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A sensitive method for extraction and determination of endocrine-disrupting compounds from wastewater using 10-ethyl-acridone-2-sulfonyl chloride as pre-column labeling reagent by high-performance liquid chromatography with fluorescence detection

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ABSTRACT

A sensitive pre-column derivatization method using 10-ethyl-acridone-2-sulfonyl chloride (EASC) as pre-column labeling reagent followed by high-performance liquid chromatography (HPLC) with fluorescence detection has been developed for the determination of eight endocrine-disrupting compounds: 4-octylphenol, 4-nonylphenol, bisphenol A, diethylstilbestrol, estrone, 17α-ethynylestradiol, 17β-estradiol and estriol in wastewater samples. Solid phase extraction (SPE) with ODS C18 cartridges was used for the extraction and purification. Derivatizing parameters including pH value, temperature and concentration of EASC, as well as types of SPE cartridges and eluents were investigated in detail. Under optimal conditions, the quantification limits for the desired compounds ranging from 1.0 to 2.0 ng L^{-1} were obtained. The recoveries were higher than 80.4% with a noticeable improvement for 4-octylphenol and 4-nonylphenol, whose recoveries were usually lower than 50% during the SPE process. The proposed method was successfully applied to the determination of the target compounds in wastewater samples with a much higher sensitivity than traditional HPLC method.

1. Introduction

Nowadays, more and more chemicals have been confirmed to possess endocrine-disrupting properties. They have been known to interfere with endocrine systems by mimicking, blocking and triggering actions of hormones and thereby influence the health and reproductive system of humans and wildlife [1–3]. Compounds identified as endocrine-disrupting compounds (EDCs) are members of different groups of chemicals, including drugs, pesticides, industrial by-products, alkylphenols, synthetic steroids, and so on. Belonging to the endocrine-disrupting phenolic family, estrogenic compounds, including xenoestrogens and endogenous estrogens, have gained increasing environmental and social concerns in recent years [4–10]. Among these estrogenic compounds, some are derived from excreta of humans and livestock (e.g. 17β-estradiol, estriol and estrone); some are synthetic estrogens added in feedstuffs for animals to promote their growth (e.g. diethylstilbestrol) or for contraceptive purpose (e.g. 17α-ethynylestradiol), and some are widely used industrial material (e.g. 4-nonylphenol, 4-octylphenol and bisphenol A). Because of the incomplete removal of these EDCs from wastewater treatment plants, they have a very high incidence to be found in environmental samples [11–14], especially for 4-nonylphenol, 4-octylphenol and bisphenol A, which were reported to be found all over the world [15–19].

The potential effects of EDCs on human health have been highlighted in recent years [20–22]. EDCs can lead to serious side effects on human health at very low concentrations and these side effects are often potent and long-lasting. They are now suspected of disrupting reproduction for humans and wildlife, increasing the incidences of cancers, and some were reported to have transgenerational effects [23,24]. These compounds usually exist simultaneously in our living environment with a self-aggravating tendency from year to year. Therefore, it is necessary to develop a rapid, simple and sensitive method for the simultaneous determination of these compounds in the environment.

With the development of analytical technologies, various approaches have been developed for the determination of EDCs, such as liquid chromatography (LC) [25–27], liquid chromatography–mass spectrometry (LC–MS) [28,29] and gas chromatography–mass spectrometry (GC–MS) [30–32]. Because of the absence of a chromophore or fluorophore group in their chemical structure (see Fig. 1), these compounds commonly exhibit low ultraviolet absorption and poor fluorescence property. Therefore, the sensitivity for the direct determination of these compounds by LC methods is usually limited,

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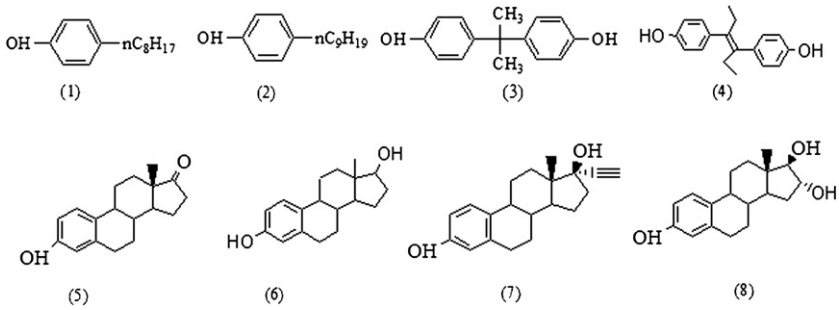

Fig.1. Structures of EDCs studied in this work. Compound: (1) 4-octylphenol; (2) 4-nonylphenol; (3) bisphenol A; (4) diethylstilbestrol; (5) estrone; (6) 17β-estradiol; (7) 17α-ethynylestradiol; (8) estriol.

while the sensitivity of LC–MS methods is also restricted as estrogenic compounds exhibit low ionization efficiency in MS ion chamber. Furthermore, the methods of GC–MS and LC–MS are expensive and not accessible in ordinary laboratories.

