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Determination of arsenic compounds using inductively coupled plasma mass spectrometry with ion chromatography

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Abstract

A combined system of inductively coupled plasma mass spectrometry (ICP-MS) with ion chromatography (IC) has been used for the determination of arsenic compounds. Arsenous acid (As^{III}), monomethylarsonic acid (MMAs), dimethylarsinic acid (DMAs), trimethylarsine oxide (TMAsO) and arsenobetaine were separated by anion-exchange chromatography. Subsequently eluates were directly introduced into ICP-MS and detected at m/z 75. Separation parameters were optimized for the arsenic compounds as follows: column, two Excelpak ICS-A35 columns (150 mm × 4.6 mm I.D. each) packed with polymer-based hydrophilic anion-exchange resin (ion-exchange capacity: 0.15 mequiv./g dry); mobile phase, $10 \cdot 10^{-3}$ *M* tartaric acid; flow-rate, 1.0 ml/min; column temperature, 50°C; injection volume, 20 μl. The detection limits for the five arsenic compounds were from 0.22 to 0.44 μg/l as an As element. The repeatability was better than 5% (relative standard deviation) for all arsenic compounds. The IC–ICP-MS system was applied to the determination of arsenic compounds in the urine of DMAs-exposed rats. As^{III}, MMAs, DMAs and TMAsO were detected in the urine.

1. Introduction

Arsenic compounds have been documented as a human carcinogen to the skin and lungs [1]. Most mammals including humans are able to methylate inorganoarsenic compounds to monomethylarsonic acid (MMAs) and dimethylarsinic acid (DMAs) [2]. In experimental rats, necrosis of proximal tubules and necrosis of renal papilla were observed in rats by oral administration of DMAs [3]. On the other hand, AsBe, which is regarded as a non-toxic organoarsenic compound, is rich in sea food and is directly eliminated with urine. Therefore, speciation analysis of arsenic compounds is required in order to evaluate the exposure.

At present, several analytical procedures for arsenic compounds have been reported. For speciation of arsenic compounds, the most commonly used technique is the application of chromatography with different detection systems [4–10]. Inductively coupled plasma mass spectrometry (ICP-MS) is a sensitive, accurate and precise analytical tool for ultra-trace multielemental and isotopic analysis. However, this method does not give any information on specia-

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tion. On the other hand, ion chromatography (IC) is a good separation method for the speciation study of inorganic ions, but lack of sensitivity is a problem for determination of arsenic compounds in biological samples. Because of the ease of combination of ICP-MS with high-performance liquid chromatography, several researchers have applied ICP-MS as a liquid chromatography detector [8–10]. Shibata and Morita [10] have reported a separation of fifteen arsenic compounds in the natural samples, using ion-pair liquid chromatography.

Urine is troublesome to handle because of its higher salt concentration, compared with arsenic compounds' concentrations. The urine matrix causes column overloading, and which results in peak broadening. Furthermore, interference from the polyatomic ion $^{40}Ar^{35}Cl^{+}$ at m/z 75 due to a high content of chloride in the urine has been observed [11–13]. In this case, high dilution of the urine with pure water might be necessary in order to solve this problem [13].

In this paper, we applied IC as a separation device, and inorganic and organic arsenic compounds were separated by an anion-exchange mode. The IC eluate was directly introduced into the ICP-MS system, and the arsenic compounds were detected. The separation parameters were optimized for the five arsenic compounds. The combination of IC and ICP-MS was applied to the determination of the five arsenic compounds in the urine from DMAs-exposed rat.

2. Experimental

2.1. ICP-MS

The ICP-MS instrument used in this experiment was Model PMS 2000 from Yokogawa Analytical Systems (Tokyo, Japan) and operational conditions are described in Table 1. A Scott-type spray chamber, maintained at 0°C by means of Peltier type thermoelectric module, Fassel type torch (Fujiwara Seisakusyo, Tokyo, Japan) and concentric glass nebulizer (Precision Glassblowing, CO, USA) were used in the experiment. For data acquisition of IC–ICP-MS, the selected ion monitoring (SIM) mode was used. For tuning of ICP-MS, 0.01 mg/l of yttrium (Y) solution was analyzed. The system was tuned to get maximum signal for Y by monitoring m/z 89 and changing a bias of lenses.

