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# Simultaneous determination of degradation products related to chemical warfare agents by high-performance liquid chromatography/mass spectrometry†

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**Chemical munitions that include organoarsenic chemical agent were manufactured by Japanese imperial forces and abandoned in various locations of Japan and China at the end of World War II. These organoarsenic compounds and various decomposition products have caused environmental contamination and damage to health. For the analysis of chemical warfare agents (CWA) and related compounds in environmental samples, determination was carried out using high-performance liquid chromatography/tandem mass spectrometry of nine CWA-related compounds: 2-chlorovinylarsonic acid (CVAOA), phenylarsonic acid (PAA), thiodigricol (TDG), phenylmethlarsinic acid (PMAA), 2-chlorovinylarsine oxide (CVAO), phenylarsine oxide (PAO), diphenylarsenic acid (DPAA), bis(2-chlorovinyl)arsinous acid (BCVAA) and bis(diphenylarsine)oxide (BDPAO). TDG and eight arsine compounds could be simultaneously measured by LC/MS/MS equipped with a reversed-phase column ($C_8$). The limits of detection of CVAOA, PAA, PMAA, DPAA and other compounds were 0.5, 0.05, 0.001, 0.0001 and 0.01 μg/ml, respectively. Copyright © 2006 John Wiley & Sons, Ltd.**

**KEYWORDS:** chemical warfare agents; organoarsenic compounds; degradation products; Lewisite oxide; Diphenylarsinic acid; LC/MS/MS

## INTRODUCTION

During World War II, the Japanese Imperial Forces produced blister agents and sternutators for chemical weapons, termed Yellow and Red agents, respectively. Yellow agents include sulfur mustard (HD), Lewisite or mustard–Lewisite mixture. Lewisite consists of a toxic main component Lewisite 1 (L1) and byproduct Lewisite 2 (L2). Red agents include diphenylarsine chloride (DA, Clark I) and diphenylarsine cyanide (DC, Clark II). These chemical warfare agents were abandoned as chemical shells and toxic canisters at various locations in China and Japan. They are generally unstable in the environment and decompose to a variety of degradation products. Therefore, a method of analyzing these degradation compounds should be established to investigate the contamination caused by chemical warfare agents manufactured by the Japanese Imperial Forces.

Thiodigricol (TDG) is one of the hydrolysates of HD, and it is also a common industrial material. TDG has a lower toxicity than HD; however since it is a precursor compound of HD, it is considered as a Schedule 2 chemical in the Chemical Weapons Convention (CWC). L1 is hydrolyzed rapidly in environment to 2-chlorovinylarsenous acid (CVAA), and then oxidized gradually to form the stable and highly water-soluble 2-chlorovinylarsonic acid (CVAOA). As CVAA is also the hydrated form of 2-chlorovinylarsenious oxide (Lewisite oxide: 2-chlorovinylarsine oxide, CVAO) in aqueous solution, we used CVAO to prepare the CVAA solution. These L1 related compounds are also considered to have potential blistering action. Whereas L2 is more

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stable than L1, it is hydrolysed gradually in water to form bis(2-chlorovinyl)arsinous acid (BCVAA). DA and DC are decomposed to bis(diphenylarsine)oxide (BDPAO) and diphenylarsenic acid (DPAA), but it is not confirmed whether phenylarsine oxide (PAO) and phenylarsonic acid (PAA) are the degradation products of DA or DC. PAA, PAO, DPAA and BDPAO are known as to be raw materials or intermediate precursors of DA and DC. It is possible that both Red agent and its precursor compounds are pollution sources, and these compounds may also cause health damage for humans. In fact, a serious health hazard occurred for inhabitants who drank well water polluted with BDPAO, DPAA and PAA[1] in Kamisu city, Ibaraki prefecture. Although the production pathway and toxicity are not known yet, PMAA was detected in rice cooked with the polluted well water and also in hair and nail of inhabitant who ate the rice in Kamisu city. The cause of environmental contamination related to chemical warfare agents (CWA) produced by Japanese Imperial forces may be Yellow agent, Red agent or both. The degradation products may also cause contamination, so a simultaneous analysis method for various contaminants is required. Typically, a derivatization method followed by gas chromatography mass spectrometry (GC/MS) analysis has been employed for analysis of degradation compounds of CWA.[2,3] However derivatization-GC/MS methods are not ideal for the identification of the individual chemical species. Some studies on the analysis of the CWA related compounds in environmental and biological samples using liquid chromatography (LC), have been published.[4–13] Because the arsine is selectively detected as an atomic ion, high-performance liquid chromatography–inductive plasma mass spectrometry (LC-ICP/MS) has better sensitivity for organic arsine analysis. For LC-ICP/MS, the retention time is the only information to identify the compound; therefore separation from the other arsenic compounds is essential. By contrast, LC/MS/MS has an advantage for qualitative analysis, because it is possible to obtain structural information on the compound by spectrum. However, there are few reports of LC analysis for simultaneous analysis of blister agents and sternutators and related compounds. We made a study of LC/MS/MS analysis to develop the available

