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Gas chromatographic determination of the lewisite hydrolysate, 2-chlorovinylarsonous acid, after derivatization with 1,2-ethanedithiol

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ABSTRACT

The primary hydrolysate of the toxic military agent lewisite (2-chlorovinylarsonous acid, or CVA) can be determined in trace concentrations by gas chromatography with flame-photometric detection after the CVA has been derivatized with 1,2-ethanedithiol to form a stable cyclic disulfide. The method has been shown to be applicable to the analysis of water samples that contain CVA in low ppb (10^9) concentrations. In addition, the method was demonstrated to be at least potentially useful for lewisite vapor determinations in air at sub-ppb levels in situations where any CVA found in the sampler can be assumed to have been formed from lewisite. Relative to the other procedures that are available for determining lewisite or CVA, this procedure is more sensitive, more specific for the analyte or simpler to perform.

INTRODUCTION

The high toxicity of lewisite (2-chlorovinyldichloroarsine) and its potential for use as a military chemical agent have prompted numerous attempts to develop analytical methods for determining this compound at trace levels in environmental matrices. However, lewisite's thermal lability and its nearly instant hydrolysis to the non-volatile 2-chlorovinylarsonous acid (CVA) on contact with moisture virtually preclude the use of gas chromatography for the direct determination of the agent [1].

The hydrolysis of lewisite is complex, involving several products that are in equilibrium with one another [2]. But CVA is always formed rapidly in the first step at pH levels above 1:

$$\underset{\text{(lewisite)}}{Cl\text{–}CH=CH\text{–}AsCl_2} + 2H_2O \overset{\text{(fast)}}{\rightleftharpoons} \underset{\text{(CVA)}}{Cl\text{–}CH=CH\text{–}As(OH)_2} + 2HCl \qquad (1)$$

$$Cl\text{–}CH=CH\text{–}As(OH)_2 \overset{\text{(slow)}}{\rightleftharpoons} H_2O + Cl\text{–}CH=CH\text{–}AsO \overset{\text{(slow)}}{\rightleftharpoons} (Cl\text{–}CH=CH\text{–}AsO)_3$$

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CVA is essentially non-volatile, and its occurrence has never been reported except as a hydrolysis product of lewisite. Indeed, lewisite almost certainly hydrolyzes to CVA rapidly after contract with the human body, so that many (if not most) of the toxic properties associated with lewisite can be presumed to be, in reality, those of CVA. Accordingly, efforts to develop analytical methods for determining "lewisite" are typically based on the detection of either intact lewisite or CVA, whichever is most appropriate to the circumstances at hand.

Waters and Williams [2] found that CVA would decompose in cold caustic alkali as follows:

$$Cl\text{–}CH=CH\text{–}As(OH)_2 + OH^- \rightarrow As(OH)_3 + Cl^- + C_2H_2 \qquad (2)$$

In cold solution (16°C), only alkaline conditions of pH 10.5 or greater would bring about this decomposition, but at 50°C, solutions of pH 9 or greater were effective. Prolonged boiling with water alone brings about some decomposition [2].

Thus, one of the most sensitive and specific methods now available for determining lewisite is an indirect approach based on the gas chromatographic (GC) determination of the acetylene that forms during the alkaline decomposition of CVA [1,3]. However, we have found that quantitative recovery and introduction of the liberated acetylene into a gas chromatograph is difficult. Moreover, the method is not useful for any samples that contain acetylene as a background constituent.

A more rugged and reliable method for determining lewisite is based on the reversed-phase high-performance liquid chromatographic (HPLC) determination of CVA with either electrochemical or ultraviolet (UV) spectrometric detection at 225 nm [4]. The detection limit of this method for CVA, based on a signal-to-noise ratio of 3 ($S/N = 3$), is approximately 2 ng in an injected 100-μl water sample (20 ng/ml) when UV detection is used.

Other less specific methods have been used to determine lewisite and CVA. For example, graphite-furnace atomic absorption spectrometry (GFAAS) provides a sensitive response to decomposed CVA [5], and molybdenum-blue spectrophotometry accurately quantifies the arsenite ion (as arsenate) that is released in the alkaline decomposition of CVA according to reaction 2 above [6]. But these techniques are not applicable to trace determinations when interfering substances are present.

