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Note

High-performance liquid chromatography analysis of alkyl methylphosphonic acids by derivatization

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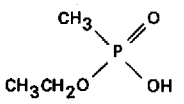
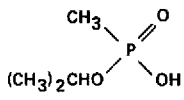
The simultaneous detection, separation and quantitation of trace amounts of alkyl methylphosphonic acids in aqueous solutions is of relevance and potential use in environmental studies as well as in general analytical methodology.

Phosphonic acids are currently analyzed using ion chromatography[1]. By this simple, direct and sensitive technique, aqueous samples of phosphonic acids can be analyzed directly. Separation (and quantitation) is dependent on degree of dissociation of the phosphonate analyte.

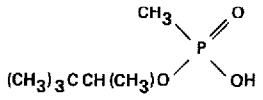
However, the alkyl methylphosphonic acids, namely, ethyl methylphosphonic acid (I), isopropyl methylphosphonic acid (II) and pinacolyl methylphosphonic acid



I



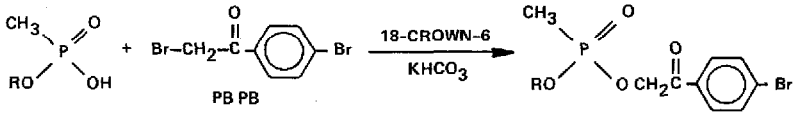
II



III

(III), with only discrete structural differences in the alkyl side chain and only slight difference in dissociation are separated and analyzed with difficulty by this method.

The alkyl methylphosphonic acids do not absorb or fluoresce in the ultraviolet (UV) or visible spectral range. They are thus not amenable by themselves to high-performance liquid chromatography (HPLC) analysis using an UV or fluorescent detector.



WHERE R = CH_3CH_2- = I → IV
R = $(CH_3)_2CH$- = II → V
R = $(CH_3)_3CCH(CH_3)$- = III → VI

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Described here is the development of an enhancement reaction technique of analysis for the reversed-phase HPLC of alkyl methylphosphonic acids. The technique involves the esterification of I–III separately and in a combined mixture with *b*-bromophenacyl bromide (PBPB), an UV chromophore, on a microscale to attain an UV absorbing phosphonate species. The esters are then separated by reversed-phase HPLC and quantitated by UV detector response.

MATERIALS*

Chemicals

Water used for HPLC was distilled and deionized (10–14 MΩ cm). Acetonitrile was HPLC grade (Burdick & Jackson Labs., Muskegon, MI, U.S.A.). Derivatization grade *p*-bromophenacyl bromide (PBPB) and 18-crown-6 catalyst were obtained from Regis (Morton Grove, IL, U.S.A.). All other solvents and chemicals were of analytical regent quality.

Compounds I–III were prepared in-house and gave analytical data consistent with their chemical structure.

Instrumentation

Infrared spectra were recorded on a Perkin-Elmer 283-B spectrophotometer. ^{1}H NMR spectra were recorded using a Varian A-60-D spectrometer. ^{31}P NMR spectra were recorded using a PT80A spectrometer. Gas chromatographic–mass spectrometric (GC–MS) analysis was carried out using a Hewlett-Packard 5985A equipped with a 10 m × 0.25 mm I.D. glass, WCOT, SP2100 column.

HPLC analyses were carried out using a Waters Associates high-performance liquid chromatograph consisting of two Model 6000A pumps, a Model 660 solvent programmer, a Model 440 UV detector and a WISP 710A automatic injector. This system was connected to a Houston Instrument Omniscribe 5000 recorder and a Shimadzu Chromatopac-E1A integrator which measured detector response in terms of peak area. Separation was carried out using a Waters Associates 30 × 0.4 cm I.D. μBondapak C_{18} column.

METHODS

Preparation of the p-bromophenacyl esters, IV–VI, for HPLC

Quantities of each of the three novel esters were prepared for use as standards to determine optimum chromatographic conditions and effectiveness of analytical derivatization. The acid (1.1 mmole), *p*-bromophenacyl bromide (1.0 mmole), 18-crown-6 (0.05 mmole) and potassium bicarbonate (600 mg) in dry acetonitrile (5 ml) were heated at 60°C with stirring for 1 h. Upon cooling, the acetonitrile was removed under reduced pressure and the residue was taken up in dichloromethane (15 ml). After shaking three times with 5-ml portions of water, the dichloromethane solution was taken to dryness under reduced pressure. In all three cases the product as a

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viscous yellow oil remained. The yield was better than 95%. The structure and purity (>97%) were determined by IR, NMR (^1H and ^{31}P) and GC–MS.

