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Highly selective dummy molecularly imprinted polymer as a solid-phase extraction sorbent for five bisphenols in tap and river water

Xiaoli Sun, Jincheng Wang, Yun Li, Jing Jin, Baoqin Zhang, Syed Mazhar Shah, Xueli Wang, Jiping Chen*

Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China

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ABSTRACT

A simple and fast method for both dummy template selection and polymer composition optimization is proposed here. A series of dummy templates for bisphenols imprinting were screened by running them on a non-imprinted polymer (NIP) column with porogen solvent as mobile phase. The tested dummy templates mainly involved bisphenol S (BPS), bromobisphenol A (TBBPA), bisphenol F (BPF), bisphenol E (BPE), bisphenol B (BPB), bisphenol AF (BPAF), 2,2′,6,6′-tetramethyl-4,4′-sulfonyldiphenol (BS-TM) and 4,4′-diaminodiphenylmethane (DADPM). Different monomers and porogens were also investigated for BPS and DADPM using the same method. BPS dummy template was finally selected with acetonitrile and 4-VP as porogen and monomer. The resulting dummy molecularly imprinted polymer (DMIP) achieved superior affinities for BPF, BPE, BPA, BPB and BPAF with imprinting factors 14.5, 13.8, 8.7, 5.7 and 4.2, respectively. An efficient method based on BPS-DMIP-SPE coupled with HPLC-UV was developed for selective extraction of BPF, BPE, BPA, BPB and BPAF in water samples. The method showed excellent recoveries (89.4–102.0%) and precision (RSD 0.3–4.8%, $n = 5$) for tap and river water samples spiked at three concentration levels each (40, 200 and 1000 ng L^{-1}). The detection limits ranged between 2.2 and 3.8 ng L^{-1} with a sample volume of 500 mL. The result demonstrated the superiority of the optimized method for selective extraction of BPs in water samples at the ng L^{-1} level.

1. Introduction

Bisphenol A (BPA) is a weak estrogenic chemical that is widely used as an intermediate in the production of polycarbonate plastic resin and epoxy resin [1]. It has been reported that BPA can induce a decrease in daily sperm count and fertility [2], disrupt chromosome alignment [3] and affect sexual maturation [4] or synaptogenesis [5] in humans. These potential health risks have raised serious concerns over its use in some consumer products and strict regulations have been implemented to minimize its adverse effects [6–8]. Subsequently, a series of structural analogs as the substitutes of BPA emerged, such as bisphenol B (BPB), bisphenol E (BPE), bisphenol F (BPF), bisphenol AF (BPAF), and bisphenol S (BPS). However, these substitutes also share the similar dilemma of acute toxicity, genotoxicity, and estrogenic activity with BPB reported to be even more toxic than BPA [9–11].

BPA is present ubiquitously in various environmental matrices and human samples such as air, surface water, waste water, sewage sludge, sediments, house dust, foodstuffs, urine, blood, and breast milk [1,12–14]. While related reports about the occurrence of other bisphenol analogs are scarce. One study reported the occurrence of BPF in surface water, sewage, dump water and sediments [15]. BPS, BPA and BPF have been reported to be present in surface water at 6.71–250.30 ng L^{-1} level [16]. The ultra trace amounts of BPF were reported in two kinds of soft drinks [17] while BPA, BPS, and BPF were the major contributors accounting for >98% of the total BPs found in the indoor dust samples from the United States and several Asian countries [18].

The ultra low concentrations and complex nature of environmental samples are the bottlenecks for analysis of BPs in real samples [19]. Pretreatment and enrichment processes are indispensable for their analysis even by LC–MS or LC–MS/MS. A series of solid-phase extraction (SPE) cartridges have been used for BPs such as reversed-phase (C18) [20], hydrophilic–hydrophobic balance (HLB) [21] and mixed-mode cationic exchange (MCX) cartridges [16,22]. Although traditional SPE sorbents have high capacities and

* Corresponding author. Tel.: +86 411 84379562; fax: +86 411 84379562.
E-mail address: chenjp@dicp.ac.cn (J. Chen).

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can trap a wide range of analytes, they suffer from low efficiencies due to their low selectivity toward a specific target molecule. It can cause ion suppression and enhancement during LC–MS analysis or a drop in sensitivity of HPLC analysis [23]. These problems necessitate the development of an effective sample pretreatment approach featuring high selectivity, sensitivity, precision and accuracy.

Molecular imprinting is a rapidly developing technique for the preparation of polymers with excellent molecular recognition properties, promoting its applications in molecular imprinted solid-phase extraction (MISPE) [24,25]. BPA-imprinted polymers have been prepared using various methods, such as surface imprinting on silica [26] and magnetic particles [27–29], precipitation polymerization [30,31], supercritical polymerization [32] or spherical polymers by using microfluidic device [33]. All of these MIPs were prepared using target molecule as template. However, the possible leakage of template molecules even after exhaustive washing steps can have a serious impact on the accuracy of analytical method [34]. This becomes one of the major areas of concern in recent sample pretreatment methods employing MISPE [35].

The use of a dummy template offers an easy way to circumvent this problem as any leakage will be different from the analyte [36]. Structurally related analogs, fragments [37–43] and isotope labeled compounds[44] such as 4,4-dihydroxybiphenyl (DHBP), BPE, BPF, BPB, TBBPA, 2,6-bis(trifluoromethyl)benzoic acid (BTFB), *p-tert*-butylphenol (PTBP), 4,4-methylenebisphenol (MBP) and [^{2}H16]bisphenol A (BPA-d16) have been used as dummy templates for BPs imprinting. However, most of those dummy molecularly imprinted polymers (DMIPs) could be used for high sensitive detection of only a single compound, but not for a group of BPs [45]. Nevertheless, the main drawback of template analog imprinted polymers is the resulting inferior molecular recognition ability compared to those prepared using analytes as templates. On the other hand, despite the excellent molecular recognition ability of isotope imprinted polymer, it still has serious limitations such as the use of expensive isotopic templates and mass spectrometric (MS) detection. Therefore, the selection of a dummy template for BPs with higher class selectivity, lower toxicity and cost is a very crucial step which can affect the final result and outcome of MISPE procedure.

