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Simultaneous screening of estrogens, progestogens, and phenols and their metabolites in potable water and river water by ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry

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ABSTRACT

In this study, a method employing ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS) was developed to simultaneously screen for 36 endocrine-disrupting chemicals (EDCs; e.g., estrogens, progestogens, phenols, and their metabolites) both in potable and river water. From the selected compounds, 21 target compounds, for which reference standards were available, were used as model compounds for method development and optimization. The other target compounds, for which reference standards were unavailable, were investigated in post-target analysis on the basis of their theoretical molecular masses. The solid-phase extraction and chromatographic separation steps were optimized. For this method, limits of detection for the target compounds were less than 0.72 ng L^{-1}, and the overall recoveries varied between 46% and 134% with relative standard deviations ranging from 7% to 35%. The mass errors between theoretical and experimental mass for all resulting precursor and characteristic fragment ions ranged from −1.9 to 2.8 mDa. The method developed was successfully used to analyze the composition of potable and river water in Shanghai City; in addition, some compounds of interest (estriol, estrone, and bisphenol A) were identified accurately. Further, a post-target analysis was performed and an estrogen metabolite was hypothesized in the water samples due to the excellent sensitivity of the method in full-spectrum acquisition mode and the valuable accurate mass information in MS and tandem MS mode. Therefore, UPLC-Q-TOF-MS has proven to be a powerful technique for wide-scope screening and identification of relevant EDCs in environmental water sources.

1. Introduction

Endocrine-disrupting chemicals (EDCs) are an extensive group of natural and anthropogenic compounds that can act as hormone-like substances to influence the regulation of development and growth in animals and humans. In addition, EDCs have been associated with prostate cancer, reproductive tract disorders, low sperm counts, and breast cancer [1–5]. In the past twenty years, various types of EDCs have been found in the environment [6–10]. Therefore, the effect of environmental exposure of EDCs on population health has raised concerns, and a comprehensive investigation of the environmental exposure level of EDCs became a pressing issue [11,12].

Estrogenic steroid compounds are of particular interest because they possess the greatest potency of all estrogenic compounds and also occur ubiquitously in the environment [13,14]. It has been well documented that both estrogen and progestogen can modulate hormonal effects at concentrations measured in nano- or picograms per liter [15–18]. These compounds, predominantly derived from human or livestock sources [19–21], enter the environment directly or through effluents from wastewater treatment plants (WTPs). These compounds have been detected in the environment in concentrations that were sufficient to induce active hormonal effects [22,23]. Alkylphenols, such as 4-nonylphenol (4-NP) and 4-octylphenol (4-OP), and bisphenol-A (BPA) are typical examples of EDCs [24]. Although their estrogenic potency is three orders of magnitude lower than that of a steroid hormone [25], they still garner significant attention due to their widespread use in domestic products [26]. Alkylphenols and BPA in the environment are mainly derived from the degradation or direct release of the corresponding products, such as alkylphenol ethoxylates (APEOs), polycarbonate plastic resin, and epoxy resin [27].

Identification and determination of EDCs is a challenging task because of the extremely low levels at which they are present in the environment (nano- or picograms per liter). Previously, gas chromatography with mass spectrometric detection (GC–MS) was most commonly used for analysis of EDCs [28,29]. However, due to the need for derivatization and in consideration of the lower sensitivity of GC–MS when compared with liquid chromatography coupled with triple quadrupole mass spectrometry (LC–QqQ MS), LC–QqQ MS has gradually replaced GC–MS for

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analysis of EDCs in environmental samples [30–34]. However, confirmation and sensitivity are compromised when using LC–QqQ MS because the qualitative information required to support the structural elucidation of analytes is lost in the multiple reaction monitoring (MRM) mode and, in the full-scan mode, the qualitative information can be obtained, but with a loss of sensitivity. Hybrid quadrupole time-of-flight mass spectrometry (Q-TOF-MS) can resolve this limitation by its ability to provide accurate mass measurements of full-product ions, thus assuring accurate identification of analytes, and the sensitivity obtained is better than that of QqQ MS in the full-scan mode. UPLC overrides conventional liquid chromatography as it generates narrow peaks, facilitates resolution of analytes and matrix interference, and shortens chromatographic runs [35]. Combining UPLC with Q-TOF-MS offers a method with high chromatographic resolution and exact mass measurement for MS and MS/MS; therefore, it provides significant advantages with regard to selectivity, sensitivity, accuracy, and speed for rapid screening for organic contaminants in complicated environmental samples. However, only a few reports in the literature have focused on the screening of organic contaminants in the water environment by using UPLC-Q-TOF-MS [36–40].

The objective of this study was to develop a sensitive and accurate screening method for estrogens, progestogens, and phenols in potable and river water by using UPLC-Q-TOF-MS and to demonstrate its reliability in identifying target compounds at low levels in complicated water samples. To these authors' knowledge, the analytical method described in this study is the first to use UPLC-Q-TOF-MS to simultaneously screen for estrogens, progestogens, and phenols in environmental water samples. This method was successfully applied to screen for all the three groups of estrogens, progestogens, and phenols in potable and river water in Shanghai City (China).

2. Materials and methods

2.1. Chemicals

The standard reference samples (>95% in purity) of nine estrogens, seven progestogens, and five phenols were purchased from Sigma-Aldrich (Shanghai, China). Individual stock solutions of these reference standards were prepared to a concentration of 1 mg mL^{-1} in methanol and stored at −20 °C until further use. Working standard mixtures of the test compounds were prepared in a solution of acetonitrile (ACN) and water (1:9, v/v) at different concentrations by appropriate dilution of the individual stock solutions. Some characteristics of the 21 target compounds are listed in Table 1.

The LC–MS grade reagents used in this study include: water and ethyl acetate from J.T. Baker (Phillisburg, NJ, USA); methanol (MeOH) and ACN from Fisher (Fair Lawn, NJ, USA); and acetic acid, ammonium hydroxide solution, and ammonium formate from Sigma-Aldrich. Deionized water was obtained from Milli-Q-Plus (Millipore, Bedford, MA, USA).

2.2. Sample collection and preservation

Potable water samples were collected from four local residential areas, representative of four different potable water-supply plants in Shanghai City, located in the Yangtze River Delta along China's eastern coast. River water samples were collected from four different sampling sites along the Huangpu River, which flows across Shanghai City into the Yangtze River. All the water samplings were performed in March 2011.

Table 1
Characteristics of 21 target compounds and 15 post-target compounds.

Group of compounds	Name	Abbreviation	CAS registry number	Production way	Molecular formula	Molecular weight
Target estrogens	Estriol	E_3	50-27-1	Natural	$C_{18}H_{24}O_3$	288.1725
	β-Estradiol	β-E_2	50-28-2	Natural	$C_{18}H_{24}O2$	272.1776
	α-Estradiol	α-E_2	57-91-0	Natural	$C_{18}H_{24}O_2$	272.1776
	Equilin	EQ	474-86-2	Natural	$C_{18}H_{20}O_2$	268.1463
	17α-Ethinylestradiol	EE_2	57-63-6	Synthetic	$C_{20}H_{24}O_2$	296.1776
	Estrone	E_1	53-16-7	Natural	$C_{18}H_{22}O_2$	270.1620
	Diethylstilbestrol	DES	56-53-1	Synthetic	$C_{18}H_{20}O_2$	268.1463
	Dienestrol	DE	84-17-3	Synthetic	$C_{18}H_{18}O_2$	266.1307
	Hexestrol	HES	84-16-2	Synthetic	$C_{18}H_{22}O_2$	270.1620
Target phenols	Bisphenol A	BPA	80-05-7	Synthetic	$C_{15}H_{16}O_2$	228.1150
	4-tert-Butylphenol	4-t-BP	98-54-4	Synthetic	$C_{10}H_{14}O$	150.1045
	4-tert-Octylphenol	4-t-OP	140-66-9	Synthetic	$C_{14}H_{22}O$	206.1671
	4-n-Octylphenol	4-n-OP	1806-26-4	Synthetic	$C_{14}H_{22}O$	206.1671
	4-n-Nonylphenol	4-n-NP	25154-52-3	Synthetic	$C_{15}H_{24}O$	220.1827
Target progestogens	Norethindrone	NTD	68-22-4	Synthetic	$C_{20}H_{26}O_2$	298.1933
	17-Hydroxyprogesterone	17-HPT	68-96-2	Natural	$C_{21}H_{30}O_3$	330.2195
	21-Hydroxyprogesterone	21-HPT	64-85-7	Natural	$C_{21}H_{30}O_3$	330.2195
	D(−)-Norgestrel	NGT	797-63-7	Synthetic	$C_{21}H_{28}O_2$	312.2089
	Chlormadinone-17-acetate	CMA	302-22-7	Synthetic	$C_{23}H_{29}ClO_4$	404.1754
	Megestrol-17-acetate	MTA	595-33-5	Synthetic	$C_{24}H_{32}O_4$	384.2301
	Progesterone	PGT	57-83-0	Natural	$C_{21}H_{30}O_2$	314.2246
Post-target estrogens	16-Epiestriol	16-epiE_3	547-81-9	Natural	$C_{18}H_{24}O_3$	288.1725
	17-Epiestriol	17-epiE_3	1228-72-4	Natural	$C_{18}H_{24}O_3$	288.1725
	16α-Hydroxyestrone	16α-OHE_1	566-76-7	Natural	$C_{18}H_{22}O_3$	286.1569
	2-Methoxyestrone	2-$MeOE_1$	362-08-3	Natural	$C_{19}H_{24}O_3$	300.1725
	4-Methoxyestrone	4-$MeOE_1$	58562-33-7	Natural	$C_{19}H_{24}O_3$	300.1725
	3-Methoxyestrone	3-$MeOE_1$	5976-63-6	Natural	$C_{19}H_{24}O_3$	300.1725
	2-Hydroxyestrone	2-OHE_1	362-06-1	Natural	$C_{18}H_{22}O_3$	286.1569
	4-Hydroxyestrone	4-OHE_1	3131-23-5	Natural	$C_{18}H_{22}O_3$	286.1569
	2-Methoxyestradiol	2-$MeOE_2$	362-07-2	Natural	$C_{19}H_{26}O_3$	302.1882
	4-Methoxyestradiol	4-$MeOE_2$	26788-23-8	Natural	$C_{19}H_{26}O_3$	302.1882
	16-Ketoestradiol	16-ketoE$_2$	566-75-6	Natural	$C_{18}H_{22}O_3$	286.1569
	2-Hydroxyestradiol	2-OHE_2	362-05-0	Natural	$C_{18}H_{24}O_3$	288.1725
Post-target progestogens	Medroxyprogesterone acetate	MPA	71-58-9	Synthetic	$C_{24}H_{34}O_4$	386.2457
	6α-Methyl-11β-hydroxyprogesterone	MHPT	2668-66-8	Natural	$C_{22}H_{32}O_3$	344.2351
	17α,20β-Dihydroxy-4-pregnene-3-one	DPO	1662-06-2	Natural	$C_{21}H_{32}O_3$	332.2351

