ELSEVIER

Journal of Chromatography A, 849 (1999) 197–207

JOURNAL OF CHROMATOGRAPHY A

Liquid chromatography analysis of phosphonic acids on porous graphitic carbon stationary phase with evaporative light-scattering and mass spectrometry detection

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

[a]*Université d'Orleans, Institut de Chimie Organique et Analytique, CNRS UPRES-A 6005, BP 6759, 45 067 Orleans cedex 2, France*
[b]*Direction des Centres d'Expertises et d'Essai, Etablissement Techniques Central n° 4, Centre d'Etudes du Bouchet, BP 3, 91710 Vert le Petit, France*

Received 9 February 1999; received in revised form 16 April 1999; accepted 20 April 1999

Abstract

The analysis of several phosphonic acids has been investigated by liquid chromatography (LC), using porous graphitic carbon as stationary phase with mass spectrometry (MS) or evaporative light-scattering detection (ELSD). In both detection modes (MS and ELSD), the mobile phase must be volatile and, due to the porous graphitic carbon (PGC) properties, should promote electronic interactions. Among the various hydrogeno- and perfluorocarboxylic acids tested, trifluoroacetic acid (0.1%, v/v) was selected as electronic competitor for solute retention. The baseline resolution of a phosphonic acids mixture required a trifluoroacetic–acetonitrile gradient elution. This methodology was then applied to the identification of phosphonic acids in a spiked tap water sample. Quantitative analyses are successfully achieved with a good correlation coefficient.

Keywords: Stationary phases, LC; Phosphonic acids; Porous graphitic carbon

1. Introduction

The implementation of the Organization for the Prohibition of Chemical Weapons (OPCW) requires the continuous development of analytical methods in order to identify chemical warfare agents. Nerve agents belonging to the forbidden chemical agents list have been identified by their hydrolysis breakdown products such as the phosphonic acids described in Table 1. The analysis of these compounds by LC has been widely described in the literature since 1983 [1].

Since phosphonic acids are devoid of a significant chromophore group, studies with derivatization steps (fluorophore derivatization agent) have been investigated on a reversed stationary phase (C_{18}) [1,2]. Direct fluorimetric detection of these phosphonic acid derivatives allows a detection limit of 5–200 μg/l.

Even though phosphonic acids are moderately strong acids in an aqueous medium ($pK_a \sim 2$ [3]), ionic chromatography has been studied on polymeric (PRP1 and PRPX 100) [4–8] and silica [5–7] stationary phases. The separation of phosphonic acids is better achieved on polymeric packing, due to the combination of ionic and apolar interactions between solutes and the stationary phase, than on

*Corresponding author. Tel.: +33-38-41-70-74; fax: +33-38-41-71-54.

0021-9673/99/$ – see front matter
PII: S0021-9673(99)00556-7

Table 1
Formulae and $[M-H]^-$ fragment of investigated phosphonic acids

Acid	Abbreviation	MW	$[M-H]^-$ (m/z)
Alkylphosphonic acids			
Methylphosphonic acid	MPA	96	95
Ethylphosphonic acid	EPA	110	109
Propylphosphonic acid	PPA	124	123
Isopropylphosphonic acid	iso-PPA	124	123
Butylphosphonic acid	BPA	138	137
tert.-Butylphosphonic acid	*tert*.-BPA	138	137
Alkyl methylphosphonic acids			
Ethyl methylphosphonic acid	EMPA	124	123
Isopropyl methylphosphonic acid	iso-PMPA	138	137
(2-Methoxyethyl) methylphosphonic acid	MEMPA	154	153
Cyclopentyl methylphosphonic acid	CPMPA	164	163
Cyclohexyl methylphosphonic acid	CMPA	178	177
Pinacolyl methylphosphonic acid	PMPA	180	179
Alkyl ethylphosphonic acids			
(2-Methoxyethyl) ethylphosphonic acid	MEEPA	154	153
Cyclohexyl ethylphosphonic acid	CEPA	192	191

silica packings, where MPA, EMPA and EPA coelutions are observed (predominance of ionic interactions). Moreover, a preconcentration step on a microcolumn (SAX packing) is necessary to reach a detection limit of 20 μg/l.

Conductometric [4–9] and indirect UV [10] detection are the two major detection modes used, but do not allow accurate identification of phosphonic acids. MS detection is the only method by which solutes can be reliably identified by their molecular mass. Thus, Wils and Hulst [11] developed a LC–thermospray method to identify five phosphonic acids at the 20 μg/l level. Recently, Black and Read [12,13] developed a methodology for the analysis and identification of phosphonic acids by coupling LC with ionspray MS detection. Without any preconcentration or derivatization steps, a mixture of eight compounds was resolved and identified on a $C_{8/18}$ mixed column with formic acid–methanol–water as mobile phase. Ionspray MS detection makes it possible to reach a detection limit of 10 μg/l.

