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Determination of diphenylarsinic acid and phenylarsonic acid, the degradation products of organoarsenic chemical warfare agents, in well water by HPLC–ICP-MS

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Diphenylarsinic acid (DPAA) and phenylarsonic acid (PAA), which were degradation products of organoarsenic chemical warfare agents used as sternutatory gas, were detected in the well water at Kamisu, Ibaraki Prefecture, Japan. The standard material of DPAA was synthesized with aqueous arsenic acid and phenylhydrazine in order to determine organic arsenic compounds in well water. The DPAA showed a protonated ion at *m/z* 263 $[M+H]^+$ and a loss of H_2O ion at *m/z* 245 $[M+H-H_2O]^+$ from protonated ion by the electrospray ionization time-of-flight mass spectrometry. The quantitative analysis of DPAA and PAA was performed by high-performance liquid chromatography inductively coupled plasma mass spectrometry and the system worked well for limpid liquid samples such as well water. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: diphenylarsinic acid; phenylarsonic acid; HPLC–ICP-MS; chemical warfare agents; arsenic

INTRODUCTION

In April 2003 in Japan, unexpected information was reported that certain drinking well water in an area of Kamisu, Ibaraki, Japan, contained a high concentration of arsenic and that the level of arsenic was 450 times above the Japanese national drinking water quality standard (0.010 mg dm^{-3}).[1] A few inhabitants in the area exhibited disorders and symptoms including with a central nervous system poisoning incident, which indicated the possibility that a large amount of arsenic could enter to the human body via the drinking well water.[2,3] Furthermore, the chemical speciation of arsenic in the well water was investigated at Ibaraki Prefectural Public Health Laboratories, and diphenylarsinic acid (DPAA) was detected in the well water by gas chromatography–mass spectrometry (GC–MS) as a propane thiol derivative.[4] DPAA is considered to be a degradation product of organoarsenic warfare chemical agents, diphenylchloroarsine and diphenylcyanoarsine, which were prepared as strong irritants to decrease a soldier's will to fight in World War II (Fig. 1). Phenylarsonic acid (PAA) was the degradation compound of the irritant phenyldichlorarsine (Fig. 1). Although it was not reported to be responsible, we decided to analyze it simultaneously. There is not much known about the biological aspects in the environment and the toxicity of PAA and DPAA for humans.[5] It is possible that these chemical agents or their degradation products can cause such environmental pollution and health damage; therefore, the

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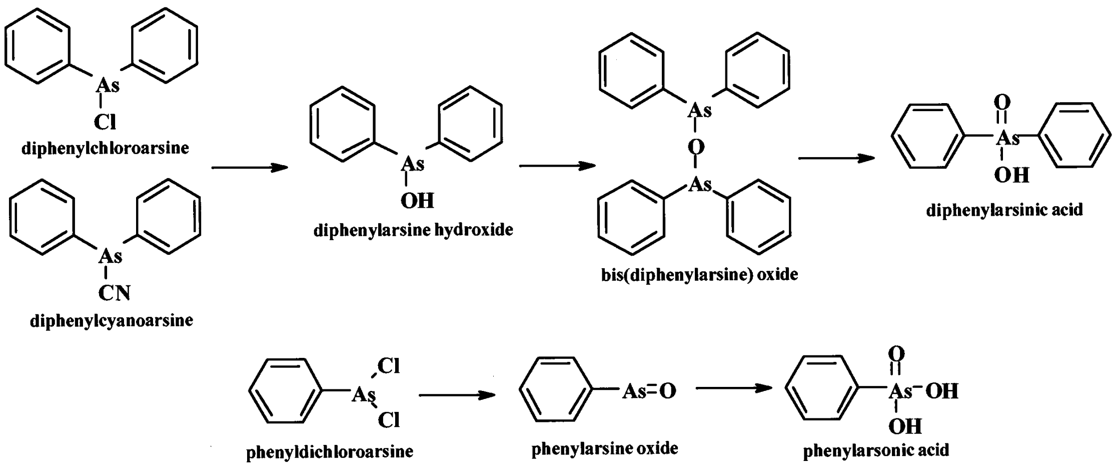

Figure 1. The production pathway of DPAA and PAA.

measurements for the pollution of DPAA in groundwater environment continue to be pressing and they were needed for the prevention of polluted expansion of DPAA. In this study, we reported a method for the determination of DPAA and PAA in well water samples by high-performance liquid chromatography (HPLC)–inductively coupled plasma (ICP)-MS.

