Journal of Chromatography, 630 (1993) 371-377
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 707

Determination of alkylphosphonic acids by capillary zone electrophoresis using indirect UV detection

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(First received August 12th, 1992; revised manuscript received October 23rd, 1992)

ABSTRACT

Capillary zone electrophoresis with indirect UV detection was used for the determination of a series of alkylphosphonic acids. For this purpose, a few UV-absorbing background electrolytes were tested and phenylphosphonic acid, which has a mobility close to that of the analysed compounds, was shown to be the most suitable. The influence of several parameters such as concentration of the UV-absorbing background electrolyte and concentration of borate on both sensitivity and efficiency was investigated. An increase in the borate concentration produced an improvement of the signal-to-noise ratio. Conversely, the sensitivity decreased with increasing concentration of the phenylphosphonic acid. The reproducibility of the method was very satisfactory and limits of detection were less than 0.21 pmol injected.

INTRODUCTION

Alkylphosphonic acids represent a great variety of components which are widely used as herbicides, insecticides and antibiotics. The determination of these compounds is of relevance not only in the environmental field, owing their biocidal potency, but also in biological fluids for trace determinations of antibacterial (fosfomycin) or antiviral compounds (foscarnet). However, the alkylphosphonic acids do not absorb or fluoresce in the UV or visible spectral range, which is why many attempts have been made by different workers to convert these compounds into ester derivatives to allow their separation by GC [1–4] or to produce fluorescing [5] or UV-absorbing species [6] for their analysis by RP-HPLC. Schiff et *al.* [7] described an ion chromatographic method for the analysis of phosphonic acids, but the technique is limited by the poor efficiency and the low sensitivity achieved using conductimetric detection. Other direct detection methods such as mass spectrometry [8] and dual flame photometry–phosphorus-selective detection [9] have also been mentioned.

Here we report the separation of linear alkylphosphonic acids using capillary zone electrophoresis (CZE). A series of homologous alkylphosphonic acids was selected to evaluate the applicability of the method to separate related compounds and to obtain more information on the selectivity of this technique. No derivatization was required as indirect UV detection was used to monitor these compounds. Parameters affecting both efficiency of separation and sensitivity, such as the nature of the UV-absorbing background electrolyte and its concentration, were investigated. Finally, the method

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was validated in terms of limits of detection, linearity of the response and reproducibility.

EXPERIMENTAL

Chemicals

The formulae of the alkylphosphonic acids used are shown in Fig. 1. Methylphosphonic acid (MPA), ethylphosphonic acid (EPA), propylphosphonic acid (PPA) and butylphosphonic acid (BPA) were obtained from Aldrich (St. Quentin Fallavier, France). Standard solutions of each alkylphosphonic acid were prepared in distilled water at a 0.5 mg ml-’ concentration. The working solution consisted of a mixture of the four alkylphosphonic acids at a 0.1 mg ml^{-1} concentration. Boric acid and phthalic acid were purchased from Sigma (St. Quentin Fallavier, France), benzoic acid from Prolabo (Paris, France) and phenylphosphonic acid from Fluka (Mulhouse, France). A 2 A4 NaOH solution used to adjust the pH of the buffer was obtained from Merck (Nogent-sur-Marne, France). All other chemicals were of analytical-reagent grade.

Apparatus

CZE experiments were performed using a P/ACE 2000 system (Beckman, Gagny, France), at 30 kV. The temperature was set at 30°C. A fused-silica capillary with an effective length of 50 cm (total length 57 cm) and 75 μm I.D. was used for the separation. Samples were introduced into the capillary by hydrodynamic injection for 3 s. Two wavelengths of UV detection were chosen according to the nature of the UV-absorbing background electrolyte in order to obtain the best sensitivity: 200 nm for indirect detection when phenylphosphonic acid was added to the buffer, otherwise 254 nm. Direct UV analyses, performed to calculated the electrophoretic mobilities of benzoic acid, phenylphosphonic acid, phthalic acid and sorbic acid, were carried out at 200 nm.