In this work, a pre-column derivatization method for the simultaneous determination of eight EDCs using EASC as labeling reagent was developed. In order to obtain lower detection limits and assure better recoveries for all the target compounds, the pretreatment conditions for the extraction and purification of eight EDCs from wastewater samples using various types of the SPE cartridges were optimized. At the same time, the derivatization conditions were modified to enhance sensitivity. Based on the development of the previously proposed method in our laboratory [33], a simple and sensitive LC method was built for the simultaneous determination of eight EDCs in water samples. The established method was successfully applied to the analysis of EDCs in wastewater samples.

2. Materials and methods

2.1. Chemicals

Analytical standards of 4-octylphenol (OP), 4-nonylphenol (NP), bisphenol A (BPA), diethylstilbestrol (DES), estrone (E1), 17α-ethynylestradiol (EE2), 17β-estradiol (E2), and estriol (E3) were all obtained from Dr. Ehrenstorfer (Ausburg, Germany) with purity higher than 99%. Methanol, dichloromethane, ethyl acetate, n-hexane and acetonitrile were of HPLC grade (Shandong Yuwang Industrial Co., Ltd., China). Water was purified on a Milli-Q system (Millipore, Bedford, MA, USA). All other reagents used were of HPLC grade or at least of analytical grade. ODS C18 cartridges (500 mg, 6 mL) were obtained from Chrome Expert (CA, USA), Oasis HLB cartridges (60 mg, 3 mL) were obtained from Waters (Milford, MA, USA), SLH cartridges (500 mg, 6 mL) were purchased from Hangzhou Fuyu Technology Services Co., Ltd., China and Bond Elute Carbon cartridges (500 mg, 6 mL) were purchased from Agilent (CA, USA), respectively. 10-Ethyl-acridone-2-sulfonyl chloride was prepared according to the method previously described in our laboratory [33].

Individual stock solutions of 100 mg L^{-1} for all compounds were prepared in HPLC-grade acetonitrile and stored at 4 °C in the dark. Standard solutions containing all compounds were mixed and diluted with acetonitrile, and working solutions of all compounds and calibration concentrations were prepared by appropriate dilution of the stock solutions on the day of analysis.

The derivatizing reagent solution (1.0×10^{-3} mol L^{-1}) was prepared by dissolving 3.21 mg EASC in 10 mL of anhydrous acetonitrile. When not in use, all reagent solutions were stored at 4 °C in a refrigerator.

2.2. Instrumentation

The HPLC analysis was performed using an Agilent 1100 Series HPLC system, equipped with an on-line-degasser, a quaternary pump, an autosampler and a thermostated column compartment. A fluorescence detector (model G1321A, Agilent, USA) was adjusted at wavelengths 262 and 430 nm for excitation and emission. Chromatographic separation was achieved on a Hypersil C18 column (200×4.6 mm, 5 μm i.d., Dalian Elite Analytical Instruments Co., Ltd., China). A Paratherm U2 electronic water-bath (Hitachi, Tokyo, Japan) was used to control the temperature.

2.3. Extraction and purification

Wastewater samples were collected from a local river to which domestic sewage was discharged. Before extraction, water samples were filtered through 0.45 μm pore size cellulose filters to remove fine particles, and then adjusted to pH 3.0 with 6 M HCl solution to ensure that all the compounds existed in their molecular form. Three extraction solvents including ethyl acetate, n-hexane and dichloromethane were, respectively, applied for the liquid–liquid extraction of EDCs from water samples. A 50 mL of extraction solvent was added into a separating funnel containing 500 mL of water sample. After vigorous shaking, the produced cloudy solution was allowed to stand undisturbed until the two layers separated, then the organic layer was collected and another 30 mL of extraction solvent was added into the same water sample for further extraction. The organic layers of the two times were pooled and evaporated to near 1 mL in a rotary vacuum evaporator at 40 °C, then it was transferred into a 2-mL vial and evaporated to near dryness for later derivatization.

