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GAS CHROMATOGRAPHY OF THE TRIMETHYLSILYL DERIVATIVES OF ARSENIC, ARSENIOS, AND DIMETHYLARSINIC ACIDS

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SUMMARY

The formation and chromatographic behavior of trimethylsilyl (TMS) derivatives of arsenic, arsenious and dimethylarsinic acids is reported. Evaluation of several silylation reagents and solvent systems revealed that most suitable derivatization took place using N,O-bis(trimethylsilyl)trifluoroacetamide in dimethylformamide. The rate of formation and stability of the derivatives was investigated. Stationary phases including SE-30, OV-17, OV-225, and OV-275 were tested for their ability to separate the derivatives. Columns of OV-225 coated on Gas-Chrom Q (100-120 mesh) were most effective in resolving the TMS-arsenicals from each other and from the TMS derivatives of CO_3^{2-} , SO_4^{2-} , and PO_4^{3-} . Detection limits were 0.1 ng of injected As(V) and dimethylarsinic acid, and 1 ng of injected As(III). Working curves were linear over three to five orders of magnitude. The identities of the derivatives were confirmed by gas chromatography-mass spectrometry.

INTRODUCTION

An important group of toxic and potentially carcinogenic compounds are those containing arsenic^{1,2}. These compounds exist in natural systems in a variety of chemical forms including: arsenic (As(V)), arsenious (As(III)), dimethylarsinic (DMA) and monomethylarsonic (MMA) acids¹. Furthermore, the distribution of the arsenicals among these species may vary markedly depending upon the prevailing chemical, physical and biological conditions¹. Adequate definition of the identities and quantities of the forms of arsenic in a natural medium requires more than a determination of total arsenic by analytical methods such as atomic absorption, neutron activation, polarography or spectrophotometry^{2,3}. The application of gas-liquid chromatography (GLC) to the speciation of chemically similar substances at trace levels in environmental, clinical and other natural samples has been motivated by the high separation efficiency of GLC and by the availability of sensitive and selective chromatographic detectors. However, efforts to determine As(III), As(V),

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DMA and MMA by GLC have been hampered by difficulties in preparing suitable derivatives.

Soderquist *et al.*⁴ determined DMA as iododimethylarsine. By using an electron-capture detector they detected less than 50 ppb of DMA in aqueous solution. Lodmell⁵ has reported the detection of as little as 5 ng of DMA as an allylthiourea derivative. Attempts to form similar derivatives of other compounds of arsenic were not successful. GLC has been employed to determine the condensation product formed by reaction of methylarsenic acid with ethylene glycol⁶. Microwave plasma emission or alkali flame detection permitted measurement of submicrogram amounts of the arsenical.

Recently, Talmi and Bostick⁷ and Andreae⁸ incorporated the arsine generation procedure of Braman and Foreback⁹ into a gas chromatographic method for determination of arsenicals. Inorganic arsenic was reduced to arsine in an acid solution of sodium borohydride; As(III) and As(V) were distinguished by control of pH during the reduction. DMA and MMA were reduced to dimethyl- and methylarsine, respectively. While all of the arsenicals of interest were determined chromatographically at nanogram and subnanogram levels, molecular rearrangements and consequent losses of accuracy have been reported⁷.

Butts and Rainey¹⁰ reported the formation and gas chromatographic separation of the trimethylsilyl (TMS) derivatives of nine inorganic oxyanions. Among these were the ammonium salts of arsenic and arsenious acids. Their work was concerned solely with the qualitative aspects of determining inorganic oxyanions by GLC.

This paper enlarges upon the work of Butts and Rainey¹⁰. It describes the preparation of the TMS derivative of DMA and discusses the quantitative GLC measurement of TMS derivatives of DMA, arsenic acid (As(V)) and arsenious acid (As(III)).

EXPERIMENTAL

The following sections present the methods for chemical derivatization along with the chromatographic conditions required for the determination of the TMS derivatives of the arsenicals.

