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Determination of phenolic pollutants in drinking water by capillary electrophoresis in the sample stacking mode

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Abstract

A method for the determination of phenol, chlorophenols and nitrophenols at levels below those allowed by international legislation in water from the public supply is proposed. Ten of the compounds studied are included on the USA Environmental Protection Agency priority list of aquatic pollutants. Samples are concentrated off-line on cross-linked polystyrene and subsequently subjected to capillary electrophoresis using the sample stacking procedure to remove the matrix. The recoveries afforded by the off-line concentration process and the behaviour of the phenols in relation to sample stacking injection were examined. © 1997 Elsevier Science B.V.

Keywords: Sample stacking; Water analysis; Phenols; Chlorophenols; Nitrophenols

1. Introduction

Phenol and chlorophenols are polluting substances present in the aquatic environment as byproducts of the coal and oil industry [1], or as the result of pesticide and drug decay [2].

Such international bodies as the US Environmental Protection Agency (EPA) [3] and the European Union (EU) [4] have included phenol and various chlorophenols and nitrophenols in their lists of priority pollutants to be monitored in the aquatic environment. The maximum allowed level for these compounds in publicly supplied water is 0.5 μg/ml.

The need to determine such low concentration levels calls for a sample concentration step prior to analyses proper. This can be accomplished by using various solid-phase absorbents including C_{18} resin [5,6] and graphitized carbon [7,8].

This type of compound is most often determined by gas chromatography with various kinds of detectors [9,10]. However, the typically high polarity of phenols gives rise to poor chromatographic peaks. This is particularly true of pentachlorophenol and nitrophenols, which are especially acid. This shortcoming can be circumvented by derivatization with acetic anhydride [11,12], diazomethane [13], pentafluorobenzoyl chloride [14] or heptafluorobutyric acid [15], among other reagents. In addition to introducing an additional step in the process, the derivatization rarely provides adequate yields for nitrophenols [10].

Capillary electrophoresis, which exploits polarity differences between phenolic species for their separation [16], allows one to dispense with the derivatization step. By using an untreated capillary surface, anodic injection and a buffered alkaline solution, phenols are swept to the cathode by the electroosmotic flow (EOF) of the buffer. Size and charge differences between species result in electrophoretic mobility differences (in the opposite direc-

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tion to EOF) that facilitate their effective separation [17].

The low sensitivity of UV detection in combination with capillary electrophoresis [17,18] makes it inappropriate for determining phenols in tap water [16], even if the sample is preconcentrated. This shortcoming can be circumvented by using a sample stacking technique for matrix removal, which affords injection of large amounts of sample without compromising separation efficiency. In this way, the sensitivity can be increased over 100 times [19].

This operating mode involves injecting the target analytes into an aqueous matrix or one with a very low conductivity relative to the separation buffer. Under these conditions, charged species rapidly migrate to the separation zone between the matrix and buffer, and concentrate over a narrow band. If, in addition, the system polarity is reversed after injection —pressurized anodic injection— then not only do anions (phenolates) migrate to the separation zone but also the EOF sweeps the matrix and positively charged species out of the capillary. When the current reaches about 95% of its normal level (*viz.* the current provided by the electrolyte used in conventional injections), the initial polarity is restored and phenols separated [17–21].

This paper reports a method for the determination of phenol and several chlorophenols and nitrophenols included on the EPA and EU lists of priority pollutants at parts-per-billion and lower levels in waters. Samples are concentrated off-line on polystyrene cartridges [22] that are eluted with methanol and the extracts thus obtained are diluted with water to a methanol–water (6:4, v/v) composition of the matrix, which make them suitable in terms of conductivity for electrophoretic injection in the sample stacking mode.

2. Experimental

2.1. Apparatus

All experiments were carried out on an HP^{3D} CE instrument from Hewlett-Packard (Palo Alto, CA, USA) equipped with an on-column diode array detector. Spectra were acquired over the wavelength range 200–400 nm and signals monitored at 230, 210 and 370 nm.

Separations were accomplished with fused-silica capillaries coated with polyimide. The capillaries were 70 cm long (effective length 62 cm)×50 μm I.D. They were constructed from Composite capillary tubing supplied by Tecnokroma (Barcelona, Spain). On-column detection windows were opened by burning a small section (0.8 cm) of the capillary's outer coating and carefully removing residual burnt polyimide with isopropanol. Capillaries were conditioned in 1 *M* NaOH for 10 min prior to use on a daily basis.

