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Short communication

Picogram per liter level determination of hydroxylated polybrominated diphenyl ethers in water by liquid chromatography–electrospray tandem mass spectrometry

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ABSTRACT

Hydroxylated polybrominated diphenyl ethers (OH-PBDEs) have obtained increasing attention; however, few analytical methods are available for sensitive identification of these compounds in water. In this paper, we developed a highly sensitive method for simultaneous determination of nine OH-PBDEs in water by using hydrophilic–lipophilic balanced cartridge extraction, silica cartridge purification, and the combination of derivatization with liquid chromatography–electrospray tandem mass spectrometry. The effective sample pretreatment process and greatly increased instrumental sensitivity by derivatization allow for the quantification of nine OH-PBDEs at a method detection limit of 0.04–3.5 pg/L with 1-L wastewater treatment plant effluent or river water. This method was applied to wastewater effluent and river water samples collected in Beijing, China, where two OH-tetraPBDEs were detected at concentrations ranging from 0.63 to 1.0 pg/L (5-OH-BDE-47) and from 0.92 to 1.3 pg/L (6-OH-BDE-47).

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are widely used as flame retardants, and have attracted attention because of their ubiquitous distribution in the environment and potential for bioaccumulation [1]. The hydroxylated (OH-) PBDEs are structural analogues of the PBDEs, and have recently obtained special attention because they are more potent than PBDEs or methoxylated (MeO-) PBDEs for some endpoints [2,3]. OH-PBDEs can disrupt thyroid hormone homeostasis, oxidative phosphorylation, and estradiol synthesis, and are neurotoxic [2,7–10]. To date, there have been many studies on OH-PBDEs in biological samples such as marine algae, mussel, fish [4,5] and human blood [6]. However, few studies have investigated their occurrence, fate and transport in abiotic environmental samples, especially in water matrices. One of the main reasons for this is the lack of an effective analytical method for these types of samples.

A number of analytical methods have been reported for analysis of OH-PBDEs in biological samples. The most common method is measurement of their MeO-derivatives by gas chromatography–mass spectrometry (GC–MS) with electron-capture-negative ionization (ECNI) or high resolution mass spectrometry (HRMS) after diazomethane derivatization [4,5,11–13]. Using this method, Uneo et al. [14] detected 18 OH-PBDEs in surface water and precipitation from Ontario, Canada, after enrichment and extraction of the OH-PBDEs by XAD-2 resin. This study reported very low detection limits (0.01–0.8 pg/L) for the target OH-PBDEs in water, but a large sample volume (50–100 L) was required. The reaction efficiency for diazomethane derivatization is usually poor, and the analysis can be complicated by the presence of interfering MeO-analogues. Alternative methods based on LC–MS/MS have been developed for OH-PBDEs [15–19]. Hua et al. [15] proposed an electrospray (ESI)-LC–MS/MS method for 2′-OH-BDE-28 in wastewater effluent and surface water after extraction of 500 mL of water with a hydrophilic–lipophilic balanced (HLB) cartridge. The method detection limits (MDLs) for direct measurement of 2′-OH-BDE-28 in the sample extract were 6 ng/L and 3 ng/L for wastewater effluent and surface water, respectively. Comparison to the reported total concentrations of OH-PBDEs in surface water and precipitation from Ontario, Canada (1.8–25 pg/L) [14], indicates that these MDLs are probably much higher than the OH-PBDE levels in the environment. The high instrumental detection limit and/or

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matrix effects of LC–MS/MS would contribute to the high MDLs. Consequently, a method with lower MDLs is required for detection of OH-PBDEs in environmental samples.

Recently, derivatization with dansyl chloride has been utilized to significantly enhance the ionization efficiencies of phenolic chemicals in LC–ESI-MS (/MS) analysis. The introduction of a dansyl moiety into phenolic group lowers the pK_a of the molecule and thereby enhances ionization under acidic conditions [20]. In our previous study, we established a LC–ESI-MS/MS method for detection of OH-PBDEs and other related phenolic compounds in blood plasma after derivatization with dansyl chloride [21]. This method has high sensitivity compared to the method without derivatization, which allows for determination of OH-PBDEs in 0.3–1 mL of blood samples [16]. However, this method cannot be suitable for water matrices with pg/L levels of OH-PBDEs. For water samples, 0.1–2 L of sample volume is generally used to obtain low MDLs, but effective sample extraction and purification process is required to remove or decrease the highly concentrated matrix components. To our knowledge, no attempt has been made for analyzing pg/L levels of OH-PBDEs in water based on LC–MS/MS method.

