Journal of Chromatography B, 874 (2008) 64–76

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

A SEC-HPLC-ICP MS hyphenated technique for identification of sulfur-containing arsenic metabolites in biological samples

Badal Kumar Mandal[a,*,1], Kazuo T. Suzuki[b], Kazunori Anzai[c], Kentaro Yamaguchi[d], Yoshihisa Sei[d]

[a] Environmental and Analytical Chemistry Division, School of Science and Humanities, VIT University, Vellore 632014, India
[b] Graduate School of Pharmaceutical Sciences, Chiba University, Chiba 263-8522, Japan
[c] Redox Regulation Research Group, National Institute of Radiological Sciences, Chiba 263-8555, Japan
[d] Chemical Analysis Center, Chiba University, Chiba 263-8522, Japan

ARTICLE INFO

Article history:
Received 7 December 2007
Accepted 3 September 2008
Available online 7 September 2008

Keywords:
Dimethyldithioarsinous acid
Dimethyldithioarsinic acid
Methylmonothioarsonous acid
Methyltrithioarsonic acid and biomolecules

ABSTRACT

The present study describes the synthesis and characterization of thioarsenicals using electro-spray ionization-MS and time of flight-MS. Separation of thioarsenicals was found to be better by size-exclusion column compared to anion exchange column coupled with HPLC-inductively coupled argon plasma mass spectrometer (ICP MS). Although four thioarsenicals were confirmed as dimethylthioarsinous acid (m/z = 138), methylmonothioarsonous acid (m/z = 122), dimethyldithioarsinic acid (m/z = 170) and methyltrithioarsonic acid (m/z = 188), it is noted that HPLC-ICP MS alone were not sufficient for their identification. Also, none of them was stable with time. This is the first report detailing the synthesis and identification of methyltrithioarsonic acid. Both dimethyldithioarsinic acid and dimethylthioarsinous acid were detected in human nail samples while dimethyldithioarsinic acid was found in urine samples. So, the above technique could be applicable to the identification of sulfur-containing biomolecules in the biological samples.

1. Introduction

The toxicity and bioavailability of arsenic (As) depend on its chemical form and oxidation states [1–3]. Speciation of arsenicals in biological samples is an essential tool to get an insight into its distribution in tissues and its species-specific toxicity to target organs. Recently multidisciplinary research with latest hyphenated analytical instrumental techniques has proven that toxicity of As in humans depends not only on different chemical species, but mostly on oxidation states of As [4–8]. All studies on cytotoxicity of arsenicals in several human cell types suggested that regardless of the cell type, cytotoxicity increases in the order of methylarsine oxide ($MeAs^{III}O$) > iododimethylarsine ($DMeAs^{III}$-I); $MeAs^{III}O$ > arsenite (iAs^{III}) in most cell types [5,7,8].

Interestingly, As inhibits several enzymes, such as glutathione reductase [9], thioredoxin reductase [10], and pyruvate dehydrogenase [11]. Arsenic binding with proteins via sulfhydryl groups may be responsible for the observed effects of As on these proteins. As a result, up to six arsenite (iAs^{III}), 10 mthylarsonous acid ($MeAs^{III}$) or 20 dimethylarsinous acid ($DMeAs^{III}$) could bind to each metallothionein (MT) via sulfhydryl groups when iAs^{III}, $MeAs^{III}$, and $DMeAs^{III}$ are in excess [12]. In addition, arsenite can conjugate with glutathione (GSH) and form arsenotriglutathione ($iAs^{III}(GS)_3$), methylarseno-diglutathione ($MeAs^{III}(GS)_2$) and dimethylarsenoglutathione ($DMeAs^{III}(GS)$) [2]. Furthermore, GSH depletion causes essentially nontoxic $MeAs^{V}$ to become toxic [13]. Thus, GSH depletion may also induce the accumulation of toxic inorganic arsenicals in As poisoning patients [14]. Since $MeAs^{III}(GS)_2$ is highly cytotoxic compared to pentavalent organic arsenicals, the effects of extracellular GSH on the cytotoxicity of $MeAs^{V}$ may be due to a small amount of $MeAs^{III}(GS)_2$ generated in the culture medium [15].

