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Simultaneous determination of seven bisphenols in environmental water and solid samples by liquid chromatography–electrospray tandem mass spectrometry

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ABSTRACT

This article presents a simple and universal analytical method for the simultaneous analysis of bisphenol S (BPS), bisphenol F (BPF), bisphenol A (BPA), bisphenol B (BPB), bisphenol AF (BPAF), tetrachlorobisphenol A (TCBPA), and tetrabromobisphenol A (TBBPA) in environmental water (river water, sewage) and solid samples (sediment, sludge) based on liquid chromatography–electrospray tandem mass spectrometry (LC–MS/MS). Analytes were extracted from water samples using hydrophilic lipophilic balanced (HLB) solid-phase extraction (SPE) cartridges, and the extracts were further purified using MAX SPE cartridges. For the solid samples, a combination of ultrasonic extraction with the same SPE clean-up procedures used for the water samples was employed. The absolute recoveries for all analytes in the water and solid samples ranged from 57.1 to 114.3%. Good method reproducibility was achieved in terms of intra- and inter-day precision, yielding relative standard deviations (RSDs) less than 16.9 and 18.1%, respectively. The method limits of quantitation (MLOQ) for the seven compounds in environmental water and solid samples ranged from 0.05 to 4.35 ng/L and from 0.06 to 2.83 ng/g (dry weight, d.w.), respectively. Finally, this method was successfully applied to real environmental sample analysis, which revealed that all of the tested BPs were present, with the exception of BPB.

1. Introduction

Bisphenol A (BPA), a high-production volume industrial chemical used to make polymers, is recognized as an endocrine disruptor [1–3]. Faced with growing concern that exposure to BPA has a wide range of adverse health issues, Health Canada, Denmark, and European Union have banned BPA use in baby bottles [4–6]. Since July 2013, BPA use in the coating of infant formula packaging has been prohibited by the United States FDA [7]. Unfortunately, a group of chemicals that are structurally similar to BPA is also utilized in the manufacture of resins and plastics. These chemicals, which consist of two phenolic rings joined through a bridging carbon or other chemical structures, are called BPA-related compounds or bisphenols (BPs), and some are considered able to partially replace BPA in industrial applications. Recently, the limitations regarding the use of BPA have led some manufacturers to replace it with bisphenol S (BPS) in thermal paper and other products [8,9]. Bisphenol F (BPF) and bisphenol B (BPB) maybe developed as alternatives to BPA in the production of epoxy resin and polycarbonate for food contact materials [10,11] and have been detected in canned foods and soft drinks [11–14]. In addition to these analogs, bisphenol AF (BPAF) has broad application in the manufacture of phenolic resins or fluoroelastomers, with an annual production of approximately 10,000–500,000 pounds in the USA [15]. Tetrabromobisphenol A (TBBPA) and tetrachlorobisphenol A (TCBPA) are organic flame retardants, and the former was reported to comprise approximately 60% of the total brominated flame retardant market [16,17]. The molecular structures of selected bisphenol analogs are shown in Fig. 1.

Of more significant concern is the fact that these BPs do not seem to be safer than BPA. Limited studies have shown that BPS, BPB, and BPF possess estrogenic activity similar to that of BPA [21–23]. Our recent study demonstrated that BPAF can cause testosterone reduction by directly affecting testis function in adult male rats [24]. TBBPA and TCBPA are candidate thyroid hormone-disrupting chemicals [25]. In addition, some BPs are much less biodegradable than BPA, which renders them more hazardous to humans [26].

The widespread occurrence of BPA in the environment has inspired significant research interest in other BPs. In the past few years, TCBPA and TBBPA have been investigated in water [27,28],

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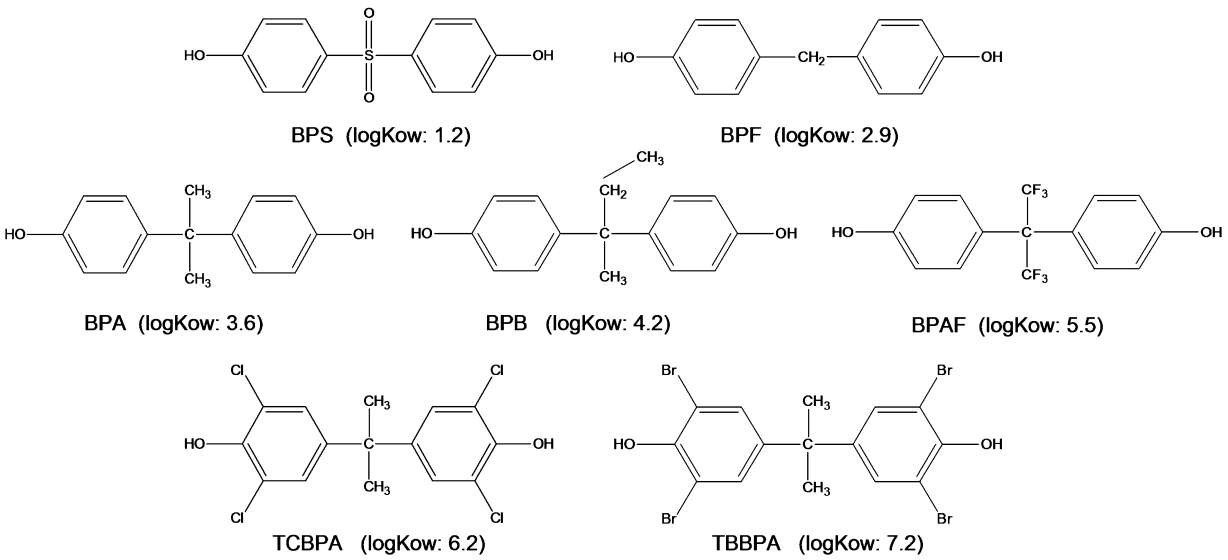