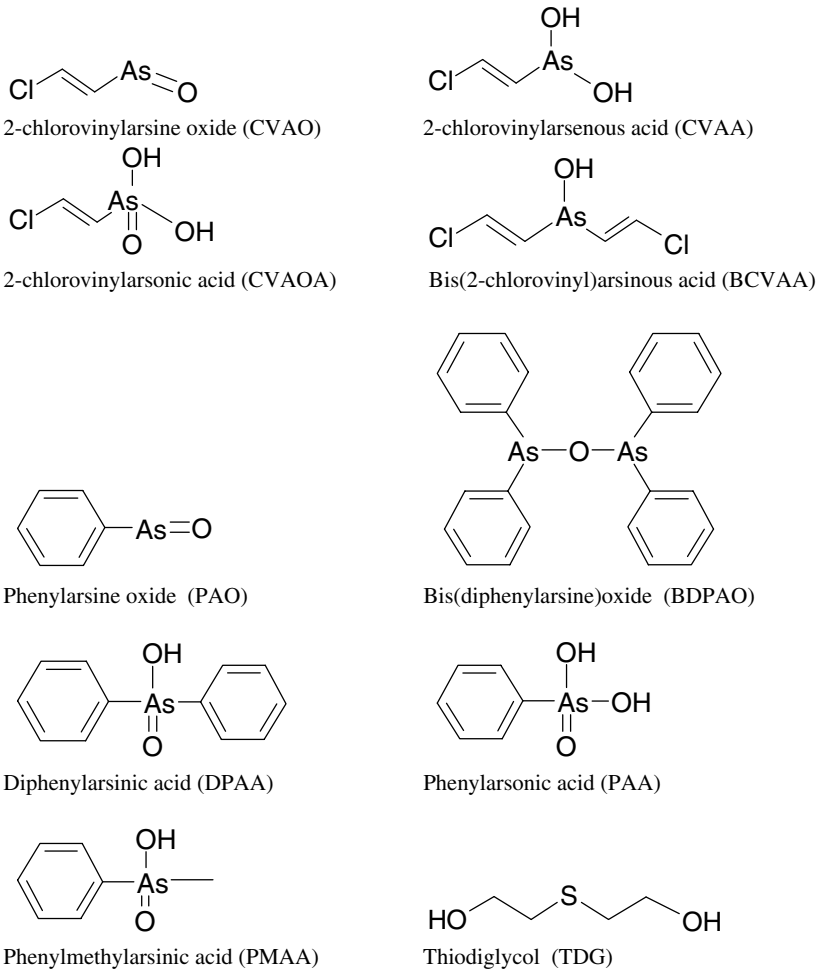


**Figure 1.** Chemical structures of investigated compounds.



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screening technique for investigation of the contamination condition caused by blister agents and sternutators and related compounds. This method enables us to elucidate the form of CWA-related compounds that exist in the environment.