The supporting buffer used was 40 m*M* sodium borate adjusted to pH 9.80 with NaOH. The temperature of the capillary cartridge was set at 25°C and that of the sample carousel at 20°C, both with water circulated from an external bath. The voltage used in the separation step was 30 kV. Samples were injected under a pressure of 50 mbar over an interval of 600 s. The assembly was programmed to reverse the polarity (from negative to positive) 2.5 min after injection in order to effect the separation.

2.2. Reagents

Methanol was supplied by Merck (Darmstadt, Germany). Isopropanol, sodium hydroxide and sodium borate were purchased from Aldrich (Milwaukee, WI, USA). Standards of phenol, 4-chloro-3-methylphenol, 4-chlorophenol, pentachlorophenol, 2,3,5,6- tetrachlorophenol, 2,4,6-trichlorophenol, 2,4-dichlorophenol, 2-methyl-4,6-dinitrophenol, 2,3,6-trichlorophenol, 2-chlorophenol, 2,4-dinitrophenol, 4-nitrophenol and 2-nitrophenol were also supplied by Aldrich. Stock solutions containing 3.0 μg/l of each analyte were made in methanol — nitrophenols are sparsely soluble in this alcohol, so their working concentration range was only 0.8–1 mg/ml. These solutions were stored at 4°C in the dark. Working strength solutions were prepared from the individual stocks in methanol–water mixtures of variable composition.

Tap and ultrapure water samples were concentrated by passage through International Sorbent Technology 200 mg/6 ml Isolute Env+polystyrene cartridges from MicroBeam (Barcelona, Spain).

Standards, concentrated sample extracts, buffer

and all solutions used to condition the capillary were passed through filters of 0.22-μm pore size prior to injection into the electrophoretic system.

2.3. Sample preparation

Pre-filtered ultrapure (Milli-Q) and tap water samples —spiked with appropriate amounts of the above-mentioned phenol solutions when required— were adjusted to pH 2.5–2.8 with 1 *M* HCl and passed through an Isolute Env+ cartridge containing 200 mg of polystyrene at a flow-rate of 100 ml/min. The cartridges were previously rinsed with 4 ml of methanol and activated with 4 ml of Milli-Q water at pH 2–3. After each sample was passed, the cartridge was eluted with 10 ml of Milli-Q water, dried by passage of a dry nitrogen stream for 30 min and turned upside down for elution with 4 ml of methanol (in the opposite direction to the sample). The volume of the final extract was measured and diluted with Milli-Q water to a methanol–water (6:4, v/v), the diluted solution being filtered through a membrane of 0.22-μm pore size and injected into the capillary electrophoresis system.

3. Results and discussion

3.1. Optimization of phenol separation by capillary electrophoresis

Initially, the target compounds were separated by using 40 m*M* sodium tetraborate buffer that was adjusted to pH 10.0 with NaOH. Under these conditions, several chlorophenols were effectively separated [16]; however, 2-chlorophenol and 2,4-chlorophenol, both included on the EPA priority pollutant list, could not be efficiently resolved. A tetraborate buffer of the same concentration but pH 9.8 (adjusted with 1 *M* NaOH) effectively resolved all the compounds studied (see Fig. 1).

All 13 phenols could be detected at both 230 and 210 nm. Also, 2-methyl-4,6-dinitro phenol, 2-dinitrophenol and 4-nitrophenol exhibit an absorption maximum at 370 nm. These three wavelengths were selected for monitoring with the diode array detector used in order to record the spectra for the phenols. In addition, the complete spectra for the region from 200 to 400 nm were available at any time during the electrophoretic separation, which increased the reliability of identifications.

3.1.1. Influence of the methanol content in the samples on the separation efficiency

The polystyrene cartridges used exhibited excellent retention properties for chlorophenols in pre-acidified samples: they afforded sample passage rates of 100 ml/min and were quantitatively eluted by methanol [22].

In sample stacking injection, both standard and samples must be diluted in an aqueous matrix or one with a much lower conductivity than the buffer where the separation is effected. Because methanol is the ideal solvent for eluting polystyrene cartridges, the influence of its content in the eluent on the efficiency of sample stacking injection was investigated.

Pressurized injection of standards made from a methanol–water matrix in variable proportions for 10 min revealed that the time needed for complete removal of the matrix was directly proportional to the methanol content in it (see Table 1).

The matrix removal time needed for matrices containing over 60% methanol was inordinately long and resulted in losses before removal was complete. Fig. 1 shows the electrophoregram obtained for a standard containing the compounds studied in methanol–water (6:4, v/v) that was pressure-injected for 10 min.