EXPERIMENTAL

Reagents

Nitric acid (analytical grade) and acetonitrile (HPLC grade) were purchased from Kanto Chemical Co. Tokyo, Japan, which were used as the mobile phase for HPLC. PAA ($C_6H_5AsO(OH)_2$) and phenylarsine oxide (C_6H_5AsO; PAO) were obtained from Acros Co. Ltd, New Jersey, USA. These standard stock solutions at an arsenic concentration of 10 mg dm^{-3} were prepared by dissolving in[9] small amount of methanol and add Milli-Q water. Sodium arsenite ($NaAsO_2$), obtained from Trichemical Lab., Yamanashi, Japan, was dissolved in Milli-Q water at an arsenic concentration of 1000 mg dm^{-3} as standard stock solution. Phenylhydrazine (reagent grade), aqueous arsenic acid (reagent grade, 60%), copper(II) oxide (analytical grade) and dichloromethane (analytical grade) were purchased from Wako Pure Chemicals Co. Osaka, Japan.

Synthesis of DPAA

DPAA was synthesized by the modification method reported by Barker *et al.*[6] Phenylhydrazine (25 g) was dropped into the mixture of aqueous arsenic acid (60 g), copper(II) oxide (0.4 g) and 150 cm^3 of water, and the mixture was stirred in a water bath at 75 °C overnight. Arsenic compounds were extracted with dichloromethane (130 cm^3) from the mixture solution twice and the dichloromethane layer was washed with water twice. Hydrogen peroxide (30%, 25 g) was added to the dichloromethane phase and then dichloromethane was evaporated under reduced pressure. Crude crystals of DPAA were obtained. DPAA was extracted with hot water three times and was recrystallized with water three times.

Apparatus

The melting point was determined with a TAS 200 model thermogravimetry differential thermal analyzer (Rigaku, Tokyo, Japan). The determination of molecular weight and exact mass spectra were performed by electrospray ionization time-of-flight (ESI-TOF) MS (Q-Tof 2, Micromass, Manchester, UK). ^{1}H and ^{13}C NMR spectra were recorded on a DRX 500 NMR spectrometer (Bruker Biospin, Germany) in $CDCl_3$. The chemical shifts are given as parts per million from a signal of $CHCl_3$ (7.26 ppm for ^{1}H NMR) in $CDCl_3$ and a signal of $CDCl_3$ (77 ppm for ^{13}C NMR). The following abbreviations are used: singlet, (s); doublet, (d); triplet, (t).

The HPLC system was composed of a CR670 carrier reservoir and dual PU611 series pumps (GL Sciences Inc., Tokyo, Japan). The separation column for arsenic compounds (Inertsil C4, 150 mm × 2.1 mm × 5 μm; GL Sciences Inc., Tokyo, Japan) was equipped with a PU611 pump and a MIDAS (Spark, Netherland) autosampler with integrated column oven was used to give a column temperature of 40 °C at an elution flow rate of 0.2 cm^3 min^{-1}. Another pump was used as a carrier for water to the ICP nebulizer at a flow rate of 1.0 cm^3 min^{-1}.

An ELAN DRC-*e* (Perkin Elmer SCIEX Inc., Ontario, Canada) ICP-MS instrument was used as an arsenic-specific detector. The effluent from the separation column was hyphenated to the ICP nebulizer. To decrease the damage to the ICP-MS nebulizer by acetonitrile, 1.0 cm^3 min^{-1} of water was mixed with the eluate as a mobile-phase modifier. The signal intensity of arsenic was monitored at m/z 75 with time (seconds) using ICP-MS. Total Chrom Workstation Ver 6.2.0 (Perkin Elmer Inc.) was used to process the chromatographic data signals.

Samples and analytical procedure

The groundwater samples were pumped out of six wells at 5 or 6 m below ground in Kizaki area, Kamisu, Ibaraki, Japan, in summer 2003. The water samples were filtered with a 0.45 μm membrane filter (GL Chromatodisc 4A; GL Sciences Inc., Tokyo, Japan) before injection. Samples were transferred to the vials of the autosampler, which were made of amber-colored glass, polypropylene or polyethylene in order to avoid contamination by trace amount of arsenic from low-quality glass. 5 mm^3 of filtered sample was injected into the HPLC system by the auto injector. The mobile phase used for HPLC consisted of acetonitrile and water (30 : 70) at pH 2.0 with nitric acid. The analytical conditions of HPLC–ICP-MS are shown in Table 1.