The aim of this work is to present a LC–MS analysis of phosphonic acids in spiked tap water using preliminary experiments performed with a specific stationary phase (porous graphitic carbon; PGC) and the help of an evaporative light-scattering detector (ELSD) where volatile eluents are required:

- PGC has been selected as stationary phase, because this strong reversed-phase packing material is more hydrophobic and stable than C_{18} [14]. Nevertheless, hydrophilic solutes [15] are totally retained on this stationary phase due to the specific electronic properties of the graphitic planar surface and delocalized π-electron density. Therefore, PGC is particularly useful for the separation of inorganic anions when a volatile electronic interaction additive (formic, acetic or perfluoro-carboxylic acid) is added to the aqueous mobile phase [16,17]. Moreover, this stationary phase has been successfully used for the separation of enantiomers and geometrical isomers [14–19]. Thus, PGC will be evaluated for the separation of a series of phosphonic acids (Table 1) and particularly for isomers (PPA/iso-PPA) that have never been resolved on C_{18} stationary phase.
- Due to the volatility requirement of LC coupled with MS, ELSD was chosen in order to develop LC conditions before coupling. Less expensive and more widely used in laboratories than an MS detector, ELSD is generally considered to be a very convenient and universal LC detector for analytes which are less volatile than the chromatographic effluent [18,19]. ELSD was therefore

evaluated as an alternative detection mode of choice to MS detection in order to optimize the separation of phosphonic acids.

2. Experimental

2.1. Chemicals

All mobile phases were prepared with de-ionized water, supplied by an Elgastat UHQ II apparatus (Elga, Antony, France). LC-grade acetonitrile was provided by J. T. Baker (Noisy Le Sec, France). Formic acid (HCOOH), acetic acid (CH_3COOH), trifluoroacetic acid (CF_3COOH), heptafluorobutyric acid (C_3F_7COOH) and nonafluoropentanoic acid (C_4F_9COOH) were purchased from Merck (Darmstadt, Germany), whereas 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. Liquid chromatographic conditions

Isocratic and gradient elutions were performed on a Thermo Separation apparatus (Les Ulis, France) LC system consisting of Spectra Series P100 and P200 pumps and a manual 7010 Rheodyne valve. The injection loop has an internal volume of 20 μl.

A Sedex 45 Evaporative Light-Scattering Detector (Sedere, Alfortville, France) was used. The detector temperature was set at 40°C, the photomultiplier at gain 10 and the pressure of the nebulization gas (dried and filtered nitrogen) regulated at 2.2 bar.

The PGC column was Hypercarb S (150×2.1 mm I.D., 7 μm) from Hypersil SA (Runcorn, UK). The mobile phase was delivered at 0.2 ml/min flow-rate (inlet pressure 110 bar) by the pumping system. In MS conditions a split system 1/10 (Perkin-Elmer Sciex, Toronto, Canada) was used to deliver around 20 μl/min to the mass analyzer. LC data were processed using a Shimadzu Model CR 5A integrator (Kyoto, Japan).

2.3. Mass spectrometry conditions

MS detection was carried out on an API 300 triple quadrupole mass spectrometer apparatus (Perkin-Elmer Sciex) 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 per step of 0.2 a.m.u. A Macintosh computer was used for instrument control and MS acquisition processing data were calculated with LC_2 Tune software (Perkin-Elmer Sciex).

3. Results and discussion

3.1. Selection of the volatile mobile phase by LC–ELSD analysis

The phosphonic acids investigated were totally retained on PGC using water as mobile phase; MPA, the more hydrophilic compound and the less retained solute, has a retention factor (k') >30, while inorganic cations (mono and di) eluted in the void volume. Indeed, the retention of phosphonic acids was probably due to charge transfer interactions between the negative charge of the analyte and the PGC surface. Retention times of solutes on PGC are governed by the competition of electronic interaction between the carboxylate additive present in the mobile phase and analytes with a delocalized π-electron density on the PGC surface (as phosphonic acids are totally dissociated at pH 2.2 [3]). Hence, several electronic interaction competitors have been investigated in the mobile phase and the k' values are reported Table 2. The k' values of phosphonic acids determine the elution strength scale of different volatile carboxylic acids used as electronic competitors as follows:

$$C_4F_9COOH > C_3F_7COOH > CF_3COOH > HCOOH > CH_3COOH$$

The separations are illustrated in Fig. 1. Hydrogenocarboxylic acids (HCOOH and CH_3COOH) have a

Table 2
Retention factors (k'), efficiencies (N), selectivities (α) and resolutions (R_s) of phosphonic acid peaks studied by LC–ELSD with hydrogeno- or perfluorocarboxylic acids used as volatile mobile phases

Eluent		MPA	EPA	iso-PPA	PPA	*tert.*-BPA	BPA
HCOOH,	k'	0.8	1.3	2.1	2.6	2.6	7.6
1% (v/v),	N	500	700	1300	800	900	1700
265 mM	α	1.4	1.2	2.9			
	R_s	1.0	1.0	5.9			
CH_3COOH,	k'	2.4	3.1	4.5	5.1	6.0	12.0
1% (v/v),	N	600	900	900	700	700	500
175 mM	α	1.3	1.1	2.0			
	R_s	1.3	0.5	2.6			
CF_3COOH,	k'	0.6	1.0	1.7	2.1	2.3	7.1
0.1% (v/v),	N	1000	1500	1300	1700	1800	5100
13.1 mM	α	1.6	1.3	3.4			
	R_s	1.7	1.3	11.0			
C_3F_7COOH,	k'	0.5	0.7	1.4	1.8	1.8	5.5
0.1% (v/v),	N	400	500	800	500	700	2800
7.69 mM	α	1.4	1.3	3.1			
	R_s	0.6	0.8	7.6			
C_4F_9COOH,	k'	0.4	0.6	1.2	1.7	1.7	5.2
0.1% (v/v),	N	300	500	400	600	800	1700
6.49 mM	α	1.7	1.4	3.1			
	R_s	0.9	1.1	5.9			