Eagle and Doak [7] reviewed the large amount of work on thioarsenites derived from the reaction of alkyl arsonous oxides and alkyl mercaptan. This reaction is given by reaction 3:

$$RAsO + 2R'SH \rightleftharpoons RAs(SR')_2 + H_2O \qquad (3)$$

The analytical method reported here is an indirect GC determination of CVA based on its reaction with 1,2-ethanedithiol (EDT) to form the more stable and volatile cyclic disulfide, 2-(2-chlorovinyl)-1,3,2-dithiarsenoline (CDA) [8]:

$$\underset{\text{(CVA)}}{Cl\text{–}CH=CH\text{–}As(OH)_2} + \underset{\text{(EDT)}}{HS\text{–}CH_2\text{–}CH_2\text{–}SH} \rightarrow$$

$$\underset{\text{(CDA)}}{Cl\text{–}CH=CH\text{–}As\text{–}S\text{–}CH_2\text{–}CH_2\text{–}S} + 2H_2O \qquad (4)$$

A sulfur-specific flame-photometric detector (FPD) is used to provide a sensitive and selective response to CDA. The detection limit of the method —about 5.5 ng of CVA per milliliter of aqueous sample (based on $S/N = 3$)— is about fourfold lower than that given for the HPLC method. However, the detection limit undoubtedly could be lowered significantly in situations where some loss of precision and accuracy can be tolerated.

EXPERIMENTAL

Neat liquid lewisite was supplied by the US Army as Lot No. L-U-6206-CTF-N; the purity of this material was given as 95.5 wt%. A stock standard solution of lewisite in cyclohexane or isopropanol was prepared by weighing to the nearest 0.1 mg about 40 mg of lewisite in a tared 50-ml volumetric flask and diluting to the mark with the appropriate solvent. Working standard solutions were then prepared as needed by serial dilution from the stock standard solution.

A water sample containing CVA to be used either for calibration of the GC instrument or for test purposes was prepared by injecting a few microliters of a cyclohexane or isopropanol solution of lewisite into a 5.0-ml aliquot of deionized water and agitating the resulting mixture for 15 s on a vortex mixer. During this operation, the mixture was contained in a 125 × 16 mm I.D. glass screw-capped culture tube with a PTFE liner in the cap.

A typical 5.0-ml water sample in a culture tube, fabricated as described above, was treated for analysis as follows. A 0.8-μl aliquot of neat liquid EDT (Aldrich, Milwakee, WI, USA), the purity of which was stated as 99 wt%, was first added to the sample in a fume hood, and the solution was then agitated for 15 s on a vortex mixer. After a 1.0-min waiting period to ensure that the EDT–CVA reaction had gone to completion, a 1.0-ml portion of a 2.0-mg/ml aqueous soltion of $AgNO_3$ (Morton Thiokol, Danvers, MA, USA) was pipetted into the sample solution to precipitate most of the remaining excess EDT. This was followed by another 15-s vortex-mixing step.

Next, a 1.0-ml aliquot of toluene was added to the sample for extraction of the CDA. This mixture was vortex-mixed for 30 s and allowed to equilibrate for an additional 1 min. It was then centrifuged for 2 min in a desk-top centrifuge to separate the aqueous and toluene layers and to settle out a greenish precipitate formed by the reaction between $AgNO_3$ and EDT. Amounts (μl) of the toluene layer were withdrawn by syringe and injected into the GC instrument to carry out the analysis step. The GC conditions are summarized in Table I.

A sample extract prepared in the above manner was analyzed for CDA by injection into a VG 70S GC–mass spectrometry (MS) system. The Hewlett-Packard 5890 GC that was interfaced to the mass spectrometer was equipped with a 25 m × 0.32 mm I.D. DB-5 fused-silica capillary column bearing a 0.52-μm-thick coating of the stationary phase. The injection port and transfer line were both maintained at 150°C. The column temperature was held at 45°C for 3 min, then programmed at 8°C/min to 300°C, where it was held for up to 30 min. The carrier gas (helium) flow-rate was approximately 1 ml/min.

