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Simultaneous identification and quantification of bisphenol A and 12 bisphenol analogues in environmental samples using precolumn derivatization and ultra high performance liquid chromatography with tandem mass spectrometry

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A method for the identification and quantification of bisphenol A and 12 bisphenol analogues in river water and sediment samples combining liquid–liquid extraction, precolumn derivatization, and ultra high-performance liquid chromatography coupled with tandem mass spectrometry was developed and validated. Analytes were extracted from the river water sample using a liquid–liquid extraction method. Dansyl chloride was selected as a derivatization reagent. Derivatization reaction conditions affecting production of the dansyl derivatives were tested and optimized. All the derivatized target compounds were well separated and eluted in 10 min. Dansyl chloride labeled compounds were analyzed using a high-resolution mass spectrometer with electrospray ionization in the positive mode, and the results were confirmed and quantified in the parallel reaction monitoring mode. The method validation results showed a satisfactory level of sensitivity. Linearity was assessed using matrix-matched standard calibration, and good correlation coefficients were obtained. The limits of quantification for the analytes ranged from 0.005 to 0.02 ng/mL in river water and from 0.15 to 0.80 ng/g in sediment. Good reproducibility of the method in terms of intra- and interday precision was achieved, yielding relative standard deviations of less than 10.1 and 11.6%, respectively. Finally, this method was successfully applied to the analysis of real samples.

KEYWORDS
bisphenol A, bisphenol analogues, dansyl chloride, liquid–liquid extraction, mass spectrometry

1 | INTRODUCTION

Bisphenol A (BPA), one of the most common endocrine-disrupting chemicals [1], is used as a monomer for manufacturing polycarbonate plastics, food packaging, baby bottles, epoxy resins, food and beverage cans, thermal paper, and dental sealants [2–4]. As shown in our previous study, even very low levels of BPA exposure may exert potential adverse effects on health, including peripheral insulin resistance,

Article Related Abbreviations: BPA, bisphenol A; BPs, bisphenol analogues; BPAF, bisphenol AF; BPAP, bisphenol AP; BPB, bisphenol B; BPC, bisphenol C; BPE, bisphenol E; BPF, bisphenol F; DHBP, 4,4′-dihydroxybenzophenone; DNSC, dansyl chloride; dw, dry weight; IStd, internal standard; MTBE, methyl *tert*-butyl ether; PRM, parallel reaction monitoring; TBBPA, tetrabromobisphenol A; TCBPA, tetrachlorobisphenol A; TDP, 4,4′-thiodiphenol; TMBPA, tetramethylbisphenol A

Conflict of interest: The authors declare no potential conflict of interest.

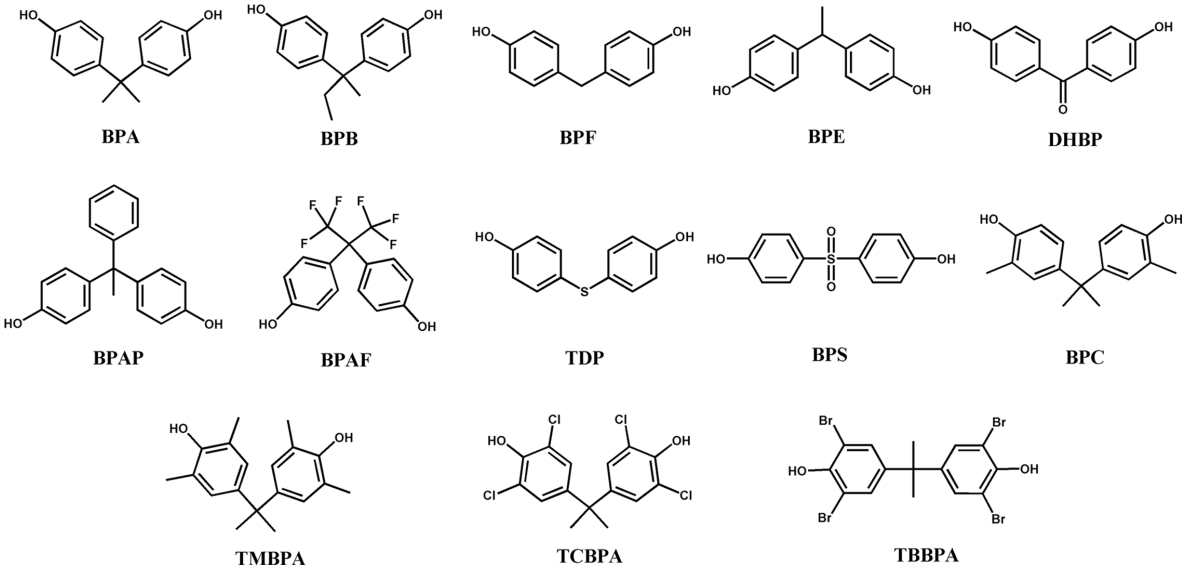

FIGURE 1 Chemical structures of the bisphenol analogues analyzed in the present study

central nervous system insulin resistance, reproductive toxicity, and Alzheimer's-like pathological changes [5].

