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# Novel modes of capillary electrophoresis for the determination of endocrine disrupting chemicals

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**Abstract**

The synthesis and usage of a wide range of organic chemicals has increased dramatically over the last five decades. These compounds sometimes termed endocrine disrupting chemicals include agricultural pesticides, industrial solvents, dyes, plasticisers, detergents and heat exchangers. Concerns have been raised about the potential adverse effects of these compounds on humans and wildlife species. Our objectives are to develop a method to identify, using novel capillary electrophoretic techniques, the endocrine disrupting compounds that are reported to be present in environmental samples. The CE modes, capillary zone electrophoresis, micellar electrokinetic chromatography (MEKC), cyclodextrin-modified MEKC (CD-MEKC) and electroosmotic flow-suppressed CD-MEKC were investigated for the determination of a range of endocrine disrupting chemical compounds. This paper shows some initial results obtained.


*Keywords:* Water analysis; Endocrine disruption; Steroids; Phenols

## 1. Introduction

Several chemicals have been suspected of having endocrine disrupting effects [1]. For the assessment of human exposure to these chemicals, the development of analytical methods for the identification of these compounds is necessary. At present gas chromatography–mass spectrometry (GC–MS) is the main analytical method [2–4]. However, non-volatile or thermally degradable chemicals cannot be analysed directly by GC–MS. Capillary electrophoresis (CE) has high separation efficiency and can be easily applied for the analysis of non-volatile or thermally degradable chemicals [5,6]. This work shows the potential of different modes of CE in separating a wide range of compounds termed endocrine disrupters. While detection sensitivity is not adequate for environmental samples, it will be possible to utilise these separations in combination with MS as the detection mechanism to increase sensitivity. In addition, the sample matrices being investigated are complex, therefore solid phase extraction methods already demonstrated by the authors, can be employed for clean-up and preconcentration to further improve sensitivity and decrease background interferences [7]. Separations of both charged and neutral compounds can be realised through the utilisation of capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC). The selectivity and resolution of these separations are directly controlled by using various different buffer additives [8–10]. CZE and MEKC have gained popularity in

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the analysis of environmental samples and in the identification of pesticides, inorganic and organic pollutants in environmental matrices [11–13]. However, the varied nature of the endocrine disrupting chemicals (EDCs) have challenged the analyst and until recently it has been difficult to analyse a wide range of chemicals, likely to be present in an environmental sample, in a single run.

While CE has well recognised limitations in environmental analysis (sensitivity, sample matrix, sample ionic strength), it has distinct advantages for dealing with the EDC compounds, notably ease of operation of CE modes i.e. CZE, MEKC and cyclodextrin-modified (CD) MEKC for the separation of neutral compounds in the presence of more polar species. The development of a single method for determination of a wide range of EDC groups will help to alleviate the labour intensive steps associated with HPLC fractionation analysis commonly used for the determination of EDCs in sewage sludge and sewage treatment waste effluent [14].

The objectives of the work are (i) to demonstrate the simultaneous screening of oestrogens and phenols in environmental samples and (ii) to demonstrate the potential of CE in separating a wide range of priority endocrine disrupting chemicals. This paper demonstrates that these objectives can be achieved by the proposed procedures. The procedures shown here, mean real steps forward in the direction of developing a method, which can be applied to relevant environmental samples.

## 2. Experimental

### 2.1. Instrumentation

Separations were performed using a Beckman P/ACE 5500 system (Beckman Coulter, Fullerton, CA, USA), equipped with a photo-diode array detection (DAD) system. The DAD detector range was 190–300 nm. The CE instrument was operated using Windows P/ACE Station Software version 1.21. Integration data was calculated by P/ACE Station, using the USP (United States Pharmacopoeia) method. The fused silica capillaries (Beckman) used were 57 cm long with an internal diameter of 50 μm.

### 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). Cyclohexylamino-1-propane sulfonic acid (CAPS), disodium tetraborate, sodium acetate and sodium phosphate were purchased from Sigma–Aldrich. HCl, NaOH, methanol (HPLC-grade), acetonitrile (ACN, HPLC grade) and sodium dodecyl sulphate (SDS) were purchased from Sigma–Aldrich. CAPS buffer pH was adjusted using 0.1 *M* HCl and 0.1 *M* NaOH. α- and β-cyclodextrins (Sigma–Aldrich) were used without further purification. Structures of some of the compounds being tested are shown in Fig. 1.

### 2.3. CE separation conditions

Stock solutions for CE determinations of the EDCs were prepared in 100% ACN with the exception of oestriol and oestrone (prepared in 100% methanol). Stock solutions were stored at 4 °C in the fridge to minimise evaporative loss and were covered in foil to minimise photo degradation of the analytes. Aliquots of the stock solutions were taken and diluted with buffer prior to separation.

#### 2.3.1. CZE

Separations were carried out at 30 kV constant voltage and performed in triplicate. Samples were injected hydrodynamically (5 s at high pressure). Analytes were detected using the DAD scanning from 190 to 300 nm.

