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Optimisation of derivatisation for the analysis of estrogenic compounds in water by solid-phase extraction gas chromatography–mass spectrometry

Z.L. Zhang [a,b], A. Hibberd [a], J.L. Zhou [a,c,*]

[a] *Department of Biology and Environmental Science, School of Life Sciences, University of Sussex, Falmer, Brighton BN1 9QJ, UK*
[b] *Department of Environmental Science and Engineering, POPs Research Centre, Tsinghua University, Beijing 100084, China*
[c] *State Key Laboratory of Water Environment Simulation, School of Environment, Beijing Normal University, Beijing 100875, China*

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Abstract

An optimisation of derivatisation methods for the simultaneous determination of endocrine disrupting chemicals (EDCs) in water by solid-phase extraction (SPE) gas chromatography–mass spectrometry (GC–MS) was developed in this study. Seven highly potent EDCs including 17β-estradiol (E2), estrone (E1), 16α-hydroxyestrone, 17α-ethynylestradiol (EE2), bisphenol A, 4-nonylphenol and 4-*tert*-octylphenol were selected as the target compounds. The SPE technique, followed by the derivatisation with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) was used for the extraction recoveries of compounds from water and effluent samples. The stability of the silylation derivatives under different reaction conditions was investigated. The combined use of BSTFA and pyridine as derivatisation reagents, together with the use of hexane as the final solvent, was preferred in order to generate more stable derivatives of EDCs. The relative response factor (RRF) of all derivatives except that of EE2 was stable 120 h after derivatisation. The addition of pyridine as derivatisation reagent with BSTFA can prevent the conversion of EE2 to other products during the reaction. Several parameters that may affect the recovery of EDCs, such as the SPE flow rate, and water properties including aquatic colloid content and surfactant concentration were tested. The results showed that the flow rate (1–25 mL min^{-1}), colloid concentration (0–50 mg L^{-1}) and surfactants concentration (0–10 μg L^{-1}) did not cause significant decrease in the EDCs recovery.

Keywords: Endocrine disrupting chemicals; Solid-phase extraction; Steroids; Phenolic compounds; Gas chromatography–mass spectrometry

1. Introduction

The presence in the environment of compounds with estrogenic and androgenic properties has become a major concern worldwide. Endocrine-disrupting chemicals (EDCs) are environmental contaminants that interfere with the function of the endocrine system of wildlife and humans. The range of substances reported to cause endocrine disruption is diverse and includes both natural and synthetic chemicals. Naturally produced estrogens such as estrone (E1) and 17β-estradiol (E2) are mainly derived from excreta of humans and livestock, and 16α-hydroxyestrone is the hepatic metabolite of the natural estrone by 16α-hydroxylation pathway [1]. Man-made substances include synthetically produced hormones, e.g. 17α-ethynylestradiol (EE2) and industrial chemicals, e.g. bisphenol A, and alkylphenols associated with plastics, household products and industrial processes [2,3]. As some EDCs can cause biological effects, e.g. feminisation of male fish, abnormal reproductive processes and the development of testicular and prostate cancer even at low concentrations down to ng L^{-1} range [4,5], it is therefore critical that reliable analytical methods are developed for EDCs analysis at trace level.

Recently, many studies have been carried out for the analysis of EDCs such as phenolic compounds, estrogens and steroids in various environmental samples by gas chromatography–mass spectrometry (GC–MS) technique [6–9], following extraction and derivatisation. Two popular reagents used to derivatise compounds bearing hydroxyl groups are *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA), which lead to the formation of trimethylsilyl (TMS) and *t*-butyldimethylsily (TBS) derivatives, respectively. Catalysts such as trimethylchlorosilane (TMCS), trimethylsilylimidazole (TMSI) and *tert*-butyldimethylsilylchlorosilane (TBCS) are

* Corresponding author. Tel.: +44 1273 877318; fax: +44 1273 677196.
E-mail address: j.zhou@sussex.ac.uk (J.L. Zhou).

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widely used to enhance derivatisation [10,11]. From the evaluation of a number of similar reagents for detecting both natural and synthetic estrogens by GC–MS, Ding and Chiang [12] concluded that BSTFA with 1% TMCS operating at 70 °C for 30 min was the best derivatisation reagent. However, Shareef et al. [10,11] reported a problem with the use of BSTFA or MTBSTFA to derivatise E1 and EE2, as the resulting TMS and TBS derivatives of EE2 could partially be converted to their respective E1 derivatives. It is therefore important to stress that using these reagents alone may not be suitable for the simultaneous determination of estrogens in environmental samples. The data from previous studies [8,13,14] that used such a method may need to be re-evaluated, as they could have overestimated E1 and underestimated EE2 as a result of inter-conversion between them. In addition, when analysing large numbers of samples following fieldwork, the use of auto-sampler in GC–MS is much preferred, therefore the stability of the silylated EDCs is also very important for environmental analysis, an aspect that is not well studied so far.

In this work, 17β-estradiol (E2), estrone (E1), 16α-hydroxyestrone, 17α-ethynylestradiol (EE2), bisphenol A, 4-nonylphenol and 4-*tert*-octylphenol were chosen as the target compounds. The aim was to investigate the stability of silylated EDC derivatives and to optimise the derivatisation conditions, especially through the addition of pyridine together with BSTFA (1% TMCS) as the derivatisation reagents. The purpose was to prevent the inter-conversion between EE2 and E1 during derivatisation. Furthermore, the effects of SPE flow rate and properties of water samples including colloid and surfactant concentrations on the extraction efficiency of EDCs, which have not been studied before, were also determined.

