Journal of Chromatography A, 1278 (2013) 126–132

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

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

Simultaneous determination of steroidal and phenolic endocrine disrupting chemicals in fish by ultra-high-performance liquid chromatography–mass spectrometry/mass spectrometry

Aili Ye[a], Yi Yang[a,b,*], Jing Zhang[b], Min Liu[a], Lijun Hou[b], Jun Liang Zhou[b]

[a] Shanghai Key Lab for Urban Ecological Processes and Eco-Restoration, School of Resource and Environmental Sciences, East China Normal University, 3663 North Zhongshan Road, Shanghai 200062, China
[b] State Key Laboratory of Estuarine and Coastal Research, East China Normal University, 3663 North Zhongshan Road, Shanghai 200062, China

ARTICLE INFO

Article history:
Received 5 November 2012
Received in revised form 2 January 2013
Accepted 4 January 2013
Available online 9 January 2013

Keywords:
Fish
EDCs
UHPLC–MS/MS
PLE
Matrix effect

ABSTRACT

A sensitive and reliable analytical method based on pressurized liquid extraction (PLE) and ultra-high-performance liquid chromatography equipped with tandem mass spectrometry (UHPLC–MS/MS) has been developed for simultaneously determining the steroidal and phenolic endocrine disrupting chemicals (EDCs) in fish. The most effective extraction of the target EDCs is achieved by using PLE with on-line purification and the parameters have been optimized as follows: extraction solvent – methanol–acetonitrile (1:1, v/v), on-line purification material – 5 g alumina (5% water), extraction – 3 cycles, static extraction time – 5 min and extraction temperature – 60 °C. Compared to the Oasis hydrophilic–lipophilic balance (HLB) solid phase extraction (SPE), freezing-lipid filtration combined with n-hexane defatting clean-up obtains much better recoveries of the target compounds and provide cleaner extracts. The matrix effect (ME) is generally eliminated by using an internal standard method. At spiking levels of 5, 50, and 100 ng/g, the mean recoveries vary from 71.2% to 108% for the target EDCs with a relative standard deviation (RSD) less than 16%. The method limit of detection (LOD) and limit of quantitation (LOQ) are 0.04–0.08 ng/g dw and 0.07–0.27 ng/g dw, respectively. The established method has been successfully applied to fish samples from the local market to determine the target EDCs.

1. Introduction

In recent decades, endocrine disrupting chemicals (EDCs) have been widely studied around the world due to their ubiquitous occurrence in the aquatic environment and adverse effects on the growth and development of wildlife or even humans, especially disruption to endocrine, reproductive and immune systems [1–5]. Among these compounds, steroidal and phenolic EDCs with estrogen properties have attracted the most attention. Steroids, such as estrone (E1), 17β-estradiol (E2) and estriol (E3) which are naturally derived from the excreta of livestock and humans [6,7] and 17α-ethynyl estradiol (EE2) which is man-made and known as the main component of oral contraceptive pills [8], and phenols, such as bisphenol A (BPA) which is mainly used for manufacturing of epoxy resin and polycarbonate plastic, have been widely exposed to humans in daily life [9,10]. It has been demonstrated that the alteration of living beings with direct or indirect exposure to EDCs, may be permanent and irreversible [1,11,12] even at trace levels.

The research on EDCs, such as their environmental fate, bioaccumulation and risk assessment, demands a sensitive and simultaneous detection method. In the literature, the determination of EDCs has mainly focused on steroids [13–15] or phenols [16–18] separately. Recently, several studies have carried out the simultaneous determination of steroidal and phenolic EDCs in water [19,20], soil [21,22], sediment [23] and sludge [24,25]. Limited studies focus on the simultaneously determining of steroidal and phenolic EDCs in biological tissues (like fish-muscle) due to matrix complexity and very low concentrations of EDCs in biological tissues. Reported analysis methods of EDCs in environmental matrix [26–31] are mainly based on gas chromatography–mass spectrum (GC–MS). Liquid chromatographic methods (LC, HPLC and UHPLC) can avoid tedious and time-consuming derivatization steps and thus prevent thermal degradation. In addition, more effective extraction and clean-up procedures are imperative for solid environmental sample pretreatment. Previous studies on EDCs analysis in biological samples are generally based on ultra-sonicated extraction (USE), microwave-assisted extraction (MAE), Soxhlet extraction (SE), and pressurized liquid extraction

* Corresponding author at: State Key Laboratory of Estuarine and Coastal Research, East China Normal University, 3663 North Zhongshan Road, Shanghai 200062, China. Tel.: +86 21 62232439; fax: +86 21 62232887.
E-mail address: yyang@geo.ecnu.edu.cn (Y. Yang).

0021-9673/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2013.01.008

Table 1
Analysis methods of target EDCs in biological samples in other studies.

