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PREPARATION, STABILITY AND QUANTITATIVE ANALYSIS BY GAS CHROMATOGRAPHY AND GAS CHROMATOGRAPHY–ELECTRON IMPACT MASS SPECTROMETRY OF *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES OF SOME ALKYLPHOSPHONIC AND ALKYL METHYLPHOSPHONIC ACIDS

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SUMMARY

Studies of *tert.*-butyldimethylsilylation of several alkyl methylphosphonic, alkylphosphonic and chlorine-substituted alkylphosphonic acids by several derivatizing reagents under differing reaction conditions are reported and a simple procedure for formation of these derivatives is identified. Determination of the stabilities of representative derivatives are reported and capillary column gas chromatographic (GC) retention data for all the derivatives studied are tabulated. General sensitivities for detection and quantitation of these compounds by GC–flame photometric detection and GC–electron impact mass spectrometry in both full-scan and multiple-ion detection modes are presented.

INTRODUCTION

To facilitate the negotiations of disarmament treaties in support of a potential ban on the development, production and stockpiling of chemical warfare (CW) weapons, sensitive methods of detection and analysis are required for verification of the use of these compounds. Consequently, it will be necessary *a priori* to have in place methods for the recovery, work-up and analysis of samples suspected of containing chemical warfare agents and their degradation products[1]. The sensitive identification and verification of the presence of organophosphorus nerve agents and similar materials by methods such as gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) is generally a straightforward procedure after separation from the environmental matrix has been effected[1–9]. Due to their instability in water, however, these compounds hydrolyse readily to form alkyl methylphosphonic acids and, ultimately, methylphosphonic acid[3,8,10–13]. These materials cannot be chromato-

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graphed readily because of their low volatility and polar nature. Therefore, chemical derivatization is necessary if GC is to be employed. Similar restrictions apply to the analysis of agricultural organophosphorus compounds which form alkylphosphoric acids on hydrolysis[12].

Traditionally, the derivatization procedure employed is diazoalkylation to form the alkyl esters which then can be chromatographed[3,4,11–17]. This approach, while effective, employs reagents which are hazardous and potentially explosive[11,15]. In addition, there have been reports that the methylation, at least in the case of alkyl methylphosphonic acids, may be incomplete[13].

Another approach which has also been used is trimethylsilylation of phosphorus acids or salts, although this has the disadvantage that the trimethylsilyl derivatives are readily hydrolysed by traces of moisture sometimes requiring that a carefully-sealed reaction vessel be used for quantitative measurements[18–21].

A third approach which has been examined is that of pentafluorobenzylation[8,13]. The advantages include enhanced sensitivity for electron-capture detectors in GC, for ultraviolet detectors in high-performance liquid chromatography (HPLC), and for negative ion chemical ionization in MS. The derivatization reaction however, requires the use of sodium hydride and 18-crown-6 at 45°C for several hours to ensure complete conversion[8].

Liquid chromatographic analysis has also been reported[22]. By employing *p*-bromophenacyl bromide derivatization, Bossle *et al.*[22] could detect levels of alkyl methylphosphonic acids down to 40 ng and analyse for the materials in aqueous media, a desirable feature for the analysis of disarmament-related samples.

We report here the application of an improved approach to derivatization for this class of compounds. *tert.*-Butyldimethylsilylation has been applied to the GC, GC–MS and MS analyses of compounds such as prostaglandins[23,24], deoxynucleosides[25], fatty acids[26], steroids[27–30] and recently, to inorganic oxyanions such as sulfates, phosphites and phosphates[31]. *tert.*-Butyldimethylsilyl (TBDMS) derivatives offer the benefits of hydrolytic stability, ease of preparation, and high sensitivity for MS detection[31]. Recently we reported a GC–MS investigation of the TBDMS derivatives of several alkyl methylphosphonic- and alkylphosphonic acid derivatives[32]. It was shown that the electron impact mass spectra of these compounds are dominated by 3–4 peaks which account for more than 40% of the total ionization of the molecule[32]. In addition, most of these dominant ions occur at relatively high m/z, suggesting that sensitive detection of these classes of compounds by GC–MS multiple ion detection (MID) methods should be free from interference. In this investigation, we report on methods of preparation and GC analysis of these compounds as well as their stabilities and detection sensitivities by GC–flame photometric detection (FPD) and GC–electron impact (EI)MS in both full-scan and MID modes.

