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Arsenic Speciation of Aqueous Environmental Samples by Derivatization with Thioglycolic Acid Methylester and Capillary Gas–Liquid Chromatography–Mass Spectrometry

Fred A. Claussen
EPL Bio-Analytical Services, Inc., P.O. Box 109, Harristown, IL 62537

Abstract

Procedures are described for the determination of dimethylarsinic acid, monomethylarsonic acid, arsenate, and arsenite in aqueous samples. The arsenicals are reacted with thioglycolic acid methyl ester to form volatile trivalent derivatives. The reaction products are extracted into benzene and separated by capillary gas–liquid chromatography. A low-resolution electron-ionization quadrupole mass spectrometer operated in the selective ion-monitoring mode is used to detect the derivatives after chromatographic separation. Method detection limits range from 0.1 ng/mL (ppb) for the alkylarsenic acids to 3 ppb for arsenate and arsenite. The method is shown to be simple, rapid, and quantitative for the determination of selected arsenic species in aqueous environmental samples and drinking water.

Introduction

Arsenic ranks 20th in abundance among the elements in the earth's crust (1). It occurs naturally in igneous and sedimentary rocks, where it is associated primarily with sulfur in mineral form. Phenomena such as weathering, biological activity, and volcanism result in the release of arsenic into the surrounding environment. Anthropogenic inputs from nonferrous smelting operations, combustion of fossil fuels, and arsenical pesticide applications also contribute to the global arsenic cycle. Arsenic is present in the environment as several chemical species but is rarely found in its elemental form. However, the term *arsenic* appears in the literature as a descriptor of the entire collection of arsenicals. The major arsenic compounds found in air, soil, sediment, and water include dimethylarsinic acid (DMAA) ($[CH_3]_2AsOOH$), monomethylarsonic acid (MMAA) ($CH_3AsO[OH]_2$), arsenate (As[V]) ($AsO[OH]_3$), and arsenite (As[III]) ($As[OH]_3$). Surface and groundwater, which are the focus of this paper, display a wide range of arsenic concentrations but typically yield sub-parts-per-billion to low parts-per-billion levels in areas where anthropogenic influences are minimal. Knowledge of the presence and concentration of specific arsenic compounds in aqueous systems is important because of the pronounced differences in toxicity and mobility between the major species (2). Thus, laboratory procedures that provide chemical speciation of arsenic have become increasingly in demand.

Several techniques have been described that allow for the determination of specific arsenic compounds in environmental samples. The most common methods are based on the conversion of the arsenicals to volatile arsines by reaction with sodium borohydride (3). After the hydriding reaction, the arsines can be cryogenically trapped, released, and separated for detection using various instruments (3–5). Detection limits in the low parts-per-trillion range have been achieved with some hydride techniques. However, the various procedures described are tedious and require very experienced analysts and specialized equipment to achieve accurate results. Because of these difficulties, much effort has been exhausted in the pursuit of simpler techniques that utilize more common laboratory instrumentation and do not require the high skill level needed for hydride techniques. In light of this, a great number of procedures have been described for the reactions of DMAA, MMAA, As(V), and As(III) with various chemical reagents to form volatile derivatives amenable to gas–liquid chromatographic (GLC) analysis (6–13). Several classes of arsenic derivatives have been reported. Some of these include trimethylsilyl derivatives (6), diethyldithiocarbamate chelates (7–9), and halogenated arsines (10–11). However, rapid hydrolysis of these derivatives precludes their use in the development of reliable arsenic speciation methods. Another class of arsenic derivatives described by Beckermann, the thioglycolic acid methyl esters (TGMs), were found to be stable for several days in cyclohexane extracts and sufficiently volatile for GLC analysis (12). The method developed by Beckermann utilized a packed GLC column (2.5% XE-60 on Chromosorb Q, 100–120 mesh) and a flame-ionization detector (FID). The detection limit for DMAA and MMAA in urine and blood was estimated at 10 ppb. Recovery experiments were conducted at a much higher concentration of 20 µg/mL (ppm). Dix et al. reported the analysis of TGM derivatives of DMAA, MMAA, As(V), and As(III) using megabore (0.53-mm i.d.) capillary columns and an electron-capture detector (ECD) (13). Instrument sensitivity for each derivative was reported at 0.1 ng/µL based on a signal-to-noise ratio of 3. Recovery and detector linearity experiments were not conducted.