Fig. 1. The molecular structures of selected bisphenol analogs (LogKow is from [18–20]).

soil, sediment, and sewage sludge [16,17,29–31]. BPF is reported to occur in surface water, sewage, and sediment [32]. More recently, several studies were conducted on the occurrence of BPAF, BPS, and BPB in dust [33], water, and sediment [34,35]. Liquid chromatography [29–31,33–35] or gas chromatography [27,28,32] with mass spectrometric detection are commonly used for the analysis of these compounds. Solid-phase extraction (SPE) is the most common method for extraction for water samples [32,34], and other methods, such as solid-phase micro-extraction, have also been reported [27]. For solid sample handling, the most common approach is a combination of accelerated water extraction (ASE) or ultrasonic extraction with an SPE clean-up step [29–31,33–35]. To the best of our knowledge, most of the present analytical methods are focused on a single type of environmental matrix or limited BPs. Regarding the environmental fate and the transforming behavior of BPs, it is essential to develop a simple and universal analytical method for varied environmental samples.

The aim of this study was to develop a selective, sensitive, and universal analytical method for the simultaneous determination of seven BPs in environmental water and solid samples using liquid chromatography–tandem mass spectrometry (LC–MS/MS). The recovery efficiency, precision, linearity of the calibration curves, and method limits of quantitation (MLOQ) were all assessed for method validation. Finally, the optimized method was successfully applied to the quantitation of BPs in real samples.

2. Experimental

2.1. Chemicals and reagents

BPS (purity >98.0%), BPF (purity >99.0%), BPA (purity 98.5%), BPA-d_4 (purity >97.8%), BPB (purity >98.0%), and BPAF (purity 98%) were all purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). TCBPA (purity >99.0%), TCBPA-$^{13}C_{12}$ (purity >99.0%), TBBPA (purity >99.0%), and TBBPA-$^{13}C_{12}$ (purity >99.0%) were obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). BPF-d_{10} was purchased from Toronto Research Chemicals Inc. (Ontario, Canada). HPLC-grade methanol, acetonitrile, ethyl acetate, and acetone were supplied by Dickma (Lake Forest, CA, USA). Ultrapure water was obtained from a Milli-Q Ultrapure water system (Millipore, Bedford, MA, USA). LC–MS grade water and methanol were purchased from Sigma–Aldrich (St. Louis, MO, USA). Formic acid (99%) and ammonium hydroxide for analysis (28–30 wt% solution of NH_3 in water) were purchased from Acros Organics (Morris Plains, NJ, USA). Stock standard solutions (1000 mg/L) were individually prepared by dissolving in methanol and were stored at −20 °C. Working solutions were prepared by serial dilution of the stock solutions with methanol/water (50:50, v/v).

The extraction and clean-up of the samples were performed using a GF/A glass fiber membrane (1.6 μm; Whatman, Maidstone, UK), Oasis HLB cartridge (200 mg, 6 mL; Waters, Milford, MA, USA), and MAX cartridge (150 mg, 6 mL; Waters, Milford, MA, USA).

2.2. Sample collection

Three types of water samples for the spiking procedure were collected in 1-L pre-cleaned amber glass bottles. The river water was collected from a city moat in Beijing, China. The influent and effluent samples were collected from a local sewage treatment plant (STP) in Beijing. Formaldehyde (1%, v/v) was immediately added to each sample to decrease the biological activity. Water samples were transported in coolers to the laboratory and filtered with a Buchner funnel under vacuum with a GF/A glass fiber membrane and stored in a freezer at −18 °C. A portion of the collected samples was analyzed prior to spiking to determine the possible background concentrations.

Samples of surface sediment (0–5 cm) were collected from Hangzhou Bay in Zhejiang, China (121°2′2.15″ E, 30°32′51.15″ N) using a grab sampler, and samples of sludge were collected from a secondary sedimentation tank of a sewage treatment plant (STP) located in Beijing. The solid samples were collected in amber glass bottles, and 1% (v/v) formaldehyde was added to inhibit microbial activity. Once in the laboratory, the solid samples were freeze-dried and ground to particles smaller than 40-mesh, which were stored at 4 °C until analysis.

2.3. Sample pretreatment

2.3.1. Water samples

After adjusting to pH 3–7 using 1 mol/L hydrochloric acid, water samples (100 mL STP influent, 300 mL STP effluent or river water) were spiked with 5.0 ng of mixed internal standards and passed through HLB cartridges that were pre-conditioned sequentially with 3× 6 mL methanol and 6 mL water at a flow

Table 1
Selected MRM transitions and optimized potentials of the target compounds.

Compound	Precursor (m/z) $[M-H]^-$	Quantitation		Confirmation	
		Product (m/z)	CE (eV)	Product (m/z)	CE (eV)
BPA	227.2	212.1	18	133.0	26
BPA-d_4[a]	231.2	216.0	20	–	–
BPS	248.9	107.9	28	155.9	22
BPF	199.1	105.0	21	92.9	21
BPF-d_{10}[a]	208.7	109.8	20	–	–
BPB	241.1	212.1	20	147.0	30
BPAF	335.1	265.0	20	197.0	35
TCBPA	365.1	314.0	25	286.0	30
TCBPA-$^{13}C_{12}$[a]	377.1	297.0	30	–	–
TBBPA	542.7	445.8	35	419.7	40
TBBPA-$^{13}C_{12}$[a]	554.9	459.8	33	–	–

[a] Only one fragment ion was selected for the isotope internal standard.

rate of 5 mL/min. Then, the cartridges were washed with 6 mL methanol/water (50:50, v/v) to remove interferences. The analytes were eluted from the HLB cartridges using 6 mL 2% ammonia in 100% methanol.