Given the potential toxicity of BPA, the Canadian Government, United States, and European Union have prohibited the import and sale of polycarbonate baby bottles containing BPA [6,7]. Due to the increasing public attention to BPA, concerns about its potential harm, and restrictions on its production and use by government organizations, manufacturers and factories are interested in developing alternative materials that are structurally similar to BPA for use in the manufacture of resins and plastics [8]. These chemical compounds, which consist of two phenolic rings joined by a bridging carbon or other chemical structures, are called BPA analogues or bisphenols (BPs), and some chemicals are considered to partially replace BPA in industrial applications. These replacement compounds include bisphenol S (BPS), bisphenol F (BPF), bisphenol AP (BPAP), and bisphenol AF (BPAF) [9–11]; their chemical structures are shown in Figure 1.

Recently, BPs have received increasing attention because of their potential toxicity [12]. These compounds also exert potential endocrine-disrupting effects on mammals [10]. For example, BPS, a major BPA alternative, has become widely used and is present in beverage containers, canned food [13], and thermal paper [14]. Moreover, BPS has been detected in 81% of the urine samples collected in the United States and Asia, indicating that BPS is distributed throughout the human body following exposure [15]. However, because the exposure level and the potential toxicity remain poorly understood, no laws or administrative guidelines are currently in place to manage and limit the usage of BPS [16]. Compared to BPA, BPS has a greater capacity to penetrate the skin and a longer half-life; in addition, as BPA is gradually replaced by BPS, the cumulative effect on the human body might become more serious [17]. Another type of BPA analogue, BPF, is mainly used in the production of epoxy resins and polymer polycarbonates, coatings, structural adhesives, electrical varnishes, and lining materials for food containers, baby bottles, and other products [18]. In one recent epidemiological study, BPS, BPF, and BPA detection rates were 78, 55, and 95%, respectively, in 100 nonoccupational urine samples collected in the United States [19]. According to the environmental monitoring survey, BPS and BPF were detected in environmental samples collected from the vast majority of provinces in China, including surface water, sewers, and silt sediment [20], and the concentrations were equal to that of BPA [21].

BPA and its analogues have been detected using several techniques, such as GC–MS [18,22,23], the molecularly imprinted polymer method [24], and LC–MS/MS [20,25–28]. However, to the best of our knowledge, the published methods for analyzing BPs almost always involve a process in which the samples are first purified and enriched on SPE columns before the identification and quantification of the analytes by LC–MS/MS. One of the most distinctive features of this method is that the ionization mode used for MS is negative because the ionization of BPs before injection in the mass spectrometer involves the loss of one hydrogen atom. Although some reports have described the quantification of BPA by derivatization with dansyl chloride (DNSC), no study has reported the simultaneous identification and quantification of BPs using LC–MS/MS after precolumn derivatization. In the present study, we propose a novel method for the identification and quantification of BPs, in which analytes are first extracted and derivatized simultaneously and then quantified using LC–MS/MS. The positive ionization mode is used for the ESI source. The introduction of the

derivatization step would be useful in substantially reducing the detection limit for BPs and improving the detection sensitivity.

The aim of the present study was to develop a method with sufficient sensitivity and accuracy for the simultaneous identification and quantification of BPA and 12 BPs in environmental samples collected from river water and sediment using the derivative reagent DNSC.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