weaker elution strength than their perfluoro homologous; indeed, even at a 10 times greater concentration (1% v/v instead of 0.1% v/v), phosphonic acids always have a greater k' value. The comparison between CF_3COOH and CH_3COOH shows the greater elution strength of perfluoro-carboxylic acids than hydrogeno-carboxylic acids, due to the greater hydrophobic behavior of the fluorine atom with PGC compared with the hydrogen atom. Besides, in contrast to reversed-phase LC, where the retention of solute increases with ion-pairing concentration and therefore its adsorption on the stationary phase [17,20–22], with this system (no ion-pairing reagent) the phosphonic acid retention time on PGC depends on the length of the perfluoro-alkyl chain of the additive. The longest alkyl chain length of the perfluoro-carboxylic acids gives the smallest phosphonic acid retention time; for example, BPA has a k' value of 7.1 with CF_3COOH compared with 5.2 with C_4F_9COOH.

Peak efficiencies are reported in Table 2. With 13.1 mM CF_3COOH concentration in the mobile phase, phosphonic acid peak efficiencies were higher than with the other electronic additives; peak efficiencies ranged from 1000 (MPA) to 5100 (BPA) theoretical plates with CF_3COOH, which corresponds to classical theoretical plate values of less retained compounds ($t_r < 10$ min, $1000 < N < 6000$ [14–16]), but remain lower than those currently observed in ion chromatography.

As expected, the selectivities between two consecutive analytes was higher with C_4F_9COOH (it varied from 1.4 for iso-PPA/PPA to 3.1 for *tert.*-BPA/BPA) than with the other additives, but with CF_3COOH selectivities were acceptable, ≥ 1.3 (iso-PPA/PPA).

Consequently, the resolution between two peaks was always higher with CF_3COOH than with the other carboxylic acids ($R_s > 1.3$) and the resolution between two geometrical isomers such as iso-PPA/PPA and *tert.*-BPA/BPA was 1.3 and 11.0, respectively, whereas the resolution between two homologous phosphonic acids such as MPA/EPA was 1.6.

Whatever the electronic carboxylic acid competitor used, branched solutes (iso-PPA and *tert.*-BPA) eluted before their linear homologous (PPA and BPA). Electronic interactions between the PGC and branched solutes (iso-PPA and *tert.*-BPA) were