Two-liter water samples were obtained in duplicate for every sampling site and collected into amber glass bottles, which were flushed at least thrice by the same water sample before collection. The river water was collected at a depth of 0.5 m below the water surface. To eliminate the possibility of any interference, the sampling bottles were prepared by methanol-rinsing and then baked at 450 °C for 2 h. All the water samples collected were kept on ice during transportation to the laboratory and then stored at 4 °C in the dark. Sample analysis was carried out within 24 h of sample collection to minimize any degradation of target compounds.

2.3. Solid-phase extraction (SPE)

One liter of each water sample was filtered through 1 μm glass fiber mesh (Whatman, Maidstone, UK) to remove particulate matter that could otherwise clogging the cartridge, and then loaded onto an Oasis HLB cartridge, which was previously preconditioned by alternating 10 mL methanol and 10 mL pure water at a flow rate of 10 mL min^{-1}. During the subsequent washing step, basic interferences were reduced by washing the cartridge with 8 mL of 5% methanol aqueous solution (v/v) containing 2% acetic acid (v/v); thereafter, the acidic interferences were removed by washing the cartridge with 8 mL 5% methanol aqueous solution (v/v) containing 2% ammonium hydroxide (v/v). Next, the cartridge was rinsed by 8 mL of 65% methanol aqueous solution (v/v) and dried under vacuum for 30 min. The compounds of interest were eluted by using 10 mL methanol from the cartridge. The methanol eluate was concentrated into a dry powder under a gentle stream of nitrogen, and then reconstituted in 0.5 mL ACN:water (10:90, v/v) solution. The final solution was filtered through a 0.45-μm membrane filter into a 2-mL amber glass vial and stored at 4 °C until analysis.

2.4. Instrumentation

A Waters Acquity UPLC system (Waters, Milford, MA, USA) was coupled to a hybrid quadrupole-orthogonal acceleration-TOF mass spectrometer (SYNAPT G2; Waters Micromass, Manchester, UK) using an orthogonal Z-spray-ESI interface. The entire operation of this apparatus as well as processing of data was done by MassLynx V4.1.

Chromatographic separation was carried out on an Acquity UPLC HSS T3 column (100 mm × 2.1 mm × 1.8 μm). The injection volume was 10 μL. Progestogens analyzed in the positive ion (PI) mode were eluted with a mobile phase comprising (A) 10 mmol L^{-1} aqueous ammonium formate and (B) methanol at a flow rate of 500 μL min^{-1}. The percentage composition of organic modifier (B) was changed linearly as described: 0.1 min, 5% B; 2 min, 60% B; 10 min, 65% B; 11 min, 95% B; 12 min, 95% B; 12.5 min, 5% B; and 14 min, 5% B. Estrogens and phenols analyzed under negative ion (NI) conditions were eluted by (A) water and (B) ACN at a flow rate of 700 μL min^{-1}. The gradient of organic modifier (B) varied linearly as described: 0.1 min, 5% B; 5 min, 30% B; 6 min, 40% B; 9 min, 55% B; 10 min, 95% B; 10.5 min, 5% B; and 12 min, 5% B. The temperature of the column was set to 40 °C. The precursor ion chromatograms of 21 target compounds, extracted at a mass window of 0.05 Da from a 1-L sample of river water that spiked at a 50 ng L^{-1} level, are shown in Fig. 1.

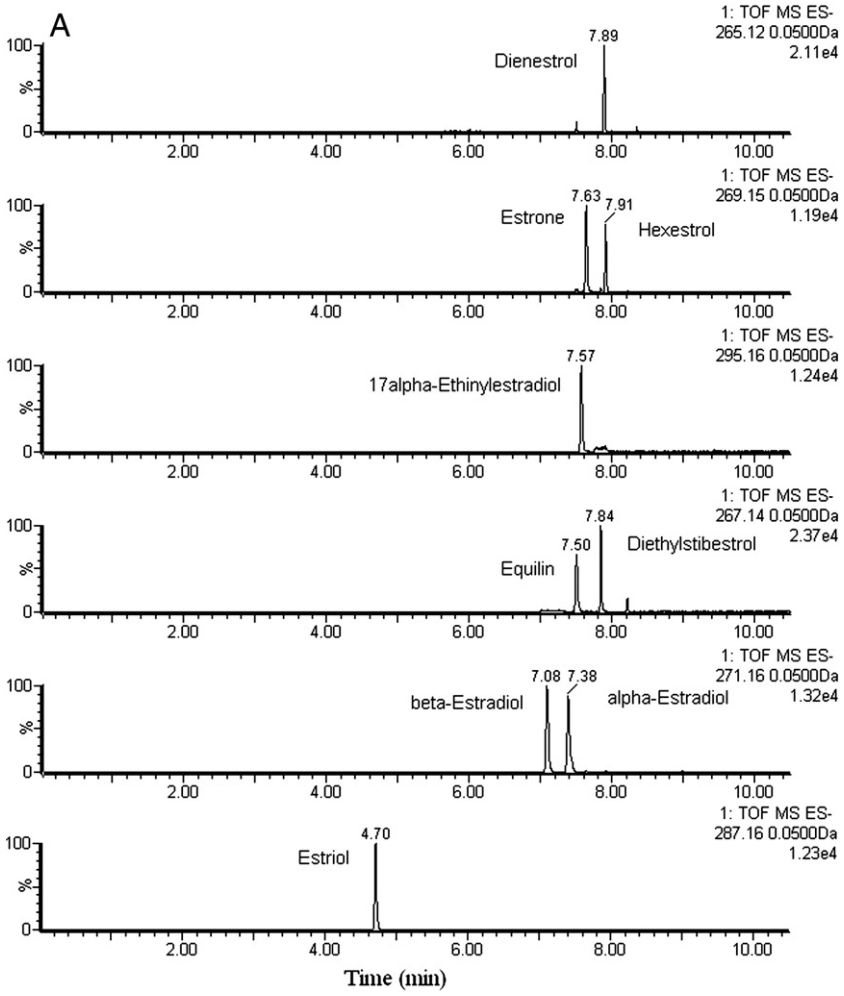

Fig. 1. Extracted precursor ion chromatograms of 21 target compounds in extract of one liter of river water samples spiked at 50 ng L^{-1} level (A: estrogens, B:phenols, C: progestogens).