EXPERIMENTAL

Reagents and instruments

N-Methyl-N-(*tert.*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) and MTBSTFA with 1% *tert.*-butyldimethylsilyl chloride (TBDMSCl) were obtained from Regis (Morton Grove, IL, U.S.A.). Derivatization kits (1 ml) of TBDMSCl and imidazole (1:2.5 mol/mol) in dimethylformamide (DMF)[23] were obtained from

Applied Science Division (State College, PA, U.S.A.). Silylation-grade acetonitrile, DMF, and toluene were obtained from Pierce (Rockford, IL, U.S.A.). Methylphosphonic (MPA), ethylphosphonic (EPA), *n*-propylphosphonic (nPPA) and *n*-butylphosphonic (nBPA) acids were obtained from Fairfield Chemical (Blythewood, SC, U.S.A.) while samples of ethyl methylphosphonic (EMPA) and isopropyl methylphosphonic (iPMPA) acids were provided courtesy of Dr. E. W. Sarver (Chemical Research, Development and Engineering Center, Aberdeen Proving Ground, MD 21010, U.S.A.). Samples of pinacolyl methylphosphonic acid (PinMPA) were generated by controlled hydrolysis of the corresponding fluoridate using aqueous hydrochloric acid for purposes of retention time determination and mass spectral generation. Diisopropyl methylphosphonate (DPMP), used as an internal standard in some experiments, and 2-chloroethylphosphonic acid were obtained from Fairfield Chemical.

GC analyses were carried out on a Varian Model 3700 gas chromatograph equipped with Varian dual flame photometric and flame ionization detectors, a fused-silica capillary column (30 m × 0.322 mm I.D.) DB5-30N (J&W Scientific, Rancho Cordova, CA, U.S.A.) and, in some preliminary studies, a shorter column (15 m × 0.32 mm I.D.) SE-30 (Varian). Injections were effected using a Varian Model 8000 autosampler adjusted to deliver 1-μl samples into a Varian fused-silica capillary direct injector body (Model 1085). Integration and instrument control were performed on Varian 111 CDS data systems. Conditions for analysis were typically: carrier gas, helium at 2.4 ml/min at 250°C; injector, 220°C; detector, 250°C; column oven program, 60°C for 2 min, 10°/min to 230°C and hold for the 30 m column; and 50°C for 2 min, 25°/min to 100°C followed by 5°C/min to 250°C and hold for the shorter column.

GC–MS analyses were carried out on a Finnigan 4023 gas chromatograph–mass spectrometer–data system equipped with a fused-silica capillary column (30 m × 0.258 mm I.D.) DB5-30N (J&W Scientific). Injections were effected manually using the Finnigan split/splitless Grob injector in the splitless mode. Conditions for analysis were typically: carrier gas, helium at 1.4 ml/min at 50°C; injector, 260°C; transfer line, 270°C; ion source, 190°C; column oven program, 50°C for 1.6 min, 22°/min to 140°C, 5°/min to 200°C, 10°/min to 260°C and hold, injection at 0.8 min, split at 1.6 min; full scan parameters were sensitivity, 10^{-7} V/A; scanning 45–1000 a.m.u. at 2.5 s/scan; MID parameters were: sensitivity, 10^{-8} V/A; scanning 1 mass unit window for 0.105 s each with a total of 0.25 s for two ions with time delay/settling time.

Derivatization procedure

Generally, derivatization involved combining carefully-measured quantities of the materials in the Reactivials, heating for specific periods of time at constant temperature and allowing to cool, followed by analysis and storage. Volumetric measurements were made using Hamilton syringes and sample preparation and storage were carried out in screw cap glass vials with PTFE/silicone septa (Pierce). Heating and temperature control were effected in a Pierce Reacti-therm heating/stirring module. No precautions were taken to exclude air or moisture from the vials and no extraction or clean-up was carried out. Stability experiments involved storage at room temperature and at 0°C.

RESULTS AND DISCUSSION

Derivatization reactions

The reagent often employed for TBDMS-derivative syntheses has been a mixture of TBDMSCl and imidazole in DMF[23–30]. The introduction of imidazole as a catalyst and DMF as the reagent solvent was found to increase the reactivity of TBDMSCl considerably when used to derivatize alcohols[23]. Recently other reagents and approaches have become available including MTBSTFA in acetonitrile[31,33], in DMF[31] and in tetrahydrofuran (THF)[31,33]. Accordingly, several derivatization procedures were examined to determine the effect of solvent, temperature, time, and reagents on the yields of TBDMS derivatives of alkyl methyl- and alkylphosphonic acids in organic solvents.

