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Journal of Chromatography A, 1038 (2004) 19–26
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Microwave-assisted extraction followed by gas chromatography–mass spectrometry for the determination of endocrine disrupting chemicals in river sediments

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Received 17 December 2003; received in revised form 9 March 2004; accepted 9 March 2004

Available online 15 April 2004

Abstract

In this study, microwave-assisted extraction (MAE) followed by gas chromatography (GC)–mass spectrometry (MS) analysis has been successfully developed for the simultaneous extraction and determination of contrasting endocrine disrupting chemicals (EDCs) including 17β-estradiol, estrone, 17α-ethynylestradiol, 16α-hydroxyestrone, 4-nonylphenol, 4-*tert*-octylphenol and bisphenol A in river sediments. For MAE, the effects of various parameters on the extraction efficiency were investigated. It is shown that the most efficient extraction (recovery >74%) of the target compounds was achieved by using methanol as the solvent, an extraction temperature of 110 °C and 15 min of holding time. The cleanup of extracts was carried out by passage through a non-deactivated silica gel column, and a satisfactory elution efficiency of all compounds was achieved using a solvent mixture of ethyl acetate–hexane (4:6, v/v). The spiking experiments show that the mean recovery of the target compounds exceeded 61% at a spiking level of 5 ng/g dry mass, and 73% at 10, 40 and 100 ng/g dry mass with a good reproducibility. The method developed was applied to the determination of target EDCs in river sediments collected from rivers Uck and Ouse, UK, and results revealed the presence of the chosen compounds at low ng/g level.

Keywords: Environmental analysis; Sediments; Extraction methods; Endocrine disruptors; Steroids; Alkylphenols; Phenols; Bisphenol A

1. Introduction

Endocrine disrupting chemicals (EDCs) are defined as exogenous substances that alter the functions of the endocrine system and consequently cause adverse health effects in an intact organism, or its progeny. EDCs are of global concern due to their widespread occurrence, persistence, bioaccumulation and potential adverse effects on ecosystem functioning and human health. These chemicals may originate from natural processes and industrial activities. Natural hormones such as 17β-estradiol and estrone are derived from excreta of humans and livestock, and 16α-hydroxyestrone from the hepatic metabolite of the natural estrone. Man-made substances include synthetically produced hormones, e.g. 17α-ethynylestradiol and industrial chemicals, e.g. bisphenol A, 4-nonylphenol and 4-*tert*-octylphenol associated with plastics, household products and industrial processes. In recent years, there has been increasing attention toward the potential effects of EDCs in aquatic environments on human and wildlife endocrine systems, e.g. the feminisation of male fish, abnormal reproductive processes and the development of testicular and prostate cancer even at the low concentrations down to 1 ng/l [1–4].

Reliable environmental analysis of EDCs is a prerequisite for their risk assessment. To date, most analytical efforts have focused on the determination methods for EDCs in aqueous matrices [5–8], which are primarily based on solid phase extraction, silylation and detection by gas chromatography (GC)–mass spectrometry (MS) or LC–MS. Hydrophobic organic pollutants in aquatic environments tend to deposit and accumulate on the solid phases such as sediments, although the magnitude of which is dependent on EDCs and sediment properties. Limited study is devoted to the analysis of EDCs from solid samples because of the complexity of sample processing and requirement of low detection limit. Recently, different extraction and

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doi:10.1016/j.chroma.2004.03.030

determination methods for EDCs in solid phases have been developed using conventional extraction systems coupled with chromatography detection technique. Soxhlet extraction is most commonly used in the extraction of organic pollutants from solid matrices. The investigations into the quantitative extraction and determination of nonylphenol polyethoxylate surfactants in marine sediments [9] and xenoestrogens 4-nonylphenol and bisphenol A from sewage sludge [10], using Soxhlet extraction system followed respectively by normal-phase LC–electrospray MS and high-performance LC–fluorescence detection, have been described by Shang et al. and Naassner et al. Sonication and LC–MS technique was used for the extraction and detection of estrogens (estriol, estradiol, ethynyl estradiol, estrone and diethylstilbestrol) and progestogens (progesterone, norethindrone and levonorgestrel) in sediments [11] and phenolic compounds (nonylphenol, octylphenol and bisphenol A) in sewage sludge [12]. By sonication extraction and GC–MS–MS analysis, the two methods have been proposed for the determination of estrone, 17β-estradiol, 17α-ethynylestradiol and mestranol down to 2 ng/g in activated sludge and down to 0.2 ng/g in freshwater sediments [13]. Pressurised liquid extraction (PLE) has been described to be very promising in the recovery of 4-nonylphenol and 4-nonylphenol ethoxylates from sediments [14], and a new method based on PLE followed by LC–MS has also been presented for the simultaneous determination of alkylphenol ethoxylate and their degradation products, alkylphenol and alkylphenol carboxylates in sediment samples [15]. Meesters and Schröder [16] have applied the different extraction techniques e.g. Soxhlet extraction, supercritical fluid extraction and accelerated solvent extraction followed by GC–MS to the simultaneous extraction and determination of 4-nonylphenol and bisphenol A.