Solid phase extraction was also used for the extraction and purification of EDCs. Water samples (500 mL) were forced to pass through the SPE cartridges at a flow rate of approximately 5 mL/min^{-1}. After washing with appropriate solution, the cartridges were dried under vacuum for 10 min and then the analytes were eluted with corresponding solutions. The SPE systems and their corresponding procedure are listed in Table 1. The eluted solution was evaporated to near 1 mL under a gentle stream of nitrogen gas at 40 °C. It was then transferred into a 2-mL vial and further evaporated to dryness for derivatization.

2.4. Derivatization procedure

The derivatization of EDCs with EASC proceeded in aqueous acetonitrile as previously described [33] with a little modification. To a solution containing an appropriate amount of standard or sample in a 2-mL vial, 80 μL 0.1 M $NaHCO_3$ buffer (pH 10.2), 200 μL acetonitrile and 50 μL EASC acetonitrile solution were added. The vial was sealed and vortexed for 1 min and then allowed to react at 60 °C for 5 min in a water bath. After the reaction was completed, the mixture was cooled to room temperature; 20 μL 50% acetic acid solution was then added to neutralize pH close to 7.0. The derivatized sample solution was then diluted to 500 μL with water–acetonitrile (1/1, v/v) and

Table 1
Overview of SPE systems tested for extraction and purification.

Number	SPE cartridges	Pre-conditioning	Washing	Elution
1	HLB	10 mL methanol, 10 mL water	10 mL methanol–water (1/9, v/v)	10 mL methanol
2	HLB	10 mL ethyl acetate, 10 mL methanol, 10 mL pH = 3 water	10 mL methanol–water (1/9, v/v)	10 mL ethyl acetate
3	HLB	10 mL ethyl acetate, 10 mL methanol, 10 mL pH = 3 water	10 mL methanol–water (1/9, v/v)	10 mL ethyl acetate–methanol (9/1, v/v)
4	HLB	10 mL methanol, 10 mL water	10 mL methanol–water (1/9, v/v)	7 mL methanol, 5 mL dichloromethane
5	HLB	10 mL acetonitrile, 10mLwater	10 mL water	10 mL acetonitrile–dichloromethane (8/2, v/v)
6	HLB	5 mL ethyl acetate, 5 mL methanol, 5 mL water	10 mL methanol–water (1/9, v/v)	5 mL dichloromethane–n-hexane (1/9, v/v), 7 mL ethyl acetate
7	C18	10 mL ethyl acetate, 10 mL methanol, 10 mL pH = 3 water	10 mL methanol–water (1/9, v/v)	10 mL ethyl acetate–methanol (9/1, v/v)
8	C18	10 mL acetonitrile, 10mLwater	10 mL water	10 mL acetonitrile–dichloromethane (8/2, v/v)
9	C18	10 mL methanol, 10 mL water	10 mL methanol–water (1/9, v/v)	10 mL methanol
10[a]	C18	5 mL ethyl acetate, 5 mL methanol, 5 mL water	10 mL methanol–water (1/9, v/v)	5 mL dichloromethane–n-hexane (1/9, v/v), 7 mL ethyl acetate
11	Bond Elute Carbon	10 mL methanol–dichloromethane (7/3, v/v)	10 mL water	10 mL methanol–dichloromethane (7/3, v/v)
12	Bond Elute Carbon	10 mL ethyl acetate–acetone (5/5, v/v)	10 mL water	10 mL ethyl acetate–acetone (5/5, v/v)
13	SLH	10 mL ethyl acetate, 10 mL methanol, 10 mL pH = 3 water	10 mL methanol–water (1/9, v/v)	10 mL ethyl acetate

[a] SPE system applied in this method.

injected directly for HPLC analysis. The derivatization process is shown in Fig. 2.

2.5. HPLC conditions

HPLC separation was carried out on a Hypersil C18 column in combination with a gradient elution. Eluent A was 5% acetonitrile and B was acetonitrile. The flow rate was constant at 1.0 mL min^{-1} and the column temperature was kept at 30 °C. The elution conditions were as follows: 50–95% B from 0 to 15 min and then held for 5 min. The column was equilibrated with the initial mobile phase for 5 min before the next injection. The injection volume was 10 μL. The fluorescence excitation and emission wavelengths were set at λ_{ex} 262 nm and λ_{em} 430 nm, respectively.

2.6. Quantification

Quantitative analysis was carried out by a series of injections of target compounds in the concentration range from 2.0 to 400 ng mL^{-1}. Calibration curve was constructed for each compound by plotting peak area versus concentration, and all target compounds from extracted water samples were measured using the external standard method.