#### 2.3.2. MEKC and CD-MEKC

The separation buffer contained CAPS and SDS. Acetonitrile and methanol were added in quantities as shown in the figure legends. All buffers were prepared with deionised water and filtered through a 0.2-μm filter (Gelman) prior to use. Buffers were prepared fresh prior to use due to precipitation of SDS during storage. For all the separation modes, a standard capillary conditioning method was used. All buffers were filtered through a 0.2-mm filter prior to use. A daily start-up procedure involved a 3-min

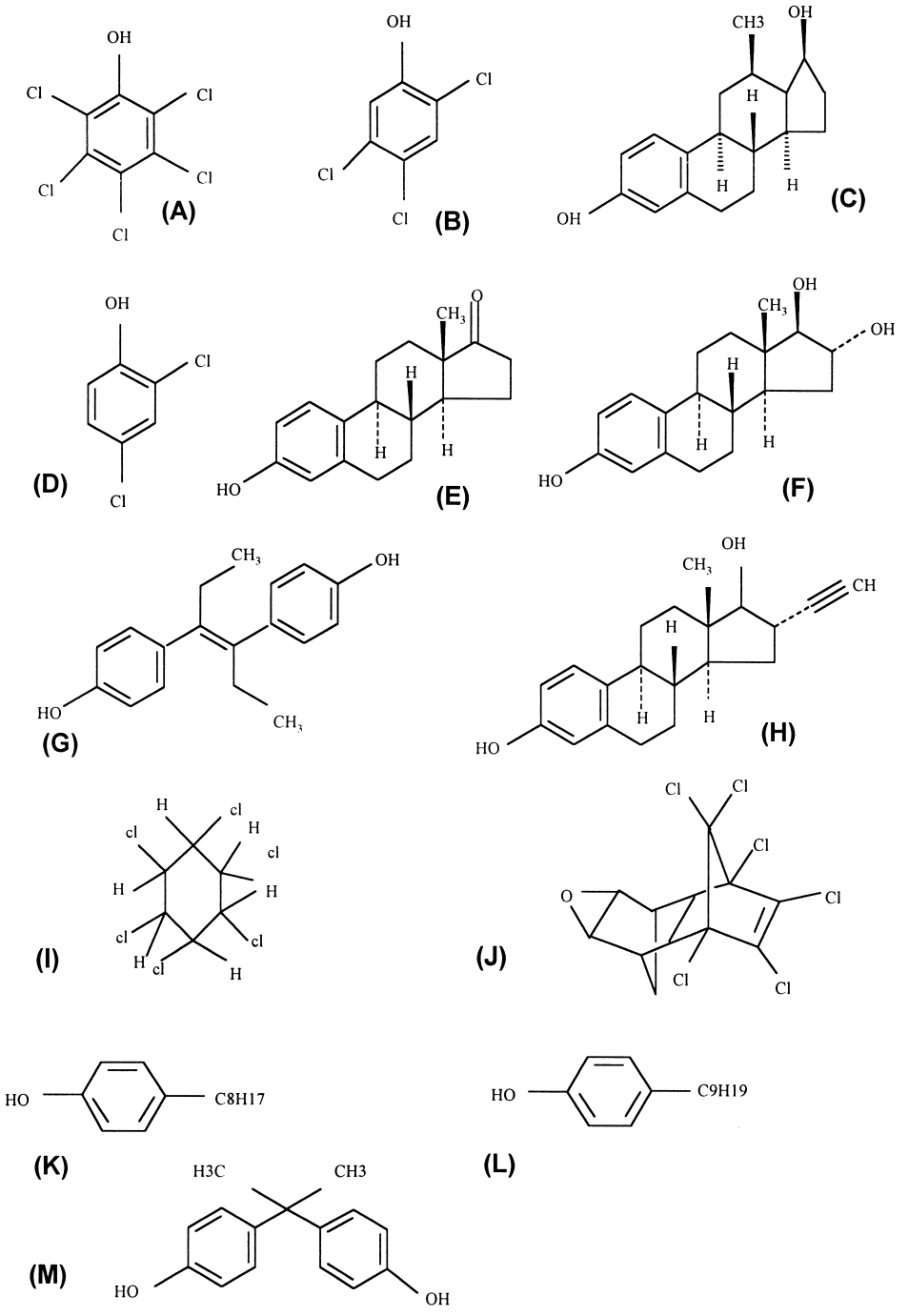


Fig. 1. Structures of some typical EDCs used in this study. (a) pentachlorophenol (PCP); (b) trichlorophenol (TCP); (c) 17β-oestradiol; (d) dichlorophenol (DCP); (e) oestrone; (f) oestriol; (g) diethylstilbestrol (DES); (h) ethynyloestradiol (EO); (i) lindane; (j) dieldrin; (k) octylphenol (OP); (l) nonylphenol (NP); (m) bisphenol-A (BPA).

rinse with 0.1 M NaOH or HCl as appropriate, followed by a 10-min rinse with buffer solution. Prior to each separation a routine rinse of 3 min each of 0.1 *M* NaOH, deionised water and buffer. Separations were performed at 20 kV constant voltage unless otherwise stated. Samples were injected hydrodynamically for 5 s at high pressure. Separations were performed in triplicate. Analytes were detected using a diode array detector scanned from 190 to 300 nm.

