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Determination of Lewisite oxide in soil using solid-phase microextraction followed by gas chromatography with flame photometric or mass spectrometric detection

Bruce A. Tomkins*, Gary A. Sega, C.-h. Ho

Organic Chemistry and Separations Section, Chemical and Analytical Sciences Division, Oak Ridge National Laboratory, P.O. Box 2008, Oak Ridge, TN 37831-6120, USA

Abstract

A rapid, sensitive, and convenient method is described for determining Lewisite oxide in soil. Samples are initially fortified with phenylarsine oxide (surrogate), then both species are extracted using ascorbic acid solutions containing 1,3-propanedithiol (derivatizing reagent). The corresponding filtered supernatant is sampled using a solid-phase microextraction fiber. Collected analytes are thermally desorbed in a heated gas chromatographic inlet, separated using fused-silica capillary columns (''primary'' and ''confirmatory''), and detected with either a mass spectrometric (selected ion monitoring mode) or flame photometric (sulfur-selective mode) detector. Two independent statistically-unbiased procedures were used to evaluate the detection limit for Lewisite oxide; the values range between 0.1 and 0.5 $\mu g\ g^{-1}$ soil.

Keywords: Soil; Warfare agents; Environmental analysis; Lewisite oxide; Organoarsenic compounds; Arsenic; Chlorovinyl arsonous acid; Chlorovinyl arsenous oxide; Chlorovinyldichloroarsine

1. Introduction

Lewisite (*syn*. 2-chlorovinyldichloroarsine or L, CAS registry No. [541-25-3]) is an organoarsenical chemical blister agent originally developed during World War I and produced worldwide ever since by various agencies, including the US Department of Defense [1–3]. Because many of these manufacturing sites are being remediated, particularly in the United States, there is a need for rigorously tested and validated analytical methods which will: (a) demonstrate the presence or absence of Lewisite at regulatory levels in soil samples, (b) be readily implemented by most commercial analytical laboratories, (c) be rapid and convenient to use, and (d) generate minimal quantities of chemically-hazardous waste. The determination of traces of the decomposition products of Lewisite is crucial to support efforts in the remediation of contaminated sites at many military installations and the verification of arms control agreements.

Lewisite per se is never found in the environment. Fig. 1 shows that this compound hydrolyses rapidly to 2-chlorovinyl arsonous acid (CVAA), which in turn slowly dehydrates to Lewisite oxide (*syn*. 2-chlorovinyl arsenous oxide or CVAO, CAS registry No. [3088-37-7]), culminating in a polymerized form of CVAO [4–7], which exhibits both a poorly-characterized composition and structure. For this reason, while it may be preferred to determine the various species individually, the customary practice is to

*Corresponding author. Fax: +1-865-576-7956.

E-mail address: tomkinsba@ornl.gov (B.A. Tomkins).

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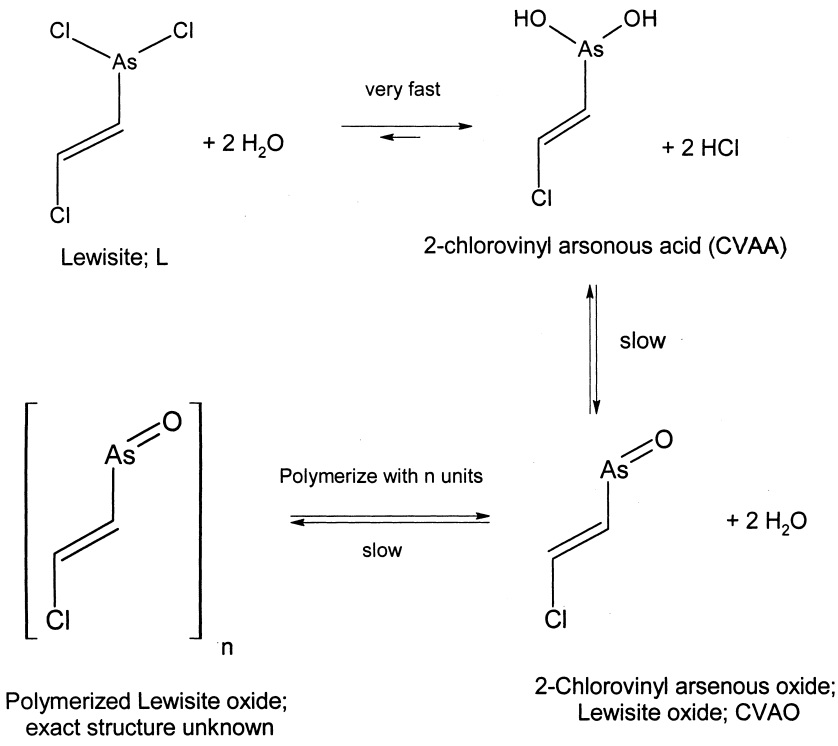

Fig. 1. Conversion pathways for Lewisite to related species.

report ''CVAO'', which in reality is the sum of the CVAA, CVAO, and extractable polymerized CVAO in a given sample. The presence of CVAO in the environment is therefore a positive indication of Lewisite contamination.

Because the prevailing Lewisite degradation products are nonvolatile, the literature describes only two direct methods for quantitating them. Bass et al. [8] described an high-performance liquid chromatography–inductively coupled plasma mass spectrometry (HPLC–ICP-MS) method capable of distinguishing the various Lewisite-related species. However, this method is based upon an instrument which will not be found in most analytical laboratories. Rewick et al. [9] studied the UV absorption of Lewisite, sulfur mustard, four chemical warfare nerve agents (GA, GB, GD, VX) and two alkylphosphonate simulants in cyclohexane. He noted that Lewisite exhibited the greatest absorptivity of the compounds tested. However, the nominal test wavelengths employed in this work were 200, 230 and 250 nm. Many organic species absorb at these wavelengths, therefore minimal discrimination against interfering species is obtained. To compound the difficulty, Lewisite is soluble in cyclohexane, but CVAO is not.

In general, analytical procedures for CVAO rely on indirect measurements, in which the analytes are either decomposed or derivatized prior to quantitation. One approach, less commonly used, decomposes CVAO in the presence of strong base to form acetylene, which may be measured in the solution headspace [10,11]. The sensitivity of these methods ranges between 0.5 and 5 μg CVAO g^{-1} soil [12,13]. The more common approach is to derivatize CVAO and its related species with a mercaptan, thereby forming a species which is both volatile and thermally-stable, and thus amenable to gas chromatographic analysis. The derivatized product may be monitored and quantitated using either mass spectrometric or flame photometric (sulfur-selective mode) detectors. A variety of monofunctional mercaptan reagents have been evaluated, including 1-ethanethiol [14], 1-propanethiol, thioglycolic acid

ethyl ester, and thioglycolic acid methyl ester [15,16]. The reagent 2-mercaptopyridine, which is also a monofunctional mercaptan, has been employed for post-column derivatization of CVAA followed by HPLC–MS [17].

Several authors have studied and reported the highly-successful reaction of small alkyl dithiols, particularly 1,2-ethanedithiol (EDT) and 1,3-propanedithiol (PDT), with CVAA to form cyclic, volatile, and thermally-stable derivatives in either water or dilute ascorbic acid, as shown in Fig. 2 [4,6,13]. The procedure described in Fowler et al. [6], which was both highly-sensitive and selective, was optimized for aqueous samples, not soils, and featured an extensive micro liquid–liquid extraction sequence which would generate a modest to substantial volume of chemically hazardous waste with time.