Until now, there are no general rules to select a dummy template for DMIP. Generally, dummy templates are selected only on the basis of molecular structures, and the final imprinting effect can not be predicted until the resulting DMIPs are obtained and tested, which makes such work really adventurous and time consuming. Hence, there is a dire need to summarize the basic rule for the selection of dummy template. In this way, the imprinting effect of one DMIP prepared using a certain dummy template can be anticipated and this may assist computational modeling [46].

For a long time, the properties of non-imprinted polymers (NIPs) have been overlooked. Since the imprinting effect is coming from the presence of a template molecule that enhance the pre-existing binding properties of a polymer, positive correlation has been found between the binding properties of non-imprinted and imprinted polymers for the non-covalent imprinting approach. Thus, the functional monomers and cross-linkers can be selected by screening a NIP library, making the optimization of polymer formulations faster and easier [47,48]. We propose here, a new alternative approach for selecting the dummy template for DMIPs. The non-imprinted polymers (NIPs) are used as packing materials in HPLC columns and the capacity factors of potential dummy templates are obtained using porogen solvents as the mobile phase. The template molecule which has the highest capacity factor can have the most significant imprinting effect. At the same time, the polymer composition can be optimized by preparing NIPs with varying polymer compositions and analyzing their effect on the capacity factors of the dummy template.

To verify this hypothesis, different DMIPs were prepared using BPS, TBBPA, BPF, BPA, BS-TM and DADPM as dummy templates and their properties were evaluated by chromatographic experiments. The results demonstrated that the capacity factors of template molecules on NIP columns were related to their imprinting factors. Bisphenol S was found to be the best dummy template with extraordinary class selectivity and low toxicity compared with the other BPs [9]. The BPS-DMIP was applied for the selective extraction and determination of BPA, BPF, BPE, BPB and BPAF in tap and river water samples with high recoveries, precision and accuracy.

2. Experimental

2.1. Reagents and chemicals

Bisphenol F (BPF), bisphenol S (BPS), bisphenol E (BPE), bisphenol A (BPA), bisphenol B (BPB), bisphenol AF (BPAF), 4,4′-diaminodiphenylmethane (DADPM), 4-*n*-nonylphenol (NP), 2,2′,6,6′-tetramethyl-4,4′-sulfonyldiphenol (BS-TM), dienestrol (DIEN), ethylene dimethacrylate (EDMA), methacrylic acid (MAA) and trifluoroacetic acid (TFA) were purchased from J&K Chemical Ltd. (Beijing, China). The initiator 2,2′-azobisisobutyronitrile (AIBN) and bromobisphenol A (TBBPA) were supplied by Aladdin Chemical (Shanghai, China). 4-vinylpridine (4-VP), 2,4,6-trichlorophenol (TCP) and 1,2-benzenediol (CAT) were obtained from Acros (NJ, USA). HPLC grade acetonitrile, methanol and formic acid were purchased from Fisher (Fair Lawn, NJ, USA). Analytical grade chloroform was purchased from Tianjin Bodi Chemical Engineering Co., Ltd. (Tianjin, China), and was purified by washing it with water, drying it over anhydrous sodium sulfate and then distilling it from calcium hydride.

2.2. Preparation of imprinted and non-imprinted polymers

Dummy molecularly imprinted polymers were prepared as follows: the dummy template (1 mmol), the functional monomer 4-VP (0.42 mL, 4 mmol) or MAA (0.35 mL, 4 mmol), the cross-linking monomer EGDMA (3.8 mL, 20 mmol), and the initiator AIBN (0.04 g) were dissolved in 5.6 mL porogen solvent (acetonitrile, methanol or chloroform) in a 10 mL thick walled glass tube. The pre-polymerization solution was sonicated and saturated with dry nitrogen for 15 min, the glass tube was then sealed under nitrogen and kept at 4 °C for 2 h. Afterwards the glass tube was placed in a water bath at 60 °C for 24 h. The obtained DMIP was crushed, ground, and sieved, and particles in the size range of 38.5–63.0 μm were collected. The particles were then allowed to sediment in acetone to remove the fine particles and the resulting DMIP was dried under vacuum at 60 °C for 24 h. The template was Soxhlet extracted for 48 h with methanol–acetic acid (9:1, v/v) and methanol. Non-imprinted polymers were prepared simultaneously using the same protocol in the absence of the template molecules.

2.3. Chromatographic evaluation of the polymers

The DMIPs and NIPs particles were suspended in methanol by sonication and then slurry packed into stainless steel HPLC columns (100 × 4.6 mm id) at 3000 psi using an air-driven fluid pump (Haskel, Burbank, CA, USA) with ethanol as the pushing solvent.

The chromatographic evaluation of the polymers were carried out using a Waters 515 HPLC pump equipped with a Waters 2487 dual wavelength absorbance detector at room temperature. The polymers binding affinities were studied using acetonitrile as the mobile phase. A 20 μL aliquot of the analyte (20 ppm) was injected for the analysis with a mobile phase flow rate of 1 mL min^{-1} and

the UV detector was set at 220 nm. The capacity factor (k), was calculated as $k=(t_R-t_0)/t_0$, where t_R and t_0 are the retention times of the analyte and the void marker (methanol), respectively. The molecular imprinting factor (IF) was calculated by the equation $IF=k_{MIP}/k_{NIP}$, where k_{MIP} and k_{NIP} are the capacity factors of the analyte on the imprinted and non-imprinted polymer, respectively.

2.4. Binding experiment

The binding capacities and the dissociation constants of the BPS-DMIP and the corresponding NIP were analyzed by binding experiments using BPA as a model compound. BPA standard solutions with different concentrations (0.005–4.0 mM) were prepared in ACN. One milliliter aliquots of each solution were mixed with 20 mg BPS-DMIP particles in 10 mL flasks. The flasks were then shaken at 150 rpm for 24 h at 25 °C. After binding, the mixtures were filtered through a 0.22 μm membrane. The free concentrations of BPA in the filtrates were determined by HPLC-DAD (Agilent 1200, USA). The HPLC experimental conditions were as follows: the mobile phase consisted of water–methanol (30:70, v/v); the column temperature was kept at 25 °C; the flow rate was 1 mL min^{-1}; the detection wavelength was set at 270 nm; and the inject volume was 5 μL.

