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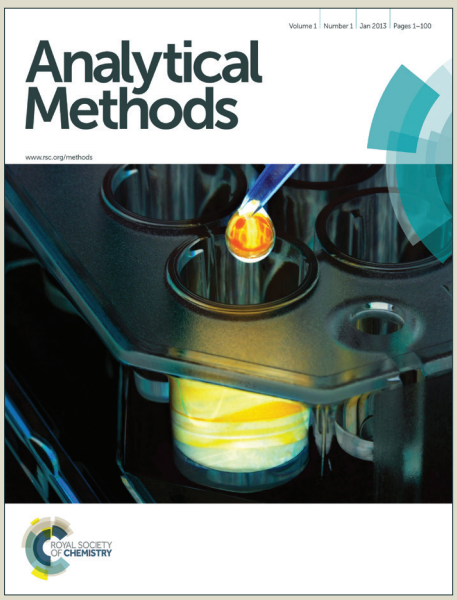





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# Use of core-shell nanoring amino-functionalized superparamagnetic molecularly imprinted polymer for matrix solid phase dispersion extraction and preconcentration of BPA in water samples at ultratrace levels water

Ping-Ping Zhan[a], Wen-Jie Gong[b], Yong-Gang Zhao[c,d,*]

([a] School of Marine Sciences, Ningbo University, Ningbo, Zhejiang, 315211, China; [b] Cixi Municipal Center for Disease Control and Prevention, Ningbo, Zhejiang, 315300, China; [c] Zhejiang Provincial Key Laboratory of Health Risk Appraisal for Trace Toxic Chemicals, Ningbo Municipal Center for Disease Control and Prevention, Ningbo, Zhejiang, 315010, China; [d] Ningbo Key Laboratory of Poison Research and Control, Ningbo Municipal Center for Disease Control and Prevention, Ningbo, Zhejiang, 315010, China)

**Abstract:**

A new method that utilizes core–shell nanoring amino-functionalized magnetic molecularly imprinted polymer (CS-NR-MMIP) as an adsorbent for matrix solid phase dispersion extraction (MSPD) has been developed for highly selective and sensitive analysis of ultratrace BPA (Bisphenol A) by ultra-fast liquid chromatography–tandem mass spectrometry (UFLC–MS/MS). The adsorption and desorption conditions including the pH value, shaking time, and sample volume were investigated. The results showed the adsorption property of the CS-NR-MMIP was highly pH dependent. Under the optimal experimental conditions, the enhancement factor of the CS-NR-MMIP MSPD procedure was 250, the limit of quantitation (LOQ) was 1 $ng \cdot L^{-1}$, the linear range was between 1 $ng \cdot L^{-1}$ and 200 $ng \cdot L^{-1}$ with a correlation coefficient of 0.9992. The results showed that the developed method was faster, easier and

*Corresponding author. Tel.: 086-574-87274559, E-mail address: zhyg91213@163.com (Y.G. Zhao).

more selective for extracting and enriching ultratrace BPA (nanogram per liter level) from water samples than the commercial C18 powder.

**Keywords:** Core-shell nanoring amino-functionalized magnetic molecularly imprinted polymer (CS-NR-MMIP), Matrix solid phase dispersion extraction (MSPD), Bisphenol A (BPA), Ultra-fast liquid chromatography–tandem mass spectrometry (UFLC–MS/MS), Water

## 1. Introduction

*In vitro* and *in vivo* bioassay studies have shown that bisphenol A (BPA), which is used to fabricate polycarbonate plastics, has estrogenic potency and chronic toxicity [1,2]. BPA is found in clinical (blood, urine, and saliva), food, and water samples in microgram per liter levels [3,4]. In China, BPA is widely present at high concentrations in the urban riverine waters of Guangzhou (65 $ng \cdot L^{-1}$) and the Liao River system in Northeast China (755.6 $ng \cdot L^{-1}$) [5,6]. Thus, the preconcentration and analysis of BPA, especially at ultratrace levels in environmental water, is urgently needed for environmental protection, as well as food and agricultural chemistry. However, the direct preconcentration and analysis of BPA in environmental water, especially in drinking water sources, is limited. Therefore, an enrichment and/or separation procedure is necessary to improve BPA preconcentration efficiency, as well as sensitivity and selectivity of analysis.

MSPD is a simple, fast, and inexpensive common technique used for separating and preconcentrating various organic analytes. In the MSPD procedure, the selection of an appropriate adsorbent is a critical factor for obtaining full recovery and high enrichment. For

phenols and BPA, many adsorbents have been developed, such as multifunctional biomagnetic capsules [7], polyethersulfone–organophilic montmorillonite hybrid particles [8], activated carbon [9], modified carbon nanotubes [10], acetylaniline-modified hyper-cross-linked polymeric adsorbents [11] and molecularly imprinted particles [12,13]. However, only a few of these adsorbents are used for the preconcentration and analysis of BPA in water samples at ultratrace levels. A kind of molecularly imprinted layer-coated silica nanoparticles was developed for the extraction and preconcentration of BPA in cosmetic cream samples [14]. SPE using molecularly imprinted layer-coated silica nanoparticles yields satisfactory analyte recovery. However, the use of adsorbents in MSPD involves difficulties with regard to the quick separation of spent adsorbent and shortening of separation time from the solution. Therefore, exploring more suitable adsorbents that can overcome these difficulties, have strong adsorption ability for MSPD, and can enrich BPA at ultratrace levels has become a great challenge.

Magnetic separation is a useful tool in several areas of applications, such as carriers, immobilizers and separators of biomolecules and drugs, because of its fast recovery, high efficiency, and low cost [15,16]. In our previous work, a kind of core–shell nanoring amino-functionalized magnetic molecularly imprinted polymer adsorbents, named as CS-NR-MMIP had been prepared by ultrasound-assisted suspension polymerization [17]. The CS-NR-MMIP had been verified to be very promising particles for the removal of BPA in wastewater. The maximum adsorption capacity of CS-NR-MMIP to BPA was found to be 750.2 $mg \cdot g^{-1}$. And so, CS-NR-MMIP is expected to be more selective and much stronger adsorption ability than other adsorbents reported in the literature for the MSPD extraction of

BPA at ultratrace levels. However, to the best of our knowledge, few such studies have been reported so far.

The focus of the present research is development and validation of rapid methods for the analysis of BPA present in the water samples at ultratrace levels using MSPD followed by UFLC-MS/MS analysis. In MSPD, the solid phase adsorbent is thoroughly mixed with sample solutions. Dynamic mixing uses less adsorbent and provides faster extractions compared to classical SPE. BPA is concentrated on the adsorbent and can be eluted in concentrated solution. In this work, the CS-NR-MMIP used as selective adsorbent in MSPD for separation and preconcentration of BPA. Parameters that can affect the adsorption and recovery efficiency of BPA, such as pH value, the shaking time, the sample volume *etc*, were assessed and optimized from laboratory batch tests. Then, the method was applied to the analysis of water samples with satisfactory results, which suggests that this method can be complimentary to MSPD for separation and preconcentration of phenols in water samples.

## 2. Experimental

### 2.1 Reagents and materials

BPA (> 99%), hexestrol (HEX, > 99%), 4-(tert-octyl)-phenol (4-tOP, > 99%), diethylstilbestrol (DES, > 98%), dienestrol (DE, > 99%), and 4-nonylphenol (4-NP, > 99%) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Structural formulas are shown in Fig. S1. Ultrapure water was obtained using a MilliQ gradient ultrapure water system.

