ELSEVIER

Journal of Chromatography A, 896 (2000) 95–104

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Highly selective and efficient determination of US Environmental Protection Agency priority phenols employing solid-phase extraction and non-aqueous capillary electrophoresis

S. Morales, R. Cela*

Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Química, Universidad de Santiago de Compostela, Avda de las Ciencias s/n, 15706 Santiago de Compostela, Spain

Abstract

Non-aqueous capillary electrophoresis has been used in the separation of a complete list of 26 priority phenols included in the 8041 US Environmental Protection Agency method and the 76/464/EEC European Union directive. A highly selective and efficient separation was obtained when the background electrolyte used was 150 m*M* ammonium acetate dissolved in *N*-methylformamide–acetonitrile (75:25). Solid-phase extraction was successfully assayed as an enrichment strategy for the analysis of low-concentration samples. A styrene–divinylbenzene functionalized cartridge provided excellent recoveries of phenols from water samples at neutral pH. The limits of quantification obtained permit the application of the proposed method to the determination of priority phenols in wastewater samples.

Keywords: Non-aqueous capillary electrophoresis; Buffer composition; Phenols; Methylformamide; Chlorophenols; Nitrophenols

1. Introduction

Nowadays there is no reason to consider water as the only useful solvent in capillary electrophoresis (CE). Organic solvents have been used successfully in background electrolytes, extending the application range of the technique. Non-aqueous capillary electrophoresis (NACE) may offer advantages in many experimental parameters including a wider range of acid–base properties, increased solubility for compounds that are not readily soluble in water, reduced sorption of hydrophobic substances onto capillary walls and reduced Joule heating.

If it is considered that the properties of the electrophoretic medium are frequently adjusted by the addition of salts and inorganic acids or bases, then the attainable diversity in background electrolyte is limited and very much determined by the physico–chemical parameters of the solvent. Therefore, by changing the solvent, all-important features of CE separations related to the background electrolyte can be influenced. Resolution, selectivity, efficiency and analysis time can be readily adjusted by selecting the right solvent or solvent mixture [1]. Solvation of solutes should be considerably different from one solvent to another, changing the size of the solvated particles and their acid–base properties, expressed as a variation in pK_a values [2]. The solvent also alters the composition of the electric double-layer near the capillary wall and around the analyte and this affects the zeta potential [3]. All

*Corresponding author. Tel.: +34-81-563-100; fax: +34-81-547-141.

E-mail address: qnrctd@usc.es (R. Cela).

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00581-1

these factors may have profound effects on the electrophoretic separation.

In selecting the proper electrophoretic medium, several characteristics of the solvent should be considered including viscosity (η), dielectric constant (ϵ), electrical and thermal conductivity, self-dissociation constant, polarity and boiling point [4]. Methanol [5,6], acetonitrile [7,8] and its combinations [9–13] formamide [14,15], dimethylformamide [16,17], *N*-methylformamide (NMF) [3,18], dimethylacetamide [19], and dimethyl sulfoxide [20–23] have been used and probably many other solvents could be applied in CE separations.

However, *N*-monosubstituted amides are especially attractive since these solvents show unusually high dielectric constants, particularly NMF with a relative dielectric constant of 182 (at room temperature) compared to 80 for water. It is well known that solvents with high ϵ^2/η values may provide better efficiency, according to Jansson and Roeraade [24] who described the efficiency by unit of time (N/t) as:

$$\frac{N}{t} \propto \frac{r}{kT\eta} \cdot (2\zeta_{\text{ion}} - 3\zeta_{\text{wall}})^2 \cdot (\epsilon_0 \epsilon_r E)^2 \quad (1)$$

where r is the Stokes radius of the analyte, k is the Boltzmann constant, T is the temperature, η is the viscosity, ϵ_0 is the permittivity in vacuum, ζ_{ion} and ζ_{wall} are the zeta potentials of the analyte and capillary wall, respectively, ϵ_r is the relative dielectric constant of the solvent and E is the electric field strength.

In this sense, NMF – having an approximately threefold higher ϵ^2/η value than water – could be one of the preferred separation media, thus allowing for potentially faster migrations. Other advantageous properties of this solvent are its high solubilizing power, low conductivity and its amphiprotic character [24].

Generally, organic solvents absorb light in the UV region more than water does; this is clearly a disadvantage for NMF and related solvents used in CE. Indirect UV detection and alternative detection methods have been used in order to overcome the strong background absorbance of organic electrolytes [25,10].

Phenolic compounds were classified as priority pollutants owing to their high toxicity and widespread environmental occurrence [26,27]. These compounds have been widely analyzed by CE techniques using aqueous electrolyte [28,29] and buffer additives [23,30–33]. None of these studies included the complete list of phenolic derivatives to be analyzed following the 8041 US Environmental Protection Agency (EPA) method and the 76/464/EEC European Union directive, concerning dangerous substances discharged into the aquatic environment.

