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Novel PEP-PAN@PSF rods extraction of EDCs in environmental water, sediment, and fish homogenate followed by pre-column derivatization and UHPLC-MS/MS detection

Sabour Issofa Bonfoh[1], Dan Li[1], Xiang Xiong, Zhifeng Du, Chaomei Xiong*, Hongliang Jiang

Department of Pharmaceutical Analysis, School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, PR China

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ABSTRACT

In the study, novel polysulfone hollow fiber membrane (PSF) supported polar enhanced phase (PEP) particles-polyacrylonitrile (PAN) polymer thin-film rods (PEP-PAN@PSF rods) were developed for the extraction of five steroidal endocrine disrupting chemicals (EDCs) (estrone (E1), estradiol (E2), estriol (E3), ethinyloestradiol (EE2), 2-methoxyestradiol (2ME2)) and four phenolic EDCs (bisphenol A (BPA), hexestrol (HEX), diethylstilbestrol (DES), dienestrol (DE)) in environmental water, sediment, and fish muscle homogenates, followed by pre-column derivatization and ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). The traditional preparation method for thin-film rods was improved by introducing a supporting membrane PSF between the external thin-film coating and the internal bare rod, avoiding the conventional pre-corrosion by strong acid/base. The commercial PEP prepared rods showed competitive capacity for both polar and nonpolar EDCs. In addition, pre-column derivatization with dansyl chloride (DNS-Cl) was adopted for the phenolic analytes prior to UHPLC-MS/MS detection, leading to a significant enhancement of sensitivity via analyzing the dansylated derivatives under positive electrospray ionization (ESI) mode instead of the analytes under negative ESI mode. The protocol was validated in four matrices including environmental water, sediment and two fish species. No matrix effects were observed in four matrices. The limits of detection (LODs) for the analytes were in the range of 0.002–0.072 μg L^{-1} for environmental water, 0.032–0.734 ng g^{-1} for sediment, and 0.011–0.435 ng g^{-1} for two fish species, respectively. Appropriate linearity was observed for all the analytes with correlation coefficients (R^2) above 0.997. The intra-day trueness of the approach at low, medium and high levels was in the range of 86.6–116.1% with relative standard deviations (RSDs) lower than 15.4%. And the inter-day trueness was in the range of 84.6–114.8% with RSDs lower than 16.3%. The proposed method was successfully applied for the analysis of nine EDCs in environmental water, sediment, and fish muscle homogenates.

1. Introduction

Endocrine disrupting chemicals (EDCs), also named environmental estrogens and environmental hormones, are exogenous compounds that interfere with the normal physiological behaviors of natural hormones in human body, such as their synthesis, secretion, transport, metabolism, binding action or elimination [1,2]. As emerging unregulated contaminants, EDCs are associated with the altered reproductive function in males and females, increasing the risk of breast cancer, abnormal growth patterns and neurodevelopment delays in children as well as changes in immune functions [3–5]. As chemically stable and fat-soluble compounds, EDCs have a long half-life and can be accumulated in organisms and human bodies through the simple food chain [4–8]. To date, EDCs, including agricultural chemicals, bisphenols and alkylphenols, phthalates, metallic compounds, steroids, and others, have been widely found in various environment (surface water, effluent, sediment, sewage sludge, etc.) and food samples (milk, chicken, fish, etc.) [9–19]. Revealing the exposure levels of EDCs in environment and food samples has become an issue of global concern [20–24].

To analyze EDCs in complex matrix samples, such as sediment, plasma, or tissue, besides the common extraction processing for sample pretreatment, an additional clean-up is highly recommended prior to

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* Corresponding author. Department of Pharmaceutical Analysis, School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, No. 13 Hangkong Road, Wuhan, 430030, Hubei Province, PR China.

E-mail address: cmxiong2002@hust.edu.cn (C. Xiong).

[1] Authors have the same contribution to this manuscript.

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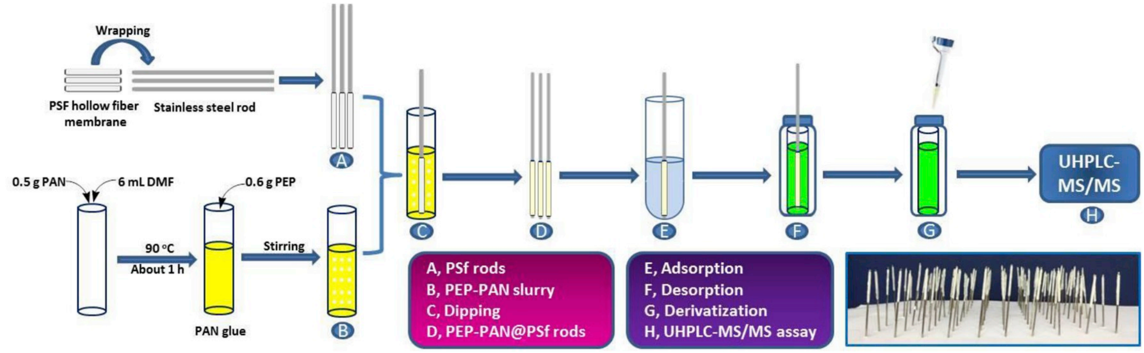

Fig. 1. Diagram of the proposed PEP-PAN@PSf rods extraction of EDCs followed by pre-column derivatization coupled with UHPLC-MS/MS detection.

the final instrumental analysis, which can significantly decrease the possible interference from the complex extract in the following UV or MS detection system [25–30]. To our knowledge, solid phase extraction (SPE) was the most widely applied technique in recent years [4,7,10,12–16,30–34]. In addition, seeking green alternative with low-solvent even non-solvent consumption during extraction has become a hotspot of research in recent years. For instance, liquid-liquid micro-extraction (LLME) [35–38] and solid-phase microextration (SPME) [39,40] were proposed for the extraction and clean-up of EDCs, leading to a significant minimization of organic solvent, and a high potential for small size sample treatment. As a branch of SPME, thin-film micro-extraction (TFME) has received much attention since it was proposed in 2001 [41–43]. By contrast, TFME can remarkably improve the sensitivity of method due to the relatively high surface area-to-volume ratio of the thin-film. It allows a time-saving sample treatment with fast kinetic equilibrium and high extraction efficiency [41–43]. There are many extraction modes based on different types of TFME. One of them typically consists of two parts, an external polymeric thin-film as the extraction phase and an internal support, such as stainless-steel rod/mesh, or blade-shaped framework [43]. With the aid of internal supports, the extraction phase of thin-films can be easily transferred by handle, while the thin-films without supports have to be moved by a tweezers, increasing the risk of damage and contamination of thin-films [43].

In general, in order to stick thin-films on supporting frameworks tightly, a preliminary treatment of the supporting frameworks is basically required for obtaining a rough surface. For instance, a pre-corrosion processing by concentrated acid was subjected to stainless-steel rods or meshes before the construction of external thin-films. To get high and stable extraction efficiency of thin-films, commercial sorbents were widely used for the preparation of external thin-films. In brief, sticky glue with the well-dispersed commercial sorbents was spayed or dipped on the rough surface of the supporting frameworks with uniform thickness. After drying, polymeric thin-films embedded with sorbent particles were tightly stuck on the surfaces of supports [41–43]. Among the reported methods, polyacrylonitrile (PAN) was widely used as the binder for the preparation of thin-films due to its good biocompatibility [42]. The commercial ostadecylsilane (ODS) was the most frequently used sorbent in the development of thin-films due to its unassailable adsorption capacity for nonpolar, neutral, even some polar compounds. The commercial Oasis hydrophilic-lipophilic balance (Oasis HLB) sorbent also manifested high potential in application due to its good dual-interactions with nonpolar and polar compounds [42]. Similar with Oasis HLB, the commercial polar enhanced phase (PEP) particles is a mixed-mode polymeric sorbent synthesized by hydrophobic poly (styrene-divinylbenzene) (PS-DVB) polymer and surface-modified by urea functional groups, allowing wide adsorption for both nonpolar and polar compounds [44].

In our previous works, PEP-PAN thin-films were prepared without any supports and applied for the analysis of three estrogens in aqueous tea extract and environmental water samples by HPLC-UV assay [44]. In that case, the thin-films had to be transferred by a tweezers. To simplify the operation for convenience, magnetic ODS-PAN thin-films were then prepared for the microextraction of quetiapine and clozapine in plasma and urine samples [45]. In this case, PEP-PAN thin-films were presently stuck on the stainless-steel rods. It made the transfer of thin-films much easier via directly holding the bare end of rods without coating. Furthermore, an additional hollow membrane was introduced between the bare rod and the thin-film as supporting material. The hollow membrane serves as two roles in this case. On the one hand, it can be tightly stuck on the surface of the stainless-steel rods directly as melting, avoiding any pre-corrosion of stainless-steel rods. On the other hand, the external thin-film can be tightly immobilized on the surface of the membrane because of the porous structure of the membrane. The prepared rods were used for the enrichment and clean-up of nine natural and synthetic estrogenic EDCs in environmental water, sediment and fish muscle homogenate. The influencing factors on extraction were investigated systematically. To further improve the sensitivity of the target compounds in MS, pre-column derivatization was introduced prior to UHPLC-MS/MS [26,27], by converting less sensitive negative ESI mode for the compounds to more sensitive positive ESI mode for the dansylated derivatives. Under the optimal conditions, the proposed PEP-PAN@PSF rods coupled with pre-column derivatization and UHPLC-MS/MS was validated and applied for the determination of the analytes of interest in environmental water, sediment and fish muscle homogenate samples.

The diagram of the proposed PEP-PAN@PSF rods extraction of EDCs followed by pre-column derivatization coupled with UHPLC-MS/MS detection is shown in Fig. 1.