## EXPERIMENTAL

### Reagents

Figure 1 shows chemical structure of the investigated compounds. Bis(2-chlorovinyl)arsine chloride (L2), CVAOA and CVAO were synthesized and provided by TNO-Prins Maurits Laboratory (The Netherlands). BDPAO and DPAA were synthesized and provided by Hodogaya Contract Laboratory (Japan). TDG, PAA, PMAA and PAO were purchased from Kanto Chemical Co. Inc., (Japan), Tokyo Kasei Kogyo Co. Ltd (Japan), Hayashi Pure Chemical Industries Ltd (Japan) and Wako Pure Chemical Industries Ltd. (Japan), respectively. Ultrapure water and methanol were LC/MS grade and purchased from Kanto Chemical Co. Inc. (Japan), and Wako Pure Chemical Industries Ltd (Japan), respectively. Acetic acid, ammonium acetate and ammonia were purchased from Kanto Chemical Co. Inc. (Japan).

### Preparation of standard solutions

Primary standard solutions of CVAO and PAO were prepared with methanol. Primary standard solutions of CVAOA, PAA, TDG, PMAA and DPAA were prepared with ultrapure water. These solutions were mixed and diluted with ultrapure water. Primary standard and working solutions of BDPAO were prepared individually with methanol. BCVAA was obtained by the hydrolysis of L2 and diluted with ultrapure water.

### Chromatographic instruments and condition

LC/MS/MS analysis was conducted on a Finnigan TSQ Quantum Discovery mass spectrometer (Thermo Electron Corporation, USA), using an ESI interface. An Agilent Technologies 1100 series HPLC system consisting of a G1379A degasser, a G1312A binary pump, a G1313A autosampler and G1316A column compartment was coupled to the mass spectrometer. Nitrogen was used as sheath gas and auxiliary gas, and argon gas was used as collision gas. The temperature of the capillary was set at 250 °C. Other MS parameters were optimized for individual compound. CVAA and BCVAA were measured in a negative mode, and other compounds were measured in a positive mode.

LC separation was performed with a L-column C8 (250 × 2.1 mm i.d., 5 μm particle size; Chemicals Evaluation and Research Institute, Japan). The following eluent compositions