The mass spectrometer was operated in the electron-impact mode at 30 eV. The ion source was maintained at 200°C, and the instrument was set to provide a resolution

TABLE I

GAS CHROMATOGRAPHIC INSTRUMENTAL CONDITIONS FOR DETERMINATION OF LEWISITE AFTER ITS CONVERSION TO CDA

Instrument	Hewlett-Packard Model 5890, series II
Detector	Flame-photometric detector in the sulfur-specific mode
Column	30 m × 0.53 mm I.D. DB-5 fused-silica capillary column with a 1.5-μm-thick coating of the stationary phase
Temperatures	
Column oven	150°C for 6 min, then ramp at 60°C/min to 300°C, then hold for 3 min
Injection port	225°C
Detector	225°C
Gas flow-rates	
Carrier gas (He)	17 ml/min
Air	97 ml/min
Hydrogen	74 ml/min
Integrator/recorder chart speed	0.5, 1.0 cm/min

of 5000 (based on a 2% valley), although a problem with instrument stability precluded the full attainment of this resolution. Mass spectra were obtained by scanning from *m/z* 700 to *m/z* 35 at 1 s/decade with a 0.30-s interscan time. Sample injection volume was 0.3 μl. The original 5-ml water sample had been fortified with 455 μg of lewisite.

To test the proposed method for possible use in determining lewisite vapor collected from air, a four-day test was conducted involving the use of glass impingers (*i.e.*, bubblers) as sampling devices. The reservoir of each bubbler was filled with 5-mm-O.D. glass beads to facilitate mixing of the entrained air with the liquid sample-collection medium. With the glass beads in place, each bubbler reservoir held 15 ml of liquid collection medium.

On each of four days, therefore, each of twelve glass bubblers was charged with 15 ml of deionized water containing 100 mg of ascorbic acid to neutralize any oxidative species that could otherwise interfere with the analyses. The water aliquots had previously been fortified with lewisite to produce duplicate test solutions containing CVA at each of the following six concentrations: 0 (blank), 11.8, 59.1, 89.5, 119 and 178 ng/ml.

Each day, the charged bubblers were immersed in an aqueous ice bath, connected to a suction sampling pump, and permitted to sample room air (free of lewisite) for 12 h at a rate of 1.0 l/min, for a total of 720 l of air. After the sampling period, the bubblers were removed from the ice bath and allowed to equilibrate to room temperature before proceeding with the analysis step. Where necessary, aliquots of bubbler fluid were diluted back to their original volume with deionized water to compensate for evaporative losses during sampling; such corrections were invariably less than 1 ml per bubbler. A 5.0-ml portion of each bubbler aliquot was then taken for analysis by the procedure described above.

During this test, the GC was calibrated daily by analyzing one aqueous lewisite solution at each of the six test concentrations given above and by then performing a linear-regression analysis of the resulting response data. A 2.0-μl sample-injection volume was used throughout the test.

In all of the work reported here, all reagents and solvents were of reagent grade except as otherwise noted.

RESULTS AND DISCUSSION

One of the first tasks in the development of this method was to confirm the presence, identity and elution time of CDA in an extract of a typical lewisite-containing sample that had been treated with EDT as described here. This was accomplished by analyzing a sample extract by GC/MS. The resulting total-ion chromatogram is shown in Fig. 1, where the peaks that appear to be due to the *cis* and *trans* isomers of CDA are identified. The peak at scan No. 541 appears to be the cyclic dimer of EDT ($C_4H_8S_4$).

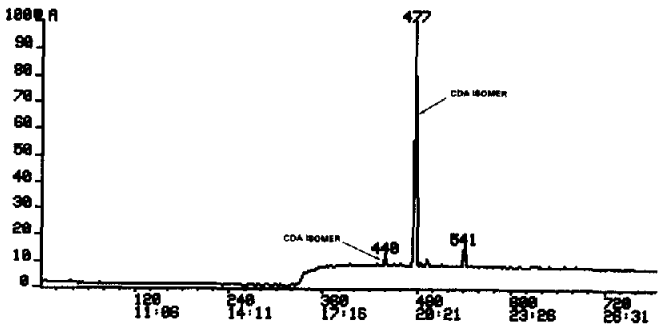

Fig. 1. Total-ion chromatogram for a water-sample extract containing CDA. Ordinate: instrumental response (arbitrary units). Abscissa: upper scale, scan No.; lower scale, time in min:s.

The mass spectra recorded at scan Nos. 440 and 477 (in Fig. 1) are presented in Fig. 2. It is to be noted that the major mass fragments in these spectra are consistent with the postulated fragmentation pattern of Fig. 3. (Although Fig. 3 depicts the *cis* isomer, essentially the same pattern is predicted for either isomer, and there appears to be no basis for identifying the isomers unambiguously from these data.) Further support for the assignments was obtained by checking the isotope peak ratios for conformity with the proposed elemental compositions of the principal ions, as synopsized in Table II.