3.1.2. Influence of the injection time

The improved detection limits obtained with sample stacking injection are a result of the increased sample volumes that are passed through the capillary. Fig. 2 shows the variation of the peak area for a phenol standard in methanol–water (6:4, v/v) as a function of the injection time. Increasing such a time also obviously increased that required to fully remove the matrix; this entailed reversing the polarity by hand — in routine sample analyses, reversals can be done automatically as described under Section 2.

With the exception of phenol, 4-chloro-3-methylphenol and 4-chlorophenol, which are discussed in greater detail later on, the peak areas for all the compounds studied increased with increasing injection time between 3 and 10 min; on the other

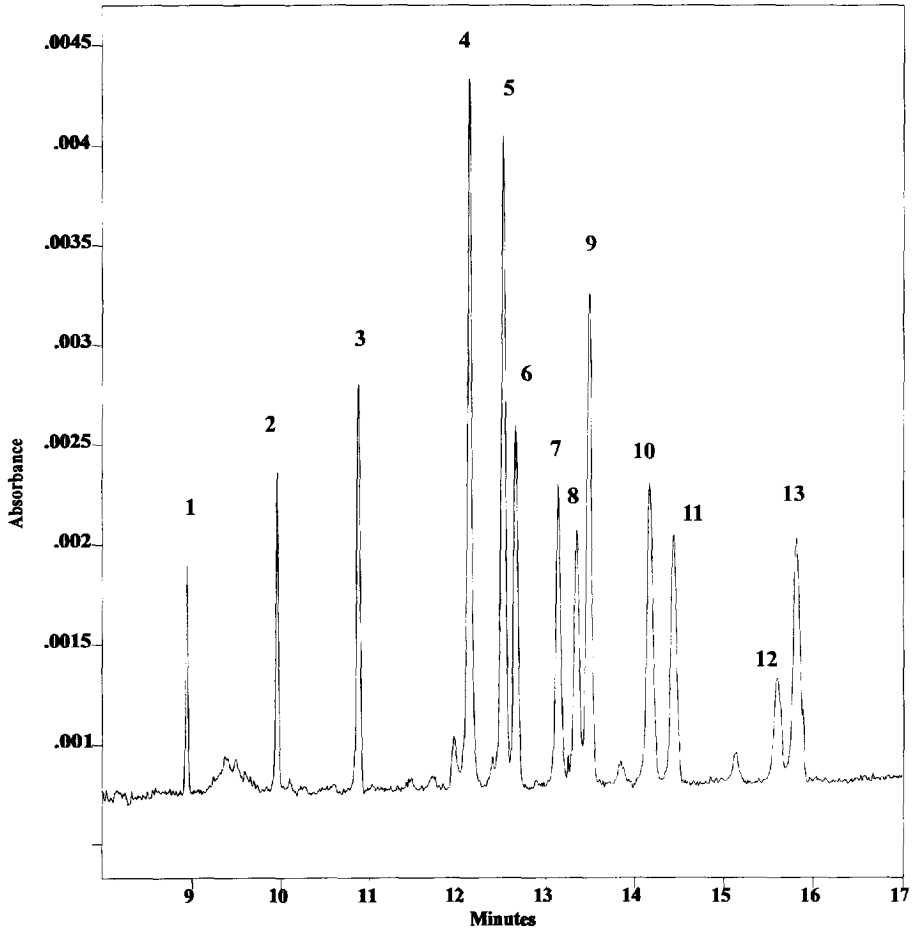

Fig. 1. Electrophoregram for a standard solution containing the species studied in methanol–water (6:4, v/v). 1=Phenol (232.7 μg/l), 2=4-chloro-3-methylphenol (228.1 μg/l), 3=4-chlorophenol (228.2 μg/l), 4=pentachlorophenol (111.6 μg/l), 5=2,3,5,6-tetrachlorophenol (117.7 μg/l), 6=2,4,6-trichlorophenol (95.0 μg/l), 7=2,4-dichlorophenol (113.4 μg/l), 8=2-methyl-4,6-dinitrophenol (32.6 μg/l), 9=2,3,6-trichlorophenol (106.7 μg/l), 10=2-chlorophenol (143.8 μg/l), 11=2,4-dinitrophenol (38.4 μg/l), 12=4-nitrophenol (29.5 μg/l), and 13=2-nitrophenol (30.6 μg/l).

