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Simultaneous determination of bisphenol A, bisphenol AF, tetrachlorobisphenol A, and tetrabromobisphenol A concentrations in water using on-line solid-phase extraction with ultrahigh-pressure liquid chromatography tandem mass spectrometry

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An on-line solid-phase extraction (SPE) ultrahigh-pressure liquid chromatography tandem mass spectrometry method was developed and validated for the simultaneous determination of the bisphenol A, bisphenol AF, tetrachlorobisphenol A, and tetrabromobisphenol A concentrations in water samples. The on-line SPE system used a 10 μm particle size SPE column to concentrate the analytes and was coupled with a 1.7 μm particle size chromatographic column for analysis. The total analysis time for each sample using this process was only 12 min, and the method limit of quantification (MLOQ) ranged from 1.5 to 53 ng/L for different water matrices when a 2.5 mL sample was injected. Isotopic internal standard calibration was used to compensate for matrix effects in the trace-level analysis using mass spectrometry. The method has good precision (RSD < 14.1%) and accuracy (spiked recoveries ranging from 85.1% to 110.8% at three spiking levels). Finally, this method was successfully used to analyse real environmental water samples from the Jiaxing region of Hangzhou Bay.

Keywords: halogenated analogues; liquid-chromatography tandem mass spectrometry; on-line solid-phase extraction; water samples

1. Introduction

Bisphenol A (BPA) is widely used as a monomer for the production of polycarbonate plastics and polystyrene resins. Approximately 8 billion pounds of BPA are produced annually [1,2]. Bisphenol AF (BPAF), 1,1,1,3,3,3-hexafluoro-2,2-bis (4-hydroxyphenyl) propane, is a homologue of BPA in which the methyl groups are perfluorinated. BPAF has broad applications in food processing equipment, electronic devices, and optical fibres and especially as a vulcaniser in fluoroelastomers owing to its excellent stability and hot tear strength [3]. Tetrabromobisphenol A (TBBPA) has been reported to be the brominated flame retardant (BFR) with the highest production volume, accounting for approximately 60% of the total BFR market [4–6], and another compound, tetrachlorobisphenol A (TCBPA), has been reported to be used as a flame retardant, but in much lower quantities (< 10,000 tons/year) [7,8]. The molecular structures of these compounds are presented in Figure 1.

Previous studies have indicated that these chemicals can be released into the environment during manufacturing processes and through leaching from consumer products or polymer

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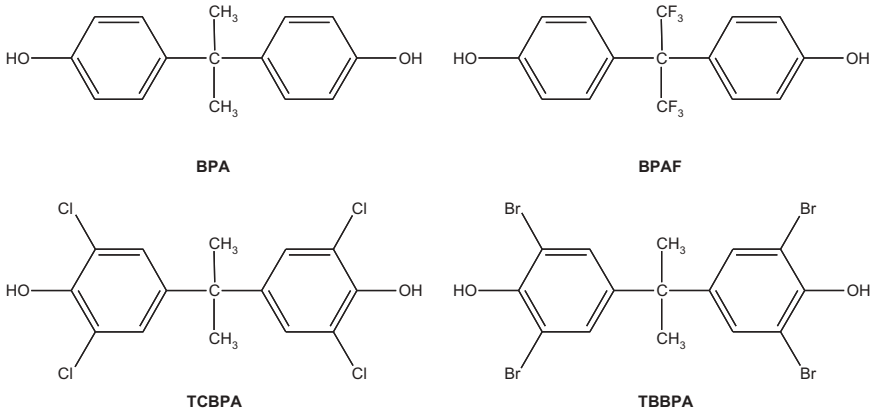

Figure 1. Molecular structures of BPA, BPAF, TCBPA and TBBPA.

matrices after incorporation [6,9], with important implications for human exposure. Recently, concerns about these chemicals were exacerbated by toxicity test studies showing various adverse effects on humans and animals [10–16].

Because of its widespread use, BPA has been detected in wastewater, surface water, and drinking water, with levels in the ng/L to µg/L range [17–21]. In contrast, reports on the presence of BPA's halogenated analogues in water samples are relatively scarce. Gallart-Ayala *et al.* [22] found TCBPA in the effluent of a paper recycling plant (460 ng/L), and Suzuki and Hasegawa [23] reported that the concentrations of TBBPA in the leachates from industrial waste landfill sites and treated water samples were up to 540 ng/L and 7.7 ng/L, respectively. More recently, BPAF was detected in rivers around a BPAF manufacturing plant, with levels ranging from below the limit of quantification (LOQ) to 1.53×10^4 ng/L [24].

