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Development of comparative methods using gas chromatography–mass spectrometry and capillary electrophoresis for determination of endocrine disrupting chemicals in bio-solids

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Abstract

Two analytical separation techniques are being investigated for their potential in determining a wide range of endocrine disrupting chemicals (EDCs) in the environment. Capillary electrophoresis (CE) in the micellar mode in conjunction with a cyclodextrin (CD) modifier is shown to have potential for determination of alkylphenol breakdown products. Gas chromatography with mass spectrometric (GC–MS) detection is being utilised for validation of the CE method development and in addition as a separation technique to optimise preconcentration using solid-phase extraction. GC has demonstrated potential for the separation of 26 priority chemicals suspected as being endocrine disrupting compounds. The challenge of the method development process lies in the fact that these compounds are of differing polarities, size and charge and therefore are difficult to separate in a single run. Capillary electrophoresis in the CD–MEKC (micellar electrokinetic chromatography) mode is showing potential in this regard. Limits of determination are in the low mg/l range for CE and GC, however, using preconcentration it is possible to improve detection sensitivity with >80% recovery for some analytes and up to 100% recovery for most target species.

Keywords: Bio-solids; Endocrine disrupting chemicals

1. Introduction

Evidence has been accumulating for over half a century which indicates that humans, domestic and wildlife species have suffered severe adverse effects on health from exposure to environmental chemicals that interact with the endocrine system. To date these health problems have been identified primarily in domestic or wildlife species with relatively high exposures to organochlorine compounds, polychlorinated biphenyls and dioxins, or to naturally occurring plant oestrogens [1]. An environmental endocrine disruptor has been defined as 'an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behaviour' [2]. Observed effects in wildlife populations have led to concerns over possible health effects in humans [3].

The endocrine and reproductive effects of environmental contaminants are now believed to be due to a variety of effects, notably, the mimicking endogenous hormones such as oestrogens and an-

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drogens; antagonising normal endogenous hormones; altering the pattern of synthesis and metabolism of natural hormones and modifying hormone receptor levels. Compounds identified as endocrine disrupting chemicals (EDCs) are members of distinct groups of chemicals including pesticides, certain polychlorinated biphenyls (PCBs), dioxins, furans, alkylphenols, synthetic steroids and natural products.

Wastewater and sewage contains the vast proportion of the environmental load of these chemicals and is a major source of a mixture of EDCs as a result of direct effluent discharge [4]. Alkylphenols are used as antioxidants and in the synthesis of detergents (alkylphenolpolyethoxylates; APEs). These synthetic surfactants are used in many applications in households, institutions and industries. APEs are not oestrogenic per se; however, APE degradation products which have been shown to have oestrogenic properties [5], have been detected in drinking water [6,7]. These findings demonstrate the general need for analytical monitoring of endocrine disrupting chemicals in sewage and surface water.

This work examines the potential of two comparative separation methods applied to the determination of a wide range of endocrine disrupting chemicals including the alkylphenols, nonyl- (NP) and octylphenol (OP). Capillary electrophoresis (CE) is being examined in the micellar mode with cyclodextrin additives. CE is being developed due to its potential in the separation of compounds of varying charge and size in a single run [8]. The individual determination of groups of EDCs such as phenols [9] alkylphenols [9–11] or oestrogens [10,11] has been achieved using MEKC. Our group has previously demonstrated the potential of MEKC for the simultaneous separation of a selection of priority endocrine disrupting compounds [12]. The use of a surfactant in conventional CE analysis has been shown to enhance the selectivity of a separation and aid the solubility of hydrophobic compounds. The hydrophobic nature of many of the EDCs can however, demand the use of high concentrations of surfactants and also organic modifiers to maintain their solubility throughout the separation and to achieve resolution of structurally similar compounds [13,14].

Cyclodextrin (CD)-modification of MEKC separations can lead to reduced analysis times while improving the selectivity of the analysis. Cyclodextrins are cyclic oligosaccharides containing from six (α), seven (β) or eight (γ) glucose units, forming a cavity into which hydrophobic analytes are included. CD–MEKC has previously been applied to the individual separation of oestrogens [15,16] and also alkylphenols [17]. The addition of hydroxypropyl groups to native CDs improves their solubility in water while providing additional selectivity allowing resolution of structurally similar hydrophobic compounds. This was achieved by He et al. [18] who resolved several structurally similar alkylphenols using hydroxylpropyl-beta-cyclodextrin (HP-β-CD).

In separations using an anionic surfactant [19], compounds that are strongly associated with the micelles will migrate last, leading to long analysis times and poor peak efficiencies. However, when electro-osmotic flow (EOF) is suppressed the most hydrophobic compounds migrate first. The use of a low pH buffer eliminates EOF, and upon the application of a negative voltage, negatively charged surfactant molecules are drawn to the anode under the influence of electrophoresis. Associated analytes and cyclodextrins are also drawn to the anode where detection occurs. EOF suppression has been successfully applied to the analysis of compounds in conjunction with CD–MEKC analysis [19]. This technique is proving to have potential for more rapid separations of alkylphenols as shown in this paper.