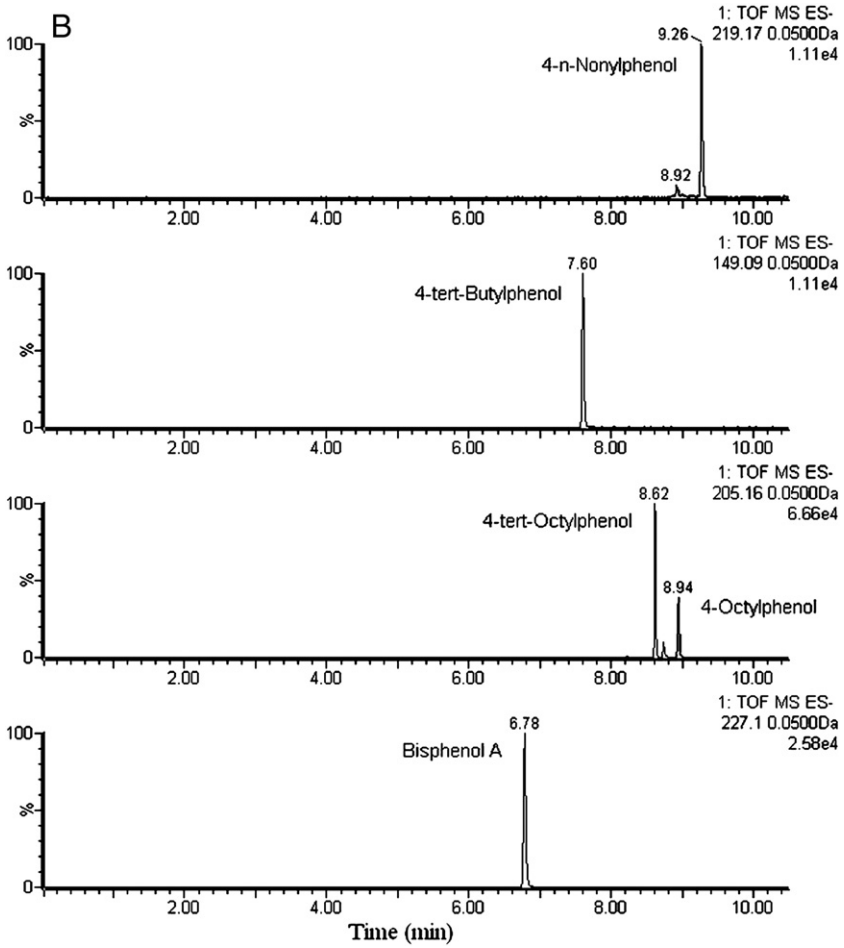

Fig. 1. (*continued*).

Mass spectrometry was performed on a Q-TOF mass spectrometer. Nitrogen was used as the nebulization gas and gas flow was set to 800/1000 (PI/NI) L h^{-1} at a temperature of 450 °C. The flow rate of cone gas was set to 40 L h^{-1} at a source temperature of 120 °C. The capillary voltage was set to 2.5/2.8(PI/NI) KV and the sampling cone voltage to 30/45(PI/NI) V. MS data was acquired across an *m/z* range of 50–600 Da in the centroid mode. The data-acquisition rate was set to 0.2 s for estrogens and phenols and 0.4 s for progestogens to gain 15–20 detection points in one chromatographic peak. MS/MS experiments were performed using variable collision energy (18–50 eV), which was optimized for each individual compound (Table 2).

Before analysis, mass calibration was conducted in the range of 50–1200 *m/z* with a 1:1 (v/v) mixture of 0.05 mmol L^{-1} NaOH:5% HCOOH, diluted 25 times by ACN:water (80:20, v/v), at a flow rate of 10 μL min^{-1}. The procedure was mandatory for both PI and NI. During analysis, 2 μg mL^{-1} of leucine–enkephalin solution in ACN: water (50:50, v/v) was injected into the lock-spray probe automatically at a flow rate of 10 μL min^{-1} for 15-s analysis intervals. It can be ionized into an $[M+H]^+$ ion weighing 556.2771 Da in the PI mode or an $[M-H]^-$ ion weighing 554.2615 Da in the NI mode, and used as the lock mass for real-time recalibration of the mass axis and to ensure accurate mass measurement.

3. Results and discussion

This study used 21 target compounds as model compounds, for which reference standards were available in our laboratory, to develop the screening method. The 21 compounds, comprising nine estrogens, five phenols, and seven progestogens, were typical compounds that were previously studied in a variety of environmental media [28,33,41,42].

3.1. Optimization of solid phase extraction

As previously described in the literature, HLB cartridges were commonly used to extract steroid hormones from environmental samples [29,30,32,33]. HLB sorbent, a macroporous copolymer prepared from a balance ratio of two monomers—the lipophilic divinylbenzene and the hydrophilic *N*-vinylpyrrolidone—can absorb a wide range of polar and no-polar compounds [43]. Because of the extensive polarity of the analytes used in this study, HLB cartridges were used, and the cleanup procedure, elution solution, and loading flow rate were optimized individually.

3.1.1. Selection of cleanup procedure

The washing of sorbent was a critical step in the sample cleanup. At first, a typical stepwise washing procedure, which applied 8 mL methanol:water (5:95, v/v) solution containing 2% acetic acid (v/v) followed by 8 mL methanol:water (5:95, v/v) solution containing 2% ammonium hydroxide (v/v), was investigated for efficacy in washing the Oasis HLB SPE cartridge [44]. As shown in Supplementary Fig. S1, the intensities (peak area) of the 21 target compounds spiked in samples of river water at 50 ng L^{-1} level before SPE were comparable to those that spiked with the same amount into the extract after SPE, and both of these readings accounted for 2–65% of the intensities obtained from the equivalent standard solutions (100 ng mL^{-1}). We hypothesized that the losses of recoveries of the target compounds were mostly attributed to the matrix interference (ion suppression),

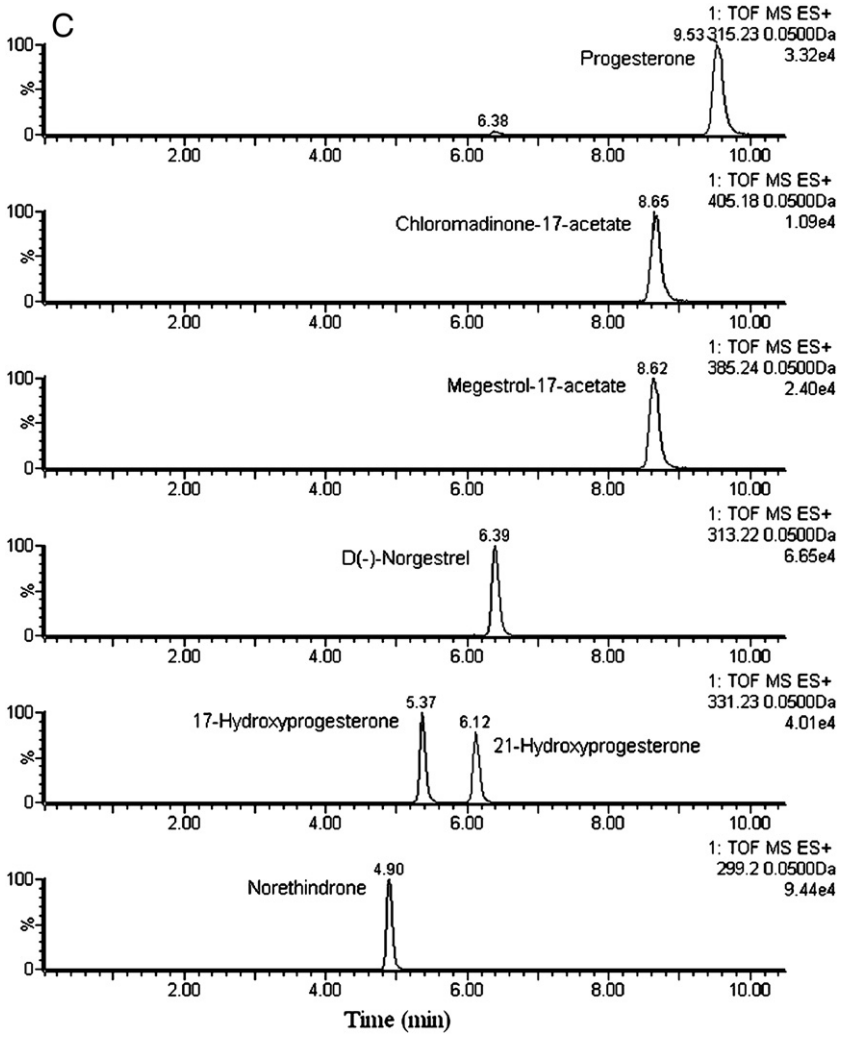

Fig. 1. (*continued*).

and the washing procedure described above was not insufficient to remove matrix interferences.

Next, an aqueous washing solution with higher methanol percentage was added to improve the stepwise washing procedure. A protocol employing gradual increase of methanol content in the washing solution to enhance the elution power of impurities was designed to identify the optimal point at which matrix interference could be minimized. As shown in Supplementary Fig. S2, with an increase in the methanol content in the washing solution, the absolute recoveries of 21 compounds were gradually improved by reducing matrix interferences, and were optimal when methanol content in the washing solution was between 60% and 70%. However, with further increase in the methanol content of washing solution, the recoveries of most target compounds began to decrease and, for the washing solution with 90% methanol, the recoveries of 14 of the 21 compounds were less than 50% as a result of elution of the target compounds from the HLB cartridge. Therefore, a washing step with 8 mL 65% methanol aqueous solution (v/v) was included after the typical washing procedure described above.

3.1.2. Selection of elution solution

Several elution solvents described in previous reports in the literature [30,43,44] were tested for their elution effects on the target compounds from the HLB cartridge. Absolute recoveries from these different solvents for 1-L of river water sample that spiked at 50 ng L^{-1} are shown in Supplementary Fig. S3. The recoveries ranged from 55% to 100% for estrogens and phenols, and from 33% to 61% for progestogens with an ethyl acetate (EtOAC):methanol (90:10, v/v) mixture as the elution solvent. These values are comparable to those obtained by using ethyl acetate as the elution solvent. Although the recoveries using the methanol as elution solvent were between 74% and 107% for estrogens and inferior to those between 87% and 100% obtained with the ethyl acetate:methanol (90:10, v/v) mixture, recoveries from 73% to 109% for progestogens were superior to those between 33% and 61% obtained using an ethyl acetate:methanol (90:10, v/v) mixture. Following a comprehensive screening of all target compounds, methanol was selected as the optimal elution solution.