For further clean-up, the extracts were diluted by the addition of 1.5 mL water, and the mixture was directly applied to the Oasis MAX cartridges, which were preconditioned with 3 × 6 mL methanol and 6 mL water. The target compounds were finally eluted by 6 mL 100% methanol containing 2% formic acid. This eluate was evaporated to dryness under a gentle stream of nitrogen, and the residual material was reconstituted with 1 mL methanol/water (50:50, v/v) for LC–MS/MS analysis.

2.3.2. Solid samples

A total of 0.2 g (dry weight, d.w.) sludge or 0.5 g (d.w.) sediment was precisely weighed in 15-mL tubes. For method development and validation, the dried samples were spiked with 500 μL of methanolic solution containing the different analytes. This volume ensured contact of the analytes with the entire sample to attain sorption equilibrium. The mixtures were left to stand for 12 h at room temperature in the dark before analysis. The solid samples were treated twice with ultrasonic extraction. For each cycle, 5 mL of methanol/acetone (50:50, v/v) was used, and ultrasonication was continuously applied for 15 min. The slurry was centrifuged at 9000 × *g* for 5 min to collect the supernatant. The mixed supernatant (approximately 9.5 mL) was evaporated to 1 mL under a gentle stream of nitrogen and diluted to 10 mL with LC–MS grade water. Subsequently, the extraction solvent was directly applied to HLB and MAX cartridges for concentration and purification according to the procedure described above.

2.4. Instrument and analytical conditions

Analyte identification and quantitation were performed with an Acquity ultra performance liquid chromatography system (UPLC) coupled to a Xevo TQ-S triple quadrupole mass spectrometer (Waters, Milford, MA, USA). UPLC separation was conducted on an Acquity BEH C18 column (2.1 mm × 100 mm; 1.7 μm; Waters). The mobile phases were LC–MS grade methanol and water. The flow rate was set at 0.4 mL/min, and the injection volume was 5 μL. The initial gradient conditions were 40% methanol for 1 min, followed by a linear increase to 80% methanol over 5 min. Methanol was increased to 100% at 6.1 min, held for 2.0 min, and finally returned to the initial state to equilibrate for 2 min before the next injection.

MS/MS acquisition was operated in negative-ion mode with multiple reaction monitoring (MRM). The capillary voltage was 2.9 kV. The source temperature and desolvation temperature were 150 °C and 400 °C, respectively. Nitrogen gas (purity 99%) was used as the cone gas and desolvation gas at flow rates of 150 L/h and 1000 L/h, respectively. For each analyte, two transitions were selected for identification, and the corresponding cone voltage and collision energy were optimized for maximum intensity. The optimized MS/MS parameters for the target compounds are listed in Table 1.

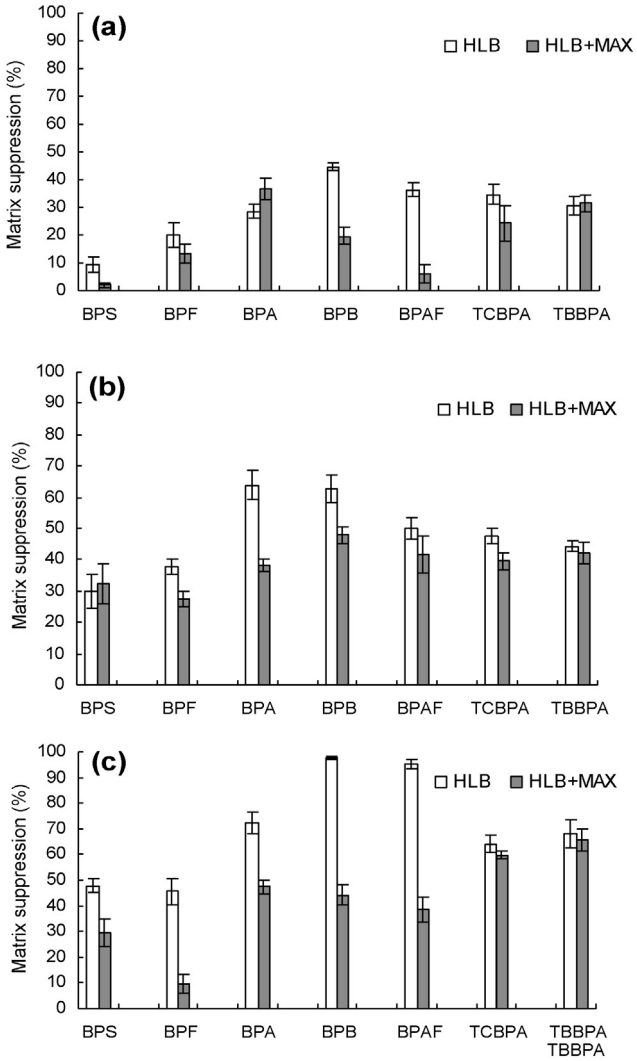

Fig. 2. Matrix suppression of analytes with and without a clean-up step on MAX cartridges (mean ± SD, $n = 3$): (a) river water; (b) STP effluent and (c) STP influent.

Table 2
Matrix effect obtained from comparison of injection to LC–MS of extracts with and without clean-up by SPE (mean ± SD, $n = 3$).