Reagents and chemicals

Silylation grade N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), N,O-bis(trimethylsilyl)acetamide (BSA), N-trimethylsilylimidazole (TSIM), N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA), dimethylformamide (DMF), pyridine, dimethylsulfoxide (DMSO) and tetrahydrofuran (THF) were obtained from Pierce (Rockford, Ill., U.S.A.). Reference purity dimethylarsinic acid (99.8%) was supplied by the Ansul Company (Weslaco, Texas, U.S.A.). Arsenic trioxide (99.99% pure) was obtained from ROC/RIC (Sun Valley, Calif., U.S.A.). All other chemicals were reagent grade.

Preparation of trimethylsilyl derivatives

BSTFA, BSA, TSIM and MSTFA were evaluated as potential derivatizing agents; pyridine, DMF, DMSO, and THF were evaluated as potential solvents.

Arsenic(III) oxide and DMA were derivatized directly. Arsenate, carbonate, sulfate, and phosphate were derivatized as the ammonium salts. Ammonium arsenate was prepared by the method of Butts and Rainey¹⁰. The other salts used for derivatization were obtained commercially.

The procedure adopted following optimization of conditions is as follows. The species of interest (1–2 mg) is placed in a septum-capped vial. Aliquots of 400 μ l of DMF and 600 μ l of BSTFA are added, the vial is shaken briefly, and the mixture is allowed to stand for an appropriate length of time: As(V) and DMA for 8 h at room temperature and As(III) for at least 24 h at 70–80°. The derivatives are then ready for GLC analysis.

The solutions used to obtain calibration curves were prepared by adding microliter quantities of the derivative–BSTFA–DMF mixture to known volumes of toluene or DMF. These diluents were freed from water which might hydrolyze the arsenical derivatives by addition of 200 μ l of BSTFA prior to the dilution step.

Gas chromatography

A Varian (Palo Alto, Calif., U.S.A.) Model 1400 gas chromatograph equipped with a flame ionization detector and on-column injection was employed for all GLC analyses. Glass columns, 1.8 m \times 2 mm. I.D., were packed with Gas-Chrom Q (100–120 mesh) coated with one of the following stationary phases: 4% SE-30, 3% OV-17, 5% OV-225 or 4% OV-275. These packings were prepared by the fluidized drying procedure of Kruppa *et al.*¹¹. Chromatographic columns were silylated extensively prior to use by repeated injection of Silyl-8 column conditioner (Pierce). Injection port and detector temperatures were maintained at 175° and 225°, respectively. Nitrogen was used as the carrier gas at a flow-rate of 30 ml/min. The flame ionization detector was operated with Zero grade air and hydrogen at flow-rates recommended by the manufacturer. All gases were dried by passing them through Drierite/molecular sieve gas purifiers (Alltech, Arlington Heights, Ill., U.S.A.).

Gas chromatography–mass spectrometry

The gas chromatography–mass spectrometry (GC–MS) system used to identify the As(V) and DMA derivatives consisted of a Varian 1740 gas chromatograph interfaced with a Hitachi Perkin-Elmer RMU-7 (Norwalk, Conn., U.S.A.) double focusing mass spectrometer via a Watson-Biemann separator. The injection port was maintained at 175°. Helium was used as the carrier gas at a flow-rate of 26 ml/min. The 1.8 m \times 2 mm I.D. glass column was packed with 5% OV-225 on Gas-Chrom Q (100–120 mesh). The column was operated at 120° for the As(V) derivative and at 150° for the DMA derivative. The separator temperature was 175°. The main reservoir oven was held at 210°. The acceleration and ionization potentials were set at 1800 V and 22 eV, respectively. Eluent from the chromatograph was split 9:1 between the mass spectrometer and the flame ionization detector of the chromatograph.

The identity of the TMS derivative of arsenic(III) was confirmed by MS of a sample collected as it exited the GC column. The solute was cold trapped in a capillary tube that was inserted into the detector base of the Varian Model 1400 chromatograph described previously. Dry ice surrounded the capillary and maintained a temperature of –78°. The trapped sample was introduced into a Hitachi Perkin-

Elmer RMU-6 mass spectrometer via the volatile liquid-solid inlet system. The reservoir oven was maintained at 200°. The acceleration and ionization potentials were 1800 V and 12 eV, respectively.

