Off-Line Concentration of Bisphenol A and Three Alkylphenols by SPE then On-Line Concentration and Rapid Separation by Reverse-Migration Micellar Electrokinetic Chromatography

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Abstract

A method for separation of four environmentally important compounds (three alkylphenols and bisphenol A) by cyclodextrin-modified reverse-migration micellar electrokinetic chromatography (CD-RM-MEKC) has been optimized. On-line concentration, stacking using reverse-migrating micelles and a water plug (SRW), was used to improve the limit of detection. Two main factors affecting the efficiency of enhancement by SRW, injection sample length and water-plug length, are discussed in detail. Compared with the normal 2-s injection, more than 121-fold enhancement of peak area and more than 71-fold enhancement of peak height were achieved with 120-s sample injection after 120-s water-plug injection. With this optimized separation method and SRW technology, limits of detection were found to be 0.089, 0.030, 0.0091, and 0.033 mg L⁻¹ for 4-nonylphenol, 4-*tert*-octylphenol, bisphenol A, and 4-*tert*-butylphenol, respectively. Calibration plots ($R^2 \geq 0.9968$) and relative standard deviations (RSD, %) of corrected peak areas and migration time were also satisfactory. Combined with off-line concentration by solid-phase extraction (SPE) this method might be used to determine these contaminants at ppb levels in groundwater samples.

Keywords

Electrokinetic chromatography
Solid phase extraction
Alkylphenols
Bisphenol A
Ground water samples

Introduction

Alkylphenols (AP) and bisphenol A (BPA) are widely used industrial chemicals—BPA is used in the production of polymers and AP are widely used to make alkylphenol ethoxylates, widely used as nonionic surfactants. During their manufacture AP and BPA can enter

the environment by leaching or metabolism. Three AP—4-*tert*-butylphenol (4-tBP), 4-nonylphenol (4-NP), and 4-*tert*-octylphenol (4-tOP)—and BPA have frequently been found in waste water, natural water, and sewage effluents [1–4]. These compounds have been closely studied in recent years because they have been shown to have estrogenic effects in

fish, avian, and mammalian cells and are suspected of having endocrine-disrupting toxicity [5, 6]. The compounds have been listed in ‘Strategic Programs on Environmental Endocrine Disruptors ’98’ (SPEED’98) by the Environmental Agency of Japan. Wide investigation of AP and BPA in the environment is therefore important, and a rapid, sensitive, effective, and economical monitoring method is necessary.

GC and HPLC are the most suitable analytical methods for these compounds [7–10]. Capillary electrophoresis (CE) is usually described as a complementary technique to HPLC. Micellar electrokinetic chromatography (MEKC) has most often been used for these compounds because they are hydrophobic and neutral [11–14]. When tetradecyltrimethylammonium bromide (TTAB) was used as micelle and a sweeping technique was used for on-line concentration, the effective sensitivity enhancement for the compounds was more than fiftyfold. On-line concentration was, however, performed by gravity injection. The injection time was too long (2400 s) and 4-NP could not be detected because its peak shape was broadened [13]. When sodium dodecyl sulfate (SDS) was used as micelle all the compounds could be detected, and a lower limit of detection was obtained, but the long injection process (20 min) meant the method lost the advantage of rapid CE separation [13]. Reverse-migration micellar electrokinetic chromatography (RM-MEKC) is useful for rapid separation of some hydrophobic

endocrine disruptors in much shorter analysis times than conventional MEKC [15–18]. Combined with RM-MEKC, stacking using reverse migrating micelles and water plug (SRW) has proved to be an effective on-line concentration technique for some hydrophobic compounds. More than 100-fold gain in detection sensitivity has been achieved [19]. It is therefore possible to separate the four target compounds rapidly by RM-MEKC with efficient enhancement of sensitivity by SRW. After off-line concentration, for example by liquid–liquid extraction (LLE) or solid-phase extraction (SPE), trace target compounds in environmental samples might be detected.

The purpose of this study was to develop a method for rapid separation and highly sensitive determination of the target compounds. The CE separation conditions were optimized and on-line and off-line concentration (SRW and SPE) techniques were examined. For validation of the method it was used to determine the target compounds in a groundwater sample.