Few studies have been done on the application of NACE to phenols determination in environmental samples [34]. Okada [35] used an acetonitrile-based electrophoretic media for the quantitative study of heteroconjugated anion formation of Brønsted acids, such as phenols, with some simple anions.

On the other hand, to reach the levels specified by regulatory organizations for monitoring priority pollutants, a pre-concentration step must be frequently applied in order to improve the low sensitivity of traditional UV detection, determined by the short optical path length of the detection cell (capillary inner diameter).

Solid-phase extraction (SPE) and liquid–liquid extraction (LLE) have been successfully used for off-line enrichment of diluted samples before electrophoretic determination. Even today, LLE is the recommended procedure in standard and official methods for determination of phenols in water [26]. However, SPE and solid-phase microextraction (SPME) are replacing LLE due to their advantages in terms of solvent consumption, analysis time and possibilities of integration into on-line systems [36].

This paper reports a method for the determination of a wide range of phenolic derivatives (EPA Method 8041 and 76/464/EEC priority phenols) in wastewater. The method combines SPE of phenolic compounds and UV in a NACE system. Two SPE cartridges and three eluents were assayed. Better results were obtained when the CE background electrolyte was used as the solid-phase eluent; it makes the extraction process compatible with the analytical system.

2. Experimental

2.1. Chemicals

Acetonitrile (HPLC grade), 2-methyl-4,6-dinitrophenol, hydrochloric acid and 2,4-dichlorophenol

were obtained from Merck (Merck, Darmstadt, Germany). NMF, ammonium acetate, 4-nitrophenol, 2-chlorophenol, 2,6-dichlorophenol, 2,4,5-trichlorophenol, pentachlorophenol, phenol, 4-chloro-3-methylphenol, 3-methylphenol, 2,4-dimethylphenol, 2-nitrophenol, 2,4-dinitrophenol, 2-amino-4-chlorophenol, 3-chlorophenol, 2,3,5-trichlorophenol and 4-chlorophenol were obtained from Aldrich (Madrid, Spain), 2-methylphenol and 4-methylphenol were from Fluka (Buchs, Switzerland).

Dinoseb was obtained from Sigma (Madrid, Spain); 2,3,4,5-trichlorophenol was from Supelco (Bellefonte, PA, USA); 2,3,4,6-tetrachlorophenol, 2,4,6-trichlorophenol, 2,3,4-trichlorophenol, 2,3,6-trichlorophenol and 2,3,5,6-tetrachlorophenol were from Riedel-de Haën (Seelze, Germany). Sodium hydroxide was supplied by BDH (Poole, UK). Ultrapure water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA).

Stock solutions of each phenol derivative were prepared at 2000 mg/l. Chemical standards were dissolved in the solvent or solvent mixture corresponding to the electrophoresis medium. All solutions were refrigerated at 4°C and protected against daylight. These solutions were used to make working standards solutions by appropriate dilution.

2.2. Capillary electrophoresis

CE was performed using a HP^{3D} system (Hewlett-Packard, Waldbronn, Germany) equipped with an on-column diode array detection (DAD) system. A detection wavelength of 280 nm (direct UV detection) was used for all samples unless otherwise stated.

Uncoated narrow-bore silica capillaries (supplied by Composite Metal Services, UK) of 70 cm (effective length 62.5 cm)×75 μm I.D. were used. The capillary was thermostated to 20°C. A Techne RB-5A external water bath was used for thermostatting the samples to 20°C.

Samples were injected by applying a pressure of 50 mbar for 5 s unless otherwise stated. The applied voltage for separation was 30 kV.

New capillaries were rinsed with 1 *M* sodium hydroxide for 20 min. Before injections, capillaries were conditioned by washing with 0.1 *M* sodium hydroxide for 5 min, water for 5 min, organic solvent corresponding to the electrophoresis medium for 10 min and 20 min with the separation electrolyte. After each run – once the electrophoretic separation has finished – the capillary was flushed for 5 min with water.

NMF and its mixtures with acetonitrile and methanol were assayed as solvent for non-aqueous background electrolyte preparation. Ammonium acetate, being readily soluble in all solvents investigated, was used as electrolytic salt.

All solutions were filtered before use through a membrane of 0.22 μm pore size.

2.3. Solid-phase extraction

Water samples were preconcentrated using either an Oasis HLB (0.060 g) cartridge obtained from Waters (USA) or an Isolute ENV+ (0.20 g) cartridge from International Sorbent Technology (IST House, UK), both polymeric sorbents with a styrene–divinylbenzene (PS–DVB) structure.

Before use, each cartridge was conditioned by flushing with 3 ml of NMF–acetonitrile (75:25) and activated with Milli-Q water, either at pH 2.5 (Isolute) or neutral pH (Oasis).