The simultaneous analysis of BPA and its halogenated analogues in water samples represents a difficult task owing to the great complexity of the matrices and the low concentrations of the target compounds. Most published analytical methods developed to monitor these chemicals in environmental water rely on off-line solid-phase extraction (SPE) coupled with gas chromatography-mass spectrometry (GC-MS) [21,25] or liquid chromatography tandem mass spectrometry (LC-MS/MS) [23,26,27]. In the past several years, studies have reported a new method for the analysis of environmental endocrine disruptors in water samples based on on-line SPE-LC-MS/MS, which requires less time for sample preparation and has increased productivity for the analysis of nonylphenol, octylphenol, testosterone, BPA, and BPA's chlorinated derivatives [19,22,28]. In addition, this procedure is a good way to reduce potential background contamination resulting from complicated pretreatment procedures [22,29]. Because of these benefits, we developed a highly sensitive analytical method using on-line SPE with ultrahigh-pressure liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) to measure the concentrations of BPA, BPAF, TCBPA, and TBBPA in water samples.

2. Experimental

2.1 *Chemicals and reagents*

Standard BPA (98.5%) was purchased from Dr Ehrenstorfer GmbH (Augsturg, Germany). Standard BPAF (98%) was purchased from Tokyo Chemical Industry Co. Ltd (Tokyo, Japan). Standard TBBPA (99%) and TCBPA (98%) were purchased from Cambridge Isotope Laboratories, Inc.

(Andover, MA, USA). BPA-d_4 was purchased from CDN (Quebec, Canada), and TCBPA-$^{13}C_{12}$ and TBBPA-$^{13}C_{12}$ (purity >98%) were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA); these compounds were used as internal standards. Methanol (MeOH) and acetonitrile (ACN) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). LC-MS grade water was purchased from J.T. Baker (Phillipsburg, NJ, USA). Ammonium hydroxide for analysis (28–30 wt% solution of NH_3 in water) was obtained from Acros Organics (Morris Plains, NJ, USA).

Individual stock solutions (1000 mg/L) were prepared by dissolving an appropriate amount of each substance in MeOH, and these solutions were stored at –20°C in amber glass vessels. Working standard mixtures were prepared by combining the stock solutions and diluting them with an appropriate volume of water containing 10% MeOH.

2.2 *On-line SPE and chromatographic conditions*

On-line extraction and analysis were performed using a Dionex Ultimate 3000 UHPLC system (Sunnyvale, CA, USA) equipped with an Ultimate 3000 Degasser, two Ultimate 3000 binary pumps, an Ultimate 3000 RS autosampler fitted with a 2500-μL loop, an Ultimate 3000 RS column compartment incorporating a six-port switching valve, and Chromeleon® chromatography data system software. The SPE column used in this study was a Direct Connect HP XBridge™ C_{18} column (30 mm × 2.1 mm, 10 μm, Waters, Milford, MA, USA), and the UHPLC chromatographic column was an Acquity Shield RP $_{18}$ column (100 mm × 2.1 mm, 1.7 μm, Waters, Milford, MA, USA). LC-MS grade water and MeOH were used to prepare the LC mobile phase.

The on-line SPE procedure consisted of three steps: loading, transfer, and elution. In the loading step, the six-port valve was switched to the loading position, and a 2500 μL water sample was directly injected into the system. The mobile phase solution provided by the SPE pump (MeOH/water, 20:80, v/v) flowed at a rate of 2.0 mL/min for 4.0 min to wash out the matrix, and the analytes were trapped on the SPE column. In the transfer step, the six-port valve was switched to the injection position to connect the SPE column and the analytical column in series. The gradient elution provided by the UHPLC pump transferred the analytes from the SPE column to the analytical column to achieve separation at a flow rate of 0.3 mL/min for 4.5 min using the back-flush mode. Following separation, the valve was returned to the loading position, and the SPE column and the analytical column were both flushed with 100% MeOH to prevent cross-contamination. The columns were then conditioned with the initial mobile phase prior to the enrichment of the next sample.

2.3 *Mass spectrometry*

Compound identification and quantification were performed using a Xevo triple quadrupole mass spectrometer (Waters, Milford, MA, USA) equipped with an electrospray ionisation source (ESI) operating in negative mode. The capillary voltage was –3.0 kV. The source temperature and desolvation temperature were set to 150°C and 400°C, respectively. The nitrogen flow rate was 800 L/h, and ultra-pure argon was used as the collision gas at a flow rate of 0.13 mL/min. Mass data acquisition was conducted in selected reaction monitoring (SRM) mode. The ion transitions used for quantification and confirmation were monitored together with the retention time for each analyte to guarantee the presence of the target compounds in the real samples.

