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MCX based solid phase extraction combined with liquid chromatography tandem mass spectrometry for the simultaneous determination of 31 endocrine-disrupting compounds in surface water of Shanghai

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ABSTRACT

A novel analytical method employing MCX (mixed-mode cationic exchange) based solid phase extraction (SPE) coupled with liquid chromatography tandem mass spectrometry (LC–MS/MS) was developed to detect 31 endocrine-disrupting compounds (EDCs) in surface water samples simultaneously. The target EDCs belong to five classes, including seven estrogens, eight androgens, six progesterones, five adrenocortical hormones and five industrial compounds. In order to simultaneously concentrate the target EDCs and eliminate matrix interferences in the water samples, MCX SPE cartridges were employed for SPE, and then followed by a simple and highly efficient three-step sequential elution procedure. Two electrospray ionization (ESI) detection modes, positive (ESI+) and (ESI−), were optimized for HPLC–MS/MS analysis to obtain the highest sensitivity for all the EDCs. The limits of detection (LODs) were 0.02–1.9 ng L^{-1}, which are lower than or comparable to these reported in references. Wide linear ranges (LOD-100 ng L^{-1} for ESI+ mode, and LOD-200 ng L^{-1} for ESI− mode) were obtained with determination coefficients (R^2) higher than 0.99 for all the compounds. With five internal standards, good recoveries (84.4–103.0%) of all the target compounds were obtained in selected surface water samples. The developed method was successfully applied to investigate the EDCs occurrence in the surface water of Shanghai by analyzing surface water samples from 11 sites. The results showed that nearly all the target compounds (30 in 31) were present in the surface water samples of Shanghai, of which three industrial compounds (4-t-OP, BPA, and BPF) showed the highest concentrations (median concentrations were 11.88–23.50 ng L^{-1}), suggesting that industrial compounds were the dominating EDCs in the surface water of Shanghai, and much more attention should be paid on these compounds. Our present research demonstrated that SPE with MCX cartridges combined with HPLC–MS/MS was convenient, efficient and reliable for multiclass analysis of EDCs in surface water.

1. Introduction

Endocrine disrupting compounds (EDCs) have generated a considerable amount of attention in the past two decades [1]. EDCs can disturb the endocrine system of animals and human beings and induce many severe problems, such as induction of vitellogenin in male fish and the occurrence of intersex in wild fish, abnormality of animals and human beings [2]. Some typical EDCs, such as estrone (E1), 17β-estradiol (E2), estriol (E3), bisphenol A (BPA) and 4-t-nonyl phenol (NP) have been found in sewage treatment plants (STPs) and surface water bodies [3–5]. Besides, many pharmaceuticals such as prednisone and methylprednisolone, were used to treat endocrine diseases of human beings. These pharmaceuticals can get into the wastewater with the excretion of human beings, and finally get into the environment water; some of them can even get into the drinking water and threat the health of human beings [6]. EDCs in the surface water usually come from the domestic, industrial and livestock wastewater [7–9]. In these EDCs, natural estrogens and industrial compounds draw much attention and they were often detectable from surface water. Meanwhile, other EDCs, such as androgens, progesterones and adrenocortical hormones have less research on and generally were omitted for the surface water analysis, while they may be present and threat the health of human beings [10]. Therefore, it is of great importance to establish a convenient and reliable

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method to simultaneously detect different classes of EDCs including natural estrogens (e.g. E1, E2), androgens (e.g. testosterone), progesterones (e.g. progesterone), adrenocortical hormones (e.g. dexamethasone) and industrial compounds (e.g. BPA) in surface water.

The low concentrations of EDCs in the surface water are really a challenge to the analysis. Gas chromatography tandem mass spectrometry (GC–MS) and liquid chromatography tandem mass spectrometry (LC–MS/MS) have been employed for the analysis of these compounds [11–13]. Many analytical methods employing GC–MS have been developed for several kinds of EDCs in various samples [14–17]. Generally, derivatization of the compounds is a necessary step prior to GC–MS in order to improve selectivity and sensitivity in these methods. However, derivatization is time-consuming and complicated, which restricts the application of GC–MS to the simultaneous determination of several classes of EDCs. Compared to GC–MS, LC–MS/MS does not require target compound derivatization and has the characteristics of simple operation, high sensitivity, high selectivity and specificity. Therefore, LC–MS/MS has been developed to determine steroids [18,19]. Some methods have been optimized for simultaneously determination of different kinds of EDCS at low concentrations in complex matrices of environmental samples. Liu et al. [10] developed a LC–MS/MS method to simultaneously analyze 28 compounds (four estrogens, fourteen androgens, five progestagens and five glucocorticoids), Chang et al. [20] developed an analytical method for monitoring 30 compounds (five estrogens, nine androgens, nine progestogens, six glucocorticoids, and one mineralocorticoid) using LC–MS/MS. In these methods, the industrial compounds which were always present in the surface water and other environment water were not included. Therefore, the object of this study was to develop a new analytical method which can simultaneously determine five classes of EDCs (estrogens, androgens, progestogens, adrenocortical hormones and industrial compounds) with LC–MS/MS.

