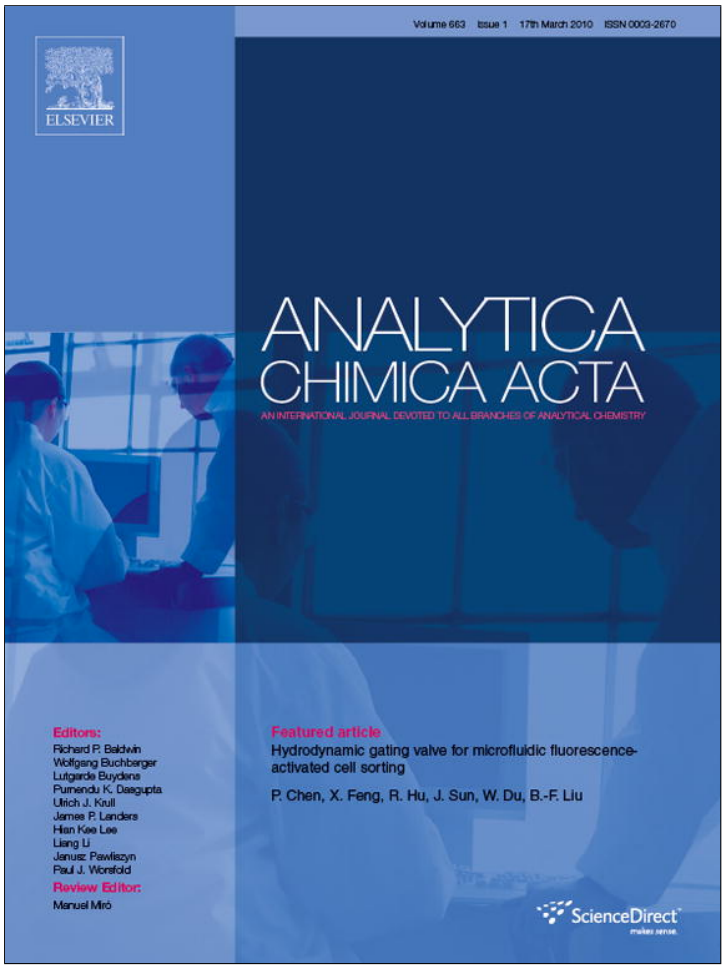

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Synthesis and applications of diethylstilbestrol-based molecularly imprinted polymer-coated hollow fiber tube

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ABSTRACT

A novel molecularly imprinted polymer-coated polypropylene hollow fiber tube (MIP-HFT) was photoinitiated for the copolymerization of diethylstilbestrol (DES) as a template molecule, α-methacrylic acid as a functional monomer, and ethylene glycol dimethacrylate as a crosslinking agent. The characteristics and applications of the MIP-HFT were investigated. In order to compare its characteristics with those of a non-imprinted polymer-immersed hollow fiber tube, the selectivity of the MIP-HFT was investigated using dienestrol and hexestrol as the structural analogues of a DES template, and phenol and methylbenzene were taken as reference compounds. The MIP-HFT was employed in the HPLC analysis of spiked milk samples. The detection limits of the method were found to be in the range 2.5–3.3 $\mu g\,L^{-1}$ for DES, dienestrol and hexestrol and the RSD% were in the range 6.4–8.9. The limits of quantitation were found to be in the range 8.7–9.4 $\mu g\,L^{-1}$ in milk for DES, dienestrol and hexestrol, and their average recoveries were 83.7–90.6% in the spiked milk samples. The experimental results revealed that the MIP-HFT provides a good carrier for the selective adsorption of DES and its chemical structure analogs, and can be used for the preconcentration of these compounds in complicated samples.

1. Introduction

Molecularly imprinted polymer (MIP), first introduced by Wulff [1,2], allows specific recognition sites for a desired target molecule (templates) to be formed in synthetic polymers because of its characteristics of high selectivity, chemical stability and easy preparation. The molecular imprinting technique has proved to be a powerful tool in the preparation of recognition materials used in many fields such as chromatography, solid-phase extraction and chemical sensors [3]. For example, Koster et al. first reported MIP as fiber coating materials for solid-phase microextraction and obtained satisfactory results in the extraction of brombuterol from urine samples [4].

