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Simultaneous determination of neutral, anionic and cationic compounds within one chromatographic run using an inductively coupled plasma mass spectrometer as element-specific detector†

Tetsushi Sakai,[1]* Yoshinori Inoue,[1] Yukiko Date,[1] Tetsuya Aoyama,[2] Kaoru Yoshida[3] and Ginji Endo[3]

[1]Division of R&D, Yokogawa Analytical Systems Inc., 15-5 Naka-cho 1-chome, Musashino-shi, Tokyo 180-8543 Japan

[2]Yamazaki R&D Group, Chemical Product Division, Hitachi Chemical Co. Ltd, 13-1 Higashi-cho 4-chome, Hitachi-shi, Ibaraki, 317-8555 Japan

[3]Department of Preventive Medicine and Environmental Health, Medical School, Osaka City University, 4-54 Asahi-machi 1-chome, Abeno-ku, Osaka 545-8585 Japan

Two simple methods for the simultaneous separation of anionic and cationic arsenic species in a single injection were developed. One method was a dual column system connected with anion and cation exchange columns; the other was a dual mode system. The dual mode system was a combination of ion exclusion and cation exchange modes. The cation exchange resin, functionalized by dicarboxylic acid, was used for the dual mode column. Separation conditions for the two separation methods were optimized for eight arsenic species. The eight arsenic standards were completely separated within 40 min in both methods. On the dual column system, a good reproducibility (peak area reproducibility was 5% and under) for arsenic species was obtained by applying the column switching method, although the retentions of anionic arsenic species did fluctuate by interference of strong adsorptive anions. Inductively coupled plasma mass spectrometry as an element-selective detector was used. Detection limits for the eight arsenic compounds ranged from 0.1 to 0.7 μg As dm^{-3}, and reproducibility (relative standard deviation, *n* = 8) ranged from 2.4 to 8.0% at 0.01 mg As dm^{-3}. The simultaneous separation methods developed were applied to the determination of arsenic species in human and rat urine. Good agreement on the quantified values by the two separation methods was obtained for all arsenic species in the urine samples. Copyright © 2001 John Wiley & Sons, Ltd.

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INTRODUCTION

The importance of speciation of arsenic species in biological and environmental samples is well recognized, because the different arsenic compounds have different chemical and toxicological properties. Inorganic arsenic species are carcinogenic compounds to humans, giving rise to both skin and lung cancer.[1] Monomethylarsonic acid (MMAA), dimethylarsinic acid (DMAA) and trimethylarsine oxide (TMAO), produced by methylation in mammals,[2–4] are less toxic than inorganic arsenic species.[5] Arsenobetaine (AB) and arsenocholine (AC), regarded as non-toxic, are

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* Correspondence to: Tetsushi Sakai, R&D, Yokogawa Analytical Systems Inc., 15-5 Naka-cho 1-chome, Musashino-shi, Tokyo 180-8543, Japan.

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Table 1 ICP-MS operational conditions

Instrument	Model HP4500
Nebulizer	Concentric nebulizer
Radio frequency forward power	1.45 kW
Plasma gas flow	Ar, 15 l min^{-1}
Auxiliary gas flow	Ar, 1.0 l min^{-1}
Carrier gas flow	Ar, 1.21 l min^{-1}
Sampling depth	7.7 mm from load coil
Monitoring mass	$m/z = 75$
Dwell time	0.5 s

present in sea food, but are not produced in mammals.[6]

Simultaneous separation of oppositely charged ionic arsenic species by high performance liquid chromatography (HPLC) is troublesome. Two analyses, one for anions and the other for cations, inhibit the requirement to improve sample throughput. In order to attain a requirement for the simultaneous separation of inorganic anions and cations in a single analysis, several techniques have been reported. One of these is a dual column system, such as the use of anion and cation separation columns.[7] The dual column system is the simplest of the simultaneous separation techniques. Ding *et al.*[8] have also reported simultaneous separation using a mixed-bed column packed with anion and cation exchange resin. Tanaka *et al.*[9] have reported simultaneous separation by a dual mode system, using a cation exchange column in which cations were separated by cation exchange mode and anions were separated by ion exclusion mode. Hu and Haraguchi[10] have developed a bimodal packing material coated with weakly/strongly charged zwitterionic bile salt micelles. Using the bimodal packing material, ions were separated by electrostatic attraction and repulsion of analyte ions.