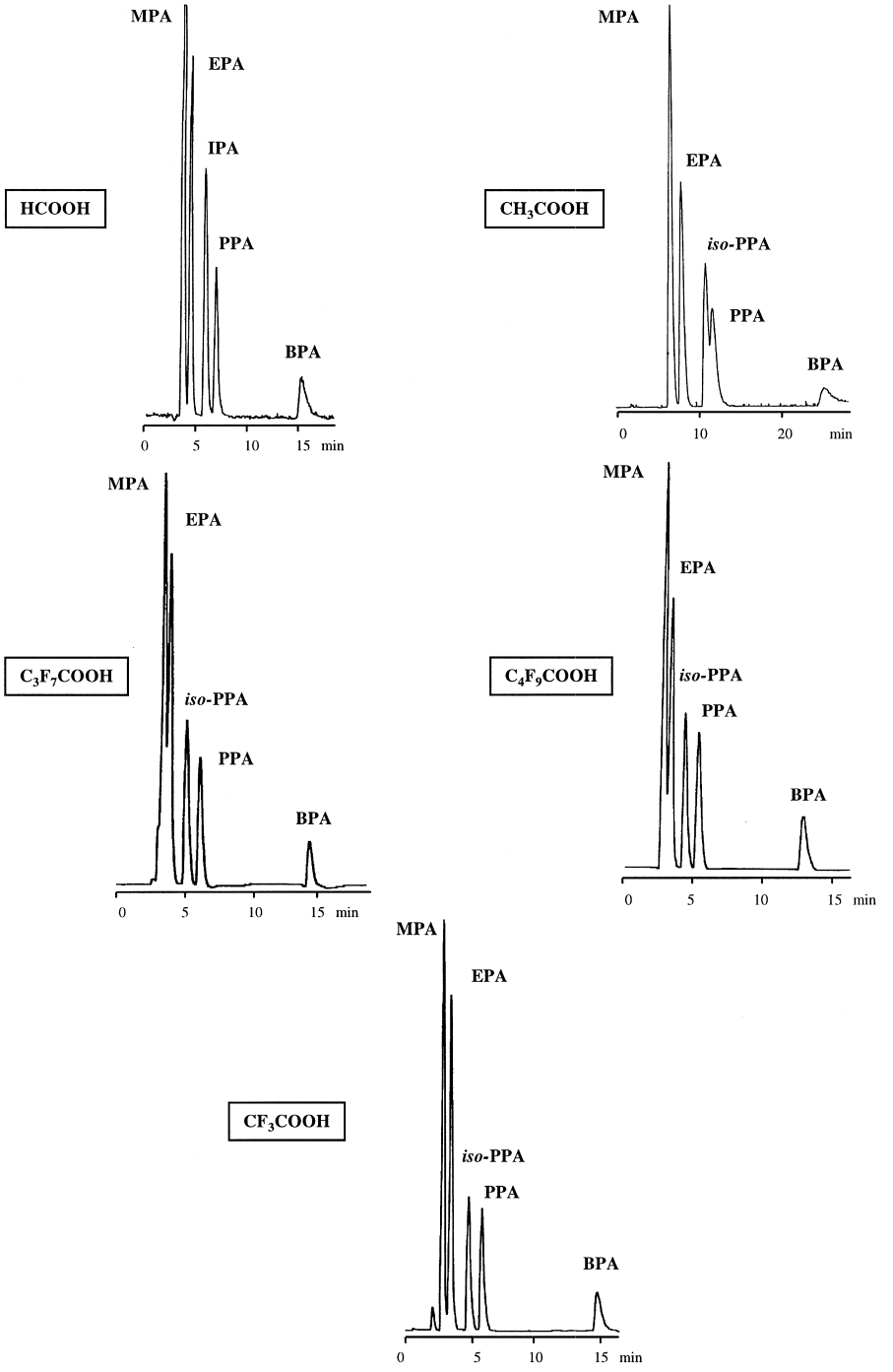

Fig. 1. LC–ELSD separations of alkylphosphonic acids with various volatile electronic interaction competitors. Mobile phase, 0.1% (v/v) carboxylic acid in water (for composition, see Table 2). Hypercarb S (150×2.1 mm I.D., 7 μm) column. Isocratic elution at 0.2 ml/min. Solute concentration, 50 mg/l; injection volume, 20 μl; Sedex 45 evaporative light-scattering detector; drift tube temperature, 40°C; photomultiplier gain set at 10; pressure of nebulization gas (dried and filtered nitrogen), 2.2 bar.

lower due to steric hindrance compared with electronic interactions with their linear homologues. Consequently, the elution strength of branched phosphonic acids was greater. As a result, CF_3COOH was selected as the additive to the mobile phase.

However, elution of the most hydrophobic compounds (CPMPA, CMPA) requires increasing the elution strength of the mobile phase by adding an organic solvent such as acetonitrile (ACN). ACN decreases the retention times of phosphonic acids by decreasing the hydrophobic interactions between PGC stationary phase and analytes. In order to improve the efficiency of separation between CMPA and CEPA, a gradient elution was carried out (Fig. 2).

3.2. LC–MS analysis

The previous mobile phase (0.1% v/v CF_3COOH–water–ACN) is sufficiently volatile to be compatible with ionspray MS detection. Direct transposition of these chromatographic conditions to MS detection is illustrated in Fig. 3(a).

Firstly, using the chromatographic optimization previously developed in LC–ELSD, a split of the flow-rate was adapted in order to reduce the LC flow towards the MS detector (20 μl/min).

Secondly, the ionspray MS conditions for phosphonic acid detection were optimized in a previous study during the development of adequate buffers for capillary electrophoresis (CE) coupling [23]. In MS negative-ion mode, using a volatile mobile phase, the soft ionization mode of ionspray exhibits minimal fragmentation, with the production of very abundant negative ion, $[M-H]^-$ (Table 1), corresponding to the loss of one proton.

Fig. 3(a) shows a single-ion monitoring (SIM) ionspray LC–MS analysis of a standard mixture of alkyl methylphosphonic acids. MS detection was set at each m/z of the analytes. Fig. 3(b) illustrates the LC–MS separation of diacids with the same elution gradient as in Fig. 3(a). As expected, separation between branched and linear acids was easily obtained; the resolution was 1.2, 1.7 and 10.5 for MPA/EPA, iso-PPA/PPA and *tert*.-BPA/BPA, respectively. Although coelution of PPA and *tert*.-BPA was observed, this did not disturb the identification of the two compounds since under ionspray MS conditions these two analytes give different fragmentation ions at m/z 123 and 137 for PPA and *tert*.-BPA, respectively (Table 1). Thus, a reconstructed ion chromatogram at each m/z (123 and 137) allows accurate identification. This procedure shows the advantages of MS detection compared with ELSD detection since each analyte could be identified by mass determination.

3.3. Analysis of spiked tap water by LC–MS

This procedure has been applied to identify phosphonic acids contained in a spiked tap water sample prepared by the Centre d'Etudes du Bouchet (France). Two samples (blank and spiked) of tap water were analyzed for the benefit of the Third Official PTS/OPCW Proficiency Test Analysis [23,24].

Direct studies by MS and tandem MS of the matrix and the blank have previously been published [19] and have enabled the molecular mass of each compound to be determined (**1**, $M_r = 124$ and **2**, $M_r = 208$) by the production of very abundant negative ions ($[M-H]^-$, **1**, m/z 123 and **2**, m/z 207) corresponding to the loss of one proton.

