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Simultaneous determination of endocrine disrupting phenolic compounds and steroids in water by solid-phase extraction–gas chromatography–mass spectrometry

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Abstract

A solid-phase extraction (SPE)–gas chromatography (GC)–mass spectrometry (MS) analytical method for the simultaneous separation and determination of endocrine disrupting chemicals (EDCs) from water samples is described in detail. Important and contrasting EDCs including estrone, 17β-estradiol, 17α-ethynylestradiol, 16α-hydroxyestrone, 4-nonylphenol, bisphenol A and 4-*tert*-octylphenol were selected as the target compounds. The SPE technique, followed by the derivatisation with bis (trimethylsilyl) trifluoroacetamide was used for the extraction recoveries of target compounds from water samples. A number of parameters that may affect the recovery of EDCs, such as the type of SPE cartridges, eluents, as well as water properties including pH value, and concentration of salts and humic substances were investigated. It is shown that the Oasis cartridges produced the best recoveries of target EDCs while ethyl acetate was efficient in eluting EDCs from SPE cartridges. The recovery of some EDCs was enhanced by the addition of salt, but reduced by the increase in pH value and humic acid concentration. The optimised method was further verified by performing spiking experiments in natural river water and seawater matrices, with good recovery and reproducibility for all the selected compounds. The established method was successfully applied to environmental water samples from East and West Sussex, UK, for the determination of the target EDCs.

Keywords: Water analysis; Endocrine disruptors; Steroids; Phenolic compounds

1. Introduction

Recently, a wide variety of chemicals that have been identified to disrupt endocrine system of higher life forms, such as fish, wildlife and even humans, have attracted considerable attentions worldwide. The so-called endocrine disrupting chemicals (EDCs) fall into two main categories: those that occur naturally and those that are man-made. Among these compounds, naturally produced estrogens, such as 17β-estradiol and estrone are mainly derived from excreta of humans and livestock, and 16α-hydroxyestrone is the hepatic metabolite of the natural estrone by 16α-hydroxylation pathway. It has been shown that woman can excrete 7 μg of estrone and 2.4 μg of 17β-estradiol per day [1]. The synthetic female hormone 17α-ethynylestradiol has been used as an oral contraceptive pill. Apart from these steroids, the endocrine disrupting phenolic compounds, e.g. bisphenol A, 4-nonylphenol and 4-*tert*-octylphenol have been widely used in household and industrial processes (textile, paper, metal working fluids, detergents and polymeric material production). The EDCs may be released directly or indirectly to the aquatic environment, leading to the alternations of normal hormone function and physiological status in wildlife and humans, e.g. the development of testicular and prostate cancer and decreased sperm reproduction in humans, and feminisation and hermaphroditism in wildlife [2–5].

Generally, the analysis of EDCs has been accomplished by electrochemical method [6–9] and chromatographic techniques, such as high-performance liquid chromatography (HPLC) equipped with ultraviolet [10,11], fluorescence [12–15], electrochemical [16], or mass spectrometry (MS)

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detection [17–21], as well as gas chromatography (GC) coupled with sensitive and specific detection systems, such as MS or MS–MS. To our knowledge, the determination of phenolic compounds, bisphenol A, nonylphenols and 4-*tert*-octylphenol from natural and wastewaters has been performed using GC–MS and LC–MS methods [22–32]. A large number of studies have also been carried out for the analysis of estrogens in various environmental samples by GC–MS technique [33–37]. Most studies have focused on the determination of single group of compounds, i.e. phenolics compounds or steroids. Only a few papers have been published on the simultaneous determination of EDCs with a wide range of polarity. Recently, Jeannot et al. [18] used GC–MS and GC–MS–MS technique to determine the concentration of 4-nonylphenol, 4-*tert*-octylphenol, bisphenol, 17β-estradiol, estriol, estrone and 17α-ethynylestradiol in both surface water and wastewater after derivatisation with bis(trimethylsilyl)trifluoroacetamide (BSTFA). Quantification limits for the target compounds reached ppt levels. But extraction recovery by solid-phase extraction (SPE) technique with copolymeric sorbent (Oasis HLB) was only 25% for 4-*tert*-octylphenol and 50% for 4-nonylphenol. Kuch and Ballschmiter [38] simultaneously determined phenolic compounds and estrogens in surface and drinking water in pg/l range using SPE–GC–MS technique with satisfactory reproducibility. The spiking experiments showed that recovery of steroids was in the range between 71 and 79% with the exception of estradiols (56–67%), and phenols were found to be in the range of 70–92% with relative standard deviation (R.S.D.) of 9–15%. Using GC–MS combined with SPE method, the determination of 4-octylphenol, 4-nonylphenol, 2,4-dichlorophenol, 4-*tert*-butylbenzoic acid, bisphenol A, 17β-estradiol and 17α-ethynylestradiol has also been carried out from surface water samples, recoveries (except for 17β-estradiol) were 58–106% with R.S.D. of 6–16%, and the limits of detection were 4–6 ng/l but higher for 17β-estradiol and 17α-ethynylestradiol (50–300 ng/l) [39]. As different EDCs of varying concentrations tend to occur simultaneously in natural waters, it is essential to develop an effective method that can extract multiple EDCs simultaneously from water samples.

The objective of this work is to develop a SPE–GC–MS method for the simultaneous separation and determination of a wide range of EDCs including phenolic compounds and steroids in water samples. The extraction recoveries of target compounds from water samples were assessed by using different types of SPE cartridges and elution solvents. The effects of water properties including pH, salts and dissolved organic matter (as modelled by humic substances) on extraction efficiency were also determined. A derivatisation step was carried out with BSTFA to enhance selectivity and sensitivity of this analysis. The silylated derivatives were determined by GC–MS. The method developed was applied to quantification of target compounds from natural river water and seawater.