Cation exchange chromatography has been applied to the separation of arsenic species.[11–14] In this method, anionic species such as arsenic(V) and MMAA was separated by ion exclusion mode. However, their separation is insufficient because it depends on column efficiency only. Pongratz[15] has reported a unique separation of six arsenic species, i.e. arsenic(III), arsenic(V), DMAA, MMAA, AB and AC using a ratex-type anion exchange resin. The ratex-type anion exchange resin was prepared by electrostatic interaction between anion exchange ratex and cation exchange resin. Consequently, anion and cation arsenic species were separated by a dual mode based on anion and cation exchange sites on the resin.

The purpose of the present study was to develop a simple, selective and highly sensitive method for the simultaneous separation of anionic and cationic arsenic species. For the separation of arsenic species, a dual column system and a dual mode system were evaluated. Inductively coupled plasma mass spectrometry (ICP-MS) was used as an element-selective detector. The method developed was applied to the determination of arsenic species of urine samples.

EXPERIMENTAL

Reagents

Arsenous acid [arsenic(III)] sodium salt and arsenic acid [arsenic(V)] disodium salt were purchased from Wako Pure Chemical (Osaka, Japan), and DMAA was purchased from Sigma (St. Louis, MO, USA). Other arsenic species were purchased from Tri Chemical Laboratory (Yamanashi, Japan). Stock solutions (100 mg As dm^{-3}) of each arsenic compound were prepared by dissolving with deionized water, and they were diluted for the analytical solution to the required concentration just prior to use. Other reagents were purchased from Wako Pure Chemical. Deionized water obtained from a Milli-Q system (Nihon Millipore, Tokyo, Japan).

Instrumentation

HPLC was carried out using an HP1100 series chromatography (Hewlett-Packard Co., Wilmington, DE, USA). The ICP-MS system used as element-selective detector was an HP4500 (Hewlett-Packard). ICP-MS operation conditions are described in Table 1. A 500 mm × 0.3 mm i.d. poly[ethylenetetrafluoroethylene] tube was used to connect the separation column to the nebulizer of the spectrometer.

For the dual column system, a Gelpack GL-IC-A15 and GL-IC-C75 (Hitachi Chemical Co. Ltd, Tokyo, Japan) were used. The IC-A15 (150 mm × 4.6 mm i.d.) is packed with an anion exchange resin (anion exchange capacity of 50 μeq g^{-1}). The IC-C75 (150 mm × 4.6 mm i.d.) is packed with a dicarboxylic-acid-type cation exchange resin (cation exchange capacity of 2.5 meq g^{-1}). For the dual mode system with the cation exchange

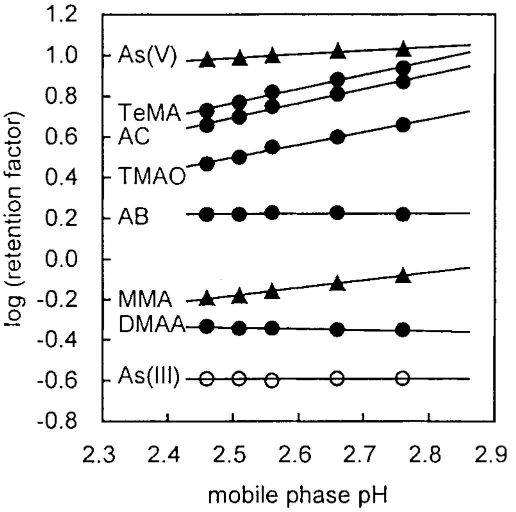

Figure 1 Effect of the mobile phase pH on the retention of arsenic species on the dual column system. Column, Gelpack GL-IC-A15 + C75; mobile phase, 4 mmol dm^{-3} phosphate buffer; flow rate, 1.2 ml min^{-1}; column temperature, 40 °C; detector, ICP-MS *m/z* = 75. Samples, 0.1 mg As dm^{-3} each; injection volume 50 μl.

resin, a Gelpack GL-IC-C75L (250 mm × 6.0 mm i.d.), which is a large column of IC-C75, was used. A Gelpack GL-IC-EM, which was packed with dihydroxymethacrylate gel, was used as a Guard column of the IC-C75L.

