for qualitative analysis.

(150 mm × 3 mm, 5 μm) (Agilent), Acclaim 120 C18 column (150 mm × 4.6 mm, 5 μm) (Dionex), Acclaim 120 C18 column (250 mm × 4.6 mm, 5 μm) (Dionex), Acclaim RSLC 120 C18 column (50 mm × 2.1 mm, 2.2 μm) (Dionex) and Acclaim RSLC 120 C18 column (100 mm × 2.1 mm, 2.2 μm) (Dionex). Among the tested columns, the Dionex Acclaim RSLC 120 C18 column (100 mm × 2.1 mm, 2.2 μm) presented the best performance for the separation of 10 OH-PBDEs. Fig. 2 shows the total ion chromatogram (TIC) and the individual MRM chromatograms of ten OH-PBDEs. Different mobile phase, as well as various gradient slopes was also considered. Comparing to the mixture of methanol and water, the mixture of acetonitrile and water are more appropriate to be used as mobile phase to solve the coelution of 6-OH-BDE47 and 2′-OH-BDE68. Since acetonitrile tended to carbonize the corona pin of APCI ion source and reduce the sensitivity consequently, ESI in negative ion mode was selected as the most suitable ionization. The installation of Agilent Jet Stream can help the ionization of analytes by sheath gas and hence increased the sensitivity.