Three solvents or solvent mixtures were assayed as eluents: (a) pure acetonitrile (ACN), (b) NMF–ACN (75:25) and (c) the CE electrolyte: 150 m*M* ammonium acetate dissolved in NMF–acetonitrile (75:25). In all cases, in order to determine the optimum elution volume, each potential eluent was subdivided in 1-ml fractions and the individually collected eluate fractions subjected to CE analysis to determine the solute content.

2.4. Sample preparation

A known volume of Milli-Q water or prefiltered wastewater (up to 500 ml) was spiked with 10 μg/l of phenolic standards. For the extraction on the Isolute cartridge, the pH of spiked sample was adjusted to 2.5 with hydrochloric acid. No pH adjustment was necessary with the Oasis cartridge.

Once the sample has passed through, the cartridges were dried for 20 min in a nitrogen stream and eluted with 1 ml of the proper eluent. This final extract was directly injected into the CE system.

Recoveries were calculated by relating the concentration determined in the sample water versus the

calibration standards prepared in CE background electrolyte.

3. Results and discussion

3.1. Capillary electrophoresis

Previous work on the separation of complex mixtures of phenolic compounds in NACE [34] was used to set some experimental parameters such as capillary characteristics (diameter and length) and conditioning sequence. Owing to the sample complexity, 26 compounds, a 70 cm×75 μm I.D. capillary was chosen. For best UV detection limits, inner diameters from 50 to 100 μm are recommended [37].

For capillary wall conditioning, as described in the Experimental section, a simple sequence, similar to that used in aqueous systems, was used. Special care should be taken of the compatibility between aqueous and organic solutions used for the conditioning process. This sequence, consisting in successively flushing with 0.1 *M* NaOH aqueous solution, organic solvent and a step with electrolyte solution has proven to provide a reproducible capillary conditioning process [34]. Once these operational parameters have been established, all efforts were focussed on the background electrolyte composition.

In NACE, the nature and properties of the organic solvent have the strongest influence on the separation efficiency and resolution. Advantageous properties of NMF such as its high dielectric constant, solubilizing power, amphiprotic character and low conductivity make this solvent very interesting for non-aqueous electrophoretic separations.

The number of resolved peaks measured the effectiveness of NMF-based electrolytes in the separation of the 26-component mixture.

Pure NMF, without added electrolyte, was assayed and even at the highest attainable field strength (400 V/cm) the system maintained a stable current level and no signs of electrical breakdown were observed. Nevertheless, poor resolution was obtained; only 12 peaks were resolved from the total mixture. Further additions of ammonium acetate – from 50 to 200 m*M* – and capillary temperature variations – from 15 to 35°C – did not significantly improve the resolution.

Better results were reached by combining NMF with acetonitrile and/or methanol at different concentrations of ammonium acetate. As could be expected, the resolution improved with the increment in the ionic strength whereas – by the same reason – the solute mobility decreased.

As experimental results suggested (electropherograms not shown) the highest resolution may be obtained with ionic strength values from 100 to 200 m*M* and about 75% NMF in a binary mixture of solvents. Highest ionic strength leads to loss of resolution and increased baseline noise, presumably as a consequence of Joule heating, since the current increases in direct proportion to the ionic strength. Ternary mixtures of solvents did not provide better resolution.

An excellent resolution was achieved by using 150 m*M* ammonium acetate in NMF–acetonitrile (75:25) as the background electrolyte. As shown in Fig. 1, which corresponds to the injection of the mixture of 26 phenolic derivatives, the system successfully resolves 23 peaks. Under these conditions, only three ''couples'' of compounds co-migrate: *o*-cresol–*p*-cresol, 4-chloro-3-methylphenol–2-amimo-4-chlorophenol and 2-chlorophenol–3-chlorophenol.

Attending to the sample complexity – 26 components – and the structural similarity between some of the priority phenolic derivatives – several are positional isomers with similar charge to mass ratios – it is evident that the use of non-aqueous background electrolytes provides a surprising resolution power for electrophoretic separations. For the compounds under study the proposed method reaches a level of resolution not seen previously even in chromatographic separations. In this sense, NACE may be a good alternative to the official gas chromatographic determination of priority phenols [38].

The results obtained from the study of the linearity of the response for each compound, limits of detection (LODs) and quantification (LOQs) and response repeatability of the method are summarized in Tables 1 and 2. Within the concentration range studied (2–10 mg/l for compounds 1–23 and 0.5–3 mg/l for compounds 24–26) there was a good correlation between normalized peak area and concentration for every compound.