2. Materials and methods

2.1. Chemicals and materials

Estrone (E1), 17β-estradiol (E2), estriol (E3) and diethylstilbestrol (DES) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Ethinyloestradiol (EE2), hexestrol (HEX), dienestrol (DE), 2-methoxyestradiol (2ME2) and dansyl chloride (DNS-Cl) were purchased from TCI Shanghai, China. Bisphenol A (BPA) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The isotopically labeled compounds estrone-d4 (d4E1), estradiol-d4 (d4E2), estriol-d3 (d3E3), ethinyloestradiol-d4 (d4EE2), diethylstilbestrol-d8 (d8DES) and bisphenol A-d16 (d16BPA) were purchased from Cambridge Isotope Laboratories (USA). The properties of analytes indicated in SciFinder and the chemical structures of six isotopically labeled compounds are shown in Table S1 and Fig. S1, respectively. The PEP particles (40–60 μm) were purchased from Agela Technologies (Tianjin, China). The ODS particles (50 μm) were obtained from H&E Co., Ltd. (Beijing, China). The polyacrylonitrile (PAN) polymer was bought from Sigma-Aldrich (MO, USA). The polysulfone hollow fiber membrane (PSF, 0.9 mm i.d.) was kindly obtained from School of Environmental and Chemical Engineering of Tianjin Polytechnic University (TJPU). Acetonitrile (ACN) and methanol (MeOH) were of HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ, USA). *N,N*-dimethylformamide (DMF), formic acid (FA), ammonium formate (NH_4FA), acetone, triethylamine (TEA) and all the salts were of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Stock standard solutions of the analytes and the internal standards (1.0 g L^{-1}) were prepared individually in pure methanol and stored at 4 °C. Working solutions were prepared daily by diluting the stock solutions with deionized water. The mixed internal standard solution containing 75 μg L^{-1} of estrone-d4 and estradiol-d4, 225 μg L^{-1} of estriol-d3 and diethylstilbestrol-d8, 150 μg L^{-1} of ethinyloestradiol-d4 and 500 μg L^{-1} of bisphenol A-d16 was prepared daily by diluting the stock internal standard solutions with methanol.

2.2. *Instrumentation*

A Navo Nano SEM 450 scanning electron microscopy (FEI, Holland) was used for the characterization of the surface and section of the thin-films. A Delta 320 pH meter (Mettler Toledo Instruments Co. Ltd., Shanghai, China) was adopted for the adjustment of pH. A HY-4 speed multi-function oscillator (Jintan Instruments Co., Ltd., Jiangsu, China) was applied to accelerate the equilibrium of extraction/desorption. A SC-06 low speed centrifuge (Anhui USTC Zonkia Scientific Instruments Co., Ltd., Anhui, China) was performed for the centrifugation of extracts. A KH3200B ultrasonic cleaner (40 kHz, 150 W, Kunshang Hechuang ultrasonic instruments Co. Ltd., China) was used for the extraction of samples.

A Hitachi HPLC-UV system (Hitachi High-Technological Corporation, Tokyo, Japan) was particularly used for the optimization of parameters of PEP-PAN@PSF rods as well as the sample extraction of sediment and fish homogenate, due to its low-cost and high accessible. The HPLC-UV system was equipped with a Model L-2130 pump, an auto-sampler with 20 μL sample loop and an L-2400 UV detector. Isocratic elution was carried out on a Welch Ultimate LP-C18 column (4.6 mm × 250 mm, 5.0 μm). Seven analytes were chosen for the optimizations. They were separated in 20 min with good shape and resolution by a modified mobile phase system of acetonitrile, methanol and water (43:12:45, v/v/v), without salt addition and pH control in comparison with the reported method by Chen et al. [36]. The detection wavelength was 230 nm. The flow rate was 1 mL min^{-1}. A typical chromatogram is depicted in Fig. S2.

A hyphenated UHPLC-MS/MS system (a Shimadzu UHPLC system (Kyoto, Japan) coupled with a 4000 QTRAP Linear Ion Trap Quadrupole mass spectrometer (MDS-AB SCIEX, Concord, Ontario, Canada)) was particularly used for the final determination of the analytes in various matrices to achieve higher specificity and sensitivity. The UHPLC system consists of a LC-30AD solvent delivery system, a SIL-30AC autosampler, a CTO-30A column oven, a DGU-20A3 degasser and a CBM-20A controller. A Welch Ultimate UHPLC XB-C18 column (50 mm × 2.1 mm, 1.8 μm) was conducted in gradient elution as follows: 70% B (0.01–0.80 min), 70%–90% B (0.80–1.30 min), 90%–98% B (1.30–4.30 min), 98% B (4.30–4.80 min), 98%–70% B (4.80–5.00 min), 70% B (5.00–5.50 min). Here, mobile phase A was deionized water containing 5 mM of ammonium formate, and mobile phase B was acetonitrile. The flow rate was 0.4 mL min^{-1}. The column temperature was 40 °C. The sample was kept at 4 °C and automatically analyzed by injecting 4 μL. To exclude the interference from salts, only the eluent between 0.80 min and 5.00 min was introduced into MS for analysis. The mass spectrometer was conducted for the analysis of analytes in positive ESI mode under multiple reactions monitoring (MRM) after the DNS-Cl derivatization. The MS/MS parameters are as follows: ion spray voltage, 5500 V; ESI temperature, 500 °C; curtain gas (CUR), 30 psi; nebulizer gas (GS 1), 40 psi, auxiliary gas (GS 2), 60 psi, collision cell exit potential (CXP), 4 V. Collision energy (CE) and decluttering potential (DP) were optimized for each compound. The individual MRM transitions are listed in Table S2.

2.3. *Preparation of PEP-PAN@PSF rods*

The common way for the preparation of thin-film rods is by spray or dipping, and spray is highly recommended by some references [41–43]. So in the experiment, the preparation methods of PEP-PAN@PSF rods by spray and dipping were compared. The dipping method is almost the same as the latter description but with 5 dipping times. The spray method is as below. The PEP-PAN slurry was transferred to a 100 mL bottle sprayer. Under the flow of nitrogen, the suspension was sprayed to form a thin-film on the PSF wrapped rods and dried in an oven at 90 °C. The two steps of spraying and drying were repeated for 25 times to obtain the thin-films with uniform and appropriate thickness. After that the extraction efficiencies of the rods were compared, and no significant difference (data not shown) was found between the rods prepared by spray and dipping. Considering the spray processing was much more complicated and led to the waste of slurry, the thin-film rods were prepared by dipping in this study. It was carried out by three steps as follows.

Step 1: Preparation of PSF wrapped rods (Fig. 1A). One meter of stainless steel rod (0.9 mm, diameter) was cut into short rods (7 cm each), and washed by methanol to remove contaminants. PSF hollow fiber membrane (0.9 mm i.d.) was cut into 2 cm pieces, and wrapped tightly around one side of the cleaned rod, but left 1–2 mm margin to the top, which can avoid possible damage of the thin-films during future use. The PSF wrapped rods were then washed by methanol and dried at 60 °C.

Step 2: Preparation of PEP-PAN slurry (Fig. 1B). The slurry was prepared according to Cai et al. and Li et al. with minor optimization [44,45]. The yellow clear PAN glue was prepared by dissolving 500 mg of PAN particles into 6 mL of *N,N*-dimethylformamide via heating at 90 °C for about 1 h. After cooling, 600 mg of PEP particles were added and mixed well by stirring.

Step 3: Preparation of PEP-PAN@PSF rods by dipping (Fig. 1C). The PSF wrapped rods were inserted in the PEP-PAN slurry for several seconds, and transferred to an oven at 90 °C immediately. The PSF membrane was melted and tightly adhered to the rods due to the synergic action of *N,N*-dimethylformamide and high temperature. After drying about 5 min, the dipping was repeated in three replicates totally. The obtained PEP-PAN@PSF rods were washed with methanol for three times, and then kept in methanol for future use.

2.4. *Sample collection*

Three environmental water samples were collected at a depth of 0.5 m by a stainless-steel bucket from the East Lake (Wuchang district, Wuhan, China), the North Lake (Jianghan district, Wuhan, China), and the Han River (Qiaokou district, Wuhan, China), respectively. The collected surface water samples were filtered through 0.45 μm PTFE filters and stored in cleaned amber glass bottles at 4 °C, respectively, before analysis.

Two sediment samples were collected from the East Lake (Wuchang district, Wuhan, China) and the North Lake (Jianghan district, Wuhan, China). They were dried at 90 °C and ground into powder. After sieving through a 180-mesh sieve (0.088 mm), the powder was collected carefully in a sealed amber glass bottle and stored at 4 °C.

Six living fish samples (three Snakeheads (*Ophiocephalus argus*), three Grass Carps (*Ctenopharyngodon idellus*)) were purchased from the local market and killed immediately by fishmonger. Only fish muscle pieces were collected and immediately transferred to the lab. The muscle pieces were carefully cleaned with deionized water. After drying the surface water, the muscle pieces were homogenized by a blender. The homogenate was divided into 15 mL centrifuge tubes, each contained 1 g of homogenates. The packed tubes were stored in −20 °C for future analysis. The experiment was approved by Tongji Medical College Ethical Committee of Huazhong University of Science and Technology.

All the above samples were collected in the winter, 2019. The brief pretreatments of fish and sediment are shown in Fig. S3.

2.5. Sediment pretreatment

The general extraction procedure for sediment samples is as follows. 0.4 g of sediment was added in a 15 mL centrifuge tube. It was spiked with 20 μL of the mixed internal standard solution, as for the spiking samples, 20 μL of the mixed standard solution at appropriate concentration was added as well. 3 mL of pure methanol was added and vibrated on an oscillator for 20 min. After centrifuging at 2433 × *g* for 5 min, the supernatant was transferred into a 10 mL centrifuge tube and evaporated to dryness under a flow of nitrogen at 40 °C. The residue was reconstituted in 50 μL of methanol and vortexed for several seconds, and then dispersed by 4 mL of deionized water and ultrasonicated for 10 min. The obtained solution was subjected to the further clean-up of PEP-PAN@PSF rods.

2.6. Fish homogenate pretreatment

The frozen fish homogenate was thawed at room temperature. After spiking with 20 μL of the mixed internal standard solution as well as with 20 μL of a mixed standard solution if necessary, the homogenate was vortexed well and incubated for 1 h at room temperature. 5 mL of acetonitrile was then added for the extraction of the analytes, first vigorously vortexed for seconds and then mechanically overturned for 20 min. The obtained extract was centrifuged for 15 min at 2433 × *g*. The supernatant was transferred to a 10 mL centrifuge tube, and evaporated to dryness under a stream of nitrogen at 40 °C. The residue was reconstituted in 100 μL of acetonitrile and vortexed for several seconds, then dispersed by 4 mL of deionized water and ultrasonicated for 10 min. The obtained solution was subjected to the treatment of PEP-PAN@PSF rods for the further clean-up and enrichment.