Analytes	Sample	Pretreatment	Detection system	Recovery (RSD)%	LOD/LOQ (ng/g)	Reference
E1, E2, E3, EE2	Meat	USE with methanol–water Freezing-lipid filtration Triple SPE	GC–MS	68–129 (5.0–16)	MDL: 0.1–0.4	[32]
E2	Fish muscle	USE with acetonitrile N-hexane defatting SPE clean-up	GC–MS	68.5–100 (4.8–10)	LOQ: 0.2	[13]
BPA	Fish	USE with methanol Freezing-lipid filtration SPE twice	GC–MS	105–120 (5.0–17)	MDL: 0.41	[33]
BPA	meat	PLE with acetone SPE clean-up	LC–MS/MS	91.5–99.9 (2.9–7.9)	LOD: 0.3 LOQ: 1	[16]
E1, E2, E3, EE2, BPA	Mollusk tissues	Automatic SE with dichloromethane SPE clean-up	GC–MS	79.7–91.7 (8.5–12)	LOD: 0.27–0.48 LOQ: 0.62–1.06	[34]
E2	Fish	USE with acetone SPE clean-up	GC–MS	≈125 (31)	LOD: 1.5	[35]
E1, E2, E3, EE2, BPA	Fish	MAE with methanol GPC, SPE clean-up	GC–MS	60.2–101 (2.3–13)	MDL: 0.4–0.7	[36]
E1, E2, E3, EE2, BPA	Fish muscle	PLE with methanol–acetonitrile Freezing-lipid filtration N-hexane defatting	UHPLC–MS/MS	71.2–108 (0.92–16)	LOD: 0.04–0.08 LOQ: 0.07–0.27	Present study

(PLE) (Table 1). Among these, PLE shows great extraction efficiency, requires less time and solvent compared to other extraction techniques, and can also provide on-line purification. Although expensive, solid phase extraction (SPE) is most frequently used for sample clean-up. In general, the reported recovery of EDCs is as low as 60.2% and up to 125% with a RSD of 31% (Table 1). Therefore, it is necessary to develop a more simplified, sensitive and reliable method for the determination of EDCs from biological samples, such as fish.

In the present study, PLE with an on-line purification technique is optimized for sample extraction. A simple and low cost clean-up method using freezing-lipid filtration combined with n-hexane defatting is compared with SPE method. In addition, the developed method is validated by assessing the matrix effect (ME) as well as the precision and accuracy, and further applied to determine the concentrations of the target EDCs in fish samples from a local market. The objective of the present work is to develop a more simplified, sensitive and reliable method for the simultaneous determination of steroid and phenolic EDCs, including E1, E2, E3, EE2 and BPA in fish by UHPLC–MS/MS.

2. Experimental

2.1. Chemicals and standards

Methanol and acetonitrile were of gradient grade for the liquid chromatography, purchased from Merck (Darmstadt, Germany), and the n-hexane was of HPLC grade, supplied from ANPEL (Shanghai, China). The ultrapure water used in the sample pretreatment and instrumental analysis was prepared from a Milli-Q Gradient system (Millipore, Billerica, MA, USA). The target compound standards (E1, E2, E3, EE2 and BPA) and internal standards (E2-d_2 and BPA-d_{16}) were of the highest purity commercially available (purity > 99%, except for E2 > 96.8%) and all were supplied by Dr. Ehrenstrofer GmbH (Augsburg, Germany). The stock solutions of individual standards were prepared in methanol at 1000 mg/l and then diluted with methanol to 10 mg/l. All standard solutions were stored at −24 °C prior to use. The SPE cartridges (Oasis HLB, 200 mg, 6 ml) were supplied by Waters (Milford, MA, USA). Neutral alumina (75–147 μm) and quartz sand (AR) purchased from Sinopharm Chemical Reagent (Shanghai, China) were heated in a muffle furnace at 450 °C for 4 h. The glass microfiber filters (GF/B, Whatman, Maidstone, UK) were soaked in methanol for an hour and washed twice with clean methanol to remove interferences before usage. All glassware used in the experiment were washed and heated at 450 °C for 4 h prior to use.

2.2. Pressurized liquid extraction

Fish samples were purchased from the local market. Fish-muscle was immediately separated from the bones, homogenized with a hand blender and stored at −24 °C prior to freeze-drying. The dried fish-muscle was ground into powder and stored in a desiccator before extraction. Sample extraction was conducted by an automated ASE 350 system (Dionex, USA). The extraction cell (stainless steel, 33 ml) was loaded from bottom to top as shown in Fig. 1: glass microfiber filters, quartz sand, alumina, glass filter, 1.5 g of fish sample mixed with quartz sand, and quartz sand on the top. PLE on-line purification was applied using alumina with different water contents. Moreover, the tested PLE parameters mainly included solvent (methanol, acetonitrile, and methanol–acetonitrile 1:1), temperature (40–100 °C, 10 °C interval), holding time of static extraction (3, 5, 7 and 9 min) and the number of static extraction cycles (1, 2 and 3), with a default pressure at 1500 psi, heated for 5 min, and purged into nitrogen for 120 s. All of the extracts (about 40 ml) were

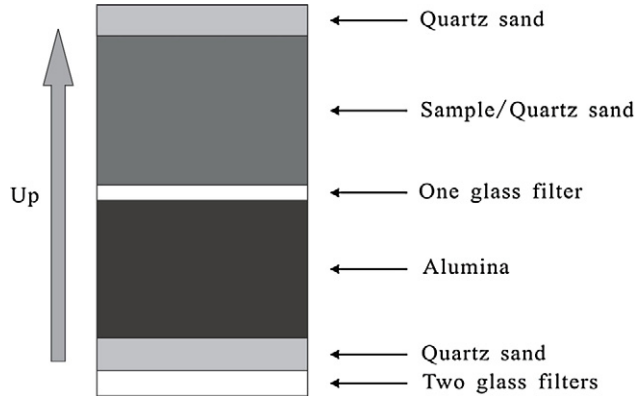

Fig. 1. Composition of the extraction cell.
Modified from [22, Fig. 1].