2. Experimental

2.1. Chemicals and standard solution

All the solvents used including methanol, ethyl acetate, acetone and dichloromethane (DCM), purchased from Rathburns, were of distilled-in-glass grade. And 17β-estradiol, estrone, 17α-ethynylestradiol, 16α-hydroxyestrone, [$^2H_2$] 17β-estradiol (17β-estradiol-d_2) and 4-nonylphenol were purchased from Sigma, UK, and bisphenol A, 4-*tert*-octylphenol, [$^{16}H_2$] bisphenol A (bisphenol A-d_{16}) and BSTFA containing 1% of trimethylchlorosilane (TMCS) were supplied by Aldrich (Dorset, UK). Separate stock solutions of individual compounds were made up at a level of 1000 mg/l by dissolving an appropriate amount of each substance in methanol. From these standards, a mixture of working standards containing each compound at 10 mg/l (except for bisphenol A at 5 mg/l) was prepared weekly by diluting the stock solution in methanol, and used to spike the water solutions. Internal standard solutions (10 mg/l) of bisphenol A-d_{16} and 17β-estradiol-d_2 were prepared in methanol. All the standard solutions were stored at −18 °C prior to use. Humic acid was purchased from Sigma-Aldrich, UK. Ultrapure deionised water was supplied by a Maxima Unit from USF Elga, UK.

2.2. Solid-phase extraction

The target compounds were extracted from water samples by different SPE cartridges (Table 1). One hundred nanogram of bisphenol A and 200 ng of 4-*tert*-octylphenol, 17β-estradiol, estrone, 17α-ethynylestradiol, 16α-hydroxyestrone, and 4-nonylphenol were spiked (four replicates) in 500 ml of ultrapure water for the recovery test. All the cartridges were conditioned with 5 ml of ethyl acetate to remove residual bonding agents, followed by 5 ml of methanol, in which methanol was drawn through the cartridges under very low vacuum to ensure that the sorbents were soaked in methanol. After 5 min of soaking in methanol, ultrapure water (3 ml × 5 ml) was passed through the cartridges at a rate of 1–2 ml/min. Then, water samples were extracted at a flow rate less than 5 ml/min. After washing the cartridges with 10 ml of deionised water–methanol (9:1), the cartridges were dried under vacuum for 30 min, and then the analytes were eluted to 20 ml vials from the sorbents with 10 ml of solvents (e.g. ethyl acetate) at a flow rate of 1 ml/min. The solvents were blown down to 1 ml under a gentle flow of nitrogen at 45 °C. For natural water samples, 1 l was filtered through a pre-combusted GF/F filter (0.7 μm) and spiked with 50–1000 ng of the target EDCs. These samples were then extracted using SPE and analysed by GC–MS to check recoveries in natural matrices.

2.3. Derivatisation procedure

The high polarity of some compounds gave rise to poor chromatographic peaks, and derivatisation was done to

Table 1
A summary of the different types of SPE cartridges being studied

Cartridges	Descriptions	Manufacturer
Strata CN (1 g, 6 ml)	Cyanopropyl	Phenomenex
Strata X (0.2 g, 6 ml)	Patent-pending polymeric material	Phenomenex
Strata SI-1 (1 g, 6 ml)	Silica sorbent	Phenomenex
DSC-18 (1 g, 6 ml)	Polymerically bonded, octadecyl (18% C)	Supelco
DSC-Si (1 g, 6 ml)	Unbonded acid washed silica sorbent	Supelco
DPA-6S (0.5 g, 6 ml)	Polyamide resin	Supelco
Isolute C18 (1 g, 6 ml)	Octadecyl	International Sorbent Technology
Isolute C18/ENV$^+$ (0.4 g, 6 ml)	C18 Hydroxylated polystyrene-divinylbenzene	International Sorbent Technology
Oasis HLB (0.2 g, 6 ml)	Poly(divinylbenzene-co-*N*-vinylpyrrolidone)	Waters

reduce the polarity of these compounds. The standards or extracts from SPE were transferred into 3 ml reaction vials followed by the addition of 100 ng each of bisphenol A-d_{16} and 17β-estradiol-d_2 as internal standards, and further evaporated to dryness under a gentle nitrogen stream. The dry residues were derivatised by the addition of 50 μl each of pyridine (dried with KOH solid) and BSTFA (1% TMCS), which were heated in a heating block at 60–70 °C for 30 min. The derivatives were cooled to room temperature and subjected to GC–MS analysis.

2.4. GC–MS analysis

GC–MS analysis was performed using a gas chromatograph (Trace GC 2000, Themoquest CE Instruments, TX, USA) coupled with an ion trap mass spectrometer (Polaris Q, Themoquest CE Instruments, Texas, USA) and an autosampler (AS 2000). A ZB5 (5% diphenyl–95% dimethylpolysiloxane) capillary column of 30 m × 0.25 mm i.d. (0.25 μm film thickness) was used. Helium carrier gas was maintained at a constant flow rate of 1.5 ml/min. The GC column temperature was programmed from 100 (initial equilibrium time 1 min) to 200 °C via a ramp of 10 °C/min, 200–260 °C via a ramp of 15 °C/min, 260–300 °C via a ramp of 3 °C/min and maintained at 300 °C for 2 min. The MS was by electron impact ionisation and operated in full-scan mode from *m/z*, 50–600 for qualitative analysis or selected ion monitoring mode for quantitative analysis. The inlet and MS transfer line temperatures were maintained at 280 °C, and the ion source temperature was 250 °C. Sample injection (1 μl) was in splitless mode. Examples of chromatograms for the identification of target compounds are shown in Fig. 1. The ions monitored for each compound are listed in Table 2.

3. Results and discussion

3.1. Mass spectra of the silylation derivatives

The full-scan mass spectra of the silylated EDCs are shown in Fig. 2. The TMS^+ ion (*m/z*, 73) was obtained for all the compounds tested. For 4-*tert*-octylphenol, the only major ion was found at *m/z*, 207 (abundance 100%) corresponding to $[(CH_3)_3Si–O–C_6H_4–C(CH_3)_2]^+$. The molecular ion at *m/z*, 292 and the ion at *m/z*, 179 due to the loss of $–C_8H_{17}$ group from molecular ion were present in mass spectra of 4-nonylphenol derivatives. For bisphenol A, the ion at *m/z*, 357 with an abundance of 100% could be attributed to the fragment $\{[(CH_3)_3Si–O–C_6H_4–C(CH_3)–C_6H_4–O–Si(CH_3)_3]^+\}$, indicating the formation of bis-TMSi ethers at both hydroxyl groups. In the case of the

Table 2
Ions for the quantitative analysis of silylation derivatives of target EDCs and internal standards

Compound	Retention time (min)	Molecular mass	Quantitative ion	Confirmation ions
4-*tert*-Octylphenol	9.19	206	207	278
4-Nonylphenol	12.02	220	179	292
Bisphenol A	14.27	228	357	372
Bisphenol A-d_{16}	14.22	244	368	386
Estrone	17.31	270	342	257 218
17β-Estradiol	17.70	272	285	416 326
17α-Ethynylestradiol	18.85	296	285	425 232
16α-Hydroxyestrone	18.97	286	286	430
17β-Estradiol-d_2	17.68	274	287	418

