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Monitoring of selected estrogen mimics in complicated samples using polymeric ionic liquid-based multiple monolithic fiber solid-phase microextraction combined with high-performance liquid chromatography

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ABSTRACT

A convenient, rapid, sensitive and environmentally friendly method for simultaneous monitoring of six estrogen mimics (bisphenol A, diethylstilbestrol, dienestrol, hexestrol, octylphenol and nonylphenol) in water and milk samples was developed by coupling multiple monolithic fiber solid-phase microextraction (MMF-SPME) to high performance liquid chromatography with diode array detection. The MMF-SPME based on polymeric ionic liquid-based monolith as extractive medium was used to concentrate target analytes. Because there were multiple interactions between adsorbent and analytes, the MMF-SPME exhibited a high extractive capability toward analytes. To obtain optimum extraction performance, several extraction parameters including desorption solvent, pH value and ionic strength in sample matrix, extraction and desorption time were investigated and discussed. Under the optimized extraction conditions, the limits of detection (S/N = 3) of the proposed method were 0.040–0.11 μg/L in water and in milk samples. Satisfactory linearity was achieved for analytes with the correlation coefficients (r) above 0.99. Excellent method reproducibility was achieved by evaluating the repeatability, intermediate precision and MMF-to-MMF reproducibility with relative standard deviations (RSDs) of both less than 10%. Finally, the proposed method was successfully applied to the determination of estrogen mimics in several milk and environmental water samples. Recoveries obtained for the determination of six target analytes in spiking samples ranged from 75.6% to 118%, with RSD below 10% in all cases.

1. Introduction

Estrogen mimics (EMs), such as bisphenol A (BPA), diethylstilbestrol (DES), dienestrol (DS), hexestrol (HS), octylphenol (OP) and nonylphenol (NP) are classified as potential endocrine disruptor chemicals and have attracted widespread attention in recent years [1–3]. The EMs can enter into the environment waters through all kinds of ways such as the excretion of human and animals, pharmaceutical wastewater and the aquaculture wastewater. At the same time, several studies have showed that some EMs residues were found in milk because of illegal application of EMs (such as DES, DS and HS) to promote growth rate of animals [4,5]. EMs can transport in blood and then are synthesized by the mammary glands and finally excreted in the milk. Studies have reported that EMs can mimic or block the actions of natural hormones in living organisms, including human, and impair their normal functioning, such as growth, metabolism and reproduction even at ultra-trace level [6,7]. Therefore, development of highly sensitive, rapid and accurate analytical method for monitoring of trace EMs in water and milk samples is necessary.

So far, gas chromatography (GC), high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) are the main analytical techniques for the determination of EMs. GC is rapid and sensitive, but derivatization step is needed in order to convert the analytes into more volatile derivatives [8,9]. The derivatization process is tedious and may cause the sample loss. CE possesses high separation performance, but it lacks stability and sensitivity for real samples with complicated matrices [10]. Compared with GC and CE, HPLC is simple and convenient. HPLC can analyze EMs directly without derivatization and exhibit good reproducibility

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[11–14]. However, due to the very low levels of EMs and the complicated matrices in the real samples, sample pretreatment steps are required before the chromatographic analysis.

There are a few of sample pretreatment methods such as liquid–liquid extraction (LLE) [15,16], liquid phase microextraction (LPME) [17,18], hollow fiber liquid phase microextraction (HF-LPME) [11], solid phase extraction (SPE) [19,20] and stir bar sorptive extraction (SBSE) [21,22] that have been used to extract EMs from complicated samples. However, the LLE method demands time-consuming extraction and cleanup procedures, as well as large volumes of sample and toxic organic solvent. Multi-step processes including extraction, elution, evaporation and sample reconstitution steps are involved in SPE method. For SBSE, the friction between stir bar and the bottom of the vial during extraction can easily cause the loss of coating. At the same time, the extraction time is relatively long. The extraction capacity is limited for LPME and HF-LPME because low extractant is employed.

Solid phase microextraction (SPME), a solvent-free extraction method invented by Pawliszyn and co-workers in the 1990s, is another promising sample preparation technique [23,24]. SPME combines sampling and concentrating into a single step. At the same time, SPME has the merits of rapidness, convenience, easy-to-operate, low organic solvent consumption and environmental friendliness. As for the other extraction format, the extraction medium is the key in SPME; it determines the extraction targets and performance. At the same time, the sensitivity and precision of the analysis are also affected strongly by the extraction medium of SPME. So far, a number of materials have been prepared and used as the coating of SPME [25]. Typically, the thickness of coating is about dozens of micrometers, and the total volume of extraction medium is only at microlitre level. Therefore, the extraction capacity of SPME is limited. At the same time, the coatings of SPME may suffer from insufficient chemical/thermal stability during extraction. Up to now, coupled with GC [26–28] or HPLC [12,29–32], SPME has been successfully applied to the extraction and determination of several EMs from environmental samples. Pocurull et al. [32] used 85 μm polyacrylate coated fiber to extract estrogenic compounds from water samples. Expected extraction results were achieved, but the extraction equilibrium was long. For bisphenol A (BPA), it did not reach equilibrium and even the extraction time was prolonged to 60 min. The same circumstance also has been reported by Jiang et al. [33]. They used commercial 50 μm carbowax/templated resin (CW/TPR) coated fiber to extract BPA, 4-*n*-nonylphenol (NP), and 4-tert-octylphenol (OP) in environmental water samples. Results showed that the time of extraction equilibrium for BPA was 60 min. However, for NP and OP, the time was as long as 180 min. Therefore, to use SPME to extract EMs effectively and quickly, developing new extraction fibers is highly desired.

