Talanta 115 (2013) 787–797

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Simultaneous analysis of eight phenolic environmental estrogens in blood using dispersive micro-solid-phase extraction combined with ultra fast liquid chromatography–tandem mass spectrometry

Yong-Gang Zhao [a], Xiao-Hong Chen [a,b], Sheng-Dong Pan [a], Hao Zhu [b], Hao-Yu Shen [c], Mi-Cong Jin [a,b,*]

[a] Ningbo Key Laboratory of Poison Research and Control, Ningbo Municipal Center for Disease Control and Prevention, Ningbo, Zhejiang 315010, China
[b] Medical school, Ningbo University, Ningbo, Zhejiang 315211, China
[c] Ningbo Institute of Technology, Zhejiang University, Ningbo, Zhejiang 315100, China

ARTICLE INFO

Article history:
Received 12 March 2013
Received in revised form
17 May 2013
Accepted 21 May 2013
Available online 11 June 2013

Keywords:
Tetraethylenepentamine (TEPA)-functionalized magnetic polymers (TEPA-MPs)
Dispersive micro-solid-phase extraction (d-μ-SPE)
Ultra fast liquid chromatography–tandem mass spectrometry (UFLC–MS/MS)
Multiple reaction monitoring (MRM)
Environmental estrogens
Blood

ABSTRACT

A novel, simple and sensitive method was developed for the simultaneous determination of eight phenolic environmental estrogens in blood by using the dispersive micro-solid-phase extraction (d-μ-SPE) procedure combined with ultra-fast liquid chromatography–tandem quadrupole mass spectrometry (UFLC–MS/MS). The excellent nanomaterials tetraethylenepentamine-functionalized magnetic polymer was used as an adsorbent, and the main factors affecting the extraction efficiency were investigated in detail. All target compounds showed good linearities in the tested range with correlation coefficients (r) higher than 0.999. The mean recoveries were in the range of 85.0–105.0%. The intra-day and inter-day relative standard deviations (RSDs) were lower than 4.9% and 5.2%, respectively. The limits of quantification for the eight phenolic environmental estrogens were between 0.075 and 0.42 μg L^{-1}. The developed method can be applied to the routine analyses for the determination of the eight phenolic environmental estrogens in blood samples.

1. Introduction

In recent years, worldwide scientific and public concern in the estrogenic potential of endocrine-disrupting chemicals in the environment has been increased with each passing day [1,2]. Many chemicals have been reported to possess estrogenic properties and a variety of reproductive disorders in wildlife and humans have been tentatively attributed to these estrogenic pollutants [3–5]. Among these substances, the presence of phenolic environmental estrogens, i.e., alkylphenol (AP) and bisphenol A (BPA) have demonstrated estrogenic potency and chronic toxicity by in vitro and in vivo bioassay studies [6–9]. Thus, there is a need to develop sensitive methods to measure a broad range of phenolic environmental estrogens in a wide variety of biological and environmental matrices.

To the best of our knowledge, phenolic compounds have previously been quantified by liquid chromatography (LC) with a variety of detection systems, such as fluorescence [10] or mass spectrometry [11–14], and gas chromatography coupled to mass spectrometry (GC/MS) based on derivatization [15]. Among these methods, LC coupled with tandem quadrupole mass spectrometry (LC–MS/MS) is an attractive alternative due to its simplicity, excellent sensitivity and selectivity for multiresidue determination of trace-level phenolic compounds [2,16,17]. However, matrix effect, a phenomenon of ion suppression or enhancement of the analyte of interest, would significantly affect the accuracy and precision of a LC–MS/MS method [18,19]. Endogenous matrix components co-eluting with the analyte of interest are believed to be the primary cause of ionization matrix effects. In the case of blood sample, blood phospholipids have been identified as a major contributing source of matrix effects in LC–MS/MS based

* Corresponding author at: Ningbo Municipal Center for Disease Control and Prevention, Ningbo Key Laboratory of Poison Research and Control, Ningbo, Zhejiang 315010, China. Tel.: +86 574 87274559; fax: +86 574 87277153.
E-mail addresses: jmcjc@163.com, jinmc@163.com (M.-C. Jin).

0039-9140/$ - see front matter Crown Copyright © 2013 Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.05.053

bioanalytical methods [20–23]. Zeng et al. reported a zirconia-based hybrid absorbent SPE cartridge for the removal of phospholipids from blood samples after protein precipitation [24]. Chang et al. reported a silica-gel SPE cartridge for the effective removal of the blood plasma complex matrix [17]. Although SPE had been verified to be effective for cleaning the biological samples, it is relatively expensive, time-consuming and tedious. Several other cleanup methods based on the traditional SPE technique, such as dispersive solid-phase extraction (dSPE), micro-solid-phase extraction (μ-SPE) and dispersive micro-solid-phase extraction (d-μ-SPE), have been widely used for multi-residue analysis of pesticides [25–32]. Based on the amino-propyl SPE method [2], we inspirited that amino-functionalized superparamagnetic polymers with magnetic separation would probably become a powerful adsorbent to carry out d-μ-SPE method.

In this work, an inexpensive and effective adsorbent namely tetraethylenepentamine-functionalized Fe_3O_4 magnetic polymer (TEPA-MP) has been synthesized, which possesses much stronger adsorbilities for the removal of phospholipids with a convenient magnetic separation. The object of this study was to prepare a novel amino-functionalized magnetic polymer (TEPA-MP), and substantiate its d-μ-SPE ability for the analysis of phenolic environmental estrogens, i.e., bisphenol A (BPA), diethylstilbestrol (DES), dienestrol (DE), hexestrol (HEX), 4-(tert-octyl)-phenol (4-tOP), 4-nonylphenol (4-NP), estrone (E1) and estradiol valerate (EV) (structural formulas are listed in Fig. 1), in blood. Furthermore, this paper also aimed to develop a rapid and efficient analytical method for the simultaneous determination of eight phenolic environmental estrogens by ultra fast liquid chromatography coupled with tandem quadrupole mass spectrometry (UFLC–MS/MS).

2. Experimental

2.1. Reagents and materials

Ferric chloride, ferrous sulfate, oleic acid (OA), methyl methacrylate (MMA), tetraethylenepentamine (TEPA), glycidylmethacrylate (GMA), divinylbenzene (DVB), polyvinyl alcohol (PVA 217) and benzoyl peroxide (BPO) of analytical grade were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Bisphenol A (BPA, > 99.0%), diethylstilbestrol (DES, > 98.0%), dienestrol (DE, > 99.0%), hexestrol (HEX, > 99.0%), 4-(tert-octyl)-phenol (4-tOP, > 99.0%), 4-nonylphenol (4-NP, > 99.0%), estrone (E1, > 99.0%) and estradiol valerate (EV, > 99.0%) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Acetonitrile, ammonium acetate (AmAc), trifluoroacetic acid (TFA) of HPLC grade were purchased from Merck Company (Darmstadt, Germany). Blood was collected from the bullfrog. Bullfrogs were acquired from local markets (Ningbo, China).

2.2. Equipment

Characterization of TEPA-MP was performed by using a ThermoFisher Flash-1112 elementary analyzer (EA) (Thermo Fisher, USA), a Hitachi H-7650 transmission electron microscope (TEM)