Bisphenol B (BPB, purity > 98.0%), bisphenol C (BPC, purity > 99.0%), bisphenol E (BPE purity > 99.0%), BPF (purity > 99.0%), BPS (purity > 98.0%), BPAF (purity 98%), BPAP (purity > 99.0%), 4,4′-dihydroxybenzophenone (DHBP, purity > 99.0%), 4,4′-thiodiphenol (TDP, purity > 99.0%), tetramethylbisphenol A (TMBPA, purity>99.0%), tetrabromobisphenol A (TBBPA, purity > 99.0%), and tetrachlorobisphenol A (TCBPA, purity > 99.0%) were obtained from Tokyo Chemical Industry (Tokyo, Japan). BPA (purity > 99.0%) was obtained from the laboratory of Dr. Ehrenstorfer (Augsburg, Germany). The isotopically labeled internal standard (IStd) BPA-$^{13}C_{12}$ (purity > 99.0%) was obtained from Cambridge Isotope Laboratories (Andover, MA, USA). HPLC-grade methanol, ethyl acetate, and acetone were supplied by Sigma–Aldrich (St. Louis, MO, USA). LC–MS-grade formic acid, methanol, and acetonitrile were purchased from Merck (Darmstadt, Germany). Ultrapure water was obtained from a Milli-Q Ultrapure water system (Millipore, Bedford, MA, USA). Stock standard solutions (10 mg/mL) were individually prepared by dissolving the compounds in methanol and were stored at −20°C. Working solutions were prepared by serially diluting the stock solutions with methanol/water (50:50, v/v).

2.2 | Sample collection

River water and sediment samples were collected from the Qinhuai River in Nanjing, Jiangsu Province, China (118°47′16.03″E, 32°01′25.42 N). Five water samples and five sediment samples were collected from several different and independent locations. Sediment samples were collected in 15 mL polypropylene EP tubes; river water samples were collected in 100 mL glass bottles. Solid samples were freeze-dried, ground into particles, and then stored at 4°C until further analysis.

2.3 | Sample pretreatment

River water samples (10 mL) were pipetted into 20 mL polypropylene EP tubes, spiked with 25 μL of the BPA IStd solution (20 ng/mL), and then thoroughly mixed. Next, 5 mL of ethyl acetate were added to the tube and then thoroughly vortex-mixed for 5 min. A total of 0.3 g (dry weight [dw]) of sediment was precisely weighed into 2.0 mL polypropylene EP tubes, spiked with 50 μL of the IS solution (20 ng/mL), and then 1 mL of methanol was added and thoroughly vortexed. Then, the sediment sample was centrifuged at 18 000 rpm for 5 min to precipitate out the solid. The upper organic phase was then transferred to another tube to obtain river water and sediment samples before being evaporated to dryness using a CentriVap Labconco centrifugal concentrator under a vacuum. The derivatization step was performed in the following steps: 50 μL of sodium bicarbonate buffer (0.2 M, pH 9) and 50 μL of DNSC in acetone (2 mg/mL) were added to the dry residues and then briefly vortexed (30 s). The mixture was then incubated at 60°C for 8 min before being cooled to room temperature. Subsequently, the mixture was centrifuged at 18 000 RPM for 10 min; 80 μL of the resulting supernatant was transferred into an autosampler vial containing a 100 μL glass insert, and then 5 μL was injected into a UHPLC–ESI-MS/MS for analysis. In our pretreatment process, the river water was concentrated by a factor of 100, changing the volume from 10 mL to 100 μL.

2.4 | LC

Chromatographic separation was performed using a Thermo Hypersill GOLD (2.1 × 100 mm, 1.9 μm) column with a column temperature of 35°C on a Thermo Scientific Dionex Ultimate 3000 RSLC system. Components were eluted with mobile phase A (water/formic acid (99.9:0.1, v/v)) and B (acetonitrile/formic acid (99.9:0.1, v/v)) at a flow rate of 0.25 mL/min using the following gradient: 20% B (0 min) → 60% B (linear increase in 1 min) → 85% B (linear increase in 2 min) → 95% B (linear increase in 2 min) → 95% B (hold for 3 min) → 20% B (linear decrease 0.1 min) → 20% B (hold for 1.9 min), and a total run time of 10 min.

2.5 | MS

A Thermo Q Exactive Orbitrap mass spectrometer equipped with a heated ESI source (HESI-II) was used in positive ionization mode. Typically, source conditions were optimized as follows: a spray voltage of 3 kV, capillary temperature of 350°C, heater temperature of 280°C, S-lens RF level of 50, sheath gas flow rate of 46, auxiliary gas flow rate of 10, and sweep gas 3 (arbitrary units). Nitrogen was used as the collision gas in the HCD cell and damping gas in the C-trap. The mass spectrometer acquired data in parallel reaction monitoring (PRM) (or also known as targeted-MS/MS) mode, with data scanned at a resolution of 17 500 (full width at half maximum at *m/z* 200). The precursor ion was selected in the quadrupole with a 3 *m/z* isolation window, subsequently

enriched in the C-trap and fragmented in the HCD cell. After fragmentation, all product ions were captured by the Orbitrap. The full product ion mass spectra for all the DNSC labeled analytes were obtained in positive ion mode. Instrument control, data acquisition, and analysis were performed with Thermo XCalibur 2.2 software.