RESULTS AND DISCUSSION

Derivatization reaction

Four compounds containing the TMS moiety were screened as potential derivatizing agents: TSIM, MSTFA, BSA, and BSTFA. Large sections of the chromatogram of the TSIM blank were obliterated by eluting reagent or reaction products. These areas included those in which the TMS derivatives of As(V), As(III), and DMA were expected to elute. Consequently, it is not known if the derivatives were formed. The MSTFA blank, while free of the major interferences found with TSIM, produced a chromatogram containing many peaks which interfered with the identification of the As(V), As(III), and DMA derivatives. BSA and BSTFA blanks produced relatively simple chromatograms. Fig. 1 is a representative chromatogram for the derivatives formed after reaction with BSTFA. Each arsenical produces a unique peak. Chromatograms obtained from BSA derivatizing mixtures were essentially identical to those for BSTFA with the exception that there was more pronounced tailing of the solvent peak. BSTFA was employed in all subsequent studies to minimize interference of the solvent front with the peak for the TMS derivative of As(V).

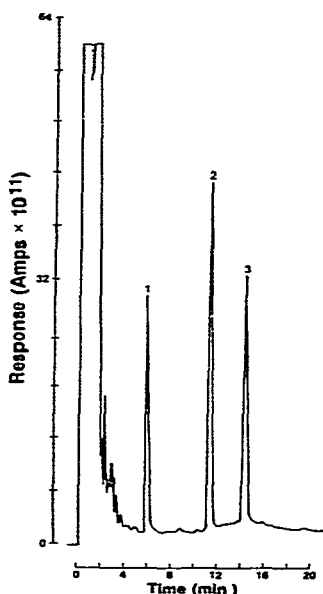


Fig. 1. Chromatogram of TMS-arsenicals on OV-225. Peaks: 1, TMS-As(V); 2, TMS-As(III); 3, TMS-DMA. Sample: 4 μ l BSTFA-DMF (3:2) containing 260 μ g/ml As(V), 500 μ g/ml As(III), 940 μ g/ml-DMA. Column: 5% OV-225 on Gas-Chrom Q (100/120 mesh), 1.8 m \times 2 mm I.D. Conditions: injector, 170°; detector, flame ionization, 230°; initial temperature 100°, 4°/min to 150°; carrier gas nitrogen, 30 ml/min.

The effects of solvent on the derivatization of As(V) and DMA with BSTFA were examined using DMF, pyridine, DMSO and THF. DMSO was the only solvent found unsatisfactory. Initially, two immiscible phases were formed on addition of BSTFA to DMSO. Over a period of 6 h a single, homogeneous, phase appeared. Chromatographic analysis of the upper phase prior to the formation of the homogeneous solution revealed that the derivative was preferentially partitioned into the upper, BSTFA, phase. DMF, THF, and pyridine were all satisfactory solvents for preparation of the TMS derivatives of As(V) and DMA. DMF was also a suitable solvent for derivatization of As(III).

Fig. 2 illustrates the rate of formation and the stability of the three arsenic-containing derivatives as a function of time for the BSTFA-DMF reaction mixture. The extent of formation was evaluated in terms of the height of the chromatographic peak for each derivative at the time of measurement relative to the maximum height of the peak observed during the experiment. Formation of the As(V) derivative was complete after 90 min, and it was stable for at least 48 h. The DMA derivative was formed to its maximum extent after 4 h, and decomposition became evident after 24 h. The As(III)-TMS compound formed much more slowly. This is thought to be due to the low solubility of arsenic trioxide in the reaction mixture. Direct derivatization of As_4O_6 was accomplished by heating 1-2 mg of the oxide in BSTFA-DMF (3:2) at 70-80°. After 24 h formation of the derivative was complete. The derivative remained stable for at least 36 h (Fig. 2).