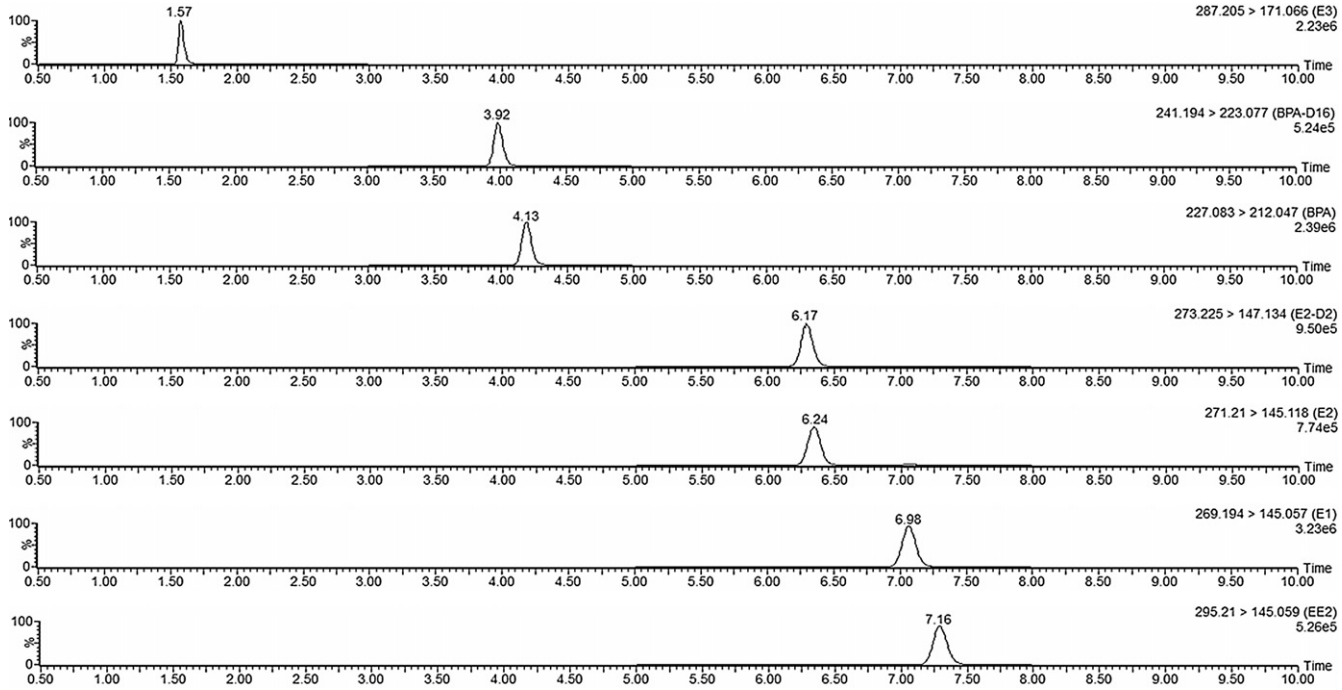

Fig. 2. MRM chromatograms of five target EDCs and two internal standards of 50 ng/ml.

collected and evaporated down to approximately 5 ml at 40 °C with a vacuum evaporator before the clean-up procedure.

The PLE with on-line purification technique was optimized by assessing the recoveries of target compounds. 50 ng of the target EDCs standards was spiked initially into each blank sample (quartz sand) and fish sample, and 50 ng of the internal standards was added into the PLE extract. Each experimental group included five replicates, and two blank controls were tested for a batch.

2.3. Clean-up of extracts

Two protocols were proposed to cleanup the extracts. For the sake of comparison, the recoveries of target compounds and purification effects were taken into account in each protocol.

Protocol 1: The concentrated extract (5 ml) was stored at −24 °C for 24 h. When most of the lipids were precipitated or suspended, they could be easily removed by filtration. The filtered extract was mixed with 10 ml of n-hexane and vigorously shaken for 1 min. After standing for stratification, the supernatant n-hexane was discarded. After defatted twice with n-hexane, the extract was transferred into a 5 ml tube and evaporated to near dryness under a gentle stream of N_2 at 40 °C.

Protocol 2: The concentrated extract (5 ml) was mixed with 100 ml of ultrapure water, and pumped through an HLB cartridge (rinsed with 15 ml of methanol and pre-conditioned with 10 ml of water) at a flow rate of 2 ml/min. After washed with 10 ml of water–methanol (9:1, v/v), the SPE cartridge was washed with 20 ml of n-hexane, and then dried under vacuum for 30 min. The analytes were eluted with 10 ml of methanol at a flow rate of 1 ml/min. The eluent was evaporated to near dryness under a gentle stream of N_2 at 40 °C.

The dry extract residue was redissolved in 1 ml of acetonitrile–water (1:1, v/v), and filtered through a 0.22 μm PES filter prior to the UHPLC–MS/MS analysis. Fifty nanogram EDC and internal standards were spiked into 5 ml of blank solutions to estimate the recovery of the two protocols. Each experimental group replicated five times and two blank controls were set for each experiment batch. Finally, the two protocols were also applied onto fish samples as well to compare the performance of the impurity elimination.