The development of a GC–MS method enables both the validation of the CE separation of the EDCs while also providing an analytical mechanism for developing a suitable solid-phase extraction (SPE) preconcentration technique. In this study a GC–MS method is demonstrated for the determination of 26 chemicals that have been identified in the literature as having endocrine disrupting capacity. The method involves an optimised temperature programme which enables the inclusion of the alkylphenol compounds octyl- and nonylphenol. A solid-phase extraction procedure for preconcentration of the phenolic compounds is demonstrated and preliminary applications to a range of sample matrices are shown.

The ultimate aim of this on-going research is to design a separation technique that can be used to identify EDCs present in sewage sludge and treated sludge. This is necessary in order that these matrices can be characterised and assessed for risk. This information is important for two reasons: to aid in

the optimisation of the sewage treatment process in order to reduce the levels of EDCs in drinking waters; and to assess the risks associated with land spreading of treated or untreated sludge. This paper demonstrates (i) the potential of CD–MEKC and suppressed EOF in CE for determination of EDCs and (ii) the potential of GC–MS as an analytical validation technique and SPE method development technique.

2. Experimental

2.1. Instrumentation

CE separations were carried out using a Beckman P/ACE 5500 system (Beckman Coulter, London, UK) which is equipped with a diode array detection (DAD) system. The DAD range was 190 nm to 300 nm. The capillary electrophoresis instrument was operated using Windows P/ACE Station software version 1.21 (Beckman Coulter). The fused-silica capillaries (Beckman Coulter) used were 57 cm×50 μm I.D. and 87 cm×75 μm I.D.

The GC–MS separations were carried out using a 5890 Hewlett-Packard series II Gas Chromatograph coupled with a 5971 Hewlett-Packard mass spectrometer with a 5973A autosampler. The carrier gas used was helium (GC grade), which was supplied by BOC gases (Limerick, Ireland). A HP-5M5 capillary column (30 m length×0.25 nm diameter×0.25 μm I.D.) coated with a 5% biphenyl siloxane stationary phase, was used for the analysis. The injection volume used was 1 μl and the flow-rate of the carrier gas was 1 ml/min. The detector was set to detect mass ions with an m/z in the range of 50–550 and the E_{mv} averaged at approximately 2000 V throughout the time the instrument was in use for this experiment.

2.2. Reagents

All analyte compounds investigated in the separations were purchased from Sigma– Aldrich, (Dublin, Ireland) and had a purity of equal or greater than 97% with the exception of technical grade 4-Nonylphenol (~85% content of *p*-isomers). Acetonitrile (ACN) and methanol, both high-performance liquid chromatography (HPLC) grade were purchased from Sigma–Aldrich. All compounds were used without further purification. Structures of some of the compounds used in the study are shown in Fig. 1.

Cyclohexylamino-1-propane sulfonic acid (CAPS) buffer for CE was purchased from Sigma–Aldrich. HCl, NaOH, methanol (HPLC grade), ACN (HPLC grade), sodium dodecyl sulphate were purchased from Sigma–Aldrich (Dublin, Ireland). CAPS buffer pH was adjusted using 0.1 *M* HCl and 0.1 *M* NaOH. The HP-β-CD (MS 0.8; Sigma–Aldrich) was used without further purification.

2.3. CE separation conditions and standard preparation

Stock solutions for CE determinations of the phenolic compounds and the synthetic oestrogen DES, 17 β-oestradiol and ethynyloestradiol were prepared in 100% ACN. Oestriol and oestrone were prepared in 100% methanol. Aliquots of these stocks were taken in a 50:50 ACN:buffer ratio for the CE separation. All endocrine disrupting target chemicals were purchased from Sigma Aldrich.

2.4. GC–MS separation conditions and standard preparation

Stock solutions were prepared in 100% ACN. For the GC–MS separation the desired standard concentrations were prepared by dilution of the stock solution (100 μg/ml) with ACN.

During the chromatographic method development many GC parameters were modified. The injection port temperature was maintained at 240 °C and the detector temperature at 280 °C. The carrier gas flow-rate was 1 ml/min. The remaining parameters were modified to optimise the separation of the compounds of interest.

2.5. Sampling

As the ultimate application of this technique would be in the analysis of environmental samples, sewage influent and effluent samples have been spiked with the target analytes in order to investigate possible sample matrix effects. Samples were collected in borosilicate amber glass containers. Con-

