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Selective solid-phase extraction of bisphenol A using molecularly imprinted polymers and its application to biological and environmental samples

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Abstract Molecularly imprinted polymers (MIPs) were prepared using bisphenol A (BPA) as a template by precipitation polymerization. The polymer that had the highest binding selectivity and ability was used as solid-phase extraction (SPE) sorbents for direct extraction of BPA from different biological and environmental samples (human serum, pig urine, tap water and shrimp). The extraction protocol was optimized and the optimum conditions were as follows: conditioning with 5 mL methanol–acetic acid (3:1), 5 mL methanol, 5 mL acetonitrile and 5 mL water, respectively, loading with 5 mL aqueous samples, washing with 1 mL acetonitrile, and eluting with 3 mL methanol. MIPs can selectively recognize, effectively trap and preconcentrate BPA over a concentration range of 2–20 μ M. Recoveries ranged from 94.03 to 105.3 %, with a relative standard deviation lower than 7.9 %. Under the optimal condition, molecularly imprinted SPE recoveries of spiked human serum, pig urine, tap water and shrimp were 65.80, 82.32, 76.00 and 75.97 %, respectively, when aqueous samples were applied directly. Compared with C18 SPE, a better baseline, better high-performance liquid chromatography separation efficiency and higher recoveries were achieved after molecularly imprinted SPE.

Keywords Molecularly imprinted polymers · Solid-phase extraction · Bisphenol A · Biological and environmental samples

Introduction

Bisphenol A (BPA), a known endocrine disrupter, has been shown to induce abnormal differentiation and development of reproductive organs by mimicking or interfering with the action of endogenous gonadal steroid hormones. BPA is the monomer used for the production of polycarbonate plastics and epoxy resins, such as baby bottles, foodstuff containers and dental sealants [1–3]. It is widely used in industry and dentistry. Therefore, BPA exists widely in the environment and can easily migrate into the human body to produce adverse effects on health. Analytical methods must ensure individual quantitative determination of BPA in order to monitor the accurate concentration of trace BPA in biological or environmental samples. To date, liquid chromatography [4–7], gas chromatography [8] and electrophoresis [9] are normally used for BPA identification and quantification.

Unfortunately complex sample matrices in biological and environmental samples often affect the accuracy of BPA determination. Solid-phase extraction (SPE) is a routine sample preparation technique for extracting analytes from a complex matrix. It has many attractive features, such as ease of operation, high load abilities, high recoveries, lower consumption of organic solvents, and a wide spectrum of stationary phases, automation capabilities and so forth [10, 11]. However, SPE is usually based on nonselective interactions between the analyte molecule and the sorbent, which often leads to coextraction of many matrix components.

To enhance the extraction efficiency, new extraction sorbents involving reversible and selective antigen–antibody interactions, called immunosorbents (IS), have been synthesized in order to trap target analytes. They give extracts which are almost free of interfering substances [12–14]. But antibody isolation is expensive and time-consuming, and the antibodies are easily denatured in the presence of organic solvents. These drawbacks limited the usage of IS in sample pretreatment.

Molecularly imprinted polymers (MIPs) are synthetic cross-linked polymers formed by the presence of a target

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molecule (template). The template is then removed, which produces a cavity with molecular recognition sites that can bind selectively to the original template. MIPs have advantages such as physical robustness, high strength, resistance to elevated temperatures and pressures, and inertness towards organic solvents, acids or bases. Sellergren [15] first introduced MIPs into the SPE procedure, namely molecularly imprinted SPE (MISPE). Since then, MISPE has been widely applied in biology, pharmacy and environmental fields [16–21]. The application of MIPs in SPE has become the most promising development of the selective SPE method for trace analysis.

It has been reported that BPA-imprinted MIPs prepared in suspension polymerization were used in MISPE of river water samples [22]. The selectivity of different BPA-imprinted MIPs shows that these MIPs can recognize and bind BPA more specifically than other steroidal and nonsteroidal analogues [23–26]. However, the usage of MISPE for BPA pretreatment of biological samples has not been reported yet. In this work, MIPs with BPA as a template were used in MISPE. Under the optimal conditions, loading, washing and eluting protocols, MISPE was successfully applied to the extraction of BPA from a large volume of spiked pig urine, human plasma, shrimp and tap water for the first time.