2. Experimental

2.1. Reagents

All the solvents used including methanol, ethyl acetate, acetone and dichloromethane (DCM), purchased from Rathburns, were of distilled-in-glass grade. Compounds E1, E2, EE2, 16α-hydroxyestrone, E2-d_2 and 4-nonylphenol were purchased from Sigma, UK, and bisphenol A, 4-*tert*-octylphenol, bisphenol A-d_{16} and BSTFA containing 1% of TMCS were supplied by Aldrich (Dorset, UK). Separate stock solutions of individual compounds were made up at a level of 1000 mg L^{-1} 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^{-1} was prepared weekly by diluting the stock solution in methanol, and used to spike the water solution. Internal standard solutions (1 mg L^{-1}) of bisphenol A-$d_{16}$and E2-d_2 were prepared in methanol. All standard solutions were stored at −18 °C prior to use. Aquatic colloids which were isolated from the River Ouse, East Sussex, UK through cross-flow ultrafiltration [15,16], were used to assess effects of water properties on EDC recovery. A pure surfactant (dodecylbenzesulfonic acid) from Aldrich was used to assess its effect on EDC recovery. Ultrapure 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 SPE technique. One hundred nanogram of E1, E2, EE2, 16α-hydroxyestrone, 4-nonylphenol, bisphenol A, and 4-*tert*-octylphenol were spiked (in triplicate) in 500 mL of ultrapure water for the recovery test. The Oasis SPE cartridges (0.2 g HLB, Waters) were conditioned with 5 mL of ethyl acetate to remove residual bonding agents, followed by 5 mL of methanol which was drawn through the cartridges under very low vacuum to ensure that the sorbents were soaked in methanol for 5 min. Then ultrapure water (3× 5 mL) was passed through the cartridges at a rate of 1–2 mL min^{-1}. Water samples were extracted at a flow rate less than 5 mL min^{-1}, except when the effect of flow rate on EDC recovery was studied. The cartridges were dried under vacuum and then the analytes were eluted to vials (20 mL) from the sorbents with 10 mL of ethyl acetate at a flow rate of 1 mL min^{-1}. The solvents were blown down to 1 mL under a gentle flow of nitrogen at less than 50 °C. In addition, the effects of water properties such as the presence of aquatic colloids and surfactants on EDC recovery were assessed by spiking different amount of colloids and surfactants to test samples. The method developed was further verified by checking recoveries in natural matrices, where river water and sewage effluent samples (1 L) were filtered through pre-combusted GF/F filters (0.7 μm) and spiked with 50–500 ng of the target EDCs. These samples were then extracted using SPE and analysed by GC–MS.

2.3. Derivatisation procedures

Due to the high polarity of some compounds, which gave rise to poor chromatographic peaks, derivatisation was necessary to 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 E2-d_2 as the internal standards. The mixtures were further evaporated to dryness under a gentle nitrogen stream. The dry residues were derivatised by using four different protocols (Fig. 1), in order to optimise the derivatisation procedures and to enhance the stability of TMS derivatives.

2.4. GC–MS analysis

GC–MS analysis was performed using a gas chromatograph (6890N network GC, Agilent Technologies, USA) interfaced with a tandem quadrupole mass spectrometer (Quattro Micro, Micromass, USA). A HP-5 capillary column (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 1.0 mL min^{-1}. The GC column temperature was programmed from 100 (initial equilibrium time 1 min) to 200 °C via a ramp of 10 °C min^{-1}, 200–260 °C via a ramp of 15 °C min^{-1}, 260–300 °C via a ramp of 3 °C min^{-1} 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

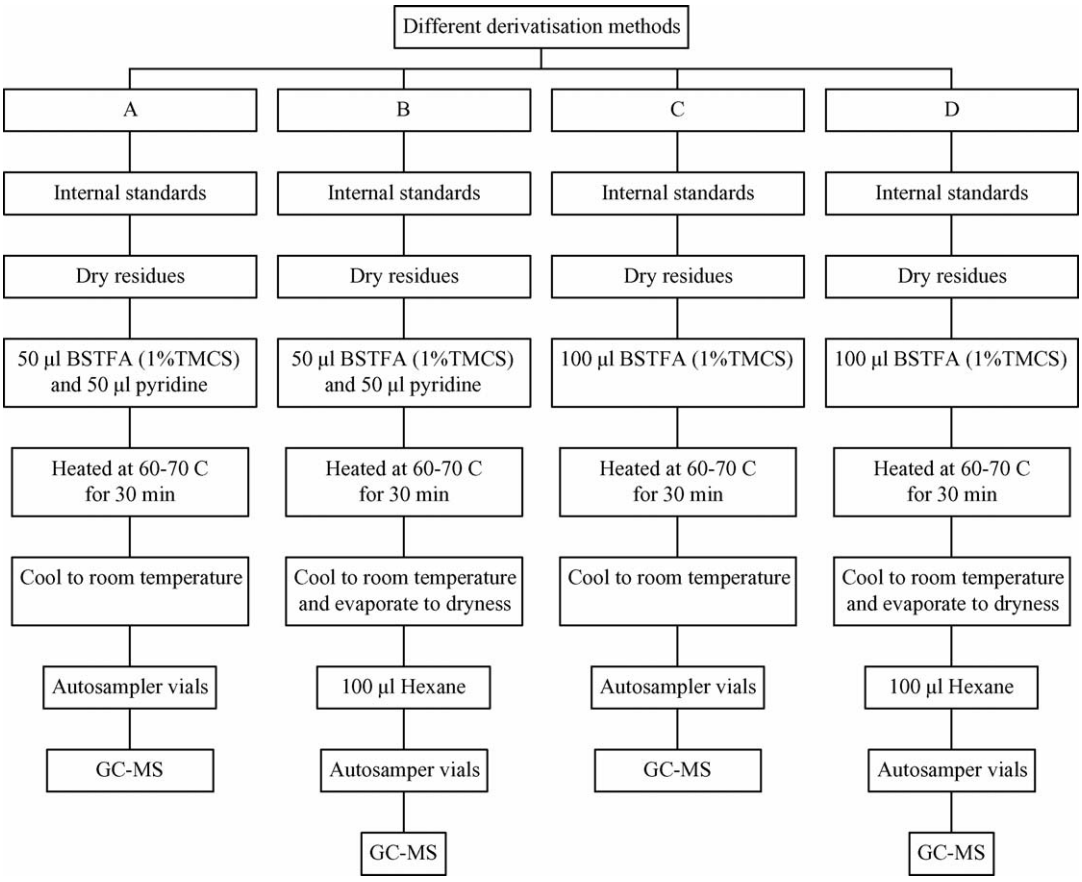

Fig. 1. Schematic diagram of different derivatisation protocols A, B, C and D.

temperature was 250 °C. Sample injection (1 μL) was in splitless mode.

