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Combination of LC-MS and CE-MS Analysis for the Separation and the Identification of Phosphonic Acids

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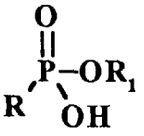
Abstract. The analysis of phosphonic acids in a spiked tap water has been investigated by capillary electrophoresis-mass spectrometry (CE-MS) and liquid chromatography-mass spectrometry (LC-MS) as part of the Third Official Proficiency Test of the Organization for the Prohibition of Chemical Weapons. The use of a non-volatile electrolyte (5 mM sorbic acid/ammonia, pH 6.5) in CE and a volatile mobile phase (water/0.1 *v/v* trifluoroacetic acid/acetonitrile) in LC achieves the baseline resolution of studied phosphonic acids. Tandem-MS detection analysis allows an accurate identification of these solutes by screening their characteristic fragmentations.

1. Introduction

The identification and the separation of phosphonic acids are important topics since 1992 due to the Geneva convention. The Organization for the Prohibition of Chemical Weapons (OPCW) requires the continuous development of analytical methods, in order to identify chemical warfare agents and their hydrolysis products such as phosphonic acids [1][2].



Gas chromatography (GC) [3–9], liquid chromatography (LC) [10–20], and capillary electrophoresis (CE) [21–26] are the main analytical methods developed for the separation of these compounds. Recently, also mass spectrometry (MS) combined with LC [27][28] and CE [30] [31] has been applied.

Black and *Read* [27][28] have investigated a methodology for the analysis and the identification of phosphonic acids by coupling LC to ionspray MS detection. Without preconcentration or derivatization steps, a mixture of eight compounds was resolved and identified on a $C_{8/18}$ mixed column with formic acid/water as mobile phase. In a previous study [29], using a porous graphitic carbon (PGC) stationary phase with trifluoroacetic acid/water/acetonitrile as mobile phase, the baseline resolution of phosphonic acids was successfully achieved. Moreover, this stationary phase allowed the resolution of isopropyl/propyl isomers, which has never been possible on the classical reversed-phase C_{18}.

In CE-MS, only two methods have been investigated [30][31]. In the negative ionization mode, ionspray ionization exhibits minimal fragmentation with production of very abundant $[M-H]^-$ ions. *Kostiainen et al.* [30] have developed a method with volatile electrolytes such as ammonium acetate. The other study [31] employed an electrolyte composed of 5 mM of sorbic acid/ammonia (pH 6.5) optimized for the simultaneous indirect UV and MS detection of phosphonic acids. The non-volatile sorbic acid did not perturb MS detection.

The aim of this work is to demonstrate that phosphonic acids can be analyzed by two different separation methods (LC and CE) coupled to MS and MS-MS detection, which allows accurate identification and quantification.

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2. Experimental Procedures

2.1. *Chemicals*

All mobile phases were prepared with deionized water, supplied by an *Elgastat UHQ II* apparatus (*Elga*, Antony, France). LC-Grade acetonitrile was provided by *J.T. Baker* (Noisy Le Sec, France). Trifluoroacetic acid (CF_3COOH) was purchased from *Merck* (Darmstadt, Germany). Electrolytes were prepared from analytical-quality products. Sorbic acid, (98% purity) ammonia and pentanol were obtained from *Fluka* (Buchs, Switzerland). All electrolytes and mobile phases were filtered before use through a polypropylene filter membrane (0.22 µm porosity (*Prolabo*, Paris, France)).

Methyl-, ethyl-, propyl-, and butylphosphonic acids were purchased from *Aldrich* (St. Quentin Fallavier, France). The other phosphonic acids were supplied by the CEB (Centre d'Etudes du Bouchet, Vert le Petit, France).

2.2. *LC and CE Conditions*

LC isocratic and gradient elutions were performed on a *Perkin Elmer* (Toronto, Canada) apparatus (*P 200 series*). The injection loop had an internal volume of 20 µl. The PGC column was *Hypercarb S* (150 × 2.1 mm i.d., 7 µm particle size) from *Hypersil SA* (Runcorn, UK). The mobile phase was delivered at a flow rate of 0.2 ml/min (inlet pressure 110 bar). In the MS study, a split system *1/10* (*Perkin*

Elmer Sciex, Toronto, Canada) was used to deliver around 20 µl/min to the mass analyzer.