2.6 | Preparation of standard solutions and QC samples

Aliquots of each standard solution were mixed to generate a stock solution containing 10 μg/mL BPA and BPs in methanol, which was then stored at −20°C. Before use, stock solutions were serially diluted with methanol/water (50:50, v/v) to obtain a series of working solutions. The internal standard was prepared at a concentration of 10 μg/mL and then diluted to 20 ng/mL for use with methanol during sample analysis as the working solution. A fixed volume of BPs and 25 μL of the internal standard working solution were spiked in blank river water (laboratory water) to establish the calibration curves (BPS, BPF, BPE, BPA, TDP, BPAF, BPC, TCBPA, and TMBPA: 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 ng/mL; DHBP, BPB, and TBBPA: 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 ng/mL; BPAP: 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 ng/mL). For sediment, the desired amount of analytes and a fixed amount of internal standard (1 ng) were spiked in sediment (baked for 3 h at 500°C) to establish the calibration curves (BPS, BPA, and TDP: 0.03, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 25.0, and 50.0 ng; BPF, BPE, and BPAF: 0.06, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 25.0, and 50.0 ng; DHBP and BPC: 0.15, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 25.0, and 50.0 ng; BPB, TMBPA, BPAP, TCBPA, and TMBPA: 0.18, 0.5, 1.0, 2.0, 5.0, 10.0, 25.0, and 50.0 ng). QC samples were prepared using the same procedure to obtain low, medium, and high analyte concentrations. QC samples were analyzed after every ten test samples to minimize errors due to instrumental variations. Following the analysis of the calibration standards and QC samples, a blank sample of methanol was injected to wash the injector to avoid any possible carryover effect.

2.7 | Method validation

The LOD, which is defined as the concentration with an S/N of 3, and the LOQ, which is defined as the concentration with a S/N of 10, were evaluated in fortified blank river water and sediment samples with decreasing analyte concentrations. The accuracy and precision of the method were validated by analyzing QC samples in three analytical runs ($n = 5$ at each concentration) accompanied by measurements of a set of calibration samples in each run. The increase in concentration was determined by subtracting the spiked concentration from the measured concentration of each QC sample. The accuracy was determined as the percent difference between the increase in concentration and the spiked concentration, which was expressed as the recovery. The following formula was used: (increased concentration/spiked concentration) × 100%. The precision was calculated as the RSD of the intra- and interday analytical results.

Five batches of calibration samples and five blank samples were prepared and analyzed using the method described above to determine the dynamic range of the method. The linear range was investigated in water and sediment matrixes. The peak/area ratio between the analytes and internal standard of the blank sample was subtracted from the ratio of the corresponding spiked sample to yield the increase in the peak/area ratio. The calibration curves were then constructed by plotting the increase in the peak/area ratio against the spiked concentration for each compound using a linear regression analysis.

The matrix effect was evaluated by comparing the peak area of the postextracted spiked sample with the value for the corresponding standard solution. The matrix effect was calculated according to previously reported assessment strategies [29] using the following formula: $C\ (\%) = (1 - Am/A0) \times 100\%$, where Am is the peak area of the matrix-matched standard and $A0$ is the peak area of the pure standard at the same concentration.

The stability of the derivatization products was assessed to evaluate the stability of the 13 BPs after derivatization under autosampler conditions. The postpreparative stability was measured by reanalyzing the treated standard samples maintained under the autosampler conditions (in the dark at 4°C) for 24 h. Stability was considered acceptable if the percentage difference was calculated to be within ±20% of the freshly prepared samples. Moreover, the stability results were compared using the paired sample t-test to determine the significance of any differences.

3 | RESULTS AND DISCUSSION

3.1 | Extraction efficiency

In the present study, river water samples were extracted using a LLE method, and the extraction parameters affecting extraction efficiency were evaluated.

