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Determination of degradation compounds derived from Lewisite by high performance liquid chromatography/inductively coupled plasma-mass spectrometry†

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A method for determination of 2-chlorovinylarsenous acid (CVAA) and 2-chlorovinylarsonic acid (CVAOA), which are degradation compounds of the chemical warfare agent Lewisite, was examined by high-performance liquid chromatography/inductively coupled plasma-mass spectrometry (HPLC/ICP-MS). Inertsil C_8 was suitable as the column and the mobile phase was consisted of 0.1% formic acid-acetonitrile (80 : 20). These compounds were detected sensitively in a short time and separated from inorganic arsenicals and diphenylarsinic acid (DPAA) and phenylarsonic acid (PAA), which are degradation compounds of diphenylchloroarsine and phenyldichloroarsine, respectively. The detection limits of CVAA and CVAOA were 0.2 and 0.1 ngAs/ml, respectively. In addition, a dynamic reaction cell and oxygen as the reaction gas were applied, and then arsenic was detected as AsO^+ (*m/z* 91) in order to prevent interference by $ArCl^+$ (*m/z* 75). This method was applied to the analysis of urine obtained from a CVAA-administered mouse and CVAOA was detected as the main metabolite. Thus, the speciation analysis of arsenic compounds derived from chemical warfare agents was achieved by HPLC/ICP-MS. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: chemical warfare agents; Lewisite; 2-chlorovinylarsenous acid (CVAA); 2-chlorovinylarsonic acid (CVAOA); HPLC/ICP-MS; speciation analysis

INTRODUCTION

Arsenic exists ubiquitously in the environment and has been studied for a long time. Additionally, it is a highly toxic element and has been used in homicide or as an insecticide, and recently, it has been used in the treatment of acute promyelocytic leukemia (APL).[1] Thus, arsenic is one of the most widely known elements; moreover, it is also a chemical warfare agent (Fig. 1). Lewisite is a type of organoarsenic chemical warfare agent. Goldman *et al*. reported the chemical features, toxicity, etc., of this compound.[2] In Japan, mustard gas, Lewisite, diphenylchloroarsine, diphenylcyanoarsine and others were produced at Okuno Island in the Seto Island Sea during World War II, and these weapons were abandoned at the end of the war.[3] Recently a number of accidents have occurred and workers have been injured due to these abandoned agents during road construction at Samukawa and Hiratsuka, Kanagawa Prefecture.[4] In 2003, some of the residents of Kamisu, Ibaraki Prefecture, exhibited disorders resulting from damage to the central nerve system; it was considered that this was caused by well water polluted by diphenylarsinic acid (DPAA) and

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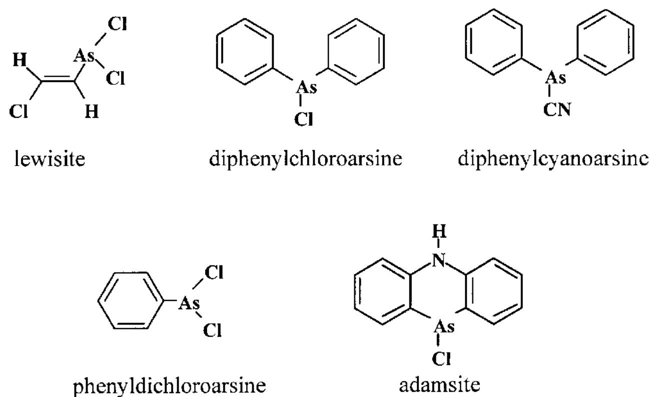

Figure 1. Structures of arsenic chemical warfare agents.

phenylarsonic acid (PAA).[5] The original agents or canisters were not recovered from the ground, and it is possible that such accidents will occur again in the future. Arsenic agents appear to have toxicity even after degradation,[6] particularly 2-chlorovinylarsenous acid (CVAA), which retains a high level of toxicity. Therefore, it is important to develop an analytical method that is able to carry out rapid determination with a high sensitivity.