(A) MTBSTFA + 1% TBDMSCl in acetonitrile and toluene. Samples of EMPA and iPMPA (42 ng/μl) in acetonitrile were treated with an excess of MTBSTFA + 1% TBDMSCl both at 60°C and at room temperature for 1 h. GC analyses using the shorter column revealed two sharp peaks not present in blank derivatization solutions whose areas were similar for treatments at both temperatures. GC–MS analysis of the 60°C sample, although indicating that the components contained ions expected for TBDMS derivatives of these compounds[32], was inconclusive because of poorly-shaped GC peaks. Mixtures of MPA, EPA, nPPA and nBPA (*ca.* 50 ng/μl) were treated with the MTBSTFA–TBDMSCl combination in acetonitrile at 60°C for 30 min and, again, GC–MS indicated that TBDMS derivatives of all the acids had been formed but poorly-shaped GC peaks were observed. Subsequently, it was determined that increasing the initial column temperature to above 100°C significantly improved the chromatography and that substitution of toluene for acetonitrile as the predominant solvent resulted in clean well-separated peaks, provided that the solvent mixture contained at least 90% toluene.

Mixtures of iPMPA (to represent alkyl methylphosphonic acids) and MPA (to represent alkylphosphonic acids) (*ca.* 40–60 ng/μl each) were employed to examine the extents of derivatization and to define optimum reaction conditions in the toluene–acetonitrile (90:10) solvent system. Reactions were carried out at room temperature, and at 60°C for 30-, 60- and 150-min periods; yields are summarized in Table I. The quantities produced at 60°C and 60 min using no catalyst (see B below) are taken as representing 100% conversion for basis of comparison. Within the margin of analytical error, derivatization of both acids at both temperatures and all three reaction times appears to be complete.

(B) MTBSTFA in toluene. A mixture of the phosphonic acids (excluding pinacolyl methyl- and chloroethylphosphonic acids), some originally prepared in acetonitrile, some in chloroform, was reacted with MTBSTFA in an excess of toluene (toluene > 95%, v/v; final concentrations approximately 45 ng/μl) for 1 h at 60°C. Sharp, well-defined peaks were obtained and complete mass spectra corresponding to the acid derivatives were recorded[32]. Derivatizations of iPMPA and MPA mixtures (40–60 ng/μl) were carried out using MTBSTFA in toluene–acetonitrile (100:1) at both room temperature and at 60°C for 30-, 60- and 150-min periods; yields are given in Table I. As in A above, both phosphorus acids appear to be quantitatively derivatized at both temperatures and all three reaction times.

Derivatization of a sample of pinacolyl methylphosphonic acid in acetonitrile

TABLE I

EFFECTS OF REACTION TIME AND TEMPERATURE ON DERIVATIZATION OF ISOPROPYL METHYLPHOSPHONIC (iPMPA) AND METHYLPHOSPHONIC ACID (MPA) MIXTURES IN TOLUENE USING MTBSTFA WITH/WITHOUT 1% *tert.*-BUTYLDIMETHYLSILYL CHLORIDE

Reaction temperature (°C)	*Reaction duration (min)*	*Yields of derivatives (%)*[a]	
		iPMPA	*MPA*
24.5	30	97 [98]	95 [97]
24.5	60	95 [97]	93 [96]
24.5	150	102 [96]	101 [94]
60	30	99 [98]	99 [102]
60	60	100 [96]	100 [98]
60	150	101 [100]	97 [97]

[a] Values for solutions with added catalyst given in square brackets.

was carried out using MTBSTFA in an excess of toluene at 60°C for 1 h and formation of the PinMPA derivative confirmed by GC–MS analysis[32]. Separation of the two stereoisomers of the derivative on the capillary column was essentially baseline and both isomers of the unhydrolysed fluoridate as well as some MPA derivative, the final product of complete hydrolysis of the fluoridate ester, were detected.

A sample of 2-chloroethylphosphonic acid, dissolved in acetonitrile, was derivatized by reaction with MTBSTFA in an excess of toluene at 60°C for 1 h; GC–MS analysis confirmed the formation of this derivative as well[32].