Experimental

Chemicals and reagents

BPA, hydroquinone (HQ), *p*-nitrophenol (p-NP), diethylstilbestrol (DES), hexestrol (HEX) (Fig. 1), 4-vinylpyridine (4-VP) and trimethylolpropane trimethacrylate (TRIM) were from Sigma (St. Louis, MO, USA). A 0.1 mmol/L stock solution of BPA and its analogues was prepared in acetonitrile and diluted with water to the final concentration when used. Azobis(isobutyronitrile) (AIBN), high-performance liquid chromatography (HPLC) grade organic solvents (methanol, acetonitrile) and analytical reagents (toluene, acetic acid) were obtained from Tianjing Chemical Reagent Company (Tianjing, P.R. China). 4-VP and TRIM were purified prior to use via general distillation methods in vacuo under argon protection to remove the polymerization inhibitor. AIBN was recrystallized from methanol and then dried at room temperature in a vacuum prior to use. Toluene was distilled before use. Fourfold-distilled water was used.

Apparatus and analytical conditions

HPLC analyses were performed using a Varian PROSTAR 230 (USA) HPLC system with a UV variable-wavelength detector operating at 281 nm. All analyses were performed using a Waters Symmetry reversed-phase octadecylsilane column (5 μ m, 250 mm \times 4.6-mm inner diameter). The mobile phase was a mixture of acetonitrile, water and acetic acid (50:47:3). The mobile phases were degassed by

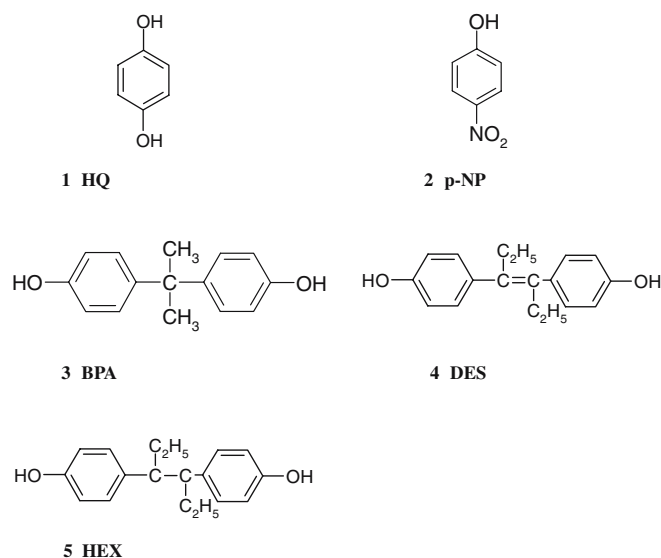


Fig. 1 Structures of hydroquinone (HQ), *p*-nitrophenol (*p*-NP), bisphenol A (BPA), diethylstilbestrol (DES) and hexestrol (HEX)

sonication and pumped at a flow rate of 0.6 mL/min. The injected sample volume was 10 μ L. The oven temperature was set at 25 $^{\circ}$ C. The linear range was from 5 to 100 μ mol/L. Good linearity was obtained for all five compounds, with a determination coefficient (r^2) higher than 0.999. The assay of BPA, DES, HEX, p-NP and HQ had a limit of detection of 0.305, 0.563, 0.685, 0.280 and 0.731 μ mol/L (signal-to-noise ratio of 10), respectively, and a limit of quantitation of 3, 5, 5, 3 and 10 μ mol/L respectively.

SPE was performed with a Supelco (Supelco, Park Bellefonte, PA, USA) 12-position SPE manifold equipped with vacuum control valve and PTFE cartridge adapters. Commercial C18 SPE columns (100 mg, 3 mL) were from Supelco.