2.4 *Sampling and preparation*

Water samples were collected in 250 mL pre-cleaned amber glass bottles and transported in coolers to the laboratory. After collection, 10% MeOH was added to the water samples to

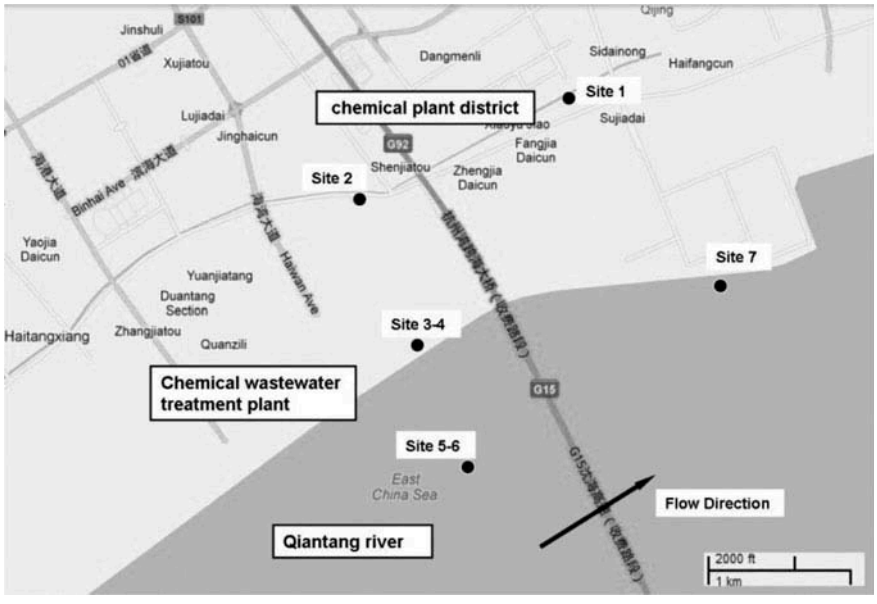

Figure 2. Map of the water sampling locations in the Jiaxing region of Hangzhou Bay.

prevent the loss of the analytes and to facilitate the elimination of particulate matter [22,30]. All water samples were centrifuged for 10 min at 7000 g and 4°C and then filtered with a GF/A glass fibre membrane (1.6 μm; Whatman, Maidstone, Kent, UK) to eliminate particulate matter. The filtered solutions were stored in brown amber bottles at 4°C, and the analyses were conducted within 48 h. The internal standards were added to the samples prior to analysis.

In this study, four types of water were used to validate the method: source water collected from Dalian, river water collected from the Huangpu River in Shanghai, effluent water collected from a local sewage treatment plant (STP) in Beijing, and the tap water from our laboratory. A portion of the collected samples were analysed prior to spiking to determine the possible background concentrations of these compounds.

Seven water samples collected from an aqueous system close to a BPAF manufacturer in the Jiaxing region of Hangzhou Bay in China (Figure 2), where substantial BPAF contamination was recently reported [24], were used in the real sample analysis. Samples 1 and 2 were stagnant river water collected near the plant, samples 3 and 4 were collected from low-lying areas near the effluent vents, and sampling sites 5~7 were located downstream of the effluent vents in the Qiantang River. All samples were collected in December 2011, which was during the dry season in southern China.

2.5 *Method validation*

The method was evaluated in terms of linearity, accuracy, precision, and sensitivity. The recovery of the target compounds using the on-line SPE procedure was assessed using the ratio of the peak areas of the target compounds in spiked LC-MS water determined by on-line SPE to the peak areas of standards containing the same absolute amount of analytes directly injected into the analytical column (i.e., without on-line SPE). To assess linearity, six-point calibration curves were constructed by performing a linear regression analysis of the ratio of the standard solution areas to the internal standard areas versus the concentration. This analysis was performed with five different types of water (LC-MS grade water, river water, source water, STP

effluent, and tap water). In this study, the calibration curves using LC-MS water spiked at different concentration levels were used for the quantification of the real samples. The concentrations of BPA ranged from 20 ng/L to 2000 ng/L (20, 50, 100, 200, 1000, and 2000 ng/L), the concentrations of BPAF and TCBPA ranged from 2 ng/L to 200 ng/L (2, 5, 10, 20, 100, and 200 ng/L), and the concentration of TBBPA ranged from 5 ng/L to 500 ng/L (5, 12.5, 25, 50, 250, and 500 ng/L). All samples were spiked with a 100 ng/L internal standard. Accuracy (evaluated by the spiked recovery based on the ratio of the measured concentration to the fortified level × 100%) and precision (relative standard deviation, RSD%) were assessed by six replicate analyses of source water samples spiked at three levels.