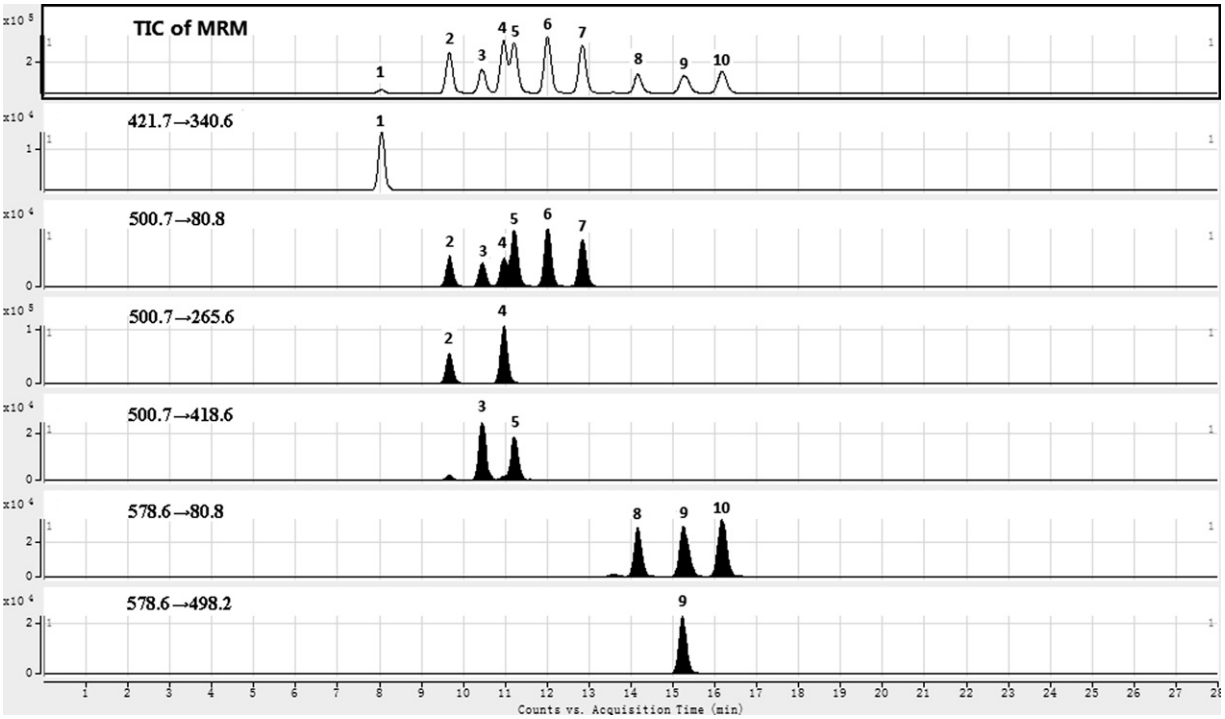

Fig. 2. Total ion chromatogram (TIC) for all the MRM channels and the individual MRM chromatograms of 30 ng/mL OH-PBDEs standard mixture for (1) 3′-OH-BDE-28, (2) 4-OH-BDE-42, (3) 3-OH-BDE-47, (4) 4′-OH-BDE-49, (5) 5-OH-BDE-47, (6) 6-OH-BDE-47, (7) 2′-OH-BDE-68, (8) 6-OH-BDE-85, (9) 5′-OH-BDE-99 and (10) 6′-OH-BDE-99.

Table 3
Method performance: linearity (concentration range from 2 to 200 ng/mL), intra- and inter-assay variation (using blank soil sample spiking with each analyte at 5 ng), EDL[a] and MDL[b] (S/N = 3) of all the target analytes for six environmental matrices.

Compounds	r^2 [c]	Intra-assay variation (%RSD, n = 5)	Inter-assay variation (%RSD, n = 5)	EDLs (ng/mL)	MDLs					
					Water (pg/L)	Soil (pg/g dw)	Sediment (pg/g dw)	Plant (pg/g dw)	Mollusk (pg/g dw)	Fish (pg/g dw)
BDE-28	0.9994	7	9	0.27	53.5	56.8	68.7	55.6	69.3	70.7
BDE-47	0.9995	6	7	0.24	48.8	46.5	57.3	49.5	56.4	59.2
BDE-66	0.9991	6	6	0.25	61.2	55.2	62.4	67.9	69.2	66.5
BDE-68	0.9992	5	6	0.28	80.2	68.4	78.4	54.8	67.9	78.3
BDE-85	0.9984	8	8	0.46	64.5	85.5	80.8	76.4	66	78.4
BDE-99	0.9982	8	9	0.41	70.4	78.8	69.4	68.4	90.5	84.6
BDE-138	0.9965	7	6	0.48	85.5	80.9	93.8	84.5	85.2	99.2