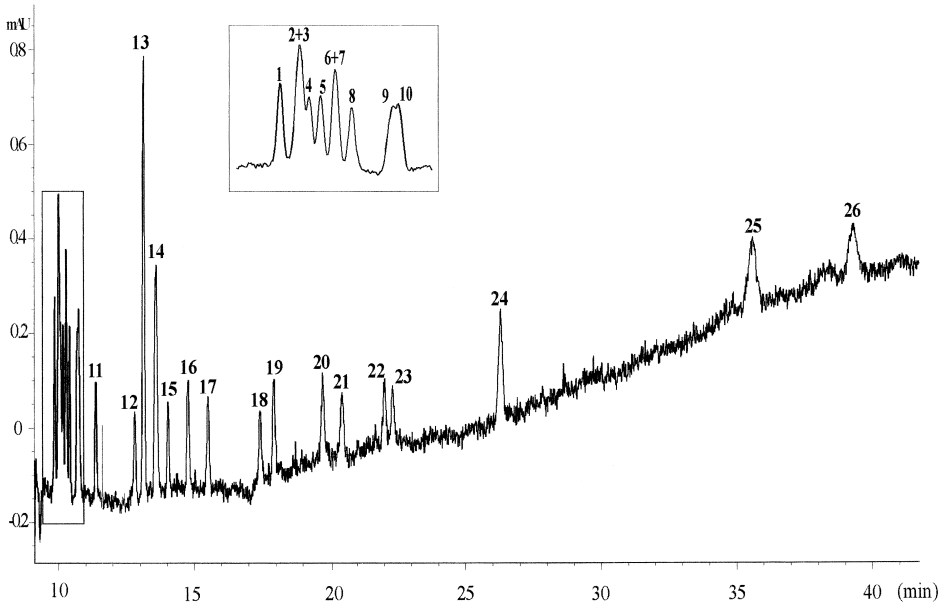

Fig. 1. Electropherogram of the mixture of 26 phenols in the study (5 mg/l of each compound) diluted with electrolyte. Injection: hydrodynamic (50 mbar by 5 s); running buffer: 150 m*M* ammonium acetate dissolved in NMF–acetonitrile (75:25); capillary: 70 cm×75 μm I.D.; applied voltage: 30 kV. $I=88$ μA. For peak numbers see Table 1.

As NMF strongly absorbs low-wavelength UV radiation, 280 nm was chosen for analyte detection. For dinoseb, 2-methyl-4,6-dinitrophenol and 2,4-dinitrophenol detection at 370 nm provides higher sensitivity, so the wavelength of 370 nm was chosen for their determinations.

Repeatability was examined by performing six replicate injections of each compound at a concentration corresponding to the 5 mg/l. For peak area, the relative standard deviation (RSD) was between 2.7 and 12.8%.

LODs and LOQs were calculated considering signal-to-noise ratios (S/N) of 3 and 10, respectively.

3.2. Solid-phase extraction

Retention of phenols from water samples in a PS–DVB polymeric cartridge takes place by a reversed-phase mechanism based on Van der Waals interactions and also by π–π interactions.

Functionalized polymeric sorbents incorporate additional mechanisms – ion-exchange and hydrogen bond formation – improving the retention of polar phenols. However, few details have been published about the extension of the derivatization process and its influence on the retention of phenols [39–41].

In this paper two PS–DVB polymeric cartridges – Isolute and Oasis – were assayed in the concentration process of water samples spiked with priority phenolic derivatives.

3.2.1. Cartridge elution

Optimal elution volumes were determined as described in the Experimental section: the lack of analyte signals in any eluate fraction signify the complete elution with previous fractions.

Volumes of 2 ml for Isolute and 1 ml for Oasis of all three potential eluents were found to thoroughly elute the phenolic compounds from the respective cartridge. Nevertheless, both ACN and NMF–ACN (75:25) were discarded. Once injected into the CE system, the low conductive acetonitrile-based eluate leads to current breakdown whereas the NMF–ACN mixture induces peak shape changes in some compounds, perhaps as a consequence of pH differences between the CE background electrolyte and the eluate.