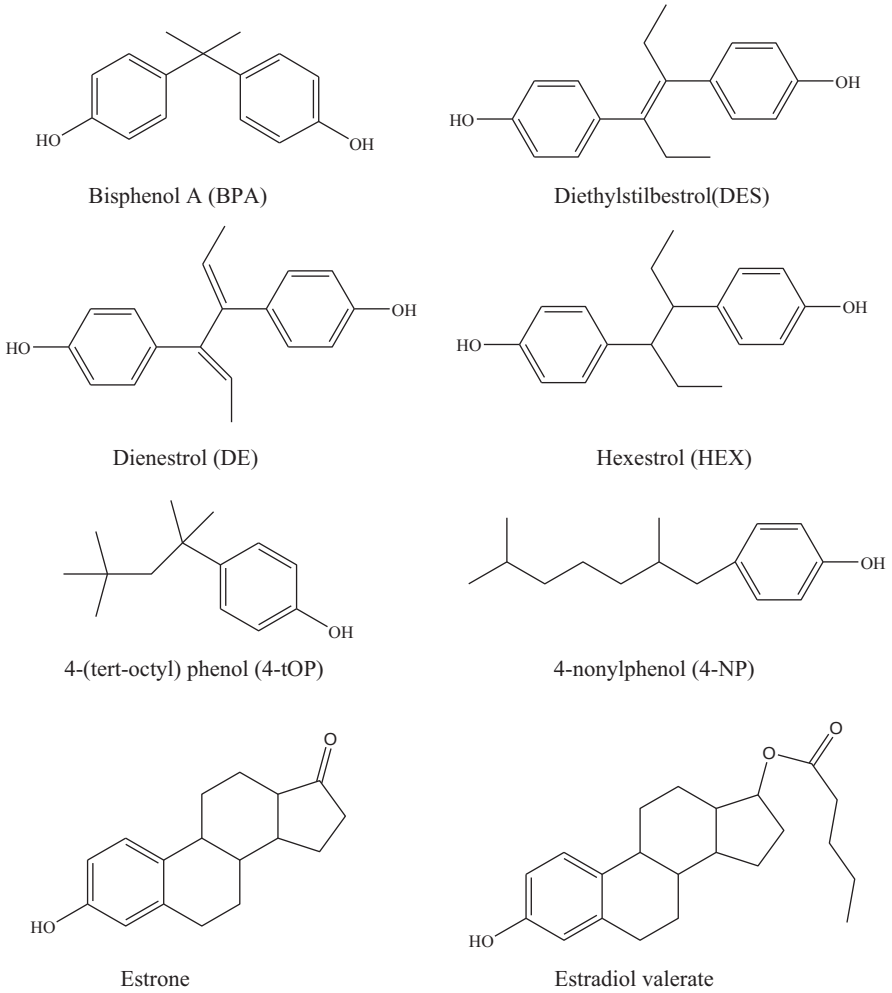

Fig. 1. Structural formulas of eight phenolic environmental estrogens.

(Hitachi, Japan), a NEXUS-470 Thermo Nicolet FTIR spectrometer (Thermo Nicolet, USA) and a Lake Shore 7410 vibrating sample magnetometer (VSM) (Westerville, USA).

A Hualida WH-866 vortex mixer (Taicang, China) was used for extraction. A 1.5-T NdFeB permanent magnet (MCE Products, Torrance, CA) was used for magnetic separation. Ultra-fast liquid chromatography-tandem quadrupole mass spectrometry (UFLC–MS/MS) analyses were performed using a Prominence UFLC XR system equipped with a DGU-20A_3 degasser, a CTO-20AC column oven, a LC-20AD pump, a SIL-20AC autosampler (Shimadzu Corporation, Tokyo, Japan) and an AB SCIEX TRIPLE QUAD™ 5500 mass spectrometer (Applied Biosystems, Foster City, CA, USA). The UFLC–MS/MS system was controlled and data were analyzed on a computer equipped with Applied Biosystems/MDS Sciex Analyst 1.5.1 (Applied Biosystems, Foster City, CA, USA).

2.3. Synthesis of TEPA-MP

TEPA-MP was prepared *via* suspension polymerization according to our previously reported procedures [33,34] after minor modification, in which tetraethylenepentamine (TEPA) was used as amino-functionalized groups replacement of the ethylenediamine (EDA). The paramagnetic Fe_3O_4 was firstly coated with oleic acid. Methyl methacrylate (MMA), divinylbenzen (DVB) and glycidyl-methacrylate (GMA) were then co-polymerized *via* the suspension polymerization procedure over the magnetic core to obtain epoxyl-functionalized magnetic polymer. Finally, TEPA was grafted onto the surface of the polymer *via* ring-opening reaction. Thus, the target TEPA-functionalized magnetic polymer was obtained. The preparation procedure is illustrated in Fig. 2.

2.4. Characterization of TEPA-MP

The TEM at 80 kV was used to examine the morphology and dimensions of the synthesized TEPA-MP. The VSM was used to analyze the magnetic behavior of the particles. The EA was used to analyze the nitrogen percentage of TEPA-MP. Each sample was prepared by placing a very dilute particle suspension onto 400 mesh carbon grids coated with copper film.

2.5. UFLC-QqQ-MS/MS analysis

The ultra fast chromatographic separation was performed on a Shim-pack XR-ODSII (100 mm × 2.0 mm i.d., 2.2 μm) by using 0.02% ammonia (v/v) in methanol as eluent (A), and 0.02% ammonia (v/v) in water as eluent (B). The linear gradient program was as follows: 0–2.00 min, 40.0–70.0% (A); 2.00–5.00 min, 70.0–85.0% (A); 5.00–6.00 min, 85.0% (A); 6.00–8.00 min, 85.0–40.0% (A); 8.00–9.00 min, 40.0% (A). Chromatographic separation was accomplished at a constant flow of 0.45 mL/min, and the injection volume was 5.0 μL. The column temperature was held at 40 °C. The mass spectrometer was performed using an electrospray ionization source in negative multiple reaction monitoring (MRM) mode, which was used for quantification. The operation conditions were as follows: ion spray voltage, −4500 V; curtain gas (CUR), 40 psi; interface heater, on; collision gas, medium; nebulizer gas (gas 1), 50 psi; heater gas (gas 2), 50 psi; turbo spray temperature, 500 °C; entrance potential (EP), −10 V; collision cell exit potential (CXP), −10 V. Nitrogen was used in all cases. The mass spectrometric information of the precursor ion, product ion, corresponding declustering potential (DP) and collision energy (CE) are shown in Table 1. The dwell time was set to 50 ms in negative mode.

2.6. TEPA-MP d-μ-SPE of bullfrog plasma samples

Exactly 100.0 μL of bullfrog blood was transferred to a polypropylene centrifuge tube (2.0 mL) and then 500.0 μL of 0.1% formic acid (v/v) in methanol was added. The contents were homogenized for 1.0 min using an Ultra Turraxmixer, and the mixture was immediately machine-shaken for 1.0 min, then centrifuged at 15000 rpm for 5.0 min. Afterwards, the supernatant was transferred to another polypropylene centrifuge tube (2.0 mL). The residues were repeat extracted with 500.0 μL of 0.1% formic acid (v/v) in methanol for two times. Subsequently, 20 mg of TEPA-MP were added, and the mixture was immediately machine-shaken for 1.0 min, then separation under a magnetic field for 2 min. The supernatant concentrated to dryness with a nitrogen stream and was redissolved with 100.0 μL of 0.02% ammonia (v/v) in methanol/0.02% ammonia (v/v) in water (4:6, v/v) and filtered using a 0.22 μm membrane prior to its injection into the UFLC–MS/MS system.

In order to investigate the effect of organic solvents on the cleanup properties, series of experiments were performed by mixing 20 mg of TEPA-MP with 100 μL of bullfrog blood sample spiked at 10.0 μg L^{-1} with the various volume of formic acid in methanol from 0.01% to 0.5% (V/V) in a 2.0 mL polypropylene centrifuge tube.

To investigate the effect of extraction time, 20 mg of TEPA-MP with 100 μL of bullfrog blood sample spiked at 10.0 μg L^{-1} with the various contact times ranging from 1.0 min to 30.0 min. When the effect of the TEPA-MP was investigated, seven dosages of TEPA-MP (i.e., 5, 10, 20, 30, 40, 50 and 60 mg for 100 μL of bullfrog blood sample) on the cleanup properties were investigated to develop an efficient cleanup procedure for the eight phenolic environmental estrogens.

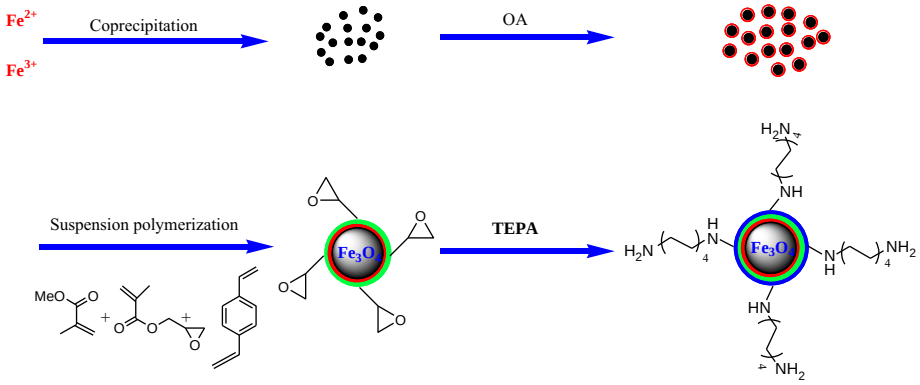

Fig. 2. The preparation procedure of the TEPA-MP.

Table 1
Q1/Q3 ion pairs, declustering potential (DP), collision energy (CE) of MRM and retention time for the eight phenolic environmental estrogens.