Table 1
ICP-MS operational conditions

Instrument	PMS 2000
Radio frequency forward power	1.3 kW
Radio frequency reflected power	< 5 W
Plasma gas flow	Ar 18 l/min
Auxiliary gas flow	Ar 1.0 l/min
Carrier gas flow	Ar 0.88 l/min
Sampling depth	5 mm from load coil
Monitoring mass	m/z 75
Dwell time	0.5 s
Times of scan	1 time

2.2. Ion chromatography

The ion chromatograph used in this experiment was Model IC 7000 from Yokogawa Analytical Systems. As to the separation column, Excelpak ICS-A35 (Yokogawa Analytical Systems) was chosen. The ICS-A35 column is 150 mm × 4.6 mm I.D., packed with polymer-based hydrophilic anion-exchange resin (a diameter of 10 μm) with 0.15 mequiv./g dry. Unless otherwise mentioned, the ion chromatograph was operated under the following conditions: mobile phase flow-rate 1.0 ml/min and injection volume 20 μl. A 800 mm × 0.3 mm I.D. poly(ethylenetetrafluoroethylene) (ETFE) tube was used for connection between the column and the nebulizer of ICP-MS. The IC–ICP-MS system is schematically illustrated in Fig. 1.

2.3. Reagents

Arsenic compounds used during this experiment are listed in Table 2. Trimethylarsine oxide (TMAsO) was obtained by oxidation of trimethylarsine (TMAs) with 30% hydrogen peroxide. Pure water was obtained from Milli-Q/SP system (Nihon Millipore, Tokyo, Japan). Stock solutions (100 mg/l) of each arsenic compound were prepared by dissolving each reagent with

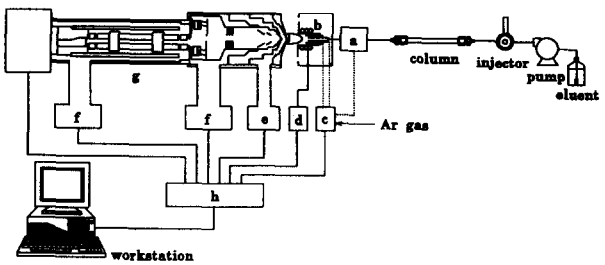

Fig. 1. Schematic flow diagram of IC–ICP-MS system. a = Nebulizer/spray chamber; b = ICP torch; c = gas controller; d = radio frequency power; e = rotary pump; f = oil diffusion pump; g = quadrupole mass filter; h = system controller.

pure water and stored in a refrigerator. Analytical solutions were prepared by diluting the stock solutions to the adequate arsenic compound concentration. Analytical-reagent grade of tartaric acid, 25% ammonium hydroxide and 30% hydrogen peroxide were purchased from Wako (Osaka, Japan).

3. Results and discussion

3.1. Separation of arsenic compounds

First, the effect of mobile phase pH was examined. Fig. 2 shows the relationship between the retention time of five arsenic compounds and the mobile phase pH on Excelpak ICS-A35. The retention time of MMAs, DMAs and TMAsO was increased as the mobile phase pH increased. On the other hand, the retention time of As^{III} and AsBe was not changed as the mobile phase pH increased. That means that As^{III} and AsBe do not fully exchange to the stationary phase, because As^{III} (p*K*a 8.78) does almost not ionize