BDE-153	0.9935	6	10	0.56	110.4	90.2	123.5	95.2	104.7	104.8
BDE-154	0.9938	7	8	0.52	124.5	93.7	130.2	113.4	120.6	137.1
BDE-183	0.9923	9	11	0.75	150.3	143.5	170.8	152.7	149.2	163.6
2′-MeO-BDE68	0.9996	5	7	0.32	67.4	63.5	64.9	53.2	78.3	69.5
6-MeO-BDE47	0.9994	5	7	0.28	55.1	50.7	58.7	66.3	70.5	84.5
3-MeO-BDE47	0.9997	7	6	0.35	65.2	67.4	70.7	74.2	66.8	62.6
5-MeO-BDE47	0.9994	6	10	0.39	74.3	70.7	88.2	83.2	67.3	75.5
4′-MeO-BDE49	0.9992	4	6	0.43	75.6	74.8	79.8	84.3	87.4	88.3
4-MeO-BDE42	0.999	7	9	0.35	69.3	65.5	78.3	73.6	59.6	68.5
6′-MeO-BDE99	0.9981	6	8	0.48	90.4	85.4	105.3	96.4	83.2	96.4
5′-MeO-BDE99	0.998	8	12	0.44	87.2	82.4	88.6	99.3	75.7	82.5
6-MeO-BDE85	0.9958	6	9	0.46	83.6	94.3	96.5	89.5	81.3	90.4
3′-OH-BDE28	0.9922	10	12	0.1	11.6	10.4	18.4	11.3	12.7	14.4
4-OH-BDE42	0.9934	7	9	0.04	7.2	6.2	11.7	6.9	7.7	8.3
3-OH-BDE47	0.9931	4	5	0.05	5.5	4.3	12.2	5.5	6.8	7.5
4′-OH-BDE49	0.998	5	8	0.02	4.9	3.7	5.6	7.6	5.8	6.2
5-OH-BDE47	0.9962	5	6	0.01	3.2	2.8	3.8	5.1	4.4	4.9
6-OH-BDE47	0.9964	7	9	0.03	3.8	4.9	6.8	4.2	5.6	5.7
2′-OH-BDE68	0.9926	6	8	0.04	7.8	5.3	7.1	4.6	6.9	9.5
6-OH-BDE85	0.9947	7	6	0.05	8.2	4.5	5.9	9.4	6.7	5.5
5′-OH-BDE99	0.9951	9	9	0.05	4.3	8.5	6.5	7.9	9.3	6.1
6′-OH-BDE99	0.9963	8	10	0.04	6.9	7.7	8.8	6.5	9.7	10.8

