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Highly class-selective solid-phase extraction of bisphenols in milk, sediment and human urine samples using well-designed dummy molecularly imprinted polymers☆

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ABSTRACT

Dummy molecularly imprinted polymers (DMIPs) towards bisphenols (BPs) were prepared employing 1,1,1-tris(4-hydroxyphenyl)ethane (THPE) and phenolphthalein (PP) as dummy templates. The selectivity of the resulting DMIPs was evaluated by high-performance liquid chromatography (HPLC). Both PP-DMIP and THPE-DMIP showed excellent class selectivity towards bisphenols. THPE-DMIP prepared using the template molecule with three hydroxyphenyl functionalities achieved higher imprinting factors (IF) for the bisphenols over a range of 7.9–19.8. An efficient approach based on dummy molecularly imprinted solid phase extraction (DMISPE) coupled with HPLC-DAD was developed for selective extraction of eight bisphenols in sediment, milk and human urine samples using THPE-DMIP as sorbents. The method showed good recoveries (82–102%) and precision (RSD 0.2–4%, $n = 3$) for these samples spiked at two concentration levels (25 and 250 ng g^{-1} or ng mL^{-1}). The detection limits ranged between 0.6 and 1.1 ng g^{-1} or ng mL^{-1}. Efficient removal of sample matrix and interferences was also achieved for these samples after DMISPE process. The results demonstrated great potential of the optimized methods for sample preparation in the routine analysis of trace BPs in complex samples.

1. Introduction

Bisphenol A (BPA) is an industrially important chemical that is abundantly and widely used as a primary raw material for the production of polycarbonate plastics, epoxy resins, and lacquer coatings [1]. The release of BPA into food and environment matrices [2] has drawn great attention all over the world because of its estrogenic and antiandrogenic activities. BPA has been reported to occur in various foodstuffs, environmental matrices and human samples [3–5]. Moreover, several chemicals that are structurally similar to BPA, with two hydroxyphenyl functionalities, have been used to perform the same function of BPA. The production and consumption of these bisphenol analogs such as bisphenol F and bisphenol S have increased recently [6]. Other bisphenols (BPs) like bisphenol AF, bisphenol Z, bisphenol AP bisphenol E and bisphenol B were also used in foodstuffs [7].

Several methods for quantitative analysis of bisphenols have been developed such as HPLC-UV [8], HPLC-FLD [9] and LC-MS/MS [10–13]. For the analysis of complex samples, the methods generally require a sample pretreatment step to separate and/or pre-concentrate the analyte prior to analysis. Solid-phase extraction (SPE) is an effective sample treatment technique for BPs analysis in view of its high enrichment efficiency. However, the application of traditional sorbents is to some extent limited due to their inefficient selectivities. The use of molecularly imprinted polymers (MIPs) as SPE sorbents allows not only pre-concentration and cleanup of the sample but also selective extraction of the target analyte, which are particularly important when the sample is complex and the impurities can interfere with quantification.

Generally, MIPs are obtained by polymerizing functional monomers and cross-linkers around template molecules, leading to highly cross-linked three-dimensional network polymers. The resulting imprinted polymers have high selectivity toward template molecules and are stable, robust and resistant to a wide range of pH, solvent and temperature [14]. However, at present, molecular imprinting still faces great challenges relating to its application involving molecularly imprinted solid-phase extraction (MISPE),

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such as template leakage and low binding capacity [15]. In this paper, we mainly focus on the template bleeding problem.

During the imprinting process, imprinted sites are formed not only on the surface but also deeply in the cross-linked polymer network structure, where organic solvent for removing template can hardly reach [16]. Thus, the possible leakage of template molecules even after exhaustive washing steps can happen and cause a serious impact on the accuracy of an analytical method, especially for trace analysis [14,17]. This problem can be solved by use of a dummy template [18] as any leakage will be different from the analyte [19], and the resulting MIP is defined as "dummy molecularly imprinted polymer (DMIP)". Up to now, structurally related analogs [20–24], fragments [25,26] and isotope labeled compounds [27] such as 3,3′,5′5-tetrabromobisphenol A (TBBPA), BPF, 2,6-bis(trifluoromethyl)benzoic acid (BTFB), *p-tert*-butylphenol (PTBP) and [2H16]bisphenol A (BPA-d16) have been reported for BPs imprinting. However, most of these DMIPs show much lower imprinting efficiency toward BPA as compared to the BPA-MIP, and their selectivities for other BPs were rarely investigated. Although the BPA-d16 imprinted material showed remarkable recognition ability toward BPA, it had been limited by the high cost and limited availability of mass spectrometric (MS) detection. The select of dummy template with high imprinting factors for a group of BPs as well as low cost was of great challenge due to the lack of effective screening method [28].

Previously, we proposed a simple and fast screening method for dummy templates by combing the non-imprinted polymer (NIP) column method and the computational modeling of molecular structure [29]. In that work, the selected BPS-template DMIP achieved high affinities towards BPs. And the imprinting factors (IFs) achieved were much higher than those reported in the literatures [30,31]. However, despite its ultra high selectivity for BPF, BPE and BPA, BPS-DMIP showed low efficiency for imprinting BPB and BPAF due to their larger molecular sizes. Moreover, BPS was one of the most important substitutions of BPA, and determination of BPS in environmental and biological samples should also be very meaningful. Therefore, for practical application of DMIPs in the class detection of BPs, further work still needed to be done for screening dummy templates that do not belong to BPs but guarantee higher class-selectivity for the entire BPs.

In this paper, two structural analogs of BPs named 1,1,1-tris(4-hydroxyphenyl)ethane (THPE) and phenolphthalein (PP) were selected as the dummy template molecules for BPs imprinting. The class-selectivity and binding affinity of the prepared polymers were examined using the chromatographic and binding experiments. The polymer with higher recognition ability for BPs was used as the selective extraction sorbents for BPs from sediment, milk and human urine samples. The selectivity, accuracy and precision of the developed method were also evaluated.