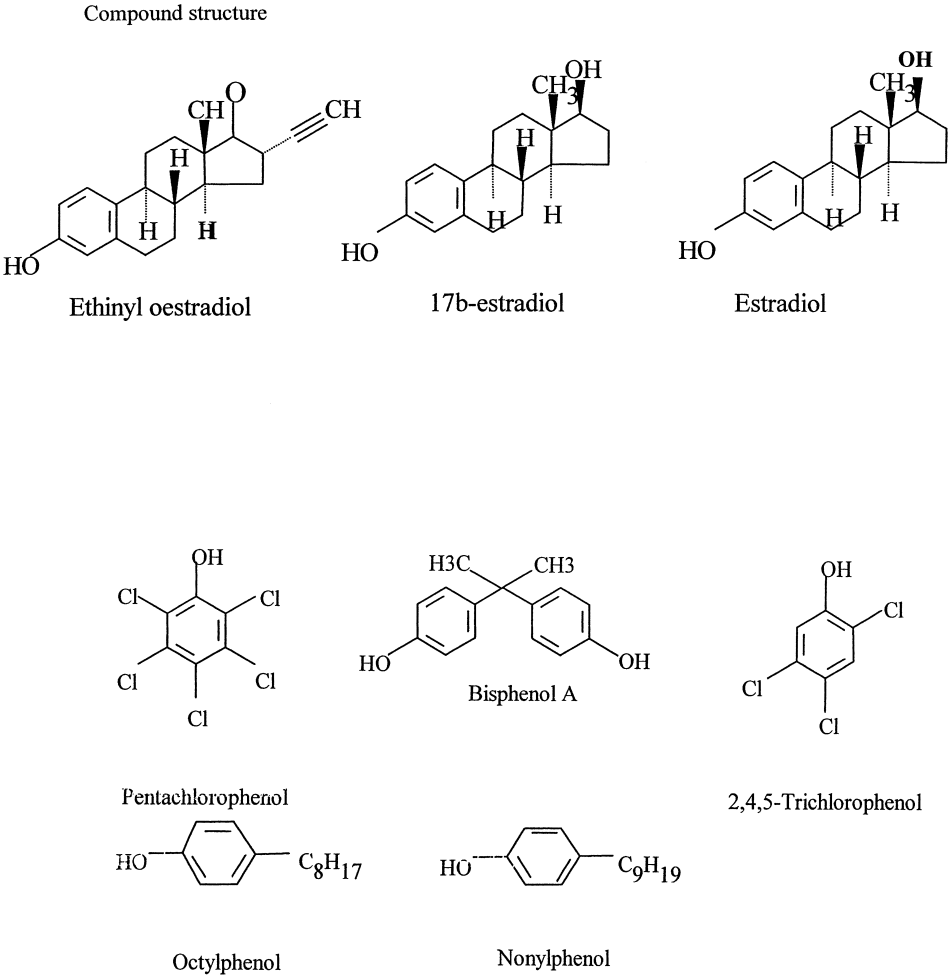

Fig. 1. Structures of some typical endocrine disrupting chemicals.

tainers were rinsed with distilled water and ultrapure water prior to sample addition. Samples were preserved by storing the bottles in the refrigerator at 4 °C immediately after sampling. Sample analysis was carried out as soon as possible in order to avoid degradation or addition of chemical preservatives.

2.6. Preconcentration using solid-phase extraction (SPE)

Due to the low concentrations at which these compounds exist in the environment a preconcentration step was required. Preconcentration of analytes was performed using C_{18} solid-phase material (Bondelut 500 mg) in 2.8 ml capacity cartridges (JVA Analytical, Dublin, Ireland). Prior to extraction each SPE cartridge was conditioned by rinsing with a series of solvents. The optimum conditioning method was designed to achieve the optimum recovery for a variety of analytes. The SPE methods investigated are highlighted in Table 1. Two target analytes of differing polarity were chosen in order to define the method for the wider range of analytes. The optimum method chosen involved a conditioning rinse of 5 ml acetone, 5 ml ACN and 5 ml deionised water. This was followed by a 30-min drying time.

Table 1
Solid phase extraction methods for preconcentration of nonylphenol and dichlorophenol (DCP)

SPE cartridge	Composition	Rinse	Drying time	Eluting solvent	% Recovery
C_{18}	Nonylphenol DCP	5 ml acetone 5 ml ACN 5 ml DI water	30 min	1 ml acetone 1 ml ACN	NP—75% DCP—124%
C_{18}	Nonylphenol DCP	5 ml acetone 5 ml MeOH 5 ml ACN 5 ml DI water	30 min	1 ml acetone 1 ml ACN	NP—75% DCP—124%
C_{18}	Nonylphenol DCP	5 ml acetone 5 ml ACN 5 ml DI water	30 min	1 ml acetone 1 ml ACN	NP—64.5% DCP—125%
C_{18}	Nonylphenol DCP	5 ml 50:50 acetone:ACN 5 ml DI water	30 min	2 ml 50:50 acetone:ACN	NP—49.8% DCP—109%
C_{18}	Nonylphenol DCP	5 ml acetone 5 ml ACN 5 ml DI water	30 min	0.5 ml ACN 1.5 ml acetone	NP—92% DCP—109%

C_{18} SPE cartridge. GC–MS separation conditions as described in Section 2.4.

Up to 1 l of analyte was preconcentrated by passing through the SPE column. To elute the analytes an elution solvent containing 0.5 ml ACN and 1.5 ml acetone was used.

For analysis of spiked effluent and influent samples, samples were adjusted to pH 2 and subsequently treated in the same manner as the standards for SPE.