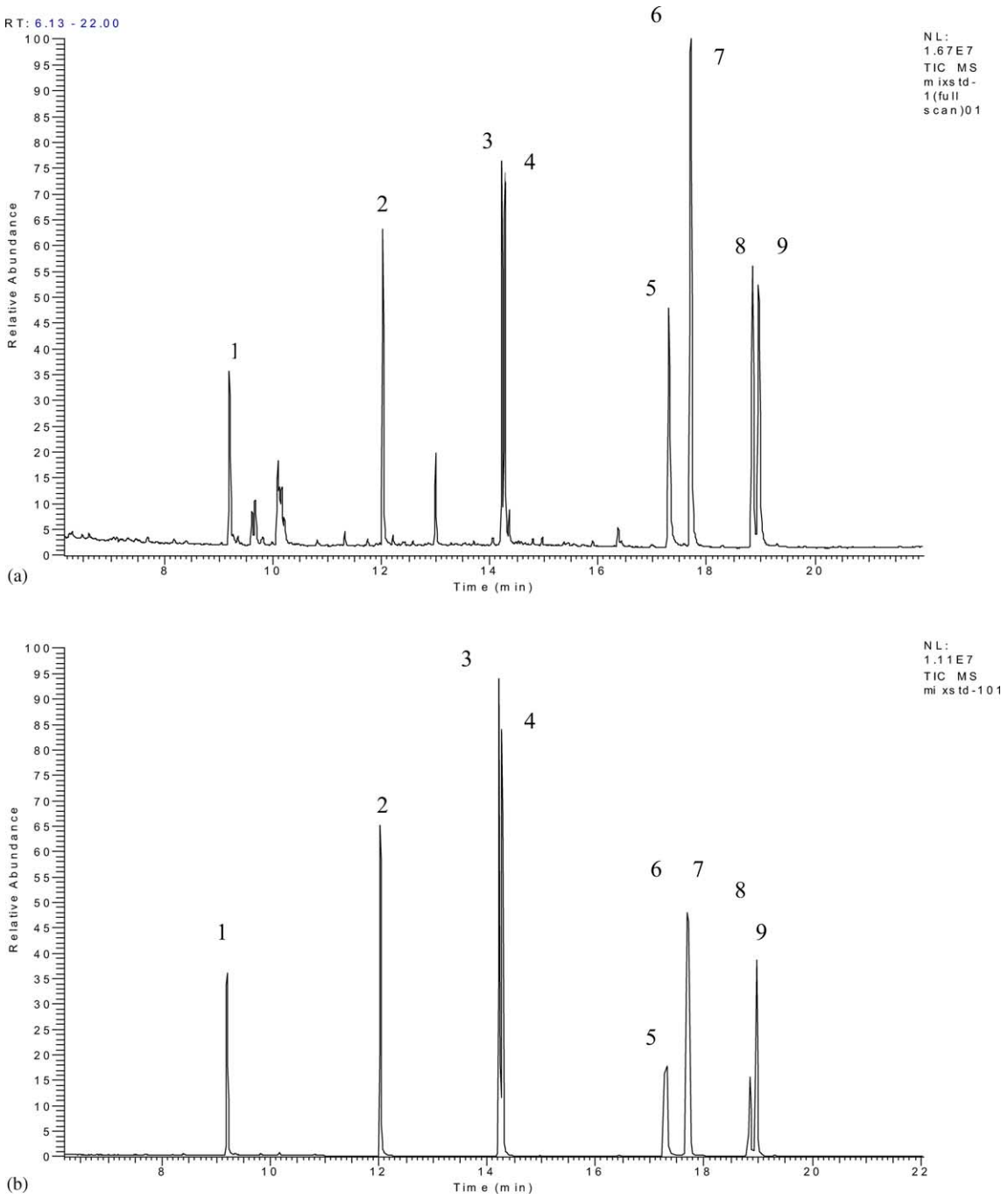

Fig. 1. Full scan chromatogram (a) and SIM chromatogram (b) of target EDCs. Peak numbers refer to (1) 4-*tert*-octylphenol, (2) 4-nonylphenol, (3) bisphenol A-d_{16}, (4) bisphenol A, (5) estrone, (6) 17β-estradiol-d_2, (7) 17β-estradiol, (8) 17α-ethynylestradiol, (9) 16α-hydroxyestrone.

four estrogens, complete derivatisation of all free hydroxyl groups was achieved. The mono-TMSi derivative was formed for estrone, which was evidenced by the presence of molecular ion at *m/z*, 342. In addition to ion fragment of *m/z*, 285, molecular ion at *m/z*, 416 with 60% abundance was shown in the mass spectra of derivative for 17β-estradiol. Also, di-TMSi derivatives of 17α-ethynylestradiol can produce the $[M - 15]^+$ ion (*m/z*, 425) as the major ion and ion fragment of *m/z*, 285 with 92% abundance. In the case of 16α-hydroxyestrone, the mass spectrum of the di-TMSi derivatives can be characterised by fragment ion at *m/z*, 286 (100%) and minor molecular ion (*m/z*, 430, 15%).

3.2. Solid-phase extraction

3.2.1. Extraction recovery with various cartridges

The optimisation of an appropriate SPE cartridge with different sorbent materials plays a key role in the

achievement of high and reproducible recovery for contaminants. The most commonly used sorbents are porous silica particles surface-bonded with C18 or other hydrophobic alkyl groups and polymeric sorbents, such as styrene–divinylbenzene and activated carbon. Furthermore, some hydrophilic groups, i.e. sulfonic acid and *N*-vinylpyrrolidone group are often added into the polymeric sorbents to enhance water movement which make the sorbent more effective. In this study, several types of cartridges from different manufacturers were selected for the evaluation of extraction efficiency of EDCs. As shown in Fig. 3, when methanol was used as elution solvent, poor recoveries for all of the compounds except for 4-nonylphenol were observed on Strata Si-1 (1 g, 6 ml) and discovery DSC-Si (1 g, 6 ml) cartridges in which silica gel as packing material can strongly retain the polar compounds. Strata