[a] EDL, equipment detection limit.
[b] MDL, method detection limit.
[c] r, correlative coefficient.

Table 4
Spike recoveries and standard deviation (%$R \pm$ SD, n = 3) at 0.3, 2 and 5 ng for each target analyte using three different environmental matrices (water, soil, sediment).

Compounds	Water %$R \pm$ SD			Soil %$R \pm$ SD			Sediment %$R \pm$ SD		
	3 ng/mL	20 ng/mL	50 ng/mL	3 ng/mL	20 ng/mL	50 ng/mL	3 ng/mL	20 ng/mL	50 ng/mL
BDE-28	81 ± 7	85 ± 5	89 ± 2	83 ± 10	92 ± 12	90 ± 8	76 ± 8	80 ± 9	84 ± 7
BDE-47	79 ± 8	84 ± 7	93 ± 5	78 ± 8	91 ± 6	95 ± 6	81 ± 13	91 ± 6	91 ± 2
BDE-66	83 ± 5	92 ± 2	94 ± 2	85 ± 6	89 ± 7	89 ± 5	83 ± 6	88 ± 8	86 ± 4
BDE-68	78 ± 5	104 ± 7	94 ± 6	76 ± 5	90 ± 8	92 ± 5	74 ± 9	109 ± 7	87 ± 4
BDE-85	84 ± 8	89 ± 6	93 ± 8	81 ± 8	103 ± 7	97 ± 5	73 ± 10	83 ± 5	86 ± 5
BDE-99	80 ± 7	95 ± 8	92 ± 4	83 ± 7	85 ± 9	89 ± 5	80 ± 8	90 ± 8	94 ± 5
BDE-138	85 ± 9	91 ± 5	95 ± 5	91 ± 14	96 ± 4	109 ± 6	82 ± 12	92 ± 8	95 ± 7
BDE-153	82 ± 8	93 ± 4	88 ± 5	90 ± 5	94 ± 8	91 ± 8	113 ± 9	87 ± 5	84 ± 6
BDE-154	111 ± 6	87 ± 5	85 ± 7	81 ± 9	90 ± 9	95 ± 3	82 ± 7	83 ± 7	87 ± 3
BDE-183	80 ± 5	84 ± 7	85 ± 6	80 ± 9	86 ± 8	89 ± 6	83 ± 14	87 ± 6	93 ± 5
2′-MeO-BDE68	77 ± 6	93 ± 4	90 ± 4	90 ± 6	94 ± 5	93 ± 8	74 ± 8	88 ± 8	87 ± 2
6-MeO-BDE47	90 ± 8	91 ± 6	96 ± 5	90 ± 6	92 ± 7	95 ± 8	76 ± 11	94 ± 12	92 ± 7
3-MeO-BDE47	84 ± 12	93 ± 7	91 ± 8	73 ± 13	95 ± 7	91 ± 7	83 ± 5	91 ± 5	94 ± 3
5-MeO-BDE47	85 ± 9	88 ± 6	94 ± 4	108 ± 6	92 ± 8	97 ± 6	84 ± 9	90 ± 7	93 ± 7
4′-MeO-BDE49	86 ± 4	89 ± 11	96 ± 3	79 ± 15	91 ± 7	96 ± 2	80 ± 9	86 ± 9	108 ± 7
4-MeO-BDE42	81 ± 12	84 ± 6	92 ± 6	85 ± 9	90 ± 6	93 ± 3	85 ± 13	87 ± 8	89 ± 6
6′-MeO-BDE99	85 ± 6	90 ± 6	93 ± 4	76 ± 12	88 ± 8	95 ± 8	91 ± 9	91 ± 7	96 ± 3
5′-MeO-BDE99	77 ± 8	93 ± 9	95 ± 6	83 ± 7	87 ± 4	97 ± 3	77 ± 10	90 ± 16	94 ± 6
6-MeO-BDE85	84 ± 9	91 ± 13	97 ± 6	87 ± 9	85 ± 7	91 ± 7	82 ± 9	87 ± 9	86 ± 12
3′-OH-BDE28	74 ± 6	84 ± 7	77 ± 8	84 ± 8	86 ± 8	89 ± 12	71 ± 7	74 ± 12	78 ± 10
4-OH-BDE42	85 ± 13	89 ± 8	87 ± 8	87 ± 5	87 ± 14	85 ± 8	76 ± 6	79 ± 3	77 ± 9
3-OH-BDE47	86 ± 7	92 ± 7	104 ± 4	77 ± 8	97 ± 8	93 ± 10	86 ± 13	90 ± 10	91 ± 5
4′-OH-BDE49	86 ± 6	86 ± 9	92 ± 5	85 ± 8	82 ± 5	88 ± 5	76 ± 8	83 ± 7	86 ± 4
5-OH-BDE47	79 ± 15	91 ± 7	95 ± 7	77 ± 8	83 ± 7	93 ± 4	83 ± 8	86 ± 8	82 ± 5
6-OH-BDE47	90 ± 5	92 ± 5	94 ± 7	79 ± 11	87 ± 5	84 ± 5	83 ± 11	93 ± 6	90 ± 8
2′-OH-BDE68	81 ± 12	87 ± 11	94 ± 6	84 ± 8	86 ± 8	94 ± 5	73 ± 8	90 ± 7	93 ± 9
6-OH-BDE85	83 ± 7	89 ± 10	88 ± 6	80 ± 13	82 ± 9	85 ± 8	82 ± 9	85 ± 10	88 ± 12
5′-OH-BDE99	77 ± 9	84 ± 5	94 ± 4	82 ± 14	85 ± 8	92 ± 8	82 ± 7	87 ± 13	89 ± 9
6′-OH-BDE99	85 ± 13	87 ± 8	95 ± 3	80 ± 9	86 ± 7	94 ± 6	74 ± 11	82 ± 6	86 ± 6