Table 1
Influence of the sample matrix composition (proportion of methanol) on the time needed to remove the matrix after stacking

Matrix composition (methanol–water)	Time to change polarity (min)
4:6	1.70
5:5	2.10
6:4	2.50

Results obtained by using a capillary of 70-cm×0.5-mm I.D. and a pressure-injection time of 10 min.

hand, a further increase from 10 to 13 min had little effect — a plot of peak height instead of peak area led to the same results.

An injection time of 10 min was thus selected for further experiments. Based on the Haegen-Poiseuille equation [23], the volume injected into the capillary over an interval of 10 min was 600 nl (i.e. the inner volume of a capillary length of ca. 30 cm or half its effective length). This is consistent with previously reported results for pentachlorophenol and a capillary of different length [16].

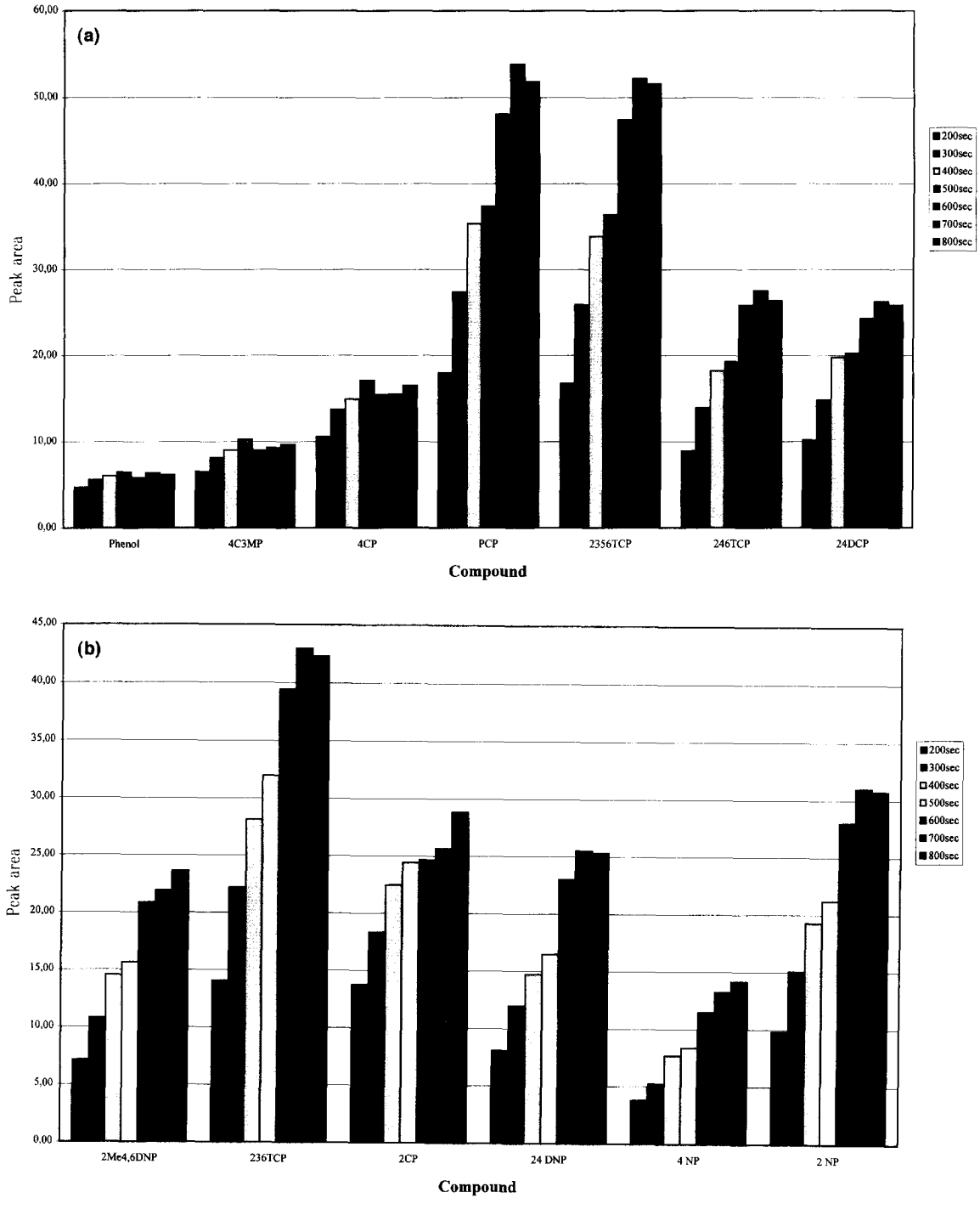

Fig. 2. Influence of the injection time on peak area for a standard solution of the analytes in methanol–water (6:4, v/v).