3. Results and discussion

3.1. Stability of derivatisation

The stability of the silylated EDCs was investigated by four different derivatisation protocols (Fig. 1) over a duration of 120 h. In terms of peak area (Fig. 2), the silylated EDCs were most stable by derivatisation in the presence of pyridine and using hexane as the final solvent (i.e. protocol B) within 48 h of derivatisation. In addition, the use of hexane improved the sensitivity of the method when no pyridine was used (Fig. 2C versus 2D). Over long durations beyond 48 h, the stability of silylated EDCs became more variable and less satisfactory. It was interesting to observe that the area abundance for silylated derivatives of EE2 in the presence of pyridine (Fig. 2A and 2B) was significantly higher (4–18 times) than that in the absence of pyridine (Fig. 2C and 2D), suggesting pyridine favoured the formation of TMS-EE2 derivatives.

In our method, deuterated internal standards E2-d_2 and bisphenol A-d_{16} were used for quantifying the concentrations of the target compounds E1, E2, 16α-hydroxyestrone, EE2, 4-nonylphenol, bisphenol A and 4-*tert*-octylphenol, based on the use of relative response factor (RRF):

$$\mathrm{RRF} = \frac{\text{response of a compound/amount of a compound}}{\text{response of an internal standard/ amount of an internal standard}} \quad (1)$$

RRF is very important when analysing environmental samples, as it is used to trace the potential loss or recovery of target compounds during the whole or part of a procedure. Ideally RRF should be a constant, but in reality it may change with time. To ensure high quality of data, RRF was calculated for each of the EDCs (Fig. 3). For the four protocols, the RRF of all compounds except EE2 was much more stable than the area abundance throughout the duration of 120 h after derivatisation. The variability of RRF values for all compounds except EE2 (represented by R.S.D.) within 120 h of derivatisation is between 0.8% and 12.6%, 1.5% and 21.5%, 2.3% and 9.3% and 1.9% and 27.6% for protocols A, B, C and D, respectively. For EE2, its RRF is more stable when the duration is less than 48 h, with a corresponding R.S.D. value ranging from 3.4% to 10.5% for protocols A, B and C, except for protocol D (R.S.D. = 20.6%). From GC–MS response, area abundance and RRF stability, it can be concluded that protocol B represents the best conditions when determining EDCs in environmental samples.

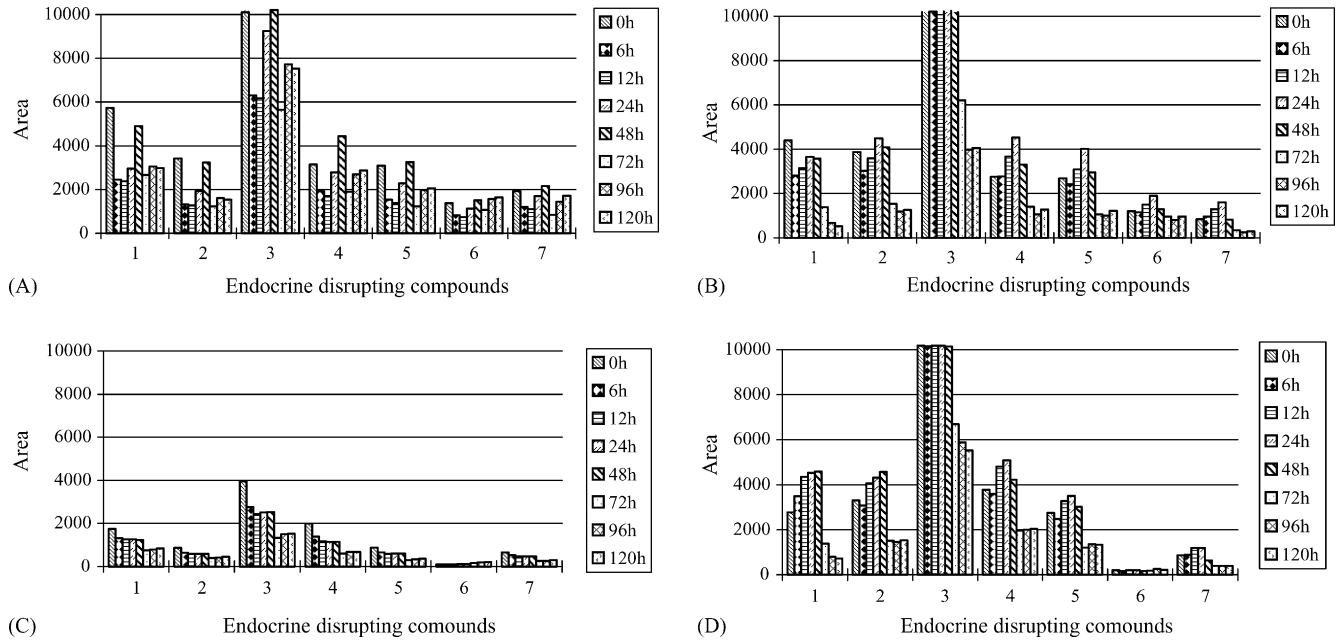

Fig. 2. Comparison of area abundance for individual EDCs by different derivatisation methods A–D for different durations. Compounds 1–7 represent 4-*tert*-octylphenol, 4-nonylphenol, bisphenol A, E1, E2, EE2 and 16α-hydroxyestrone, respectively.