The aim of the present study was to develop a sensitive and specific LC–ESI-MS/MS based method for detection of low pg/L concentrations of OH-PBDEs in water. Water samples (1 L) were spiked with nine OH-PBDEs, which were then extracted using HLB cartridges. After dansyl chloride derivatization, the sample extracts were purified through silica cartridges before analysis. The developed method was applied to detection of OH-PBDEs in wastewater effluent and surface water samples.

2. Experimental

2.1. Sample collection

Water samples from the Qing River, which receives water from a Qinghe wastewater treatment plant (WWTP) in Beijing, China, were collected in August 2011. The sampling sites were situated 0.5, 4, and 8 km downstream of the Qinghe WWTP. An effluent sample was also collected. All samples were treated within 4 h of collection.

2.2. Sample preparation

To avoid blocking the solid-phase extraction cartridges, suspended materials were removed from the samples by filtration through a glass fiber pad (GF/F 0.7 μm, Whatman, Maidstone, UK) before cartridge extraction. After filtration, 1 L samples of effluent and river water spiked with 5 μg/L of 2′-OH-6′-Cl-BDE-68 (100 μL) and adjusted to pH 3 with HCl were extracted with Oasis HLB cartridges (6 mL, 500 mg, Waters, Milford, MA). The cartridges were pre-conditioned with 6 mL of EtOAc, 6 mL of acetonitrile, and 12 mL of acidified water (pH 3). After passing the water samples through the cartridges, they were washed with 10 mL of distilled water and then dried under a flow of nitrogen. The analytes were eluted from the cartridges with 15 mL of EtOAc. The eluates were evaporated to dryness, and the residues were redissolved in 100 μL of aqueous sodium bicarbonate and 100 μL of dansyl chloride. Each sample was vortex-mixed for 1 min and incubated at 60 °C for 5 min. Next, 1 mL of HPLC grade water and 2× 3 mL of hexane were added. The organic layer was removed and passed through a silica cartridge (6 mL, 500 mg, Waters), which was pre-conditioned with 6 mL of n-hexane. The cartridge was eluted with 6 mL of n-hexane/DCM (1:1, v/v), and the eluate was evaporated to dryness and reconstituted with 100 μL of acetonitrile for LC–MS/MS analysis.

2.3. LC–MS/MS analysis

Analyses were conducted using an Agilent 1200 series LC system (Santa Clara, CA) connected to an API 5000 triple-quadrupole MS/MS system (Applied Bioscience, Foster City, CA). Both the LC and mass spectrometer were controlled by AB Sciex Analyst 1.4.2 software (Applied Bioscience). All analytes were separated using an XBrige C18 column (150 mm × 2.1 mm I.D., 3.5 μm particle size) from Waters. Separations were conducted at room temperature with 10 μL of sample. The mobile phase consisted of acetonitrile (Solvent A) and 0.1% formic acid in water (Solvent B) with a flow rate of 0.25 mL/min. The mobile phase gradient of A:B was 60:40 (0–1 min) to 95:5 (1–15 min) and to 95:5 (15–20 min).