CE Separations were carried out on a *P/ACE 5000* apparatus (*Beckman Instruments*, Fullerton, CA, USA) with a fused-silica capillary of 85 cm × 50 µm (20 cm to the detector). Indirect UV detection was performed using a 254-nm UV filter; the detector's time constant was 1 s and the data-acquisition rate was 20 Hz. The capillary was kept at constant temperature (25°) by immersion into a cooling liquid circulating in the cartridge. Analytes were injected at the anode by hydrodynamic injection and nitrogen overpressure (3.45 × 10^7 Pa).

2.3. *MS Conditions*

MS Detection was carried out on an *API300* triple-quadrupole mass spectrometer (*Perkin Elmer- Sciex*, Toronto, Canada) *via* an ionspray interface operating at room temperature and atmospheric pressure. Mass-spectrometry parameters were fixed as follows: spray voltage (–4 kV), orifice voltage (–25 V), ring voltage (–400 V) and air as nebulization gas. Mass spectra were acquired using a dwell time of 1 ms. A *Macintosh* computer was used for instrument control, and MS-acquisition processing data were calculated with the *LC_2 Tune* software (*Perkin Elmer-Sciex*, Toronto, Canada).

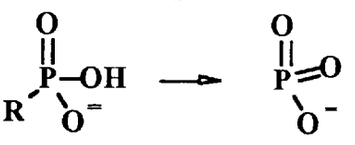
3. Results and Discussion

The procedures described above have been applied to identify some phosphonic acids contained in spiked tap-water samples prepared by the Centre d'Etudes du Bouchet (France) for the benefit to the Third Official PTS/OPCW Proficiency Test Analysis [31][32]. The levels and the chemical structure of compounds were unknown.

3.1. *MS and MS-MS Analysis*

The mass spectra of the sample and the blank were recorded after transfer by the syringe pump (*Fig. 1a* and *1b*). The comparison between the two figures shows two negative ions caused by the matrix (*m/z* 97, 111) and two supplementary negative ions from the spiked compounds at *m/z* 123 (MW = 124) and *m/z* 207 (MW = 208).

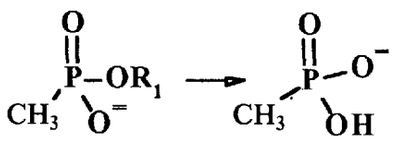
The identity of the added phosphonic acids was confirmed by tandem-MS studies. *Fig. 1c* shows the MS/MS spectrum of the ion *m/z* 123. By increasing in the collision energy (15 to 25 eV), the intensity of the peak at *m/z* 123 decreased leading to an increase in the intensity of the peak at *m/z* 79, which corresponds to the ion product characteristic for the fragmentation of an alkylphosphonic acid [31].



m/z 207 *m/z* 79

The loss of an alkyl group at *m/z* 43 (123–1–79) corresponds to a C_3H_7 alkyl group (propyl or isopropyl).

Fig. 1d shows the fragmentation of the ion *m/z* 207 resulting in an ion at *m/z* 95, which corresponds to the characteristic product of an alkyl methylphosphonic acid [31].



