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Analytical Methods

Selective solid-phase extraction using molecular imprinted polymer for the analysis of diethylstilbestrol

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Abstract

A simple imprinted amino-functionalized silica gel material was synthesized by combining a surface molecular imprinting technique with a sol–gel process for solid-phase extraction–high performance liquid chromatography (SPE–HPLC) determination of diethylstilbestrol (DES). Activated silica gel was used as the supporter and non-imprinted silica sorbent was synthesized without the addition of DES using the same procedure as that of DES-imprinted silica sorbent. Compared with non-imprinted polymer particles, the prepared DES-imprinted silica sorbent showed high adsorption capacity, significant selectivity, good site accessibility and fast binding kinetics for DES. The maximum static adsorption capacity of the DES-imprinted and non-imprinted silica sorbent for DES was 62.58 mg g^{-1} and 19.89 mg g^{-1}, respectively. The relatively selective factor value of this DES-imprinted silica sorbent was 61.7 at the level of 50 mg L^{-1}. And the uptake kinetics was fairly rapid so that the adsorbent equilibrium was achieved within 10 min. Furthermore, the DES-imprinted polymers were used as the sorbent in solid-phase extraction to determine DES in fish samples. The MIP–SPE–HPLC method showed higher selectivity and good recoveries higher than 87.5% (R.S.D. 11.6%).
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Keywords: Molecularly imprinted polymers; Surface imprinted technique; Diethylstilbestrol; Solid-phase extraction; High performance liquid chromatography

1. Introduction

Molecular imprinted polymers (MIPs) are tailor-made materials with high selectivity for a target molecule. In the last few years, the selectivity of MIPs has been exploited in several applications, e.g., chromatography and electrochromatography, sensors, etc. (Shughart, Ahsan, Detty, & Bright, 2006; Wang, Jiang, Ma, & Pang, 2006; Zhou, Lai, & Miller, 2004). One of the most exciting applications of MIPs is as sorbent for solid-phase extraction (da Costa Silva & Augusto, 2006; Tamayo, Casillas, & Martin, 2005; Yang, Liu, Wang, Liu, & Chen, 2005; Zhu, Yang, Shu, Cai, & Gao, 2005). The use of MIPs in SPE (MIP–SPE) is advantageous mainly when a selective extraction must be performed and the commonly used sorbents lack selectivity. MIP–SPE allows not only the analyte to be pre-concentrated but also the other compounds present in the sample matrix to be removed (Caro, Marce, Borrull, Cormack, & Sherrington, 2006).

MIPs can be synthesized following three different imprinting approaches: the non-covalent, the covalent, and the semi-covalent. In all these protocols, a template molecule interacts with an appropriate functional monomer to establish specific interactions. In recent years, surface molecular imprinting technique was proposed. This technique provides, after polymerization, the complementary recognition sites to the target molecules at the inner cavity surfaces of the imprinted polymer. The polymer obtained is ground to appropriate particles to interact with the target molecules (Han, Fang, & Yan, 2005; Lu & Yan, 2004; Shiomi, Matsui, Mizukami, & Sakaguchi, 2005; Yoshida, Hatate, Uezu, Goto, & Furusaki, 2000). The polymer with binding sites situated at the surface shows many

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advantages including high selectivity, more accessible sites, fast mass transfer and binding kinetics (Fang, Tan, & Yan, 2005).

DES is a synthetic form of estrogen, a female hormone. In the past few years a number of papers have highlighted the potentially dangerous consequences to humans and wildlife of the presence of estrogens in the aquatic environment (Almeida & Nogueira, 2006; Lee et al., 2004; Liu et al., 2005; Yang, Luan, & Lan, 2006). In contrast to natural estrogen, estrogenic drugs such as DES are more stable and remain in the body longer than natural estrogen (Tapiero, Bo, & Tew, 2002). However, DES is often used as the agent to increase the heft of flesh for cattle, sheep, chook and fish, etc. It is important to determine the leavings of DES in meat.

