Capillary electrophoresis separation of alkylphosphonic acid monoesters with indirect ultraviolet detection

J.-P. Mercier[a], Ph. Morin[a,*], M. Dreux[a], A. Tambute[b]

[a]Université d'Orléans, Institut de Chimie Organique et Analytique, CNRS UPRES-A 6005, B.P. 6759, 45067 Orléans Cedex 2, France
[b]Direction de la Recherche et de la Technologie, Centre d'Etudes du Bouchet, B.P. 3, 91710 Vert le Petit, France

Abstract

The separation of two series of alkylphosphonic acid monoesters (methyl and ethyl) by capillary electrophoresis (CZE) with indirect UV detection is described. In order to get short run times and high peak efficiencies the electroosmotic flow (EOF) is reversed by flushing the fused-silica capillary with 0.2% aqueous solution of polybren. The optimisation of several experimental parameters (running voltage, temperature) has been carefully investigated, and resolution, peak efficiency were studied. The signal-to-noise ratio and the repeatability of the EOF were investigated by adding hydroxylamine to the electrolyte system, to improve the buffer capacity. Thus, the electrolyte, composed of 5 mM sorbate anion (pH 6) as background absorbance co-anion and 1.6 mM hydroxylamine, allows the separation of these alkylphosphonic acid monoesters in less than 6 min. © 1997 Elsevier Science B.V.

Keywords: Ethylphosphonic acid monoesters; Methylphosphonic acid monoesters; Alkylphosphonic acids

1. Introduction

Analysis of alkylphosphonic acid monoesters is a topical question for environmental protection. Since they are breakdown products (Fig. 1) of chemical warfare agents such as G agents or VX, and since the convention on chemical weapons negotiated in Geneva in 1992 is close to being ratified, army inspectors need analytical methods to allow identification of these products. Moreover, phosphonic acids such as fosfomycin [2] were used as a bactericidal antibiotics in severe infections.

Several analytical methods have been studied with regard to their trace level determination in different matrices. The monoester derivatives are the first

compounds of hydrolysis of chemical warfare agents and alkylphosphonic acids (diacids) are the residue from the complete hydrolysis degradation. Ion liquid chromatography appears to be the main analytical method for the determination of phosphonic compounds in anionic form at a trace level. As they do not contain a UV-absorbing group, three detection modes were investigated: indirect UV spectrometry [3,4], fluorescence [5,6] and conductometry [7]. Other authors [8] have developed a chiral separation by liquid chromatography of (±)-VX. In order to lower the GC–MS detection limit, LC–MS and MS methods [9–11] were studied; a 50 pg detection limit was reached by using chemical derivatization.

Capillary electrophoresis has been reported in several papers for the analysis of these solutes. Pianetti et al. [12] resolved in 10 min a mixture of

*Corresponding author.