Preparation of MIPs

BPA-imprinted MIPs as well as nonimprinted polymeric particles (NMIPs) were prepared by precipitation polymerization based on the method reported by Li and Stover [25]. Table 1 shows the molar amounts of the template molecule used for the preparation of MIPs and NMIPs in this study. For a general polymerization procedure, different molar amounts of template BPA, monomer 4-VP (6.0 mmol), cross-linker TRIM (12 mmol) and free-radical initiator AIBN (40 mg) were dissolved in a mixture of acetonitrile and toluene (128 mL, 3:7 v/v) in a 250-mL round-bottomed flask. The solution was degassed in an ultrasonic bath for 5 min then purged with oxygen-free nitrogen for 10 min. The flask was then attached to the rotor arm and rotated slowly (about 50 rpm). The polymerization was induced by heating 65 $^{\circ}$ C for 24 h. After centrifugation, the particles were extracted using a Soxhlet apparatus in methanol–acetic acid 9:1 (v/v) for 24 h to remove the template. Then the particles were washed by acetonitrile five times and dried in vacuo

Table 1 The preparation conditions, binding and polarity characteristics of molecularly imprinted polymers (MIPs) and nonimprinted polymeric particles (NMIPs)

Polymer	Amount of BPA (mmol)	Equation	$K_d \times 10^{-5}$ (mol/L)	$B_{\max} \times 10^{-2}$ (mmol/g)	N ₂ adsorption		
					Surface area (Brunauer–Emmett–Teller) (m ² /g)	Pore volume (cm ³ /g)	Pore diameter (Å)
P1	0.5	B/F = -0.89 × 10 ⁵ B + 0.0082	1.12	0.92	335.1	0.40	49.1
P2	1	B/F = -1.03 × 10 ⁵ B + 0.0117	0.97	1.14	333.7	0.40	48.3
P3	3	B/F = -1.09 × 10 ⁵ B + 0.0140	0.92	1.29	335.9	0.39	48.6
P4	6	B/F = -1.12 × 10 ⁵ B + 0.0157	0.89	1.40	343.5	0.40	46.8
P5	8	B/F = -1.16 × 10 ⁵ B + 0.0165	0.86	1.42	339.03	0.40	47.9
P0	0	B/F = -0.85 × 10 ⁵ B + 0.0065	1.18	0.77	340.21	0.40	49.2

BPA bisphenol A

overnight at 25 °C. NMIPs were prepared under identical conditions except that the template was omitted.

Pore volumes and the surface area of the polymers were determined by nitrogen adsorption on an ASAP 2020M system (Micromeritics, USA).

Measurement of binding ability

BPA toluene solution (2 mL 0.5 mmol/L) was added to 20 mg MIPs. The samples were shaken at 25 °C for 24 h. The concentration of BPA on the supernatant (free BPA) was analyzed by HPLC. The amount of BPA bound to the imprinted particles was calculated by subtracting the amount of free BPA from the amount of BPA initially added. A Scatchard plot was constructed by plotting the ratios of the concentrations of bound to free BPA against the concentration of bound BPA [26]. The dissociation constant and the maximum absorption ability were determined from the equation $\frac{B}{[Free]} = -\frac{B}{K_d} + \frac{B_{\max}}{K_d}$, where K_d is the equilibrium dissociation constant, B is the concentration of bound BPA, $[Free]$ is the concentration of free BPA and B_{\max} is the maximum absorption ability [27]. K_d was calculated from the Scatchard plot.

Selectivity of MIPs

A range of structural analogues of BPA (2 mL, 0.25 mmol/L in toluene) were added to 20 mg MIPs. The samples were shaken at 25 °C for 24 h. The imprinting-induced promotion of binding $[IPB; IPB = (C_{MIP} - C_{NMIP})/C_{NMIP}]$ was used to demonstrate the specificity of the MIPs due to the molecular imprinting effect. C_{MIP} is the amount of the analyte that was bound to MIPs, and C_{NMIP} is the corresponding value for the nonimprinted polymers [28].