In this study, DES was used as the template, 3-aminopropyltrimethoxysilane (APTES) as a functional monomer and tetraethoxysilicane (TEOS) as a reticulating agent to prepare a new and simple molecular imprinted amino-functionalized silica gel sorbent by a surface imprinted technique in combination with a sol–gel process. Then, the silica gel sorbent was characterized by FT-IR, static adsorption experiment, uptake kinetics experiment, selectivity experiment and repeated utilization experiment. Finally, the silica gel sorbent was applied to SPE coupled with high performance liquid chromatography for the determination of DES in fish samples. The proposed method presented high selectivity and adsorption capacity for DES.

2. Experimental

2.1. Materials and chemicals

Silica gel (80–120 mesh, Qingdao Ocean Chemical Company, Qingdao, China) was used as the supporter to prepare the DES-imprinted functionalized sorbent. 3-Aminopropyltrimethoxysilane (APS), tetraethoxysilicane (TEOS) (Qingdao Ocean University Chemical Company, Qingdao, China), diethylstilbestrol (DES) (Ying Fa Peng Na, Ningbo, China), bisphenol A (BPA) (Sinopharm Group Chemical Reagent Co., Shanghai, China), chloramphenicol (Sigma, Beijing, China) were used in this study. Ultra pure water used throughout the experiments was obtained from a purification system (MILLI-Q).

The mobile phase used for HPLC experiments was a mixture of acetonitrile/water (55:45, v/v), and was filtered through a 0.45 μm filter prior to use.

2.2. Instrumentation

The chromatographic system consisted of a Model 210 HPLC pump and a UV detector (VARIAN PROSTAR). All separations were achieved on an analytical reversed-phase column (Hanbon Science & Technology, C_{18} column, 4.6×150 nm) with a flow rate of 0.8 mL min^{-1} at room temperature. The UV detector was operated at 254 nm.

The MIP–SPE study was developed in an off-line mode using a solid-phase extraction cartridge supplied by Dalian institute of Chemical Physics, Chinese Academy of Sciences. Two hundred milligrams of DES-imprinted silica gel sorbent and non-imprinted silica gel sorbent was packed into this SPE cartridge, respectively.

2.3. Procedures for the preparation of the DES-imprinted amino-functionalized silica gel sorbent

The silica gel surfaces were activated by refluxing 8 g of silica gel (80–120 mesh) with 60 mL of 6 mol L^{-1} hydrochloric acid while stirring for 8 h, then the activated silica gel was filtered and washed with ultra pure water to neutral and dried under vacuum at 70 °C for 8 h.

To prepare the DES-imprinted amino-functionalized silica gel sorbent, 0.5 g of DES was dissolved in 5 mL of methanol while stirring, and 2 mL of APS was added into the mixture. After the solution was stirred and refluxed for 20 min, 4 mL of TEOS was added. After stirring for 10 min, 1.0 g of activated silica gel and 1 mL of 1.0 mol L^{-1} HAc were added. Then, the mixture was stirred for 15 h at room temperature.

The product was filtrated and dried at 100 °C for 12 h. The sorbent was washed with 30 mL of methanol and 30 mL of 1.0 mol L^{-1} HCl while stirring for 3 h to remove the DES. The product was recovered by filtration, washed with 50 mL of mixture of methanol and 6 mol L^{-1} HCl (1:1, v/v) and ultra pure water, neutralized with 0.05 mol L^{-1} KOH and washed with ultra pure water again. Finally, the sorbent was dried at 100 °C for 12 h. Then, the above-mentioned experiment was repeated once to remove DES. The non-imprinted functionalized silica gel sorbent was also prepared using an identical procedure without adding DES.

2.4. Static adsorption test

Ten millilitres of various concentrations of DES was dissolved in methanol, then 50 mg of DES-imprinted silica sorbent was added. The mixture was shaken for 1 h at room temperature to facilitate the adsorption of DES onto the DES-imprinted sorbent. After the solution was centrifuged, the concentrations of the DES in the supernatants were determined by HPLC. The adsorption of DES to the non-imprinted sorbent was also tested using an identical procedure.

2.5. Selectivity experiment

Adsorption and competitive recognition studies were performed with DES and structurally similar compound BPA.

Two DES and BPA mixture solutions were prepared. Ten millilitres of the mixture solutions was dissolved in methanol, then 50 mg of DES-imprinted silica sorbent was added. The mixture was shaken for 1 h at room tem-

perature. After the solution was centrifuged, the concentrations of the DES and BPA in the supernatants were determined by HPLC.