1. **2.** **3.**

4. **5.** **6.**

7. **8.** **9.**

10. **11.** **12.**
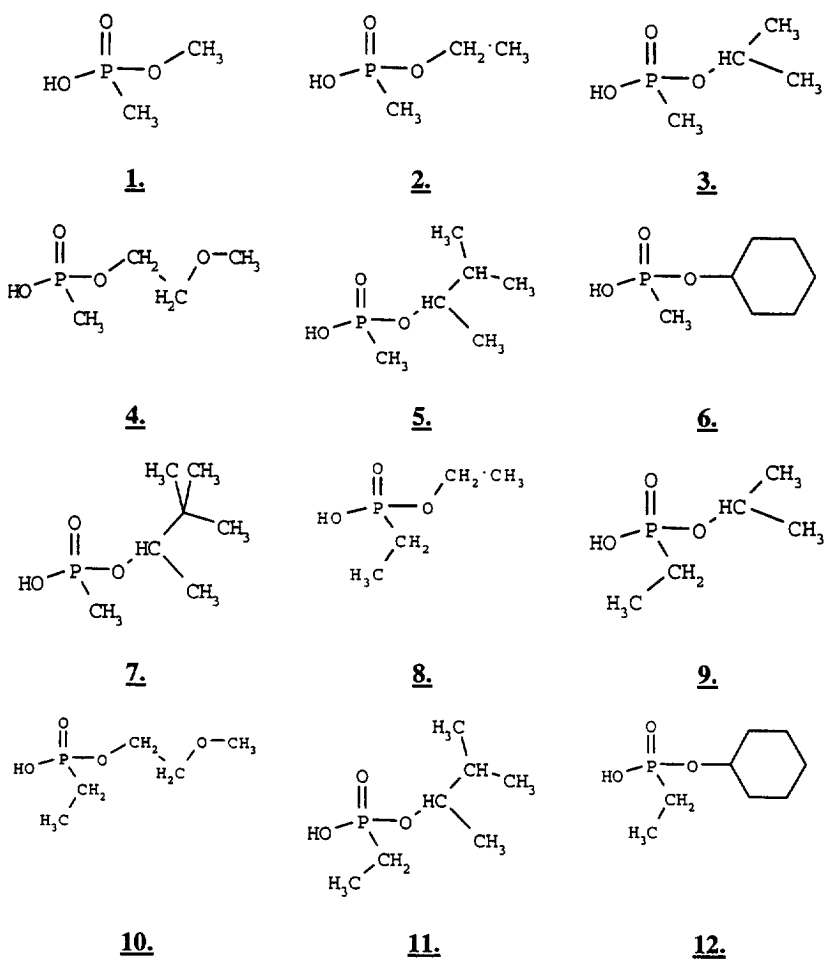
N°	NAME	Abbreviation
1	Methyl methylphosphonic acid	*MMPA*
2	Ethyl methylphosphonic acid	*EMPA*
3	Isopropyl methylphosphonic acid	*MEMPA*
4	[1-Methoxyethyl] methylphosphonic acid	*IMPA*
5	1,2-Dimethylpropyl methylphosphonic acid	*DMPA*
6	Cyclohexyl methylphosphonic acid	*CMPA*
7	Pinacolyl methylphosphonic acid	*PMPA*
8	Ethyl ethylphosphonic acid	*EEPA*
9	Isopropyl ethylphosphonic acid	*MEEPA*
10	[2-Methoxyethyl] ethylphosphonic acid	*IEPA*
11	1,2-Dimethylpropyl ethylphosphonic acid	*DEPA*
12	Cyclohexyl ethylphosphonic acid	*CEPA*

Fig. 1. Formulae of alkylphosphonic acid monoesters.

four alkylphosphonic compounds (500 mg/l) by CZE with indirect UV detection, using phenylphosphonic acid as UV marker and boric acid as ionic strength control. In order to increase the sensitivity of detection, we proposed in a previous report [1] a counter-electroosmotic mode separation of a series of organophosphorus compounds (monoesters and diacids) using sorbate anion and only some mg/l were determined. Using a co-electroosmotic mode, Oehrle et al. [13] resolved only one phosphonic acid and three ester derivatives with chromate as chromophore. The EOF was reversed by adding an organic flow modifier in the electrolyte, that induces shorter run times. Recently, Cheicante et al. [14,15] resolved the separation of organophosphorus compounds, using CZE and micellar electrokinetic chromatography.

Here, we report a new rapid CZE analysis of a series of 12 alkylphosphonic acid monoesters which involves a co-electroosmotic mode by flushing the capillary with an organic cationic polymer (polybren) before each injection.

Moreover, sorbate anion has been selected as UV marker both for its high molar absorbance coefficient (which increase the dynamic reserve) and its electrophoretic mobility close to that of the analytes.

2. Instrumental

2.1. Apparatus

The capillary electrophoresis system employed was a P/ACE 5000 (Beckman Instruments, Fullerton, CA, USA) using a fused-silica capillary of 57 cm× 50 μm (50 cm to the detector). Indirect UV detection was performed at a wavelength close to the maximum absorption of chromophore electrolyte, using 254 nm UV filters; detector time constant was 1 s and data acquisition rate was 20 Hz.

The capillary was kept at constant temperature by immersion in a cooling liquid circulating in the cartridge. Analytes were injected at the anode by hydrodynamic injection under nitrogen overpressure (0.5 p.s.i.=34 450 Pa). Data were collected using an IWT computer with an electrophoresis data calculation program.