It was proven that the above-mentioned volumes

Table 1
Regression analysis

Compound no.	Name	Slope ($\cdot 10^{-2}$)	Slope standard error ($\cdot 10^{-4}$)	Intercept ($\cdot 10^{-3}$)	Intercept standard error ($\cdot 10^{-3}$)	Correlation coefficient (r)
1	2,4-Dimethylphenol	3.87	6.50	2.05	4.81	0.9995
2	*o*-Cresol	2.89	12.32	14.16	9.12	0.9973
3	*p*-Cresol	3.60	7.62	−1.31	3.79	0.9995
4	*m*-Cresol	3.39	14.74	23.09	7.28	0.9981
5	Phenol	2.81	9.18	8.47	6.79	0.9984
6	2-Amino-4-chlorophenol	2.74	11.44	14.84	9.39	0.9983
7	4-Chloro-3-methylphenol	2.59	9.82	−9.24	4.85	0.9986
8	4-Chlorophenol	2.55	13.05	15.94	9.66	0.9961
9	2-Chlorophenol	3.43	15.55	1.46	11.51	0.9969
10	3-Chlorophenol	2.88	7.11	−2.46	3.51	0.9994
11	2,4-Diclorophenol	2.22	7.13	13.03	5.28	0.9985
12	2,3,4-Trichlorophenol	1.65	5.12	2.07	2.53	0.9990
13	4-Nitrophenol	4.94	11.62	−13.77	5.73	0.9994
14	2-Nitrophenol	6.80	19.44	−6.80	14.39	0.9988
15	2,4,5-Trichlorophenol	1.57	3.98	7.01	1.96	0.9994
16	2,6-Dichlorophenol	2.24	4.80	10.43	3.55	0.9993
17	2,3,5-Trichlorophenol	1.74	8.84	11.02	6.54	0.9962
18	2,3,4,5-Tetrachlorophenol	0.89	1.56	20.91	1.15	0.9995
19	2,4,6-Trichlorophenol	1.24	2.61	17.47	1.49	0.9996
20	2,3,6-Trichlorophenol	1.13	4.59	12.07	2.62	0.9984
21	2,3,4,6-Tetrachlorophenol	0.98	5.46	18.33	3.11	0.9969
22	2,3,5,6-Tetrachlorophenol	0.87	5.83	20.98	3.32	0.9955
23	Pentachlorophenol	0.74	4.41	4.84	2.18	0.9964
24[a]	Dinoseb	10.43	53.21	4.85	10.04	0.9974
25[a]	2-Methyl-4,6-dinitrophenol	10.35	47.01	2.08	8.87	0.9974
26[a]	2,4-Dinitrophenol	10.25	41.08	8.64	7.75	0.9984

[a] For compounds 24, 25 and 26: detection at 370 nm.

of CE electrolyte effectively remove phenolic compounds from these cartridges and ensure the compatibility between extraction and analytical determination phases. In all subsequent assays, the CE electrolyte – 2 ml for Isolute and 1 ml for Oasis – was used as eluent.

3.2.2. Influence of the acidity of the matrix sample

Milli-Q water – spiked with all phenols under study – and Isolute polymeric cartridges were used in preliminary extraction assays. As the analyte's pK_a values recommend, the pH of the water samples was adjusted to 2.5 in order to enhance the phenol retention. However, this procedure provides an acidic character to the eluate which induces changes in the degree of ionization of phenols and in their UV spectrum. This effect may lead to an erroneous interpretation of recovery results obtained by methods such as CE, based on UV detection; in order to determine the content of phenols in a extract, both the eluate and the calibration standard should have equivalent pH values.

Fig. 2 shows the effect of the pH on peak areas of three nitrophenols: dinoseb, 2-methyl-4,6-dinitrophenol and 2,4-dinitrophenol. Electropherograms – registered at 370 nm – were obtained from two parallel samples at a concentration of 4 mg/l for each compound, dissolved in CE electrolyte. Sample B contains 10% (v/v) of 1 *M* hydrochloric acid whereas an equivalent volume of water was added to sample A. It is evident that UV spectra of nitrophenols are pH-dependent. Despite the fact that both samples were prepared at the same concentration, peak areas in case B are almost twice as large as those in case A.

However, adjusting pH values in organic media is a relatively complicated task, so SPE without any modification of matrix acidity was assayed.

Table 2
Limits of determination and response repeatability

Compound no.	Limit of quantification (μg/l) $S/N=10$	Limit of detection (μg/l) $S/N=3$	Peak area repeatability RSD (%)
1	890	267	6.8
2	1038	311	2.8
3	863	258	2.7
4	1320	396	10.9
5	1161	348	7.6
6	2099	629	6.7
7	1330	399	6.6
8	1140	342	10.3
9	989	296	9.5
10	1050	315	9.6
11	1120	336	10.5
12	1419	425	10.5
13	563	168	11.5
14	364	109	11.6
15	1142	342	7.7
16	851	255	10.9
17	922	276	10.9
18	950	285	12.8
19	800	240	6.7
20	949	284	8.5
21	950	285	6.8
22	782	234	5.5
23	1298	389	12.3
24[a]	142	42	5.7
25[a]	94	28	10.2
26[a]	93	28	11.9

[a] For compounds 24, 25 and 26: detection at 370 nm.

It was supposed that the two cartridges – both containing functionalized polymeric sorbents – would be capable of retaining phenols even at neutral pH. However low recovery values – less than 60% – were found with the Isolute cartridge. Better results were obtained when samples of Milli-Q water spiked with all phenols under study were concentrated on the Oasis cartridge.

Table 3 shows average recoveries of phenols from two independent samples of Milli-Q water (500 ml each one) spiked with 10 μg/l of each compound. Recoveries in the table are the means of individual values calculated for every compound once both samples had been analyzed.