The present authors have reported a determination method for DPAA and PAA,[7] and this paper examines the compound Lewisite. However, since Lewisite is easily hydrolyzed by contact with water, its degradation compounds, namely CVAA and 2-chlorovinylarsonic acid (CVAOA), were instead dealt with in this study. The putative degradation pathway is shown in Fig. 2. The analytical methods have been reported in a number of studies. Schoene *et al.* analyzed CVAA using GC/MS accompanying derivatization by thioglycolic acid methyl ester.[8] Creasy employed HPLC/APCI-MS accompanying post-column derivatization with 2-mercaptopyridine.[9] Shanmao *et al.* used HPLC/UV for the determination of CVAA and CVAOA without derivatization. Here the detection limits for CVAA and CVAOA were 20 and 1 ppb, respectively.[10] We employed HPLC for separation and applied ICP-MS for the specific detection of arsenic compounds. After this method was carried out, for the analysis of biological samples, a urine sample of a CVAA-administered mouse was obtained and investigated.

EXPERIMENTAL

Reagents

Acetonitrile (HPLC-grade) and hydrogen peroxide (AAS-grade) were purchased from Kanto Chemical Co., Tokyo. Formic acid (analytical grade) was obtained from Wako Pure Chemicals Co., Osaka. PAA was purchased from Acros Co. Ltd, NJ, USA. DPAA and inorganic arsenicals were obtained from Trichemical Lab., Yamanashi, Japan.

Lewisite 1 (2-chlorovinyldichloroarsine, m.p. 98 °C) was imported from TNO Prins Maurits Laboratory, The Netherlands. Lewisite 1 in hexane solution (Laboratory 1% v/v, 19 mg/ml) was hydrolyzed, and then a 10 ppm stock solution of CVAA was prepared. CVAOA was prepared by the oxidization of CVAA with 0.05% of H_2O_2.

Apparatus

The HPLC system comprised a carrier reservoir (CR670), pump (PU611, GL Sciences Inc., Tokyo, Japan), and autosampler with a built-in column oven (MIDAS, Spark, The Netherlans). The following columns were examined: Inertsil C_4, C_8, Ph (2.1 × 150 mm, 5 μm), and CHEMCOSORB 3-Dph (2.1 × 150 mm, 3 μm). Arsenate was eluted from transparent glass vials made of amber-colored glass or polyethylene. The ICP-MS system was an ELAN DRC-e, and the retention times and peak areas were determined with a TotalChrom Workstation version 6.2.0 (PerkinElmer SCIEX Inc., Ontario, Canada).

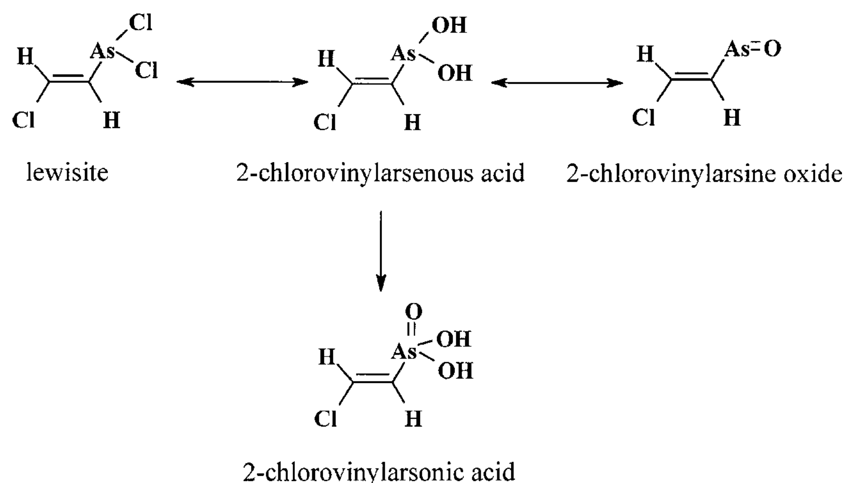

Figure 2. Degradation pathway of Lewisite.

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Examination of separation method for CVAA and CVAOA

Firstly, the selection of analytical column was carried out. Based on the data from our previous report,[7] a reversed-phase mode and water–acetonitrile mobile phase were applied. The columns examined were C_4 and C_8, and Ph and Dph, which were useful for the separation of compounds containing phenyl groups. Following this, CVAA, CVAOA, PAA and DPAA were separated. For application to biological samples, those arsenicals were added to human urine; the samples were then compared with normal standard samples, followed by a confirmation of whether the retention times shifted and co-eluted with the urinary components. Each retention time was compared with that of the inorganic or methylated species, present in urine. In addition, the injection volume was examined.