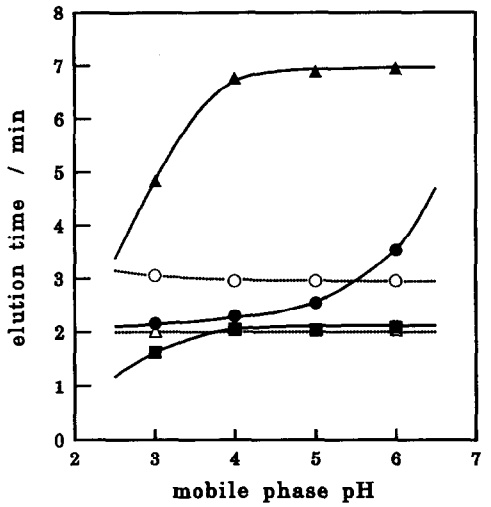

Fig. 2. Dependence of the elution time on the mobile phase pH for five arsenic compounds. Column, Excelpak ICS-A35; mobile phase, $1 \cdot 10^{-3}$ *M* tartaric acid, pH adjusted by 30% ammonium hydroxide; flow-rate, 1.0 ml/min; column temperature, 40°C. Sample (injection volume 20 μl): ▲ = MMAs; ● = DMAs; ■ = TMAsO; △ = AsBe; ○ = As^{III}.

and AsBe is a cationic compound in this pH range. As^{III} does not seem to be retained by ion-exchange interaction with ion-exchange groups, but is retained by hydrophilic interaction with hydrophilic groups on packing materials. At pH ≥ 4.0, TMAsO was not separated from AsBe. Lower pH demonstrated the best separation for the five arsenic compounds.

Second, the effect of the mobile phase concentration was examined at a fixed mobile phase pH of 3. The concentration of tartaric acid as the mobile phase was varied from $1 \cdot 10^{-3}$ to $5 \cdot 10^{-3}$ *M*. The retention time of MMAs was rapidly decreased as the mobile phase concentration

Table 2
Arsenic compounds

Compounds	Manufacturer
Arsenous acid (As^{III}) monosodium salt	Wako, Osaka, Japan
Arsenic acid (As^{V}) disodium salt	Wako
Monomethylarsonic acid (MMAs)	Tri Chemical Lab., Kanagawa, Japan
Dimethylarsinic acid (DMAs)	Sigma, St. Louis, MO, USA
Trimethylarsine (TMAs)	Strem Chemicals, Newburyport, MA, USA
Arsenobetaine (AsBe)	Tri Chemical Lab.

increased. The separation between DMAs and AsBe was not improved by changing the mobile phase concentration. These operational conditions were not suitable for this study because of lack of separation capability. Therefore, the ICS-A35 column was combined with another ICS-A35 column to increase the number of theoretical plates.

Fig. 3 shows the relationship between the retention time of five arsenic compounds and the mobile phase concentration on two ICS-A35 columns. The retention times of arsenic compounds were hardly affected by the mobile phase concentration except for the retention time of MMAs and As^{III}. However, the retention time of MMAs rapidly decreased and the retention time of As^{III} increased as the mobile phase concentration increased. The order of their retention was changed at $4.5 \cdot 10^{-3}$ *M* tartaric acid. On the other hand, the retention time of AsBe was slightly decreased and the separation between AsBe and DMAs was improved.

Next, the effect of column temperature was examined. The column temperature hardly affected the retention time of arsenic compounds. However, a higher column temperature improved the resolution and the peak shape of the arsenic compounds.

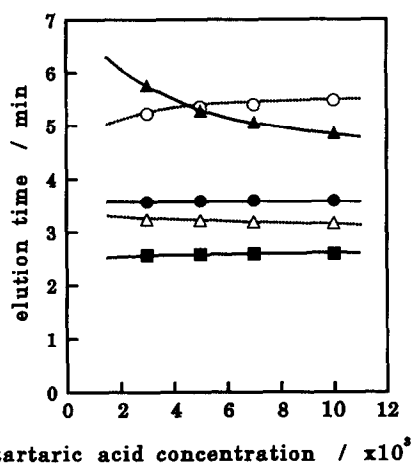

Fig. 3. Dependence of the elution time on the mobile phase concentration for five arsenic compounds. Column, two Excelpak ICS-A35 columns; mobile phase pH, 3.5; flow-rate, 1.0 ml/min; column temperature, 50°C. Sample and injection volume as in Fig 2.