Table 2
Limits of quantitation (μg/l) obtained at a signal-to-noise ratio of 10 by sample stacking injection of 1000 ml of water concentrated by using the proposed procedure, as a function of the monitoring wavelength

Compound	LOQ		
	230 nm	210 nm	370 nm
Phenol	0.16	0.21	–
4-Chloro-3-methylphenol	0.14	0.06	–
4-Chlorophenol	0.10	0.07	–
Pentachlorophenol	0.01	0.01	–
2,3,5,6,-Tetrachlorophenol	0.01	0.01	–
2,4,6-Trichlorophenol	0.02	0.01	–
2,4-Dichlorophenol	0.02	0.01	–
2-Methyl-4,6-dinitrophenol	0.01	0.01	0.01
2,3,6-Trichlorophenol	0.01	0.01	–
2-Chlorophenol	0.02	0.02	–
2,4-Dinitrophenol	0.01	0.02	0.02
4-Nitrophenol	0.01	0.02	0.02
2-Nitrophenol		0.01	0.02

3.2. Linearity of the response

3.2.1. Limits of quantitation

Because the repeatability of the migration times for phenol standards in methanol–water (6:4, v/v) pressure-injected for 10 min with automatic polarity reversal after 2.5 min, was about 1%, the linearity of the response was examined by plotting the concentration of each compound against each peak area without the need to use the peak area/migration time ratio. The calibration curves thus obtained exhibited excellent linearity over the range 20–700 μg/l for all the phenols studied.

Table 2 lists the limits of quantitation obtained at a signal-to-noise ratio of 10 and the three wavelengths used to monitor the absorbance of the phenols studied. The low sensitivity for phenol, 4-chloro-3-methylphenol and 4-chlorophenol relative to the other compounds studied was a result of their anomalous behaviour during the sample stacking injection step (see Fig. 2).

3.3. Application to water samples

Water samples were concentrated by passage through polystyrene cartridges and the eluate, 3 ml in volume, was made to 5 ml with Milli-Q water in order to obtain the same composition as for the standards. The concentration factor obtained for 1 l of water was thus 200:1. This, together with the limits of quantitation given in Table 2, allows all the compounds studied to be determined at levels below 0.5 μg/ml. Such favourable limits of detection can be further lowered (by a factor of 2–3) by using bubble capillaries.

Table 3 shows the recoveries obtained from 500- and 1000-ml samples of ultrapure water spiked with known amounts of the phenols studied. Samples

Table 3
Average recoveries (n=4, duplicate injection) obtained by using polystyrene cartridges of 200 mg at a flow-rate of 100 ml/min

Compound	500 ml of water			1000 ml of water		
	Conc. (μg/l)	Recovery (%)	R.S.D. (%)	Conc. (μg/l)	Recovery (%)	R.S.D. (%)
Phenol[a]	2.33	86.6	4.0	1.16	93.8	10.0
4-Chloro-3-methylphenol[a]	2.28	102.9	4.4	1.14	102.1	2.1
4-Chlorophenol	2.28	96.1	8.8	1.14	92.8	2.6
Pentachlorophenol[a]	1.12	95.0	6.9	0.56	92.7	6.0
2,3,5,6,-Tetrachlorophenol	1.18	102.0	5.5	0.59	93.9	9.2
2,4,6-Trichlorophenol[a]	0.95	88.0	8.3	0.48	96.8	5.3
2,4-Dichlorophenol[a]	1.13	105.6	3.7	0.57	104.7	3.6
2-Methyl-4,6- dinitrophenol[a]	0.33	78.9	5.8	0.17	75.8	4.9
2,3,6-Trichlorophenol	1.07	96.8	2.5	0.54	99.7	4.4
2-Chlorophenol[a]	1.44	86.2	7.8	0.72	89.1	2.9
2,4-Dinitrophenol[a]	0.38	81.5	5.4	0.19	78.1	5.5
4-Nitrophenol[a]	0.30	97.6	11.1	0.15	100.0	6.6
2-Nitrophenol[a]	0.31	98.2	7.9	0.16	95.3	8.2

[a] EPA priority pollutants.

were all passed through the cartridges at a rate of 100 ml/min, so the preparation process is quite expeditious in relation to commonplace methods for determining phenols. Except for 2-methyl-4,6-dinitrophenol and 2,4-dinitrophenol, recoveries were in the region of 90% — estimated by measuring the sample absorbance at 230 nm and reading the corresponding concentration off a calibration curve constructed from standards.