3. Results and discussion

3.1. Separation of EDCs using CD–MEKC

3.1.1. Injection sample for CD–MEKC

The composition of the injection sample is crucial in CD–MEKC analysis and therefore was investigated. Due to the hydrophobic nature of many of the analytes under investigation, stock standards of each analyte were prepared in ACN. Injection of the analytes in pure organic solutions was found to lead to the disruption of the micelle in the buffer surrounding the sample zone. This is illustrated in Fig. 2, which shows that additional selectivity is achieved through dilution of the injection sample with the corresponding run buffer solution.

3.1.2. Degree of CD substitution

The concentration of the CD was set at 1 m*M* as higher concentrations have previously been seen to allow resolution of the NP isomers [18] which was not seen as a priority for this study. The degree of substitution of the HP-β-CD was investigated. As HP-β-CD has been successfully employed in the resolution of OP and NP it was investigated for the separation of target analytes. Two CDs were investigated. The CD used by He and Lee [18] had an average molecular substitution (MS) of 0.8. This was applied to the separation of the synthetic oestrogen ethynyloestradiol and OP and NP. HP-β-CD with an average substitution of (4–11) was also investigated to observe if an increase in selectivity occurs due to additional substitution of the CD groups. This was found to be not the case as illustrated in Fig. 3. It was determined that the CD with a MS of 0.8 gave a more desirable response with a minor increase in migration times.

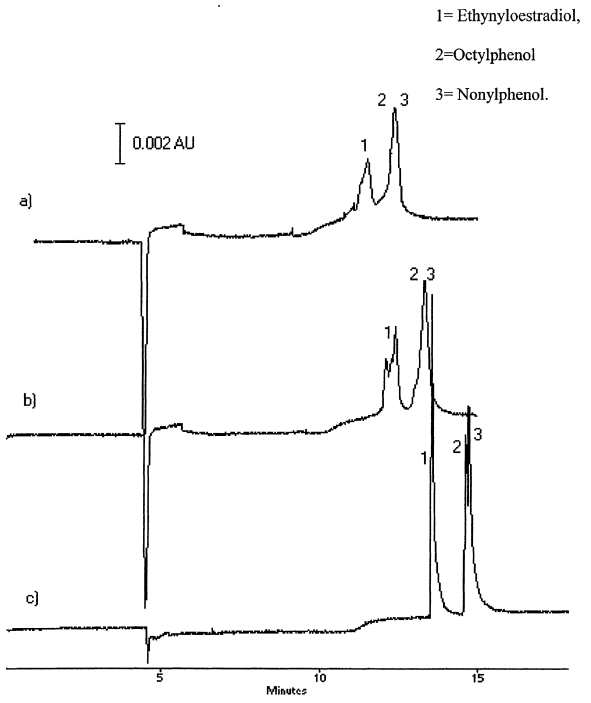

Fig. 2. Separation of an oestrogen and two alkylphenols using CD–MEKC with different sample injection compositions. Run buffer: 12.5 m*M* boric acid pH 9.0, 25 m*M* SDS, 1 m*M* HP-β-CD (MS 4–11), 8% ACN. Concentration of analytes 0.5 m*M*. Analyte injection: (a) 100% ACN, (b) 50% ACN and 50% buffer, (c) 94% buffer, 6% ACN.

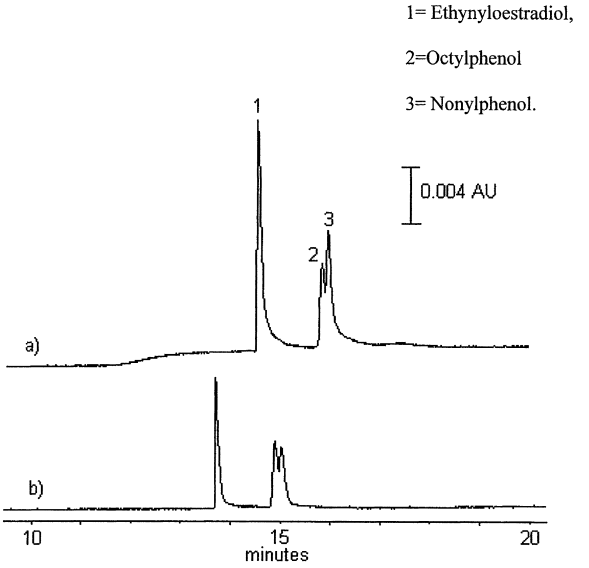

Fig. 3. Comparison of degree of cyclodextrin substitution at pH 9. Run buffer: 12.5 m*M* boric acid pH 9.0, 25 m*M* SDS, 8% ACN. (a) MS 0.8, (b) MS 4–11. Concentration of analytes 0.5 m*M*; concentration of CD 1 m*M*.

3.2. Separation of nine priority EDCs

As the ultimate object of this method development is to enable the analysis of environmental samples, several priority environmental EDCs were included in the separation. As illustrated in Fig. 4, some of those compounds included were bisphenol-A (BPA), pentachlorophenol (PCP) and ethynyloestradiol (EO) which is a major component of the contraceptive pill. The introduction of additional analytes demanded a re-optimisation of the method reported in the literature [18] to observe the effects on the analytes, and to optimise the method to allow resolution of all components while maintaining peak integrity. A number of buffer parameters were modified to achieve an optimum separation of the target analytes.