Chlorine is often present in biological samples, especially urine. It reacts with argon to produce $ArCl^+$ (m/z 75), which interferes with As^+ (m/z 75). In order to prevent this interference, the DRC (dynamic reaction cell) mode was applied. In general, ammonia and methane are often used as the reaction gas, in this case, however, oxygen was selected, and then the arsenic compounds were detected as AsO^+ (m/z 91). The analytical conditions are listed in Table 1.

Analysis of mouse urine sample

The mouse urine sample was prepared by the following procedure. After oral administration of 1 ml of stock solution, urine was collected over a period of 24 h. It was adjusted to

Table 1. Analytical conditions of HPLC/ICP-MS

[HPLC]		[ICP-MS]
Column	Inertsil C_4/C_8/Ph (2.1 × 150 mm, 5 μm)	RF power, 1500 (W)
	CHEMCOSORB 3-Dph (2.1 × 150 mm, 3 μm)	Nebulizer gas flow, 0.94 (l/min)
Mobile phase	0.1% HCOOH–CH_3CN = 80 : 20	Auxiliary gas flow, 1.15 (l/min)
Flow rate	0.2 ml/min	Plasma gas flow, 18 (l/min)
Temperature	40 °C	Reaction gas, oxygen
Injection volume	5 μl	Cell gas flow, 0.8 (ml/min)
		m/z, 90.9165 (AsO)

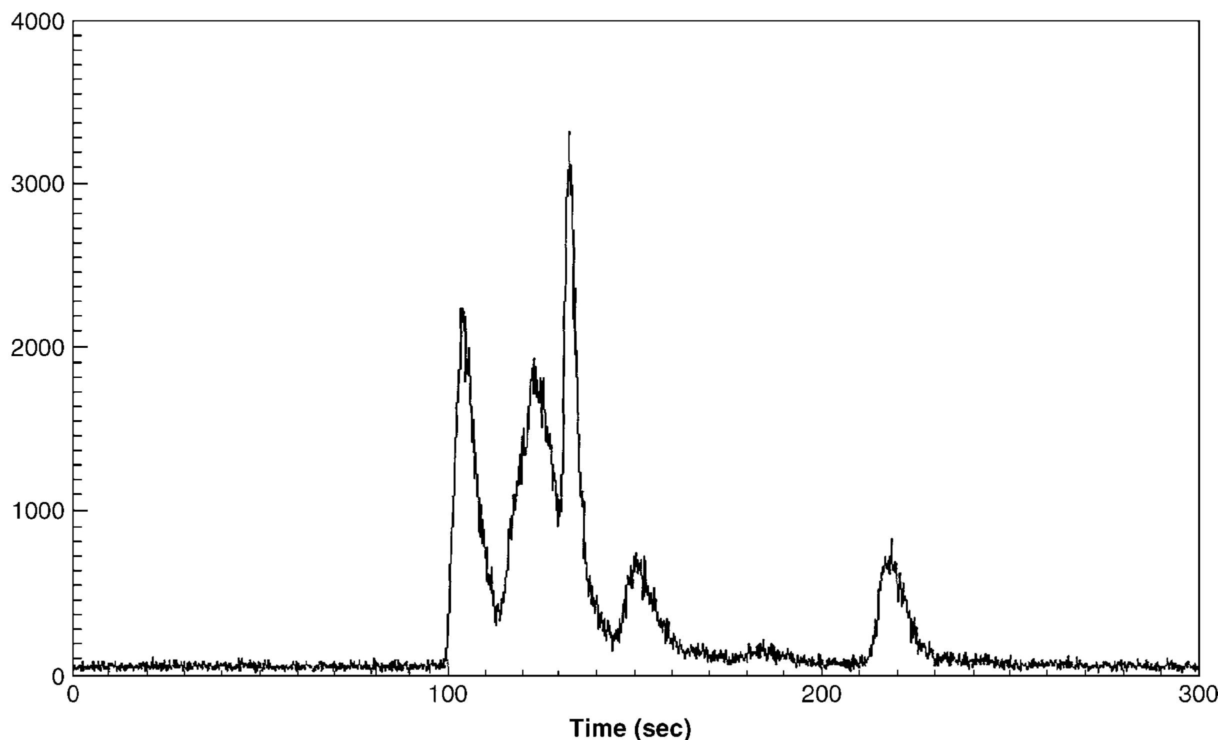

Figure 3. Chromatogram of arsenic compounds by C_4 column. Each peak indicates arsenate, CVAOA, PAA, CVAA and DPAA. The analytical conditions of HPLC was as follows: mobile phase, H_2O (pH 2.0)–CH_3CN = 70 : 30; flow rate, 0.2 ml/min; temperature, 40 °C, and injection volume, 5 μl.

