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Speciation of Arsenic Compounds in the Urine of Rats Orally Exposed to Dimethylarsinic Acid Ion Chromatography with ICP–MS as an Element-Selective Detector

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A combined ion chromatography (IC) with inductively coupled plasma mass spectrometry (ICP—MS) system as an element-selective detector has been used for the determination of arsenic compounds. Seven arsenic compounds were separated by cation-exchange chromatography. Subsequently, the separated arsenic compounds were directly introduced into the ICP—MS and were detected at $m/z=75$. Detection limits for the seven arsenic compounds ranged from 0.8 to 3.8 μg As/l. The IC–ICP–MS system was applied to the determination of arsenic compounds in the urine of dimethylarsinic acid (DMAA)-exposed rats. DMAA was the most abundant arsenic compound detected. Arsenous acid, monomethylarsonic acid and trimethylarsine oxide were also detected.

Keywords: arsenic compounds; speciation; rat urine; ion chromatography (IC); inductively coupled plasma mass spectrometry (ICP–MS); Element-selective detector

INTRODUCTION

Inorganic arsenic compounds have been documented as carcinogens of the skin and lungs.[1] Most mammals, including humans, are able to methylate inorganic arsenic compounds to monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA).[2,3] It is well known that the biological availability and toxicological effects of arsenic compounds depend upon the chemical forms. The methylated arsenic compounds have a lower toxicity and a lower affinity for tissue constituents than the inorganic arsenic compounds. However, recent *in vitro* studies indicate that DMAA may be a potent clastogenic agent and induce chromosome abberations, such as tetraploid formation.[4,5]

A combined inductively coupled plasma mass spectrometry (ICP–MS) and ion chromatography (IC) system is a sensitive and precise tool for speciation studies on trace metal compounds.[6–10] We have reported that anionic and nonionic arsenic compounds in the urine can be determined by a combined IC–ICP–MS system with an anion-exchange mode.[11,12] Five arsenic compounds were separated by the anion-exchange mode within 8 min. The proposed IC–ICP–MS method was applied to the determination of arsenic compounds in the urine of rats chronically exposed to DMAA in studies on cancer induction by DMAA.[13,14] Arsenous acid as the sodium salt [As(III)], MMAA, DMAA and trimethylarsine oxide (TMAO) were detected in the urine of DMAA-exposed rats.

However, changes in retention time of arsenic compounds owing to the large amount of chloride in the urine were observed during the measurements because chloride was very strongly retained in the anion-exchange mode. Furthermore, TMAO often overlapped with an unknown peak eluted at the void volume, because TMAO was weakly retained on the anion column. Qualitative analysis using only one separation mode is not sufficient for other applications, because many forms of arsenic compound are metabolized in the urine. A more accurate toxicological evaluation of arsenic exposure, therefore, should be based on data obtained by several separation modes.

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In this study, cation-exchange chromatography was used instead of anion-exchange chromatography as the separation device. The effectiveness of the IC–ICP–MS system in the cation-exchange mode on the determination of arsenic compounds was evaluated. The IC–ICP–MS system was applied to the determination of arsenic compounds in the urine of DMAA-exposed rats.

MATERIALS AND METHODS

Reagents

Arsenic compounds used in this experiment are listed in Table 1. TMAO was prepared by the oxidation of trimethylarsine (TMA) with 30% hydrogen peroxide. Stock solutions (100 mg As l^{-1}) were prepared by dissolution in pure water. Analytical solutions were prepared by diluting the stock solutions to the required concentration. Other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Instruments

The ion chromatograph used in this experiment was a Model IC7000 from Yokogawa Analytical Systems Inc. (Tokyo, Japan). For the separation of arsenic compounds, three separation modes — ion exclusion chromatography (IEC), size exclusion chromatography (SEC) and cation-exchange chromatography (CEC) — were used. All columns were purchased from Yokogawa Analytical Systems Inc. Excelpak CHA-E11 (300 mm × 7.8 mm i.d.), which is packed with sulphonated polystyrene resin with 3.5 mequiv/g dry wt was chosen as the IEC column. Excelpak SEC-W12 (300 mm × 7.8 mm i.d.) which is packed with non-ionic hydrophilic polymer resin, was chosen as the SEC column. Excelpak ICS-C45 (150 mm × 4.6 mm i.d.) packed with polymer-based hydrophililc cation-exchange resin with 2.3 mequiv/g of dry wt was chosen as the CEC column. Nitric acid was chosen as the mobile phase.

The ICP–MS instrument used in this experiment was a Model HP4500 from Hewlett–Packard (DE, USA). ICP–MS operational conditions are described in Table 2. A 500 mm × 0.3 mm i.d. poly(ethylene tetrafluoroethylene) tube was used to connect the IC column and the nebulizer of the ICP–MS.