Solid samples	Matrix effect (%)[a]						
	BPS	BPF	BPA	BPB	BPAF	TCBPA	TBBPA
Sediment	−9.9 ± 3.9	26.6 ± 3.1	8.6 ± 4.0	48.7 ± 1.4	62.8 ± 2.0	23.0 ± 5.2	43.5 ± 3.0
	22.9 ± 3.6	*14.7 ± 4.6*	*10.1 ± 1.8*	*23.0 ± 2.0*	*30.4 ± 1.1*	*18.6 ± 0.8*	*38.1 ± 4.9*
Activated sludge	80.4 ± 1.5	−158.0 ± 6.4	87.0 ± 4.2	100.0	100.0	100.0	93.7 ± 0.5
	15.1 ± 2.5	*−53.6 ± 9.7*	*14.3 ± 3.3*	*58.0 ± 1.3*	*20.8 ± 3.5*	*74.3 ± 1.0*	*82.0 ± 1.4*

[a] Matrix effect (%) = $(1 - A_m/A_0) \times 100\%$, where A_m is the peak area of the background-subtracted matrix-matched standard and A_0 is the peak area of the pure standard at the same concentration. The signal was enhanced if the value was negative, whereas the signal was suppressed if the value was positive.
Normal font represents the matrix effect obtained without clean-up by SPE; italic font represents the matrix effect obtained with clean-up by SPE.
Spiked with 10 ng for BPF, BPB, and TBBPA; 5 ng for BPA and TCBPA; 1 ng for BPS and BPAF.

2.5. Quality control

The accurate determination of BPs at ultra-low concentrations is frequently hindered by many factors. Background contamination can occur at trace levels because some of the targets are inherently ubiquitous in the laboratory environment due to the widespread use of polycarbonate plastics and epoxy resins. Previous reports have indicated that BPA can be introduced from plastic components of the SPE cartridges that are generally employed to extract and pre-concentrate BPA in water analysis and to clean up sediment and sludge extracts [36]. In this experiment, the HLB and MAX cartridges were washed with methanol to investigate the potential contamination from SPE cartridges. The results showed that after washing with 6 mL methanol 3 times, BPA could be effectively removed. Simultaneously, all glassware used in the experiment was washed with ethanol, placed in an ultrasonic bath for 2 h, rinsed with Milli-Q water, and baked for 4 h at 400 °C. LC–MS grade water and methanol (Sigma–Aldrich, St. Louis, MO, USA) that were free of analytes were used to prepare the LC mobile phase and in the SPE preconditioning. Procedural blanks were also conducted for each sample batch to account for background contamination, and trace levels of BPA (approximately 0.06 ng, $S/N < 3$) were found in the procedural blanks.

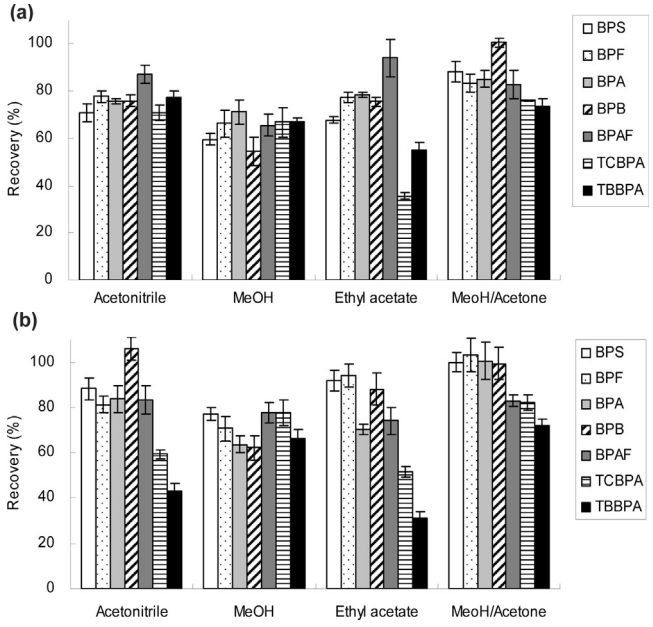

Fig. 3. Recoveries of the target compounds in solid samples after ultrasonic-assisted extraction with different organic solvents (mean ± SD, $n = 3$): (a) sediment and (b) activated sludge.

2.6. Method validation

To ensure that the samples could be accurately analyzed, a desired amount of analyte and a fixed amount of internal standard (BPS, BPAF: 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 ng; BPA, TCBPA: 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0 ng; BPF, BPB, TBBPA: 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 ng; internal standards: 5 ng) were spiked in river water and sediment to establish the calibration curves. Internal standard calibration curves for BPA, BPF, TCBPA, and TBBPA were obtained by performing a linear regression analysis of the ratio of standard-solution areas to internal-standard areas versus concentration. Matrix-fortified standard curves were constructed for the quantitation of BPS, BPB, and BPAF because isotopically labeled compounds were not commercially available and matrix effects were observed with significant difference. For highly contaminated samples, the extracts were diluted with LC–MS grade water to keep the level of analytes within the linear range of the method to ensure accurate quantitation.

Each water sample and solid sample was spiked 6 times with a mixture of analytes at 3 levels to test the recovery and measure its precision. After the spiked samples were treated using the aforementioned procedures, the concentrations of compounds were analyzed by LC–MS/MS during the same day, and the intra-day precision was calculated (expressed as the relative standard deviation, %). Simultaneously, the background concentrations of BPs in the original samples were determined and then subtracted from the total concentrations to determine the absolute recovery. The inter-day precision was quantified by performing twenty analyses of spiked activated sludge samples on five different days.