Abbreviations: As, arsenic; iAs^{V}, arsenate; iAs^{III}, arsenite; AsC, arsecocholine; AsB, arsenobetaine; DMeAs, dimethylated As; $DMeAs^{III}$, dimethylarsinous acid; $DMeAs^{III}$-I, dimethylarsinous iodide; $DMeAs^{V}$, dimethylarsinic acid; MeAs, monomethylated As; $MeAs^{III}$, methylarsonous acid; $MeAs^{III}$-I_2, methylarsonous diiodide; $MeAs^{V}$, methylarsonic acid; $DMeSAs^{III}$, dimethylthioarsinous acid; $DMeSAs^{V}$, dimethylthioarsinic acid; $DMeDSAs^{V}$, dimethyldithioarsinic acid; $MeSAs^{III}$, methylthioarsonous acid; $MeDSAs^{V}$, methyldithioarsonic acid; $MeTSAs^{V}$, methyltrithioarsonic acid; HPLC, high performance liquid chromatography; ICP MS, inductively coupled argon plasma mass spectrometry; ESI-MS, electro-spray ionization-MS; TOF-MS, time of flight-MS.

* Corresponding author at: Chemistry Division, School of Science and Humanities, Vellore Institute of Technology University, Vellore 632014, Tamil Nadu, India. Tel.: +91 416 2202339/9442344774; fax: +91 416 2240411.
E-mail addresses: badalmandal@vit.ac.in, mbadal@hotmail.com (B.K. Mandal).

[1] The author carried out this research work at National Institute of Radiological Sciences and Chiba University in Japan as STA cum JSPS and JSPS Foreign Research Postdoctoral Fellow, respectively.

1570-0232/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2008.09.004

Although the concentration of $MeAs^V$ added to the culture medium was quite high (10 mM), the generation of $MeAs^{III}(GS)_2$ in culture medium is relevant to in vivo As metabolism, as $MeAs^{III}(GS)_2$ is a major metabolite of inorganic arsenicals in bile and concentration of GSH in bile fluid has been shown to increase to more than 10 mM in As-injected rats [16]. Kala et al. [16] reported that intravenously and orally administered iAs^{III} was excreted into bile as two As-GSH (As-GSH) complexes, As triglutathione [$As(GS)_3$] and methylarsenic diglutathione [$CH_3As(GS)_2$], and that these complexes were transported by multidrug resistance associated protein 2/canalicular multispecific organic anion transporter (MRP2/cMOAT) [16]. Also, As has a great affinity to keratin tissue that has high percentage of cysteine, which can bind As via sulfhydryl groups [17].

Recent studies suggested that a few minor As metabolites were also found in urine and organs [17–28]. Among these minor thiolated As metabolites, dimethylthioarsinic acid ($DMeSAs^V$) and dimethyldithioarsinic acid ($DMeDSAs^V$) were the most recent As metabolites discovered in the urine of man and animals [19–22] and rat organs [23–25] in *in vivo* as well as *in vitro* studies. Moreover, marine organisms could transform inorganic As to arsenosugars and arsenobetaines (AsB) and several studies have identified them as minor marine thiolated arsenometabolites [26–28]. Thus, it was proposed that trivalent dimethylated arsenical ($DMeAs^{III}$) was thiolated to the assumed intermediate $DMeSAs^{III}$, and then oxidized to $DMeSAs^V$ and $DMeDSAs^V$ [29]. However, it is not known till now where (i.e., in which organs and subcellular locations) or how these thioarsenicals were produced in the body, and it is also not known how thioarsenicals were metabolized, i.e., similar to or different from the corresponding non-thiolated trivalent arsenicals [29]. As $DMeDSAs^V$ was almost quantitatively excreted in urine in its intact form, this seems to be a more efficiently excreted form than $DMeAs^V$, and hence may be a less toxic form than $DMeAs^V$ [25]. On the other hand, $DMeSAs^V$ is delivered to organs/tissues and then to RBCs as in the case of $DMeAs^{III}$, and is thought to be more toxic than $DMeAs^V$ [21,29,30]. Although some information is available in literature [19–30] on metabolism and toxicity of dimethylated thioarsenicals in humans and animals there is no related literature on monomethylated thioarsenicals. Also, standard monomethylated thioarsenicals are not available commercially.

The main objectives of this research were to (i) synthesize and characterize monomethylated thioarsenicals; (ii) develop a high performance liquid chromatography-inductively coupled argon plasma mass spectrometric technique (HPLC-ICP MS) coupled with a size-exclusion column for their separation and identification and (iii) screen human biological samples such as urine and nails to detect their presence.