The highly-successful method of Szostek and Aldstadt [4] emphasized the determination of derivatized CVAO and its related products in mildly-acidic (10 m*M* hydrochloric acid) aqueous extracts of soil or sediment; comparatively little work was performed on contaminated soil samples themselves. This method was unique in that it featured solid-phase microextraction (SPME) sampling of the aqueous extract prior to a final separation and quantitation of the derivatized CVAO using gas chromatography (GC)–MS. Sampling of the aqueous soil extract was performed using a coated fiber which concentrated the derivatized products without the need for additional liquid–liquid extraction followed by concentration procedures such as nitrogen ''blow-down''. The products so collected could then be desorbed conveniently into the inlet of a gas chromatograph, separated using an appropriate fused-silica chromatographic column, and detected using either mass spectrometric or element-selective detection. The latter includes both conventional flame photometric detection (FPD) in its sulfur-selective mode or the newer pulsed-flame photometric detection (PFPD), which may be tuned to respond to either sulfur or arsenic [18,19].

The current work expands the procedure of Szostek and Aldstadt [4] to provide a rapid, convenient, sensitive, and rigorously-tested procedure for quantitating CVAO in soil. Small (2-g) soil samples are fortified with phenylarsine oxide (PhAsO), a candidate surrogate compound, then extracted with 10 ml of 0.66% (w/v) ascorbic acid containing 100 μl PDT l^{-1}. After the initial extraction is completed (30 min), the samples are centrifuged. The supernatant is passed through a 0.45-μm porosity nylon syringe filter, then sampled using an SPME fiber for 20 min.

The derivatized products so collected are ther-

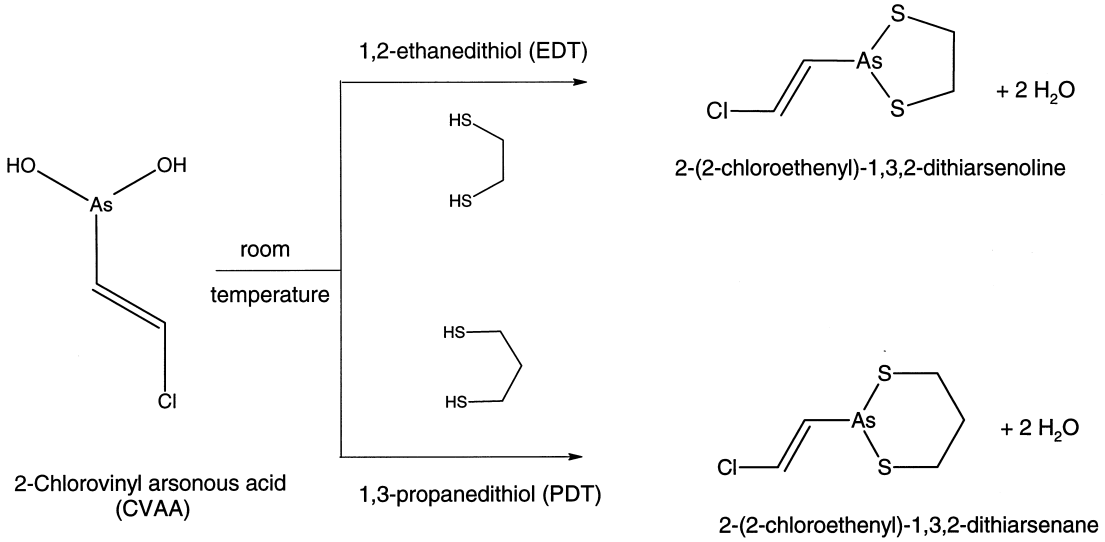

Fig. 2. Formation of cyclic disulfide derivatives of CVAA using 1,2-ethanedithiol and 1,3-propanedithiol.

mally desorbed in the inlet of a gas chromatograph, separated, and detected using FPD in its sulfur-selective mode. The detection limits and recoveries for both CVAO and PhAsO were rigorously determined using protocols mandated by both Rocky Mountain Arsenal (US Army) and the US Environmental Protection Agency (EPA).

The resulting chemical waste is less hazardous and more easily treated than that produced using some of the other procedures cited.

2. Materials and methods

2.1. Chemicals

Phenylarsine oxide (technical grade, CAS registry No. [637-03-6]), 1,2-ethanedithiol (technical grade, 90% purity, CAS registry No. [540-63-6]), and 1,3-propanedithiol (99% purity, CAS registry No. [109-80-8]) were purchased from Aldrich (Milwaukee, WI, USA). Ascorbic acid (99+% purity, CAS registry No. [50-81-7] was purchased from MCB (Cincinnatti, OH, USA) and Sigma (St. Louis, MO, USA). Water and methanol (HPLC grade, CAS registry No. [67-56-1]) were obtained from J.T. Baker (Phillipsburg, NJ, USA).

CVAO, sample MRI 8-4-99, lot 21093 (technical grade) was kindly provided by Dr. John Witt, Midwest Research Institute, Kansas City, MO, USA. *Caution: CVAO is a vesicant. Wear gloves when handling neat material.*

2.2. Reagents

The standard matrix solution, 0.66% (w/v) ascorbic acid in water, was prepared by diluting 6.6 g ascorbic acid to a final volume of 1 l water. The extracting solution was prepared in a similar fashion, and also includes 100 $\mu l\ l^{-1}$ PDT. Both solutions are stored at room temperature in amber bottles and replaced every 30 days.

2.3. Stock and spiking solutions

''Master stock'' solutions of CVAO and PhAsO were prepared by dissolving 10 mg of each compound into 0.66% (w/v) ascorbic acid solution and methanol, respectively, with vigorous stirring. (This may take several hours; the solubility of each is approximately 1 mg ml^{-1} in the solvent listed). ''Master calibrating'' and ''master spiking'' solutions (10 and 40 μg each analyte ml^{-1}, respectively) were prepared by diluting portions of the ''master stock'' solutions in 0.66% (w/v) ascorbic acid. The shelf life of the ''master stock'' solutions is approximately 30 days at 4°C. ''Master calibrating'' and ''master spiking'' solutions were stored at 4°C and replaced every 7 days.

2.4. Soil samples

Sieved dried standard soil was provided by the Laboratory Support Division, Rocky Mountain Arsenal, Commerce City, CO, USA. Clean washed laboratory sand was purchased from J.T. Baker.

2.5. Solid-phase microextraction equipment

SPME fibers (100 μm polydimethylsiloxane, part No. 57300-U), the corresponding holder for manual sampling (part No. 57330-U), a sampling stand (part No. 57333-U), and heat/stir plate (part No. Z262129-1) were purchased from Supelco (Bellefonte, PA, USA). Micro stirrer bars (''fleas'') were obtained from VWR (USA).