3.1.3. Effect of flow rate on the recoveries of analytes

The flow rate for loading the sample onto the SPE sorbent was another important factor for recoveries of the analytes. A loading rate that was too fast could result in low recoveries due to breakthrough of the cartridge, whereas a slow loading rate would increase the sample-pretreatment time. The optimization of flow rates was carried out on 1 L Milli-Q pure water spiked at 50 ng L^{-1} level. The results showed that the recoveries of analytes had no significant differences at flow rates of 5 and 10 mL min^{-1} (data not shown). Thus, a flow rate of 10 mL min^{-1} was selected for all further experiments.

Table 2
Collision energy, elemental composition and error between theoretical and experimental mass of precursor and product ions of 21 target compounds in spiked river sample using the Q-TOF mode.

Group	Compound	Collision energy (ev)	Elemental composition	Theoretical mass (*m*/*z*)	Experimental mass (*m*/*z*)	Error	
						mDa	ppm
NI mode estrogens	E_3	45	$C_{18}H_{23}O_3$[a]	287.1647	287.1647	0.0	0.0
			$C_{12}H_{11}O$	171.0810	171.0822	1.2	7.0
			$C_{10}H_9O$	145.0653	145.0658	0.5	3.5
	β-E_2	50	$C_{18}H_{23}O_2$[a]	271.1698	271.1679	−1.9	−7.0
			$C_{13}H_{11}O$	183.0810	183.0793	−1.7	−9.3
			$C_{10}H_9O$	145.0653	145.0658	0.5	3.5
	α-E_2	50	$C_{18}H_{23}O_2$[a]	271.1698	271.1686	−1.2	−4.4
			$C_{17}H_{19}O$	239.1441	239.1424	−1.7	−7.1
			$C_{10}H_9O$	145.0653	145.0658	0.5	3.5
	EQ	35	$C_{18}H_{19}O_2$[a]	267.1385	267.1411	2.6	9.7
			$C_{14}H_{15}O$	199.1123	199.1138	1.5	7.5
			$C_{10}H_9O$	145.0653	145.0658	0.5	3.4
	EE_2	40	$C_{20}H_{23}O_2$[a]	295.1698	295.1694	−0.40	−1.4
			$C_{14}H_{15}O$	199.1123	199.1138	1.50	7.5
			$C_{10}H_9O$	145.0653	145.0658	0.5	3.5
	E1	40	$C_{18}H_{21}O_2$[a]	269.1542	269.1561	1.9	7.0
			$C_{11}H_{11}O$	159.0810	159.0831	2.1	13.2
			$C_{10}H_9O$	145.0653	145.0658	0.5	3.5
	DES	28	$C_{18}H_{19}O_2$[a]	267.1385	267.1410	2.5	9.4
			$C_{17}H_{15}O_2$	251.1072	251.1100	2.8	11.2
			$C_{16}H_{13}O_2$	237.0916	237.0921	0.5	2.1
	DE	30	$C_{18}H_{17}O_2$[a]	265.1229	265.1228	−0.1	−0.38
			$C_{17}H_{13}O_2$	249.0916	249.0934	1.8	7.2
			$C_{16}H_{11}O_2$	235.0759	235.0770	1.1	4.9
	HES	22	$C_{18}H_{21}O_2$[a]	269.1542	269.1559	1.7	6.3
			C_9H_9O	133.0653	133.0657	0.4	3.0
			C_8H_7O	119.0497	119.0499	0.2	1.7
NI mode phenols	BPA	28	$C_{15}H_{15}O_2$[a]	227.1072	227.1078	0.6	2.6
			$C_{14}H_{11}O_2$	211.0759	211.0759	0.0	0.0
			C_9H_9O	133.0653	133.0653	0.0	0.0
	4-t-OP	28	$C_{14}H_{21}O$[a]	205.1592	205.1591	−0.1	−0.5
			C_9H_9O	133.0653	133.0652	−0.1	−0.8
	4-OP	35	$C_{14}H_{21}O$[a]	205.1592	205.1618	2.6	12.7
			C_8H_7O	119.0497	119.0501	0.4	3.4
			C_7H_6O	106.0419	106.0418	−0.1	−0.9
	4-t-BP	28	$C_{10}H_{13}O$[a]	149.0966	149.0966	0.0	0.0
			C_9H_9O	133.0653	133.0657	0.4	3.0
			C_8H_5O	117.0340	117.0346	0.6	5.1
	4-n-NP	35	$C_{15}H_{23}O$[a]	219.1749	219.1755	0.6	2.7
			C_8H_7O	119.0497	119.0505	0.8	6.7
			C_7H_6O	106.0419	106.0417	−0.2	−1.9
PI mode progestogens	NTD	25	$C_{20}H_{27}O_2$[a]	299.2011	299.2013	0.2	0.7
			$C_{16}H_{23}O$	231.1749	231.1748	−0.1	−0.4
			C_7H_9O	109.0653	109.0659	0.6	5.5
	17-HPT	25	$C_{21}H_{31}O_3$[a]	331.2273	331.2274	0.1	0.3
			C_7H_9O	109.0653	109.0651	−0.2	−1.8
			C_6H_9O	97.0653	97.0648	−0.5	−5.2
	21-HPT	25	$C_{21}H_{31}O_3$[a]	331.2273	331.2278	0.5	1.5
			C_7H_9O	109.0653	109.0652	−0.1	−0.9
			C_6H_9O	97.0653	97.0654	0.1	1.0
	PGT	24	$C_{21}H_{31}O_2$[a]	315.2324	315.2333	0.9	2.9
			C_7H_9O	109.0653	109.0654	0.1	0.9
			C_6H_9O	97.0653	97.0654	0.1	1.0
	CMA	18	$C_{23}H_{30}O_4Cl$[a]	405.1833	405.1821	−1.2	−3.0
			$C_{21}H_{26}O_2Cl$	345.1621	345.1623	0.2	0.6
			$C_{21}H_{25}O_2$	309.1855	309.1851	−0.4	−1.3
	MTA	22	$C_{24}H_{33}O_4$[a]	385.2379	385.2365	−1.4	−3.6
			$C_{22}H_{29}O_2$	325.2168	325.2167	−0.1	−0.3
			$C_{19}H_{23}O$	267.1749	267.1745	−0.4	−1.5
	NGT	26	$C_{21}H_{29}O_2$[a]	313.2168	313.2165	−0.3	−1.0
			$C_{17}H_{25}O$	245.1905	245.1896	−0.9	−3.7
			C_7H_9O	109.0653	109.0654	0.1	1.2

[a] Precursor ion.

3.2. Optimization of UPLC-Q-TOF-MS

Good chromatographic separation of the compounds is another efficient way to decrease matrix interference. Selection of the separation column is a key step in chromatography. Two types of UPLC columns, BEH C18 column (100 mm × 2.1 mm × 1.7 μm; Waters) and HSS T3 (100 mm × 2.1 mm × 1.8 μm; Waters), were tested. The results showed that difficulties were encountered with the BEH C18 column for separation of estrogens and phenols from the matrix interferences in river water by variation of mobile phase composition (methanol:water mixture or ACN:water mixture), gradient elution program, mobile phase pH, and flow rate. However, good separation was achieved by using

the HSS T3 column after elaborate adjustment of gradient elution and flow rate of mobile phase. A comparable chromatographic separation of progestogens could be obtained on both BEH C18 and HSS T3 columns. Therefore, in this study, HSS T3 was selected.

Because the ionization efficiency of ESI source is also affected by solvent conditions, the mobile phase composition and the additive were investigated. From Fig. 2a, it is apparent that the signal intensities of estrogens and phenols in the mobile phase of ACN:water mixture, without additive, were obviously higher than those in the rest of the mobile phase compositions, including methanol:water, ACN:10 mmol L^{-1} ammonium formate (AF), methanol:10 mmol L^{-1} ammonium formate, ACN:0.1% acetate acid (AA), and methanol:0.1% acetate acid solutions. For progestogens, signal intensities in the mobile phase mixture of both methanol:water and methanol:10 mmol L^{-1} ammonium formate were significantly higher than the other mobile phase compositions (Fig. 2b). In consideration of the easy formation of $[M+Na]^+$ precursor ions of progestogens in the PI mode in the methanol:water mixture, the mobile phase mixture of methanol:10 mmol L^{-1} ammonium formate was used for analysis of progestogens.

The optimization of the key parameters (capillary voltage, sampling core voltage, source temperature, desolvation temperature, and desolvation gas flow) of Q-TOF-MS was done by directly infusing a mixture of standard solutions (100 ng mL^{-1}) into the mass spectrometer. The optimal parameters have been described in Section 2.4. For MS/MS analysis, the collision energy for each analyte was optimized by complete analysis of UPLC-Q-TOF (Table 2).