CS-NR-MMIP used in the experiment was prepared in our laboratory according to the reported procedure [17]. The paramagnetic $Fe_3O_4$ was firstly coated with oleic acid. Methyl methacrylate (MMA), divinylbenzen (DVB) and glycidylmethacrylate (GMA) were then co-polymerized *via* the ultrasound-assisted suspension polymerization procedure over the magnetic core to obtain epoxyl-functionalized magnetic polymer. Then self-assembly through hydrogen bond interaction was conducted by stirring the template (BPA) and active groups, i.e., tetraethylenepentamine (TEPA) in methanol. Finally, BPA@TEPA was grafted onto the surface of the polymer *via* ring-opening reaction. Thus, the BPA-imprinted CS-NR-MMIP was obtained. The template molecules were ultrasonically cleaned with 10% (v/v) acetic acid in methanol and methanol. Control core-shell nanoring amino-functionalized magnetic nonimprinted polymer (CS-NR-MNIP) nanoparticles were prepared using the same procedures described above but without addition of the template. The preparation procedure of CS-NR-MMIP was illustrated in Scheme 1. The adsorption capacities of CS-NR-MMIP and CS-NR-MNIP for BPA were found to be 750.2 and 125.0 mg/g, respectively [17].

**<Insert Scheme. 1>**

The CS-NR-MMIP obtained was ringed and exhibited a well-defined core-shell configuration. The $Fe_3O_4$ nanoparticles exhibited a uniform morphology with an average particle size of about 20 nm. The inside and outside diameters of the ringed CS-NR-MMIP were 70 and 150 nm, respectively, and the coated shell had an average thickness of

approximately 10 nm. CS-NR-MMIP showed a saturation magnetization value of 7.12 emu·g$^{-1}$.

**2.2 Equipment**

Ultra-fast liquid chromatography-tandem quadrupole mass spectrometry (UFLC-MS/MS) analyses were performed using a Prominence UFLC XR system equipped with a DGU-20A3 degasser, a CTO-20AC column oven, a LC-20AD pump, a SIL-20AC autosampler (Shimadzu Corporation, Tokyo, Japan) and an AB SCIEX TRIPLE QUAD$^{TM}$ 5500 mass spectrometer (Applied Biosystems, Foster City, CA, USA). The UFLC-MS/MS system was controlled and data were analyzed on a computer equipped with Applied Biosystems/MDS Sciex Analyst 1.5.1 (Applied Biosystems, Foster City, CA, USA).

**2.3 UFLC–MS/MS analysis**

UFLC analysis was performed on a Shim-pack XR-ODS II (100 mm × 2 mm, 2.2 μm) using 0.08% ammonia (v/v) in methanol (A) and 0.08% ammonia (v/v) in water (B) as eluents in gradient elution. The linear gradient elution was conducted as follows: 0 min to 2 min, 40% to 70% (A); 2 min to 5 min, 70% to 85% (A); 5 min to 6 min, 85% (A); 6 min to 8 min, 85% to 40% (A); and 8 min to 9 min, 40% (A). Chromatographic separation was conducted at a constant flow rate of 0.45 mL·min$^{-1}$, with an injection volume of 5 μL. The column was thermostated at 40 °C. Mass spectrometry was performed using an electrospray ionization source in negative multiple-reaction monitoring (MRM) mode. The operation conditions were as follows: -4500 V ion spray voltage, 40 psi curtain gas (with the interface heater on), medium collision gas, 50 and 50 psi nebulizer gas (gas 1) and heater gas

(gas 2), 500 °C turbo spray temperature, 10 V entrance potential, 10 V collision cell exit potential, and 50 ms dwell time. Nitrogen was used in all cases. The mass spectrometric information of BPA are shown as follow, the precursor ion: 227.0 (m/z), product ion: 133.0 (m/z) and 211.0 (m/z), corresponding declustering potential (DP): 150 V, and collision energy (CE): 34 eV and 40 eV, respectively. The dwell time was set to 50 ms in the positive mode. Applied Biosystems/MDS Sciex Analyst software (version 1.5.1) was used for data acquisition and processing. Furthermore, the MRM parameters of the five structural analogs, *i.e.*, HEX, 4-tOP, DES, DE and 4-NP for quantification are shown in Table S1.

**2.4 Sample preparation**

River water was collected from the Yaojiang River, Yongjiang River, and Fenghua River, in Ningbo, China. The pH value of the water sample was adjusted to 7±2 with 0.1 $mol \cdot L^{-1}$ HCl and 0.1 $mol \cdot L^{-1}$ NaOH, filtered through a polytetrafluoroethene membrane (0.45 μm), and then stored in pre-cleaned polyethylene bottles prior to use. Tap water samples were also collected from our laboratory and analyzed without pretreatment.

**2.5 Evaluation of BPA-imprinted CS-NR-MMIP as MSPD adsorbents**

2.5.1 Adsorption procedure

Batch adsorption studies were performed by mixing 20 mg of CS-NR-MMIP with 50 mL of BPA solution with varying concentrations from 2 $ng \cdot L^{-1}$ to 100 $ng \cdot L^{-1}$ in a 100 mL stopper conical flask. HCl (0.1 $mol \cdot L^{-1}$) and NaOH (0.1 $mol \cdot L^{-1}$) solutions were used for pH adjustment. To investigate the effect of pH, 50 mL of 2, 10, and 100 $ng \cdot L^{-1}$ BPA with pH ranging from 2 to 11 were mixed with 20 mg CS-NR-MMIP for 24 h to reach equilibrium. To determine the effect of shaking time, 20 mg CS-NR-MMIP was added into 50 mL of 2, 10,

and 100 ng·L$^{-1}$ BPA under pH 5 to 9, with contact time ranging from 1 min to 120 min. The BPA concentration of the samples was measured at specific time intervals. For thermodynamic studies, 20 mg CS-NR-MMIP was added into 50 mL of 2, 10, and 100 ng·L$^{-1}$ BPA, with temperature ranging from 283.15 K to 323.15 K. When the maximum volume was investigated, different volumes of 100 ng·L$^{-1}$ BPA (5 mL to 100 mL) at pH 5 to 9 were mixed with 20 mg CS-NR-MMIP for 30 min to reach equilibrium.

2.5.2 Elution procedure

Under adsorption conditions, 20 mg CS-NR-MMIP was mixed with 50 mL of 2, 10, and 100 ng·L$^{-1}$ BPA solution in a 100 mL stopper conical flask. After adsorption, the resulting adsorbents (BPA-imprinted CS-NR-MMIP) were isolated under the magnetic field and washed with 2 mL of water, and then was finally eluted by 200 μL of 0.5% ammonia/methanol (v/v) under ultrasound for 1 min. The supernatant concentrated to dryness with a nitrogen stream and was redissolved with 200.0 μL of initial mobile phase and filtered using a 0.22 μm membrane prior to its injection into the UFLC-MS/MS system.

**.2.6 Selective extraction comparison**

To evaluate the effectiveness of CS-NR-MMIP for the selective extraction of BPA, comparative studies were conducted among three MSPD procedures using CS-NR-MMIP, CS-NR-MNIP, and C18 as absorbents. The various extraction procedures are presented below.