Since the first application of a microwave oven to the extraction of analytes from solid matrices using organic solvents [17], growing interest has been attracted to the use of microwave energy in the extraction of various organic pollutants from various matrices, including the extraction of polycyclic aromatic hydrocarbons (PAHs) [18–20] from soil and marine sediments, pesticides from sediments and soils [21–23], and polychlorinated biphenyls (PCBs) from soils [24–26]. Furthermore, many articles have been published on the extraction of phenols [27,28] and herbicides [29] from environmental samples. The advantages of this technique include the reduction of solvent consumption and considerable saving in processing time. However, little work has been completed on the microwave-assisted extraction of EDCs from environmental particulate samples such as sediments.

In the previous work from our group, we developed an analytical method for EDCs from natural water based on solid-phase extraction and GC–MS [30]. In this study, we focus our effort on the development of a reliable microwave-assisted extraction technique for the simultaneous recovery of 17β-estradiol, estrone, 17α-ethynylestradiol, 16α-hydroxyestrone, 4-nonylphenol, 4-*tert*-octylphenol and bisphenol A from river sediment samples, followed by GC–MS analysis. Various extraction and elution conditions were tested in order to achieve best conditions for the simultaneous extraction recovery of the EDCs. The newly developed method was also compared with sonication extraction technique, and applied to quantify the concentration levels of target compounds from river sediments in the UK.

2. Experimental

2.1. Chemicals and standards

All standards were of the highest purity commercially available. 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, [$^2H_{16}$]bisphenol A (bisphenol A-d_{16}) and bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% of trimethylchlorosilane (TMCS) were supplied by Aldrich (Dorset, UK).

Stock solutions (1000 mg/l) for each of the standards were prepared by dissolving an appropriate amount of pure standards in methanol. Working solutions of mixture containing each compound at 10 mg/l (except for bisphenol A at 5 mg/l) were obtained by diluting the stock solution with methanol. 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. Distilled-in-glass grade solvents methanol, ethyl acetate, acetone, hexane and dichloromethane (DCM) were purchased from Rathburns (Walkerburn, UK). High-purity deionised water was supplied by a Maxima Unit from USF Elga, UK. The silica gel 40 (0.063–0.2 mm particle size was obtained from Fluka, Buchs, Switzerland). Sodium sulphate anhydrate was from Fisher Chemicals, UK.

2.2. Microwave-assisted extraction

Sediment samples from River Ouse, East Sussex, UK were used for spiking experiments. They were first of all extracted to determine the levels of target compounds in the matrix. The results show that except for 17β-estradiol (2 ng/g dry mass) and 17α-ethynylestradiol (3.2 ng/g dry mass), all the other compounds were below the limit of quantification. Wet sediment samples (approximately 5 g dry mass) were weighed into PTFE-lined extraction vessels and spiked with 100 ng of bisphenol A and 200 ng of the other compounds. The dry mass of each sediment sample was determined by drying in an oven at 100 °C for 4 h, and all concentration values are reported on a dry mass basis. The samples were carefully mixed with a spatula and allowed to stand for 2 h in the MAE vessels prior to addition of solvents. Copper granules were added for removing sulphurous compounds. Extractions were performed by MARS-X (CEM

Corp., USA) at 90, 110 or 130 °C for 5, 15, 25 or 40 min with 100% of power, 1200 or 600 W, and 200 p.s.i. (1378 kPa) with 25 ml of a solvent or solvent mixture. After extraction, the vessels were cooled to room temperature before they were opened. The supernatants were transferred to the round-bottomed flasks (250 ml), and the sediment samples were washed with 15 ml of the same solvent or solvent mixture for three times and centrifuged at 2500 rpm (700 × *g*) for 5 min. The supernatants were combined, then evaporated to nearly 1 ml by rotary evaporation, and subjected to clean-up procedure.