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The research reported in the present paper expands on the past work with TGM arsenic derivatives. The specific goals of the research were to provide improved sensitivity for the TGM derivatives and, hence, lower detection limits for the arsenicals over that previously reported and to evaluate the usefulness of the technique in the speciation of aqueous environmental samples. It was thought that the use of a thin-film, narrow-bore capillary column (0.25-mm i.d., 0.25-µm film thickness) would decrease chromatographic peak widths, thus improving signal-to-noise ratios. It was also hypothesized that a mass spectrometer operated in selective ion-monitoring (SIM) mode would provide superior sensitivity and selectivity over the FID and ECD.

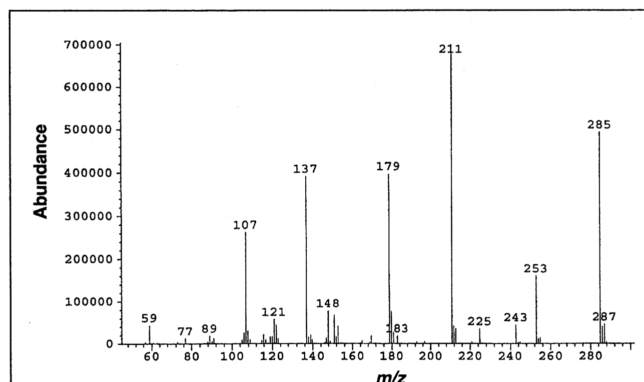

Figure 1. Mass spectrum of the TGM derivative of As(III). Detection in full scan mode; scan range from *m/z* 50 to 500. Background subtraction applied.

Experimental

Chemicals

DMAA and MMAA were obtained from Johnson Matthey Alfa Aesar (Ward Hill, MA). Sodium arsenite (meta) and sodium arsenate were obtained from Fisher Scientific (St. Louis, MO). Neat standards were used as received. Organic solvents were either Optima, Pesticide grade, or HPLC grade and were purchased from Fisher Scientific. TGM was obtained from Aldrich Chemical (Milwaukee, WI). Concentrated hydrochloric acid (HCl) was obtained from Fisher and was TraceMetal grade. Stock solutions of each arsenical were prepared by dissolving 0.01 g, corrected for purity and hydration, of the neat standard in 100 mL of 0.01M HCl to yield solution concentrations of 100 µg/mL. Single- or multi-compound working solutions of the arsenicals were prepared by appropriate dilution of the stock solutions in 0.01M HCl. Stock solutions were prepared on a weekly basis. Working solutions were prepared daily. Stock and working solutions were stored in polypropylene containers in a refrigerator. Hexachlorobenzene (HCB), used as an internal standard (IS), was purchased from Aldrich Chemical. A stock IS solution was prepared by dissolving 0.01 g of neat HCB in 100 mL of hexane. The HCB stock solution was diluted 1:100 in 2-propanol to yield a working IS solution containing 1 µg/mL HCB. IS stock and working solutions were stored in glass vials in a refrigerator.