2.6.1 Extraction with CS-NR-MMIP

Equal concentrations (10 ng·L$^{-1}$) of BPA and its five structural analogs, namely, HEX, 4-tOP, DES, DE, and 4-NP were prepared by appropriate dilution of stock solutions (1

mg·L$^{-1}$) with double-distilled water. Batch adsorption studies were performed by mixing 20 mg CS-NR-MMIP with 50 mL of the BPA solution mentioned earlier in a 100 mL stopper conical flask. The pH of the BPA solution was adjusted to 7±2 with 0.1 M HCl and 0.1 mol·L$^{-1}$ NaOH. The mixture was vigorously shaken for 30 min to facilitate the adsorption of BPA onto the adsorbents. After adsorption, the BPA-imprinted CS-NR-MMIP was isolated under the magnetic field for 1 min and washed with water. Then, the BPA-imprinted CS-NR-MMIP was eluted using 200 μL of 0.5% formic acid (v/v) in methanol prior to the determination of BPA in aqueous solution by UFLC–MS/MS.

2.6.2 Extraction with CS-NR-MNIP

The overall procedure was similar to that of CS-NR-MNIP MSPD. However, CS-NR-MMIP was replaced with CS-NR-MNIP.

2.6.3 Extraction with C18

The overall procedure was similar to that of CS-NR-MMIP MSPD. CS-NR-MMIP was replaced by C18. After the C18 MSPD procedure, the residues and supernatant were separated by centrifugation rather than by magnetic separation under a magnetic field.

**2.7 Method validation**

UFLC–MS/MS coupled with CS-NR-MMIP MSPD was developed to determine BPA levels because of the low concentration of BPA and the complicated matrix in real samples. The matrix-matched calibration curve was constructed by measuring the elution of BPA standards in a blank matrix of water after CS-NR-MMIP MSPD in seven different concentrations ranging from 1.0 ng·L$^{-1}$ to 200 ng·L$^{-1}$. The limit of detection (LOD) and limit of quantitation (LOQ) were defined as three- and ten-fold of the signal-to-noise ratio,

respectively. Both the method accuracy and precision were estimated by BPA spiked at concentrations of 2.0, 10.0, and 100.0 $ng \cdot L^{-1}$ in blank samples. The method accuracies were expressed as the recoveries, and the method precisions were expressed as the intra-day and inter-day relative standard deviations (RSDs). The intra-day RSDs were obtained by repeating the three levels of spiked samples nine times within a day, and the inter-day RSDs were obtained by repeating the three levels of spiked samples in triplicate on 6 separate days within a 2-week period.

## 3. Results and discussion

### 3.1 FT-IR analysis

The infrared spectra of CS-NR-MMIP and CS-NR-MNIP nanoparticles were obtained, and the results are shown in Fig. 1. The main functional groups of the predicted structures can be observed from corresponding infrared absorption peaks. A broad absorption band at 3440 $cm^{-1}$ in the spectra of CS-NR-MMIP and CS-NR-MNIP corresponded to stretching vibrations of N–H bonds in the amino groups of TEPA molecules. Typical bands of the CS-NR-MMIP nanoparticles were observed at 2994 and 2920 $cm^{-1}$, whereas CS-NR-MNIP nanoparticles showed similar bands at 2990 and 2930 $cm^{-1}$ due to C–H stretching vibrations. Other absorption bands, such as those at 1730 (stretching vibrations of C=O bonds on carbonyl groups), 1638 (stretching vibrations of C–N bonds), and 1270 and 1150 (stretching vibrations of C–O–C bonds of GMA) $cm^{-1}$ matched the minor peaks of CS-NR-MNIP nanoparticles. Furthermore, an absorption band at 563 $cm^{-1}$ in the spectra of CS-NR-MMIP and CS-NR-MNIP corresponded to the Fe–O bond of $Fe_3O_4$ particles.

**<Insert Fig. 1>**

### 3. 2 Effect of pH

To investigate the effect of pH on recovery, 50 mL of 2, 10, and 100 $ng \cdot L^{-1}$ BPA with pH values ranging from 2 to 11 were mixed with 20 mg CS-NR-MMIP for 24 h to reach equilibrium. The adsorption experiments were conducted in triplicate, and the results are shown in Fig. 2. The adsorption quantity of BPA gradually increased from 22.3% to 94.6%, 35.5% to 97.1%, and 74.1% to 98.4%, with an increase in pH from 2 to 5 at 2, 10, and 100 $ng \cdot L^{-1}$ BPA, respectively. Above pH 9, the adsorption quantity of BPA decreased with the increase in pH within the studied pH range. The adsorption quantity of BPA increased with the increase in initial BPA concentration from 2 $ng \cdot L^{-1}$ to 100 $ng \cdot L^{-1}$, which resulted from the high maximum adsorption capacity of CS-NR-MMIP for BPA (750.2 $mg \cdot g^{-1}$).

**<Insert Fig. 2>**

The primary driving forces for the rebinding process, *i.e.*, hydrogen bonding, were strongly related to the sample pH. The state of BPA (p*K*a = 9.7) and the amino groups ($-NH_2$, -NH-) of CS-NR-MMIP was influenced by the pH value of the sample. Under acidic conditions (pH < 5.0), the amino groups were easily protonated and in an ionic state, whereas most of the BPAs were in a molecular state. With the increase in pH from 2 to 5, the concentration of $H^+$ rapidly decreased. The ability of $-NH_2$ to be protonated considerably

weakened, thereby increasing the adsorption quantity of BPA. Under alkaline conditions (pH > 9.0), most of the BPAs were in an ionic state, which reduced their adsorption by CS-NR-MMIP. Hydrogen bonding was suppressed in strongly acidic or alkaline solutions because of the ionic state of BPA and amino groups. Therefore, various pH values ranging from 5 to 9 were selected for subsequent experiments.

**3.3 Effect of shaking time**

Shaking time is an important factor in determining the possibility of applying CS-NR-MMIP for the selective extraction of BPA. To investigate the effect of shaking time on adsorption properties, dynamic adsorption test was performed at different time intervals (1.0-60.0 min). When the shaking time increased from 1.0 min to 20.0 min, the adsorption quantity of BPA increased from 46.86% to 99.22%, 37.80% to 99.92%, and 38.81% to 98.26% for 2, 10, and 100 ng·$L^{-1}$ BPA, respectively (Fig. S2). The results show that the rate of BPA uptake was initially quite high. Then, a considerably slower adsorption rate was observed, which gradually led to an equilibrium condition.

The adsorption kinetic data obtained from batch experiments were analyzed using a pseudo-second-order rate equation [18] as follows:

$$t/q_t = 1/k_2 q_{e.c}^{\ 2} + t/q_{e.c} \qquad (3)$$

where $q_t$ is the amount of BPA adsorbed onto the adsorbent at time $t$ (mg/g) and $k_2$ is the second-order rate constant at equilibrium (g/(mg·min)). By plotting $t/q_t$ against $t$, the values of $k_2$ (slope$^2$/intercept), $q_{e.c}$ (1/slope), and $k_2 q_{e.c}^{\ 2}$ (initial adsorption rate (mg/(g·min)), 1/intercept) can be determined from the slope and intercept of the revealed plots.

In this work, the pseudo-second-order rate equation for the adsorption of BPA onto CS-NR-MMIP nanoparticles was $t/q_t = 0.0226t + 0.0481$ ($R^2$ = 0.9996), which indicates that CS-NR-MMIP possessed rapid adsorption kinetics. BPA adsorption reached the equilibrium within 20 min. Compared with previous studies that suggest a duration of more than 3 h, an incubation period of 30 min was considered sufficient for subsequent studies [8,12,19]. The rapid rebinding kinetics of BPA-imprinted CS-NR-MMIP nanoparticles was attributed to the numerous recognition sites on the surface or near the surface of the particles, which provided easy diffusion of target analytes into the imprinting cavities. Given this feature, BPA-imprinted CS-NR-MMIP nanoparticles are suitable MSPD adsorbents.

