**Preparation of a stir bar sorptive extraction coating based on molecularly imprinted polymer and its application in the extraction of dienestrol and hexestrol in complicated samples**

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**Abstract:** A new stir bar sorptive extraction (SBSE) coating based on molecularly imprinted polymer (MIP) with diethylstilbestrol as replaced template molecule was prepared. The influences of the contents of template molecule and monomer in the polymerization mixture on the extraction performance of MIP-SBSE were investigated thoroughly. The MIP was characterized by elemental analysis, scanning electron microscopy and infrared spectroscopy. In order to evaluate the usability of the new coating, the MIP-SBSE was combined with high performance liquid chromatography (HPLC) and diode array detector (DAD) with dienestrol (DS) and hexestrol (HS) as detected solutes. To achieve optimally selective extraction performance for DS and HS, several parameters, including extraction and desorption times, desorption solvent, ionic strength and pH value in sample matrix were investigated. The results showed that under the optimized experimental conditions, the present method has high selectivity and sensitivity. When drying-redissolving procedure was taken during sample preparation, the limits of detection for DS and HS were as low as 0.04 μg/L and 0.14 μg/L, respectively. Good linearities were obtained for analytes with the correlation coefficients (R²) above 0.99. Finally, the proposed method was successfully applied to the determination of DS and HS in wastewater, honey and cow urine samples. The recoveries of spiked target compounds in real samples ranged from 61.3% to 120%. The developed method is simple, selective, sensitive and applicable for the analysis of trace DS and HS in complicated samples.

**Key words:** stir bar sorptive extraction (SBSE); diethylstilbestrol; molecularly imprinted polymer; extraction; dienestrol; hexestrol

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**分子印迹固相萃取搅拌棒的制备及对复杂样品中双烯雌酚和己烷雌酚的萃取性能**

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**摘要：** 以己烯雌酚为替代模板，利用整体材料的“原位”合成技术制备了分子印迹聚合物，并将其用于固相萃取搅拌棒的涂层（MIP-SBSE）制备了新的搅拌棒。详细考察了分子印迹聚合物制备条件中模板分子及功能单体用量对MIP-SBSE选择性萃取性能的影响，同时利用元素分析、扫描电镜和红外光谱对聚合物进行表征。以双烯雌酚（DS）和己烷雌酚（HS）为目标化合物，将MIP-SBSE与高效液相色谱-二极管阵列检测器联用，建立起复杂样品中DS和HS的分离分析方法。考察了吸附和解吸时间、解吸溶剂、离子强度和样品pH值等萃取条件对MIP-SBSE选择性萃取性能的影响。结果表明，在最佳萃取条件下，MIP-SBSE对DS和HS具有较高的选择性萃取性能，线性范围分别为1.0～400.0 μg/L和5.0～400.0 μg/L，利用氮吹再定容的方法，对DS和HS的检出限（S/N = 3）分别可低至0.04和0.14 μg/L。在对实际污水、蜂蜜和牛尿样品的分析中取得了良好的加标回收率，其值为61.3%～120%。所建方法具有简单、高选择性和灵敏等特点，可用于复杂样品中双烯雌酚和己烷雌酚的分析监测。

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Diethylstilbestrol (DES), dienestrol (DS) and hexestrol (HS) are synthetic estrogen that was widely used in livestock production to promote growth rate and as a treatment for estrogen-deficiency disorders in veterinary medicine [1]. Due to their adverse effects to human and animals, the use of DES, DS and HS as growth promoters has been banned in many countries [2]. Although the use of these compounds as growth promoters has been banned for years, some studies still found the existence of these compounds in rivers and foods [3,4]. In line with this, residue analysis of these estrogens has been given attention in order to control their illegal use.