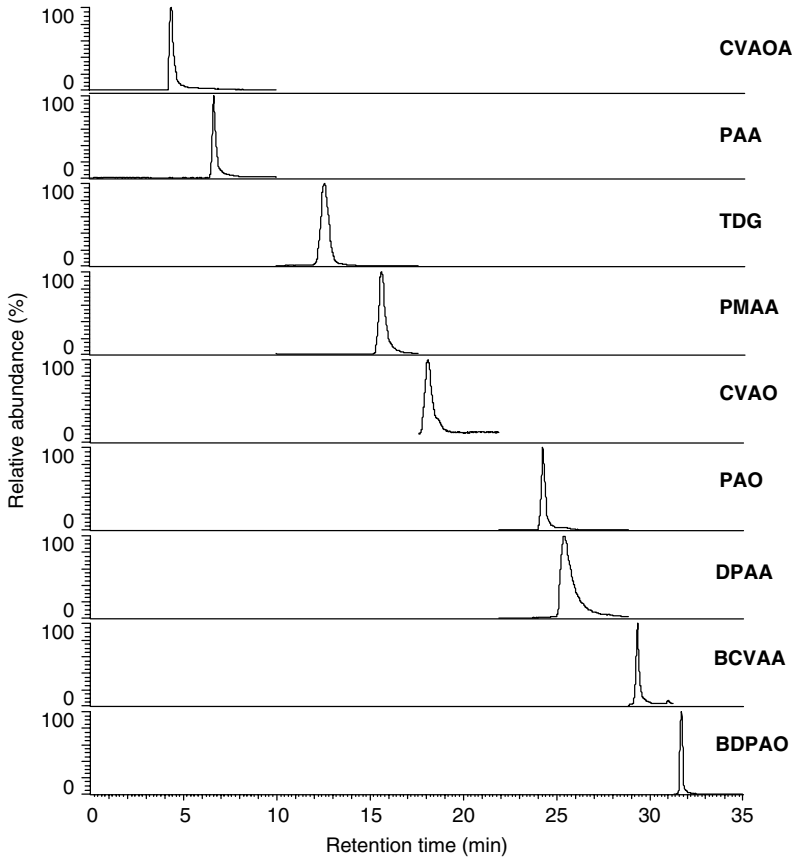


**Figure 2.** Typical LC/MS/MS SRM chromatograms of CWA-related compounds.



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were prepared for the mobile phase: eluent A [10 mM ammonium acetate in water, pH 8.0 (adjusted with diluted ammonia solution)] and eluent B (0.4% acetic acid, 10 mM ammonium acetate in methanol). Elution was performed using a stepwise gradient program of 5–60% B (10 min) then 60–100% B (15 min). The column compartment temperature, injection volume and flow rate were adjusted at 35 °C, 20 μL and 0.2 ml/min, respectively.

## Stability of BDPAO

The aqueous solutions of BDPAO were prepared at the concentrations of 0.05, 0.1, 0.5 and 1 μg/ml, and kept at 5 °C. The concentration of DPAA was measured by LC/MS at immediately after, one day after, 5 days after and 14 days after preparation. Decomposition rate of BDPAO was calculated from the concentration of DPAA in the BDPAO solution.

## Effect of sample matrices

In order to examine adequacy for this method, a recovery test of nine compounds in soil elute was performed. Ten grams of the soil mined from Kamisu city including no organoarsenic compounds was shaken with 100 ml of ultra pure water for 6 h. Then the supernatant obtained by centrifugation (3000 rpm, 10 min) was filtered with 0.2 μm

