The Determination of Degradation Products of Lewisite and/or Mustard Gas in Water by High Performance Liquid Chromatography

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ABSTRACT

Lewisite (L) and mustard gas (HD) are highly toxic vesicant warfare agents that are very sparingly soluble in water and thereby converted quantitatively to the stable and soluble degradation products 2-chlorovinylarsonous acid (CVAA), 2-chloro vinylarsonic acid (CVAOA), 2,2′-dihydroxyethyl sulphide and 2,2′-dichlorodiethyl sulphoxide. A new method based on reversed-phase high performance liquid chromatography (RP-HPLC) has been developed for the simultaneous detection of CVAOA, CVAA, 2,2′-dichlorodiethyl sulphoxide, 2,2′-dihydroxyethyl sulphide. The effects of eluent and pH on the separation efficiency were studied. UV spectra of CVAOA, CVAA, 2,2′-dichlorodiethyl sulphoxide and 2,2′-dihydroxyethyl sulphide were recorded. Good separation was achieved by HPLC using a 250×4.6 mm column with 5 μm ODS C18 after optimization of all relevant parameters. The calibration curves of CVAOA, CVAA, 2,2′-dichlorodiethyl sulphoxide and 2,2′-dihydroxyethyl sulphide showed high linearity over a concentration range of 5–500, 2–500, 5–500, 50–1000 mg L^{-1}, respectively. The detection limits at a signal-to-noise ratio of 3 were 0.001, 0.2, 2, 20 mg L^{-1}. The method may be beneficial for studying the distribution of lewisite-and/or mustard gas and their degradation products in the environment.

KEYWORDS

High performance liquid chromatography, 2-chlorovinylarsonic acid, 2-chlorovinylarsonous acid, 2,2′-dichlorodiethyl sulphoxide, 2,2′-dihydroxyethyl sulphide.

1. Introduction

Lewisite and mustard gas are highly toxic vesicant warfare agents, that are very sparingly soluble in water and hereby converted quantitatively to stable and soluble degradation products. Lewisite is hydrolysed to 2-chlorovinylarsonous acid (CVAA), which is the hydrated form of 2-chlorovinylarsenious oxide (CVAO) that exists only in aqueous solution. It can be oxidized to 2-chlorovinylarsonic acid (CVAOA)[1]. These derivatives of lewisite are all potent vesicant agents. Mustard gas is hydrolysed to harmless 2,2′-dihydroxyethyl sulphide or oxidized to 2,2′-dichlorodiethyl sulphoxide, itself a potent vesicant. These metabolites are more likely to be encountered in the natural environment than lewisite and mustard gas themselves. Because CVAOA, CVAA and 2,2′-dichlorodiethyl sulphoxide are all potent vesicants, they should be part of any environmental screening protocols for lewisite and mustard gas.

Lewisite and mustard gas are often used together and therefore their derivatives are also often found together. A suitable method for the simultaneous and quantitative analysis of CVAOA, CVAA, 2,2′-dichlorodiethyl sulphoxide and 2,2′-dihydroxyethyl sulphide does currently not exist.

Previously used methods for the detection of lewisite and mustard gas and their derivatives[2–5] include thin layer chromatography (TLC), capillary electrophoresis and NMR spectroscopy[6,7]. But these methods did not give good results. Gas chromatography (GC) was efficient in the separation of mustard gas and its decomposition products after derivatisation[8–12,] but was ineffective in separating L from its degradation products

because they are derivatized to the same compound[13–16]. Furthermore, the water sample containing the analytes used for GC analysis must be extracted with a volatile solvent prior to injection and analysis.

HPLC has been introduced in many areas from 1960 onwards. In the 1980s, P.C. Bossle used reversed-phase high performance liquid chromatography to determine CVAOA[17] and CVAA[18], respectively, with detection limits of 0.2 mg L^{-1}. W.R. Creasy used postcolumn derivatization liquid chromatography/mass spectrometry for the detection of lewisite and CVAA with 2-mercaptopyridine[19]. This led to a significant improvement in sensitivity when air pressure chemical ionization (APCI) or electrospray ionization (ESI) was used for detection.

This laboratory has now developed a new method for the simultaneous determination of CVAOA, CVAA, 2,2′-dichlorodiethyl sulphoxide and 2,2′-dihydroxyethyl sulphide in water by using reversed-phase high performance liquid chromatography combined with an ultraviolet (UV) detector.