There are several analytical methods including gas chromatography (GC) [5,6], capillary electrophoresis (CE) [7] and high performance liquid chromatography (HPLC) [8–10] have been applied to the determination of estrogen. Mass spectrophotograph (MS) and ultraviolet detectors can be used to detect estrogen. Higher sensitivity can be obtained on MS but higher cost will be paid in instrument and analysis procedure. Therefore, HPLC with ultraviolet detection still plays a key role in the analysis of estrogen [9,10]. In order to lower the detection limits and eliminate the disturbance of environmental matrices, pre-concentration and clean-up steps are usually required. Solid-phase extraction (SPE) is the popular sample preparation for the analysis of estrogen because SPE possesses some advantages such as speediness, good reproducibility and cleaner extracts [10,11]. However, the main problems of SPE for the determination of estrogen in the environmental matrices are the lack of selectivity. Hereby, non-targeted substances are also extracted when the target analytes are enriched, the non-targeted substances will disturb the extraction of target analytes and lead to low recoveries of the analytes [12]. The above problems can be circumvented by the use of molecularly imprinted polymers (MIPs) as sorbents. MIPs are stable synthetic polymers possessing selective molecular recognition sites, which have been shown to have promising applications in many fields, such as chiral separation [13], biological sensors [14] and SPE [9]. However, template leaking is a potential problem, especially for the extraction of trace substances. Using a structurally analogue analyte as a replaced template to the prepared MIPs can overcome this problem [15]. There are several studies that using DES-based MIPs as the SPE sorbents [9,16]. The MIP-SPE showed high selectivity to template molecule and structural analogues such as DS and HS. However, several steps which are time-consuming and also affect recoveries are involved in MIP-SPE. Recently, Chen et al. [17] synthesized a diethylstilbestrol-based molecularly imprinted polymer-coated hollow fiber tube. The fiber tube could selectively extract DES, DS and HS with simple operation, but the extraction capacities were not as high as expected. Hereby, it is necessary to develop simple, highly selective, efficient and robust pretreatment methods for the monitoring of estrogen.

Stir bar sorptive extraction (SBSE) was introduced in 1999 by Sandra et al. [18] and has been employed as an “environmental friendly” sample preparation technique based on the same principle as those of solid-phase microextraction (SPME) [19]. Because SBSE possesses many advantages such as high sensitivity, good reproducibility and high recovery, well in the removal of matrix interference, it has been successfully applied to the trace analysis of various target analytes in environmental and biological samples. In our previous studies, a serial of new coatings based on monoliths for SBSE [20–24] have been prepared and applied to the extraction of organic pollutants [20–25] and inorganic ions [26,27] in all kinds of matrices. The results well indicated that using monolithic materials as the extraction media possesses many advantages such as simple preparation, good permeability, high extractive capacity and low cost. However, these coatings suffer from unsatisfactory extraction selectivity. In
order to improve the extraction selectivity of SBSE and supply a simple sample pretreatment for the analysis of estrogen in complicated samples, in the present study, the flexibility and simplicity of SBSE were combined with the high selectivity and good permeability of MIPs. Furthermore, DES whose structure was analogous to the structures of DS and HS was selected as template molecule for the avoidance of template leaking. The sorptive performance of the MIP-SBSE was evaluated in detail. Then, a methodology combined the MIP-SBSE and liquid desorption (LD), followed by HPLC with diode array detector (MIP-SBSE-LD-HPLC/DAD) for the direct analysis of traces of DS and HS in wastewater, honey and cow urine was developed. The results showed that the present method was very simple and sensitive in the determination of DS and HS.

1 Experimental

1.1 Chemicals

4-Vinylpyridine (VP) (99%), ethylene dimethacrylate (EDMA) (97%) and 3-(trimethoxysilyl)propyl methacrylate (γ-MAPS) (95%) were supplied by Alfa Aesar (Tianjin, China); azobisisobutyronitrile (AIBN) (97%, recrystallized before use) was purchased from Shanghai Chemical Co. (China); diethylstilbestrol (DES) (99%), dienestrol (DS) (98%) and hexestrol (HS) (99%) were supplied by Sigma Company (Shanghai, China). The chemical structures of DES, DS and HS are shown in Fig. 1. HPLC-grade acetonitrile (ACN) and methanol were purchased from Tedia Company (Fairfield, USA); water used throughout the study was purified using a Milli-Q water purification system (Millipore, USA). A standard solution of 100 mg/L of DS and HS was prepared in methanol and renewed monthly. The standard mixtures of DS and HS were prepared by dissolving 2.00 mg of each compound in methanol in 100 mL volumetric flask. The stock solution was stored at 4 °C and diluted with Milli-Q water to give the required concentration.

![Chemical structures of DES, DS and HS](image)

**Fig. 1** Chemical structures of DES, DS and HS

1.2 Equipments

HPLC analyses were carried out on an LC chromatographic system (Shimadzu, Japan) equipped with a binary pump (LC-20AB) and a diode array detector (SPD-M20A). Sample injection was carried out using an RE3725i manual sample injector with a 20 μL loop (Rheodyne, Cotati, CA, USA); all experiments were performed at room temperature.