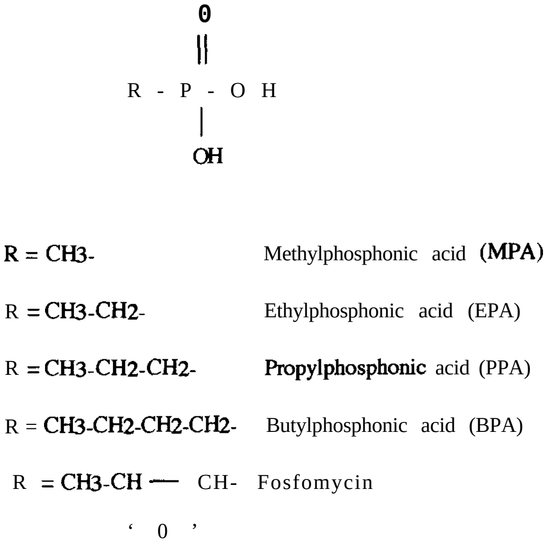

Fig. 1. Structural formulae of the alkylphosphonic acids used.

Procedures

A 200 m*M* sodium borate buffer (pH 6.0) was employed for direct UV analyses while various concentrations of the UV-absorbing background electrolyte were added to perform the indirect UV measurements. For comparison studies, 10 m*M* of either sorbic acid, phenylphosphonic acid or benzoic acid were added to 100 m*M* borate buffer (pH 6.0). All buffers were filtered through a 0.22-μm membrane (Millex-Millipore, St. Quentin-en-Yvellines, France) prior to use. Before each run, the capillary was rinsed with 0.1 *M* NaOH and then filled with the running buffer.

RESULTS AND DISCUSSION

As shown in Fig. 2, the four alkylphosphonic acids were separated in less than 9 min. The anionic species were transported to the cathode by the competition of the electroosmotic flow and the electrophoretic flow. The separation was carried out with the monoionic acid alkylphosphonic acids, which permitted a shorter analysis time than with dianionic species. At the chosen pH of 6.0, the monoionized form was predominant for all the investigated alkylphosphonic acids with respect to their pK_{a2} values (MPA = 7.10, EPA = 8.05, PPA = 8.18 and BPA = 8.19 at 25°C). Moreover, at this pH, the amplitude of electroosmotic flow was high enough to allow their migration towards the cathode. As expected, the order of migration is inversely related to the number of CH_2 groups as the electrophoretic mobility decreases with increasing relative molecular mass. As shown in Fig. 3, a linear relationship was observed between the charge/mass ratio of the com-

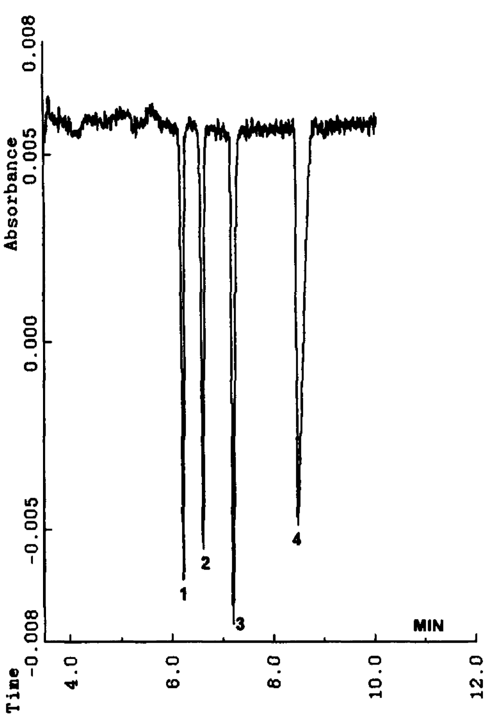

Fig. 2. Separation of alkylphosphonic acids by CZE. Conditions: 200 m*M* sodium borate buffer (pH 6.0)–10 m*M* phenylphosphonic acid; fused silica, 50 cm × 75 μm I.D.; applied voltage, 30 kV; temperature, 30°C. Peaks: 1 = BPA; 2 = PPA; 3 = EPA; 4 = MPA.