Phenolic environmental estrogen	Precursor ion (Q1, m/z)	Fragment ion (Q3, m/z)	DP(V)	CE (eV)	Retention time (min)
BPA	227.0	133.0*, 211.0	150, 150	34, 40	3.07
DES	267.1	251.1*, 237.1	140, 140	32, 37	3.46
E1	269.1	145.1*, 159.1	180, 180	56, 46	3.48
DE	265.2	93.0*,171.0	130, 130	31, 25	3.59
HEX	269.1	134.1*,119.0	130, 130	20, 51	3.69
4-tOP	205.0	133.0*,93.0	120, 120	32, 60	5.54
EV	355.3	101.1*, 145.0	180, 180	38, 63	6.56
4-NP	219.0	106.0*,119.0	100, 100	26, 55	7.09

* Quantitative ion

2.7. Stability, recycling and reproducibility of TEPA-MP

In order to investigate their stability and recyclability, all the TEPA-MP particles, used for the d-μ-SPE cleanup procedure, were collected and soaked in sodium hydroxide solution at a concentration of 1.0 mol L^{-1} for at least 0.5 h. Then the particles were washed with water and methanol to pH 7.0 to remove the superfluous sodium hydroxide and dried under a vacuum at 60 °C for 12 h.

In order to evaluate their preparative reproducibility, three batches of the TEPA-MP, namely TEPA-MP-1, TEPA-MP-2 and TEPA-MP-3, were synthesized. The recoveries of the eight phenolic environmental estrogens in bullfrog blood using TEPA-MP as an adsorbent for the d-μ-SPE were determined.

2.8. Comparison of different cleanup procedures

In order to evaluate the effectiveness of the TEPA-MP for the cleanup properties for eight phenolic environmental estrogens, comparative studies were carried out among three d-μ-SPE procedures used C18, PSA and TEPA-MP as an absorbent, respectively. The various extraction procedures were performed as follows.

2.8.1. Cleanup via d-μ-SPE with C18 (approach □)

In a 2.0 mL polypropylene centrifuge tube, 100.0 μL of bullfrog blood and 500.0 μL of 0.1% formic acid (v/v) in methanol were added. The contents were homogenized for 1.0 min using an Ultra Turraxmixer, and the mixture was immediately machine-shaken for 5.0 min, then centrifuged at 15000 rpm for 5.0 min. Afterwards, the supernatant was transferred to another 2.0 mL polypropylene centrifuge tube. The residues were extracted with 500.0 μL of 0.1% formic acid (v/v) in methanol twice. Subsequently, 20 mg of C18 were added, and the mixture was immediately machine-shaken for 1.0 min and centrifuged at 6800 rpm for 5.0 min. The supernatant was concentrated to dryness under a nitrogen stream. Then, the residues were redissolved with 100.0 μL of 0.02% ammonia (v/v) in methanol/0.02% ammonia (v/v) in water (4:6, v/v) and filtered through a 0.22 μm membrane prior to its injection into the UFLC–MS/MS system.

2.8.2. Cleanup via d-μ-SPE with PSA (approach □)

The overall procedure was similar to that of *approach* □. Only the C18 was replaced by the PSA.

2.8.3. Cleanup via d-μ-SPE with TEPA-MP (approach □)

The overall procedure was similar to that of *approach I*. The C18 was replaced by the TEPA-MP. Furthermore, after TEPA-MP d-μ-SPE procedure, the residues and supernatant were separated by magnetic separation under a magnetic field rather than centrifugation.

2.9. Method validation

2.9.1. Standard preparation

Individual stock standard solutions were prepared at a concentration of 1000 mg L^{-1} by exact weighing and dissolution in water–acetonitrile (1:1, v/v). These solutions were stored at 4 °C in the dark. Working standard mixture solution at a concentration of 100.0 mg L^{-1} was prepared by appropriate dilution of the stock solutions with water–acetonitrile (1:1, v/v).

2.9.2. Spiked samples

Spiked recoveries were performed at concentrations of 1.0, 20.0 and 50.0 μg L^{-1} for eight phenolic environmental estrogens in the samples. For each spiked sample, the stock mixture solution of the standards was added to 100.0 μL of bullfrog blood, which was free from the target compounds. The spiked samples were stored at 4 °C for about 12 h to let the phenolic environmental estrogens permeate uniformly into the samples. Recoveries at each level were run along with both reagent and a sample blank.

2.9.3. Validation parameters

The method was evaluated by linearity, LOD and LOQ, precision and accuracy. Calibration standards in acetonitrile with concentrations at 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0 μg L^{-1} were prepared for the calibration curves. Calibration curves of peak area vs. concentration (μg L^{-1}) were used to calibrate the UFLC–MS/MS system and spike samples in recovery experiments. LOD and LOQ were determined based on a signal-to-noise ratio of 3 (S/N=3) and 10 (S/N=10), respectively. Both the method precision and accuracy were estimated. The intra-day and inter-day precision were investigated by injection of the three levels of spiked samples (1.0, 20.0 and 50.0 μg L^{-1}) with six replicates and replicated on six different days, respectively. Accuracy of the method was also checked by spiking 1.0, 20.0 and 50.0 μg L^{-1} of eight phenolic environmental estrogens in the samples.

3. Results and discussion

3.1. Characterization of TEPA-MP

The TEM image of the TEPA-MP particles reveals that the TEPA-MP particles synthesized in this study are multidispersed with an average diameter of approximately 800 nm, as shown in Fig. 3. The paramagnetic properties of the TEPA-MP were verified by the VSM, and the saturation moments obtained from the hysteresis loop was found to be 9.98 emu/g. Therefore, the TEPA-MP was responded well to magnetic fields without any permanent magnetization for quick and easy separation in two-phase (solid phase and liquid phase) systems.

The Fourier transform infrared (FT-IR) spectra of M-co-poly (MMA-DVB-GMA) have the characteristic absorptions of C=O groups at ~1731 cm^{-1}, C–O–C groups at ~1273 cm^{-1} and ~1148 cm^{-1}, and Fe_3O_4 at ~580 cm^{-1}. After further amino-functionalization, the characteristic peaks of –NH– and $–NH_2–$ groups at ~1570 cm^{-1} and ~3425 cm^{-1} appeared in the FT-IR spectra of TEPA-MP. These results revealed that the epoxyl- of M-co-poly (MMA-DVB-GMA) had been functionalized successfully with the amino groups *via* ring-opening reaction, and the nitrogen percentage of TEPA-MP obtained from EA was 9.6%.

3.2. Optimization of UFLC–MS/MS conditions

In order to get optimization of triple quadrupole MS/MS conditions, the choice of precursor ions, product ions, and optimization of collision energies for best response were required for

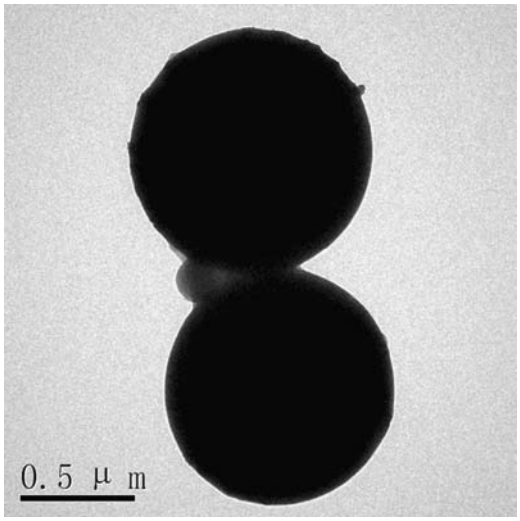

Fig. 3. TEM image of TEPA-MP.

each target compound. Suitable precursor-to-product MS–MS transitions of eight phenolic environmental estrogens were selected through the optimization procedure. The final MS/MS conditions are detailed in Table 1, and an extract ion chromatogram (XIC) of the target compounds at 10.0 μg L^{-1} is illustrated in Fig. 4.

3.3. TEPA-MP d-μ-SPE procedure and its optimization

In this work, sample preparation was a critical part of the method due to the highly complex blood composition with containing large amounts of phospholipids. The main challenge of developing a cleanup method was the separation of the analytes of interest from the matrix, the TEPA-MP d-μ-SPE was used for the cleanup.