2. Experimental

2.1. Chemicals and reagents

1,1,1-Tris(4-hydroxyphenyl)ethane (THPE), phenolphthalein (PP), bisphenol F (BPF), bisphenol S (BPS), bisphenol E (BPE), bisphenol A (BPA), bisphenol B (BPB), bisphenol AF (BPAF), bisphenol AP (BPAP), bisphenol Z (BPZ), 2,2′,6,6′-tetrameth-yl-4,4′-sulfonyldiphenol (BS-TM), dienestrol (DIEN), diethylstilbestrol (DES), 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 tetrabromobisphenol A (TBBPA) were supplied by Aladdin Chemical (Shanghai, China). 4-Vinylpyridine (4-VP) and 2,4,6-trichlorophenol (TCP) were obtained from Acros (NJ, USA). HPLC grade acetonitrile, methanol and formic acid were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

2.2. Preparation of imprinted and non-imprinted polymers

Dummy molecularly imprinted polymers (DMIPs) were synthesized by the method described previously [29]. Briefly, 1,1,1-tris(4-hydroxyphenyl)ethane (THPE) and phenolphthalein (PP) were used as dummy templates, with 4-vinylpridine as functional monomer and acetonitrile as polymerization solvent. Non-imprinted polymers (NIPs) were obtained by performing the same procedure in the absence of template molecules.

2.3. Chromatographic evaluation of the prepared polymers

The DMIPs and NIP particles were slurry-packed in methanol into stainless steel HPLC columns (100 mm × 4.6 mm id) at 3000 psi using ethanol as the pushing solvent. The analyses were carried out using BPs dissolved in acetonitrile on an HPLC system which included a manual injector (Rheodyne, 7725, Park Court, CA, USA), a Waters 515 HPLC pump and a Waters 2487 dual wavelength absorbance detector (Milford, MA, USA). Acetonitrile at a flow rate of 1 mL min^{-1} was used as mobile phase and 20 μL of the analyte (20 ppm) was injected for analysis. The UV detector was set at 220 nm. 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 for the imprinted and non-imprinted polymers, respectively.

2.4. Binding experiments

The binding capacities and dissociation constants of THPE-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 acetonitrile. One milliliter aliquots of each solution were mixed with 20 mg of THPE-DMIP particles in a 10 mL flask. The mixtures were incubated at 150 rpm for 24 h (25 °C) in a water bath, and then rapidly filtrated. The BPA concentration in the filtrate was measured by HPLC.

The adsorption capacity and dissociation constant K_d (mmol L^{-1}) were calculated according to the Eqs. (1) and (2) [32]:

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

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

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. DMISPE procedures

Solid-phase extraction cartridges with a 3 mL volume were packed with 200 mg of the THPE-DMIP and NIP sorbents.

2.5.1. DMISPE of spiked sediment sample

Sample extraction was based on the method proposed by Liao et al. [12]. In brief, 2 g of spiked sediment was extracted with 5 mL of methanol–water mixture (5:3, v/v) by shaking for 60 min. After centrifugation (4500 g for 5 min; Sorvall Biofuge Stratos,

Thermoelectron LED GmbH, D-37520, Osterode, Germany), the supernatant was transferred into a glass tube. The extraction step was repeated twice, and the extracts were combined and concentrated to ~ 4 mL under a gentle stream of nitrogen. After dilution to 10 mL with ultrapure water, the extract was loaded onto the THPE-DMIP cartridge (preconditioned sequentially with 3 mL of acetonitrile and 3 mL of water). The cartridge was then rinsed with 3 mL of water and dried under vacuum for 30 min. After drying, the cartridge was further washed with 5 mL of acetonitrile and eluted with 6 mL of methanol–trifluoroacetic acid (98: 2, v/v). The eluent was dried under a stream of nitrogen gas and then reconstituted in 0.5 mL methanol–water (35: 65, v/v). An aliquot of 20 μL was injected into the HPLC system for analysis.

2.5.2. DMISPE of spiked milk sample

Sample extraction was based on the method proposed by Cao et al. [33]. In brief, 2 g of spiked milk (3.5% fat) was deproteinated by addition of 4 mL of acetonitrile. After vortexing for 1 min, the sample was then centrifuged at 3000 g for 5 min at 4 °C. The supernatant was transferred into a glass tube. The extraction step was repeated twice, and the extracts were combined and concentrated to ~ 4 mL under a gentle stream of nitrogen. After dilution to 10 mL with ultrapure water, the extract was loaded onto the THPE-DMIP cartridge. The following washing and elution steps are the same with sediment preparation.

2.5.3. DMISPE of spiked human urine

After collection, human urine was centrifuged at 5000 g for 10 min to eliminate precipitates. 2 mL of spiked human urine sample (adjusted to pH 12.0 by dilute sodium hydroxide) was directly applied to the THPE-DMIP cartridge. The cartridge was then rinsed with 2 mL of water (pH = 12.0) and dried for 30 min. After further washing with 2 mL of acetonitrile, the cartridge was eluted with 6 mL of methanol–trifluoroacetic acid (98:2, v/v). The eluent was dried under a stream of nitrogen gas and then reconstituted in 0.5 mL of 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 system (Agilent Technologies, Palo Alto, CA, USA) equipped with a vacuum degasser, a quaternary pump, an autos ampler 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.

Table 1
The non-imprinted capacity factors (k_{NIP}) and imprinting factors (IF) of the prepared DMIPs toward dummy template, bisphenols (BPs) and other structurally related compounds.

Analyte	k_{NIP}	$IF_{BPS-DMIP}$[a]	$IF_{PP-DMIP}$	$IF_{THPE-DMIP}$
THPE	3.67			33.4
PP	1.66		22.7	
BPAP	1.59	–	11.4	19.8
BPB	1.49	5.7	7.8	17.4
BPA	1.49	8.7	8.1	16.4
BPAF	1.37	4.2	10.6	14.4
BPZ	1.62	–	8.0	11.6
BPE	1.60	13.8	6.1	10.8
BPS	3.97	34.6	6.5	8.5
BPF	1.79	14.5	4.5	7.9
TBBPA	3.7	3.9	12.9	2.2
DIEN	1.46	2.6	2.0	3.2
BS-TM	0.60	2.9	2.1	1.8
CAT	1.57	1.7	3.0	1.4
TCP	2.25	3.3	1.7	1.2

[a] Data from reference [29].

3. Results and discussion

3.1. Preparation and evaluation of DMIPs

3.1.1. Dummy template selection and chromatographic evaluation

As we summarized before, both of the structural similarities between the dummy template and target molecules and the imprinting effect for the dummy template were important for a successful dummy molecularly imprinting process [29]. High non-imprinted capacity factor (k_{NIP}) of dummy template obtained using porogen solvent as the mobile phase can result in high imprinting factor (IF) for the dummy template itself.