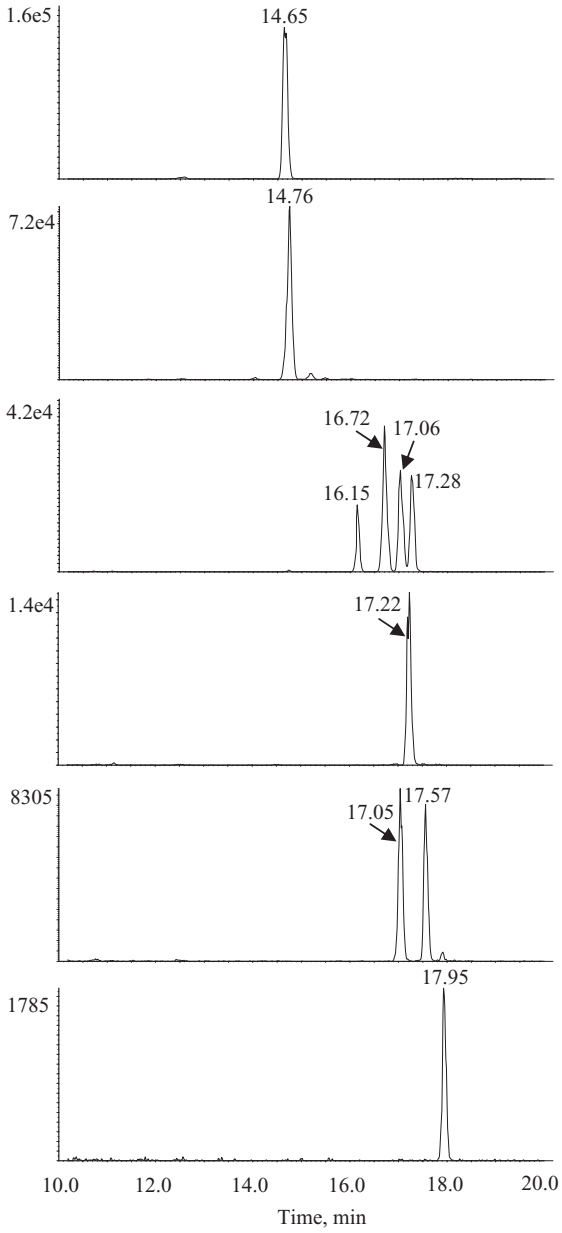

Fig. 1. LC–MS/MS MRM chromatograms of target OH-PBDEs and internal standard (2′-OH-6′-Cl-BDE-68) spiked in a river sample. 2′-OH-6′-Cl-BDE-7 (14.65 min), 6′-OH-BDE-17 (14.76 min), 3-OH-BDE-47 (16.15 min), 5-OH-BDE-47 (16.72 min), 6-OH-BDE-47 (17.06 min), 4′-OH-BDE-49 (17.28 min), 2′-OH-6′-Cl-BDE-68 (17.22 min), 6-OH-BDE-90 (17.05 min), 2-OH-BDE-123 (17.57 min), and 6-OH-BDE-137 (17.97 min).

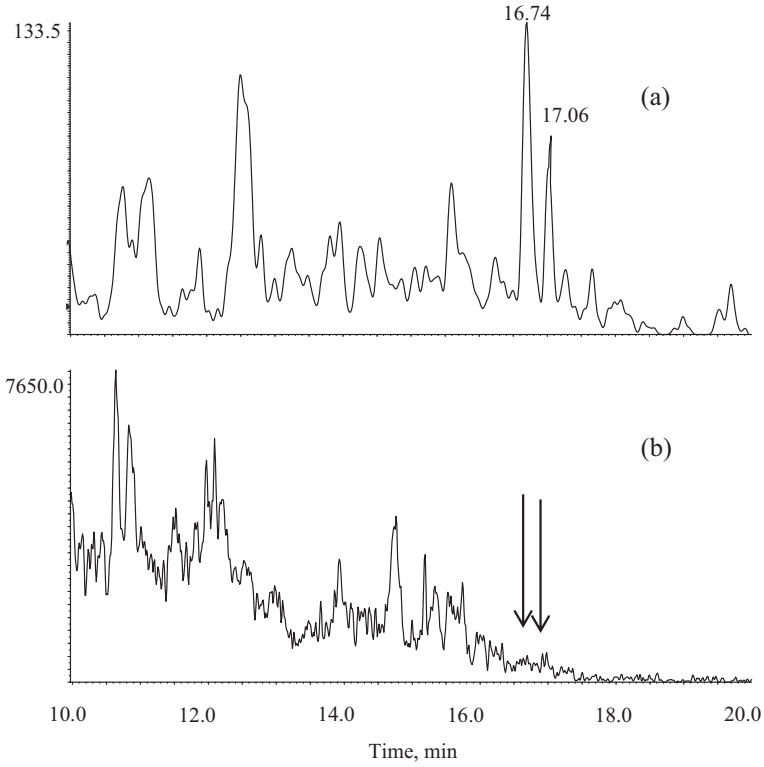

Fig. 2. LC–MS/MS MRM chromatograms of OH-PBDEs detected in a river sample of WWTP downstream 4 km: (a) with purification and (b) without purification.