Molecularly imprinted solid-phase extraction

Optimization of MISPE with standard solutions

Empty SPE cartridges (3 mL) were dry-packed with 100 mg pf corresponding polymers or nonimprinted polymers. The frit of the SPE columns was 0.5 μm. A vacuum was applied to force the solutions through the particle bed at a flow rate of 0.2 mL/min. Different SPE protocols were applied, utilizing different solvents during loading, washing and eluting. Mixed solutions of BPA and its analogues over a concentration range of 2–20 μM (5 mL) were loaded. The fractions eluted from the cartridges were collected separately and the amount of the compounds recovered was quantified by HPLC.

MISPE of spiked pig urine

After collection, pig urine was centrifuged at 50,00 rpm for 10 min to eliminate precipitates. Mixed stock solution (400 μL; BPA, DES, HQ, p-NP and HEX, 0.1 mmol/L each) was added to 9.6 mL pig urine. The mixture was vortex mixed for 1 min and 5 mL of the mixture was directly applied to the MISPE cartridge.

Table 2 Imprinting-induced promotion of binding (%) of various compounds on MIPs

Solute	P1	P2	P3	P4	P5
BPA	138	152	227	307	455
DES	171	167	162	209	233
HEX	178	156	173	167	194
p-NP	148	115	153	156	172
HQ	118	125.5	138	169	170

DES diethylstilbestrol, HEX hexestrol, p-NP p-nitrophenol, HQ hydroquinone

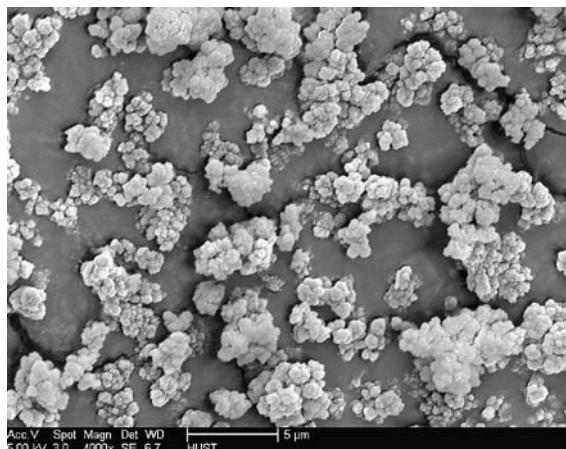


Fig. 2 Scanning electron micrographs of the particles prepared

MISPE of spiked human plasma

Human plasma samples from anonymous patients were obtained from Tongji Hospital, Wuhan, China. To 1 mL human plasma, 400 μL mixed stock solution was added and next 600 μL cold acetonitrile was added. The mixture was vortex-mixed for 1 min and centrifuged at 5,000 rpm for 10 min. The supernatant was diluted with water to 10 mL, and 5 mL of the mixture was applied to the MISPE cartridge [18].

MISPE of spiked shrimp samples

Smashed shrimp (1 g) and 2 mL acetonitrile were homogenized for 1 min and then centrifuged for 10 min at 5,000 rpm. Stock solution (400 μL) was added to the supernatant and diluted with water to 10 mL, and then 5 mL of the mixture was applied to the MISPE cartridge.

MISPE of spiked tap water samples

Mixed stock solution (400 μL) was added to tap water samples and the total volume was 10 mL. Then, 5 mL of the mixture was directly applied to the MISPE cartridge.

SPE on C18

5 mL spiked pig urine was also applied to commercial C18 columns (Supelclean LC-18, Supelco). C18 SPE extraction conditions were optimized by modifying Inoue's protocol [29]: conditioning with 15 mL of methanol and 3 mL of water in turn, loading with spiked urine, washing with 3 mL of water-methanol (90:10) and eluting with 3 mL methanol. All the applied fractions were collected and evaporated to dryness (at 45 $^{\circ}\text{C}$ under a stream of N_2). The residues were dissolved with 200 μL mobile phase and analyzed by HPLC. Each sample was assayed three consecutive times.