2.6. Instrumentation

A Varian 3400 gas chromatograph (Varian Associates, Sunnyvale, CA, USA) equipped with a flame photometric detector in its sulfur-selective mode and a splitless septum programmable injector (SPI) was used. Two fused-silica capillary columns were evaluated: ''primary'', 30 m×0.53 mm I.D., 1.0 μm film thickness, Rtx-5; ''confirmatory'', 30 m× 0.32 mm I.D., 0.5 μm film thickness, Rtx-35. Both are products of Restek, Bellefonte, PA, USA. The linear velocity of the carrier gas (99.999% helium) for both columns was set to approximately 40 cm s^{-1} at the initial oven temperature of 100°C. The total flow of the carrier and make-up gas (99.999% nitrogen) at the detector was 30 ml min^{-1}. The flows of breathing air and hydrogen (>99.999%) were

optimized to produce maximum detector selectivity and sensitivity for sulfur. A splitless injection liner was employed for each column. Pre-drilled high-temperature 11 mm diameter septa (catalog No. 23168, Supelco) were employed for all SPME determinations.

A Hewlett-Packard Model HP 5989A mass spectrometer interfaced to a HP Model 5890 Series II gas chromatograph was employed for all GC–MS determinations. The gas chromatograph was equipped with a 30 m×0.25 mm I.D., 0.25 μm film thickness, HP-5MS column. The head pressure of the carrier gas (99.999% helium) was maintained at 54 kPa (7.8 p.s.i.).

2.7. Instrument operating parameters

The injector and detector temperatures for the Varian 3400 were maintained at 250°C and 300°C, respectively. The column oven temperature was increased as follows: (a) from the initial value of 100°C (hold for 2 min) linearly to 175°C at 20°C min^{-1}; (b) from 175°C linearly to 200°C at 4°C min^{-1}; (c) from 200°C to 250°C linearly at 50°C min^{-1} (hold for 5 min). The SPME fiber remained in the injector port throughout a given analysis.

The injector and detector temperatures for the Hewlett-Packard Model 5890 Series II gas chromatograph were maintained at 225°C and 280°C, respectively. The column oven temperature program was identical to that employed for the Varian 3400. Once again, the SPME fiber remained in the injector port throughout a given analysis. The ionization source voltage was 70 eV. The mass spectrometer was operated in its selective ion monitoring (SIM) mode. The m/z values chosen to monitor and quantitate derivatized CVAO were 242, 181, 149, 132, 107, 78, 58 and 45. The corresponding values for derivatized PhAsO were 258, 216, 184, 149, 107, 91 and 77.

2.8. Filtration apparatus

All soil extracts were filtered through 10-ml capacity polypropylene syringe barrels (Becton-Dickinson 309604) equipped with 25 mm diameter, 0.45 μm porosity, nylon syringe filters (Gelman 4438 or equivalent). PTFE syringe filters of similar diameter and porosity may be substituted.

2.9. Calibration procedure

An aliquot of ''master calibrating'' solution (5 to 250 μl) was added to a 10-ml portion of extracting solution in a precleaned 20-ml screwcap vial. A micro stirring bar was added to the diluted solution, which was then immediately sampled for 20 min with the SPME fiber. The derivatized CVAO and PhAsO so collected was immediately desorbed in the injection port of either gas chromatograph, separated, and detected as noted above. These ''working'' calibrating solutions must be prepared fresh daily and analyzed immediately.

2.10. Soil extraction procedure: analysis with flame photometric detection

Independent sets of experiments optimized the soil extraction procedure for the following parameters: (a) quiescent vs. ultrasonic extraction; (b) pH; (c) hydrochloric vs. ascorbic acid; (d) extraction time; (e) residence time of the spike on the soil surface. Based on these results, the following optimized soil extraction procedure was employed:

Aliquots (2±0.05 g) of Rocky Mountain Arsenal standard soil or clean washed laboratory sand in a precleaned 40-ml screwcap vial were fortified with ''master spiking'' solution (5–250 μl) added to the sample surface. The spiked soil was shaken briefly (<10 s), then *immediately* extracted with 10 ml soil extracting solution. The solutions were shaken briefly by hand, then allowed to stand undisturbed for 30 min, with brief gentle hand shaking (end-over-end) after 15 min. After the extraction was completed, the sample was centrifuged for approximately 5–10 min using an International Equipment (Needham, MA, USA) Model CL centrifuge (3600 rpm, corresponding to ~2000 *g*). The supernatant was filtered using a 0.45-μm porosity nylon syringe filter, then collected in a precleaned 10-ml screwcap vial. The filtered supernatant was sampled and analyzed in the same manner as the diluted calibrating solutions. Soil extracts should be sampled and analyzed as soon as possible after preparation. If necessary, they should

be stored at room temperature, then discarded after 24 h.

2.11. Soil extraction procedure: analysis with mass spectrometric detection

Aliquots of Rocky Mountain Arsenal standard soil were fortified as described in Section 2.10. A 10-ml portion of extracting solution was *immediately* added to the precleaned vial, which was gently rocked back and forth by hand for 2 min. The samples were then centrifuged for 1 min using the instrument described. The supernates were filtered, collected, and analyzed in the same manner as the diluted calibration solutions. Total sample processing time was approximately 5 min per sample.

2.12. Calculations

The measured integrated peak area data from the flame photometric detector were fit to a calibration curve of the form $\ln A = \ln B + n \ln C$, where A is the measured integrated peak area, C is the analyte concentration in ng ml^{-1}, n is the slope of the line, and B is the intercept. (The nominal value of ''n'' is 2 for a flame photometric detector operated in its sulfur-selective mode [20]).

The measured integrated peak area data from the GC–MS-SIM analyses were fit to a linear calibration curve of the form $y = mx + b$, where y is the measured integrated peak area, x is the analyte concentration in ng ml^{-1}, m is the slope of the line, and b is the intercept.

3. Results and discussion

3.1. Optimization of the calibration procedure

One of the standard approaches for demonstrating that an analytical procedure is working properly is to add a ''model'' or ''surrogate'' compound to each sample prior to the initial preparation. PhAsO was a reasonable surrogate for CVAO because it contains the same organoarsenical moiety and is commercially available. The derivatives of PhAsO prepared using various dithiols, including EDT and PDT, have been reported and are well-characterized [4,21,22].

Several authors have successfully derivatized CVAO with both EDT and PDT [3,4,6,13]. However, Szostek and Aldstadt [4] noted that the purity of PDT was much greater than that of EDT. Hence, chromatograms of samples which had been reacted with PDT were always simpler and easier to interpret than those employing EDT. For that reason, the work described in this paper focused on PDT as the reagent of choice. Szostek and Aldstadt [4] also employed a derivatization reagent concentration of 1 μl per 2.5 ml extract (400 μl reagent l^{-1}). In our experience, both CVAO and PhAsO were successfully derivatized with 100 μl PDT l^{-1} with a concomitant reduction in the mass of reagent collected on the SPME fiber.

Szostek and Aldstadt [4] recommended the 100 μm thick polydimethylsiloxane fiber in part because of its ruggedness and reliability compared to the 85 μm thick polyacrylate and 65 μm thick poly(ethylene glycol)–poly(divinylbenzene) (i.e., Carbowax–DVB) fibers. Our experience agrees with these authors, in that we have obtained reliable extractions with individual fibers for at least 250 cycles of extraction and desorption. A 7 μm thick polydimethylsiloxane fiber was also evaluated; its sensitivity was considerably poorer than that of its 100 μm counterpart, as expected. On the other hand, those results suggested that the thinner coating would actually be preferred for samples which contained very high concentrations of derivatized CVAO. Such a sampling would be preferred to, for example, direct aqueous injection of the derivatized product for the following three reasons. First, potential degradation of the gas chromatographic bonded phases caused by exposure to the aqueous matrix would be avoided. Second, vapor-phase water is immiscible with the surface of any of the aforementioned bonded phases. For that reason, the chromatography of derivatized Lewisite oxide and phenylarsine oxide, i.e., peak shape and resolution from contaminant materials, would be adversely affected. Third, a direct aqueous injection would introduce unwanted contaminants from the soil into the injection sleeve. All three of these potential problems are avoided when SPME sampling, rather than direct aqueous injection, is employed.