2.7. Sample extraction by PEP-PAN@PSF rods

Four milliliter of environmental water sample or the above prepared redissolutions were carried out as follows. The PEP-PAN@PSF rods were first preconditioned in consecutive methanol and water. After conditioning with water, the rods were directly immersed into the individual solutions and mechanically shaken for 90 min. After that, each rod was transferred into a 300 μL sample cell with 200 μL of acetone for desorption. The cell was hold in a 1.5 mL sample vial in advance. The elution processing lasted for 10 min by shaking. As for the complex sediment and fish samples, an additional water washing (1 mL, shaking 10 min) was suggested before the desorption process for removing the possible residue on the thin-films.

2.8. Pre-column derivatization

The analytes in the desorption solution was derivatized by adding 50 μL of 3.0 g L^{-1} DNS-Cl and 50 μL of 0.1 M $NaHCO_3$ buffer (pH 9.0). After shaking well, it was kept in 60 °C oven for 10 min. The obtained solution was then subjected to UHPLC-MS/MS analysis.

2.9. Statistical analyses

Results are presented as means ± SD of tree replicates. One-way ANOVA was used for the statistical analysis by the Student-Newman-Keuls test. Differences were considered as significant as $p < 0.05$.

3. Results and discussion

3.1. Preparation of PEP-PAN@PSF rods

3.1.1. Optimization of dipping times

To obtain PEP-PAN@PSF rods with good performance, the number of dipping times for the preparation of rods was optimized. As shown in Fig. S4A, only one dipping time showed high variability on the recoveries of analytes, while almost constant recoveries were obtained for all analytes as dipping times were 3, 6, 10 and 20. So the optimized number of dipping times was three.

3.1.2. Comparison of extraction efficiency of PEP-PAN@PSF rods

The extraction efficiency of PEP-PAN@PSF rods was particularly compared with commercial ODS (C18) prepared PAN@PSF rods, owing to ODS was the most frequently used sorbent for neutral and nonpolar compounds. As shown in Fig. S4B, PEP-PAN@PSF rods shows better extraction efficiency than C18-PAN@PSF rods especially for the relatively high polar compounds like estriol (E3) and bisphenol A (BPA), owing to the fact that PEP is a mixed-mode polymeric sorbent of PS-DVB surface-modified by urea functional groups, indicating good adsorption for both polar and nonpolar compounds. In contrast with PEP-PAN@PSF rods, bare PSF coated rods showed much lower adsorption efficiencies for the targets, even though PSF hollow fiber membrane is a widely used water-treated membrane for contaminants. While after they were coated by the PAN slurry without the introduction of PEP, the extraction efficiencies become even worse owing to the blocking effect of PAN coating.

3.1.3. Characterization of PEP-PAN@PSF rods

To verify the surface configuration of thin-films, 10 nm of gold was deposited on the surface of thin-films and SEM images were recorded. As shown in Fig. 2, in the field of view, two different layers are observed on the rod. The middle porous PSF membrane is sticking on the surface of the rod, and meanwhile serves as a support for the external PEP-PAN thin-film coating. Plenty of PEP particles are embedded in PAN constructive layer. The average thickness of the PEP-PAN thin-film coating is estimated to be about 200 μm.

3.2. Optimization of extraction parameters of PEP-PAN@PSF rods

To obtain the optimal adsorption and desorption conditions of PEP-PAN@PSF rods for the targets, various factors including pH values, extraction time, desorption solvent, desorption volume, desorption time, ion strength and sample volume were optimized.

3.2.1. Effect of pH

The pH of sample solution shows critical effect on the extraction efficiency of analyte on sorbent, since the analyte might exist as ionic or neutral form at appropriate pH values. In the study, the pH of solution was adjusted to the desired value by diluted HCl and NaOH solutions. The influence of pH ranged 2–10 on the extraction efficiency was evaluated, and the results are displayed in Fig. 3A. As can be seen, no obvious difference for the recoveries of analytes was obtained for all pH values. Therefore, no pH adjustment was subjected to the following experiment.

3.2.2. Effect of adsorption time

To investigate the effect of adsorption time on extraction efficiency, a series of adsorption times (5, 10, 20, 30, 40, 60, 90, 120, 150 min) were evaluated. As can be seen in Fig. 3B, the extraction efficiencies of all analytes kept increasing prior to 60 min. After 60 min, the extraction efficiencies kept almost constant. So 90 min of adsorption time was chosen for the following experiment.

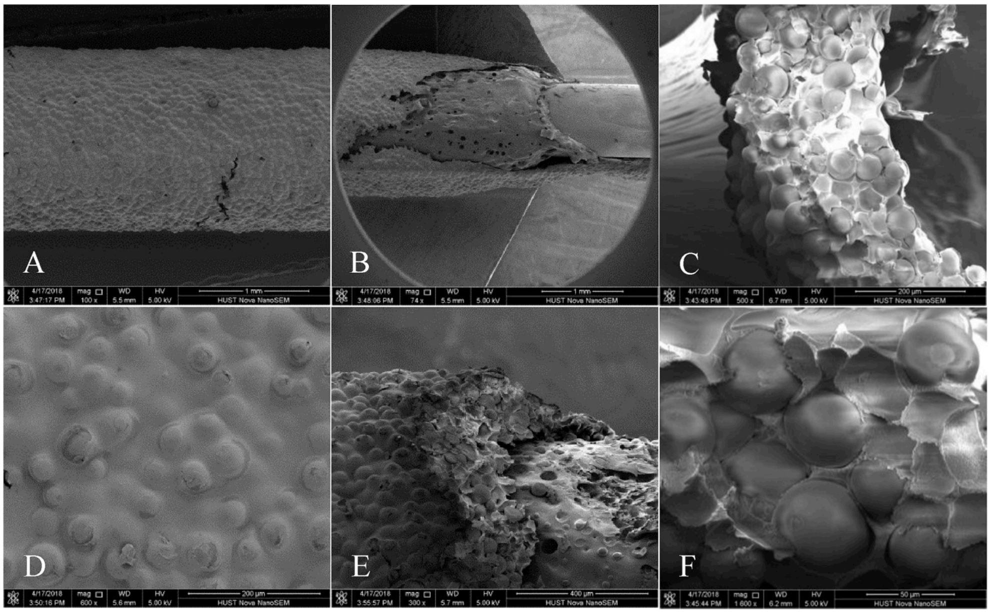

Fig. 2. SEM images of PEP-PAN@PSf rods. A, B and C are the surface of rods, the section of PSf supported PEP-PAN thin-film coating, and the section of PEP-PAN coating, and D, E and F are the relative amplifier SEM images.

3.2.3. Effect of desorption solution

A satisfactory desorption solution should give a full consideration for desorption efficiency of analyte and compatibility with the following instrumental detection system. In the study, seven desorption solutions, including methanol, acetonitrile, acetone, methanol or acetonitrile containing 1% formic acid, methanol or acetonitrile containing 1% triethylamine, the optimal mobile phase (acetonitrile/methanol/water, 43:12:45, v/v/v) have been evaluated as the desorption volume was fixed at 1 mL. As shown in Fig. 3C, pure methanol, acetonitrile and acetone showed comparative high recoveries (> 80%) for all analytes. By contrast, methanol containing 1% triethylamine or formic acid showed better recoveries for all analytes except for estriol (E3), whose peak was partially overlapped by the peak of formic acid, and the same phenomenon was observed as using acetonitrile (1% formic acid) as desorption solution. It indicates that the desorption solution containing formic acid cannot be adopted in HPLC-UV system due to the serious spectral interference of formic acid to estriol (E3). So as using HPLC-UV for the following optimization, methanol (1% triethylamine) was adopted for desorption. While it should be noted that methanol containing triethylamine cannot be used in UHPLC-MS/MS system due to the strong carryover of triethylamine in MS system. Hence, an additional optimization was subjected to the UHPLC-MS/MS system as using methanol with/without 1% formic acid, acetonitrile and acetone as desorption solution (section 3.4.2.4).

3.2.4. Effect of desorption time

Desorption time was changed between 2 min and 10 min and was examined as using 200 μL of methanol (1% triethylamine) for

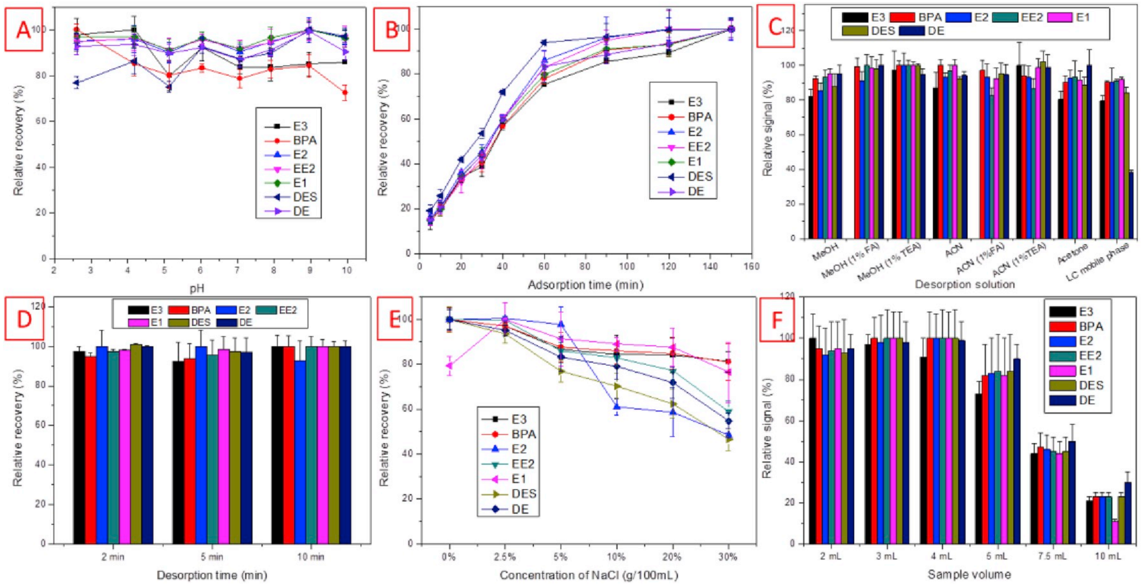

Fig. 3. Optimization of extraction parameters of PEP-PAN@PSf rods (A: pH; B: adsorption time; C: desorption solution; D: desorption time; E: ionic strength; F: sample volume) (n = 3). Conditions: concentration of the analytes was 5 mg L^{-1}; sample volume for A–E was 1 mL; adsorption time for A was 60 min, for C–F was 90 min; desorption solution for A and B was 1 mL methanol, for D–F was 200 μL methanol (1% TEA); desorption time for A–C was 30 min, for E and F was 5 min (n = 3). The abbreviations: estriol (E3), bisphenol A (BPA), 17β-estradiol (E2), ethinyloestradiol (EE2), estrone (E1), diethylstilbestrol (DES), dienestrol (DE).

desorption. It was found that in Fig. 3D, even within a very short time of 2 min, the analytes were well recovered. So, a relatively short desorption time of 5 min was chosen. In addition, considering that pure acetone was finally used for the desorption prior to the pre-column derivatization, the effect of desorption time was also evaluated by using 200 μL of acetone as desorption solvent, and similar result as methanol (1% triethylamine) was obtained.