The morphologies of monolithic materials were examined by a Model XL30 scanning electron microscopy (SEM) instrument (Philips, Eindhoven, The Netherlands). Elemental analysis (EA) was carried out on a PerkinElmer (Shelton, CT, USA) Model PE 2400 instrument. Fourier transform infrared spectrometry (FT-IR) was performed on an Avatar-360 FT-IR instrument (Thermo Nicolet, Madison, WI, USA).

1.3 Chromatographic conditions

All separations were performed on a Phenomenex LC-18 column (250 mm x 4.6 mm i. d. , 5 μm particle size). The mobile phase was a 66% (v/v) methanol aqueous solution. The detector was set at 230 nm; the flow rate was 1.0 mL/min and injection volume was 20 μL.

1.4 Preparation of MIP-SBSE

In the present study, the molecularly imprinted monolithic material was prepared by an in situ polymerization process. In order to increase the stability of the coating of SBSE, some pretreatment procedure of glass stir bar should be taken.
The procedure of preparation of glass bar, pretreatment and chemical modifications by γ-MAPS was described previously [20–22]. The MIP was prepared through the thermal radical copolymerization of VP and EDMA in the presence of DES as template molecule. AIBN was used as the polymerization initiator (1% (w/w) of the total monomer amount) in the all polymerization reaction. Different template and monomer contents were used to prepare different polymers (Table 1). The monomer mixtures and porogen (ACN) were mixed ultrasonically into a homogenous solution and then the reactant solution was purged with nitrogen for 5 min. Subsequently, the reactant mixture was poured into a glass tube with a definite diameter (3 mm i.d.). The stir bar that has been pretreated was vertically immersed into the reactant mixture. The tube was sealed with septum and kept at 70 °C for 24 h. After the polymerization, the glass tube was carefully cut off with grindstone. The monolithic material on the bar was dipped into acetic acid/acetonitrile (10/90, v/v) solution for 48 h to remove the template molecules, residue monomer, porogen, uncrosslinked polymer and initiator. A blank stir bar coated with non-imprinted polymer (NIP-SBSE) was prepared under the same polymerization conditions in the absence of the template. Before use, the SBSE was rinsed with a plenty of water for at least 24 h.

1.5 Sample preparation of wastewater, honey and cow urine

In this study, stirring extraction and stirring liquid desorption modes were used for the pretreatment of wastewater, honey and cow urine samples.

The wastewater samples including inlet and outlet were obtained from the treatment plant in Xiamen city. The samples spiked with DS and HS were filtered through filters (pore size, 0.22 μm). Then 100 mL volume of the sample was stirred at 300 r/min at room temperature for a certain time. After the extraction, the MIP-SBSE was removed and immersed in 3.0 mL desorption solvent, stirred for a certain time to release the extracted analytes. The stripping solution was used directly for HPLC/DAD analysis.

For the honey sample obtained from the local market, the pretreatment procedure was as follows: DS and HS were directly spiked into 5.0 g analyte free honey samples. Thirty minutes were allowed for equilibration at room temperature. Then, the samples were diluted with Milli-Q water to 100 mL (the mass concentration of each analyte was 100 μg/L). The MIP-SBSE was directly put into the spiked sample and stirred at 300 r/min at room temperature for a certain time. The subsequently operational process was the same as the procedure of wastewater samples. For cow urine samples, DS and HS were spiked into 10.0 mL samples, ten minutes were allowed for equilibration at room temperature. Then, the samples were diluted with Milli-Q water to 100 mL (the mass concentration of each analyte was 100 μg/L). The other operational processes were the same as the procedure of wastewater samples. Each extraction was repeated at least twice to evaluate the repeatability of the extraction protocol; analyte recovery was the average of the three measured values.

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<th>Table 1 Extraction performance of different MIP-SBSE for HS and DS</th>
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2 Results and discussion

2.1 Preparation and characterization of MIP-SBSE

In the preparation of MIPs, the amount of template and functional monomer affect the extraction performance strongly. In order to optimize the extraction performance of MIP-SBSE, a series of MIP-SBSE were prepared with different ratios of template and functional monomer. DS and HS were selected as detected solutes. The data in Table 1 shows that when the quantities of DES is 20 mg, the proportion of 4-VP is kept 25% in the monomer mixture, while the ratio of monomer mixture to porogen is 45/55 (w/w) (bar 4), the MIP-SBSE shows the expected extraction performance for solutes. Under the optimized preparation conditions, the MIP-SBSE exhibited good longevity and it can be reused at least 60 times for the extraction of DS and HS spiked in Milli-Q water.