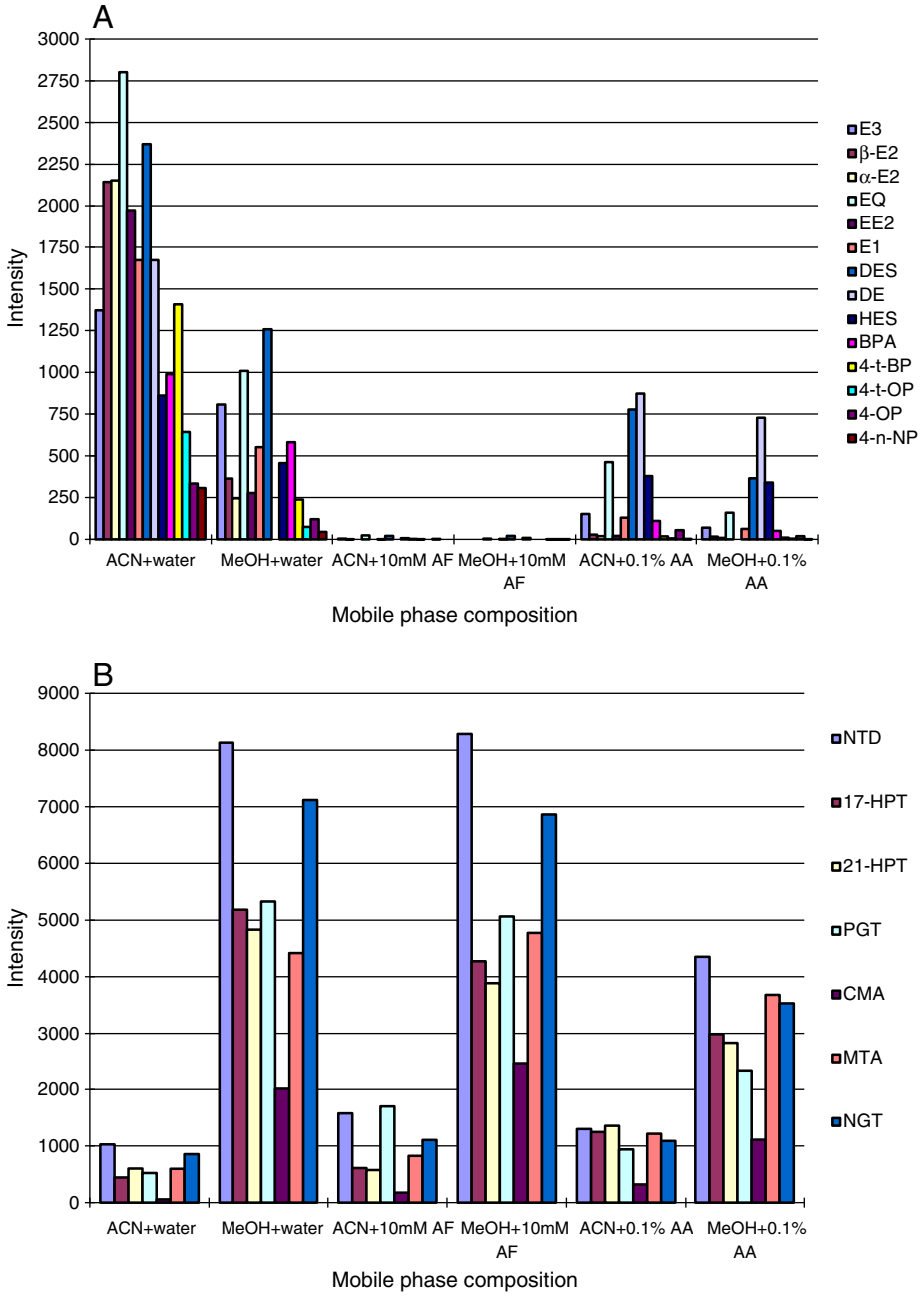

Fig. 2. Effects of different mobile phase compositions on ionization efficiency of target compounds (a: estrogens and phenols, b: progestogens, AF: ammonium formate, AA: acetate acid, ACN: acetonitrile, MeOH: methanol).

Table 3
Parameters of the analytical method performance.

Compound	r^2	Drinking water							River water						
		Spiked 5 ng L^{-1}		Spiked 50 ng L^{-1}		ME (%)	LOD (ng L^{-1})	LOQ (ng L^{-1})	Spiked 5 ng L^{-1}		Spiked 50 ng L^{-1}		ME (%)	LOD (ng L^{-1})	LOQ (ng L^{-1})
		Rec (%)	RSD (%)	Rec (%)	RSD (%)				Rec (%)	RSD (%)	Rec (%)	RSD (%)			
E_3	0.997	75	11	82	9	10	0.27	0.91	67	16	78	12	13	0.28	0.95
β-E_2	0.9997	78	14	86	10	16	0.38	1.27	69	18	72	15	18	0.42	1.40
α-E_2	0.9998	68	15	81	13	17	0.26	0.85	62	21	75	17	20	0.30	1.00
EQ	0.9998	85	12	96	8	11	0.16	0.53	73	16	82	11	17	0.34	1.13
EE_2	0.9998	76	14	82	11	18	0.35	1.17	71	18	79	13	19	0.29	0.96
E_1	0.998	73	13	86	7	−12	0.25	0.83	76	19	87	9	13	0.31	1.03
DES	0.998	83	17	94	13	−15	0.33	1.10	72	22	81	14	−18	0.38	1.28
DE	0.993	76	18	106	15	−19	0.35	1.17	121	23	109	18	−31	0.31	1.03
HES	0.997	83	15	97	9	−11	0.48	1.60	115	26	96	18	−26	0.51	1.70
BPA	0.998	76	16	87	10	15	0.22	0.74	68	19	83	13	18	0.34	1.13
4-t-OP	0.975	56	22	67	19	25	0.35	1.17	46	31	52	25	31	0.43	1.43
4-OP	0.997	76	14	83	10	16	0.52	1.73	65	16	74	12	21	0.72	2.40
4-t-BP	0.995	55	21	63	18	29	0.30	0.99	50	27	56	22	32	0.32	1.08
4-n-NP	0.994	69	22	76	11	−10	0.41	1.37	114	30	86	19	−28	0.54	1.80
NTD	0.996	61	19	74	13	13	0.22	0.73	56	23	68	16	21	0.23	0.77
17-HPT	0.995	76	15	86	10	16	0.14	0.46	72	18	82	11	19	0.28	0.93
21-HPT	0.993	71	13	83	9	14	0.28	0.93	111	16	97	13	−23	0.35	1.17
PGT	0.992	78	24	86	15	−15	0.50	1.67	134	35	116	29	−56	0.42	1.40
CMA	0.976	74	22	85	12	−14	0.51	1.70	125	29	109	23	−33	0.45	1.50
MTA	0.983	72	18	82	14	14	0.26	0.87	121	23	113	19	−26	0.34	1.13
NGT	0.995	79	18	86	13	16	0.23	0.77	63	21	75	15	23	0.25	0.83

ME: matrix effects, Rec: recovery, RSD: relative standard deviation.

3.3. Implementation of the method

3.3.1. Q-TOF screening of target compounds

Due to the superior resolving power and mass-measurement accuracy provided by Q-TOF-MS, the method presented was efficient in isobaric interference reduction and analyte determination. The primary screening could be achieved in TOF-MS mode by two techniques. The first technique involved matching the retention time of the analyte with that obtained for a standard solution. The other technique involved accurate mass measurement of molecular ions of the target compounds. The accurate mass data for the molecular ions were processed by MassLynx, a software program which provided the corresponding elemental composition and the mass errors (i.e., differences between experimental and theoretical masses). Mass errors for all 21 molecular ions obtained in the TOF mode in spiked river water were between −1.0 and 1.6 mDa or −2.4 and 4.7 ppm (data not shown), which fell within the scope of the widespread acceptable accuracy threshold of 5 ppm.

Further validation of the detected compounds can be accomplished in Q-TOF-MS/MS mode by accurate mass measurement and relative abundance of the obtained product ions. Table 2 lists accurate mass data for precursor and main product ions of all 21 target compounds and their corresponding elemental compositions at optimized Q-TOF-MS/MS conditions in spiked river water samples. The errors between experimental and theoretical masses of precursor and product ions of all target compounds were between −1.9 and 2.8 mDa. The results of this study conform to EU regulations (EU Commission Decision 2002/657/EC), which require at least three identification points (IPs) for identification of organic residues. For the majority of target compounds analyzed in this study, a total of 7 IPs could be earned by accurate mass measurements of one precursor and two product ions (Q-TOF mode). For compounds such as 4-t-OP, only one product ion is available, but the 4.5 IPs were still achieved on the basis of accurate mass measurements of one precursor and one product ion. The Q-TOF-MS/MS spectra of three representative compounds (estrone, BPA, and progesterone) and their tentative interpretations of fragmentation pattern are shown in Supplementary Fig. S4.