The inter-day precision (n = 9) was quantified by performing nine analyses of the same sample on three different days. The sensitivity of the method was evaluated by the method limit of quantification (MLOQ) and the method limit of detection (MLOD). The MLOQ represents the lowest spiked concentration that can yield a signal-to-noise ratio (S/N) greater than 10, and the MLOD represents the lowest spiked concentration that can yield an S/N greater than three in the selected product-ion chromatogram.

Carryover is a major problem in on-line SPE processes. The level of carryover was evaluated by injecting LC-MS water spiked with 3000 ng/L of four analytes in six samples. Two LC-MS water samples were injected following the highly concentrated sample to determine the levels of the target chemicals still present. The peak areas of the first blank samples were divided by those of the high-concentration standards to obtain a quantitative measure of carryover. No carryover was detected for any these four compounds.

3. Results and discussion

3.1 *Optimisation of the mass parameters*

The SRM parameters were optimised in negative ESI mode with the direct infusion of a standard solution (1000 μg/L) via the syringe pump at a flow rate of 20 μL/min and a T piece to mix this solution with MeOH/water (50:50, v/v) at a flow rate of 0.1 mL/min. This process provided a stable and continuous response during optimisation. Diagnostic product ions were selected, and the relevant parameters were optimised to obtain the maximum sensitivity. The product ions obtained for each compound and the collision energies are shown in Table 1. BPA gave the ion $[M - H]^-$ at *m/z* 226.9, and the main fragmentation of this ion led to the formation of two ions, one at *m/z* 211.9 and another at *m/z* 132.7, which were assigned as $[M - H - CH_3]^-$ and $[M - H - C_6H_6O]^-$, respectively. The precursor ion of BPAF was $[M - H]^-$ (*m/z* 335.2), and the corresponding product ions were $[M - H - CHF_3]^-$ at *m/z* 265.0 and $[M - H - (CF_3)_2]^-$ at *m/z* 197.0. For TCBPA, the precursor ion at *m/z* 365.0 corresponded to $[M\ (^{35}Cl_3\ ^{37}Cl) - H]^-$, and it provided a quantification ion at *m/z* 314.0, which could have originated from the loss of $CH_4{}^{35}Cl$ from $[M\ (^{35}Cl_3\ ^{37}Cl) - H]^-$, and a conformation ion at *m/z* 286.0, corresponding to the loss of $C_2H_4O^{35}Cl$. For TBBPA, the ion at *m/z* 542.7 was selected as the precursor ion $[M\ (^{79}Br_2{}^{81}Br_2) - H]^-$ to maximise the signal, and the product ion at *m/z* 445.8, which corresponds to $[M\ (^{79}Br_2{}^{81}Br_2) - H - CH_4{}^{81}Br]^-$, was chosen as the quantification ion. The optimised SRM parameters are listed in Table 1 and are in agreement with those observed in previous studies [31,32].

3.2 *Optimisation of the on-line SPE and chromatographic conditions*

Various mobile phases and concentrations of ammonium hydroxide (usually employed in reversed-phase chromatography with negative ESI mode mass spectrometry) were compared in these experiments. When MeOH-water was used as the mobile phase, the response of the target compound was greater for all of the compounds than when using ACN-water. The use of an aqueous

Table 1. MS/MS parameters for the analysis of the target compounds.

Compound	Retention time	SRM transition	Cone voltage (V)	Collision energy (eV)
BPA	1.80	226.9 > 132.7	35	18
		226.9 > 211.9		28
BPAF	2.28	335.1 > 197.0	30	35
		335.1 > 265.0		20
TCBPA	4.38	365.0 > 286.0	40	25
		365.0 > 314.0		25
TBBPA	5.11	542.7 > 419.7	40	40
		542.7 > 445.8		35
BPA-d_4	1.77	231.1 > 216.1	36	20
TCBPA-$^{13}C_{12}$	4.38	376.5 > 296.7	40	30
TBBPA-$^{13}C_{12}$	5.11	554.2 > 459.4	40	30

Notes: Quantitative ion transitions are underlined.

mobile phase containing ammonium hydroxide (0.1%) instead of pure water resulted in a greater than 50% increase in BPA; however, the responses for BPAF and TBBPA decreased by more than 50% relative to those for the MeOH-water mobile phase. Similar results were also obtained in previous studies [8,33]. In addition, when using ammonium hydroxide (0.1%) in the mobile phase, TCBPA and TBBPA co-eluted, and the retention time was shorter than that for BPA. This result was attributed to the fact that the ammonium hydroxide is prone to promote the ionisation of TCBPA and TBBPA, which are more acidic than BPA.