The literature also varies widely on the properties of the soil extraction solution, which should also be

used as the calibration solution. Choices include water [13], 0.66% (w/v) ascorbic acid (pH 2.76) [13], hydrochloric acid (pH 2) [4], and pH 0.20 (2%, v/v) hydrochloric acid [12], each containing PDT. The work described in Section 3.2 demonstrated that 0.66% (w/v) ascorbic acid containing PDT was the preferred solution medium.

The optimized SPME equilibration time was determined by preparing fresh individual 10 ml solutions of extracting solution containing 100 ng ml^{-1} in each of CVAO and PhAsO, then sampling them with vigorous stirring for periods ranging between 1 and 40 min. The results, summarized in Fig. 3, demonstrated clearly that the SPME fiber was equilibrated with derivatized CVAO in approximately 20 min, but was never equilibrated with derivatized PhAsO. Because the focus of this work was the determination of CVAO alone, not CVAO and PhAsO, the optimized SPME sampling time was 20 min. The sampling time was carefully monitored to ensure reproducible sampling and collection of PhAsO.

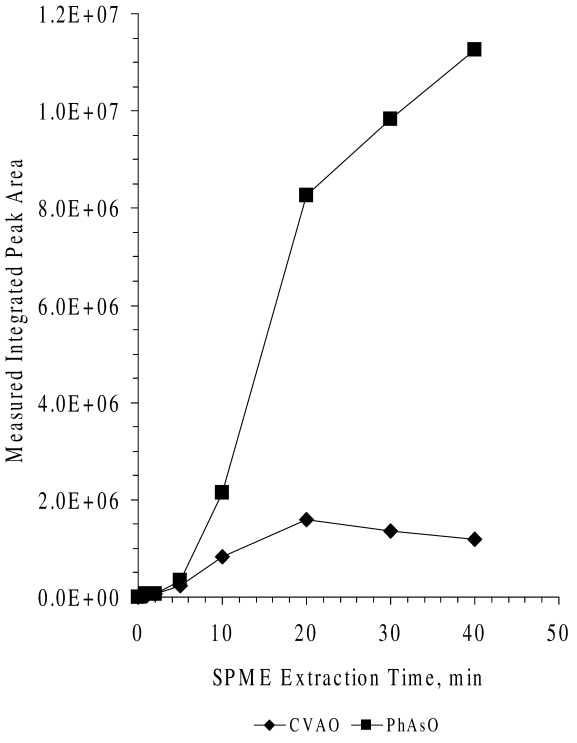

Fig. 3. Determination of the optimized SPME extraction time. Solution medium, 0.66% (w/v) ascorbic acid with 100 μl PDT l^{-1}. Test concentration, 100 ng each CVAO and PhAsO ml^{-1}. Other conditions described in the text.

3.2. Selection of the soil extraction solution

The optimization of the soil extraction procedure included evaluations of medium, time and condition. Extraction ''condition'' refers to a potential preference for ultrasonic extraction of sample and solution, rather than merely letting the sample sit undisturbed for 30 min, as described by Parks [3].

The choice of solvent medium was performed using a set of 2-g aliquots of Rocky Mountain Arsenal standard soil which had been fortified to 2.5 μg each CVAO and PhAsO g^{-1}. A given sample was extracted ultrasonically for either 5 or 60 min in 40-ml precleaned vials with 10 ml of water, hydrochloric acid (pH 2 or 0.2), or 0.66% (w/v) ascorbic acid. Following each extraction, the samples were centrifuged, then filtered through a 0.45-μm porosity nylon syringe filter. Each supernatant was fortified with 1 μl PDT, then subjected to SPME sampling and analysis, as described previously. The extracts containing only water did not clear easily upon centrifugation and could not pass through the nylon syringe filter. For that reason, water was immediately rejected as an extracting solvent.

When the standard soil sample was contacted with pH 0.20 hydrochloric acid, gas was generated immediately. This solvent was partially dissolving the soil sample – a potentially undesirable characteristic. No gas generation occurred when either the 0.66% (w/v) ascorbic acid or pH 2 hydrochloric acid extraction solvents were tested. All of the acidic extracts were easily centrifuged and passed readily through the nylon syringe filter. The solution pH was tested prior to SPME sampling, and was considerably greater compared to that immediately prior to extraction. The pH of the ascorbic acid solution, for example, rose from 2.76 to 6.6; that for water increased to 8.9. In general, the recovery of CVAO was greater at 5 min than at 60 min for all three acidic test mixtures. Furthermore, with 5 min extraction time, the recovery of CVAO declined in the following order: ascorbic acid (53%)>pH 2 hydrochloric acid (43%)>pH 0.20 hydrochloric acid (14%). For this reason, ascorbic acid was selected as the optimal

extraction medium. Because ascorbic acid is also a powerful antioxidant, its presence would also reduce the conversion of the As(III) of CVAO into an As(V)-containing moiety.

Additional work demonstrated that the extractions could be made more effective and convenient by adding the required volume of PDT to the solution prior to, rather than immediately after, extraction, as noted by Parks [3]. PhAsO was recovered at approximately 25% from both the ascorbic acid and pH 2 hydrochloric acid solutions after a 5-min extraction.

3.3. Optimization of the soil extraction time and method

A second set of 2-g soil aliquots of Rocky Mountain Arsenal standard soil in pre-cleaned 40-ml vials which had been fortified to 2.5 μg each CVAO and PhAsO g^{-1} were extracted using 10-ml portions of the optimized extracting solvent [0.66% (w/v) ascorbic acid containing 100 μl PDT l^{-1}] for periods ranging from 15 min to 6 h. Duplicate samples were extracted using either a water-cooled ultrasonic bath (nominal temperature was ambient) or a quiescent procedure, in which the samples were initially mixed gently by hand, then allowed to stand undisturbed on the laboratory benchtop. The latter were further mixed gently, end-over-end, by hand every 15–20 min until the selected extraction period was completed.

The results, summarized in Fig. 4, clearly demonstrated greater scatter when the ultrasonic bath was employed compared to the quiescent procedure. Furthermore, the quiescent procedure exhibited a modest linear dependence of recovery with time, as evidenced by a coefficient of determination, r^2, ~0.8, for each analyte. By contrast, the ultrasonic extraction procedure suggested only random scatter for each analyte (r^2~0.3). Even more significant, the sets of data for ultrasonic and quiescent extractions largely overlapped for both CVAO and PhAsO, thereby demonstrating that the ultrasonic bath did not offer a significant advantage compared to the quiescent extraction. Finally, the extraction profiles for both extraction procedures suggested a maximum recovery after approximately 30 min for both CVAO and PhAsO.