A phosphate buffer solution was used as the mobile phase for the dual column system. The phosphate buffer solution was made with H_3PO_4, and the pH adjusted with 1 mol dm^{-3} NaOH. A 5 mmol dm^{-3} oxalic acid solution was used as the mobile phase for the dual mode system. HPLC was carried out under the following conditions: mobile phase flow rate, 1.2 ml min^{-1}; column temperature, 40 °C; and injection volume, 50 μl.

Urine samples

Adult male F344/DuCrj rats were obtained from Charles River Japan (Hino, Japan). Rats were given 100 mg dm^{-3} of DMAA in drinking water. Urine was collected by forced urination after 12 weeks of administration. The urine samples were centrifuged to remove particulate materials and stored at −20 °C until analysis. The urine sample for analysis was diluted 50 times by deionized water, and then was passed through an ODS cartridge to eliminate hydrophobic compounds. 50 μl of the eluate was injected into the LC–ICP-MS system for analysis.

Human urine samples for analysis were diluted ten times by deionized water, and then treated by the same procedure as rat urine. 50 μl of the treated human urine was injected into the LC–ICP-MS system for analysis.

RESULTS AND DISCUSSION

Optimization of separation conditions for arsenic species

First, the separation conditions on the dual column system were optimized. With ion exchange chromatography, dissociation control of species is an important process, because retention and resolution of analyte depend on their ionicity. In arsenic species, when the mobile phase pH rises, the retention times of anions on the anion exchange resin will increase, and those of cations on the cation exchange resin will decrease. The effect of mobile phase pH on the retention factor k' ($k' = (t_r - t_0)/t_0$, where t_r is the retention time, and t_0 is the dead volume ratio) of the arsenic species was investigated. Acidic phosphate buffer was chosen as the mobile phase because DMAA, TMAO and AB act as cationic species due to protonation under acidic conditions.[12,13]

The retention behavior of arsenic species under 4 mmol dm^{-3} of phosphate buffer in the pH 2.4 to 2.8 region is given in Fig. 1. The retentions of cationic species such as TMAO, TeMA and AC increased as pH rose. That of MMAA also increased, but that of DMAA decreased. On the other hand, those of arsenic(V) and AB did not vary. An increase of the retention for cationic species will be caused by an increase in the ionization of the dicarboxylic group on the IC-C75. The behavior of MMAA and DMAA can be interpreted as an increase of anionicity and decrease of cationicity respectively. Acid dissociation constants pK_a of arsenic(V) (pK_{a_1} 2.20, pK_{a_2} 6.97, pK_{a_3} 11.53) are almost the same as those of the phosphate (pK_{a_1} 2.15, pK_{a_2} 7.20, pK_{a_3} 12.38) mobile phase.[16] Consequently, an increase in the ionization of arsenic(V) did not contribute to an increase in retention, because phosphate acted equally. The retention behavior of AB in this pH region was appropriate, considering its pK_a of 2.18.

A good separation for eight arsenic species was obtained by using 4 mmol dm^{-3} phosphate buffer at

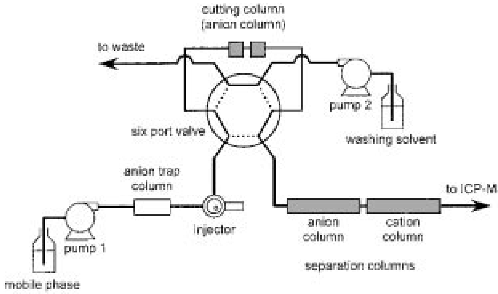

Figure 2 Schematic flow diagrams of column switching system (dual column system).