3.3.1. The effect of organic solvents on the cleanup properties

In order to get optimization of protein precipitation conditions, pure methanol and acetonitrile were used for the test. The

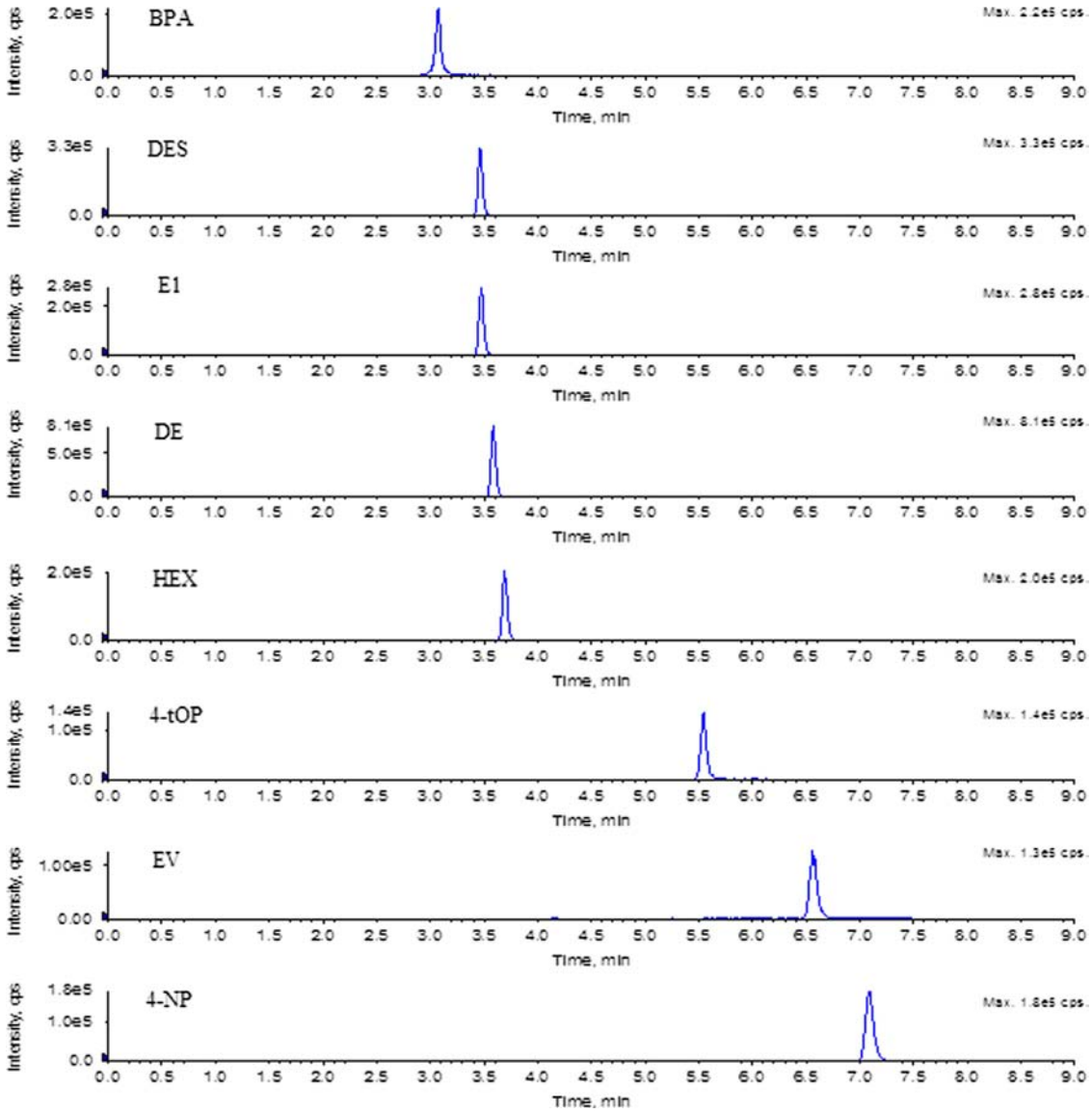

Fig. 4. Extract ion chromatograms (XIC) of eight phenolic environmental estrogens.

obtained results showed that the precipitate dispersed uniformly in methanol with much higher recoveries of analytes ranged from 54.2% to 76.9% than acetonitrile.

In order to obtain satisfactory recoveries of eight phenolic environmental estrogens, the effect of the ratio of formic acid in methanol on the recoveries was studied. Series of experiments were performed by mixing 20 mg of TEPA-MP with 100 μL of blood sample spiked at 10.0 μg L^{-1} with the different ratios of formic acid in methanol from 0.01% to 0.5% (V/V) in a 2.0 mL polypropylene centrifuge tube. The results are shown in Fig. 5. It can be seen that with the increasing of the ratio of formic acid in methanol, the recoveries of BPA, DES, DE, HEX, 4-tOP, 4-NP, E1 and EV increased significantly. The satisfactory recoveries (> 95%) of all analytes were obtained by using formic acid–methanol (0.1%, V/V) as the organic solvent for protein precipitation.

The dependence of the recoveries of BPA, DES, DE, HEX, 4-tOP, 4-NP, E1 and EV on the ratio of formic acid in methanol could be due to the perspective of surface chemistry of TEPA-MP used as adsorbent in the d-μ-SPE procedure. The TEPA-MP surface is generally covered with amino groups (–NH–, $–NH_2$) with different charges at different pH levels. Generally, the amino groups are more easily protonated under acidic conditions than under basic condition. In the case of methanol used as organic solvent for protein precipitation, hydrogen-bonding interaction happens between amino groups (–NH–, $–NH_2$) of TEPA-MP and –OH groups of phenolic environmental estrogens. With the increasing of the ratio of formic acid in methanol, the concentration of H^+ was increased, and amino groups were much easier to be protonated resulting in the less chance of hydrogen-bonding interaction between amino groups (–NH-, $–NH_2$) of TEPA-MP and –OH groups of phenolic environmental estrogens. And interaction mechanism among TEPA-MP, formic acid in methanol, and phenolic environmental estrogens, e.g., BPA, are shown in Fig. S1. Therefore, formic acid–methanol (0.1%, v/v) was selected as the optimal organic solvent for protein precipitation for further studies.

3.3.2. *The effect of extraction time on the cleanup properties*

When designing the TEPA-MP d-μ-SPE cleanup optimization experiments, one of the primary considerations was to develop an easy and fast cleanup procedure without affecting the analytes recoveries. Therefore, the effect of extraction time on the recovery was studied. Batch experiments were performed by mixing 20 mg of TEPA-MP with 100 μL of blood sample spiked at 10.0 μg L^{-1} with various extraction time from 1.0 min to 30.0 min in a 2.0 mL polypropylene centrifuge tube. With the increasing of the extraction time from 1.0 min to 30.0 min, the satisfactory recoveries of BPA, DES, DE, HEX, 4-tOP, 4-NP, E1 and EV were kept in the range of 91.6–94.8%, 93.6–97.2%, 89.5–93.2%, 90.8–92.5%, 89.7–92.3%, 93.2–96.8%, 98.6–101.3% and 92.5–94.3%, respectively. This indicates the recoveries of the phenolic environmental estrogens are independent of extraction time. Therefore, short extraction time of 1.0 min was chosen as the optimal extraction time for further studies. Furthermore, the similar results were also obtained in the case of low concentration level of 1.0 μg L^{-1} and high concentration level of 100 μg L^{-1}, respectively.

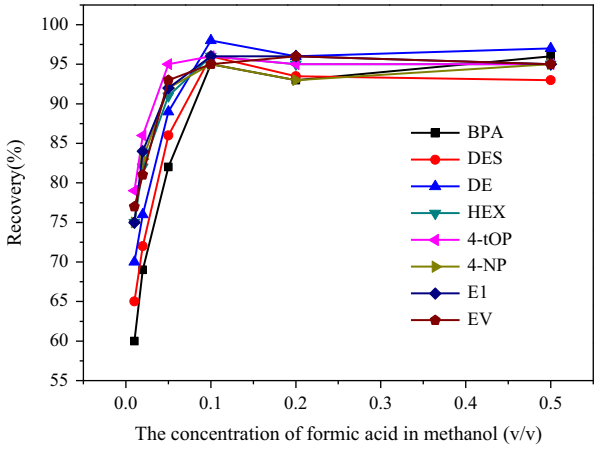

Fig. 5. Effect of the concentration of formic acid in methanol on the recoveries of eight phenolic environmental estrogens.