2.3. Sonication extraction of sediment sample

Wet sediment samples (5 g dry mass) from River Ouse were spiked with 200 ng of the target compounds, and extracted for three times in an ultrasonication bath (Decon Lab., UK) with 25 ml of methanol. For each extraction step, the sample was sonicated for 20 min, and centrifuged at 2500 rpm (700 × *g*) for 5 min. The supernatant was collected in a 250 ml round-bottomed flask. The three solvent extracts were combined and concentrated using rotary evaporation to approximately 1 ml.

2.4. Preparation of silica gel column and cleanup of extracts

The silica gel was heated in an oven at 130 °C overnight (at least 18 h), and cooled down to room temperature. For the purpose of deactivation, a desired amount of the silica gel was weighed in a conical flask, and stoppered immediately. Different amount of high-purity water was added, shaken for 10 min, and stored at room temperature overnight for equilibration.

Sodium sulphate anhydrate in ceramic dish was ashed at 450 °C for 5 h, cooled and stored in a desiccator till use. Glass wool (pre-ashed at 500 °C for 3 h) was inserted into the bottom end of a cartridge (6 ml), followed by filling cartridge half full with ethyl acetate–hexane (4:6, v/v). The slurry was made up by stirring 1 g silica gel in ethyl acetate–hexane (4:6, v/v) and transferred to the cartridge. Sodium sulphate (3 g) was packed to the top of silica gel. The column was conditioned with 25 ml of ethyl acetate–hexane (4:6, v/v), and soaked in the solvent mixture for use.

The sediment extracts were quantitatively transferred to the above column using a Pasteur pipette. The analytes were allowed to pass into the column and eluted with 20 ml of ethyl acetate–hexane (4:6, v/v). The elutions were evaporated to nearly 0.5 ml under a gentle nitrogen stream, and transferred to 3 ml reaction vials for derivatisation. The extracts in 3 ml reaction vials were spiked with 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). After a reaction of 30 min at 60–70 °C, the derivatives were cooled to room temperature, and 1 μl was injected for GC–MS analysis.

Table 1
Ions for the quantitative analysis of silylation derivatives of target EDCs and internal standards

Compound	Molecular mass	Confirmation ions	Quantitative ion
4-*tert*-Octylphenol	206.3	208.1	207
4-Nonylphenol	220.3	292.2	179
Bisphenol A	228.3	358.1	357
Bisphenol A-d_{16}	244.3	369.3	368
Estrone	270.4	257.2, 218.2	342
17β-Estradiol	272.4	416.2, 326.1	285
17α-Ethynylestradiol	296.4	425.2, 232.2	285
16α-Hydroxyestrone	286.4	429.9	286
17β-Estradiol-d_2	274.4	418.1	287

2.5. GC–MS analysis

The separation and detection of analytes were achieved using a gas chromatograph (Trace GC 2000, Themoquest CE Instruments) coupled with an ion trap mass spectrometer (Polaris Q, Themoquest CE Instruments, TX, USA) and an autosampler (AS 2000). The gas chromatograph was equipped with A ZB5 (5% diphenyl:95% dimethylpolysiloxane) capillary column of 30 m × 0.25 mm i.d. (0.25 μm film thickness). Helium carrier gas was maintained at a constant flow rate of 1.5 ml/min.

Following the injection of 1 μl extracts, the GC column temperature was programmed as follows: 100 °C isothermal for 1 min, 10 °C/min to 200 °C, 15 °C/min to 260 °C, 3 °C/min to 300 °C and 300 °C isothermal for 2 min. MS was by electron impact ionisation and operated in full-scan mode from *m*/*z* 50 to 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 ion source temperature was 250 °C. The ions monitored for each compound are listed in Table 1.

2.6. Analysis of environmental samples

Sediment samples were collected in pre-cleaned glass jars using a grab sampler from the sewage outfall, and from 30 to 50 m upstream or downstream away from the outfall along rivers Ouse and Uck, UK, in April 2003. The oxic fraction was removed by a stainless steal spoon, transferred to the laboratory, and frozen at −18 °C till extraction. The wet sediment samples (approximately 5 g dry mass) were spiked with 100 ng of internal standards (bisphenol A-d_{16} and 17β-estradiol-d_2), then well mixed with a spatula and left to stand for 2 h. They were subjected to microwave-assisted extraction, clean-up procedure, derivatisation and GC–MS analysis. Examples of chromatograms for the identification of target compounds are shown in Fig. 1.

