

Capillary electrophoresis analysis of chemical warfare agent breakdown products

I. Counterelectroosmotic separation of alkylphosphonic acids and their monoester derivatives

J.-P. Mercier^a, Ph. Morin^{a,*}, M. Dreux^a, A. Tambute^b

^aUniversité d'Orléans, Institut de Chimie Organique et Analytique, URA 499, B.P. 6759, 45067 Orléans Cedex 2, France

^bDirection de la Recherche et de la Technologie, Centre d'Etudes du Bouchet, B.P. 3, 91710 Vert le Petit, France

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Abstract

An analytical method has been developed for the analysis of chemical warfare agents by capillary zone electrophoresis (CZE). Sorbate anion has been selected as indirect UV-absorbing agent and decamethonium cation reduces the electroosmotic flow. The optimized electrolyte (5 mM sorbic acid, 0.1 mM decamethonium bromide pH 6) allows the simultaneous separation of alkylphosphonic acids and their monoester derivatives in less than 15 min. The measurement of phosphonic solutes has been achieved in spiked soils using calibration curves in the 5–50 mg/l concentration range.

Keywords: Chemical warfare agents; Alkylphosphonic acids; Capillary electrophoresis

1. Introduction

Phosphonic acids and derivatives are of considerable interest in chemistry, as pesticides, herbicides, insecticides and antibiotics [1]. They are also of great importance, since they are breakdown products of chemical warfare agents (G agents and VX). Table 1 gives the formulae of the different compounds studied.

Since implementation of the chemical weapons convention (in Geneva in 1992), the authorities in charge of disarmament control need to have at their disposal a rugged, efficient and accurate analytical method to allow identification of these breakdown

products, which may be encountered on site inspection. As the breakdown products are more stable than their parent compounds, they have been used as surrogates, to indicate that chemical warfare agents may have been used recently.

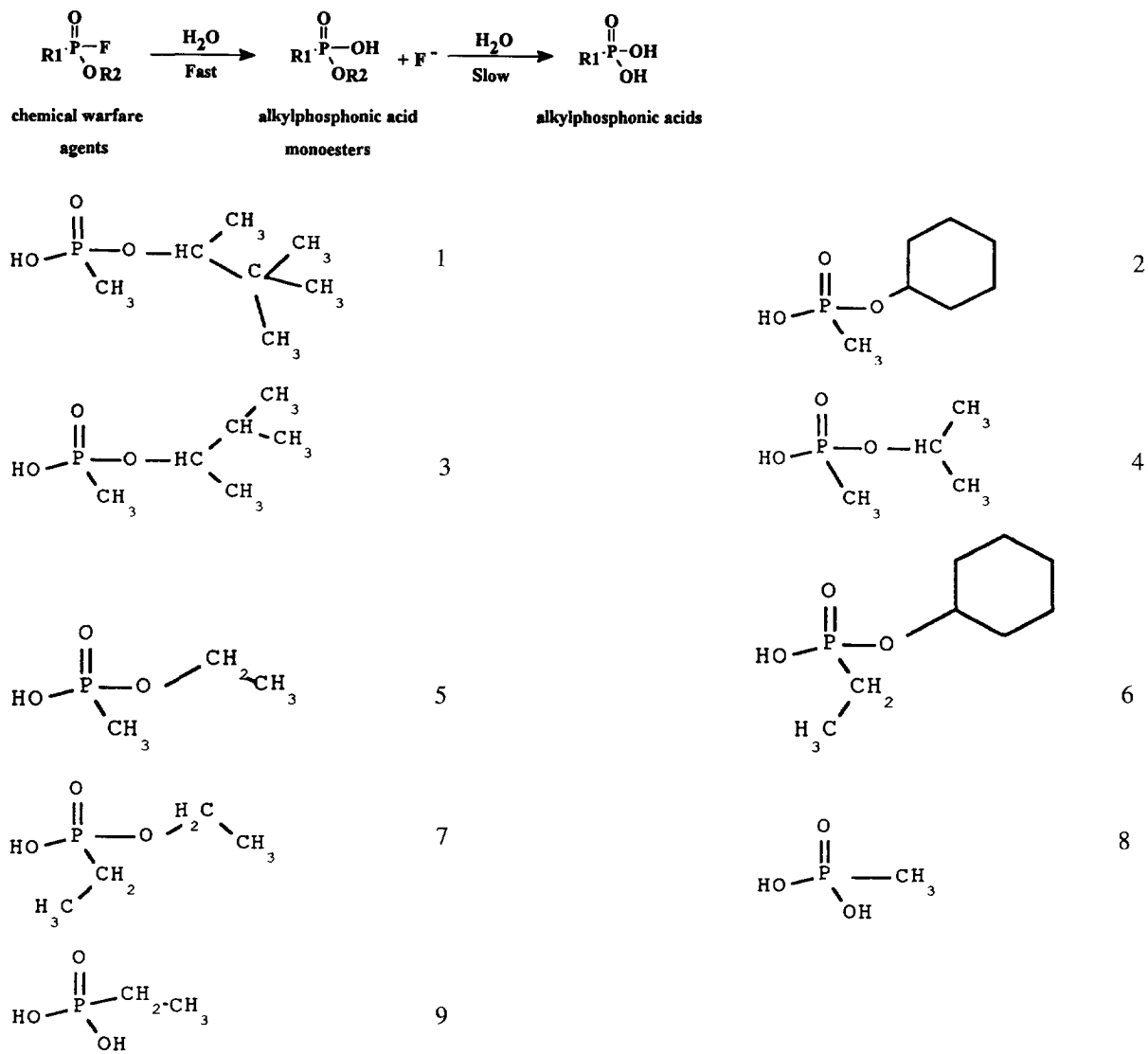
Ion chromatography is the obvious method of choice for the analysis of chemical warfare agent breakdown products as ionic solutes.