SIM LC–MS analysis under optimized chromatographic conditions (PGC–CF_3COOH–water–ACN) of the matrix and the blank sample, in negative-ion mode, was then carried out as illustrated in Fig. 4(a and b). Compared with the LC–MS blank analysis (Fig. 4(a)), two phosphonic acids at m/z 123 and 207 appear in the LC–MS chromatogram of the spiked sample (Fig. 4(b)). The k' values of the analytes (**1**, $k' = 1.7$ and **2**, $k' = 8.0$) correspond to the order of magnitude of their molecular masses.

Compound **1** ($k' = 1.7$) corresponds to a solute with moderate hydrophobicity. Fragmentation studies have confirmed this observation and revealed the loss of an alkyl chain group at m/z 43, which may be due to a C_3H_7 alkyl group (linear or branched, propyl or isopropyl). Compound **1** is therefore either an iso-PPA or PPA. The matrix was then spiked with PPA (Fig. 5(a)) and IPA (Fig. 5(b)) ($M_r = 124$) and further analyzed by LC–MS. Separation and resolution between compound **1** and PPA are shown in Fig. 5(a), whereas solute number **1** coeluted with iso-PPA (Fig. 5(b)). Compound **1** was then identified to be the isopropylphosphonic acid (iso-PPA).

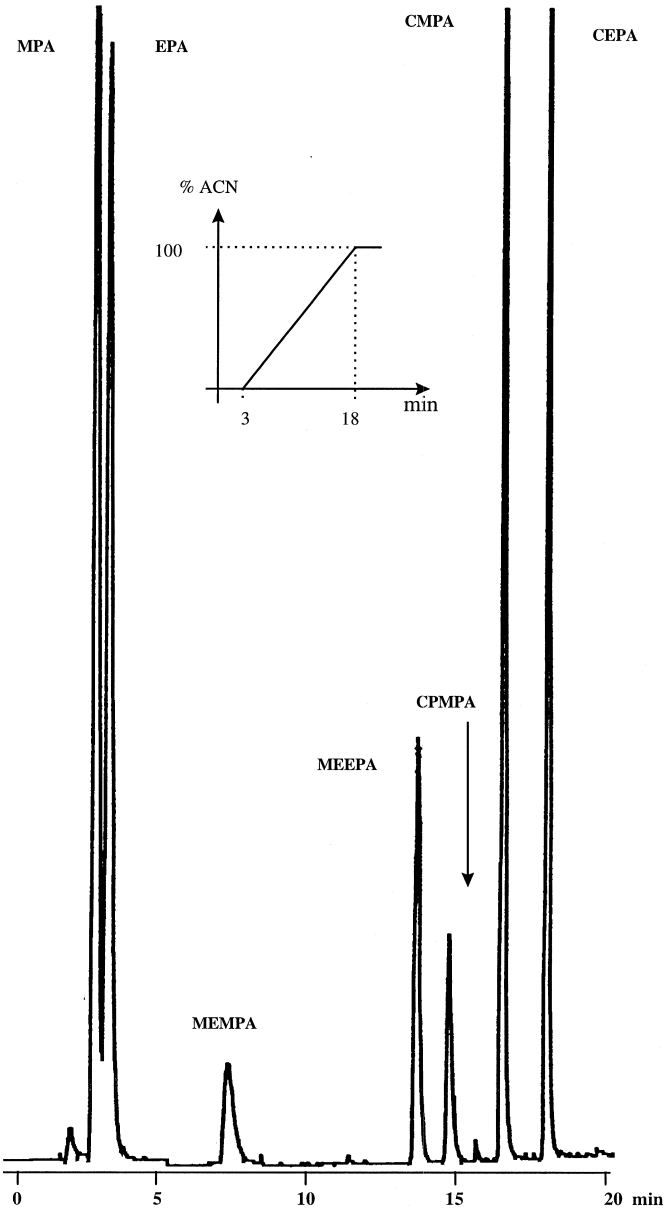

Fig. 2. LC–ELSD separation of phosphonic acids using 0.1% (v/v) trifluoroacetic acid. LC conditions as in Fig. 1 except for the mobile phase: gradient elution, 0–3 min, 0.1% (v/v) trifluoroacetic acid in water; 3–18 min, 0.1% (v/v) trifluoroacetic acid in water to 0.1% (v/v) trifluoroacetic acid in ACN.

Concerning compound **2** (m/z 207; $M_r = 208$), its k' value ($k' = 8.0$) revealed that it was a solute with high hydrophobic behavior and probably not included in our standard collection (Table 1). Previous studies by CE–MS [23] of this compound have shown it to be an alkyl-methylphosphonic acid, but only the alkyl chain (C_8H_{17}) has been determined. Fragmentation studies using other ionization sources are necessary to identify compound **2**, which is the (2-ethylhexyl) methylphosphonic acid (EHMPA).