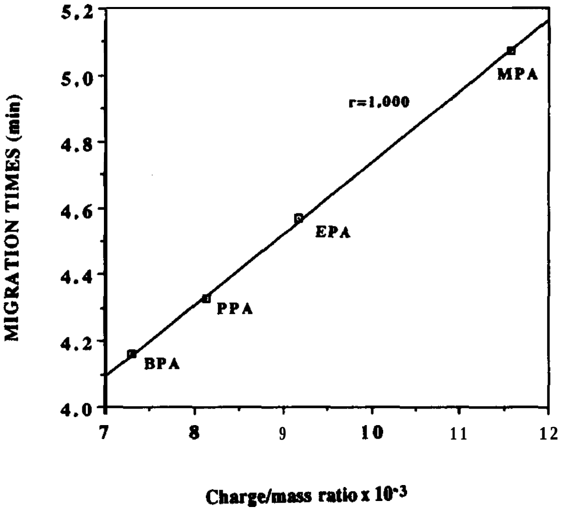

Fig. 3. Plot of the charge/mass ratios of alkylphosphonic acids versus their migration times.

pounds and their migration time for MPA, EPA, PPA and BPA (r = 1.000). The migration time of methanol used as the neutral marker was 2.35 min. At the temperature set for the analysis (30°C) the pK_{a2} value of MPA is 7.0 and is therefore the lowest of all the investigated alkylphosphonic acids. As the separation was carried out at pH 6.0, we assumed that the diionized form of MPA represents 10% of the total MPA. The presence of this diionized form leads to a shift of the monoionized MPA to a higher apparent mobility. This feature could be corrected by applying to MPA a charge of 1.1 (10% of diionized and 90% of monoionized) instead of 1.0.

Choice of the UV-absorbing background electrolyte

Selection of the most suitable UV-absorbing background electrolyte is conditioned by a few parameters that affect both the sensitivity of detection and the efficiency of separation. These two factors depend mostly on the electromigration dispersion. The highest sensitivity can be achieved for compounds having apparent mobilities close to the mobility of the UV-absorbing background electrolyte. The sensitivity of detection can be greatly increased by using a low-concentration but highly UV-absorbing background electrolyte [10]. Efficiencies are improved by using UV-absorbing background ions with a low mobility in order to diminish the dispersion phenomenon due to Joule heating [11]. The selection of a UV-absorbing background ion with an apparent mobility close to those of the analysed compounds will lead to symmetrical peaks and a negligible band-broadening, resulting in higher efficiency [10,12]. In fact, anionic compounds which exhibit, for example, a higher mobility than that of the UV-absorbing background ion will give rise to broad peaks with a diffuse front and sharp rear boundary. Consequently, the detection response will be lower for these compounds.

The electrophoretic mobilities of various electrolytes were determined at 30 kV using borate buffer (pH 6.0). Methanol was used as the marker of the electroosmotic flow. The calculated electrophoretic mobilities were $-33.25 \cdot 10^{-5}$, $-33.42 \cdot 10^{-5}$, $-30.87 \cdot 10^{-5}$ and $-30.50 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} for benzoic acid, phthalic acid, sorbic acid and phenylphosphonic acid, respectively. As the electrophoretic mobilities of alkylphosphonic acids ranged from $-27.17 \cdot 10^{-5}$ (BPA) to $-34.33 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}