Table 1. Arsenic compounds used in this study

Compound	Manufacturer
Arsenous acid [As(III)] Na salt	Waka Pure Chemical Industries (Osaka, Japan)
Arsenic acid [As(V)]Na_2 salt	Wako Pure Chemical Industries
Monomethylarsonic acid (MMAA)	Tri Chemical Laboratory (Yamanashi, Japan)
Dimethylarsinic acid (DMAA)	Sigma (MO, USA)
Trimethylarsine (TMA)	Strem Chemicals (MA, USA)
Arsenobetaine (AsBe)	Tri Chemical Laboratory
Arsenocholine (AsC)	Tri Chemical Laboratory
Tretramethylarsonium iodide (TMAI)	Tri Chemical Laboratory

RESULTS AND DISCUSSION

Selection of separation column

The separation of the arsenic compounds by three separation modes was first examined. 0.005 mol/l nitric acid was used as the mobile phase, and the concentrations of nitric acid were changed to suit each column.

On IEC separation, inorganic arsenic compounds such as AS(III) and arsenic acid [AS(V)] were completely separated, but organoarsenic compounds except MMAA and DMAA were not eluted. On SEC separation, five arsenic compounds, i.e. As(III), As(V), MMAA, DMAA and TMAO, were separated, but cationic arsenic compounds overlapped with these arsenic compounds. The SEC mode will be used for the determination of arsenic compounds in the urine, because these five arsenic compounds are the

Table 2. ICP–MS operational conditions

Radio-frequency forward power	1300 W
Radio-frequency refracted power	< 1 W
Plasma gas flow	16.0 l min^{-1}
Auxiliary gas flow	1.00 l min^{-1}
Carrier gas flow	1.06 l min^{-1}
Sampling point	6 mm from load coil
Detection mass	m/z=75 (As) and m/z=77 ($^{40}Ar^{37}Cl$)
Dwell time	0.5 s (m/z=75) and 0.05 s (m/z=77)
Number of scans	1

main metabolic arsenic compounds in urine. However, it was decided that the SEC mode was not suited to the determination of arsenic compounds in urine of the DMAA-administered rats, (which was the purpose of this study) because of lack of resolution of MMAA and DMAA. On the other hand, seven arsenic compounds, not including As(V), were completely separated by the CEC mode. Nitric acid was used as a mobile phase, and the concentrations of nitric acid for IEC, SEC, and CEC modes were chosen to be 0.002 mol l^{-1}, 0.005 mol l^{-1} and 0.01 mol l^{-1}, respectively. The ion chromatograph was operated with the following conditions: flow rate of the mobile phase 1.0 ml/min, column temperature 40°C, and injection volume 50 μl. According to the results of preliminary experiments, Excelpak ICS-45 was chosen as the separation column.

Separation of arsenic compounds in cation-exchange mode

Several parameters, such as concentration of mobile phase, mobile phase flow rate and column temperature, were examined in order to produce optimal conditions on the CEC model. The optimized IC operational conditions were determined to be as follows: mobile phase 0.008 mol l^{-1} nitric acid; flow rate 1.0 ml min^{-1}; column temperature 50 °C.

A chromatogram of the seven standard arsenic compounds is shown in Fig. 1. The concentration of each of the seven standards was 0.1 mg As l^{-1}. Arsenobetaine (AsBe), arsenocholine (AsC) and tetramethylarsonium iodide (TMAI) were separated based on the cation-exchange mechanism. TMAO was also clearly retained. It is speculated that the cationic character of TMAO is due to protonation of the As=O bond under acidic conditions.[10] On the other hand, As(III) and MMAA showed poor retention due to ion exclusion interaction based on the charge of the packing materials. Although As(V) was not added to the sample solution, a small peak of As(V), which might be produced by oxidation of As(III), was observed immediately before the MMAA peak.

Method statistics

The detection limits and the reproducibility (RSD) for the seven arsenic compounds were calculated using 0.1 mg As l^{-1} standard solutions by injecting a 50 μl sample. The detection limits were calculated from three times the baseline noise. The reproducibility for each standard was obtained from five replicates of the peak area. Table 3 gives the detection limits and the reproducibility for the arsenic compounds.