2. Experimental

2.1. Reagents

All reagents were of analytical grade. Milli-Q water (Millipore) was used throughout. Trizma® HCl and Trizma® Base were purchased from Sigma (St. Louis, MO, USA). Hydrogen peroxide (30%), sodium sulfide (Na_2S), concentrated sulfuric acid, nitric acid, hydrogen chloride, ammonium acetate, acetic acid, a 28% ammonia solution, L-cysteine (Cys), arsenite (iAs^{III}) as $NaAsO_2$, arsenate (iAs^V) as $Na_2HAsO_4{\cdot}7H_2O$, and dimethylarsinic acid ($DMeAs^V$) as $(CH_3)_2AsO(OH)$ were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Methylarsonic acid ($MeAs^V$) as $CH_3AsO(OH)_2$ was purchased from Tori Chemicals Ltd. (Yamanashi, Japan). Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Arsenobetaine (AsB) was purchased from Tri Chemical Laboratories, Inc. (Yamanashi, Japan). The As standard solution (1000 μg/mL) for ICP MS was purchased from SPEX CentiPrep (Metuchen, NJ, USA). Iodide salts of $MeAs^{III}$ and $DMeAs^{III}$ were gifted by Dr. Kenzo Yamanaka (Nihon University College of Pharmacy, Funabashi, Chiba 274-8555, Japan). Stock solutions of all As compounds (100 mM) were prepared in purified water, stored in the dark at 4 °C, and diluted every time prior to use.

2.2. Instruments

The HPLC system consisted of a PU-610 liquid chromatograph solvent delivery pump and a DG 660B-2 degasser (GL Sciences Co., Tokyo, Japan). A polymer-based gel filtration column (Shodex Asahipak GS 220 HQ, 300 mm × 7.6 mm i.d.; Showa Denko, Tokyo) with an exclusion limit of 3 kDa was used to separate low and high molecular weight constituents. A 20 μL aliquot of a sample solution was applied to the column and the column was eluted with 50 mM ammonium acetate buffer (pH 6.5 at 25 °C). An anion exchange column (Shodex Asahipak ES-502N 7C, 100 mm × 7.6 mm i.d., Showa Denko, Tokyo) was used. The column was equilibrated with the mobile phase (15 mM citric acid monohydrate, pH 2.0, at 25 °C) at a flow rate of 1.0 mL min^{-1} for at least 1 h before any sample injection. The pH of the mobile phase was adjusted to 2.0 at 25 °C with 10% HNO_3. Arsenic in the eluate was monitored with a HP 4500 ICP MS (Yokogawa Analytical Systems, Hachiouji, Japan) at $m/z = 75$. The signal at $m/z = 77$ was also monitored to compensate for the molecular interference by $^{40}Ar^{35}Cl^+$. On-line ICP MS data were processed with software developed in-house.

2.3. ESI MS analyses

ESI MS analyses were performed in both negative and positive modes using a four-sector (BE/BE) tandem mass spectrometer (JMS-700T, JEOL) equipped with a cold-spray ionization (CSI) source [31]. Nitrogen was used as a nebulizing gas at a pressure of 50 psi and a temperature of 200 °C. Typical ESI measurement conditions were as follows: acceleration voltage, 5.0 kV; needle voltage, 1.6 kV; needle current, −100-0 nA; orifice voltage, 40–73 V; resolution (10% valley definition), 1000; sample flow rate, 0.5 mL/h; solvent, methanol:ethanol (1:9); concentration, 0.25 mmol; spray temperature, 20 °C; ion source temperature, 200 °C for negative mode (ESI-); and acceleration voltage, 5.0 kV; needle voltage, 3.6 kV; needle current, 6000–7000 nA; orifice voltage, 70–94 V; resolution (10% valley definition), 1000; sample flow rate, 0.5 mL/h; solvent, chloroform; concentration, 0.20 mmol; spray temperature, 20 °C; ion source temperature, 200 °C for positive mode (ESI+). Simultaneously, all compounds were checked using a CSI source. MS instrument was calibrated accurately using reserpine ($C_{33}H_{40}N_2O_9$ as m/z: 608.69) as standard reference material. Peak intensity was 100% against m/z 608.69 (data not shown).