Commercial porous material, such as hollow fiber membrane (HFM) or tube, has high flow flux and permeability, but no selectivity for target molecules. However, MIP membranes with high permeability seem to be very attractive for affinity separation [5,6]. Recently, it has been reported that a composite thin layer MIP membrane can be prepared using a hollow fiber member as the carrier. Kochkodan et al. [7] developed molecularly imprinted composite membranes for the selective binding of desmetryn from aqueous solutions. Hilal and Kochkodan [8] obtained surface modified microfiltration membranes with molecular recognising properties. However, the extraction device was complicated and hard to operate and, furthermore, the amount of analyte extraction is limited.

Diethylstilbestrol (DES) is medically a potent regulatory reagent of physiological response, and has been widely used as a growth promoter in livestock and as a treatment for estrogen-deficiency disorders in veterinary medicine [9]. Since the abuse of estrogens is known to be very harmful to human health owing to their potential carcinogenic properties, the use of estrogens in food-producing animals and other products has been prohibited in many countries. Synchronously, residue analyses of these estrogens have been used to control their illegal usage [10–13]. Generally, the determination of DES in water has been carried out using gas chromatography–mass spectrometry [14,15], liquid chromatography–mass spectrometry [16,17] or high performance liquid chromatography (HPLC) equipped with diode array detection [18–20]. Since the concentration of DES in environmental matrices is usually very low, a preconcentration step, such as solid-phase extraction and liquid-phase microextraction, is usually required. However, the main problems associated with the use of the most common technique for sample preconcentration for the determination of DES are the lack of selectivity and the low recovery [18–21].

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Hollow fiber tube (HFT) is a cheap, chemical properties-stable and porous material, which is of greater surface area than HFM. HFT is widely used in water-purifying processes and, in recent years, the application of HFT in liquid-phase microextraction for sample pre-treatment is reported [9,22]. However, there are few reports concerning the study of HFT and MIP. To overcome this, in this paper, a novel MIP-HFT photoinitiated copolymerization of DES as a template molecule, with α-methacrylic acid (MAA) as a functional monomer and ethylene glycol dimethacrylate (EGDMA) as a crosslinking agent. The synthesis process was simple and low cost. The novel MIP-HFT presented good adsorption and selection abilities to DES and its structural analogues, which provided a simple microfiltration approach in the fast preconcentration step for the determination of these compounds.

2. Experimental

2.1. Chemicals and materials

DES (100%), dienestrol (DS, 100%) and hexestrol (HS, 98%) solutions were purchased from Sigma Aldrich (St. Louis, MO, USA). The chemical structures of phenol and methylbenzene (reference compounds), DES and its structural analogues are shown in Fig. 1. Methanol and acetic acid (HPLC grade) were from Sinopharm Chemical Reagent Co. Ltd. (China). MAA, azo(bis)-isobutyronitrile (AIBN), trimethylolpropane trimethacrylate and EGDMA were obtained from Sigma–Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade. All solutions used for HPLC were prepared using ultra-pure water obtained from a Millipore purification system and filtered through a nylon 0.45 μm filter before use.

Individual stock solutions of DES, DS, HS, phenol and methylbenzene were prepared at a concentration of 200 mg L^{-1} in methanol with iso-proportional mass, and lower concentration solutions were prepared by serial dilution of the stock solutions with water. All the stock and standard solutions were stored in a refrigerator and the standard solutions were re-prepared every 2 weeks.

Commercial porous polypropylene HFT was purchased from Membrana GmbH (Wuppertal, Germany, the physical properties and performance characteristics of the HFT in Table 1).