Only two compounds – phenol and 2,4-dinitrophenol – have recoveries below 80%. Losses of these compounds come from cartridge breakthrough with such sample volume. By using a tandem of two Oasis cartridges, the latter was found to contain traces of both compounds, although the peaks were not strong enough to reach the LOQ.

Losses due to incomplete elution were discarded owing to the lack of analyte signal in the second eluate fraction from the first cartridge.

In this way, it was proven that effective retention of phenolic compounds on the Oasis cartridge could take place even without adjusting the pH value of the aqueous sample matrix.

3.2.3. Limits of quantification

Table 2 shows the LOQs ($S/N=10$) obtained from direct injections of standards under the conditions specified in the Experimental section. For spiked samples of Milli-Q water, LOQs were determined by dividing such values into the highest concentration factor obtained with the SPE on the Oasis cartridge (500:1). These LOQs are shown in Table 4.

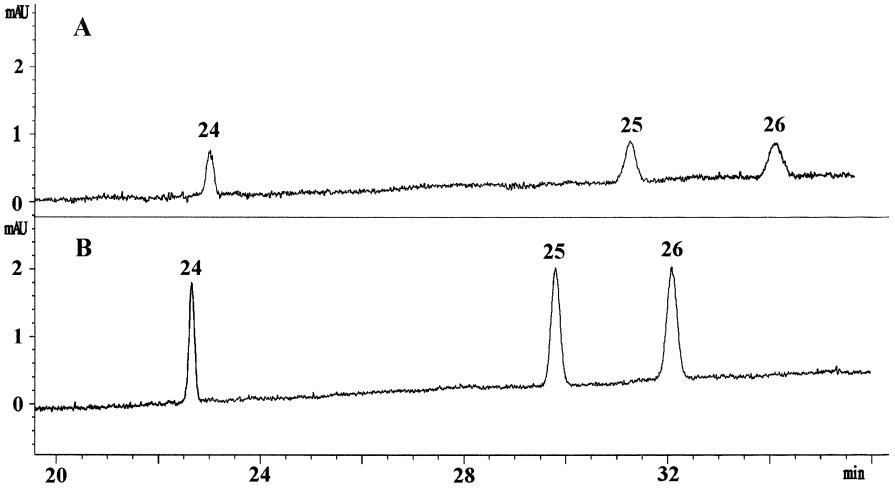

Fig. 2. Electropherogram of three nitrophenols at different pH. Concentration: 4 mg/l of each compound; (A) contains 10% (v/v) of water and (B) 10% (v/v) of 1 *M* hydrochloric acid. Injection: hydrodynamic (50 mbar by 5 s); running buffer: 150 m*M* ammonium acetate in NMF–acetonitrile (75:25); capillary: 70 cm×75 μm I.D.; applied voltage: 30 kV. $I=88$ μA. Detection at 370 nm. For peak numbers see Table 1.

Table 3
Recoveries of phenols on the Oasis cartridge from spiked samples of Milli-Q water at neutral pH

Compound no.	Added (μg/l)	Recovery (%)	RSD (%)
1	10	99.4	11.5
2	10	109.7	4.6
3	10	103.5	10.3
4	10	96.9	7.2
5	10	70.1	2.2
6	10	83.4	7.6
7	10	89.8	13.9
8	10	100.9	3.2
9	10	98.3	8.4
10	10	108.0	3.9
11	10	95.8	4.8
12	10	97.5	10.9
13	10	109.3	8.6
14	10	100.6	8.4
15	10	102.8	3.3
16	10	99.4	2.6
17	10	104.1	5.1
18	10	94.1	4.4
19	10	102.2	11.2
20	10	106.1	8.4
21	10	116.3	7.7
22	10	108.5	9.2
23	10	99.9	9.9
24[a]	10	103.5	5.9
25[a]	10	120.5	7.1
26[a]	10	63.9	7.1

[a] For compounds 24, 25 and 26: detection at 370 nm.

Table 4
Limits of quantification in water samples

Compound no.	LOQ (μg/l) in water samples
1	1.8
2	2.1
3	1.7
4	2.6
5	2.3
6	4.2
7	2.7
8	2.3
9	2.0
10	2.1
11	2.2
12	2.8
13	1.1
14	0.73
15	2.3
16	1.7
17	1.8
18	1.9
19	1.6
20	1.9
21	1.9
22	1.6
23	2.6
24[a]	0.28
25[a]	0.19
26[a]	0.19

[a] For compounds 24, 25 and 26: detection at 370 nm.

