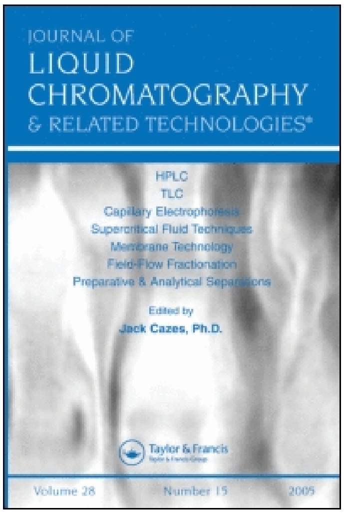

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SIMULTANEOUS DETERMINATION OF INORGANIC ANIONS AND METHYL AND ETHYLPHOSPHONIC ACIDS BY ANION EXCHANGE CHROMATOGRAPHY USING EVAPORATIVE LIGHT SCATTERING DETECTION

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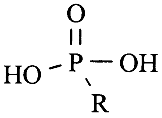
ABSTRACT

Anion exchange chromatography with evaporative light scattering detection was investigated for the direct and simultaneous analysis of methyl and ethylphosphonic acids and inorganic anions in aqueous media. The chromatographic system made up of a Dionex IonPac AS4A-SC column and an aqueous mobile phase containing NH_4HCO_3 25 mM (pH 7.8) allows the resolution of the two phosphonic acids in less than 10 minutes without coelution of chloride, phosphate, and sulfate.

This methodology has been successfully applied to the direct analysis of aqueous matrix containing low levels of phosphonic acids and high levels of inorganic anions (chloride 1700 mg/L, phosphate 1000 mg/L, or sulfate 1200 mg/L) without any baseline perturbation and matrix pretreatment. Quantitative analysis of phosphonic acids is achieved with a good correlation coefficient, and a 200 ng (200 µL of 1 mg/L) detection limit of MPA or EPA is obtainable.

INTRODUCTION

Phosphonic acids are hydrophilic and moderately strong acid solutes, of which, the two major representatives are methylphosphonic and ethylphosphonic acids (respectively MPA and EPA).



R = $-CH_3$ MPA R = $-C_2H_5$ EPA

These compounds are the final hydrolysis products of chemical warfare agents such as nerve agents. The identification and separation of these analytes is, therefore, of relevance in environmental studies.

Ion exchange chromatography is the obvious method of choice for the analysis of hydrophilic organic and inorganic ions in aqueous media. Previous methods based upon ion exchange chromatography[1-5] have shown that the separation of alkylphosphonic acids which have identical ionic moieties, discrete structural differences in the alkyl side chain, and consequently quite similar pKa's,[3,6] is difficult to obtain by a pure ion exchange mechanism. A more satisfactory separation of phosphonic acids, is achieved on a polymer-based material rather than on a silica-based column, due to the combination of ionic and apolar interactions between solutes and the polymer-based packing. Furthermore, as the monoionized form is poorly retained, an eluent pH value set at 8.5, in order to obtain the phosphonic acids mainly as dianions, results in better retention. Nevertheless, on a Waters 4.6 x 50 mm IC-Pak anion column,[3,4] MPA and EPA coeluted whatever the nature of the eluent at pH 8.5 (borate/gluconate buffers or potassium phthalate).

The analysis of alkylphosphonic acids has been also investigated by ion-pair reversed phase chromatography. With tetrabutylammonium hydroxide used as ion-pairing reagent,[1] the separation of hydrophobic phosphonic acids is achieved, but coelution of the hydrophilic MPA and EPA is observed. However, with pentylammonium formate as volatile ion-pairing reagent,[7] a satisfactory resolution (Rs = 2) between MPA and EPA was obtained.