2.2. Preparation of MIP-coated HFT

HFT was treated with these chemicals in turn: (1) water, (2) 0.1 mol L^{-1} HCL, (3) water, (4) 0.1 mol L^{-1} NaOH, (5) water and (6) methanol. The MIP-HFT was prepared using DES as template, MAA as functional monomer and EGDMA as cross-linker. A 1.2 mL acetonitrile solution in a transparence reagent bottle containing 0.1 mmol DES, 0.4 mmol MAA, 2.1 mmol EGDMA and 3.9 mg AIBN. was degassed for 10 s in an ultrasonic bath. To produce the imprinted polymer layer of the HFT, a 50 mm length of pretreated HFT bound with a string at one end were coated by soaking in the mixture solution. After the mixture was sparged with nitrogen for 2 min, the HFT was removed from the mixture and suspended in the reagent bottle. At the same time, the transparence reagent bottle was sealed. Then, the HFT was modified using a UV-induced polymerization procedure. The UV irradiation was performed using an 18 W mercury lamp (Beltron GmbH, Germany) with a maximum wavelength of 360 nm. In the experiment, the depth to which the HFT was dipped into the mixture was kept constant during the preparation of the MIP-HFT. In order to remove the template molecule after UV irradiation, the MIP-HFT was washed repeatedly with methanol:acetic acid (9:1) solution until no DES was detected in the eluent solution using HPLC. Finally, the obtained MIP-HFT was dried under vacuum at room temperature. Non-imprinted polymer-immersed hollow fiber tube (NIP-HFT) was prepared using the same procedure in the absence of the template molecule.

2.3. DES extraction using MIP-HFT

The experimental setup for the MIP-HFT application was performed as follows: an MIP-HFT was connected at one end to the needle tip of a micro-syringe, and heat sealed at the other end. Then the micro-syringe connecting to the MIP-HFT was immersed in a 4 mL sample vial containing analytes on a magnetic stirrer. After the sample solution was stirred at the rate of 800 rpm for 30 min, the solution in the inner cavity of the MIP-HFT was drawn into the micro-syringe and prepared for HPLC analysis directly (the collected solution was called A solution). At the same time, the MIP-HFT was eluted and the collected eluent solution was dried

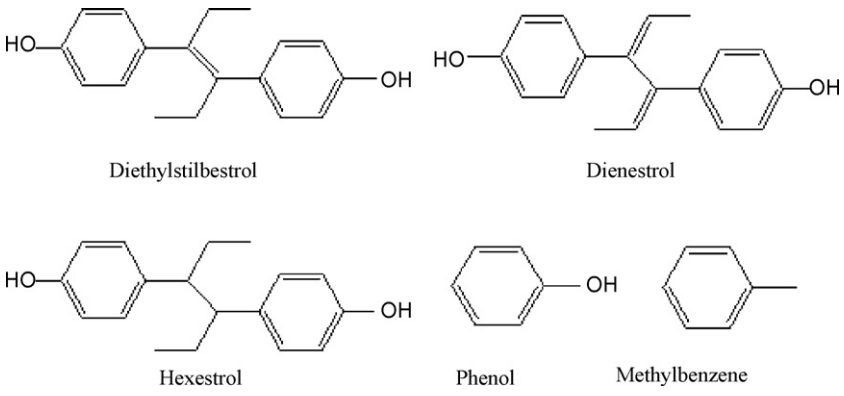

Fig. 1. Chemical structures of phenol and methylbenzene (reference compounds), diethylstilbestrol and its structural analogues.

Table 1
Physical properties and performance characteristics of hollow fiber tube.

	Wall thickness (μm)	Inner diameter (μm)	Pore size (calculated from BP)	Transmembrane flow (isopropylalcohol, 37 °C)
m	150	330 μm	0.47 μm	9.3 ml/[min × cm^2 × bar]
d	±25	±50 μm	+0.07 μm −0.06 μm	±2.3 ml/[min × cm^2 × bar]

m = mean value and *d* = deviation of the mean value. "+"and "−" mean size uniformity range. BP, bubble point.

with nitrogen, and then dissolved in 10 μL methanol before being injected for HPLC (the collected solution was called B solution). The eluting procedure was done as following: first the MIP-HFT was washed with 3 mL of methanol:acetic acid (99:1), and then eluted with 3 mL of methanol:acetic acid (9:1) repeatly. At a lower concentration of acetic acid (methanol:acetic acid, 99:1), acetic acid could be applied to destroy non-specific binding between MIP materials and foreign matter, resulting in lower non-specific binding as well as background interference, but acetic acid at lower concentration could not remove the specific binding between MIP materials and target analytes. Acetic acid at a higher concentration could destroy both specific binding and non-specific binding between MIP materials and objective molecules, the target analytes could be eluted under this condition.

2.4. HFT characterization

Morphological evaluation of the MIP-HFT and unmodified HFT was performed using an XL-30 SEM (Philips, Eindhoven, Netherlands).