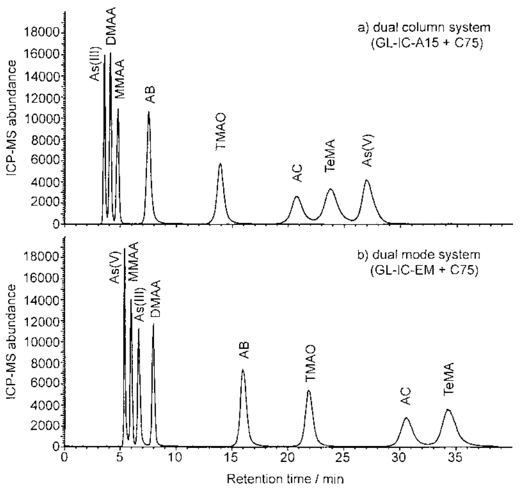

Figure 3 Chromatograms of eight standard arsenic species on the dual column system (a) and dual mode system (b). Conditions (a): column, Gelpack GL-IC-A15 + C75; mobile phase, 4 mmol dm^{-3} phosphate buffer pH 2.6; flow rate, 1.2 ml min^{-1}; column temperature, 40 °C; detector, ICP-MS $m/z = 75$. Samples: As(III), MMAA, DMAA, 0.05 mg As dm^{-3} each; AB, TMAO, AC, TeMA, As(V), 0.1 mg As dm^{-3} each; injection volume 50 µl. Conditions (b): column, Gelpack GL-IC-EM + C75L; mobile phase, 5 mmol dm^{-3} oxalic acid; flow rate, 1.2 ml min^{-1}; column temperature, 40 °C; detector, ICP-MS $m/z = 75$. Samples: As(V), As(III), MMAA, DMAA, 0.05 mg As dm^{-3} each; AB, TMAO, AC, TeMA, 0.1 mg As dm^{-3} each; injection volume 50 µl.

pH 2.60. However, the retentions of arsenic(V) and MMAA decreased sharply in the repeatability test using a test sample that included 100 mg dm^{-3} of chloride ions. It was estimated that the ion exchange capacity of the anion exchange column was temporarily decreased by adsorption of chloride ions, because the retentions of cation species did not vary. It was confirmed that chloride ions were eluted at 96 min by injecting chloride ions at 1000 g dm^{-3} into this system. In addition, impurity anions in the mobile phase might also have affected the decreases of retention for arsenic species.

For elimination of inorganic anions, a column switching system (Fig. 2) was applied. Two IC-A13G (10 mm × 4.6 mm i.d.), with the same packings as IC-A15, were chosen as the cutting column. Strong adsorptive anions were trapped on the cutting column, and then washed out by washing solvent (the same as the mobile phase) after the six-port valve was switched. The 3.8 min switching time was determined by considering the resolution between arsenic(V) and chloride at 1000 mg dm^{-3}. Furthermore, an anion trap column (100 × 4.6 mm i.d.) with the same packings as IC-A15 was used for removing impurity anions in the mobile phase. The repeatability of arsenic species was markedly improved by applying the column switching method.

Table 2 Repeatability[a] ($n = 8$) and system detection limits of arsenic species

	Repeatability	(%)	Detection limit	(µg As dm^{-3})
	A15/C75	EM/C75L	A15/C75	EM/C75L
As(III)	3.29	7.90	0.13	0.17
As(V)	2.28	6.98	0.69	0.09
MMAA	2.95	5.49	—	—
DMAA	4.85	5.58	0.12	0.16
TMAO	3.44	3.58	0.46	0.64
TeMA	3.20	2.57	—	—
AB	4.95	3.46	0.29	0.46
AC	4.74	2.67	—	—

[a] RSD % of peak area (reproducibility).

Table 3 Quantified values of arsenic species in urine samples

	Human urine	(µg As dm^{-3})	Rat urine	(µg As dm^{-3})
	A15/C75	EM/C75L	A15/C75	EM/C75L
As(III)	ND	ND	ND	ND
As(V)	17.0	18.0	Trace	15.3
MMAA	5.65	6.77	ND	8.90
DMAA	57.8	58.3	31 800	33 000
TMAO	ND	ND	16 200	17 500
TeMA	ND	ND	546	561
AB	41.9	43.0	177	178
AC	ND	ND	ND	ND
M-2	ND	ND	15.9	16.8

For the dual mode system, oxalic acid was chosen as the mobile phase, considering its masking ability for metals. The IC-EM, packed with dihydroxymethacrylate gel, was connected, because MMAA and arsenic(III) were completely inseparable using only the IC-C75. The retention of arsenic(III) was increased by hydrophilic interaction,[12,13] and separated from MMAA at a mobile phase concentration of 5 mmol dm^{-3} oxalic acid.