As a general rule, one of the difficulties encountered in ion chromatography analysis of aqueous samples, is the possible interference of inorganic anions (Cl^-, SO_4^{2-}, phosphate…) endemic to natural waters, with the hydrophilic anionic contaminants analysed. In ion-pair reversed phase chromatography, it has been noted[7] that co-elutions between chloride and MPA and between nitrate and EPA, respectively, could not be avoided. In ion chromatography, the proposed method[5] is based on the use of a Dionex Omnipac PAX-100 column and a suppressed conductimetric detection. Thus, when natural waters are analyzed, a pretreatment and a clean up of the sample before analysis is necessary, in order to limit the interferences due to a high concentration of background inorganic ions. This methodology is limited by the lack of a suitable confirmatory detector.

The aim of this work is to evaluate a direct, isocratic and simultaneous analysis of alkylphosphonic acids and inorganic anions in aqueous samples by anion exchange chromatography using an Evaporative Light Scattering (ELS) detection. ELSD is generally considered to be a very convenient and universal LC detector for analytes without chromophore groups and which are less volatile than the chromatographic eluent. Furthermore, this detection mode has proved useful for inorganic anion[7,8] and phosphonic acid[7,9] determination. ELSD needs a volatile mobile phase when LC-ELSD coupling is investigated, in order to avoid high background noise. This is also a requirement for mass spectrometry. As a consequence, LC methodology previously developed with ELSD is directly compatible with MS detection if a further and complementary identification of solutes is necessary.

Anion exchange chromatography has been investigated in this work at a working pH where the difference in the apparent charge of MPA and EPA is maximal, to promote the separation between MPA and EPA by ionic interactions, in addition to some reverse phase interactions with the polymeric anion exchanger. An application of the preliminary method to spiked water containing high levels of inorganic anions is also shown.

EXPERIMENTAL

Instrumentation

Isocratic elution was performed with a Gilson (Villiers Le Bel, France) HPLC system consisting of a 302 model equipped with a manual Rheodyne valve (7125). The injection loop has an internal volume of 20 µL (or 200 µL for the LOD determination).

A Sedex 55 Evaporative Light-Scattering Detector (Sedere, Alforville, France) was used. The detector temperature was set at 40°C, the gain set at 8, and the pressure of nebulizing gas (dried and filtered nitrogen) at 2.2 bar.

Separations were performed on a Satisfaction P 4000-SAX 50 x 4.6 mm I.D (Cluzeau, Sainte-Foy-La-Grande, France) column; a Dionex IonPac AS4A-SC 250 x 4 mm I.D (Sunnyvale, CA, USA) column, and a Hamilton PRPX-100 100 x 4.1 mm I.D. (Bonaduz, Switzerland) column.

Data were processed using Shimadzu (Kyoto, Japan) model CR 6 A calculator.

In a typical procedure, the necessary amount of ammonium hydrogen carbonate to produce the desired salt concentration in the mobile phase was weighed and dissolved in distilled water. The pH of each mobile phase was checked on a Beckman pH meter, model ϕ 10 (Gagny, France) and a value of pH equal to 7.8 was measured for the three salt concentrations studied (7.5 mM, 12.5 mM, and 25 mM). The apparent charge of the eluent anion, the ionic strength, and the buffer capacity of each eluent were calculated with the help of Phoebus software (Sedere Co., Franklin MA, USA).

The mobile phase was delivered at a flow rate of 1 $mL.min^{-1}$. Standard solutions were injected at a concentration of 100 $mg.L^{-1}$ during the measurement of the retention factors.

Chemicals

Ammonium hydrogen carbonate (NH_4HCO_3) was purchased from Fluka (St. Quentin Fallavier, France). Chloride, nitrate, phosphate, and sulfate were standard sodium salts from Sigma (St. Quentin Fallavier, France) and methylphosphonic acid (MPA) and ethylphosphonic acid (EPA) were purchased from Aldrich (St. Quentin Fallavier, France). All standard mixtures of analytes were prepared in purified water (HPLC-quality) obtained with Elgastat UHQ II System (Elga, Antony, France).