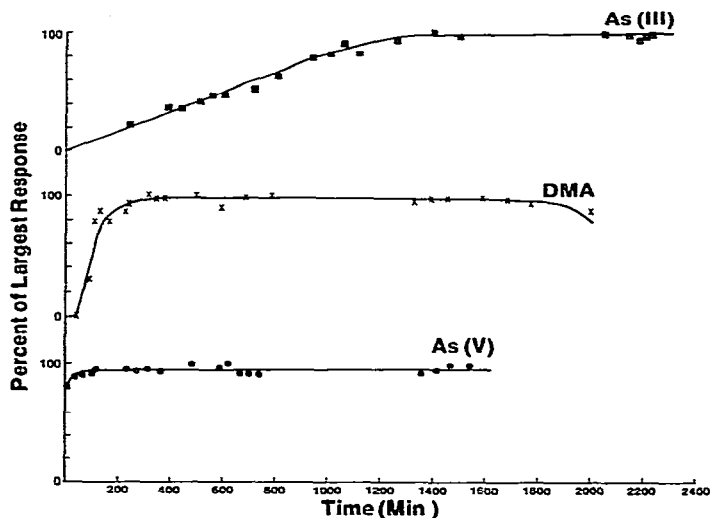


Fig. 2. Extent of TMS derivative formation for As(III), As(V), and DMA as a function of time for BSTFA-DMF reaction mixture.

Butts and Rainey¹⁰ reported similar difficulties with the production of TMS derivatives when sodium or potassium salts of the oxyanions were used as starting materials. The ammonium salts were found to be more satisfactory, due primarily to their higher solubility in DMF-BSTFA. We observed that conversion of arsenic acid (As(V)) to its ammonium salt was readily accomplished, as was derivatization

of this salt. However, arsenious acid and DMA produced compounds of irreproducible or indeterminate form when attempts were made to convert them to ammonium salts via the ion-exchange procedure of Butts and Rainey¹⁰. Consequently, DMA and As(III) were derivatized directly.

Gas chromatographic separations

Separations of the TMS-arsenicals were evaluated on a variety of liquid phases ranging from non-polar SE-30 to highly polar OV-275. Chromatograms obtained from As(V) and DMA derivatives on OV-275 were indistinguishable from the chromatograms of BSTFA-DMF blanks. It could not be determined whether the deriv-