2. Experimental

2.1. Reagents and Materials

The water used for standards was first distilled twice from deionized water using an automatic dual-distilling apparatus (Ya Rong Co., Shanghai), and then filtered through a 0.45 μm HPLC membrane. Acetonitrile was of HPLC grade (Fisher, U.S.A.) and potassium dihydrogen phosphate, which was used to adjust the pH, was of spectrophotometric grade (Ya Rong Co., Shanghai). Phosphoric acid was PRA grade (85%, Red Star Co.,

Peking). CVAOA (98%), CVAA (98%), 2,2′-di-chlorodiethyl sulphoxide (97%), 2,2′-dihy- droxy-ethyl sulphide (97%) were obtained from the analytical centre of chemical defense of China, which is an authorized laboratory of the Organization for the Prohibition of Chemical Weapons (OPCW).

2.2. Instruments

A HP 1050 high performance liquid chromatograph with diode array detector (HPLC-DAD) was used for all measurements.

General chromatographic procedure and parameters were: ODS C18, 250×4.6 mm $5\,\mu$m; flow rate: 1 mL min^{-1}; sample volume: $10\,\mu$L; detection wavelength: 215 nm; gradient programmes for mobile phase on HPLC-DAD are shown in Table 1, A: 1% acetonitrile in buffered solution; B: 10% acetonitrile in buffered solution; C: acetonitrile.

2.3. Sample Preparation

2.3.1. Buffer Solution

0.27 g KH_2PO_4 were added to a 1 L volumetric flask which was filled up with dual-distilled water to the mark, resulting in a buffer solution of pH 4.45. When necessary, the pH was adjusted to a different pH by adding 1 M H_3PO_4.

2.3.2. Standard Solutions

Standard solutions of CVAOA, CVAA, 2,2′-dichlorodiethyl sulphoxide and 2,2′-dihydroxy ethyl sulphide with a concentration range of 5–500, 2–500, 5–500, and 50–1000 mg L^{-1}, respectively, were prepared by adding the four compounds to a 100 mL volumetric flask and adding mobile phase to the mark.

3. Results and Discussion

3.1. Optimization of Experimental Conditions

The complete spectra of CVAOA, CVAA, 2,2′-dichlorodiethyl sulphoxide and 2,2′-dihydroxyethyl sulphide were obtained using the scan mode of HPLC-DAD. As shown in Fig. 1, 215 nm was selected as the detection wavelength of choice.

3.1.1. Effect of pH Value on k′

The parameter k′ is called the capacity factor. It is equal to the ratio of the relative retention time divided to the dead time. The parameter is a measure of the capability of the solid phase to adsorbed analyte. Figure 2 was obtained by plotting the k′ values of CVAOA, CVAA and 2,2′-dichlorodiethyl sulphoxide versus the pH of the eluent which was a mixture of 20% acetonitrile in aqeous buffer solution.

A good separation of the four compounds was obtained for pH = 4.45. KH_2PO_4 was chosen to adjust the pH of the solution.

3.1.2. Effect of Eluent on k′

Figure 3 was obtained by plotting the k′ values of CVAOA, CVAA and 2,2′-dichlorodiethyl sulphoxide versus percentage acetonitrile at a constant pH of 4.45.

It was found that the signal for 2,2′-dihydroxyethyl sulphide split up and could not be completely separated when the concentration of acetonitrile was above 10%. Using the procedure described in Table 1, it is possible to separate the four compounds.
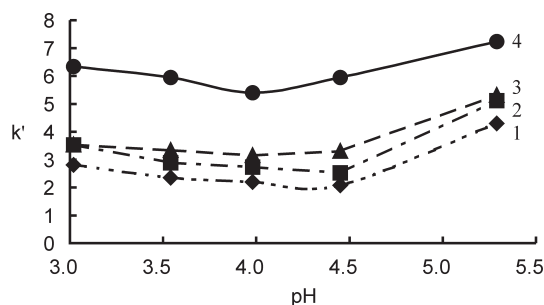
3.2. Chromatogram