RESULTS AND DISCUSSION

Choice of the Chromatographic System

MPA and EPA are two diprotic acids with pKa values[3] equal to:

	pKa_1	pKa_2
MPA	2.35	7.10
EPA	2.43	8.05

Calculations have been undertaken to evaluate the apparent charge (z_i) of these phosphonic acids versus pH. z_i can be defined as:[10]

$$z_i = \Sigma n[AP^n] \big/ [AP]_0$$

where $[AP^n]$ is the concentration of the phosphonic acid form at a n charge and $[AP]_0$ the total concentration of phosphonic acid. The variation of the apparent charge of the two phosphonic acids versus pH is shown in Figure 1a. At pH 0, phosphonic acids are in their neutral form, their apparent charge increases with an increase in pH, and the difference of their apparent charge is illustrated in Figure 1b. The difference between EPA and MPA charge reaches a maximum at pH 7.6 (Figure 2b) and a minimum between pH 4 to 6 and above pH 10. A working pH closely related to 7.6 was therefore selected. According to the anion exchange chromatography principle, at this pH, MPA will be analyzed as a di-anion (apparent charge -1.8) and EPA as a mono-anion (apparent charge -1.3); thus, MPA will be more retained than EPA using a pure ion exchange mechanism.

ELSD was chosen instead of the usual detection for ions such as indirect/direct UV absorbance or conductivity, as universal detection mode. The principle of the detection is based on (i) nebulization of the chromatographic effluent, (ii) evaporation of the chromatographic eluent, and then, (iii) scattering of the light by the residual particles ideally constituting the analytes.[11,12] Hence, the addition in the aqueous mobile phase of a common competing agent for anion exchange chromatography should be compatible with ELSD volatility requirements. Ammonium hydrogen carbonate (NH_4HCO_3) was selected to compose the volatile mobile phase for several reasons. First, the mobile phase preparation was easy. When this volatile salt is dissolved in water, a value of pH equal to 7.8 is measured for a respectively, 7.5 mM, 12.5 mM, or 25 mM salt aqueous solution. Then, no adjustment of the eluent pH was necessary to obtain a value closely related to pH 7.6. Furthermore, the buffer capacity of the eluent, which varied from 1.1 mM/pH unity for the lowest salt concentration studied to 3.6 mM/pH unity for the highest salt concentration, was sufficient to avoid any variation of pH buffer into the chromatographic system and, consequently, allowed to obtain reproducible capacity factors even with different preparations of mobile phase.

Three anion exchangers, with different exchange capacities, were selected for this study. A polymeric backbone packing (Table 1) was chosen since silica backbone cannot be used at 7.8 without bonding hydrolysis. Moreover, such an anion exchanger induces both ionic and hydrophobic interactions due to the specific properties of the polymer surface (apolar interactions).