3.2.1. Variation of % organic modifier

The % concentration of the organic modifier, in this case ACN, was found to be important due to its

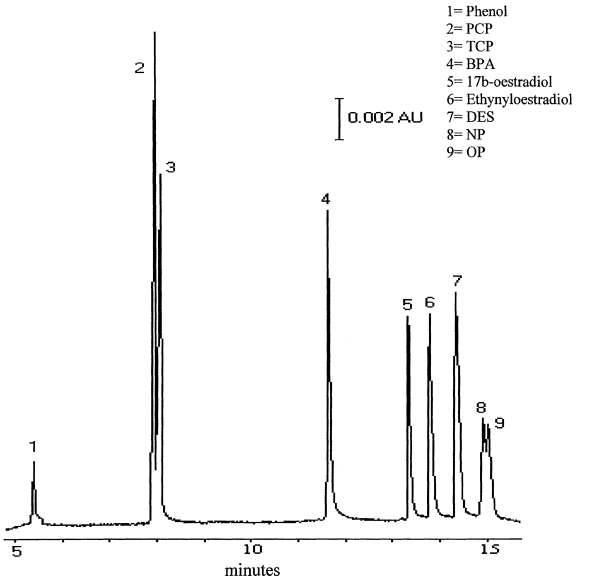

Fig. 4. Separation of several priority EDC's using CD–MEKC. Run conditions: 12.5 m*M* boric acid buffer pH 9.0, 25 m*M* SDS, 1 m*M* HP-β-CD (MS 0.8), 8% ACN. Analytes dissolved in 18% ACN, 82% run buffer.

influence on the partitioning of the analytes between the CD and the SDS. While the increase in the % ACN concentration aided the resolution of the more hydrophobic compounds it also led to the co-migration of the more polar phenols and peak splitting was observed for BPA.

At the highest concentration of organic modifier (15%) baseline resolution of OP and NP was achieved at the expense of the polar phenols and oestrogens. As illustrated in Fig. 5, the oestrogens showed peak tailing. Higher concentrations of organic modifier also led to increases in the migration of each analyte. The % organic concentration was optimised for the separation at 8%.

3.2.2. Variation of surfactant concentration

The surfactant used was the anionic sodium dodecyl sulphate (SDS). As expected an increase in the concentration of the SDS was found to result in a corresponding increase in the migration of the analytes. Lower concentrations of SDS resulted in a better response for phenol, the most polar analyte, while peak splitting was observed for BPA. As the concentration of SDS was increased co-migration of several compounds was observed. The SDS concentration was optimised at 25 m*M*.

3.2.3. Variation of buffer concentration

Slight increases in the buffer concentration did not affect the resolution dramatically. When the buffer concentration was increased to 100 m*M* a dramatic improvement in the resolution of OP and NP was observed. As illustrated in Fig. 6 this was at the expense of increased migration times which were also accompanied by increased current generation. Buffer concentration was optimised at 100 m*M*.

3.2.4. EOF suppression

The possibility of reducing analysis time for OP and NP was investigated to develop a method for

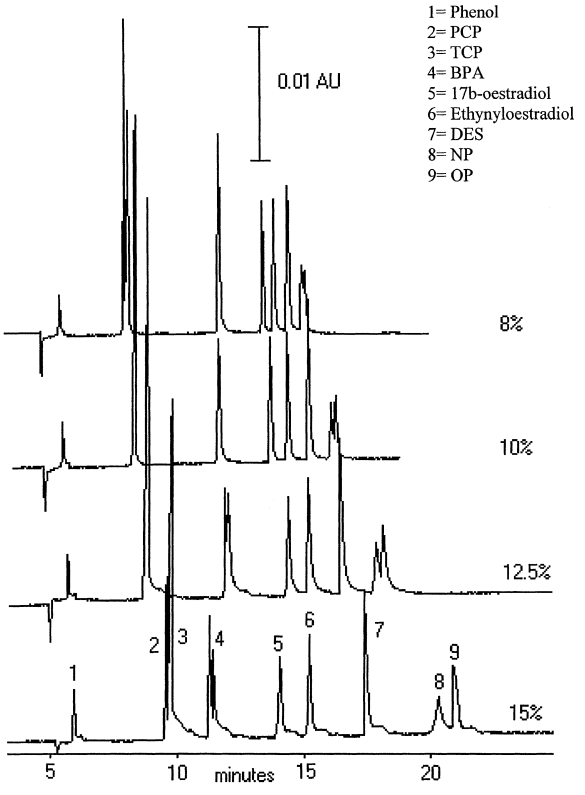

Fig. 5. Variation of % organic in buffer. Run conditions: 12.5 m*M* boric acid buffer pH 9.0, 25 m*M* SDS, l m*M* HP-β-CD (MS 0.8), various % ACN. Analytes dissolved in 18% ACN, 82% run buffer.