3. Result and discussion

3.1. Optimization of derivatization parameters

3.1.1. Effect of sodium bicarbonate buffer on derivatization

Several kinds of buffers were tested in this study for derivatization, including sodium bicarbonate buffers, phosphate buffers and borate buffers. The results indicated that sodium bicarbonate buffers are superior to the other two buffers. The effect of pH on the derivatization reaction was then evaluated with sodium bicarbonate buffer (0.1 M) in the pH range of 7–11.5. The maximum derivatization yields were achieved in the pH range of 10.0–10.5. The detector responses for EDC derivatives obviously decreased when pH value was lower than 8 or higher than 11. With pH > 11, the low responses should be attributed to the hydrolysis reaction of products. As a result, 0.1 M sodium bicarbonate buffer with pH 10.2 was applied for all subsequent derivatization.

3.1.2. Effect of EASC concentration on derivatization

BPA and DES have two phenolic hydroxyl groups to easily react with EASC under the proposed conditions. It was believed that the mono-substituted and disubstituted forms were significantly affected by the amount of EASC added. The effects of EASC concentrations in the range of 1.0×10^{-4}–1.0×10^{-3} mol L^{-1} on the mono-substituted and disubstituted forms were investigated for BPA and DES derivatives. The results indicated that the completely disubstituted derivatives could be obtained when the EASC concentration was $\geq 1.0\times10^{-3}$ mol L^{-1}; increasing the excess of EASC beyond this level had no significant effect on the yields of disubstituted derivatives. With EASC concentration lower than 2.0×10^{-4} mol L^{-1}, BPA and DES gave mainly mono-substituted derivatives, but derivatization for other compounds was incomplete. To obtain complete disubstituted derivatives, a large excess of labeling reagent was used. On the other hand, when EASC concentration was beyond 1.0×10^{-3} mol L^{-1}, a serious overload with a large negative peak for fluorescence detector was observed. To avoid such a serious overload, EASC concentration of 1.0×10^{-3} mol L^{-1} was selected for all subsequent experiments. In this case, BPA and DES gave mainly

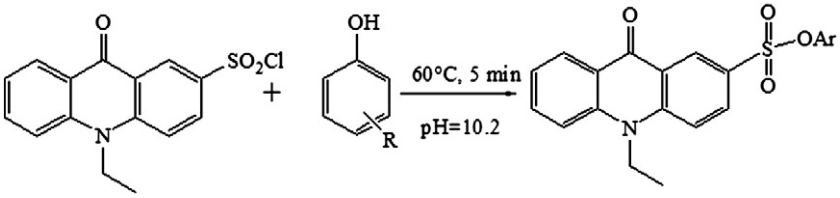

Fig. 2. Derivatization scheme of EASC with EDCs.