Experimental

Apparatus

All separations were performed with a P/ACE 5510 system (Beckman, Palo Alto, CA, USA) equipped with a UV detector. A 50 μm i.d. fused-silica capillary column (Yongnian Photoconductive Fiber Factory, Heibei Province, China) of 67 cm total length was used. The effective length to the detector was 60 cm. The system was controlled by P/ACE Station software. The pH was measured by means of a pH-meter with Sure-Flow Combination electrode (pH meter model 410A plus, Thermo Orion, Beverly, USA). Supelclean LC-18 cartridges (500 mg/3 mL) from Supelco (MA, USA) were used for solid-phase extraction.

Reagents and Solutions

The bisphenol A (BPA, purity >99.0%) and 4-nonylphenol (4-NP; mixture of compounds with branched sidechains) used in this study were obtained from Tokyo Kasei (TCI, Tokyo, Japan). 4-*tert*-Octylphenol (4-tOP, purity >97%), 4-*tert*-butylphenol (4-tBP, purity >99%),

and hydroxypropyl- β -cyclodextrin (HP- β -CD, MS = 0.8) were from Aldrich (Milwaukee, WI, USA). Sodium dodecyl sulfate (SDS, purity >95% based on total alkyl sulfate) was acquired from Sigma (St Louis, MO, USA). Sulfated- β -cyclodextrin (S- β -CD) and β -cyclodextrin (β -CD, purity >99%) were purchased from Fluka (Buchs, Switzerland). All other solid reagents were analytical reagent-grade and the solvents were HPLC-grade. Water used for preparation of the samples and buffers was purified by means of a Milli-Q system (Millipore, Bedford, MA, USA).

Running solutions were prepared by dissolving appropriate amounts of SDS, CD, and acetonitrile in a mixture of 100 mM phosphoric acid (H_3PO_4) and 20 mM sodium dihydrogen phosphate (NaH_2PO_4). These solutions were filtered through a 0.45 μm pore size membrane filter (Millipore) before use. Stock solutions of BPA and AP (2000 mg L^{-1}) were prepared in methanol and were diluted to 50 mg L^{-1} in the running buffer for optimization of the separation. Sample solution for on-line concentration was prepared in a solution containing 4 mM H_3PO_4 and 10 mM SDS.

Electrophoresis and On-Line Concentration Procedure

Before its first use the capillary tube was treated by flushing with 1.0 M HCl for 10 min, purified water for 5 min, 1.0 M NaOH for 20 min, and purified water for 5 min. Between two runs, a rinse-cycle of 1 M NaOH for 1 min, water for 2 min, and running buffer for 3 min was used (high pressure, 172 kPa). Samples were injected into the capillary by means of hydrodynamic pressure (4.3 kPa). The external temperature of the capillary was 25°C. UV detection was performed at 214 nm. The solutions were injected five times and average corrected peak areas (area/migration time) were used for quantification.

For SRW the sample was prepared in a low-conductivity matrix containing 10 mM SDS and 4 mM H_3PO_4 . The sample zone and water plug lengths selected were those obtained from 120-s injections. In the procedure, the capillary was first conditioned with running buffer at acid pH. A water plug was then introduced into the capillary before the sample zone. The vial was changed to

that of running buffer and a high voltage at negative polarity was applied [19, 20].

Solid-Phase Extraction Procedure

Supelclean LC-18 Cartridges were placed on a vacuum manifold and activated by flushing with 5 mL isopropanol, 5 mL methanol, and 5 mL water. The water sample was adjusted to pH 6 and 100 mL was then passed through cartridge at a flow rate of 5 mL min^{-1} , with the aid of a vacuum. After loading of the sample the SPE cartridge was washed with 5 mL 10% methanol aqueous solution to remove co-adsorbed matrix materials. The solid-phase material was then dried by means of a gentle stream of nitrogen gas and the target compounds retained in the cartridge were then eluted with 3 mL isopropanol at 1.0 mL min^{-1} . The eluent was evaporated to dryness by use of a gentle stream of nitrogen gas and the dry residue was dissolved in 500 μL sample solution and filtered before CE analysis.

A ground water sample from a Beijing suburb was used for method validation. Before SPE concentration the sample was filtered through a 0.45 μm filter to remove suspended particulate matter.