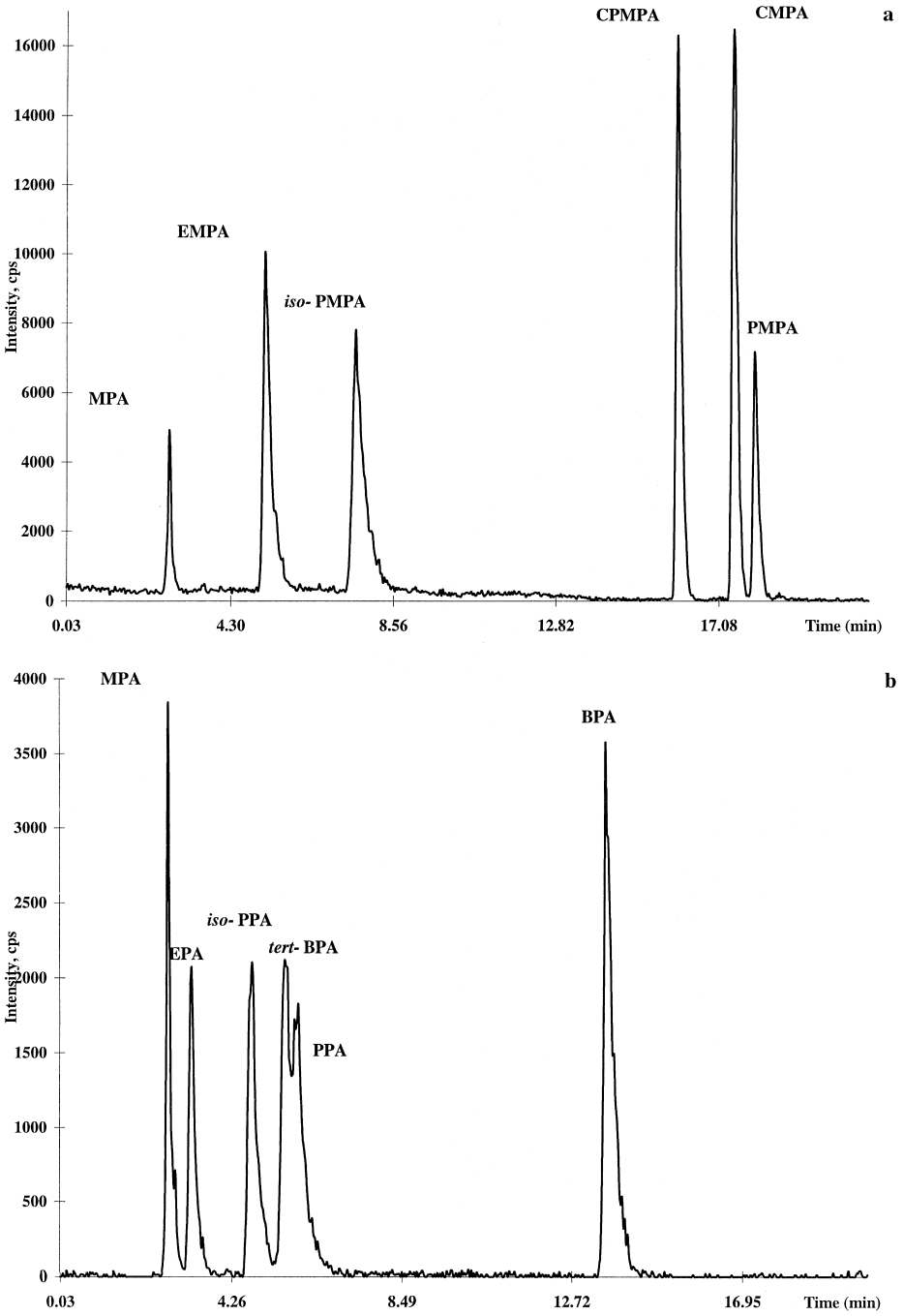

Fig. 3. LC–MS analysis of several phosphonic acids. LC conditions as in Fig. 2, solute concentration 50 mg/l. MS conditions: split system 1/10; nebulization gas, air; ionspray voltage, −4 kV; orifice voltage, −25 V; ring voltage, −400 V. (a) Alkyl-methylphosphonic acid; (b) diacids.