Kingery and Allen [2] and Bossle et al. [3], described an ion pair reversed-phase chromatographic method. This technique was found to give satisfactory repeatability and stability, but to suffer from low efficiency and interference by sample matrix components. Moreover, Bossle et al. show a low selectivity for separation of monoesters, particularly between EMPA and IMPA with elution close to the dead volume. Capillary electrophoresis may be ex-

*Corresponding author.

Table 1

Phosphonic acids and phosphonic acid esters resolved by CZE



No.	Name	Abbreviation
<i>Alkylphosphonic acid monoesters:</i>		
<i>Methylphosphonic acid and ethylphosphonic acid derivatives</i>		
1	Pinacolyl methylphosphonic acid	PMPA
2	Cyclohexyl methylphosphonic acid	CMPA
3	1,2-Dimethylpropyl methylphosphonic acid	DMPA
4	Isopropyl methylphosphonic acid	IMPA
5	Ethyl methylphosphonic acid	EMPA
6	Cyclohexyl ethylphosphonic acid	CEPA
7	Ethyl ethylphosphonic acid	EEPA
<i>Alkylphosphonic acids</i>		
8	Methylphosphonic acid	MPA
9	Ethylphosphonic acid	EPA

pected to be best suited to the analysis of the breakdown products as shown by Oehrlé [4].

The separation of four alkylphosphonic acids (methyl, ethyl, propyl, butyl) was achieved with indirect UV detection by Robins and Wright [1] and Pianetti et al. [5].

The best separation of alkylphosphonic acids in CZE was obtained with an electrolyte composed of 200 mM sodium borate and 10 mM phenylphosphonic acid as indirect UV absorbing agent at pH 6, and the detector set at 200 nm. Alkylphosphonic acids moved to the cathode by competition between electroosmotic flow and electrophoretic flow. Thus, the electroosmotic mobility was high enough to transport the organophosphorus compounds towards the cathode. The migration order is inversely related to the length of the alkyl chain; as expected, the higher the length of the alkyl chain linked at the phosphorus atom, the lower the electrophoretic mobility. The separation of chemical warfare agent breakdown products (phosphonic acid monoester derivatives), which are more hydrophobic compounds than phosphonic acids, requires optimization of the electrolyte composition with respect to UV marker and ionic strength.