The pH of each solution was checked on a

Beckman pH meter (Model Ø 10, Fullerton, CA, USA). The capillary was conditioned by rinsing with 1 M sodium hydroxide (5 min), then water (5 min) and finally with the electrolyte buffer during 20 min. Between two consecutive analyses, the capillary was flushed during 3 min with 0.2% (w/w) aqueous solution of polybren and equilibrated during 5 min with the electrolyte buffer in order to improve the EOF and solute migration time reproducibilities.

2.2. Chemicals

Electrolytes were prepared from analytical quality products; sorbic acid, hexadimethrin bromide (polybren) and hydroxylamine (98% purity) were obtained from Fluka, (Buchs, Switzerland).

Standard mixture solutions of analytes and electrolytes were prepared in purified water (HPLC Quality) obtained with an Elgastat UHQ II system (Villeurbanne, France).

All electrolytes were filtered before use through a polypropylene filter membrane with a 0.22 μm porosity (Prolabo, Paris, France).

Phosphonic diacids were purchased from Aldrich (Milwaukee, WI, USA) and phosphonic acid monoesters were supplied by the CEB (Centre d'Etudes du Bouchet, Vert le Petit, France).

3. Results and discussions

3.1. Co-electroosmotic flow

Capillary electrophoresis allows the separation of phosphonic anions according to the two following modes: counter-electroosmotic conditions reported in [1] or co-electroosmotic mode. In co-electroosmotic flow mode, the electroosmotic mobility is oriented in the same direction as the electrophoretic mobility (EPM) of anions. The capillary was dynamically coated with an organic cationic polymer (polybren) as first described by Terabe and co-workers [16,17].
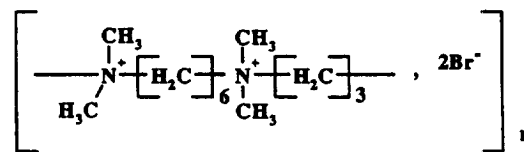
We selected 0.2% (w/w) aqueous solution of polybren as cationic polymer. In fact, by electrostatic and hydrophobic interactions between the polybren and the capillary, the silica surface becomes positively coated. Thus, the electroosmotic mobility and the electrophoretic mobilities of alkylphosphonic anions have the same direction, which induces faster separation than those observed in counter-electroosmotic mode.

3.2. Influence of the applied voltage and the temperature upon resolution

In our previous publication [1], 5 mM sorbate electrolyte (pH 6) was selected to resolve alkylphosphonic mixtures by capillary electrophoresis using counter-electroosmotic mode. Sorbate anion (pK_a = 4.75) has been selected as chromophore anion because of its high molar absorbance coefficient (ϵ = 25 000 l mol^{-1} cm^{-1}) and its electrophoretic mobility close to these of analytes ($3 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} determined in 5.5 mM borax-9 mM KH$_2$PO$_4$ (pH 6) buffer at 25°C). Here, a study on the influence of two physical parameters (running voltage and temperature) on peak efficiency and resolution has been undertaken. The test mixture contained five phosphonic acid esters (EMPA, IMPA, IEPA, DMPA, CEPA) at 20 mg/l.

3.2.1. Applied voltage

High running voltage has well-known effects; while it increases sample migration and EOF, as well as decreasing analysis time, it may increase the efficiency of the peaks and improves resolution.

As expected, peak efficiency of each organophosphorus compound linearly increases with the running negative voltage in the 5–15 kV range and reaches 100 000–140 000 theoretical plates for a maximum around 20 kV. In the 5–30 kV range the resolution between two consecutive analytes always reaches optimum at 20 kV and the resolution values are greater than 2.0.

3.2.2. Temperature

Temperature variations affect physical parameters such as electrolyte viscosity, analyte diffusion coefficient and consequently the electroosmotic mobility and the electrophoretic mobilities of the analytes.

The present study was carried out at seven different temperatures (18, 20, 25, 30, 35, 35, 40, 45°C) with sorbate anion electrolyte.