In this study, three types of 2.1 mm × 100 mm, 1.7 μm UHPLC columns (Acquity CSH™ Fluoro-Phenyl column, Acquity UPLC BEH C_{18} column, and Acquity UPLC Shield RP_{18} column, Waters, Milford, MA, USA) were compared for the chromatographic separation of the analytes. This experiment was conducted by direct injection without on-line SPE. The Acquity UPLC Shield RP_{18} column was selected as the analytical column because of its superior separation, better responses, and lower baseline for BPA and TBBPA (Figure 3).

Using the analytical column described above, two types of SPE columns, the Direct Connect HP XBridge™ C_{18} column (2.1 mm × 30 mm, 10 μm, Waters, Milford, MA, USA) and the SolEx™ HRP reversed-phase perfusion cartridge (2.1 × 20 mm, 12–14 μm, Dionex, CA, USA), were investigated for the fully automated on-line SPE enrichment step. The HRP column, which has a larger particle size, has a lower column backpressure, but it resulted in poor separation and distorted peaks. Compared with the HRP column, the XBridge™ C_{18} column offered better separation, sharper peak shapes, and good recoveries (83.8~110.8%, Table 2) for all of the analytes. This column was selected as the on-line SPE column.

The sample-loading flow rate and the composition of the wash solvent were also investigated. A flow rate of 2.0 mL/min was selected to load the water samples. This flow rate ensured sufficient enrichment and equilibration of the SPE column in a short time. The best composition was found to be 20:80 ($MeOH/H_2O$, v/v), which achieved the best purification without compromising the SPE recovery. The on-line SPE UHPLC-MS/MS procedure is described in Table 3. The SRM chromatograms of the target analytes in a pure mixed standard under the optimised conditions are shown in Figure 4(a).

3.3 *Sample pretreatment*

To maximise the lifetimes of the SPE and UHPLC columns, a simple preliminary treatment was needed to eliminate particulate matter. We tried using three types of membrane filters (polyether – 0.2 μm, cellulose – 0.2 μm, and nylon – 0.45 μm) to perform this filtration step.

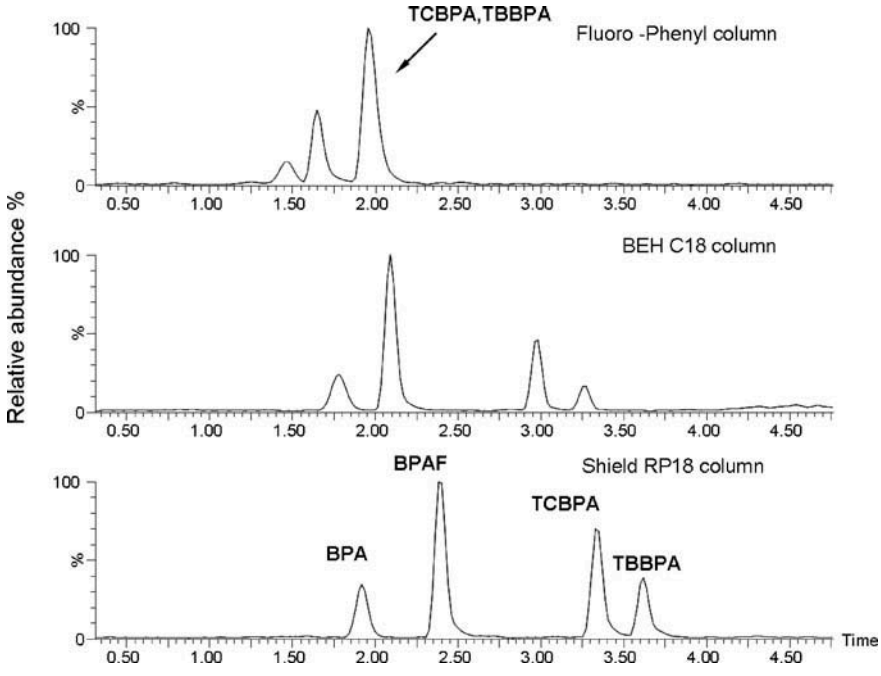

Figure 3. Chromatograms of the separation observed with three types of analytical columns. Notes: This experiment was conducted using direct injection without on-line SPE. The LC working conditions for the UHPLC pump (4–12 min) in Table 3 were employed for this separation.