Table 5
Spike recoveries and standard deviation (%$R \pm$ SD, $n = 3$) at 0.3, 2 and 5 ng for each target analyte using three different environmental matrices (plant, mollusk, fish).

Compounds	Plant %$R \pm$ SD			Mollusk %$R \pm$ SD			Fish %$R \pm$ SD		
	3 ng/mL	20 ng/mL	50 ng/mL	3 ng/mL	20 ng/mL	50 ng/mL	3 ng/mL	20 ng/mL	50 ng/mL
BDE-28	84 ± 7	84 ± 6	89 ± 9	85 ± 8	86 ± 7	93 ± 2	83 ± 7	84 ± 7	86 ± 8
BDE-47	83 ± 10	91 ± 5	93 ± 5	74 ± 8	86 ± 8	89 ± 6	77 ± 6	91 ± 8	93 ± 3
BDE-66	79 ± 8	87 ± 8	91 ± 5	87 ± 7	96 ± 5	92 ± 4	85 ± 9	88 ± 5	94 ± 4
BDE-68	75 ± 10	96 ± 7	92 ± 6	75 ± 5	109 ± 7	95 ± 6	90 ± 8	91 ± 8	96 ± 7
BDE-85	83 ± 6	104 ± 6	85 ± 7	83 ± 6	85 ± 9	92 ± 7	81 ± 5	86 ± 6	89 ± 6
BDE-99	82 ± 5	94 ± 5	93 ± 9	77 ± 6	90 ± 9	92 ± 11	73 ± 9	88 ± 7	84 ± 4
BDE-138	79 ± 9	91 ± 7	94 ± 4	84 ± 7	88 ± 5	93 ± 5	83 ± 6	90 ± 8	92 ± 6
BDE-153	84 ± 8	84 ± 6	92 ± 5	86 ± 9	88 ± 8	85 ± 6	110 ± 8	86 ± 8	89 ± 13
BDE-154	92 ± 6	85 ± 5	91 ± 7	83 ± 6	92 ± 6	93 ± 8	78 ± 9	92 ± 6	95 ± 6
BDE-183	82 ± 6	92 ± 8	95 ± 2	84 ± 12	89 ± 9	87 ± 6	82 ± 10	88 ± 9	85 ± 7
2′-MeO-BDE68	76 ± 9	90 ± 6	94 ± 8	76 ± 6	91 ± 8	95 ± 9	73 ± 7	84 ± 5	91 ± 5
6-MeO-BDE47	87 ± 8	86 ± 7	93 ± 7	83 ± 10	95 ± 11	92 ± 6	91 ± 8	96 ± 8	94 ± 4
3-MeO-BDE47	79 ± 8	88 ± 8	98 ± 5	103 ± 5	92 ± 5	95 ± 4	86 ± 6	90 ± 7	93 ± 5
5-MeO-BDE47	74 ± 7	104 ± 6	94 ± 3	84 ± 8	85 ± 7	89 ± 2	83 ± 7	85 ± 6	89 ± 4
4′-MeO-BDE49	87 ± 7	83 ± 4	93 ± 6	76 ± 7	91 ± 6	95 ± 5	78 ± 9	91 ± 15	95 ± 3
4-MeO-BDE42	90 ± 6	91 ± 5	94 ± 5	81 ± 7	88 ± 5	85 ± 4	82 ± 8	90 ± 8	92 ± 12
6′-MeO-BDE99	73 ± 12	90 ± 10	95 ± 2	84 ± 6	91 ± 6	94 ± 6	84 ± 12	92 ± 7	96 ± 6
5′-MeO-BDE99	81 ± 7	90 ± 8	90 ± 8	83 ± 9	93 ± 8	95 ± 7	76 ± 9	107 ± 6	96 ± 5
6-MeO-BDE85	90 ± 5	96 ± 5	93 ± 7	85 ± 6	92 ± 6	94 ± 3	74 ± 5	94 ± 9	91 ± 8
3′-OH-BDE28	78 ± 4	87 ± 8	83 ± 4	82 ± 8	86 ± 8	83 ± 12	83 ± 14	87 ± 7	87 ± 7
4-OH-BDE42	89 ± 8	86 ± 8	86 ± 11	77 ± 8	87 ± 14	79 ± 5	76 ± 5	89 ± 4	76 ± 6
3-OH-BDE47	90 ± 9	88 ± 13	85 ± 6	79 ± 5	88 ± 8	95 ± 11	79 ± 8	91 ± 6	97 ± 4
4′-OH-BDE49	83 ± 6	93 ± 8	94 ± 4	84 ± 7	91 ± 6	94 ± 2	78 ± 7	87 ± 7	81 ± 4
5-OH-BDE47	82 ± 6	85 ± 11	87 ± 13	83 ± 13	86 ± 7	83 ± 6	76 ± 11	88 ± 9	85 ± 6
6-OH-BDE47	75 ± 7	92 ± 5	96 ± 5	81 ± 8	87 ± 11	82 ± 7	85 ± 7	96 ± 8	92 ± 5
2′-OH-BDE68	81 ± 5	91 ± 14	93 ± 4	77 ± 7	85 ± 4	83 ± 14	77 ± 6	91 ± 6	95 ± 7
6-OH-BDE85	79 ± 14	89 ± 6	81 ± 5	83 ± 8	87 ± 12	85 ± 10	82 ± 9	85 ± 8	88 ± 7
5′-OH-BDE99	85 ± 6	87 ± 7	88 ± 14	75 ± 7	87 ± 5	83 ± 8	79 ± 7	86 ± 11	81 ± 8
6′-OH-BDE99	81 ± 8	86 ± 10	95 ± 6	81 ± 6	85 ± 7	89 ± 8	84 ± 13	89 ± 7	86 ± 6