**3.4 Adsorption thermodynamic studies**

To evaluate the thermodynamic parameters for the adsorption of BPA onto CS-NR-MMIP nanoparticles, adsorption studies were conducted from 283.15 K to 323.15 K. When the temperature increased from 283.15 K to 323.15 K, the adsorption quantity of BPA increased from 96.36% to 99.68%, 97.82% to 99.86%, and 98.88% to 99.89% for 2, 10, and 100 ng·L$^{-1}$ BPA, respectively. The adsorption thermodynamic data obtained from batch experiments were analyzed using Eq. (4) [20],

$$lnK_D = -\frac{\Delta H^\theta}{RT} + \frac{\Delta S^\theta}{R} \qquad (4)$$

where $\Delta H^\theta$ and $\Delta S^\theta$ are the values of standard enthalpy change and standard entropy change, respectively. $K_D$ is the distribution coefficient defined by Eq. (5)

$$K_D = \frac{\text{Amount of BPA adsorbed on SCS-Mag-MIP}}{\text{Amount of BPA in solution equilibrium}} \times \frac{V}{m} \qquad (5)$$

In this study, the adsorption thermodynamic equation for BPA adsorbed onto

CS-NR-MMIP nanoparticles was $lnK_D = -1793.7/T + 6.0865$ ($R^2 = 0.9879$). The adsorption quantity of BPA increased with increased temperature, which indicates that the adsorption of BPA onto CS-NR-MMIP nanoparticles was endothermic and entropy-favorable in nature. Given that BPA can be measured at 303.15 K, this temperature was selected as the optimum temperature for further studies.

**3.5 Elution conditions**

In order to obtain satisfactory recoveries of BPA, a series of eluting agents including 50% methanol/water (v/v), 60% methanol/water (v/v), 70% methanol/water (v/v), 80% methanol/water (v/v), 90% methanol/water (v/v), methanol, 0.5% ammonia/methanol (v/v) and 0.5% acetic acid/methanol (v/v) were evaluated. The results showed that the best recoveries (76.2–86.3%) were obtained when 0.5% ammonia/methanol (v/v) and 0.5% acetic acid/methanol (v/v) were used as the elution solvent. Furthermore, because 0.08% ammonia (v/v) in methanol (A) and 0.08% ammonia (v/v) in water (B) are used as eluents in gradient elution, we chose 0.5% ammonia/methanol (v/v) as the elution solvent.

The eluting solvent volume of 100 μL, 200 μL, 300 μL, 400 μL, 500 μL and 600 μL was also investigated. The results showed that 200 μL of 0.5% ammonia/methanol (v/v) was enough for the elution of BPA from the adsorbents, and the recoveries are in the range of 79.0-90.2% at the three spiking levels (Fig. S3). In order to improve the recoveries, the CS-NR-MMIP capturing BPA was subjected to ultrasound during elution process. The ultrasonic time length (0.5-5.0 min) had also been studied and satisfactory recoveries (89.2-102.6%) were obtained when 1 min was applied. Therefore, the eluting procedure was

carried out with 200 μL of 0.5% ammonia/methanol (v/v) under ultrasound for 1 min after optimization.

### 3.6 Maximum of sample volume and enrichment factor

To obtain reliable and reproducible analytical results and a high concentration factor, obtaining satisfactory recoveries of the analyte studied from a large volume of sample solutions is important. The maximum volume was studied using CS-NR-MMIP MSPD procedure with BPA solutions (100 $ng \cdot L^{-1}$) ranging from 5 mL to 100 mL. Following the experimental procedure, the recoveries of the analyte at different volumes were obtained, and the results indicate that the maximum volume was as high as 50 mL with recovery > 99.8%. Furthermore, the similar results were also obtained in medium level of 10.0 ng/g and low level of 2.0 ng/g, respectively (Fig. S4). Therefore, 50 mL of the sample solution was adopted for the preconcentration of analytes from sample solutions. A high enrichment factor of 250 was obtained because the final elute solution was 200 μL in these experiments.

### 3.7 Effect of adsorbent mass and reusability of CS-NR-MMIP

In the design of CS-NR-MMIP MSPD procedure, a suitable mass of CS-NR-MMIP that had no effect on recovery should be primarily considered. Accordingly, the effects of various adsorbent mass on recoveries were studied with tap water sample spiked with 2, 10, and 100 $ng \cdot L^{-1}$ BPA. The spiked simples were preconcentrated using CS-NR-MMIP with different masses. The results are shown in Fig. 3. The mass of CS-NR-MMIP adsorbents affected BPA recoveries, and a clear trend in the recovery was observed when the adsorbent mass increased from 5 mg to 30 mg.

**<Insert Fig. 3>**

With the increase in the mass of CS-NR-MMIP adsorbents from 5 mg to 30 mg, the recoveries of BPA increased gradually from 85.0% to 107.6% for the three spiked levels. Thus, the lowest mass of CS-NR-MMIP adsorbents (20 mg) ensured a satisfactory recovery (100.9% to 102.8%) for all concentrations of BPA in this study.

To determine the longevity of the adsorbent, CS-NR-MMIP was subjected to several adsorption and elution experiments. The capacity of the adsorbent was practically constant (variation of 0.8% to 6.2%) after repeated use of more than 50 times. Thus, multiple use of the adsorbent is feasible.

### 3.8 Selective extraction comparison

The selective extraction of BPA from solutions of BPA and its five structural analogs (HEX, 4-tOP, DES, DE, and 4-NP) was studied. Fig. 4 shows that only BPA was selectively retained on CS-NR-MMIP with almost 100% recovery. C18 showed no retaining selectivity for BPA, as shown by the recoveries for BPA and its structural analogs in the range of 72.6% to 100.2%. BPA and its structural analogs were all poorly retained on CS-NR-MNIP with recoveries of less than 60%. Therefore, BPA-imprinted CS-NR-MMIP adsorbent exhibited high selectivity and molecular recognition function. Although the concentration of each analog was five times that of BPA, the average recovery of BPA was approximately 100%. This result demonstrates that the BPA-imprinted CS-NR-MMIP adsorbent possessed an anti-interference ability. Moreover, the nonspecific binding of HEX to CS-NR-MNIP was very strong, and its recoveries were highest among HEX, 4-tOP, DES, DE, and 4-NP in all

situations. Given the presence of two –OH groups in the HEX structure compared with that in 4-tOP and 4-NP, HEX easily formed hydrogen bonds with the amino group in CS-NR-MNIP. Compared with DES and DE, no −C=C− and −C=C−C=C− groups were present in the HEX structure, and HEX was easily adsorbed by CS-NR-MNIP without stereospecific blockade. Therefore, HEX presented high recoveries of 42.8% ± 2.6% and 48.9% ± 3.9% with CS-NR-MNIP. HEX did not affect the recovery of BPA by CS-NR-MMIP adsorbent despite its strong nonspecific binding. The BPA recoveries corresponding to the mixed solutions were 102.2% ± 2.7% (1:1) and 99.8% ± 1.8% (1:5), respectively. The experimental results show that the BPA recoveries in samples containing incremental concentrations of HEX were almost 100%.