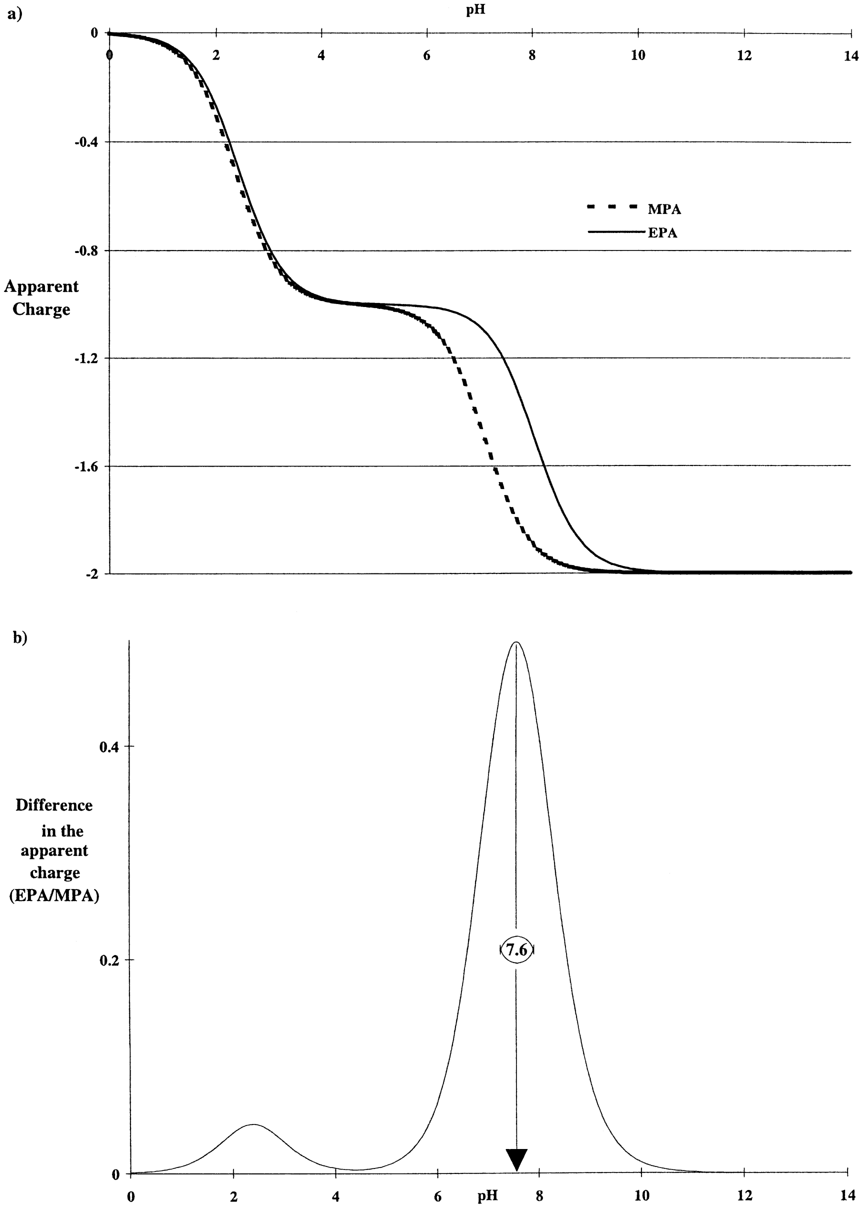


Figure 1: Variation in the apparent charge of MPA and EPA versus the pH. a) Comparison of MPA and EPA apparent charge versus the pH. b) Difference in the apparent charge (MPA-EPA) versus the pH.