The analytes were detected using a turbo ion spray ion source operated in positive ion mode with multiple reaction monitoring (MRM). The most intense MRM transitions were $[MH]^+ \rightarrow 171$ and $[MH]^+\text{-}2 \rightarrow 171$ because of the presence of bromine isotopes in these compounds. The ion at m/z 171 originates from cleavage of a C—S bond in the dansyl portion of the molecule. All of the source and instrument parameters were optimized by infusing purified dansyl derivatives of the analytes into the mass spectrometer. Nitrogen was used as the collision (setting 10) and curtain gas (setting 15). The ion spray voltage was set at 5000 V, and the turbo ion spray interface was maintained at 525 °C. A dwell time of 50 ms was used for each of the MRM transitions. The declustering potential, entrance potential, collision energy, and collision cell exit potential were optimized at 65 V, 7 V, 48 V, and 17 V, respectively, for the selected MRM transition of the analytes and isotope-labeled standards.

3. Results and discussion

3.1. Method development and validation

Although OH-PBDEs in the aquatic environment have attracted increasing attention, few studies have reported effective analytical methods for sensitive determination of the OH-PBDEs in water [14,15]. In the current study, an HLB cartridge was used to enrich and extract the target OH-PBDEs from water. Several solvents (DCM, EtOAc, acetone, methyl *tert*-butyl ether, and methanol) were tested for elution of the target compounds from the HLB cartridge. EtOAc-acetone (50:50, v/v) has been used to recover 2′-OH-BDE-28 from HLB cartridges [15], and the sample recovery was 91–100% for WWTP effluent and surface water samples. However, in our experiments, the recoveries of the tetra- to hexa-OH-PBDEs eluted with EtOAc-acetone (50:50, v/v) were only 20–30%, which suggests this solvent is not suitable for many OH-PBDEs. Among all the solvents tested, EtOAc gave the best recoveries (85–98%) for all the target compounds (Table S1 in Supporting Information).

In our previous study, LC–MS/MS of dansyl derivatives of OH-PBDEs was more sensitive than direct LC–MS/MS analysis of OH-PBDEs [21]. Derivatization will decrease the matrix effects caused by co-elution of matrix components because it will change the retention behavior of the analytes on the separation column and increase the selectivity of the analytes with mass spectrometry. However, for complicated matrices such as wastewater, further purification of the derivatives was needed to reduce the matrix effects and lower the MDLs. In this study, a silica cartridge purification process was developed for the removing the pre-concentrated target OH-PBDE dansyl derivatives from the matrix components of the wastewater and surface water. Fig. 1 shows the MRM LC–MS/MS chromatograms of the target OH-PBDEs and internal standard spiked in WWTP effluent. The internal standard-corrected mean recoveries of the target OH-PBDEs from WWTP effluent and river water ranged from 78% to 95% with an RSD less than 16% (Table 1), showing good accuracy of this method. The between-day RSD (<14%) was also assessed by replicate analyzing the spiked effluent and river sample in a 10-day period.

Throughout the whole procedure, no contamination of the blank samples was detected. The linear ranges of the internal standard calibration curves were 10–10,000 ng/L for di- to tetra-OH-PBDEs and 100–100,000 ng/L for penta- to hexa-OH-PBDEs. The coefficients of determination were typically greater than 0.99, which indicates the linearity is acceptable for all the target compounds over the environmentally relevant concentration range. Ten replicate determinations of 100 ng/L of standard solution were carried out on the same day under the optimum conditions to determine the run-to run precision of LC–ESI-MS/MS analysis. The RSD was typically less than 10%. The MDLs, defined as the amount of analyte that produced a signal-to-noise ratio of three (peak to peak), were 0.04–3.5 pg/L in WWTP effluent and surface water. The MDLs were

Table 1
Recoveries, instrumental detection limits (IDLs) and method detection limits (MDLs) in the WWTP effluent and river water.

Compounds	IDL (ng/L)	Effluent				River water			
		Recovery[a] (%)	Within-day RSD (%)	Between-day RSD (%)[b]	MDL (pg/L)	Recovery[a] (%)	Within-day RSD (%)	Between-day RSD (%)[b]	MDL (pg/L)
2′-OH-6′-Cl-BDE-7	0.20	96	8.6	8.0	0.04	95	7.6	9.1	0.05
6′-OH-BDE-17	0.30	90	9.2	7.8	0.12	88	9.4	10	0.12
3-OH-BDE-47	1.0	78	10	9.7	0.41	80	8.5	5.9	0.42
5-OH-BDE-47	0.35	80	7.5	5.8	0.13	84	11	8.5	0.21
6-OH-BDE-47	0.87	90	9.8	12	0.35	87	13	11	0.40
4′-OH-BDE-49	0.85	81	8.9	6.4	0.35	79	10	14	0.41
6-OH-BDE-90	3.5	90	14	10	1.3	91	16	7.8	1.2
2-OH-BDE-123	3.0	78	6.9	7.8	1.2	78	7.9	6.2	1.4
6-OH-BDE-137	8.2	85	9.1	11	3.4	82	8.6	9.6	3.5