2.4. ESI-TOF MS analyses

Electrospray ionization-time of flight MS (ESI-TOF MS) analyses were performed in negative mode using a TOF mass spectrometer (JMS-T100LC, JEOL). Nitrogen was used as a nebulizing gas at a pressure of 50 psi and a temperature of 250 °C. Typical ESI-TOF measurement conditions were as follows: acceleration voltage, 2.0 kV; needle voltage, −200 V^{-1}; orifice voltage, −50 V; orifice temperature, 80 °C; resolution, 6000; sample flow rate, 0.5 mL/h; solvent, methanol; concentration, 0.25 mmol; spray temperature, 20 °C; ion source temperature, 200 °C for negative mode (ESI-) using syringe pump.

2.5. Preparation of dimethylthioarsenicals from dimethylarsinic acid ($DMeAs^V$)

2.5.1. Reay and Asher method

The reduction of $DMeAs^V$ by sodium thiosulfate and sodium disulfite was carried out according to Reay and Asher [32]. Typically, an equivolume of a 10 mM $DMeAs^V$ solution and a reducing solution were mixed and kept at room temperature for 1 h. The reducing solution was prepared by adding 2 mL of 1% sodium thiosulfate and 0.1 mL of concentrated H_2SO_4 to a 280 mg of sodium metabisulfite solution in 15 mL of water.

2.5.2. Hydrogen sulfide gas (H_2S) or sodium sulfide (Na_2S) and sulfuric acid (H_2SO_4) reduction

Dimethylthioarsinous acid [$DMeSAs^{III}$ (DMeAs-A)] was prepared by stepwise addition of H_2SO_4 to a purified water solution-containing $DMeAs^V$ and Na_2S at a final molar ratio of $DMeAs^V$:Na_2S:H_2SO_4 (1:1.6:1.6) in a nitrogen atmosphere and was allowed to stand for 1 h or by bubbling H_2S gas into an ethanol solution of $DMeAs^V$ for 24 h. $DMeSAs^{III}$ was extractable in organic solvents such as diethyl ether, while $DMeAs^V$ was not. Dimethyldithioarsinic acid [$DMeDSAs^V$ (DMeAs-B)] was prepared similarly to $DMeSAs^{III}$ except for the molar ratio of $DMeAs^V$:Na_2S:H_2SO_4 (1:7.5:7.5) and for a prolonged reaction time (1 day) in an air atmosphere or simply by bubbling H_2S gas into an ethanol solution of $DMeAs^V$ for 1 day. $DMeDSAs^V$ was prepared by stepwise addition of concentrated H_2SO_4 to an aqueous solution of 10 mM $DMeAs^V$ and 75 mM Na_2S with a final molar ratio of $DMeAs^V$:Na_2S:H_2SO_4 (1:7.5:7.5), followed by standing for 1 day. The final solution was kept for 3–4 h at −80 °C and then freeze-dried (VD-80, TAITEC, Tokyo). The freeze-dried residue was dissolved in Milli-Q water, centrifuged, filtered, and analyzed by HPLC-ICP MS.

2.5.3. L-Cysteine (Cys) reduction

10 mM $DMeAs^V$ was incubated with 50 mM cysteine at 72 °C for 1 h to prepare $DMeAs^{III}$-Cys after purging nitrogen gas for 10 min. After cetrifugation at 1500 × *g* for 10 min the supernatant was analyzed by HPLC-ICP MS.

2.6. Preparation of methylthioarsenicals from methylarsonic acid ($MeAs^V$)

2.6.1. Reay and Asher method

The reduction of $MeAs^V$ by sodium thiosulfate and sodium disulfite was carried out according to Reay and Asher [32]. Typically, an equivolume of a 10 mM $MeAs^V$ solution and a reducing solution were mixed and kept at room temperature for 1 h. The reducing solution was prepared were prepared by adding 2 mL of 1% sodium thiosulfate and 0.1 mL of concentrated H_2SO_4 to a 280 mg of sodium metabisulfite solution in 15 mL of water.