Table 1. Analytical conditions of HPLC–ICP-MS

[HPLC]	
Eluent	acetonitrile: water = 30 : 70, at pH 2.0
Column	Inertsil C4 (150 mm × 2.1 mm, 5 μm)
Column oven (°C)	40
Flow rate ($\text{cm}^3\ \text{min}^{-1}$)	0.2
[ICPMS]	
Nebulizer gas flow ($\text{dm}^3\ \text{min}^{-1}$)	0.91
Auxiliary gas flow ($\text{dm}^3\ \text{min}^{-1}$)	1.325
Plasma gas flow ($\text{dm}^3\ \text{min}^{-1}$)	18
Lens voltage (V)	7.5
ICP RF power (W)	1500
Dwell time per AMU (ms)	200

RESULTS AND DISCUSSION

DPAA

DPAA was obtained as white prism crystals (12 g). The yield was 34% and the crystals showed a definite melting point at 169.6 °C. DPAA was dissolved with a small amount of acetonitrile and diluted with water (500 $\mu\text{g dm}^{-3}$), and a 200 mm^3 of aqueous solution of DPAA was applied to ESI-TOF-MS by microsyringe at a flow rate of 200 $\text{mm}^3\ \text{min}^{-1}$. A protonated ion of DPAA was detected at m/z 263 $[\text{M} + \text{H}]^+$, the ion due to the loss of H_2O from the protonated ion was also detected at m/z 245 $[\text{M} + \text{H} - \text{H}_2\text{O}]^+$ on the ESI-TOF-MS spectrum of DPAA. The mass spectrum of DPAA is shown in Fig. 2.

The exact mass number showed at m/z 263.0075 (Calc.: 263.0053, $[\text{C}_{12}\text{H}_{11}\text{O}_2\text{As} + \text{H}]$). ^{1}H NMR spectra: 2, 6, 2′, 6′ of phenyl protons, 7.44 ppm, 4H, (d); 3, 5, 3′, 5′ of phenyl protons, 7.52 ppm, 4H, (t); 4, 4′ of phenyl protons, 7.76 ppm, 2H, (t); OH, 8.68 ppm, 1H, (s). ^{13}C NMR spectra: 2, 6, 2′, 6′ of phenyl carbon, 130.6 ppm; 3, 5, 3′, 5′ of phenyl carbon atoms, 129.1 ppm; 4, 4′ of phenyl carbon atoms, 132.5 ppm; 1, 1′ of phenyl carbon atoms, 133.2 ppm. No other signals were observed in the ^{1}H and ^{13}C NMR spectra. Also, DPAA showed a single peak on the HPLC–ICP-MS chromatogram. It was thought from these data that DPAA had enough high purity to use as a standard for the HPLC–ICP-MS analysis. DPAA was dissolved in small amounts of acetonitrile and Milli-Q water at an arsenic concentration of 10 mg dm^{-3} as standard stock solutions.

HPLC–ICP-MS analysis

Arsenite, PAA, PAO and DPAA were detected within 4 min. The retention times of the arsenic compounds are as follows: arsenite, 1.81 min; PAA, 2.25 min; PAO, 2.98 min; DPAA, 3.36 min. Also arsenite, PAA, PAO and DPAA were quantified with each calibration curve. The HPLC–ICP-MS chromatograms and calibration curves of arsenite, PAA, PAO and DPAA are shown in Figs 3 and 4.

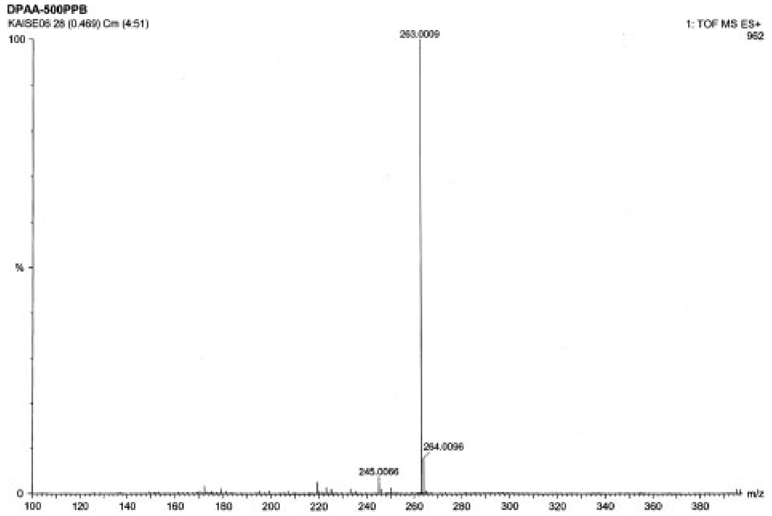

Figure 2. ESI-TOF-MS spectrum of DPAA.