3.3.3. *The effect of the amount of TEPA-MP on the cleanup properties*

According to the discussion above, the TEPA-MP was very effective to remove various matrix, i.e., phospholipids and other substances. When designing the TEPA-MP d-μ-SPE cleanup optimization experiments, it is the primary consideration to employ a suitable amount of the TEPA-MP without affecting the analytes recoveries. For this purpose, the effectiveness of various amounts of adsorbents on cleanup efficiency and analytes recoveries were studied with the blood sample spiked with each of the eight phenolic environmental estrogens at 10.0 μg L^{-1}. The spiked samples were purified by using different amounts of the TEPA-MP with constant amounts of the rest of adsorbents. The results are shown in Fig. 6. The dispersive TEPA-MP adsorbents had an impact on recoveries of the studied phenolic environmental estrogens, and it was easy to discern a clear trend in the recovery when increasing the amount of adsorbents from 5 mg to 60 mg. When using TEPA-MP adsorbents of 5 mg, the high recoveries of the analytes were in the range of 110.3–133.0%, which resulted from the matrix interfering. With the increasing of the amount of TEPA-MP adsorbents from 10 mg to 30 mg, the satisfactory recoveries of the analytes were consistent in the range of 83.0–116.8%. It could be seen that the least amounts of TEPA-MP adsorbents of 20 mg could be used to ensure the removal of the various matrix, i.e., phospholipids and other substances. However, quantification using amounts between 40 and 60 mg of TEPA-MP seemed to be inappropriate for satisfactory reproducibility of recoveries (43.9–83.0%). Based on the results of the experiments, it could be concluded that the combination of the least amounts of TEPA-MP adsorbents in the proportion of 10–20 mg per 100 μL of bullfrog blood sample would ensure efficient and robust cleanup while maintaining quantitative recovery of the target phenolic environmental estrogens.

3.4. *Stability, recycling and reproducibility of TEPA-MP*

The chemical stability is considered as a prior condition for the invariant magnetic property under wide pH condition for the recycling of TEPA-MP. In this paper, the chemical stability of TEPA-MP under acidic and alkaline conditions was tested. TEPA-MP was dispersed in different concentrations of hydrochloric acid (HCl) solution with pH values ranging from 0 to 3 for 12 h. The results indicate that Fe_3O_4 could not be dissolved out from the core of the particles and the TEPA-MP still maintained its original magnetic ability. TEPA-MP was also dispersed in sodium hydroxide (NaOH) solution with pH values from 11 to 14 for 12 h. The results indicated that the polymer was efficient to resist the high pH solution and there was no Fe_3O_4 leakage under alkaline conditions. This unique feature will broaden the application of TEPA-MP in various fields.

The results of recycling experiment showed that TEPA-MP could be reused at least six times without much sacrifice of the cleanup efficiency. Furthermore, the preparative reproducibility of the TEPA-MP used for the cleanup procedure was deeply evaluated. The recoveries of the eight phenolic environmental estrogens by the three batches of TEPA-MP are shown in Fig. 7. The results

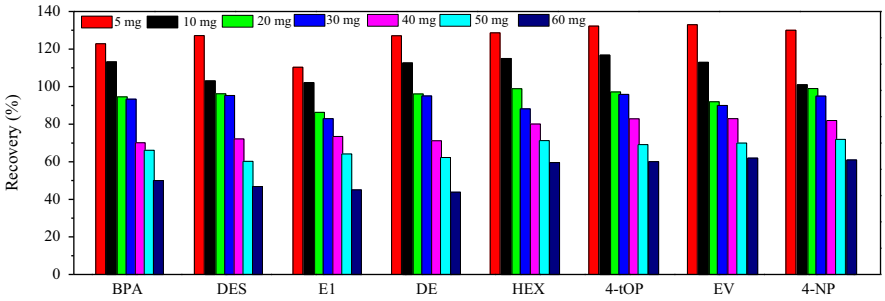

Fig. 6. Effect of the amount of TEPA-MP on the cleanup properties.

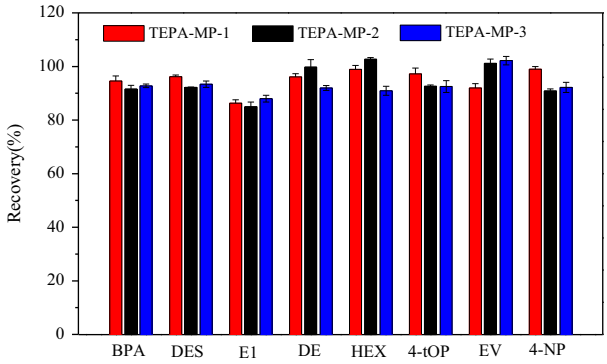

Fig. 7. Recoveries of eight phenolic environmental estrogens under the cleanup procedure *via* d-μ-SPE with three batches of TEPA-MP.

show that the absolute deviations of the recoveries for each phenolic environmental estrogen by the three batches are less than 5.0%. This indicates that the preparation procedure of TEPA-MP has good repeatability and reproducibility.

3.5. Matrix effect

Matrix effects, a phenomenon of ion suppression or enhancement of the analyte of interest, and the absolute matrix effect may be quantitatively assessed by comparing the response of the analyte spiked into extracted blank matrix with the response of the analyte spiked into matrix-free reconstitution solution [18]. Using this method, the matrix effect for the determination of phenolic environmental estrogens was evaluated, and the initial results showed that absolute matrix effects ranged from 128% to 201% at the tested concentrations (Table 2).

Plasma phospholipids have been identified as a major contributing source of matrix effects in LC–MS/MS based bioanalytical methods. Since attempts to clean up the interference components from phenolic environmental estrogens by protein precipitation were not successful, our attention turned toward methodology to eliminate the presence of these components during sample preparation. Thus TEPA-MP d-μ-SPE was evaluated for application to the phenolic environmental estrogens assay.

Our results showed that TEPA-MP d-μ-SPE procedure after protein precipitation dramatically reduced the level of phospholipids in plasma samples, and lead to a significant reduction in absolute matrix effects (Table 2). The recovery could be determined by comparing the response of the analyte spiked into matrix with the response of the analyte spiked into extracted blank matrix [18]. The recovery of phenolic environmental estrogens utilizing protein precipitation/TEPA-MP d-μ-SPE for sample preparation was near 100% (Table 2).

Table 2
Absolute matrix effect of phenolic environmental estrogens in protein precipitated bullfrog blood before and after TEPA-MP d-μ-SPE with the corresponding recovery.

Phenolic environmental estrogen	Added ($\mu g\ L^{-1}$)	Mean absolute matrix effect[a] Qbefore TEPA-MP d-μ-SPE (%, $n=6$)	Mean absolute matrix effect[a] after TEPA-MP d-μ-SPE (%, $n=6$)	Mean recovery[b] after TEPA-MP d-μ-SPE (%, $n=6$)
BPA	1.0	186	100.0	101.0
	20.0	138	92.1	97.6
	50.0	136	90.6	99.8
DES	1.0	190	96.1	95.6
	20.0	142	88.2	96.2
	50.0	139	89.9	96.8
D1	1.0	162	87.3	90.2
	20.0	129	83.2	89.6
	50.0	128	85.6	90.8
DE	1.0	192	102.0	93.6
	20.0	152	93.2	93.9
	50.0	142	92.9	94.1
HEX	1.0	180	98.7	97.2
	20.0	132	90.6	98.1
	50.0	139	91.3	92.9
4-tOP	1.0	196	105.0	98.2
	20.0	145	95.2	92.8
	50.0	139	96.0	100.0
EV	1.0	201	102.0	88.6
	20.0	152	95.9	89.2
	50.0	149	96.2	90.6
4-NP	1.0	198	103.0	101.0
	20.0	149	94.0	99.6
	50.0	136	92.8	100

[a] Expressed as (the mean peak area of analyte spiked after extraction/the mean peak area of the neat analyte standard) × 100%.
[b] Expressed as (the mean peak area of analyte spiked before extraction/the mean peak area of analyte spiked after extraction) × 100%.