Sample preconcentration is utmost important for EDC analysis because of their low concentration in the environment. Extraction and enrichment of EDCs from water samples is usually performed by SPE utilizing different types of sorbent materials. Several different kinds of SPE cartridges have been used for EDCs such as hydrophilic–hydrophobic balance (HLB) and mixed-mode cationic exchange (MCX) cartridges. HLB, with its hydrophilic–lipophilic balance, is versatile and efficient for the extraction of EDCs with a wide range of polarities and pH values. HLB has been used in many studies with different kinds of water samples [21,22]. However, the universality of HLB also makes it less selective [23]. MCX, which is built upon HLB copolymer with additional presence of sulfonic groups to make it a strong cation-exchanger, can overcome the shortcomings of HLB. Therefore, it has been successfully employed to extract a wide range of pharmaceuticals and synthetic hormones from water matrices [23,25,26]. However, the applicability of MCX SPE cartridges to simultaneously concentrates various classes of EDCs with different physical and chemical properties need to be tested.

To our knowledge, most of the currently available analytical methods usually can simultaneously analyze less than ten EDCs, all of which belong to the same class or a few classes [27]. For the case of analyzing many kinds of classes of EDCs, more than one SPE cartridges were usually needed for the enrichment and separation of compounds [20]. The use of too many SPE cartridges makes the sample preparation process tedious and costly. Furthermore, too many steps in the sample preparation may increase the loss of the compounds in the water and reduce the recovery and analysis accuracy. There were few studies which can simultaneously concentrate more than five classes of EDCs simultaneously with a single SPE cartridge. Therefore, a simple SPE analytical method only employing MCX cartridge is advantageous for these various kinds of EDCs in our present research.

The objective of this research was to develop a novel MCX based SPE (SPE with a single MCX cartridge) combined HPLC–MS/MS method for the simultaneous analysis of 31 EDCs including seven estrogens, eight androgens, six progestogens, five adrenocortical hormones and five industrial compounds in surface water. MCX based SPE provided a more convenient basis for the analysis of various kinds of EDCs in surface water. After that, the developed method was applied to detect these EDCs in 11 surface water samples of Shanghai.

2. Experimental

2.1. Reagents and materials

All the test compounds and five internal standards including estrone-D2, diethylstilbestrol-D8, testosterone-D3, progesterone-D9, norgestrel-D6 (Table 1) were purchased from Sigma–Aldrich (St. Louis, MO, USA) and Dr. Ehrenstorfer (Augsburg, Germany). Milli-Q water was obtained from a Millipore system (Billerica, MA, USA). Individual stock solutions of the studied compounds were prepared in methanol and stored in amber glass vials at −20 °C. Methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Glass fiber filters (GF/F) were supplied by Whatman (Middlesex, UK). MCX extraction cartridges (500 mg, 6 mL) were purchased from Anpelclean (Shanghai, China). Ammonia water and ammonium acetate were purchased from Sigma–Aldrich (St. Louis, MO, USA).

The compounds were divided into two groups in the analysis based on their performance in the ionization and were tested under their corresponding mode and separation method. The categorizations of these compounds and their corresponding internal standard are shown in Table 1.

2.2. Instrumentation

LC–MS/MS analysis was performed using a Waters Xevo TQ MS Instrument Platform (Milford, MA, USA). The platform was consisting of ultra performance liquid chromatography, binary pump, gradient elution system, sample plate (4 °C), autosampler and BEH C_{18} column (100× 2.1 mm i.d., 1.7 μm, Waters, Milford, MA, USA). The platform was operated by Masslynx (V4.1).

For positive ion electrospray ionization (ESI+) mode, the capillarity voltage was set at 3.5 kV, the cone voltage at 30 V, the desolvation temperature at 450 °C, the source temperature at 120 °C. Nitrogen (99.5% purity) was used as the desolvation gas at a flow of 800 L h^{-1}, the cone gas flow at 50 L h^{-1}.

For negative ion electrospray ionization (ESI−) mode, the capillarity voltage was set at 3.2 kV, the cone voltage at 40 V, the desolvation temperature at 450 °C, the source temperature at 120 °C. Nitrogen (99.5% purity) was used as the desolvation gas at a flow of 800 L h^{-1}, the cone gas flow at 45 L h^{-1}.

2.3. The mobile phases in the analytical method

Two groups of mobile phases were used in the analysis of these compounds. For the compounds which were tested under ESI+ mode, 0.1% formic acid/water solution and 0.1% formic acid/acetonitrile solution were used as the mobile phase. For the compounds which were tested under ESI− mode, acetonitrile, water with 2 mM ammonium acetate and 0.5% ammonia were used as the mobile phase. It takes 14 min for one analysis, the time sequences are shown in Table 2.

Table 1
The tested EDCs and their internal standards, detection parameters for HPLC–MS/MS.