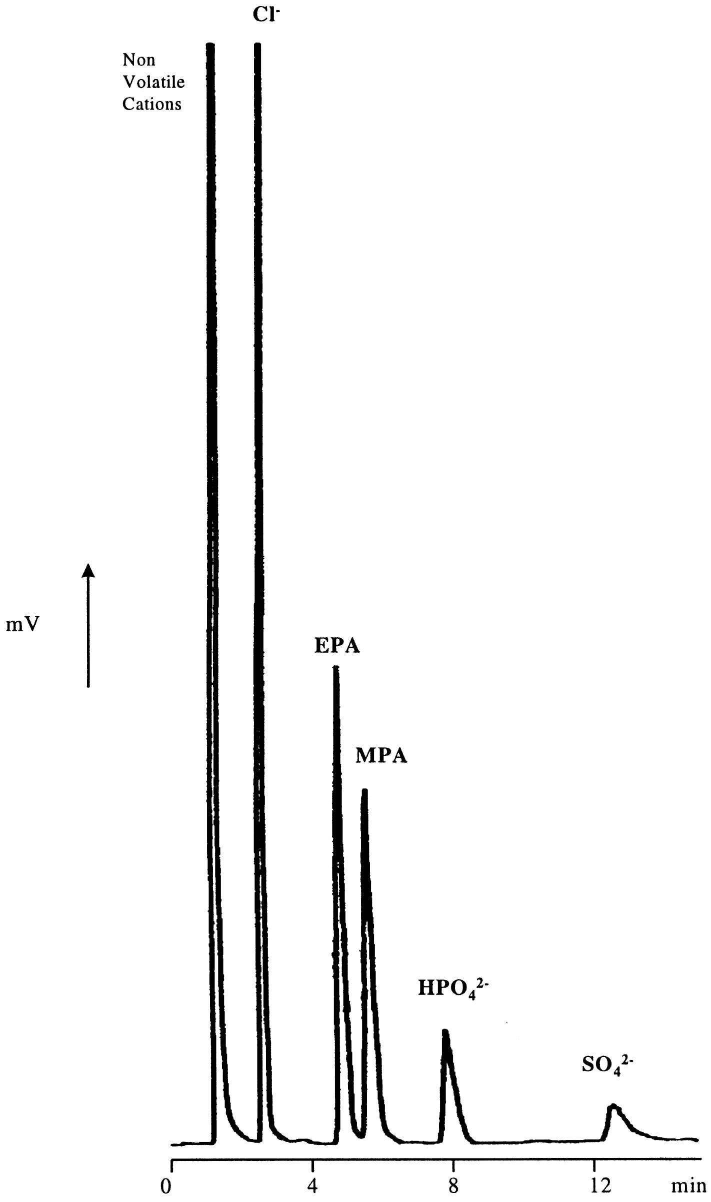


Figure 2: Separation of MPA, EPA and inorganic anions. Column: Dionex, IonPac AS4A-SC (250 x 4 mm); isocratic elution at 1 mL.min^{-1} with 25 mM ammonium hydrogen carbonate; injected volume, 20 μL; solute concentration 100 mg/L. Detection: DEDL Sedex 55, 40°C, PM 8, P_{N2}, 2.2 bar.

Table 1

Characteristics of the Commercial Anion-Exchange Packing Materials Studied

Stationary Phase	Dimensions (mm)	pH Range	Ion-Exchange Capacity	Particle Diameter (µm)	Type of Packing Material
Hamilton PRPX-100	100 x 4.1	1 - 13	190 µeq/g	10	Macroporous poly(styrene-divinylbenzene) co-polymer with trimethylammonium groups
Satisfaction P4000-SAX	50 x 4.6	1 - 14	*	*	Crosslinked polystyrene-divinylbenzene coated with quaternary aminomethyl
Dionex IonPac AS4A-SC	250 x 4	0 - 14	20 µeq/column	12	Highly cross-linked poly-ethylvinylbenzene/ divinyl-benzenesubstrate agglomerated with anion exchange latex that has been completely aminated

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* No data available.

Retention and Selectivity

The retention factors of four common inorganic anions (Cl^-, NO_3^-, HPO_4^{2-}, SO_4^{2-}) and of MPA and EPA have been determined at different ammonium hydrogenocarbonate concentrations in the aqueous mobile phase (25, 12.5, and 7.5 mM) and are reported in Table 2. As usually observed in ion chromatography, a decrease in the competing ion concentration in the eluent (25 to 7.5 mM) involves an increase in the anion retention factor. On Satisfaction P 4000-SAX and Dionex IonPac AS4A-SC column, the elution strength of the three mobile phases studied is suitable to elute the anions selected, whereas, on Hamilton PRPX-100 column, even with the mobile phase containing the highest concentration of the eluent anion (25 mM) it is not possible to elute the solutes selected, except Cl^- (k' = 6.5). The exchange capacity of Hamilton support is about ten times greater than that of the other ion exchangers (Table 1). Consequently, as well established in an ion exchange mechanism, the greater the exchange capacity of the packing is, the more retentive the support is. Furthermore, as a low concentration of additive in eluent is favorable to a low ELSD background noise, the Hamilton column was not retained for further studies.

