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Separation of eleven priority phenols by capillary zone electrophoresis with ultraviolet detection

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Abstract

The separation and detection of eleven priority phenols using capillary zone electrophoresis with UV detection are described. Using a 70 cm × 75 μm I.D. capillary at 15 kV and an electrophoretic buffer of 20 m*M* sodium borate (pH 9.9 ± 0.1), the eleven compounds could be completely separated in less than 14 min. The different variables that affect separation were studied and optimized, and good linearity was obtained between 0.5 and 20 mg l^{-1} for 2,4-dimethylphenol (2,4-DMP) and between 0.25 and 20 mg l^{-1} for the other compounds, and the detection limits were 0.1 mg l^{-1} for all compounds except 2,4-DMP (0.25 mg l^{-1}). Previous analyses of real samples, an off-line solid-phase extraction process with a highly cross-linked styrene–divinylbenzene copolymer cartridge was used to determine the priority phenolic compounds at low μg l^{-1} levels. The method was tested with tap and Ebro river waters.

Keywords: Water analysis; Environmental analysis; Phenols

1. Introduction

Phenol and substituted phenols are common by-products of many industrial processes. They are generated, for instance, in the manufacture of dyes, plastics, drugs, antioxidants and paper and in the petroleum industry [1].

The presence of phenol and substituted phenols is not significant in natural waters because they are only present as a result of the decomposition of some types of vegetation. They only increase if waste water from industrial or mineral processes in which phenol and substituted phenols are used is not properly treated [2].

Under environmental conditions they can persist, depending on temperature and pH, at mg l^{-1} levels in ground water for a number of days or weeks. Phenols have been found to be toxic to most aquatic organisms and even at low concentrations (<1 μg l^{-1}) have an adverse effect on the taste and odour of water [3]. Therefore, if such ground waters are to be used as sources of drinking water, it is necessary to screen them for contamination by these organic pollutants. Moreover, the disinfection process with chlorine increases the effect of odour and taste because of the reaction of phenol to form chlorinated compounds [4–6]. The US Environmental Protection

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Agency (EPA) lists eleven phenols as priority organic pollutants [7].

There are various methods to determine phenolic compounds in water samples. Gas chromatography (GC) and high-performance liquid chromatography (HPLC) have been commonly used for the determination of individual phenolic compounds [8,9]. In order to enhance the volatility and detectability of phenols, sample derivatization is usually necessary before GC analysis [10–12]. On the other hand, HPLC has advantages for the determination of phenols because no derivatization process has to be carried out. Isocratic or gradient elution is widely used to determine these compounds; isocratic elution in HPLC is mainly applied when electrochemical detection is used because it allows these compounds to be determined at low $\mu g\, l^{-1}$ levels [13,14]. However, it is necessary to carry out two injections in order to determine the eleven EPA priority phenolic compounds, one for more polar compounds and the other for less polar compounds [14].

If the eleven EPA compounds are to be determined with only one injection, gradient elution is required. In this case, a UV detector is preferred because of the better stability of signal [15–17]. Some workers determine some of the phenolic compounds by gradient elution with an electrochemical detector, but in this case the detection limits obtained are higher than in isocratic elution [18].

In the past few years, capillary electrophoresis (CE) has been shown to be a rapid, powerful and efficient technique for separating various compounds [19]. Most of the environmental applications of CE are restricted to demonstrating high-resolution separations for specific pollutants and only a few relate to the determination of pollutants in real matrices [20–24].

Capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MECC) have both been used for the separation of phenolic compounds [23–28] with different detection techniques. The analysis of eleven phenols using MECC with UV detection has been established [28]. In this case a micellar solution is necessary and an analysis time of ca. 45 min is required to separate the eleven priority phenols. If CZE is used with indirect fluorescence detection, a non-interacting fluorescing ion needs to be added to the running buffer to create a constant fluorescence background [24].

In this paper, a method for the separation and detection of the eleven priority phenols using free solution CZE coupled with on-column UV detection is described, using a simple solution of sodium tetraborate as running buffer. Detection limits, linearity and repeatability are examined.