Multiple monolithic fiber SPME (MMF-SPME) with porous monolithic materials as extractive media is a new extraction format which was developed in our group [34,35]. Compared with conventional SPME fiber, there are several outstanding properties of MMF-SPME. Firstly, the MMF-SPME consisted of four independent substrateless thin monolithic fibers. Therefore, the total amount of extraction medium is higher than that of coated fiber, and high extraction capacity can be obtained. Secondly, the aqueous samples can form convection within MMF-SPME during extraction because there are gaps between thin fibers. The formation of convection favors the diffusion of analytes and accelerates the interaction between analytes and sorbent. Thus, MMF-SPME possesses quick extraction speed. Thirdly, the MMF-SPME uses porous monolithic materials as extraction media. Monoliths possess many intrinsic merits such as simple preparation, fast mass-transfer, varied chemical properties and so on. The extraction medium of MMF-SPME is very flexible. According to the character of target analytes, the monolithic fiber can be conveniently designed and prepared to realize effective extraction of analytes. In the present study, six EMs including bisphenol A, diethylstilbestrol, dienestrol, hexestrol, octylphenol and nonylphenol were selected as target analytes. A novel home-made MMF-SPME based on poly (1-allyl-3-methylimidazolium bis [(trifluoro methyl) sulfonyl] imide-*co*-ethylene dimethacrylate) (AMED) monolith was used to extract the target analytes. After optimization of the factors affecting the MMF/AMED-SPME of six target EMs (such as desorption solvent, extraction and desorption time, pH value and ionic strength in sample matrix), a combination of MMF/AMED-SPME with liquid desorption (LD), followed by high performance liquid chromatography with diode array detection (MMF/AMED-SPME-LD-HPLC/DAD) for the direct analysis of trace EMs in water and milk samples was developed.

2. Experimental

2.1. Chemicals

1-Allyl-3-methylimidazolium bis [(trifluoro methyl)sulfonyl]-imide (AM) (98%) was purchased from Cheng Jie Chemical Co., Ltd. (Shanghai, China); ethylene dimethacrylate (ED) (98%) was supplied by Alfa Aesar Ltd. (Tianjin, China); azobisisobutyronitrile (AIBN) (97%, recrystallized before use) and *N*,*N*-dimethylformamide (DMF) (98%), were purchased from Shanghai Chemical Co. (China); 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).

BPA (99%) was purchased from TCI Co. (China), DES (99%), DS (98%) and HS (99%) were supplied by Sigma–Aldrich (Germany); OP (97%) and NP (96%) were purchased from Chemservice Co. (USA). The chemical properties of the above-mentioned analytes are shown in Table 1. Water samples were collected from Xiamen city and filtrated through 0.45 μm membranes. Different brands of milk were purchased from local retail markets. All samples were stored at −4 °C before use. Individual stock solutions of EMs were prepared at a concentration of 10.0 mg/L by dissolving methanol and renewed monthly. The standard mixtures of EMs were prepared by dissolving 2.00 mg of each compound in methanol in 100 mL volumetric flask. The stock solutions were stored at 4 °C and diluted with ultrapure water to give the required concentration.

2.2. Equipments and materials

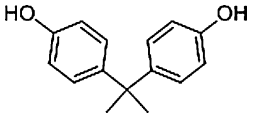
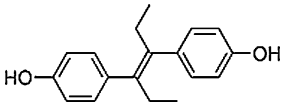
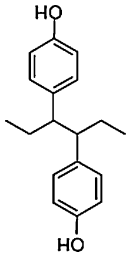
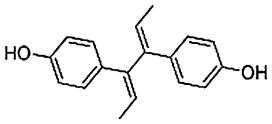
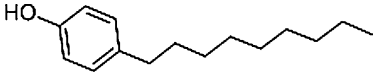
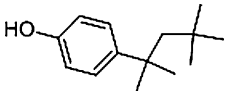
HPLC analyses were carried out on Agilent 1260 LC chromatographic system (USA) equipped with a binary pump (1260 Quat Pump) and a diode array detector (1260-DAD). Sample injection was carried out using a RE3725i manual sample injector with a 20 μL loop (Rheodyne, Cotati, CA, USA), all experiments were performed at room temperature.