**<Insert Fig. 4>**

**<Insert Table 1>**

Furthermore, specific selectivity of the CS-NR-MMIP was also carried out with BPA and its analogs [21]. Adsorbent (20.0 mg) was dispersed in 50 mL of 0.5 $g \cdot L^{-1}$ of BPA and the five analogs. Distribution coefficient ($K_D$), selectivity coefficient ($k$) and relative selectivity coefficient ($k'$) were obtained and the results were listed in Table 1. $K_D = (C_0 - C_e)V/mC_e$, where $C_0$ and $C_e$ ($g \cdot L^{-1}$) represent the initial and equilibrium BPA and its analogs concentration in the solution. The selectivity coefficient of the adsorbent suggested the otherness of two substances adsorbed by one adsorbent, $k = K_D$ (BPA)/$K_D$ (HEX); the relative selectivity coefficient suggested the otherness of two adsorbents, $k' = k$ MIP/$k$ NIP. BPA and

its analogs had similar $K_D$ on the CS-NR-MNIP, but the CS-NR-MMIP showed BPA adsorption capacity about five times greater than similar compounds. As shown in Table 1, the *k* value of the CS-NR-MMIP (3.66 for HEX, 7.16 for 4-tOP, 5.30 for DES, 6.42 for DE and 7.41 for 4-NP) was larger than that of the CS-NR-MNIP (0.709 for HEX, 1.83 for 4-tOP, 1.73 for DES, 1.36 for DE and 3.04 for 4-NP), which showed that the CS-NR-MMIP had high selectivity for BPA over the analogs. The relative selectivity coefficient was 5.2 for HEX, 3.9 for 4-tOP, 3.1 for DES, 4.7 for DE and 2.4 for 4-NP, which shows the high selectivity of the CS-NR-MMIP than the CS-NR-MNIP.

### 3.9 Method validation and application to real samples

Under the optimized conditions for UFLC–MS-MS coupled with CS-NR-MMIP MSPD, the correlation coefficient of the calibration curve was excellent ($r$ = 0.9992) in the concentration range of 1 $ng \cdot L^{-1}$ to 200 $ng \cdot L^{-1}$. LOD and LOQ were 0.3 and 1 $ng \cdot L^{-1}$, respectively. The accuracy of the method was estimated by determining river water and tap water samples spiked with BPA at three different concentration levels. The method robustness has been indicated by two analysts who used two analytical columns and two LC-MS-MS plate-forms during the analysis of real samples.

**<Insert Table 2>**

**<Insert Table 3>**

The proposed MSPD UFLC–MS-MS method was used to determine ultratrace BPA in river water and tap water samples. The results are listed in Table 2. The average recoveries by

the MSPD method for river water and tap water were 90.0% to 107% with relative standard deviation < 10%. These results indicate the suitability of CS-NR-MMIP for selective MSPD and determination of ultratrace BPA in environmental water samples. Furthermore, a comparison study among different methods for the determination of BPA applied in water samples was also outperformed, and the results are shown in Table 3. The CS-NR-MMIP need not to be packed into the SPE cartridge but dispersed in the sample instead. Extraction and enrichment, the two most time-consuming procedures in sample pretreatment, could be fulfilled simultaneously by simply blending and stirring the sample, and the use of CS-NR-MMIP. As is shown in the table, the proposed method gives a simpler and faster way for the extraction of BPA in water samples and provides a relatively lower LOD, higher recoveries of BPA and better precisions.

## 4. Conclusion

For the first time, magnetic isolate was used in the MSPD with magnetic polymeric particles(CS-NR-MMIP) for separation/preconcentration of BPA at ultratrace in water samples, which can separate the adsorbed adsorbent quickly and shorten the separation time from the solution. This simple, fast and cost effective procedure is based on the adsorption of BPA onto CS-NR-MMIP by hydrogen bond interaction. The enrichment method allowed BPA determination in different aqueous samples with good accuracy and reproducibility. The proposed MSPD UFLC–MS/MS method was highly sensitive and selective for BPA in environmental water samples, with LOD at nanogram per liter level.

## Acknowledgements

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**Tables Captions**

**Table 1** Distribution coefficient ($K_D$), selectivity ($k$) and relative selectivity ($k'$) obtained by competitive adsorption of BPA and its five structural analogs on CS-NR-MMIP and CS-NR-MNIP

**Table 2** Determination of ultratrace levels of BPA in river water and tap water samples using CS-NR-MMIP MSPD UFLC–MS-MS

**Table 3** Comparison of the analytical features of current LC-MS-based methodologies for the determination of BPA in water samples

**Table 1** Distribution coefficient ($K_D$), selectivity ($k$) and relative selectivity ($k'$) obtained by competitive adsorption of BPA and its five structural analogs on CS-NR-MMIP and CS-NR-MNIP

| Analyte | $C_0$(g·L$^{-1}$) | $C_e$(g·L$^{-1}$) | | $K_D$ | | $k$ | | $k'$ |
|---|---|---|---|---|---|---|---|---|
| | | CS-NR-MMIP | CS-NR-MNIP | CS-NR-MMIP | CS-NR-MNIP | CS-NR-MMIP | CS-NR-MNIP | |
| BPA | 0.500 | 0.351 | 0.468 | 1.06 | 0.171 | —— | —— | —— |
| HEX | 0.500 | 0.448 | 0.456 | 0.29 | 0.241 | 3.66 | 0.709 | 5.2 |
| 4-tOP | 0.500 | 0.472 | 0.482 | 0.148 | 0.0933 | 7.16 | 1.83 | 3.9 |
| DES | 0.500 | 0.463 | 0.481 | 0.200 | 0.0988 | 5.30 | 1.73 | 3.1 |
| DE | 0.500 | 0.469 | 0.476 | 0.165 | 0.126 | 6.42 | 1.36 | 4.7 |
| 4-NP | 0.500 | 0.473 | 0.489 | 0.143 | 0.0561 | 7.41 | 3.04 | 2.4 |

**Table 2** Determination of ultratrace levels of BPA in river water and tap water samples using CS-NR-MMIP MSPD UFLC–MS-MS

| | CS-NR-MMIP MSPD UFLC–MS-MS | | | |
|---|---|---|---|---|
| Sample | Added ($ng \cdot L^{-1}$) | Found ($ng \cdot L^{-1}$) | Accuracy[a] (%) | Precision (RSD, %) |
| Yaojiang water | 0 | 20.3 | - | - |
| | 1 | 21.2 | 90.0 | 2.9[b], 4.2[c] |
| | 10 | 31.0 | 107 | 5.9, 5.3 |
| | 100 | 123 | 103 | 3.6, 6.3 |
| Fenghuajiang water | 0 | 16.3 | - | - |
| | 1 | 17.3 | 100 | 2.6, 3.9 |
| | 10 | 26.2 | 99.0 | 5.3, 6.1 |
| | 100 | 120 | 104 | 2.8, 5.5 |
| Tap water | 0 | 2.26 | - | - |
| | 1 | 3.28 | 102 | 5.6, 6.2 |
| | 10 | 13.0 | 107 | 4.6, 7.6 |
| | 100 | 100 | 97.7 | 8.2, 9.2 |

[a] The mean value was determined in one day ($n$ = 9 replicates).

[b] Intra-day, $n$ = 9 replicates.

[c] Inter-day, $n$ = 3 replicates × 6 days within a 2-week period.