In order to identify the phosphonic compounds and to lower the detection limit, liquid chromatography, gas chromatography and capillary electrophoresis have been coupled with mass spectrometry [4–8].

This paper presents some results which enable the simultaneous analysis of degradation products of chemical warfare agents: alkylphosphonic acids, alkylphosphonic acid monoesters.

2. Experimental

Capillary electrophoresis separation was carried out on a P/ACE 2100 apparatus (Beckman Instrument, Fullerton, CA, USA), using a fused-silica capillary of 67 cm × 50 μm I.D., with a length to the detector of 60 cm. The detection aperture was 100 μm × 800 μm.

Phosphonic acids and esters were injected at the anode by hydrodynamic injection for 5 s, under nitrogen overpressure (0.5 p.s.i.; 1 p.s.i. = 6894.76 Pa). Separations were carried out at constant temperature by immersion in a cooling liquid circulating

in the cartridge (35°C) under constant applied voltage (+30 kV).

Indirect UV detection was performed at 254 nm, which is close to the maximum absorption of the sorbate anion. Detector time constant was 1 s and data acquisition rate was 20 Hz. All data were collected using an IBM PS/2 computer with an electrophoresis data calculation program. The pH of each solution was checked on a Beckman pH meter (Model φ10, Fullerton, CA, USA).

The capillary was conditioned by rinsing with a solution of 1 M sodium hydroxide (15 min), then water (15 min) and finally with electrophoretic buffer during 30 min. Between analyses, the capillary was equilibrated with electrolyte buffer for 5 min.

A soil sample (2 g) was spiked with 1 ml of a solution containing phosphonic acids and/or phosphonic acid esters (400 mg/l and 750 mg/l). Extraction with 4 ml distilled water was repeated 5 times to give 20 ml of extract which was then centrifuged at 9000 g during 30 min, and filtered respectively through 1.0 and 0.45 μm membrane filters (Whatman, Maidstone, UK).

3. Reagents and products

Sorbic acid (95% purity) was obtained from Fluka (Buchs, Switzerland), and decamethonium bromide from Sigma (St Louis, MO, USA).

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

A solution of 0.1 M sodium hydroxide (Aldrich, Milwaukee, WI, USA) was used to adjust the pH of the electrolyte. All solutions were filtered before use through a 0.25-μm polypropylene filter membrane (Prolabo, Paris, France). Phosphonic acids and monoester derivatives were obtained from the Centre d'Etudes du Bouchet (Vert-le Petit, France).

4. Results and discussion

4.1. Choice of the electrolyte and indirect UV chromophore anion

Sorbate anion ($pK_a=4.75$) has been selected as chromophore anion because of its high molar ab-

sorbance coefficient ($\epsilon=25\,000\text{ l mol}^{-1}\text{ cm}^{-1}$ at 253 nm [9]) and its electrophoretic mobility close to that of the analytes, in order to maximize the dynamic detection range and also to decrease the electro-migration dispersion.

Decamethonium bromide (0.1 mM) was added to the electrolyte in order to reduce the EOF; as reported in the literature [10], surfactant cations mask the activity of residual silanols by electrostatic and hydrophobic interactions, thus lowering the EOF.

The pH is an important parameter for CZE, since it governs the electrophoretic mobility of ionizable compounds and also the electroosmotic flow by modification of the negative charge density of the silica.

The optimum electrolyte pH was determined between 5.5 and 7 with a mixture of five phosphonic acids. In the pH range 5.5–7, ethylphosphonic and sorbic acids were in the monoionized form ($\text{p}K_{\text{a}}$ values are 3, 7.5 and 4.75, respectively [6,11]).