3.3.2. Matrix effect in UPLC-Q-TOF-MS

Suppression or enhancement of ionization in the ESI source caused by co-elute from chromatography column, known as matrix effects (ME), was a common problem when analyzing compounds of interest by LC–MS/MS. In this study, the impact of matrix effects on signal intensity was investigated in both potable and river water extracts that were unspiked or spiked with all target standards at a concentration of 50 ng mL^{-1}. The extent of suppression or enhancement of signal intensity was calculated for each individual compound using Eq. (1) [45], and the result was expressed as a percentage, as follows:

$$\frac{A_s-\left(A_{sp}-A_{usp}\right)}{A_s}\times 100\% \quad (1)$$

where A_s is the peak area for standards in pure solvent, A_{sp} is the peak area for standards spiked after extraction into water extracts, and A_{usp} is the peak area obtained in unspiked water extracts. As shown in Table 3, the MEs of most of the target compounds ranged from −33% to 32%; however, −56% of PGT and the MEs in river water samples were generally more severe than those in potable water samples, where the negative values denotes the ion enhancement and the positive values represents ion suppression.

3.3.3. Quantitative analysis

The limits of detection (LODs) and limits of quantitation (LOQs) of the method were calculated by analysis of spiked potable or river water, with minimum concentrations of each individual compound at a signal-to-noise ratio of 3 and 10, respectively. The LODs and LOQs of the newly developed method in river water ranged between 0.23 and 0.72 ng L^{-1} and between 0.77 and 2.40 ng L^{-1}, respectively (Table 3).

Quantitation was carried out using peak area of the extracted molecular ion chromatogram for each individual compound from a total ion chromatogram at a mass window of 0.05 Da in the TOF-MS mode. All the target compounds were quantified using external standard calibration. Eight-point calibration curves were calculated for the concentration range from the LOQs to 500 ng L^{-1} (1000 ng L^{-1} for phenols).

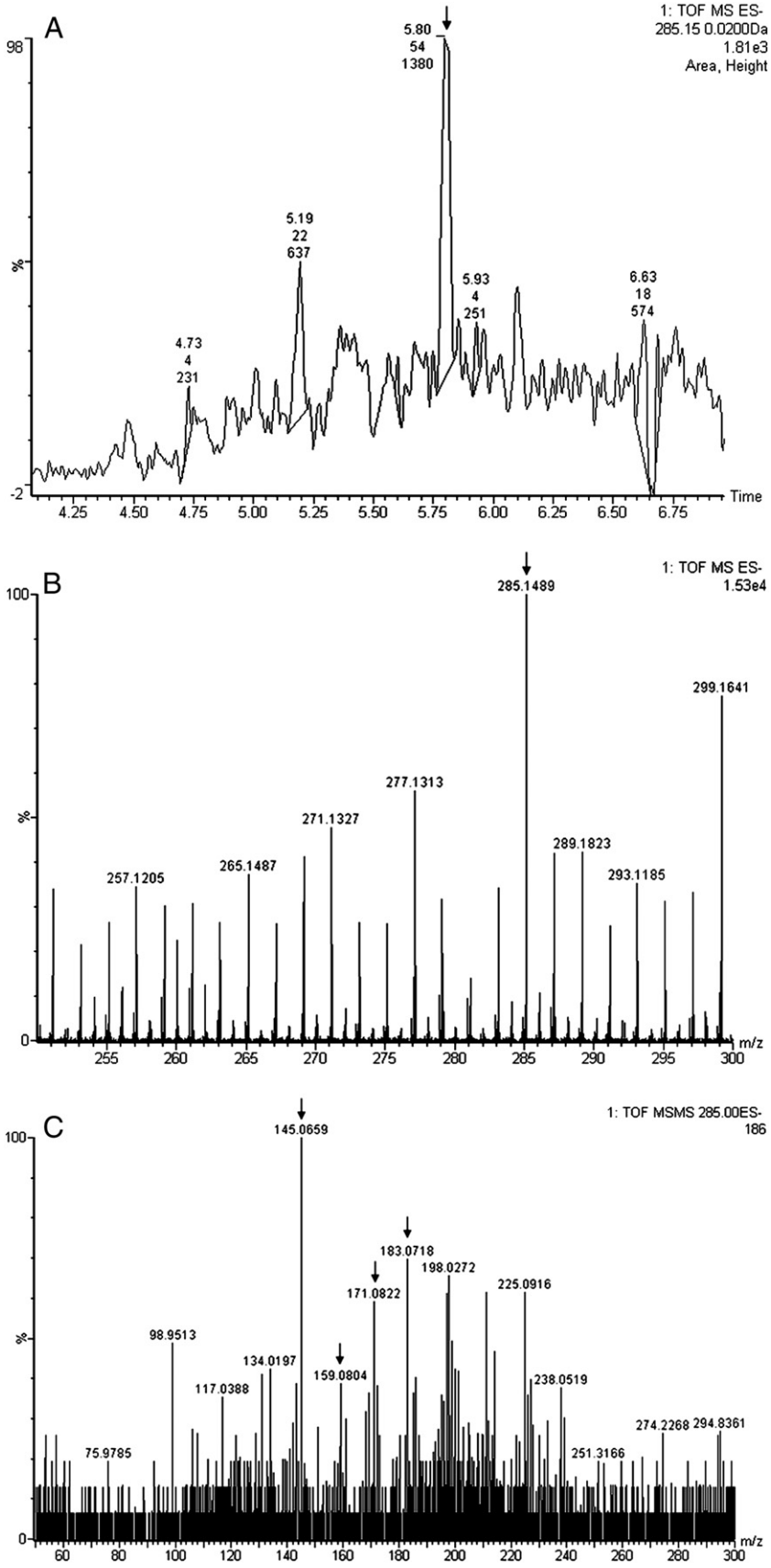

Fig. 3. Exploration of one post-target compound in river water sample.

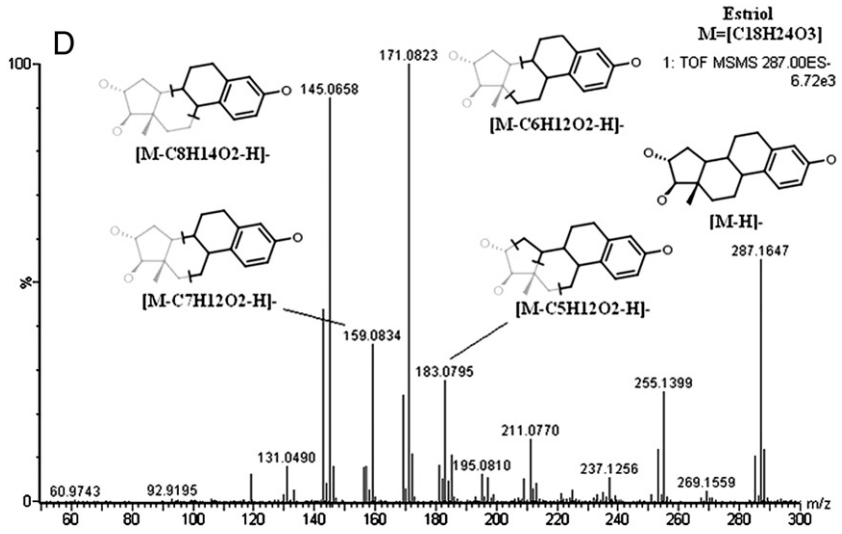

Fig. 3. (*continued*).

The coefficients of determination (r^2) of calibration curves for all compounds were greater than 0.990, with the exception of 4-t-OP (r^2: 0.975) and CMA (r^2: 0.976; Table 3).

Five replicates ($n = 5$) of 1-L potable or river water samples spiked with the standard mixture at two different concentration levels (5 and 50 ng L^{-1}) were used to estimate the accuracy and repeatability of the method (Table 3). Average method recoveries of the compounds obtained from potable water samples range between 55% and 85% at a low spiked level and were better than those in the range between 46% and 134% for river water samples; this was matched with relative standard deviations (RSDs) of 11–24% for potable water versus 16–35% for river water at low spiked level. The overall recovery of the compounds varied from 46% to 134%, with RSDs ranging from 7% to 35%.

3.4. Application of real water samples

Sixteen water samples (8 river water samples and 8 potable water samples) were analyzed by the proposed method. Three (E_1, E_3, and BPA) of the 21 target compounds were identified in water samples. For positive confirmation of these compounds, strict criteria developed based on the EU Commission Decision 2002/657/EC standards were met: the chromatographic retention time of the compounds in the water sample were not to vary more than 2%, the relative abundance of the characteristic ions had to be within the 25% margin compared with the calibration standards, and the IPs obtained had to be greater than four. Estriol, estrone, and BPA were found in river water samples, and their concentration ranges were 1.0–1.6, 1.6–5.2, and 229–690 ng L^{-1}, respectively. Similarly, BPA was identified in potable water samples, with concentrations ranging from 6.4 to 423 ng L^{-1}. Levels of estrogens and BPA detected in river water samples were the similar to levels reported, specifically 0.1–3.9 for E_1 and 13.9 ng L^{-1} for E_3 in Beijing, China [46] and 19.1–830 ng L^{-1} for BPA in Haihe, China [47]. The BPA levels in potable water in this study were comparable to those identified in Guangzhou, China [48].