The MMF/AMED-SPME was prepared in our lab [35]. It consisted of four independent substrateless thin monolithic fibers (the dimension for each thin monolithic fiber was 20 mm in length and 0.5 mm in diameter). The chemical structure of the polymer monolithic material is displayed in Fig. 1.

2.3. Chromatographic conditions

The separation of six EMs was performed on a Hypersil BDS C18 column (5 μm particle size, 250 mm × 4.6 mm i.d.). Optimum separation was obtained with a binary mobile phase composed of ultrapure water (solvent A) and ACN (solvent B). The gradient elution program was as follows: 0.0–5.0 min = 55% B, 5.0–10.0 min = 55% B–100% B and kept to 12.0 min, 12.0–17.0 min = 100% B–55% B and

Table 1
The basic properties of six estrogen mimics.

Compounds	Molecular formula	Molecular mass	Chemical structures	Log K_{ow}	pK_a
Bisphenol A	$C_{15}H_{16}O_2$	228.29		3.32	9.59
Diethylstilbestrol	$C_{18}H_{20}O_2$	268.35		5.07	10.2
Hexestrol	$C_{18}H_{22}O_2$	270.37		5.60	10.1
Dienestrol	$C_{18}H_{18}O_2$	266.33		5.32	10.5
Nonylphenol	$C_{15}H_{24}O$	220.35		5.99	10.7
p-tert-Octylphenol	$C_{14}H_{22}O$	206.32		5.28	10.4

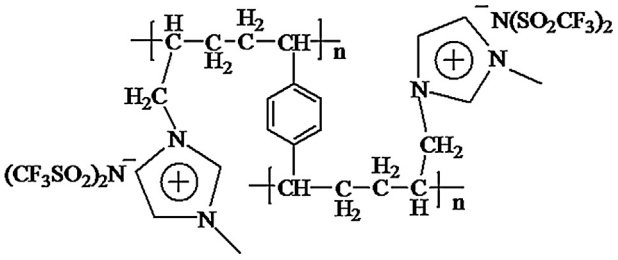


Fig. 1. The structure of poly (AM-*co*-ED).

kept to 20.0 min. The detector wavelength was set at 230 nm. The flow rate was 1.0 mL/min and injection volume was 20 μL.

2.4. MMF/AMED-SPME procedure

Stirring extraction and LD modes were used in this work. The MMF/AMED-SPME was activated with methanol and ultrapure water in sequence. A volume of 20 mL of sample solution was added into a 25 mL vial containing an 8 mm × 2 mm stirring bar. MMF/AMED-SPME was performed by direct immersion of the fiber bunch in the sample solution for some time under low stirring (a vortex just appeared) using a magnetic stirrer. After extraction, the MMF/AMED-SPME was removed and desorbed with 400 μL desorption solvent (methanol) in a 0.4 mL vial insert. For increasing the sensitivity, the stripping solvent was evaporated to dryness under a gentle stream of nitrogen. The dried residue was redissolved in 0.1 mL methanol for HPLC analysis. Between samples, fiber bunch was reconditioned in two consecutive steps of 15 min by immersion in methanol and ultrapure water, respectively. The MMF/AMED-SPME procedure is shown in Fig. S1.

2.5. Preparation of water and milk samples

Tap, lake and river water samples were collected in 2.5 L amber glass bottles and stored in the dark at 4 °C until analysis. All the samples were vacuum-filtered through a 0.45 μm nylon filter to remove suspended matter. The pH values of sample solutions were adjusted to 4.0 by 0.1 mol/L HCl, and ionic strength did not be adjusted. Subsequently, MMF/AMED-SPME procedure was used to extract EMs from the above-mentioned water samples.

Milk sample (20 mL) was transferred into a centrifuge tube and 1.0 mL TFA was added. Subsequently, the milk was centrifuged (3000 rpm, 10 min). A 4 mL volume of supernatant was transferred into a vial (25 mL) and the milk was diluted with ultrapure water to 20 mL. The subsequent extraction and analytical procedure were the same as in the case of water samples.

2.6. Method validation

The limit of detection (LOD) and limit of quantification (LOQ) values of each analyte were considered as the concentration giving a signal to noise ratio of 3 and 10, respectively. The calibration curves were made by fortified with the analytes at each of eight concentrations from 0.25 to 200 μg/L. The spiked samples were performed with complete MMF/AMED-SPME procedure. The calibration curves were calculated using the linear least squares regression analyses of the peak area to concentration ratios. To evaluate the repeatability of proposed method, four replicates samples with 100 μg/L spiking concentration were extracted and