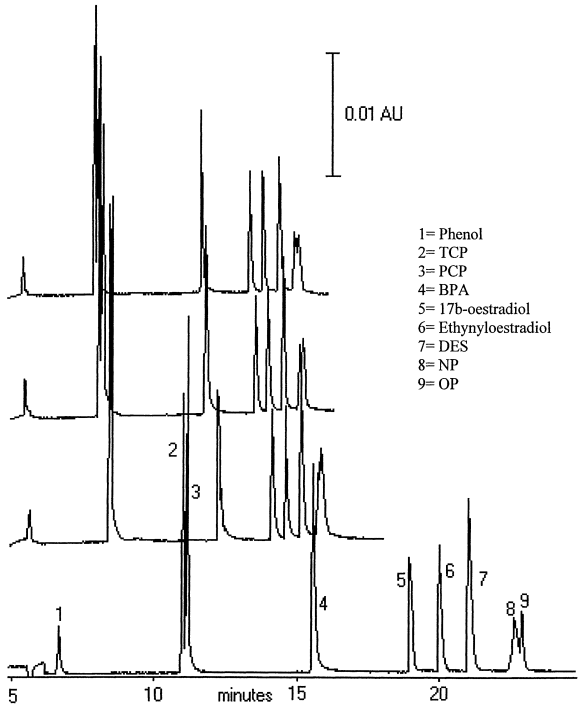

Fig. 6. Variation of buffer concentration. Run conditions: boric acid buffer, various concentrations, pH 9.0, 25 m*M* SDS, 1 m*M* HP-β-CD (MS 0.8), 8% ACN. Analytes injected in 18% ACN, 82% run buffer.

rapid screening of environmental samples. EOF suppression allowed a dramatic decrease in the migration times of the analysis while maintaining partial resolution of OP and NP (Fig. 7). As outlined in the introduction the more hydrophobic compounds that are included in the micelles migrate last using conventional CD–MEKC. Analysis times are further increased by the need for conditioning pre-rinses prior to analyte separation. It is therefore necessary to minimise analysis times as much as possible. To this end, pH suppression has been successfully employed to allow a faster separation of two of the priority EDCs, OP and NP. The separation was found to be very reproducible and was repeated several times (<2% RSD, $n=10$). This result was achieved through the use of a low pH buffer and the reversal of electrode polarity. This preliminary work shows the potential of the EOF-suppressed technique for OP and NP and some additional analytes.

3.3. GC–MS separation of EDCs

3.3.1. Separation of target compounds

A GC–MS separation mechanism was investigated for the determination of a diverse group of endocrine disruptor compounds. Initially this work focussed on a group of 11 priority environmental oestrogens. The analytes measured ranged from the phenols to the natural and synthetic oestrogens. In this instance GC–MS was monitored as a validation technique for the MEKC method developed by Fogarty et al. [12] for the determination of the same compounds. This separation was then developed further to incorporate a more diverse group of compounds which included the phenols and oestrogens but also pesticides and the alkylphenols. Fig. 8 illustrates the separation of 26 target EDCs using GC–MS in the scan mode.

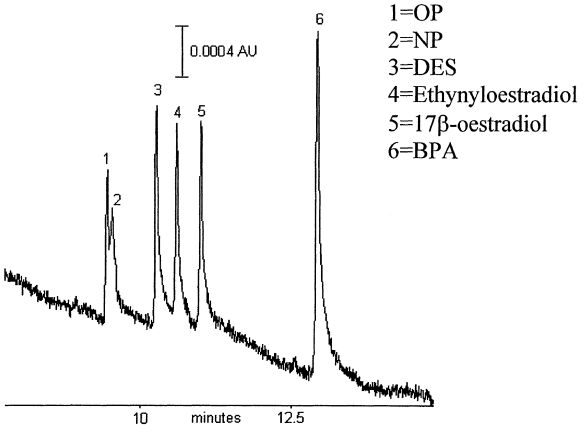

Fig. 7. Separation of OP, NP, DES, ethynyl oestradiol, 17-β-oestradiol and BPA using pH suppressed EOF at different voltages. Run buffer: 100 m*M* phosphate pH 1.8, 25 m*M* SDS, 12.5% ACN, 1 m*M* HP-β-CD (MS 0.8). Run conditions: +ve polarity, capillary 57 cm, 50 μm I.D. 25 °C, Applied voltage 20 kV. UV detection at 214 nm. Analytes dissolved in 10% ACN, 90% run buffer. Analyte concentration 50 mg/l.