### *2.4. Sample preparation*

A river water sample was spiked with six test analytes (NP, OP, NPEO, DES, ES and BPA) for analysis using CD-MEKC in the electroosmotic flow (EOF)-suppressed mode. In order to minimise differences between the sample zone and surrounding buffer zone, some of the key buffer components were added directly to the river water sample prior to analysis, which included cyclodextrin, surfactant and buffer. Due to the limited solubility of analytes in aqueous samples at high concentrations, 10 ml of acetonitrile was added to aid analyte solubility. The analyte concentration was equivalent to 20 mg/l. An un-spiked sample was analysed in order to identify possible sample matrix effects.

## 3. Results

The CE modes of CZE, MEKC and CD-MEKC in the EOF-suppressed mode have been investigated. The aim of this study includes the generation of a separation mechanism that will allow rapid determination of several species present in an environmental sample. The proposed method will offer advantages over current laborious fractionation methods in addition to preconcentration, to isolate particular groups of compounds. Due to the nature CE, this analytical technique has the potential of separating a variety of different compounds in a single run making CE suitable for the analysis of complex environmental matrices. Separation parameters such as buffer pH, buffer concentration, organic modifier concentration etc. were optimised to obtain a satisfactory separation of EDCs (in particular oestrogens, phenols and alkylphenols) using CE, MEKC and CD-MEKC.

### *3.1. Separation of EDCs using CZE*

The technique of CZE involves the use of a simple electrolyte buffer system for separation of charged compounds. To demonstrate the potential of CZE the oestrogen and phenol groups of EDCs were studied.

#### *3.1.1. Selection of a buffer pH*

A variety of buffers were studied in the range pH 4–11.5. From the study of buffer pH, the resulting electropherograms pointed to a reduction in the magnitude of the EOF when using a buffer above pH 10. The results showed that longer migration times resulted at high pH. The electrolyte chosen was a standard sodium borate buffer adjusted to a pH of 9.5. The efficiency and peak shape of the oestrogen was not optimum at this pH, i.e. a broad peak resulted. Oestrogens are difficult to separate by CZE at this pH, as they are neutral below pH 11.5. The optimum pH therefore for CZE separation is below pH 10.

#### *3.1.2. Buffer concentration and applied voltage*

A decrease in the magnitude of EOF with increasing buffer concentration has been observed previously by Van Orman et al. [15], and was correlated to a decrease in the zeta potential of colloidal silica. The effect of increasing buffer concentration (10–50 mM borate) was investigated for the separation of estriol, 17β-estradiol and estrone. Increasing concentrations of run buffer subsequently increased the viscosity. This led to increased analyte migration times due to a decrease in the magnitude of the EOF.

#### *3.1.3. Organic modification of run buffer*

The hydrophobic nature of the analytes under investigation means that they are not readily amenable to analysis using an aqueous system such as CZE. To overcome their reduced solubility in the buffer system, the organic modifier methanol was added [16]. From the investigations it was found that the addition of the organic modifier aided compound solubility, but due to the poor peak shape of the oestrogen, an alternative CZE method was investigated for their analysis. The CZE separation reported by Potter et al. [12] was applied to the analysis of three natural oestrogens and buffer pH, buffer concentration and organic concentration were further

optimised. A high-pH buffer (100 mM CAPS, pH 11.5) was used.

### 3.1.4. Optimisation of separation of oestrogens

The pH of the run buffer has a significant impact upon the separation of the oestrogens. Deprotonation of the oestrogen molecules, which occurred above pH 11.5, allowed baseline resolution of these otherwise uncharged compounds (Fig. 2). At values below the p$K_a$, the analytes exhibit reduced efficiencies and peak shape. The p$K_a$ values for oestrone and 17β-oestradiol have been reported as 10.77 and 10.71, respectively [17]. Below pH 11.5, the separation time reduced from 7 min at pH 11.5 to 2 min at pH 10. At pH 10, all of the analytes are neutral and co-migrate, with the solvent front identified by a trough in the baseline. The hydrophobic nature of these analytes, which are practically insoluble in water [18], probably led to their precipitation in the capillary or adsorption to the capillary wall.

The increase in buffer concentration from 10 to 100 mM CAPS zwitterionic buffer, resulted in an increase in the overall analysis time, but improved the resolution of the analytes. The buffer concentration was optimised at 100 mM. The electrophoretic buffer was modified with methanol, which allows increased solvation of analytes as well as improved resolution through the reduction of the EOF. The addition of methanol was optimised at 20%.

### 3.1.5. Separation of phenols

Following optimisation of the oestrogen separation, an investigation into the separation of several priority phenols was carried out. Conditions for the separation of both groups of compounds were then compared in order to determine if simultaneous analysis of both compound groups was possible. A change in the order of migration of the phenols was observed at different pH values. This was attributed to a change in the extent of ionisation of analytes due to differing p$K_a$ values (i.e. p$K_a$ of phenol 9.89) [19]. A split peak was observed for phenol red. The p$K_a$ of this compound is 7.9 [18] so the stability of the compound may have been altered at an extremely alkaline pH. As the ultimate aim was to separate the oestrogens and phenols together, the zwitterionic buffer CAPS, pH 11.5 (used for separation of oestrogens) was chosen for further investigation.