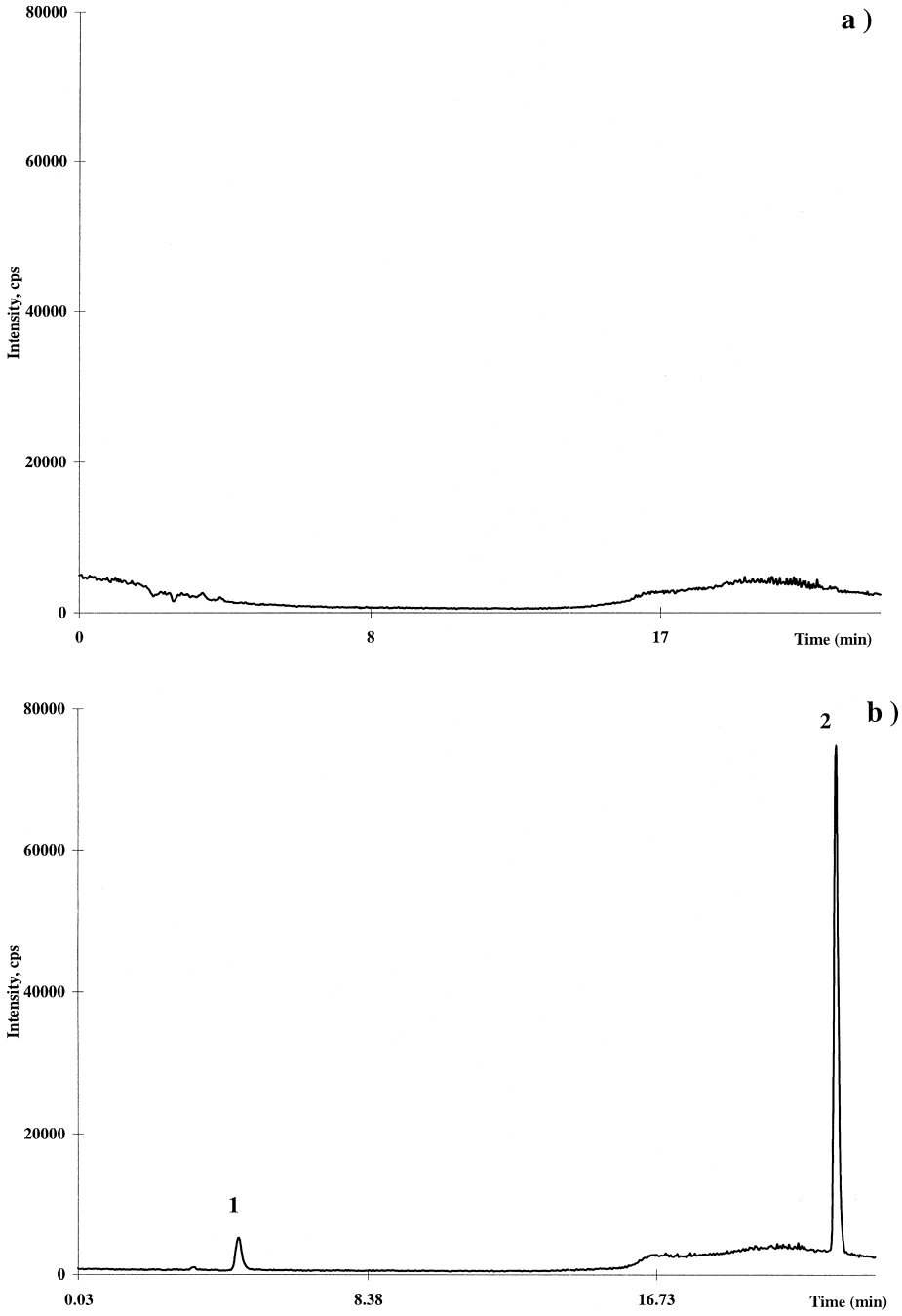

Fig. 4. LC–MS analysis of the spiked sample. LC and MS conditions as in Fig. 3. (a) Blank sample; (b) spiked sample.

Finally, quantitative analysis was performed by LC–MS. The linear calibration curve of two phosphonic acids shows a good correlation coefficient ($r = 0.999$ and 0.998 for IPA and EHMPA, respectively) in the 1–100 mg/l concentration range. Concentrations in spiked tap water were found to be

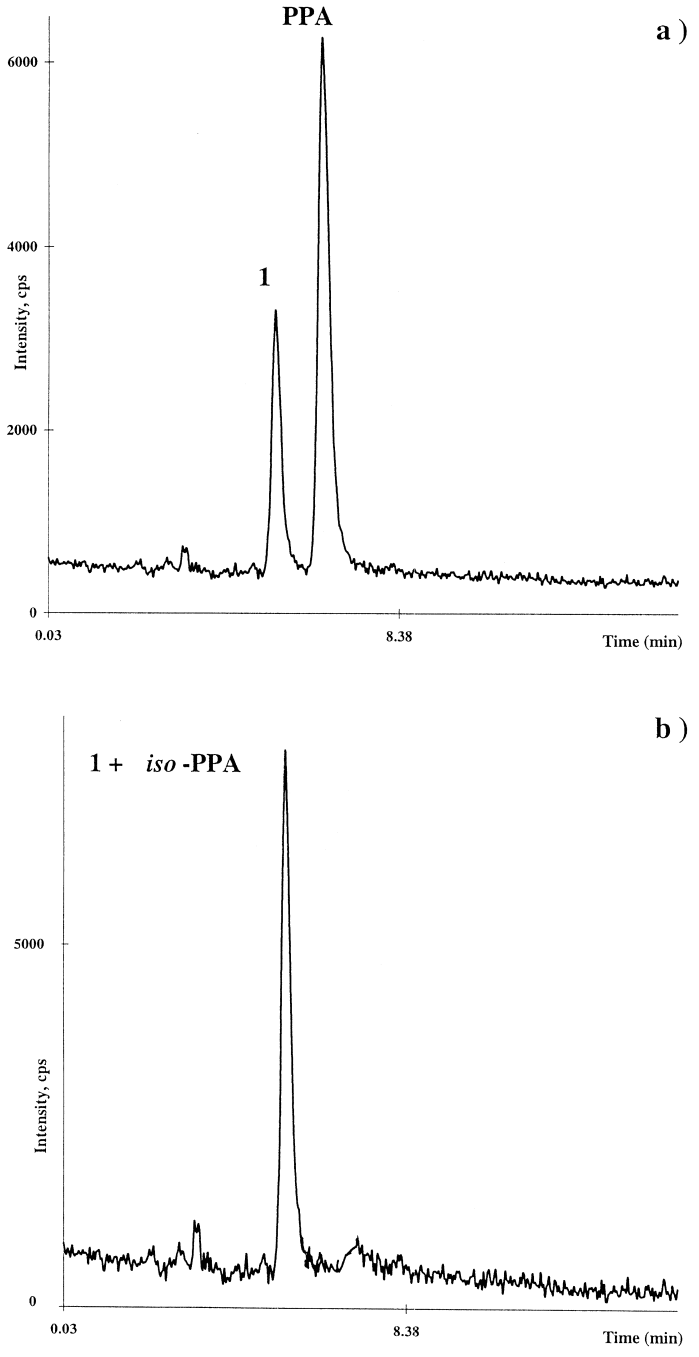

Fig. 5. LC–MS identification of compound **1**. LC and MS conditions as in Fig. 3.