Table 2. Summary of the on-line SPE recovery and spiked recovery at three levels (n = 6).

	On-line SPE recovery (%)			Spiked recovery (%)		
Analyte	Spiking level (ng/L)	Recovery (%)	RSD (%)	Spiking level (ng/L)	Recovery (%)	RSD (%)
BPA	10	83.8	8.5	20	95.3	14.1
	50	103.3	6.9	100	110.8	7.1
	500	101.9	6.4	500	106.7	6.8
BPAF	1.5	110.8	9.0	1.5	88.7	7.7
	4	108.5	8.4	8	95.5	5.3
	40	106.2	7.7	40	93.1	11.2
TCBPA	1.5	107.4	8.5	1.5	85.1	11.4
	20	84.0	12.9	40	108.9	7.7
	200	95.7	10.2	200	108.7	5.4
TBBPA	5	93.4	6.9	5	97.6	6.3
	50	88.9	13.3	100	97.7	6.5
	500	96.7	9.6	500	89.3	7.5

Notes: On-line SPE recovery: calculated as the ratio of the peak areas of the target compounds in spiked LC-MS water determined by on-line SPE to the peak areas of standards containing the same absolute amounts of analytes directly injected into the analytical column; the spiked recovery corrected by internal standards was tested in source water.

Unfortunately, more than 90% of the analytes disappeared when spiked samples were filtered through these membranes. In addition, some researchers have found that filtration can introduce interference and background noise [22,29]. Hence, a centrifugation step at 7000 g for 10 min at

Table 3. Parameters for the on-line SPE UHPLC-MS/MS procedure.

		SPE pump			UHPLC pump		
Time (min)	Valve position	Flow rate (mL/min)	MeOH (%)	Water (%)	Flow rate (mL/min)	MeOH (%)	Water (%)
0	Loading	2.0	20	80	0.3	75	25
4	Injection	2.0	20	80	0.3	75	25
8	Injection	2.0	100	0	0.3	100	0
8.5	Loading	2.0	100	0	0.3	100	0
10	Loading	2.0	100	0	0.3	100	0
12	Loading	2.0	20	80	0.3	75	25