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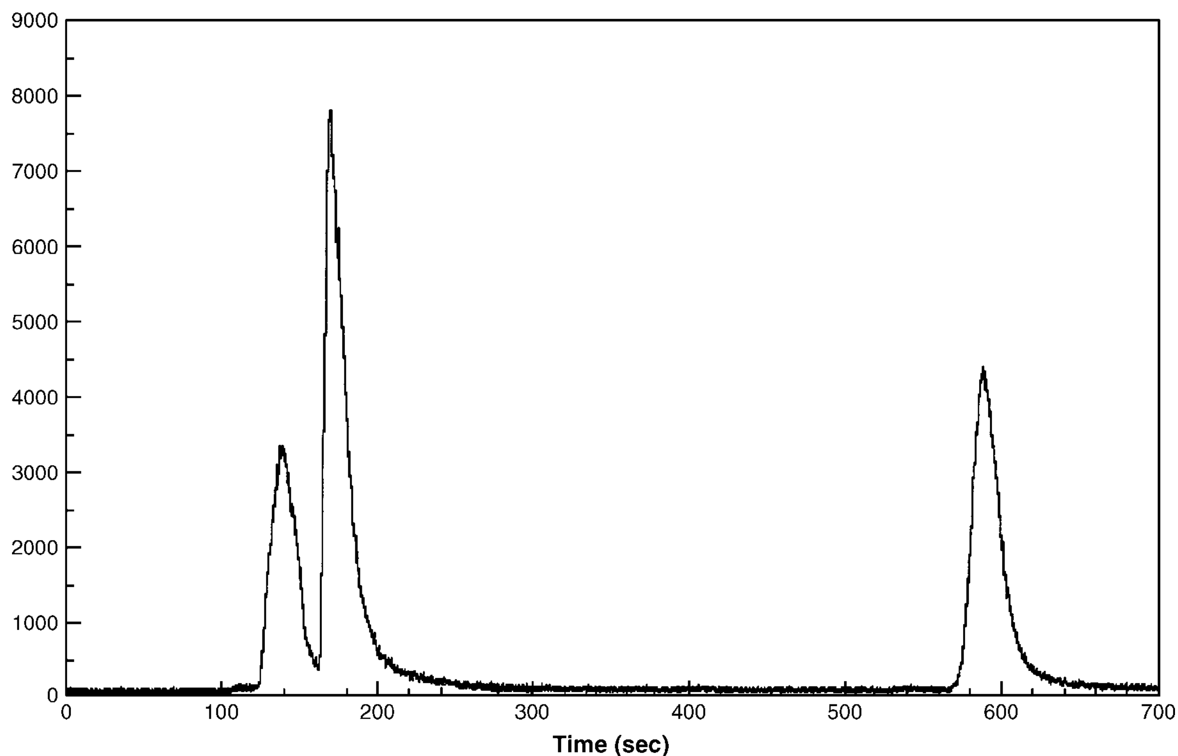

Figure 4. Chromatogram of arsenic compounds by Ph column. Each peak indicates CVAOA, PAA-CVAA mix and DPAA. The analytical conditions of HPLC was as follows: mobile phase, 0.1% HCOOH–CH_3CN = 80 : 20; flow rate, 0.2 ml/min; temperature, 40 °C; and injection volume, 5 µl.