Apparatus

Sample analyses were performed on a Hewlett-Packard 5890 series II Plus gas chromatograph equipped with a 5972 mass selective detector (MSD) and a 6890 automatic liquid sampler. The chromatographic separation utilized a crosslinked 5% phenyl silicone–95% methyl silicone liquid phase (HP-5MS, Hewlett-Packard). The column dimensions were 30 m × 0.25-mm i.d.; the film

Table I. SIM Ions, Typical Retention Times, and Structural Formulas for TGM Arsenic Derivatives and the Internal Standard

Analyte	Target ion (m/z)	Qualifier ion (m/z)	Retention time (min)	Structural formula
DMAA–TGM	195	121	3.65	$(CH_3)_2AsSCH_2COOCH_3$
MMAA–TGM	195	285	6.35	$CH_3As(SCH_2COOCH_3)_2$
As(III)/As(V)–TGM	285	211	8.40	$As(SCH_2COOCH_3)_3$
Internal standard (HCB)	282	249	5.85	C_6Cl_6

Table II. Recovery of Added Arsenicals from Aqueous Samples

Sample type	Analyte	Amount added (ppb)	Mean amount found* (ppb)	Mean recovery (%)	Standard deviation (%)
Tap water	DMAA	5.2	5.3	102	4.9
	MMAA	5.2	5.0	96	14
	Total inorganic As	40	36	90	17
	DMAA	1.0	1.2	120	7.6
	MMAA	1.0	0.91	91	5.6
	Total inorganic As	17	14	82	15
River water	DMAA	5.2	5.0	96	15
	MMAA	5.2	5.5	106	12
	Total inorganic As	40	36	90	5.0
	DMAA	1.0	1.1	110	7.6
	MMAA	1.0	1.2	120	13
	Total inorganic As	17	18	106	3.2
Well water	DMAA	5.2	5.5	106	15
	MMAA	5.2	4.0	77	6.6
	Total inorganic As	40	31	78	19
	DMAA	1.0	1.3	130	16
	MMAA	1.0	1.3	130	19
	Total inorganic As	17	20	118	9.4

* Analysis of three separate spiked samples.

thickness was 0.25 µm. The carrier gas was helium maintained at a constant flow rate of 0.75 mL/min using electronic pressure programming. The oven program for the gas chromatograph began at 75°C and was held at this temperature for 0.5 min. The oven was then raised to a final temperature of 280°C at a rate of 30°C/min and held at the final temperature for 1 min. Sample injections were made into a single-taper glass-lined split–splitless inlet held at 280°C. Injections were made in the splitless mode. The septum purge valve was turned on automatically 30 s after injection. The MSD was operated in the EI mode at 70 eV. The MSD interface temperature was 280°C. Data acquisition and processing were controlled by a Hewlett-Packard computer system with Chemstation software. The MSD was tuned daily prior to use by invoking the Maximum Sensitivity Autotune command in the Chemstation software. Perfluorotributylamine was used as the tuning compound. Optimized parameters for the EI source, mass analyzer, and electron multiplier established during the tuning procedure were saved and used for instrument calibration and sample analysis.

Methods

All glassware and plasticware were cleaned by soaking in a hot detergent solution followed by a tap water rinse and five rinses with deionized water. The clean glassware was dried in an oven at 120°C prior to use. Tap water, river water, and nonpotable well water were collected in 500-mL polyethylene bottles. Upon arrival at the laboratory, water samples were acidified to pH 2 using concentrated HCl and stored in a refrigerator until needed for analysis. For derivatization, a 5-mL aliquot of the acidified water sample was transferred to a 15-mL glass test tube. Using glass syringes, 0.05 mL of IS working solution and 0.25 mL of TGM were added under a fume hood. The contents of the test tube were thoroughly mixed on a vortexing apparatus for 30 s and allowed to react at room temperature for an additional 2 min after mixing. The TGM derivatives were extracted with 1 mL of benzene by vortexing for 1 min. After phase separation, approximately 0.5 mL of the benzene layer was removed and dried over 100 mg of anhydrous sodium sulfate in a clean test tube. The dried benzene was placed in an autosampler vial for GLC–MSD analysis.