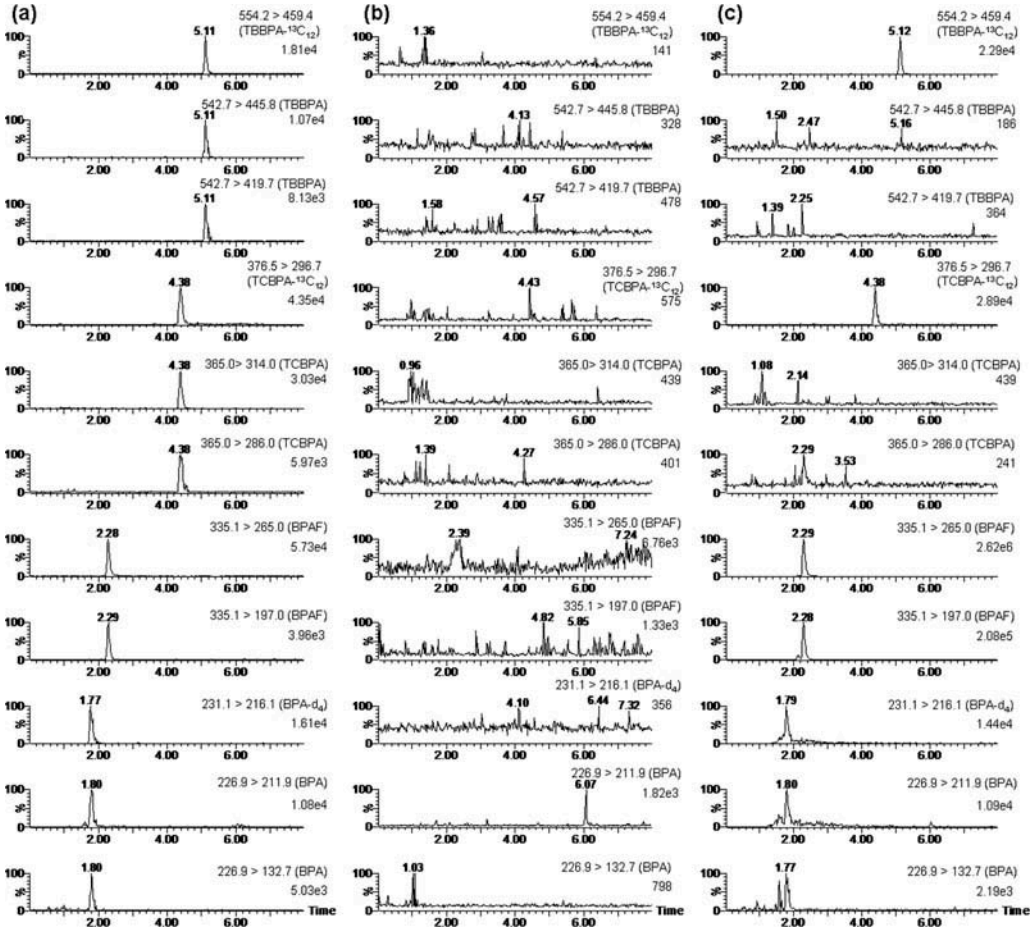

Figure 4. SRM chromatograms of the target compounds. Notes: (a) a pure mixed standard sample (BPAF, 5 ng/L; TCBPA, 25 ng/L; BPA and TBBPA, 50 ng/L; internal standards, 100 ng/L); (b) a blank sample (LC-MS water); and (c) sample 4 from Hangzhou Bay (BPA, 53 ng/L; BPAF, 273 ng/L).

4°C followed by filtration through a glass fibre membrane (without any loss of analytes) was adopted to remove the particulate matter. In this study, the lifetime of the on-line SPE column was observed to exceed more than 400 injections of real water samples of 2500 μL each.

3.4 *Quality control*

Contamination must be avoided throughout the entire analytical procedure because BPA can leach from plastic and epoxy resins in the laboratory equipment, and serious contamination problems may be encountered owing to the presence of BPA in purified water [19]. In this study, various quality controls were applied to ensure the accuracy of the method. Plastics were not used, and the glassware was baked for 4 h at 400°C in a muffle furnace (L9/11/B 170, Nabertherm Industrial Furnaces Limited, Lilienthal/Bremen, Germany) before use. Because the trace level in purified water may not be negligible, LC-MS water (LC mobile phase), which contained no target compounds, was injected after every fifth sample injection to monitor sample carryover. As shown in Figure 4(b), no significant peaks (S/N < 3) were observed in these blank samples.

3.5 *Method validation*

Calibration curves were constructed using linear regression by spiking different water samples with analytical standards and internal standards. The water samples that were used for method validation were analysed initially, and only BPA was found in the river water (20 ng/L) and STP effluent samples (29 ng/L). The background-subtracted response of BPA was used when creating the calibration curves. The linear range of each analyte is provided in Section 2.5, and acceptable linearities for all the target compounds were obtained, with correlation coefficients of $r^2 > 0.99$.

In general, typical signal suppression or enhancement effects are often observed when determining the concentrations of analytes in water samples using an LC-ESI-MS/MS system [34,35]. In this study, the matrix effects were evaluated using the formula applied by Matuszewski *et al.* [36] with slight modifications, as follows: subtract the ratio between the slope of the matrix-matched standard curves (spiked matrix samples) and the slope of the neat standard curves (spiked LC-MS water) and then multiplying by 100 to obtain a percentage. The signal was enhanced if the value was negative, whereas the signal was suppressed if the value was positive. In light of the significant matrix effects found in different water matrices (Table 4), isotopic internal standards were employed to compensate for these matrix effects. In this study, the BPA-d_4 was selected as the internal standard for BPAF because no labelled BPAF was commercially available and because it proved sufficient to compensate for the matrix effects (the slopes of the BPAF calibration curves corrected using BPA-d_4 in different matrices were very

Table 4. Matrix effects of different water samples.

	Matrix effect (%)			
Analyte	Source water	River water	Effluent	Tap water
BPA	–15.6	48.4	46.4	–19.8
BPAF	–24.3	56.5	37.7	–18.5
TCBPA	–67.4	–65.0	–21.0	–1.1
TBBPA	23.9	12.1	35.8	37.6

Notes: The matrix effect calculated as [1-(slope of the matrix-matched standard curve/slope of the neat standard curve using spiked LC-MS water)] × 100%.