Elemental analysis, FT-IR and SEM were used to characterize the MIP. Elemental analysis on the MIP before the removal of template molecule (DES) demonstrated that its carbon content was 57.95% (w/w) and nitrogen content was 3.41% (w/w), while the corresponding values were 55.74% and 3.24% (w/w), respectively, when the template molecules were removed. The decrease of carbon content and no obvious change of nitrogen content after the removal of DES indicated that DES was imprinted in the MIP.

Fig. 2 shows the FT-IR spectra of MIP (Fig. 2a) and NIP (Fig. 2b). The main functional groups of the predicted MIP and NIP coatings could be found with corresponding infrared absorption peaks. A broad absorption band at 3406.8 cm\(^{-1}\) on the MIP coating corresponded to several overlapped peaks of infrared absorption such as the stretching vibration of O–H bonds of VP molecules (functional monomer), the hydrogen bonding and electrostatic binding interactions between DES and VP. However, for NIP, just a weaker absorption peak around 3406.8 cm\(^{-1}\) was found because of the lack of hydrogen bonding or electrostatic binding interaction between template molecules and functional monomers. The sharp peaks at 1715.4 and 1594.8 cm\(^{-1}\) were observed on both coatings, owing to the stretching vibration of C=O in EDMA and C=N in VP, respectively. In comparison of the spectra of MIP and NIP, two unique peaks were found on MIP. The observed features around 1630.5 and 1516.6 cm\(^{-1}\) indicated the C–C stretching vibrations of the phenyl were present in DES. The FT-IR spectra proved the success of MIPs preparation.

Fig. 3 shows the SEM image of the MIP at 20 000 magnification. It can be seen that the pore size and microglobules of the monolithic material were very even. The existence of even pore size distribution ensured that the new monolith possessed good permeability and favorable mass transfer in the extraction applications. The total surface area of the MIP was calculated from Brunauer-Emmett-Teller (BET) plot, and the area was
13.5 m²/g. The relatively large surface area ensured that the new MIP-SBSE possessed high extraction capacity.

2.2 Optimization of the MIP-SBSE method

In order to obtain the optimal extraction efficiency of MIP-SBSE for DS and HS, several main parameters such as extraction and desorption times, desorption solvent, ionic strength and pH value in sample matrix were studied in detail. Fig. 4a shows the effect of extraction time on the extraction efficiencies for DS and HS. The extraction time was varied from 0.5 h to 3.5 h. The results indicated that the extraction efficiencies increased with the increase of time and the extraction reached equilibrium at 3.0 h. Therefore, extraction time of 3.0 h was selected for further studies. The effect of liquid desorption time on the results was also studied. It was found that the 0.5 h was enough for desorption of target compounds from MIP-SBSE when the extraction time was 3.0 h. Consequently, 3.0 h and 0.5 h were adopted for extraction and desorption procedure, respectively, in the following research.

Different content of acetic acid in ACN solvent were used to desorb the analytes from MIP-SBSE. It can be seen from Fig. 4b that the increase of percentage of acetic acid in ACN favored the desorption of DS and HS from the coating. The reason is that acetic acid can disrupt the hydrogen bond between analytes and functional monomer in MIP. The data in Fig. 4b shows that ACN solvent containing 10% (v/v) acetic acid can desorb DS and HS from MIP-SBSE effectively.

Typically, the increase of ionic strength in sample matrix can enhance extraction performance because of salting-out effect. However, the present research results showed that the extraction efficiencies decreased with the increase of content of NaCl (Fig. 4c). The main reason may be that the overmuch ion will disrupt the hydrogen bond between the analytes and functional monomer in MIP and lead to the decrease of interaction be-

Fig. 4 Optimization of the extraction conditions

a. extraction time; b. desorption solvent; c. content of NaCl; d. pH value.