The adsorption capacity and the dissociation constant (K_d (mmol L^{-1})) were calculated according to Eqs. (1) and (2) [49]:

$$Q=\frac{(C_0-C_f)v}{m} \quad (1)$$

$$\frac{Q}{C_f}=-\frac{1}{K_d}Q+\frac{Q_{max}}{K_d} \quad (2)$$

where C_0 (μmol L^{-1}) and C_f (μmol L^{-1}) are the initial and final concentrations of BPA, v (L) is the total volume of the sample, m (g) is the mass of DMIP, Q and Q_{max} (μmol g^{-1}) are the amount of BPA adsorbed at equilibrium and saturation, respectively.

2.5. DMIP-SPE procedure

Solid-phase extraction cartridges with a 3 mL volume were packed with 200 mg of the BPS-DMIP sorbents. The cartridges were equilibrated with 3 mL acetonitrile and 3 mL water. Water samples (pH = 9) were percolated at a constant flow rate of 5 mL min^{-1} with the aid of a peristaltic pump. The cartridges were rinsed with 2.5 mL acetonitrile after drying for 30 min under vacuum. Finally, BPs were eluted with 4 mL methanol–trifluoroacetic acid (99:1, v/v). The eluent was dried under a stream of nitrogen gas and then reconstituted in 250 μL methanol–water (35:65, v/v). An aliquot of 20 μL was injected into the HPLC system for analysis.

2.6. HPLC analysis

HPLC analysis was performed on an Agilent 1200 (Agilent Technologies, Palo Alto, CA, USA) equipped with a vacuum degasser, a quaternary pump, an autosampler, and a diode array detector (DAD) connected to a reversed-phase column (Agilent ZORBAX SB-C18, 250 × 4.6 mm id, 5 μm). A gradient program was used at a flow rate of 1 mL min^{-1}, by combining solvent A (water) and solvent B (methanol) as follows: 35–100% B (25 min), 100% B (2 min). The column temperature was kept at 25 °C. The injection volume was 20 μL, and the DAD wavelength was set at 225 nm.

2.7. Characterization

The specific surface areas and pore volumes of BPS-DMIP and NIP were measured by nitrogen adsorption method using a Nova Station A 4200e (Boynton Beach, FL, USA). Typically, a polymer sample of around 50–60 mg was degassed at 100 °C for 4 h. The specific surface areas and pore volumes were calculated by Brunauer–Emmett–Teller (BET) and Barrett–Joyner–Halenda (BJH) method [50]. The surface morphology of the BPS-DMIP was observed by scanning electron microscopy (SEM) (Zeiss Supra 55, Germany).

3. Result and discussion

3.1. Selection of the dummy template

3.1.1. Screening of the alternative dummy templates and their polymer compositions on the NIP columns

Acetonitrile and 4-VP were the most optimal porogen and monomer for BPA imprinted materials according to the literature [51]. So, the 4VP–EDMA–ACN polymer formulation was used for the screening of BPs dummy templates. Dummy template molecules including BPS, BPF, BPE, BPB, BPA, BPAF, TBBPA, BS-TM and DADPM were run on the 4VP–EDMA–ACN-NIP column using porogen as the mobile phase, and their capacity factors (k_{NIP}) were calculated. To explore the correlation between k_{NIP} and IF, the corresponding DMIPs using BPS, TBBPA, BPF, BPA BS-TM and DADPM as templates were prepared and their IF values were calculated. To further prove the validity of this approach to optimize the composition of DMIPs, different monomers and porogens were investigated for BPS and DADPM using the same method, including BPS–4VP–EDMA–MeOH, BPS–MAA–EDMA–ACN, DADPM–4VP–EDMA–$CHCl_3$ and DADPM–MAA–EDMA–ACN.

As can be seen in Table 1, the k_{NIP} values were highly related with their IF values, and the correlation and regression analysis showed a positive linear correlation between k_{NIP} and IF values with a correlation coefficient of 0.9619. Higher k_{NIP} values resulted in better imprinting effects during the polymerization process. BPS-DMIP and TBBPA-DMIP with the highest k_{NIP} values achieved ultrahigh imprinting factors of 34.55 and 28.56, respectively. DADPM-DMIP′ with chloroform and 4-VP as porogen and monomer show the lowest imprinting effect resulted from very low k_{NIP} value.

The capacity factors of BPS, TBBPA, BPF, BPE, BPA and BPB on the 4VP–EDMA–ACN-NIP column increased with a reduction of pK_a value [52] because the analytes with low pK_a values undergo stronger interactions with 4-VP via acid–base interactions [53]. However, the capacity factors of BPAF, BS-TM and DADPM did not follow this trend. The most possible reason is that the repulsive electrostatic forces due to the lone pair electrons on nitrogen atoms of amino groups (DADPM) or the fluorine atoms of trifluoromethyl groups (BPAF) on the 4VP-NIP column. And the low capacity factor of BS-TM possibly resulted from the stereo-hindrance effects of *ortho*-substituted methyl groups on both sides of hydroxyl groups. No obvious relationship was found between $\log K_{ow}$ and k_{NIP} values since hydrophobic interactions were suppressed when pure organic solvent was used as the mobile phase.

Great differences for the imprinting of BPS and DADPM have been found when different porogens and monomers were used. 4VP–EDMA–ACN and MAA–EDMA–ACN were most suitable for BPS and DADPM, respectively. The most possible reason is that BPS and 4-VP can form strong acid–base interaction in acetonitrile rather than in methanol. And the hydrogen-bonding interaction between BPS and MAA was broken or weakened in acetonitrile solvent. The basic character of amino groups in DADPM made it unable to form pronounced interaction with 4-VP both in acetonitrile and chloroform, meanwhile promoting the formation of strong acid–base interaction between DADPM and MAA in acetonitrile.

The above results confirm that the imprinting effect of a dummy template can be predicted based on its k_{NIP} value. The selection of dummy template and its polymer composition can be achieved by

Table 1
The acid dissociation constant (pK_a), octanol–water partition coefficient ($\log K_{ow}$) and the non-imprinted capacity factors (k_{NIP}) of the tested dummy templates and the imprinted factors (IF) of the prepared DMIPs.