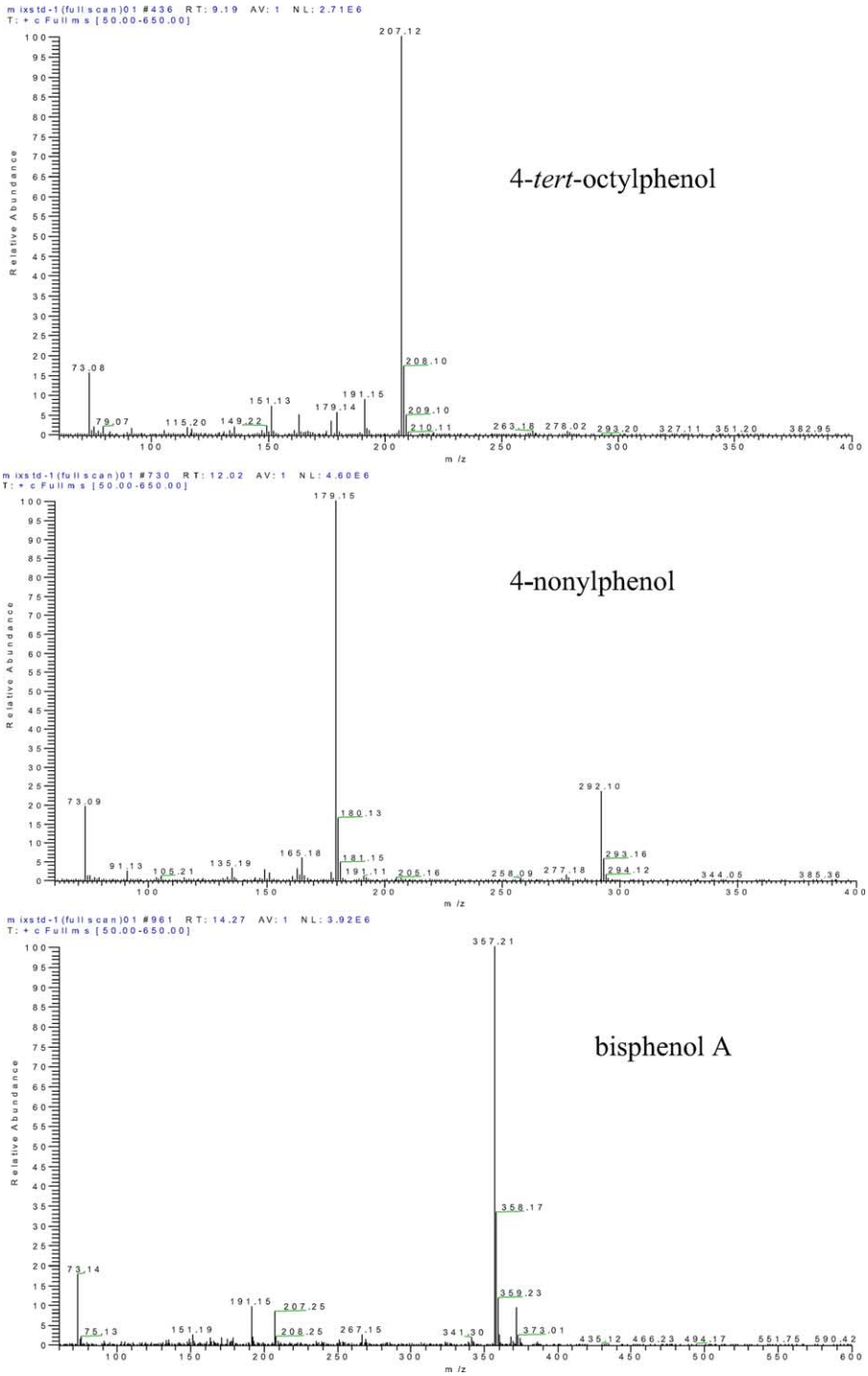

Fig. 2. Mass spectra of the EDCs being studied.

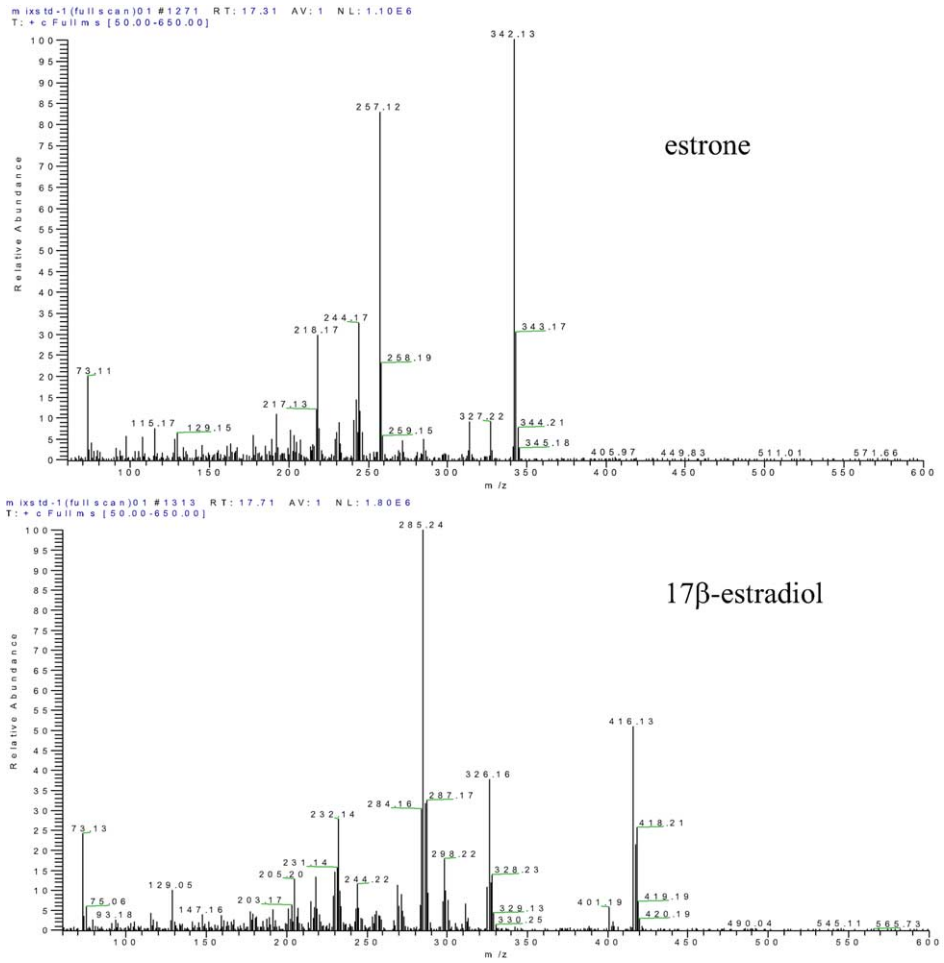

Fig. 2. (*Continued*)