Category	Compounds	Abbreviation	Internal standards	Mode	Parent	Quantification		Confirmation		Retention time (min)
						Ion	CE (V)	Ion	CE (V)	
Estrogens	Estrone	E1	E1-D2	ESI−	269.2	145.1	36	159.0	34	3.58
	Estrone-D2[a]	E1-D2	–	ESI−	271.3	185.1	36	171.1	34	3.53
	17β-Estradiol	E2	E1-D2	ESI−	271.0	182.9	38	145.0	34	3.17
	Estriol	E3	E1-D2	ESI−	287.1	171.0	32	158.8	30	1.87
	Diethylstilbestrol	DES	DES-D8	ESI−	267.2	221.9	37	237.0	30	3.69
	Diethylstilbestrol-D8[a]	DES-D8	–	ESI−	275.0	244.8	24	259.0	24	3.65
	Dienoestrol	Dieno	DES-D8	ESI−	265.0	92.8	26	171.0	18	3.78
	Hexestrol	Hexe	DES-D8	ESI−	269.1	119.0	36	134.0	18	3.79
	Estradiol benzoate	E2-ben	Proges-D9	ESI+	377.4	105.0	24	77.0	50	5.80
Androgens	19-Nortestosterone	Nortes	TES-D3	ESI+	275.3	82.9	27	109.0	26	3.16
	Trenbolone	Tren	TES-D3	ESI+	271.2	106.9	41	91.0	42	2.92
	Testosterone	TES	TES-D3	ESI+	289.2	97.0	20	253.3	22	3.43
	Testosterone-D3[a]	TES-D3	–	ESI+	292.4	97.0	27	109.0	28	3.35
	Methyl testosterone	Me-TES	TES-D3	ESI+	303.4	97.0	28	109.0	26	3.69
	Nandrolone Phenylpropionate	Nan-phen	TES-D3	ESI+	407.1	91.0	79	104.9	37	6.19
	Testosterone propionate	TES-pro	TES-D3	ESI+	345.3	109.3	35	97.2	32	5.75
	Boldenone	Bold	TES-D3	ESI+	287.2	121.0	22	135.1	16	3.05
	Epitestosterone	Epite	TES-D3	ESI+	289.2	109.3	34	97.2	30	3.81
Progesterones	Norethisterone	Noreth	Proges-D9	ESI+	299.2	109.0	30	91.0	40	3.44
	D(−) Norgestrel	Norges	Nogres-D6	ESI+	313.2	109.0	26	245.6	22	3.99
	Norgestrel-D6[a]	Norges-D6	–	ESI+	319.5	251.7	20	301.5	10	3.90
	Medroxy progesterone	Me-pro	Proges-D9	ESI+	345.3	123.0	30	97.0	33	4.18
	Progesterone	Proges	Proges-D9	ESI+	315.4	97.0	20	109.0	16	4.70
	Progesterone-D9[a]	Proges-D9	–	ESI+	324.4	100.1	32	113.1	35	4.67
	Megestrol acetate	Me-ace	Proges-D9	ESI+	385.3	224.2	32	267.2	14	4.62
	Hydroxyprogesterone	Hydrop	Proges-D9	ESI+	429.3	253.5	28	271.3	24	5.84
Adrenocortical hormones	Prednisone	Predn	Proges-D9	ESI+	359.5	146.8	30	313.1	12	2.17
	Cortisone	Corti	Proges-D9	ESI+	361.3	121.3	36	90.9	58	2.21
	Dexamethasone	Dexa	Proges-D9	ESI+	393.4	373.2	8	147.0	24	2.63
	Prednisolone	Prednln	Proges-D9	ESI+	361.4	147.1	38	307.2	14	2.21
	Methylprednisolone	Me-prednl	Proges-D9	ESI+	375.4	161.0	28	120.9	44	2.52
Industrial chemicals	Bisphenol S	BPS	DES-D8	ESI−	248.4	108.0	31	91.9	42	0.76
	4-t-Nonyl Phenol	NP	DES-D8	ESI−	219.3	106.0	17	119.1	42	6.35
	4-n-Octyl Phenol	OP	DES-D8	ESI−	205.2	189.1	30	133.3	33	5.56
	Bisphenol A	BPA	DES-D8	ESI−	227.3	212.0	20	133.3	16	3.06
	Bisphenol F	BPF	DES-D8	ESI−	199.1	93.0	25	197.4	27	2.46

[a] Internal standards.

Table 2
The mobile phase in the analysis.

Mode	Mobile phase		
	Time (min)	A: 0.1% formic acid/acetonitrile	B: 0.1% formic acid/water
ESI+	0	30%	70%
	5	95%	5%
	10	95%	5%
	10.5	30%	70%
	14	30%	70%
Mode	Mobile phase		
	Time (min)	A: acetonitrile	B: 2 mM NH_4CH_3COO/0.5% NH_3/water
ESI−	0	30%	70%
	5	95%	5%
	10	95%	5%
	10.5	30%	70%
	14	30%	70%