The GC–MS retention times for the CDA isomers (*ca.* 19–21 min) were much too long for routine GC analyses; accordingly, GC conditions (Table I) were chosen to reduce the CDA retention times to less than about 5 min. This approach also had the desirable effect of merging the *cis* and *trans* isomers into a single peak so that both forms were quantified together. A typical chromatogram from the GC analysis of a lewisite-fortified water sample under the conditions of Table I is shown in Fig. 4. All of the peaks in Fig. 4 except the CDA peak were present in chromatograms obtained from blank (*i.e.*, lewisite-free) water samples. None of these extraneous peaks were identified.

The chromatographic response to residual EDT reagent (Fig. 4) was always very broad, so that the tail of the EDT peak tended to run under the CDA peak. That is, the chromatographic base line beneath the CDA peak was significantly elevated by the tail of the EDT peak. Because the sulfur-specific FPD does not respond linearly to sulfur

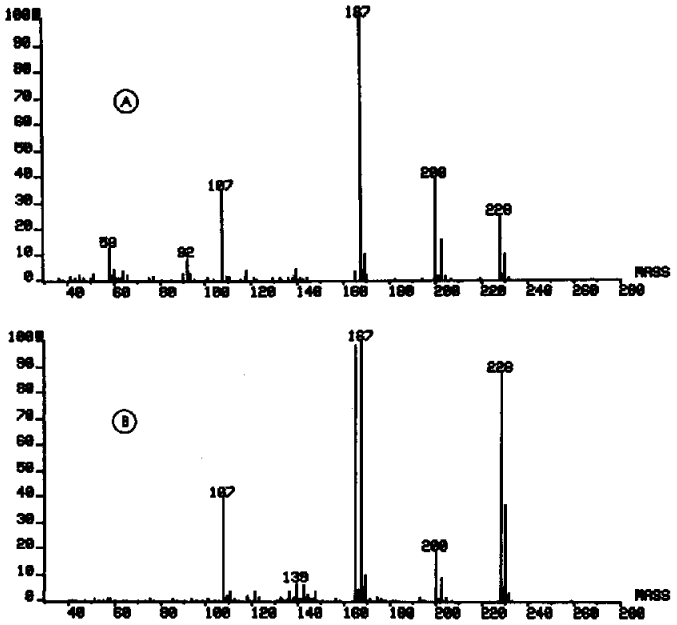

Fig. 2. Mass spectra of sample components corresponding to (A) scan No. 440 and (B) scan No. 477 in the chromatogram of Fig. 1.

compounds, the deconvolution of the CDA response from the EDT response is not a simple subtraction, as would be the case for a linear detector.

Consequently, the CDA peaks must be quantified by computing, for each CDA peak, the quantity $Z = (H_t)^{\frac{1}{2}} - (H_e)^{\frac{1}{2}}$, where H_e is the height of the response or upward baseline displacement due to EDT at the retention time of CDA and H_t is the sum of H_e and the height of the CDA peak above the EDT peak tail. Thus, the analyst must estimate the location of the true chromatographic baseline beneath the CDA peak in each chromatogram. Values of Z are directly proportional to the CDA concentrations

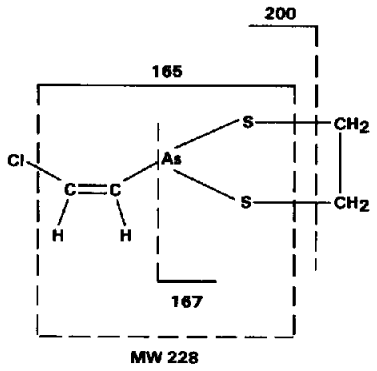

Fig. 3. Mass-spectral fragmentation of CDA. MW = Molecular weight.

TABLE II

ISOTOPE RATIO MEASUREMENTS FOR CDA[a]

Nominal mass	Proposed formulation	Relative intensity	
		Calculated	Found
228	$C_4H_6AsClS_2$	100.00	100.00
229		4.94	5.80
230		40.92	41.5
231		1.83	2.55
232		3.06	3.09
233		0.14	0.092
200	$C_2H_2AsClS_2$	100.00	100.00
201		2.24	3.67
202		40.85	46.6
203		0.92	0.787
204		3.04	5.83
167	$C_2H_4AsS_2$	100.00	100.00
168		2.24	5.16
169		8.87	9.52
170		0.20	0.21
165	$C_2H_2AsS_2$	100.00	100.00
166		2.24	3.94
167		8.87	(102)[b]

[a] These measurements were taken from the chromatographic peak appearing at scan No. 477 in Fig. 1.
[b] The value in parentheses is high because of interference from other ions.

in the solutions from which they are derived, and only when this procedure is used are data linearity and reproducibility likely to be acceptable at low CVA concentrations. Note also that the tail of the EDT peak probably enhances the instrumental detection limit for CDA to some degree because of the non-linear detector response.