Fig. 3 shows the electrophoregram for a 1-l sample of tap water that was spiked with a 0.5 μg/l concentration of each analyte. The ability to record

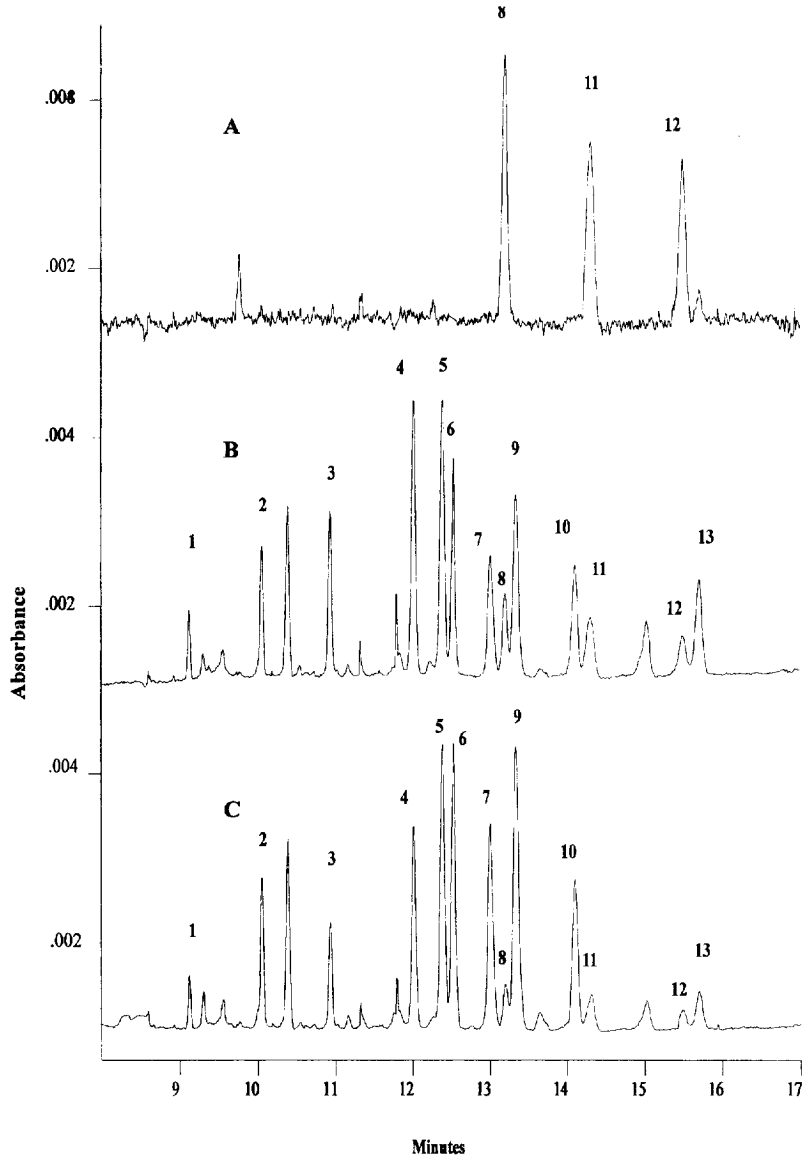

Fig. 3. Electrophoregram for a 1000-ml tap water sample spiked with the phenols studied: 1=Phenol (1.16 μg/ml), 2=4-chloro-3-methylphenol (1.14 μg/ml), 3=4-chlorophenol (1.14 μg/ml), 4=pentachlorophenol (0.56 μg/ml), 5=2,3,5,6-tetrachlorophenol (0.59 μg/ml), 6=2,4,6-trichlorophenol (0.48 μg/ml), 7=2,4-dichlorophenol (0.57 μg/ml), 8=2-methyl-4,6- dinitrophenol (0.17 μg/ml), 9=2,3,6-trichlorophenol (0.54 μg/ml), 10=2-chlorophenol (0.72 μg/ml), 11=2,4-dinitrophenol (0.19 μg/ml), 12=4-nitrophenol (0.15 μg/ml), and 13=2-nitrophenol (0.16 μg/ml). Signals at 370 nm (A), 230 nm (B) and 210 nm (C).