Lupton et al. [33] proposed an LC–MS/MS method and the separation of nine OH-PBDEs achieved in 35 min. In another paper [34], eight OH-PBDEs can be separated by using a ternary mixture of 5 mM ammonium acetate, acetonitrile and methanol as the mobile phases. Chang et al. [35] used acetonitrile and 0.1% formic acid in water as the mobile phases for the separation of ten OH-PBDEs after derivatization with dansyl chloride. In our developed method, ten OH-PBDEs were separated completely in only 20 min without derivatization and no buffer was used in the mobile phase.

The separation of isomer on LC chromatographic column was known to be difficult. In the published work, only 2–4 OH-tetraBDE isomer were analyzed and the separating degree was not satisfied [29,33,34]. While using the improved methods in this work, six most frequently detected OH-tetraBDE isomer, including two *ortho*-hydroxylated PBDEs (6-OH-BDE47 and 2′-OH-BDE68), two *para*-hydroxylated PBDEs (4′-OH-BDE49 and 4-OH-BDE42), two *meta*-hydroxylated PBDEs (3-OH-BDE47 and 5-OH-BDE47) [36], can be simultaneously identified, which were shown in Fig. 2.

MS/MS parameters were optimized by automatic procedures. In MS2 scan mode, the $[M-H]^-$ base peak formed at m/z 578.6 for OH-pentaBDE, m/z 500.7 for OH-tetraBDE and m/z 420.7 for OH-triBDE. Following the successful detection of the $[M-H]^-$ ion, fragmentor voltage was optimized to obtain the most intense signal. Subsequently, product ion scan mode was used to choose product ions and optimize collision energy. The typical fragment ions of analytes were consistent with the results in other papers [29,33,34]. The formation of specific fragment ions allowed the efficient analytical method based on the MRM mode. Two specific MRM transitions were used for each compound, one for qualitative analysis and another for quantification. The $[M-H]^-$ precursor ion, the product ions, the chosen MRM transitions with optimized fragmentor (frag) and collision energy (CE) for each OH-PBDEs are summarized in Table 2.

3.3. Performance of the method

To evaluate the performance of the developed method, linearity, recovery, precision, repeatability and sensitivity were examined (Tables 3–5). Good linearity was obtained with r^2 over 0.992 for PBDEs, 0.995 for MeO-PBDEs and 0.992 for OH-PBDEs under a concentration range from 2 to 200 ng/mL. The equipment detection limits (EDLs) ranged from 0.24 to 0.75 ng/mL for PBDEs and MeO-analytes and from 0.01 to 0.10 ng/mL for OH-PBDEs. According to the work of Lacorte et al. [37], blank samples were used to evaluate the performance of the optimized approach because there is no reference material available presently for MeO- and OH-PBDEs. For OH-PBDEs, MDLs were 3.2–11.6 pg/L in water sample and 2.8–18.4 pg/g dry weight (dw) in solid samples. For PBDEs and MeO-PBDEs, MDLs were 48.8–150.3 pg/L in water sample and 46.5–170.8 pg/g dry weight in solid samples. The MDLs of hydroxylated analytes determined by LC–MS/MS were over one order of magnitude lower than other reported non-derivatization LC–MS/MS methods [29,34], and similar to the results for OH-PBDEs after derivatization with dansyl chloride by LC–MS/MS method [35]. This high sensitivity could be very helpful in detecting trace OH-PBDEs in aquatic system and for studying the metabolic mechanism in organisms [36]. Recoveries for all the analytes in six different environmental matrices were assessed by spiking with each compound. The mean ($n = 3$) recoveries for 29 target analytes in all the matrices ranged from 71% to 113%. Repeatability was evaluated by intra- and inter-assay variation. At the spiking level of 5 ng, repeatability, as measured by relative standard deviation (%RSD), was between 4–12% for all analytes. The precision of the method, obtained as the relative standard deviations (RSDs) of analyte recoveries, were 2–16% in all the spiking experiments. Because of absence of certified reference materials (CRMs), reported method was used [37] for the accuracy assessment. The results of *Pseudosciaena polyactis* sample showed that the detected concentrations by our method (1.5 ng/g dw for 6-MeO-BDE-47) close to that by

Table 6
Concentrations (ng/g dry weight) of MeO-PBDEs detected in marine animals obtained from the Bohai Sea and the Donghai Sea, China.