Interference from chloride

In ICP–MS with the current introduction method, interference of the polyatomic ion $^{40}Ar^{37}Cl^{+}$ at $m/z=75$ due to high chloride content in the sample solution has been observed.[9,15,16] In order to check the $ArCl^{+}$ ion interference at $m/z=75$, a 1000 mg l^{-1} chloride solution was

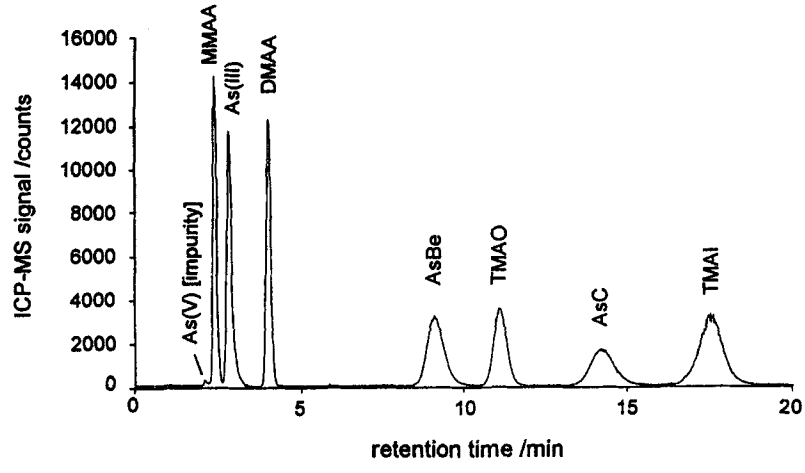

Figure 1 Chromatogram of seven standard arsenic compounds. Separation conditions are described in the text. ICP–MS operational conditions are as given in Table 2. Sample, 0.1 mg As l^{-1} each; injection volume, 50 μl. As(V) was not added to the sample solution.

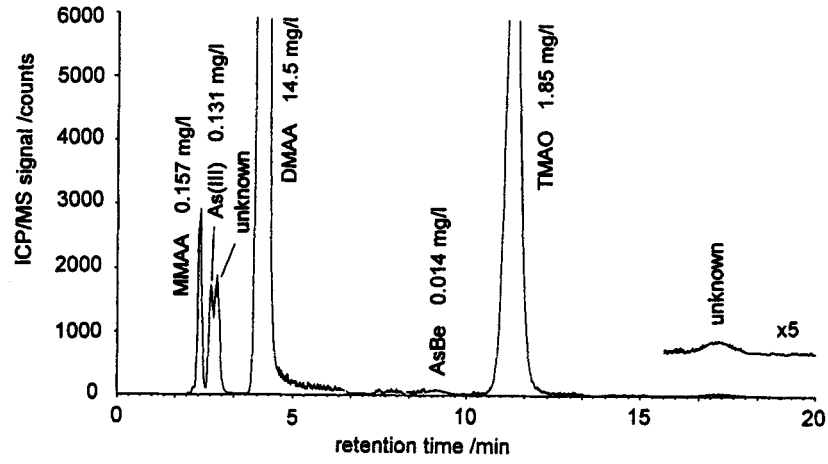

Figure 2 Chromatogram of arsenic compounds in the diluted urine of DMAA-exposed rats at 4 h after dosage. Conditions are as in Fig. 1.

analysed and was detected at $m/z=75$ and 37. An $ArCl^+$ ion was observed immediately before the peak of MMAA. The peak area of the $ArCl^+$ ion produced from 1000 mg l^{-1} chloride corresponded to that of 1.3 μg As l^{-1}

Application to the determination of arsenic compounds in rat urine

The IC–ICP–MS system was applied to the determination of metabolic arsenic compounds in the urine of rats to which DMAA had been administered. Rats were given 50 mg DMMA/kg body wt by a single oral administration. Urine samples collected by forced urination at 4 h after dosage were used. The urine was diluted 20-fold with pure water and 50 μl of the diluted urine was injected into the IC–ICP–MS system.

As(III), MMAA, DMAA, TMAO and AsBe were clearly detected in the urine at 4 h after the dosage, while trace DMAA and AsBe was detected in the urine at 0 h, that is the blank urine. Chromatogram of the diluted urine at 4 h after the dosage is shown in Fig. 2. DMAA was the most abundant arsenic compound in the urine. Relatively high proportions of TMAO were observed in the chromatogram. Lesser amounts of MMAA and As(III) were also detected. Furthermore, two unknown peaks were detected in chromatogram. It is estimated that the former and latter unknown peaks are anionic and cationic arsenic compounds, respectively, due to their retention. Even though it has been reported that AB was not produced in mammals,[17] AsBe was detected in the urine of DMAA exposed rats. It is concluded that AsBe came from the feed, because the concentration of AsBe in the urine at 4 h was equivalent to that in the blank urine.

In conclusion, the IC–ICP–MS system with cation-exchange chromatography as the separation device was found to be a sensitive speciation method for metabolic arsenic compounds in the urine of DMAA-exposed rats. The present method not only demonstrated good detection limits, but also facilitated the sample preparation process. The IC–ICP–MS will be useful for biological monitoring and toxicological evaluation of arsenic compounds.

Table 3. Comparison of detection limits and reproducibility

Arsenic compound	Detection limit (μg As l^{-1})	RSD ($n=5$) (%)
As(III)	0.83	0.74
MMAA	0.73	1.25
DMAA	0.81	0.32
TMAO	2.20	1.62
AsBe	2.09	1.03
AsC	3.88	1.07
TMAI	3.27	2.75

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