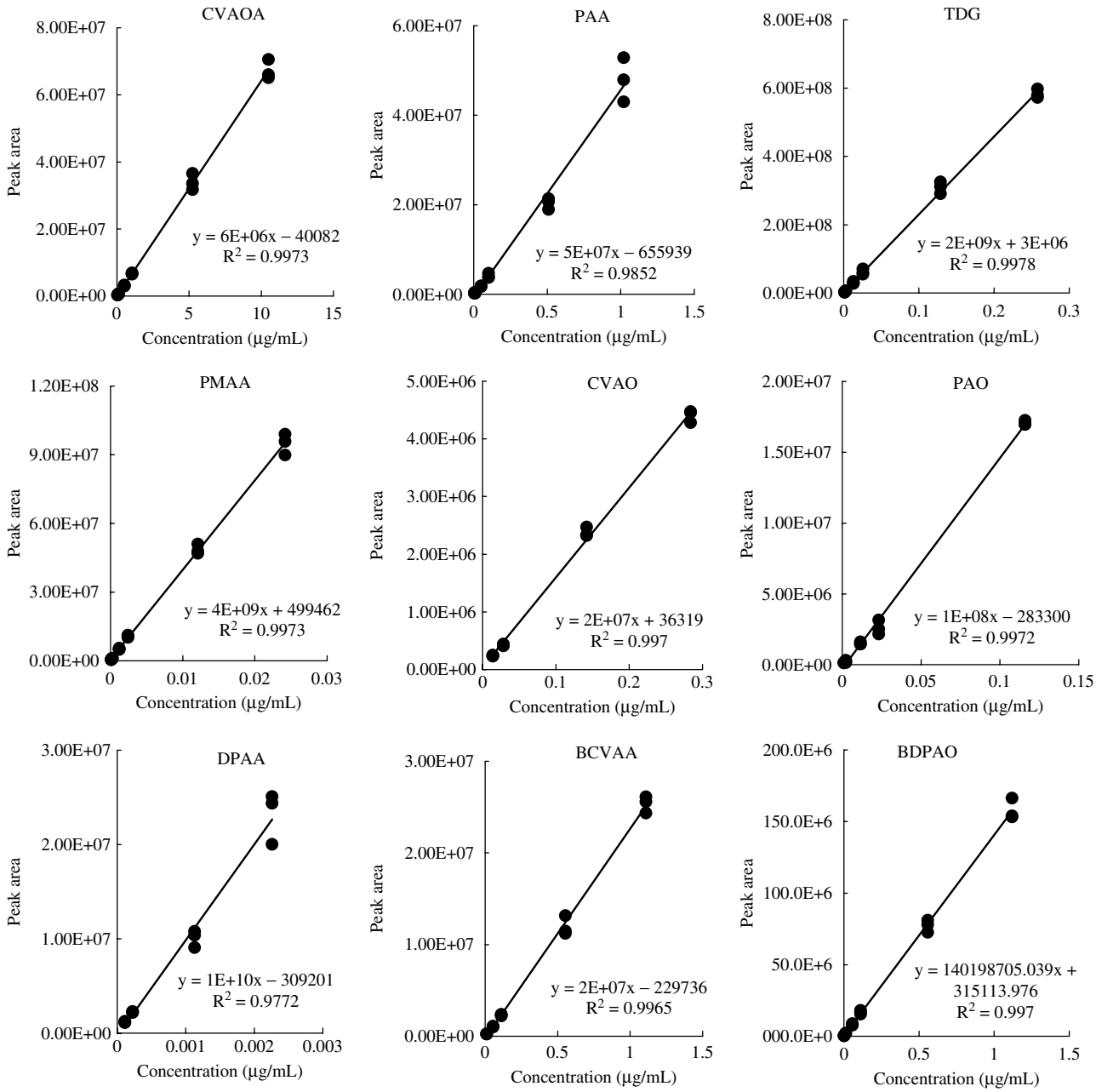


**Figure 3.** Intraday reproducibility of calibration curves for CVAOA, PAA, TDG, PMAA, CVAO, PAO, DPAA, BCVAA and BDPAO.



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micropore membrane filter (GL Chromatodisc; GL Sciences Inc., Tokyo, Japan). A mixed solution of nine compounds was added to the extract, and analyzed by LC/MS/MS directly.

## RESULT AND DISCUSSION

### Chromatographic analysis

Previously, we have reported the LC/MS method that used atmospheric pressure chemical ionization (APCI) mode for the analysis of DPAA, PAA, and BDPAO.[2] However, APCI mode gave lower sensitivity, narrow detection range and poor linearity for DPAA analysis. In this study, we employed the electro spray ionization (ESI) mode, as can be applied to more kinds of compounds. The sensitivity decrease due to the interference with the sample matrix is a well-known problem in the LC-ESI-MS analysis. When two or more compounds are ionized at the same time, competition of protonation may occur. Therefore, LC separation is also important for LC/MS/MS analysis. We established the separation condition in selected ion monitoring (SIM) mode prior to investigation of the LC/MS/MS analysis. The ions for monitoring were selected from LC-ESI-MS full scan spectra of the analytes that measured using mixed media of ammonium acetate aqueous solution and methanol. The positive mode was suitable for ionization of CVAOA, PAA, TDG, PMAA, PAO, DPAA and BDPAO, and the negative mode was suitable for CVAA and BCVAA. The spectrum of CVAOA was dominated by protonated molecule ion at $m/z$ 187 and ammonium adduct ion at $m/z$ 204 due to $[M + NH_4]^+$. Protonated molecule ions at $m/z$ 203 and $m/z$ 263 were observed as specific peaks in the spectra of PAA and DPAA, respectively. The spectrum of TDG contained protonated molecule ion $m/z$ 123 $[M + H]^+$, product ion due to loss of $H_2O$ $m/z$ 105 $[M + H - H_2O]^+$ and significant ammonium adduct ion $m/z$ 140 $[M + NH_4]^+$. The spectrum of PMAA contained protonated molecule ion $m/z$ 201 $[M + H]^+$ and dimer cluster ion $m/z$ 401 $[M_2 + H]^+$. An acetate adduct ion at $m/z$ 229 due to $[M + CH_3COO]^-$ was observed on CVAA spectrum. PAO exhibited a protonated molecule ion at $m/z$ 168 and ammonium adduct ion at $m/z$ 186 due to $[M + NH_4]^+$. The mass spectrum of BCVAA contained deprotonated molecule ion at $m/z$ 213 and acetate adduct ion at $m/z$ 273 due to $[M - H]^-$ and $[M + CH_3COO]^-$, respectively. Because the fragmentation of BDPAO happens easily, intensity of the protonated molecule ion at $m/z$ 475 is smaller than significant product ions observed at $m/z$ 229, $m/z$ 246 and $m/z$ 261.