3.3.2. Preconcentration by solid-phase extraction (SPE)

Due to the low concentrations at which the EDCs exist in the environment and the sensitivity limitations of the separation technique, it is necessary to perform a sample preconcentration step prior to analysis. This was carried out off-line using C_{18} SPE cartridges. Conditioning and elution solvents were chosen based on the analytes of interest and various modifications were made in order to achieve optimum recoveries of the target analytes. Subsequent

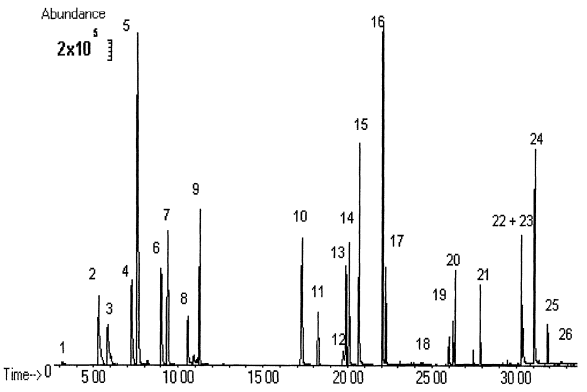

Fig. 8. Separation of 26 EDCs using GC–MS in scan mode. Temperature ramp conditions—80 °C/min; ramp—8 °C to 150; 150 for 8 min; ramp—11 to 280; 280 for 5 min. Run time—34.57. Sample composition—% ACN. (1) Phenol; (2) ethylphenol; (3) dichlorophenol; (4) 4-propylphenol; (5) 2-sec Butylphenol; (6) 2,4,5 trichlorophenol; (7) biphenyl; (8) methylparaben; (9) 2,3 *tert.*butyl-4-hydroxyanisol; (10) 4-hexloxyphenol; (11) hexachiorobenzene; (12) pentaclorophenol; (13) lindane; (14) octylphenol; (15) 4-heptyloxylphenol; (16) octylphenol 2 polyethoxylate; (17) nonylphenol; (18) nonylphenol 2 polyethoxylate; (19) bisphenol A; (20) delidrin; (21) DES; (22) estrone; (23) 17-β-oestradiol; (24) ethynyl oestradiol; (25) NP 12 polyethoxylate; (26) oestriol. Time schedule on the *x*-axis in min.

analysis was carried out on the eluate using GC–MS. SPE has seen widespread use as a preconcentration technique [20] due to its lower solvent consumption and possibility of automated operation. This technique also has the ability to extract polar and ionic species and to handle large sample volumes. It may also be a very fast technique providing the sample volume is small. SPE also avoids the formation of emulsions which often occurs in the solvent extraction of many sewage and wastewater samples [21,22].

For the purpose of this study two cartridge types were investigated for initial tests using DCP as the test analyte. These cartridges were octadecylsilane (C_{18}) and the polystyrene copolymer resin ENV as used in the study by Bolz et al. [23]. From the preliminary investigation ENV was found to be unsuitable for DCP and it was not investigated further in this study. C_{18} showed some promise and therefore it was chosen for further investigation. The conditioning and eluting solvents were investigated. It was found that a conditioning step of ACN followed by water and an elution step of ACN provided 100% recovery of DCP from a standard ultrapure water sample.

The DCP preconcentration method was employed with a range of real samples to investigate the effect of sample matrices. The results in the form of % recoveries are shown in Table 2. A total of five different sample matrices were investigated. The samples were spiked with 10 ppb DCP and eluted to give a final concentration factor of 100. The eluate was measured using the GC–MS method developed as shown in Fig. 8. Selective ion monitoring (sim) mode was used for accurate detection of the compound. The percentage recovery was determined by comparing the preconcentrated solution with that of a stock, were the area of the stock represents 100% of analyte concentration. It was found that samples of sewage effluent, influent and river water showed elevated recoveries. This may be due to the presence of the target analyte in the sample already or another interfering ion. No ion of a molecular mass of 162 was identified at the same retention time as DCP.

3.3.3. SPE for alkylphenol compounds

As alkylphenols in the environment are a main concern of this research, a further optimisation of the preconcentration method was required for the alkylphenols which included octylphenol and nonylphenol. Using the DCP method described above did not achieve adequate recovery of the more hydrophobic alkylphenols. Modification of the SPE method achieved recoveries of 96 and 97%, respectively, for OP and NP as shown in Table 1. It was observed from this investigation that modification of the conditioning and elution solvents can effect improved analyte recoveries and the SPE procedure requires careful modification for particular analytes. To demonstrate the effectiveness of the SPE and GC–MS procedure influent and effluent samples from a local wastewater treatment plant (WWTP) were analysed.

Table 3 shows a range of other endocrine disrupters can be determined using this SPE approach. The average % recoveries ($n=3$) from the analysis of

Table 2
Investigation of five real sample matrices using SPE

Sample	Preconcentration step	Enrichment	% Recovery
Distilled water	Standard method	1/100	102
Tap water	Standard method	1/100	112
River water	Standard method —Adjustment to pH 2 —Filtered	1/100	137
Effluent	Standard method —Adjustment to pH 2 —Filtered	1/100	115
Influent	Standard method —Adjustment to pH 2 —Filtered	1/100	186

% Recoveries from samples spiked with 10 ppm DCP. Concentration factor=100.