Results and discussion

Preparation and evaluation of NMIPs and MIPs

In this study, particles with a diameter of about 2-10 μm (Fig. 2, Table 1) were obtained. The particles had a large surface area and pore diameter (Table 1), indicating they were porous particles which can be directly used as affinity-type sorbents. Similar pore characteristics of MIPs were obtained as the same porogen was used (Table 1). As the particles had similar morphology and surface characteristics (Fig. 2, Table 1), the binding isotherms and B_{max} values (Table 1) were determined to evaluate the binding ability, and the IBP values (Table 2) were used to evaluate binding selectivity.

The binding ability of the imprinted polymers (Table 1) showed that B_{max} of MIPs increased with an increase of the molar amounts of the template. This may be because in precipitation polymerization excess porogen (typically 95

Table 3 Recoveries of BPA and different analogues (4 $\mu\text{mol/L}$)

Fraction	Recovery (%)									
	BPA		HEX		DES		HQ		p-NP	
	P5	NMIP	P5	NMIP	P5	NMIP	P5	NMIP	P5	NMIP
Load 5mL water	ND	17.28 \pm 2.10	ND	5.64 \pm 1.71	ND	2.16 \pm 2.31	31.09 \pm 3.65	40.77 \pm 2.56	ND	4.23 \pm 1.41
Wash 1 mL acetonitrile	9.95 \pm 1.28	52.15 \pm 3.19	53.91 \pm 3.33	54.36 \pm 1.71	55.4 \pm 5.35	48.15 \pm 1.73	40.10 \pm 6.95	40.26 \pm 1.86	62.96 \pm 4.16	63.76 \pm 1.79
Elute 3 mL methanol	98.66 \pm 3.16	32.72 \pm 4.39	48.7 \pm 3.42	43.59 \pm 3.21	54.4 \pm 4.76	50.31 \pm 3.02	41.90 \pm 2.87	38.46 \pm 3.93	51.85 \pm 4.54	51.85 \pm 2.17
Total	108.6 \pm 3.00	102.15 \pm 2.86	102.61 \pm 3.11	103.59 \pm 2.92	109.8 \pm 4.89	100.62 \pm 2.99	113.09 \pm 3.35	119.49 \pm 3.87	114.81 \pm 4.46	119.84 \pm 2.56

Triplicate experiments were performed for each compound
 ND not detected

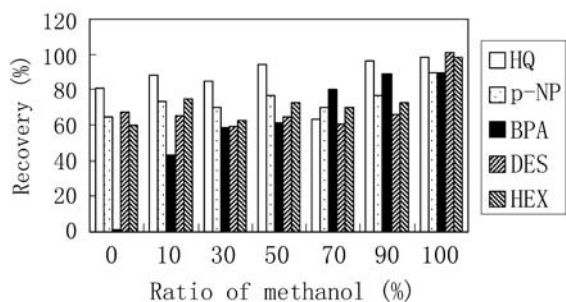


Fig. 3 Recoveries of BPA on molecularly imprinted polymer cartridges: 5 mL of a 4 $\mu\text{mol/L}$ mixed solution was loaded; then, acetonitrile solution including different percentages of methanol was used as the washing solvent

% of the total reaction volume) was used [25, 26]; therefore, much of the template dissolves in the porogen instead of reacting with the functional monomers to form a prepolymerization complex. An increase in the amount of template would result in an increase in the formation of the prepolymerization complex and the efficiency of imprinting. P5 had the highest B_{max} , which was higher than that of other MIPs recently reported [30–34]. Ikegami et al. [35] evaluated the binding properties of a BPA-imprinted polymer in chloroform and B_{max} was 0.0107 mmol/g, which was lower than B_{max} of P3, P4 and P5.

High ligand selectivity and affinity are characteristics of MIPs [16, 28, 31, 32]. IPB for BPA and its analogues (Table 2) showed that selectivity of MIPs increased as the molar amounts of the template used increased. IPB for BPA was higher than that of its analogues for P3, P4 and P5. P3, P4 and P5 could selectively recognize BPA among the phenolic compounds tested. As P5 exhibited the highest binding ability and binding selectivity, it was chosen as the MISPE sorbent.