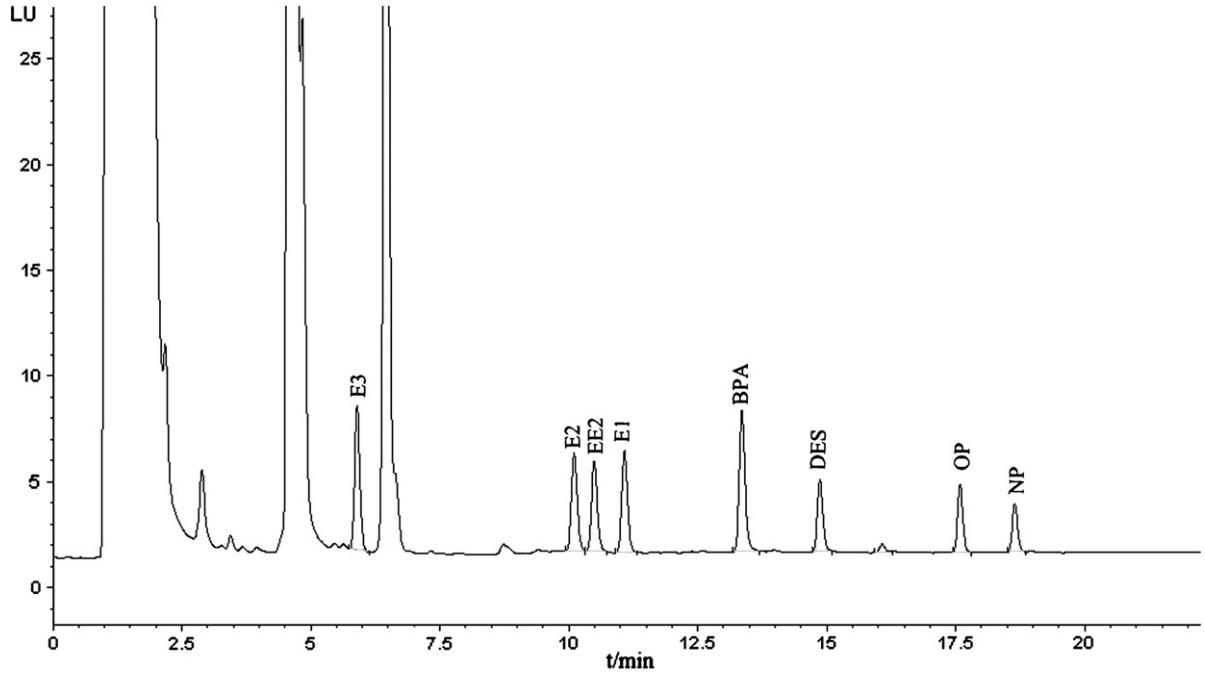

Fig. 3. HPLC chromatogram of 10 μg L^{-1} derivatized EDCs. E3: estriol; E2: 17β-estradiol; EE2: 17α-ethynylestradiol; E1: estrone; BPA: bisphenol A; DES: diethylstilbestrol; OP: 4-octylphenol; NP: 4-nonylphenol.

disubstituted derivatives, but their mono-substituted derivatives were also slightly observed. The amounts of mono-substituted BPA and DES derivatives were sufficiently small compared to those obtained from their disubstituted derivatives (peak area ratio<1.0%), therefore there was no obvious effect on the quantitative results for the determination of BPA and DES.

3.1.3. Effect of reaction temperature on derivatization

The effect of reaction temperature on the fluorescence spectra of EDC derivatives was tested over the temperature range from 20 °C to 80 °C. The results indicated that the complete derivatization could be achieved at 60 °C for 5 min. With reaction temperature <60 °C, a long derivatization time was needed to obtain a constant response. As expected, a high temperature (>70 °C) needed a relatively short derivatization time, but an obviously low response was also observed. This should be attributed to the fact that high temperature results in the hydrolysis of the derivatives in basic media. Based on these results, derivatization was performed at 60 °C for 5 min with pH of 10.2.

Table 2
Performance of SPE systems tested for extraction and purification (n = 3).

Number [a]	Recovery (%)							
	OP	NP	BPA	DES	E1	EE2	E2	E3
1	24	31	90	37	93	85	97	119
2	32	36	109	54	94	76	91	75
3	47	36	96	80	103	100	102	91
4	20	40	56	149	114	90	84	140
5	30	48	124	99	106	97	96	102
6	46	34	80	88	92	86	87	86
7	50	40	92	90	95	89	88	101
8	42	31	82	94	119	101	89	104
9	40	31	90	83	89	99	98	105
10	92	80	91	92	96	90	88	103
11	40	29	48	49	75	72	64	99
12	5.1	3.8	0	0	4.4	9.5	8.9	19
13	15	10	98	99	95	90	91	96

[a] Number is proportional to Table 1.

3.1.4. Stability of EDC derivatives

Anhydrous acetonitrile solution of EASC could be stored at 4 °C for 1 week without obvious decrease in derivatization yields for EDCs compared to those newly prepared EASC solution. The stabilities of the corresponding derivatives were also investigated. A standard solution containing 20 μg L^{-1} EDCs was derivatized and neutralized according to the procedure described in Section 2.4. This solution was repeatedly analyzed by HPLC-FLD after being placed at room temperature for 0, 4, 8, 12, 24, 48 and 72 h, respectively. The corresponding derivatives were stable for normalized peak areas with relative standard deviations (RSDs) of less than 3.5%, and therefore it can be concluded that the stability of EASC-EDC derivatives is sufficient for chromatographic analysis.

3.2. Sample extraction and purification

Liquid–liquid extraction is a very popular method for water sample handling due to its simplicity and low-costing. In this study, the extraction efficiency of EDCs from different water samples was, respectively, evaluated using ethyl acetate, n-hexane and dichloromethane as extraction solvents. The results indicated that n-hexane and ethyl acetate gave total recoveries, respectively, less than 20% and 50% for all eight estrogenic compounds. Dichloromethane gave acceptable recoveries for the extraction of OP and NP, but recoveries for the other six compounds were still relatively low (<70%). Liquid–liquid extraction using other

Table 3
Calibration curves, LODs and LOQs for EDC derivatives.