2.4. Preparation of water samples

The samples (1000 mL, adjust pH to 3 with 40% H_2SO_4) were filtered through pre-ashed 0.7 μm glass fiber filters (GF/F) and then spiked with internal standards at an absolute amount of 1 or 5 ng (1 ng for ESI+ compounds and 5 ng for ESI− compounds). After that, SPE utilizing MCX SPE cartridges were performed on a Supelco (Madrid, Spain) vacuum manifold for 12 cartridges connected to a vacuum pump for the isolation and concentration of target EDCs. The cartridges were placed on a SPE element and conditioned sequentially with 6 mL of methanol, 6 mL of pure water and 6 mL of 0.1 N HCl at a flow rate of 1 mL min^{-1}. Then, samples were loaded through the cartridges at a flow rate of 2–2.5 mL min^{-1}. After all the samples were filtered, the cartridges were dried under nitrogen and cleaned sequentially with 6 mL of 0.1 M HCl, 6 mL of pure water and 6 mL of methanol/water solution (1:1, v/v) at a flow rate of 1 mL min^{-1}. After that, the EDCs were eluted with 9 mL of 0.6 M ammonia/methanol solution at a flow rate of 1 mL min^{-1}. The extracts were dried under a gentle stream of nitrogen and redissolved in 1 mL methanol. Then the solutions were filtered through 0.22 μm filter unit (Millex, Billerica, MA, USA) and prepared for analyzing with LC–MS/MS with the corresponding mobile phase.

2.5. Identification-quantification

The tested compounds were identified by means of matching their retention times with those of calibration standards and by the ratio of target ions. The quantification of the compounds was performed by comparing the peak area which was corrected by the internal standard to the peak area of the standard curve with MassLynx V4.1 (Waters, America). Mixtures solution of target compounds spiked with internal standards with an absolute concentration of 1 or 5 ng (1 ng for ESI+ compounds, 5 ng for ESI− compounds) were used as the standard curve to quantify the compounds in samples. For the chemicals which were tested under ESI+ mode, the concentrations of calibration standards were from 0.01 ng L^{-1} to 100 ng L^{-1} (0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 0.75, 1.0, 2.5, 5, 10, 25, 50, 100 ng L^{-1}), for the compounds which were tested under ESI− mode, the concentrations of calibration standards were from 0.1 ng L^{-1} to 200 ng L^{-1} (0.1, 0.25, 0.5, 0.75, 1.0, 2.5, 5, 10, 25, 50, 100, 200 ng L^{-1}).

2.6. Quality control

Calibration curves were obtained by plotting the ratios of each compound to internal standard in detector responses versus their concentrations. Also, the internal standards (1 ng for ESI+ compounds, 5 ng for ESI− compounds) were spike to the water samples to automatically compensate for the loss of compounds during sample preparation and the matrix induced change in ionization and variations in instrumental response. The compounds and their relevant internal standards are shown in Table 1.

The linearity, sensitivity, reproducibility and precision of the method were evaluated. Linearity of the method was tested with standards at 10 concentration levels (0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 0.75, 1.0, 2.5, 5, 10, 25, 50, 100 ng L^{-1} for compounds tested with ESI+ mode, and 0.1, 0.25, 0.5, 0.75, 1.0, 2.5, 5, 10, 25, 50, 100, 200 ng L^{-1} for compounds tested with ESI− mode). Sensitivity was evaluated by determining the limit of detection (LOD). Low concentration EDCs (10 times of estimated LOD) were spike into the surface water, and the LODs were calculated from the S/N (signal to noise) 3:1. Reproducibility and precision were evaluated by spiking target EDCs (10 times of LODs) to the surface water sample from a river in Ningbo. Three parallel samples were prepared. The preparation was according to Section 2.4. The recoveries and their LODs were present in Table 3.

2.7. Application to real surface water samples

Surface water samples were sampled from Shanghai in May, 2010. Eleven sample sites cover the upstream (TP, XT), middle reached (SP, DP, CQ), downstream (SZ, YSP, ZB, WS) of Huangpu River and another two important water sources (SG is effluent river from Yangtse river, CH is lake for drinking water) were chosen. The distribution of these sample sites can represent the occurrences of the tested compounds in the surface water of Shanghai. Four liters of water samples were taken from each site, then, samples were brought back to laboratory at low temperature and prepare for the SPE. For each site, 3 L water sample was used (1×3) for the SPE and three parallel samples were prepared for each site. At the same time, another 1 L water samples from each site were used for the recoveries of EDCs from these samples. The preparation of the samples was according to Section 2.4.

3. Results and discussion

3.1. SPE conditions

With the increase of number and type of target compounds, it was difficult to simultaneously preconcentrate and separate the compounds with SPE. SPE process with two or more cartridges was usually employed in previous studies. Chang et al. [20] employed both HLB and Florisil cartridges to preconcentrate and separate 30 EDCs (five estrogens, nine androgens, nine progestogens, six glucocorticoids, and one mineralocorticoid). However, the excessive steps are tedious and may cause loss of compounds and reduce the recoveries. SPE with HLB cartridge has been employed in the detection of natural and synthetic estrogens and industrial compounds [3,14,16]. Therefore, SEP with MCX cartridge was employed in this study. MCX provides both ion-exchange and reversed-phase retention and can adsorb different kinds of compounds simultaneously from aqueous media [24]. And it has been successfully employed to extract many pharmaceuticals and synthetic hormones from water samples. Therefore, MCX cartridge was chosen and a three-step sequential elution procedure was tested for the adsorption and elution efficiency of these 31 compounds in the water sample. The results showed that the MCX cartridge has a good performance.