Variation of the buffer concentration did not appear to change the order of analyte migration. Increasing buffer concentrations did lead to co-migration of some phenols, which is attributed to the reduction in EOF. This was not observed with

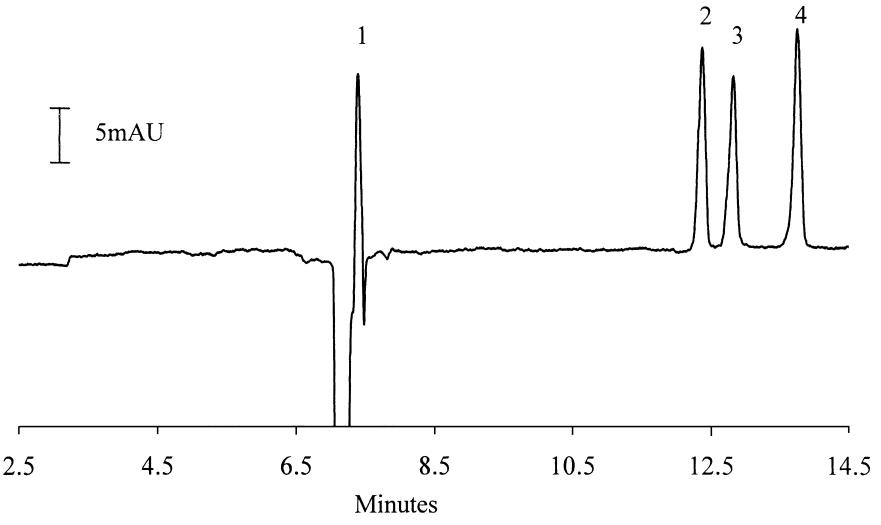


Fig. 2. Separation of three natural oestrogens. Separation conditions: 100 mM CAPS buffer pH 11.5 and 20% MeOH. Applied voltage 30 kV; detection UV 210 nm. Analytes dissolved in 100% MeOH. Peak identification 1=MeOH, 2=0.5 mM oestriol, 3=1 mM 17β-oestradiol, 4=0.5 mM oestrone.

previous separations, as increasing concentrations of buffer in the oestrogen optimisation led to improved resolution of analytes. The use of a zwitterionic buffer also facilitated the use of higher electrolyte concentrations without excessive current generation. A 15 mM CAPS was chosen as the optimum buffer concentration as it gave the best compromise between peak shape and separation time.

The concentration of organic modifier (MeOH) was varied in order to observe the effect on the peak shape and separation of the phenolic target analytes. Increasing MeOH concentration also influenced the selectivity of the separation. This was observed by a change in the migration order of the phenols, which was not observed previously with oestrogens. A compromise between peak shape and peak migration times was reached at 15% MeOH, which provided the most satisfactory separation conditions.

### *3.1.6. Calibration curves for phenol mixture*

Calibration curves for each analyte were prepared in the range zero to 1 mM. A linear correlation ($r^2$=0.99) was observed for all analytes between peak area and decreasing analyte concentration.

### *3.1.7. Separation of oestrogens and phenols using cze*

The method used for the analysis of the oestrogens was applied to the determination of several environmentally relevant phenols. The separation time is greater than 20 min and peak splitting was observed for the compound phenol-red. Some correlation between molecular weight and migration time was observed with larger compounds migrating first. While a lower pH value would allow a faster analysis of phenols, a simultaneous separation of several oestrogens and the phenols by CZE below pH 11.5 would not be possible due to oestrogen co-migration.

## *3.2. Separation of EDCs using MEKC*

Using the optimised conditions shown for the CZE separation, further modifications of this mechanism were investigated, i.e. micellar electrokinetic chromatography. MEKC can be used to separate both charged and uncharged analytes and compounds with hydrophobic characteristics. Separation conditions were not re-optimised in this study but rather an investigation of separation potential for a range of 19 key EDC compounds is shown.

### *3.2.1. Migration time as a function of molecular mass*

The size of the solute is reported to have a significant effect on its retention in the micelle, with separation selectivity influenced by hydrogen-bond interactions [18]. Fig. 3 graphs the peak migration time and molecular weight of each compound. While the relationship is not strictly linear, a trend of similar migration times for compounds with similar structures was observed. This implies that the larger compounds (oestrogens) interact with the micelles to a greater extent than the smaller phenols attributed to differences in their hydrophobicity and charge.