3.5. Post-target analysis

As data acquisition was performed at full-scan mode for accurate mass measurement with Q-TOF-MS, a post-target screening approach was undertaken, where searching and identification of other interesting compounds could be done at any time without performing additional analyses or without using reference standards.

In this study, the post-target analysis, including 12 estrogen metabolites and other three progestogens, was performed. The investigation of those post-target compounds in water samples has been described in some reports in the literature [30,49,50]. Some of their characteristics are listed in Table 1. The screening procedure comprised the following steps. In the TOF-MS mode, using theoretical molecular mass of the selected compounds, chromatograms of extracted precursor ions in a narrow mass window (0.02 Da) were gained from total ion chromatograms. Peaks with an area greater than 50 a.u. (arbitrary units) were selected to evaluate experimental masses of these compounds. The mass error between experimental and theoretical molecule mass was less than 3 mDa, which implied that the selected compounds may occur in the samples. Further confirmation should be performed by accurate mass measurement of characteristic fragment ions of suspected compounds in Q-TOF-MS/MS mode.

During the application of the above-described screening procedure to river water-sample analysis, an estrogen metabolite was identified. As shown in Fig. 3a and b, in the TOF-MS mode, a suspected compound with an area of 54 a.u. was found at a retention time of 5.80 min, close to that of 4.70 min for estriol (Fig. 1a), and the mass error for its precursor ion of experimentally accurate mass ($[M-H]^-$, 285.1489) was 0.2 mDa, compared with the theoretical deprotonated molecular mass ($[C_{18}H_{22}O_3$-H]-, 285.1491) of four post-target compounds with identical elemental composition ($C_{18}H_{22}O_3$), including 16α-OHE_1, 2-OHE_1, 4-OHE_1, and 16-ketoE$_2$ (the structures are shown in Fig. 4). In the Q-TOF-MS/MS spectrum for the suspected compound (Fig. 3c), four fragment ions (*m/z* 145.0659, 159.0804, 171.0822, and 183.0718) were detected, and they also existed in the MS/MS spectrum of estriol (Fig. 3d). The similar fragmentation pattern suggested that the suspected compounds may share a common structural fragment $C_{10}H_9O$ with estriol (Fig. 4); therefore, two candidates without the common structural fragment, 2-OHE_1 and 4-OHE_1 (Fig. 4), were ruled out. In addition, a fragment ion of *m/z* 171.0822 has been reported as the monitoring ion of 16α-OHE_1 and 16-ketoE$_2$ in triple quadrupole mass spectrometry [49]; however, it is difficult to determine which one is the suspected compound unless their reference standards are available. The concentration was estimated to be ~5 ng L^{-1} by the peak area of the extracted precursor ion chromatogram using the calibration coefficient of estriol.

4. Conclusion

In this study, an accurate, sensitive, and reliable method for screening of estrogens, phenols, and progestogens in potable and river water samples using off-line SPE followed by UPLC-Q-TOF-MS was established and successfully applied to actual water samples.

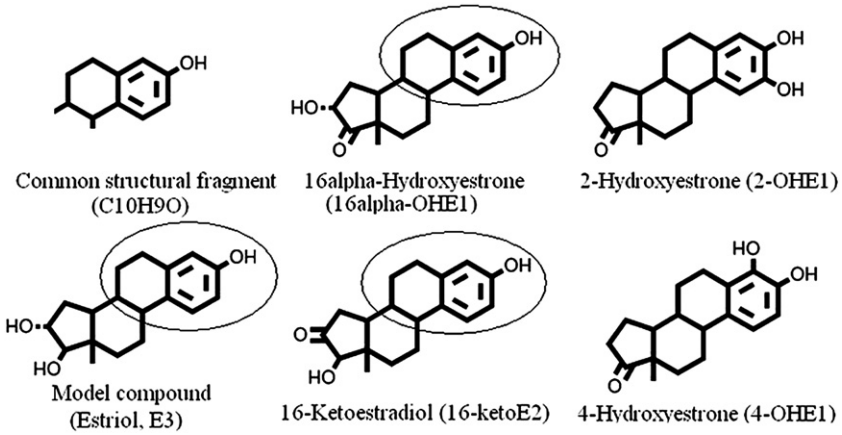

Fig. 4. Structures of 5 estrogen metabolites (16α-OHE_1, 2-OHE_1, E_3, 16-ketoE_2 and 4-OHE_1) and common structural fragment ($C_{10}H_9O$).

This method performed well, with an LOD less than 0.72 ng L^{-1} and recoveries between 46% and 134% for all target compounds. Estriol, estrone, and BPA were detected in river water, but only BPA was identified in potable water samples. Post-target screening was undertaken to search for 12 estrogen metabolites and other three progestogen compounds in the water samples studied, and the presence of an estrogen metabolite was hypothesized. The method described in this article has been shown to be a powerful tool for target or post-target screening and confirmation of the presence of estrogens, phenols, progestogens, and their metabolites at low concentrations in environmental water samples.

Supplementary materials related to this article can be found online at doi:10.1016/j.microc.2011.09.010.

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References

[1] T.M. Crisp, E.D. Clegg, R.L. Cooper, W.P. Wood, D.G. Anderson, K.P. Baetcke, J.L. Hoffmann, M.S. Morrow, D.J. Rodier, J.E. Schaeffer, L.W. Touart, M.G. Zeeman, Y.M. Patel, Environmental endocrine disruption: an effects assessment and analysis, Environ. Health Persp. 106 (1998) 11–56.
[2] T. Manning, Endocrine-disrupting chemicals: a review of the state of the science, Australas. J. Ecotoxicol. 11 (2005) 1–52.
[3] A.K. Hotchkiss, C.V. Rider, C.R. Blystone, V.S. Wilson, P.C. Hartig, G.T. Ankley, P.M. Foster, C.L. Gray, L.E. Gray, Fifteen years after "Wingspread" — environmental endocrine disrupters and human and wildlife health: where we are today and where we need to go, Toxicol. Sci. 105 (2008) 235–259.
[4] J. Gray, N. Evans, B. Taylor, J. Rizzo, M. Walker, State of the evidence the connection between breast cancer and the environment, Int. J. Occup. Environ. Health 15 (2009) 43–78.
[5] E. Diamanti-Kandarakis, J.P. Bourguignon, L.C. Giudice, R. Hauser, G.S. Prins, A.M. Soto, R.T. Zoeller, A.C. Gore, Endocrine-disrupting chemicals: an endocrine society scientific statement, Endocr. Rev. 30 (2009) 293–342.
[6] G. Pojana, A. Gomiero, N. Jonkers, A. Marcomini, Natural and synthetic endocrine disrupting compounds (EDCs) in water, sediment and biota of a coastal lagoon, Environ. Int. 33 (2007) 929–936.
[7] H.M. Hwang, E.K. Park, T.M. Young, B.D. Hammock, Occurrence of endocrine-disrupting chemicals in indoor dust, Sci. Total. Environ. 404 (2008) 26–35.
[8] R.A. Rudel, L.J. Perovich, Endocrine disrupting chemicals in indoor and outdoor air, Atmos. Environ. 43 (2009) 170–181.
[9] F.A. Caliman, M. Gavrilescu, Pharmaceuticals, personal care products and endocrine disrupting agents in the environment — a review, Clean-Soil Air Water 37 (2009) 277–303.
[10] Z.H. Liu, Y. Kanjo, S. Mizutani, A review of phytoestrogens: their occurrence and fate in the environment, Water Res. 44 (2010) 567–577.
[11] A. Kortenkamp, Ten years of mixing cocktails: a review of combination effects of endocrine-disrupting chemicals, Environ. Health Perspect. 115 (2007) 98–105.
[12] S. Swan, C. Nellemann, Mixture effects of endocrine disrupting compounds in vitro Panel discussion, Int. J. Androl. 33 (2010) 433.
[13] G.G. Ying, R.S. Kookana, Y.J. Ru, Occurrence and fate of hormone steroids in the environment, Environ. Int. 28 (2002) 545–551.
[14] A. Pal, K. Gin, A. Lin, M. Reinhard, Impacts of emerging organic contaminants on freshwater resources: review of recent occurrences, sources, fate and effects, Sci. Total. Environ. 408 (2010) 6062–6069.
[15] J. Gascon, A. Oubina, D. Barcelo, Detection of endocrine-disrupting pesticides by enzyme-linked immunosorbent assay (ELISA): application to atrazine, Trac-Trend. Anal. Chem. 16 (1997) 554–562.
[16] J.H. Clemons, L.M. Allan, C.H. Marvin, Z. Wu, B.E. Mccarry, D.W. Bryant, T.R. Zacharewski, Evidence of estrogen- and TCDD-like activities in crude and fractionated extracts of PM10 air particulate material using in vitro gene expression assays, Environ. Sci. Technol. 32 (1998) 1853–1860.
[17] E.J. Routledge, D. Sheahan, C. Desbrow, G.C. Brighty, M. Waldock, J.P. Sumpter, Identification of estrogenic chemicals in STW effluent. 2. In vivo responses in trout and roach, Environ. Sci. Technol. 32 (1998) 1559–1565.
[18] D. Larsson, M. Adolfsson-Erici, J. Parkkonen, M. Pettersson, A.H. Berg, P.E. Olsson, L. Forlin, Ethinyloestradiol — an undesired fish contraceptive? Aquat. Toxicol. 45 (1999) 91–97.
[19] T.A. Hanselman, D.A. Graetz, A.C. Wilkie, Manure-borne estrogens as potential environmental contaminants: a review, Environ. Sci. Technol. 37 (2003) 5471–5478.
[20] S. Combalbert, G. Hernandez-Raquet, Occurrence, fate, and biodegradation of estrogens in sewage and manure, Appl. Microbiol. Biotechnol. 86 (2010) 1671–1692.
[21] A.O. Ifelebuegu, The fate and behavior of selected endocrine disrupting chemicals in full scale wastewater and sludge treatment unit processes, Int. J. Environ. Sci. Technol. 8 (2011) 245–254.
[22] V. Gabet, C. Miege, P. Bados, M. Coquery, Analysis of estrogens in environmental matrices, Trac-Trend. Anal. Chem. 26 (2007) 1113–1131.
[23] Z.H. Liu, Y. Kanjo, S. Mizutani, Urinary excretion rates of natural estrogens and androgens from humans, and their occurrence and fate in the environment: a review, Sci. Total. Environ. 407 (2009) 4975–4985.
[24] S.C. Laws, S.A. Carey, J.M. Ferrell, G.J. Bodman, R.L. Cooper, Estrogenic activity of octylphenol, nonylphenol, bisphenol A and methoxychlor in rats, Toxicol. Sci. 54 (2000) 154–167.
[25] M. Petrovic, E. Eljarrat, M. de Alda, D. Barcelo, Endocrine disrupting compounds and other emerging contaminants in the environment: a survey on new monitoring strategies and occurrence data, Anal. Bioanal. Chem. 378 (2004) 549–562.
[26] K. Guenther, V. Heinke, B. Thiele, E. Kleist, H. Prast, T. Raecker, Endocrine disrupting nonylphenols are ubiquitous in food, Environ. Sci. Technol. 36 (2002) 1676–1680.
[27] E.L. Teuten, J.M. Saquing, D. Knappe, M.A. Barlaz, S. Jonsson, A. Bjorn, S.J. Rowland, R.C. Thompson, T.S. Galloway, R. Yamashita, D. Ochi, Y. Watanuki, C. Moore, H.V. Pham, T.S. Tana, M. Prudente, R. Boonyatumanond, M.P. Zakaria, K. Akkhavong, Y. Ogata, H. Hirai, S. Iwasa, K. Mizukawa, Y. Hagino, A. Imamura, M. Saha, H. Takada, Transport and release of chemicals from plastics to the environment and to wildlife, Philos. Trans. R. Soc. 364 (2009) 2027–2045.
[28] H. Mol, S. Sunarto, O.M. Steijger, Determination of endocrine disruptors in water after derivatization with N-methyl-N-(tert.-butyldimethyltrifluoroacetamide) using gas chromatography with mass spectrometric detection, J. Chromatogr. A 879 (2000) 97–112.
[29] J.B. Quintana, J. Carpinteiro, I. Rodriguez, R.A. Lorenzo, A.M. Carro, R. Cela, Determination of natural and synthetic estrogens in water by gas chromatography with mass spectrometric detection, J. Chromatogr. A 1024 (2004) 177–185.
[30] H. Chang, S.M. Wu, J.Y. Hu, M. Asami, S. Kunikane, Trace analysis of androgens and progestogens in environmental waters by ultra-performance liquid chromatography–electrospray tandem mass spectrometry, J. Chromatogr. A 1195 (2008) 44–51.
[31] E. Vulliet, L. Wiest, R. Baudot, M.F. Grenier-Loustalot, Multi-residue analysis of steroids at sub-ng/L levels in surface and ground-waters using liquid chromatography coupled to tandem mass spectrometry, J. Chromatogr. A 1210 (2008) 84–91.
[32] H.C. Chen, H.W. Kuo, W.H. Ding, Determination of estrogenic compounds in wastewater using liquid chromatography–tandem mass spectrometry with electrospray and atmospheric pressure photoionization following desalting extraction, Chemosphere 74 (2009) 508–514.