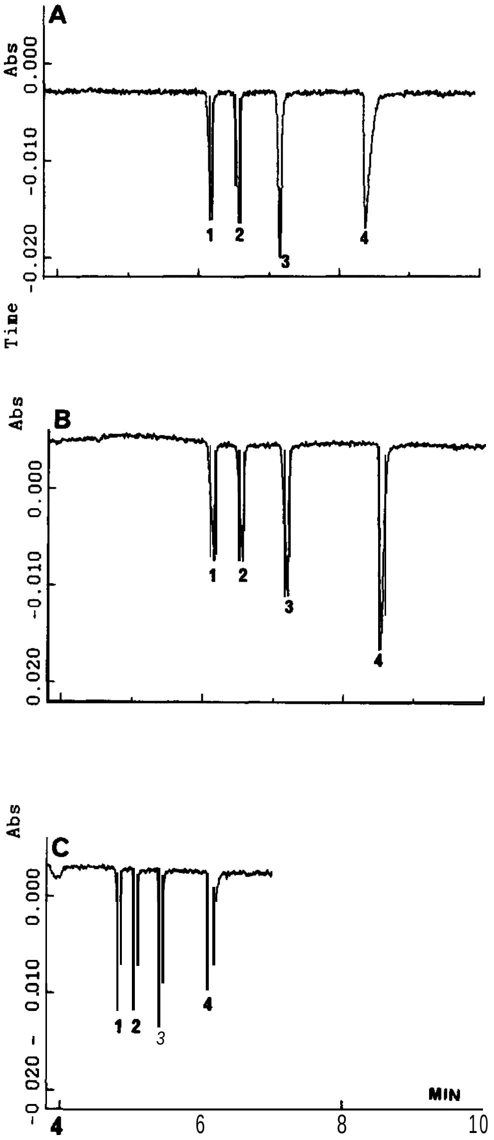

Fig. 4. Electrophoretic profiles obtained in three different buffer systems: 100 m*M* sodium borate buffer (pH 6.0) containing 10 m*M* of (A) sorbic acid, (B) benzoic acid and (C) phenylphosphonic acid. Other conditions as in Fig. 2.

(MPA), we selected three of them, benzoic acid, sorbic acid and phenylphosphonic acid, to investigate the sensitivity, efficiency and time of the separation. Fig. 4 shows the electrophoretic profils obtained using 100 m*M* sodium borate buffer (pH 6.0) with the three UV-absorbing background electrolytes.

Fig. 5 compares the performances of the three UV-absorbing background electrolytes in terms of resolution, migration time and sensitivity of detection. For the purpose of comparison, experimental conditions such as pH, concentration of borate and concentration of the UV-absorbing background ion were kept constant. The applied voltage was 30 kV and the temperature was set at 30 ± 1°C. The sample consisted of a mixture of the four alkylphosphonic acids, each at 0.1 mg ml^{-1}. Resolution factors calculated for each adjacent peaks were above 2.5, indicating that the resolution was very satisfactory regardless of the nature of the UV-absorbing background electrolyte. In contrast to what is commonly observed using chromatographic methods, the resolution between adjacent peaks increases with the migration times and is therefore higher between EPA and MPA than between BPA and PPA. On the other hand, we achieved the best resolution with benzoic acid. The shortest analysis time was obtained when phenylphosphonic acid was added to the buffer. Accordingly, peaks were broader using the two other UV-absorbing ions.

A more important feature is the comparison between the sensitivity, expressed as the signal-to-noise ratio, achieved for the various alkylphosphonic acids with the three different systems. Three of the four compounds studied showed a much better sensitivity using phenylphosphonic acid, whereas for MPA the sensitivity was of the same order with benzoic acid and phenylphosphonic acid. When phenylphosphonic acid was employed, a slightly higher sensitivity of detection was observed for EPA, which could be attributed to its closer electrophoretic mobility to that of phenylphosphonic acid. As a consequence of the short eluting period (1.2 min), the EPA, PPA and BPA response coefficients are very similar. MPA, which is the slowest ion, produces an asymmetric peak with a sharp front and a diffuse rear boundary, giving rise to a poorer sensitivity. In contrast, using benzoic acid as the absorbing electrolyte, the sensitivity is higher for MPA than for the other alkylphosphonic acids investigated because it has roughly the same electrophoretic mobility as benzoic acid.