2.4. UHPLC–MS/MS analysis

The target EDCs were analyzed by a Waters Acquity™ ultra high performance liquid chromatograph–tandem mass spectrometer (UHPLC–MS/MS) system with an HSS T3 (2.1 mm × 100 mm, 1.7 μm particle size) column. The column temperature was set at 40 °C and the flow rate was 0.4 ml/min. Milli-Q water and acetonitrile–methanol (1:2, v/v) were used as the mobile phases, A and B, respectively. The UPLC gradient program was performed as follows: 45% A (0 min), 50% A (7.6 min), 100% A (8 min) and finally 45% A (10 min). The sample volume injected was 4 μl. The entire analysis time was 10 min, with 2 min for flushing the column and reestablishing the initial conditions. A mass spectrometric analysis was conducted on a Waters triple quadrupole tandem mass spectrometer with a Z-spray electrospray interface (Waters Corp., Manchester, UK). All target EDCs in this study are measured in negative ion mode $[M-H]^-$. The flow rate and temperature of the desolvation gas (N_2) were 800 l/h and 500 °C, respectively. The flow rate of the collision gas (Ar) was 0.17 ml/min, and the capillary voltage was 2.8 V.

3. Results and discussion

3.1. UHPLC–MS/MS conditions

The optimization of the MS/MS conditions for each target compound was performed by the direct infusion of 50 ng/ml standard solution in combination with 1:1 eluent flow (eluents A and B). Parameter tuning was performed in negative ion mode to maximize sensitivity. Two product ions were selected. The most stable and intense fragment ion was used for quantification, and the second transition was used for confirmation. The dwell times were established to obtain at least 12 data points across the narrowest peak in the window. The selected multiple reaction monitoring (MRM) transitions as well as the individual cone voltages and collision

energy applied to each compound are shown in Table S1 (Supplementary material). All the precursor ions were $[M-H]^-$ except for BPA-d_{16}. This is caused by the transformation of BPA-d_{16} into BPA-d_{14} in water, as the two acidic deuterium atoms of BPA-d_{16} are immediately exchanged against protons when dissolved in a protic medium [37]. The selection of the conditions in ESI-MS/MS detection is dependent on the separation efficiency of the compounds. The mobile phase parameters were optimized in order to achieve effective separation and maximum sensitivity for detection. In comparing methanol–water with acetonitrile–water as the mobile phase, the former gave significantly higher signal for the standard solution, but relatively worse separation of compounds, especially for E1 and E2; while acetonitrile–water produced better separation of compounds, but lower signal. This is probably due to the higher boiling point of acetonitrile in comparison to methanol. Therefore, the desolvation of the droplets formed is probably less favorable in the presence of acetonitrile [38]. Consequently, acetonitrile–methanol (1:2, v/v) was chosen as the optimum organic mobile phase to obtain effective separation and relatively high signal (Fig. 2).

3.2. Optimization of the PLE process

Methanol and acetonitrile were selected as the extraction solvents, due to their good solubility of the target EDCs and immiscibility with n-hexane, which was used in following the clean-up step. The fraction of methanol was not more than 50% due to its great extracting capacity for polar interferences. As shown in Fig. 3, the water content in alumina is very critical for extraction efficiency, but an increase in the alumina amount shows no significant effect on the on-line purification and recoveries of the target EDCs. Compared to acetonitrile, methanol–acetonitrile generally produces better extraction efficiency, especially for EE2 (Fig. 3). Therefore, 5 g alumina with 5% water and acetonitrile–methanol (1:1, v/v) were considered as the optimum conditions for PLE on-line purification with an extraction recovery of 69.6–89.3%. When applied to the fish samples, it was obvious that the extract from the PLE on-line purification was cleaner than that without on-line purification.

The PLE parameters were selected based on the optimized on-line purification conditions. The extraction percentage of the target EDCs was more than 80% in cycle 1, about 5–10% in cycle 2, and less than 3% in cycle 3 (Fig. S1, Supplementary material). Therefore, three cycles are sufficient for the complete extraction of target EDCs from fish samples. As shown in Fig. 4a, the recovery rates for E1, E2 and EE2 are all more than 90% and show no significant difference in various durations of static extraction. However, for E3 and BPA, 5 min of static extraction shows the best recoveries. The recovery rates of target EDCs at different extraction temperatures are shown in Fig. 4b. For E1, E2 and EE2, the recoveries slightly decrease with an increase in the temperature from 40 to 100 °C; while for E3 and BPA, the recoveries first increase and then decrease when the extraction temperature was set above 60 °C. In the extraction process, heating was generally considered to increase the kinetics of the extraction [39], but it was shown that E3 and BPA experience thermal degradation when the temperature is above 60 °C. Therefore, the extraction temperature was set at 60 °C to achieve >80% recovery for each target EDC.

3.3. Optimization of the cleanup procedure

Freezing-lipid filtration was reported to be used in the clean-up of biological samples (such as fish and meat), which can eliminate about 90% of lipids in extracts without any significant loss of the target compounds [32,33,40]. N-hexane was usually applied to eliminate non-polar lipid in analysis of polar compounds. Recently, SPE was most frequently applied to cleanup complex matrix, among all the SPE cartridges, HLB SPE cartridge was known to have the unique ability to retain a wide spectrum of compounds, and reported to show the best recoveries of phenolic and steroidal EDCs in water [41].