3.2.5. Effect of ionic strength

The effect of ionic strength of sample solution on the recoveries of the analytes has been investigated experimentally. As shown in Fig. 3E, at low ionic strength (≤ 2.5%, m/v), only estrone (E1) increased slightly with the increase of ionic strength, and the others remained almost constant, however, as ionic strength further rising, the extraction efficiencies of almost all the compounds showed different degrees of decline. Considering that the exact ionic strength of the specific analyzed object could not be accurately known, subsequent experiments were not intended to control the ionic strength of sample.

3.2.6. Effect of sample volume

Sample volume on extraction efficiency was evaluated by spiking same amount of analytes in different volumes of water solution. It was found that in Fig. 3F, almost all the analytes showed comparative recoveries before 4 mL, but with a further increase, the extraction efficiencies showed a sharp decline, owing to the increased liquid-solid ratio of water and coating. So the sample volume was controlled at 4 mL.

3.3. Optimization of extraction conditions for fish homogenate and sediment

3.3.1. Extraction solution for fish homogenate

Pure acetonitrile, acetonitrile containing 1% formic acid, acetone, methanol and the 1:1 mixture of methanol and 0.1 M HAc (pH 4) have been reported for the extraction of fish homogenate [28,36]. Hence, the extraction performance of five solutions was compared in the case. 5 mL of the evaluated solutions were subjected to the same spiking fish homogenates and vibrated by a turn-over oscillator for 20 min. After collecting the supernatant by centrifuge (2433 × g, 15 min), the residue was subjected to the second extraction with the corresponding extraction solution. The obtained extracts were individually analyzed. As shown in Fig. 4A, acetonitrile and acetonitrile containing 1% formic acid showed better extraction for all the tested analytes. The first extraction took around 90% of total extracted amount from fish homogenate. In a full consideration of the time-cost (20 min for another extraction and even more time for drying process), one time of extraction by acetonitrile was adopted for fish homogenate.

3.3.2. Acetonitrile volume for fish homogenate

The volume of extraction solvent is a very important factor to interfere the extraction efficiency. Therefore, different volumes (3, 4, 5, 8 mL) of acetonitrile were applied for the extraction of the analytes from the spiking fish homogenates. As shown in Fig. 4B, 5 and 8 mL of acetonitrile showed comparative extraction performance for the tested analytes. 5 mL of extraction volume was chosen for time-saving during drying.

3.3.3. Extraction time for fish homogenate

As using 5 mL of acetonitrile as extraction solvent for fish homogenate, the extraction time (10, 20, 30, 40 min) was evaluated in the experiment. As shown in Fig. 4C, no difference for most analytes was found for the evaluated extraction time, except for bisphenol A (BPA), which showed a little lower extraction at 10 min. 20 min was thus chosen for the extraction by 5 mL of acetonitrile for fish homogenate.

3.3.4. Extraction solvent for sediment

According the reference [36], acetonitrile and methanol were the most commonly used extraction solvents for sediment samples. Therefore, the extraction efficiencies of acetonitrile and methanol for sediment samples were compared in the experiment. 3 mL of acetonitrile and methanol were subjected to the same spiking sediment samples by vibrating for 20 min on an oscillator. After collecting the supernatant by centrifuge (2433 × g, 5 min), the residues were subjected to the same extraction in three replicates. The obtained extracts were analyzed individually. As seen in Fig. 4D, both solvents showed comparative extraction performance, but methanol, by contrast, showed a little better extraction for relatively polar compound estriol (E3). The first extraction took nearly 90% of total extraction amount for bisphenol A (BPA) and 17β-estradiol (E2), and over 90% for the rest analytes, indicating that an exhaustive extraction can be achieved by one extraction processing as using methanol as extraction solvent.

3.4. Optimization of pre-column derivatization and UHPLC-MS/MS system

3.4.1. UHPLC-MS/MS system

In UHPLC-MS/MS system, the constituents of mobile phase not only show influence on the retention of analytes in column but also have an effect on ionization efficiencies of analytes in MS system. Different types of water phase including pure water, 0.1% formic acid solution, 5 mM ammonium formate solution, 10 mM ammonium formate solution, the equal volume mixed buffer of 0.1% formic acid and 10 mM ammonium formate, and the equal volume mixed buffer of 0.1% formic acid and 5 mM ammonium formate have be evaluated by using acetonitrile as organic phase, and the results are shown in Fig. S5A. It was found that the natural estrogens estrone (E1), 17β-estradiol (E2) and estriol (E3) and the metabolite 2-methoxyestradiol (2ME2) showed the best response in the system of acetonitrile–0.1% formic acid, while the half-synthetic estrogen ethinyloestradiol (EE2) and the complete synthetic estrogens hexestrol (HEX), dienestrol (DE) and diethylstilbestrol (DES) showed the best response in acetonitrile–5 mM ammonium formate system. Only bisphenol A (BPA) showed the best response as using the 1:1 mixed buffer of 0.1% formic acid and 5 mM ammonium formate as water phase. Considering that the natural estrogens can show competitive sensitivity even in acetonitrile − 5 mM ammonium formate system than the synthetic estrogens at the same concentration level, acetonitrile–5 mM ammonium formate system was finally used as the mobile phase.

According to Capriotti et al. and Athanasiadou et al. [26,27], phenolic compounds are mainly detected under negative ESI mode, showing relatively lower sensitivity in comparison with other compounds that can be detected under positive ESI mode. To improve the sensitivity of analytes in ESI-MS system, an alternative strategy is to convert phenolic compounds to their derivatives with appropriate derivatization reagent, which can be detected under positive ESI mode instead. To the best of our knowledge, DNS-Cl is the most practical derivatization reagent for phenolic compounds [46–49]. The schematic diagram of the dansylation of phenolic compounds with DNS-Cl is shown in Fig. S6. The dansylated products mainly form positive 171 collision products due to the cleavage reaction at position 1, which can further form positive 156 collision product owing to the cleavage reaction at position 2.

The responses of the analytes were compared as with/without the dansylation. As shown in Fig. S7, estrone (E1) was found to have two derivatives due to the equal chance to form cis-trans isomers. 33 $\mu g\ L^{-1}$ of the dansylated derivatives showed much competitive responses in positive ESI-MS/MS to 1000 $\mu g\ L^{-1}$ of the undansylated analytes in negative ESI-MS/MS. The responses of the dansylated analytes were preliminarily estimated to be 10–1000 times (data not shown) higher than those of undansylated ones, and the obtained enhancement of sensitivity in this case is in a middle position among the methods reviewed by Higashi and Ogawa [46], but much higher than the method

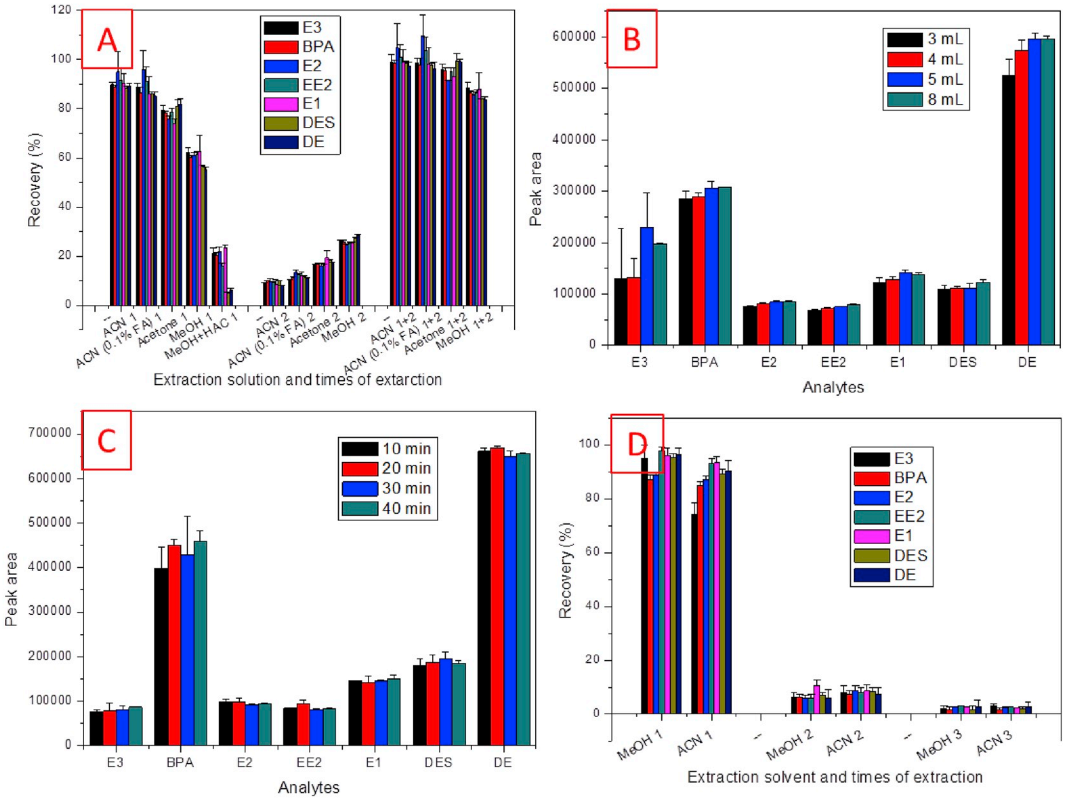

Fig. 4. Optimization of extraction conditions for fish homogenate (A: extraction solution and repeated times; B: acetonitrile volume; C: extraction time) and sediment (D: extraction solvent and repeated times) (n = 3). Conditions: sample A–C was 3 g of fish homogenate spiked 30 μg of analytes; sample D was 0.4 g of sediment spiked with 30 μg of analytes; incubation was lasted for 1 h at room temperature (10–20 °C in this study); volume of extraction solvent for A and C was 5 mL, for D was 3 mL; vibrating time for A, B and D was 20 min; power of centrifuge was 2433 × g; centrifuge time for A–C was 15 min, for D was 5 min. The abbreviations: estriol (E3), bisphenol A (BPA), 17β-estradiol (E2), ethinyloestradiol (EE2), estrone (E1), diethylstilbestrol (DES), dienestrol (DE).

reported by Wang et al. [48].