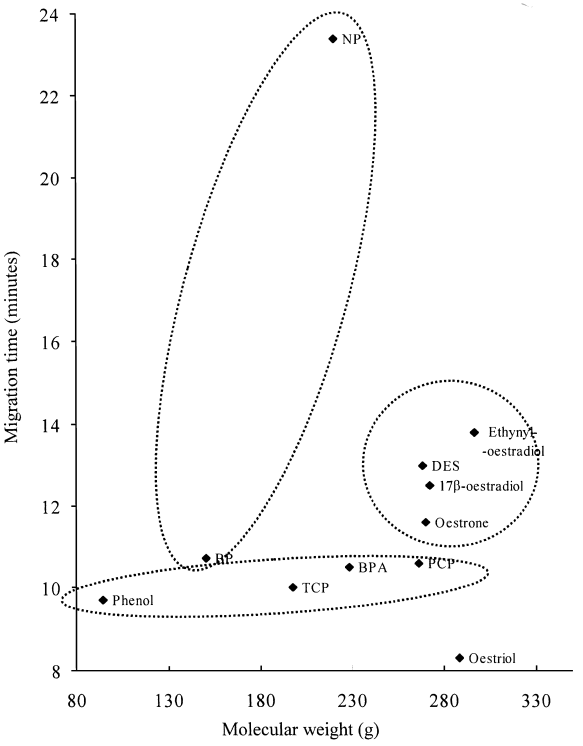


Fig. 3. Peak migration time as a function of molecular weight. Separation conditions: 20 mM CAPS pH 11.5 with 15% ACN and 25 mM SDS; voltage 20 kV; detection at 200 nm; Injection sample 12.5 mM SDS and 1:1 ACN/$H_2O$. Peak identification: 1=oestriol, 2=phenol, 3=TCP, 4=BPA, 5=PCP, 6=butylphenol, 7=oestrone, 8=17β-oestradiol, 9=DES, 10=ethynyloestradiol, 11=nonylphenol.

*3.2.2. Separation of target EDCs*

In order to solve a real environmental problem it is necessary to develop a method that can determine a range of relevant EDCs. Fig. 4 shows the MEKC separation of 19 compounds that are reported to occur in environmental samples at low concentrations [20,21]. While this separation shows potential, a limitation is the reproducibility of the method and the apparent variation in migration time. When separations are carried out for compounds individually, good reproducibility can be achieved (<3.5% RSD, $n=3$), and when a larger mixture (Fig. 4) is presented, reproducibility is also good (average RSD<2%, $n=3$). Table 1 shows the migration time results for analytes determined as part of a mixture of 19 compounds. Compounds numbered 16 and 17 are not baseline resolved and therefore are difficult to quantitate however, most other species can be measured. When comparing the migration times for a selection of these analytes measured individually (Table 2), it can be seen that there is a difference in migration times for the same compounds in a mixture. For example the analyte number 1, ethylphenol, elutes at 5.6 min when in solution alone but in a mixture its migration time increases to 8.3 min. The variation in injection composition due to additional analytes can cause a change in sample viscosity and analyte solubility, therefore changing the interaction of analytes with the micelle.

*3.3. CD-MEKC separation of EDCs*

From previous work carried out by the authors it was found necessary to add a cyclodextrin (hydroxypropyl (HP)-β-CD) to the separation buffer in MEKC to aid the separation of EDCs [7].

*3.3.1. Cyclodextrin type*

Cyclodextrins are cyclic oligosaccharides produced from the enzymatic degradation of the amylose fraction of starch, and comprise six (α), seven (β) or eight (γ) glucopyranose units whose arrangement forms a cavity. The α-CDs have the smallest cavity. By using a cyclodextrin to aid the separation of the larger number of compounds, it was found that migration times varied depending on the cyclodextrin

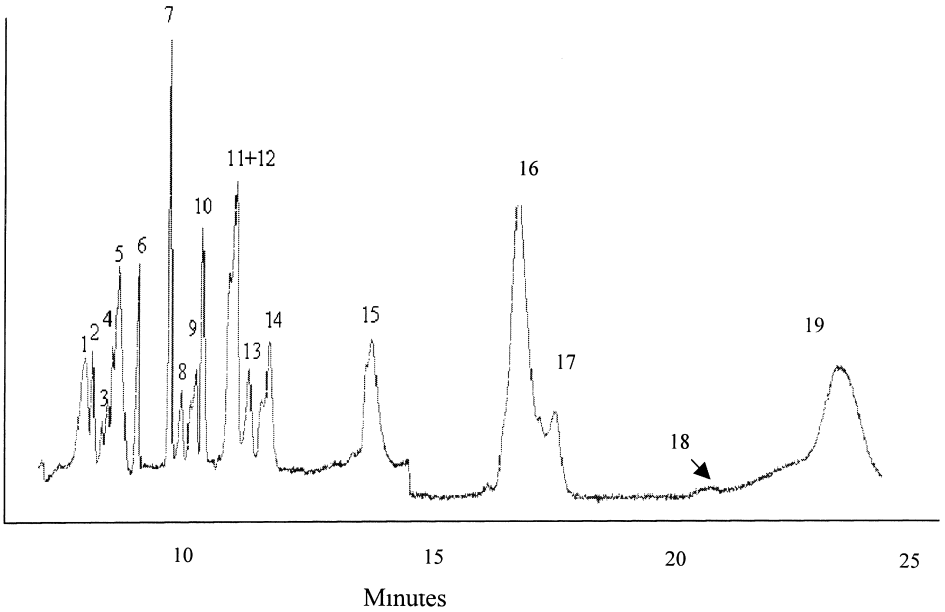


Fig. 4. Separation of 19 target EDCs using MEKC. Separation conditions: 20 mM CAPS pH 11.5 with 15% ACN and 25 mM SDS; voltage 20 kV; detection at 200 nm; Injection sample MeOH–buffer (50:50, v/v). Peak identification: 1=ethylphenol, 2=oestriol, 3=methylparaben, 4=phenol, 5=propylphenol, 6=lindane, 7=TCP, 8=bisphenol-A, 9=pentachlorophenol, 10=butylphenol, 11=oestrone, 12=17β-oestradiol, 13=DES, 14=hexylphenol, 15=dieldrin, 16=ethynyloestradiol, 17=NP12, 18=NP2EO 19=nonylphenol.