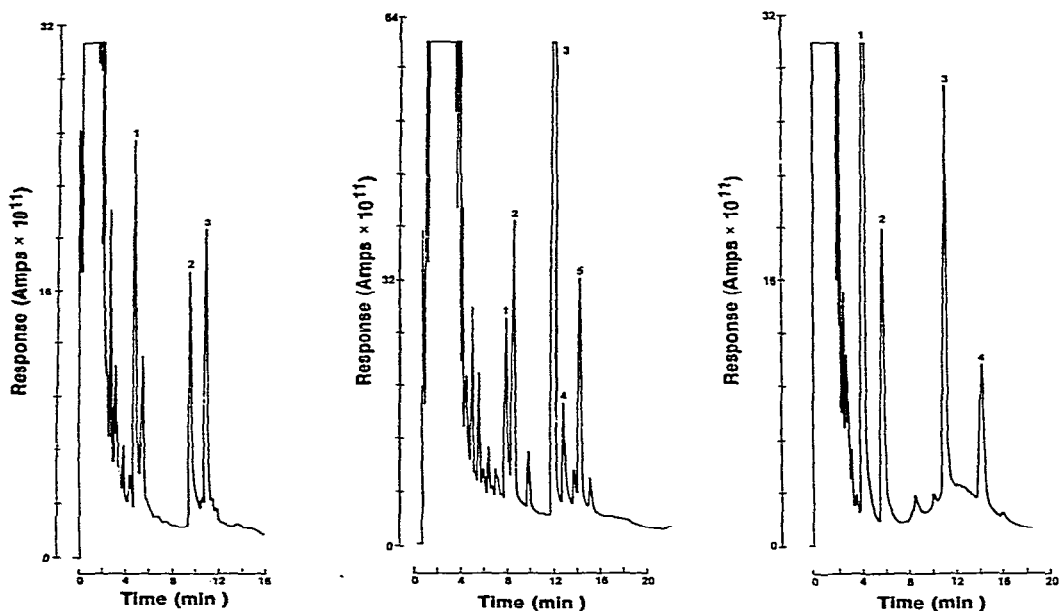


Fig. 3. Chromatogram of TMS-arsenicals on SE-30. Peaks: 1, TMS-As(III); 2, TMS-DMA; 3, TMS-As(V). Sample: 4 μ l BSTFA-DMF (3:2) containing 190 μ g/ml As(III), 500 μ g/ml DMA, 255 μ g/ml As(V). Column: 4% SE-30 on Gas-Chrom Q (100-120 mesh), 1.8 m \times 2 mm I.D. Conditions: injector, 175 $^{\circ}$; detector, flame ionization, 220 $^{\circ}$; initial temperature 70 $^{\circ}$, 4 $^{\circ}$ /min to 120 $^{\circ}$; carrier gas nitrogen, 30 ml/min.

Fig. 4. Chromatogram of TMS-arsenicals and TMS-PO₄³⁻, TMS-SO₄²⁻, and TMS-CO₃²⁻ on SE-30. Peaks: 1, TMS-As(III); 2, TMS-SO₄²⁻; 3, TMS-PO₄³⁻; 4, TMS-DMA; 5, TMS-As(V). Sample: 4 μ l BSTFA-DMF (3:2) containing 78 μ g/ml As(III), 510 μ g/ml SO₄²⁻, 550 μ g/ml PO₄³⁻, 190 μ g/ml DMA, 98 μ g/ml As(V), 360 μ g/ml CO₃²⁻. Column: 4% SE-30 on Gas-Chrom Q (100-120 mesh), 1.8 m \times 2 mm I.D. Conditions: injector, 175 $^{\circ}$; detector, flame ionization, 200 $^{\circ}$; initial temperature 60 $^{\circ}$, 4 $^{\circ}$ /min to 120 $^{\circ}$; carrier gas nitrogen, 30 ml/min.

Fig. 5. Chromatogram of TMS-arsenicals and TMS-PO₄³⁻, TMS-SO₄²⁻ and TMS-CO₃²⁻ on OV-225. Peaks: 1, TMS-SO₄²⁻ + TMS-PO₄³⁻; 2, TMS-As(V); 3, TMS-As(III); 4, TMS-DMA. Sample: 4 μ l BSTFA-DMF (3:2) containing 980 μ g/ml SO₄²⁻, 1100 μ g/ml PO₄³⁻, 160 μ g/ml As(V), 340 μ g/ml As(III), 280 μ g/ml DMA, 710 μ g/ml CO₃²⁻. Column: 5% OV-225 on Gas-Chrom Q (100-120 mesh), 1.8 m \times 2 mm I.D. Conditions: injector, 170 $^{\circ}$; detector, flame ionization, 230 $^{\circ}$; initial temperature 100 $^{\circ}$, 4 $^{\circ}$ /min to 150 $^{\circ}$, carrier gas nitrogen, 30 ml/min.

atives were irreversibly adsorbed on the column or whether they failed to be separated from the solvent front. OV-17 resolved the three derivatives adequately but produced extensive solvent tailing. This adversely affected the limit of detection for the TMS-As(V) derivative which was the first arsenical eluted. OV-17 was, therefore, judged to be an unsatisfactory stationary phase. OV-225 (Fig. 1) and SE-30 (Fig. 3) separated all of the arsenical derivatives and provided adequate resolution from the solvent front. On SE-30 the TMS derivatives were eluted in the order: As(III), DMA and As(V); on OV-225 the order was As(V), As(III) and DMA. Similar variability in the elution order of the TMS derivatives of inorganic oxyanions was reported by Butts and Rainey¹⁰.