If an increase of the temperature obviously induces an increase of EPM and EOF, the selectivity doesn't change significantly. On going from 18°C up to 45°C, a significant EPM increase (41%) was observed for IMPA compared to the non-significant selectivity variation between IMPA and IEPA (α = 1.03 down to α = 1.02).

Peak efficiencies were slightly better at low running temperature due to a slower diffusion kinetic.

Resolution between two analytes always decreases (20%) with an increase in temperature. Thus, working at 20°C appears to get high peak efficiency and resolution without too long analysis time.

Therefore, the optimum experimental conditions using the sorbate electrolyte (5 mM at pH 6) were a negative voltage of 20 kV and a temperature of 20°C.

3.3. Addition of hydroxylamine to the sorbate anion electrolyte

The buffer capacity of the 5 mM sorbate electrolyte (pH 6) has been improved by adding hydroxylamine (pK_a 5.94). Several hydroxylamine concentrations were tested. Whereas in the 2–10 mM range system peaks appear, at a concentration lower than 1.6 mM, the repeatability of the analysis (EOF and solute electrophoretic mobilities) is not satisfactory; so, 1.6 mM hydroxylamine concentration has been selected.

The influence of 1.6 mM hydroxylamine concentration added to the sorbate electrolyte (pH 6) on the peak efficiency, resolution, signal-to-noise ratio and on the repeatability of the EOF and the EPM was studied. Whatever the phosphonic monoesters, peak efficiency has been improved (25–50%) when 1.6 mM hydroxylamine is added to the electrolyte. It seems that hydroxylamine in cationic form reduces the adsorption of phosphonic anion with positively charged capillary surface by creating an ion-pair with phosphonic anion. Besides, an EOF increase (1.5%) and an average decrease for each analyte electrophoretic mobility (3%) were observed when 1.6 mM hydroxylamine was added to the 5 mM sorbate electrolyte (pH 6).

As expected, the resolution is slightly better

(+20%) in the presence of 1.6 mM hydroxylamine for all the couples. The signal-to-noise ratio of each organophosphorus analyte significantly (+52%) increases with 1.6 mM hydroxylamine addition due to

the increase of peak efficiency and to the buffer capacity (Fig. 2a).

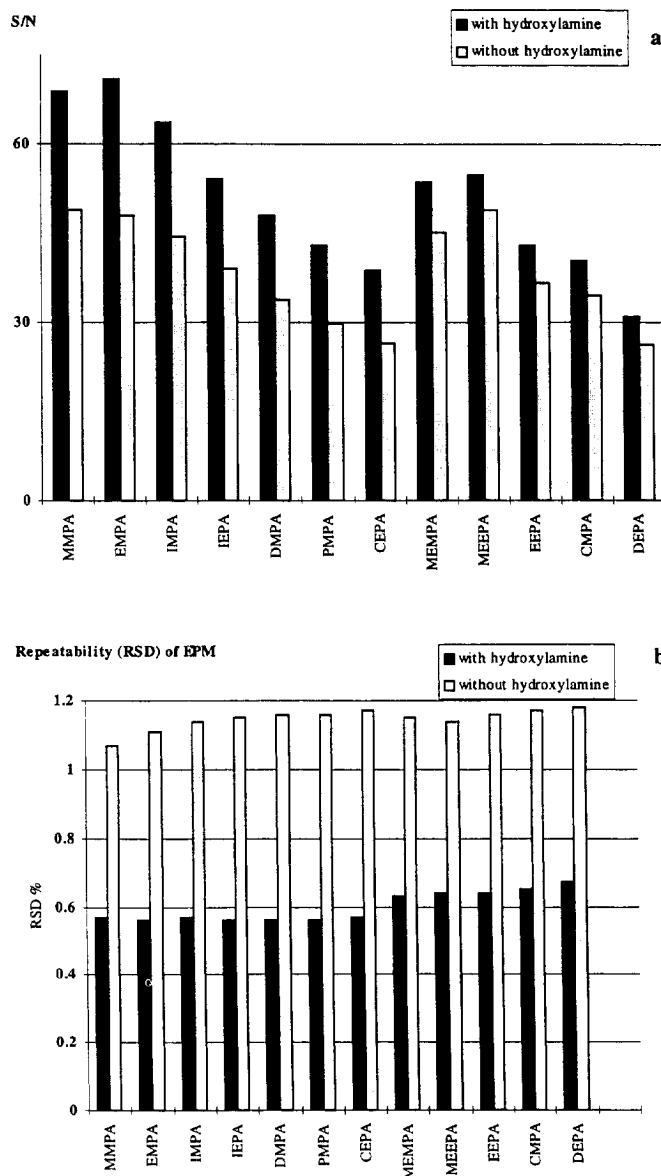Lastly, Fig. 2b compares the repeatability of the electrophoretic mobility of each analyte with and