3.4.2. Optimization of parameters of derivatization

To our knowledge, the medium of solution, the concentration of DNS-Cl, the pH, the ionic strength and the temperature can show critical effect on the derivatization. Therefore, all the parameters were considered in the study.

3.4.2.1. Concentration of derivatization reagent. The concentration of derivatization reagent is critical for the derivatization efficiency. As shown in Fig. 5A, with the increase of concentration of DNS-Cl from 0.5 to 3.0 g L^{-1}, the signals of the analytes kept increasing. But with a further increase of DNS-Cl, the solution was found to easily generate precipitate crystal. Therefore, to avoid the possible interference of the precipitate for the following detection of UHPLC-MS/MS, a relatively high concentration of DNS-Cl (3.0 g L^{-1}) was chosen for the derivatization.

3.4.2.2. pH value of $NaHCO_3$ solution. It was found that the medium of derivatization was most occurred in basic 0.1 M $NaHCO_3$ solution, but the pH values were to some extent different [48,49]. So the pH value was evaluated in the study, which is considered to mainly influence the ionization efficiency of analyte. As shown in Fig. 5B, different analytes manifested different effect at pH 9.0 and pH 9.5. Most of the analyzed steroids (17β-estradiol (E2), estriol (E3), 2-methoxyestradiol (2ME2), ethinyloestradiol (EE2)) except for estrone (E1) showed a little higher signal at pH 9.5 than pH 9.0, while the complete synthetic estrogens (hexestrol (HEX), dienestrol (DE), diethylstilbestrol (DES)) and bisphenol A (BPA) showed better response at pH 9.0. With a further increase of pH, the signal of all analytes decreased. Considering that the complete synthetic estrogens showed much lower sensitivity than the tested steroids at the same concentration level, the pH was controlled at pH 9.0 for the complete synthetic estrogens and bisphenol A (BPA) to achieve relatively higher sensitivity.

3.4.2.3. Mixing proportion of DNS-Cl and $NaHCO_3$ buffer. The optimal DNS-Cl concentration and pH values of $NaHCO_3$ were obtained by adding 50 μL of the individual solutions in desorption solution. In this study, the other mixing proportions of DNS-Cl solution and $NaHCO_3$ buffer were evaluated, and the result is shown in Fig. 5C. As can be seen, 1:1 of mixing proportion showed the best derivatization efficiencies for all the analytes. Increasing the proportion of DNS-Cl could not enhance the derivatization efficiencies, might due to the low ionization efficiencies at low ionic strength. Increasing the proportion of $NaHCO_3$ buffer still lowered the response of analyte, might due to the decreased derivatization efficiencies at lower level of derivatization reagent.

3.4.2.4. Desorption solution on derivatization. According to the results obtained in section 3.2.3, four kinds of solution mediums including methanol, acetonitrile, acetone and methanol (1% formic acid) were subjected to the derivatization processing by directly spiking the same amount of the analytes in the corresponding desorption solutions, since those four solutions showed comparative recoveries for all the analytes. As shown in Fig. 5D, methanol showed very low derivatization efficiencies as compared with acetonitrile and acetone. In addition, methanol containing 1% formic acid showed even worse derivatization, probably due to the change of pH of the reaction medium. By contrast, acetonitrile showed a little better derivatization efficiencies than acetone as without the interference of matrix.

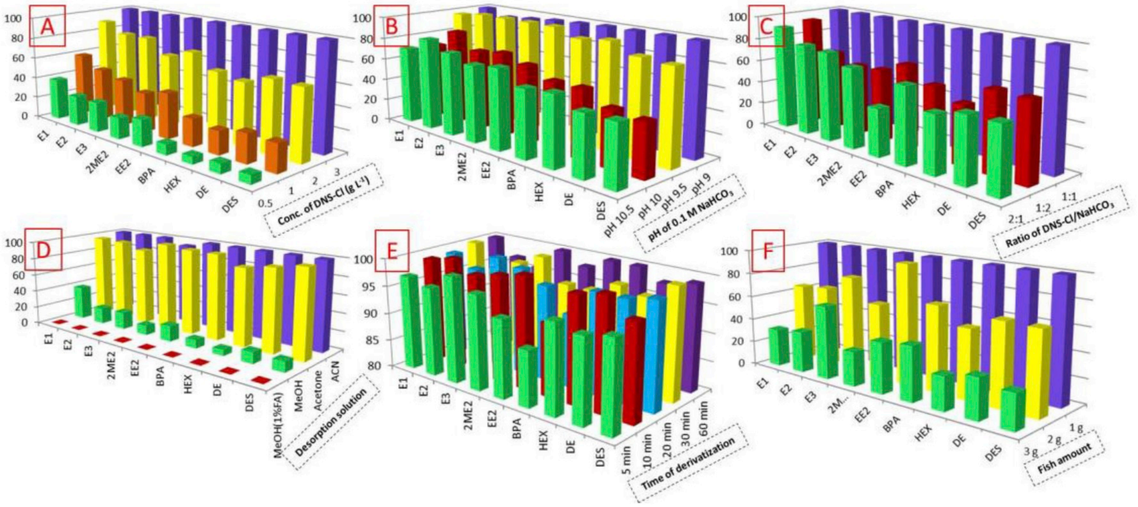

Fig. 5. Optimization of the parameters of pre-column derivatization (A: concentration (g L^{-1}) of DNS-Cl; B: pH of 0.1 M $NaHCO_3$; C: ratio of DNS-Cl/$NaHCO_3$; D: desorption solution; E: derivative time of heating; F: amount of fish homogenate) (n = 3). Conditions: spiking amount of analytes was 18 ng; concentration of DNS-Cl for B–F was 3.0 g L^{-1}; pH of 0.1 M $NaHCO_3$ except for B was 9.0; ratio of DNS-Cl/$NaHCO_3$ except for C was 1:1; temperature was 60 °C; derivative time of heating for A–D was 30 min, for F was 10 min. The abbreviations: estrone (E1), 17β-estradiol (E2), estriol (E3), 2-methoxyestradiol (2ME2), ethinyloestradiol (EE2), bisphenol A (BPA), hexestrol (HEX), dienestrol (DE), diethylstilbestrol (DES), dansyl chloride (DNS-Cl).

To further confirm the possible effect of desorption solution on derivatization as the matrix existed, pure acetonitrile and acetone were used as the desorption solutions for fish homogenate. As shown in Fig. S5B, only estrone (E1), 17β-estradiol (E2) and ethinyloestradiol (EE2) showed better derivatization efficiencies as using acetonitrile as the desorption solution for fish homogenate. By comparison, acetone manifested better repeatability than acetonitrile during the derivatization. Therefore, acetone was chosen as the desorption solution prior to the derivatization.

3.4.2.5. Heating mode on derivatization. The temperature for the derivatization was controlled at 60 °C and normal heating mode was water batch [48,49]. Considering that operation of water bath is more complicate than oven heating. So the derivatization efficiencies were compared between water bath and oven heating. It was found that 20 min heating by oven showed a bit higher signal than the same time control by water batch (Fig. S5C). So convenient oven heating was chosen for the derivatization.

3.4.2.6. Heating time on derivatization. The time of heating for the derivatization was evaluated by keeping the solution in an oven at 60 °C. It was found that, as shown in Fig. 5E, the signals just increased a little from 5 min to 10 min, and then kept unchanged with a further increase of time. It indicates that the speed of derivatization was very fast, and was completed within 10 min. Therefore, 10 min was chosen for the derivatization.

3.4.2.7. Fish amount. The amount of biological sample can show critical effect on the analysis of analytes. It might lower the extraction efficiency during sample preparation and cause interference during UV or MS analysis. To evaluate the effect of fish amount, same amount of the analytes was spiked in 1, 2 and 3 g of fish homogenates, respectively. They were subjected to the same processing of acetonitrile extraction and thin-film rods clean-up. The obtained signals of each analyte were compared as shown in Fig. 5F. Same amount of the analytes showed the highest signals as spiking in 1 g of fish homogenate. Almost all the analytes were found to have a decreased tendency with the increase of fish amount. The reason might be that more fish amount allows stronger interactions with the analytes, probably leads to much serious competitive adsorption during the treatment by the thin-film rods, as well as stronger matrix effect during the MS analysis.

In spite of 1 g of fish homogenate showed the highest signals for the analytes, it does not mean the higher sensitivity for analysis because of the difference of concentration. Therefore, the relative signals of the analytes at the concentration of 1 ng g^{-1} were calculated and compared. As shown inFig. S5D, six out of nine compounds showed higher or comparative response as spiking same amount of analytes in 1 g of fish homogenates. Estriol (E3) and bisphenol A (BPA) showed better response in 3 g of fish homogenate. And ethinyloestradiol (EE2) showed better response in 2 g of fish homogenate. Eventually, 1 g of fish homogenate was used for sample analysis.

3.4.2.8. Effect of the content of organic solvent in redissolution. To efficiently dissolute the analytes from the residues of extract, adding a certain amount of organic solvent in redissolution was recommended in many references. In the study, the signals of the analytes were compared with/without the addition of organic solvent in the redissolution. As shown in Fig. S8, no significant difference was observed for the sediment residue with and without the addition of methanol (50 μL). But the signals of most analytes, especially estrone (E1), 17β-estradiol (E2), bisphenol A (BPA), hexestrol (HEX) and diethylstilbestrol (DES), were significantly enhanced in the fish residue by the addition of acetonitrile (100 μL) in the redissolution. Therefore, in the redissolution processing, 50 μL of methanol or 100 μL of acetonitrile (the corresponding concentrations were about 1.25% and 2.5% in total redissolution, respectively) was first added to reconstitute the residue by vortexing for a while, prior to the ultra-sonication dispersion by 4 mL of water.