Polymer formulation	Dummy templates	pK_a[a]	$\log K_{ow}$[a]	k_{NIP}	IF	Polymer code
4VP–EDMA–ACN	BPS	8.13	1.387	3.97	34.55	BPS-DMIP
	TBBPA	8.50	9.693	3.70	28.56	TBBPA-DMIP
	BPF	9.91	2.764	1.79	19.44	BPF-DMIP
	BPE	10.10	3.230	1.60	–	–
	BPA	10.29	3.641	1.49	14.70	BPA-DMIP
	BPB	10.27	4.150	1.45	–	–
	BPAF	8.74	3.975	1.37	–	–
	BS-TM	8.74	3.091	0.63	12.30	BS-TM-DMIP
	DADPM	5.32	1.697	0.35	2.04	DADPM-DMIP
4VP–EDMA–MeOH	BPS	–	–	0.50	4.15	BPS-DMIP′
4VP–EDMA–$CHCl_3$	DADPM	–	–	0.10	1.37	DADPM-DMIP′
MAA–EDMA–ACN	BPS	–	–	0.27	2.33	BPS-DMIP″
	DADPM	–	–	0.86	14.1	DADPM-DMIP″

[a] Software calculated value from SciFinder Scholar database 2008: http://www.cas.org/products/sfacad/.

simply running the NIP columns. The limitation of this method was the inability to predict dummy template molecules which has extra strong interaction groups that do not contribute to the dummy molecular imprinting effect. For example, the carboxylic acid group on position 7 of diphenolic acid can result in a very strong retention on 4-VP-NIP column but did not contribute to the imprinting of the main structure of BPs (data not shown).

3.1.2. *Influence of molecular structure on the dummy molecular imprinting effect*

The chemical structures of the BPs and other structurally related compounds are shown in Fig. 1. The lowest energy conformation of BPS, BPF, DADPM, TBBPA and BPA were optimized (MM2 function in Chem3D Pro 8.0, CambridgeSoft Corporation, Cambridge, MA, USA). The bond angles of C(5)—S(7)—C(8) for BPS and C(5)—C(7)—C(8) for

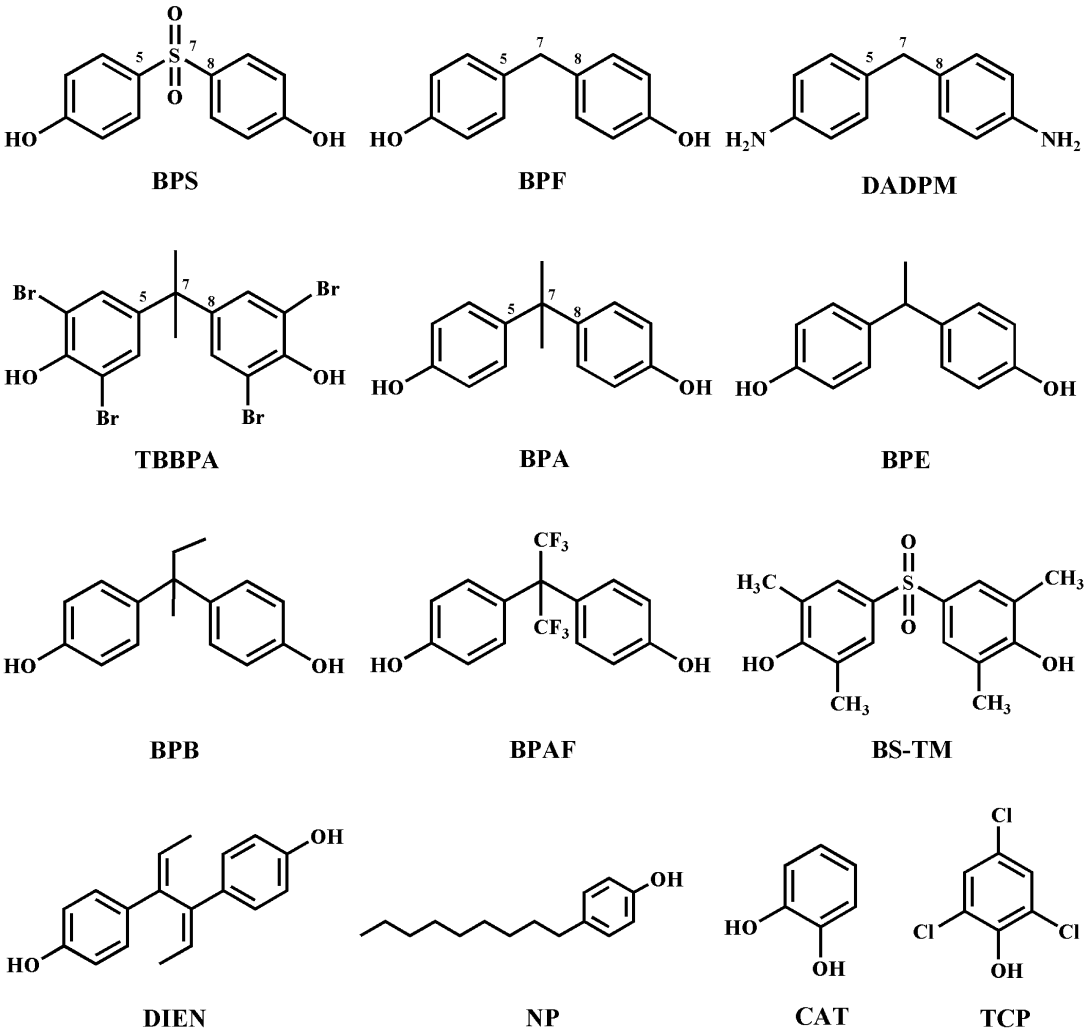

Fig. 1. Schematic molecular structures of investigated compounds.

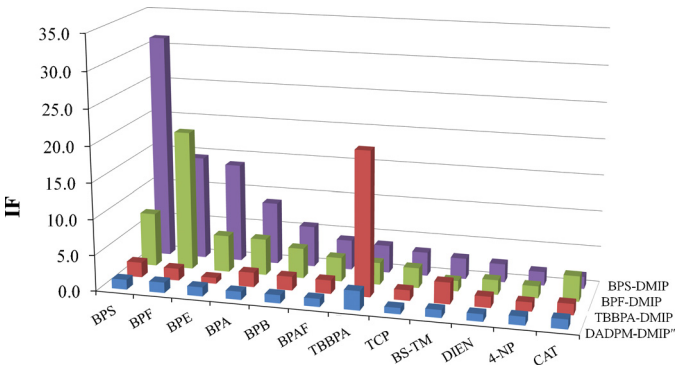

Fig. 2. Evaluation of the cross-selectivity of the DMIPs in terms of imprinted factors (IFs) toward BPs and structure related compounds.