The four calibration curves always maintained a high linearity (correlation coefficient over 0.99) and the detection limits of these arsenic compounds are as follows: arsenite, 4 pg; PAA, 2.5 pg; PAO, 2.5 pg; DPAA, 2.8 pg. The reproducibility was investigated by measuring arsenic concentrations of 5, 10, 20, 50, 100 ng cm^{-3} for each compound 10 times, and each calculated relative standard deviation (RSD) was found to be good, ranging from 1.7 to 3.0% for arsenite, 1.9 to 6.0% for PAA, 1.7 to 2.8% for PAO, and 1.8 to 3.2% for DPAA (Table 2). In addition, the stability of long time analysis was also examined and the instrument for arsonic measurements was stable during 7 h (RSD 1.3–5.8%, $n = 24$), and each peak area was not correlated with the passage of time.

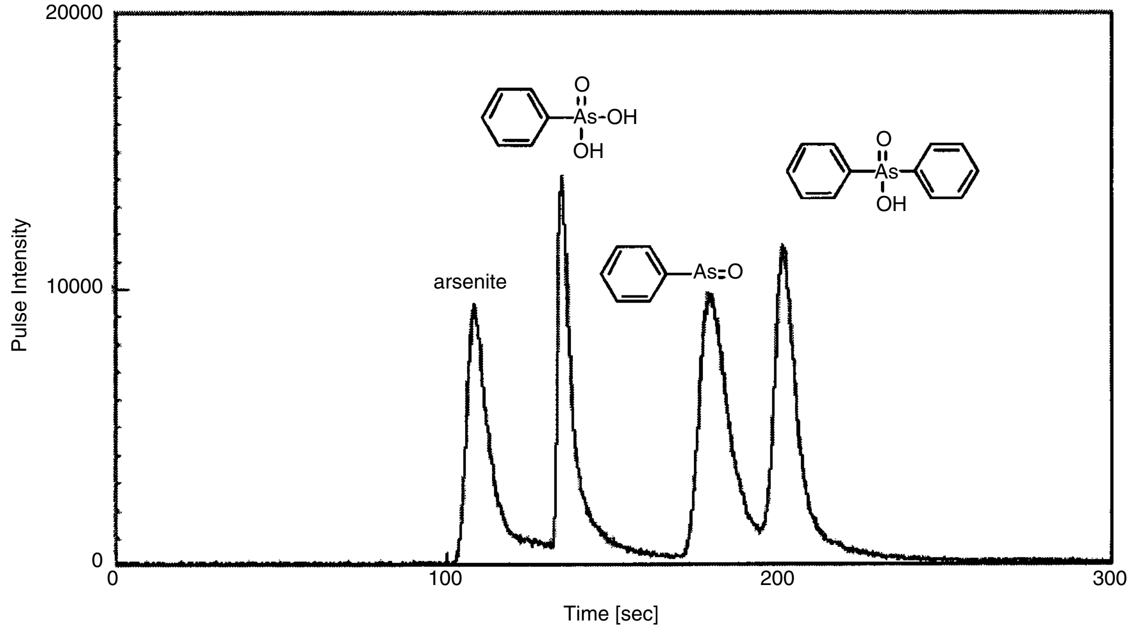

Figure 3. HPLC–ICP-MS chromatogram of arsenite, PAA, PAO and DPAA.