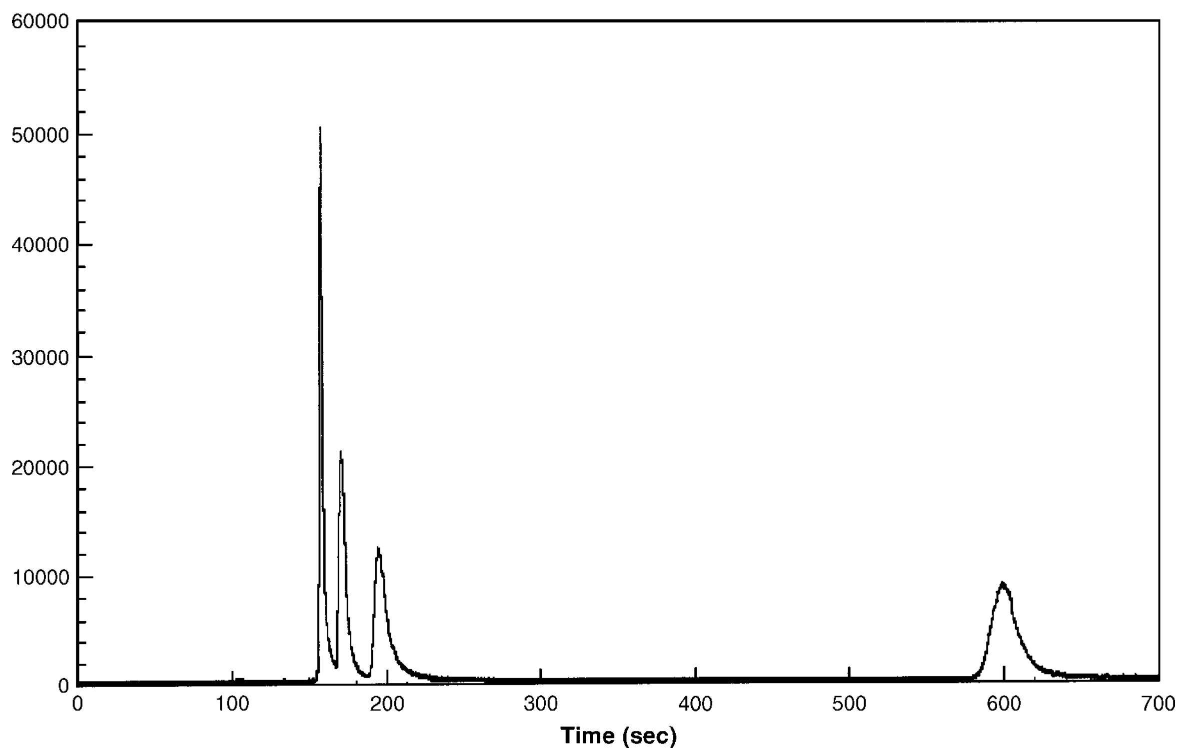

Figure 5. Chromatogram of arsenic compounds by C_8 column. Each peak indicates CVAOA, PAA, CVAA and DPAA. The analytical conditions of HPLC was as follows: mobile phase, 0.1% HCOOH–CH_3CN = 80 : 20; flow rate, 0.2 ml/min; temperature, 40 °C; and injection volume, 20 µl.

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10 ml by ultrapure water and then filtered using a 0.45 μm membrane filter (GL Sciences Inc., Tokyo).

RESULTS AND DISCUSSION

Separation of CVAA and CVAOA

In addition to CVAA and CVAOA, DPAA and PAA, which are derived from phenylarsenic chemical warfare, were also taken into consideration, and then the analytical column was selected. When the C_4 column was employed under the previous analytical conditions, the separation of CVAOA and PAA was not complete (Fig. 3); additionally, CVAOA was not separated from the urinary components. Then, the concentration of acetonitrile was reduced (Table 1). When the C_4 column was used, the peak shape of CVAOA was not sharp and it was not separated from PAA. In contrast, when the C_8 column was used, the peak shape of CVAOA was very sharp, and the separation of four compounds was achieved, although DPAA was not eluted very rapidly. On the other hand, the phenyl-group columns (Ph and Dph) resulted in the co-elution of CVAA and PAA. The chromatogram is shown in Fig. 4. Next, the C_8 column was used for the human urine sample to which arsenic was added. In this case, the