Table 3
The analytical parameters of the method and comparison with references.

Compounds*	Linner range ($ng\,L^{-1}$)	Recovery (%)	RSD[a] (%)	LOD[b] ($ng\,L^{-1}$)	Determination Coefficient (R^2)	LOD from references ($ng\,L^{-1}$)	Method from reference
E1	0.27–200	0.9972	98.7	5.0	0.27	0.20 [20]	SPE+LC–MS/MS
E2	0.30–200	0.9944	99.1	2.1	0.30	0.24 [10]	SPE+RRLC–MS/MS
E3	0.58–200	0.9917	100.3	3.8	0.58	0.44 [28]	SPE+GC–MS
DES	0.15–200	0.9956	101.1	7.8	0.15	0.14 [10]	SPE+RRLC–MS/MS
Dieno	0.76–200	0.9984	97.7	9.1	0.76	450 $ng\,kg^{-1}$ [29]	SPE+LC–MS/MS
Hexe	0.39–200	0.9907	92.5	4.7	0.39	40 $ng\,kg^{-1}$ [29]	SPE+LC–MS/MS
E2-ben	0.04–100	0.9990	97.7	4.8	0.04	1920 $ng\,kg^{-1}$ [30]	SPE+GC–MS
Nortes	0.11–100	0.9950	95.6	3.2	0.11	0.10 [10]	SPE+RRLC–MS/MS
Tren	0.02–100	0.9996	95.0	6.4	0.02	0.11 [10]	SPE+RRLC–MS/MS
TES	0.04–100	0.9964	100.3	3.7	0.04	0.05 [10]	SPE+RRLC–MS/MS
Me-TES	0.06–100	0.9946	93.5	9.6	0.06	0.20 [20]	SPE+LC–MS/MS
Nan-phen	0.04–100	0.9999	96.3	3.7	0.04	100 $ng\,kg^{-1}$ [31]	SPDE+LC–MS/MS
TES-pro	0.18–100	0.9923	88.8	6.2	0.18	500 [32]	SPDE+LC–MS/MS
Bold	0.04–100	0.9951	92.6	6.8	0.04	100 $ng\,kg^{-1}$ [31]	SPDE+LC–MS/MS
Epite	0.02–100	0.9988	95.4	6.1	0.02	0.14 [33]	SPE+LC–MS/MS
Noreth	0.38–100	0.9938	92.9	3.4	0.38	250 [34]	SPE+GC–MS
Norges	0.06–100	0.9942	93.2	5.7	0.06	0.08 [20]	SPE+LC–MS/MS
Me-pro	0.05–100	0.9950	96.1	5.0	0.05	0.04 [10]	SPE+RRLC–MS/MS
Proges	0.02–100	0.9958	103.0	7.0	0.02	0.05 [10]	SPE+RRLC–MS/MS
Me-ace	0.02–100	0.9923	96.8	5.6	0.02	0.02 [20]	SPE+LC–MS/MS
Hydrop	0.02–100	0.9994	84.4	4.7	0.02	0.10 [20]	SPE+LC–MS/MS
Predn	0.30–100	0.9932	99.3	3.5	0.30	0.05 [10]	SPE+RRLC–MS/MS
Corti	0.19–100	0.9982	94.6	4.5	0.19	0.07[10]	SPE+RRLC–MS/MS
Dexa	0.05–100	0.9929	95.6	3.5	0.05	0.04[10]	SPE+RRLC–MS/MS
Prednln	0.09–100	0.9963	101.6	3.8	0.09	0.03[10]	SPE+RRLC–MS/MS
Me-prednl	0.09–100	0.9975	92.6	6.9	0.09	0.02[20]	SPE+LC–MS/MS
BPS	0.17–200	0.9916	95.5	2.5	0.17	22 [35]	SPME–GC–MS
NP	1.20–200	0.9937	92.8	6.2	1.20	3.8 [36]	SPE+GC–MS
OP	0.57–200	0.9921	95.6	5.3	0.57	2.0 [37]	DLLME+GC–MS
BPA	1.90–200	0.9937	90.0	6.9	1.90	14 [36]	SPE+GC–MS
BPF	0.26–200	0.9982	101.0	4.4	0.26	0.1 [38]	SPE+GC–MS

* The names of the compounds were abbreviated for convenience according to Table 1.
[a] RSD: relative standard deviation, $n = 3$.
[b] LOD were estimated based on the lowest detectable peak that had signal/noise = 3.

3.2. ESI detection modes and HPLC separation

ESI source was selected for the determining of 31 target compounds in this study. Because it can fill the detection request of the determining at low concentration, and the operation and maintenance are easier than atmospheric pressure chemical ionization (APCI) source [31]. In all of the tested compounds, some can only get ionization under ESI+ or ESI− mode, i.e. estrogens, androgens, progesterones and industrial compounds, Therefore, their test mode can be set. However, some compounds (e.g. adrenocortical hormones) can get ionization under both modes, so the ionization efficiency was tested under both the ESI+ and ESI− mode and the better one was set as their test mode. To enhance the ionization efficiency, formic acid and ammonia were added to the mobile phase respectively. A 14-min gradient of mobile phases was used to separate these compounds.