Apparently, the use of phenylphosphonic acid provides satisfactory results. With regard to both sensitivity and efficiency this UV-absorbing background ion seems to be the most suitable. As expected, the number of theoretical plates obtained

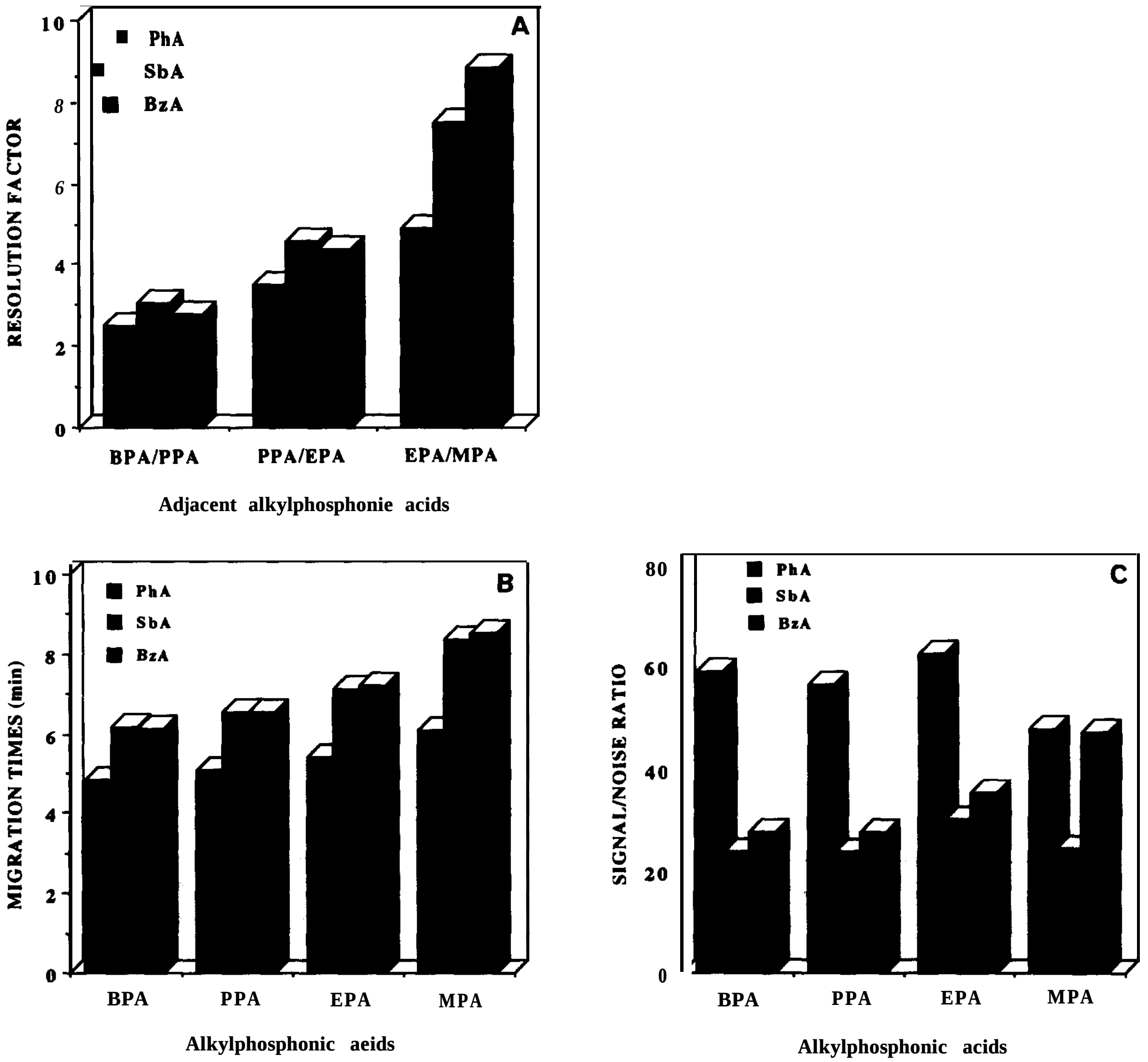

Fig. 5. Comparison of (A) resolution factors, (B) migration times and (C) signal-to-noise ratios determined with buffer systems containing either sorbic acid (SbA), benzoic acid (BzA) or phenylphosphonic acid (PhA) as the UV-absorbing background electrolyte. For conditions, see Experimental.