[a] Spiked at 20 pg/L level of each target OH-PBDE in WWTP effluent and river water ($n = 3$). Based on the within-day mean of three samples analyzed.
[b] Based on three samples analyzed in a 10-day period.

much lower than those reported by Hua et al. [15] (3–10 ng/L for 2′-OH-BDE-28 in 500 mL of water) using LC–MS/MS, and similar to those reported by Ueno et al. [14] (0.01–0.8 pg/L in 50–100 L water) using GC–HRMS.

In LC–ESI-MS (/MS) analysis, matrix effects such as signal suppression and isobaric interference occur because of co-eluting interferences, and can considerably reduce the detection sensitivity and reliability. Hua et al. [15] reported a large degree of signal suppression for 2′-OH-BDE-28 in extracts of 500 mL of WWTP effluent (approximately 80%) and surface water (approximately 50%), when analyzing 2′-OH-BDE-28 directly by ESI(-)-MS/MS. In this study, we investigated the matrix effects on the signal intensity of OH-PBDE dansyl derivatives by adding 0.1 ng of target OH-PBDE to the concentrated extract of 1 L of WWTP effluent. The response varied by 40–55% of that of standard solution.

3.2. Environmental samples

The developed method was applied to environmental water samples collected in Beijing, China in August 2011. Fig. 2 shows the typical MRM LC–MS/MS chromatograms obtained from a river water sample. Although peaks for 5-OH-BDE47 and 6-OH-BDE47 were detected in the purified extract, no peaks were detected in the chromatogram obtained without purification. This indicates that the purification procedure largely decreased the background noise.

OH-tetraBDEs (5-OH-BDE-47 and 6-OH-BDE-47) were the major compounds detected in the wastewater effluent sample and three river water samples. 5-OH-BDE-47 was present at 0.63, 1.0, 0.62, and 0.95 pg/L in the WWTP effluent and 0.5, 4, and 8 km downstream river water samples, respectively. The 6-OH-BDE-47 concentrations were 0.92, 1.2, 0.94, and 1.3 pg/L for the WWTP effluent and 0.5, 4, and 8 km downstream river water samples, respectively. These concentrations are similar to those found for OH-tetraBDEs in water samples collected from Southern Ontario, Canada (<0.01–8.2 pg/L) [14]. For OH-triBDEs, Hua et al. [15] estimated OH-triBDEs to be at μg/L or high ng/L levels based on the response parameters of 2′-OH-BDE-28. In this study, 6′-OH-BDE-17, which is an OH-triBDE, was under the MDL (<0.1 pg/L), and this result is similar to that in the Southern Ontario, Canada study [14].

5-OH-BDE-47 and 6-OH-BDE-47 were the two main metabolites in plasma and feces of rodents exposed to PBDEs [22,23], and the dominant OH-tetraBDEs found near WWTPs. Therefore, these two compounds are thought to be human and animal metabolites and oxidation products of PBDEs in WWTPs [14]. Recently, demethylation of 6-MeO-BDE-47 was reported to be the primary transformation pathway leading to formation of 6-OH-BDE-47 [24,25]. Consequently, the 6-OH-BDE-47 detected in the current study may have been formed by biotransformation of MeO-PBDEs (e.g. 6-MeO-BDE-47) in humans and animals. The sources and fate of OH-PBDEs in the urban environment needs to be investigated further.

4. Conclusions

An LC–ESI-MS/MS method was developed for simultaneous detection of nine OH-PBDEs in water. The key advantage of the method is that the effective sample pretreatment process and greatly increased instrumental sensitivity by derivatization significantly decreased the MDLs, allowing for the determination of pg/L level OH-PBDEs in environmental waters. The recovery and repeatability of this method were validated within acceptable ranges. The method was successfully applied to one wastewater effluent and three river water samples collected from Beijing, China, and provided an effective tool to obtain data of OH-PBDEs in environmental waters.

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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.2011.12.075.

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