Table 2
The mean recoveries of target EDCs based on two cleanup protocols.

Compound	Protocol 1		Protocol 2	
	Recovery rate %	RSD %	Recovery rate %	RSD %
E1	98.5	1.7	72.1	5.5
E2	105	2.4	93.4	2.4
E3	93.0	5.1	66.4	17
EE2	106	2.8	107	1.0
BPA	96.3	3.7	165	26

Freezing-lipid filtration combined with n-hexane defatting (Protocol 1) and SPE with n-hexane washing (Protocol 2) were used as two clean-up alternatives. As shown in Table 2, protocol 1 obtains satisfactory recoveries of 93.0–106% with RSDs of 1.7–5.1%. The freezing points of the target EDCs are far below −24 °C, at which most lipids could be precipitated or condensed and easily removed by filtering. However, in protocol 2, the recovery of E3 declines to 66.4% with an RSD of 17%, and BPA recovery is 165% with an RSD of 26%. It is probably due to the contamination from SPE cartridge. In addition, the efficiency of SPE is generally sensitive to the elution solvent, flow rate, and sample properties [41]. The SPE technique is generally effective for the concentration and clean-up of water or sediment samples. However, when it is applied to fish samples, lipid elimination is not as effective and further clean-up is required. Therefore a simple and low cost clean-up procedure described in protocol 1 is suggested for fish samples.

3.4. Method validation

Blank samples were inserted into each batch to check for background contamination and no target EDCs were detected from the blank controls in our experiments. The ME was evaluated by a comparison of the responses of standard compounds in a neat standard solution and spiked fish sample (after pretreatment), and replicated five times. The ME was calculated with formula (1) [42].

$$\text{Matrix effect (ME)\%} = 100 \times \left(\frac{B}{A}\right) \quad (1)$$

A is the corresponding peak area of each analyte in the neat standard solution and B is the peak area of each analyte obtained in the spiked blank fish samples.

The MEs of target EDCs are 115, 113, 101, 114 and 111% for E1, E2, E3, EE2 and BPA, with RSDs of 3.1–5.0%. The ME of each target EDC is greater than 100% except for E3, which is approximately equal to 100%, indicating that after preparation, the fish samples exhibit a certain ME on signal enhancement for E1, E2, EE2 and BPA. However, when quantified by an internal standard method, the MEs of the target compounds could be modified. According to a method described by Maragou et al. [38], the MEs were examined by constructing a correlation curve based on two sets of analytical parameters (ratio of analyte peak area to internal standard peak area). These parameters were obtained from spiked fish samples after sample preparation (plotted on the y axis) and the standard solutions of target EDCs (plotted on the x axis) at five fortification levels (5, 10, 50, 100 and 200 ng/ml) spiked with 50 ng/ml internal standard, respectively. The statistical parameters of the correlation curves for the ME evaluation are presented in Table 3. It turns out that the confidence interval of slope (b) contains the value 1 and the confidence interval of the intercept (a) contains the value 0. Therefore, the regression equation of the correlation curve could

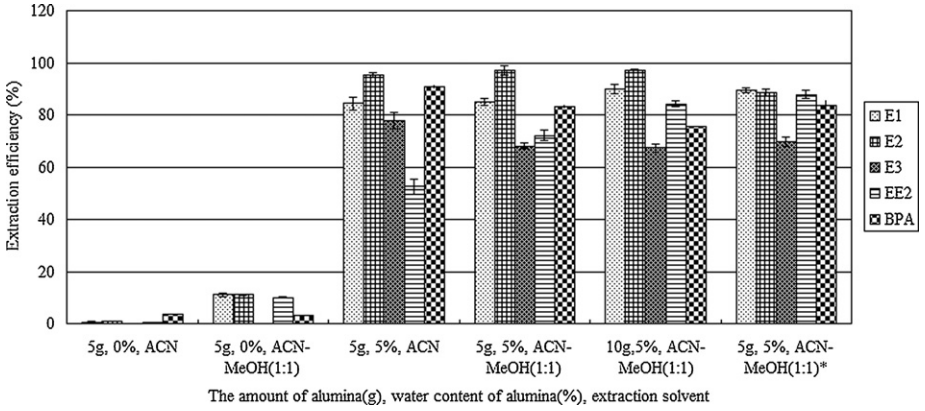

Fig. 3. Extraction efficiencies of target EDCs from spiked blank and fish samples* under different on-line purification conditions.