**Table 3** Comparison of the analytical features of current LC-MS-based methodologies for the determination of BPA in water samples

| Sample | Sample preparation (main steps) | Detection | Recovery (%) | R.S.D.(%) | Method LOD ($ng \cdot L^{-1}$) | reference |
|---|---|---|---|---|---|---|
| Waste water treatment plant effluents and surface waters | HLB SPE | LC–MS/MS | ____ | ____ | 2.0 | [22] |
| Sewage treatment plants and natural waters | HLB SPE | LC–MS/MS | 99.0-102% | 2.0-4.0 | 0.2-3.0 | [23] |
| Sewage samples | $C_{18}$ SPE | LC–MS/MS | 73.0-93.0 % | 7.0-13 | 1.9-9.8 | [24] |
| River | RAM-MIP SPE | LC–MS | 86.4-103% | 0.83-3.3 | 5.0 | [25] |
| River | CS-NR-MMIP MSPD | LC-MS/MS | 96.0-120% | 2.6-9.2 | 0.30 | This work |

**Scheme and Figures Captions**

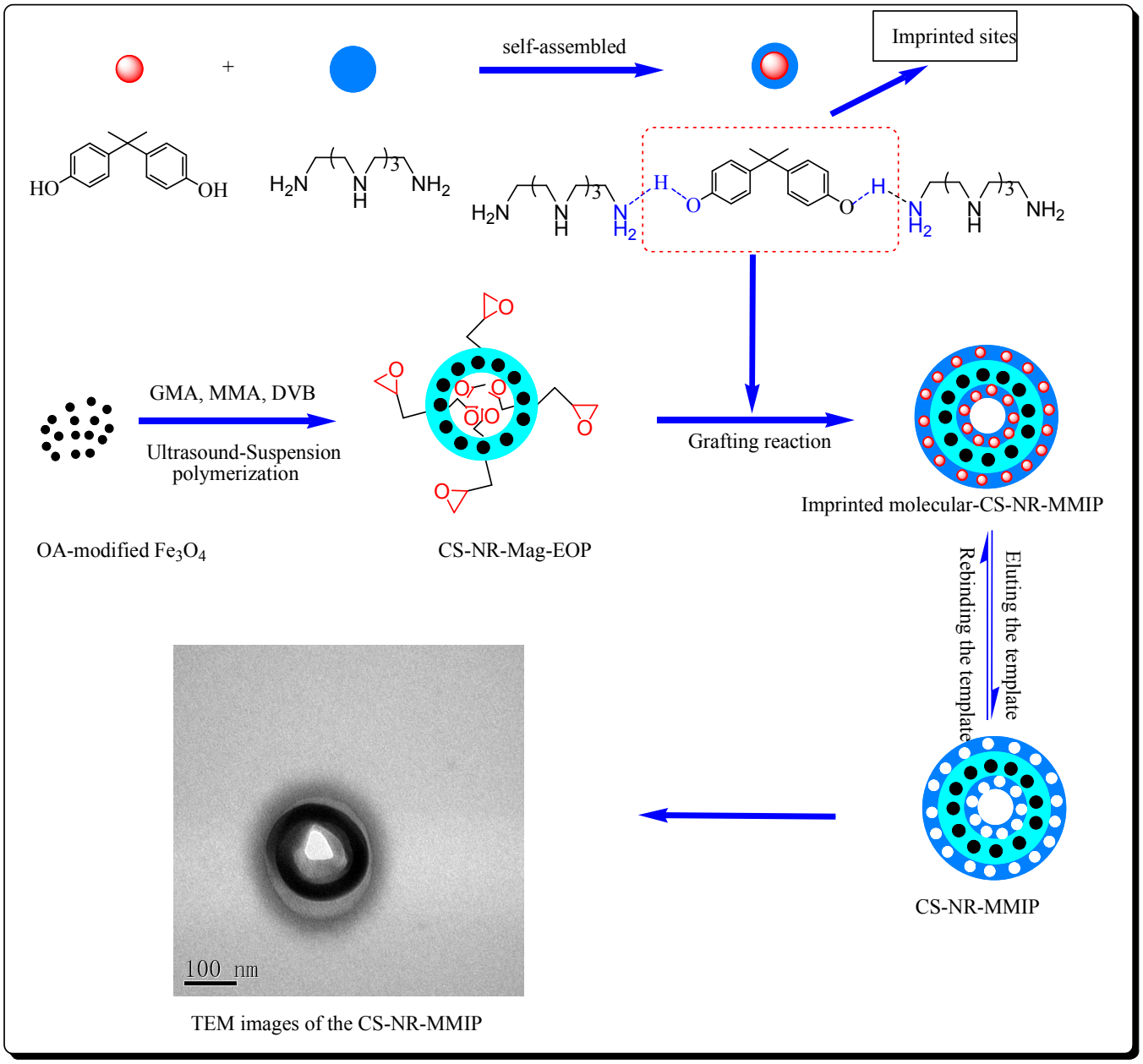
**Scheme 1** Schematic procedures for the self-assembly of surface bisphenol A-imprinted CS-NR-MMIP nanoparticles.

**Fig. 1** FTIR spectra of CS-NR-MMIP and CS-NR-MNIP nanoparticles

**Fig. 2** Effect of pH on BPA recovery using CS-NR-MMIP.

**Fig. 3** Effect of the amount of CS-NR-MMIP on BPA recovery.

**Fig. 4** Recoveries of BPA and its analogs after C18 MSPD, CS-NR-MMIP MSPD, and CS-NR-MNIP MSPD(the concentration of each analyte was 10 $ng \cdot L^{-1}$)