Overall, the optimized soil extraction conditions included a 30-min period during which the soil sample would be allowed to stand undisturbed on the laboratory benchtop, except for occasional gentle

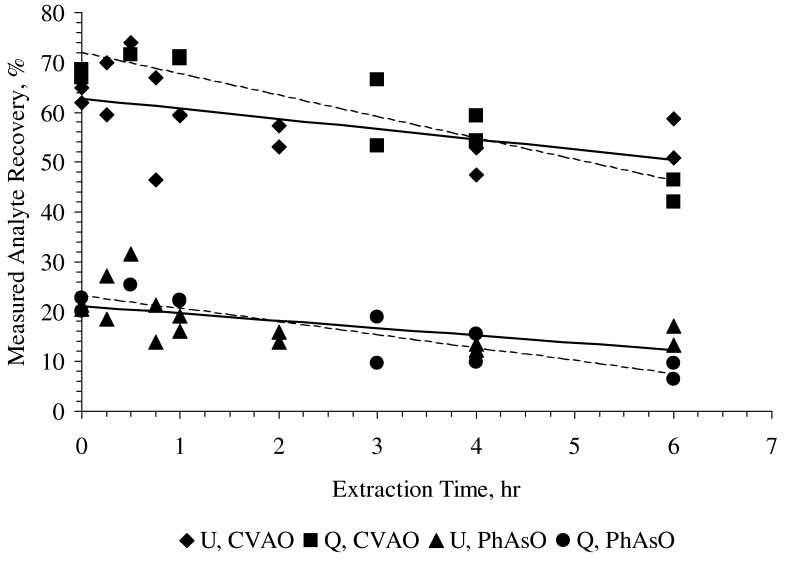

Fig. 4. Determination of the optimized soil extraction conditions. Solution medium, 0.66% (w/v) ascorbic acid with 100 μl PDT l^{-1}. Solid lines represent extractions in a water-cooled ultrasonic bath (U); dashed lines represent quiescent extractions (Q) performed on the laboratory benchtop.

mixing by hand. This approach was also adopted by Parks [3], who extracted Lewisite from soil using an extracting solution containing cyclohexane and PDT, after initially mixing the sample and solution vigorously and briefly using both a vortex mixer and a water-cooled ultrasonic bath. Because the observed recovery maximum was both small and poorly defined, we considered the possibility in the GC–MS-SIM method that shorter extraction (2 min) and centrifugation (1 min) times would produce satisfactory results.

3.4. Optimization of the spike residence time for CVAO and PhAsO on the soil surface

A third set of 2-g soil aliquots of Rocky Mountain Arsenal standard soil or clean washed laboratory sand in pre-cleaned 40-ml vials were initially spiked with 2.5 μg each CVAO and PhAsO g^{-1}. The ascorbic acid/PDT extraction solvent (10 ml) was not added until a ''spike residence time'' ranging from 0 (i.e., virtually no residence time whatsoever) to 180 min had elapsed. The soil samples were then extracted and processed as described in Sections 3.1 and 3.2. The extract concentrations in ng ml^{-1} of both CVAO and PhAsO were calculated and plotted as their natural logarithms against time, as shown in Fig. 5.

Fig. 5 clearly demonstrates that both soil type and analyte residence time on the soil surface are important variables which affect analyte recovery. Clean washed laboratory sand is a neutral medium; the pH of the ascorbic acid extracting solution did not vary from its original value of 2.8 throughout the 3-h test period. Furthermore, the recoveries of both CVAO (98%) and PhAsO (32%) were virtually invariant and residence time-independent when extracted from sand. On the other hand, when CVAO and PhAsO were extracted from Rocky Mountain Arsenal standard soil, there was a clear dependence of analyte recovery upon analyte residence time. The extraction solvent was partially neutralized, as noted previously. The greatest recoveries for CVAO and PhAsO, obtained at or near $t=0$ min, were 51% and 12%, respectively. Furthermore, the decrease in analyte recovery appeared to exhibit first-order degradation behavior; the coefficient of determination, r^2, exceeded 0.8 for both analytes. The half-lives for CVAO and PhAsO were estimated from the rate constant (slope of each regression line), and were 30 min and 20 min, respectively.

Because it may be inconvenient and/or inappro-

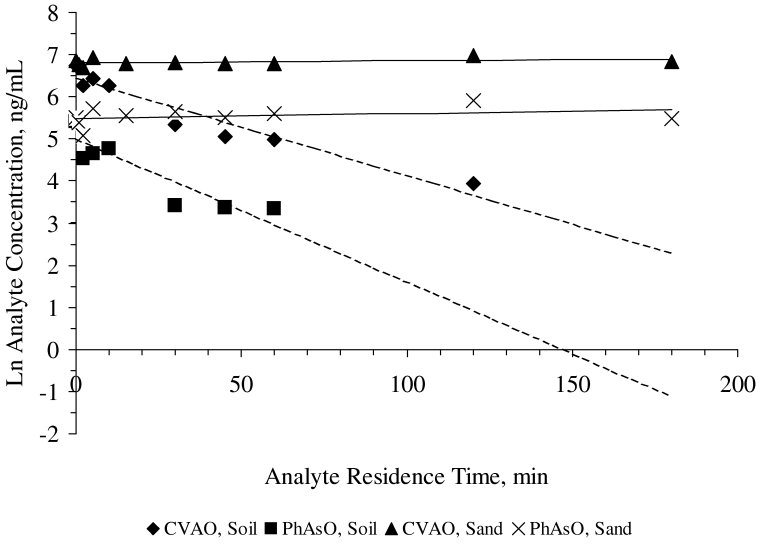

Fig. 5. Dependence of extraction recovery upon ''spike residence time'' for CVAO and PhAsO on the surface of Rocky Mountain Arsenal standard soil and clean washed laboratory sand. Solid lines represent extractions performed using clean washed laboratory sand; dashed lines represent extractions performed using Rocky Mountain Arsenal standard soil.

priate to determine whether a given soil sample is sandy, neutral or basic, the analytical chemist should proceed as though the sample were basic and resembled Rocky Mountain Arsenal standard soil. This assumption means that all soil extractions must begin not later than 5 min after spiking.

3.5. Summary and rationale for the optimized soil extraction conditions

The optimized soil extraction procedure requires that sample extractions begin immediately (<5 min) after spiking with PhAsO (surrogate) and/or CVAO with 10 ml 0.66% (w/v) ascorbic acid containing 100 μl PDT l^{-1}. The sample and extraction solvent are mixed gently by hand, then allowed to stand undisturbed for 30 min, during which time they may be mixed once more in the same manner. Afterwards, the extracts are centrifuged using a simple laboratory benchtop centrifuge and the supernatant filtered through a 0.45-μm porosity nylon filter attached to a 10-ml capacity syringe barrel. The filtered supernatant is then ready for SPME sampling, as described in Section 3.1.

These rather stringent extraction conditions may be explained by recognizing that Lewisite (and, probably, CVAO as well) will react with base to form acetylene and trisodium arsenate [2]. Hence, when CVAO is spiked onto a basic soil – and Rocky Mountain Arsenal standard soil is clearly basic – the analyte probably begins degrading immediately. For that reason, the contact time between CVAO and basic soil should be minimized. In addition, reactive soil particles will contact CVAO more frequently, and therefore promote degradation, during an ultrasonic bath extraction than a quiescent extraction. For that reason, a quiescent extraction would provide greater recovery and reproducibility than an ultrasonic bath extraction. The fundamental ground rules for method certification using the protocol developed by the US Army, discussed in Section 3.7 (below), did not allow for a change of soil matrix which would eliminate the problems caused by using a basic soil.