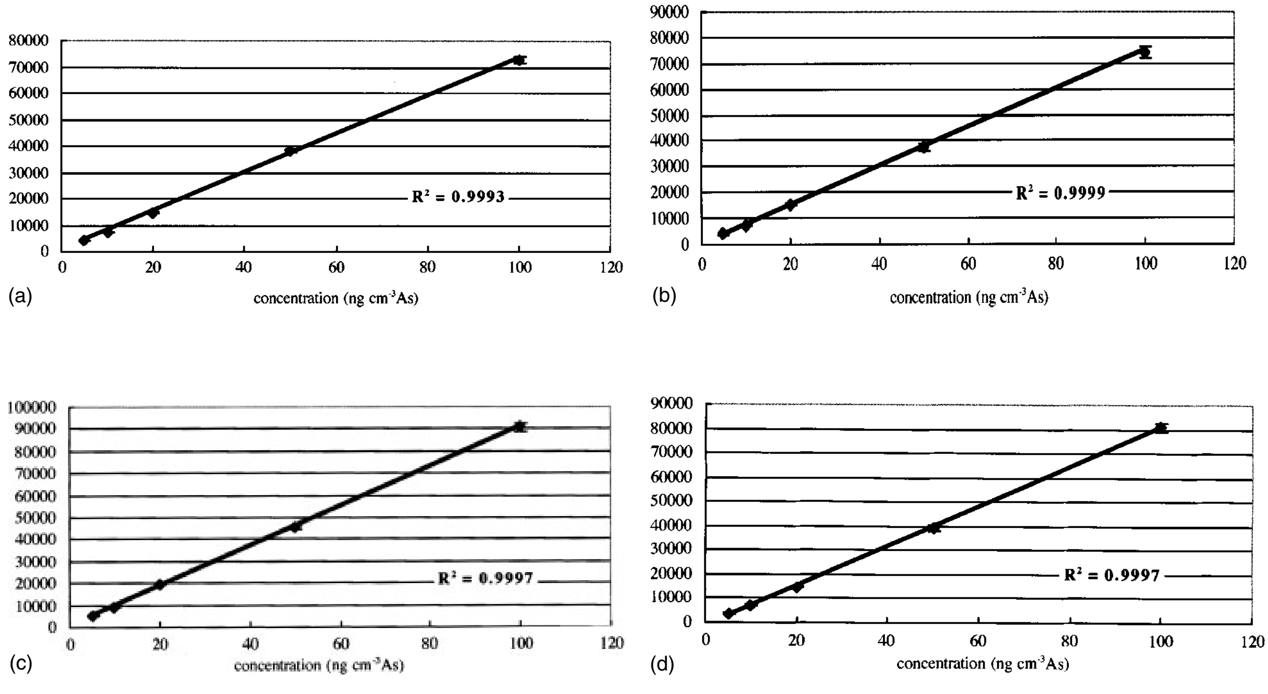

Figure 4. Calibration curves of (a) arsenite, (b) PAA, (c) PAO and (d) DPAA.