Chromatographic procedure

Analytical separations were performed under the following conditions: sample size, 200 μl; flow-rate, 2.0 ml/min; column temperature, ambient; mobile phase, water–acetonitrile; elution mode, No. 6 gradient, 20–55% acetonitrile, 25 min; UV detector, 254 nm.

Standard solutions of the *p*-bromophenacyl esters were injected onto the column and their retention times determined. Calibration curves conforming to Beer's Law were obtained by injecting known concentrations (0.2, 0.4, 1.0, 2.0, 4.0 and 10.0 μg/ml) of the acids as esters onto the column in triplicate and measuring the resulting peak areas.

Analytical derivatization

Two equivalents of *p*-bromophenacyl bromide and 0.1 equivalent of 18-crown-6 in acetonitrile were added to one equivalent of each of the alkyl methylphosphonic acids in 1 ml of acetonitrile. Potassium bicarbonate (5 mg) was then added to each reaction flask, and the mixture was brought to 3 ml volume with acetonitrile–water (4:1). The mixture was heated with stirring at 60°C for 2 h. After cooling, the mixture was poured into a 4-ml WISP sample vial and fitted with a cap having a self-sealing PTFE faced silicone septum. The WISP was then programmed so that three 200-μl samples of the solution were injected into the liquid chromatograph.

In this way, concentrations of each alkyl methylphosphonic acid were prepared at 0.2, 0.4, 1.0, 2.0, 4.0 and 10.0 μl/ml for detection as the ester species.

RESULTS AND DISCUSSION

Alkyl methylphosphonic acids are currently analyzed with difficulty by ion chromatography. Separation of analytes by this method is a function of the difference in their dissociation constants or pK_a values. The pK_a values of I, II and III are nearly identical, being 2.75, 2.38 and 2.50 respectively, with concomitant difficulty in separation and analysis.

Another difficulty encountered in ion chromatography analysis is the interfering or "smothering" effect of inorganic anions (Cl^-, F^-, etc.) endemic to natural waters.

p-Bromophenacyl bromide is a highly reactive esterifying agent as well as a strong UV absorbing chromophore. 18-Crown-6 solubilizes and chemically activates potassium bicarbonate in the reaction medium. This permits the ready synthesis of the novel chromophore bearing *p*-bromophenacyl ester derivatives in acetonitrile–water. In addition, esterification decreases the polarity of the analytes permitting ready separation by reversed-phase HPLC.

The qualitative capability of this technique is illustrated in Fig. 1 in which the complete separation of IV–VI and excess PBPB is achieved in 25 min. The PBPB peak in the chromatogram is well separated from that of V and VI, eliminating any interference in the analysis from excess PBPB.

The retention time of the *p*-bromophenacyl ester derivative (IV–VI) increases

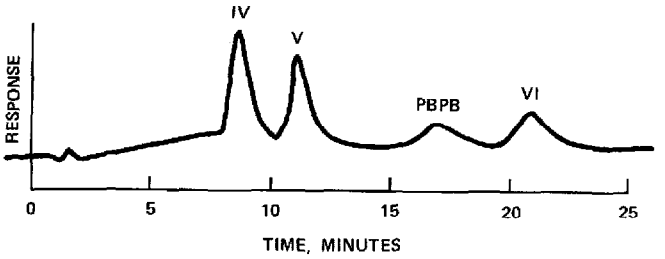

Fig. 1. HPLC separation of *p*-bromophenacyl alkyl methylphosphonic esters.

proportionately as the alkyl side chain (R–) of the methylphosphonic acid moiety increases. As seen in Table I, retention time which is a measure of affinity for lipophilic μBondapak is proportional to the size of the R moiety and its corresponding π value[2]. The π value is a measure of lipophilicity of a chemical group.

Quantitation was also readily achieved by this analytical method. The overall efficiency of the derivatization reaction for the three acids was determined to be 84–99% by comparison with standards. The reactions were reproducible and detector response was linear for I–III, as the *p*-bromophenacyl ester species, in concentrations

TABLE I

π VALUE–RETENTION TIME RELATIONSHIP FOR IV–VI

Compound	*R–*	*π value*	*Retention time (min)*
IV	CH_3CH_2–	1.0	8.7
V	$(CH_3)_2CH$–	1.3	11.2
VI	$(CH_3)_3CCH(CH_3)$–	2.6	21.2

of 0.2–10.0 μg/ml. The detection limits[3] for I, II and III were 43, 59 and 62 ng, respectively.

The limits of detection are based on the method described by Hubaux and Vos[3] wherein 5% of the results are allowed to be higher than expected and 5% lower.

Previous HPLC analysis of phosphonic acids[4], using the relatively insensitive refractive index detector, achieved detection limits in the tenths of milligram level.

ACKNOWLEDGEMENTS

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