3.6. Comparison of cleanup procedure via d-μ-SPE with three kinds of adsorbents

An overview of the three procedures for analysis of phenolic environmental estrogens is shown in Fig. 8. The aim of the present study was to investigate the cleanup procedure *via* three different approaches, kept up the other variables unchanged. Bullfrog blood sample spiked with eight phenolic environmental estrogens at a concentration of 10.0 $\mu g\ L^{-1}$ was used to compare the cleanup procedures, as shown in Fig. 9. It can be seen that the analytes are prone to strong matrix enhancement interactions in samples, which could not be avoided well by using *Approach I*, since the recoveries of all the analytes were obviously low, ranged from 107.8% to 133.7%. With the use of *Approach II* and *Approach III*, the

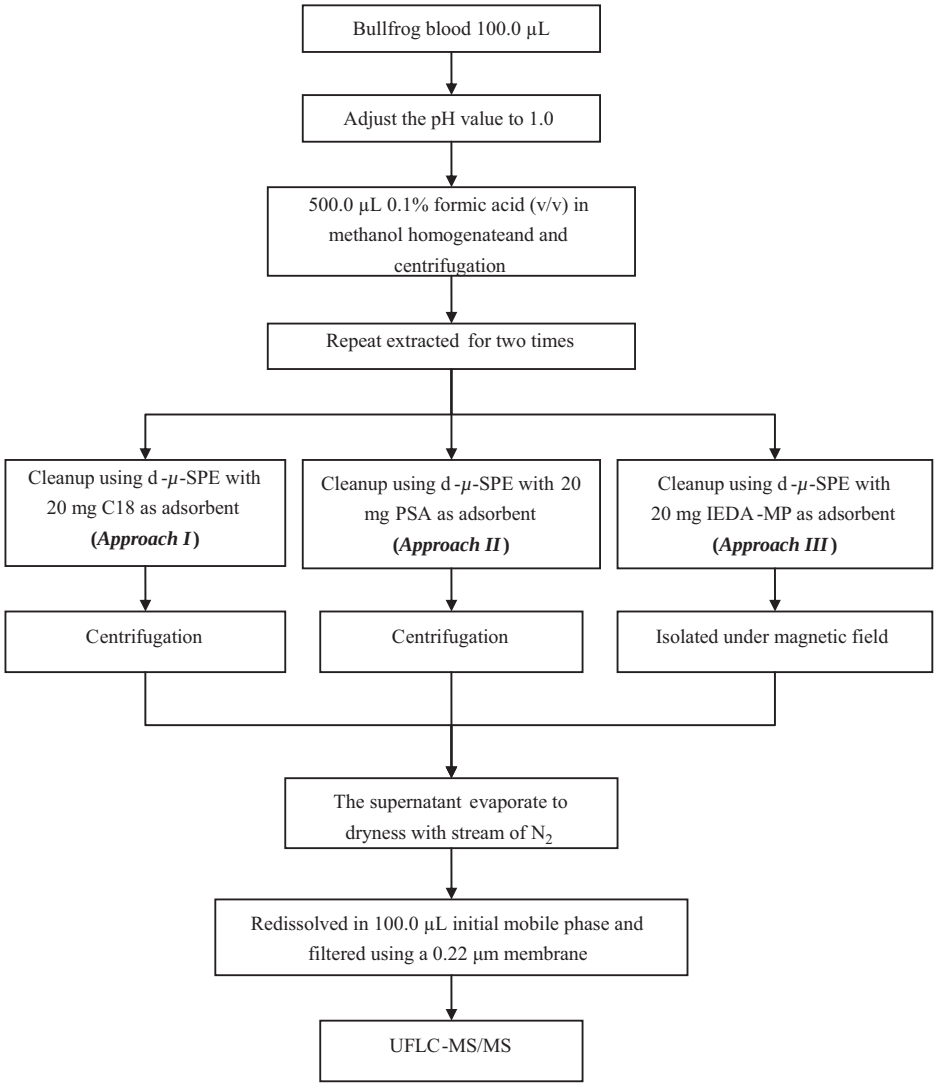

Fig. 8. Overview of the procedure for sample cleanup procedure for determination of eight phenolic environmental estrogens.

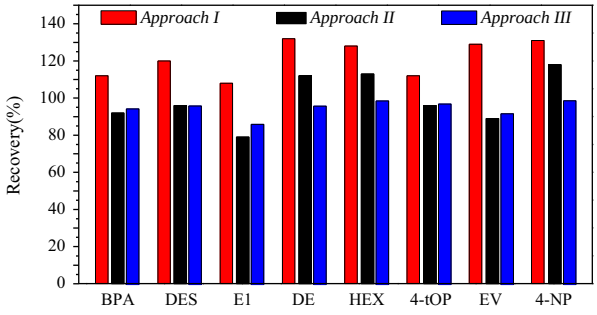

Fig. 9. Recoveries of eight phenolic environmental estrogens under three cleanup procedures *via* d-μ-SPE with C18, PSA and TEPA-MP.

recoveries of all the analytes were ranged from 79.6% to 118.2% and 86.3% to 99.2%. This reveals that the cleanup property of TEPA-MP is comparable with that of PSA materials. In addition, the advantages of using the TEPA-MP d-μ-SPE procedure were obvious, namely a short sample preparation time and an easy cleanup procedure for the removal of various matrix in bullfrog

Table 3
Linear equations, correlation coefficients (r), linear anges, LODs and LOQs of eight phenolic environmental estrogens.

Phenolic environmental estrogen	Linear equation[a]	r	Linear range ($\mu g\ L^{-1}$)	LOD ($\mu g\ L^{-1}$)	LOQ ($\mu g\ L^{-1}$)
BPA	$A=6.96\times10^4$ $C+8.90\times10^3$	0.9996	0.5～100.0	0.090	0.31
DES	$A=1.16\times10^5$ $C-4.92\times10^4$	0.9996	0.5～100.0	0.082	0.27
E1	$A=1.02\times10^5$ $C-6.80\times10^4$	0.9999	0.5～100.0	0.036	0.12
DE	$A=2.69\times10^5$ $C-1.01\times10^4$	1.0000	0.5～100.0	0.022	0.075
HEX	$A=7.12\times10^4$ $C-1.43\times10^3$	0.9996	0.5～100.0	0.083	0.28
4-tOP	$A=5.61\times10^4$ $C+1.95\times10^3$	0.9996	0.5～100.0	0.12	0.40
EV	$A=8.64\times10^4$ $C-1.35\times10^3$	0.9992	0.5～100.0	0.14	0.42
4-NP	$A=1.09\times10^5$ $C-4.25\times10^4$	0.9991	0.5～100.0	0.075	0.25

[a] A: peak area; C: mass concentration.

Table 4
Precision and accuracy of eight phenolic environmental estrogens ($n=6$, $\bar{x} \pm s$).

Phenolic environmental estrogen	Added ($\mu g\ L^{-1}$)	Found ($\mu g\ L^{-1}$)		Accuracy (%)		Precision (RSD, %)	
		Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
BPA	1.0	0.98 ± 0.05	0.99 ± 0.02	98.3	99.3	4.9	2.1
	20.0	19.03 ± 0.91	19.57 ± 0.45	95.2	97.8	4.8	2.3
	50.0	52.50 ± 2.10	49.58 ± 0.91	105.0	99.2	4.0	1.8
DES	1.0	0.96 ± 0.02	0.97 ± 0.02	96.3	97.0	1.8	2.1
	20.0	18.80 ± 0.54	18.53 ± 0.46	94.0	92.7	2.9	2.5
	50.0	48.17 ± 1.60	48.91 ± 0.29	96.3	97.8	3.3	0.6
E1	1.0	0.85 ± 0.02	0.86 ± 0.02	85.0	86.5	2.9	2.2
	20.0	17.63 ± 0.32	17.53 ± 0.26	88.2	87.7	1.8	1.5
	50.0	44.17 ± 0.53	43.92 ± 0.65	88.3	87.8	1.2	1.5
DE	1.0	0.92 ± 0.03	0.96 ± 0.05	91.7	95.8	3.4	5.2
	20.0	18.53 ± 0.69	18.70 ± 0.72	92.7	93.5	3.7	3.8
	50.0	47.08 ± 1.01	47.08 ± 1.00	94.2	94.2	2.1	2.1
HEX	1.0	0.93 ± 0.01	0.95 ± 0.02	93.0	95.0	1.5	2.1
	20.0	18.80 ± 0.17	19.03 ± 0.33	94.0	95.2	0.9	1.7
	50.0	46.92 ± 0.46	48.09 ± 0.55	93.8	96.2	1.0	1.1
4-tOP	1.0	0.93 ± 0.02	1.00 ± 0.02	92.7	100.0	1.8	2.0
	20.0	18.67 ± 0.19	18.97 ± 0.37	93.3	94.8	1.0	1.9
	50.0	47.25 ± 0.78	51.30 ± 1.94	94.5	102.7	1.6	3.8
EV	1.0	0.86 ± 0.03	0.86 ± 0.02	86.2	85.5	3.3	3.0
	20.0	17.07 ± 0.52	17.40 ± 0.22	85.3	87.0	3.1	1.3
	50.0	44.58 ± 0.52	44.08 ± 1.09	89.2	88.2	1.2	2.5
4-NP	1.0	0.96 ± 0.03	1.04 ± 0.05	95.8	104.2	3.5	4.8
	20.0	19.33 ± 0.66	19.57 ± 0.23	96.7	97.8	3.4	1.2
	50.0	46.67 ± 1.71	50.12 ± 1.02	93.3	100.2	3.7	2.0

Table 5
Comparison of the analytical features of current LC-MS-based methodologies for the determination of phenolic environmental estrogens in food and blood samples.