3.2. Suitability of BSTFA as derivatisation reagent

Shareef et al. [10,11] reported that EE2 could be partially converted to E1 during the derivatisation and chromatography, when using BSTFA (containing 1% TMCS) as the derivatisation reagent. In our experiments, it was observed that the peak area for silylated derivatives of EE2 in protocols A and B (in the presence of pyridine) was higher than that in protocols C and D (in the absence of pyridine), suggesting beneficial effect of pyridine in enhancing the signal of derivatised EE2 through some mechanism.

In order to elucidate how BSTFA and pyridine affect the derivatives of EE2 and E1, the derivatisation reactions of EE2 and E1 were investigated in a series of experiments when E2-d_2 was used as the internal standard. Typical total ion chromatograms (TIC) of the TMS derivatives for E1, EE2 and E2-d_2 are shown in Fig. 4, and the mass spectra from individual peaks are shown in Fig. 5. Peak 1 is the TIC of TMS-E1

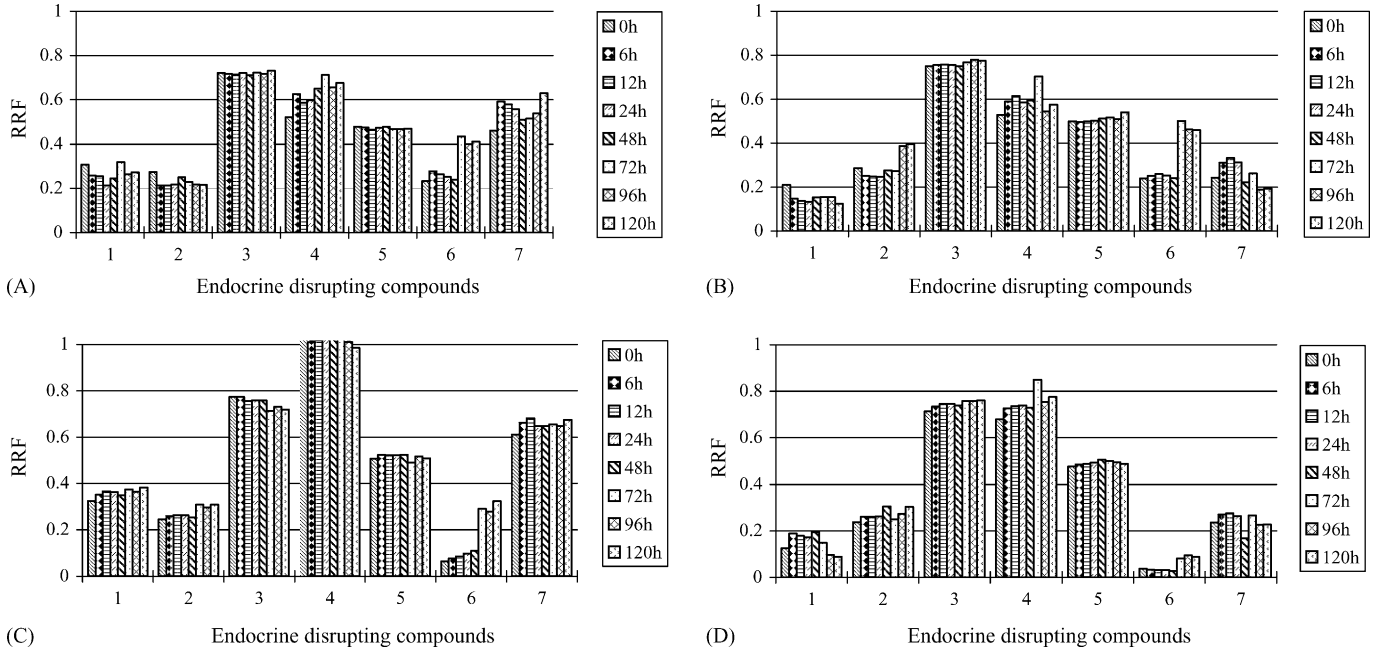

Fig. 3. Comparison of the RRF values for individual EDCs by different derivatisation methods A–D for different durations. Compounds 1–7 represent 4-*tert*-octylphenol, 4-nonylphenol, bisphenol A, E1, E2, EE2 and 16α-hydroxyestrone, respectively.