Extraction solvents that have been utilized in other studies for the extraction of BPA from water samples, including *n*-hexane, dichloromethane, methyl *tert*-butyl ether (MTBE), and ethyl acetate, were evaluated [30]. Extraction efficiency tests were performed by spiking the investigated BPs and the IS in water samples to select the appropriate extraction solvent. As shown in Figure 2, compared to *n*-hexane, which showed relatively poor extraction efficiency, dichloromethane showed a very good extraction efficiency for most analytes, with the exception of BPS, DHBP, and TMBPA. On the

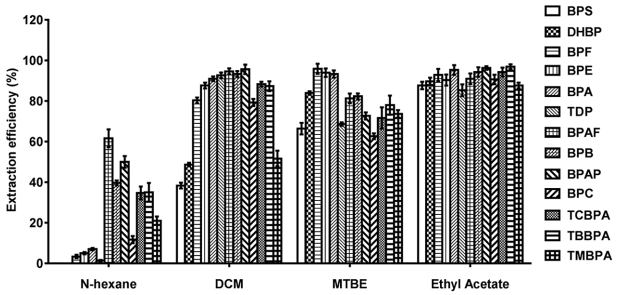

FIGURE 2 Extraction efficiency of the target compounds in river water samples following LLE with different organic solvents (mean ± SD, $n = 5$)

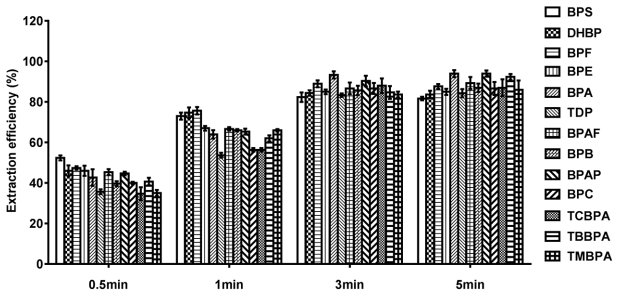

FIGURE 3 Effect of the extraction time on extraction efficiency of 13 types of analytes

other hand, MTBE and ethyl acetate yielded good extraction efficiencies for all BP analytes, but the extraction efficiencies obtained using ethyl acetate were higher than the values obtained using MTBE for most substances. Therefore, ethyl acetate was selected as the extraction solvent due to its satisfactory extraction efficiencies for all compounds analyzed.

The extraction time was also characterized to ensure that the analytes were completely extracted into the extraction solution. The corresponding results are depicted in Figure 3, showing that an ideal extraction efficiency was obtained with ethyl acetate in 5 min.

3.2 | Effects of derivatization conditions on the yield of the derivation reaction of BPs

Under alkaline conditions, DNSC reacts with phenolic hydroxyl groups [31]. The polarity of the derivatization products is lower than that of BP compounds; thus, the ionization efficiency and sensitivity of the analytes can be improved in the MS assay. The formation of dansylated BP compounds is affected by the following parameters: the concentrations of DNSC and sodium bicarbonate, pH, reaction time, and temperature. Therefore, the derivatization conditions were optimized, and the optimal parameters were always used in subsequent examinations of the next parameter.

3.2.1 | Effect of reaction temperature

The reaction temperature is one of the most important factors in the derivatization procedure. Different temperatures were studied to select the appropriate reaction temperature. In our study, the reaction temperature was evaluated in the range of 20–90°C. As shown in Figure 4A, the maximum peak area for BPs was obtained at 60°C; therefore, the most suitable reaction temperature was 60°C for the whole mixture of BPs. This finding is consistent with the derivatization temperatures reported by Pernica et al. [32] and Vitku et al. [33].

3.2.2 | Effect of pH

Dansyl chloride reacts with phenolic hydroxyl groups under alkaline conditions, with the strength of the alkaline environment affecting the yield of the derivatization reaction. A series of experiments were performed to test the effect of pH. The reaction temperature was set to 60°C. The 0.2 M sodium bicarbonate solution was adjusted to a pH of 8–12 using 1 M NaOH. The maximum peak area for BPs was obtained at pH 9, as shown in Figure 4B. Therefore, the pH of the buffer added to the samples was 9 for the actual sample derivatization procedure.

3.2.3 | Effect of the concentration of DNSC

The effect of the concentration of DNSC on the yield of the derivatization procedure was investigated at concentrations ranging from 0.1 to 3 mg/mL. The concentration of each BP used in this study was 100 ng, which is representative of environmental samples such as sediment and river water. As shown in Figure 4C, a DNSC concentration of 2 mg/mL is sufficient for the reaction.

3.2.4 | Effect of the reaction time

The length of the derivatization reaction will have a significant effect on the efficiency of derivative formation. In the present study, the reaction time was tested in the range of 1–60 min using a reaction temperature of 60°C, the buffer having pH 9, and a DNSC concentration of 2 mg/mL. The results are shown in Figure 4D. Initially, the amount of reaction products increased with time and reached a peak in approximately 8 min, but as the reaction continued under these conditions (water bath at 60°C), the amount of product was reduced. Based on these results, a reaction time of 8 min was selected for further experiments.