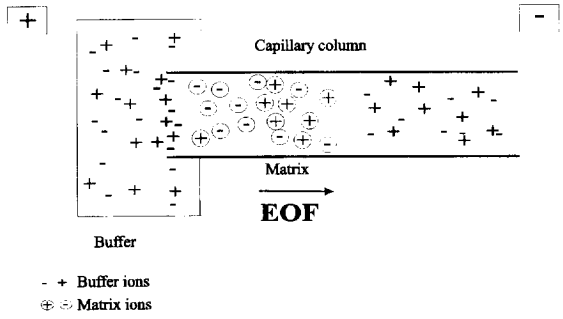

Fig. 4. Scheme of the situation inside the capillary while positive polarity is maintained following injection of a large sample volume in the stacking mode.

the absorbance of the analytes at different wavelengths facilitates their identification and ensures the obtainment of the stated limits of quantitation. Highly polluted samples will give a large number of peaks. By recording electropherograms at the three wavelengths and comparing the spectrum for each peak with that for a pure standard, one can reliably confirm identifications.

3.4. Interpretation of the anomalous behaviour of some phenols

As can be seen in Fig. 2, the peak area for phenol, 4-chloro-3-methylphenol and 4-chlorophenol did not increase with increasing injection time in the same proportion as those for the other compounds studied. In fact, these three phenols exhibited the shortest migration times, so their electrophoretic flows —in the opposite direction to the electroosmotic flow in a capillary with an untreated surface— ran at a lower rate than those of the other phenols studied. We may thus hypothesize that, when the polarity is reversed, the EOF itself not only causes the matrix to be removed, but also the loss of some molecules of the three compounds at the injection end of these anomalous phenols cannot migrate rapidly enough to

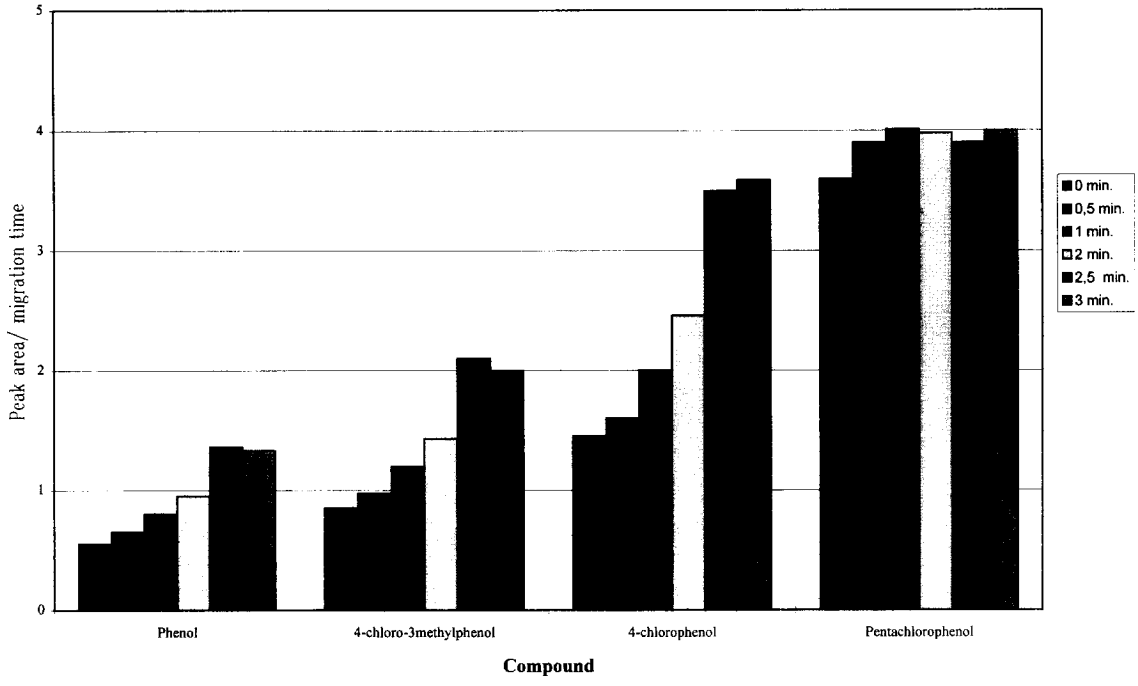

Fig. 5. Variation of peak area as a function of the time during which positive polarity is maintained prior to matrix removal. Results obtained for a methanol–water (6:4, v/v) standard containing phenol (116.4 μg/l), 4-chloro-3-methylphenol (114.1 μg/l), 4-chlorophenol 114.1 μg/l) and pentachlorophenol (55.8 μg/l), injected for 10 min.

the separation zone between the buffer and the methanol–water matrix.