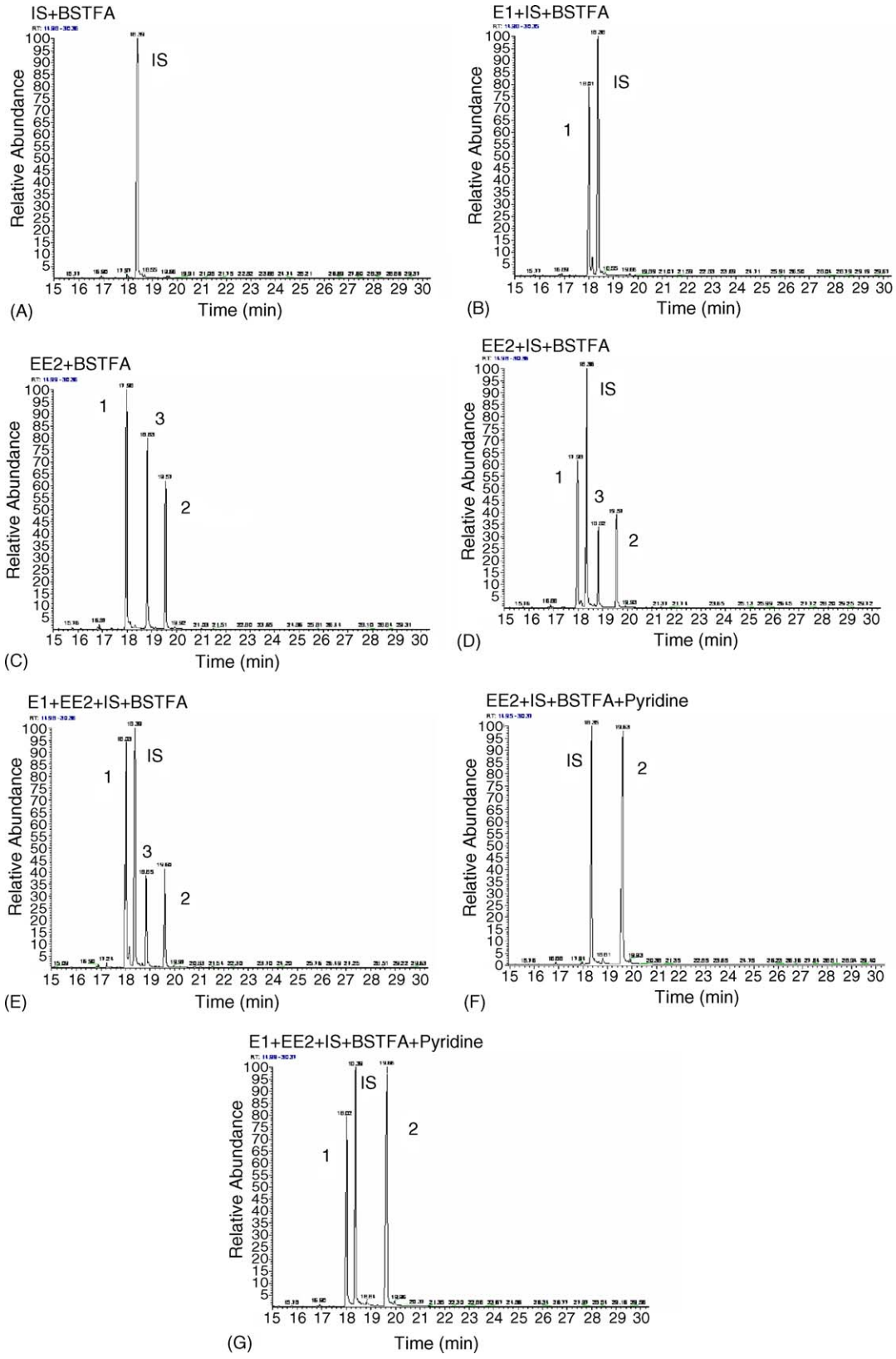

Fig. 4. TIC from GC–MS analysis of TMS derivatives of EE2 and E1 under different reactive conditions. (1) TMS-E1, (2) di-TMS-EE2 and (3) mono-TMS-EE2. The internal standard (IS) was E2-d_2.