Results and Discussion

Optimization of Separation Conditions for RM-MEKC

Effect on the Separation of Organic Modifier Type and Concentration

The effect of the type of organic solvent on resolution was examined using 25 mM surfactant (SDS) in a mixture of 100 mM H_3PO_4 and 20 mM NaH_2PO_4 buffer, pH 1.85, containing 12.5% organic modifier, at a potential of -20 kV. Methanol, ethanol, acetone, and acetonitrile were investigated as organic modifiers. Short migration times and good resolution of the critical pair 4-tOP and 4-NP were used as criteria for optimization. Results showed that the compounds were eluted in the time order 4-NP < 4-tOP < BPA < 4-tBP; i.e. the alkylphenol with the largest molecular weight and the highest hydrophobicity was eluted first. Addition of ethanol resulted in the longest migration time (17.5 min) and poor resolution of 4-tOP and 4-NP

Fig. 1. Diagrams showing the enhancement of efficiency obtained for the four target compounds after SRW with different injection times when the ratio of the length of the water plug to that of the sample was 1:1. (A) effect on corrected area; (B) effect on peak height. Other conditions: running buffer, 25 mM SDS and 12.5% acetonitrile in 100 mM H_3PO_4 and 20 mM NaH_2PO_4 solution containing 1.0 mM β -cyclodextrin (pH 1.85); applied potential, -30 kV; detection wavelength, 214 nm; temperature, 25°C. Peak identity: 1 = 4-NP; 2 = 4-tOP; 3 = BPA; 4 = 4-tBP

($R_s = 0.9$). Use of acetone and acetonitrile resulted in better resolution of 4-tOP and 4-NP ($R_s = 1.2$) but acetonitrile resulted in the shortest migration time (12 min). Obviously, acetonitrile was the best of the four organic solvents whether resolution or migration time was used as criterion.

The effect of acetonitrile concentration was investigated at 5.0, 12.5, and 25.0%. It was observed that both the resolution of 4-tOP and 4-NP and the migration times of the compounds were increased by increasing the concentration of acetonitrile. With these acetonitrile concentrations both of BPA and 4-tBP were well separated but 4-NP and 4-tOP were not separated by use of 5.0% acetonitrile and only partially separated by use of 12.5% ($R_s = 1.2$) but were baseline-separated by use of 25.0% acetonitrile ($R_s = 4.9$) in a separation time of approximately 20 min. The time required for this resolution enhancement, however, was not appropriate for rapid separation of the target compounds and 12.5% acetonitrile was selected for further investigation.

Effect of Cyclodextrin Type and Concentration on the Separation

Cyclodextrins (CD) are well known for their potential to increase the aqueous solubility of compounds of low polarity and to enhance the separating power of MEKC [21–24]. The effects of S- β -CD, β -CD, and HP- β -CD on the separation of the compounds were tested with an acetonitrile concentration of 12.5%. The initial CD concentration was set to 1.0 mM.

Results showed that addition of the three CD improved the resolution of 4-tOP and 4-NP. The resolution of 4-tOP and 4-NP obtained by use of β -CD was the most satisfactory; the resolution was similar to that obtained by use of buffer containing 25% acetonitrile without CD. Addition of CD resulted in a much shorter migration time (approx. 13 min) than the latter (approx. 20 min) under the same conditions. This showed that introduction of CD effectively reduced migration time and improved resolution in this study.

For this reason 1.0 and 2.0 mM β -CD were used to investigate the effect of β -CD concentration on the separation. Although better resolution of 4-tOP and 4-NP was obtained by use of 2.0 mM β -CD ($R_s = 3.8$) than use of 1.0 mM β -CD ($R_s = 2.8$), the peak shape of 4-NP clearly became wider and peak splitting occurred when 2.0 mM β -CD was used. Use of 1.0 mM β -CD was more suitable for quantitative and qualitative analysis of 4-NP on the basis of satisfactory resolution.

Effect of SDS Concentration and Applied Potential on the Separation

The concentration of surfactant SDS was set to 15, 25, or 40 mM with a β -CD concentration of 1.0 mM. It was found that migration times and resolution of 4-tOP and 4-NP were inversely proportional to micelle concentration. The migration time was shortest when 40 mM SDS was used but resolution of 4-tOP from 4-NP and of BPA from 4-tBP was worse. When 25 mM SDS was used resolution of all the target compounds

was satisfactory and the analysis time (13 min) was a good compromise. The intermediate SDS concentration (25 mM) in the running buffer was therefore selected.

The effect of applied potential on resolution was examined from -15 to -30 kV with an SDS concentration of 25 mM. When a potential of -30 kV was applied the whole separation could be completed in less than 9 min and R_s for the critical pair 4-tOP/4-NP (2.2) was still satisfactory. A potential of -30 kV was therefore a suitable compromise between resolution and shorter migration time.