2.6. Uptake kinetics experiment

In a typical uptake kinetics text, 50 mg of the sorbent was added to 10 mL of 200 mg L^{-1} DES solution. The mixture was mechanically shaken for 5–35 min at room temperature. After the solution was centrifuged, the concentration of the DES in the supernatants was determined by HPLC.

2.7. MIP–SPE procedure in extraction of DES from fish samples

2.7.1. Selectivity of the MIP–SPE column

Two hundred milligrams of DES-imprinted silica gel sorbent and 200 mg of the non-imprinted silica sorbent were placed into two empty SPE cartridges, respectively. After they were pretreated with 10 mL of methanol and 10 mL of pure water, 1.0 mL of a 10 μg mL^{-1} DES, BPA and chloramphenicol mixture solution was loaded onto the MIP–SPE column and the NIP–SPE (non-imprinted SPE) column with the speed of 2 mL min^{-1}. Then the columns were washed with 2 mL of water/methanol (98:2, v/v) solution, then, eluted with 2 mL of methanol. The elution was analyzed by HPLC and UV detection at 254 nm.

2.7.2. Determination of DES in spiked water samples

According to the procedure in Section 2.7.1, an MIP–SPE column was prepared for the determination of DES in water samples. Two millilitres of the DES solutions was loaded onto the MIP–SPE column and the NIP–SPE column with the speed of 2 mL min^{-1}. Ultra pure water was spiked with DES at three concentration levels with 10.0 μg mL^{-1}, 1.0 μg mL^{-1} and 0.1 μg mL^{-1}. Then, the columns were eluted with 2 mL of methanol. The elution was analyzed by HPLC.

2.7.3. Recycling of the MIP–SPE column

In this experiment, the MIP–SPE column was used to extract DES through five cycles. According to Section 2.7.2, 2 mL of 10.0 μg mL^{-1} DES solutions was used in this experiment.

2.7.4. Determination of DES in fish samples

In this experiment, three kinds of fish were selected. The three kinds of fish were cyprinoid, hairtail and herbivorous fish. After the fresh fish was wringed, four portions of 10 g of samples were added to four 100 mL containers, respectively. One of the samples was blank sample; the other three samples were added with DES standard. Then, the volume of the solution was made up to 50 mL with methanol. After adequate shaking, the samples were extracted by ultrasonics for 30 min. Then the supernatants were obtained by centrifuging at 10,000 rpm for 5 min. After evaporation, the residue was dissolved in 5 mL of methanol. The solution (1.0 mL) was diluted to 50 mL with ultra pure water. Twenty millilitres of the diluted solution was loaded onto the MIP–SPE column at the rate of 2 mL min^{-1}. Then, the column was eluted with 2 mL of methanol. The analytes in the elution were analyzed by HPLC.

3. Results and discussion

3.1. Preparation of the DES-imprinted amino-functionalized silica gel sorbent

Silica gel is an amorphous inorganic polymer having siloxane groups (Si–O–Si) in the bulk and silanol groups (Si–OH) on its surface. The surface silanol groups facilitate the introduction of the organic groups which covalently bind to the silica surface. Because commercial silica gel contains a low concentration of surface silanol groups suitable for modification, the activation of silica gel surface is necessary (Jiang, Chang, Zheng, He, & Hu, 2006).

The complex was formed between DES and APS, then grafted to the activated silica gel. The template was thought to be bound using non-covalent interaction owing to the functional monomers used and the template functionalities present. The proposed monomer–template interaction in this MIP was considered as hydrogen bonding interaction. A similar mechanism has been hypothesized by Han et al. (2005). After the residue of APS and DES was removed, the imprinted functionalized silica gel sorbent, which contained a tailor-made cavity for DES, was formed.

3.2. Characteristic of the FT-IR spectra

FT-IR spectra were obtained from activated silica gel, DES-imprinted and non-imprinted amino-functionalized silica gel sorbents, respectively. As shown in Fig. 1, the observed features around 1102.32 and 957.97 cm^{-1} indicated Si–O–Si and Si–O–H stretching vibrations, respectively. The presence of adsorption water was reflected by υ_{OH} vibration at 3434.85 and 1641.57 cm^{-1}. The bands around 807.52 and 469.89 cm^{-1} resulted from Si–O vibrations. Fig. 1b and c shows that a characteristic feature of the DES-imprinted and non-imprinted silica gel sorbent compared with activated silica gel is N–H band around 1553.19 cm^{-1} and C–H band around 2940.74 cm^{-1} in APS. These results suggest that $-NH_2$ has been grafted onto the surface of silica gel after modification. There was a significant feature in the frequencies of IR spectra of DES-imprinted. The observed features around 1508.55 cm^{-1} indicate C–C stretching vibrations of the phenyl in DES. The feature proved that the DES has been grafted successfully onto the imprinted silica gel sorbent.