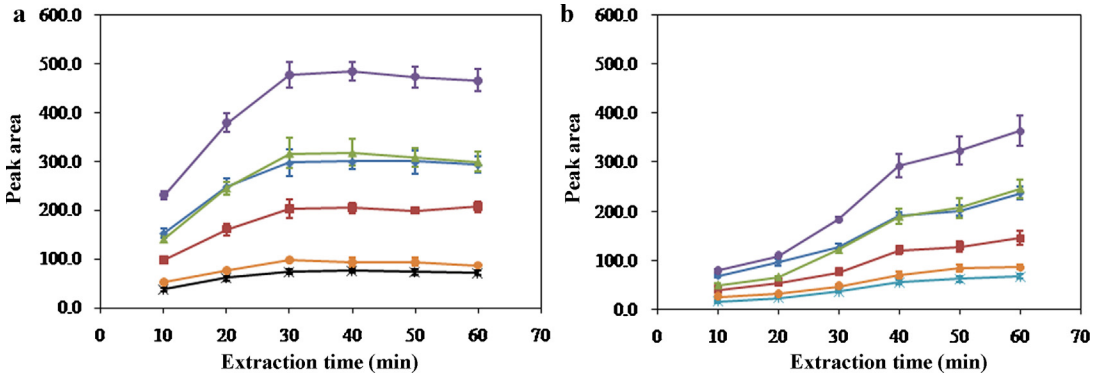


Fig. 2. The effect of extraction time on extraction efficiency. (a) MMF/AMED-SPME; (b) thick fiber. Conditions: methanol was used as desorption solvent; desorption time was 0.5 h; No salt was added in the sample and the pH values of sample matrix were not adjusted. The spiked concentration was 100 μg/L for each estrogen mimics. Symbols: (◆) BPA; (■) DES; (▲) DS; (●) HS; (✶) OP; (●) NP.

analyzed within 1 day. The intermediate precision of the method was assessed at l00 μg/L spiking concentration during a period of four consecutive days.

3. Results and discussion

3.1. Optimization of MMF/AMED-SPME method

To obtain expected extraction performance, several parameters including desorption solvent, extraction and desorption time, pH value and ionic concentration in the sample solution, were investigated and optimized in detail by coupling MMF/AMED-SPME to HPLC/DAD.

3.1.1. The effect of desorption solvent

In this study, methanol/water binary solvent was selected as desorption solvent. The content of methanol in desorption solvent varied from 80% to 100% (v/v) (Fig. S2). Results show that the desorption efficiency enhances with the increase of the usage of methanol. There is no obvious difference between 95% and 100% content of methanol. Considering the analytical time and convenience of experiment, methanol was used as desorption solvent in the following research.

3.1.2. The effect of extraction and desorption time

Extraction time plays a key role in SPME. The extraction time profiles of six EMs were monitored by varying the extraction time from 10 min to 60 min. As shown in Fig. 2a, the extraction performance of MMF/AMED-SPME for EMs increased rapidly with the increase of the extraction time from 10 to 30 min, and no obvious change was observed when extraction time prolonged continuously. To further demonstrate the extraction speed of MMF/AMED-SPME, the extraction time profile of thick fiber (1.0 mm in diameter and 20 mm in length; at the same time, the weight of the monoliths was equal to the weight of the monoliths in MMF/AMED-SPME) was also studied for comparison (Fig. 2b). It can be seen from the profile that the extraction performance enhanced with the increase of extraction time, but the equilibrium did not be reached; even the extraction time was prolonged to 60 min. At the same time, the extraction performance of thick fiber was obviously lower than that obtained with MMF/AMED-SPME when the extraction time was 30 min. The reasons are that most part of sorbents in the thick fiber had not contacted with analytes, and longer time should be needed for target analytes to diffuse into the inner of sorbents. The comparison obviously indicates that MMF/AMED-SPME can enrich EMs effectively and quickly. The reason is the formation of convection between the thin fibers in MMF/AMED-SPME during extraction. Therefore, the analytes can reach the sorptive sites quickly [34]. The effect of liquid desorption time on extraction performance was also studied. A minimum time of 10 min was found to be optimal for efficient desorption of all target compounds from MMF/AMED-SPME when the extraction time was 30 min (Fig. S3). Therefore, 30 min and 10 min were adopted for extraction and desorption procedure, respectively, in the subsequent studies.