CN (1 g, 6 ml) cartridge with bond-cyano group showed slightly better but still unsatisfactory recovery (<60%). Much improved recoveries (>80%) for 17β-estradiol, estrone, 17α-ethynylestradiol and bisphenol A were obtained on the C18- type cartridges (DSC-18, 1 g, 6 ml; Isolute C18-, 1 g, 6 ml; isolute C18-/ENV$^+$, 0.4 g, 6 ml). Good recoveries for most compounds were also achieved on Strata X (0.2 g, 6 ml) and Discovery DPA-6S (0.5 g, 6 ml). Of all the cartridges, Waters Oasis HLB (0.2 g, 6 ml) copolymer cartridges showed the best recoveries overall (57–118%) and were therefore used for further testing.

3.2.2. Elution by different solvents

The recovery of organic compounds by SPE is highly dependent on the polarity of the eluents. Acetone, DCM, ethyl acetate and methanol as eluents were tested for the elution recovery of EDCs from Oasis HLB cartridges, which were spiked at 200 ng/l for bisphenol A and 400 ng/l for the other compounds. The results (Fig. 4) show that DCM produced poor recovery for bisphenol A (8%) and 4-*tert*-octylphenol (27%), which may be due to the relatively polar nature of these two compounds. Better recoveries (between 37 and 116%) were obtained with acetone as the elution solvent. The best recoveries were achieved with elution by ethyl acetate or methanol. Accordingly, ethyl acetate was chosen as the solvent for the simultaneous extraction of all EDCs.

3.2.3. Effect of solution parameters on extraction recovery

Natural waters can have different salt content (e.g. freshwater, seawater). It is well known that the aqueous solubility of many organic compounds decreases with increasing salt concentration, thus their extraction efficiency in SPE is likely to increase. As shown in Fig. 5, the extraction efficiency for 4-*tert*-octylphenol and 4-nonylphenol was enhanced in the presence of NaCl, although the effect is minimal for the other compounds.

The effect of pH on the extraction efficiency was studied by adjusting the pH value of water sample with diluted solutions of sodium hydroxide and hydrochloric acid. Generally, the speciation of weakly acidic compounds in aqueous solutions depends on the solution properties, such as its pH value. Acidification of water solution is likely to decrease the dissociation of weakly acidic analytes, this may lead to increasing extraction efficiency of the target compounds if the non-dissociated form binds strongly to the SPE cartridges. The results (Fig. 6) show that the extraction recovery for all

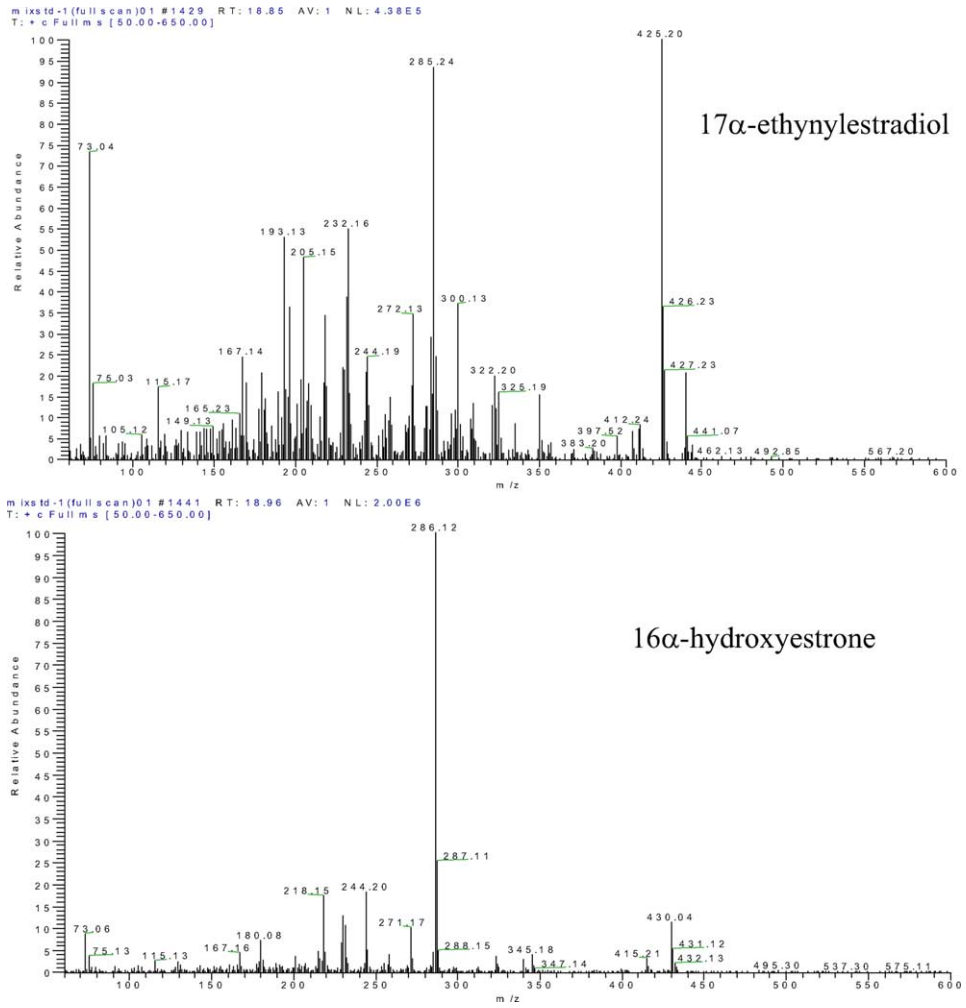

Fig. 2. (*Continued*).