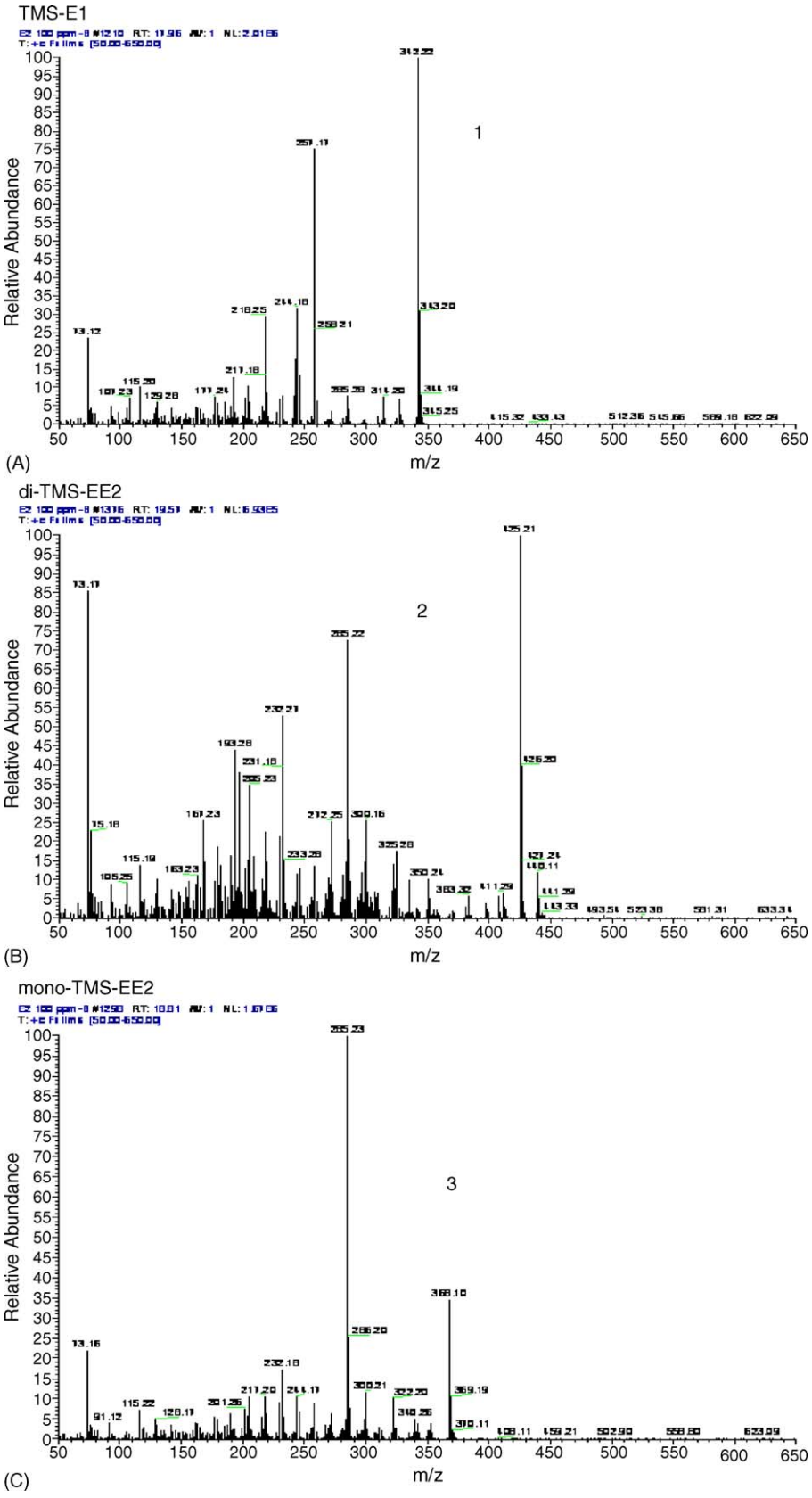

Fig. 5. Mass spectra of TMS derivatives: (1) TMS-E1, (2) di-TMS-EE2 and (3) mono-TMS-EE2.

as shown in Fig. 4B–E and G which all had the same retention time and identical mass spectra as shown in Fig. 5A. The TIC of peak 2 is the di-TMS-EE2, which was formed by its reaction with TMS at both the 3-OH and 17-OH positions. It had the same retention time as shown in Fig. 4C–G, and the mass spectrum of di-TMS-EE2 is shown in Fig. 5B. The peak 3 is the mono-TMS-EE2 as a result of its reaction with TMS only at 3-OH position, which was only produced when EE2 was being derivatised by BSTFA (containing 1% TMCS) alone. The mass spectrum of mono-TMS-EE2 is shown in Fig. 5C.

The derivatisation reactions show clearly that when E1 was derivatised, only one product (TMS-E1) was formed. But when EE2 was derivatised by adding BSTFA (containing 1% TMCS) only as reactive reagent, three products were formed, TMS-E1, mono-TMS-EE2 and di-TMS-EE2 (Fig. 4C). The same pattern occurred when internal standard E2-d_2 was added (Fig. 4D) and when E1 was added, with an enhanced signal for TMS-E1 (Fig. 4E). The results support an earlier report by Shareef et al. [10] who observed the formation of TMS-E1, although there was no mention of mono-TMS-EE2 observed during the reactions in their study until more recently [11]. The identical retention times and mass spectra, together with fragmentation patterns that match those expected for peaks 1 (TMS-E1), 2 (di-TMS-EE2) and 3 (mono-TMS-EE2), indicate that two types of TMS derivatives of EE2 plus a transformed product of E1 derivatives are formed.

When pyridine was used together with BSTFA (containing 1% TMCS) as derivative reagents, it is interesting to find that only one EE2 derivative was formed (i.e. di-TMS-EE2), and there was no formation of TMS-E1 and mono-TMS-EE2 during the reaction (Fig. 4F and G). As a result, the addition of pyridine inhibited the conversion of EE2 to TMS-E1 or mono-TMS-EE2. It is therefore concluded that the combination of BSTFA and pyridine as reactive reagents is essential if quantitative analysis of EDCs is to be performed. Previous work based on BSTFA derivatisation in the absence of pyridine should be ex-assessed, in the light of this new finding of potential EE2 conversion to two EE2 derivatives and more seriously, to E1 derivative.

The EE2 conversion was calculated by comparing Fig. 4E and G, when EE2 and E1 together with E2-d_2 were derivatised by BSTFA in the absence or presence of pyridine, respectively. The original amount of EE2 and E1 for derivatisation was 10 μg. Based on the reaction in Fig. 4G which has no conversion and from the relative peak areas for the E1 and EE2 derivatives (Fig. 4G and E), it was estimated that about 72% of EE2 was converted to the TMS-E1 and mono-TMS-EE2 after reaction with BSTFA when pyridine was not present (Table 1). The conversion here is higher than that found by Shareef et al. [10] with 42% conversion, due to the fact that they did not consider the conversion to mono-TMS-EE2. When the same amount of E1 and EE2 were derivatised by BSTFA in a reaction, it can be estimated that 38% of E2 was derivatised to TMS-E1, while the rest (34%) being transformed to mono-TMS-EE2.

Table 1
Estimation of the conversion of EE2 to TMS-E1 and mono-TMS-EE2

	Peak area normalised to internal standard	Peak area normalised to internal standard	Conversion of EE2 (%)
Pyridine	Yes	No	
Figure	Fig. 4g	Fig. 4e	
Di-TMS-EE2	1.34 ± 0.02 ($n = 3$)	0.38 ± 0.01 ($n = 3$)	−72
TMS-E1	0.80 ± 0.01 ($n = 3$)	1.10 ± 0.02 ($n = 3$)	+38
Mono-TMS-EE2	0	0.38 ± 0.06 ($n = 3$)	+34

"−" means conversion and "+" means production.

3.3. Effect of SPE flow rate on EDC recovery

When SPE was used to extract trace organic compounds from water samples, relatively low flow rates of water passing through the SPE cartridges were adopted due to concern of analyte loss. In common practice, low flow rates such as either less than 5 mL min^{-1} or between 5 and 10 mL min^{-1} were used [7,9,17,18]. This reflects little understanding of the potential impacts of SPE flow rates on recovery. In order to investigate whether the flow rate has any negative effect on the recovery of target EDCs, the SPE system vacuum was adjusted to test the recovery of target compounds under different SPE flow rates ranging from 1 to 25 mL min^{-1}. As shown in Fig. 6, the effect of SPE flow rate on EDC recovery was variable and compound-dependent. Overall, the recovery was always greater than 70% except in one case. The results would suggest that the rate of EDC extraction from the moving aqueous phase by the Oasis SPE cartridges was very fast; hence a short residence time was needed for the compounds to be kept in the column.