While the recovery of CVAO typically exceeded 75%, that for the candidate surrogate PhAsO usually ranged between 15 and 20%. These poor recoveries for PhAsO may be explained both by the degradation behavior on the soil surface and its poor solubility in the ascorbic acid solution. PhAsO is modestly soluble in methanol, but virtually insoluble in both acetonitrile and ascorbic acid solutions. By contrast, CVAO is modestly soluble in ascorbic acid solutions, but virtually insoluble in methanol and acetonitrile. Because the focus of this work is to extract and quantitate CVAO, the method conditions have been selected to optimize the recovery of CVAO and may not be optimized for PhAsO.

3.6. Explanation of the storage condition for derivatized extracts and the master calibration and spiking solutions

The storage of the derivatized soil extracts is complicated by the precipitation of a waxy-white solid, presumably CVAO- or PhAsO-related, upon storage at 4°C even after just a few days. For that reason, extract storage at room temperature is preferred. Even then, the concentration of derivatized CVAO and PhAsO appears to change after approximately 24 h, although no precipitate is observed. For that reason, the recommended storage conditions for derivatized extracts are 24 h at room temperature. Extract re-analysis past that time should commence with a freshly-spiked sample.

The ‘‘master’’ spiking and calibration solutions are inherently unstable, probably because of the formation of polymerized CVAO (Fig. 1), which is insoluble in water and precipitates. The loss of CVAO and PhAsO in these solutions is apparent when they are stored at room temperature even for as short a period as 48 h. For that reason, the ‘‘master’’ calibration and spiking solutions should be stored at 4°C and prepared fresh weekly. The ‘‘working’’ calibration solutions, containing 5–250 ng each CVAO and PhAsO ml^{-1}, should be prepared fresh daily and analyzed immediately [4].

The stability of the individual concentrated ‘‘master’’ stock solutions, like that of the ‘‘master’’ calibration and spiking solutions, is clearly of concern. The initial recommendation is to prepare fresh ‘‘master’’ stock solutions monthly in 0.66% (w/v) ascorbic acid, and to store them in a refrigerator maintained at 4°C.

Table 1
Comparison of ''found'' versus ''true'' concentrations of CVAO and PhAsO using the GC–FPD method (Rtx-35 analytical column) in method reporting limit (MRL) certification samples

''True'' concentration (μg g^{-1})	''Found'' CVAO (μg g^{-1})		''Found'' PhAsO (μg g^{-1})	
	Day 1	Day 2	Day 1	Day 2
0.00	0.00	0.00	0.00	0.00
0.05	0.03	0.03	0.00	0.00
0.10	0.07	0.07	0.02	0.00
0.20	0.14	0.13	0.03	0.04
0.50	0.36	0.28	0.06	0.06
1.00	0.69	0.81	0.08	0.22
2.00	1.33	1.63	0.19	0.41
4.00	T[a]	T	0.70	0.75
MRL (μg g^{-1})	0.20		1.12	
Estimated recovery (%)	75		18	

[a] Measured peak areas exhibited ''analog–digital conversion'' or integrator overrange errors, and were not used.

3.7. Method evaluation and determination of the method reporting limits

The performance of the proposed method was evaluated using two statistically-unbiased protocols, viz., those of the US Army Rocky Mountain Arsenal [23] and the US EPA [24], to determine the method reporting limit (MRL) and the method detection limit (MDL), respectively. The former is equivalent to determining a ''found'' concentration so that both the false positive and the false negative errors are both 5%, as discussed in Hubaux and Vos [25] and Grant et al. [26]. By contrast, the latter is the minimum concentration that can be measured and reported with 99% confidence that the analyte concentration is greater than zero [24].

The MRL was evaluated using a procedure established by the US Army [23] and discussed in detail elsewhere [27]. Briefly, portions of Rocky Mountain Arsenal standard soil are fortified with both CVAO and PhAsO to concentrations ranging between 0.05 and 5 μg g^{-1} in each compound, or 0.5–50 times the ''target reporting limit'' (TRL) of 0.1 μg g^{-1} (range of 0.5–20 times the TRL required). Samples were spiked, extracted, and analyzed as described above, and the resulting soil concentrations calculated using calibration data obtained on each of two method certification days. (An extra certification day may be

Table 2
Comparison of ''found'' versus ''true'' concentrations of CVAO and PhAsO using the GC–MS-SIM analytical method in method reporting limit (MRL) certification samples

''True'' concentration (μg g^{-1})	''Found'' CVAO (μg g^{-1})		''Found'' PhAsO (μg g^{-1})	
	Day 1	Day 2	Day 1	Day 2
0.00	0.011	0.023	0.008	0.010
0.05	0.034	0.041	0.012	0.013
0.10	0.060	0.065	0.015	0.017
0.20	0.14	0.14	0.026	0.024
0.50	0.48	0.45	0.11	0.10
1.00	0.88	0.90	0.22	0.19
2.00	1.75	1.48	0.45	0.40
MRL (μg g^{-1})	0.31		0.20	
Estimated recovery (%)	82		22	

needed to obtain consistent results because of the chemical reactivity of the soil matrix). The MRL values were calculated using the current software recommended by the Program Manager Rocky Mountain Arsenal [28].

This procedure was performed for both the ''primary'' (Rtx-5) and ''confirmatory'' (Rtx-35) analytical columns used with the GC–FPD method. The spiked (''true'') and analyzed (''found'') concentrations for CVAO and PhAsO obtained using the Rtx-35 analytical column employed in the GC–FPD method are given in Table 1. The slope of the calculated linear regression line representing the relationship between the ''found'' and ''true'' values may be taken as a measure of analyte recovery. The calculated MRL values using the ''primary'' analytical column for CVAO and PhAsO were 0.49 and 1.09 μg g^{-1}, with recovery values of 51% and 10%, respectively. The same figures of merit obtained using the ''confirmatory'' analytical column for the same analytes, as shown in Table 1, were 0.20 and 1.12 μg g^{-1}, with recovery values of 75 and 18%, respectively. Chromatograms obtained at a soil concentration of 0.5 μg g^{-1} in each analyte for both columns used in the GC–FPD analysis are presented in Fig. 6, and demonstrate excellent resolution between peaks from the derivatized analytes and the reagent.

A similar evaluation of the GC–MS-SIM method, employing the HP-5MS column, yielded the certification data presented in Table 2. The calculated MRL values for CVAO and PhAsO were 0.31 and 0.20 μg g^{-1} soil, with recovery values of 82% and 22%, respectively. The mass spectra of the PDT-derivatized CVAO and PhAsO and the respective ions used for quantitation and identification are shown in Figs. 7 and 8, respectively. These target ions were used to quantitate the derivatized species in a 2-g soil sample fortified to 1 μg g^{-1} in each analyte (Fig. 9B) and a matching aqueous standard containing each compound at 0.2 μg ml^{-1} (Fig. 9A). The two SIM chromatograms would appear identical if the recovery of each analyte were 100%.