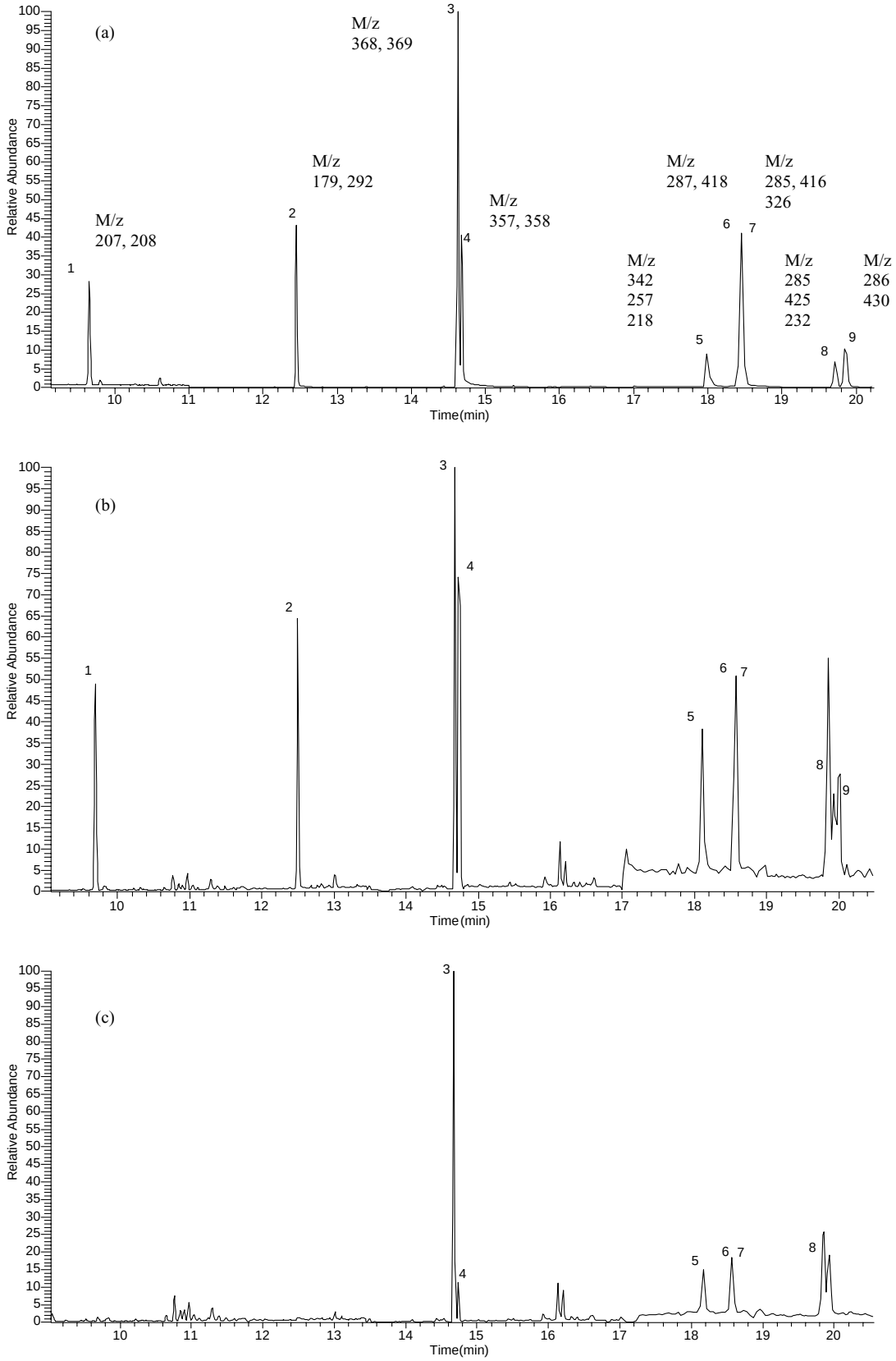

Fig. 1. SIM chromatogram of target EDCs in (a) standard solution (1 ng injection), (b) sediment sample spiked with 20 ng/g dry mass of the target compounds and (c) sediment sample. Peak numbers refer to (1) 4-*tert*-octylphenol, (2) 4-nonylphenol, (3) bisphenol A-d_{16}, (4) bisphenol A, (5) estrone, (6) 17β-estradiol-d_2, (7) 17β-estradiol, (8) 17α-ethynylestradiol and (9) 16α-hydroxyestrone.