2.6.2. Hydrogen sulfide gas (H_2S) or sodium sulfide (Na_2S) and sulfuric acid (H_2SO_4) reduction

Methylthioarsonous acid [$MeSAs^{III}$ (MeAs-A)] was prepared by stepwise addition of Na_2S aqueous solution to a mixture of 100 mM H_2SO_4 and 20 mM $MeAs^V$ at a final molar ratio of $MeAs^V$:Na_2S:H_2SO_4 (1:1:5) and allowed for 1 h for the completion of reaction after purging nitrogen gas for 10–20 min. $MeSAs^{III}$ was extractable in organic solvents such as chloroform, while $MeAs^V$ was not. Methyldithioarsonic acid [$MeDSAs^V$ (MeAs-B)] was prepared by stepwise addition of Na_2S aqueous solution to a mixture of 100 mM H_2SO_4 and 20 mM $MeAs^V$ at a final molar ratio of $MeAs^V$:Na_2S:H_2SO_4 (1:1.5:5) and allowed for 1 h for the completion of reaction after purging nitrogen gas for 10–20 min. Methyltrithioarsonic acid [$MeTSAs^V$ (MeAs-C)] was prepared by stepwise addition of Na_2S aqueous solution to a mixture of 100 mM H_2SO_4 and 20 mM $MeAs^V$ at a final molar ratio of $MeAs^V$:Na_2S:H_2SO_4 (1:7.5:5) and allowed for 1 day for the completion of reaction after purging nitrogen gas for 10–20 min. The final solution was kept for 3–4 h at −80 °C and then freeze-dried (VD-80, TAITEC, Tokyo). The freeze-dried residue was dissolved in Milli-Q water, centrifuged, filtered, and analyzed by HPLC-ICP MS.

2.6.3. L-Cysteine (Cys) reduction

30 mM $MeAs^V$ was incubated with 50 mM cysteine at 72 °C for 1 h to prepare $MeAs^{III}$-Cys after purging nitrogen gas for 10–20 min. After centrifugation at 1500 × *g* for 10 min the supernatant was analyzed by HPLC-ICP MS.

2.7. Protocol (study group)

The subjects of the present study were from Sahadiarh village of Domkol block in an As-affected Murshidabad district, West Bengal, India, and were drinking As-contaminated water for about 3–10 years during our sample collection. We selected those places where seafoods were not available. They were also asked not to take seafood within 3 days of the collection of urine, hair and fingernail samples.

2.8. Urine collection

Spot urine samples were collected from all studied groups in pre-washed (with Milli-Q water) new polyethylene bottles within daytime (around 10.00 a.m. to 3.00 p.m.). Immediately after collection, the samples were stored in a salt–ice mixture and the samples were kept frozen during the return to home. The frozen urine samples were transferred by air in a cool-fisher to Chiba University, Japan, where they were stored at −28 °C. All urine samples were transported in a salt–ice mixture for ∼72 h.

2.9. Fingernails collection

Fingernail clippings from all fingers were collected both from As-affected population as well as control population using stainless steel scissors and ceramic blade, respectively. All samples were stored individually in sealed plastic bags at room temperature.

2.10. Biological sample preparations for HPLC-ICP MS

All fingernail samples were incubated in Milli-Q water at 90 °C for 0.5 h. After incubation all extracts of fingernails were filtered through 0.45 μm filter (Millex®-HV, Millipore, Japan) prior to injection. Similarly, all urine samples were diluted five-fold with Milli-Q water and filtered through 0.45 μm filter (Millex®-HV) prior to injection.