Table 3
Optimized IC operational conditions

Instrument	IC7000
Column	Two Excelpak ICS-A35 columns (150 mm × 4.6 mm I.D. each)
Mobile phase	$10 \cdot 10^{-3}$ *M* tartaric acid
Flow-rate	1.0 ml/min
Column temperature	50°C
Detection	ICP-MS
Monitoring mass	*m/z* 75
Injection volume	20 μl

The optimized IC operational conditions based on these results are described in Table 3. A chromatogram of the five standard arsenic compounds is shown in Fig. 4. The concentration of the five arsenic compounds were 1 mg/l each as As. Arsenate (As^{V}) was not added to the sample solution. The five arsenic compounds and arsenate were completely separated within 15 min.

3.2. Detection limit and reproducibility

The detection limits and reproducibility of the IC–ICP-MS method were determined for the five arsenic compounds. Table 4 gives the detection limits and the reproducibility for the five arsenic compounds calculated from 1.0 mg/l standard solution by injecting a 20-μl sample. The detection limits were calculated from 10 times the square root of blank signal. The reproducibility for 0.1 mg/l standard arsenic compound was obtained from three replicates of the peak area.

Since the temperature of plasma is very high (over 6000 K), arsenic compounds are decomposed and turned into As, O, H and C ions. That means that the sensitivity of arsenic compounds as As does not depend upon the structure of arsenic compounds. When the concentrations of arsenic compounds as As are the same, each compound has to give the same sensitivity at *m/z* 75, giving the same area on the chromatogram. Good agreement was obtained for TMAsO, AsBe and DMAs, but MMAs and As^{III} were smaller than expected. When a standard solution of each arsenic compounds, *i.e.* TMAsO, AsBe and DMAs was injected, each

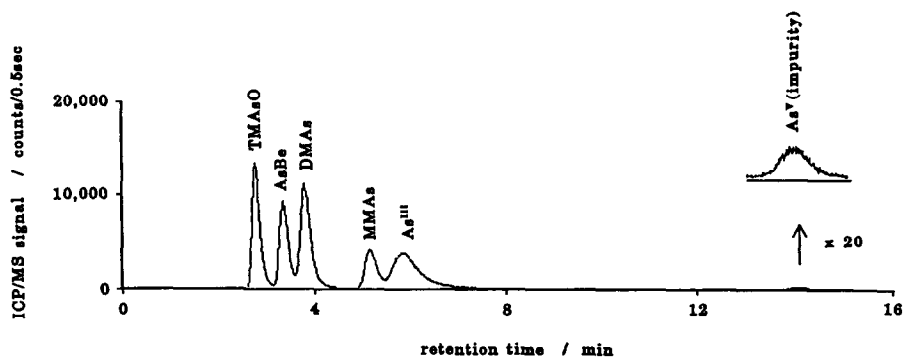

Fig. 4. Chromatograms of five standard arsenic compounds. Conditions as in Tables 2 and 3. Sample concentration: 1 mg/l each.

arsenic species gave almost the same peak area. But when these standard solutions were mixed, TMAsO gave a relatively larger peak area and AsBe a relatively smaller peak area. We assume that AsBe was decomposed in the mixed standard solution, and turned into TMAsO. In the case of MMAs, lack of purity seems to be a main reason. As^{III} was ultimately oxidized to AsV by oxygen in the solution.

3.3. Interference of chloride

In ICP-MS, interference of the polyatomic ion $^{40}Ar^{35}Cl^{+}$ at m/z 75 due to high chloride content in the sample solution has been observed [11–13]. The ArCl ion is formed by combination of chloride in sample solution with argon as the plasma gas. In order to decrease the ArCl ion, addition of a few percent nitrogen to the plasma gas has been employed [14]. However, formation of the ArCl ion cannot be completely depressed by plasma gas control on ICP such as diluting with a few percent of nitrogen. To solve the interference of ArCl, chloride in the sample solution should be removed or separated by some pretreatment before introducing into ICP-MS. Another approach was to separate the arsenic compounds from chloride in sample solution by using liquid chromatography [10,12,13,15,16]. However, the retention time of chloride was close to those of MMAs and TMAsO on an ODS column with ion-pair chromatography [10] and the chloride in urine affected the determination of several μg/l of arsenic compounds. So anion-exchange chromatography was used to separate the arsenic compounds from chloride in the sample solution. In order to check the ArCl ion interference at m/z 75, a 1000 mg/l chloride solution was analyzed under the same conditions. Under these conditions, ArCl ion was not detected and no peaks at m/z 35 and 37 were observed.