The matrix effect was also determined in this experiment. The matrix effect was evaluated according to the strategy applied by Matuszewski et al. [37] following the formula matrix effect (%) = $(1 - A_m/A_0) \times 100\%$, where A_m is the peak area of the background-subtracted matrix-matched standard and A_0 is the peak area of the pure standard at the same concentration. The signal was enhanced if the value was negative, whereas the signal was suppressed if the value was positive. The MLOQ and the method limits of detection (MLOD) were defined as the analyte concentrations corresponding to signal-to-noise ratios of 10:1 and 3:1, respectively.

3. Results and discussion

3.1. Optimization of sample preparation

3.1.1. Water sample

In this study, 3 types of SPE cartridges were tested to obtain good recoveries for the widest range of compounds in a single extraction step. The extraction methods that were tested included an Oasis HLB SPE cartridge (200 mg, 6 mL, Waters, Milford, MA, USA), a Sep-Pak C18 cartridge (500 mg, 6 mL; Waters), and an ENVI-Carb graphitized carbon black (GCB) cartridge (500 mg, 6 mL; Supelco, Bellefonte, PA, USA). The test was conducted by spiking

Table 3
Recoveries, intra-day precision, and MLOQ (MLOD) of target compounds in environmental samples ($n = 6$).

Analytes	Spiked level[a]	River water			Effluent			Influent			Sediment			Activated sludge		
		Rec. (%)[b]	Intra-pre.[c] (%)	MLOQ/MLOD[d]	Rec. (%)[b]	Intra-pre.[c] (%)	MLOQ/MLOD[d]	Rec. (%)[b]	Intra-pre.[c] (%)	MLOQ/MLOD[d]	Rec. (%)[b]	Intra-pre.[c] (%)	MLOQ/MLOD[d]	Rec. (%)[b]	Intra-pre[c] (%)	MLOQ/MLOD[d]
BPS	L	86.9	16.9		99.5	6.0		/	/		80.1	11.7		/	/	
	M	107.3	4.8	0.12/0.04	101.8	6.9	0.19/0.06	96.1	2.9	0.55/0.16	103.2	9.5	0.06/0.03	94.2	6.7	0.24/0.07
	H	97.7	3.7		90.0	13.5		90.2	6.1		98.9	3.5		101.9	7.6	
BPF	L	87.2	9.7		89.9	8.2		/	/		66.1	16.8		/	/	
	M	97.0	6.3	1.49/0.45	100.3	5.5	1.65/0.5	87.0	6.4	4.35/1.32	70.9	7.5	0.55/0.20	85.0	13.6	2.83/0.86
	H	95.7	3.9		103.7	3.2		94.2	6.2		67.4	14.5		81.5	5.1	
BPA	L	87.6	15.4		97.1	10.1		/	/		88.7	11.0		/	/	
	M	97.3	5.3	0.72/0.21	114.3	6.0	0.78/0.23	89.8	8.9	2.69/0.82	82.7	13.7	0.24/0.08	93.9	16.8	0.90/0.27
	H	99.1	6.5		93.5	3.7		96.6	5.9		80.3	4.7		81.6	5.6	
BPB	L	86.0	12.1		95.7	12.5		/	/		75.2	10.1		/	/	
	M	93.9	2.9	0.36/0.11	90.1	6.1	0.54/0.16	92.1	5.2	1.55/0.47	72.0	7.1	0.24/0.08	66.8	16.4	0.82/0.25
	H	89.9	5.0		91.0	5.2		90.9	6.7		83.0	7.3		62.5	12.7	
BPAF	L	82.1	11.2		90.5	9.1		/	/		82.8	15.1		/	/	
	M	98.3	5.4	0.05/0.02	94.4	5.2	0.08/0.02	91.7	6.2	0.23/0.07	93.9	12.4	0.06/0.02	87.2	5.7	0.09/0.03
	H	91.6	4.0		93.5	3.6		95.6	4.2		80.4	7.5		93.5	8.2	
TCBPA	L	80.6	12.2		88.5	11.5		/	/		70.4	7.5		/	/	
	M	93.2	5.2	0.16/0.05	90.1	11.8	0.20/0.06	86.7	11.5	0.92/0.28	78.4	3.7	0.27/0.09	76.4	5.7	0.26/0.08
	H	93.9	5.3		94.4	7.5		94.1	3.7		78.0	11.1		77.3	14.6	
TBBPA	L	86.9	15.3		88.8	7.8		/	/		70.6	14.1		/	/	
	M	84.4	4.3	0.34/0.10	75.8	16.1	0.43/0.13	78.7	5.9	1.13/0.35	78.0	11.2	0.70/0.20	57.1	8.3	1.00/0.30
	H	87.0	3.4		80.8	14.3		91.0	7.5		71.3	14.1		68.9	7.9	

[a] L: the low spiked level (BPS, BPAF: 0.05 ng; BPA, TCBPA: 0.25 ng; BPF, BPB, TBBPA: 0.5 ng); M: the middle spiked level (5.0-fold higher than L); and H: the high spiked level (20-fold higher than L). Only the middle and high spiking levels were assessed for influent and activated sludge because of the endogenous concentrations of the analytes.

[b] Rec.: recoveries.

[c] Intra-pre.: intra-day precision, calculated as the relative standard deviation ($n = 6$).

[d] MLOQ: the method limits of quantitation; MLOD: the method limits of detection; the units of MLOQ (MLOD) are ng/L for water samples and ng/g (d.w.) for solid samples.