Template leakage was a problem that may affect the precision of MISPE [17, 22]. P0 and P5 were shaken in pure toluene for 24 h in order to detect the leakage. BPA was not detected in the supernatant, indicating no MIP leakage was detected in this assay. Template leakage was not a big problem that may affect the precision of MISPE when P5 was used as an MISPE sorbent.

Optimization of MISPE conditions

Because most of the environmental and biological samples were aqueous samples, water was used as the loading solvent. By the way, BPA and its analogues normally existed in trace amounts; therefore 5-mL instead of 1-mL samples were loaded. The cartridges were first conditioned with 5 mL methanol–acetic acid (3:1), followed 5 mL methanol, 5 mL acetonitrile and 5 mL water. Then, 5 mL of mixed solution (4 $\mu\text{mol/L}$ each in water) was loaded onto the cartridge. The results showed that except for HQ, no BPA and its analogues were detected in MISPE in the loading step and the breakthrough in nonimprinted SPE (NISPE) ranged from 2.16 to 17.28 % (Table 3). This indicated that the binding from aqueous phases was strong

but nonselective. Water was suitable for use as the loading solvent. This characteristic will be very useful in extracting trace BPA from environmental and biological samples.

The optimal washing step is a key point in order to get the best MISPE extraction result. In such a case, analytes nonselectively bound to the MIPs would be eluted, whereas the template would remain trapped in the polymer by selective interactions. In contrast, in the control polymer, quantitative elution of the template is expected owing to the disturbance of the hydrophobic interactions retaining the molecule [18, 20, 22]. Generally speaking, MIPs exhibit better molecular recognition in the solvent used as a porogen in the polymerization process [18, 34]. Therefore, mixed solutions of different ratios of methanol and acetonitrile were used in order to find the most appropriate washing ratio (Fig. 3). It can be seen that the amount of BPA eluted from the columns increased as the proportion of methanol increased. Several analogues were largely washed off the MIPs cartridges in pure acetonitrile, while BPA was still retained in the cartridges; therefore, pure acetonitrile was chosen as the washing solvent.

Considering the high eluting ability of methanol (Fig. 3), methanol was investigated as the eluting solvent. The results showed that methanol can effectively elute BPA. Therefore, loading with water, washing with 1 mL acetonitrile and eluting with 3 mL methanol was the optimal MISPE protocol. This protocol resulted in satisfactory extraction recovery and also in the highest recovery differences between the MIPs and the NMIPs (Table 3).

Under the optimal MISPE conditions, different concentrations of mixed BPA and analogues solutions were loaded on the MISPE column. Table 4 shows that the recoveries ranged from 94.03 to 105.3 % for 2–20 $\mu\text{mol/L}$ concentrations of BPA, with a relative standard deviation lower than 7.9 %. MISPE assay was quite reproducible and accurate.

Specificity of MISPE

To evaluate the cross-reactivity of the MISPE method, mixed solution of several BPA structural analogues (Fig. 1) were loaded on the MISPE and NISPE cartridges, respectively (Table 3). Under the optimal MISPE condi-

Table 4 Repeatability and recovery of molecularly imprinted solid phase extraction

Spiked concentration ($\mu\text{mol/L}$)	Repeatability (RSD %, $n=5$)		Recovery (%) ^a ($n=5$)
	Intraday	Interday	
2	1.2	7.6	94.03
4	3.2	5.7	98.66
10	4.5	7.9	105.3
20	2.1	3.6	99.4

RSD relative standard deviation

^aRecovery=(measured spiked sample concentration–measured blank sample concentration)/initial spiked sample concentration $\times 100$ %

Table 5 Recoveries of BPA from spiked samples (4 $\mu\text{mol/L}$)

Sample	Recovery (%)	RSD (%)
Spiked urine	82.32	9.24
Spiked plasma	65.80	6.50
Spiked tap water	76.00	5.09
Spiked shrimp	75.97	8.80

Results of three experiments

tions, the recovery of BPA on the MISPE cartridge was as high as 98.66 %, much higher than that of other analogues and NMIPs. This indicated that BPA-imprinted polymers can selectively separate and enrich BPA from its analogues in MISPE.