m/z 207 *m/z* 95

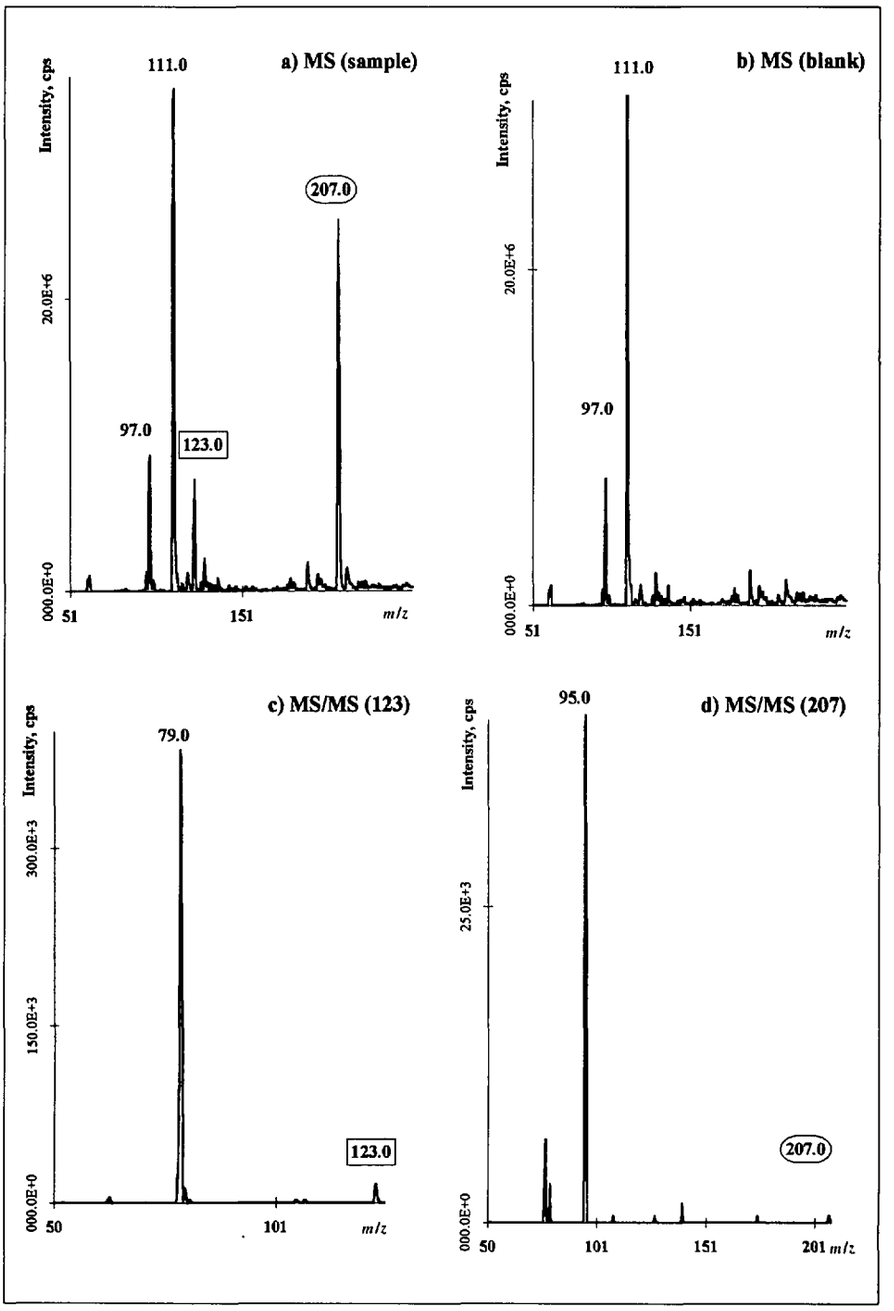


Fig. 1. *MS and tandem-MS analysis of spiked tap water.* MS conditions: nebulizer gas, air; ionspray voltage, –4 kV; orifice voltage, –25 V; ring voltage, –400 V; collision energy set at 15 eV; tandem-MS conditions: collision energy set at 25 eV.

The loss of the alkyl group (*m/z* 113) leads to a tentative formula of C_8H_{17} (linear or branched).

3.2. *CE Analysis*

On-line CE-UV-MS analysis resulted in the CE electropherogram of the sample recorded with indirect UV detection as shown in *Fig. 2a*. Despite some baseline drift due to matrix effects, a complete separation and resolution of the two unknown compounds could be achieved (detection was performed at 20-cm capillary length).