Analyte	Calibration equation	R	LOD (ng L^{-1})	LOQ (ng L^{-1})
OP	$Y = 0.94768681X + 4.1315569$ [a]	0.99744	0.5	1.5
NP	$Y = 0.72017766X + 2.799617$	0.99765	0.7	2.0
BPA	$Y = 2.24586316X + 10.596039$	0.99636	0.3	1.0
DES	$Y = 1.59305591X + 0.0192509$	0.99984	0.5	1.5
E1	$Y = 1.81344554X + 2.732754$	0.99963	0.4	1.4
EE2	$Y = 1.77407351X + 1.1483922$	0.99982	0.4	1.5
E2	$Y = 1.68398144X + 4.5506835$	0.99933	0.4	1.4
E3	$Y = 2.11069828X + 6.4817736$	0.99907	0.3	1.2

[a] X = theoretical concentration of EDC, Y = peak area.

Table 4
Recoveries of spiked samples with the proposed analytical method (n = 3).

Compound		OP	NP	BPA	DES	E1	EE2	E2	E3
Spiked level (ng L^{-1})	10	90.6 ± 3.6[a]	83.5 ± 4.5	96.4 ± 3.0	89.6 ± 5.0	93.5 ± 4.1	95.0 ± 4.6	90.5 ± 3.6	105 ± 4.7
	20	92.0 ± 2.5	80.4 ± 3.0	91.5 ± 3.6	92.8 ± 4.0	96.2 ± 3.6	90.6 ± 2.8	88.7 ± 3.4	99.2 ± 5.8
	100	88.6 ± 3.2	85.2 ± 4.3	93.2 ± 2.8	91.0 ± 3.6	92.6 ± 4.4	92.3 ± 3.7	91.6 ± 4.2	98.2 ± 5.0

[a] Data are expressed as mean recovery (%) ± S.D.

extraction solvents, such as n-butanol, ethyl pentanoate, chloroform and so on, still could not provide satisfactory results for the simultaneous extraction of all these compounds.

Different types of SPE cartridges were evaluated in order to obtain good recoveries with low organic solvent-consuming. As shown in Table 2, SLH cartridges provided good recoveries for the extraction of EDCs, with the exception of OP and NP, whose recoveries were lower than 20%. With Bond Elute Carbon cartridges, acceptable recoveries for E1, EE2 and E3 could be obtained, while the recoveries for other compounds were lower than 65%. HLB cartridges have been used more frequently than other cartridges for the extraction of EDCs as it could provide good recoveries for most of the target compounds. However, the recoveries for OP and NP were usually less than 50% [8,10,34]. The similar results were also observed in our experiment (see Table 2). To enhance recoveries of OP and NP on HLB cartridges, different eluents such as methanol, acetonitrile, ethyl acetate, dichloromethane and their mixture were also investigated. No significant improvement for their recoveries was observed.

C18 cartridges have similar properties for the extraction of organic compounds as do of HLB cartridges. However, they were more suitable for the extraction of low polar compounds as they were less polar than HLB cartridges. After a series of tests, an ideal elution for NP and OP had been obtained using a mixed solvent of n-hexane and dichloromethane (9/1, v/v) with recoveries of higher than 80%. Subsequently, other compounds could be eluted by using a more polar solvent, such as methanol and ethyl acetate or their mixture. Based on these results, the extraction of EDCs using C18 cartridges was as follows: elution of NP and OP with 5.0 mL of mixed solvent of n-hexane/dichloromethane (9/1, v/v) followed by 7.0 mL of ethyl acetate to elute other more polar estrogenic compounds. Under the proposed conditions, satisfying recoveries were obtained for all the target compounds (>80%).

3.3. HPLC separation

The complete HPLC separation of the derivatized EDCs could be easily accomplished using a Hypersil C18 column in combination with a gradient elution with water and acetonitrile as mobile phase composition (see Fig. 3). As expected, all the derivatives were separated within 19 min with a good baseline resolution. Here, BPA and DES were monitored by their disubstituted forms by using an excess of labeling reagent. Derivatization increased the hydrophobicity of BPA and DES, therefore they were eluted at increased retention times with less interference.

3.4. Linearity and detection limit

Linearity for each compound was established by analysis of standard solution ranging from 2.0 to 400 μg L^{-1}. The standard curves were found to be linear with correlation coefficients of >0.996. Limits of detection (LODs) and limits of quantification (LOQs) for all EDCs were calculated at a signal-to-noise (S/N) ratio of 3 and 10, respectively. As can be seen from Table 3, LOQs for eight EDCs ranged from 1.0 to 2.0 ng L^{-1}, lower than the US EPA recommended water quality criteria. The proposed method could be well used to monitor the residual concentration of those pollutants in water.