BPF, TBBPA, DADPM and BPA were similar, with values of 108.3, 112.1, 108.5, 109.7 and 111.0 degrees, respectively. So the spatial locations of OH and NH_2 groups in the alternative template molecules were similar.

The cross-selectivity of the four DMIPs with the highest imprinting factors (BPS-DMIP, TBBPA-DMIP, BPF-DMIP and DADPM-DMIP″) were investigated toward BPs and structurally related compounds, the results are shown in Fig. 2. The BPS-DMIP showed considerably higher affinities for other BPs than TBBPA-DMIP, BPF-DMIP, DADPM-DMIP″ and reported BPs-DMIPs [44,45,54]. The IF values of BPS-DMIP obtained for BPS, BPF, BPE, BPA, BPB and BPAF (IF = 34.6, 14.5, 13.8, 8.7, 5.7 and 4.2, respectively) were much higher than that for TCP, BS-TM, DIEN, 4-NP and CAT (IF = 3.3, 2.9, 2.6, 1.9 and 1.7, respectively), further demonstrating its high class selectivity for the BPs. Compared to BPS-DMIP and BPF-DMIP, TBBPA-DMIP and DADPM-DMIP showed very poor dummy molecular imprinting performance due to large-size template and amino-functional template, respectively. Moreover, similar degree of drop in imprinting factors of BPS-DMIP and BPF-DMIP toward other BPs were observed even with very similar molecular structures. The BPS-DMIP with superior imprinting factor for BPS (34.6) retained higher imprinting factors for other BPs than BPF-DMIP.

In conclusion, the similarity of molecular structure between dummy template and target molecules and the high IF value for the dummy template itself (achieved by the NIP column method described in Section 3.1.1) were both important for a successful dummy molecularly imprinting process. The dummy molecular imprinting effect of one DMIP prepared using a certain dummy template can be anticipated by combining the NIP column method and computational modeling of molecular structure.

3.2. Characterization of BPS-DMIP

3.2.1. Binding capacities and affinities of BPS-DMIP

Equilibrium absorption and Scatchard analysis experiments were used to investigate the binding properties of the obtained BPS-DMIP. BPA with a concentration range of 0.005–4.0 mM was used as the target molecule. Fig. 3A shows the experimental adsorption isotherms of BPA on BPS-DMIP and NIP sorbents. BPS-DMIP displayed an obviously higher capacity than NIP. Moreover, in Fig. 3B, two different straight lines were obtained corresponding to the high and low affinity binding sites, indicating that the binding sites in BPS-DMIP were heterogeneous. The corresponding dissociation constants (K_{d12}) and saturation capacity (Q_{max12}) values can be calculated according to the slopes and intercepts of the two linear portions of Scatchard analysis. The K_{d12} and Q_{max12} values were calculated as 0.205 mmol L^{-1} and 5.900 μmol g^{-1} for the high-affinity

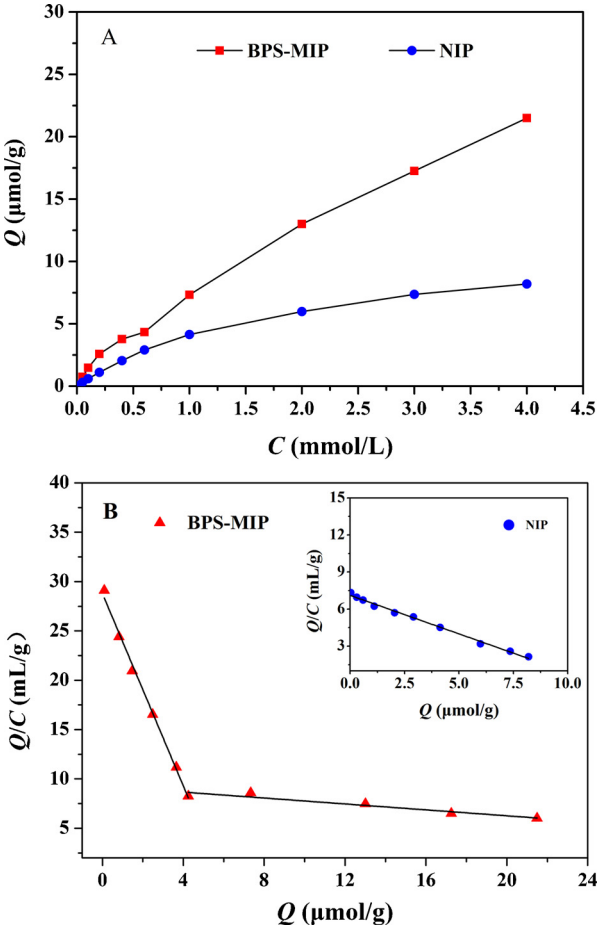

Fig. 3. (A) Adsorption isotherms of BPS-DMIP and NIP for BPA; (B) Scatchard plot analysis of the binding of BPA to the BPS-DMIP and NIP. Sorbent weight: 20 mg.

binding sites, and 6.266 mmol L^{-1} and 50.10 μmol g^{-1} for the low-affinity binding sites.

The K_{d1} and Q_{max1} for high affinity binding sites were the most important constants during the selective washing process of SPE. Usually, lower K_{d1} and higher Q_{max1} value means higher binding affinity and capacity. The result of static adsorption capacity showed that 200 mg BPS-DMIP could selectively absorb 269.4 μg of BPA.

3.2.2. Morphological analysis

Both the BPS-DMIP and NIP particles show "type IV" nitrogen adsorption isotherm, which is associated with capillary condensation taking place in mesoporous materials. The BET area (S_{BET}) value for the BPS-DMIP was 224.37 m^2 g^{-1} with a total pore volume (V_t) of 0.653 cm^3 g^{-1}. The values for NIP were a little higher than BPS-DMIP (S_{BET} = 244.05 m^2 g^{-1} and V_t = 0.737 cm^3 g^{-1}). Similar pore size distributions with one sharp peak were found, which consists of a narrow distribution at 3.69 nm (BPS-DMIP) and 3.48 nm (NIP) and a wider distribution between 2 and 50 nm without obvious peaks. The retention of BPs on BPS-DMIP was much stronger than the NIP even though the NIP has a larger surface and pore volume, further confirmed the existence of the imprinting effect.

The polymer morphologies of the BPS-DMIP are given in Fig. 4. The SEM image displays particles with a size range of 40–60 μm, which is suitable for packing the SPE columns. Both large and small pores were observed, which were consistent with the BJH results.