The reaction times for the CVA–EDT reaction and the EDT–$AgNO_3$ reaction were experimentally optimized. For the CVA–EDT reaction, a reaction period of 1 min yielded essentially the same response to spiked lewisite as did reaction periods of 7, 15 and 30 min. Thus, the time allotted for this reaction need not exceed 1 min. The EDT–$AgNO_3$ reaction was optimized by visual observation, since this reaction produces a visible precipitate. Because no further precipitation was observed after the addition of the $AgNO_3$ and the completion of the ensuing 15-s vortex-mixing step (described above), no additional reaction time beyond the mixing step is required.

We found that if a precipitate was not formed on addition of $AgNO_3$ to the sample mixture, then there was not enough EDT in the solution, and neat liquid EDT was therefore added in 0.2-μl increments (with vortex-mixing between increments) until the precipitate was observed. Only when a net stoichiometric excess of EDT was carried into the extraction step was a linear response to the CDA obtained from GC. Furthermore, the precipitate had to be carefully excluded from the syringe whenever an aliquot of the toluene layer was taken for analysis. Otherwise, the resulting response to CDA was found to be excessively variable. This operation was greatly facilitated by

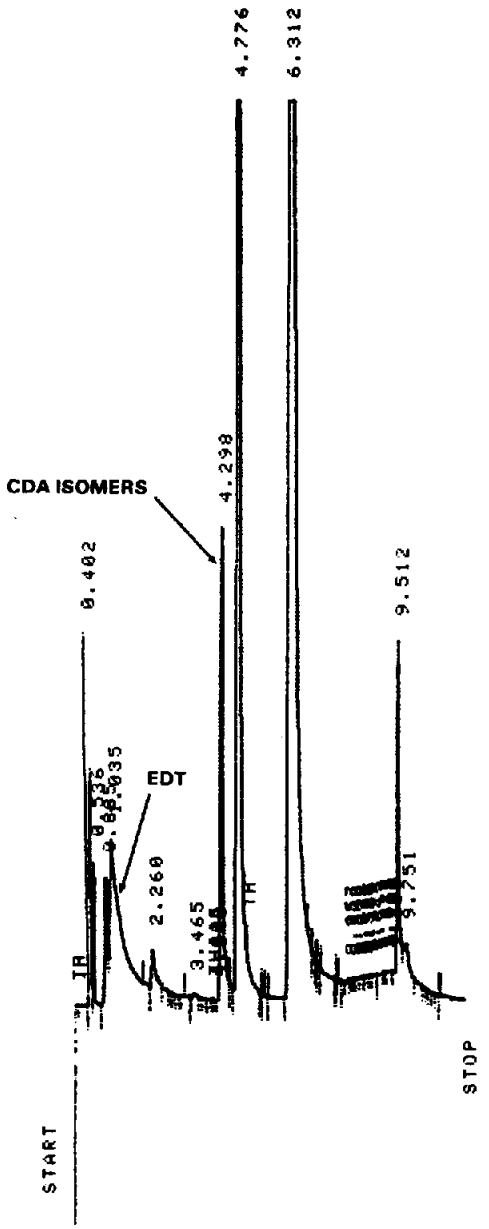

Fig. 4. Typical chromatogram from the GC–FPD analysis of the extract of a lewisite-fortified water sample after conversion of the lewisite to CDA. The lewisite concentration in the water sample was 726 ng/ml. Retention times (in min) are displayed in the chromatogram.

the prior centrifuging step, although the precipitate eventually settled spontaneously if allowed to stand for a day or so.

The efficiency with which the CDA is extracted by toluene was also estimated.