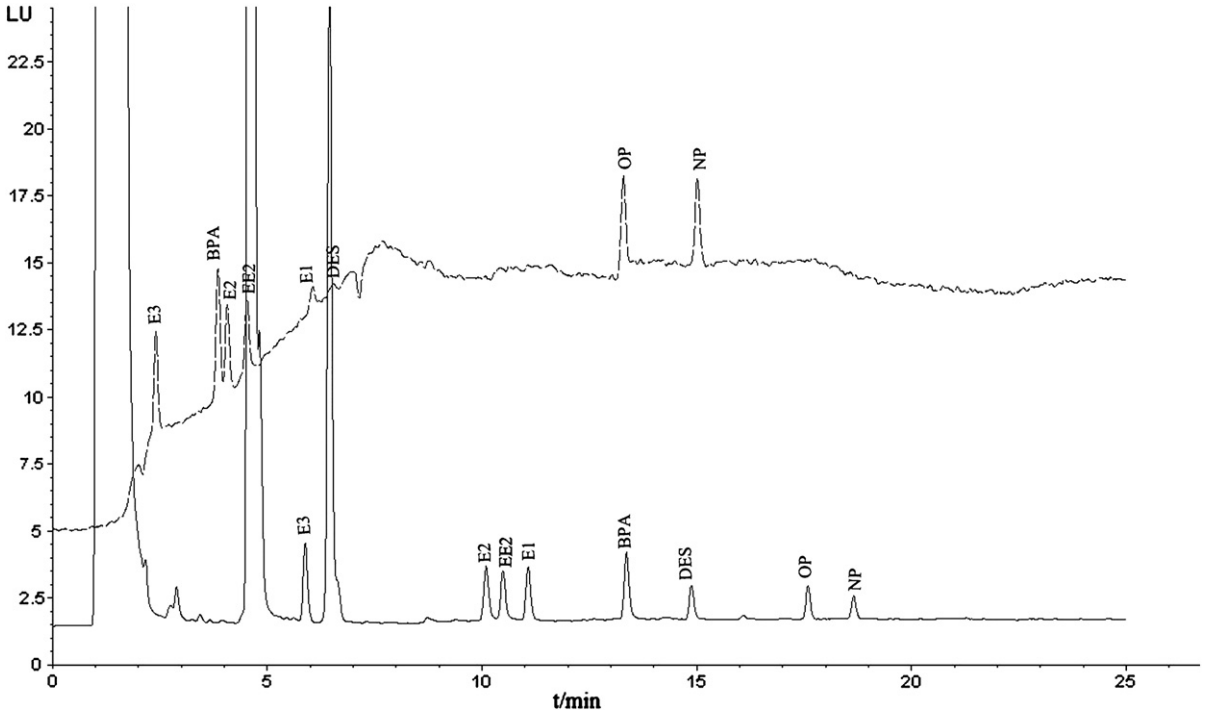

Fig. 4. Comparison of HPLC chromatograms of EDCs (continuous curve: 10 μg L^{-1} of EDC derivatives; interrupted curve: 1000 μg L^{-1} of EDCs without derivatization.).

Table 5
Comparison of the methods used before to the proposed method.

Article	Method	Volume of water	LOQ (ng L^{-1})							
			OP	NP	BPA	DES	E1	EE2	E2	E3
Quintana[13]	GC–MS	5 L	–[a]	–	–	2	3	5	3	6
Liu[32]	GC–MS	500 mL	–	2.6	17.4	–	5.6	2.6	11.2	–
Jeannot[10]	GC/MS/MS	250 mL	–	1	0.5	–	2	20	3	12
This article	HPLC	500 mL	1.5	2.0	1.0	1.5	1.4	1.5	1.4	1.2

[a] –: not included in the method.

3.5. Recoveries and precision

Recoveries were carried out by spiking blank samples with three different concentrations of standard solutions. Satisfactory recoveries were obtained for all the tested compounds (80.4%–105%), and the results are summarized in Table 4. Intra-day precision was determined by running a sample with spiked standards at a level of 20 ng L^{-1} with six replicates, and inter-day precision was determined by running a sample with spiked standards at the same level with three replicates on three different days over a period of 1 week. The intra-day precision for the tested water samples was in the range of 2.7%–5.9%, while the inter-day precision was between 6.5% and 9.8%.

3.6. Comparison of the proposed method with other methods

Because of the weak fluorescence property and low ionization efficiency of these compounds, the sensitivities of LC and LC–MS methods are usually limited [25,29]. With the introduction of EASC into the EDC molecules, the sensitivity of LC method for the determination of EDCs was enhanced 50- to 100-folds. Chromatographic separation of EDCs using this method was compared with that obtained by HPLC with direct fluorescence detection (see Fig. 4). In addition, the sensitivity of this proposed method was also compared to some sensitive GC–MS methods published before, and the results are summarized in Table 5. Though the proposed method is much easier and cheaper, its sensitivity is comparable to those of GC–MS methods with a noticeable improvement for EE2 and E3, whose sensitivity is usually much lower than other compounds when analyzed by GC–MS.