Sample	Phenolic environmental estrogens	Sample preparation (main steps)	LC column	LC parameters	Detector parameters	Linear range (LR), LOD or LOQ, recoveries (R)	Reference
Cereals	4-n-nonylphenol 4-tert-butylphenol 2,4-dichlorophenol 2,4,5-trichlorophenol pentachlorophenol 4-tert-butylbenzoic acid BPA	PLE–SPE	XTerra MS C18 100 mm × 2.1 mm, 3.5 μm	Mobile phase: 0.0025 M ammonium formate buffer (pH 3.1, adjusted with formic acid)/methanol with gradient elution, 0.2 mL min^{-1}, Inj: 50 μL	ESI(-)–MS: capillary voltage: 3.0 kV, cone voltage: 40 V, source temperature: 120 °C, desolvation temperature: 250 °C,	LR: 0.08–0.8 mg kg^{-1}; LOD: 3–43 μg kg^{-1}; R: 81–104%	[35]
Meat	Octylphenol Nonylphenol	PLE–SPE	Symmetry C18 150 mm × 2.1 mm, 3.5 μm	Mobile phase: water (0.1% ammonia)/methanol with gradient elution, 0.2 mL min^{-1}, Inj: 10 μL	ESI(-)–MS/MS: capillary voltage: 3.5 kV, cone voltage: 70 V, source temperature: 100°C	LR: 1–500 μg L^{-1}; LOQ: 0.2–1.0 μg kg^{-1}; R: 92–97%	[2]
Milk	BPA	SPE	LiChrospher100 RP-18 250 mm × 4 mm, 5 μm	Mobile phase: water/ methanol (30:70, v/v), 0.9 mL min^{-1}, Inj: 10 μL	ESI(-)–MS: capillary voltage: 3.5 kV, cone voltage: 70 V, source temperature: 500 °C	LR: 5–700 μg L^{-1}; LOQ: 2.2 μg · L^{-1}; R: 52% (absolute recovery), 101% (relative recovery)	[36]
Dry milk powder	BPA	SPE	Eclipse DB-C8 150 mm × 4.6 mm, 5 μm	Mobile phase: water (0.025% ammonia)/methanol (0.025% ammonia) with gradient elution 0.5 mL min^{-1}, Inj: 25 μL	ESI(-)–MS/MS: capillary voltage: 4.0 kV, cell accelerator voltage: 7 V, source temperature: 350 °C	LR: 100–1000 μg L^{-1}; LOQ: 8.8 μg kg^{-1}; R: 95-99%	[37]
Fish	Estrone 17β-estradiol estriol 17α-ethynylestradiol BPA	PLE	HSS T3 100 mm × 2.1 mm, 1.7 μm	Mobile phase: water /acetonitrile–methanol (1:2, v/v) with gradient elution, 0.4 mL min^{-1}, Inj: 4 μL	ESI(-)–MS/MS: capillary voltage: 2.8 kV, source temperature: 500 °C	LR: 1–500 μg L^{-1}; LOQ: 0.07–0.27 μg kg^{-1}; R: 71.2-108%	[38]
Meat	Estrone 17β-estradiol estriol 17α-ethynylestradiol BPA Octylphenol Nonylphenol *trans* diethylstilbestrol *cis* diethylstilbestrol	SPE	SB-C18 50 mm × 2.1 mm, 1.8 μm	Mobile phase: water (0.1% formic acid, 0.5% acetonitrile)/acetonitrile (0.1% formic acid) with gradient elution, 0.3 mL min^{-1}, Inj: 2 μL	ESI(+)–MS/MS: capillary voltage: 4 kV,source temperature: 300 °C	LR: 0.5–200 μg L^{-1}; LOQ: 0.15–0.50 μg kg^{-1}; R: 75.3–92.5%	[39]

Table 5 (*continued*)

Sample	Phenolic environmental estrogens	Sample preparation (main steps)	LC column	LC parameters	Detector parameters	Linear range (LR), LOD or LOQ, recoveries (R)	Reference
Blood	Diethylstilbestrol dienestrol hexestrol 4-(tert-octyl)-phenol 4-nonylphenol estrone estradiol valerate BPA	d-μ-SPE	Shim-pack R-ODSII 100 mm × 2.0 mm, 2.2 μm	Mobile phase: water (0.02% ammonia)/methanol (0.02% ammonia) with gradient elution, 0.45 mL min^{-1}, Inj: 5 μL	ESI(-)–MS/MS: capillary voltage: 4.5 kV, cone voltage: 10 V, source temperature: 500 °C	LR: 0.5–100 μg L^{-1}; LOQ: 0.075–0.42 μg L^{-1}; R: 85–105%	This work

Abbreviations: PLE, pressurized liquid extraction; SPE, solid-phase extraction; d-μ-SPE, dispersive micro-solid-phase extraction Inj., injection volume, ESI, electrospray ionization.

blood because of the magnetic separation instead of centrifugation in the PSA d-μ-SPE procedure.

3.7. Method linear range, precision, accuracy, LOD and LOQ

The linearity of the calibration curves made by peak area (y) vs. concentration (x, μg L^{-1}) was studied using calibration standards in solvent at seven concentrations of 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0 μg L^{-1}. The response function was found to be linear with correlation coefficients (r) higher than 0.999 in the tested range listed in Table 3 for eight phenolic environmental estrogens.

Precision and accuracy were assessed based on the analysis of eight phenolic environmental estrogens spiked at concentrations of 1.0, 20.0 and 50.0 μg L^{-1} in blank bullfrog blood. The results are summarized in Table 4. It shows that the majority of mean recoveries are in the range of 85.0–105.0% at the three spiking levels with associated intra-day relative standard deviations (RSDs) ranging from 0.9 to 4.9% and inter-day relative standard deviations (RSDs) ranging from 0.6 to 5.2%.

The LODs and LOQs values for the analyzed phenolic environmental estrogens are shown in Table 3. The LODs and LOQs, which were defined as the lowest analyte concentration that yielded a signal-to-noise (S/N) ratio of 3 and 10 in blank extracts were in the range of 0.022–0.14 μg L^{-1} and 0.075–0.42 μg L^{-1}, respectively. Compared with the LC–MS methods in literatures for the determination of phenolic environmental estrogens in various samples, as shown in Table 5, the developed method had lower LOQs, simpler pretreatment, and faster run.

3.8. Sample analysis

Three batches of bullfrog (ten samples for each batch) were analyzed by the developed method. Each batch of samples was processed together with a matrix blank (phenolic environmental estrogens-free sample), which was confirmed by using UFLC–MS/MS method. The matrix blank eliminated the false positive as result of contamination in the extraction process, instrument or chemicals. A blank extract spiked at the calibration level (10.0 μg L^{-1}) was used to control the extraction efficiency. The results showed the presence of BPA and 4-tOP in two of the thirty collected samples with concentrations of 55.29 and 2.86 μg L^{-1}, respectively, and other six phenolic environmental estrogens were not detected because of lower than the LOQs in the analyzed samples. The typical UFLC–MS/MS chromatogram is shown in Fig. 10.

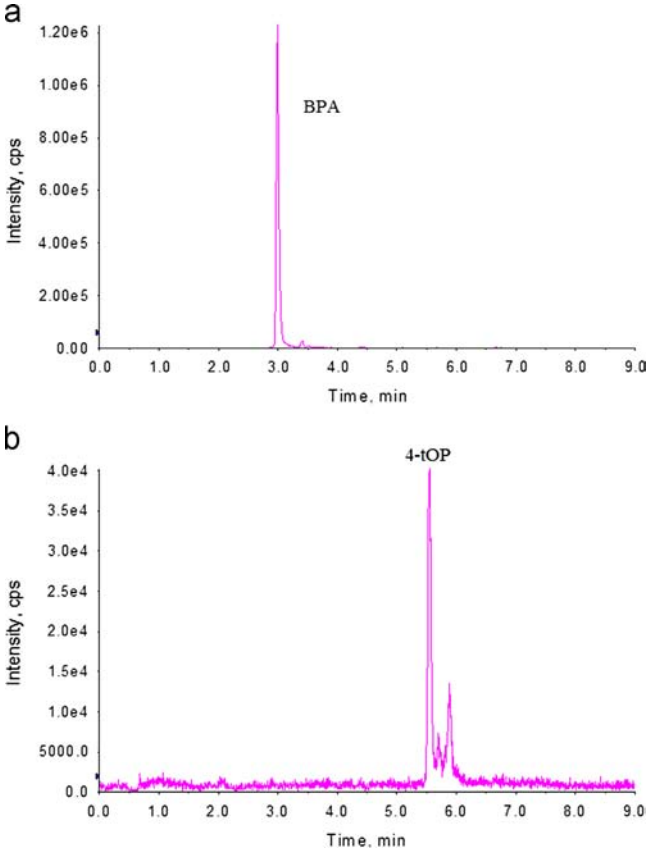

Fig. 10. Extract ion chromatogram (XIC) for two examined samples.