3.1.3. The effect of pH value

It can be seen from the structures that the target analytes have abundant polar phenolic hydroxyl groups. At the same time, there are nitrogen atoms in the imidazole groups of sorbent. Therefore, sample pH value will influence the existing form of target analytes and sorbent. To enhance the affinity of the analytes toward the sorbent and to improve extraction efficiency, the pH value should be controlled to avoid EMs becoming ionic forms which will largely weaken the interactions between analytes and sorbent. In the present study, the effect of sample pH on the extraction efficiency was investigated in the range from 2.0 to 8.0. As shown in Fig. 3, the pH value affects the extraction performance of MMF/AMED-SPME for EMs. The results showed that the extraction efficiencies improved with the increasing pH value from 2.0 to 4.0, and the extraction efficiencies decreased when pH values increased continuously. The above-mentioned variation trend may be explained as follows: at low sample pH values, the protonation procedure happened on imidazole groups of sorbent. Hereby, only π–π interaction contributed to the extraction. With the increase of pH values, the deprotonation procedure happened on imidazole groups, which leaded to the increase of hydrophobic interaction between sorbent and analytes. Furthermore, with the enhancement of pH value, hydrogen-bonding and dipole–dipole interactions produced by the polar groups between the sorbent and the analytes also contributed to the extraction. So, higher extraction performance could be achieved with the increase of sample pH values. However, when the pH values increased continuously, EMs became ionic forms gradually because of the dissociation of phenolic hydroxyl groups, which cause the increase of their solubility in aqueous solution. At the same time, the favorable hydrogen-bonding and dipole–dipole interactions were weakened by overmuch hydroxyl groups in solution when pH value was increased. The above factors resulted in the decrease of extraction performance at high pH values. The results also well indicate that multiple interactions such as hydrophobic, π–π, hydrogen-bonding and dipole–dipole interactions involve the effective extraction of MMF/AMED-SPME for EMs. According to the results, in the subsequent experiments, the samples pH value was controlled at 4.0.

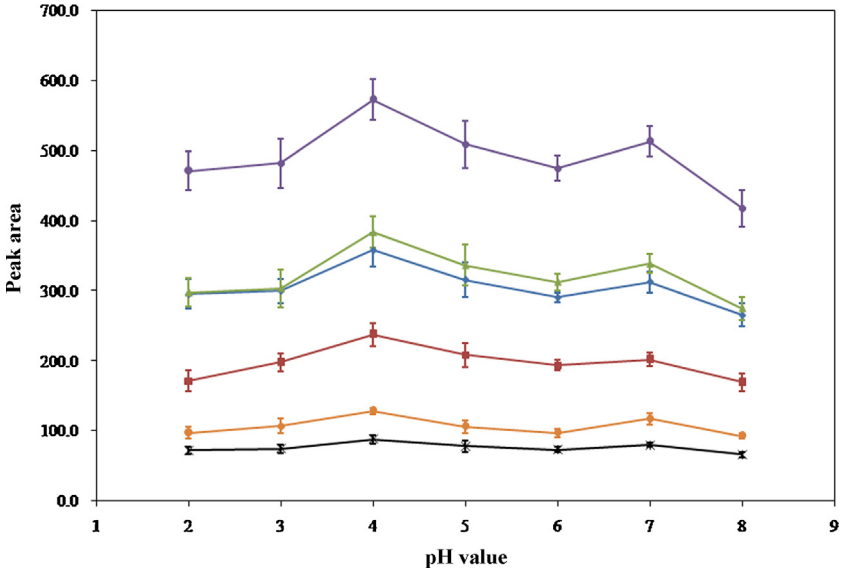


Fig. 3. The effect of pH value of sample matrix on extraction efficiency. Conditions: extraction and desorption time were 30 and 10 min, respectively; the sample pH values were adjusted by 0.1 mol/L HCl or 0.1 mol/L NaOH. The other conditions and symbols are the same as in Fig. 2.

3.1.4. *The effect of ionic strength*

For polar analytes, the ionic strength in sample matrix can affect the extraction because there are salting-out and salting-in effects when salt is added into sample solution [36]. Typically, salting-out effect favors the increase of extraction performance, and salting-in effect can decrease the extraction performance. In the present research, ionic strength of the matrix was adjusted by addition of NaCl from 0 to 25% (w/v). As shown in Fig. 4, the extraction performance decreased with the addition of NaCl. The reason might be that the salting-in effect dominated over the salting-out effect when salt was added. Therefore, the ionic strength of sample matrices could not be adjusted in the following studies.

Comprehensively considering the experimental results, the optimal MMF/AMED-SPME conditions for EMs are as follows: methanol was used as desorption solvent; extraction and desorption time were 30 min and 10 min, respectively; the pH value of sample matrix was 4.0 and the ionic strength of sample matrix could not be adjusted. Under the optimized extraction conditions, the MMF/AMED-SPME can extract EMs effectively. Fig. 5b shows the chromatogram of six target analytes after extraction. Compared with Fig. 5a (direct injection of spiked sample without extraction), it can be seen that the peak heights for all the analytes are obviously enhanced after enrichment with MMF/AMED-SPME. The enriched factors (EF) (calculated by the ratio of the slopes of the calibration curves obtained with and without extraction) for BPA, DES, DS, HS, OP and NP were 43, 45, 53, 52, 34 and 56, respectively. The satisfactory results well indicate that multiple interactions between sorbent and target analytes contribute to the effective extraction. At the same time, the new MMF/AMED-SPME possesses good lifespan, and it could be reused continuously more than 150 times, including real samples; no cracking of the monolithic fiber and no loss of their extraction efficiency were observed during usage.