Summarizing the results above, we have determined the optimized conditions for rapid separation of BPA and three AP. The analytes can be separated to baseline by use of CD-RM-MEKC in a mixture of 100 mM H_3PO_4 -20 mM NaH_2PO_4 buffer (pH 1.85) containing 25 mM SDS, 12.5% acetonitrile, and 1.0 mM β -CD. The separation was complete in 9 min when a potential of -30 kV was applied. (The electropherogram obtained from an optimized separation after a conventional 2-s pressure injection is shown in Fig. 2D.)

On-Line Concentration by Stacking by Means of Reverse-Migrating Micelles and a Water Plug (SRW)

Theory of SRW

As already mentioned, some neutral and hydrophobic compounds are separated rapidly with effective enhancement of sensitivity by use of SRW-RM-MEKC.

Fig. 2. Electropherograms obtained from the four target compounds after conventional injection and after SRW with water plugs of different length. Sample matrix for SRW, 10 mM SDS and 4 mM H_3PO_4 (pH 2.5); hydrodynamic injection. (A) SRW with a 60-s injection of water and a 120-s injection of sample in the sample matrix; (B) SRW with a 240-s injection of water and a 120-s injection of sample in the sample matrix; (C) SRW with a 120-s injection of water and a 120-s injection of sample in the sample matrix; (D) 2 s of 200 mg L^{-1} sample in the running buffer. Other conditions and peak identification as for Fig. 1

The focusing mechanism of SRW is primarily based on the abrupt change in effective analyte electrophoretic velocities at the stacking boundary. SRW was performed by preparing the sample in a matrix which has a conductance lower than that of the running buffer. As a result of the low-conductivity of the sample matrix the analyte in the sample zone reaches the water zone more rapidly, which results in effective concentration. A surfactant is added to the sample matrix at a concentration slightly higher than the critical micelle concentration. The presence of micelles in the sample matrix increases the solubility of the analytes. Injection of a water plug before injection of the sample zone eliminates extra-band broadening of the peak caused by the presence of micelles and leads to good peak shape. Sample matrix, sample length, and water length are, therefore, the three main factors affecting the efficiency of concentration of the analytes. In this study, the sample length and water plug length were studied in detail to ob-

tain the highest sensitivity for the compounds and the best peak shape.

Optimization of SRW Conditions

In our study the sample for SRW was prepared in a solution containing 10 mM SDS and 4 mM H_3PO_4 . The effect of the length of the sample zone was first examined for a ratio of sample zone to water plug of 1:1. The injection time was varied from 60 to 198 s. The corrected peak area and height obtained under different SRW conditions were used as a measure of the effect. As shown in Fig. 1, the longer the injection time, the greater the enhancement of peak area. Enhancement of peak height was different from that of peak area. Within the range of injection times selected, the heights of BPA and 4-tBP were always improved, that of 4-tOP reached a maximum when the injection time was 120 s and that of 4-NP barely changed when the injection time was set at 150 s. The change in the peak width of 4-NP was, however, more

than that of peak height when the injection time was increased. The 4-NP peak became so broad that it resulted in a bad resolution of 4-tOP from 4-NP when the injection time was too long (for example 198 s). It was known that on-line enhancement might improve the separation of isomers and that the isomers of 4-NP might begin to separate during the course of on-line concentration, so the 4-NP peak shape became broad. The rapid increase in the width of the 4-NP peak might also affect the height enhancement of 4-tOP. A 120 s sample zone resulted in the best enhancement of height and area and better resolution of all the target compounds.

The length of the water plug in SRW is an important factor that affects peak shapes and migration time. Optimizing the length of the water plug is necessary to obtain sharp peaks and short migration times [19]. In this study the effect of the length of water plug between 60 and 240 s was studied with the length of sample zone kept at 120 s. The results are

Table 1. Reproducibility, linearity of response, limit of detection (LOD), and sensitivity enhancement factor using SRW