with this buffer is better for EPA (N = 53 500), having an electrophoretic mobility close to that of phenylphosphonic acid. The number was lower for MPA (N = 37 300), which exhibits a greater difference in the electrophoretic mobilities.

Optimization of the electrophoretic purameters

In an attempt to improve the separation, we subsequently investigated the influence of the sodium borate and phenylphosphonic acid concentrations on the quality of the separation. Fig. 6 shows the influence of the concentrations of sodium borate and phenylphosphonic acid on the signal-to-noise ratio. A general trend was observed with the variation of the concentration of sodium borate from 50 to 200 m*M*, higher concentrations producing an ap-

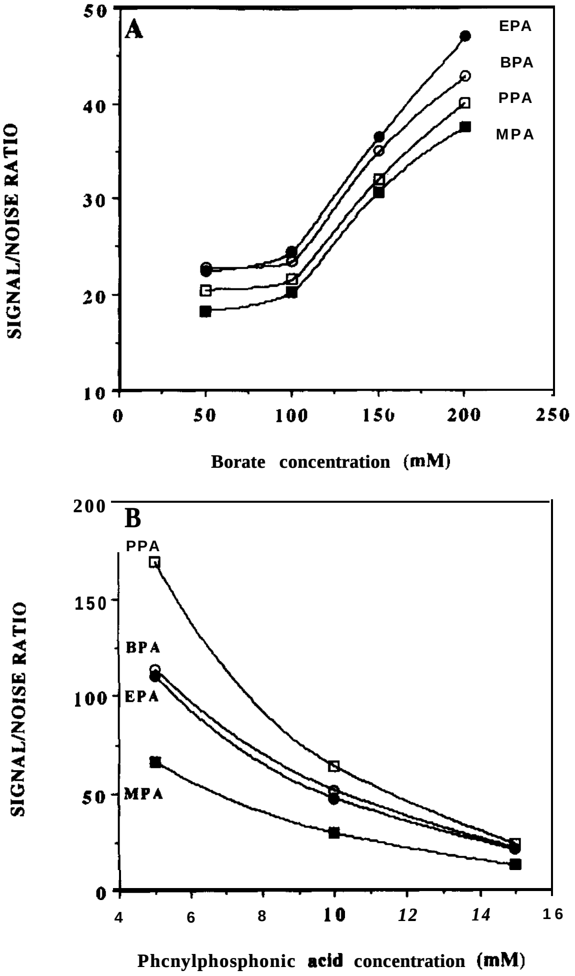

Fig. 6. Variation of the signal-to-noise ratio with increasing (A) sodium borate and (B) phenylphosphonic acid concentration. The concentration of phenylphosphonic acid was kept at 10 mM in (A) and that of sodium borate at 200 mM in (B). Other conditions as in Fig. 3.

TABLE I

REPEATABILITY AND REPRODUCIBILITY OF THE ELECTROPHORETIC MOBILITIES (μ_{ep} AND PEAK HEIGHTS EXPRESSED AS RELATIVE STANDARD DEVIATION

Factor	Parameter	BPA	PPA	EPA	MPA
Repeatability	μ_{ep}	0.93	1.80	1.66	1.47
(%) (n = 8)	Peak height	2.09	2.34	2.45	1.44
Reprodicibility	μ_{ep}	1.39	1.80	1.78	1.65
(%) (n = 17)	Peak height	3.62	3.81	2.95	1.97