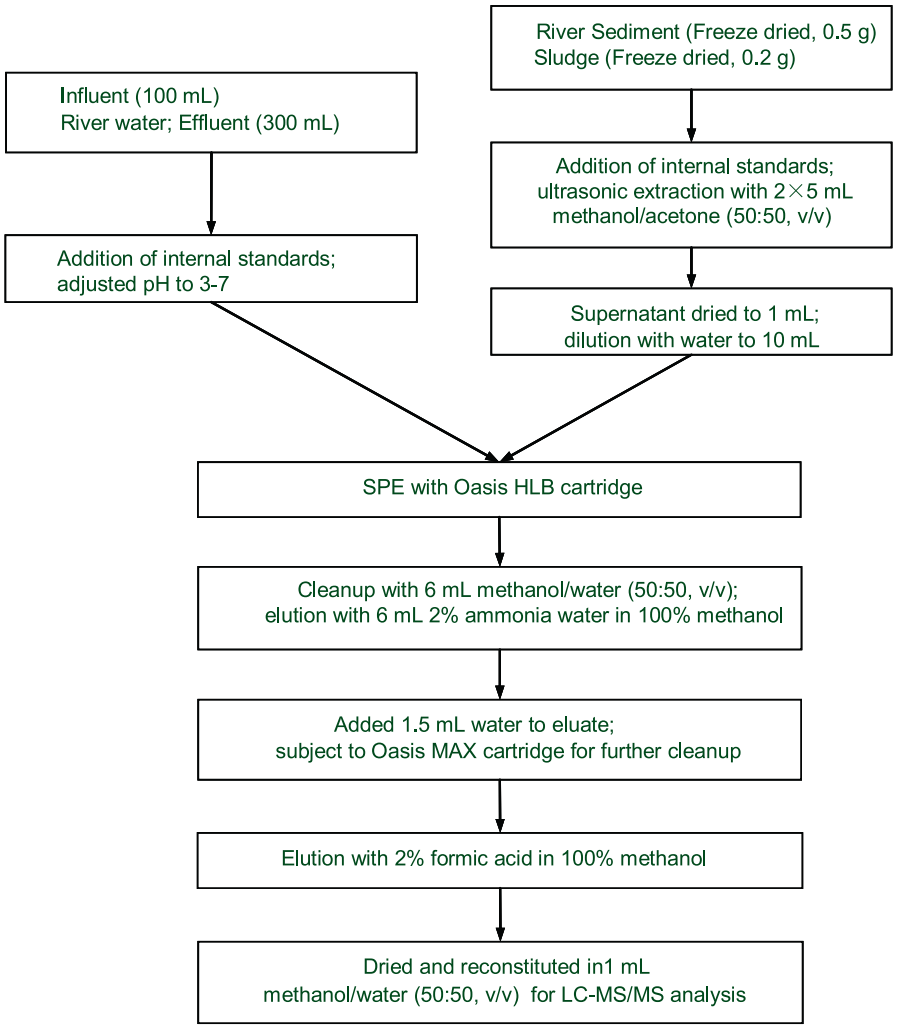

Fig. 4. Schematic diagram of the analysis of target BPs in water samples and solid samples.

analytes in 300 mL pure water samples at 50 ng/L. All cartridges were conditioned with 6 mL methanol and 6 mL water prior to use. After sample loading, all SPE cartridges were cleaned with 6 mL methanol/water (50:50, v/v) and dried by vacuum. Then, the analytes were eluted with 6 mL 2% ammonia water in 100% methanol for the HLB and C18 cartridges. For the GCB cartridge, the analytes were eluted with 6 mL 2% formic acid in 100% methanol. Of the 3 types of cartridges, the C18 cartridges resulted in the lowest recoveries (<20%) of BPS and BPF, which was attributed to the weak absorbance of the relatively polar chemicals on the C18 cartridges and the losses even occurred when methanol/water (30:70, v/v) was used during the clean-up step. The GCB cartridges had relatively poor recoveries of BPS, TCBPA, and TBBPA (<60%). This could be attributed to the strong bonding between these analytes and the sorbent. GCB is considered a reversed-phase sorbent and an anion exchanger because of the presence of positively charged chemical heterogeneities on the surface. Analytes with stronger acidity tend to bond with the sorbents for the anion exchanger, and it was difficult to elute them from the cartridge. Of all the cartridges, the Oasis HLB cartridge was the most versatile and displayed the best overall recoveries (85.4–105.8%) for the extraction of analytes of a wide range of polarities and was therefore used for further testing.

In addition, the effect of pH on the extraction efficiency was studied by adjusting the pH value of pure water samples with solutions of sodium hydroxide and hydrochloric acid. Generally, the acidification of a water solution is likely to decrease the dissociation of weakly acidic analytes, which could maximize the extraction efficiency of the target compounds if the non-dissociated form binds strongly to the reversed-phase SPE cartridges. In this study, all the target compounds had similarly high extraction recoveries at pH 3–7; increasing the pH further led to a reduction in the extraction efficiency, particularly for BPS, TCBPA, and TBBPA (Supplementary data, Fig. S1). The dissociated state under alkaline conditions could be the primary reason for the losses of these acidic analytes in the clean-up steps, and a pH between 3 and 7 was selected for SPE.