The elution order of anions depends on their apparent charge. For inorganic anions, dianions (phosphate and sulfate) were eluted after mono-anions, such as chloride and nitrate and, for phosphonic anions, MPA is more retained than EPA at pH 7.8. The elution order of the mixture is as follows: first Cl^- is eluted, then NO_3^-, EPA, MPA, $HPO_4^{2,-}$ and finally, SO_4^{2-}. It is interesting to note, that previously, an opposite elution order of MPA/EPA has been observed on silica bonded column in ion-pair reversed chromatography[7] at pH 4 and on a porous graphitic carbon[9] with carboxylic acid as electronic competitor in the mobile phase. This new methodology can be used favorably as a complementary technique to the previously described methods for identification of trace amounts, or determination of a minor compound in the presence of a major compound. A mixture of the two phosphonic acids in various proportions could be easily analyzed and accurately quantified if the LC system elutes the major compound after the minor compound.

A good selectivity between the last eluted monoanion, MPA, and the first eluted dianion, HPO_4^{2-}, is always observed. The selectivities between Cl^-/NO_3^- and between EPA/MPA (Table 2) are constant, whatever the ammonium salt concentration in mobile phase, and moreover, for the EPA, MPA pair, the value of the separation factor is similar on the two supports. The resolution between EPA/MPA and Cl^-/NO_3^- (Table 2) slowly increases with a decrease in the ionic strength, due to an increase in the retention factor. The chromatographic system constituted by a Satisfaction P 4000-SAX support and an aqueous solution of 12.5 mM as the mobile phase, proved to be a suitable system to achieve with a well-defined baseline resolution, the separation of the six selected anions.

Table 2

Retention Factor, Separation Factor, and Resolution of Inorganic Anions and Phosphonic Acids by Anion Exchange Chromatography-ELSD with Ammonium Hydrogen Carbonate as Volatile Mobile Phase

		Retention Factor (k)						Separation Factor (α)		Resolution (R)	
Columns	NH_4HCO_3 Concn. in Mobile Phase (mM)	Cl^-	NO_3^-	EPA	MPA	HPO_4^{2-}	SO_4^{2-}	Cl^-/NO_3^-	EPA/MPA	Cl^-/NO_3^-	EPA/MPA
Satisfaction P4000-SAX	25	0.9	1.5	1.3	1.5	2.8	4.8	1.8	1.2	1.7	0.5
	12.5	1.3	2.2	3.4	4.0	6.5	12.8	1.7	1.2	2.1	0.8
	7.5	2.1	3.4	7.1	8.2	---	---	1.7	1.2	2.3	1.3
Dionex Ion Pac AS4A-SC	25	1.0	2.7	2.9	3.5	5.2	8.7	2.6	1.2	6.1	1.3
	12.5	1.7	4.3	9.5	11.5	13.6	22.9	2.6	1.2	6.1	2.3

However, the retention of SO_4^{2-} is high (k' = 12.8) under these isocratic conditions. The chromatographic system made up of a column Dionex IonPac AS4A-SC and an aqueous mobile phase containing NH_4HCO_3 25 mM, allows a satisfactory resolution of the two phosphonic acids (Rs = 1.3) in less than 10 minutes without coelution of chloride, phosphate, and sulfate (Figure 2). Only nitrate coeluted with EPA on this system (Table 2).

Figures 3a, 3b, and 3c depict the direct analysis of EPA and MPA in a water sample containing a major inorganic anion, such as chloride (1700 mg/L), phosphate (1000mg/L), or sulfate (1200 mg/L). The presence of such an anion, even at high concentration, does not perturb the separation of EPA and MPA, so good resolutions have been obtained. Consequently, this methodology could be used for the determination of MPA and EPA in a matrix containing inorganic anions, such as water sample or soil sample.

Limit of Detection (LOD) and Calibration Curve

LOD and quantitative analysis have been investigated with a lake water sample spiked with MPA and EPA. Using the most sensitive range of the ELSD, a 200 ng (200 µL of 1 mg/L) detection limit of EPA or MPA is obtainable (Figure 4), at a signal-to-noise ratio of 3.