The alternative dummy templates PP and THPE were first selected based on their molecular structures. The optimized three-dimensional structures of PP, THPE and BPA are shown in Fig. 1. Both THPE and PP are structurally close to BPA with similar distance between two hydroxyl groups. Moreover, larger groups were found on the middle carbon of PP and THPE. THPE has a symmetrical structure with three hydroxyphenyl functionalities. The imprinting effects of PP-DMIP and THPE-DMIP for the dummy template itself were predicted by running PP and THPE on the non-imprinted column using acetonitrile as the mobile phase. The k_{NIP} values for PP and THPE were then calculated as described in Section 2.3. As shown in Table 1, the k_{NIP} values of THPE and PP were 3.67 and 1.66, respectively, both higher than BPA (k_{NIP} = 1.49 [29]). So, it was expected that higher imprinting factors can be achieved for THPE-DMIP and PP-DMIP as compared to BPA-MIP (IF = 14.7 [29]). Moreover, since there exist large groups on the middle carbon of PP

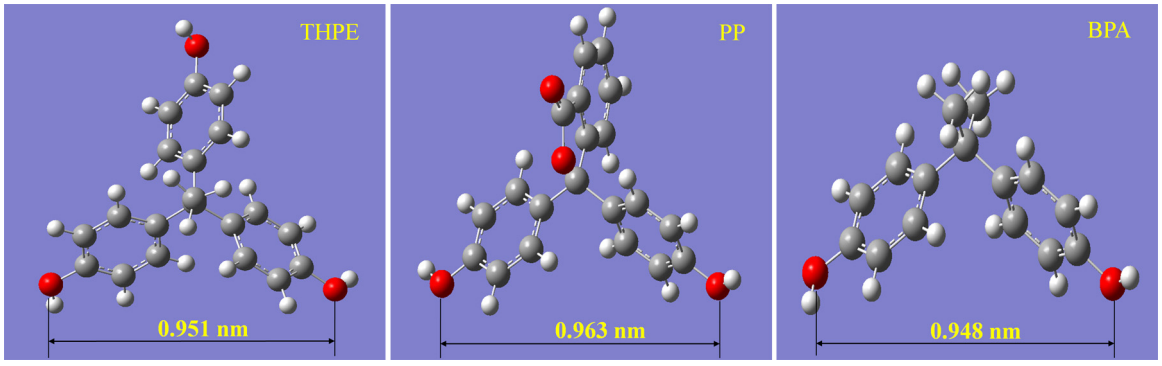

Fig. 1. Three-dimensional structures of dummy templates and BPA. The stable structure in vacuum was calculated by the molecular modeling software, Gaussian 09.

and THPE, good selectivity for large BPs may be generated by using THPE and PP as dummy template.

To demonstrate the practicability and feasibility of the prediction method, PP-DMIP and THPE-DMIP were then prepared and tested. The selectivity of THPE-DMIP and PP-DMIP were evaluated with HPLC using acetonitrile as the mobile phase. The achieved non-imprinted retention factors and imprinted factors toward dummy template, BPs and structurally related compounds are listed in Table 1. The structures of the chemicals used are shown in Fig. 2.

As can be seen in Table 1, the IF values of PP-DMIP and THPE-DMIP were 22.7 and 33.4, which were indeed much higher than BPA-MIP. Such results further confirmed the effectiveness of the NIP column method. Furthermore, the dummy molecular imprinting effect of PP-DMIP and THPE-DMIP toward BPs were also studied (Table 1). Good class-selectivity of both PP-DMIP and THPE-DMIP for BPs were obtained as compared to other structurally related compounds (such as DES, BS-TM, CAT and TCP). Compared to BPS-DMIP, more excellent recognition abilities for the large BPs (such as BPB and BPAF) were obtained on PP-DMIP and THPE-DMIP. The lowest imprinting factors of PP-DMIP and THPE-DMIP were 4.5 and 7.9 for BPF, respectively, both of which are higher than that of BPS-DMIP (4.2 for BPAF). So the overall class-selectivity of PP-DMIP and THPE-DMIP were comparable or higher than BPS-DMIP. Furthermore, PP was cheaper and safer than BPS, and both PP and THPE were non-bisphenol compounds. Therefore, PP and THPE were better choices for the dummy imprinting of BPs than BPS and other reported dummy templates [30,31]. To achieve the highest class-selectivity of BPs, THPE was selected for the further investigation.

3.1.2. Binding capacities and affinities

Both equilibrium binding experiments and Scatchard analysis were used to investigate the binding properties of the obtained THPE-DMIP and NIP. 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 THPE-DMIP and NIP sorbents. THPE-DMIP displayed an obviously higher capacity than NIP. Moreover, in Fig. 3B, two different slopes were observed corresponding to the high and low affinity binding sites. This indicates that the binding sites in THPE-DMIP are heterogeneous. The corresponding dissociation constants (K_{d12}) and saturation capacity (Q_{max12}) values were 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 0.107 mmol L^{-1} and 4.512 μmol g^{-1} for the high-affinity binding sites, and 4.226 mmol L^{-1} and 48.76 μmol g^{-1} for the low-affinity binding sites, respectively.

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 mean higher binding affinity and capacity. The affinity of high sites for THPE-DMIP (K_{d1} = 0.107 mmol L^{-1}) was about one half of the reported BPS-DMIP (K_{d1} = 0.205 mmol L^{-1}) [29] and BPE-DMIP (K_{d1} = 0.221 mmol L^{-1}) [30] towards BPA. Although the capacity of high sites for THPE-DMIP (Q_{max1} = 4.512 μmol g^{-1}) is a little lower than BPS-DMIP (Q_{max1} = 5.900 μmol g^{-1}), it is still high enough for trace analysis of BPs. The ultra high binding affinity of THPE-DMIP allows stronger washing protocol during the SPE process, thus removing the interferences from sample matrices more effectively.