The chromatograms of eight arsenic species under the optimized conditions are given in Fig. 3. The ICP-MS was used as an element-selective detector. The eight arsenic standards were completely separated within 40 min.

Method statistics

The repeatability (RSD) and the system detection limits for the eight arsenic species were calculated using 0.01 mg As dm^{-3} standard solutions by injecting a 50 µl sample. Table 2 gives the system detection limits and the reproducibility for arsenic species. The reproducibility for each standard was obtained from eight replicates of the peak area. The detection limits were calculated from three times the base-line noise ($S/N = 3$), but those of MMAA, TeMA and AC were not calculated because of their low purity.

Interference by chloride cannot be disregarded in the speciation of arsenic species using ICP-MS.[7] The molecular ion $ArCl^+$ will interfere in the detection of arsenic species at $m/z = 75$, if chloride cannot be eliminated or separated. On the dual column system there is no interference by chloride because of use of the column-switching method. On the other hand, although chloride did not interfere for detection using the dual mode system with a cation column, the retention time of arsenic(V) increased slightly as chloride concentration increased. Therefore, a standard addition method was necessary for the quantitative analysis of arsenic(V).

Application to the determination of arsenic compounds in rat urine

The simultaneous separation methods developed were applied to the determination of arsenic species in urine samples. Human urine and rat urine were diluted 10 times and 50 times respectively, and then each 50 µl of the treated urine was injected into the LC–ICP-MS system for analysis. Chromatograms of human urine are given in Fig. 4, and the

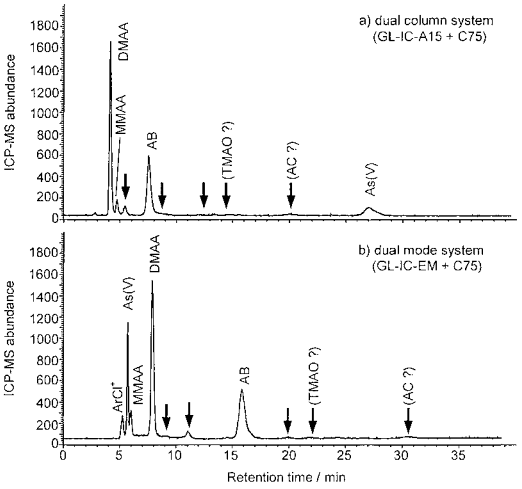

Figure 4 Chromatograms of arsenic species in human urine (ten times diluted) on the dual column system (a) and dual mode system (b). Operating conditions are the same as those given in Fig. 3. '↓' are unknown peaks.

quantified values are given in Table 3. Good results for both separation and quantification were obtained on the measurement of the human urine. For rat urine, the quantified values agreed with the two separation methods except for arsenic(V) and MMAA. Although trace arsenic(V) was observed in the chromatogram on the dual column system, a quantified value for arsenic(V) was not obtained because of the large dilution ratio and the detection limit. For MMAA, its peak was overlapped with the large DMAA peak on the dual column system.

A few unknown peaks were detected in the chromatograms of human urine (Fig. 4). Several further studies will be necessary for the elucidation of these unknown peaks. For the unknown peaks in the rat urine, one was estimated as being the same as M-2 of two unknown peaks reported in our previous paper.[18] The other unknown peak (M-1) reported in our previous paper might be overlapped with a large DMAA or AB peak.

In conclusion, the methods developed in this work, i.e. the dual column system and the dual mode system using cation exchange chromatography, were found to be effective for simultaneous separation of arsenic species in urine samples. The method presented not only demonstrated good separation, but also high sensitivity and selective determination achieved by the combination with ICP-MS. Simultaneous separation methods for anion and cation species will be useful for the biological monitoring and toxicological evaluation of arsenic species.

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