Conditions: The spiked mass concentration of each analyte was 100 μg/L. The other conditions were as follows: a. Desorption time was 2.0 h; no salt was added and the pH value in the matrix was not changed; ACN as the desorption solvent. b. Extraction and desorption times were 3.0 and 0.5 h, respectively; no salt was added and the pH value in the matrix was not changed. c. Extraction and desorption times were 3.0 and 0.5 h, respectively; ACN/acetic acid binary solvent (90/10, v/v) was used as desorption solvent and the pH value in the matrix was not changed. d. Extraction and desorption times were 3.0 and 0.5 h, respectively; ACN/acetic acid binary solvent (90/10, v/v) was used as the desorption solvent and no salt was added.
between the analytes and MIP. Hereby, no addition of any salt was adopted in the following studies. The effect of sample pH on the extraction efficiency was investigated in the range from 4.0 to 12.0. As shown in Fig. 4d, when the other conditions were constant, the extraction efficiencies improved significantly with the increase of pH value from 4.0 to 8.0 and decreased with the pH value increased continuously for DS and HS on MIP-SBSE. This interesting phenomenon can be explained as follows: The $pK_{a1}$ and $pK_{a2}$ values of DS and HS were reported to be 7.4, 10.5 and 7.2, 10.1, respectively [28]. As the pH changed, DS and HS molecules could be present as cationic, anionic or neutral forms in aqueous solution. Therefore, the pH values in the sample matrix will affect the extraction of DS and HS. More and more DS and HS molecules became neutral forms when pH value increased to 8.0 which closed to their $pK_{a1}$. So, more DS and HS molecules took part in the specific interactions between analytes and MIP coatings, and lead to the improvement of extraction performance. When the pH value increased continuously, especially, the pH value higher than 11, the DS and HS molecules became anionic forms. Their interactions with MIP coatings were weakened, resulting in lower extraction efficiency. Therefore, pH 8.0 was selected as the optimized pH for sample solution.

From the above experimental results, the optimized parameters for the extraction of DS and HS with MIP-SBSE were as follows: extraction and desorption time were 3.0 h and 0.5 h, respectively; using ACN/acetic acid binary solvent (90/10, v/v) as desorption solvent; no salt was added in the matrix; the pH value of matrix was 8.0.

### 2.3 Extraction selectivity and capacity of MIP-SBSE

In order to investigate the extraction selectivity of MIP-SBSE, the MIP- and NIP-coated SBSE were separately used for the extraction of 100 μg/L of DS, HS and three reference compounds, phenol, benzene and toluene from the aqueous solutions under the optimized extraction conditions. The results are shown in Fig. 5a by means of chromatographic peak areas. Obviously, the chromatographic peak areas of DS and HS with the MIP-SBSE were much higher than those of the NIP-SBSE. The peak areas of DS and HS obtained on MIP-SBSE were 1.64 and 1.71 times as much as those obtained on NIP-SBSE, respectively. This indicated that the MIP-SBSE provided high selectivity to the structural analogues of template molecule owing to the molecular size recognition of MIP to the structural analogues and the hydrogen bonding interaction. Fig. 5b shows the chromatograms of DS and HS extracted by MIP-SBSE and NIP-SBSE, respectively. It can be seen that the peak heights of DS and HS obtained on MIP-SBSE obviously higher than those got on NIP-SBSE, which indicated that lower detection limits can be achieved on MIP-SBSE than on NIP-SBSE. It also can be seen from Fig. 5a that extraction performance of benzene, toluene and phenol were all lower than DS and HS with MIP-SBSE since the three compounds have little structural similarity.
with the template molecule. There was no obvious difference between the MIP-SBSE and the NIP-SBSE in extracting the reference compound because of their same extraction mechanism of mainly non-specific adsorption. The ratios of peak areas obtained on MIP-SBSE to NIP-SBSE for benzene, toluene and phenol were 0.95, 1.09 and 1.05, respectively.