3.4. Effect of colloid concentration on EDC recovery

Colloids, defined as particles sized between 1 nm and 1 μm, are ubiquitous components of seawater, freshwater and groundwater as well as pore water [19,20], and make up a significant fraction (10–40%) of marine total organic carbon [21]. These colloidal material, often defined as the so-called "third phase", play a vital role in the transport and ultimate fate of trace organic pollutants in aquatic systems [22]. So the colloids are

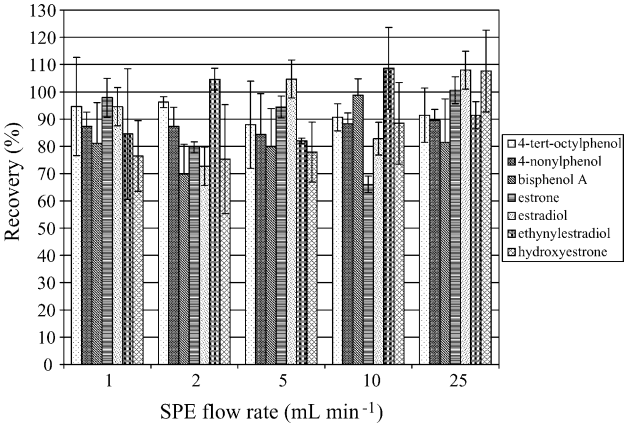

Fig. 6. The effect of SPE flow rate on the recovery of EDCs.

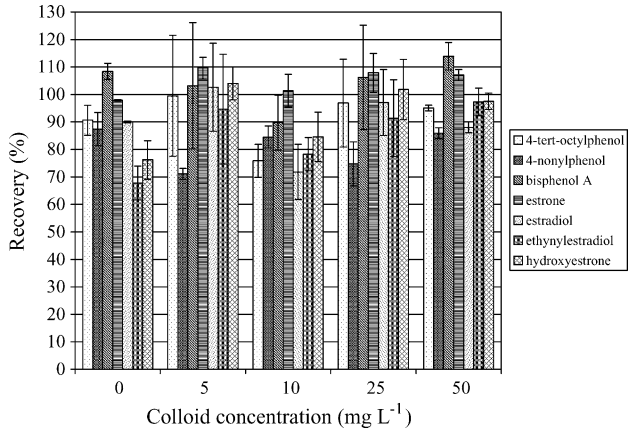

Fig. 7. The effect of colloid concentration on the recovery of EDCs.

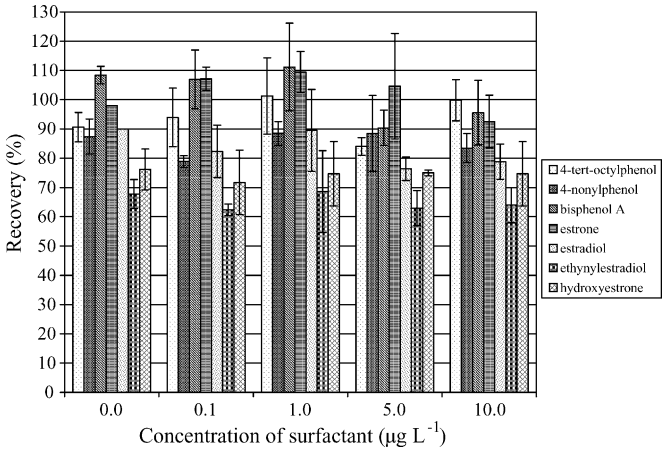

Fig. 8. The effect of surfactant concentration on the recovery of EDCs.

important in terms of binding with organic pollutants. Such colloid–pollutant interactions may interfere with SPE operation and the derivatisation reaction, hence affecting recovery. In this work, the influence of colloid on the recovery was investigated by spiking different concentrations of aquatic colloid isolated from the River Ouse in the test solutions. As shown in Fig. 7, when the colloid concentration was increased from 0 to 50 mg L^{-1}, there was no obvious trend of influence on the recovery of target compound by SPE. Although it is likely that the EDC and colloid interacted in water [16,22], both the dissolved and colloid-bound EDCs may be concentrated by the SPE sorbents. Subsequently the EDCs in both the colloidal and dissolved phases could be eluted from the SPE cartridges by solvents (e.g. ethyl acetate). Hence it can be concluded that the aquatic colloids did not significantly affect the recovery of target compound by the Oasis SPE cartridges. The results are consistent with Liu et al. [9] who showed that a commercial humic acid did not affect the recovery of EDCs during SPE-GC–MS.

3.5. Effect of surfactant concentration on EDC recovery

Natural waters such as river water are highly complex in chemical composition, containing a variety of substances such as dissolved organic matter, oil residues, industrial chemicals and surfactants, some of which may affect the recovery of EDCs using SPE. The presence of surfactant is a potentially important parameter to study, as entry of EDCs to the environment is via the sewage treatment works in the form of effluent, which will normally contain a variety of surfactants [23,24]. The chemical properties of surfactants mean that they are good solvents of sparingly soluble organic compounds [25], and can enhance the solubility of hydrophobic organic compounds as shown by West and Harwell [26]. Surfactants have also been used to increase the desorption of hydrophobic pollutants from sediments and soils [27,28]. A pure surfactant (dodecylbenzesulfonic acid) was used in this study, although in reality, at discharge sites, there would be a mixture of compounds, which are usually from a variety of different sources. The effect of this surfactant on the SPE recovery of target compounds was studied at five different surfactant concentrations, ranging from 0 to 10 μg L^{-1}. As shown in Fig. 8, the surfactant had no significant adverse effect on the recovery of EDCs by SPE, possibly due to the relatively low concentrations of the surfactant being used.