For most of the reported methods for detecting BPA in water samples by HPLC–MS/MS, the optimal SPE procedure was noted as being dependent only on the HLB cartridge step [38–40]. However, in our experiments, after extraction by HLB cartridges from the dirty influent water samples, BPB and BPAF showed considerable ion suppression (>90%). Wastewater contains a plethora of dissolved organic carbon species that are co-extracted with target compounds and can cause severe signal suppression of the target analytes, greatly reducing the LC–MS/MS sensitivity. Thus, a further clean-up procedure was needed to reduce the matrix suppression and expand this method to more complicated environmental samples. Oasis MAX is a polymeric, reversed-phase, anion exchange mixed-mode sorbent that enables the retention and release of weakly acidic compounds; this sorbent was selected for the clean-up step in the experiments. As shown in Fig. 2, matrix suppression for all target compounds was decreased by the use of the MAX procedure, indicating that the purification step is effective

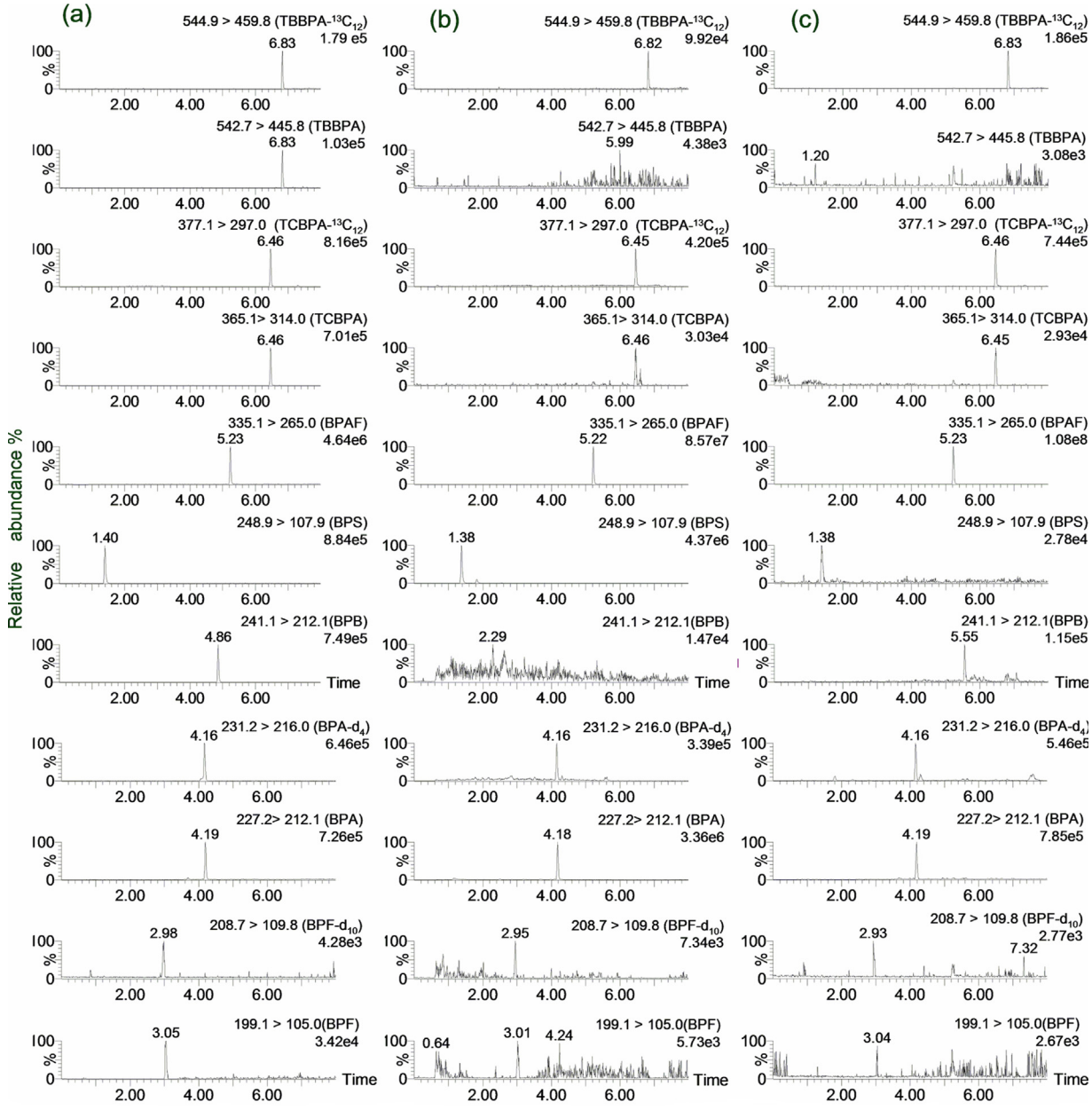

Fig. 5. LC–MS/MS chromatograms of a spiked sample and real sample: (a) spiked sediment sample (spiking level: BPF, BPB, TBBPA, 25 ng/g; BPA, TCBPA, 12.5 ng/g; BPS, BPAF, 2.5 ng/g; internal standard, 25 ng/g); (b) river water sample 1 from Hangzhou Bay and (c) sediment sample 1 from Hangzhou Bay.

and selective. In addition, the color of the SPE extract solution from influent samples changed from brown to light yellow after further clean-up.

3.1.2. Solid sample

In this study, sediment and sludge samples were extracted with ultrasonication, and the extraction parameters that affect extraction efficiency, including extraction solvent, ultrasonication time, and the number of extraction cycles, were evaluated. Numerous solvents, including methanol, acetone, and ethyl acetate, either individually or in combination, have been utilized in other studies for the extraction of BPA and steroids such as estrogen in solid samples [30,35,38,41]. To select the extraction solvent, absolute recovery tests were performed by spiking the investigated BPs in sediment and sludge. As shown in the test results in Fig. 3, methanol exhibited relatively poor extraction efficiency, and mixtures of methanol and acetone yielded the best recoveries for most analytes. Methanol/acetone (50:50, v/v) was ultimately selected as the extraction solvent due to its satisfactory extraction efficiency and good reproducibility for all the investigated compounds. The effect of the duration of ultrasonication on the extraction efficiency was also evaluated at 10, 15, and 20 min. Increasing the extraction time from 10 to 15 min resulted in a slight increase in the recoveries, but further increases in the extraction time were not effective. The influence of the extraction cycles was also evaluated by testing the recovery in spiked sediment, which revealed that 73.4–100.7% of the total extractable amounts were recovered in the first two cycles; the extraction efficiency of the compounds of the second cycle was 14.5–25.8%. Therefore, two 15-min sonication extractions were used for the sample treatment. Because the extracts from the sediment and sludge samples are dirty, a clean-up procedure is required to avoid contamination and to avoid introducing interfering co-extracting compounds into the LC–MS system. The same SPE procedures used for water samples

Table 4
Concentrations of BPs in real environmental samples from Hangzhou bay in Zhejiang Province.