2.5. Application of MIP-HFT in HPLC

An LC-10AT HPLC (Shimadzu, Japan) equipped with a Shimadzu SPD-10AV UV–vis detector and a 5 μm Microsorb-MV C_{18} (4.6 mm × 250 mm) column was used. An MIP-HFT or NIP-HFT was used to extract the target molecules following the procedure described above. The eluents obtained were used for HPLC analysis. The mobile phase of HPLC was a mixture of methanol:water (66:34), and the flow-rate was selected as 1.0 mL min^{-1}. The wavelength for UV detection was set at 230 nm. The binding of DES with MIP-HFT was evaluated by measuring the sorption of the DES. The extraction amounts of analytes were calculated using the respective area of chromatographic peaks and relative standard curves.

2.6. Application of MIP-HFT in sample analysis

Commercial milk samples without the three selected estrogens from a local supermarket were analyzed with spiking concentration for each estrogen. In a 100 mL Erlenmeyer flask, a 2 mL milk sample was mixed with estrogen standard solutions in a medium containing 0.5 mL of l.0 mol L^{-1} hydrochloric acid and 5 mL acetonitrile. After 5 min shaking, the mixed solution was centrifuged at a speed of 4000 rpm for 10 min. The supernatant liquid in the centrifuge tube was filtered through a fritted glass disk funnel, dried with a stream of nitrogen, and then dissolved in 5 mL methanol. The spiking concentration for each estrogen was selected to be 10.0 and 100.0 μg L^{-1}. The obtained sample solution was extracted with MIP-HFT and analyzed with HPLC following the same procedure and conditions as those described in Section 2.3.

3. Results and discussion

3.1. Mass transfer process of DES in MIP-HFT

In our presumption, there were two kinds of target site where the analytes could reach in mass transfer process. At the first kind of site, the analytes entered into the inner cavity of the MIP-HFT and could been drawn back into the micro-syringe (the analytes would been existed in A solution). At the other kind of site, the analytes were adsorbed in the tubal wall of the MIP-HFT and could been eluted in methanol: acetic acid solution (the analytes would been existed in B solution). According to the experiment of Section 2.3, DES could be undetectable in A solution from the inner cavity of the MIP-HFT, but about 90 pmol DES was detected in B solution. In other words, most DES was adsorbed in the MIP-HFT while not entered in the inner cavity of the MIP-HFT. This result indicated that there were amount of recognition site in the MIP-HFT so that the analytes were adsorbed in the MIP-HFT and would not resolved from the MIP-HFT.

3.2. Optimized UV exposure time

Since the membrane surface is practically inert to radical reactions, a modification by the deposition of an MIP layer onto the surface of a polymer supporter was considered [23,24]. Experimental results revealed that the porosity of modification of the HFT supporter depended on the polymerization time, when the proportion of functional monomer mixture was fixed during pre-soaking. MIP-HFT with different porosity of modification was obtained using various UV exposure times (polymerization time). The porosity of modification of an MIP-HFT affects its DES extraction capability, so the effects of various UV exposure times in the range (0–16 min) were done. Based on the experimental results, the extraction capability of the MIP-HFT increased with increase in the UV exposure time, but decreased if the UV exposure time was over 10 min. Generally, a suitable thickness of MIP layer on the HFT preserves a high flux for the MIP-HFT and a high extraction amount over a suitable UV exposure time. Correspondingly, a longer UV exposure time causes more deposition of the MIP material on the HFT, resulting in a lower extraction ability of the MIP-HFT [8].

3.3. MIP-HFT characterization

The cross-sectional SEM images for the MIP-HFT and unmodified HFT are shown in Fig. 2 under 2500-fold magnification. The specific surface area and pore volume impacted significantly on the effi-