EOF slightly increases when the pH increases from 5.5 to 7; in that case, electroosmotic mobility increases of 50% [12] without cationic surfactant were observed, compared to a 10% increase when 0.1 mM decamethonium bromide was added to the electrolyte. So, silanols were not totally masked by decamethonium bromide hemimicelles, and pH modification slightly influences the EOF. Consequently, we selected pH 6 in order to obtain acceptable migration times.

We also studied the influence of UV-absorbing agent concentration (sorbic acid) on the electrophoretic mobility of several phosphonic acids. At +30 kV applied voltage, the current increases with the ionic strength of the electrolyte. A sorbate anion concentration of 10 mM was selected for several reasons: (1) to limit current intensity and reduce the noise and baseline aberrations, and (2) to maintain the solubility of sorbic acid in the electrolyte (0.25%, w/w, in water at 30°C). The electrophoretic mobility of solutes with increasing UV-absorbing agent concentration varied in the same way as found for the pH increase.

At pH 6, the electrophoretic mobility of each analyte slightly decreases over the 1–10 mM co-ion concentration range, due to an increase of the electrolyte ionic strength; thus, a 5 mM sorbic acid concentration has been selected.

Under optimum conditions (5 mM sorbic acid, pH 6), a standard mixture of eight phosphonic monoesters and acids (concentration 20 mg/l) was resolved in less than 15 min (Fig. 1). Two families of phosphonic compounds are resolved: firstly, monoester alkylphosphonic acids (ethylphosphonic acid and methylphosphonic acid) and secondly phosphonic acids (ethyl and methylphosphonic acids). High-hydrophobicity compounds have smaller migration times than low-hydrophobicity compounds due to their weak electrophoretic mobility.

The resolution between two consecutive phosphonic solutes was always above 1.29, so all compounds were correctly separated and analysed.

The electrophoretic mobility of sorbic acid can be deduced from the tailing factor of the analytes ($-33.3\cdot 10^{-5}\text{ cm}^2/\text{V s}$); so, this co-ion has an electrophoretic mobility close that of EMPA.

The fluoride anion is also of interest in the analysis of chemical warfare agents because it is the main inorganic breakdown product of G agents. As the electrophoretic mobility of this inorganic anion is high and as the EOF is reduced with decamethonium bromide, fluoride does not migrate towards the detector outlet and was not determined under the electrophoretic conditions used.

Nevertheless, the fluoride anion can easily be determined by capillary electrophoresis using different migration conditions as reported by several authors [13–20].

4.2. Quantitation

Fig. 2a shows the analysis of a spiked soil; two organophosphorus compounds (CEPA, EPA) were identified and no significant interference due to the matrix was detected (Fig. 2b). The detector response has been determined in the 5–50 mg/l concentration range. The calibration curves of five phosphonic acid esters were obtained with sorbic acid buffer and hydrodynamic injection for 3 s. Each data point represents the average of four injections. Linear regression coefficients range from 0.9945 to 0.9979 and slopes vary from 0.0279 for DEPA to 0.0359 for EPA.

At low concentrations (5–10 mg/l), the response factor (expressed as peak height/concentration ratio) differs from the value obtained at high concentration