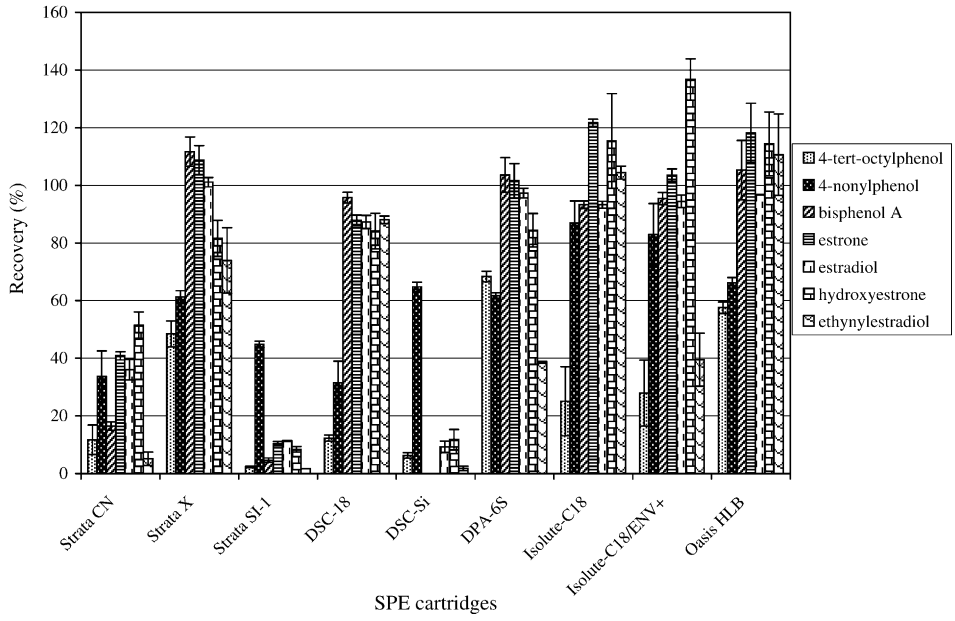

Fig. 3. The recovery of EDCs on different SPE cartridges using methanol as eluent.

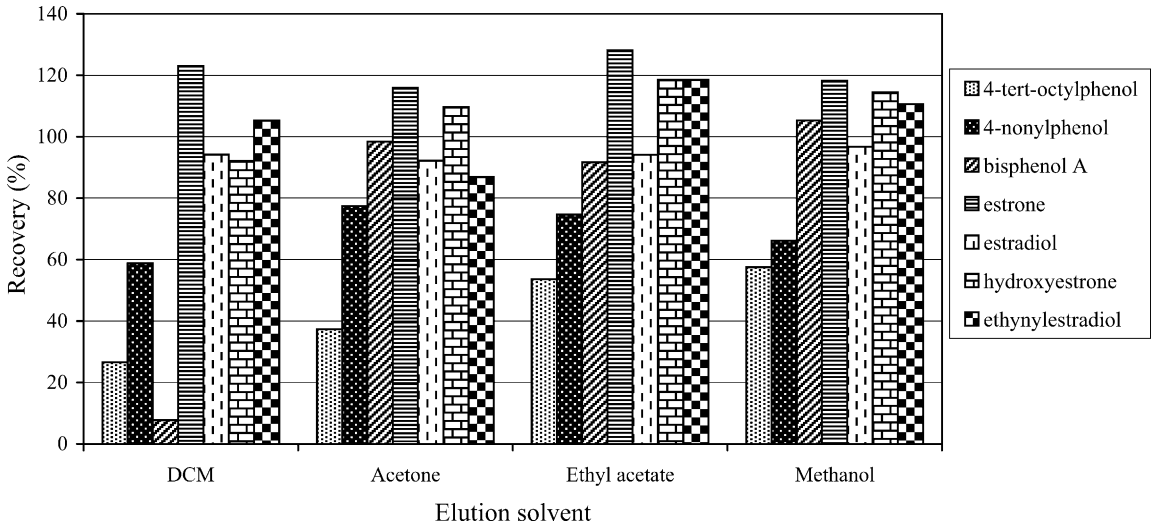

Fig. 4. The recovery of EDCs from Oasis HBL cartridges with different elution solvents.

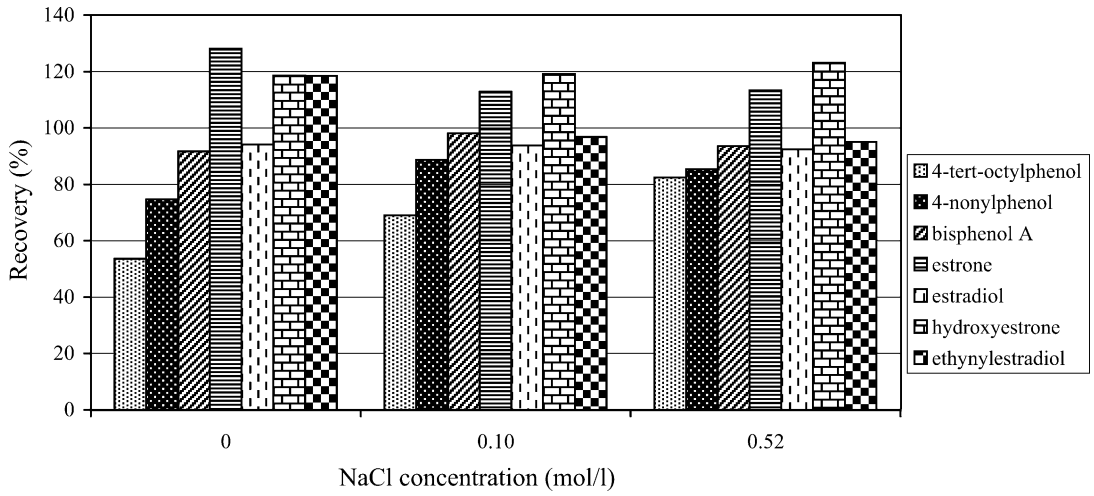

Fig. 5. The effect of NaCl concentration on the recovery of EDCs.