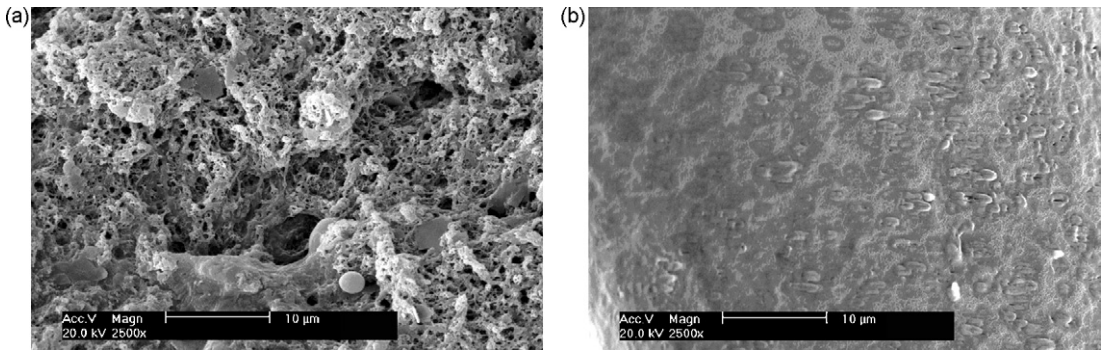

Fig. 2. Scanning electron micrographs of the unmodified HFT (a) and the DES MIP-HFT (b) under 2500 magnification (preparation conditions of MIP-HFT: solvent: acetone; monomer: MAA; cross-linker: EGDMA; initiator: AIBN; UV polymerization time: 10 min).

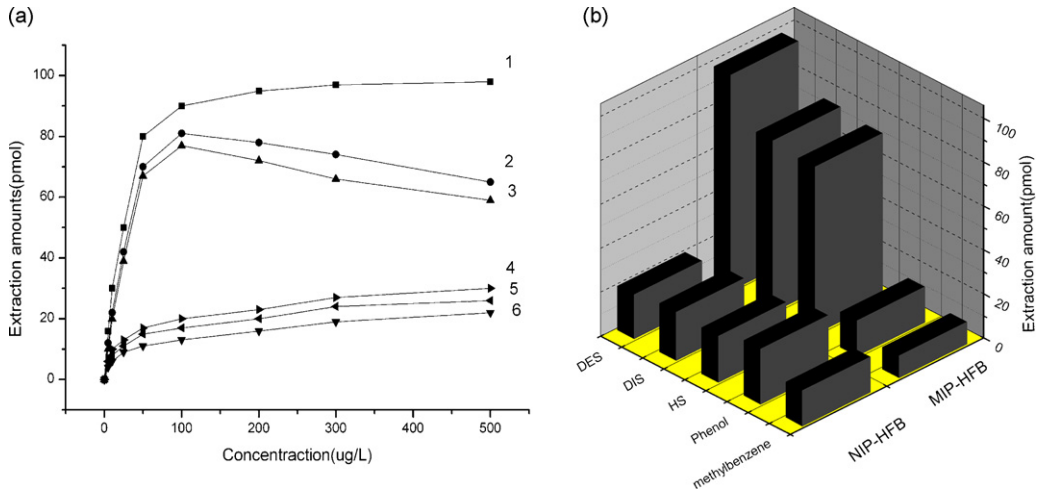

Fig. 3. (a) Extraction amount curves of MIP-HFT and NIP-HFT to DES, DS and HS mixed solution of 5.00–500 μg/L. 1: DES (of MIP-HFT), 2: DS (of MIP-HFT), 3: HS (of MIP-HFT), 4: DES (of NIP-HFT), 5: DS (of NIP-HFT), 6: HS (of NIP-HFT) and (b) extraction amounts of diethylstilbestrol (DES), dienestrol (DS), hexestrol (HS), phenol and methylbenzene with MIP-HFT and NIP-HFT at 100 μg/L level.

ciency of adsorption. The homogeneous and dense morphological structure shown in Fig. 2(b) indicated that the MIP-HFT preparation achieved a more highly crosslinked and porous structure. It thus provided a guarantee of a sufficient extraction performance of the MIP-HFT for DES.

3.4. Analytical applications of MIP-HFT

3.4.1. Comparative adsorption experiment

The extraction capabilities of the MIP-HFT and NIP-HFT were investigated using a series of estrogen standard solutions at concentrations from 5.0 to 500.0 $\mu g\,L^{-1}$ (Fig. 3(a)). It was obvious that the extraction amounts of three estrogens increased along with the increase of their concentration in the range 5.0–100.0 $\mu g\,L^{-1}$ for both MIP-HFT and NIP-HFT. In concentrations of 100.0–500.0 $\mu g\,L^{-1}$, the extraction amounts of DES increased gradually, but those of DS and HS decreased. This result indicated the presence of competitive adsorption of DES over DS and HS when MIP-HFT reached extraction equilibrium. The extraction capacities for DES, DS and HS of the MIP-HFT were higher than those for the NIP-HFT. This capacity difference between MIP-HFT and NIP-HFT fibers was probably caused by dissimilar extraction mechanisms. For the MIP-HFT, the hydrogen bonding interaction between template molecules and monomers caused specific positions and orientations of the monomer. When immobilized, the functional residual of the monomer with specific position and orientation could selectively adsorb template or structure-similar molecules. While for the NIP-HFT, though the physical properties of the NIP-HFT were similar with those of MIP, the non-specific arrangement of the functional residual of the monomer resulted in extraction being non-selective and much weaker than that of MIP-HFT [3].