(C) TBDMSCl–imidazole in DMF. Derivatizations using a combination of TBDMSCl–imidazole (1:2.5) in DMF were attempted at 60 and at 160°C for 1 h using iPMPA (22.5 ng/μl) and MPA (32.4 ng/μl) in DMF–acetonitrile (DMF > 95%) containing the internal standard DPMP. A parallel reaction using MTBSTFA in toluene–acetonitrile (100:1), carried out at 60°C for 1 h, served as a basis of comparison. Yields of iPMPA and MPA derivatives were found to be only 37 and 80% at 60°C and 72 and 48% at 160°C, respectively. Gas chromatograms of the solutions resulting from the TBDMSCl–imidazole reaction indicated the presence of a number of other components.

Derivatization of these acids using either MTBSTFA with/without 1% TBDMSCl catalyst is very effective. In both cases, reactions appear to be complete at room temperature, and at 60°C, in 30 min, although there is a suggestion that higher yields may have resulted at the higher temperature. In contrast, although TBDMS derivatives were formed when TBDMSCl–imidazole reagent was used in DMF, the conversion is incomplete under both similar and more severe conditions. Increasing the reaction temperature in this case improves the formation of the iPMPA derivative but appears to reduce the conversion of MPA derivative. Without further study, it is unclear whether the MPA derivative yield decreased because of subsequent decomposition or because of other factors, although instability of silylation product mixtures using this reagent have been reported[33]. In addition, the presence of other byproducts using this approach could present problems for quantitation of the derivatives by non-specific detection such as FID or FPD. Attempts to remove or reduce the levels of these materials by extraction or adsorption cleanup would complicate the analysis and

possibly decrease sample recovery. In summary, the first two methods of derivatization possess clear advantages over the last approach both in simplicity and completeness.

GC separation

A typical reconstructed GC–MS ion chromatogram of a mixture of six of the acid derivatives (excluding that for PinMPA and 2-chloroethylphosphonic acid) is shown in Fig. 1. The solution was prepared by derivatizing a mixture of the acids (43–47 ng/μl) with MTBSTFA for 1 h at 60°C in a toluene–acetonitrile–chloroform (100:10:1) mixed solvent. The minor components are traces of the original solvents employed to dissolve the acids. The identifications of the various components, confirmed by GC–MS[32], are listed in Table II along with retention times of all derivatives and other materials identified in the GC–MS and GC studies. Baseline resolution of the two alkyl methylphosphonic acid derivatives was achieved in a GC–MS analysis using a DB-5 capillary column, an on-column injector (J&W Scientific), a 1-m length of silylated precolumn/retention gap and an oven temperature program of 110°C for 1 min followed by a rise of 20°/min to 260°C. In summary, clean separation of the derivatives can readily be achieved using toluene as the major solvent component for splitless injection. Similar GC results can be accomplished by direct- or cool on-column injection techniques using toluene and acetonitrile as co-solvents.

The poor chromatography observed in the analysis of catalysed derivatizations (see A above) can be ascribed to solvent polarity (acetonitrile) and the method and temperature of injection being employed. The effects of using polar compounds such as methanol, water, and acetonitrile as solvents on the chromatographic elution of a variety of compounds on bonded non-polar columns, DB-1 and DB-5, have been reported[34]. On-column injection with acetonitrile as solvent led to very severe peak splitting unless the injection was effected rapidly. Co-injection of a non-polar solvent such as benzene with the polar solvent, especially if the two form an azeotrope, nearly eliminated the problem and varying the temperature of the injection improved the

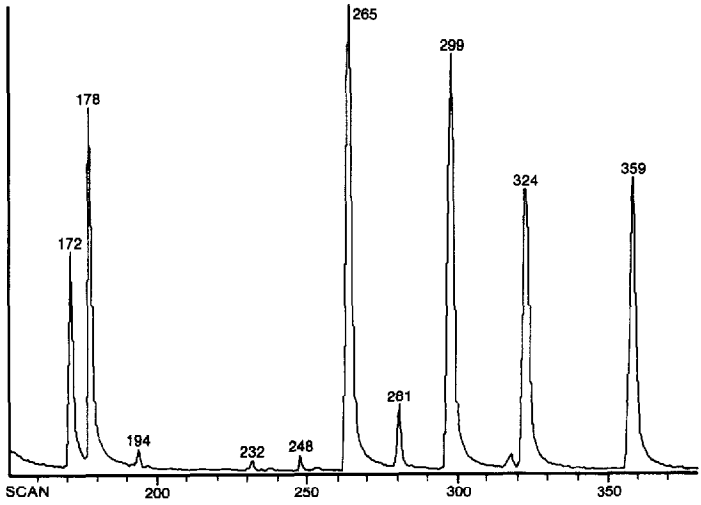

Fig. 1. GC–MS analysis of a sample of TBDMS derivatives of alkyl methyl- and alkylphosphonic acids.