[33] V. Kumar, N. Nakada, M. Yasojima, N. Yamashita, A.C. Johnson, H. Tanaka, Rapid determination of free and conjugated estrogen in different water matrices by liquid chromatography–tandem mass spectrometry, Chemosphere 77 (2009) 1440–1446.
[34] F.F. Sodre, I.C. Pescara, C.C. Montagner, W.F. Jardim, Assessing selected estrogens and xenoestrogens in Brazilian surface waters by liquid chromatography–tandem mass spectrometry, Microchem. J. 96 (2010) 92–98.
[35] D. Guillarme, D. Nguyen, S. Rudaz, J.L. Veuthey, Method transfer for fast liquid chromatography in pharmaceutical analysis: application to short columns packed with small particle. Part I: isocratic separation, Eur. J. Pharm. Biopharm. 66 (2007) 475–482.
[36] I. Bobeldijk, J. Vissers, G. Kearney, H. Major, J.A. van Leerdam, Screening and identification of unknown contaminants in water with liquid chromatography and quadrupole-orthogonal acceleration-time-of-flight tandem mass spectrometry, J. Chromatogr. A 929 (2001) 63–74.
[37] M. Petrovic, M. Gros, D. Barcelo, Multi-residue analysis of pharmaceuticals in wastewater by ultra-performance liquid chromatography–quadrupole-time-of-flight mass spectrometry, J. Chromatogr. A 1124 (2006) 68–81.
[38] M. Ibanez, J.V. Sancho, D. Mcmillan, R. Rao, F. Hernandez, Rapid non-target screening of organic pollutants in water by ultraperformance liquid chromatography coupled to time-of-light mass spectrometry, Trac-Trend. Anal. Chem. 27 (2008) 481–489.
[39] M. Ibanez, C. Guerrero, J.V. Sancho, F. Hernandez, Screening of antibiotics in surface and wastewater samples by ultra-high-pressure liquid chromatography coupled to hybrid quadrupole time-of-flight mass spectrometry, J. Chromatogr. A 1216 (2009) 2529–2539.
[40] F. Hernandez, L. Bijlsma, J.V. Sancho, R. Diaz, M. Ibanez, Rapid wide-scope screening of drugs of abuse, prescription drugs with potential for abuse and their metabolites in influent and effluent urban wastewater by ultrahigh pressure liquid chromatography–quadrupole-time-of-flight-mass spectrometry, Anal. Chim. Acta 684 (2011) 96–106.
[41] R. Meesters, H.F. Schroder, Simultaneous determination of 4-nonylphenol and bisphenol a in sewage sludge, Anal. Chem. 74 (2002) 3566–3574.
[42] Y. Koh, T.Y. Chiu, A.R. Boobis, E. Cartmell, S. Pollard, M.D. Scrimshaw, J.N. Lester, A sensitive and robust method for the determination of alkylphenol polyethoxylates and their carboxylic acids and their transformation in a trickling filter wastewater treatment plant, Chemosphere 73 (2008) 551–556.
[43] N. Fontanals, R.M. Marce, F. Borrull, New materials in sorptive extraction techniques for polar compounds, J. Chromatogr. A 1152 (2007) 14–31.
[44] J. Tso, D.S. Aga, A systematic investigation to optimize simultaneous extraction and liquid chromatography tandem mass spectrometry analysis of estrogens and their conjugated metabolites in milk, J. Chromatogr. A 1217 (2010) 4784–4795.
[45] N.M. Vieno, T. Tuhkanen, L. Kronberg, Analysis of neutral and basic pharmaceuticals in sewage treatment plants and in recipient rivers using solid phase extraction and liquid chromatography–tandem mass spectrometry detection, J. Chromatogr. A 1134 (2006) 101–111.
[46] L. Sun, W. Yong, X.G. Chu, J.M. Lin, Simultaneous determination of 15 steroidal oral contraceptives in water using solid-phase disk extraction followed by high performance liquid chromatography–tandem mass spectrometry, J. Chromatogr. A 1216 (2009) 5416–5423.
[47] X.L. Jin, G.B. Jiang, G.L. Huang, J.F. Liu, Q.F. Zhou, Determination of 4-tert-octylphenol, 4-nonylphenol and bisphenol A in surface waters from the Haihe River in Tianjin by gas chromatography–mass spectrometry with selected ion monitoring, Chemosphere 56 (2004) 1113–1119.
[48] X. Li, G.G. Ying, H.C. Su, X.B. Yang, L. Wang, Simultaneous determination and assessment of 4-nonylphenol, bisphenol A and triclosan in tap water, bottled water and baby bottles, Environ. Int. 36 (2010) 557–562.
[49] X. Xu, J.M. Roman, T.D. Veenstra, J. Van Anda, R.G. Ziegler, H.J. Issaq, Analysis of fifteen estrogen metabolites using packed column supercritical fluid chromatography–mass spectrometry, Anal. Chem. 78 (2006) 1553–1558.
[50] W. Yan, L. Zhao, Q. Feng, Y. Wei, J. Lin, Simultaneous determination of ten estrogens and their metabolites in waters by improved two-step SPE followed by LC–MS, Chromatographia 69 (2009) 621–628.