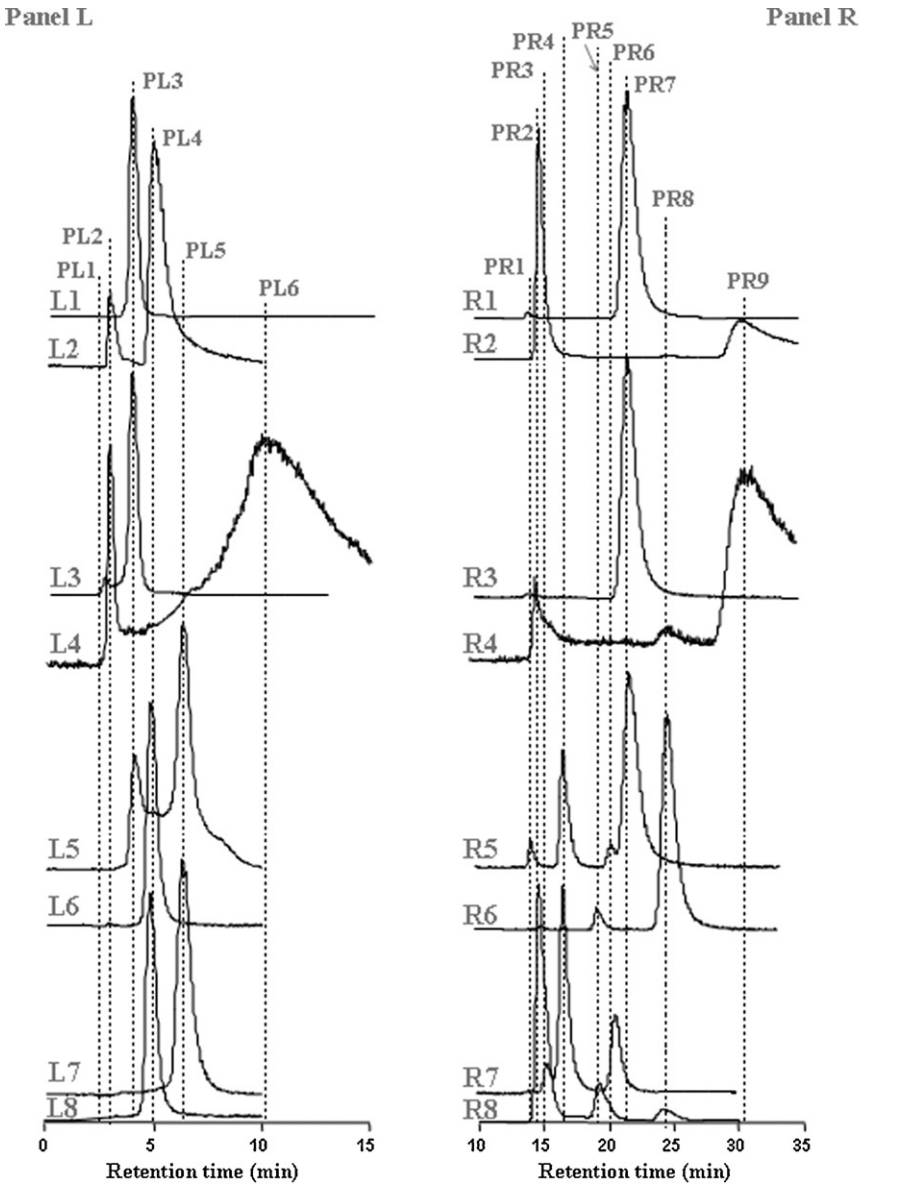
3. Results

All experiments were performed to separate thioarsenicals first by both anion exchange column (Shodex Asahipak ES-502N 7C, 100 mm × 7.6 mm i.d., Showa Denko, Tokyo) and size exclusion column (Shodex Asahipak GS 220 HQ, 300 mm × 7.6 mm i.d.; Showa Denko, Tokyo) and then identified by HPLC-ICP MS, ESI-MS and ESI-TOF MS. Thioarsenicals were synthesized by different techniques and analyzed simultaneously as follows.

3.1. Using an anion exchange column (AEC)

3.1.1. Reaction products of $MeAs^V$ and $DMeAs^V$ under different reducing conditions

Analysis of reduction products of $DMeAs^V$ with sodium thiosulfate and sodium metabisulfite by Reay and Asher method [32] or by hydrogen sulfide gas (H_2S) or sodium sulfide (Na_2S) and sulfuric acid (H_2SO_4), showed a peak at a retention time of 5 min on an AEC (Fig. 1L6 and L8). Also, the authentic sample prepared by hydrolysis of dimethylarsinous iodide ($DMeAs^{III}$-I) showed a peak at the same retention time of 5 min (Fig. 1L2). Therefore, the reaction product was assumed to be $DMeAs^{III}$ [17,18]. But analysis of reduction products of $DMeAs^V$ with cysteine produced a peak at the retention time of 10.5 min (Fig. 1L4), which posed a doubt on its identifica-



PL1 – $MeAs^V$; PL2 – $DMeAs^V$; PL3 – $MeAs^{III}$, $MeAs^{III}$-I, $MeAs^{III}$-Cys;
PL4 – $DMeAs^{III}$, $DMeAs^{III}$-I, $DMeSAs^{III}$, $DMeDSAs^V$; PL5 – $MeAs^{III}$; PL6 – $DMeAs^{III}$-Cys

