Letters to the Editor

Speciation of Arsenic by Reversed-Phase High Performance Liquid Chromatography-Inductively Coupled Plasma Mass Spectrometry

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Inductively coupled plasma (ICP) is among the most efficient exciting sources for element analysis of liquid samples. Because of the ease of coupling of the plasma with modern liquid chromatographic techniques, many reports have been published on the speciation of various elements, using a combination of high performance liquid chromatography (HPLC) and ICP atomic emission spectrometry (ICP-AES).[1]

Recently, a highly sensitive ICP-based technique, ICP mass spectrometry (ICP-MS), has been developed.[2] ICP-MS shows much higher sensitivity than ICP-AES for many elements, but has some inherent problems, such as interferences by the molecular ions or deterioration of the sensitivity by the deposit of non-volatile materials within the system. Thompson and Houk[3] have reported a separation of four simple arsenic compounds with ion-pair chromatography using tetra-*n*-butyl ammonium (TBA) phosphate for ICP-MS detection. Their system is expected to avoid some of the above problems, though the reported resolution was unsatisfactory.

We report here full separation and highly sensitive detection of fifteen arsenic compounds reported so far from the natural samples, using ICP-MS as the detector; we also report new HPLC conditions including ion-pair techniques for the separation.

Experimental

Materials

The arsenosugars, (XIII)[4] and (XV)[5], were purified from a brown alga, *Sargassum thunbergii*. Dimethylarsinoylethanol, (IX)[6] was kindly donated from Edmonds and Francesconi. The other arsenic standards were prepared as reported previously.[7] Tetramethylammonium (TMA) hydroxide, tetraethylammonium (TEA) hydroxide and malonic acid were the products of Nacalai Tesque, Japan. Nitric acid (EL SS grade; Kanto Chemicals, Japan) was distilled by a quartz sub-boiling system. 1-Butane sulfonic acid sodium salt (IPC grade; Tokyo Kasei, Japan) was recrystallized twice from ethanol/water (95:5, v/v). Water was purified by a Milli-Q water purification system (Millipore Ltd., U.S.A.).

HPLC

A Perkin Elmer 410 Bio LC system was used with an HPLC column of Asahipak GS220 (gel-permeation, 7.6×500 mm: Asahi Kasei, Japan) or Inertsil ODS-2 (reversed-phase, 4.6×250 mm: Gasukuro Kogyo, Japan). The column outlet was connected by a short Teflon tube (i.d. 0.25 mm) to the inlet of a concentric nebulizer.

Optimization of the system

The HPLC conditions were optimized for good separation by monitoring the 228.8 nm As emission line by ICP-AES (JY-38: Seiko Denshi Kogyo, Japan). The operation conditions of an ICP-MS (PMS100: Yokogawa Electric, Japan) were as follows: Ar flow rate, neb. 0.78 l/min, aux. 0.5 l/min, plasma 13 l/min; sampling height 4.5 mm from top of the induction coil; power 1.3 kW.

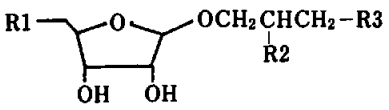
Results and Discussion

Our previous system had employed strong ion exchange columns, and therefore had needed fairly concentrated phosphate buffers (typically 50 mM).[7] To avoid high concentration of salts, especially non-volatile phosphate, from the elution buffers, we tested ion-pair chromatographic conditions. Our approach involved (1) separation of anionic arsenic compounds using tetraalkylammonium ion (TRA) as a pairing ion at neutral pH, (2) separation of cationic arsenic compounds using alkylsulfonate at pH 3, and (3) separation of arsenic compounds with different molecular size on gel-permeation. The second condition was

Table 1 Retention times of fifteen arsenic standards

Compound		Retention time/min[b] LC-1	LC-2	LC-3
(I)	AsO_4^{3-}	5.0	2.8	11.3
(II)	AsO_3^{3-}	3.8	3.7	20[c]
(III)	$CH_3AsO_3^{2-}$	4.4	3.8	11.7
(IV)	$(CH_3)_2AsO_2^-$	4.8	4.3	12.1
(V)	$(CH_3)_3AsO$	4.7	8.0	13.4
(VI)	$(CH_3)_4As^+$	3.9	8.1	14.8
(VII)	$(CH_3)_3As^+CH_2CH_2OH$	3.7	8.7	15.0
(VIII)	$(CH_3)_3As^+CH_2COO^-$	3.9	4.5	12.9
(IX)	$(CH_3)_2As(O)CH_2CH_2OH$	4.5	8.3	13.4
(X)[a]		6.0	11.3	12.7
(XI)[a]		6.9	5.3	10.8
(XII)[a]		6.7	4.6 (4.4)[d]	11.3
(XIII)[a]		4.2	5.8	12.0
(XIV)[a]		8.5	5.9	11.5
(XV)[a]		6.8	6.4	13.2
Cl^-[e]		4.5	5[c]	13.3

a. The structures are;