4. Conclusions

In this work, a novel and efficient amino-functionalized Fe_3O_4 magnetic polymer (MP) using tetraethylenepentamine (TEPA) as a coupling agent with stronger magnetism was synthesized. The d-μ-SPE extraction procedure using TEPA-MP with rapid magnetic separation was optimized for the phenolic environmental estrogens, and the matrix effects caused by phospholipids were effectively eliminated by the TEPA-MP adsorbent. Acceptable recoveries for the studied phenolic environmental estrogens were obtained in the range of 85.0–105.0%. The results demonstrate that the accuracy and precision of the proposed TEPA-MP d-μ-SPE coupled with UFLC–MS/MS method are satisfactory for analysis of the phenolic environmental estrogens in blood samples.

Acknowledgments

We would like to thank the Agriculture and Social Development Funds of Ningbo, China (No. 2011C50058), the Zhejiang Provincial Analytical Foundation of China (No. 2012C37002), the Advanced Key Program of Agriculture and Social Development Funds of Ningbo, China (No. 2011C11021), the Medical Health Foundation for Key Talents in Zhejiang Province, China (No. 2011RCB033), the Medical Science and Technology Funds of Ningbo (No. 2011A07), the Zhejiang Provincial Natural Science Foundation (No. LY12H26003) and Zhejiang Provincial Program for the Cultivation of High-level Innovative Health Talents.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.05.053.

References

[1] H. Chang, Y. Wan, J. Naile, X. Zhang, S. Wiseman, M. Hecker, M.H.W. Lam, J. P. Giesy, P.D. Jones, J. Chromatogr. A 1217 (2010) 506.
[2] B. Shao, H. Han, D. Li, Y. Ma, X. Tu, Y. Wu, Food Chem. 105 (2007) 1236.
[3] L.C. Folmar, M.J. Hemmer, N.D. Denslow, K. Kroll, J. Chen, A. Cheek, H. Richman, H. Meredith, E.Gordon Grau, Aquat. Toxicol. 60 (2002) 101.
[4] M. Cargouët, D. Perdiz, A. Mouatassim-Souali, S. Tamisier-Karolak, Y. Levi, Sci. Total Environ. 324 (2004) 55.
[5] S. Pawlowski, T.A. Ternes, M. Bonerz, A.C. Rastall, L. Erdinger, T. Braunbeck, Toxicol. In Vitro 18 (2004) 129.
[6] A.S. Al-Hiyasat, H. Darmani, A.M. Elbetieha, Eur. J. Oral Sci. 112 (2004) 267.
[7] A. Can, O. Semiz, O. Cinar, Mol. Hum. Reprod. 11 (2005) 389.
[8] T. Funabashi, T.J. Nakamura, F. Kimura, J. Neuroendocrinol. 16 (2004) 99.
[9] M. Iwata, Y. Eshima, H. Kagechika, H Miyaura, Immunol. Lett. 94 (2004) 135.
[10] C. Nerin, C. Fernandex, C. Domeno, J. Salafranca, J. Agric. Food Chem. 51 (2003) 5647.
[11] H. Gallart-Ayala, E. Moyano, M.T. Galceran, Rapid Commun. Mass Spectrom. 21 (2007) 4039.
[12] J. Sajiki, Y. Hasegawa, H. Hashimoto, Y. Makabe, F. Miyamoto, R. Yanagibori, Toxicol. Mech. Method 18 (2008) 733.
[13] M.J. Lopez de Alda, D. Barcelo, J. Chromatogr. A 892 (2000) 391.
[14] G. Ferretti, C. Ferranti, T. Crovella, M. Fiori, C. Civitareale, C. Marchiafava, F. Delli Quadri, P. Cammarata, L. Palleschi, J. Chromatogr. B 871 (2008) 135.
[15] B.D. Stanford, H.S. Weinberg, J. Chromatogr. A 1176 (2007) 26.
[16] N. Casajuana, S. Lacorte, J. Agric. Food Chem. 52 (2004) 3702–3707.
[17] H. Chang, Y. Wan, J. Naile, X. Zhang, S. Wiseman, M. Hecker, M.H.W. Lam, J.P. Giesy, P.D. Jones, J. Chromatogr. A 1217 (2010) 506.
[18] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[19] S.T. Wu, D. Schoener, M. Jemal, Rapid Commun. Mass Spectrom. 22 (2008) 2873.
[20] M. Ahnoff, A. Murzer, B. Lindmark, R. Jussila, in: Proceedings of the 51st ASMS Conference on Mass Spectrometry and Allied Topics, Montreal, Canada, 2003.
[21] P.K. Bennett, K.C. Van Horne, AAPS (American Association of Pharmaceutical Scientists) Annual Meeting and Exposition, Salt Lake City, UT, 2003.
[22] K.C. Van Horne, P.K. Bennett, AAPS (American Association of Pharmaceutical Scientists) Annual Meeting and Exposition, Salt Lake City, UT, 2003.
[23] L.Q. Pang, Q.L. Liang, Y.M. Wang, L. Ping, G.A. Luo, J. Chromatogr. B 869 (2008) 118.
[24] W. Zeng, Y. Xu, M. Constanzer, E.J. Woolf, J. Chromatogr. B 878 (2010) 1817.
[25] Y.G. Zhao, H.Y. Shen, J.W. Shi, X.H. Chen, M.C. Jin, J. Chromatogr. A 1218 (2011) 5568.
[26] R.H. Savant, K. Banerjee, S.C. Utture, S.H. Patil, S. Dasgupta, M.S. Ghaste, P. G. Adsule, J. Agric. Food Chem. 58 (2010) 1447.
[27] J.W. Wong, K. Zhang, K. Tech, D.G. Hayward, C.M. Makovi, A.J. Krynitsky, F. J. Schenck, K. Banerjee, S. Dasgupta, D. Brown, J. Agric. Food Chem. 58 (2010) 5868.
[28] S. Walorczyk, B. Gnusowski, J. Chromatogr. A 1216 (2009) 6522.
[29] A. Rashid, S. Nawaz, H. Barker, I. Ahmad, M. Ashraf, J. Chromatogr. A 1217 (2010) 2933.
[30] S.J. Lehotay, K.A. Son, H. Kwon, U. Koesukwiwat, W.S. Fu, K. Mastovska, E. Hoh, N. Leepipatpiboon, J. Chromatogr. A 1217 (2010) 2548.
[31] C. Basheer, A.A. Alnedhary, B.S. Madhava Rao, S. Valliyaveettil, H.K. Lee, Anal. Chem. 78 (2006) 2853.
[32] J.M. Jiménez-Soto, S. Cárdenas, M. Valcárcel, J. Chromatogr. A 1245 (2012) 17.
[33] X.H. Chen, Y.G. Zhao, H.Y. Shen, M.C. Jin, J. Chromatogr. A 1263 (2012) 34.
[34] Y.G. Zhao, H.Y. Shen, S.D. Pan, M.Q. Hu, J. Hazard. Mater. 182 (2010) 295.
[35] R. Carabias-Martínez, E. Rodriguez-Gonzalo, P. Revilla-Ruiz, J. Chromatogr. A 1137 (2006) 207.
[36] N.C. Maragou, E.N. Lampi, N.S. Thomaidis, M.A. Koupparis, J. Chromatogr. A 1129 (2006) 165.
[37] A. Khedr, J. Chromatogr. B http://dx.doi.org/http://dx.doi.org/10.1016/j.jchromb.2013.04.040.
[38] A. Ye, Y. Yang, J. Zhang, M. Liu, L. Hou, J.L. Zhou, J. Chromatogr. A 1278 (2013) 126.
[39] S. Zhang, J. You, S. Ning, C. Song, Y.R. Suo, J. Chromatogr. A 1280 (2013) 84.