3.8. Method evaluation and determination of the method detection limits

MDL values were calculated for both analytes using the two analytical columns in the GC–FPD method, as described in Ref. [24]. Two sets of 10 2-g soil samples (seven required) were independently fortified to 1.00 μg in each of CVAO and PhAsO g^{-1}, then processed as described above. The sample standard deviation was multiplied by 2.8210, which is the one-tailed ''Student's *t*'' value corresponding to nine degrees of freedom (df) and 99% confidence, to obtain the MDL. The resulting data are summarized in Table 3, and demonstrate typical calculated MDL values of 0.14–0.30 μg CVAO g^{-1} soil and 0.06–0.09 μg PhAsO g^{-1} soil.

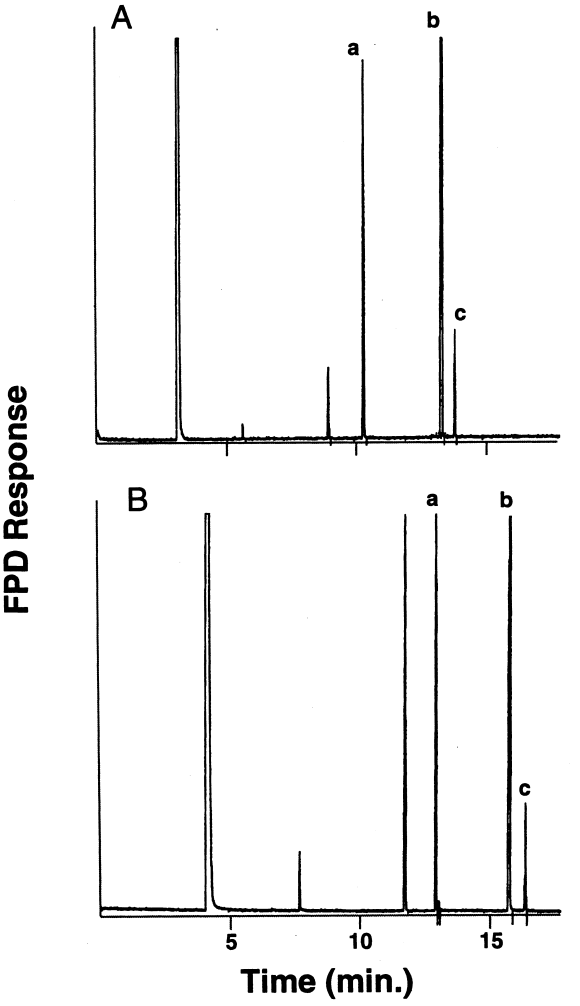

Fig. 6. Chromatograms of the derivatized extracts from soil samples fortified to 0.5 μg g^{-1} in each of CVAO and PhAsO obtained using the primary (A) and confirmatory (B) analytical columns in the GC–FPD method. Legend: (a) derivatized CVAO, (b) PDT contaminant, (c) derivatized PhAsO.

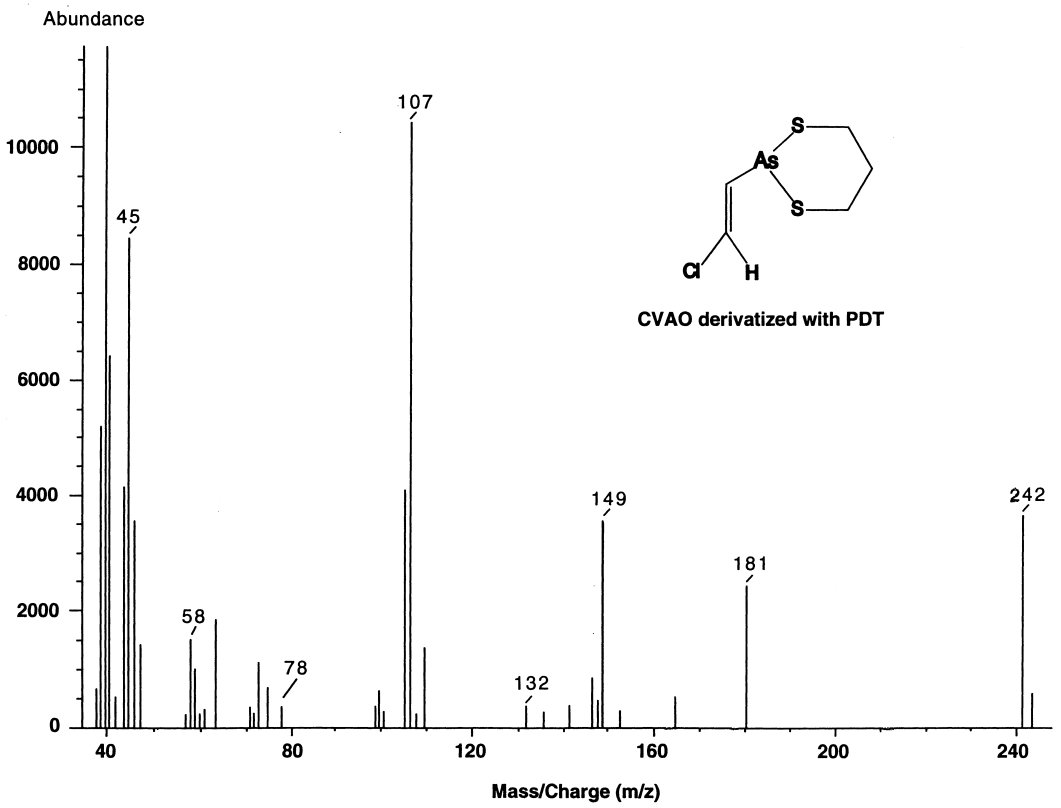

Fig. 7. Mass spectrum of PDT-derivatized CVAO. The ions used for SIM analysis are marked.

Table 3
Determination of the method detection limit (MDL) for CVAO and PhAsO using the analytical columns in the GC–FPD method[a]

Sample number	Measured CVAO ($\mu g\ g^{-1}$)		Measured PhAsO ($\mu g\ g^{-1}$)	
	"Primary"	"Confirmatory"	"Primary"	"Confirmatory"
1	0.65	0.51	0.10	0.11
2	0.85	0.57	0.14	0.14
3	0.83	0.61	0.12	0.14
4	0.89	0.63	0.13	0.15
5	0.90	0.62	0.14	0.16
6	0.95	0.61	0.17	0.14
7	0.91	0.63	0.13	0.16
8	0.93	0.63	0.16	0.16
9	1.07	0.69	0.13	0.18
10	0.95	0.68	0.22	0.15
Estimated recovery (%)	89.3	61.8	14.4	14.9
Experimental SD	0.11	0.05	0.03	0.02
Student's *t* table value[b]	2.8210	2.8210	2.8210	2.8210
MDL ($\mu g\ g^{-1}$)	0.30	0.14	0.09	0.06

[a] "True" concentration is 1.00 $\mu g\ g^{-1}$ soil for each analyte.
[b] One-tailed, 99% confidence, df=9.

Table 4
Determination of the method detection limit (MDL) for CVAO and PhAsO using the GC–MS-SIM method[a]

Sample number	Measured CVAO ($\mu g\ g^{-1}$)	Measured PhAsO ($\mu g\ g^{-1}$)
1	0.40	0.078
2	0.43	0.095
3	0.40	0.085
4	0.40	0.079
5	0.38	0.083
6	0.44	0.099
7	0.38	0.089
8	0.39	0.094
Experimental SD	0.022	0.008
Student's *t* table value[b]	2.998	2.998
MDL ($\mu g\ g^{-1}$)	0.066	0.023
Estimated recovery (%)	81	18

[a] "True" concentration is 0.50 $\mu g\ g^{-1}$ for each analyte.
[b] One-tailed, 99% confidence, df=7.