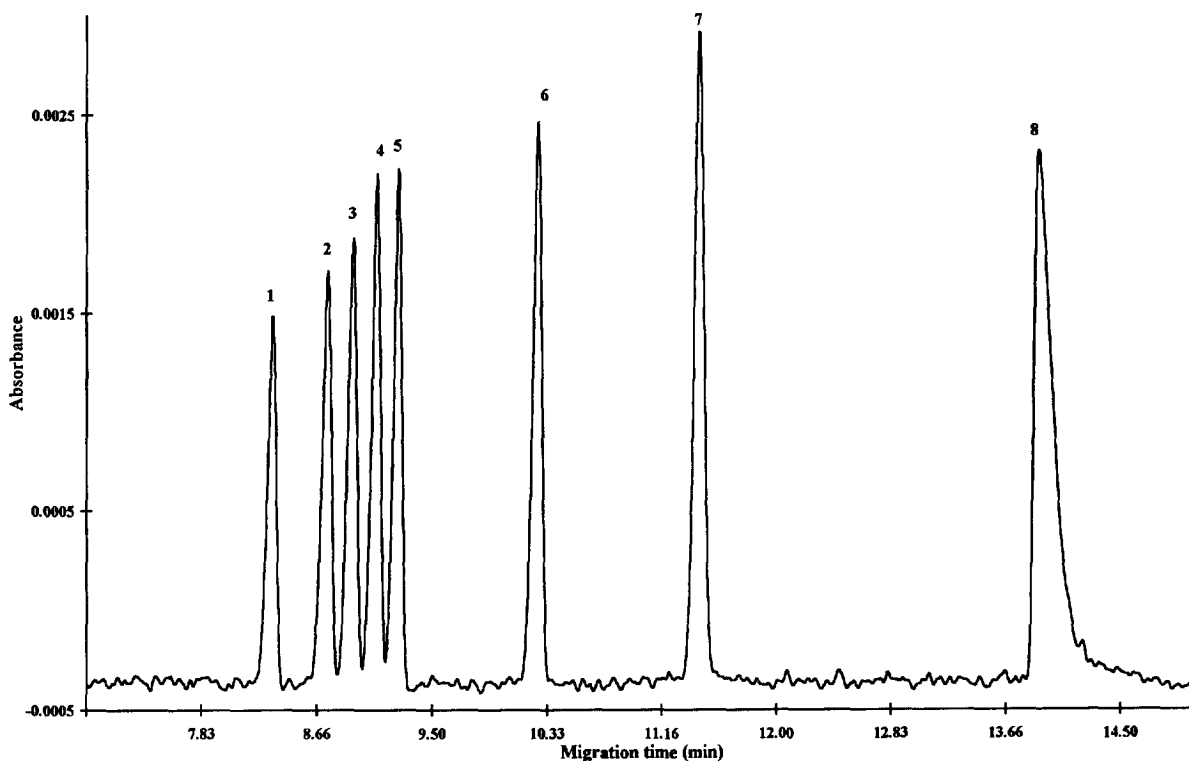


Fig. 1. Standard mixture separation of eight phosphonic acids and phosphonic acid esters by CZE. Fused-silica capillary dimensions, 67 cm (60 cm from injector to detector) \times 50 μ m I. D., 100 μ m \times 800 μ m aperture; electrolyte, 5 mM sorbic acid, 0.1 mM decamethonium bromide, pH 6; indirect UV detection set at 254 nm; applied voltage, +30 kV; temperature, 35°C; hydrodynamic injection, 5 s; solute concentration, 20 mg/l. Anions: 1=CEPA; 2=PMPA; 3=CMPA; 4=DMPA; 5=EEPA; 6=IMPA; 7=EMPA; 8=MPA.

(20–50 mg/l) (Table 2), indicating either a problem of solute degradation or a problem in the procedure.

The migration times of CEPA in standard solution (Fig. 1) differ from those in Fig. 2, showing matrix influence and hence the necessity to add a standard in the mixture to achieve quantitative measurements.

Reproducibility, repeatability and ruggedness of quantitation by this method will be investigated in the near future in order to validate the method for determination of phosphonic acids and esters in different matrices (water, soil, etc.).

5. Conclusion

The electrolyte used (sorbic acid 5 mM, 0.1 mM decamethonium bromide, pH 6) allows the simultaneous identification of alkylphosphonic acids and

their monoester derivatives as breakdown products of chemical warfare agents (G agent and VX) in soil matrices by capillary electrophoresis. A great variety of phosphonic acids and phosphonic acid esters has been resolved by this method.