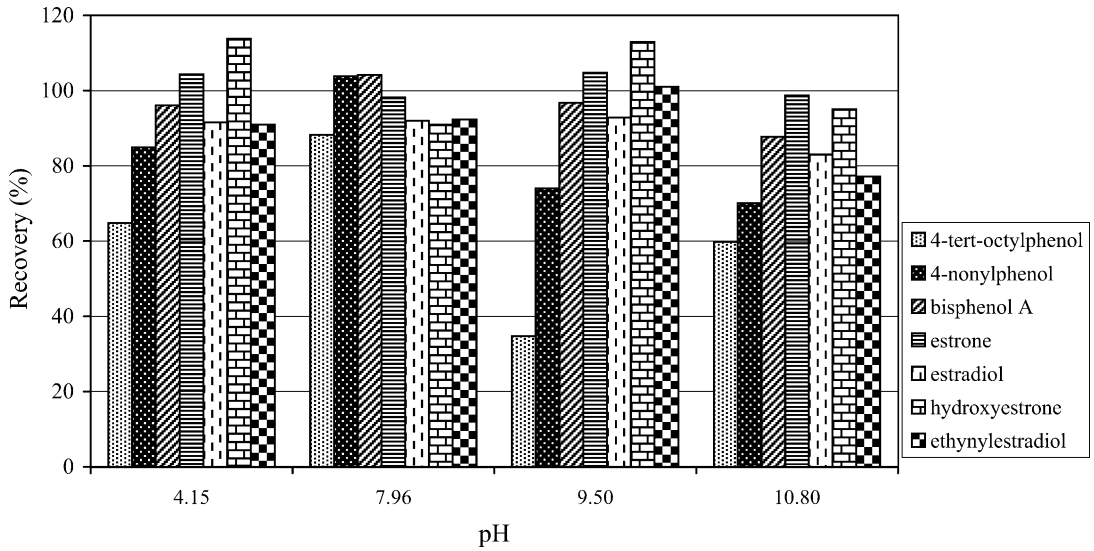

Fig. 6. The effect of pH on the recovery of EDCs.

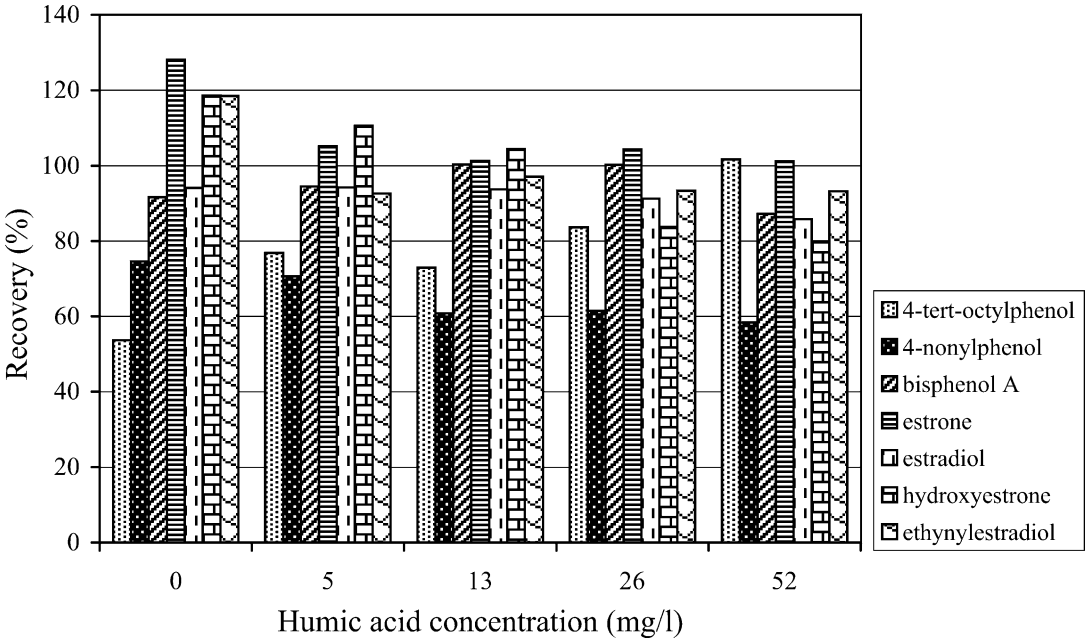

Fig. 7. The effect of humic acid concentration on the recovery of EDCs.

target compounds remains high and relatively similar at pH 4.15 and 7.96. Further increase in pH led to a reduction in the extraction efficiency, in particular for 4-*tert*-octylphenol and 4-nonylphenol.

Natural water also contains dissolved organic matter, of which the so-called humic substances are the most important in terms of binding with organic pollutants. Such humic-pollutant interactions may interfere with SPE operation and the derivatisation reaction, hence affecting recoveries. In this work, the influence of humic acid on the recovery was investigated by spiking different concentrations of humic acid in the test solutions. As shown in Fig. 7, for most compounds the presence of humic acid did not significantly affect their extraction efficiency, although for 4-nonylphenol, 16α-hydroxyestrone and 17α-ethynylestradiol, their recovery was reduced in the presence of humic acid. However, even in the presence of 52 mg/l humic acid, the recovery of all compounds except 4-nonylphenol was above 80%. As a result, it can be concluded that humic acid does not significantly affect recoveries of chosen EDCs and further clean-up of natural water samples is not required in the humic acid concentration range being tested.

It was also found that one of the ions belonging to 17α-ethynylestradiol ($[M - 15]^+$ ion; *m/z*, 425) was also produced by humic acid at exactly the same retention time, the amount of which increased quantitatively with increasing concentration of humic acid when the ion (*m/z*, 425) was used as quantitative ion. Fortunately the other ion (*m/z*, 285) characteristic of 17α-ethynylestradiol was not formed by humic acid. Thus it is possible to quantify the concentration of 17α-ethynylestradiol in the presence of humic acid by using ion *m/z*, 285.

3.3. Linearity of calibration curve

The linear range of GC–MS for the determination of EDCs was tested by increasing amounts of standards at 10, 20, 50, 100, 500, 1000, 2000, 5000, 10 000 ng/l and a fixed

Table 3
The linear range for the target EDCs in different matrices

Compound	Matrix	Linear range (ng/l)	Correlation coefficients (r^2)
4-*tert*-Octylphenol	Ultrapure water	10–2000	0.998
	River water		0.997
	Sea water		0.997
4-Nonylphenol	Ultrapure water	10–500	0.993
	River water		0.995
	Sea water		1.000
Bisphenol A	Ultrapure water	10–500	0.962
	River water		0.999
	Sea water		0.996
Estrone	Ultrapure water	10–500	0.996
	River water		0.995
	Sea water		0.999
17β-Estradiol	Ultrapure water	10–500	0.986
	River water		1.000
	Sea water		1.000
17α-Ethynylestradiol	Ultrapure water	10–500	0.992
	River water		0.998
	Sea water		1.000
16α-Hydroxyestrone	Ultrapure water	10–500	0.994
	River water		0.995
	Sea water		0.992

Table 4
Limit of detection (LOD) and limit of quantification (LOQ) for EDCs in matrix matched standards ($n = 4$)

Compound	LOD (ng/l)	LOQ (ng/l)
4-*tert*-Octylphenol	2.6	8.5
4-Nonylphenol	0.8	2.6
Bisphenol A	5.3	17.4
Estrone	1.7	5.6
17β-Estradiol	3.4	11.2
17α-Ethynylestradiol	0.8	2.6
16α-Hydroxyestrone	0.3	1.0

amount (100 ng/l) of internal standards in ultrapure water, and the analytes were extracted and derivatised as described above. The ratio of the peak area of analyte ions to that of internal standards was calculated. The experiments were repeated in natural water matrices, i.e. river water and seawater. Table 3 shows the results of linear regression using least squares fit.