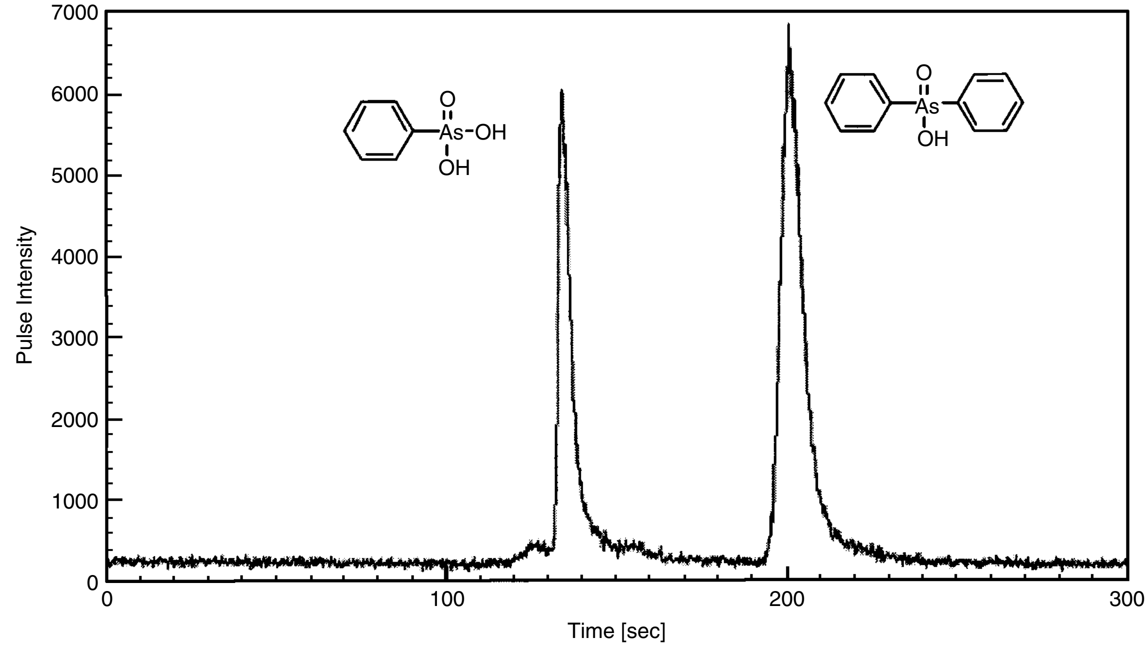

Figure 5. HPLC–ICP-MS chromatogram of PAA and DPAA in well water (Well no. 1).

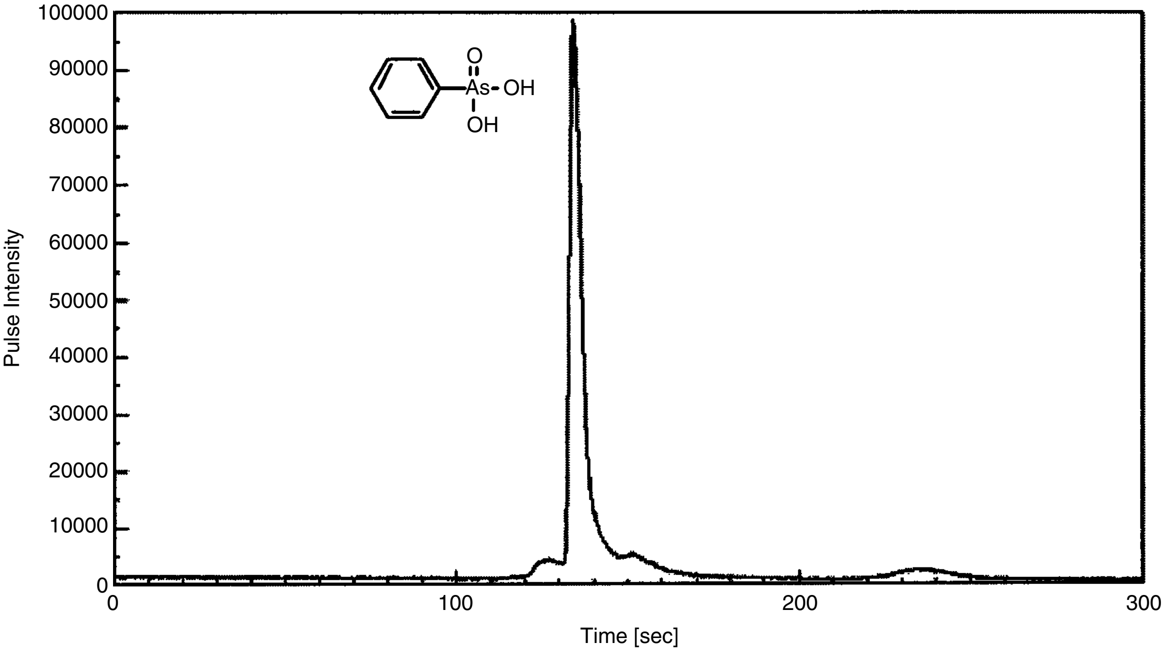

Figure 6. HPLC–ICP-MS chromatogram of PAA in well water (Well no. 2).

Ishizaki *et al.* detected bis-diphenylarsine oxide (BDAO) with solid phase extraction of well water and it was carried out GC-MS measurement.[4] Although non-volatile DPAA was detected by GC-MS as volatile thiol-derivatives with propanethiol[4] or thioglycolic acid methyl ester,[7] but these thio-reagents has having a peculiar stench, and the derivatives were unstable against heat and decomposed in the heated injection port of GC-MS, then the derivative methods were considered that there were some problems of accuracy or reliability on analysis.

Therefore, these results suggest that the HPLC–ICP-MS system is suitable for the determination of phenylarsenic compounds.