3.3. Static adsorption of DES-imprinted sorbent for DES

The adsorption capacity is an important factor because it determines how much adsorbent is required to quantitatively concentrate the analyte from a given solution. The

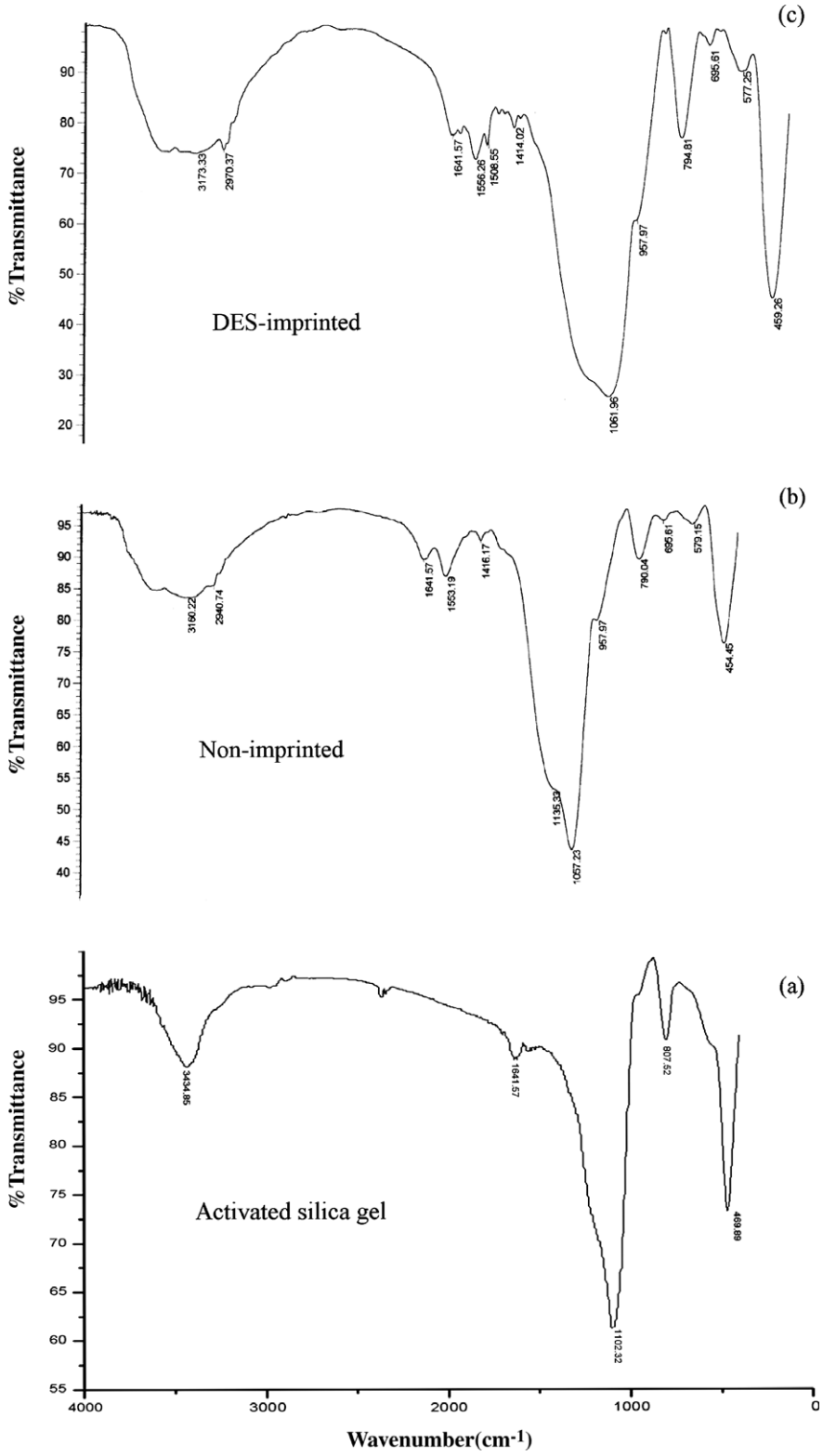

Fig. 1. FT-IR spectra of the activated silica gel, non-imprinted and DES-imprinted sorbents.