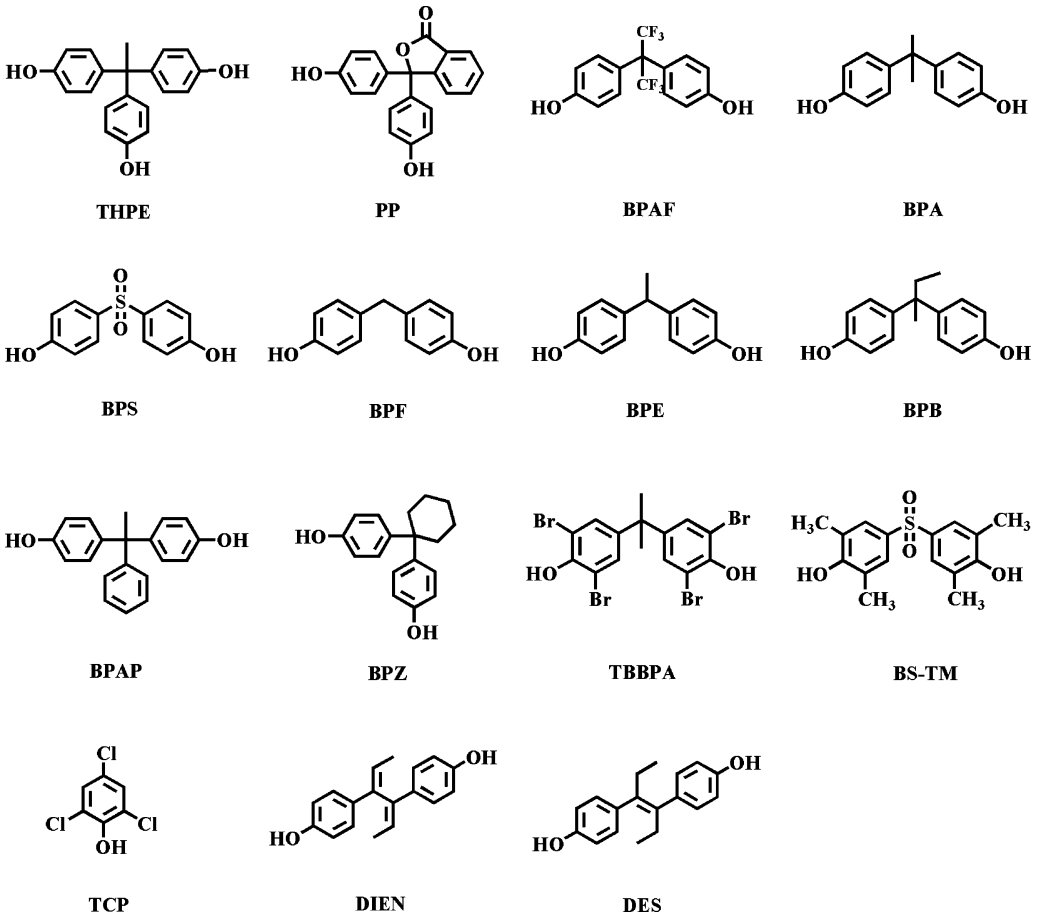

Fig. 2. Schematic molecular structures of the investigated compounds.

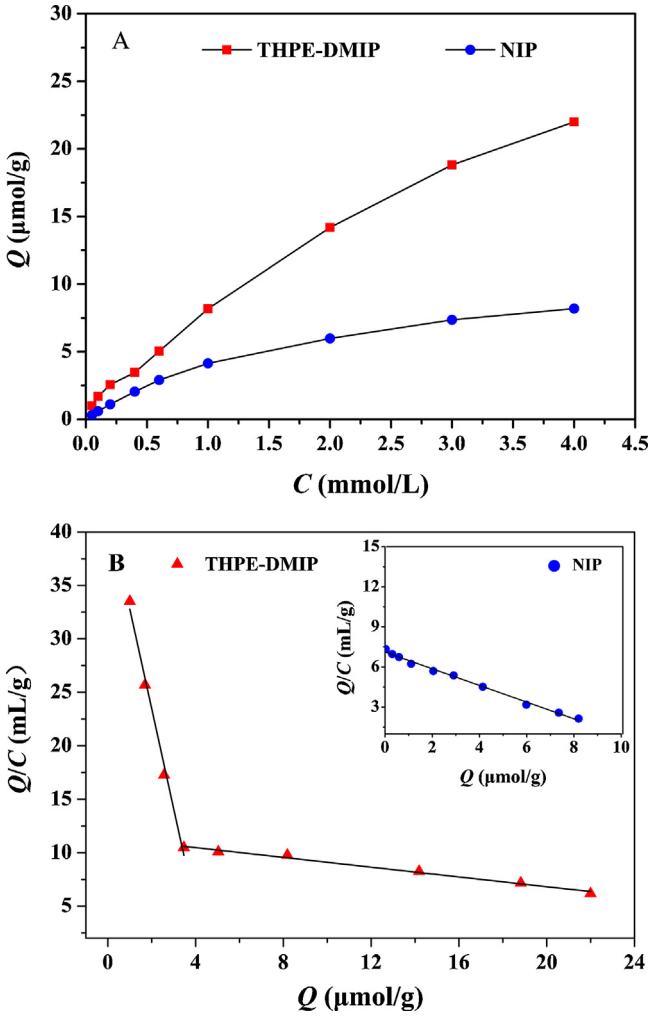

Fig. 3. (A) Adsorption isotherms of THPE-DMIP and NIP for BPA; (B) Scatchard plot analysis of BPA binding to the THPE-DMIP and NIP. Sorbent weight: 20 mg.

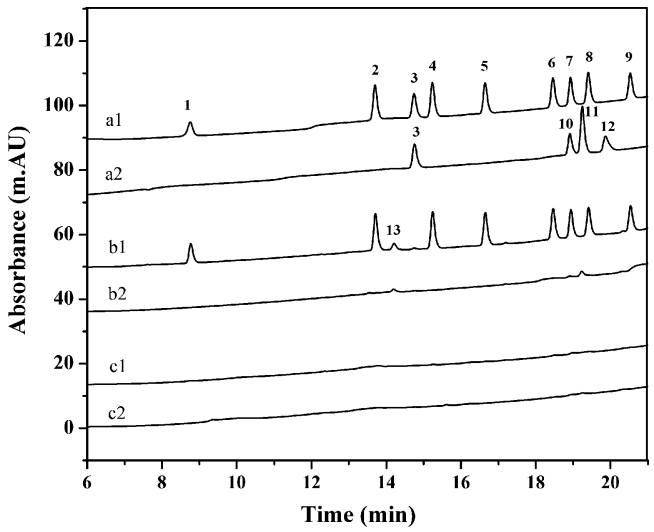

Fig. 4. HPLC-DAD chromatograms obtained at 225 nm. (a1) S1; (a2) S2; (b1) elution fraction of S1 from the THPE-DMIP; (b2) elution fraction of S2 from the THPE-DMIP; (c1) elution fraction of S1 from the NIP; (c2) elution fraction of S2 from the NIP. 1, BPS; 2, BPF; 3, BS-TM; 4, BPE; 5, BPA; 6, BPB; 7, BPAF; 8, BPAP; 9, BPZ; 10, DES; 11,DIEN; 12, TCP; 13, THPE. Conditions: loading, 10 mL of ultrapure water fortified with S1 or S2 (0.5 μg of each compound), washing solvent, 5 mL of acetonitrile; elution solvent, elution, 6 mL methanol–trifluoroacetic acid (98: 2, v/v).