Table 1
Reproducibility data for MEKC separation of 19 target compounds in a mixture as shown in Fig. 4

| Compound no. (Fig. 4) | Compound name | Migration time average (min; $n=3$) | $\sigma$ | RSD (%, $n=3$) |
|---|---|---|---|---|
| 1 | Ethylphenol | 8.30 | 0.14 | 1.70 |
| 2 | Oestriol | 8.55 | 0.07 | 0.83 |
| 3 | Methylparaben | 8.65 | 0.07 | 0.82 |
| 4 | Phenol | 8.75 | 0.07 | 0.81 |
| 5 | Propylphenol | 8.85 | 0.07 | 0.80 |
| 6 | Lindane | 9.25 | 0.07 | 0.76 |
| 7 | TCP | 9.90 | 0.00 | 0.00 |
| 8 | Bisphenol A | 10.05 | 0.07 | 0.70 |
| 9 | PCP | 10.30 | 0.00 | 0.00 |
| 10 | Butylphenol | 10.45 | 0.07 | 0.68 |
| 11 | Oestrone | 10.75 | 0.35 | 3.29 |
| 12 | β-Oestradiol | 11.20 | 0.14 | 1.26 |
| 13 | Diethylstilbestrol | 11.50 | 0.14 | 1.23 |
| 14 | Hexylphenol | 11.80 | 0.00 | 0.00 |
| 15 | Dieldrin | 13.58 | 0.31 | 2.29 |
| 16 | Ethynyloestradiol | 16.53 | 0.10 | 0.60 |
| 17 | NP12EO | 17.24 | 0.09 | 0.53 |
| 18 | NP2EO | 21.50 | 0.09 | 0.53 |
| 19 | NP | 22.75 | 0.21 | 0.93 |

Conditions of separation as for Fig. 4. Compounds numbered in order of elution.

type (Fig. 5). Both α- and β-CDs were tested initially on five of the compounds (ethylphenol, methylparaben, bisphenol A, propylphenol, pentachlorophenol) to assess their potential. It was found that β-CD had the effect of almost doubling the separation time (from 8 to ~12 min) and poor reproducibility of the separations was achieved. However, the α-CD showed improved reproducibility and still resulted in complete separation of the compounds. Due to the fact that the alkylphenol group of chemicals, e.g. nonylphenol and octylphenol are reported as being present in environmental samples [20,21] and are potent EDCs, it was decided to focus on these compounds to assess the merits of CD-MEKC. The EOF suppressed mode was used, as it showed potential for rapid separation and screening of these target species.

### *3.4. Separation of EDCs using EOF-suppressed CD-MEKC*

EOF suppression was achieved through the use of a low pH buffer (phosphate, pH 1.8) preventing ionisation of both the silanol groups necessary for

Table 2
Reproducibility data for a selection of target compounds determined individually using the same conditions as for the MEKC separation shown in Fig. 4

| Compound no. (Fig. 4) | Compound name | Migration time average (min, $n=3$) | $\sigma$ | RSD (%, $n=3$) |
|---|---|---|---|---|
| 1 | Ethylphenol | 5.6 | 0.06 | 1.14 |
| 3 | Methylparaben | 6.5 | 0.01 | 0.22 |
| 5 | Propylphenol | 7.4 | 0.08 | 1.04 |
| 6 | Lindane | 9.3 | 0.33 | 3.50 |
| 15 | Dieldrin | 10.7 | 0.10 | 0.93 |
| 17 | NP12EO | 13.9 | 0.04 | 0.31 |
| 18 | NP2EO | 14.6 | 0.14 | 0.97 |