An off-line solid-phase extraction (SPE) prior to separation is used for the determination of these compounds in water samples in order to decrease the detection limits. Ion-pair extraction with a highly cross-linked styrene–divinylbenzene copolymer is used because higher breakthrough volumes were obtained for the more polar phenolic compounds compared with other sorbents such as C_{18} or PRPL-S [29]. The separation and detection of the eleven priority phenols using CZE with UV detection is described and the selectivity of the method was checked with tap and Ebro river water.

2. Experimental

2.1. Instrumentation

A Crystal 310CE instrument (ATI Unicam, Cambridge, UK) in combination with a Unicam Model 4225 absorbance detector was used. Data were collected with an HP ChemStation version A.01.01 chromatographic data system. The separations were carried out using an uncoated fused-silica capillary (70 cm × 75 μm I.D.) supplied by Supelco (Bellefonte, PA, USA); 56 cm from the capillary inlet a detection window was obtained by burning off the polyimide coating. The injection mode was hydrodynamic.

2.2. Chemicals

The eleven phenolic compounds studied were (**1**) 2,4-dimethylphenol (2,4-DMP), (**2**) phenol (Ph), (**3**) 4-chloro-3-methylphenol (4-C-3-MP),

(**4**) pentachlorophenol (PCP), (**5**) 2,4,6-trichlorophenol (2,4,6-TCP), (**6**) 2-methyl-4,6-dinitrophenol (2-M-4,6-DNP), (**7**) 2,4-dichlorophenol (2,4-DCP), (**8**) 2-chlorophenol (2-CP), (**9**) 2,4-dinitrophenol (2,4-DNP), (**10**) 4-nitrophenol (4-NP) and (**11**) 2-nitrophenol (2-NP). All of them were obtained from Aldrich Chemie (Beerse, Belgium) except pentachlorophenol, which was obtained from Janssen Chemie (Geel, Belgium). A stock standard solution of 2000 mg l^{-1} of each compound was prepared in methanol–water (50:50) and stored in a refrigerator. Working standard solutions were prepared weekly or daily, depending on their concentration, by diluting the stock standard solutions with water purified using a Milli-Q system (Millipore, Bedford, MA, USA).

Sodium tetraborate (Fluka, Buchs, Switzerland) and sodium hydroxide (Aldrich Chemie) were used to prepare the electrolyte solution.

HPLC gradient-grade methanol (Scharlau, Barcelona, Spain), acetic acid (Merck, Darmstadt, Germany) and tetrabutylammonium bromide (TBAB) (Aldrich Chemie) as the ion-pair reagent were used in the SPE process.

2.3. Electrophoretic conditions and detection

The electrolyte solution was made by adjusting the pH of 20 m*M* sodium tetraborate solution to 9.9 ± 0.1 with sodium hydroxide. The pH of the electrolyte buffer was checked prior to use and, if necessary, brought to 9.9 ± 0.1.

Before use, the capillary was washed (1000 mbar pressurized flow) with 0.1 *M* NaOH for 15 min followed by a 10-min rinse with water and a 10-min flush with the running buffer. Every day, or when the composition of the electrolyte was changed, the capillary was equilibrated with 0.1 *M* NaOH for 5 min followed by a 2-min rinse with water and a 10-min flush with the running buffer by pressurized flow at 1000 mbar.

The applied voltage in the separation was 15 kV and the capillary temperature was kept constant at 35°C. The detector was set at 220 nm. Injection was performed hydrodynamically by pressure at 100 mbar for 0.05 min.

2.4. Extraction process

The off-line trace enrichment process was carried out using 500 mg of highly cross-linked styrene–divinylbenzene copolymer ENVIchromP (particle size 80–160 μm) (Supelco). Extraction was carried out using a Bond Elut/Vac Elut system (Varian, Harbor City, CA, USA). Prior to the extraction process, the sample was adjusted to pH 9 and different volumes of TBA were added to adjust the final concentration to 5 m*M*. Then, 500 ml of sample were extracted and the phenolic compounds retained were eluted with 5 ml of methanol acidified with 1% of acetic acid to decrease the effect of the ion-pair reagent. The 5-ml volume was concentrated with a rotary evaporator (Büchi, Flawil, Switzerland) to a volume of about 200 μl and then diluted to a final volume of 500 μl with methanol. This extraction process was described in a previous paper [29].

Tap and Ebro river water samples were filtered through a 0.45-μm filter (MSI, Westboro, MA, USA) before preconcentration.