3. Results and discussion

3.1. Cleanup and elution of analytes

Environmental samples are highly complicated in terms of chemical composition, so it is essential to remove unwanted matrix interferences from sample extracts prior to derivatisation and GC–MS analysis. However, there is no universal method for the removal of all kinds of interference compounds. The purification of EDCs from environmental matrices has been performed by means of deactivated neutral alumina phase [14,16], LiChrolute C_{18} cartridges [12] and silica gel sorbent [13]. In this study, the sediment extracts from sonication were passed through the columns of silica gel with different amount of water, and the recoveries of EDCs using ethyl acetate–hexane (4:6 or 2:8 v/v) as the elution solvent are shown in Figs. 2 and 3. In the range of water content from 0 to 15%, no statistically significant differences were observed for the recovery of 4-*tert*-octylphenol, 4-nonylphenol, bisphenol A, 17β-estradiol and estrone (all mean recovery >100%). The recovery of 17α-ethynylestradiol was the highest with 6% water in silica gel column. For 16α-hydroxyestrone, its recovery reached the highest value with 0 and 15% of water in silica gel column. The matrix of the primary silica gel particle consists of a core of silicon atoms joined together with oxygen atoms by siloxane bonds (silicon–oxygen–silicon bonds). On the surface of each primary silica gel particle, some residual uncondensed hydroxyl groups from the original polymeric silicic acid remain and confer upon silica gel its polar properties, which adsorb strongly polar compounds from matrices. As shown in Fig. 2, non-deactivated silica gel column is a better choice for the cleanup of extracts due to the relatively high recoveries, simple preparation of columns, and removal of potential interference compounds such as humic or fulvic acids in environmental

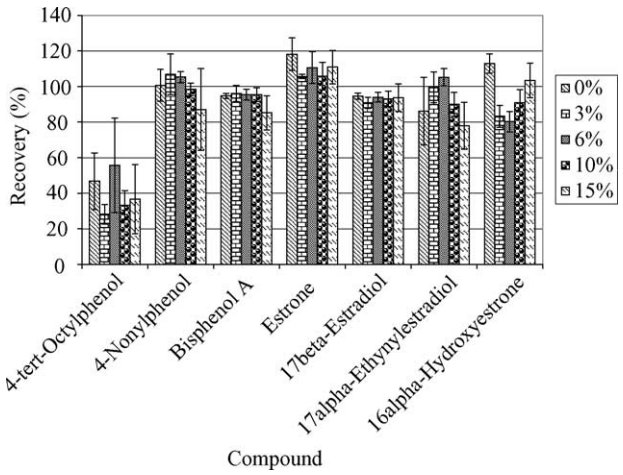

Fig. 2. The effect of water content in silica gel on the recovery of EDCs from spiked sediment (sediment spike level: 20 ng/g dry mass of bisphenol A and 40 ng/g dry mass of the other compounds).

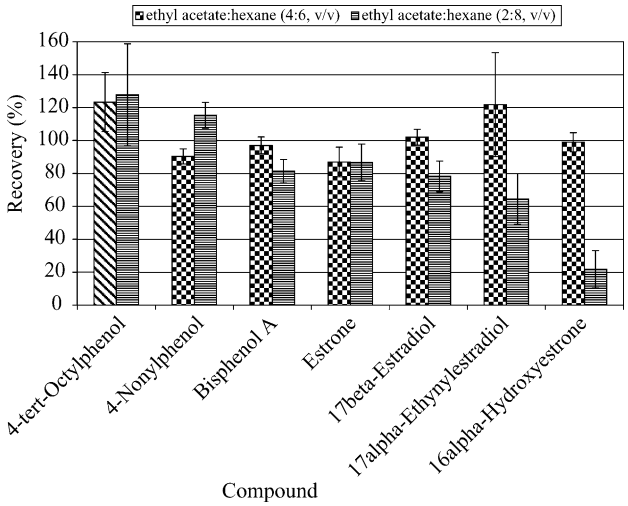

Fig. 3. The elution efficiency of EDCs in extracts from silica gel column with two types of solvent mixture (sediment spike level: 20 ng/g of bisphenol A and 40 ng/g of the other compounds).

samples that may adversely affect the analysis of target compounds. Furthermore, the elution efficiency of EDCs highly depends on the polarity of the eluting solvents and compounds, as shown in Fig. 3. For 4-*tert*-octylphenol and estrone, their recovery is almost the same no matter which solvent mixture was used for elution. For 4-nonylphenol, its recovery was higher with a less polar solvent mixture (ethyl acetate–hexane, 2:8, v/v), whilst for the rest of the compounds their recovery was enhanced with a more polar solvent mixture (ethyl acetate–hexane, 4:6, v/v).