The chromatograms of CVAOA, CVAA, 2,2′-dichlorodiethyl sulphoxide and 2,2′-dihydroxyethyl sulphide have been
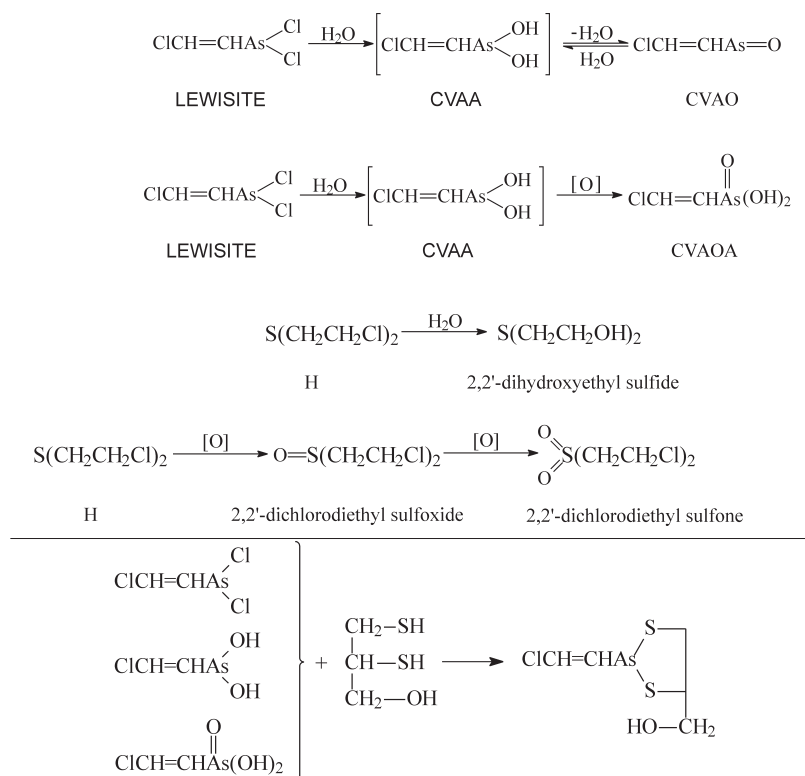
Scheme 1
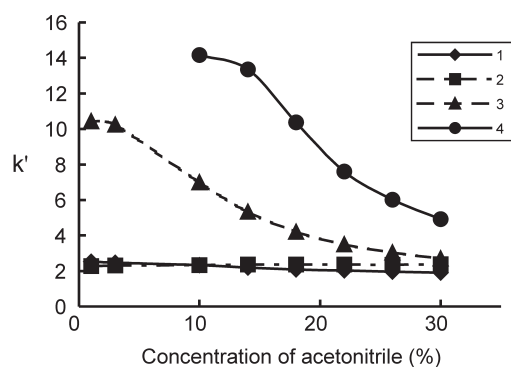Degradation of lewisite and mustard gas.
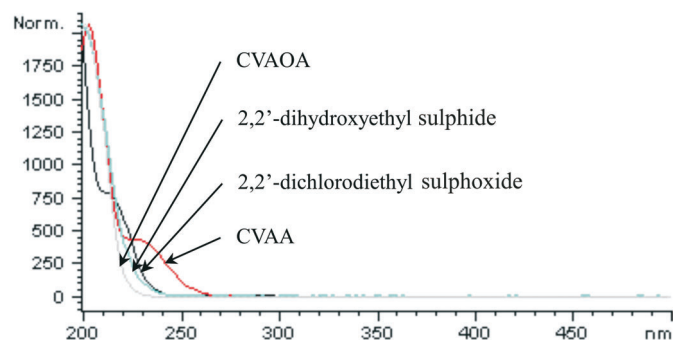
**Figure 1** UV Spectra of CVAOA, CVAA, 2,2′-dichlorodiethyl sulphoxide, 2,2′-dihydroxyethyl sulphide.

Figure 2 Relative k′ values versus pH. 1, CVAOA; 2, unknown; 3, CVAA, 4, 2,2′-dichlorodiethyl sulphoxide.

Table 1 Gradient programmes for mobile phase on HPLC-DAD.

Time (min)	A	B	C
0–3	100	0	0
3–8	0	100	0
8–15	0	50	50

Figure 3 Effect of eluent on k′. 1, CVAOA; 2, unknown peak; 3, CVAA; 4, 2,2′-dichlorodiethyl sulphoxide.
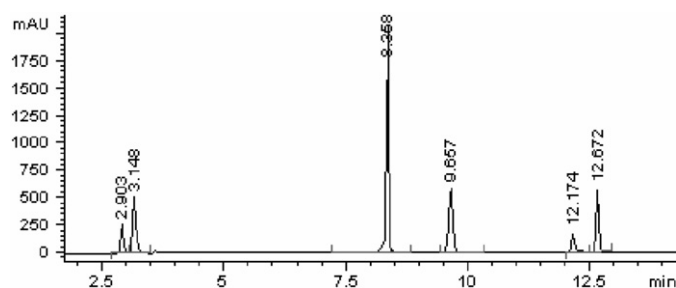
Figure 4 Chromatogram of CVAOA(3.1 min, 390 mg L⁻¹), 2,2′-dihydroxy-ethyl sulphide (8.3 min, 2075 mg L⁻¹), CVAA (9.6 min, 474 mg L⁻¹), 2,2′-dichlorodiethyl sulphoxide (12.7 min, 1085 mg L⁻¹).