**Scheme 1** Schematic procedure for the self-assembly of surface bisphenol A-imprinted CS-NR-MMIP nanoparticles.

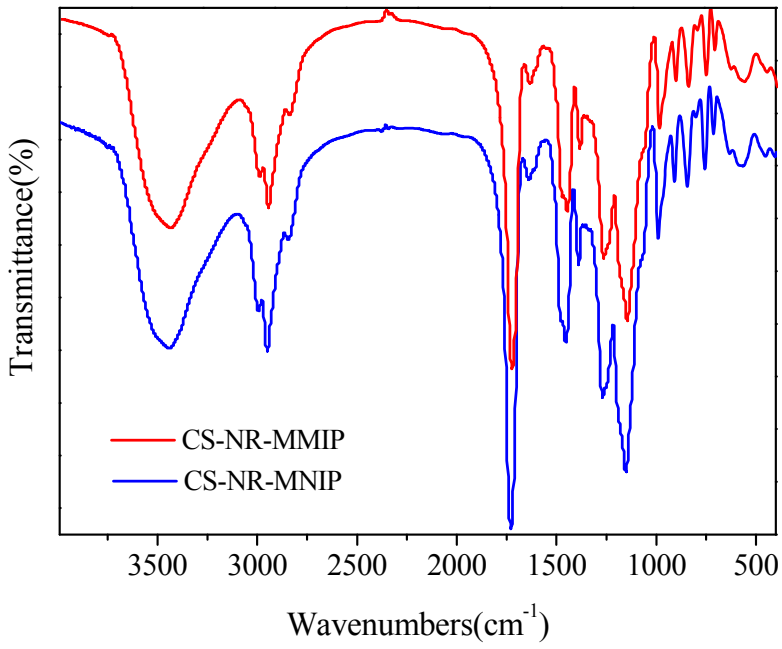



**Fig. 1** FTIR spectra of CS-NR-MMIP and CS-NR-MNIP nanoparticles

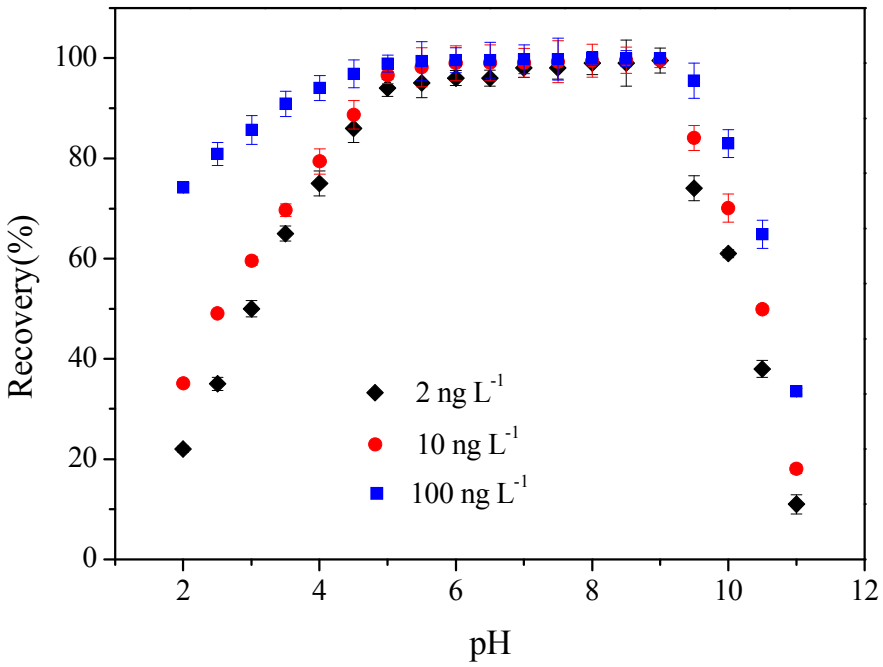



**Fig. 2** Effect of pH on BPA recovery using CS-NR-MMIP.

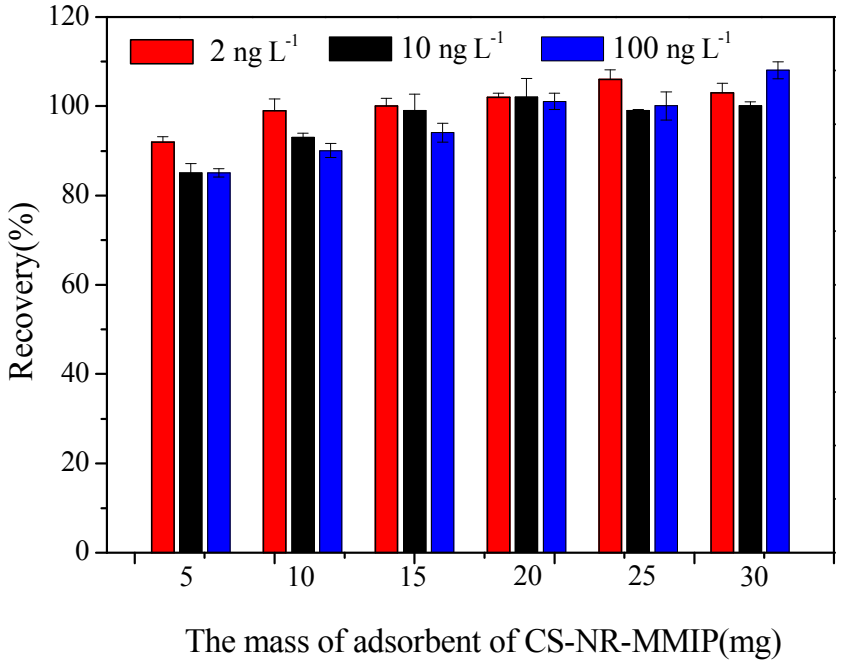



**Fig. 3** Effect of the amount of CS-NR-MMIP on BPA recovery.

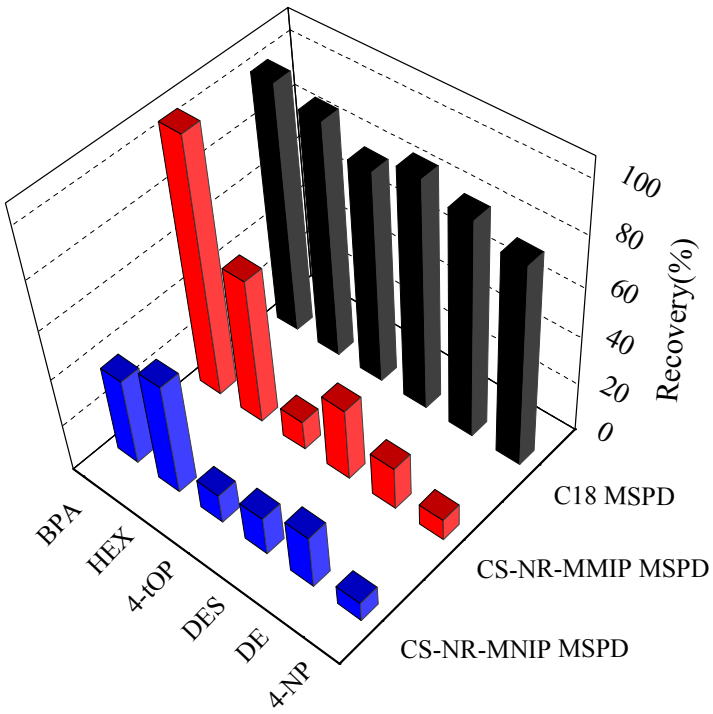



**Fig. 4** Recoveries of BPA and its analogs after C18 MSPD, CS-NR-MMIP MSPD, and CS-NR-MNIP MSPD (the concentration of each analyte was 10 ng·L$^{-1}$)

**Supporting Information**

**Table S1.** Q1/Q3 ion pairs, declustering potential (DP), and collision energy (CE) from multiple-reaction monitoring and retention time for five structural analogs.

**Fig. S1.** Structural formulas of BPA and its five structural analogs.

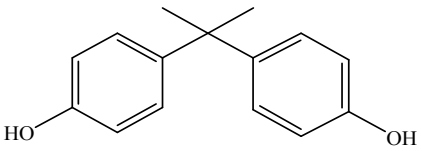
**Fig. S2.** Effect of the shaking time on BPA recovery.

**Fig. S3.** Effect of the volume of eluting solvent on BPA recovery.

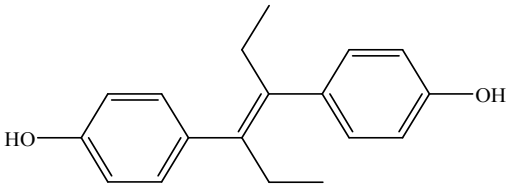
**Fig. S4.** Effect of the volume of sample on BPA recovery.