The applied electrolyte system (5 mM sorbic acid/ammonia, pH 6.5) allows a dual detection (UV and MS). The nonvolatile sorbic acid appears to be suitable as an electrolyte for achieving indirect UV as well as MS and tandem-MS detection (*Figs. 2b* and *2c*, respectively) [31]. *Fig. 2b* shows the CE mass electropherogram of the spiked sample for *m/z* 123 and 207. The two compounds are well resolved at 13.4 and 16.7 min, and good signal-to-noise ratios (S/N 600 and 60 for *m/z* 207 and 123, respectively) are obtained. Compounds of high hydrophobicity (*m/z* 207) have a smaller migration time than those of low hydrophobicity (*m/z* 123) due to their weak electrophoretic mobility (opposite to the high electroosmotic flow). As expected, the CE-MS-MS electropherogram (*Fig. 2c*) shows an increase in the signal-to-noise ratio (9 000 *vs.* 600 and 7 000 *vs.* 60 for *m/z* 95 and 79, respectively).

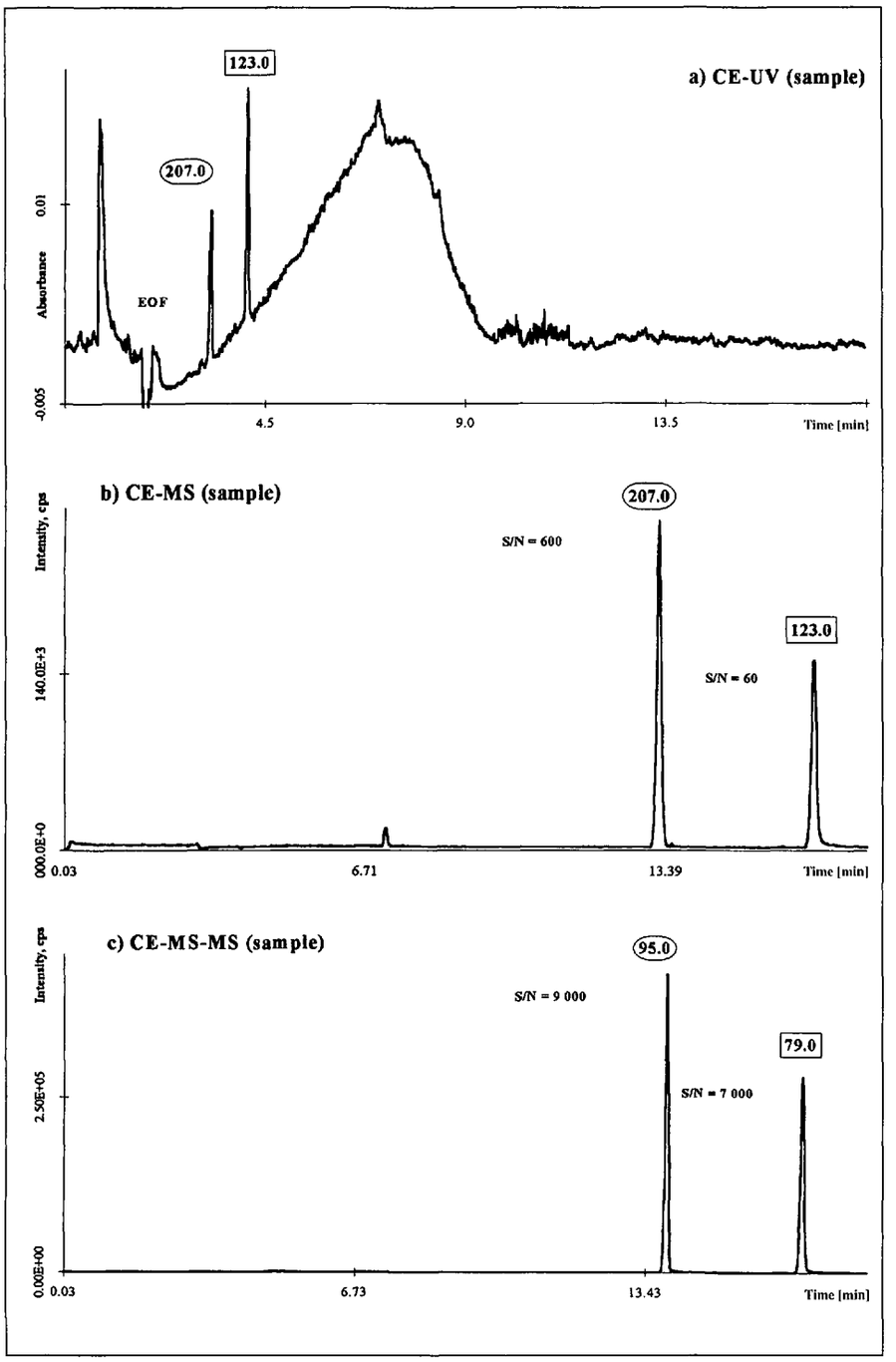


Fig. 2. *CE-UV,CE-MS, and CE-MS-MS analysis of the spiked tap water.* CE Conditions: Fused-silica capillary, dimensions, 85 cm (Indirect UV detection set at 20 cm, 254 nm) × 50 μm i. d.; electrolyte, 5 mM sorbic acid and ammonia pH 6.5; applied voltage, +30 kV; temperature, 25°; hydrodynamic injection, 5 s; capillary-conditioning step, 3 min with the electrolyte buffer. MS conditions as in *Fig. 1*.

3.3. *LC Analysis*