3.4. Validation and application of the proposed method

The limit of detection (LOD), defined as the concentration that corresponds to three times the standard deviation of blanks, was measured by integrating blank peak area for each analyte in 10 independent performances with ultrapure water as blank. The limit of quantification (LOQ) is the lowest EDC concentration that can be quantified in a sample with acceptable precision under the stated operational conditions of the method. LOQ was determined as the analyte concentration corresponding to a signal/noise ratio of 10. As shown in Table 4, LOD varied from 0.3 to 5.3 ng/l, while LOQ was between 1.0 and 17.4 ng/l.

To further validate the precision and accuracy of the method, recovery testing was carried out by spiking a known amount of the standard mixture to "clean" river water and seawater samples, which do not contain the chosen EDCs. For spiked river water samples, the recovery for 4-*tert*-octylphenol ranged from 84.9 to 113% at spiking levels of 50–500 ng/l, beyond which the recovery dropped to 60.4% (Table 5). Similarly the recovery for the other EDCs tended to be satisfactory or excellent (63–116%) when EDCs were spiked at levels up to 500 ng/l. These compounds showed similar patterns in seawater matrix. The results are consistent with data for the linear range of the EDCs, in that low recoveries generally correspond to levels exceeding the linear range for these compounds. The R.S.D. of all recovery experiments was <20%, with a large majority of samples (79%) with R.S.D. < 10%. The precision of the method is therefore very good. The results therefore demonstrate that the EDCs studied can be simultaneously separated and determined from seawater and river water samples by the proposed method, with good accuracy and precision. The SPE–GC/MS method developed therefore can be applied to water samples containing the target EDCs at a concentration up to 500 ng/l.

Table 6
Concentration of EDCs in river waters in East and West Sussex, UK

Compound	Upstream of sewage outfall (ng/l)	Sewage outfall (ng/l)	Downstream of sewage outfall (ng/l)
4-*tert*-Octylphenol	<LOD-25	6–55	<LOD-10
4-Nonylphenol	<LOD	<LOD-4	<LOD
Bisphenol A	<LOD-10	9–24	<LOD-13
Estrone	<LOD	<LOD-10	<LOD-5
17β-Estradiol	<LOD-16	14–17	<LOD-17
17α-Ethynylestradiol	<LOD	<LOD	<LOD
16α-Hydroxyestrone	<LOD	<LOD	<LOD

The method developed was applied to the determination of chosen EDCs in river water samples from East and West Sussex, UK, between February and July 2003. As shown in Table 6, the concentrations of the target EDCs are relatively low, many of which are frequently below their LOD. For the compounds that were detected, their concentrations were higher in water close to the sewage outfall than in the upstream or downstream of the outfall. The results are consistent with those found in other rivers and streams, reflecting the relatively low concentrations of the chosen EDCs in sewage effluents and the further dilution in the aquatic environment.

Table 5
Recovery data for EDCs in river water and seawater matrices ($n = 4$)

Matrix	Spiked level (ng/l)	4-*tert*-Octylphenol	4-Nonylphenol	Bisphenol A	Estrone	17β-Estradiol	17α-Ethynylestradiol	16α-Hydroxyestrone
River water	50	113 ± 5.1	64.1 ± 2.0	95.1 ± 2.7	116 ± 4.3	113 ± 5.3	112 ± 4.4	82.8 ± 2.2
	100	96.2 ± 15.0	78.1 ± 11.8	98.0 ± 4.9	101 ± 2.7	91.5 ± 3.2	91.4 ± 8.1	90.6 ± 3.4
	200	83.2 ± 4.9	62.6 ± 5.2	86.3 ± 0.3	86.9 ± 1.7	92.9 ± 3.2	93.4 ± 6.6	95.5 ± 6.6
	500	84.9 ± 16.7	63.9 ± 5.2	80.6 ± 4.0	68.8 ± 5.8	63.3 ± 3.3	74.8 ± 2.3	69.0 ± 1.0
	1000	60.4 ± 9.3	46.2 ± 8.0	–	39.6 ± 2.9	42.0 ± 3.8	53.6 ± 10.1	41.6 ± 6.9
Seawater	50	85.5 ± 12.2	61.7 ± 10.5	95.1 ± 1.9	99.0 ± 4.8	106 ± 3.9	96.4 ± 14.7	107 ± 3.8
	100	75.8 ± 19.7	62.0 ± 8.8	95.1 ± 1.9	109 ± 2.5	93.6 ± 1.3	110 ± 4.0	105 ± 8.3
	200	77.6 ± 15.1	79.4 ± 15.9	95.4 ± 1.8	104 ± 7.3	88.6 ± 1.8	114 ± 6.3	82.2 ± 8.7
	500	78.8 ± 13.5	63.9 ± 0.7	96.2 ± 1.9	64.4 ± 3.8	63.1 ± 4.2	77.9 ± 10.7	82.0 ± 3.1
	1000	75.1 ± 4.0	63.8 ± 16.6	–	49.7 ± 4.7	41.0 ± 6.0	50.1 ± 12.5	54.9 ± 4.0

Conditions, seawater: pH = 8.00, salinity = 32.7‰; river water: pH = 7.80, salinity = 0‰.

4. Conclusions

A method has been developed for the extraction and analysis of important and contrasting EDCs including 17β-estradiol, estrone, 17α-ethynylestradiol, 16α-hydroxyestrone, 4-nonylphenol, bisphenol A and 4-*tert*-octylphenol, from water samples. The target EDCs were first extracted by SPE using Oasis HLB cartridges, followed by derivatisation with BSTFA and analysis by SIM-GC/MS. The proposed method shows very good recovery and reproducibility for the target compounds at ng/l level. The method was successfully applied for the determination of these target EDCs in river water samples.

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