TABLE II

GAS CHROMATOGRAPHIC RETENTION DATA FOR SELECTED TBDMS DERIVATIVES OF ALKYL METHYL- AND ALKYLPHOSPHONIC ACIDS

Oven program: (GC–MS), 50°C for 1.6 min, 22°/min to 140°C, 5°/min to 200°C, 10°/min to 260°C and hold, injection at 0.8 min, split at 1.6 min; (GC), 60°C for 2 min, 10°/min to 230°C and hold.

Compound name	*Scan (No.)*[a]	*Retention time (min)*	
		GC–MS	*GC*
Diisopropyl methylphosphonate[b]	–	–	8.82
Ethyl methylphosphonic acid derivative	172	7.17	12.15
Isopropyl methylphosphonic acid derivative	178	7.42	12.53
Pinacolyl methylphosphonic acid derivative[c]	–	–	15.30
Methylphosphonic acid derivative	265	11.03	15.78
1,3,5-Trimethylbenzene[d]	281	–	–
Ethylphosphonic acid derivative	299	12.45	16.71
n-Propylphosphonic acid derivative	324	13.50	17.33
n-Butylphosphonic acid derivative	359	14.95	18.18
2-Chloroethylphosphonic acid derivative	–	–	18.65

[a] GC–MS results (See Fig. 1).
[b] Internal standard employed in some GC analyses.
[c] Pinacolyl methylphosphonic acid derivative synthesized by hydrolysis of the corresponding fluoridate ester and analysed separately *in situ*; retention time quoted is for first stereoisomer.
[d] Impurity found in toluene.

chromatography[34]. Another study noted that splitting of the GC peaks could be eliminated by injecting at temperatures higher than the boiling point of the solvent[35]. In the present study, elimination of the poor peak shapes when acetonitrile was used as the solvent in the splitless injector by the use of toluene as the predominant solvent (>90%) and when the injector temperature was increased, are consistent with the reported investigations[34,35]. In conclusion, GC separation of phosphonic acid TBDMS derivatives is best achieved using an excess of non-polar solvent for on-column injection or high injector temperatures for splitless or direct injection.

GC retention times

Generally, retention times increase linearly with molecular weight of the derivative within a class. For a GC analysis with a linear temperature program, the retention times of the alkyl methylphosphonic- and the alkylphosphonic acid derivatives display similar straight line relationships with molecular weight, the latter being slightly shifted to longer retention times (Fig. 2). The 2-chloroethylphosphonic acid derivative appears to fall on the line of the other alkylphosphonic acid derivatives. This linear behaviour could permit the prediction of retention times for other homologues in the two series to aid in identification of other phosphonic acid derivatives. However, although the retention times were very reproducible thus providing a possible basis for a useful screening tool for the detection of these compounds, samples submitted for verification purposes would contain an assortment of environmental components, some of which could interfere with and weaken identifications based solely on retention indices. An improvement could be realised by

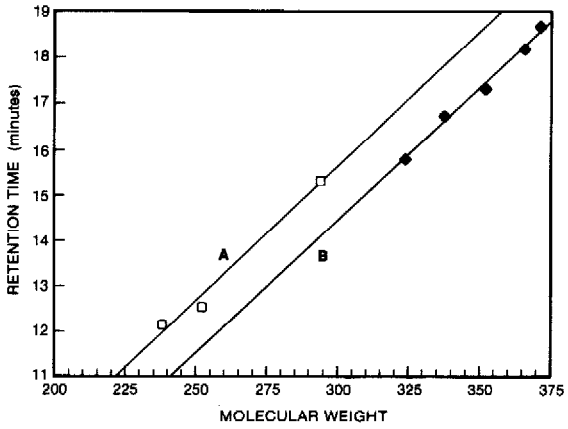

Fig. 2. Capillary column GC retention times of (A) alkyl methylphosphonic, and (B) alkylphosphonic acid TBDMS derivatives as a function of their molecular weight.

use of an FPD system with a phosphorus filter which would reduce the interference problem, compared to FID which responds to a greater number of materials. MS is clearly superior due to its unequivocal identification capability.