PR1 – $MeAs^V$; PR2 – $DMeAs^V$; PR3 – MeAs-C; PR4 – MeAs-A; PR5 – $DMeDSAs^V$;
PR6 – MeAs-B; PR7 - $MeAs^{III}$-I, $MeAs^{III}$-Cys; PR8 – $DMeSAs^V$;
PR9 – $DMeAs^{III}$-I, $DMeAs^{III}$-Cys

Fig. 1. HPLC-ICP MS chromatograms of reduced standard $MeAs^V$ and $DMeAs^V$. In Panel (L): (L1) a sample (20 μg As L^{-1}) of $MeAs^{III}$-I_2 by an anion exchange ES-502N 7C column with a mobile phase containing 15 mM citric acid at pH 2.0 with 10% HNO_3 at 25 °C at a flow rate of 1 mL/min; (L2) aqueous $DMeAs^{III}$-I sample; (L3) $MeAs^{III}$-Cys; (L4) $DMeAs^{III}$-Cys; (L5) reduced std. $MeAs^V$ by Reay and Asher; (L6) reduced std. $DMeAs^V$ by Reay and Asher; (L7) reduced std. $MeAs^V$ by Na_2S and H_2SO_4; (L8) reduced std. $DMeAs^V$ by Na_2S and H_2SO_4 and in Panel (R): (R1) an aqueous sample (20 μg As L^{-1}) of $MeAs^{III}$-I_2 analyzed by HPLC-ICP MS using a size exclusion GS-220 HQ with 50 mM ammonium acetate buffer (pH 6.5 at 25 °C) at a flow rate of 0.6 mL min^{-1}; (R2) aqueous $DMeAs^{III}$-I sample; (R3) $MeAs^{III}$-Cys; (R4) $DMeAs^{III}$-Cys; (R5) reduced std. $MeAs^V$ by Reay and Asher; (R6) reduced std. $DMeAs^V$ by Reay and Asher; (R7) reduced std. $MeAs^V$ by Na_2S and H_2SO_4; (R8) reduced std. $DMeAs^V$ by Na_2S and H_2SO_4. The injection volume of the sample was 20 μL. The ion intensities at m/z 75 and 77 were recorded with time-resolved analysis software.

tion as $DMeAs^{III}$ (Panel L of Fig. 1). Analysis of reduction products of $MeAs^{V}$ with sodium thiosulfate and sodium metabisulfite by Reay and Asher method [32] or by hydrogen sulfide gas (H_2S) or sodium sulfide (Na_2S) and sulfuric acid (H_2SO_4), showed a peak at the retention time of 6.2 min (Fig. 1L5 and L7). Therefore, the reaction product was assumed to be $MeAs^{III}$ [17,18]. But, the authentic sample prepared by hydrolysis of methylarsonous diiodide ($MeAs-I_2$) or reduction products of $MeAs^{V}$ with cysteine showed a peak at the retention time of 4 min (Fig. 1L1 and L3), which posed a doubt on its identification as $MeAs^{III}$ (Panel L of Fig. 1). For the identification and quantification of oxoarsenicals AEC was appeared to be suitable but thioarsenicals were not separated al all in the present study. Hence a size exclusion column was used to clarify this doubt.

3.2. Using a size exclusion column (SEC)

3.2.1. Reaction products of $MeAs^{V}$ and $DMeAs^{V}$ under different reducing conditions

When the authentic sample prepared by hydrolysis of $DMeAs^{III}$-I in distilled water under nitrogen atmosphere or reduction products