Selective extraction of BPA from complex matrices

In order to investigate the potential of MIPs for the selective entrapment of a target analyte from complex matrices, spiked pig urine, human plasma, shrimp and tap water were applied to MISPE cartridges. Urine and tap water samples were directly applied to MISPE cartridges. Plasma and shrimp samples had to be extracted with acetonitrile and then MISPE was performed with water. Satisfactory sample cleanup was achieved by the MISPE extraction (Table 5). The average recoveries were reproducible and in agreement with the recoveries of standard solutions.

MIPs showed the highest selectivity in organic solvents, but the majority of environmental and biological samples are aqueous in nature. In order to deal with this problem, usually the samples were first extracted into an organic solvent and then MISPE was performed using the organic extract [36, 37]. Moller et al. [38] reported direct MISPE of diphenyl phosphate analogues from aqueous spiked urine samples and the recoveries were higher than 70 %. In this study, aqueous samples were loaded onto the cartridges directly and the recoveries varied from 65.80 to 82.32 %, which is comparable to the recoveries of organic extraction loading [36, 37]. This showed that MISPE was able to separate and enrich BPA from a complex matrix accurately even when loading aqueous samples directly. MISPE was quite reproducible in biological and environmental sample pretreatment.

In order to evaluate the enriching ability of MISPE, 15 mL diluted spiked (1.33 $\mu\text{mol/L}$) urine was applied in MISPE using the optimum protocol. The BPA recoveries were a little bit different in the urine samples with two different concentrations (82.32 % for undiluted and 80.79 % for diluted). This suggested that acceptable BPA recoveries could be maintained despite the usage of a 3 times larger loading volume. This feature may have important implications for trace analytes when processing of large volumes of biological and environmental samples is required.

In order to confirm the possible usage of this method in practical samples, 150 mL spiked (0.1 $\mu\text{mol/L}$ each) tap

water samples were applied to the MISPE columns. The loading fractions were collected and evaporated to dryness. The residues were dissolved with 200 μL mobile phase and were analyzed by HPLC. Each sample was assayed three consecutive times. The results showed that no BPA was detected even when 150 mL water samples were loaded.