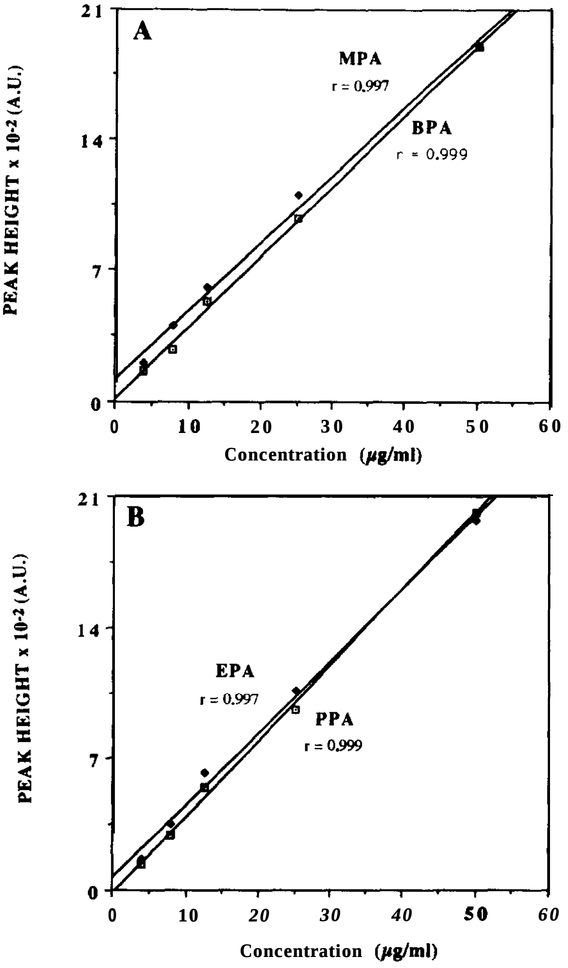

Fig.7. Calibration graphs obtained for (A) MPA-BPA and (B) EPA-PPA.

preciable improvement in the signal-to-noise ratio. In contrast, the sensitivity increased with a decrease in the concentration of phenylphosphonic acid, which is in agreement with the results reported by Foret *et al.* [10] and Nielen [12]. In addition, a substantial increase in the migration times is observed with increasing concentration of phenylphosphonic acid. Lowering the concentration to 5mM leads to baseline perturbations. Accordingly, a 10 mM concentration of phenylphosphoric acid was found to be the best compromise between high sensitivity and buffer stability and 200 mM sodium borate was chosen to produce the best signal-to-noise ratio.

Validation of the method

Detection limits, expressed as twice the baseline noise, ranged from 0.15 to 0.21 pmol injected, making this method more sensitive than some of those previously reported such as ion chromatography [7] or column liquid chromatography with detection of alkylphosphonic acids as their bromophenacyl esters [6]. The present technique is at least as sensitive as gas chromatography [3,4], while column liquid chromatography coupled with a laser fluorimetric detection allowed detection in the femtomole range [5]. Nevertheless, these techniques require a long esterification procedure and/or an extraction step with poor recoveries. However, even though the minimum amount of sample injected is very small using CZE, the small volumes injected (less than 10 nl) require solution concentrations as high as 2 μg ml-‘.

Calibration graphs obtained for MPA, EPA, PPA and BPA showed a linear response of the detector in the concentration range studied (5–50 μg ml-‘) (Fig. 7). Values of peak heights represent the average of five experimental data. The slopes were similar for all the alkylphosphonic acids investigated.

The repeatability ($n = 8$) and between-day reproducibility ($n = 17$) of the method were evaluated and the results are summarized in Table I. With regard to the relative standard deviations (less than 1.8%), we concluded that the electrophoretic mobilities are very reproducibile. The relative standard deviations for the peak heights are satisfactory (less than 3.8%), given that absolute peak heights are involved.

CONCLUSIONS

The proposed method was able to separate different alkylphosphonic acids according to their size. We stress the reliability of this technique, which is rapid and sensitive. The method can be applied to a wide range of alkylphosphonic acid derivatives and current studies are directed towards its application to the determination of fosfomycin and foscarnet in biological sera.

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