3.5. Stability, reusability and reproducibility of PEP-PAN@PSF rods

The stability of PEP-PAN@PSF rods was evaluated by keeping them in pure methanol solution for over two years. The coating was still sticking on the stainless steel rods tightly even though without the etching treatment, and showed the same extraction efficiencies for all analytes as the new prepared rods.

The reusability is one of the key parameters to reflect the performance of PEP-PAN@PSF rods. Normally complex sample, particularly biological sample is considered as a challenge for the reuse of rods, although the rods were reused more than 30 times in water samples. To evaluate the reusability of rods in sediment and fish homogenate, a set of rods were repeatedly subjected to the spiking sediment and fish homogenate samples at 10 ng g^{-1}. After each extraction, the rods were washed with methanol for more than 3 times to remove possible contaminations. It was found that the rods showed appropriate recoveries (within 80%–120%) and precisions ($\leq$ 15%) for all the targets until five times of reuse. And while with a further increase of reuse times, the variabilities of recoveries and precisions were occasionally beyond the acceptable range for some analytes. Therefore, the rods were suggested to be reused within 5 times in sediment and fish homogenate samples.

The reproducibility of preparing the rods is another critical parameter to reflect the performance of PEP-PAN@PSF rods. Three batches of PEP-PAN@PSF rods were prepared individually, and each batch had 50 rods. Random 5 rods from each batch were subjected to the analysis of spiking samples at 10 ng g^{-1}. It was found that three batches of PEP-PAN@PSF rods had appropriate precisions for all the analytes within each batch (RSDs $\leq$ 9.8%) and among three batches (RSDs $\leq$ 8.3%).

3.6. Matrix effect

An analyte especially in complex matrices like fish could suffer ionization suppression or enhancement in ESI-MS as a consequence of matrix effect, thereby leading to inaccurate assay results. According to the guidelines of China Food and Drug Administration (CFDA) on bioanalytical method validation, to evaluate possible matrix effect from the matrices of environmental water, sediment, and two fish species, a set of each kind of samples were subjected to the optimal sample treatment and extraction. The final obtained desorption solutions were separated into three sets, one was only spiked the internal standards, and the other two were spiked the internal standards as well as the analytes at low and high levels. The corresponding matrix-free samples were prepared by directly spiking the same amount of the analytes and internal standards in desorption solutions. The matrix effect was validated with the corrections of internal standards, and the internal standard-normalized matrix effect was obtained by comparing the ratio of the analyte response to the internal standard (IS) response from the matrix sample with that from the matrix-free sample at the same level. $\mathrm{ME} = \frac{A_{analyte\ in\ matrix\ sample} / A_{IS\ in\ matrix\ sample}}{A_{analyte\ in\ matrix-free\ sample} / A_{IS\ in\ matrix-free\ sample}}$. As shown in Table S3, the analytes normalized by its own isotopically labeled IS showed ideal matrix effect values within 0.85–1.15, and the analytes 2-methoxyestradiol (2ME2), hexestrol (HEX) and dienestrol (DE), which are normalized by the structural similar compound, showed a little wider deviation but still appropriate within 0.80–1.20. The results indicate that the addition of isotopically labeled compounds allows satisfactory correction of matrix effect since the adopted internal standards undergo the same/very similar behavior as the analytes during the sample treatment and instrumental analysis.

3.7. Method validation

To our knowledge, the calibration methods, including standard addition, internal standard with isotopically labeled standards and matrix-matched calibration are widely applied for the analysis of complex samples [28,50,51]. The application of standard addition is the best choice to overcome the matrix effect, because all the analysis is carried out in the same matrix. But it is time consuming as facing a big amount of samples. The internal standard with isotopically labeled standards is also highly recommended due to the appropriate correction of matrix effect, but it is expensive to purchase all isotopically labeled standards for each target analyte. The matrix-matched calibration is a good alternative if a matrix-matched sample free of the target analytes is available. Unfortunately such blank matrix was unavailable in this case. Therefore, a modified internal sample calibration was carried out in this study, which was regarded as the best approach for the analysis of EDCs in complex sample [28,51]. In brief, one sample from each matrix with relatively low levels for most analytes was chosen for the calibration curve. And then a set of samples with different concentration levels were prepared by spiking constant amount internal standards and different amount of the target analytes. The calibration curve for each analyte was plotted as a dependence of the ratio of the analyte response and the IS response to a spiking concentration.

The parameters of carryover, limit of detection (LOD), limit of quantification (LOQ), calibration curve, trueness and precision of the protocol were validated in four types of matrices, including environmental water, sediment and two fish matrices, on the basis of the guidelines of China Food and Drug Administration (CFDA) on bioanalytical method validation.

No impact of carryover was observed in UHPLC-MS/MS assay, since the residues of system after undergoing the highest calibrator showed less response than 20% of the lower limit of quantification (LLOQ) response and 5% of the IS response.

The LOD and LOQ were defined by two ways in this study. One was determined by signal-to-noise ratios of 3 and 9, respectively, which is for the anayte not found in at least one of the sample. As for the analyte with diverse concentrations in the samples, on the other hand, the LOD was calculated by the standard deviation of sample multiplied by the correct Student's t-value ($t_{(6,99)} = 3.143$, seven replicates of the sample at low concentration, six degrees of freedom with 99% confidence), and the relative LOQ was calculated by ten times of the sample standard deviation (s) [52]. As summarized in Table 1, the LODs for the analytes ranged in 0.002–0.072 μg L^{-1} for environmental water, 0.032–0.734 ng g^{-1} for sediment, and 0.011–0.435 ng g^{-1} for two fish species, respectively. Appropriate linearity was observed for all the analytes with correlation coefficients (R^2) above 0.997. The intra-day trueness (n = 6, carried out in one day) of the approach at low, medium and high levels was in the range of 86.6–116.1% with RSDs $\leq$ 15.4%. The inter-day trueness (n = 18, carried out on three consecutive different days) was in the range of 84.6–114.8% with RSDs $\leq$ 16.3%. By contrast, the present method showed very competitive sensitivity to most reported methods for fish muscle homogenate [4,13,18–20,28,32]. However, it showed relatively lower sensitivity for water samples due to the low tolerance of sample volume [4,10,13,15,17,18,20,23,31], but in spite of this, it still showed much better sensitivity to those references with fluorescence [34] or UV [36,39,40] as detector. As for sediment samples, the protocol showed comparative sensitivity to some methods [10,13,20], but competitive sensitivity to those methods [16,18,24].

The recoveries of the protocol for the target analytes were also calculated at two spiking levels in the research. To evaluate the recoveries of analytes from each kind of sample species, two sets of the samples were prepared by adding fixed internal standards, and each contains 6 samples. Three samples were spiked at a concentration level before sample extraction, and three samples were spiked with the same amount of the analytes but after the clean-up by the rods. The recoveries were determined by the peak area ratio of analyte to IS obtained from the sample spiked after the clean-up of rods *versus* that spiked before sample extraction. The results are shown in Table S4. The analytes showed appropriate recoveries in environmental samples due to the relatively simple matrix. While with the change of matrix from sediment to fish species, the recoveries become lower. In contrast, sediment showed better recoveries than two fish species, especially for the complete synthetic estrogens (hexestrol (HEX), dienestrol (DE), diethylstilbestrol (DES)). The possible reasons might be the low capacity of solvent for sample extraction and serious competitive adsorption from the interferences of the extracts of sediment and fish species.

3.8. Application

To assess the feasibility of the proposed method for real samples, three environmental water samples, two sediment samples, two fish species (Grass Carp, Snakehead) were analyzed in the study. The obtained results are listed in Table 2.

Table 1

Analytical performance of the protocol for environmental water, sediment, and two fish species.