Derivative stabilities

Mixtures of iPMPA (22.5 ng/μl), MPA (32.4 ng/μl) and an internal standard of DPMP were derivatized using MTBSTFA in toluene–acetonitrile (100:1) at 60°C for 1 h. The resulting solution was stored at room temperature without cleanup, reactant removal, or addition of drying agent. GC analyses were carried out over a period of six days at which time the solution was stored in a freezer at 0°C for a further period of 22 days; results are given in Table III.

TABLE III

STABILITIES OF ISOPROPYL METHYLPHOSPHONIC- AND METHYLPHOSPHONIC ACID DERIVATIVES

Based on GC–FPD analyses; formation reaction conditions were 60°C for 60 min using MTBSTFA in toluene.

Elapsed time (h)	*Isopropyl methylphosphonic acid derivative (% change)*	*Methylphosphonic acid derivative (% change)*
0.0	–	–
4.0	+5	−7
4.5	+4	−8
27.5	−5	+3
98.25	+1	−7
98.75	−2	−9
119.75	−2	−11
120.5	−7	+2
143.5	−3	−8
672 (freezer, 22 days)	+3	−3

A separate examination of the 2-chloroethylphosphonic acid derivative stability, conducted by reacting 2-chloroethylphosphonic acid (23.1 ng/μl) with MTBSTFA in toluene–acetonitrile (20:1) at 60°C for 1 h and analyzing by GC, demonstrated a stability of better than 96% over 24 h at room temperature. However, after a period of 4 days, the solution yellowed and a fine precipitate formed; no analysis of the latter solution was undertaken.

The behaviour observed with the iPMPA and MPA derivatives indicates that long term stability of these materials can be expected even when in contact with the derivatization reagents. Over a storage period of six days at room temperature and in a freezer for a further three weeks in a closed air atmosphere with no added drying agent, neither derivative displayed significant degradation. From the single examination of the stability of the 2-chloroethylphosphonic acid derivative, however, there is an indication that it may be less stable than its alkyl counterpart; further tests would be required to verify its behaviour.

Sensitivity and linearity

For a derivative to be useful in trace analysis, a low detection limit and a wide range of linearity of response are important characteristics. Consequently, a limited investigation of these factors was undertaken. Employing iPMPA and MPA as representatives of the two compound classes and DPMP as an internal standard, derivatives were formed using MTBSTFA in toluene–acetonitrile (100:1) at 60°C for 1 h. Solutions were prepared by successive dilution with toluene and analysed by GC. The responses to the phosphonic acid derivatives, corrected for injection volume variation using the internal standard as a reference, are displayed in Fig. 3. Each point is the average of at least four injections at each concentration.

For GC–FPD, responses for all three compounds are strictly linear over the range with correlation coefficients of greater than 0.999 and minimum detectable limits of less than 500 pg for each derivative. The response remains linear to amounts exceeding 20 to 30 ng although actual upper limits were not determined. A limited GC–MS study was conducted in both full-scan and MID EI modes. In the former,

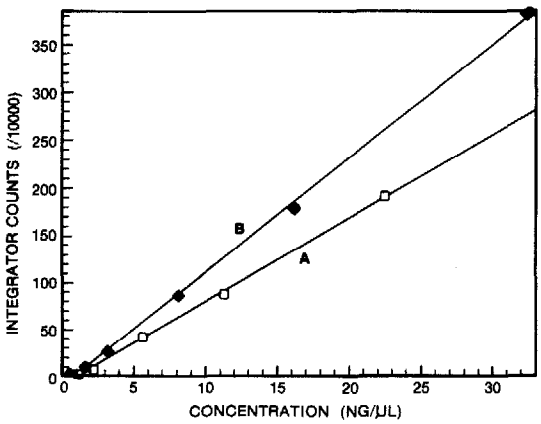

Fig. 3. Linearity and sensitivity of flame photometric capillary column gas chromatography to representative TBDMS derivatives of phosphonic acids. (A) iPMPA, (B) MPA.

a sample containing iPMPA (0.34 ng) and MPA (0.49 ng) derivatives still produced background-subtracted spectra containing sufficient information to perform library searches for identification (Fig. 4). Using these full-scan spectra, single ion chromatograms at *m/z* 153 (iPMPA derivative) and 267 (MPA derivative) indicate that accurate quantitation would be possible down to less than 200 pg and 350 pg, respectively. EI-MS in the full-scan mode, possessing approximately equivalent sensitivity to the GC–FPD, has the important advantage of simultaneous unequivocal MS identification of the compound.