9.3 and 13.5 mg/l for IPA and EHMPA, respectively, and are close to the theoretical values (respectively 8 and 12 mg/l).

4. Conclusion

The analysis of several phosphonic acids has been

achieved by LC–MS on porous graphitic carbon as stationary phase. The porous graphitic carbon surface induces both electronic and hydrophobic interactions. Therefore, a mobile phase composed of 0.1% (v/v) CF_3COOH (electronic interaction competitor) and acetonitrile (hydrophobic interaction competitor) allowed the separation of all the analytes studied. Separations of branched phosphonic acids (iso-PPA/PPA and *tert*.-BPA/BPA) and homologous compounds (MPA/EPA) are achieved with this new chromatographic system in <15 min.

ELSD was very useful for developing an adequate mobile phase composition which is also suitable for MS detection. Indeed, ELSD is an inexpensive detector which requires volatile mobile phases, as in MS detection mode. LC–MS coupling methodology was then carried out for the identification and quantification of two unknown phosphonic acids contained in a spiked tap water sample. This methodology is easy to implement since no preconcentration and derivatization steps are necessary.

References

[1] P.C. Bossle, J.J. Martin, E.W. Sarver, H.Z. Sommer, J. Chromatogr. 267 (1983) 209.
[2] M.C. Roach, L.W. Ungar, R.N. Zare, L.M. Reimer, D.L. Pompliano, J.W. Frost, Anal. Chem. 59 (1987) 1056.
[3] J.P. Mercier, Ph. Morin, M. Dreux, A. Tambuté, Chromatographia 48 (1998) 1.
[4] P.C. Bossle, D.J. Reutter, E.W. Sarver, J. Chromatogr. 407 (1987) 399.
[5] C.E. Kientz, A. Verweij, G.J. De Jong, U.A.T. Brinkman, J. High-Resolut. Chromatogr. 12 (1989) 793.
[6] C.E. Kientz, A. Verweij, H.L. Boter, A. Poppema, R.W. Frei, G.J. De Jong, U.A.T. Brinkman, J. Chromatogr. 467 (1989) 385.
[7] C.E. Kientz, A. Verweij, G.J. De Jong, U.A.T. Brinkman, J. Microcol. Sep. 4 (1992) 465.
[8] C.E. Kientz, A. Verweij, G.J. De Jong, U.A.T. Brinkman, J. Microcol. Sep. 4 (1992) 477.
[9] G.A. Pianetti, A. Baillet, F. Traore, G. Mahuzier, Chromatographia 36 (1993) 263.
[10] G.A. Pianetti, L.M. Moreira de Campos, P. Chaminade, A. Baillet, D. Baylocq-Ferrier, G. Mahuzier, Anal. Chim. Acta 284 (1993) 291.
[11] E.R.J. Wils, A.G. Hulst, J. Chromatogr. 454 (1988) 261.
[12] R.M. Black, R.W. Read, J. Chromatogr. A 759 (1997) 79.
[13] R. Black, R.W. Read, J. Chromatogr. A 794 (1998) 233.
[14] P. Ross, J.H. Knox, Adv. Chromatogr. 37 (1997) 121.
[15] P. Chaimbault, J-P. Mercier, C. Elfakir, M. Dreux, Communication presented at HPLC'97, Birmingham, June 1997.
[16] C. Elfakir, P. Chaimbault, M. Dreux, J. Chromatogr. A 826 (1998) 193.
[17] K.N. Petritis, P. Chaimbault, C. Elfakir, M. Dreux, J. Chromatogr. A 833 (1999) 147.
[18] M. Dreux, M. Lafosse, L. Morin-Allory, LC–GC Int. 9 (1996) 148.
[19] M. Dreux, M. Lafosse, LC–GC Int. 10 (1997) 382.
[20] A. Bartha, G. Vigh, H.A. Billiet, L. De Galans, J. Chromatogr. 303 (1984) 29.
[21] M. Patty, J. Chromatogr. A 660 (1994) 17.
[22] J.P. Mercier, Ph. Morin, C. Elfakir, M. Lafosse, M. Dreux, A. Tambuté, J. Liq. Chromatogr. 22 (1999) 267.
[23] J-P. Mercier, P. Chaimbault, Ph. Morin, M. Dreux, A. Tambuté, J. Chromatogr. A 825 (1998) 71.
[24] A.E.F. Nassar, S.V. Lucas, W.R. Jones, L.D. Hoffland, Anal. Chem. 70 (1998) 1085.