3.4.2. Non-competitive adsorption experiment (selectivity experiment)

The selectivity of the MIP-HFT was investigated with DS and HS as the structural analogues of the DES template and phenol and methylbenzene as reference compounds. The corresponding NIP-HFT was used for comparison. To avoid competitive adsorption, DES, its structural analogues and reference compound solutions were prepared individually with a concentration of 100.0 $\mu g\,L^{-1}$. The extraction amounts of the five analytes with the MIP-HFT and the NIP-HFT are compared in Fig. 3(b). Obviously, the extraction amounts of DES and two analogues with the MIP-HFT were much higher than those of the NIP-HFT. This indicated that the MIP-HFT provided high selectivity to the DES template and its structural analogues owing to the molecular size recognition of MIP to the template molecule and the hydrogen bonding interaction. In contrast, the extraction amounts of phenol and methylbenzene were all less than 16 pmol with the MIP-HFT since the two compounds have little structural similarity with the template molecule. There was no obvious difference between the MIP-HFT and the NIP-HFT in extracting the reference compound because of their same mechanism of mainly non-specific adsorption. In addition, we found that there were no obvious differences in extraction amounts for the three estrogens with the NIP-HFT. This result indicated that the NIP-HFT had no specific selectivity for the three estrogens.

3.5. Optimal conditions for MIP-HFT extraction

The effect of stirring speed on the extraction amounts was investigated with the 100.0 $\mu g\,L^{-1}$ standard solutions of three estrogens. The results revealed that a stirring speed of 1000 rpm enhanced the extraction amounts of the three estrogens most effectively. In the experiment, the effect of extraction time was also investigated. The extraction amounts of the three estrogens increased rapidly with the increase of extraction time and reached a maximum amount at 30 min, indicating that adsorption and desorption of the selected compounds in the extraction process became balanced at this extraction time.

3.6. Application in HPLC

The linearity of the MIP-HFT, using HPLC as the determination method, was investigated with a series concentration of estrogen standard solutions (7.5, 10, 20, 50, 100, 150, 200 $\mu g\,L^{-1}$) under optimized conditions. As shown in Table 2, good linearity for three estrogens was achieved in the concentration range from 7.5 to 200 $\mu g\,L^{-1}$ with a correlation coefficient of 0.9912–0.9927. The detection limits were from 2.5 to 3.3 $\mu g\,L^{-1}$. As compared with the reported reference, a detection limit of 6.0 $\mu g\,L^{-1}$ with MIP as a sorbent for the on-column solid-phase extraction of DES from aqueous samples [25], a lower detection limit was obtained using the MIP-HFT due to its special selectivity. The precision of the method was monitored with 10.0 and 100.0 $\mu g\,L^{-1}$ estrogen standard solutions, and it was found that the RSDs for the three estrogens varied from 6.4 to 8.9%.

Table 2
The linear range, detection limit and RSD of the MIP-HFT method coupled with HPLC for the determination of three estrogens.

Target compounds	Linearity range ($\mu g\,L^{-1}$)	Correlation coefficient (r^2)	LOD[a] ($\mu g\,L^{-1}$)	RSD (%) ($n=3$)	
				10.0 ($\mu g\,L^{-1}$)	100.0 ($\mu g\,L^{-1}$)
DES	7.5–200	0.9927	2.5	6.6	7.6
DIS	10–200	0.9915	3.3	6.4	8.3
HS	7.5–200	0.9912	3.3	7.9	8.9

[a] LOD (limit of detection) were estimated on the basis of 3:1 signal to noise ratios. The analysis was done in triplicate with different hollow fiber tubes.

Table 3
Average recovery, precision, reproducibility and limit of quantitation of the three estrogens from spiked milk samples.