3. Results and discussion

As a result of initial investigations testing several buffers, sodium borate was chosen as the background buffer for the separation of the eleven phenols. Once the buffer had been selected, the first parameter studied was the pH of the electrolyte. Fig. 1 shows the influence of the buffer pH on the migration time. The best results were obtained at pH $\approx$ 10, and the great influence of this parameter on the peak resolution made a precise study necessary. Fig. 2 shows the results obtained, from which the pH was chosen as 9.9 ± 0.1.

It is known that when the buffer concentration increases there is an improvement in the separation efficiency. In this paper, the range of borate concentration studied was limited to 20–35 m*M* because an initial study had demonstrated that a borate concentration of 10 m*M* was too low to separate the different compounds, and concentration higher than about 35 m*M* did not

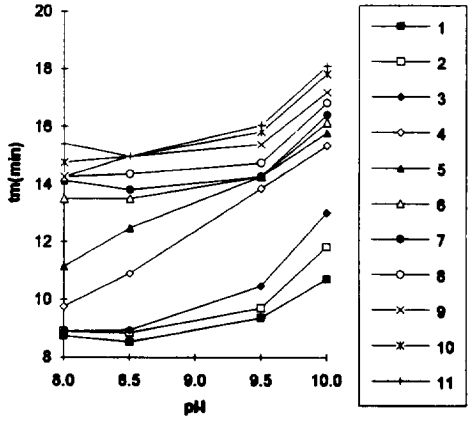

Fig. 1. Influence of the pH of the buffer on migration time. Electrolyte, 15 m*M*, sodium borate; separation voltage, 10 kV; injection, 100 mbar for 0.05 min; concentration of each phenolic compound, 5 mg l^{-1}; detection wavelength 220 nm. For compound identification, see Table 1.

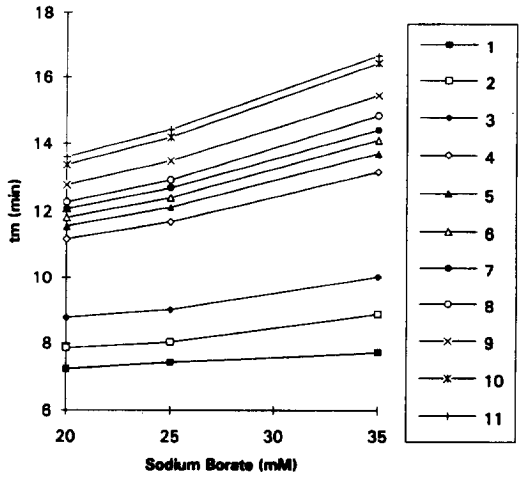

Fig. 3. Variation of migration time with sodium borate concentration. For other experimental conditions, see text.

significantly improve the resolution. Fig. 3 shows that the best separation was obtained at a borate concentration of 20 m*M*.

Adding organic modifiers to the separation buffer is known to reduce the electroosmotic flow, and may result in better resolution at the expense of a longer analysis time [30]. In this work, two organic solvents, methanol and acetonitrile, were studied at different concentrations up to 20%. In general, the electroosmotic flow decreased when the organic solvent fraction increased, but the resolution of the phenolic compounds in the range of concentrations of organic solvents studied did not improve, so an electrolyte with no organic solvent was used.

Temperature control is commonly used for efficient heat removal [31]. Fig. 4 shows the influence of capillary temperature on migration time. In the range studied, 35–60°C, a change in

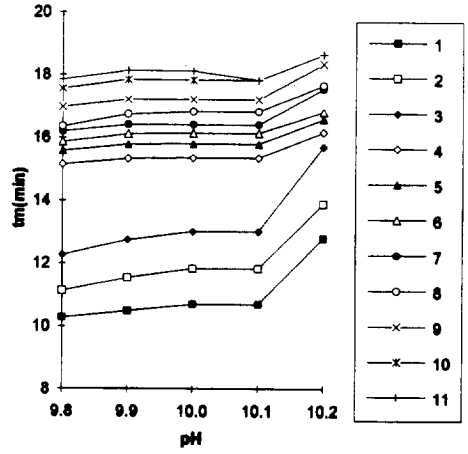

Fig. 2. Influence of the buffer pH on the migration time. For experimental conditions, see text.