Samples/number	BPs in river water or sediment (ng/L, ng/g d.w.)[a]						
	BPS	BPF	BPA	BPB	BPAF	TCBPA	TBBPA
River water/1	18.99	3.47	74.58	ND	123.44	<MLOQ	ND
River water/2	2.77	2.84	26.39	ND	245.69	4.14	ND
River water/3	0.50	ND	11.40	ND	2.17	5.45	ND
River water/4	0.51	ND	10.84	ND	1.73	1.92	ND
River water/5	0.29	ND	6.59	ND	0.90	1.84	ND
Sediment/1	0.08	<MLOQ	20.56	ND	111.65	0.58	ND
Sediment/2	0.22	30.16	42.76	ND	2009.80	ND	5.08
Sediment/3	0.07	6.24	6.63	ND	708.81	ND	1.12
Sediment/4	<MLOQ	0.60	9.09	ND	488.90	ND	ND
Sediment/5	<MLOQ	ND	1.37	ND	0.18	ND	ND

ND: not detected.
[a] The units of concentration are ng/L for river water and ng/g (d.w.) for sediment.

were applied to the clean-up of solid samples, and this was proven effective in eliminating signal suppression while achieving satisfactory sensitivity (Table 2). A schematic diagram of the analysis of target BPs in water samples and solid samples is shown in Fig. 4.

3.2. Method performance

The calibration curves for the target BPs were respectively established for the water samples and sediment samples. Linearity was assessed for all analytes using a series of increasing amounts of standards as described in Section 2.6. Satisfactory linearity was obtained with correlation coefficients greater than 0.99 for all analytes studied.

The recoveries of the analytes using this method were evaluated at 3 levels with 6 replicates. The replicate samples were extracted and analyzed according to the respective methodology. The recoveries ranged from 75.8 to 114.3% for the water samples and from 57.1 to 103.2% for the solid samples (Table 3). The recovery efficiencies of BPF, BPB, TCBPA, and TBBPA in the solid samples were lower than those in the water samples. BPF and BPB have weaker acidity, and their ability to bind to an anion exchanger would be weakened by competition from the acid interferences in the complicated extracts of solid samples. These effects could decrease the recovery in the MAX purification procedure. The relatively low recovery of TCBPA and TBBPA could be attributed to their stronger hydrophobicity, which enhances their binding to a complicated solid matrix, thus making these compounds more difficult to recover.

Precision (expressed as the relative standard deviation, RSD%) was determined from spiked samples during the same day (intra-day precision) and on five different days (inter-day precision). The intra-day precision was less than 16.9% (Table 3), and the inter-day precision was less than 18.1% (Supplementary data, Table S1), which indicates that the methods are highly reproducible.

Because some of the studied BPs usually had relatively high endogenous concentrations in the environmental matrices, it was difficult to directly determine their MLOQ. In this study, the instrument quantitation limits were first determined using a pure standard to provide a signal to noise ratio of 10:1, and then the MLOQ in the reference matrix was calculated based on the instrument quantification limits, enrichment factors, recovery, and matrix effect (Table 3) [42,43]. The MLOQ varied from 0.05 to 4.35 ng/L in water samples and from 0.06 to 2.83 ng/g (d.w.) in solid samples, providing lower limits of quantitation for BPA and its analogs than those established by other published methods [30,34,35].

3.3. Method application

The analytical method developed in the study was applied in the determination of BPs in 5 river water and 5 river sediment samples collected from an aqueous system close to an emerging chemical-producing area in the Jiaxing region of Hangzhou Bay (Zhejiang, China) in August 2012. The chromatograms of the analytes in a spiked sample and a real sample are shown in Fig. 5. Table 4 shows the different BPs that were detected, as well as their concentration levels. All of the tested BPs, except BPB, were present in the analyzed samples. In these samples, BPA and BPAF were the predominant BPs, and other tested compounds were present at lower concentrations. In particular, BPAF occurred at high levels, ranging from 0.9 to 245.7 ng/L in water and from 0.18 to 2009.8 ng/g (d.w.) in sediment, implying a substantial contamination of BPAF in the sampling areas.

4. Conclusions

A simple and universal analytical method based on SPE procedures and LC–MS/MS was developed for the determination of BPS, BPF, BPA, BPB, BPAF, TCBPA, and TBBPA in environmental water (river water, sewage) and solid samples (sediment, sludge). Analytes were extracted from water samples using HLB cartridges, and the extracts were further purified using MAX SPE cartridges. For the solid samples, a combination of ultrasonic extraction with the same SPE clean-up procedures used for the water samples was employed. The proposed method shows satisfactory precision, good recoveries for almost all the tested compounds, and adequate limits of detection for environmental monitoring. The performance of the method was demonstrated by its application to real samples, in which all target analytes except BPB were detected.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2013.12.074.

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