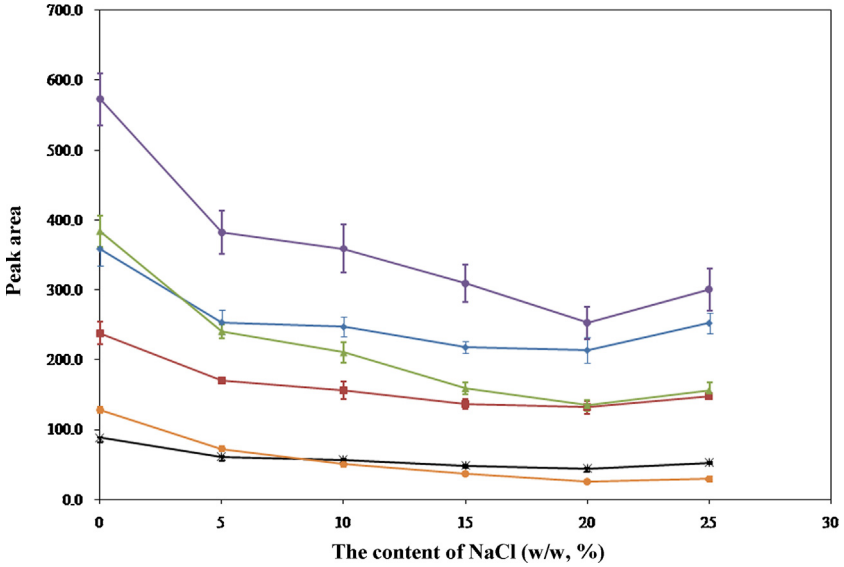


Fig. 4. The effect of salt concentration in sample matrix on extraction efficiency. Conditions: pH value of sample matrix was adjusted to 4.0. The other conditions and symbols are the same as in Fig. 3.

Table 2
Linear dynamic range, correlation coefficients, LODs and LOQs, repeatability and intermediate precision, MMF-to-MMF reproducibility achieved for six estrogen mimics.

Samples	Compounds	Linear range (μg/L)[a]	*r*	LOD (μg/L)[b]	LOQ (μg/L)[c]	Repeatability (RSD%; *n* = 4)[d]	Intermediate precision (RSD%; *n* = 4)[d]	MMF-to-MMF reproducibility (RSD%; *n* = 4)[d]
Water	BPA	0.25–200.0	0.9985	0.045	0.15	3.53	5.27	5.00
	DES	0.50–200.0	0.9985	0.10	0.34	4.30	5.48	3.72
	DS	0.25–200.0	0.9964	0.059	0.20	5.03	5.13	8.60
	HS	0.25–200.0	0.9981	0.040	0.13	3.54	5.59	8.87
	OP	0.50–200.0	0.9955	0.11	0.35	4.84	5.55	6.95
	NP	0.50–200.0	0.9990	0.075	0.24	3.81	5.97	5.42
Milk	BPA	0.50–200.0	0.9988	0.093	0.31	4.69	4.21	2.05
	DES	1.00–200.0	0.9995	0.29	0.83	4.65	7.18	3.31
	DS	0.50–200.0	0.9994	0.12	0.40	7.42	5.03	7.05
	HS	0.50–200.0	0.9992	0.091	0.30	4.52	5.82	5.56
	OP	1.00–200.0	0.9985	0.23	0.76	3.98	5.80	6.81
	NP	1.00–200.0	0.9996	0.18	0.63	5.40	3.06	9.13

[a] Spiking level included 0.25, 0.50, 1.00, 5.00, 10.0, 20.0, 50.0, 100.0 and 200.0 μg/L, respectively.
[b] S/N = 3.
[c] S/N = 10.
[d] Assays at 100.0 μg/L level.

Table 3
Results of determination and recoveries of real water and milk samples spiked with six estrogen mimics.