SIM was used for quantitative analysis. Two ions were chosen for each derivative (Table I) based on their abundance and lack of interference at the retention times of interest. A single ion for each analyte was used for calibration and quantitation (target ion). The second ion will be used in future work for qualitative confirmation of "unknown" environmental samples (qualifier ion). The MSD was calibrated by analyzing benzene extracts of derivatized aqueous arsenic standards containing from 0.5 to 25 ppb DMAA and MMAA and from 5 to 200 ppb As(III). The standards were derivatized and extracted using the same procedure already described for samples. For samples and standards, 1 µL of the benzene extract was injected into the gas chromatograph using the 6890 autosampler. Calibration curves of the response ratio (Equation 1) versus the concentration ratio (Equation 2) were plotted using linear regression analysis. For simplicity, analyte concentrations were expressed in terms of the underivatized arsenical in the starting aqueous solution. Calibration curves with a coefficient of determination greater than or equal to 0.99 were considered acceptable.

$$\text{Response ratio} = \frac{\text{analyte peak area}}{\text{IS peak area}} \qquad \text{Eq 1}$$

$$\text{Concentration ratio} = \frac{\text{analyte concentration}}{\text{IS concentration (10 ppb)}} \qquad \text{Eq 2}$$

Results and Discussion

Derivatization and extraction

The reactions of DMAA, MMAA, As(V), and As(III) with TGM have been described (12–13). TGM is a strong reducing agent and, as such, results in the conversion of arsenic from the pentavalent (oxidized) state to its trivalent (reduced) state as a first step in the reaction. Therefore, As(III) and As(V) form the same derivative upon reaction with TGM, and speciation is not possible without the isolation of one of the compounds prior to derivatization. The TGM reaction products were verified by the analysis of benzene extracts of derivatized arsenic working standards with the MSD in full scan mode. Mass spectra for the DMAA and MMAA TGM derivatives were previously published (12). The mass spectrum for the As(III) TGM derivative appears in Figure 1. The As(V) TGM spectrum (not shown) was identical to that of the As(III) TGM derivative.

The derivatization and extraction procedure described by Dix et al. (13) was used as a starting point for the method development research. Only a few minor changes were made based on preliminary experiments conducted to evaluate the ruggedness of the procedure. These changes included the use of benzene instead of cyclohexane as an extractant for the derivatives, a TGM-to-sample volume ratio of 0.1 instead of 0.05, and a sample-to-benzene volume ratio of 5 instead of 1. These changes were incorporated to provide

Table III. Recovery of Added As(III) from Aqueous Samples

	Amount added (ppb)	Mean amount found* (ppb)	Mean recovery (%)	Standard deviation (%)
Tap water	20	9.8	49	13
	10	8.4	84	6.3
River water	20	13	65	2.5
	10	7.5	75	3.3
Well water	20	< 3	0†	
	10	< 3	0†	

* Analysis of three separate spiked samples.
† As(III) oxidized to As(V) upon addition of spiking solution to sample.

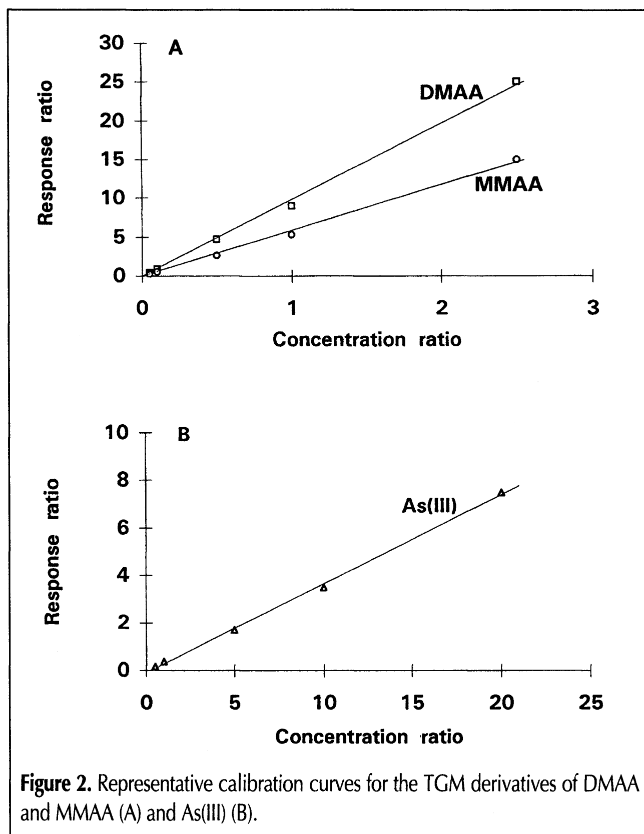

Figure 2. Representative calibration curves for the TGM derivatives of DMAA and MMAA (A) and As(III) (B).