range of DES solution with the concentration of 100–1500 mg L^{-1} was studied. As can be seen in Fig. 2, the amount of DES adsorbed per unit mass of MIPs increased with the initial concentrations of DES. The static adsorption capacity of the DES-imprinted silica sorbent and non-imprinted silica sorbent for DES was calculated as 61.71 mg g^{-1} and 19.89 mg g^{-1}, respectively. The calculated method was according to the reference (Han et al., 2005). The static adsorption capacity of the DES-imprinted silica sorbent was about three times of non-imprinted silica

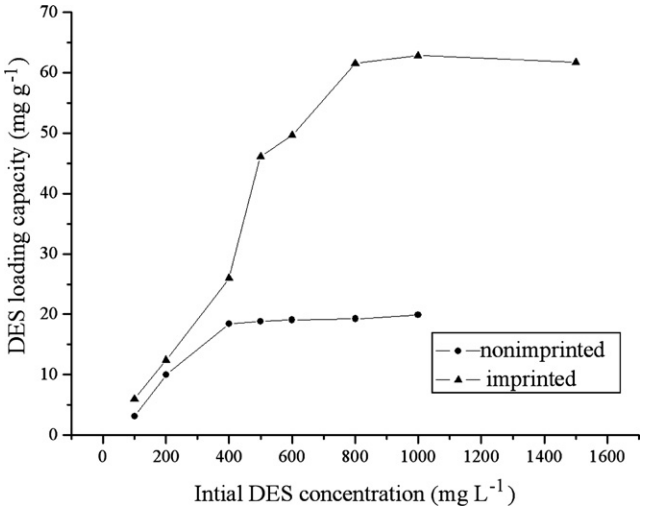

Fig. 2. Loading isotherm of DES onto the imprinted and non-imprinted sorbents.

sorbent. The results showed that the DES-imprinted silica sorbent had a higher adsorption capacity for DES. The DES-imprinted silica sorbent would be better to enrich trace DES in the samples.

3.4. Selectivity of the imprinted sorbent

The structurally similar compound BPA was chosen as the competitive species with DES for the competitive recognition study. As can be seen in Table 1, distribution coefficient (K_d), selectivity coefficient of the sorbent (k) and the relative selectivity coefficient (k') were obtained in these competitive experiments. Distribution coefficient (K_d) suggested the character of a substance adsorbed by a sorbent, selectivity coefficient of the sorbent (k) suggested the otherness of two substances adsorbed by one sorbent and relative selectivity coefficient (k') suggested the otherness of two sorbents. These factors were calculated as the following formulae (1)–(3) (Han et al., 2005). As can be seen in Table 1, DES and BPA had similar K_d on the non-imprinted silica sorbent, but the DES-imprinted silica sorbent showed about 20 times adsorbed capacity to DES than to BPA. The k (DES/BPA) values of the DES-imprinted silica sorbent (17.98 and 29.00) were greater than that of non-imprinted silica sorbent (1.14 and 0.470), which showed that the DES-imprinted silica sorbent had high selectivity for DES over the structurally similar BPA compounds. The k' values were 15.8 and 61.7, which were greater than 1.0. It showed that the DES-imprinted silica sorbent had higher selectivity than the non-imprinted silica sorbent.

$$K_d = (C_i - C_f)/C_f \times \text{volume of solution(mL)}/\text{mass of gel(g)} \quad (1)$$

where C_i and C_f represent the initial and final concentrations, respectively.

$$k = K_d\text{BPA}/K_d\text{BP} \quad (2)$$

$$K' = k_{\text{imprinted}}/k_{\text{non-imprinted}} \quad (3)$$

3.5. Uptake kinetics of DES by the DES-imprinted silica gel sorbent

The uptake kinetics of DES was experimented according to Section 2.6. The result showed that the adsorption process of the DES-imprinted silica gel sorbent was rapid. The adsorption equilibrium was achieved within 10 min. And if the concentration of DES was lower, the time to saturate became shorter. This meant that the surface imprinted polymer with binding sites situated at the surface showed the advantages of more accessible sites, fast mass transfer and binding kinetics.