Separation of the TMS-arsenicals from other oxyanions which form TMS derivatives and which are encountered in natural systems was investigated. The anions of primary concern were phosphate, sulfate and carbonate. The SE-30 column did not completely resolve the TMS-As(III) and TMS-SO₄²⁻ derivatives or the TMS-PO₄³⁻ and TMS-DMA derivatives (Fig. 4). The TMS-CO₃²⁻ derivative was not an interferent as it eluted in the later portion of the solvent front ($t_R = 2.7$ min), and produced a response with the flame ionization detector which was only 10% of that for the most rapidly eluting arsenical, TMS-As(V). OV-225 provided superior separation of the arsenic-containing derivatives from other oxyanions (Fig. 5). The TMS-SO₄²⁻ and TMS-PO₄³⁻ co-eluted, but were well resolved from the peak for TMS-As(V). TMS-CO₃²⁻ separated well after ($t_R = 21.3$ min) the most strongly retained arsenical ($t_R = 13.9$ min). In subsequent studies OV-225 was employed as the stationary phase.

Typical working curves for the three arsenic derivatives appear in Fig. 6. Correlation coefficients for the curves exceed 0.98, and y -intercepts are negligible. The minimum limit of detection (MLD) and upper limit of the linear calibration range are presented in Table I. The MLD values were calculated by the method of Sullivan¹² for a signal-to-noise ratio of 2. In practice, the detection limits are dictated by the tailing of solvent peak and the point at which this tailing renders peak area measurements unreliable. For the systems used in this investigation, injection of 0.1

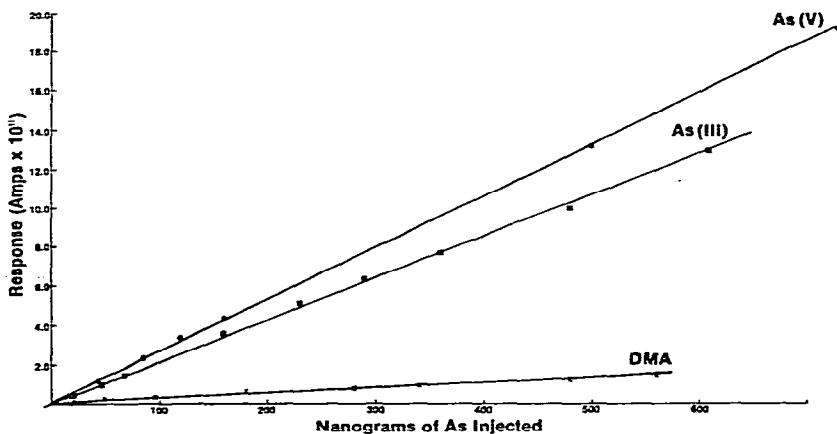


Fig. 6. Working curves for TMS-arsenicals.

ng of As(V) or As(III), or 1.0 ng of DMA represent practical limits of detection. From the upper limits of the linear calibration range and the values for the MLD it is seen that calibration curves are linear over 3 to 5 orders of magnitude.

TABLE I
CHROMATOGRAPHIC RESPONSE CHARACTERISTICS FOR TMS-ARSENICALS

Compound	Formula	Minimum limit of detection (g/sec)	Upper limit of linear calibration range (g/sec)	Relative response ratio*
DMA-TMS	$(\text{CH}_3)_2\text{AsO}(\text{OTMS})$	2.0×10^{-11}	9.3×10^{-8}	0.43
As(III)-TMS	$\text{As}(\text{OTMS})_3$	6.0×10^{-12}	1.5×10^{-7}	0.87
As(V)-TMS	$\text{AsO}(\text{OTMS})_3$	2.2×10^{-12}	2.3×10^{-7}	1.00
<i>n</i> -Octadecane	$\text{C}_{18}\text{H}_{38}$	—	—	2.27

* Relative response ratio defined with respect to As(V)-TMS.

Table I also presents the relative response of the silylated arsenicals. The As(V) derivative was assigned a ratio of 1. It is evident from these figures that the response of the flame ionization detector is considerably lower for the TMS-arsenicals than for the pure hydrocarbon, as might be expected for the smaller number of oxidizable carbons in the arsenical derivatives.

Mass spectrometry of TMS-arsenicals

The identities of the peaks attributed to the TMS-arsenicals were confirmed by MS. The spectra of the As(V)-TMS and As(III)-TMS derivatives (Figs. 7 and 8) are identical in all prominent respects to those reported previously¹⁰, and are consistent with the formation of tris(trimethylsilyl) derivatives.