Thus, the optimal values for the derivatization reaction are as follows: 2 mg/mL DNSC, 0.2 M sodium bicarbonate, pH 9.0, a reaction time of 8 min, and a reaction temperature of 60°C.

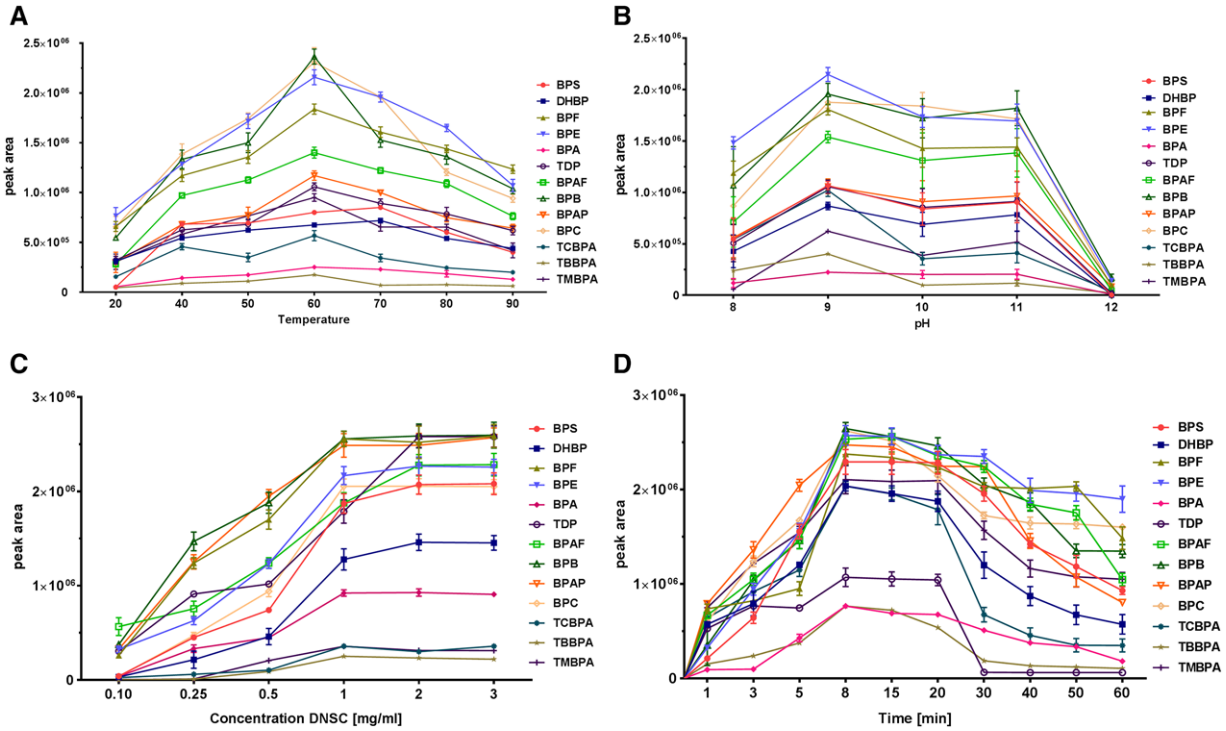

FIGURE 4 Effects of reaction properties on the derivatization yield of BPs expressed as peak areas in the LC–MS/MS analysis. Effects of (A) reaction temperature, (B) pH value, (C) DNSC concentration, and (D) reaction time are shown

3.3 | UHPLC–MS/MS optimization

The MS parameters were optimized through UHPLC with the mobile phases A and B (1:1, v/v) in ESI positive ion mode at a 0.25 mL/min flow rate. The *m/z* values for precursor ions and the normalized collision energy were optimized to obtain the strongest precursor ion and the most stable product ion signals (Table 1). After derivatization, all analytes produced $[M + H]^{2+}$ ions with the highest intensity, with the exception of BPA and BPA-$^{13}C_{12}$, which produced $[M + H]^{+}$ ions with the highest intensity. The derivatization reaction of BPA with DNSC is shown in Supporting Information Figure S1. The PRM mode was quantified by selecting *m/z* values for a precursor ion and one of the product ions. Generally, the product ion showing the most intense signal was selected for quantification, which provided maximum sensitivity.