3.3. Identification of the compounds

With Masslynx (V4.1), compounds can be analyzed with Intellistart. The parent, quantification and confirmation ions and their corresponding collision energy (CE) were determined by Masslynx and the results are shown in Table 1.

The compounds were identified by matching the retention time and the ratio of target ions with the standards. The retention time of these compounds are also shown in Table 1. For each chemical, the time window was 1 min, each 30-second before and after the retention time. For each peak, at least 12 data points were required. With the retention time and ions ratios, the compounds in the analysis can be judged.

Figure S1 shows the selected ion chromatograms of the parent ion and their corresponding quantification ion for each chemical.

3.4. Analytical performance of the method and comparison with previous studies

The method was validated by several parameters, including linearity, sensitivity, reproducibility and precision. The analytical parameters of the method are shown in Table 3.

As described in Section 2.6, the linearity of the method was tested with 10 concentrations of these compounds, the regression of the result shows that the correlation coefficients (R^2) were higher than 0.99 for all the compounds in a wide linear range (LOD-100 $ng\,L^{-1}$ for ESI+ mode, and LOD-200 $ng\,L^{-1}$ for ESI− mode). The wide linear range nearly covers the common concentrations of the compounds in the drinking water and surface water detected in many studies [16,39]. The good linearity and wide linear range ensures that the method can perform well in the quantification of these compounds in the surface water and other micro-polluted water.

For the compounds which were tested under ESI+ mode, the LODs were from 0.02 $ng\,L^{-1}$ to 0.38 $ng\,L^{-1}$, 15 of them were lower than 0.1 $ng\,L^{-1}$. For the compounds which were tested under ESI− mode, the LODs were from 0.15 $ng\,L^{-1}$ to 1.90 $ng\,L^{-1}$, nine of them were lower than 1 $ng\,L^{-1}$. In other studies, the LODs of the analytical methods are always higher than or comparable to that in our present work. The LODs of these EDCs from references are also listed in Table 3. From the table we can see that the compounds which were tested under ESI− mode have relatively higher LODs than other compounds, but their LODs are still similar to or

Table 4
The occurrence of the tested EDCs in surface water of Shanghai.