Target compound	LOQ[a] ($\mu g\,L^{-1}$)	10.0 $\mu g\,L^{-1}$			100.0 $\mu g\,L^{-1}$		
		Average recovery[b]	RSD[c] (%) ($n=3$)	RSD[d] (%) ($n=3$)	Average recovery[b]	RSD[c] (%) ($n=3$)	RSD[d] (%) ($n=3$)
DES	8.7	85.5	6.7	7.1	90.6	9.3	10.1
DIS	9.4	83.7	7.2	7.5	89.2	9.7	9.6
HS	8.9	86.4	8.1	8.2	88.5	10.4	10.7

[a] LOQ (limit of quantitation) was investigated in milk.
[b] Average recovery was done in triplicate with different hollow fiber tubes.
[c] RSD (relative standard deviation) for precision was done in triplicate with different hollow fiber tubes.
[d] RSD for reproducibility was done with the same hollow fiber tubes.

3.7. Applicability to detection in real samples

Commercial milk samples without the three selected estrogens from a local supermarket were analyzed. Spiked milk was selected for the validation of MIP-HFT selectivity. Solvent extraction with centrifugation and filtration was used and the spiking concentration for each estrogen was set with two levels of 10.0 and 100.0 $\mu g\,L^{-1}$. As shown in Fig. 4, compared with the direct HPLC analysis (chromatogram C in Fig. 4), the sensitivities of the three estrogens in milk were greatly enhanced with the MIP-HFT preparation (chromatogram A in Fig. 4), but less enhanced with the NIP-HFT preparation (chromatogram B in Fig. 4). In Table 3, The limits of quantitation were found to be in the range 8.7–9.4 $\mu g\,L^{-1}$ in milk for DES, dienestrol and hexestrol (chromatograms shown for the LOQ of three estrogens in spiked sample solutions with MIP-HFT). The average recoveries were 83.7–90.6% with different hollow fiber tubes in the spiked milk samples. The RSD% for precision was 6.7–10.4 (done in triplicate with different hollow fiber tubes) and the RSD% for reproducibility was 7.1–10.7 (done with the same hollow fiber tubes). Because of the special recognition of template molecules and structural analogues, the MIP-HFT following HPLC determination could be applied for selective and sensitive determination of traces of DES, DS and HS in complicated samples.

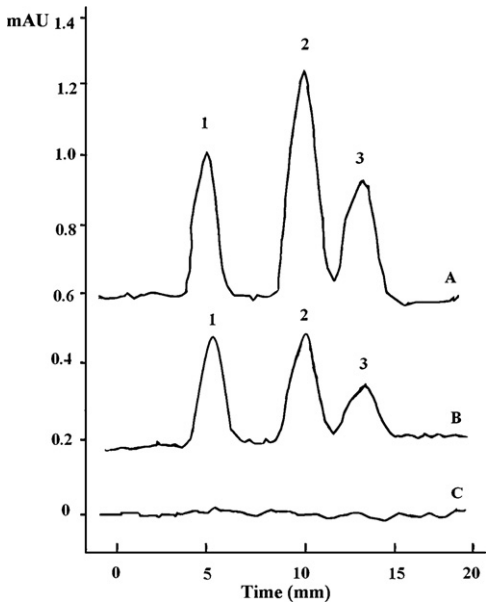

Fig. 4. Chromatograms of 100 μg/L estrogen spiked solutions of milk samples and determination of three estrogens in spiked sample solutions with MIP-HFT and NIP-HFT. (A) Spiked sample solution extracted with MIP-HFT, (B) spiked sample solution extracted with NIP-HFT, and (C) estrogens spiked sample solution without MIP-HFT and NIP-HFT. 1: DES, 2: DS, and 3: HS.

4. Conclusions

In this paper, a novel MIP-coated HFT was prepared by photo polymerization using DES as a template molecule, MAA as a functional monomer and EGDMA as a crosslinking agent. The material was homogeneous, highly crosslinked, and porous, and could provide a good adsorption and selectivity to DES and its structural analogues. The MIP-HFT could be re-used in three times and provided larger specific surface area as well as more adsorption capability than those of molecularly imprinted membrane. The analytical results revealed that the MIP-HFT could be applied in a rapid preconcentration for the determination of DES and its structural analogues in milk samples.

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