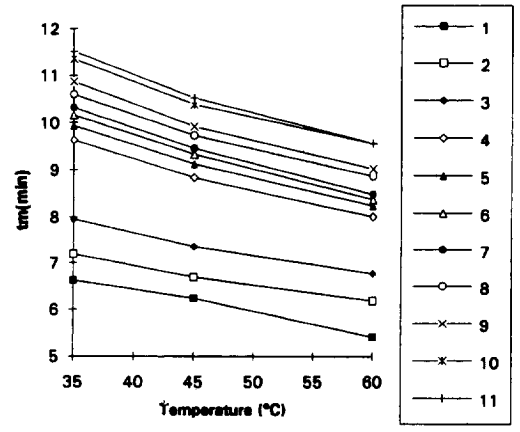

Fig. 4. Effect of capillary temperature on the separation of the eleven phenolic compounds. For experimental conditions, see text.

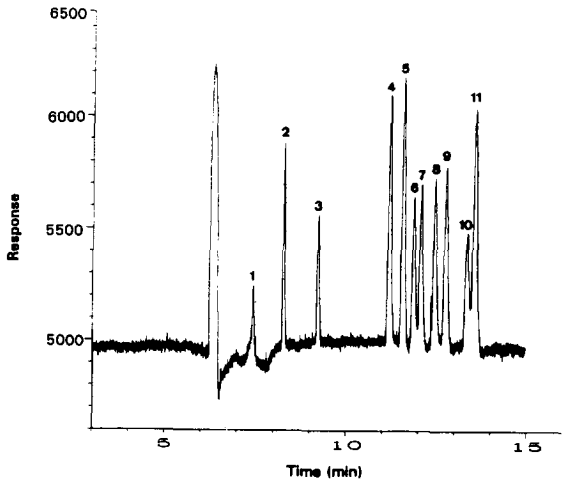

Fig. 5. Electropherogram of 1 mg l^{-1} of each phenolic compound. Electrolyte, 20 m*M* sodium borate; separation voltage, 15 kV; injection, 100 mbar for 0.05 min; detection wavelength, 220 nm. For peak designation, see Table 1.

temperature appeared to influence all migration times in a similar way. The migration sequence remained unchanged throughout the temperature range.

Hydrodynamic injection was performed. The conditions chosen for injection were 100 mbar for 0.05 min (5 mbar min). The wavelength for detection was also optimized and the best sensitivity was obtained at 220 nm.

Fig. 5 shows an electropherogram of a standard solution of 1 mg l^{-1} of each phenolic compound under the optimum conditions: 20 m*M* sodium borate, separation voltage 15 kV, injection at 100 mbar for 0.05 min and detection at 220 nm.

The results obtained from studying the linearity and response repeatability for standard solutions are shown in Table 1. Within the concentration range studied (0.25–20 mg l^{-1} for all compounds except 0.5–20 mg l^{-1} for 2,4-DMP), there was a good correlation between peak area and concentration for every compound.

Repeatability was examined by five replicate injections of each compound at a concentration corresponding to the lowest point on the calibration line and the R.S.D.s were between 11.2% for 4-C-3MP and 4.2% for 2-M-4,6-DNP. The limits of detection (LOD) were calculated using a signal-to-noise ratio of 3. It was 0.10 mg l^{-1} for all compounds except 2,4-DMP (0.25 mg l^{-1}).

To decrease the detection limit, an SPE process with a cross-linked styrene–divinylbenzene copolymer cartridge was carried out. In this case, the method optimized in a previous study [29] applied prior to the CZE analysis enabled these compounds to be detected at μg l^{-1} levels with LOD between 0.3 and 1 μg l^{-1}.

The method was applied to tap and Ebro river water. Real samples were filtered through a 0.45-μm filter and, after filtration, 300 μl of a solution of 10% Na_2SO_3 were added for each 100 ml of

Table 1
pK_a values, calibration data and precision for the eleven phenolic compounds