Samples	Spiked (μg/L)	Detected (μg/L)/recoveries (%RSD, *n* = 3)											
	BPA		DES		DS		HS		OP		NP	
Tap water	0.0	ND		ND		ND		ND		ND		ND	
	1.0	1.16	116 (8.9)	0.907	90.7 (8.9)	0.94	93.6 (8.6)	0.98	97.9 (6.7)	1.17	117 (9.7)	1.16	116 (3.5)
	10.0	11.8	118 (9.5)	11.3	113 (8.1)	10.1	111 (8.9)	110	110 (6.8)	9.85	98.5 (5.3)	10.0	100 (7.0)
	100.0	106	106 (5.2)	106	106 (5.9)	95.8	95.8 (8.0)	105	105 (7.7)	100	100 (9.0)	95.0	95.0 (6.7)
Lake water	0.0	ND		ND		ND		ND		ND		ND	
	1.0	1.11	111 (6.4)	1.11	111 (8.8)	1.08	108 (7.8)	1.02	102 (2.2)	1.06	106 (8.3)	1.06	106 (5.3)
	10.0	118	118 (6.4)	11.9	119 (8.0)	11.9	119 (6.6)	11.6	116 (7.4)	10.2	102 (7.7)	11.3	113 (3.0)
	100.0	97.4	97.4 (5.3)	95.3	95.3 (4.1)	88.0	88.0 (4.8)	94.9	94.9 (6.5)	97.6	97.6 (9.6)	88.7	88.7 (8.6)
River water	0.0	ND		ND		1.36		ND		ND		ND	
	1.0	0.794	79.4 (3.9)	1.08	108 (4.2)	2.24	87.7 (6.0)	0.79	79.1 (4.7)	0.94	93.7 (2.4)	0.96	95.7 (9.5)
	10.0	11.2	112 (7.1)	110	110 (5.0)	11.4	100 (7.8)	10.2	102 (8.3)	7.85	78.5 (9.1)	7.56	75.6 (8.8)
	100.0	106	106 (6.2)	105	105 (8.2)	101	100 (9.3)	103	103 (8.5)	75.5	75.5 (8.8)	76.0	76.0 (9.6)
Milk 1	0.0	ND		ND		ND		ND		ND		ND	
	1.0	0.748	74.8 (7.3)	0.778	77.8 (6.4)	0.925	92.5 (6.9)	0.866	86.6 (4.6)	0.858	85.8 (8.0)	0.846	84.6 (5.2)
	10.0	7.68	76.8 (6.3)	11.6	116 (8.1)	10.8	108 (5.5)	11.1	111 (6.0)	11.6	116 (4.9	11.0	110 (6.2)
	100.0	107	107 (9.7)	106	106 (9.1)	112	112 (9.8)	112	112 (9.6)	117	117 (9.7)	115	115 (8.3)
Milk 2	0.0	ND		ND		ND		ND		ND		ND	
	1.0	0.882	88.2 (8.2)	0.903	90.3 (5.7)	0.708	70.8 (7.9)	0.806	80.6 (4.1)	0.801	80.1 (3.1)	1.15	115 (6.0)
	10.0	7.99	79.9 (5.7)	11.6	116 (9.0)	11.2	112 (8.3)	10.2	102 (9.8)	11.6	116 (5.3)	8.12	81.2 (3.9)
	100.0	91.8	91.8 (9.7)	90.9	90.9 (8.8)	91.9	91.9 (9.2)	91.6	91.6 (9.4)	220	110 (9.3)	93.3	93.3 (8.1)

3.2. Validation of the MMF/AMED-SPME-LD-HPLC/DAD method

In the present study, some parameters including linear dynamic range, correlation coefficients, recoveries, LODs, LOQs and reproducibility were used to evaluate the validation of proposed method. Linear dynamic range was constructed with the EMs spiked to the ultrapure water or analyte free milk sample over the range of 0.25–200.0 μg/L. Linear regression analyses were performed using relative peak areas against the respective analytes concentration. The data of linear dynamic range, correlation coefficients, LOD, LOQ and method reproducibility for target analytes under the optimized conditions are listed in Table 2. In water sample, the linear dynamic ranges were 0.25–200.0 μg/L for BPA, DS and HS, 0.50–200 μg/L for DES, OP and NP. The corresponding values in milk sample were 0.50–200.0 μg/L and 1.00–200 μg/L, respectively. All linear dynamic ranges possess satisfactory coefficients of correlation (*r* higher than 0.99). In water sample, the LOD and LOQ values were in the range of 0.040–0.11 and 0.13–0.35 μg/L, respectively. The corresponding values in milk sample were 0.093–0.29 μg/L and 0.31–0.83 μg/L, respectively. At the same time, excellent method reproducibility was achieved, the relative standard deviations (RSDs) for repeatability and intermediate precision were both less than 8%. The MMF-to-MMF reproducibility was also investigated. The results showed that the RSD values were below 10% in all cases. The data in Table 2 well demonstrate that the developed method has good reproducibility and high sensitivity for the detection of trace EMs in water and milk samples.

3.3. Real samples analysis

The developed MMF/AMED-SPME-LD-HPLC/DAD method was applied to the determination of six target EMs in milk and real water samples including tap water, lake and river waters. The analytical results obtained by the external calibration method and recoveries for the spiked samples are listed in Table 3. The results indicated that trace DS (1.36 μg/L) was detected in river water, the other EMs were not detected in any of the water and milk samples. To further validate the feasibility of the proposed method, extraction recoveries were assessed by spiking different standards solutions (1.0, 10.0 and 100.0 μg/L, respectively). Acceptable recoveries and good reproducibility were obtained. The recoveries were in the range of 75.6–118% for all samples. The RSDs for reproducibility were less

Table 4
Comparison of the LODs, total extraction time and recoveries of present method with other methods for the determination of estrogen mimics in water samples.