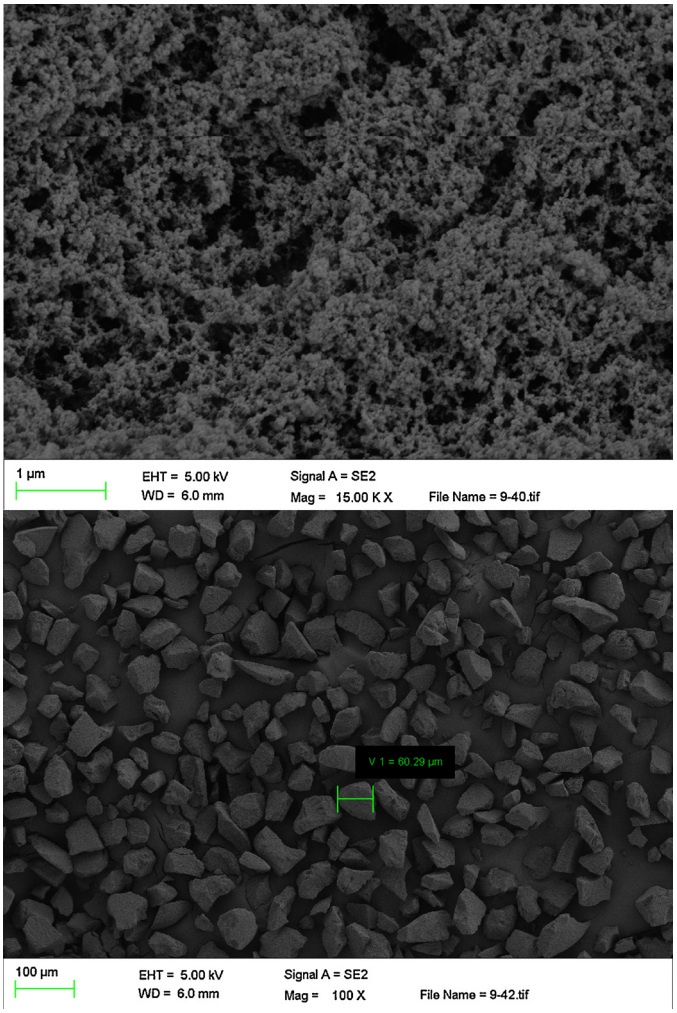

Fig. 4. Scanning electron micrographs of the BPS-DMIP sorbents.

3.3. DMISPE optimization

The factors for optimizing the DMISPE procedure include sample pH, flow rate, sample loading volume, volume of washing solvent and the composition and volume of eluting solvent. For all the steps, SPE columns packed with 200 mg BPS-DMIP were used.

3.3.1. Effect of sample loading pH and flow rate

The aqueous solution (Millipore water) was adjusted to pH 3.0, 6.0, 9.0 and 12.0 by dilute hydrochloric acid or sodium hydroxide. A volume of 100 mL of 0.05 μg mL^{-1} spiked aqueous solutions at various pH values were percolated through the BPS-DMIP cartridges at a flow rate of 2.5 mL min^{-1}, followed by eluting with methanol containing 1% (v/v) TFA. The obtained eluents were analyzed by HPLC, and the recoveries of BPs were calculated to evaluate the extraction efficiency. As shown in Fig. 5, the recoveries of BPs ranged from 90.2% to 99.2% (RSD < 3.1%, $n = 3$) in pH range of 3.0–9.0, and a large drop was observed at a pH value of 12. This was mainly related to the dissociation constants (pK_a) of the BPs. The pK_a values of the five tested BPs were between 8.7 and 10.3. Therefore, the bisphenols were completely ionized, and existed as BPs sodium salt at pH 12.0. After the ionization, the polarities of BPs were increased, thereby decreasing the hydrophobic interactions between BPs and BPS-DMIP in the loading step and resulting in low recoveries of BPs. This conclusion can be further confirmed by the positive correlation between the recoveries of BPs at pH 12.0 and their log K_{ow} values (Table 1) before the ionization. Finally, pH 9.0 was selected for subsequent experiments.

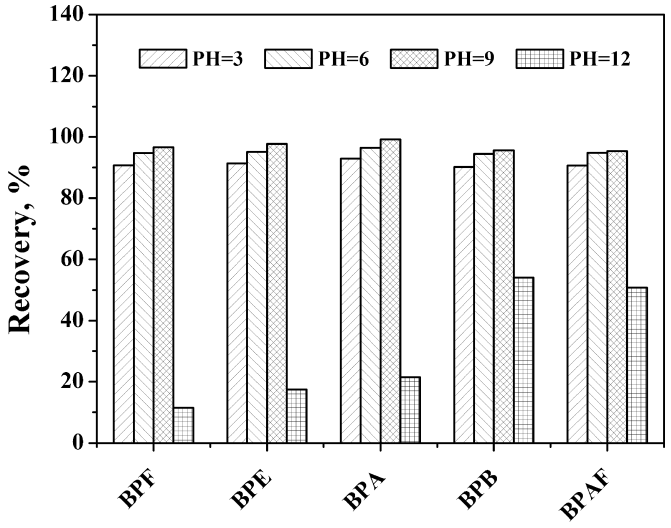

Fig. 5. Influence of sample loading pH on recoveries of BPF, BPA, BPE, BPB and BPAF from pure water. The total amount of analytes was kept constant (5 μg). Sorbent: 200 mg of BPS-DMIP. Washing and elution steps as in Section 2.5.

The effect of sample loading flow rate on BPs recoveries was studied by percolating 100 mL of 0.05 μg mL^{-1} spiked aqueous solution at various flow rates (1.0, 2.5 and 5.0 mL min^{-1}). Recoveries ranging from 93.7% to 98.6% (RSD < 4.5%, $n = 3$) were obtained up to a flow rate of 5.0 mL min^{-1}. Thus, a value of 5.0 mL min^{-1} was selected for further experiments.

3.3.2. Breakthrough volume

The breakthrough volume of BPS-DMIP cartridge was determined by percolating different volumes (100, 250 and 500 mL) of spiked ultrapure water samples (pH = 6.0), with the amount of each analyte in the sample constant (0.5 μg). BPs recoveries did not differ significantly over the tested range with values close to 100%. The exposure concentration of BPs in water sample is down to ng L^{-1} level. Thus, a volume of 500 mL was selected to increase the sensitivity of the developed HPLC method.