No.	Compound	pK_a	Linear range (mg l^{-1})	Slope	Intercept	r^2	R.S.D. (%)[a]
1	2,4-DMP	10.5	0.50–20	136.6	7.15	0.9996	8.3
2	Ph	9.9	0.25–20	291.6	16.60	0.9999	6.0
3	4-C-3-MP	9.6	0.25–20	239.0	0.60	1.0000	11.2
4	PCP	4.9	0.25–20	761.3	−277.20	0.9974	10.9
5	2,4,6-TCP	7.4	0.25–20	676.6	−144.50	0.9972	9.0
6	2-M-4,6-DNP	4.3	0.25–20	420.3	−33.80	0.9992	4.2
7	2,4-DCP	7.7	0.25–20	421.8	−10.30	0.9998	10.4
8	2-CP	8.1	0.25–20	490.8	9.60	0.9999	8.7
9	2,4-DNP	4.1	0.25–20	578.7	1.10	0.9999	5.8
10	4-NP	7.2	0.25–20	381.5	18.80	0.9999	9.8
11	2-NP	7.2	0.25–20	840.8	142.40	0.9981	9.5

[a] Obtained for the lowest point on the calibration line.

drinking water to eliminate free chlorine, which could have reacted with phenols to produce chlorophenols.

Fig. 6a shows the electropherogram obtained when 500 ml of tap water were preconcentrated 1000-fold using the copolymer cartridge and Fig. 6b shows the same sample spiked with a standard solution of phenolic compounds at 5 $\mu g\ l^{-1}$. The same analysis was carried out with Ebro river water. A 500-ml volume of water (Fig. 6c) and the same sample spiked with 5 $\mu g\ l^{-1}$ of a standard solution of phenolic compounds (Fig. 6d) were analysed using the same method. In all cases, there was good resolution between different peaks and no interference of the system's peaks.

Comparing the electropherogram obtained by this method with the chromatogram obtained when RPLC was used in the analysis of real samples, it can be deduced that phenol, one of the most important compounds, is more easily determined by CZE than by RPLC, because of the appearance of a large peak at the beginning of the RPLC trace due to the humic and fulvic acids.

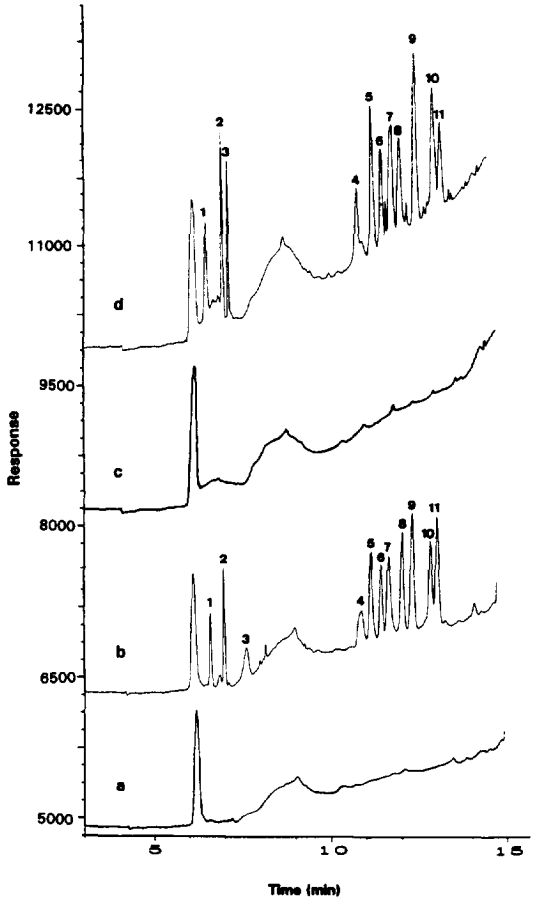

Fig. 6. Electropherograms obtained from (a) an extract of 500 ml of tap water, (b) an extract of 500 ml of tap water spiked with a standard solution of phenolic compounds at 5 $\mu g\ l^{-1}$, (c) an extract of 500 ml of Ebro river water and (d) an extract of 500 ml of Ebro river water spiked with a standard solution of phenolic compounds at 5 $\mu g\ l^{-1}$. For experimental conditions, see text.

4. Conclusions

CZE with UV detection can rapidly separate and sensitively detect the eleven priority phenols. Complete separation of the eleven compounds can be achieved in ca. 14 min using an electrophoretic buffer of 20 m*M* sodium borate (pH 9.9). The calibration graphs over two orders of magnitude of concentration were linear and the limits of detection were at the mg l^{-1} or $\mu g\ l^{-1}$ level when an SPE process was applied. The CZE method enabled these compounds, mainly phenol, to be detected with lower matrix interference than when the RPLC method was applied.

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