The LC-MS analysis of the matrix and the blank sample in the negative-ion mode under optimized chromatographic conditions (PGC/CF_3COOH/water/ACN [29]) is presented in *Fig. 3a*. The two phosphonic acids are easily observed at *m/z* = 123 and 207. The difference in the *k'* value of the analytes ($k'_{m/z\ 123}$ = 1.7 and $k'_{m/z\ 207}$ = 8.0) is caused by their different molecular mass and hydrophobicity.

The compound at *m/z* 123 could be either *iso*-PPA or PPA. Therefore, the sample was spiked with PPA and IPA (M_r = 124) and further analyzed by LC-MS. The separation of the compound with *m/z* 123 and PPA is shown in *Fig. 3b*. As shown in *Fig. 3a*, it coelutes with *iso*-PPA and is identical with this compound.

The compound with *m/z* 207 is a solute with strongly hydrophobic behavior due to its high *k'* value (*k'* = 8.0). Previous studies by CE-MS have shown that it is an alkyl-methylphosphonic acid, but only the length of the alkyl chain (C_8H_{17}) could be determined. However, this was enough according to the Third Official PTS / OPCW Proficiency Test procedure. Fragmentation studies using other ionization techniques are necessary to identify this compound which was officially identified as (2-ethylhexyl)-methylphosphonic acid (EHMPA).

3.4. *Quantitative Analysis*

Quantitative analysis was performed by LC-MS. The calibration curves of two phosphonic acids were linear between 1–100 mg/l and showed good correlation coefficients (*r* = 0.999 and 0.998 for IPA and EHMPA, respectively). Concentrations in spiked tap water were found to be 9.3 and 13.5 mg/l for IPA and EHMPA, respectively, which was close to the expected level (8 and 12 mg/l, respectively).