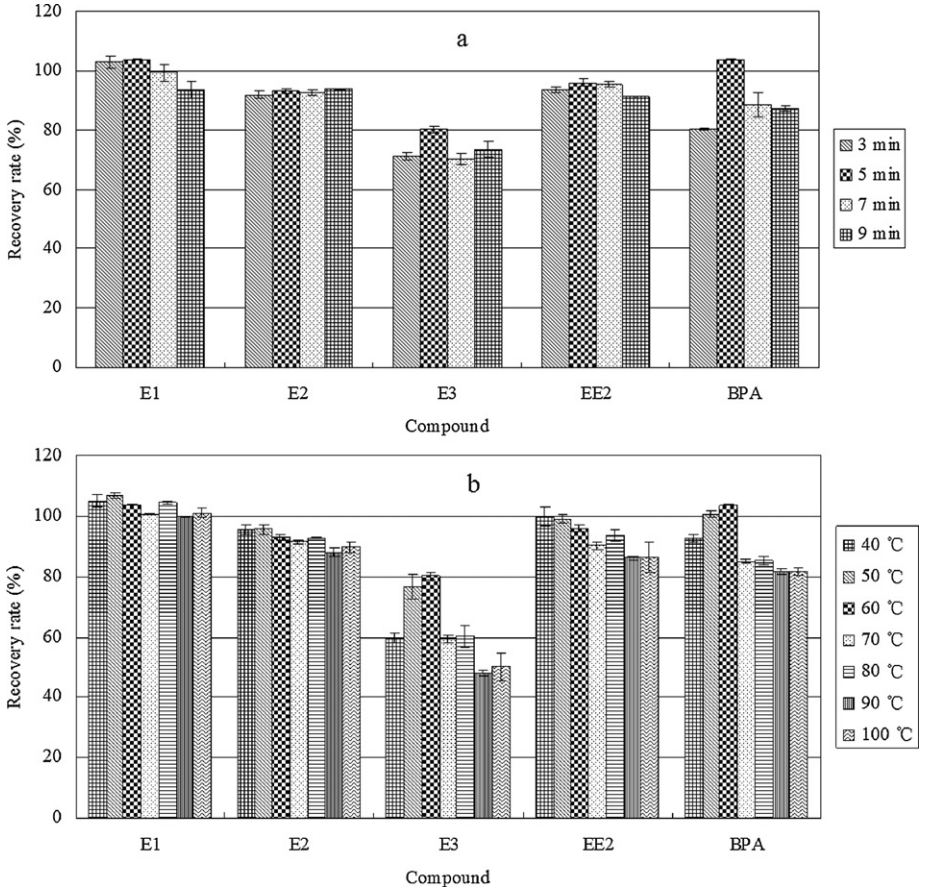

Fig. 4. Recoveries of target EDCs from spiked fish (a) under different static extraction times and (b) at different extraction temperatures.

Table 3
Statistical parameters of the correlation curve of analytical parameters of spiked fish samples and standard solutions.

Compound	E1	E2	E3	EE2	BPA
Slope (b)	1.0338	1.0147	0.9027	1.0295	1.0409
Standard deviation of slope (S_b)	0.0513	0.0432	0.0377	0.0320	0.0403
Intercept (a)	0.0548	0.0129	0.0195	0.0112	−0.0207
Standard deviation of intercept (S_a)	0.0490	0.0331	0.0226	0.0258	0.0348
Square correlation coefficient (r^2)	1	0.9999	0.9999	0.9999	0.9998
Number of test replicates (n)	5	5	5	5	5
t (confidence level: 95%, degrees of freedom: 4)	2.7764	2.7764	2.7764	2.7764	2.7764
Confidence interval 95% b: $b \pm t \times S_b/\mathrm{sqrt}(n)$	[1.0973, 0.9703]	[1.0681, 0.9613]	[0.9493, 0.8561]	[1.0691, 0.9899]	[1.0908, 0.9910]
Confidence interval 95% a: $a \pm t \times S_a/\mathrm{sqrt}(n)$	[0.1157, −0.0061]	[0.0540, −0.0282]	[0.0475, −0.0085]	[0.0432, −0.0208]	[0.0225, −0.0639]

Table 4
The mean recoveries (RSD)% for EDCs of fish samples spiked at three levels and the method LODs and LOQs.

Compound	Spiked level (ng/g dw)						LOD (ng/g dw)	LOQ (ng/g dw)
	5		50		100			
	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day		
E1	90.8 (12)	93.7(4.3)	103(0.92)	98.8(6.2)	99.4(3.5)	92.3 (11)	0.05	0.08
E2	90.0(9.0)	95.3(7.9)	96.0(3.0)	96.4(5.1)	89.9(3.1)	92.3(3.8)	0.06	0.11
E3	73.6 (16)	74.5(1.8)	72.1 (4.3)	75.7(8.4)	71.2 (10)	74.1 (5.5)	0.04	0.07
EE2	100 (13)	102 (2.8)	107 (1.0)	97.7(8.0)	103 (5.1)	100 (4.2)	0.08	0.27
BPA	88.8(6.3)	86.8 (16)	108 (11)	101 (12)	101 (5.3)	100(0.94)	0.05	0.12

be described as $y = x$ for the target EDCs except for E3, in which the y is slightly less than x. As a result, the analytical parameters of the spiked fish samples and standard solutions have no significant differences at the 95% confidence level for E1, E2, EE2 and BPA. Generally, the MEs are mainly eliminated by an internal standard method to determine target EDCs from fish samples.

The linearity of the UHPLC–MS/MS system for EDC determination was examined with a calibration curve, made by detecting seven different concentrations (1, 5, 10, 50, 100, 200 and 500 ng/ml) of standard solutions spiked with 50 ng/ml of internal standards, respectively. The linear regression analysis of the concentration and the response (analyte peak area/internal standard peak area) of the instrument obtained satisfying results with correlation coefficients exceeding 0.9999.