Method	LOD (μg/L or μg/kg)						Total extraction time (min)	Recoveries (%)	Ref.
	BPA	DES	DS	HS	OP	NP			
HF-LLLME[a]-HPLC/UV	0.055	0.20	0.11	–	1.46	–	50	73.2–117.5	[11]
SPME-HPLC/UV	0.3	0.3	–	–	–	1.1	47	–	[37]
SPME-HPLC/FD	0.43	–	–	–	0.16	0.29	60	85.5–108.6	[33]
DSPE[b]-HPLC/UV	3.0	6.1	0.2				80	70–106	[38]
SPE-UPLC/MS	0.10	–	–	–	0.15	0.10	–	80.1–109	[39]
TFME[c]-HPLC/UV	0.2		–	–	0.4	0.4	60	86.0–104	[40]
CPE[d]-HPLC/UV	–	0.1	–	–	–	–	60	85.4–104.0	[41]
SBSE-HPLC/UV	0.15	0.26	0.18	–	0.24	–	55	72.2–124.4	[21]
SBSE-GC/MS	0.002	–	–	–	0.0005	0.005	90	93.9–113.0	[42]
SBSE-HPLC/UV	–	0.6	–	–	–	–	120	98–110	[43]
SDME[e]-HPLC/UV	4	–	–	–	9	9	60	99.4–111	[44]
MMF/AMED-HPLC/DAD	0.045	0.10	0.059	0.040	0.11	0.075	40	75.6–118	Present work

[a] Hollow fiber liquid–liquid–liquid microextraction.
[b] Dispersive solid-phase extraction.
[c] Thin-film microextraction.
[d] Cloud-point extraction.
[e] Single-drop microextraction.

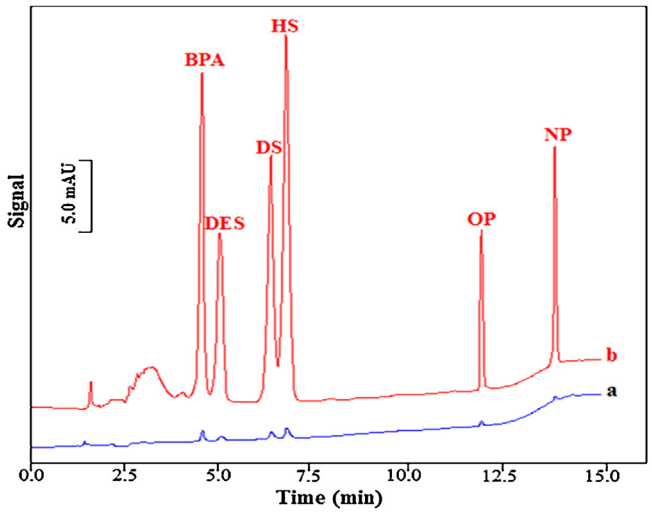


Fig. 5. HPLC chromatograms of six estrogen mimics. (a) Direct injection of spiked water sample; (b) spiked water sample with each analyte at 100.0 μg/L and treated with MMF/AMED-SPME. Conditions: methanol was used as desorption solvent; extraction and desorption time were 30 and 10 min, respectively; the pH value of sample matrix was 4.0; no salt was added in sample matrix. The spiked concentration was 100.0 μg/L for each estrogen mimics.

than 10% for all target analytes. The results further demonstrate that the established method is applicable for the monitoring of trace EMs in water and milk samples.

3.4. Comparison with other methods

A comparison of LODs, recoveries and total extraction time (including adsorption and desorption time) achieved in the present method and other reported methodologies for the determination of EMs in water samples is summarized in Table 4. As can be seen, the LODs of six target analytes obtained by the proposed method were lower than that obtained by other methods with the same kind of detector [11,21,32,37,39–41,43,44]. Typically, higher sensitivity can be achieved when high sensitivity detectors such as fluorimetric detection (FD) and mass spectrum (MS) are used. However, the LODs achieved in the proposed method are lower than that obtained with SPME-HPLC/FD [33] and SPE-UPLC/MS [38]. The SBSE-GC/MS showed better sensitivity than other methods, but in situ derivatization with acetic acid anhydride was needed. At the same time, special thermal desorption instrument was required between the combination of SBSE and GC/MS [42]. It is worthy to stress that the total extraction time in proposed method is less than other studies. In previous methods, the equilibrium time was around 50–60 min or more [11,21,32,33,37–44]. However, the corresponding time was reduced to 40 min. At the same time, the spiked recoveries achieved in the present method are comparable with other works. The comparative results well indicate that the MMF/AMED-SPME can enrich EMs effectively in short time.

4. Conclusions

In this work, a new method of polymeric ionic liquid-based MMF-SPME combined with HPLC/DAD was proposed for simultaneously analyzing selected EMs in milk and environmental water samples. The developed MMF/AMED-SPME method has the advantages of quick extraction speed, high enrichment, easy-to-operate, low organic solvent consumption and low-cost. The established MMF/AMED-SPME-LD-HPLC/DAD method provides low LODs, good reproducibility and wide linear ranges. In comparison to the existing methods for EMs determination, the proposed method was simple, rapid and environmentally friendly. Thus, the proposed method is expected to have potential applications in the screening and determination of trace EMs in various water and milk samples.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2015.01.072.

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