3.2. Choice of microwave-assisted extraction conditions

To achieve efficient extraction of the target compounds from solid samples by microwave-assisted system, the optimal extraction solvent should be selected, the polarity of which matches that of the tested compounds. The four solvents or their mixtures evaluated for the extraction efficiency of the tested compounds in this study were (1) methanol, (2) ethyl acetate, (3) hexane–acetone (1:1, v/v) and (4) hexane–acetone (1:4, v/v), and the results are shown in Fig. 4. For 17β-estradiol, 16α-hydroxyestrone and to some extent bisphenol A and estrone (all mean recovery >100%), their recovery was similar between different extraction solvents. For 4-*tert*-octylphenol and 4-nonylphenol, the best recovery was achieved with hexane–ethyl acetate (1:1) being used for their extraction. The recovery of 17α-ethynylestradiol was the highest when methanol was used for extraction. This may be due to the high polarity of this compound, which therefore favours methanol as the solvent. Overall, the mean recovery of 4-*tert*-octylphenol, 4-nonylphenol, bisphenol A, estrone and 17β-estradiol was above 80%, whilst the mean recovery of 17α-ethynylestradiol and 16α-hydroxyestrone was generally above 70%.

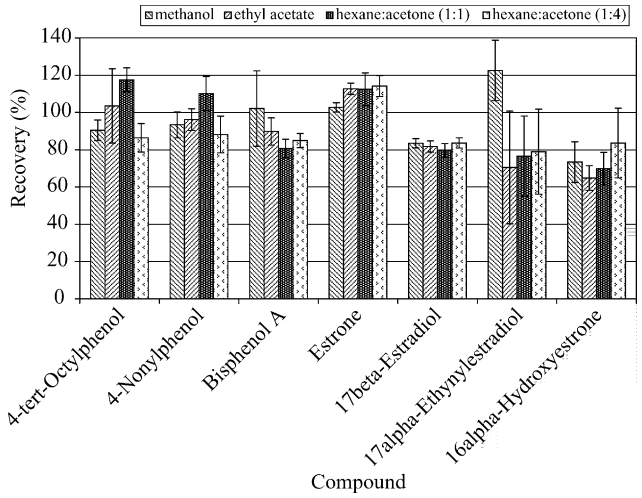

Fig. 4. The whole-procedure recovery of EDCs from sediments spiked with target compounds (20 ng/g of bisphenol A and 40 ng/g of the other compounds), followed by MAE by different solvents, clean-up, derivatisation and GC–MS analysis. MAE conditions: 110 °C, 15 min, 100% power, 600 W.

The water content of the samples has been found to be very important for the increasing extraction efficiency, resulting from the efficient heating of the samples as water absorbs microwave energy [31]. However, the use of wet sediment samples in this work might also cause inefficient contact between sample particles and solvent when ethyl acetate or hexane–acetone (1:1, v/v) was used as the extraction solvent. The satisfactory extraction efficiency (73–122%) of all the compounds was obtained using either methanol or hexane–acetone (1:4, v/v). In summary, the recovery from methanol as the solvent is comparable or even better than from other solvents. In addition, methanol is miscible with water, hence potentially more effective in mixing well with wet sediments and enhancing extraction from sediment matrices. Methanol was therefore chosen as the best solvent for further studies.

The extraction temperature and duration are potentially important parameters on the extraction efficiency of EDCs. The choice of these parameters depends on the nature of the solvent and its relative permittivity, as well as sample matrix and the properties of compounds. To obtain higher recovery and reproducible operating conditions, the extraction temperature and duration were changed from 90 to 130 °C for 5–40 min, and results listed in Table 2. Statistically insignificant changes in the mean recovery of most analytes were found in the temperature range from 90 to 130 °C except for 16α-hydroxyestrone, the recovery of which generally decreased with increasing temperature. When the temperature was increased from 90 to 130 °C, the mean recovery of 16α-hydroxyestrone decreased dramatically from 105 to 50% with a holding time of 5 min, from 77 to 67% with a holding time of 15 min, and from 102 to 49% with a holding time of 25 min. These results may be attributed to the degradation of this compound at higher temperature. As shown

Table 2
Microwave-assisted extraction recovery (%) of spiked sediments at 90, 110 and 130 °C with different holding time