Thus, three 5.0-ml water samples were each spiked with 3.63 μg of lewisite, treated with EDT and $AgNO_3$ as detailed above, combined with 3.0 ml of toluene, and analyzed in the usual way. At the same time, another set of three water samples was treated identically except the volume of toluene was only 1.0 ml. An algebraic evaluation of the resulting GC responses indicated CDA recoveries of 96% for 3.0 ml of toluene and 90% for 1.0 ml of toluene. That the CDA extraction efficiency obtainable with 1.0 ml of toluene is at least 90% was also confirmed by extracting a spiked water sample with two successive 1.0-ml aliquots of toluene and analyzing both extracts for CDA.

In another test, we injected 1-, 2-, 3-, and 4-μl amounts of a CDA-containing toluene extract into the GC system to ascertain the effect of injection volume on the response to CDA. The CDA response per unit volume of injected sample extract was essentially constant over this volume range. Hence, a volume of at least 4 μl could be used if necessary to enhance the method detection limit.

A set of water samples that had been fortified with lewisite at different levels was analyzed by the method, and the resulting response data (Z values) were subjected to linear-regression analysis to obtain an expression for response as a function of CVA concentration. These data were produced by fortifying 5.0-ml aliquots of distilled water with varying amounts of lewisite (including zero lewisite, for use as a blank) in the manner detailed previously. The resulting samples were then analyzed by the recommended procedure. A 1.4-μl injection volume into the GC instrument was used, and all Z values were normalized to a single GC attenuation setting. The non-zero CVA solution concentrations were 28.4, 58.3, 75.6, 89.5, 120, 150 and 298 ng/ml. Moreover, the toluene extract of the sample with a CVA concentration of 120 ng/ml was analyzed eight times over a 3-h period.

The resulting least-squares slope, Y-intercept, and correlation coefficient of the curve were, respectively, 0.0202 $mm^{\frac{1}{2}}$ml/ng, -0.083 $mm^{\frac{1}{2}}$ and 0.99586, where Y was the corrected instrument response (Z value) in units of $mm^{\frac{1}{2}}$. The regression analysis included all eight measurements at 120 ng/ml; the relative standard deviation (R.S.D.) of these replicate measurements was 6.2%. In addition, the sample with the lowest non-zero CVA concentration in the group, *i.e.*, 28.4 ng/ml, yielded an S/N of approximately 10. From this information, the detection limit for CVA in water was computed by extrapolation to $S/N = 3$ (assuming a qadratic response characteristic for the FPD) and found to be 15.6 ng/ml for a 1.4-μl injection volume, or 5.5 ng/ml for a 4.0-μl injection volume. The latter detection limit, when expressed in terms of lewisite rather than CVA, is 6.7 ng/ml.

Fig. 5 displays a chromatogram from the analysis of a water sample that had been spiked with lewisite to produce a 12.3-ng/ml aqueous CVA solution. The analysis was conducted under the conditions of Table I, and a 2.4-μl injection volume was employed. This figure illustrates the low detection limit of the method, as well as the need for correction of the signal for baseline displacement by EDT when the CVA concentration is low.

The results of simulating four days of air sampling with lewisite-fortified bubbler samplers are summarized in Table III. The lewisite recoveries at the lowest non-zero concentration exhibited a consistent positive bias of from 20 to 60%, whereas a generally smaller negative bias occurred at the other non-zero concentrations. The cause of the positive bias at low levels is not known; perhaps the ascorbic acid (which was present in the bubbler fluid but not in the calibration standards) played a role. But