3.2. Optimization of the DMISPE conditions

The factors for optimizing the DMISPE procedure include sample loading pH and volume, volume of washing solvent and the composition and volume of eluting solvent. For all the steps, the DMISPE column packed with 200 mg of THPE-DMIP was used.

3.2.1. Sample loading pH and volume

The spiked aqueous solutions (Milipore water) were adjusted to pH 3.0, 6.0, 9.0 and 12.0 by dilute hydrochloric acid or sodium hydroxide. Different volumes (3–20 mL) of these solutions were percolated through the THPE-DMIP cartridges. It was found that, the recoveries of BPs were close to 100% up to 20 mL in the pH range of 3.0–9.0. However, the breakthrough of BPS, BPF, BPE and BPA happened at pH 12. This was because the bisphenols were completely ionized and existed as BPs sodium salt at pH 12. After the ionization, the polarities of BPs increased, thereby decreasing the hydrophobic interactions between BPs and THPE-DMIP in the loading step. BPS and BPF with lowest log K_{ow} values (1.387 and 2.764, respectively) have the breakthrough volumes of approximately 3 mL and 10 mL, as shown in Fig. S1.

3.2.2. Washing solvent selection

Following the loading on DMI-SPE, 3 mL of water was used as washing solvent in the first washing step. This was used to remove sample constituents remaining on cartridge and also to washout the high polar interferences from the sample matrix. In the subsequent step, the SPE column was dried with the aid of a peristaltic pump for 30 min. As it has been reported, residual water on the cartridge can reduce the selectivity of MIPs toward target molecules due to the non-specific hydrophobic binding [17]. After the column was dried, a selective washing step was applied. This step was optimized in order to get the maximum selectivity of the MIP and to remove the interferences as many as possible. In the present work, acetonitrile was used as a selective washing solvent.

To estimate the selectivity of the DMISPE process, BPs and their structurally related compounds (BS-TM, DES, DIEN and TCP) were all tested. Both of DES and DIEN were hard to separate with BPAF and BPAP. So the analytes were separated into two series: standard series 1 (S1: BPS, BPF, BPE, BPA, BPB, BPAF, BPAP, BPZ and BS-TM) and standard series 2 (S2: BS-TM, DES, DIEN and NP). 10 mL of ultrapure water, fortified with S1 and S2 (0.5 μg of each compound), respectively, were loaded onto the THPE-DMIP/NIP cartridges. Different volumes (3 mL, 4 mL, 5 mL, 5.5 mL and 6 mL) of acetonitrile were applied in the washing step. In order to achieve high cleanup efficiency rather than sacrificing the recoveries of BPs, 5 mL of acetonitrile was finally selected (See Fig. S2).

After selective washing (5 mL of acetonitrile), the elution fractions of S1 and S2 on both THPE-DMIP and NIP were collected and analyzed by HPLC. The chromatograms are shown in Fig. 4. While all BPs and structurally related compounds were effectively washed off from the NIP cartridge, all BPs were selectively retained on the THPE-DMIP cartridge. DES and DIEN with similar retention times of BPAF and BPAP were also eliminated. The extraction recoveries for the bisphenols on the NIP and THPE-DMIP columns were in the range of 0.6 – 2.4% and 91 – 100%, respectively (Fig. 5). Meanwhile, low recoveries for BS-TM, DES, DIEN and TCP were obtained using either NIP (0.7–2.7%) or THPE-DMIP (3.5–7.9%) columns. These results are in agreement with those obtained in the HPLC evaluation experiments described in Section 3.1.2. The improved dummy molecularly imprinting effect of THPE-DMIP compared to BPS-DMIP [29] allowed higher removal efficiency of BS-TM, DES, DIEN and TCP on the imprinted column by using a stronger washing protocol.

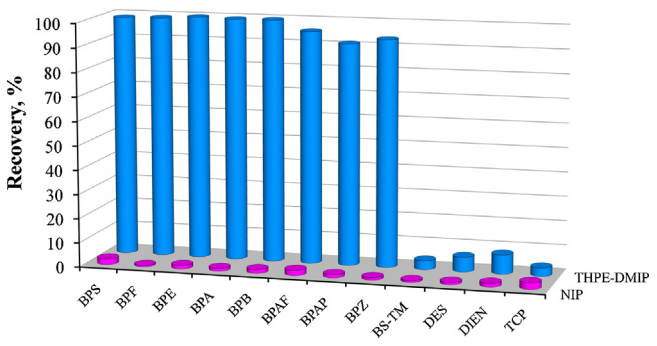

Fig. 5. Extraction recoveries (%) obtained with THPE-DMIP and NIP cartridges for eight BPs after percolation of 10 mL of pure water containing 0.5 μg of each compound using a washing step with 3 mL water and 5 mL acetonitrile.

3.2.3. Elution solvent selection

To optimize the volume of elution solvent, 10 mL of ultrapure water fortified with 0.5 μg of BPS, BPF, BPE, BPA, BPB, BPAF, BPAP, and BPZ were loaded onto the THPE-DMIP cartridge. Different elution volumes (4 mL, 5 mL and 6 mL) of methanol–TFA (98: 2, v/v) were tested. It was found that a volume of 6.0 mL was sufficient to desorb the trapped BPs from the DMISPE column. The use of less elution volume resulted in low recovery of BPAP (See Fig. S3).