	4-NP	4-tOP	BPA	4-tBP
<i>RSD</i> ($n = 5$, using 1 ppm of each sample)				
Migration time (%)	0.34	1.03	1.49	1.48
Peak height (%)	6.3	4.14	1.98	9.53
Corrected peak area (%)	3.26	4.92	2.02	1.11
Equation of line through corrected peak areas ^a	$y = 903x + 138$	$y = 1026x + 60$	$y = 2868x + 370$	$y = 1525x - 10$
LOD (mg L^{-1} , $S/N = 3$)	0.089	0.03	0.0091	0.033
Correlation coefficient	0.9975	0.9977	0.9968	0.9985
SEFheight ^b	71	167	211	95
SEFarea ^c	122	121	193	162

The conditions were the same as used for Fig. 1

^aCalibration plots were obtained for concentrations from 0.2–5 mg L^{-1}

^bSEFheight = [(peak height obtained with concentration)/(peak height obtained with usual injection)] $\times$ dilution factor

^cSEFarea = [(peak area obtained with concentration)/(peak area obtained with usual injection)] $\times$ dilution factor

Fig. 3. Electropherograms obtained from the four target compounds in ground water after SPE and SRW. (A) groundwater spiked with 1.0 $\mu\text{g L}^{-1}$ of the target compounds; (B) groundwater spiked with 1.0 $\mu\text{g L}^{-1}$ of target compounds after SPE but without SRW. Sample matrix for SRW, 10 mM SDS and 4 mM H_3PO_4 (pH 2.5). SRW conditions: 120-s injection of water before 120-s injection of sample in the sample matrix; Other conditions and peak identification as for Fig. 1

shown in Fig. 2. It was observed that migration time increased with increasing length of the water plug. When the length of water plug was short (60 s water injection, Fig. 2A) BPA and 4-tBP gave tailing peaks and the peaks of 4-NP and 4-tOP were irregular and split into three. When the length of water plug was much longer (240 s water injection, Fig. 2B) the BPA peak became sharper and higher but the efficiency for 4-NP and 4-tOP became worse, and the peak of 4-tBP became broader and the peak height decreased. Under the optimized conditions for SRW the same length, 120 s, was selected for the injection length and the water plug. This enabled better enhancement for all the target compounds (Fig. 2C) and the migration time obtained was a reasonable compromise.

Detector Response Improvement Obtainable with SRW

A 2-s injection without SRW is also included in Fig. 2D for comparison with SRW conditions. The sample used for the 2-s injection was prepared in running

buffer and the concentrations of target compounds were 200 mg L^{-1} . The reproducibility and linearity of the response for this method are summarized in Table 1. The separation time obtained was similar to that in Ref. [13]. Although limits of detection obtained were slightly higher, injection time in SRW was much shorter (total 240 s) than in Ref. [13] (1200 s).

Determination of AP and BPA in a Groundwater Sample

A groundwater sample was selected for validation of this method. Because of to the low concentration in this environmental sample, it is necessary to perform off-line sample concentration before CE analysis. Solid-phase extraction (SPE) is an effective preconcentration technique, because it uses small amounts of organic solvents, is rapid, and the enhancement factor is high. In this study, a modified SPE preconcentration process was based on the method reported in Ref. [25]. A Supelclean LC-18 Cartridge (Supelco) was used for solid-phase extraction. After

the SPE process the target compounds in the water sample were concentrated approximately 200 times. Fig. 3B shows the electropherograms obtained with the usual 2-s injection time when 100 mL groundwater was spiked with 1.0 $\mu\text{g L}^{-1}$ target compounds. It is clearly apparent that without on-line concentration the test analytes were not detected even after SPE. After SRW concentration, however, all the target compounds could be detected (Fig. 3A). The blank electropherogram obtained from the groundwater sample indicated there was no obvious interference from the groundwater matrix. Recoveries of the target compounds obtained from the ground water after SPE and SRW were satisfactory—83.5, 87.7, 78.8, and 71.3% for 4-NP, 4-tOP, BPA, and 4-tBP, respectively. *RSD* for the target compounds was 9.1, 5.0, 3.6, and 13.1, respectively.

Conclusion

An electrophoresis method has been established for rapid separation of three

alkylphenols and bisphenol A. On the basis of this rapid separation, on-line concentration by SRW was developed. By use of this procedure *LOD* obtained for the target compounds were efficiently improved by use of short injection and enhancement times. Finally, the method was validated by determination of the compounds in a groundwater sample after solid-phase extraction. In conclusion, this work shows that SRW-RM-MEKC with SPE is a feasible technique for rapid separation and highly sensitive determination of three alkylphenols and bisphenol A in environmental water samples.

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