3.6. MIP–SPE procedure in the extraction of DES from fish samples

3.6.1. Selectivity of the MIP–SPE column

Chromatograms of the DES, BPA and chloramphenicol mixture solution from the MIP–SPE and NIP–SPE are shown in Fig. 3. Fig. 3a shows that the directly injection of the standard of the mixture solution. Fig. 3b and c shows the direct outflow from NIP–SPE column and MIP–SPE column when loading the samples. It could be found that the three chemicals were retained approximately identical on NIP–SPE column. However, on the MIP–SPE column, DES was retained more than the others. Fig. 3d and e shows the washing solution obtained from NIP–SPE column and MIP–SPE column with water/methanol (98:2, v/v) solution. From Fig. 3d and e, we could find that BPA and chloramphenicol were completely removed from the two columns after the washing step but the template molecular DES was retained on the MIP–SPE column

Table 1
Competitive loading of DES and BPA by the DES-imprinted and non-imprinted polymers

Sorbents	Initial solution (mg L^{-1})		Capacity (mg g^{-1})		K_d		k	k'
	DES	BPA	DES	BPA	DES	BPA	DES/BPA	
Imprinted	200	200	8.51	0.57	54.8	3.05	18.0	15.8
	50	50	3.46	0.18	107	3.65	29.0	61.7
Non-imprinted	200	200	6.40	4.71	31.2	27.3	1.14	
	50	50	0.34	0.82	8.33	17.9	0.47	