3.3. Selective extraction of eight BPs from complex matrices

3.3.1. Matrix effects

After extraction, the concentrated extracts of spiked sediment and milk samples were diluted to 10 mL with ultrapure water and then directly applied to the DMISPE cartridges. Their matrix effects were then evaluated using the optimized washing protocol (see Section 3.3.2). The chromatograms of directly extracted sediment and milk samples are shown in Fig. 6 A and B, together with their spiked samples (both 25 and 250 ng g^{-1} or ng mL^{-1}) after DMISPE. The directly extracted sediment sample showed high background interferences when the retention time was from 15 to 20 min which can have a big influence on the quantitative analysis of BPs. Meanwhile, the background for milk sample was relatively clean. THPE-DMIP extracts for sediment and milk samples at both high and low spiked levels were very clean and, particularly, free from matrix interferences. These results compare favorably with those reported by other authors for BPs-MISPE [30,34]. Moreover, the template bleeding from THPE-DMIP at a sub-ng g^{-1} or ng mL^{-1} level was observed in the chromatographic experiments (Fig. 5, 6A and 6B). THPE bleeding can be detected by HPLC even after 20 time use of the column (Fig. 7). Here, the template bleeding problem which may have significant influence on the quantitative analysis of BPs was successfully solved by using THPE as dummy template.

The purification of human urine sample was not satisfactory using the optimized loading and washing conditions, thus necessitating further study on the removal of impurities. As can be seen in Fig. 7, the chromatograms of spiked human urine samples after DMISPE at loading pH of 6 and 9 showed poor cleanup efficiencies. As a result, complex sample matrices in urine affected the quantification of BPs. A notable phenomenon during these DMIPE processes was that a large amount of colored urinary impurities adsorbed on THPE-DMIP cartridges during the loading step. Most of these colored impurities cannot be removed during the washing step and can be easily eluted. According to Section 3.3.2, 5 mL of acetonitrile was used in the selective washing step, and neutral and low acidic impurities even with very similar molecular structure can be easily washed out. However, it was hard to remove the impurities with low pK_a values that can form strong interactions with 4-VP via acid-base interactions. When a pH value of 12 was used for

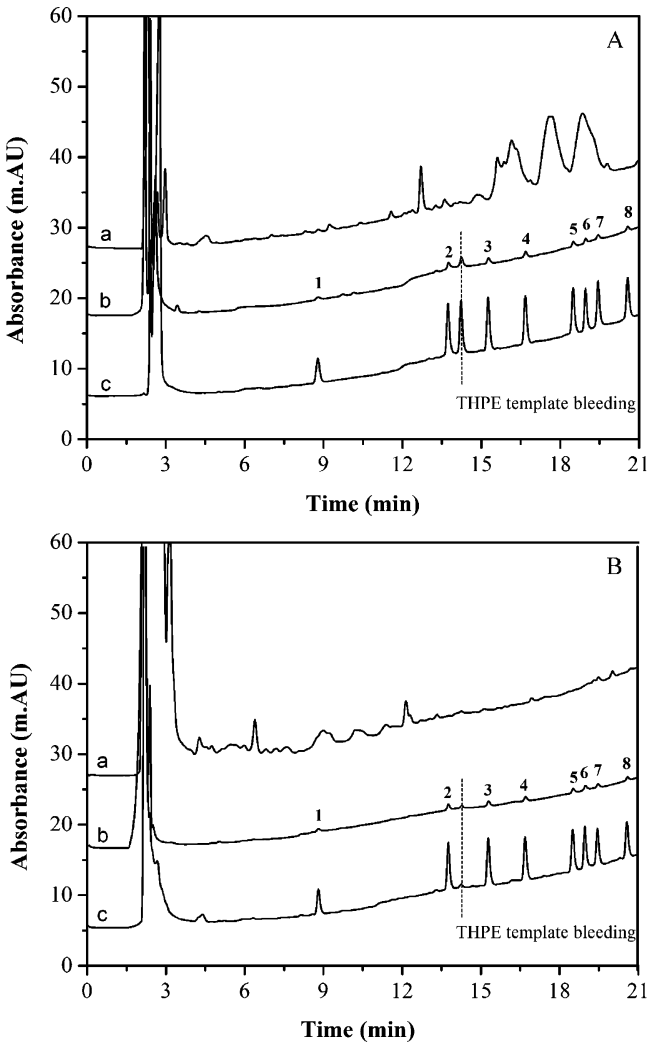

Fig. 6. HPLC-DAD chromatograms obtained at 225 nm. (a) Directly extracted samples; (b) spiked samples (25 ng g^{-1}) after DMISPE; (c) spiked samples (250 ng g^{-1}) after DMISPE. (A) Sediment sample; (B) milk sample. Peaks: 1, BPS; 2, BPF; 3, BPE; 4, BPA; 5, BPB; 6, BPAF; 7, BPAP; 8, BPZ.

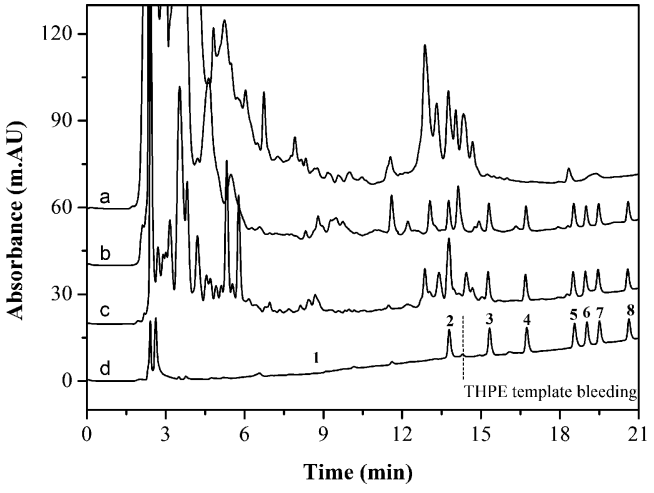

Fig. 7. HPLC-DAD chromatograms obtained at 225 nm. (a) directly injected human urine sample; (b) spiked human urine sample (250 ng mL^{-1}) after DMISPE at loading pH 6; (c) loading pH 9; (d) loading pH 12. Peaks: 1, BPS; 2, BPF; 3, BPE; 4, BPA; 5, BPB; 6, BPAF; 7, BPAP; 8, BPZ.

Table 2
Average recoveries, relative standard deviations (RSDs, $n = 3$), limits of detections (LODs) and coefficients of correlation (r) obtained after solid-extraction of sediment, milk and human urine samples spiked at 25 and 250 ng g^{-1} or ng mL^{-1} concentration levels.