Sampling site Sample name	Tianjin *Mya arenaria*	Tianjin *Mytilus edulis*	Weihai *Mytilus edulis*	Taizhou *Pseudosciaena polyactis*
2′-MeO-BDE-68	n.d.[a]	2.1	1.0	n.d.
6-MeO-BDE-47	n.d.	1.4	1.3	1.5
3-MeO-BDE-47	n.d.	n.d.	n.d.	n.d.
5-MeO-BDE-47	2.0	3.2	n.d.	n.d.
4′-MeO-BDE-49	n.d.	n.d.	n.d.	n.d.
4-MeO-BDE-42	n.d.	n.d.	n.d.	n.d.
6′-MeO-BDE-99	n.d.	n.d.	n.d.	n.d.
5′-MeO-BDE-99	n.d.	n.d.	n.d.	n.d.
6-MeO-BDE-85	n.d.	n.d.	n.d.	n.d.

[a] None-detectable.

reported method (1.7 ng/g dw for 6-MeO-BDE-47). Therefore, the method is reliable and the linearity, recovery, repeatability, and sensitivity of the method were demonstrated to be suitable for analyzing PBDEs, MeO-PBDEs, and OH-PBDEs.

3.4. Application of the method to environmental samples

Using the developed method, OH-PBDEs and MeO-PBDEs in mollusk and fish samples were studied. Three MeO-PBDEs: 5-MeO-BDE-47, 6-MeO-BDE-47 and 2′-MeO-BDE-68 were detected in *Mytilus edulis*, *Mya arenaria* and *Pseudosciaena polyactis* samples (Table 6), in which 6-MeO-BDE-47 and 2′-MeO-BDE-68 were frequently determined in wildlife [2,5,10,38]. The concentration of MeO-PBDEs in *Mytilus edulis* collected from Tianjin ranged from 1.4 to 3.2 ng/g dry weight. *Mytilus edulis* collected from Weihai presented a lower concentration than that from Tianjin, with 1.0 ng/g dry weight for 2′-MeO-BDE-68, 1.3 ng/g dry weight for 6-MeO-BDE-47. 5-MeO-BDE-47 was only detected in *Mya arenaria* collected from Tianjin with a concentration of 2.0 ng/g dry weight and in *Pseudosciaena polyactis* samples collected from Taizhou, 6-MeO-BDE-47 (1.5 ng/g dry weight) was found. The different distributions of MeO-PBDE congeners in different species might be related to the different sampling location and metabolism [35]. The concentrations were comparable to the results of *Mytilus edulis* (8.8 ng/g lipid weight for 6-MeO-BDE-47 and 2.8 ng/g lipid weight for 2′-MeO-BDE-68) collected from the Hudson Bay region of northeastern Canada [10]. All the analyzed OH-PBDEs were below the detection limits, which were also reported to be much lower than that of MeO-PBDEs in the marine food web [39].

4. Conclusions

In the present study, a reliable, robust, efficient, and sensitive analytical method was developed for the identification and quantification of 10 PBDEs, 19 potential metabolized MeO-PBDEs and OH-PBDEs in six environmental matrices including abiotic and biotic samples. A water-impregnated silica separating column was found to be most suitable to be used for the simultaneous separation of PBDEs, MeO-PBDEs and OH-PBDEs. The linearity, recovery and repeatability of the method were demonstrated in acceptable ranges. The selectivity and sensitivity of LC–MS/MS methods were further improved comparing with reported methods and made it more efficient in OH-PBDEs analysis.

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