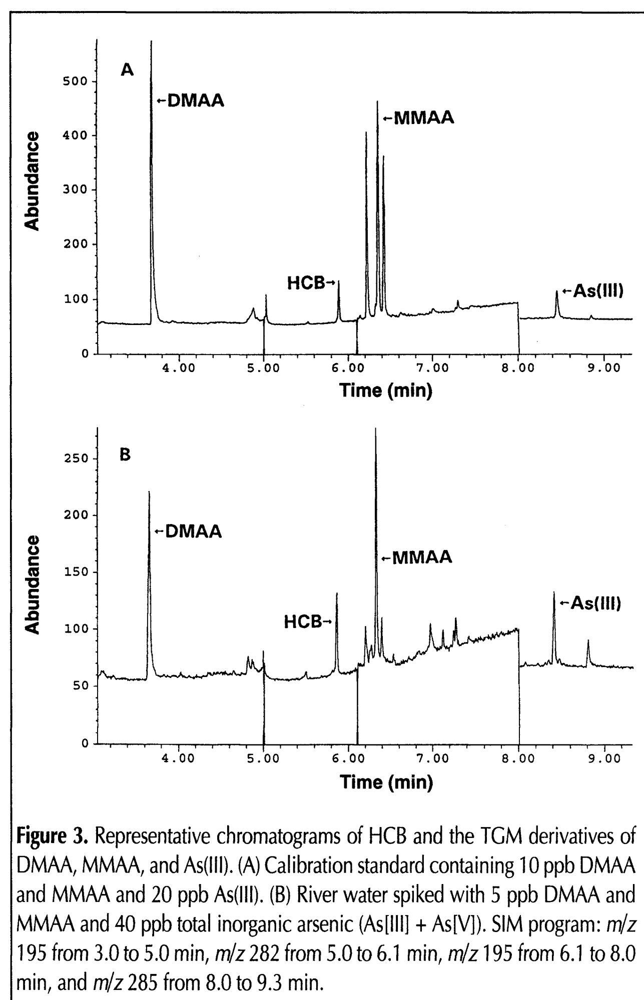

Figure 3. Representative chromatograms of HCB and the TGM derivatives of DMAA, MMAA, and As(III). (A) Calibration standard containing 10 ppb DMAA and MMAA and 20 ppb As(III). (B) River water spiked with 5 ppb DMAA and MMAA and 40 ppb total inorganic arsenic (As[III] + As[V]). SIM program: *m/z* 195 from 3.0 to 5.0 min, *m/z* 282 from 5.0 to 6.1 min, *m/z* 195 from 6.1 to 8.0 min, and *m/z* 285 from 8.0 to 9.3 min.

lower detection limits and better precision. All TGM derivatives were found to be stable in benzene solution for at least 72 h.

Detection limit, recovery, and precision

Due to the fact that As(V) and As(III) form the same TGM derivative, no initial attempt was made to determine them separately. Instead, results for recovery experiments with As(V) and As(III) were expressed in terms of total inorganic arsenic. For total inorganic arsenic, a mixture of As(III) and As(V) was spiked into the water samples. The As(V) spike concentration was converted by calculation to As(III) equivalent concentration for the purpose of determining recovery because As(III) was used as a calibration standard. Spike levels for all arsenicals were selected so that the final analyte concentrations fell within the concentration range of the respective calibration curve without dilution. The results presented in Table II indicate quantitative recovery of all analytes from the matrices evaluated. As expected, the well water spike recoveries were more variable than those obtained for tap water and river water analyses. Although no attempt was made to identify specific chemical components in the well water, a high Fe(III) concentration was suspected based on a rust-like coloration and corresponding odor prior to acidification. The high Fe(III) concentration may have contributed to increased variability by preferentially reacting with TGM and/or by binding a fraction of the arsenic and rendering it unavailable for reaction with TGM. The addition of a chelating agent prior to derivatization might improve method precision. The well water also contained relatively high levels of undissolved particulates. Depending on the chemical nature of the suspended material, sample filtration could provide some improvement in precision.