Table 4
Detection limits and reproducibility

Arsenic compounds	Detection limits (μg/l)	R.S.D. (%) ($n = 3$)
As^{III}	0.39	3.9
MMAs	0.44	4.9
DMAs	0.28	4.1
TMAsO	0.25	4.7
AsBe	0.22	3.2

3.4. Determination of arsenic compounds in rat urine

The IC–ICP-MS system was applied to the determination of arsenic compounds in urine of DMAs-exposed rats. The urine was obtained from rats which were given 50 and 100 mg DMAs/l in drinking water for 4 weeks. The urine was diluted 20 times by deionized water and 50 μl of the diluted urine were injected into the IC–ICP-MS system. Chromatograms of the diluted urine are shown in Fig. 5. As^{III}, MMAs, DMAs and TMAsO were detected in the urine of DMAs-exposed rat. Not all the DMAs seems to be directly eliminated with the urine; a part was metabolized to MMAs, TMAsO and As^{III}.

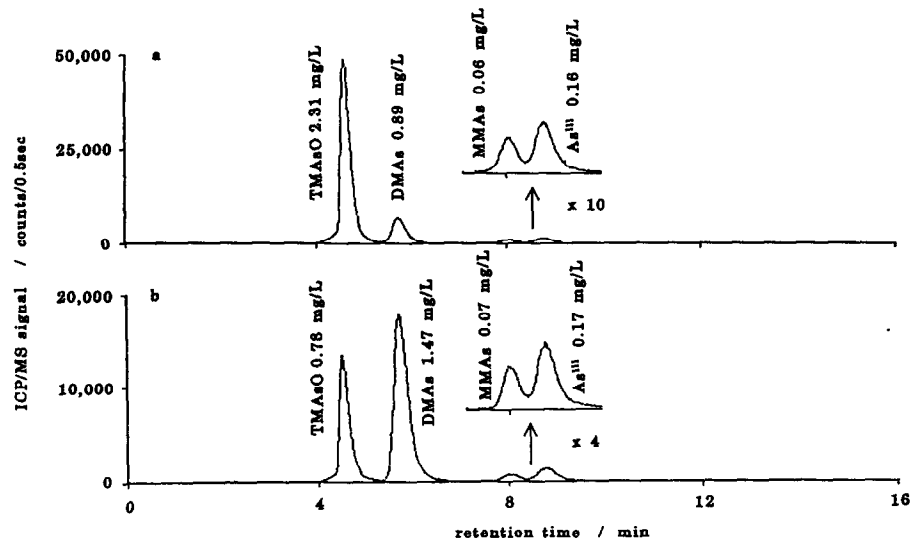

Fig. 5. Chromatograms of arsenic compounds in the urine of DMAs-exposed rats. Conditions as in Tables 2 and 3 except for the injection volume. Sample: (a) 50 mg/l DMAs and (b) 100 mg/l DMAs in drinking water. Injection volume: 50 μl each.

4. Conclusions

An analytical method for the speciation of arsenic compounds is presented. As^{III}, MMAs, DMAs, TMAsO and AsBe were completely separated within 10 min by the IC–ICP-MS method without any interference of ArCl. The detection limits of the five arsenic compounds were better than $4.5 \cdot 10^{-4}$ mg/l. As far as the reproducibility was concerned, the R.S.D. (n = 3) was better than 5%. The IC–ICP-MS system was a sensitive speciation method for arsenic compounds in the urine of DMAs-exposed rats. The IC–ICP-MS system not only demonstrated good detection limits, but also facilitated the sample preparation process. The IC–ICP-MS system will be useful for biological monitoring of arsenic compounds.

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