Table 2. RSD of each arsenic compound in various concentrations ($n = 10$)

Concentration ($ng\ cm^{-3}$)	Arsenite (%)	PAA (%)	PAO (%)	DPAA (%)
5	1.8	6.0	2.8	2.6
10	3.0	3.5	2.4	2.0
20	2.0	1.9	2.7	1.8
50	2.0	3.2	2.2	3.2
100	1.7	2.9	1.7	2.2

Table 3. Concentration (as arsenic) of arsenic compounds in well water

Well no.	Arsenite ($ng\ cm^{-3}$)	PAA ($ng\ cm^{-3}$)	PAO ($ng\ cm^{-3}$)	DPAA ($ng\ cm^{-3}$)
1	N.D.	3549.3 ± 55.1	N.D.	7980.7 ± 107.0
2	N.D.	1388.4 ± 65.6	N.D.	N.D.
3	N.D.	N.D.	N.D.	N.D.
4	5782.4 ± 23.1	N.D.	N.D.	N.D.
5	N.D.	653.9 ± 18.0	N.D.	N.D.
6	N.D.	N.D.	N.D.	162.8 ± 1.4

Analysis of well water

This system was actually applied to analysis of Kamisu well water. In advance, we investigated the precision of HPLC–ICP-MS for well water samples, and then six samples were determined several times, followed by calculating each RSD. The results of the six well water samples are shown in Table 3, and those samples with large amounts of arsenic were diluted and reanalyzed. It was not clear whether PAA was the degradation product of DPAA in the environment, the starting material of the synthesis of diphenychloroarsine and diphenylcyanoarsine, or the degradation product from phenyldichloroarsine. The HPLC–ICP-MS chromatograms of the phenylarsenic compounds detected from each well water sample are shown in Figs 5–7.

CONCLUSIONS

Synthesized DPAA had a enough high purity to use as a standard for the analysis. The method we established not only detected DPAA and PAA, but also PAO within 4 min, and the precision was good. Acetonitrile was utilized for the separation of these compounds, but water was mixed on the way and the concentration of acetonitrile became low, which kept the stability good. Such robustness will enable adaptation of our system for routine analysis of phenylarsenic compounds in a large number of samples.

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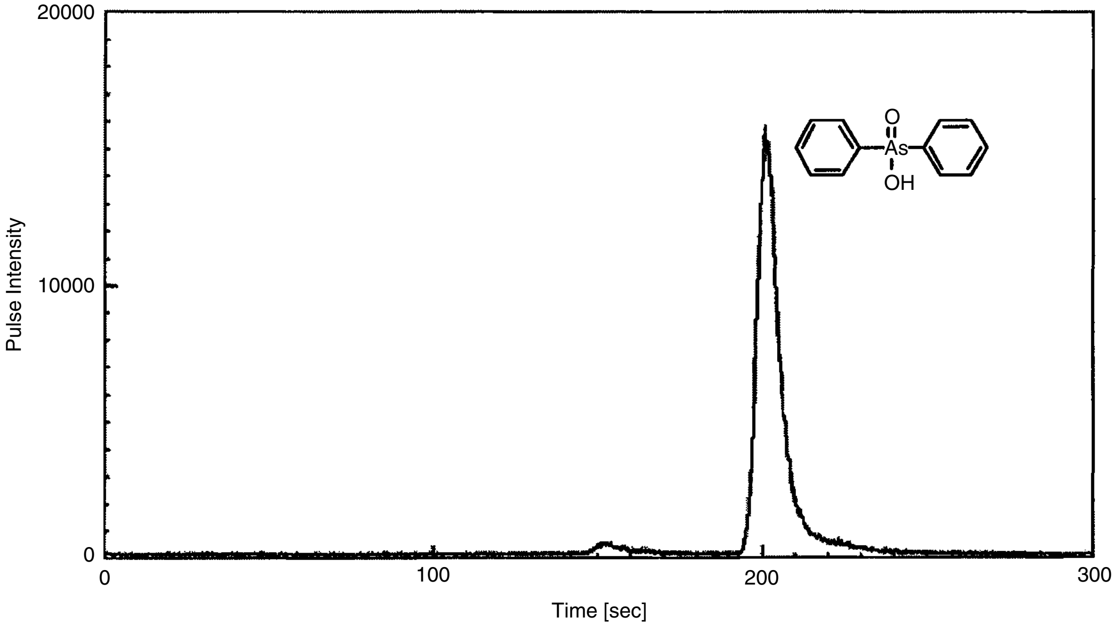

Figure 7. HPLC–ICP-MS chromatogram of DPAA in well water (Well no. 6).

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