Compounds*	EDC concentrations ($ng\,L^{-1}$) in various sampling sites													Frequency (%)	Recovery (mean ± SD, %)
	SG	CH	TP	XT	SP	DP	CQ	SZ	YSP	ZB	WS	Max	Min		
E1	6.36 ± 0.48	0.58 ± 0.04	ND	ND	0.71 ± 0.08	8.50 ± 1.09	3.82 ± 0.28	5.44 ± 0.35	6.81 ± 0.16	6.44 ± 0.66	4.30 ± 0.60	8.50	0.58	81.82	83 ± 11
E2	0.41 ± 0.08	1.08 ± 0.22	1.09 ± 0.12	0.74 ± 0.53	ND	3.90 ± 0.64	ND	4.49 ± 2.14	2.53 ± 2.53	3.16 ± 2.86	5.76 ± 5.10	5.76	0.41	81.82	82 ± 10
E3	ND	ND	1.11 ± 0.61	4.23 ± 0.52	0.72 ± 0.17	6.60 ± 0.38	ND	1.62 ± 0.24	ND	4.09 ± 0.34	6.31 ± 8.05	6.60	0.72	63.64	81 ± 15
DES	ND	ND	0.72 ± 0.51	ND	ND	0.54 ± 0.74	1.42 ± 1.97	ND	0.92 ± 0.98	0.24 ± 0.34	0.86 ± 0.61	1.42	0.24	54.55	91 ± 12
Dieno	ND	1.58 ± 0.28	2.86 ± 3.97	3.64 ± 0.36	ND	4.76 ± 2.19	ND	0.87 ± 0.07	1.42 ± 0.14	ND	1.68 ± 0.14	4.76	0.87	63.64	89 ± 12
Hexe	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.00	0.00	0.0	86 ± 6
E2-ben	ND	ND	ND	ND	ND	ND	ND	ND	0.17 ± 0.01	ND	0.16 ± 0.02	0.17	0.16	18.18	90 ± 8
Nortes	0.50 ± 0.03	ND	0.32 ± 0.02	0.38 ± 0.01	0.34 ± 0.01	0.25 ± 0.01	0.17 ± 0.01	0.68 ± 0.08	0.22 ± 0.03	0.56 0.02	0.26 ± 0.01	0.68	0.17	90.91	91 ± 15
Tren	1.04 ± 0.02	0.91 ± 0.02	0.87 ± 0.06	0.91 ± 0.08	0.99 ± 0.04	0.86 ± 0.03	1.23 ± 0.02	0.92 ± 0.03	0.91 ± 0.01	0.90 ± 0.08	1.00 ± 0.02	1.23	0.86	100.00	99 ± 18
Testo	0.46 ± 0.03	0.63 ± 0.01	0.33 ± 0.03	0.55 ± 0.01	0.65 ± 0.02	0.34 ± 0.02	0.70 ± 0.02	0.66 ± 0.01	0.80 ± 0.05	0.85 ± 0.10	0.44 ± 0.02	0.85	0.33	100.00	89 ± 11
Me-testo	0.40 ± 0.01	0.09 ± 0.01	0.16 ± 0.02	0.46 ± 0.04	0.19 ± 0.01	0.28 ± 0.03	0.94 ± 0.04	0.37 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.24 ± 0.02	0.94	0.09	100.00	88 ± 9
Nan-phen	ND	ND	0.25 ± 0.03	0.18 ± 0.09	0.22 ± 0.10	0.06 ± 0.02	ND	ND	0.19 ± 0.04	0.22 ± 0.04	0.28 ± 0.07	0.28	0.06	63.64	93 ± 15
Testo-pro	0.37 ± 0.033	ND	ND	ND	ND	ND	ND	ND	ND	0.18 ± 0.01	ND	0.37	0.18	18.18	79 ± 7
Bold	ND	ND	ND	ND	0.08 ± 0.01	0.05 ± 0.01	ND	ND	0.06 ± 0.01	0.10 ± 0.05	ND	0.10	0.05	36.36	86 ± 10
Epite	0.32 ± 0.01	0.27 ± 0.01	0.22 ± 0.03	0.25 ± 0.01	0.15 ± 0.02	0.29 ± 0.02	0.26 ± 0.00	0.22 ± 0.06	0.19 ± 0.04	0.49 ± 0.04	0.24 ± 0.01	0.49	0.15	100.00	85 ± 11
Noreth	0.41 ± 0.01	ND	0.43 ± 0.04	0.45 ± 0.04	0.46 ± 0.03	0.57 ± 0.04	ND	ND	0.81 ± 0.03	0.73 ± 0.01	ND	0.81	0.41	63.64	85 ± 10
Norges	0.58 ± 0.06	0.06 ± 0.04	0.40 ± 0.09	0.87 ± 0.06	0.65 ± 0.02	0.84 ± 0.02	0.64 ± 0.03	0.80 ± 0.12	0.83 ± 0.07	0.60 ± 0.08	0.36 ± 0.04	0.87	0.06	100.00	88 ± 14
Me-pro	0.18 ± 0.03	ND	0.14 ± 0.02	0.12 ± 0.05	0.12 ± 0.02	0.34 ± 0.01	0.20 ± 0.04	0.38 ± 0.03	0.14 ± 0.06	0.21 ± 0.01	0.12 ± 0.03	0.38	0.12	90.91	85 ± 8
Proges	0.26 ± 0.06	0.02 ± 0.01	0.14 ± 0.02	0.07 ± 0.03	0.06 ± 0.03	0.22 ± 0.02	0.14 ± 0.02	0.38 ± 0.00	0.14 ± 0.03	0.10 ± 0.02	0.10 ± 0.02	0.38	0.02	100.00	91 ± 10
Me-ace	0.02 ± 0.00	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.00	0.02 ± 0.00	0.17 ± 0.02	0.06 ± 0.02	0.06 ± 0.01	0.07 ± 0.04	0.08 ± 0.03	0.17 ± 0.07	0.17	0.02	100.00	95 ± 18
Hydrop	0.06 ± 0.02	0.07 ± 0.02	0.06 ± 0.01	0.04 ± 0.00	0.06 ± 0.00	0.13 ± 0.00	ND	0.08 ± 0.01	0.04 ± 0.01	0.08 ± 0.01	0.05 ± 0.01	0.13	0.04	90.91	94 ± 13
Predn	2.63 ± 0.14	0.38 ± 0.09	0.52 ± 0.08	1.78 ± 0.21	1.66 ± 0.34	1.99 ± 0.41	4.08 ± 0.10	2.32 ± 0.47	1.05 ± 0.16	1.81 ± 0.22	2.11 ± 0.40	4.08	0.38	100.00	85 ± 6
Corti	0.55 ± 0.05	ND	0.37 ± 0.03	0.75 ± 0.02	0.81 ± 0.09	0.29 ± 0.06	1.60 ± 0.02	1.44 ± 0.07	0.86 ± 0.14	1.29 ± 0.12	0.37 ± 0.04	1.60	0.29	90.91	84 ± 9
Dexa	0.12 ± 0.01	ND	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.02	ND	0.19 ± 0.00	0.08 ± 0.01	0.07 ± 0.00	0.09 ± 0.01	0.06 ± 0.01	0.19	0.06	81.82	93 ± 13
Prednl	0.57 ± 0.24	0.38 ± 0.01	0.21 ± 0.08	0.10 ± 0.08	0.38 ± 0.26	0.55 ± 0.06	0.66 ± 0.07	0.50 ± 0.18	0.54 ± 0.11	1.21 ± 0.06	0.43 ± 0.01	1.21	0.10	100.00	91 ± 12
Me-prednl	ND	0.59 ± 0.00	0.42 ± 0.03	0.29 ± 0.10	0.60 ± 0.22	0.47 ± 0.00	0.09 ± 0.01	ND	0.26 ± 0.05	0.95 ± 0.16	ND	0.95	0.09	72.73	80 ± ± 7
BPS	ND	ND	ND	0.67 ± 0.18	ND	6.71 ± 0.75	0.84 ± 0.01	2.57 ± 0.21	4.36 ± 1.72	2.25 ± 0.17	ND	6.71	0.67	54.55	85 ± 11
4-NP	ND	ND	ND	ND	ND	1.23 ± 1.06	ND	ND	ND	ND	2.34 ± 3.31	2.34	1.23	18.18	84 ± 13
4-OP	6.54 ± 0.33	12.53 ± 1.05	22.59 ± 1.39	28.47 ± 4.59	12.05 ± 0.92	101.03 ± 7.06	23.50 ± 1.09	164.71 ± 7.33	10.52 ± 1.71	33.69 ± 4.20	41.87 ± 4.51	164.71	6.54	100.00	91 ± 15
BPA	7.58 ± 2.77	5.62 ± 0.93	12.49 ± 0.78	24.66 ± 3.63	11.26 ± 1.91	18.37 ± 2.05	ND	16.20 ± 1.46	4.58 ± 0.69	12.76 ± 1.16	2.63 ± 0.66	24.66	2.63	90.91	86 ± 6
BPF	250.30 ± 18.60	13.98 ± 1.31	ND	5.88 ± 0.67	46.86 ± 7.81	ND	15.99 ± 0.44	38.48 ± 2.32	8.49 ± 0.80	17.65 ± 1.52	20.29 ± 1.21	250.30	5.88	81.82	96 ± 13