Reagent blanks and unspiked control samples of each matrix were analyzed concurrently with the spiked samples to determine background arsenic levels. All reagent blank and control analyses yielded results less than the method detection limit (MDL) for each analyte. MDLs were defined as the analyte concentration in the starting water sample which produced a signal-to-noise ratio of 3 after derivatization and extraction. The MDLs were established at 0.1 ppb for DMAA and MMAA and at 3 ppb for total inorganic arsenic. The linear working range (calibration range) for each analyte began at a concentration approximately five times the MDL (Figure 2). Figure 3 displays the chromatogram of a mid-level calibration standard. The chromatogram of a spiked river water sample is also shown. MDLs were determined experimentally by analysis of reagent blanks (10 replicates) and aqueous standards (10 replicates). Higher detection limits may be expected for environmental samples, depending on the nature of the matrix being evaluated. For the matrices examined in this research, the MDLs cited above appear to be reasonable estimates because no major coeluting peaks were detected in control samples. The large difference in detection limits (approximately 30 times) between the alkylarsenic acids and the inorganic arsenicals could not be explained. The most likely scenarios include incomplete reaction of As(III) and As(V) with TGM or decomposition of the derivative after injection into the gas chromatograph. Stability experiments preclude degradation in benzene solution at an ambient temperature up to 72 h after extraction. Incomplete extraction into benzene was eliminated as a possibility after multiple extractions of an

aqueous solution containing the As(III) TGM derivative yielded no detectable analyte response after the first extraction. The source of this apparent anomaly is presently being investigated.

Because of pronounced differences in toxicological properties and mobility in the environment between As(III) and As(V), methodologies that are able to distinguish between the two species are of great value. As part of this research, an initial effort to speciate As(III) and As(V) was made using a procedure described by Takamatsu et al. (14) in which As(III) was isolated from an aqueous sample by extraction with benzene after the sample was adjusted to an HCl concentration of 8N. The benzene was back-extracted with neutral phosphate buffer. The aqueous buffer was then analyzed yielding the As(III) concentration. A second sample aliquot was analyzed for total arsenic. The calculated difference between total arsenic and As(III) yielded the As(V) concentration, assuming no organic arsenicals were present in the sample. As part of this research, the TGM derivatization procedure was applied to the back-extracted As(III) after acidification of the aqueous buffer to pH 2. Recovery experiments for As(III) from water samples were carried out in the presence of an approximately equal concentration of As(V). Evaluation of As(III) recovery from well water could not be made because of the nearly instantaneous oxidation of the spiked As(III) to As(V). The oxidation was probably carried out by the Fe(III)–Fe(II) redox couple. Only partial recovery of As(III) from tap water and surface water was achieved (Table III). However, the technique appears to show some promise and will be the focus of future research.

Conclusion

The GLC arsenic speciation procedure described in this paper appears to be the first of its kind to provide accurate quantitative results for aqueous samples containing low parts-per-billion concentrations of arsenic. Superior sensitivity, and hence, detection limits, to those reported for ECD (13) and FID (12) analyses were achieved using a mass spectrometer in SIM mode for the TGM derivatives of DMAA, MMAA, As(V), and As(III). The technique has been successively applied to the analysis of spiked drinking water as well as surface and subsurface fresh water. The method is simple and rapid and employs equipment and instrumentation readily available in modern environmental monitoring laboratories. These attributes make the procedure attractive as a tool for routine arsenic speciation of selected compounds in aqueous samples.

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