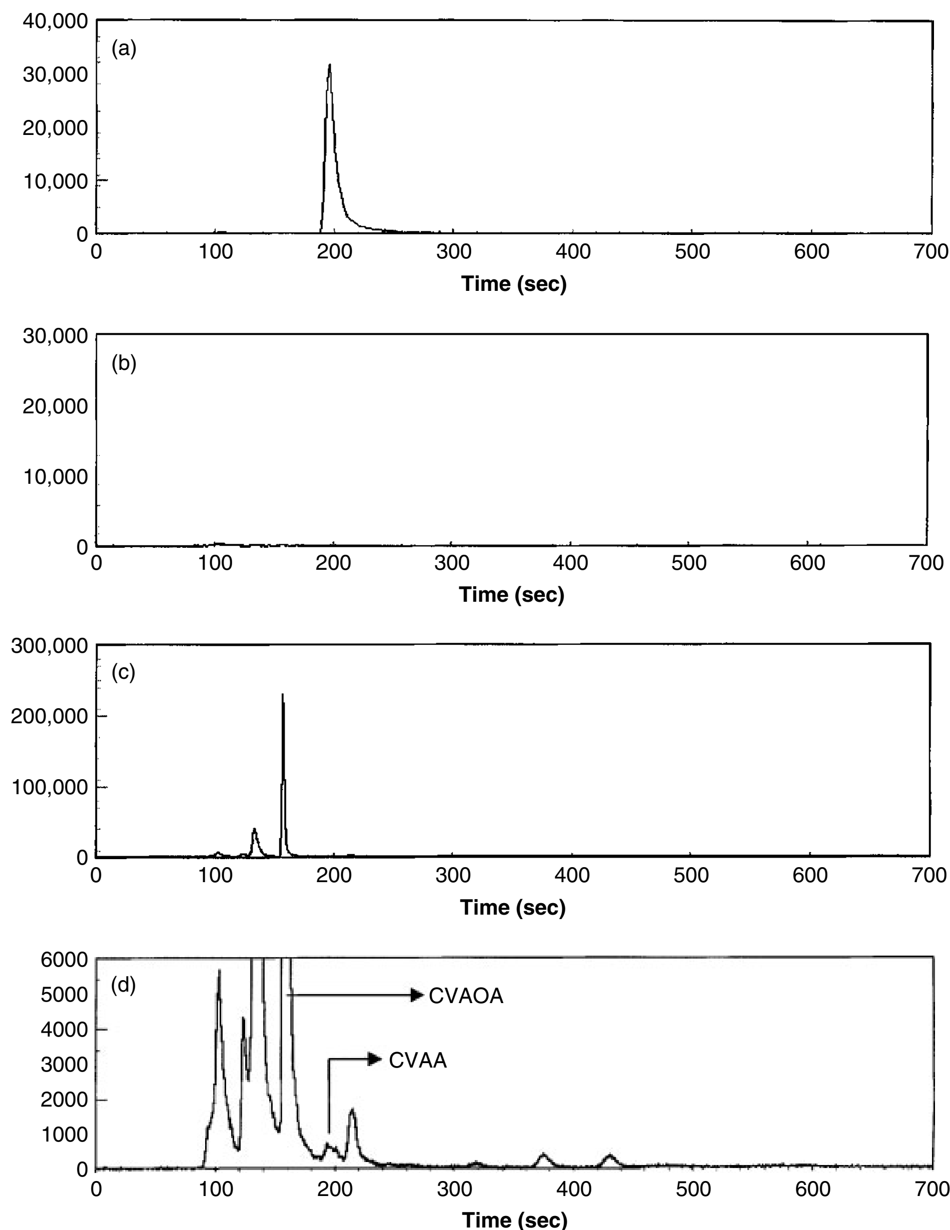

Figure 6. Chromatograms of urine of CVAA-administrated mouse. (a) CVAA alone; (b) urine, before administration; (c) urine, after administration; and (d), low-count region of chromatogram (c).

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arsenicals derived from the chemical warfare agents were separated from the urinary components and no shift was observed in the retention time. Thus, the C_8 column was selected.

Next, the injection volume was examined, which was followed by an investigation of the linearity of the calibration curve and detection limits at a signal-to-noise ratio of 3 : 1. An increase in the injection volume resulted in a decrease in the detection limits while maintaining a favorable shape of the peak; the volume was then set to 20 µl. Here, the guard column used was the C_4 column (1.5 × 10 mm, 5 µm), and the concentration range of the calibration curves of CVAA, PAA, and DPAA was 1–100 ppb, while that of CVAOA was 0.5–100 ppb. As a result, a good linearity of the calibration curve and sensitivity were achieved, as shown in Table 2. Figure 5 shows the chromatogram obtained under these improved analytical conditions; these peaks are very sharp, especially that for CVAOA. Thus, CVAA and CVAOA were detected with high sensitivity in a short time, and the process took about 12 min when DPAA was also identified.