Compound	90 °C			110 °C			130 °C			
	5 min	15 min	25 min	5 min	15 min	25 min	5 min	15 min	25 min	40 min
4-*tert*-Octylphenol	96.6 ± 29.4	88.5 ± 18.6	129 ± 9.0	80.0 ± 5.1	84.1 ± 11.9	93.9 ± 13.4	119 ± 4.3	103 ± 19.2	114 ± 29.8	146 ± 24.7
4-Nonylphenol	90.1 ± 14.2	101 ± 19.1	88.8 ± 21.9	89.0 ± 13.6	92.0 ± 21.1	108 ± 5.6	93.6 ± 7.6	96.4 ± 1.6	87.7 ± 8.3	95.2 ± 19.7
Bisphenol A	130 ± 7.8	98.5 ± 7.9	123 ± 1.3	71.8 ± 7.1	86.2 ± 24.4	84.9 ± 7.8	98.7 ± 8.8	96.4 ± 9.7	94.2 ± 10.6	135 ± 19.8
Estrone	98.2 ± 10.4	95.6 ± 3.6	99.0 ± 3.8	70.5 ± 6.7	81.5 ± 14.9	92.3 ± 18.7	114 ± 17.9	107 ± 3.6	112 ± 4.3	123 ± 5.7
17β-Estradiol	94.2 ± 5.4	89.2 ± 1.8	94.6 ± 5.6	60.2 ± 8.0	95.8 ± 12.8	88.5 ± 28.0	78.5 ± 15.4	91.7 ± 5.7	71.0 ± 5.6	89.0 ± 2.1
17α-Ethynylestradiol	86.8 ± 29.8	82.3 ± 7.9	75.1 ± 23.3	71.2 ± 31.0	74.1 ± 18.4	76.1 ± 6.7	88.3 ± 4.8	102 ± 2.9	107 ± 3.8	86.1 ± 16.3
16α-Hydroxyestrone	105 ± 29.6	76.9 ± 27.6	102 ± 9.2	77.4 ± 7.3	87.3 ± 24.5	91.0 ± 21.4	49.5 ± 17.0	67.1 ± 9.5	49.1 ± 2.2	31.2 ± 21.7

Table 3
Limits of detection (LODs) and limits of quantification (LOQs) for EDCs

Compounds	LOD (ng/g dry mass)	LOQ (ng/g dry mass)
4-*tert*-Octylphenol	0.5	1.7
4-Nonylphenol	0.5	1.7
Bisphenol A	1.0	3.4
Estrone	0.3	0.9
17β-Estradiol	0.3	0.9
17α-Ethynylestradiol	0.4	1.4
16α-Hydroxyestrone	0.2	0.5

in Table 2, recoveries in excess of 60% for all the target compounds were achieved at 90 and 110 °C for 5 min of extraction duration, and a slight increase in the recovery of all the analytes was found at 110 °C when holding time was increased from 5 to 25 min. In addition, with the highest temperature (i.e. 130 °C) and longest holding time (i.e. 40 min), it is probable that the matrix materials from sediment samples may have been co-extracted, resulting in material that can interfere with the analysis of the target compounds. This may explain the rather high recoveries (e.g. 146 and 135%) observed for 4-*tert*-octylphenol and bisphenol A, respectively. As a result, a compromise must be made between high extraction efficiency and selectivity. Thus, an extraction temperature of 110 °C with 15 min of extraction period was selected as the best operating condition for the extraction of all the analytes with mean recovery exceeding 74%.

3.3. Method validation

The analytical method was validated by the linear range, limit of detection and precision. A series of injections of target compounds in the concentration range from 0.10 to 100 μg/ml and 1.0 μg/ml of internal standards were used to determine the linear concentration range of GC–MS instrumentation. In the range between 0.10 and 5.0 μg/ml, the method was found to be linear for 4-nonylphenol, bisphenol A and 17β-estradiol with correlation coefficients from 0.96 to 0.99. The linear range was from 0.1 to 10 μg/ml for estrone and 16α-hydroxyestrone, and from 0.10 to 20 μg/ml for 4-*tert*-octylphenol and 17α-ethynylestradiol, with $r^2 > 0.99$.

The limits of detection (LODs), calculated as the concentration of three-times the standard deviation in 10 independent blank performance, are given in Table 3. The limits of quantification (LOQs) are the minimum concentrations of quantitative analysis, and determined as the analyte amount related to a signal/noise ratio of 10. The results are also listed in Table 3. In all case, LOD fell between 0.2 and 1.0 ng/g sediment, and LOQ varied from 0.5 to 3.4 ng/g.

The recovery tests were performed for the validation of this method by spiking four different levels of standard mixture in sediment samples, and results presented in Table 4. Mean recoveries of all analytes in sediments ranged from 61.5 to 133% at the spiking level of 5–100 ng/g, with R.S.D. less than 24.3%. For 4-*tert*-octylphenol, bisphenol A, estrone and 17α-ethynylestradiol, their recoveries were higher than 80% at four different spiking levels. Therefore, the results show that the method developed exhibits a satisfactory precision and reproducibility for the separation and determination of EDCs from sediment samples.