3.3.3. Washing solvent selection

During percolation of the aqueous samples, retentions of analytes and impurities were non-specific due to hydrophobic interactions. The selective retention mechanism resulting from the presence of cavities should be introduced by an appropriate washing step. Since shape recognition is often best in the solvent used as porogen in the polymerization, acetonitrile was chosen as the selective washing solvent.

The effect of washing volume on BPs recoveries and selectivity was studied by percolating 100 mL (pH = 6.0) of 0.05 μg mL^{-1} spiked aqueous solutions of five BPs and structurally related compounds (BS-TM, DIEN, NP, TCP and CAT), followed by washing with 3 mL water and different volume of acetonitrile. Before washing with acetonitrile, the SPE column was dried with the aid of a peristaltic pump for 30 min to remove the residual water as far as possible.

Recoveries obtained without any washing step were close to 100% both for BPS-DMIP and NIP due to the non-specific hydrophobic binding. But after washing with 2.5 mL of acetonitrile, the recoveries of BPs from BPS-DMIP were still near 100%, whereas the recoveries from NIP decreased to less than 5%, as shown in Fig. 6. This result confirms the good class selectivity of BPS-DMIP for BPs as discussed in Section 3.1.2. However, structurally related

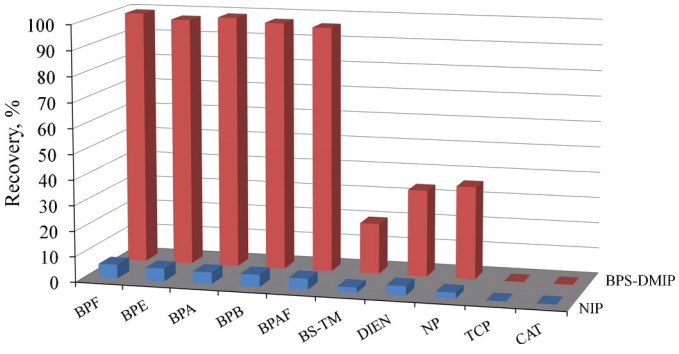

Fig. 6. Extraction recoveries (%) obtained with the BPS-DMIP and NIP cartridges for five BPs after percolation of 100 mL (pH = 6.0) of pure water containing 0.05 μg mL^{-1} of each compound using a washing step with 3 mL water and 2.5 mL acetonitrile.

compounds BS-TM, DIEN and TCP which should be washed more completely on BPS-DMIP still gave recoveries of 19.8%, 33.9% and 36.2%, respectively. This may due to the high log K_{ow} values of BS-TM, DIEN and TCP (log K_{ow} = 3.464, 4.920 and 3.69, respectively), causing significantly increased hydrophobic interactions with BPS-DMIP sorbents, since the residual water cannot be completely dried during the 30 min drying step. Further washing with acetonitrile can cause low recoveries of BPAF and BPB. Therefore, in order to achieve high cleanup efficiency rather than sacrificing the BPs recoveries, 2.5 mL of acetonitrile was selected. Higher elimination of BS-TM, DIEN and TCP should be achieved for further improvement of selectivity. The ultimate goal is to improve the imprinting factors of BPB and BPAF that have the lowest imprinted factors among the BPs.

3.3.4. Elution solvent selection

The final elution of BPs was conducted by using 5 mL methanol–acetic acid (98:2, v/v) and 4 mL methanol–TFA (99:1, v/v). Both elution solvents achieved BPs recoveries close to 100%. In order to shorten the drying time, 4 mL methanol–TFA (99:1, v/v) was selected.

3.4. Analysis of real water samples

To evaluate the applicability of the optimized DMISPE procedure to real water sample analysis, the precision and accuracy of the method were determined using ultrapure water, tap water and river water samples (500 mL) at three concentration levels (40, 200 and 1000 ng L^{-1}). The results are summarized in Table 2. The linearity of the established method was estimated in the range of 25–5000 ng L^{-1} with a correlation coefficient $r > 0.9999$ for all BPs. The limits of detections (LODs) ranged from 2.2 to 3.6 ng L^{-1} based on a signal-to-noise ratio of 3 and were lower than the earlier reported work [45]. The recoveries of BPs in these spiked samples ranged from 89.4 to 102.0% with the relative standard deviation (RSD, $n = 5$) less than 4.8%. The BPs recoveries in this study were much higher than the previously reported values [44]. The above results suggest that BPS-DMIP served as an efficient SPE sorbents for highly class-selective extraction of BPs from aqueous solutions.

Furthermore, to estimate the effect of BPS-DMIP on removal of impurities, both the washing and eluting fractions of river water sample were collected and analyzed by HPLC (Fig. 7). As was expected, the matrix components presented in river water samples were effectively removed in the selective washing step, giving a clean baseline for the chromatogram of the elution fraction of BPS-DMIP. The compounds with similar log K_{ow} values as BPs which may exist as interference peaks on a C18 HPLC column were effectively eliminated, paving the way for a highly accurate and sensitive

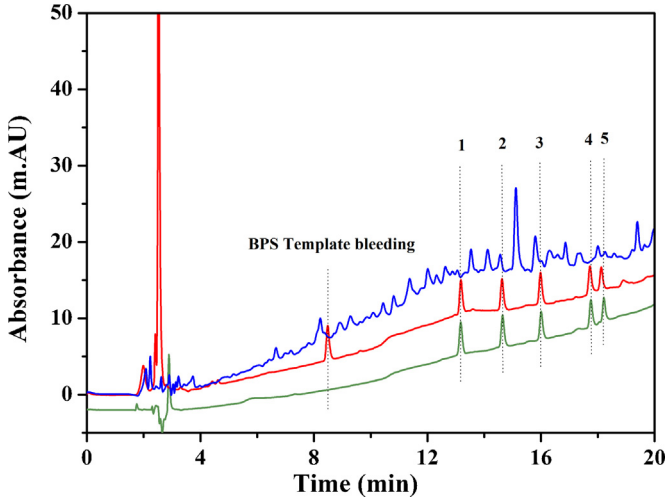

Fig. 7. HPLC-DAD chromatograms corresponding to river samples spiked with 200 ng L^{-1} of each analyte: (blue line) washing step of BPS-DMIP; (red line) elution step of BPS-DMIP; (green line) standard solution, sample: 500 mL of river water. Peaks: 1, BPF; 2, BPE; 3, BPA; 4, BPB; 5, BPAF. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

detection of BPs. BPS template bleeding was observed at similar concentration level with other BPs.