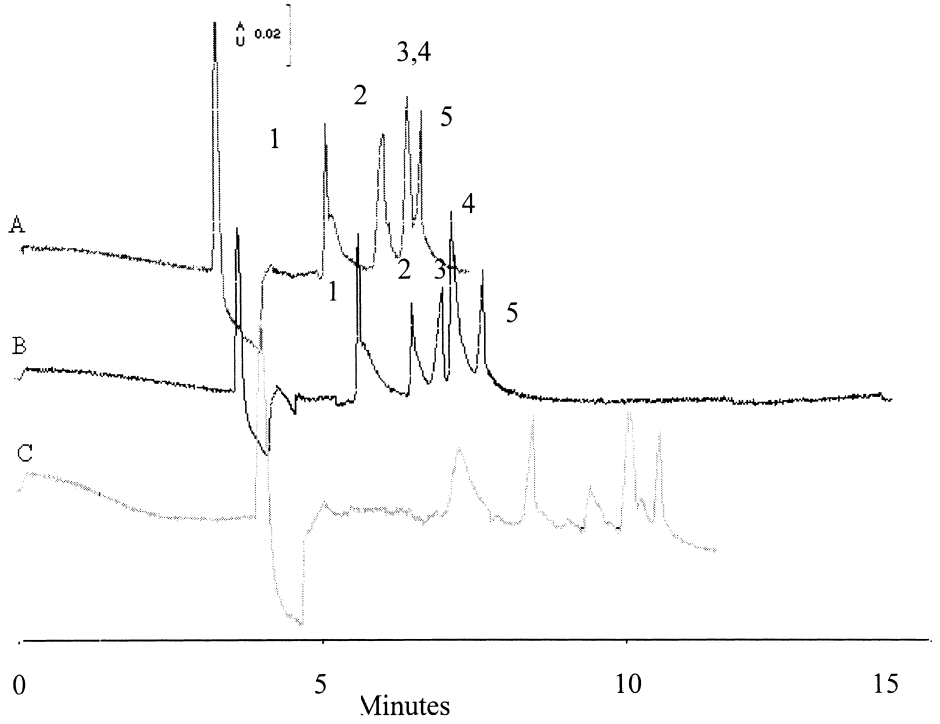


Fig. 5. Separation of five target EDCs using CD-MEKC. Separation conditions: 20 mM CAPS pH 11.5 with 15% ACN and 25 mM SDS; voltage 20 kV; detection at 200 nm; Injection sample 12.5 mM SDS and ACN–$H_2O$ (1:1, v/v). A=No CD included; B=1 mM α-CD, and C=1 mM β-CB. Compound identification: 1=ethyl phenol; 2=methyl paraben; 3=bisphenol-A; 4=propylphenol; 5=pentachlorophenol.

EOF generation and the analytes. As an anionic surfactant was investigated, conventional electrode polarity was reversed in order to ensure analyte migration to the anode located at the detection window. At low pH analyte adsorption to capillary wall should also be minimal due to protonation of both the wall and the analytes [22]. As faster migration times for hydrophobic analytes associated with anionic micelles were anticipated, 100 mM buffer was used in order to aid peak separation of OP and NP. The SDS concentration was maintained at 25 mM.

The possibility of reducing analysis time for OP and NP was investigated to develop a method for rapid screening of environmental samples. EOF suppression achieved a dramatic decrease in the migration times of OP and NP from 25 min to less than 10 min at the same voltage while maintaining partial peak separation the potential of which has been shown previously [7]. A further decrease in analysis time was observed at higher voltages without a significant impact on compound resolution or background noise. Migration order of analytes was now reversed, with the most hydrophobic migrating first due to association with the negatively charged SDS micelles.

The concentrations of the cyclodextrin of 1 and 2 mM was used to observe the effect on the separation of OP and NP in an EOF-suppressed environment. As expected, resolution of isomers the nonylphenol isomers occurred with peak splitting at a concentration of 2 mM. This has been observed previously and has been employed to allow resolution of NP isomers [22,23].

### *3.4.1. Injection sample composition*

A mismatch in the conductivities of the sample and analyte zones can promote stacking due to compression of the sample plug in the capillary, resulting in improved peak shape and efficiency [24]. The composition of the injection sample was varied in order to aid peak shape and separation. As the

presence of SDS was thought to contribute to current generation in the system, surfactant was removed from the injection plug in order to reduce the conductivity of the sample zone. A standard mixture was diluted in different solutions prior to injection for comparison. When samples were diluted in (a) 100 mM phosphate buffered to pH 1.8 with no other additives, and (b) 100 mM phosphate pH 1.8, 1 mM HP-β-CD, compound solubility was compromised and a deterioration of peak shape was observed. This illustrates the importance of the presence of the SDS and the ACN in the run buffer.

### *3.4.2. Surfactant concentration*

While a significant reduction in analysis time had been achieved through the suppression of EOF, the resolution of OP and NP was still an issue and some of the major factors that influence the selectivity in a CD-MEKC system were optimised. The negative surfactant concentration was varied due to its effect on the migration of associated uncharged analytes to the anode under the influence of electrophoresis. As expected, increasing the concentration of surfactant led to a marginally faster analysis. At lower surfactant concentrations, the analysis took slightly longer, with an improvement in peak resolution. A 25 mM SDS was used for further investigation of the method. Higher concentrations were not investigated due to concerns over further loss of resolution of OP and NP. The concentration of organic modifier (acetonitrile) in the run buffer was also varied to observe the effect on separation of the six target analytes. As the concentration was increased, an increase in the migration times of all analytes was observed, and was more pronounced for the more polar compounds. Although a slight improvement in the resolution of OP and NP was observed with 20% ACN, the overall analysis time had increased thereby offsetting the advantages gained by the use of the EOF-suppressed method. A 12.5% ACN concentration was chosen as the optimum composition for the separation.

### *3.4.3. Calibration and sample analysis*

A calibration curve was constructed for all analytes. From Table 3 it can be noted that good correlation values were achieved for each compounds using this separation method. The RSD values were in the region of $<10\%$ for all compounds tested except NP. Migration times remained reproducible for three repeated runs.