The Q Exactive mass spectrometer utilized in this study offers high resolution and exact mass numbers, ensuring the selectivity and sensitivity of the system. In the PRM mode, the third quadrupole of a triple quadrupole is substituted with an ultrahigh-resolution mass analyzer Orbitrap to enable the detection of all target product ions in parallel using a guaranteed high-resolution mass analysis [34]. However, if ion interference was detected in the product ion with the most intense *m/z* value, the measurement of the full product ion mass spectrum provides us with the opportunity to select the ion with the next intense *m/z* value for quantification, which ensures the advantages of both high specificity and sensitivity [35]. In the present study, derivatives of three compounds (DHBP, BPE, and TDP) shared the same or similar exact molecular weight due to their similar chemical structures and common fragmentation patterns. In these cases, high-resolution mass spectrometric identification was insufficient for discrimination, and chromatographic separation was required for the correct identification of the compounds. Under the optimized LC conditions, DNSC-labeled DHBP, BPE, and TDP were eluted at 5.81, 6.08, and 6.29 min, respectively. All these compounds were well separated for identification (Table 1).

3.4 | Method performance

The calibration curves for the target BPs were established for the river water and sediment samples. The linearity range was assessed for BPA and BPs using a series of increasing amounts of standards, as described in Section 2.7. Satisfactory linearity was obtained for all analytes investigated, with correlation coefficients greater than 0.99. The recoveries of the BPs were evaluated at three different levels with five replicates each using this method. The recoveries ranged from 81.1 to 119.1% for the water samples and from 80.9 to 111.5% for the solid samples (Supporting Information Table S1).

The RSD was calculated for spiked samples during the same day (intraday precision) and on five different days (interday precision) to estimate the precision of the method. The intraday precision was less than 9.7 and 10.8% and the

TABLE 1 UHPLC-ESI-HRMS/MS parameters: precursor, daughter, and retention time (RT)

Analyte	Precursor (*m/z*)	Daughter (*m/z*)	CS (*z*)	RT (min)	NCE
BPS	359.07	174.09	2	5.50	0.35
DHBP	341.09	203.09	2	5.81	0.35
BPF	334.10	203.09	2	5.92	0.35
BPE	341.11	174.09	2	6.08	0.35
BPA	695.22	174.09	1	6.28	0.4
BPA-$^{13}C_{12}$	707.26	174.09	1	6.28	0.35
TDP	343.08	217.03	2	6.29	0.35
BPAF	402.09	174.09	2	6.44	0.35
BPB	355.12	203.09	2	6.52	0.35
BPAP	379.12	203.09	2	6.59	0.35
BPC	362.13	203.09	2	6.74	0.35
TCBPA	417.04	203.09	2	6.94	0.35
TBBPA	505.93	203.09	2	7.04	0.35
TMBPA	376.15	203.09	2	7.29	0.35

CS, charge state; NCE, normalized collision energy; RT, retention time.

interday precision was less than 10.1 and 10.8% for river water and sediment, respectively (Supporting Information Table S1). Thus, the method is highly reproducible.

The LOQ was defined as the acceptable lowest concentration with S/N = 10 and acceptable chromatographic separation. In the present study, the LOQ was evaluated in real river water and sediment samples with decreasing analyte concentrations. The chromatograms of 13 DNSC-labeled BPs in river water and sediment samples at the LOQ concentration are shown in Supporting Information Figure S2. The LOQ ranged from 0.005 to 0.02 ng/mL in water samples and from 0.15 to 0.80 ng/g (d w) in sediment samples (Table S1). Yamazaki established a method for identifying and quantifying BPA, BPS, and BPF in river water from India: samples were first processed using SPE (Oasis HLB) and then analyzed with LC–ESI(−)-MS/MS. The LOQ ranged from 1.7 to 25 ng/L [36]. Yang identified and quantified seven different types of BPs in river sediments using LC–ESI(−)-MS/MS, and the LOQs ranged from 0.06 to 2.83 ng/g in sediment samples [21]. We measured 13 species of BPs, including these seven analytes, and showed that our method reliably detected a wide range of BPs in environmental samples. The derivatization steps for BPs substantially improved chromatographic separation, as well as the selectivity and sensitivity of detection. The LOQs and LODs for BPA and its analogues obtained in the present study are comparable to the values obtained using above established methods.

The matrix effects of two types of environmental samples are shown in Supporting Information Table S2. A positive result indicates that the signal was suppressed, whereas a negative result indicates that the signal was enhanced. None of the analytes displayed significant matrix effects ($-12.4\% < C\% < +21.3\%$), indicating that the extraction step used in the present study was both effective and selective. We have several explanations for this low matrix effect. First, the isotope internal standard, which is very close to the nature of the analytes being measured, greatly reduced the interference of the sample matrix. Second, samples were subjected to LLE and the analytes were extracted into the extraction solution; therefore, the matrix of the samples was purified. Third, we optimized the chromatographic elution conditions and obtained well-separated analytes from the matrix.