Extraction capacity is another important parameter for MIP. Different mass concentrations (from 1 μg/L to 1 200 μg/L) of DS and HS were used to evaluate the extraction capacity of MIP-SBSE. It can be seen from Fig. 6 that the extraction amounts of DS and HS increased along with the increase of their concentration on both MIP-SBSE and NIP-SBSE. At the same time, the extraction capacities for DS and HS on the MIP-SBSE were higher than those on the NIP-SBSE. The extraction of DS and HS on NIP-SBSE reached equilibrium when their concentrations were 1 000 μg/L. However, the extraction equilibrium did not be observed on MIP-SBSE even if the concentrations were 1 200 μg/L. The capacity difference between MIP-SBSE and NIP-SBSE was probably caused by dissimilar extraction mechanisms. For the MIP-SBSE, the hydrogen bonding interaction between structure-similar molecules and monomers caused specific positions and orientations of the monomer. When immobilized, the functional residue of monomer with specific position and orientation could selectively adsorb structure-similar molecules. While for the NIP-SBSE, though the physical properties of the NIP-SBSE were similar with those of MIP, the non-specific arrangement of the functional residue of monomer resulted in extraction was non-selective and much weaker than that of MIP-SBSE. The above results demonstrated that the template molecule was successfully imprinted on MIP coating and MIP-SBSE possessed the capability of selective extraction for DS and HS.

2.4 Method validation

The blank water samples were spiked with DS and HS and taken for analysis to evaluate the developed method. The data of linear dynamic ranges, correlation coefficients (R²), extraction efficiencies, limits of detection (LODs), limits of quantification (LOQs), and reproducibility for the analytes under the optimized experimental conditions are listed in Table 2. It can be seen from the data that the MIP-SBSE-LD-HPLC/DAD methodology presents a good performance. The linear dynamic ranges for DS and HS were 1.0 – 400 and 5.0 – 400 μg/L, respectively. Their correlation coefficients were both higher than 0.99. The LODs and LOQs for the analytes were calculated with the signal-to-noise ratios of 3 and 10, respectively. The LODs were 0.21 μg/L and 0.90 μg/L for DS and HS, and the LOQs were 0.71 μg/L and 2.97 μg/L for DS and HS, respectively. In order to improve the sensitivity, the 3.0 mL stripping solvent during desorption procedure could be evaporated to dryness under a gentle stream of nitrogen, then the dried residue was redissolved in 0.5 mL ACN for HPLC analysis. The results indicated that the LOD values for DS and HS could be further decreased to 0.04 and 0.14 μg/L, respectively, but more time was needed during drying-redissolving step.

The repeatability of the proposed method was assessed by investigating the inter-assay precisions. As shown in Table 2, the relative standard deviations (RSDs) for DS and HS were less than 3.67% and 8.78%, respectively. At the same time, the present method showed good bar to bar.

![Fig. 6 Extraction amount curves of MIP-SBSE and NIP-SBSE to DS and HS mixed solution of 1.0 – 1200 μg/L. The conditions are the same as in Fig. 5.](image)
reproducibility of preparation. The RSDs (n = 5) of enrichment factors for DS and HS were 5.25% and 6.93%, respectively.

Comparative study of the present method with other reported analytical methodologies for DS and HS was performed and the results are presented in Table 3. The results show that in comparing with other methods, lower LODs could be obtained in the present method with the same kind of detector. Determination of DS and HS by MS detector [10,16,30] was more sensitive than using HPLC method with UV detection [9,17,28,29]. However, when drying-redissolving procedure was taken during sample preparation, the sensitivity of the proposed method could be further increased and the LODs were lower than SPE-HPLC-MS.

### 2.5 Analysis of real samples

Under the optimized conditions, the proposed MIP-SBSE-LD-HPLC/DAD method was applied to the determination of DS and HS in wastewater, honey and cow urine samples. Low concentration of the DS was found in wastewater treatment plant before treatment, and no target analyte was found in other samples (Fig. 7 and Table 4). It also could be seen from Fig. 7 that there was no interference around analytes after MIP-SBSE, which indicated that the MIP could effectively selective enrich the analytes. Recovery was studied by spiked different concentrations of DS and HS in real samples. As the results are listed in Table 4, satisfactory recoveries in the range of 63.1% – 120% were obtained, with RSDs ranging from 1.96% to 8.79%. These results demonstrated that the proposed method was reliable for the analysis of DS and HS in complicated samples.

### 3 Conclusions

In this work, an in-situ polymerization was introduced to prepare molecular imprinted porous polymer with diethylstilbestrol as template molecule. The MIP was applied as SBSE coating to extract DS and HS in wastewater, honey and cow urine samples. The combination of MIP-SBSE-LD-
HPLC/DAD was successfully applied to the determination of DS and HS in different matrices, at the trace level. In comparison with the existing extraction methods for DS and HS determination, the proposed method is simple, environmentally friendly, inexpensive, highly selective and sensitive. Therefore, it will be useful and practical in the screening and determination of DS and HS in environmental and biological samples.

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