The method LODs and LOQs of target EDCs were measured by spiking blank fish samples at a series of different concentrations, and seven replicates were set for each concentration. The LOD and LOQ were determined as the analyte concentration that corresponds to a signal/noise (S/N) ratio of 3 and 10 [16,36], respectively. As shown in Table 4, the LODs are between 0.04 and 0.08 ng/g dw, while the LOQs are from 0.07 to 0.27 ng/g dw, which are far lower than previous methods (Table 1).

In order to validate the precision and accuracy of the method, blank fish samples spiked with three fortification levels (5, 50, and 100 ng/g) of target EDCs that contained 50 ng internal standard, respectively, were analyzed within one day with five replicates for the intra-day assay, and the five replicates were further determined in five different days over one week for the inter-day assay. The mean recoveries of the target EDCs in the fish samples ranged from 71.2% to 108% at spiked levels of 5, 50 and 100 ng/g, with RSDs less than 16% (Table 4). The results of the experiments confirmed that the method described above is validated with good reproducibility and satisfactory precision and accuracy for the simultaneous determination of steroidal and phenolic EDCs from fish samples.

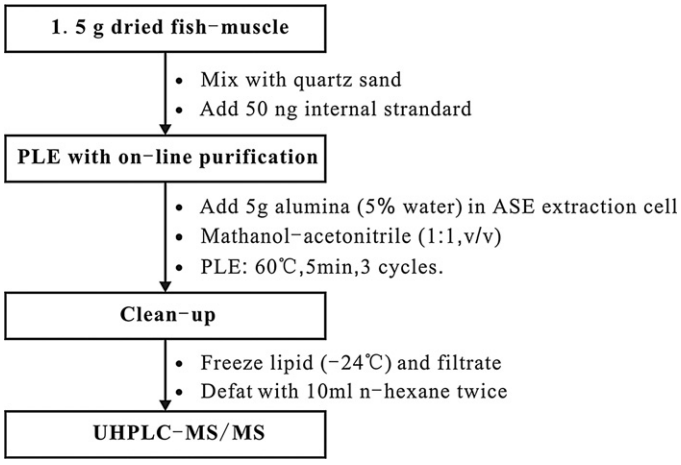

Fig. 5. Analysis procedure for EDCs in fish-muscle.

3.5. Application of the method

The method developed in this study is described in Fig. 5 and further applied to 30 fresh water fish samples purchased from Tongchuan Aquatic Product Market in Shanghai, January 2012. An example of MRM chromatograms of target EDCs in real samples is shown in Fig. S2 (Supplementary material). The testing results showed that E1, EE2 and BPA were detected (above the LOQ) in all fish samples at levels of 0.09–0.22, 0.47–0.84, and 1.97–8.47 ng/g dw, respectively, while E2 was found in 12 of the fish samples at a concentration of 0.12–0.60 ng/g dw and E3 from 0.13 to 1.13 ng/g dw in 15 of the fish samples. Obviously, BPA concentrations were relatively higher than other EDCs in fish samples analyzed in this study.

4. Conclusions

This study has developed a sensitive method based on the PLE followed by UHPLC–MS/MS for simultaneously determining steroidal and phenolic EDCs, including E1, E2, E3, EE2 and BPA, in fish samples. The PLE technique which uses on-line purification with 5 g alumina (containing 5% water) produces the best extraction efficiency, and the optimized parameters are as follows: 3 extractions with acetonitrile–methanol (1:1, v/v) solvent at 60 °C and static extraction for 5 min. The clean-up procedure of freezing-lipid filtration combined with n-hexane defatting has a better performance than HLB SPE clean-up, representing low cost and high efficiency in purification. This method is validated to give satisfactory accuracy and precision, and present no ME through the use of internal standards. Therefore, a low LOQ in the method could be realized and trace level concentrations of target EDCs are detected in the fish samples from the local market as low as 0.09 ng/g dw based on the high sensitivity of the detection system and "clean" samples after pretreatment.

Acknowledgements

The work was funded by the National Natural Science Foundation of China (41271473, 41130525, 41171376 and 40901256) and the Fundamental Research Funds for the Central Universities. Additional support was provided by the State Key Laboratory of Estuarine and Coastal Research (grant number: 2010RCDW01) and large instruments Open Foundation of East China Normal University. We thank Hao Shi and Xianbao Huang for their assistance in sample pretreatment.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2013.01.008.