**Table S1.** Q1/Q3 ion pairs, declustering potential (DP), and collision energy (CE) from multiple-reaction monitoring and retention time for five structural analogs

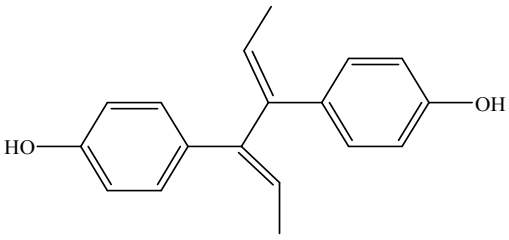
| Compounds | Precursor ion (Q1, *m/z*) | Fragment ion (Q3, *m/z*) | DP (V) | CE (eV) | Retention Time (min) |
|---|---|---|---|---|---|
| DES | 267.1 | 251.1*, 237.1 | 140, 140 | 32, 37 | 3.46 |
| DE | 265.2 | 93.0*,171.0 | 130, 130 | 31, 25 | 3.59 |
| HEX | 269.1 | 134.1*,119.0 | 130, 130 | 20, 51 | 3.69 |
| 4-tOP | 205.0 | 133.0*,93.0 | 120, 120 | 32, 60 | 5.54 |
| 4-NP | 219.0 | 106.0*,119.0 | 100, 100 | 26, 55 | 7.09 |



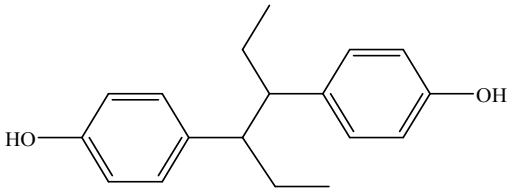

Bisphenol A (BPA)



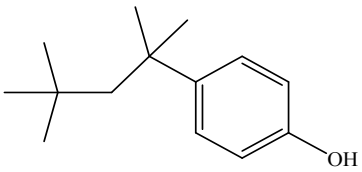

Diethylstilbestrol(DES)



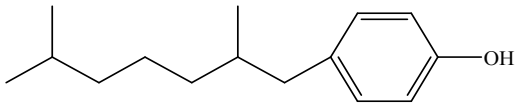

Dienestrol (DE)




Hexestrol (HEX)




4-(tert-octyl) phenol (4-tOP)




4-nonylphenol (4-NP)

**Fig. S1.** Structural formulas of BPA and its five structural analogs

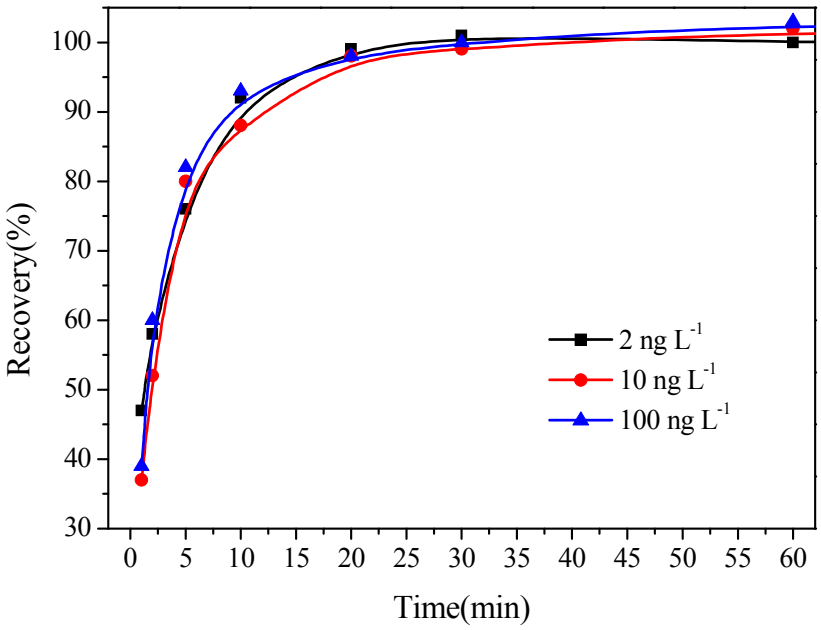



**Fig. S2.** Effect of the shaking time on BPA recovery.

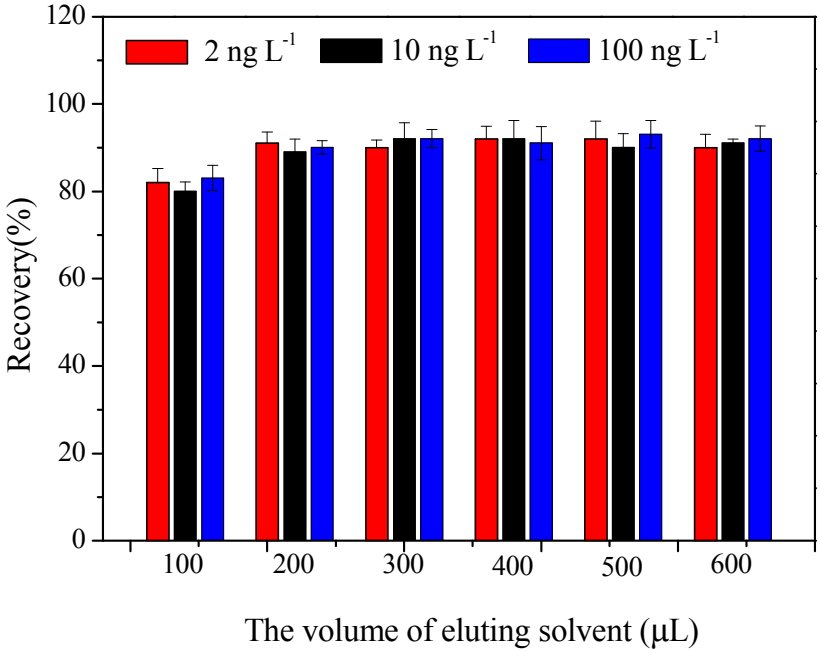



**Fig. S3.** Effect of the volume of eluting solvent on BPA recovery.

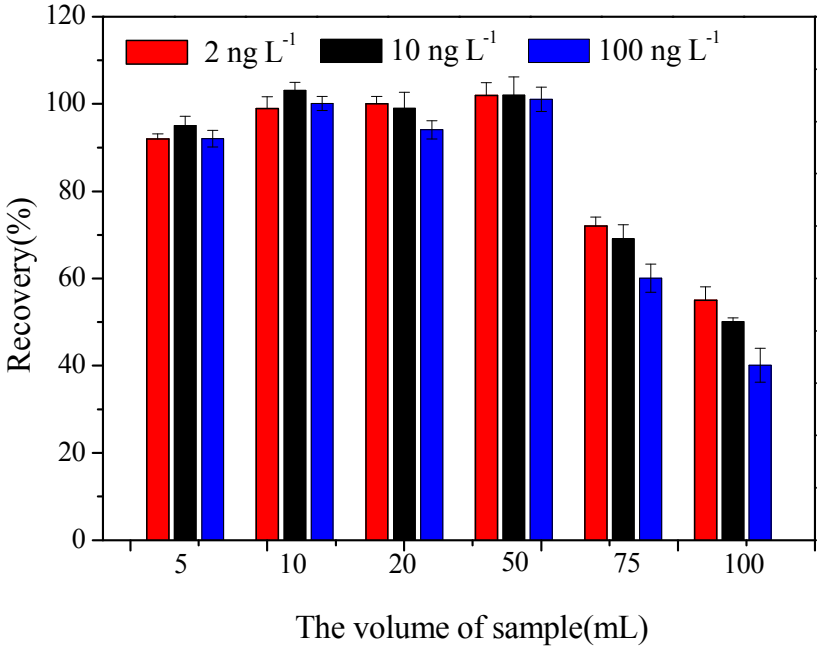



**Fig. S4.** Effect of the volume of sample on BPA recovery.