Fig. 2. Influence of the addition of 1.6 mM hydroxylamine to the sorbic acid electrolyte on several electrophoretic parameters. Fused-silica capillary dimensions, 57 cm (50 cm to detector)×50 μm I. D., (100 μm×800 μm aperture); electrolyte, 5 mM sorbic acid, pH 6; applied voltage, −20 kV; temperature, 20°C; hydrodynamic injection, 2 s; indirect UV detection, 254 nm; analyte concentration, 10 mg/l; capillary conditioned step, 3 min with 0.2% polybren aqueous solution before each injection. (a) Signal-to-noise ratio; (b) electrophoretic mobility repeatability of 12 organophosphorus compounds.

without hydroxylamine added to the electrolyte. The relative standard deviation (R.S.D.) of the analytes' electrophoretic mobility is lower with 1.6 mM hydroxylamine added to the buffer (50%). Besides, the R.S.D. of the electroosmotic mobility is more than four times lower (2.56% to 0.59%) with 1.6 mM hydroxylamine added to the electrolyte, due to an improvement of the buffer capacity of the sorbate electrolyte.

3.4. CZE separation of phosphonic acid monoesters

The optimum experimental conditions (5 mM sorbic acid, pH 6, −20 kV, 20°C and 3 min of 0.2% polybren before each analysis) were applied to the separation of two standard mixtures (methyl and ethylphosphonic series) at 10 mg/l concentration. Fig. 3 shows the separation of only six among seven methylphosphonic acid monoesters. So, the observed migration order is as follows: MMPA, EMPA, IMPA/ MEMPA, DMPA, CMPA and PMPA but IMPA and

MEMPA (compounds 3 and 4) comigrated in such electrophoretic conditions. The EPM of alkylphosphonic acids is inversely related to the length of the alkyl-chain; thus, a solute having a long alkyl-chain (PMPA, CMPA) will have a weak electrophoretic mobility.

The same migration order is reported in Fig. 4 for the ethylphosphonic acid monoester series (EEPA, IEPA/MEEPA, DEPA and CEPA) and the comigration of isopropyl and methoxy methylphosphonic acid is also observed.

4. Conclusion

The electrolyte composed of 5 mM sorbate anion (which presents a high molar absorbance coefficient and an electrophoretic mobility close to that of the monoesters) and 1.6 mM hydroxylamine at pH 6 allows the separation of a series of alkylphosphonic acid monoesters. To reverse the EOF and thus to
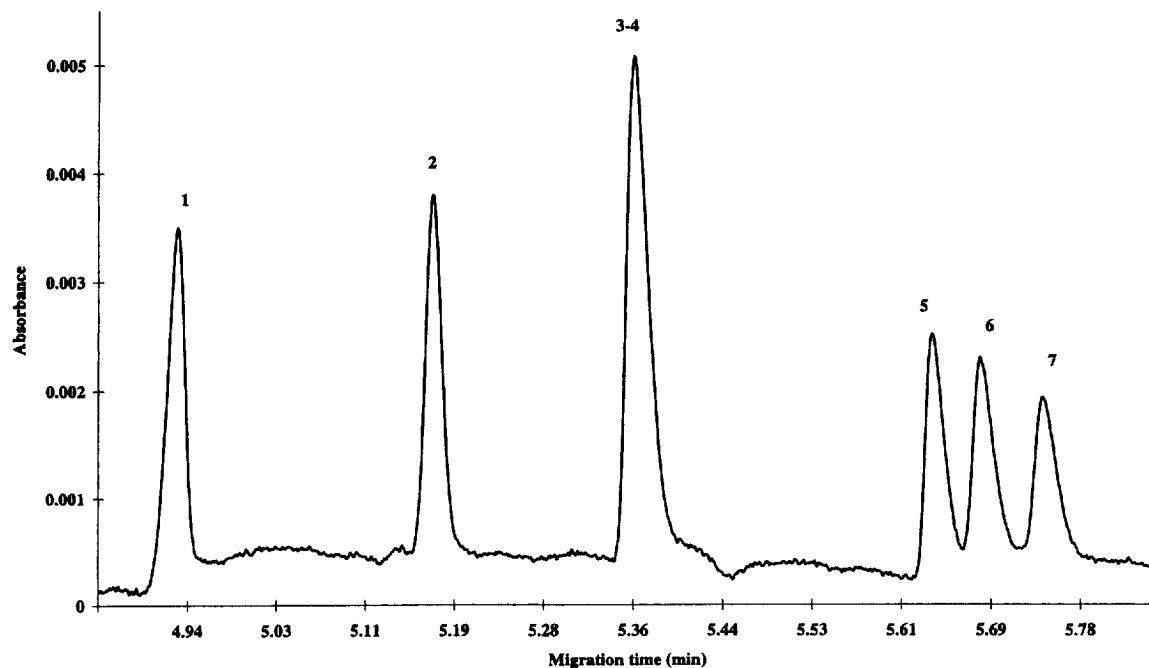