3.7. Application

The developed method was successfully applied to the determination of EDCs in wastewater samples. The content of EDCs from different water samples were summarized in Table 6. OP, NP, BPA, E1 and E2 were detected in all the samples analyzed, ranging from 2.4 to 24 ng L^{-1}. The concentration of BPA was much higher than the others, reflecting the high usage of BPA-containing substance of local people. DES, EE2 and E3 were not detected probably because their contents in samples were lower than their limits of detection. A typical chromatogram of wastewater sample is shown in Fig. 5.

4. Conclusions

A sensitive analytical method was developed for the simultaneous determination of eight EDCs in wastewater samples. The reaction of EDCs with labeling reagent EASC gave stable fluorescence derivatives under mild conditions, and the sensitivity of LC method for the

Table 6
Concentration of EDCs determined in water samples.

Sample		OP (ng L^{-1})	NP (ng L^{-1})	BPA (ng L^{-1})	DES (ng L^{-1})	E1 (ng L^{-1})	EE2 (ng L^{-1})	E2 (ng L^{-1})	E3 (ng L^{-1})
Wastewater	1	4.2	5.6	24	–[a]	6.2	–	3.0	–
	2	3.0	2.4	13	–	8.4	–	4.9	–

[a] –: not detected.

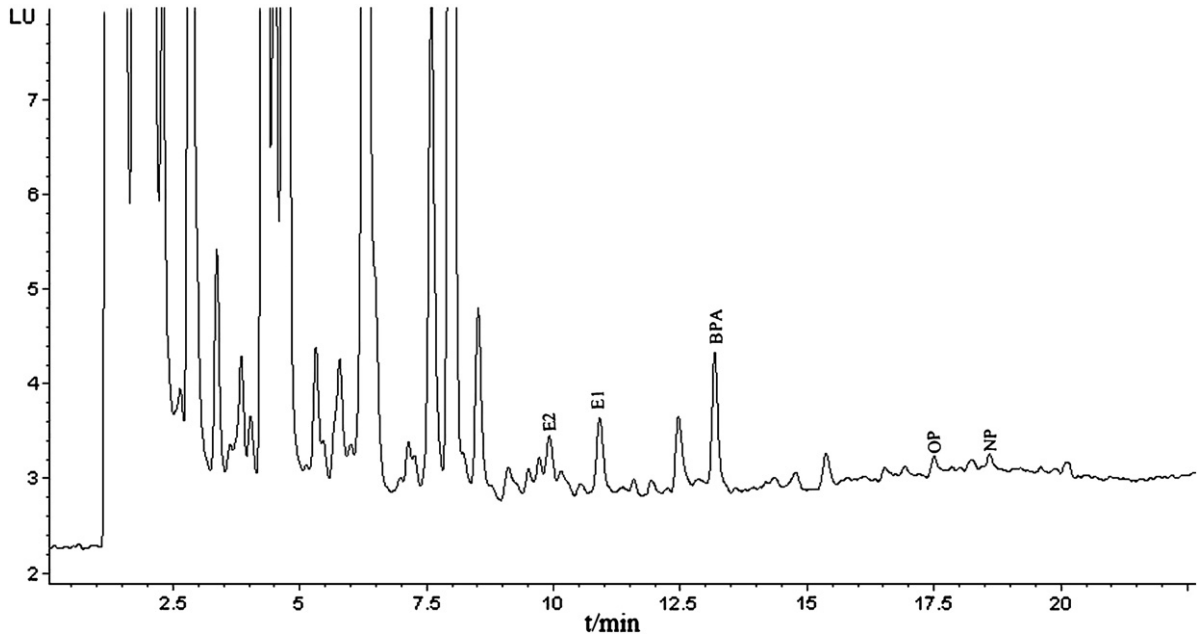

Fig. 5. Chromatogram of wastewater sample no. 2 listed in Table 5.

determination of EDCs was greatly enhanced by this derivatization. In addition, solid phase extraction with ODS C18 cartridges was applied to sample preparation and provided good recoveries for the extraction of the target compounds, especially for OP and NP, whose recoveries were usually lower than 50%. Besides, the whole extraction procedure was low organic solvent-consuming. The proposed method could be well applied to the determination of EDCs in water samples with quantification limits between 1.0 and 2.0 ng L^{-1}. A possible disadvantage of the proposed method was that the reagent of EASC could only be used in the pre-column derivatization.

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