In order to confirm this hypothesis, a standard solution of phenol, 4-chloro-3-methyl phenol, 4-chlorophenol and pentachlorophenol was prepared in methanol–water (6:4, v/v). Pentachlorophenol did not exhibit the effect studied, so it functioned as a reference for injections. The standard was repeatedly injected and after the capillary was filled with sample, positive polarity was maintained over variable intervals prior to reversal and matrix removal. The aim of these operations was to favour stacking by facilitating progress of the sample zone within the capillary before the stacking step proper was started.

With positive polarity, the current was very low and the EOF swept the matrix to the detector, as shown in Fig. 4. Under the working conditions used, the longest time positive polarity could be maintained was 3 min. Longer times resulted in some sample matrix being lost at the detection end of the capillary.

As the polarity was reversed, the EOF swept the matrix to the capillary end through which it was injected. Obviously, the time needed to remove the matrix under these conditions was longer than when the polarity was reversed immediately after injection, so the anions of phenol, 4-chloro-3-methylphenol and 4-chlorophenol had a long enough time to concentrate in the separation zone between the matrix and buffer, thereby avoiding being swept out of the capillary.

Fig. 5 shows the variation of the peak area for the compounds studied as a function of the time during which positive polarity was maintained after injection. While the peak area for pentachlorophenol (the reference) was not affected, those for the other compounds increased up to 2.5 min, when it levelled off. A similar experiment conducted at 25 rather than 30 kV led to identical results. This confirms that the extent to which the concentration of compounds with a low electrophoretic mobility in the capillary can be increased using sample stacking injection is restricted by the resulting losses through the injection end.

Therefore, if positive polarity is maintained for 2.5–3 min before the matrix is removed, the limits of quantitation for phenol, 4-chloro-3-methylphenol and 4-chlorophenol are lowered by a factor of 2–3 (Fig. 5). However, this additional step in the sample injection — capillary concentration process substantially increases the migration time for all the compounds studied, so pentachlorophenol and the analytes with the longer migration times give rise to significantly broader peaks (Fig. 6) that may eventually split if positive polarity is maintained for more than 2.5 min.

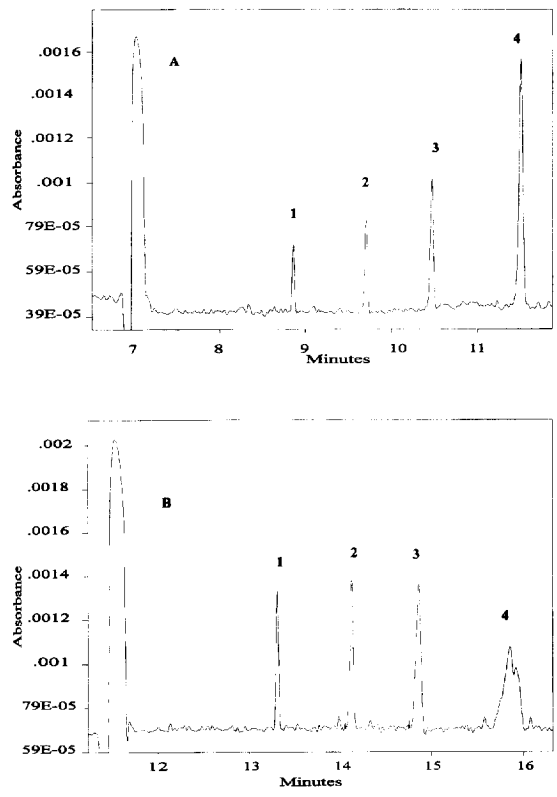

Fig. 6. Electrophoregram for a standard of phenols obtained with sample stacking injection under two different types of conditions: (A) with change to negative polarity immediately after the capillary is loaded; and (B) with positive polarity for 2.5 min after the capillary is loaded and then negative polarity. 1=Phenol (116.4 μg/l), 2=4-chloro-3-methyl phenol (114.1 μg/l), 3=4-chlorophenol (114.1 μg/l), and 4=pentachlorophenol (55.8 μg/l).

4. Conclusions

The use of cross-linked polystyrene cartridges allows water sample volumes of 1 l to be concen-

trated in 10 min, with excellent recoveries for all the phenols studied. Use of capillary electrophoresis in the sample stacking injection mode allows the phenols to be resolved in 16 min and determined at concentrations below 0.5 μg/l with straight capillaries. If needed, the limits of quantitation for phenol, 4-chloro-3-methylphenol and 4-chlorophenol (higher than those for the other analytes) can be lowered by including an additional step in the stacking process (viz. pre-stacking with positive polarity for 2.5–3 min). However, such a step is better performed in a second, separate injection in order not to detract from the analytical figures of merit for the other phenols.

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