Analyte	Spiking level[a]	Sediment		Milk		Human urine		LOD[a]	r
		Recovery (%)	RSD(%,$n = 3$)	Recovery (%)	RSD(%,$n = 3$)	Recovery (%)	RSD(%,$n = 3$)		
BPS	25	99	0.5	96	2	–	–	0.6	0.9999
	250	100	1	95	0.7	–	–		
BPF	25	96	2	95	0.3	94	0.5	1.1	0.9999
	250	93	1	93	4	90	3		
BPE	25	95	1	92	0.8	88	2	0.7	0.9999
	250	95	0.9	94	2	90	0.7		
BPA	25	101	3	96	0.4	97	1	0.9	0.9999
	250	98	0.6	96	0.6	92	3		
BPB	25	98	0.4	97	3	90	2	1.0	0.9999
	250	98	2	96	1	94	4		
BPAF	25	92	1	98	3	98	3	0.6	0.9999
	250	94	0.6	96	3	97	2		
BPAP	25	95	0.5	84	0.8	82	1	0.6	0.9999
	250	97	2	86	3	85	2		
BPZ	25	99	0.2	88	1	93	3	0.6	0.9999
	250	102	0.8	96	2	91	0.7		

[a] The units of spiking level and LOD are ng g^{-1} for sediment and milk samples and ng mL^{-1} for human urine sample.

the loading of urine sample, most of the colored impurities were not retained by the column, and further washing with 2 mL water (pH = 12) and 2 mL acetonitrile resulted in a very clean baseline. This may be due to the ionization of the acid impurities under basic conditions, which greatly increased their polarities and thus made them easily breakthrough from the column in the loading step. The only drawback of this procedure was the inability to extract BPS. As we discussed in Section 3.3.1, the breakthrough problem happened especially for the BPS with the lowest logK_{ow} value.

3.3.2. Validation

To evaluate the applicability of the optimized DMISPE procedure for real sample analysis, the precision and accuracy of the method were determined using sediment, milk and human urine samples spiked at two concentration levels (25 and 250 ng g^{-1} or ng mL^{-1}). The results are summarized in Table 2. The linearity of the established method was estimated in the range of 5–2500 ng g^{-1} or ng mL^{-1} with a correlation coefficient $r > 0.9999$ for all BPs. The limits of detections (LODs) ranged from 0.6 to 1.1 ng g^{-1} or ng mL^{-1} based on a signal-to-noise ratio of 3, and were lower than those reported in other work [35]. The recoveries of BPs in these spiked samples ranged from 82–102% with the relative standard deviation (RSD, $n = 3$) less than 4%. The BPs recoveries in this study were higher than the previously reported values [36,37]. The above results suggest that THPE-DMIP served as an efficient SPE sorbents for highly class-selective extraction of BPs from complex matrices.

4. Conclusions

In this study, highly selective dummy templates for BPs imprinting were carefully selected. THPE-DMIP with superior imprinting factors for BPs were synthesized and used as sorbents for DMISPE of BPs from sediment, milk and human urine samples. Due to high selectivity of the optimized washing step, BPs in these complex matrices were selectively isolated and matrix interferences were eliminated. The high extraction efficiency of DMISPE for different complex matrices suggested that it is a practicable solution for sample preparation in routine analysis of trace BPs in environmental and biological samples.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2014.07.055.

References

[1] M. Chen, M. Ike, M. Fujita, Acute toxicity, mutagenicity, and estrogenicity of bisphenol-A and other bisphenols, Environ. Toxicol. 17 (2002) 80–86.
[2] A.V. Krishnan, P. Stathis, S.F. Permuth, L. Tokes, D. Feldman, Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving, Endocrinology 132 (1993) 2279–2286.
[3] L.N. Vandenberg, R. Hauser, M. Marcus, N. Olea, W.V. Welshons, Human exposure to bisphenol A (BPA), Reprod. Toxicol. 24 (2007) 139–177.
[4] Y. Huang, C. Wong, J. Zheng, H. Bouwman, R. Barra, B. Wahlström, L. Neretin, M. Wong, A. Bisphenol, (BPA) in China: a review of sources, environmental levels, and potential human health impacts, Environ. Int. 42 (2012) 91–99.
[5] L.N. Vandenberg, I. Chahoud, J.J. Heindel, V. Padmanabhan, F.J. Paumgartten, G. Schoenfelder, Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A, Environ. Health Perspect. 118 (2010) 1055–1070.
[6] E. Danzl, K. Sei, S. Soda, M. Ike, M. Fujita, Biodegradation of bisphenol A, bisphenol F and bisphenol S in seawater, Int. J. Environ. Res. Public Health 6 (2009) 1472–1484.
[7] C. Liao, K. Kannan, Concentrations and profiles of bisphenol A and other bisphenol analogues in foodstuffs from the United States and their implications for human exposure, J. Agric. Food Chem. 61 (2013) 4655–4662.
[8] T. Yoshida, M. Horie, Y. Hoshino, H. Nakazawa, Determination of bisphenol A in canned vegetables and fruit by high performance liquid chromatography, Food Addit. Contam. 18 (2001) 69–75.
[9] Q. Xiao, Y. Li, H. Ouyang, P. Xu, D. Wu, High-performance liquid chromatographic analysis of bisphenol A and 4-nonylphenol in serum, liver and testis tissues after oral administration to rats and its application to toxicokinetic study, J. Chromatogr. B 830 (2006) 322–329.
[10] R. Carabias-Martinez, E. Rodriguez-Gonzalo, P. Revilla-Ruiz, Determination of endocrine-disrupting compounds in cereals by pressurized liquid extraction and liquid chromatography-mass spectrometry. Study of background contamination, J. Chromatogr. A 1137 (2006) 207–215.
[11] Y. Yang, L. Lu, J. Zhang, Y. Yang, Y. Wu, B. Shao, Simultaneous determination of seven bisphenols in environmental water and solid samples by liquid chromatography-electrospray tandem mass spectrometry, J. Chromatogr. A 1328 (2014) 26–34.
[12] H.C. Zhang, X.J. Yu, W.C. Yang, J.F. Peng, T. Xu, D.Q. Yin, X.L. Hu, MCX based solid phase extraction combined with liquid chromatography tandem mass spectrometry for the simultaneous determination of 31 endocrine-disrupting compounds in surface water of Shanghai, J. Chromatogr. B 879 (2011) 2998–3004.
[13] C. Liao, F. Liu, H.B. Moon, N. Yamashita, S. Yun, K. Kannan, Bisphenol analogues in sediments from industrialized areas in the United States, Japan, and Korea: spatial and temporal distributions, Environ. Sci. Technol. 46 (2012) 11558–11565.