In order to achieve accurate quantitative analysis of phosphonic acids, calibration curves have been carried out in the 12.5-100 mg/L concentration range (injected volume : 20 µL) with ammonium hydrogen carbonate 25 mM as aqueous mobile phase. Although the variation in the ELSD response is complex (it depends on droplet size, concentration and nature of the solute, gas and liquid flow rates, vaporization temperature, etc.), it was assumed that in a large range of sample size the measured peak area can be related to sample size by the following relationship:[12]

$$A = a\,C^{b}$$

Where b is the slope of the response line, C is the solute concentration and *a* is the response factor. As a result, the linearity between surface area response and concentration is obtained in double logarithmic coordinates ($\log A = b \log C + \log a$).[13] Slope b mentioned in the literature, has values generally comprised between 1 and 1.6 with 1.3 being the most representative value.[14] For MPA and EPA, graphs were linear with an acceptable correlation coefficient (R^2 higher than 0.99). The equation for the ELSD calibration curve is respectively ($\log A = 1.38 \log C + 3.31$) for MPA and ($\log A = 1.30 \log C + 3.39$) for EPA. Slopes obtained for MPA and EPA are contained in the expected interval and are close to the most representative value.

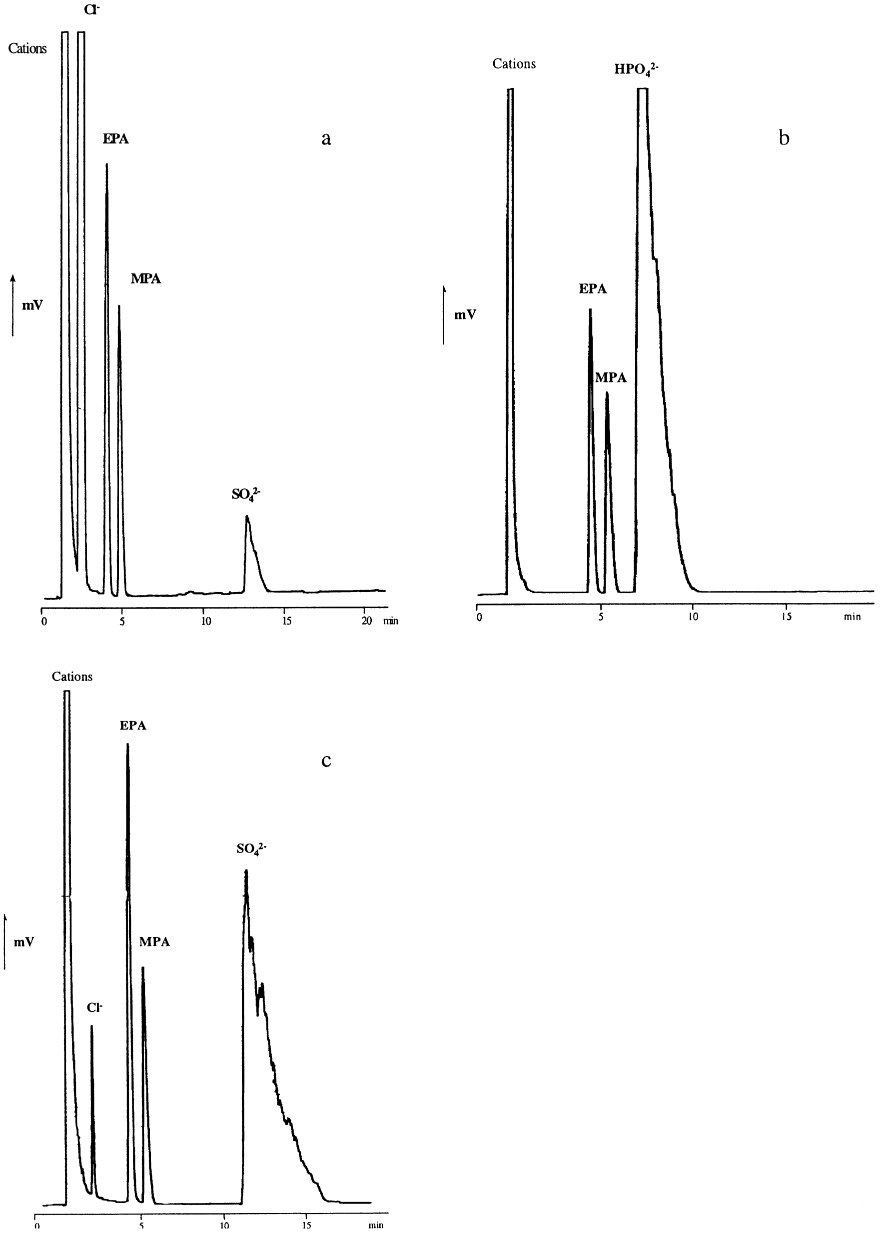