To demonstrate that the procedure shows a real step forward towards environmental application, a river water sample was analysed. The riverwater sample was spiked with six target analytes (Fig. 6). As with the MEKC analysis, the surfactant was added directly to the sample, together with the buffer, CD and 10% ACN. It was observed that the direct addition of solubilising agents aided the recovery of target compounds, which exhibit low solubility in aqueous systems, especially at the relatively high concentrations needed to ensure detection. The addition of the components of the run buffer also ensured that the sample plug was as similar to the surrounding buffer zone as possible in order to improve peak shape and resolution. The sample was then filtered and injected onto the capillary. As illustrated in Fig. 6 all six analytes

Table 3
Reproducibility data for optimised separation ($n=3$)

| Compound | Resolution | Peak area precision (RSD, %) | Peak efficiency ($N$) | Migration precision (RSD, %) | $R^2$ values for calibration curve |
|---|---|---|---|---|---|
| OP | 0 | 5.7 | 294 053 | 0 | 0.9974 |
| NP | 1 | 14.2 | 83 914 | 0 | 0.9981 |
| DES | 8.3 | 8.7 | 203 715 | 0.1 | 0.9803 |
| EO | 6 | 1.7 | 227 763 | 0.2 | 0.9978 |
| 17β-Oestradiol | 6 | 6.7 | 217 687 | 0.2 | 0.9941 |
| BPA | 19.67 | 7.3 | 184 378 | 0.3 | 0.9934 |

Separation conditions: 100 mM phosphate pH 1.8, 25 mM SDS, 12.5% ACN, 1 mM HP-β-CD (M.S. 0.8). Run conditions: +ve polarity, capillary 57 cm×50 μm I.D.; 25 °C. Applied voltage 20 kV; 214 nm. Analytes dissolved in 10% ACN 90% run buffer. Analyte concentration 20 mg/l. Analyte identification: 1=OP, 2=NP, 3=DES, 4=EO, 5=17β-oestradiol, 6=BPA.

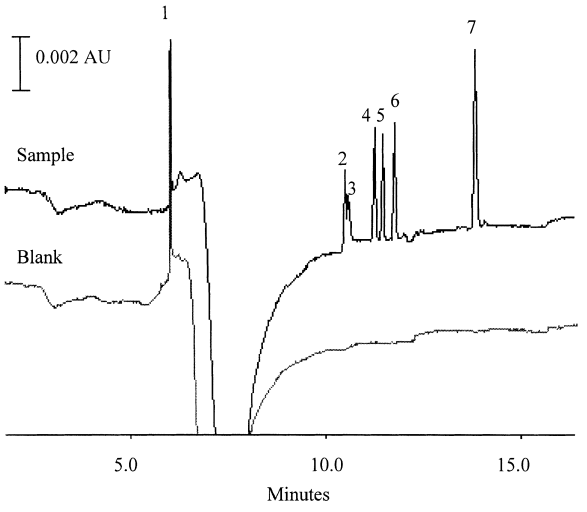


Fig. 6. Analysis of spiked river water sample. Separation conditions: 100 mM phosphate pH 1.8, 25 mM SDS, 12.5% ACN, 1 mM HP-β-CD (M.S. 0.8). +ve polarity, capillary 57 cm×50 μm I.D; 25 °C. Applied voltage +20 kV; 214 nm. Analytes dissolved in 10% ACN, 90% run buffer. Analyte concentration 20 mg/l. Peak identification: 1=sample matrix, 2=OP, 3=NP, 4=DES, 5=EO, 6=17β-oestradiol, 7=BPA.

tested were identified in the sample along with an additional peak prior to the solvent front. In order to assess if this peak was due to sample matrix effects, a blank riverwater sample was taken through the same treatment as the sample and the peak was detected once more. This peak is likely to be due to organic material present in the riverwater sample. The result compares favourably with the analysis of a standard mixture.

## 4. Conclusions

This paper demonstrates three potential modes of separation of endocrine disrupting chemicals using capillary electrophoresis. The ultimate aim of the study is the development of a separation mechanism that can (i) screen for oestrogens and phenols and (b) include a wide range of chemicals that are expected in an environmental sample. Studies are on going to develop a suitable separation method for monitoring EDC levels in the environment. This paper shows three developments using CE to achieve this:

(i) While CZE does not have the potential for separating the large compound group required it does have potential for separation of the more polar species e.g. oestrogens.

(ii) Using MEKC a separation of 19 target analytes in shown with potential windows for other analyte groups. While migration time is seen to vary as the number of analytes increases the reproducibility of the separation is such that the separation can be tailored to each individual sample and therefore migrations can be monitored.

(iii) By addition of a cyclodextrin to the separation solubility of the analytes can be improved. In addition by performing this separation in an EOF suppressed mode analysis time for alkylphenols was reduced by almost 20 min with an overall analysis time for key target species being less than 14 min.

By using the EOF suppressed method for real sample analysis following pre-concentration, sample matrix effects were not seen to impact on migration times significantly. Therefore MEKC and CD-MEKC in the EOF-suppressed mode have potential for the determination of target species in environmental waters.

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