Optimization of the LC condition was performed in SIM mode. Polarity mode was switched to negative during detection of CVAA and BCVAA, and switched to positive to detect other compounds. The hydrophilic compounds such as CVAOA, PAA and TDG are hard to retain in the reversed-phase column, and the separation of these compounds was difficult. Therefore the column of 250 mm in length was preferred to separate these compounds. It is also difficult to separate PAO from DPAA by several reversed-phase column such as ODS and C8 under the neutral mobile phase condition. There are several columns that can separate these compounds by a narrow margin, but it is dependent on the brand or the production lot, and it suggested to be the effect of residual silanol groups. We paid attention to the following to ensure separation. Pentavalent organoarsenic compounds such as CVAOA, PAA, PMAA, and DPAA are weak acids; therefore it was expected that the retention time of these compounds could be influenced by the pH of the mobile phase. To use the acidic eluent, dissociation of these compounds is controlled and retention time would be extended. On the other hand, the retention time of TDG and trivalent arsenic compounds are not influenced by the pH of the eluent. At pH 4.5, DPAA and PAO were successfully separated, and the narrower bandwidth peaks of TDG, PAO and BDPAO were obtained. However, the ionization of CVAOA, PAA and CVAO are inhibited under the lower pH condition, and the peak area of these compound decreased approximately 4-fold when the pH was lowered from 7 to 4.5. To settle these problems, it is necessary to change the acidity of mobile phase during gradient elution. Thus mobile phase was varied from neutral to acidic by changing proportion of two eluents. CVAOA, PAA, TDG, PMAA, CVAO, PAO, DPAA, BCVAA

**Table 1.** MS/MS-CID ions of investigated compounds

| Compound | MW | Precursor ion | | CID energy (V) | Product ion | |
|---|---|---|---|---|---|---|
| | | $m/z$ | Suggested ion | | $m/z$ | Suggested ion |
| CVAOA | 186 | 187 | $[M + H]^+$ | 48 | 91 | $[AsO]^+$ |
| PAA | 202 | 203 | $[M + H]^+$ | 32 | 77 | $[C_6H_6]^+$ |
| TDG | 122 | 140 | $[M + NH_4]^+$ | 10 | 105 | $[C_4H_8OS]^+$ |
| PMAA | 200 | 201 | $[M + H]^+$ | 43 | 77 | $[C_6H_6]^+$ |
| CVAA | 170 | 229 | $[M + CH_3COO]^-$ | 12 | 169 | $[M - H]^-$ |
| PAO | 168 | 169 | $[M + H]^+$ | 32 | 91 | $[AsO]^+$ |
| DPAA | 262 | 263 | $[M + H]^+$ | 34 | 141 | $[CH_5AsO_3]^+$ |
| BCVAA | 214 | 273 | $[M + CH_3COO]^-$ | 11 | 213 | $[M - H]^-$ |
| BDPAO | 474 | 246 | $[C_{12}H_{11}AsO]^+$ | 50 | 152 | $[C_6H_5As]^+$ |



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and BDPAO were successfully separated and quantitatively analyzed with the developed method.

Optimization of ionization condition of LC/MS/MS for individual compound was performed by flow injection method by mixing the individual standard solution and carrier fluid in the ratio of 1 : 10. Carrier fluid for individual compounds was prepared as similar composition to the mobile phase at the retention time of the compound, and supplied by syringe pump at the flow rate of 0.01 ml/min. Table 1 shows the selected precursor ion, collision energy and product ion for nine target compounds. Figure 2 shows LC/MS/MS selected reaction monitoring (SRM) chromatograms of standard mixture. All compounds were able to elute within 35 min. Calibration was performed on peak area and provided good linearities for each compound. Correlations ($R^2$) of calibration curve for nine compounds with triple measurements are shown in Fig. 3. Calibration curves for individual measurement show high linearity (contributing rate over 0.99); however, PAA and DPAA showed low linearity in three replications. Sensitivity of pentavalent organic arsine had tendency to vary. It is assumed that the ionization of pentavalent arsine may be easily affected by matrix and unstable in ESI. Thus $^{13}C_{12}$-DPAA and $^{13}C_6$-PAA were added to the working solution as internal standard substance to correct ionization efficiency at concentration of approximately 0.05 and 0.5 μg/ml, respectively. Relative standard deviation (RSD) of PAA and DPAA were improved by sensitivity correction for SIM measurement (Table 2). Limits of detection are calculated as three times the standard deviation on three measurements of the lowest concentration of standard solution.