Fig. 3. Separation of methylphosphonic acid monoesters by CZE using indirect UV detection. Fused-silica capillary dimensions, 57 cm (50 cm to detector)×50 μm I.D., (100 μm×800 μm aperture); electrolyte, 5 mM sorbic acid, 1.6 mM hydroxylamine, pH 6; applied voltage, −20 kV; temperature, 20°C; hydrodynamic injection, 2 s; indirect UV detection, 254 nm; analyte concentration, 10 mg/l; capillary conditioned step, 3 min with 0.2% polybren aqueous solution before each injection.
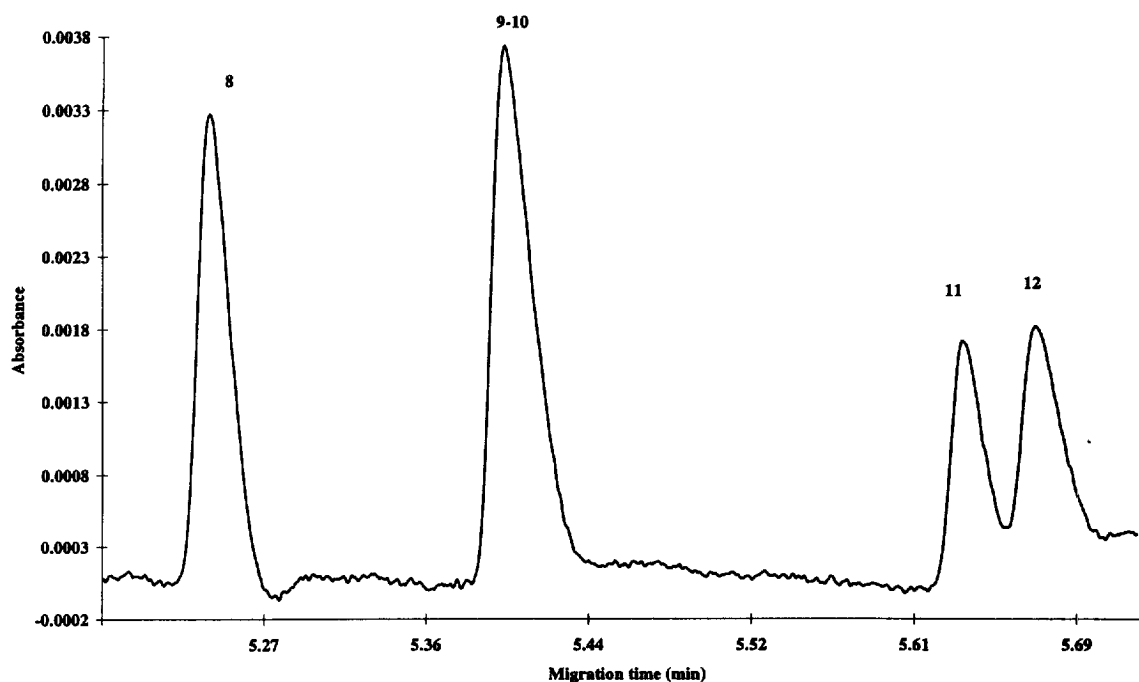
Fig. 4. Separation of ethylphosphonic acid monoesters by CZE using indirect UV detection. Fused-silica capillary dimensions, 57 cm (50 cm to detector)×50 μm I.D., (100 μm×800 μm aperture); electrolyte, 5 mM sorbic acid, 1.6 mM hydroxylamine, pH 6; applied voltage, −20 kV; temperature, 20°C; hydrodynamic injection, 2 s; indirect UV detection, 254 nm; analyte concentration, 10 mg/l; capillary conditioned step, 3 min with 0.2% polybren aqueous solution before each injection.