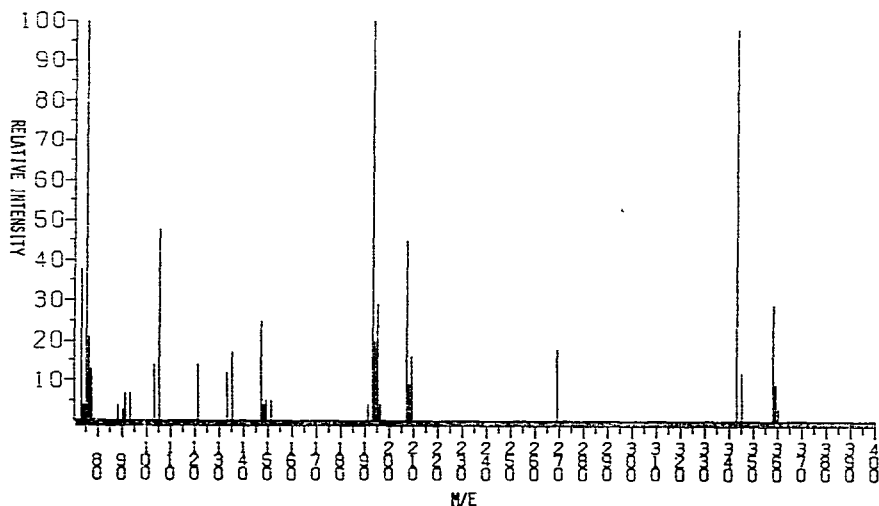


Fig. 7. Mass spectrum of TMS-As(V) derivative.

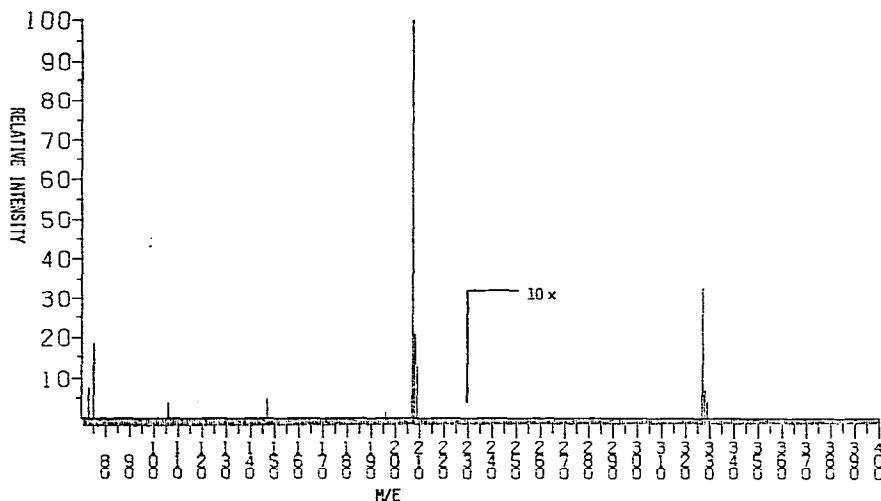


Fig. 8. Mass spectrum of TMS-As(III) derivative.

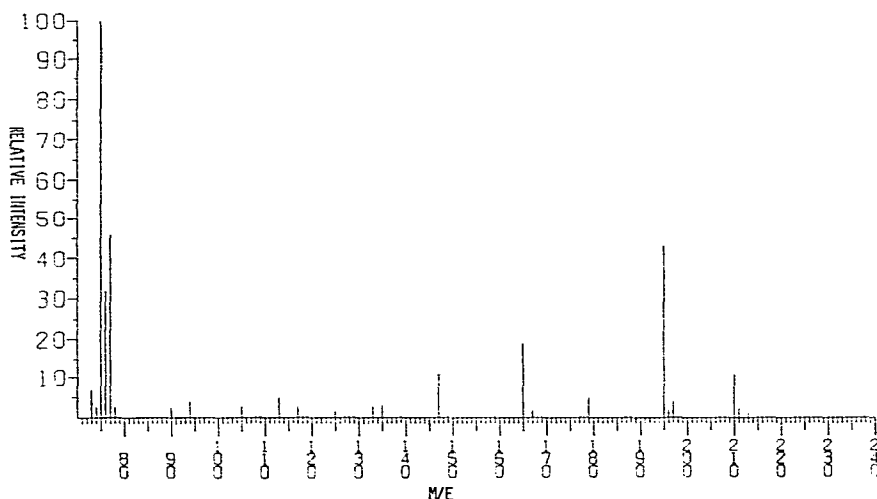


Fig. 9. Mass spectrum of TMS-DMA derivative.