[14] F.G. Tamayo, E. Turiel, A. Martin-Esteban, Molecularly imprinted polymers for solid-phase extraction and solid-phase microextraction: recent developments and future trends, J. Chromatogr. A 1152 (2007) 32–40.
[15] A. Martín-Esteban, Molecularly-imprinted polymers as a versatile, highly selective tool in sample preparation, Trends Anal. Chem. 45 (2013) 169–181.
[16] J. Haginaka, H. Sanbe, Uniform-sized molecularly imprinted polymers for 2-arylpropionic acid derivatives selectively modified with hydrophilic external layer and their applications to direct serum injection analysis, Anal. Chem. 72 (2000) 5206–5210.
[17] E. Caro, R. Marce, F. Borrull, P. Cormack, D. Sherrington, Application of molecularly imprinted polymers to solid-phase extraction of compounds from environmental and biological samples, Trends Anal. Chem. 25 (2006) 143–154.
[18] M.D. Barton, Antibiotic use in animal feed and its impact on human health, Nutr. Res. Rev. 13 (2000) 279–300.
[19] L. Andersson, A. Paprica, T. Arvidsson, A highly selective solid phase extraction sorbent for pre-concentration of sameridine made by molecular imprinting, Chromatographia 46 (1997) 57–62.
[20] W. Zhan, F. Wei, G. Xu, Z. Cai, S. Du, X. Zhou, F. Li, Q. Hu, Highly selective stir bar coated with dummy molecularly imprinted polymers for trace analysis of bisphenol A in milk, J. Sep. Sci. 35 (2012) 1036–1043.
[21] W. Zhao, N. Sheng, R. Zhu, F. Wei, Z. Cai, M. Zhai, S. Du, Q. Hu, Preparation of dummy template imprinted polymers at surface of silica microparticles for the selective extraction of trace bisphenol A from water samples, J. Hazard. Mater. 179 (2010) 223–229.
[22] Z. Lin, W. Cheng, Y. Li, Z. Liu, X. Chen, C. Huang, A novel superparamagnetic surface molecularly imprinted nanoparticle adopting dummy template: an efficient solid-phase extraction adsorbent for bisphenol A, Anal. Chim. Acta 720 (2012) 71–76.
[23] E. Takano, Y. Taguchi, T. Ooya, T. Takeuchi, Dummy template-imprinted polymers for bisphenol A prepared using a schiff base-type template molecule with post-imprinting oxidation, Anal. Lett. 45 (2012) 1204–1213.
[24] Y. Yin, Y. Chen, X.F. Wang, Y. Liu, H. Liu, M. Xie, Dummy molecularly imprinted polymers on silica particles for selective solid-phase extraction of tetrabromobisphenol A from water samples, J. Chromatogr. A 1220 (2012) 7–13.
[25] T. Kubo, K. Hosoya, T. Sano, M. Nomachi, N. Tanaka, K. Kaya, Selective separation of brominated bisphenol A homologues using a polymer-based medium prepared by the fragment imprinting technique, Anal. Chim. Acta 549 (2005) 45–50.
[26] Y. Watabe, K. Hosoya, N. Tanaka, T. Kubo, T. Kondo, M. Morita, Novel surface modified molecularly imprinted polymer focused on the removal of interference in environmental water samples for chromatographic determination, J. Chromatogr. A 1073 (2005) 363–370.
[27] H. Sambe, K. Hoshina, K. Hosoya, J. Haginaka, Simultaneous determination of bisphenol A and its halogenated derivatives in river water by combination of isotope imprinting and liquid chromatography-mass spectrometry, J. Chromatogr. A 1134 (2006) 16–23.
[28] L. Chen, S. Xu, J. Li, Recent advances in molecular imprinting technology: current status, challenges and highlighted applications, Chem. Soc. Rev. 40 (2011) 2922–2942.
[29] X. Sun, J. Wang, Y. Li, J. Jin, B. Zhang, S.M. Shah, X. Wang, J. Chen, Highly selective dummy molecularly imprinted polymer as a solid-phase extraction sorbent for five bisphenols in tap and river water, J. Chromatogr. A 1343 (2014) 33–41.
[30] J. Yin, Z. Meng, Y. Zhu, M. Song, H. Wang, Dummy molecularly imprinted polymer for selective screening of trace bisphenols in river water, Anal. Method. 3 (2011) 173–180.
[31] Y. Hiratsuka, N. Funaya, H. Matsunaga, J. Haginaka, Preparation of magnetic molecularly imprinted polymers for bisphenol A and its analogues and their application to the assay of bisphenol A in river water, J. Pharm. Biomed. Anal. 75 (2013) 180–185.
[32] J.A. García-Calzón, M.E. Díaz-García, Characterization of binding sites in molecularly imprinted polymers, Sens. Actuat. B: Chem. 123 (2007) 1180–1194.
[33] X. Cao, G. Dufresne, S. Belisle, G. Clement, M. Falicki, F. Beraldin, A. Rulibikiye, Levels of bisphenol A in canned liquid infant formula products in Canada and dietary intake estimates, J. Agric. Food Chem. 56 (2008) 7919–7924.
[34] Y. Watabe, T. Kondo, M. Morita, N. Tanaka, J. Haginaka, K. Hosoya, Determination of bisphenol A in environmental water at ultra-low level by high-performance liquid chromatography with an effective on-line pretreatment device, J. Chromatogr. A 1032 (2004) 45–49.
[35] J. O'Mahony, M. Moloney, M. McCormack, I.A. Nicholls, B. Mizaikoff, M. Danaher, Design and implementation of an imprinted material for the extraction of the endocrine disruptor bisphenol A from milk, J. Chromatogr. B 931 (2013) 164–169.
[36] J.H. Zhang, M. Jiang, L. Zou, D. Shi, S.R. Mei, Y.X. Zhu, Y. Shi, K. Dai, B. Lu, Selective solid-phase extraction of bisphenol A using molecularly imprinted polymers and its application to biological and environmental samples, Anal. Bioanal. Chem. 385 (2006) 780–786.
[37] F. Tan, H. Zhao, X. Li, X. Quan, J. Chen, X. Xiang, X. Zhang, Preparation and evaluation of molecularly imprinted solid-phase microextraction fibers for selective extraction of bisphenol A in complex samples, J. Chromatogr. A 1216 (2009) 5647–5654.