References

[1] T. Colborn, F.S. vom Saal, A.M. Soto, Environ. Health Perspect. 101 (1993) 378.
[2] L.A. Jones, R.A. Hajek, Environ. Health Perspect. 103 (Suppl. 7) (1995) 63.
[3] J.P. Sumpter, Acta Hydroch. Hydrob. 33 (2005) 9.
[4] O. Tsutsumi, J. Steroid Biochem. 93 (2005) 325.
[5] A. Casanova-Nakayama, M. Wenger, R. Burki, E. Eppler, A. Krasnov, H. Segner, Mar. Pollut. Bull. 63 (2011) 412.
[6] J.P. Sumpter, S. Jobling, Environ. Health Perspect. 103 (Suppl. 7) (1995) 173.
[7] D.G.J. Larsson, M. Adolfsson-Erici, J. Parkkonen, M. Pettersson, A.H. Berg, P.E. Olsson, L. Förlin, Aquat. Toxicol. 45 (1999) 91.
[8] C.E. Purdom, P.A. Hardiman, V.J. Bye, N.C. Eno, C.R. Tyler, J.P. Sumpter, Chem. Ecol. 8 (1994) 275.
[9] A.V. Krishnan, P. Stathis, S.F. Permuth, L. Tokes, D. Feldman, Endocrinology 132 (1993) 2279.
[10] B.S. Rubin, J. Steroid Biochem. Mol. Biol. 127 (2011) 27.
[11] C. Desbrow, E.J. Routledge, G.C. Brighty, J.P. Sumpter, M. Waldock, Environ. Sci. Technol. 32 (1998) 1549.
[12] E.J. Routledge, D. Sheahan, C. Desbrow, G.C. Brighty, M. Waldock, J.P. Sumpter, Environ. Sci. Technol. 32 (1998) 1559.
[13] Z. Long, L. Hong, J. Jie, Chin. J. Anal. Chem. 35 (2007) 983.
[14] J. Chen, Q.L. Liang, H.Y. Hua, Y.M. Wang, G.A. Luo, M.Q. Hu, Y.Q. Na, Talanta 80 (2009) 826.
[15] J. Aufartová, C. Mahugo-Santana, Z. Sosa-Ferrera, J.J. Santana-Rodríguez, L. Nováková, P. Solich, Anal. Chim. Acta 704 (2011) 33.
[16] B. Shao, H. Han, D.M. Li, Y.L. Ma, X.M. Tu, Y.N. Wu, Food Chem. 105 (2007) 1236.
[17] F. Yu, X.J. Pan, B. Wang, Int. J. Environ. Anal. Chem. 92 (2012) 1532.
[18] T. Raecker, B. Thiele, R.M. Boehme, K. Guenther, Chemosphere 82 (2011) 1533.
[19] C. Postigo, M. Kuster, M. Villagrasa, S. Rodríguez-Mozaz, R. Brix, M. la Farré, M.L. de Alda, D. Barceló, Hdb. Environ. Chem. 5J (2009) 227.
[20] D. Matějíček, J. Chromatogr. A 1231 (2012) 52.
[21] F. Chen, G.G. Ying, J.F. Yang, J.L. Zhan, L. Wang, J. Environ. Sci. Health B 45 (2010) 682.
[22] Z.L. Zhang, S.M. Rhind, C. Kerr, M. Osprey, C.E. Kyle, Anal. Chim. Acta 685 (2011) 29.
[23] A. Hibberd, K. Maskaoui, Z. Zhang, J.L. Zhou, Talanta 77 (2009) 1315.
[24] Y.F. Nie, Z.M. Qiang, H.Q. Zhang, C. Adams, J. Chromatogr. A 1216 (2009) 7071.
[25] Y. Yu, L.S. Wu, Talanta 89 (2012) 258.
[26] R. Jeannot, H. Sabik, E. Sauvard, T. Dagnac, K. Dohrendorf, J. Chromatogr. A 974 (2002) 143.
[27] A. Arditsoglou, D. Voutsa, Environ. Sci. Pollut. Res. 15 (2008) 228.
[28] M. Kuster, M.J. López-de-Alda, D. Barceló, Trends Anal. Chem. 23 (2004) 790.
[29] V. Gabet, C. Miège, P. Bados, M. Coquery, Trends Anal. Chem. 26 (2007) 1113.
[30] S. Görög, J. Pharm. Biomed. Anal. 55 (2011) 728.
[31] H. Tomšíková, J. Aufartová, P. Solich, Z. Sosa-Ferrera, J.J. Santana-Rodríguez, L. Nováková, Trends Anal. Chem. 34 (2012) 35.
[32] J. Seo, H.Y. Kim, B.C. Chung, J. Hong, J. Chromatogr. A 1067 (2005) 303.
[33] Y.G. Ahn, J.H. Shin, H.Y. Kim, J. Khim, M.K. Lee, J. Hong, Anal. Chim. Acta 603 (2007) 67.
[34] Y. Liu, Y.T. Guan, T. Mizuno, H. Tsuno, W.P. Zhu, J. Chromatogr. 69 (2009) 65.
[35] P. Navarro, J. Bustamante, A. Vallejo, A. Prieto, A. Usobiaga, S. Arrasate, E. Anakabe, E. Puy-Azurmendi, O. Zuloaga, J. Chromatogr. A 1217 (2010) 5890.
[36] J.L. Liu, X.J. Pan, B. Huang, K. Fang, Y. Wang, J.P. Gao, Int. J. Environ. Anal. Chem. 92 (2011) 1135.
[37] K. Inoue, M. Kawaguchi, Y. Funakoshi, H. Nakazawa, J. Chromatogr. B 798 (2003) 17.
[38] N.C. Maragou, E.N. Lampi, N.S. Thomaidis, M.A. Koupparis, J. Chromatogr. A 1129 (2006) 165.
[39] M.M. Schantz, Anal. Bioanal. Chem. 386 (2006) 1043.
[40] J. Hong, H.Y. Kim, D.G. Kim, J. Seo, K.J. Kim, J. Chromatogr. A 1038 (2004) 27.
[41] R. Liu, J.L. Zhou, A. Wilding, J. Chromatogr. A 1022 (2004) 179.
[42] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Trends Anal. Chem. 75 (2003) 3019.