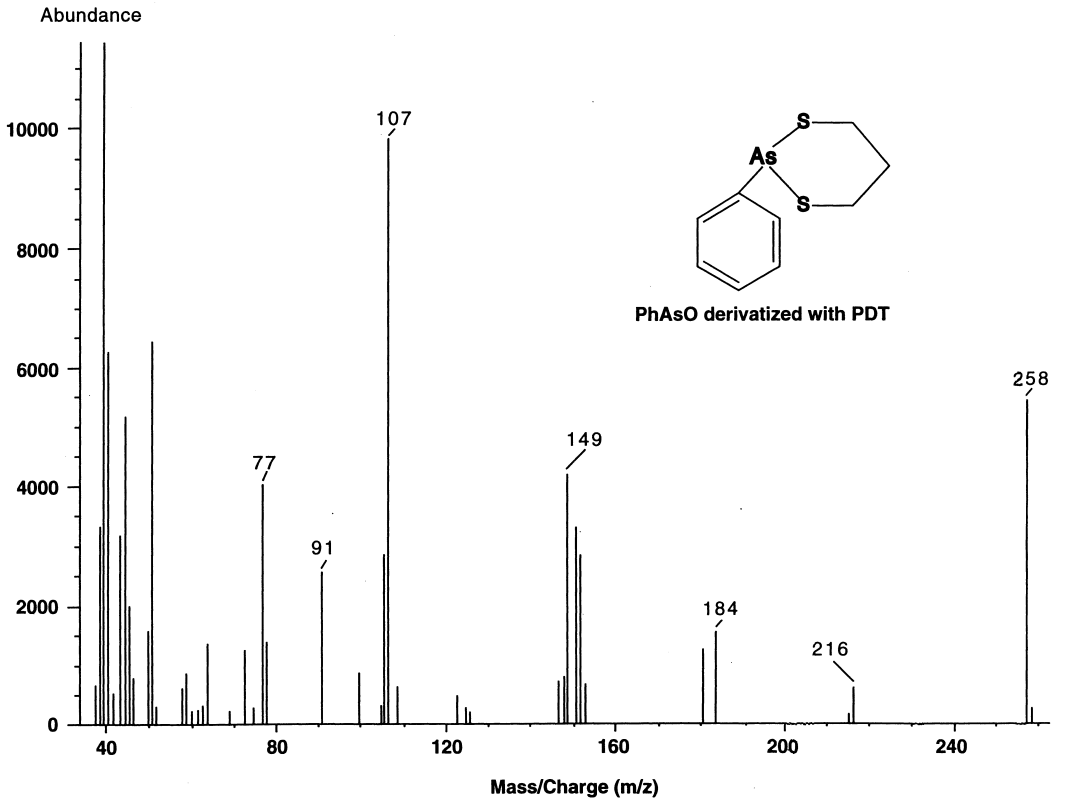

Fig. 8. Mass spectrum of PDT-derivatized PhAsO. The ions used for SIM analysis are marked.

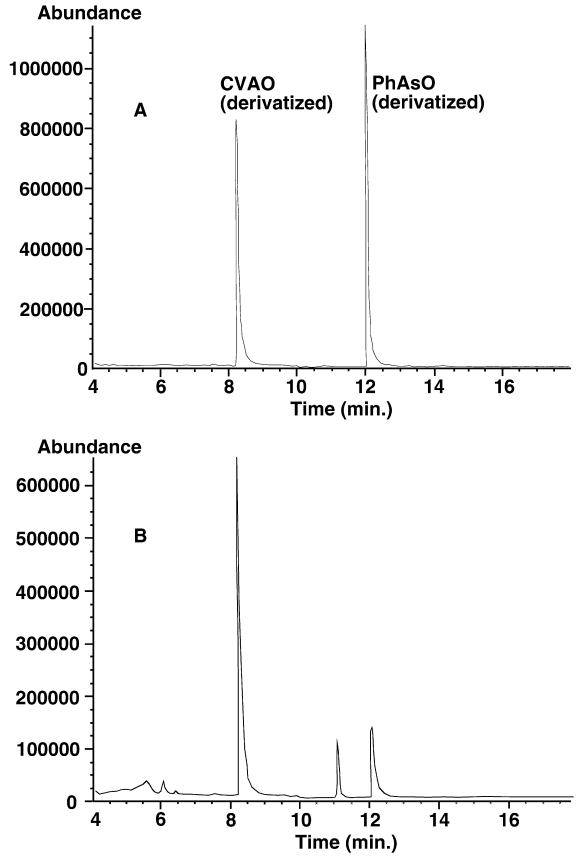

Fig. 9. GC–MS-SIM chromatograms of CVAO and PhAsO derivatized with PDT. (A) Aqueous standard containing 0.2 μg ml^{-1} of each analyte; (B) aqueous extract from a 2-g soil sample fortified to 1 μg g^{-1} soil in each analyte.

MDL values calculated for the GC–MS-SIM analytical procedure employed a single set of eight 2-g soil samples spiked with 0.5 μg g^{-1} soil in each of CVAO and PhAsO. In this case, the sample standard deviations were multiplied by 2.998, which is the one-tailed ''Student's *t*'' value corresponding to seven degrees of freedom and 99% confidence, to obtain the MDL. The resulting data, which are displayed in Table 4, exhibit comparable recovery values and improved precision compared to those shown in Table 3. The recovery for PhAsO, which remained below 20% for MDL calculations involving either the GC–FPD or GC–MS-SIM method, suggests that PhAsO be used only with caution as a surrogate compound, regardless of the outstanding MDL values.

4. Conclusions

Lewisite oxide may be extracted and simultaneously derivatized from neutral and highly basic soil samples using an extraction solvent containing ascorbic acid and PDT. After a brief extraction procedure, the derivatized product can be sampled from the filtered extract using an SPME fiber, desorbed into a gas chromatograph, and ultimately detected using either a flame photometric (sulfur-selective mode) or mass spectrometric (selected ion monitoring mode) detector. The detection limits for the procedure, calculated using two independent statistically-unbiased procedures, range between 0.1 and 0.5 μg CVAO g^{-1} soil, with a typical analyte recovery of 60%. Phenylarsine oxide has been proposed and evaluated as a potential surrogate compound; however, its poor recovery (<20%) make its usefulness questionable at best.

The typical sampling rate for the proposed method is 16 samples per 8-h working day, based on eight standards and eight unknown samples. For modest daily sampling loads (i.e., less than six per day), manual sampling and analysis may be entirely sufficient. If large daily sampling loads (i.e., greater than 12) are anticipated, the possibility of automated SPME sampling and injection using a commercially-available system [29,30] should be considered.

Note added in proof

The present work clearly demonstrated that the recovery of CVAO depends upon both the chemical characterstics of the soil matrix and the length of time that CVAO resides on the soil surface. Both of these observations were recently confirmed by Chaudot et al. [31], who employed both accelerated solvent extraction and supercritical fluid extraction of Lewisite oxide species from soil.

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