## Stability of BDPAO

Detection of BDPAO in environment should suggest that DC or DA present as a contaminants. Therefore, we attended to BDPAO as one of the analyte. Hydrophobicity and instability of BDPAO must be taken into account for preparing a solution. At the concentration of 0.01 μg/ml, more than 3% of BDPAO was decomposed to DPAA in aqueous solution immediately after the preparation. The decomposition rate of BDPAO was depended on its concentration, i.e. BDPAO tends to be resolved easily in lower concentrations. On the other hand, less than 0.8% of BDPAO was decomposed in methanol solution at the concentration of 0.01 μg/ml. And BDPAO was stable for 3 weeks in methanol solution as compared with its aqueous solution at corresponding concentrations. Furthermore, peak shape and sensitivity of BDPAO measured for methanol solution were in good accordance with those measured for aqueous solution. Consequently, it was suggested that methanol is suitable as the solvent for a standard solution of BDPAO.

**Table 2.** Improvement of reproducibility

| Compound | Concentration (μg/ml) | RSD (%) | |
|---|---|---|---|
| | | Peak area[a] | Peak area ratio[b] |
| DPAA | 0.0001 | 13.2 | 4.6 |
| | 0.001 | 13.8 | 1.6 |
| | 0.001 | 5.3 | 1.6 |
| PAA | 0.05 | 20.5 | 13.3 |
| | 0.5 | 12.1 | 8.2 |
| | 5 | 3.3 | 2.7 |

[a] Absolute calibration method.
[b] Internal standard method.

**Table 3.** Recovery of investigated compounds from soil effluent

| Compounds | Concentration[a] (μg/ml) | Recovery[b] (%) | RSD[c] (%) |
|---|---|---|---|
| CVAOA | 0.5 | 83.7 | 0.8 |
| PAA | 0.05 | 107 | 2.4 |
| TDG | 0.01 | 98.1 | 1.8 |
| PMAA | 0.001 | 101 | 3.3 |
| CVAO | 0.01 | 85.0 | 6.7 |
| PAO | 0.01 | 55.6 | 12.3 |
| DPAA | 0.0001 | 109 | 9.2 |
| BCVAA | 0.05 | 101 | 5.5 |
| BDPAO | 0.05 | 54.2 | 6.1 |

[a] Spiked concentration.
[b] Mean recovery, $n = 3$.
[c] Relative standard deviation, $n = 3$.

## Effect of sample matrices

The recoveries of CVAOA, PAA, TDG, PMAA, CVAO, PAO, DPAA, BCVAA and BDPAO from soil extract are shown in Table 3. Successful recoveries were obtained for most of these compounds excepting with PAO and BDPAO. Low recovery of these compounds may involve with their water solubility. Thus it is expected that the recoveries of these compounds would be improved by addition of organic solvent. The developed method would be applicable to the analysis of degradation products and precursors of abandoned CWAs in aqueous samples such as underground water or extract and elute of soil.

## Conclusion

We developed a LC/MS/MS method for simultaneous analysis of major compounds related to CWA manufactured by Japanese Imperial Forces in environmental samples. It allows us to separate CVAOA, PAA, TDG, PMAA, CVAO, PAO, DPAA, BCVAA and BDPAO, and detect them by practicable sensitivity. The LODs of CVAOA, PAA, PMAA and DPAA were 0.5, 0.05, 0.001 and 0.0001 mg/ml respectively. The LODs of TDG, CVAO, PAO, BCVAA and BDPAO were 0.01 mg/ml. This method can be used as screening technique for investigation of the contamination condition caused by blister agent and sternutaters related compounds. To apply to a more complex sample, additional



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work is needed to develop methods for solid phase extraction, surrogates and a better internal standard.

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