Under GC–MS MID conditions monitoring the two most intense ions for each derivative, *m/z* 153 and 195 (iPMPA) and *m/z* 225 and 267 (MPA) and quantitating on the more intense for each, quantities down to less than 17 and 24 pg respectively at a signal-to-noise ratio of 2:1 were determined without special instrumental tuning precautions. Although the useful limits for analysis performed in this manner are conservatively 30 and 60 pg respectively, monitoring single ions for each compound coupled with accurate retention time data to verify the identification, could further reduce these limits.

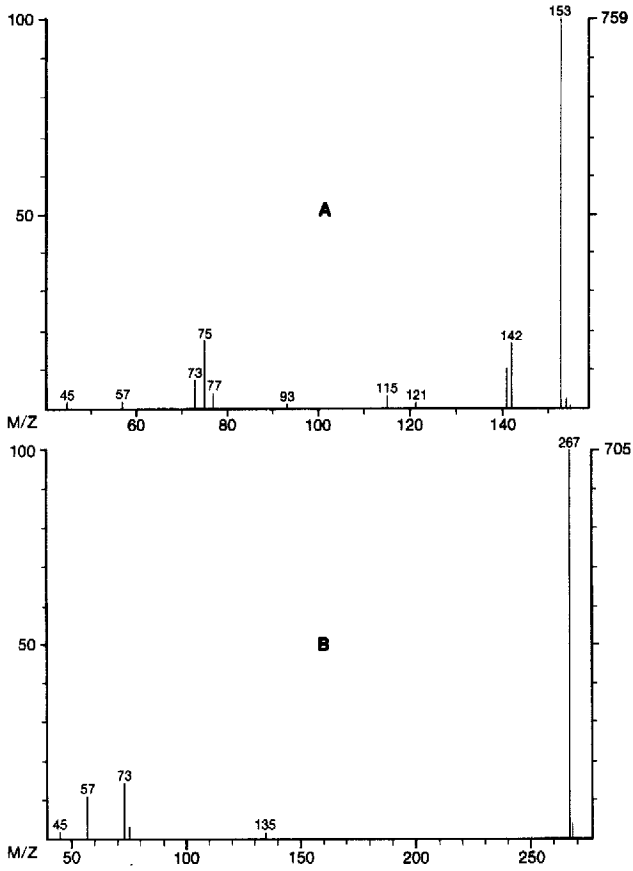

Fig. 4. Background-corrected electron impact mass spectra of (A) the iPMPA TBDMS derivative at 0.34 ng, (B) the MPA TBDMS derivative at 0.49 ng.

CONCLUSION

tert.-Butyldimethylsilyl derivatization possesses significant advantages in ease of formation, stability, excellent detection sensitivity, and clean chromatography over methylation or trimethylsilylation for the GC analysis of alkyl methylphosphonic-, alkylphosphonic and chlorinated alkylphosphonic acids. Derivatization without the use of drying agents or inert atmosphere is readily effected by the addition of commercially-available MTBSTFA, either with or without catalyst (1% TBDMSCl), to the sample and heating to 60°C for 30 min. GC analysis can be carried out immediately by direct injection of the reaction solution without further cleanup. The derivatized solutions of chlorinated alkylphosphonic acids may be safely stored without significant degradation for up to 24 h at room temperature while those of alkyl methyl- and alkylphosphonic acids can be stored for up to 6 days at room temperature or at least a month in a freezer.

GC separation of the phosphonic acid TBDMS derivatives examined in this study is readily achieved on a non-polar capillary column, provided that more than 90% of the solvent employed is non-polar for on-column injection, or high injector temperatures are employed for splitless or direct injection. Linear correlations of the retention times for each class of phosphonic acid derivative with molecular weight are excellent, permitting accurate predictions of retention times for other homologues. Quantitation down to 500 pg is possible using GC–FPD, while with EI-MS, quantitation can be carried out down to 300–500 pg for the full-scan mode and down to 30–60 pg using MID.

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