obtained by using the conditions as described above. All four compounds can be completely separated as shown in Fig. 4, i.e. there are no spectral overlaps.
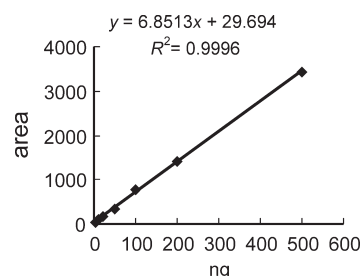
3.3. Quantitative Analysis

3.3.1. Linear Range and Detection Limits

Aqueous solutions of different concentrations of CVAOA, CVAA, 2,2′-dichlorodiethyl sulphoxide and 2,2′-dihydroxyethyl sulphide were prepared and analysed averaging the results of three repetitions. The calibration curves of CVAOA, CVAA, 2,2′-dichlorodiethyl sulphoxide and 2,2′-dihydroxyethyl sulphide showed high linearity over a concentration range of 5–500, 2–500, 5–500, 50–1000mg L⁻¹, respectively. The resulting calibration curves are shown in Figs 5–8.

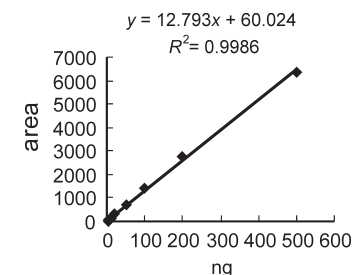Detection limits were obtained at a signal-to-noise ratio of 3. If $\overline{S}_{b1}$ is defined as the average noise, S_m as the signal which was analysed at the concentration of the detection limit, and s_{b1} the standard deviation of the blank samples, then the detection limit can be defined as $S_m - \overline{S}_{b1} = 3s_{b1}$. The detection limit of the four compounds is 0.001, 0.2, 2 and 20 mg L⁻¹, respectively.

Figure 5 Calibration curve for CVAOA.

Figure 6 Calibration curve for CVAA.
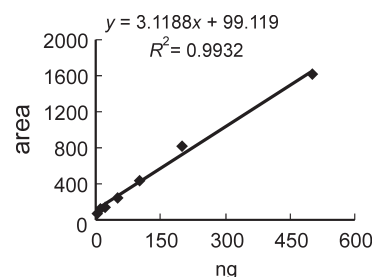
Figure 7 Calibration curve for 2,2′-dichlorodi-ethyl sulphoxide.

Figure 8 Calibration curve for 2,2′-dihydroxy-ethyl sulphide.

3.3.2. Accuracy and Relative Error

Each sample of the standard solutions of the four compounds was injected at least five times. The relative errors were within ±10%. The results show that the method has good reproducibility (Table 2).
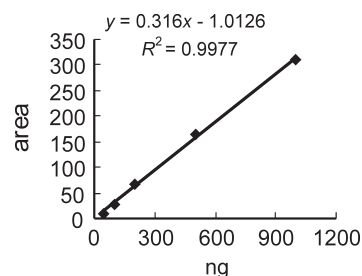
Table 2 Repeatability and relative error of quantitative analysis.

Samples	Added amount (mg L⁻¹)	Results (mg L⁻¹)					Average (mg L⁻¹)	Standard deviations	RSD (%)	Relative error (%)
		1	2	3	4	5				
CVAOA	80.00	75.56	74.81	74.20	74.35	74.47	74.68	0.48	0.65	−5.5 – −7.2
CVAA	50.00	48.50	48.25	47.97	48.43	48.13	48.26	0.19	0.40	−3.0 – −4.1
2,2′-dichloro diethyl sulphoxide	80.00	75.91	76.04	73.57	76.65	76.12	75.66	1.07	1.42	−4.2 – −8.0
2,2′-dihydroxy-ethyl sulphide	300.00	315.0	311.5	291.4	291.8	282.6	298.46	12.6	4.21	5.0 – −5.8

4. Conclusion

It is concluded that the reported method is capable of separating and quantitatively determining CVAOA, CVAA, 2,2′-dichloro diethyl sulphoxide and 2,2′-dihydroxyethyl sulphide in water. The method could be beneficial for the study of the distribution of lewisite and/or mustard and their degradation products in the environment.

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