Nevertheless, the simultaneous separation of phosphonic compounds and the fluoride anion needs further studies in the following areas:

- (1) reversing the EOF direction by using a flow modifier for coelectroosmotic separation in order to directly determine high electrophoretic mobility anions (fluoride) and medium electrophoretic mobility solutes as monoesters and phosphonic acid;
- (2) changing the chromophore anion to improve the detection limit;

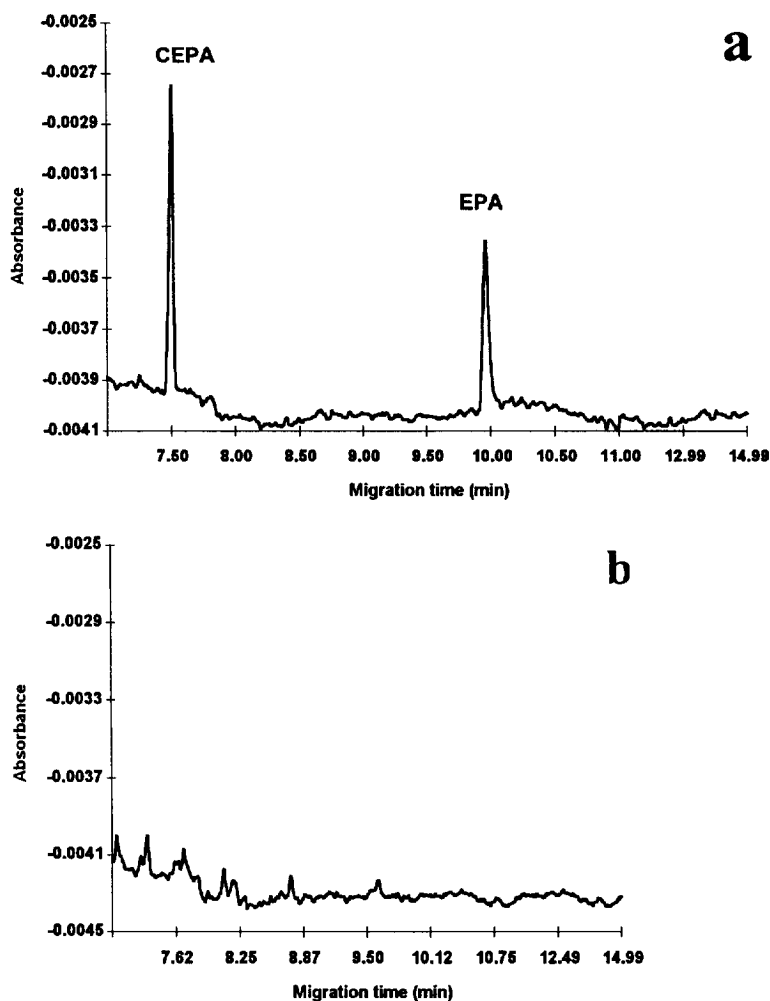


Fig. 2. Analysis of phosphonic compounds extracted from soil. Fused-silica capillary dimensions, 67 cm (60 cm from injector to detector) \times 50 μ m I. D., 100 μ m \times 800 μ m aperture; electrolyte, 5 mM sorbic acid, 0.1 mM decamethonium bromide, pH 6; indirect UV detection set at 254 nm; applied voltage, +30 kV; temperature, 35°C; hydrodynamic injection, 5 s. (a) Spiked soil; (b) blank soil.

Table 2
Response factors expressed as peak height analyte concentration (mg/l)

Concentration ^a	CEPA	PMPA	DMPA	IEPA	EPA
5	0.0676	0.0478	0.0539	0.0490	0.0429
10	0.0378	0.0369	0.0372	0.0415	0.0415
20	0.0315	0.0330	0.0352	0.0377	0.0371
30	0.0227	0.0329	0.0338	0.0357	0.0365
40	0.0299	0.0306	0.0339	0.0350	0.0373
50	0.0297	0.0301	0.0327	0.0339	0.0365

^a Expressed as mg/l.

(3) determining pK_a values of various phosphonic compounds in order to better manage the separation.

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