3.5. Comparison of DMISPE with commercial SPE cartridges

The low concentration of BPs in water samples necessitates detection at low UV wavelengths (210–235 nm). At these wavelengths, strong interferences from sample matrix are expected and enhanced purification prior to HPLC analysis becomes essential. Hence, investigation of highly selective material of BPs from complex matrices is of significant interest. In order to evaluate the real superiority of the BPS-DMIP for the selective enrichment of BPs in river water samples, the results obtained by BPS-DMIP were compared with those obtained by commercial sorbents.

The commercially available SPE cartridges tested were a reversed-phase column (Cleanert ODS C18, 12 mL, 500 mg, obtained from Agela Technologies, Beijing, China), a hydrophilic–hydrophobic balance column (OASIS HLB, 12 mL, 200 mg, obtained from Waters, Milford, MA, USA), and a strong cation exchanger column (Select SCX, 12 mL, 200 mg, obtained from Supelco, Bellefonte, PA, USA). The commercial SPE cartridges have been assessed under their own optimum conditions reported by the literatures [22,55].

In order to prove the superiority of the BPS-DMIP sorbents, the river water sample we collected was seriously polluted and the original color was light yellow. For the comparison, each cartridge was percolated with 500 mL river water sample spiked at 200 ng L^{-1} level of five BPs followed with their own washing and elution steps, and the eluents were dried under a stream of nitrogen gas and then reconstituted in 250 μL methanol:water (35:65, v/v). Fig. 8 shows the chromatograms of the elution fractions of the tested columns. In the chromatograms corresponding to the commercial sorbents, the signals of BPs were hard to identify due to the high chromatogram backgrounds and large interference peaks, making the quantification of BPs in such low concentration impossible. However, for the BPS-DMIP, all BPs were quantitatively detected, and the interference peaks were almost completely eliminated, giving a very clean baseline. These can be further confirmed by the color of the concentrated elution samples. As can be seen from the picture in Fig. 8, a colorless and transparent sample was obtained from BPS-DMIP sorbents while samples from other SPE sorbents were yellow to varying degrees. These results demonstrated the real superiority

Table 2
Average recoveries, relative standard deviations (RSDs, $n = 5$), limits of detections (LODs) and coefficients of correlation (r) obtained after solid-extraction of ultrapure water, tap water and river water spiked at 40, 200 and 1000 ng L^{-1} concentration levels.

Analyte	Spiking level (ng L^{-1})	Ultrapure water		Tap water		River water		LOD (ng L^{-1})	r
		Recovery (%)	RSD (%, $n = 5$)	Recovery (%)	RSD (%, $n = 5$)	Recovery (%)	RSD (%, $n = 5$)		
BPF	40	98.9	0.7	94.9	0.3	98.5	1.9	2.2	0.9999
	200	96.0	2.5	97.8	1.5	98.0	3.6		
	1000	96.2	1.7	97.8	1.9	98.4	3.7		
BPE	40	94.6	1.7	89.4	2.2	94.3	4.8	2.2	0.9999
	200	93.4	2.9	98.6	1.3	98.3	3.9		
	1000	96.9	2.1	98.8	2.2	99.6	3.6		
BPA	40	95.0	2.5	92.3	2.6	99.8	0.9	2.3	0.9999
	200	96.8	2.4	95.8	4.0	102.0	4.4		
	1000	97.2	0.5	98.1	2.1	101.0	4.1		
BPB	40	93.0	0.8	93.8	1.6	99.1	1.2	2.1	0.9999
	200	95.8	3.0	97.9	1.9	100.1	4.6		
	1000	96.7	0.3	96.1	2.4	98.5	2.6		
BPAF	40	96.3	1.3	94.3	2.3	96.2	3.2	3.6	0.9999
	200	94.4	2.7	98.3	4.2	94.5	3.7		
	1000	92.2	2.1	93.2	4.8	94.6	4.8		

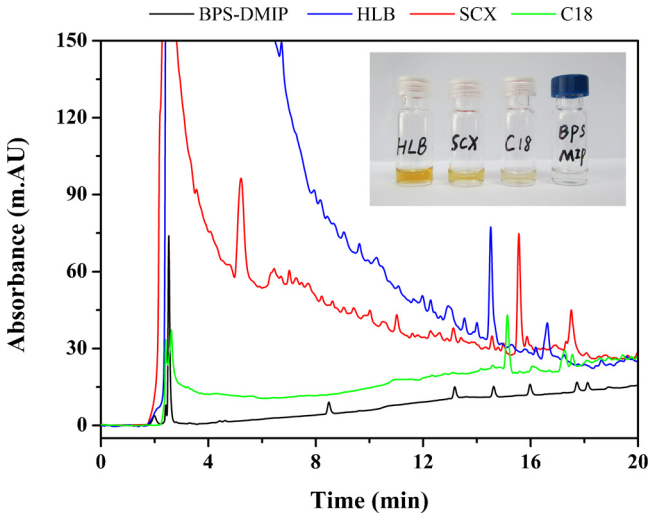

Fig. 8. HPLC-DAD chromatograms corresponding to river samples spiked with 200 ng L^{-1} of each analyte: (blue line) SPE with Oasis HLB; (red line) SPE with Select SCX; (green line) SPE with cleanert ODS C18; (black line) SPE with BPS-DMIP. Sample: 500 mL of river water. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of the BPS-DMIP for the selective enrichment of BPs from complex sample matrices. And the template bleeding problem was solved at the same time.

4. Conclusions

This work confirms the applicability of the NIP column method for the dummy template selection and polymer composition optimization. The imprinting effect of one DMIP can be predicted based on the non-imprinted capacity factors of the template. The high imprinting factor for the dummy template itself and the similarity of molecular structure between dummy template and target molecule are two vital factors for a successful dummy molecular imprinting process. Such high selective dummy molecularly imprinted polymer for BPs was prepared using BPS as template. The resulting BPS-DMIP had improved binding affinity and selectivity for BPs compared to other DMIPs reported previously. A method for extracting BPs from water samples with good recoveries and reproducibility was explored with BPS-DMIP as a specific DMISPE sorbent. The developed DMIP-SPE–HPLC method showed much higher sensitivity and selectivity than traditional SPE–HPLC methods for the analysis of complex samples such as river water.

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