In this study, the arsenic compounds were detected as AsO^+ (m/z 91) in order to prevent interference by $ArCl^+$ (m/z 75); this also lowered the level of the baseline.

Analysis of urine obtained from CVAA-administered mouse

For the analysis of a biological sample, the urine of a CVAA-administered mouse was used, and the results are presented in Fig. 6. As shown in Fig. 6(c), CVAOA was detected as the main metabolite, while CVAA was hardly detected. In addition, several unknown species were detected [Fig. 6(d)]; these included hydrophilic species as well as those that were more hydrophobic. All of these unknown species were derived from the metabolism of CVAA because the same peak was not detected in Fig. 6(a) and (b). This suggested that CVAA was metabolized in a complex manner in the body.

Table 2. LOD and correlated coefficient (r^2) of calibration curve

	CVAA	CVAOA	PAA	DPAA
LOD (ngAs/ml)	0.2	0.1	0.3	0.5
r^2	0.99993	0.99974	0.99996	0.99995

Although mouse urine was used in this study, this method can also be applied to samples from humans. Some researchers have studied the kinetics of Lewisite and CVAA, and Snider *et al*. have investigated the distribution of Lewisite in rabbits after the administration of Lewisite and BAL by total As analysis.[11] Logan *et al*. determined CVAA in urine with derivatization after a guinea pig was subcutaneously administered with Lewisite.[12] Our method makes speciation analysis possible, and it may yield useful information that cannot be obtained by total As analysis or a procedure that includes derivatization. Thus it can be applied to studies of metabolism.

In conclusion, CVAA and CVAOA, which are degradation products of Lewisite, can be determined by HPLC/ICP-MS, and a speciation analysis was successfully carried out. In the future, it is possible that this method will be quickly applied to accidents or pollution caused by chemical warfare agents.

REFERENCES

1. Shen ZX, Chen GQ, Mi JH, Li XS, Xiong SM, Qiu QY, Zhu J, Tang W, Sun GL, Yang KQ, Chen Y, Zhu L, Fang ZW, Wang YT, Ma J, Zhang P, Zhang TD, Chen SJ, Chen Z, Wang ZY. *Blood* 1997; **89**: 3354.
2. Goldman M, Dacre JC. *Rev. Environ. Contam. Toxicol.* 1989; **110**: 75.
3. Hanaoka S, Nomura K, Kudo S. *J. Chromatogr. A* 2003; **1085**: 213.
4. Hanaoka S, Nagasawa E, Nomura K, Yamazawa M, Ishizaki M. *Appl. Organometal. Chem.* 2005; **19**: 265.
5. Ishii K, Tanaoka A, Otsuka F, Iwasaki N, Shin K, Matsui A, Endo G, Kumagai Y, Ishii T, Shoji S, Ogata T, Ishizaki M, Doi M, Shimojo N. *Ann. Neurol.* 2004; **56**: 741.
6. Pitten FA, Thurow K, Koch A, Kramer A. *Trace Elements* 2000; 215.
7. Kinoshita K, Shida Y, Sakuma C, Ishizaki M, Kiso K, Shikino O, Ito H, Morita M, Ochi T, Kaise T. *Appl. Organometal. Chem.* 2005; **19**: 287.
8. Schoene K, Steinhanses J, Bruckert HJ, Konig A. *J. Chromatogr.* 1992; **605**: 257.
9. Creasy WR. *J. Am. Soc. Mass Spectrom.* 1999; **10**: 440.
10. Shanmao L, Wei L, Boli Z, Lijun Y, Qinpei W. *S. Afr. J. Chem.* 2005; **58**: 82.
11. Snider TH, Wientjes MG, Joiner RL, Fisher GL. *Fundam. Appl. Toxicol.* 1990; **14**: 262.
12. Logan TP, Smith R, Jakubowski EM, Nielson RE. *Toxicol. Meth.* 1999; **9**: 275.

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