3.2.4. Analysis of real samples

Volumes of 500 ml of previously filtered wastewater were spiked with a mixture of 12 phenolic derivatives at 10 μg/l for each one. Spiked samples were subjected to SPE on Oasis cartridges at neutral pH. The cartridge was eluted with 1 ml of CE electrolyte and the extract analyzed by NACE. Recoveries were calculated by relating the phenols content of these extracts versus calibration standards prepared in CE electrolyte. In the electrophoretic determination every extract and calibration standard was injected in triplicate.

Fig. 3 depicts an electropherogram obtained from a spiked wastewater sample.

Table 5 shows average recoveries of these 12 phenolic compounds from two independent spiked samples of wastewater. Each value is the mean of individual recoveries calculated for every sample.

When real water samples are analyzed, a decrease in recovery values is frequently observed when compared to those obtained with artificial samples, which are prepared with ultrapure water. Sample matrix components – mainly fulvic and humic acids – are normally responsible for this effect as they can saturate the load capacity of the sorbent.

However, recovery values summarized in Table 5 are in a reasonable agreement with those obtained from Milli-Q water (Table 3). It indicates that the sample matrix did not substantially affect the retention of phenols at neutral pH in the Oasis cartridges. As Di Corcia and co-workers [42–44] reported, humic and fulvic substances are retained more in polymeric sorbents at lower pH values than when working at neutral pH, as the extraction process is less prone to the interference of these substances.

Table 5
Recoveries of phenols from spiked samples of wastewater

Compound no.	Added (μg/l)	Recovery (%)	RSD (%)
7	10	109.2	13.9
10	10	108.4	1.3
11	10	109.3	13.9
13	10	96.2	3.9
15	10	109.7	4.1
17	10	109.9	10.4
19	10	105.8	8.8
20	10	106.9	6.4
23	10	97.8	17.5
24[a]	10	113.4	1.5
25[a]	10	104.2	11.4
26[a]	10	65.3	3.9

[a] For compounds 24, 25 and 26: detection at 370 nm.

4. Conclusions

The proposed methodology is a good alternative to official methods for the determination of priority

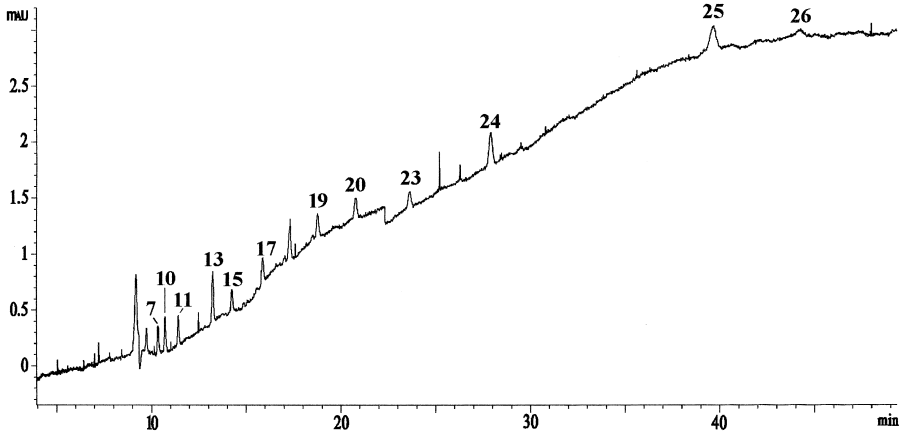

Fig. 3. Electropherogram of a spiked wastewater sample. Concentration: 10 μg/l of each compound. Injection: hydrodynamic (50 mbar by 5 s); running buffer: 150 m*M* ammonium acetate in NMF–acetonitrile (75:25); capillary: 70 cm×75 μm I.D.; applied voltage: 30 kV. $I=88$ μA. Detection at 280 nm. For peak numbers see Table 1.

phenols in wastewater samples in quantities below the levels permitted by legislation (0.5 mg/l).

The NMF-based electrolyte provides a surprising resolution to the electrophoretic separation of a complex mixture of phenolic derivatives: 23 from 26 compounds can be readily separated.

Solid-phase polymeric functionalized cartridges (Oasis) permit the extraction of priority phenols from volumes of up to 500 ml of wastewater at neutral pH with a concentration factor of 500:1. Employing the CE electrolyte as eluent, the extracts can be directly injected into the CE system without any dilution or solvent change. It allows a good compatibility between extraction and electrophoretic processes.

Acknowledgements

S.M. acknowledges a long-term fellowship from the Spanish Agency for International Cooperation (Ministerio de Asuntos Exteriores). We thank Dr. I. Rodríguez (Universidad de Santiago de Compostela) for his fruitful comments on the optimization of the solid-phase extraction process.