Analyte	Matrix	Calibration curve[b]	linear range[a]	R^2	Intra-day trueness % and RSD%[c]			Inter-day trueness % and RSD%[d]			LOD[a]	LOQ[a]
					Low	Medium	High	Low	Medium	High		
E1	Water	$y=(2.6972 \pm 0.0185)\,x + (0.0462 \pm 0.0151)$	0.005–2.5	> 0.999	100.1 (7.6)	99.9 (4.9)	99.8 (2.9)	87.6 (10.0)	101.6 (3.6)	109.2 (4.8)	0.002	0.005
	Sediment	$y=(0.3165 \pm 0.0240)\,x + (0.0107 \pm 0.0033)$	0.10–25	> 0.999	93.8 (6.9)	103.0 (4.8)	98.9 (2.7)	95.2 (7.9)	99.3 (3.4)	97.3 (3.0)	0.032	0.107
	Grass Carp	$y=(0.5752 \pm 0.0131)\,x + (0.0608 \pm 0.0007)$	0.05–10	> 0.999	100.2 (5.2)	104.4 (4.1)	98.7 (5.2)	95.2 (7.9)	98.7 (3.9)	92.6 (2.5)	0.011	0.035
	Snakehead	$y=(0.5434 \pm 0.0105)\,x + (0.0487 \pm 0.0012)$	0.05–10	> 0.998	99.7 (7.6)	100.6 (3.7)	100.0 (1.9)	101.4 (12.5)	107.4 (2.7)	99.9 (5.5)	0.015	0.047
E2	Water	$y=(2.9796 \pm 0.2466)\,x +(0.0318 \pm 0.0163)$	0.025–25	> 0.999	113.7 (2.4)	97.6 (10.1)	99.9 (5.5)	105.3 (9.7)	99.0 (7.9)	100.6 (8.2)	0.004	0.012
	Sediment	$y=(0.3080 \pm 0.0039)\,x +(-0.0194 \pm 0.0051)$	0.10–250	> 0.999	110.7 (13.7)	101.7 (3.9)	100.5 (1.9)	91.2 (7.3)	99.7 (3.9)	100.8 (1.6)	0.032	0.101
	Grass Carp	$y=(0.6459 \pm 0.0204)\,x + (0.1778 \pm 0.0096)$	0.10–100	> 0.999	94.3 (2.5)	100.0 (5.1)	98.5 (4.8)	111.1 (11.8)	99.9 (7.3)	105.9 (4.3)	0.034	0.109
	Snakehead	$y=(0.6540 \pm 0.0300)\,x + (0.3688 \pm 0.0171)$	0.1–100	> 0.999	91.6 (7.0)	107.8 (13.1)	96.6 (13.1)	89.9 (7.8)	98.2 (8.9)	92.6 (14.3)	0.029	0.091
E3	Water	$y=(1.3996 \pm 0.0859)\,x + (0.0124 \pm 0.0020)$	0.025–12.5	> 0.999	89.8 (11.6)	106.8 (9.9)	96.7 (8.8)	95.1 (14.7)	98.4 (10.0)	91.9 (5.5)	0.007	0.021
	Sediment	$y=(0.1470 \pm 0.0136)\,x + (0.0237 \pm 0.0035)$	0.5–125	> 0.999	88.2 (12.4)	99.6 (6.6)	101.2 (5.0)	113.3 (12.1)	96.3 (7.0)	94.9 (4.5)	0.116	0.370
	Grass Carp	$y=(0.2087 \pm 0.0125)\,x + (0.1041 \pm 0.0061)$	0.25–50	> 0.999	86.6 (8.8)	99.7 (8.7)	97.9 (4.1)	91.2 (13.9)	101.8 (5.4)	99.9 (6.8)	0.039	0.125
	Snakehead	$y=(0.1846 \pm 0.0192)\,x + (0.1625 \pm 0.0062)$	0.25–50	> 0.998	113.7 (10.2)	95.4 (11.1)	96.0 (7.3)	95.6 (8.0)	101.1 (10.1)	114.8 (7.6)	0.089	0.224
2ME2	Water	$y=(0.1572 \pm 0.0103)\,x +(-\,0.0094 \pm 0.0054)$	0.25–250	> 0.999	99.1 (9.1)	100.5 (8.6)	96.1 (4.5)	109.7 (9.0)	97.5 (9.2)	102.3 (5.2)	0.072	0.228
	Sediment	$y=(0.0175 \pm 0.0014)\,x +(-0.0014 \pm 0.0006)$	2.5–2500	> 0.999	104.8 (7.4)	100.3 (7.3)	100.8 (6.4)	102.8 (11.1)	97.3 (4.8)	96.8 (5.3)	0.734	2.335
	Grass Carp	$y=(0.0232 \pm 0.0011)\,x + (0.0885 \pm 0.0099)$	2.5–1000	> 0.999	105.6 (5.6)	101.4 (5.3)	99.5 (1.4)	105.3 (6.1)	93.7 (7.2)	102.7 (6.5)	0.435	1.632
	Snakehead	$y=(0.0370 \pm 0.0023)\,x + (0.2623 \pm 0.0393)$	2.5–1000	> 0.999	92.3 (6.2)	102.0 (6.7)	94.8 (9.6)	102.6 (11.7)	100.2 (11.2)	99.3 (11.4)	0.059	1.762
EE2	Water	$y=(0.7544 \pm 0.0110)\,x + (0.0046 \pm 0.0007)$	0.025–25	> 0.999	97.3 (4.6)	103.5 (4.8)	103.9 (5.5)	95.1 (3.1)	99.2 (4.2)	96.9 (7.1)	0.008	0.022
	Sediment	$y=(0.0772 \pm 0.0026)\,x +(-0.0098 \pm 0.0019)$	0.25–250	> 0.999	101.7 (5.4)	100.8 (5.5)	100.7 (4.6)	114.3 (7.2)	96.7 (6.0)	97.2 (3.9)	0.075	0.238
	Grass Carp	$y=(0.2154 \pm 0.0049)\,x +(-0.1404 \pm 0.0252)$	0.1–100	> 0.999	91.8 (5.4)	98.8 (5.4)	99.3 (8.6)	103.9 (7.2)	96.6 (5.8)	102.7 (6.6)	0.031	0.103
	Snakehead	$y=(0.2116 \pm 0.0054)\,x + (0.3221 \pm 0.0395)$	0.1–100	> 0.999	109.2 (8.5)	100.2 (6.1)	100.0 (8.4)	112.7 (8.1)	105.1 (4.3)	104.6 (5.3)	0.028	0.093
BPA	Water	$y=(0.3088 \pm 0.0040)\,x + (0.0029 \pm 0.0027)$	0.01–25	> 0.999	95.8 (7.6)	103.3 (6.9)	100.3 (4.2)	96.3 (8.5)	99.3 (6.6)	94.0 (4.3)	0.003	0.009
	Sediment	$y=(0.0312 \pm 0.0014)\,x + (0.0162 \pm 0.0027)$	0.25–250	> 0.999	102.1 (5.5)	95.9 (4.0)	101.9 (2.7)	108.1 (16.3)	97.7 (5.8)	102.2 (3.7)	0.064	0.203
	Grass Carp	$y=(0.0648 \pm 0.0010)\,x + (0.0323 \pm 0.0031)$	0.1–100	> 0.999	100.8 (6.8)	100.0 (9.9)	98.4 (2.3)	100.5 (15.8)	99.8 (7.6)	106.6 (4.7)	0.024	0.081
	Snakehead	$y=(0.0676 \pm 0.0021)\,x + (0.0335 \pm 0.0029)$	0.1–100	> 0.999	116.1 (15.4)	107.8 (4.7)	102.9 (7.8)	110.9 (14.3)	100.3 (5.5)	95.3 (3.1)	0.023	0.078
HEX	Water	$y=(0.8340 \pm 0.0552)\,x + (0.0098 \pm 0.0036)$	0.05–25	> 0.999	87.4 (5.6)	97.2 (6.6)	101.0 (7.2)	84.8 (10.2)	111.7 (3.8)	103.8 (10.2)	0.016	0.050
	Sediment	$y=(0.0885 \pm 0.0033)\,x + (0.0037 \pm 0.0011)$	0.25–250	> 0.999	105.2 (8.0)	99.6 (10.2)	105.6 (6.3)	84.6 (4.0)	101.8 (8.4)	100.0 (5.3)	0.061	0.204
	Grass Carp	$y=(0.1825 \pm 0.0143)\,x +(-0.2470 \pm 0.0267)$	0.5–100	> 0.997	93.2 (15.2)	102.0 (5.0)	98.9 (6.0)	104.8 (12.2)	91.7 (10.2)	96.3 (7.7)	0.046	0.153
	Snakehead	$y=(0.1610 \pm 0.0034)\,x +(-0.1838 \pm 0.0149)$	0.5–100	> 0.998	99.6 (11.9)	102.7 (7.3)	95.6 (6.8)	90.7 (8.3)	99.7 (7.0)	102.1 (14.3)	0.066	0.220
DES	Water	$y=(1.2400 \pm 0.0263)\,x +(0.0015 \pm 0.0012)$	0.05–25	> 0.999	89.5 (8.5)	98.0 (6.8)	105.2 (5.8)	85.0 (12.5)	108.7 (3.0)	108.1 (4.8)	0.013	0.043
	Sediment	$y=(0.1161 \pm 0.0021)\,x + (0.0112 \pm 0.0031)$	0.25–250	> 0.999	103.1 (10.8)	99.9 (5.4)	102.4 (4.2)	94.9 (14.4)	99.8 (5.1)	106.0 (4.0)	0.056	0.187
	Grass Carp	$y=(0.3253 \pm 0.0040)\,x\,(-0.0012 \pm 0.0017)$	0.25–100	> 0.999	103.9 (8.0)	99.9 (2.2)	99.4 (5.1)	90.8 (6.4)	100.3 (5.1)	102.4 (4.3)	0.048	0.161
	Snakehead	$y=(0.2956 \pm 0.0126)x +(-0.0844 \pm 0.0063)$	0.25–100	> 0.999	94.6 (6.8)	100.2 (4.7)	98.2 (5.3)	110.7 (10.5)	100.5 (4.2)	106.6 (8.1)	0.063	0.209
DE	Water	$y=(0.6864 \pm 0.0413)\,x +(-0.0051 \pm 0.0027)$	0.05–25	> 0.999	107.3 (5.5)	94.9 (4.3)	107.0 (6.5)	97.3 (8.6)	99.5 (3.9)	101.5 (4.5)	0.016	0.037
	Sediment	$y=(0.0708 \pm 0.0013)\,x +(-0.0065 \pm 0.0018)$	0.25–25	> 0.999	93.0 (15.3)	96.1 (13.0)	99.0 (4.6)	90.8 (15.9)	104.1 (8.3)	104.7 (5.6)	0.0502	0.167
	Grass Carp	$y=(0.0915 \pm 0.0057)\,x + (0.0116 \pm 0.0023)$	0.1–100	> 0.999	98.7 (6.8)	94.6 (1.5)	96.9 (4.9)	99.6 (13.2)	93.5 (2.7)	101.6 (5.5)	0.024	0.081
	Snakehead	$y=(0.1053 \pm 0.0093)\,x +(-0.1190 \pm 0.0098)$	0.1–100	> 0.998	111.3 (11.5)	95.7 (5.5)	109.5 (8.0)	95.9 (12.4)	102.0 (6.9)	103.4 (6.0)	0.026	0.083

The analytes corresponding to the abbreviations: estrone (E1), 17β-estradiol (E2), estriol (E3), 2-methoxyestradiol (2ME2), ethinyloestradiol (EE2), bisphenol A (BPA), hexestrol (HEX), diethylstilbestrol (DES) and dienestrol (DE).

For the environmental water, the spiking low, medium and high levels were 0.025, 0.25, 2.5 μg L^{-1} for E1, 1.25, 12.5, 125 μg L^{-1} for 2ME2, and 0.125, 1.25, 12.5 μg L^{-1} for other analytes.

For the sediment, the spiking low, medium and high levels were 0.25, 2.5, 25 ng g^{-1} for E1, 12.5, 125, 1250 ng g^{-1} for 2ME2, and 1.25, 12.5, 125 ng g^{-1} for other analytes.

For the fish homogenate, the spiking low, medium and high levels were 0.2, 2, 10 ng g^{-1} for E1, 10, 100, 500 ng g^{-1} for 2ME2, and 1, 10, 50 ng g^{-1} for other analytes.

[a] units for water, sediment and fish are μg L^{-1}, ng g^{-1}, and ng g^{-1}, respectively.

[b] n = 3.

[c] n = 6, analyzed in one day.

[d] n = 18, analyzed on three consecutive days.

Table 2

Results of environmental water, sediment and fish samples (mean ± SD, n = 3).