Figure 3: Separation of EPA and MPA in different aqueous media. Chromatographic conditions as in Figure 2. A) in chloride matrix (1700 mg/L for Cl^-), b) in phosphate matrix (1000 mg/L for HPO_4^{2-}), c) in sulfate matrix (1200 mg/L for SO_4^{2-}).

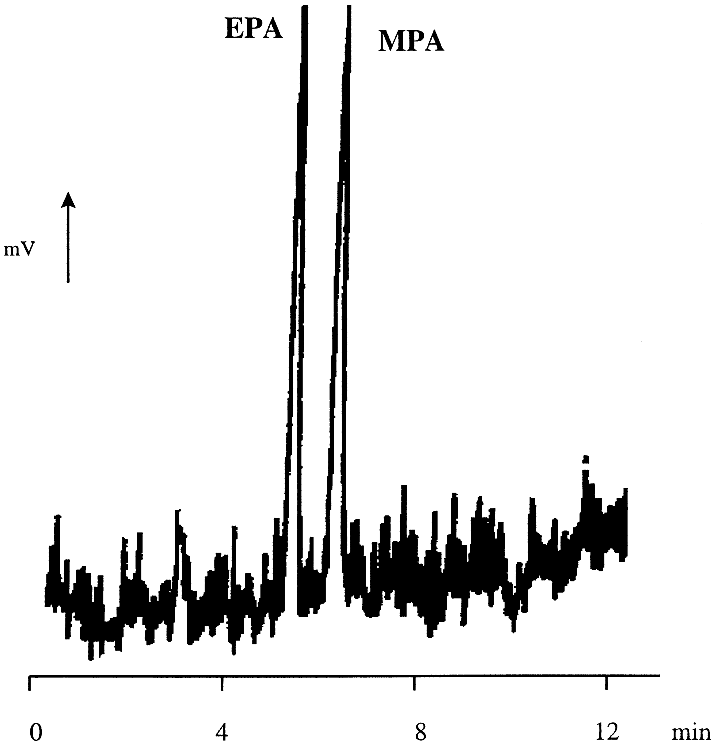

Figure 4: MPA and EPA limit of detection. Conditions as in Figure 2 except injected volume (200 µL at 1 mg/L).

CONCLUSION

This work illustrates the determination of two phosphonic hydrolysis products of chemical warfare agent, MPA and EPA, using anion exchange chromatography at controlled pH in the presence of common inorganic anions of aqueous media. The choice of adequate pH (pH 7.8) allows an elution order according to the apparent charge of the two phosphonic acids: EPA then MPA, with satisfactory baseline resolution. In comparison with previously published results, a reverse elution order is noticed. Thus, such complementary analysis methods allow accurate determination of a minor ion (MPA or EPA) in the presence of a major ion (EPA or MPA). Moreover, the proposed chromatographic system can be used for the direct and simultaneous determination of EPA and MPA in aqueous media, since inorganic anions such as chloride, sulfate, and phosphate do not coelute with phosphonic anions. The presence of such inorganic anions, even at high concentration, does not perturb the separation of EPA and MPA, thus, good resolution has been obtained.

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