create co-electroosmotic conditions, the capillary is conditioned by rinsing with 0.2% (w/w) of polybren during 3 min between each analysis. The addition of 1.6 mM hydroxylamine to the sorbate electrolyte greatly improved peak efficiencies (250 000 theoretical plates) and the repeatability of this method. Thus, this system greatly induces shorter migration times (by a factor of two) while resolution and selectivity are quite similar to those obtained by performing in counter-electroosmotic mode [1].

This method is quite new relative to the determination of alkylphosphonic acid monoesters. Nevertheless, the co-electroosmotic method is complementary to those developed in counter-electroosmotic mode for the determination of monoester compounds. The combination of these two analytical methods leading to a reverse migration time order of solute series, provides a surer determination.

Direct coupling with mass-spectrometry of those two modes are under investigation in order to make determination certain.

Acknowledgments

References

[1] J.P. Mercier, Ph. Morin, M. Dreux, A. Tambute, J. Chromatogr. A 741 (1996) 279.
[2] A. Baillet, G.A. Pianetti, M. Taverna, G. Mahuzier, D. Baylocq-Ferrier, J. Chromatogr. B 616 (1993) 311.
[3] P.C. Bossle, D.J. Reutter, E.W. Soarmer, J. Chromatogr. 407 (1987) 399.
[4] A.F. Kingery, H.E. Allen, Anal. Chem. 66 (1994) 155.
[5] M.C. Roach, L.W. Ungar, R.N. Zare, L.M. Reimer, D.L. Pompliano, J.W. Frost, Anal. Chem. 59 (1987) 1056.
[6] P.C. Bossle, J.J. Martin, E.W. Sarver, H.Z. Sommer, J. Chromatogr. 267 (1983) 209.

[7] G.A. Pianetti, A. Baillet, F. Traore, G. Mahuzier, Chromatographia 76 (1993) 263.

[8] C.E. Kientz, J.P. Langenbery, U.A.T. Brinkman, J. High. Resolut. Chromatogr. 17 (1994) 95.

[9] J.G. Purdon, J.G. Pagotto, R.K. Millet, J. Chromatogr. 475 (1989) 261.

[10] E.R.J. Wils, A.G. Hulst, J. Chromatogr. 454 (1988) 261.

[11] J.C. Ingram, G.S. Groenewold, A.D. Appelhans, J.E. Delmore, D.A. Dahl, J. Chromatogr. 67 (1995) 187.

[12] G.A. Pianetti, M. Taverna, A. Baillet, G. Mahuzier, D. Baylocq-Ferrier, J. Chromatogr. 630 (1993) 371.

[13] S.A. Oehrle, P.C. Bossle, J. Chromatogr. A. 692 (1995) 247.

[14] R.L. Cheicante, J.R. Stuff, H.D. Durst, J. Chromatogr. A 711 (1995) 347.

[15] R.L. Cheicante, J.R. Stuff, H.D. Durst, J. Cap. Elec. 4 (1995) 157.

[16] S. Terabe, T. Isemura, J. Chromatogr. 515 (1990) 667.

[17] S. Terabe, T. Isemura, Anal. Chem. 62 (1990) 650.