Table 3
The range of EDCs determined in standard solutions and a spiked influent sample using SPE followed by GC–MS

Endocrine disrupting compound	% Recovery analytes in distilled water (n=3)	% Recovery analytes in spiked influent sample (n=3)
Phenol	95.2	18.6
Ethylphenol	97.3	97.0
Dichlorophenol	107.3	95.8
Propylphenol	99.6	97.5
Butylphenol	95.5	97.5
Trichlorophenol	131.6	109.3
Hexyloxylphenol	91.6	109.8
Lindane	90.3	101.1
Octylphenol	80.0	92.5
Heptyloxylphenol	87.0	113.5
Nonylphenol	86.3	99.8
Dieldrin	94.3	79.5

Average % recoveries are shown (n=3).

spiked influent samples and that of the standard solutions (in distilled water) of each analyte are shown. The elevated recoveries for influent suggest that many of the target analytes are present in the WWTP effluent. A rigorous sample analysis regime is on-going in addition to modification of the SPE method for determining a wider range of EDCs. The SPE samples can also be applied to the CD–MEKC

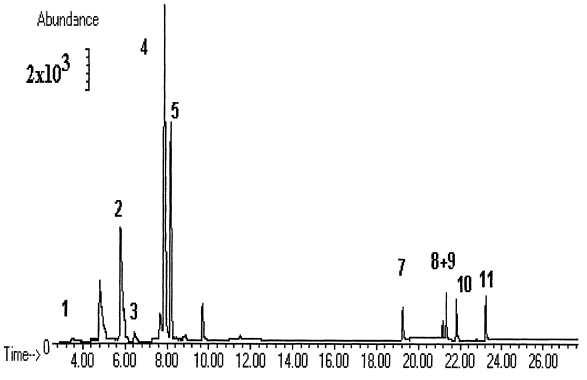

Fig. 9. GC chromatogram of unspiked influent sample. Compounds detected in selective ion monitoring (sim) mode. 1 in 50 preconcentration on C_{18} SepPak cartridge. Preconcentration method no. 5 in Table 1. Compound identification—(1) phenol; (2) ethylphenol; (3) dichlorophenol; (4) propylphenol; (5) butylphenol; (6) trichlorophenol; (7) hexyloxylphenol; (8) lindane; (9) octylphenol; (10) heptyloxyphenol and (11) nonylphenol. Time schedule on the x-axis in min.

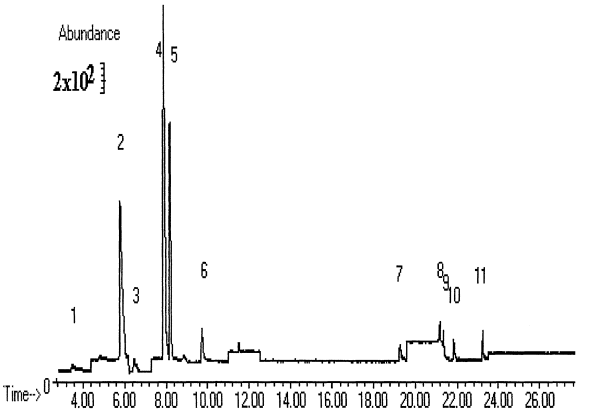

Fig. 10. Gas chromatogram of unspiked effluent sample. Compounds detected in sim mode. 1 in 50 preconcentration on C_{18} SepPak cartridge. Preconcentration method no. 5 in Table 1. Compound identification—(1) phenol; (2) ethylphenol; (3) dichlorophenol; (4) propylphenol; (5) butylphenol; (6) trichlorophenol; (7) hexyloxylphenol; (8) lindane; (9) octylphenol; (10) heptyloxyphenol and (11) nonylphenol. Time schedule on the x-axis in min.

analysis method. Fig. 9 shows a preconcentrated ($\times$50) influent sample and the compounds that were detected to be present under the existing SPE and GC–MS conditions. The effluent sample analysis using the same procedure is shown in Fig. 10. This shows that while the concentrations of each analyte are reduced in the effluent sample, they are still present in the effluent after treatment.

4. Conclusions

4.1. Comparison of CD–MEKC and GC–MS for determination of EDCs

From the initial investigations reported here it was found that both techniques have merits for the analysis of environmental samples for EDCs. The advantages of the CD–MEKC method that have become evident include: that it may be used as a rapid screening test for target species (alkylphenol compounds) if used in the EOF suppressed mode; and it also has shown potential for the separation of a variety of compounds in particular the two main target groupings, i.e. phenols and oestrogens.

The GC–MS method has shown potential in separating a wide range of target compounds and is

suitable as a validation technique for CE. The SPE method developed has demonstrated the possibility of determining these target analytes at concentrations present in the environment.

4.2. Advantages over existing techniques

Existing techniques for determination of EDCs in the environment rely on the use of bioassay for identification of a positive oestrogenic response [1,2] followed by analysis for qualitative determination of analytes. The existing analytical techniques do not provide the range of analytes shown here and the subsequent application to real samples and quantitative determinations. The GC–MS method described here has shown potential for quantitative determination of typical endocrine disrupting compounds that are present in the environment.

By employing a suppressed EOF–CE the alkylphenols can elute more rapidly and this shows potential in screening for these compounds and could prove useful for routine monitoring of WWT plants. The potential for bio-solid and effluent sample analysis has been demonstrated. Further work will be focussed in this area.

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