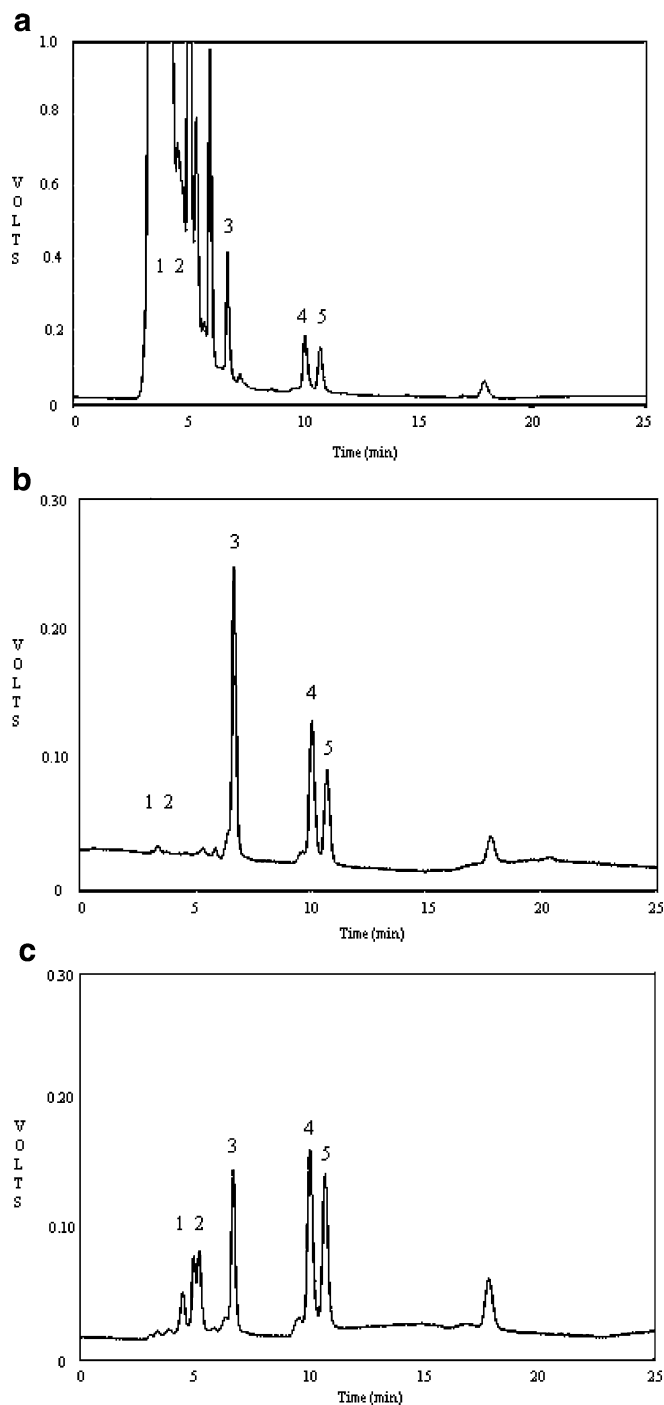


Fig. 4 High-performance liquid chromatography analysis of spiked pig urine. **a** Direct injection; **b** analysis of a sample extracted by molecularly imprinted solid phase extraction; **c** analysis of a sample extracted on a C18 column. Chromatograms of urine sample of spiked HQ (1), p-NP (2), BPA (3), DES (4) and HEX (5). Spiked concentration 4 $\mu\text{mol/L}$ per analyte

The breakthrough volume of MISPE was greater than 150 mL when 0.1 $\mu\text{mol/L}$ mixed solution was loaded.

The chromatogram of the direct assay of the spiked urine sample (Fig. 4a), after MISPE (Fig. 4b) and after C18 SPE (Fig. 4c) confirmed that better extraction efficiency were achieved after MISPE. Although the BPA peak was readable, complex sample matrices in urine affected the correct quantification of BPA (Fig. 4a). Compared with C18 SPE, fewer interfering compounds appeared after MISPE. The recovery of C18 SPE was only 59.8 %, much less than that of MISPE (82.32 %). MISPE had better baselines, better HPLC separation efficiency, higher selectivity and better recovery than were obtained after C18 SPE. This confirmed that satisfactory sample cleanup was achieved by the MISPE.

One of the major advantages of MIPs is their high chemical robustness, providing the opportunity to reactivate them under relatively harsh conditions for multiple uses in SPE applications. Generally, a single MIP cartridge could be employed for eight consecutive cycles without any treatment between the two cycles. Then, the MIP cartridge needed to be reconditioned with 5 mL methanol–acetic acid 3:1, 5 mL methanol, 5 mL acetonitrile and 5 mL water before further usage. It is important to stress that all of the samples in this work were analyzed using the same cartridge in 3 weeks and that no losses were detected.

Conclusion

In this study, BPA-imprinted MIPs were synthesized and directly used as sorbents for MISPE of several biological and environmental samples. The results indicated that the MIPs exhibited high binding ability and selectivity even when aqueous samples were applied directly. Optimal loading, washing and eluting protocols were critical for the superior MISPE procedure. With the optimized procedure, high selectivity could be obtained for all the complex sample matrices treated, and better recovery than C18 SPE could be shown. MISPE had good precision and accuracy. The high extraction efficiency of MISPE from different complex matrices suggested that it is a practicable solution for sample preparation in routine analysis of trace BPA in environmental and biological samples.

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