Phosphonic acids can easily be separated by CE and LC. The combination of

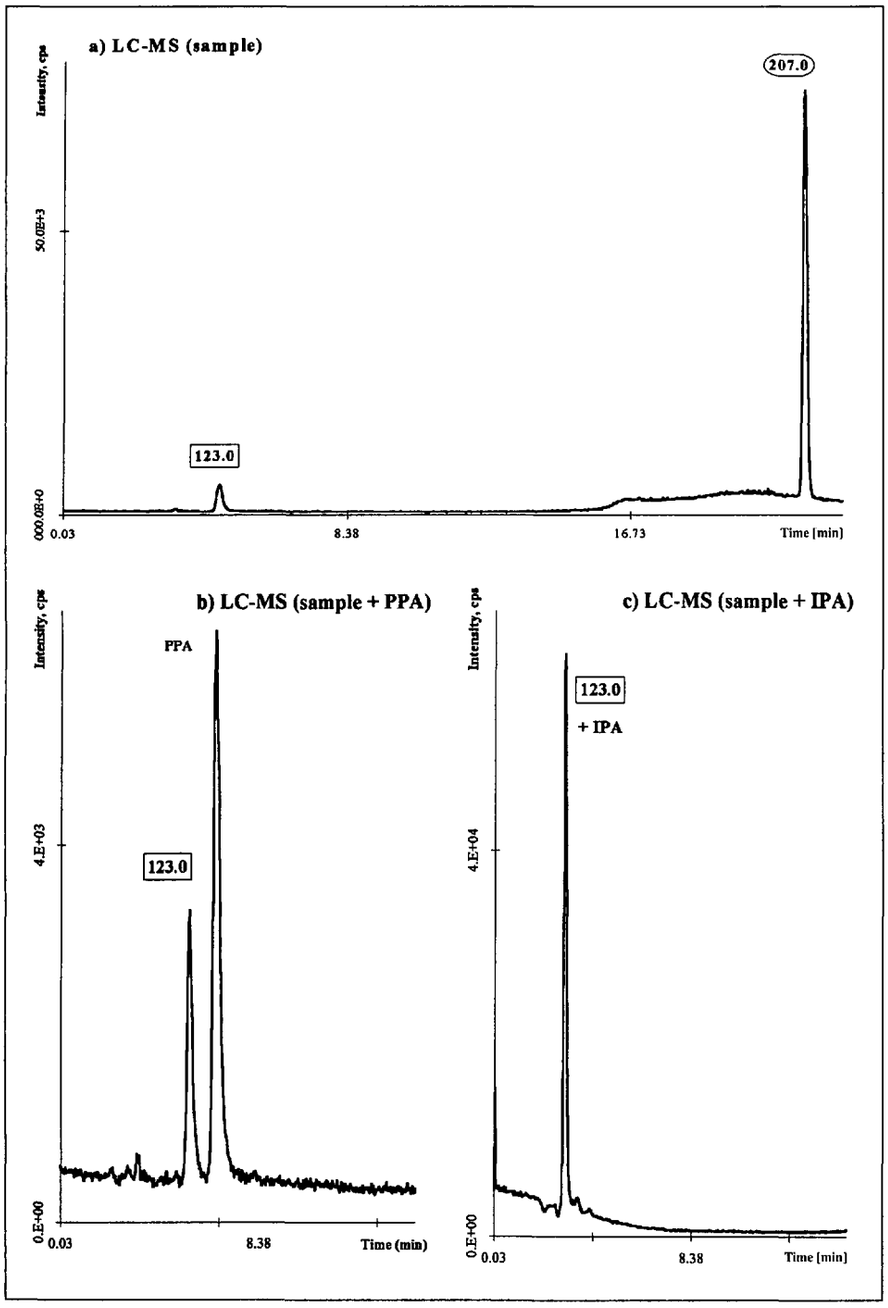


Fig. 3. *LC-MS Analysis. Hypercarb S* (150 × 2.1 mm i.d., 7 µm particle size) column. Gradient elution at 0.2 ml/min. 0 to 3 min, 0.1% (*v/v*) trifluoroacetic acid in water; 3 to 18 min, 0.1% (*v/v*) trifluoroacetic acid in water to 0.1% (*v/v*) trifluoroacetic acid in acetonitrile. MS Conditions: Split system 1/10; nebulization gas, air; ionspray voltage, –4 kV; orifice voltage, –25 V; ring voltage, –400 V; a) received spiked sample; b) addition of PPA; c) addition of IPA.

CE-MS-MS and LC-MS-MS allows their accurate structural identification and their quantification (*e.g.*, in soil samples), due to the high sensitivity of these analytical methods.

Moreover, CE and LC eluents are sufficiently volatile to be compatible with MS detection using the ionspray-detection mode. The use of sorbic acid in the electrophoretic buffer system allows a dual detection (indirect UV and MS detection).

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