The mass spectrum of the compound producing the chromatographic peak attributed to TMS-DMA contains prominent m/e 210, 195, 165, 147 and 75 ions (Fig. 9). These ions are characteristic of a DMA derivative whose single, acidic, hydrogen is replaced by the TMS group. The $P - 15$ ion (m/e 195) results from the loss of a methyl group from the parent ion. The CH_3 moiety most likely originates from fragmentation of the TMS portion of the silylated molecule¹³. However, cleavage of a CH_3-As bond in DMA is another possible, but probably less favorable, route for formation of the $P - 15$ fragment. The ion at m/e 165 may be produced from loss of two additional methyl groups from the $P - 15$ fragment. Silylated

compounds frequently yield ions of m/e 147 and m/e 75. These result from $(\text{CH}_3)_2\text{-Si}=\text{OSi}(\text{CH}_3)_3^+$ and $\text{HO}=\text{Si}(\text{CH}_3)_2^+$, respectively¹³. The mass spectrum is consistent with formation of a TMS-DMA derivative with the formula $(\text{CH}_3)_2\text{AsO}(\text{OTMS})$.

CONCLUSIONS

This work has demonstrated that arsenious, arsenic and dimethylarsinic acids may be speciated as their trimethylsilyl derivatives. The gas chromatographic behavior of these compounds, including linear ranges of calibration and detection limits suggest that this method is suitable for quantitative determination of the arsenicals at trace levels. Development of such analytical schemes requires pretreatment of real samples in order to transfer the arsenic-containing species to an organic phase where the derivatization reactions may be performed in the absence of water which otherwise would hydrolyze the TMS derivatives. Adaptation of this gas chromatographic procedure for the measurement of arsenicals in aqueous environmental samples and pharmaceuticals is now in progress. Preliminary GLC results suggest that a fourth environmentally significant arsenical, monomethylarsonic acid, can be determined as a TMS derivative, and further studies of the stability and quantitative utility of this derivative will be reported later.

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REFERENCES

- 1 E. A. Woolson, *Arsenical Pesticides*, ACS Symposium Series No. 7, American Chemical Society, Washington, D.C., 1975.
- 2 M. D. Luh, R. A. Baker and D. E. Hensley, *Sci. Total Environ.*, 2 (1973) 1.
- 3 Y. Talmi and D. T. Bostick, *J. Chromatogr. Sci.*, 13 (1975) 231.
- 4 C. J. Soderquist, D. G. Crosby and J. B. Bowers, *Anal. Chem.*, 46 (1974) 155.
- 5 J. D. Lodmell, *Ph.D. Thesis*, University of Tennessee, 1973.
- 6 L. D. Johnson, K. O. Gerhardt and W. A. Aue, *Sci. Total Environ.*, 1 (1972) 108.
- 7 Y. Talmi and D. T. Bostick, *Anal. Chem.*, 47 (1975) 2145.
- 8 M. O. Andreae, *Anal. Chem.*, 49 (1977) 820.
- 9 R. S. Braman and C. C. Foreback, *Science*, 182 (1973) 1247.
- 10 W. C. Butts and W. T. Rainey, *Anal. Chem.*, 43 (1971) 538.
- 11 R. F. Kruppa, B. S. Henly and D. L. Smead, *Anal. Chem.*, 39 (1967) 851.
- 12 J. J. Sullivan, in R. L. Grob (Editor), *Modern Practice of Gas Chromatography*, John Wiley and Sons, New York, 1977, p. 219.
- 13 A. E. Pierce, *Silylation of Organic Compounds*, Pierce Chemical Co., Rockford, Ill. 1968.