Table 5. The method limit of quantitation (MLOQ), method limit of detection (MLOD), and calibration curves for the analytes in different matrices.

	LC-MS water		Source water		River water		Effluent		Tap water		
Analyte	MLOQ/ (MLOD) (ng/L)	Calibration curve	MLOQ/ (MLOD) (ng/L)	Calibration curve	MLOQ/ (MLOD) (ng/L)	Calibration curve	MLOQ/ (MLOD) (ng/L)	Calibration curve	MLOQ/ (MLOD) (ng/L)	Calibration curve	RSD (%)
BPA	11.0 (3.5)	$y = 10.89x + 0.06$	18.0 (5.0)	$y = 10.71x + 0.16$	33.0 (10.0)	$y = 10.30x + 0.07$	53.0 (18.0)	$y = 11.54x + 0.18$	11.0 (3.0)	$y = 10.19x–0.05$	5.0
BPAF	1.5 (0.5)	$y = 45.35x + 0.07$	1.5 (0.5)	$y = 42.41x + 0.10$	3.0 (1.0)	$y = 35.81x + 0.02$	11.0 (3.5)	$y = 41.04x + 0.07$	1.5 (0.5)	$y = 37.24x + 0.01$	9.6
TCBPA	3.0 (1.0)	$y = 28.41x–0.14$	1.5 (0.5)	$y = 25.75x + 0.09$	3.0 (1.0)	$y = 26.76x + 0.01$	3.0 (1.0)	$y = 29.70x–0.02$	6.0 (2.0)	$y = 26.17x + 0.03$	6.0
TBBPA	5.0 (1.5)	$y = 7.81x + 0.05$	5.0 (1.5)	$y = 8.08x–0.03$	5.0 (1.5)	$y = 7.89x + 0.01$	5.0 (1.5)	$y = 8.52x–0.04$	10.0 (3.0)	$y = 7.60x + 0.06$	4.4

Notes: BPA-d_4 was used as the internal standard for BPAF; calibration curves: y: peak area ratio of the compound and internal standard, x: mass concentration of the compound, µg/L; the RSD represent the variation in the calibration curve slopes in different matrices, $n = 5$.

Table 6. Concentrations of BPA, BPAF, TCBPA, and TBBPA in real water samples (n = 3).

	Concentration (ng/L)			
Water sample	BPA	BPAF	TCBPA	TBBPA
1	< MLOQ	2883	ND	ND
2	77	2184	ND	ND
3	< MLOQ	948	ND	ND
4	53	273	ND	ND
5	< MLOQ	104	ND	ND
6	34	39	ND	ND
7	< MLOQ	32	ND	ND

Notes: ND: not detected.

similar, RSD <9.6% (n = 5), Table 5). The validated internal standard methods for the compensation of matrix effects ensured that the calibration curve obtained from the LC-MS water could be used for the analysis of real samples.

The accuracy of each compound ranged from 85.1% to 110.8%, with a precision (RSD) of no more than 14.1%. The inter-day precision was 5.4%~13.9%, demonstrating the good reproducibility of the method.

The MLOQ and MLOD were estimated by spiking samples at low concentration levels. For the river water and effluent samples, in which BPA was detected, the MLOQ was estimated as the 10 times S/N ratio of BPA in the sample matrices plus the BPA background level. The results are listed in Table 5. The limits of quantification for BPA and TCBPA were similar or lower than those for other published on-line SPE methods [19,22,28].

3.6 *Sample analysis*

Seven water samples collected from rivers close to a BPAF manufacturer in an emerging chemical-producing area in the Jiaxing region of Hangzhou Bay were analysed by the proposed method. Figure 4(c) shows the SRM chromatograms of the target compounds in a selected real sample, and Table 6 summarises the detected concentrations. TCBPA and TBBPA were not detected, and BPA was detected in seven samples, with concentrations ranging from <MLOQ to 77 ng/L. BPAF was detected in all samples, with levels ranging from 32 to 2883 ng/L, comparable to the concentrations of BPAF recently reported for the same location (<LOD to 1.53×10^4 ng/L) [24].

4. Conclusions

A rapid, reliable, and sensitive method for the simultaneous analysis of BPA and its halogenated analogues in water using on-line SPE-UHPLC-MS/MS has been developed. This method has better analysis efficiencies, allows complete separation, and has lower limits of quantification for analytes, without requiring complicated pretreatment steps. This new method was successfully applied to the determination of the concentrations of target compounds in water samples from the Jiaxing region of Hangzhou Bay.

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