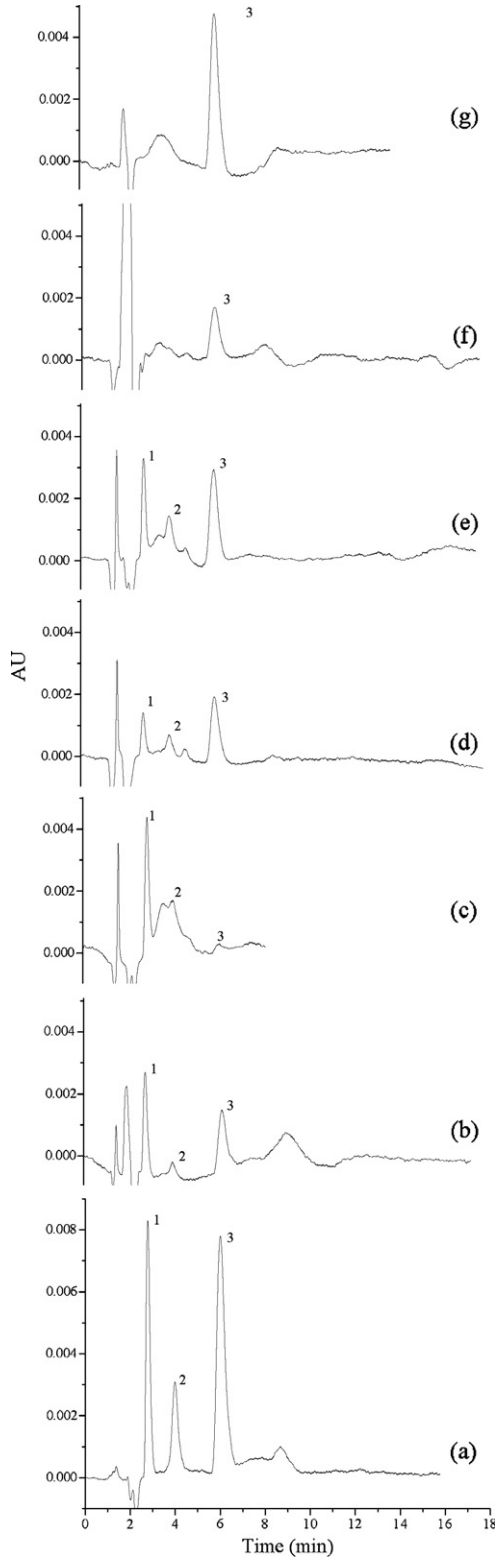

Fig. 3. Chromatograms of the DES, BPA and chloramphenicol mixture solution from the SPE columns: (a) direct injection of the standard mixture solution; (b) direct outflow from NIP–SPE column when loading the samples; (c) direct outflow from MIP–SPE column when loading the samples; (d) washing solution obtained from NIP–SPE column; (e) washing solution obtained from MIP–SPE column; (f) elution solution obtained from NIP–SPE column; (g) elution solution obtained from MIP–SPE column. (1) Chloramphenicol, (2) BPA, (3) DES. Washing step: 2 mL of water/methanol (98:2, v/v); elution step: 2 mL of methanol.

more than on the NIP–SPE. These chromatograms showed that the DES-imprinted silica gel sorbent exhibited highly selective binding affinity for DES.

Fig. 3f and g shows the elution solution obtained from column NIP–SPE and MIP–SPE column by methanol. From Fig. 3g, we can find that comparative pure DES was obtained after the washing step. So, when complicated matrix existed, the DES-imprinted silica gel sorbent was the better choice.

3.6.2. Determination of DES in spiked water samples

The samples were extracted according to Section 2.7.2. The recoveries and reproducibility of the method were calculated and summarized in Table 2. As can be seen, the average recovery of the MIP–SPE method was 100.19% at the studied levels and the average recovery of the NIP–SPE method was 84.63%. These results demonstrated that the DES-imprinted silica sorbent had good recovery than non-imprinted silica sorbent.

3.6.3. Recycling of the MIP–SPE column

To test the reusability of the MIP–SPE columns, the samples were extracted according to Section 2.7.3. The results showed that the DES-imprinted polymers have better reusability towards DES. One MIP–SPE column could be used repeatedly for two times with a recovery of 97.33% and repeatedly for five times with a recovery of 90.15%.

3.6.4. Determination of DES in fish samples

The proposed method was applied to the analysis of DES in three fish samples. The analytical results are shown in Table 3. Three DES levels were added to blank fish samples. The recoveries from the MIP–SPE method were in the range of 87.5–97.3%. The detection limit (LOD) was obtained from the signal-to-noise ratio (S/N) and calibration curve. In this work, the noise of the baseline was measured from chromatogram of blank fish sample. Three times of the noise as the signal value (S/N = 3) was used to calculate the LOD in the calibration curve. The LOD obtained was 60 ng mL^{-1}. Under the operation condition, no DES was found in the blank fish samples. The detect limit would be lower further if the sample was concentrated before the MIP–SPE operation or before the injection on HPLC.

These results indicated the suitability of DES-imprinted amino-functionalized silica gel sorbent for selective solid-

Table 2
Recoveries (%) and RSD of DES after MIP–SPE and NIP–SPE of spiked water samples

Added (mg L^{-1})	Found (mg L^{-1})		Recovery (%)		RSD (%)	
	MIP	NIP	MIP	NIP	MIP	NIP
0.1	0.10	0.88	102	88.5	3.21	2.70
1.0	0.99	0.85	99.2	85.3	2.60	2.54
10.0	0.99	0.80	99.8	80.1	3.72	3.81

Table 3
Recoveries of the determination of DES after MIP–SPE of spiked fish samples

Sample content	Added (mg g^{-1})	Found (mg g^{-1})	Recovery (%)	RSD (%)
Herbivorous fish	0.1	0.09	90.3	11.6
	1.0	0.9	87.5	8.3
	12.0	11.6	97.0	6.5
Cyprinoid	0.1	0.08	89.2	6.0
	1.0	0.9	91.5	5.12
	12.0	11.7	97.3	6.0
Hairtail	0.1	0.09	91.9	11.3
	1.0	0.9	91.6	4.3
	12.0	11.6	96.8	5.2

phase extraction and determination of DES in complicated samples.

4. Conclusions

In this paper, a novel and simple procedure was developed to synthesize DES-imprinted amino-functionalized silica gel sorbent with a surface molecular imprinting technique. The imprinted sorbent had high adsorption capacity, selectivity and good site accessibility for DES. And the polymer was used as a solid-phase extraction sorbent to determine DES in fish samples. The MIP–SPE–HPLC method showed good recoveries and higher selectivity. The precision and accuracy of the method are satisfactory.

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