3.5 | Stability of the derivatization products

The prototype compound BPA and its structural analogues are relatively stable in the environment [37]. The stability of the product after DNSC derivatization should also be evaluated to establish an analytical method for BPA and its structural analogues following precolumn derivatization. As shown in Supporting Information Table S3, the DNSC-labeled BP analytes were relatively stable in the autosampler (4°C, 24 h) after derivatization. We recommend that the sample should be analyzed within 24 h after the derivatization reaction.

3.6 | Identification and quantification of BPs in river water and sediment samples

The analytical method developed in the present study was applied to the identification and quantification of BPs in river water and sediment samples collected from the Qinhuai River in Nanjing, the capital city of Jiangsu province, in November 2016. The samples, five river water and five river sediment

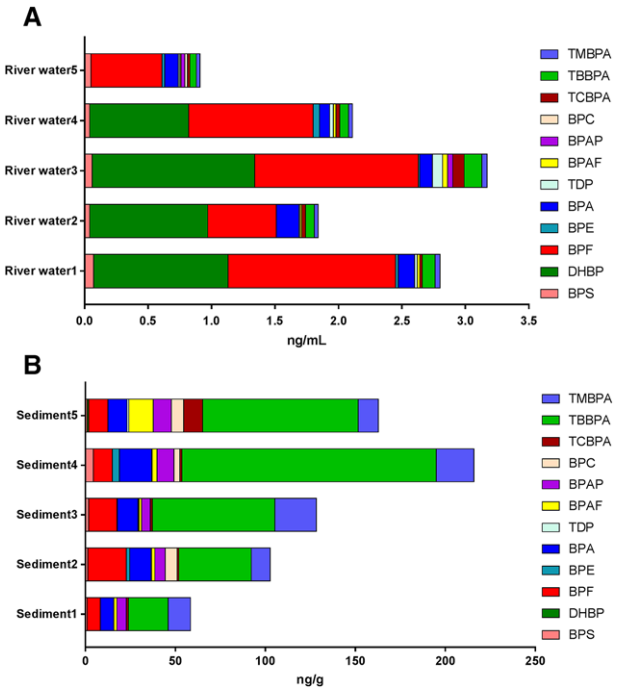

FIGURE 5 Stacked bar graphs showing the concentration of BPs detected in river water (A) and sediment (B) samples collected from the Qinhuai River in Jiangsu Province

samples, were collected from different and independent locations. The concentrations of 13 BPs are shown in stacked bar graphs in Figure 5.

All BPs were present in the samples analyzed, with the exception of BPB, which was not detected in river or sediment. The concentrations of the analytes in real samples are shown in Supporting Information Table S4. These findings are consistent with the results published by Yang et al. who analyzed several river and soil samples from the Jiaxing region of Hangzhou Bay (Zhejiang, China) and did not detect BPB [21].

The BPs analyzed in the present study were present at lower concentrations in river water samples than in sediment samples, perhaps because the water solubility of the tested substances is relatively poor, and thus, they accumulate at the bottom of the river.

In particular, BPF was present at high levels in water, ranging from 0.54 to 1.29 ng/mL, whereas TBBPA was present at high levels in sediment, ranging from 22.02 to 141.38 ng/g (dw), implying substantial contamination with BPF and TBBPA in the specific sampling areas. BPF is used to produce adhesives and coatings, and in systems requiring increased thickness and durability, such as pipe linings, industrial floors, and electrical varnishes [12]. TBBPA is the most common type of bromine flame retardant and is widely used in epoxy resins and polyurethane resins, among others. TBBPA is a ubiquitous environmental contaminant [38].

4 | CONCLUDING REMARKS

A simple and universal analytical method combining LLE, precolumn derivatization, and UHPLC–MS/MS was developed and validated for the extraction and determination of BPA and 12 other BP analogues in environmental samples (collected from river water and sediment). To the best of our knowledge, this report is the first to describe an analytical method for the simultaneous detection of a variety of BPs after precolumn derivatization with DNSC, which greatly improved the sensitivity of the detection method. Analytes were extracted from river water samples using ethyl acetate and derivatized with DNSC. The DNSC-labeled extracts were ionized in the ESI (+) mode and analyzed using a high-resolution mass spectrometer. The developed method shows satisfactory precision, good recoveries for almost all tested compounds, and adequate limits of detection for monitoring biological exposure and environmental contamination. The performance of the method was verified by applying it to real samples.

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