ND: not detected.
* The names of the compounds were abbreviated for convenience according to Table 1.

lower than the LODs from the references, especially for the industrial compounds. But for the compounds which were tested under ESI+ mode, most of them have lower LODs than that in the references. The lower LODs of this method facilitate the analysis of these EDCs in the drinking water and surface water which have low EDC concentrations.

The mean recoveries and RSDs of these compounds from one typical surface water samples were calculated and the results were presented in Table 3. For all the compounds, the recoveries were from 84.4% to 102.9%, and the recoveries of samples were consistent well (RSD < 10%). The good recoveries indicate that this method can compensate for the chemical loss in sample preparation and the varieties in the instrument analysis. In addition, this present method performs well for the simultaneous determination of the target EDCs in surface water samples.

3.5. Application to real surface water samples and occurrence of the EDCs in Shanghai

The method was applied to 11 surface water sites and the results of the analysis are shown in Table 4. In the 11 sampling sites, 10 were river and one is lake for drinking water. Although the water matrices vary from upstream to downstream and from river to lake, the recoveries (68.27–119.97%) of all the compounds in these 11 sites were good enough for the determination and the recoveries did not vary a lot between these sample sites (SD < 15%) (Table 4).

In the 31 tested compounds, only hexestrol was not detected in all the sampling sites; the detection frequency of E2-ben, testopro and 4-NP was 18.18%; for bold, the detection frequencies were 36.36%. For the other 26 compounds, the detection frequencies were higher than 50%. 10 compounds showed the highest detection frequencies of 100%. Some of the compounds with high detection frequencies have also been detected in various kinds of water in China and worldwide [40].

The median values of the detected compounds vary from 0.06 ng L^{-1} to 25.98 ng L^{-1}. For 21 compounds, the median values were lower than 1 ng L^{-1}. While, four compounds including E1, 4-t-OP, BPA and BPF have median values more than 5 ng L^{-1}, all these compounds have detection frequencies higher than 80% and have been testified to have the estrogenic effect [41,42]. The higher concentrations and higher detection frequencies of these four EDCs show that EDCs with estrogenic effect are still the key compounds and more attention should be paid on the control of them. Among these four estrogenic compounds, three industrial compounds including 4-t-OP, BPA, BPF have the highest median values. The highest maximum values of the detected concentrations were also found for the 3 industrial compounds. The above results show that the industrial compounds were the key EDCs in the surface water of Shanghai and need more strict control.

4. Conclusions

A sensitive, precise and robust analytical method was developed for the simultaneous determination of 31 EDCs, including seven estrogens, eight androgens, six progesterones, five adrenocortical hormones and five industrial compounds in the surface water samples. SPE with MCX SPE cartridges was used for the pretreatment of water samples. The results indicate that the SPE method could efficiently reconcentrate the target EDCs in the surface water sample. With the five internal standards (estrone-D2, diethylstilbestrol-D8, testosterone-D3, progesterone-D9, and norgestrel-D6), the recoveries were from 79 ± 7% to 99 ± 18% for all the EDCs in 11 real surface water samples and the SDs of the real sample determination were within 15%. This method was successfully applied to determine the concentrations of target EDCs in the surface water samples collected from 11 surface water sampling sites in Shanghai. Among the 30 detected EDCs, concentrations of three industrial compounds (4-t-OP, BPA, BPF) were much higher than other compounds. Our research indicates that industrial compounds were still the dominated EDCs in the surface water of Shanghai.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2011.08.036.

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