Furthermore, compared with the results from ultrasonication extraction experiments (shown in Table 4), the extraction efficiency by microwave-assisted system was similar for all the compounds. However, the advantages of microwave-assisted extraction include low solvent consumption (25 ml) and short extraction time (15 min).

3.4. Analysis of environmental samples

The method developed was then applied to the extraction and analysis of EDCs from natural sediment samples, collected from rivers Ouse and Uck of UK. As shown in Table 5, the results revealed the presence of the target compounds in some river sediment samples in the low ng/g range, which in many cases was below the LOQ, especially for River Ouse at Haywards Heath. For River Ouse at Lewes and River Uck at Uckfield, the concentrations of the target compounds were slightly higher in sediment samples from the sewage outfall than those upstream or downstream of the sewage outfall.

Table 4
Recovery (%) for EDCs from sediment samples ($n = 3$) after subtracting background concentrations

Compound	Microwave-assisted extraction, spike level (ng/g dry mass)				Sonication extraction, spike level
	5	10	40	100	40 (ng/g dry mass)
4-*tert*-Octylphenol	129 ± 18.3	133 ± 5.4	90.4 ± 6.1	91.6 ± 1.8	123 ± 14.6
4-Nonylphenol	61.5 ± 14.1	74.7 ± 8.0	93.4 ± 7.4	101 ± 7.4	90.3 ± 5.1
Bisphenol A	103 ± 11.9	80.2 ± 24.3	102 ± 19.8	98.8 ± 1.8	97.0 ± 5.3
Estrone	93.5 ± 7.5	107 ± 1.5	103 ± 2.5	83.2 ± 0.9	86.9 ± 10.4
17β-Estradiol	92.3 ± 13.4	109 ± 1.4	83.5 ± 2.9	77.1 ± 7.2	102 ± 4.8
17α-Ethynylestradiol	120 ± 6.1	116 ± 12.8	113 ± 13.2	123 ± 4.6	122 ± 25.9
16α-Hydroxyestrone	80.0 ± 5.2	87.8 ± 16.4	73.4 ± 14.9	75.4 ± 1.9	99.0 ± 5.8

Table 5
The mean concentrations (ng/g dry mass) and standard deviations ($n = 3$) of the target compounds measured in sediment samples from Sussex rivers, UK

Compound	Uckfield sewage outfall, River Uck		Haywards Heath sewage outfall, River Ouse			Lewes sewage outfall, River Ouse	
	Upstream of outfall	Sewage outfall	Upstream of outfall	Sewage outfall	Downstream of outfall	Sewage outfall	Downstream of outfall
4-*tert*-Octylphenol	2 ± 0.7	12	<LOQ	<LOQ	5	8 ± 1.3	4
4-Nonylphenol	4 ± 1.1	4	<LOQ	<LOQ	2	5 ± 1.5	<LOQ
Bisphenol A	8 ± 1.9	8	<LOQ	<LOQ	<LOQ	9 ± 2.7	5
Estrone	3 ± 0.8	7	<LOQ	<LOQ	<LOQ	3 ± 0.6	<LOQ
17β-Estradiol	2 ± 0.7	4	<LOQ	2	3	4 ± 1.1	<LOQ
17α-Ethynylestradiol	9 ± 1.4	12	9	2	4	<LOQ	<LOQ
16α-Hydroxyestrone	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

4. Conclusions

A method based on microwave-assisted extraction followed by GC/MS has been developed for the accurate and precise determination of important EDCs in river sediments. The best extraction conditions involve methanol as the solvent, an extraction temperature of 110 °C and a duration of 15 min. Reasonably low LOQ values are made possible by an effective clean-up step with silica gel. The method developed coupling microwave-assisted extraction and GC–MS technique provides a means for the quantitative analysis of EDCs from river sediments down to 0.5 ng/g. In addition, the determination of EDCs at trace levels in sediments is challenging as matrix interferences have to be removed by clean-up steps. The chemical composition and functionality of sediments from different origins can vary enormously. Therefore, clean-up procedure is a key step for the quantitative determination of the target compounds.

Acknowledgements

The authors would like to thank the Leverhulme Trust for funding this project (Grant number: F/00230/H). A. Wilding's contribution was supported by a DPhil Studentship from the UK Engineering and Physical Sciences Research council (award number: 00311789).

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