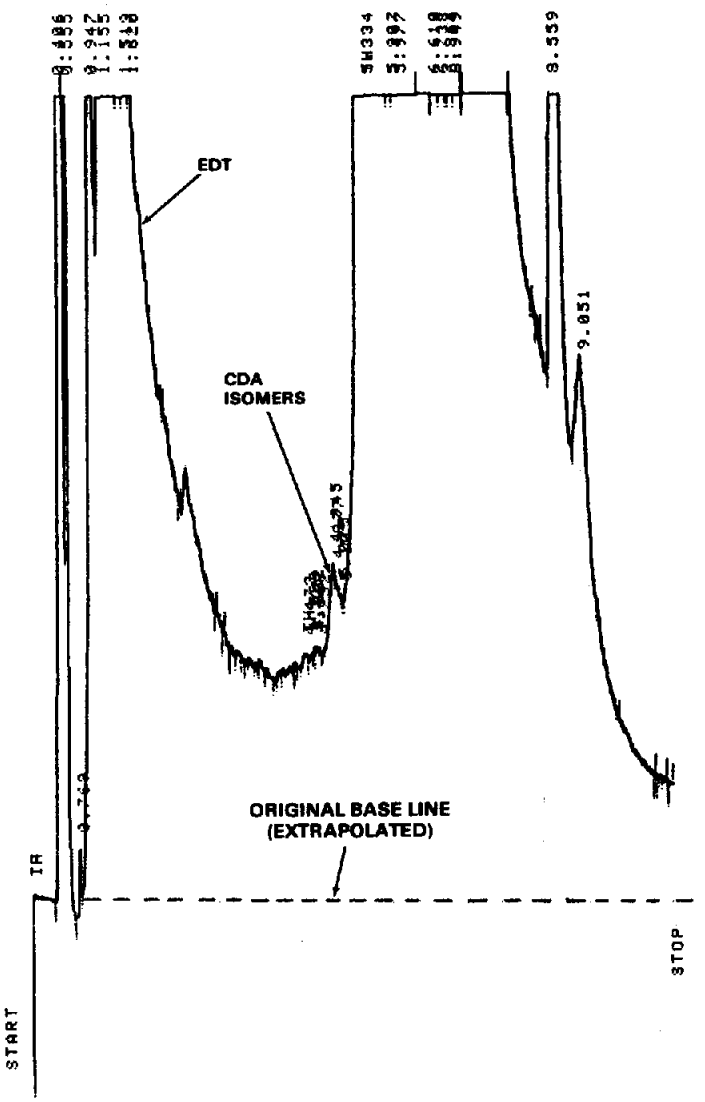

Fig. 5. Chromatogram from the analysis of a water sample that had been fortified with lewisite to produce a CVA concentration of 12.3 ng/ml. The response to the EDT derivative of CVA (*i.e.*, CDA) is indicated in the figure.

note that, because an air volume of 720 l was sampled through each bubbler, the lowest non-zero CVA concentration corresponded to the sampling of lewisite vapor at an average concentration of 0.3 ng/l, or about 35 ppt (10^{12}). For most applications at this rather low concentration level, the observed biases, although quite significant, are likely to be acceptable. Hence, the method appears to have the potential for use in the determination of lewisite vapor at trace concentration levels in air.

TABLE III

RECOVERIES OF ADDED LEWISITE FROM BUBBLERS AFTER DRAWING AIR THROUGH THE BUBBLERS

CVA solution concentration[a] (ng/ml)	Lewisite vapor concentration[b] (ng/l)	Lewisite recovery (%)			
		Day			
		1	2	3	4
0	0	–	–	–	–
0	0	–	–	–	–[c]
11.8	0.30	160	130	140	130
11.8	0.30	150	150	130	120
59.1	1.50	102	82	76	94
59.1	1.50	94	80	80	94
89.5	2.28	100	83	71	97
89.5	2.28	99	92	86	96
119	3.03	105	94	89	91
119	3.03	93	76	85	90
178	4.53	94	95	81	97
178	4.53	93	94	81	93

[a] CVA concentrations in the sample-collection media of the various bubblers.

[b] Lewisite vapor concentrations in air that would have been required in order to have collected the amounts of CVA that were actually present in the bubblers.

[c] The second blank determination on day 4 yielded a very slight non-zero response that corresponded to a CVA solution concentration of about 3 ng/ml. Note, however, that this is well below the estimated detection limit of the method.

The above study was first attempted without the use of ascorbic acid in the bubblers. But the lewisite recoveries in this case were exceedingly low (*ca.* 50%) and variable (*ca.* 25% R.S.D.). Thus, the data demonstrate that the presence of ascorbic acid in the sample-collection fluid does, indeed, improve the recovery of lewisite under these conditions, ostensibly by neutralizing an oxidizing interferant in the atmosphere.

Finally, it should be noted that the excess EDT that remains unneutralized by $AgNO_3$ going into the toluene extraction step eventually fouls the GC syringe after several hours of continuous use, causing a decrease in the GC response to CDA. But the effectiveness of the syringe can be restored by cleaning it thoroughly in a commercial acidic syringe-cleaning solution.

CONCLUSIONS

It was concluded that the method reported here is capable of determining CVA in water with high sensitivity, high specificity and adequate accuracy for most applications. An additional benefit of the method is the relatively low level of operator expertise required to use it effectively. Moreover, because of the facile hydrolysis of lewisite to the non-volatile CVA, air sampling and analysis for lewisite vapor should be possible with the use of an impinger sampler (or bubbler) charged with an aqueous sampling medium.

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