References

[1] I.E. Valkó, S. Heli, M.J. Riekkola, LC·GC Int. March (1997) 190.
[2] K. Sarmini, E. Kenndler, J. Chromatogr. A 792 (1997) 3.
[3] I.E. Valkó, H. Sirén, M.L. Riekkola, Chromatographia 43 (1996) 242.
[4] I.E. Valkó, H. Sirén, M.L. Riekkola, J. Chromatogr. A 737 (1996) 263.
[5] T. Okada, J. Chromatogr. A 695 (1995) 309.
[6] H. Salimi-Moosavi, R.M. Cassidy, J. Chromatogr. A 790 (1997) 185.
[7] F.M. Matysik, J. Chromatogr. A 802 (1998) 349.
[8] J. Tjornelund, S.T. Hansen, J. Chromatogr. A 792 (1997) 475.
[9] J. Tjornelund, S.T. Hansen, J. Chromatogr. A 737 (1996) 291.
[10] H. Salimi-Moosavi, R.M. Cassidy, Anal. Chem. 68 (1996) 293.
[11] R. Carabias-Martínez, E. Rodríguez-Gonzalo, J. Domínguez, J. Hernández-Méndez, Anal. Chem. 69 (1997) 4437.
[12] H. Salimi-Moosavi, R.M. Cassidy, J. Chromatogr. A 749 (1996) 279.
[13] S.H. Hansen, I. Bjornsdottir, J. Tjornelund, J. Chromatogr. A 792 (1997) 49.
[14] Y. Mori, K. Ueno, T. Umeda, J. Chromatogr. A 757 (1997) 328.
[15] R.S. Sahota, M.G. Khaledi, Anal. Chem. 66 (1994) 1141.
[16] J. Tjornelund, S.T. Hansen, J. Chromatogr. A 779 (1997) 235.
[17] H. Salimi-Moosavi, R.M. Cassidy, Anal. Chem. 67 (1995) 1067.
[18] M. Jansson, J. Roeraade, Chromatographia 40 (1995) 163.
[19] I. Bjornsdottir, S.H. Hansen, J. Chromatogr. A 711 (1995) 313.
[20] V.L. Ward, M.G. Khaledi, J. Chromatogr. B 718 (1998) 15.
[21] F. Wand, M.G. Khaledi, J. Chromatogr. A 817 (1998) 121.
[22] P. Britz-Mckibbin, D.L. Y Chen, Anal. Chem. 70 (1998) 907.
[23] L. Xueliang, F. Hartmut, J. High Resolut. Chromatogr. 21 (1998) 309.
[24] M. Jansson, J. Roeraade, Chromatographia 40 (1995) 163.
[25] M. Chiari, E. Kenndler, J. Chromatogr. A 716 (1995) 303.
[26] Fed. Reg., EPA Method 604, Phenols, Part VIII, 40 CFR Part 136, Environmental Protection Agency, 26 October 1984, pp. 58–66.
[27] Fed. Reg., EPA Method 625, Base/Neutrals and Acids, Part VIII, 40 CFR Part 136, Environmental Protection Agency, 26 October 1984, pp. 153–174.
[28] D. Martínez, E. Pocurull, R.M. Marcé, F. Borrull, M. Calull, Chromatographia 43 (1996) 619.
[29] A. Hilmi, J.H.T. Luong, A.L. Nguyen, J Chromatogr. A 761 (1997) 259.
[30] S.M. Masselter, A.J. Zemann, Anal. Chem. 67 (1995) 1047.
[31] A. Zemann, D. Volgger, Anal. Chem. 69 (1997) 3243.
[32] F. Bedia Erim, J. Chromatogr. A 768 (1997) 161.
[33] G. Li, D.C. Locke, J. Chromatogr. A 734 (1996) 357.
[34] S. Morales, R. Cela, J. Chromatogr. A 846 (1999) 401.
[35] T. Okada, J. Chromatogr. A 771 (1997) 275.
[36] E.M. Thurman, M.S. Mills, Solid-Phase Extraction – Principles and Practice, Wiley, 1998.
[37] G.M. McLaughlin, A. Weston, K.D. Hauffe, J. Chromatogr. A 744 (1996) 123.
[38] EPA Method 8041, Phenols by Gas Chromatography, Environmental Protection Agency, December 1996.
[39] P.J. Dumont, J.S. Fritz, J. Chromatogr. A 691 (1995) 123.
[40] V. Piangerelli, F. Nerini, S. Cavalli, Ann. Chem. 83 (1993) 331.
[41] J.S. Fritz, P.J. Dumont, L.W. Schmidt, J. Chromatogr. A 691 (1995) 133.
[42] A. Di Corcia, A. Bellioni, M.D. Madbouly, S. Marchese, J. Chromatogr. A 733 (1996) 383.
[43] C. Crescenzi, A. Di Corcia, G. Passariello, R. Samperi, M.I. Turnes, J. Chromatogr. A 733 (1996) 41.
[44] A. Di Corcia, S. Marchese, R. Samperi, J. Chromatogr. A 642 (1993) 175.