3.6. Further validation and application to environmental samples

To ensure that the method is fit for purpose, further recovery experiments were conducted by spiking a known amount of the standard mixture to "clean" river water samples, which do not contain the chosen EDCs. For spiked river water samples, the recovery for 4-*tert*-octylphenol and 4-nonylphenol ranged from 74.1% to 87.2% at spiking levels of 50–500 ng L^{-1} (Table 2).

Table 2
Recovery data for EDCs in river water ($n = 4$)

Matrix	Spiked level (ng L^{-1})	4-*tert*-Octylphenol	4-Nonylphenol	Bisphenol A	Estrone	17β-Estradiol	17α-Ethynylestradiol	16α-Hydroxyestrone
River water	50	87.2 ± 6.1	74.1 ± 7.1	91.1 ± 8.7	102 ± 5.3	94.2 ± 9.3	90.2 ± 7.8	78.2 ± 5.8
	100	86.2 ± 12.5	77.1 ± 10.2	92.0 ± 3.9	97.2 ± 8.7	90.1 ± 8.8	89.1 ± 7.2	86.3 ± 6.2
	200	81.2 ± 3.9	78.6 ± 6.2	88.3 ± 2.3	88.9 ± 3.2	93.2 ± 7.4	91.4 ± 10.1	88.4 ± 7.2
	500	78.9 ± 13.7	82.9 ± 7.2	81.8 ± 5.2	75.2 ± 7.2	70.1 ± 8.9	78.5 ± 7.1	76.7 ± 6.7
Sewage effluent	50	77.6 ± 7.6	76.2 ± 6.8	82.3 ± 4.5	90.2 ± 10.1	81.3 ± 7.0	82.1 ± 8.1	81.3 ± 4.5
	100	76.6 ± 9.1	72.1 ± 2.2	80.0 ± 9.1	88.1 ± 9.2	78.8 ± 6.5	86.2 ± 8.8	82.8 ± 3.2
	200	71.2 ± 2.5	85.5 ± 8.9	75.3 ± 5.5	76.5 ± 8.6	82.7 ± 9.1	84.7 ± 7.1	75.6 ± 8.1
	500	82.9 ± 4.5	80.1 ± 6.5	72.8 ± 11.1	72.0 ± 3.3	77.5 ± 4.5	72.8 ± 6.5	71.4 ± 6.5

Table 3
Concentrations of the target EDCs in water samples from the River Ouse, East Sussex, UK in 2004

Compound	Upstream of sewage outfall ($ng\,L^{-1}$)	Sewage outfall ($ng\,L^{-1}$)	Downstream of sewage outfall ($ng\,L^{-1}$)
4-*tert*-Octylphenol	<0.3–25	8–65	<0.3–30
4-Nonylphenol	<0.2	<0.2–8	<0.2
Bisphenol A	<0.1–8	10–48	<0.1–12
Estrone	<0.3	<0.3–41	<0.3–17
17β-Estradiol	<0.7–2	10–22	<0.7–9
17α-Ethynylestradiol	<0.5	<0.5–5	<0.5
16α-Hydroxyestrone	<0.4	<0.4	<0.4

Similarly the recovery for the other EDCs was shown to be satisfactory or excellent (70–102%) when EDCs were spiked at levels up to $500\,ng\,L^{-1}$. The R.S.D. of all recovery experiments was less than 14%, with a large majority of samples (86%) with R.S.D. < 10%. The precision of the method is therefore very good. The results demonstrate that the EDCs studied can be simultaneously separated and determined from 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 concentrations up to $500\,ng\,L^{-1}$. The recovery of EDCs in sewage effluent samples is generally lower than that in river samples, due to the highly complex matrix in wastewater samples (Table 2). Nevertheless, the recovery in effluent samples varied between 71% and 90%, with R.S.D. of all samples less than 15%. The findings therefore confirm that the extraction and analytical method developed can be applied to wastewater samples. It is expected that the recoveries for water samples close to sewage outfalls (mixture of effluent and river water) will lie between those for river water samples and effluent samples, which will be fit for the purpose based on the recovery data in Table 2.

After validation, the method was applied to the analysis of target EDCs in river water samples from the Ouse, East Sussex, UK. As shown in Table 3, the concentrations of the target compounds are low, as many of which are below the limits of detection (LOD). In addition, higher levels of EDCs were measured in water samples close to the sewage outfall than those upstream or downstream of the sewage outfall, suggesting that sewage discharge is a major source of these EDCs in rivers, consistent with many previous reports of fish feminisation near or downstream of sewage outfalls [2,3].

4. Conclusions

The optimisation of derivatisation methods based on SPE-GC–MS has been developed for the determination of contrasting and highly potent EDCs including E1, E2, 16α-hydroxyestrone, EE2, 4-nonylphenol, bisphenol A and 4-*tert*-octylphenol, in water samples. The stability of derivatives under different reaction conditions was investigated, which showed that the combination of BSTFA and pyridine was essential for a successful derivatisation reaction. The use of hexane as the final solvent for the derivatisation products was also beneficial for improving sensitivity and stability. The RRF of most derivatives was considered stable within 120 h of reaction except for EE2. A key finding was that the inter-conversion between EE2 and E1 during derivatisation was halted by using pyridine together with BSTFA (1% TMCS) as derivatisation reagents. For most compounds, the various SPE flow rate between 1 and $25\,mL\,min^{-1}$ did not significantly affect their extraction efficiency. In addition, neither the colloid concentration ($0–50\,mg\,L^{-1}$) nor surfactant concentration ($0–10\,\mu g\,L^{-1}$) showed any significant effect on the EDC recovery. The method has been successfully applied to the analysis of river water and effluent samples.

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