	R1	R2	R3
(X)	$(CH_3)_2As(O)$–	–OH	–OH
(XI)	$(CH_3)_2As(O)$–	–OH	$-OP(O)_2^-OCH_2CHCH_2OH$ OH
(XII)	$(CH_3)_2As(O)$–	–OH	$-SO_3^-$
(XIII)	$(CH_3)_2As(O)$–	$-NH_3^+$	$-SO_3^-$
(XIV)	$(CH_3)_2As(O)$–	–OH	$-SO_4^-$
(XV)	$(CH_3)_3As^+$–	–OH	$-SO_4^-$

b. Chromatographic conditions; LC-1: Column=Inertsil ODS-2, buffer=10 mM tetraethylammonium hydroxide in water/methanol (99.95:0.05, v/v), pH 6.8 adjusted by malonic acid, 0.75 ml/min; LC-2: Column=Inertsil ODS-2, buffer=10 mM 1-butane sulfonic acid sodium salt–4 mM tetramethylammonium hydroxide–4 mM malonic acid in water/methanol (99.95:0.05, v/v), pH 3.0 adjusted by HNO_3, 0.75 ml/min; LC-3: Column=GS220, buffer=25 mM tetramethylammonium hydroxide–25 mM malonic acid in water, pH 6.8 adjusted by ammonia, 1 ml/min.

c. Broad peak.

d. The minor component (in parentheses) could be separated.[8]

e. The molecular ion, $(^{40}Ar^{35}Cl)^+$, may cause a ghost peak at this position when a large amount of chloride ion is present.

selected because the dimethylarsinoyl group ($(CH_3)_2As(O)$–) protonates at around pH 4.[8]

The final conditions and the retention times are listed in Table 1. Among TMA, TEA, tetra-*n*-propyl ammonium (TPA) and TBA ions, TEA was the most suitable for the separation of anions. The arsenosugar (X) showed some hydrophobicity. It was eluted from the column later than the strong anion, arsenate (I), when TEA was used as a pairing ion. When TPA was used instead of TEA, (X) was eluted earlier, while (I)

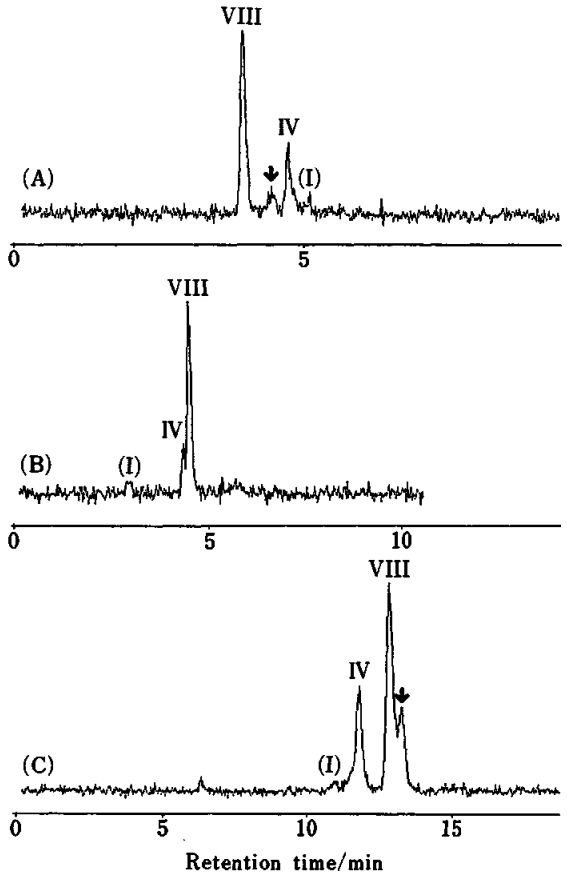


Fig. 1 Chromatograms of human urine sampled after eating a fish. (A) LC-1, (B) LC-2 and (C) LC-3. The sample was centrifuged to remove precipitates, and an aliquot (3 µl for LC-1 and LC-2, and 20 µl for LC-3) of it was injected. The arrows in (A) and (C) indicate the positions of chloride ion (see Table 1, footnote e).

was retained strongly in the column. Combination of TBA with water/acetonitrile mixture did not improve the separation. Among C2 to C4 alkylsulfonates, 1-butane sulfonate was the most appropriate for the separation of cations. A small amount of TMA was added to improve the separation. Malonic acid was used as a buffer. Addition of 0.05% methanol was effective to maintain the column efficiency.

An application of this system is shown in Fig. 1. A human urine sampled after eating a fish was analyzed. Arsenobetaine (VIII) and cacodylate (IV) were detected clearly. Detection limits were 20–50 pg As for LC-1 and LC-2, and 100–150 pg As for LC-3. No noticeable differences of the ICP-MS response among the arsenic species could be observed.[3]

As reported in this study, ion-pair chromatography, if carefully optimized by the standards, seems to be promising as a suitable choice to separate ions and hydrophilic ionic compounds for the HPLC-ICP-MS analysis. Accumulation of the experimental data will be important for further improvement of the system.

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