Analyte	Environmental water (μg L^{-1})			Sediment (ng g^{-1})		Grass Carp (ng g^{-1})			Snakehead (ng g^{-1})		
	HR	NL	EL	NL	EL	#1	#2	#3	#1	#2	#3
E1	ND[c]	ND	ND	0.153 ± 0.025	0.141 ± 0.013	0.076 ± 0.010	0.090 ± 0.011	0.038 ± 0.003	0.037 ± 0.007	0.037 ± 0.004	0.045 ± 0.009
E2	ND	ND	ND	< LOQ	< LOQ	0.225 ± 0.011	0.244 ± 0.035	0.539 ± 0.030	0.096 ± 0.015	0.184 ± 0.017	1.026 ± 0.079
E3	ND	< LOQ[a]	ND	ND	ND	ND	ND	0.124 ± 0.021	0.357 ± 0.025	0.077 ± 0.015	0.225 ± 0.036
2ME2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
EE2	ND	ND	ND	0.240 ± 0.048	1.123 ± 0.091	11.035 ± 0.557	0.336 ± 0.040	0.168 ± 0.030	1.695 ± 0.222	0.550 ± 0.085	0.133 ± 0.014
BPA	0.072 ± 0.014	ND	< LOQ	0.941 ± 0.107	4.664 ± 0.732	0.402 ± 0.023	0.667 ± 0.069	2.051 ± 0.148	0.445 ± 0.075	0.638 ± 0.091	0.462 ± 0.037
HEX	ND	ND	ND	< LOQ	< LOQ	ND	ND	ND	ND	ND	ND
DES	ND	ND	ND	< LOQ	0.278 ± 0.052	ND	ND	ND	ND	ND	ND
DE	ND	ND	ND	ND	< LOD[b]	0.100 ± 0.004	0.115 ± 0.015	0.070 ± 0.014	ND	ND	0.096 ± 0.016

The analytes corresponding to the abbreviations: estrone (E1), 17β-estradiol (E2), estriol (E3), 2-methoxyestradiol (2ME2), ethinyloestradiol (EE2), bisphenol A (BPA), hexestrol (HEX), diethylstilbestrol (DES) and dienestrol (DE).

NL: the North Lake; EL: the East Lake; HR: the Han River; Grass Carp: *Ctenopharyngodon idellus*; Snakehead: *Ophiocephalus argus*.

[a] detectable but the value was lower than the reported LOQ.

[b] detectable but the value was lower than the reported LOD.

[c] not detected.

For the environmental water samples, only bisphenol A (BPA) was quantified to be 0.072 μg L^{-1} in the surface water of the Han River, showing comparative levels with the surface water from the Pearl River [4,13,14] and the Shaying River [17], but a little higher than the surface water from the Taihu Lake [7] and the Panlong River [18]. As a brunch of the Yangtze river at Wuhan section, the surface water from the Han River contained lower bisphenol A (BPA) than the surface water collected from the Yangtze river at Nanjing section whose mean value was 119 ng L^{-1} [12], however, it showed much higher level than the surface water from the Yangtze Estuary which manifested the highest concentration at 11.96 ng L^{-1} [10]. The surface water samples from the East Lake and the North Lake were found to only contain bisphenol A (BPA) and estriol (E3), respectively, with levels < LOQs. The limit results demonstrated that bisphenol A (BPA) was most frequently detected in surface waters [4,7,10,12–14]. Most analytes were not detected in this case probably due to the limit of sensitivity of the protocol as a consequence of small sample consumption. But it can be concluded that the levels of the analytes were at least lower than the reported LODs (0.002 μg L^{-1} for estrone (E1) – 0.072 μg L^{-1} for 2-methoxyestradiol (2ME2)). By contrast, comparatively high levels (several to hundreds of ng L^{-1}) of estrone (E1), 17β-estradiol (E2), estriol (E3) and ethinyloestradiol (EE2) were found in the Dianchi Lake catchment [20]. A little higher concentrations (mean 1.9, maximum 8.3 ng L^{-1}) of estrone (E1) were reported in the United States, as the most commonly detected estrogen (13 of 38 water samples) in that case [22].

Only natural estrogen estriol (E3) and metabolite 2-methoxyestradiol (2ME2) were not detected in two collected sediment samples, in spite of the fact that estriol (E3) was the only analyte detected in the surface water of the North Lake. Most analytes were found to be sub ng g^{-1} levels even lower than the reported LOQs, except for ethinyloestradiol (EE2) and bisphenol A (BPA), which were found to be 1.123 and 4.664 ng g^{-1} in the East Lake sediment, respectively. Similarly ethinyloestradiol (EE2) and bisphenol A (BPA) were found to be at relatively high concentrations in the North Lake sediment, which can be considered as a consequence of human's behaviors. Ethinyloestradiol (EE2) is widely taken by woman for contraception, and bisphenol A (BPA) is widely used for manufacturing digital media and reusable bottles [10]. Both were confirmed to show low removal efficiency in WWTPs [21]. In comparison with references, the high detected value of bisphenol A (BPA) (4.664 ng g^{-1}) in this case was still lower than the highest value measured in the Pearl River Estuary (2.31–13.16 ng g^{-1}) [13], and much lower than the values measured in the Pearl River Delta (1.7–430 ng g^{-1}) [16], the Panlong River (6.6–124 ng g^{-1}) [18] and the Humen Estuary (8.0 ng g^{-1}) [35]. Additionally, bisphenol A (BPA) was found to be at very high levels in other countries, such as Auckland (maximum 145 ng g^{-1}) [24]. By contrast, estrone (E1), 17β-estradiol (E2) and estriol (E3) showed much lower even nondetectable values in the sediment samples in comparison with the reported values (not detectable – 114.8 ng g^{-1}) in the Dianchi Lake catchment [20], while ethinyloestradiol (EE2) showed comparative values in the Dianchi Lake catchment (≤ 21.2 ng g^{-1}) [20] and the Humen Estuary (4.4 ng g^{-1}) [35]. The measured values of the complete synthetic hexestrol (HEX), dienestrol (DE) and diethylstilbestrol (DES) were much lower (not detectable – 0.278 ng g^{-1}) than the values reported in the Humen Estuary (1.5–3.0 ng g^{-1}) [35].

As for the fish muscle homogenates, the metabolite 2-methoxyestradiol (2ME2) and the complete synthetic estrogenic hexestrol (HEX) and diethylstilbestrol (DES) were not detected in six fish homogenates. Among three natural estrogens, 17β-estradiol (E2) was found to be at high levels from 0.096 to 1.026 ng g^{-1}, and estriol (E3) was found to be at low levels from not detectable to 0.357 ng g^{-1}, the measured values were in accordance with the values reported in the wild fish of the Pearl River [4] and the caged fish in the Dianchi Lake [19]. Among three completed synthetic estrogens, only dienestrol (DE) was found in four out of six fish homogenates, with measured levels from 0.070 to 0.115 ng g^{-1}. By contrast, much higher values were reported in the wild fish collected from the Gernika (located downstream of WWTP) [32] and the Xiangjiang River (near Changsha city) [38], particularly diethylstilbestrol (DES) was reported to be 2.2 ± 1.3 ng g^{-1} in white bream and 3.6 ± 1.4 ng g^{-1} in catfish [38]. No measured values were reported in fish collected from the Sand Lake (Wuhan) [36], probably due to the limit of sensitivity of that protocol (LODs: 0.20 μg L^{-1} for diethylstilbestrol (DES) and 0.11 μg L^{-1} for dienestrol (DE)). Similar to the sediment samples, half-synthetic ethinyloestradiol (EE2) and chemical material bisphenol A (BPA) showed relatively high concentration in fish muscle homogenates. Ethinyloestradiol (EE2) was ranged from 0.133 to 11.035 ng g^{-1}, and bisphenol A (BPA) was ranged from 0.402 to 2.051 ng g^{-1}. The former ethinyloestradiol (EE2) showed significant difference among fish homogenates, showing big variability among organisms. No measured values for ethinyloestradiol (EE2) were reported in wild fish [4, 36, 38] and lower than 8 ng g^{-1} of ethinyloestradiol (EE2) was ever found in adult thicklip grey mullets in the Gernika [32]. The average values (0.402–2.051 ng g^{-1}) of bisphenol A (BPA) measured in the commercial fish of this case were in accordance with the biota (0.19–4.51 ng g^{-1}) collected from the Pearl River Estuary [13], while they were less than the wild fish (0.25–1973 ng g^{-1}) from the Pearl River [4], the aquatic organisms (0.220–14.5 ng g^{-1}) from the Taihu Lake [7], the Marine organism (not detectable – 396 ng g^{-1}) from the Pearl River Estuary [14], the wild fish (1.9–69 ng g^{-1}) from the Panlong River [18], the wild fish (10.1–83.5 ng g^{-1}, dry weight) from the Dianchi Lake [19], the fish (59.09 ± 8.12 and at a maximum of 223.91 ng g^{-1}) from the Mediterranean rivers [28], the adult thicklip grey mullets (20–28 ng g^{-1}) captured in the Gernika [32], and three fish species (8.3–24.3 ng g^{-1}) collected from the Xiangjiang River (near Changsha city) [38].

To further evaluate the method, three levels of the target analytes were spiked in the above analyzed samples. The recoveries were found to be 84.4–117.0% for the environmental water samples (Table S5), 85.1–116.8% for the sediment samples (Table S6), 80.9–118.5% for the fish homogenates (Table S7).

4. Conclusion

In the study, the preparation of traditional thin-film rods was improved by introducing PSF as supporting material between the internal stainless steel rod and the external thin-film coating, avoiding conventional pre-corrosion of stainless steel rods by strong acid. The improvement makes the preparation of thin-film rods much easier and also makes thin-film coatings more tightly on the rods. As a dual functional sorbent, commercial PEP particles were introduced to prepare PEP-PAN@PSF rods, which allow competitive adsorption for nonpolar and polar estrogens. The rods also showed good biocompatibility and long-term stability even in complex samples. In addition, to further improve the sensitivity of estrogens in ESI-MS, pre-column derivatization was carried out to significantly enhance the signal response by converting negative ESI mode for the analytes to positive ESI mode for their derivatives. At last, the PEP-PAN@PSF rods coupled with pre-column derivatization and UHPLC-MS/MS assay was successfully applied for the analysis of nine estrogens in environmental water, sediment, and fish homogenates.

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Declaration of competing interest

The authors declared that they have no conflicts of interest to this work.

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Appendix A. Supplementary data

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