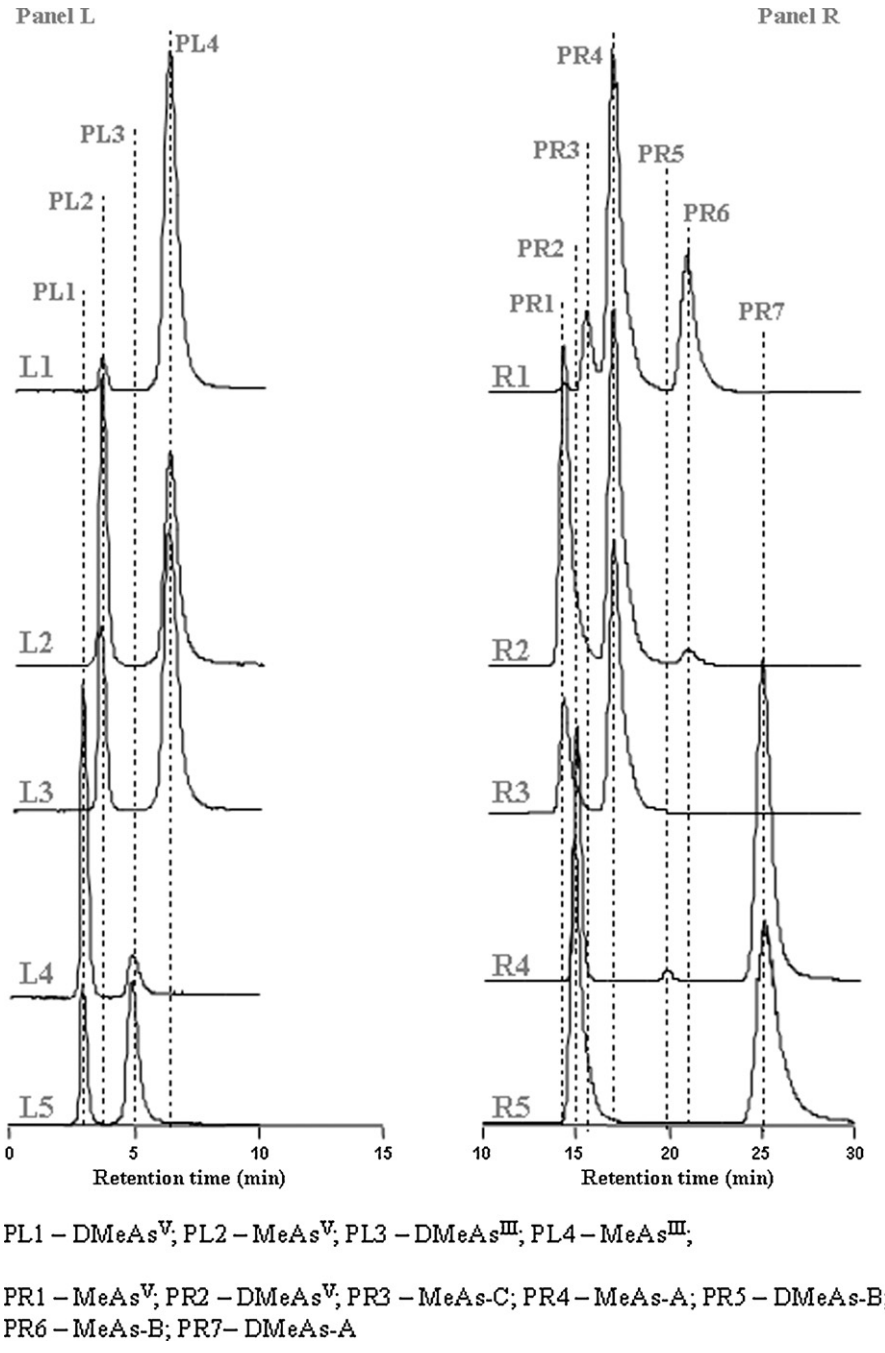


Fig. 2. HPLC-ICP MS chromatograms of reduced standard $MeAs^{V}$ and $DMeAs^{V}$ by Na_2S and H_2SO_4. In Panel (L): (L1) a mixture of 20 mM $MeAs^{V}$ and 100 mM H_2SO_4 was reduced by 150 mM Na_2S and analyzed by an anion exchange; (L2) $MeAs^{V}$ reduced by 30 mM Na_2S; (L3) $MeAs^{V}$ reduced by 20 mM Na_2S; (L4) a mixture of 20 mM $DMeAs^{V}$ and 100 mM H_2SO_4 was reduced by 30 mM Na_2S; (L5) $DMeAs^{V}$ reduced by 20 mM Na_2S. In Panel (R): (R1) a mixture of 20 mM $MeAs^{V}$ and 100 mM H_2SO_4 was reduced by 150 mM Na_2S and analyzed by a size exclusion column; (R2) reduced by 30 mM Na_2S; (R3) reduced by 20 mM Na_2S; (R4) a mixture of 20 mM $DMSAs^{V}$ and 100 mM H_2SO_4 was reduced by 30 mM Na_2S; (R5) reduced by 20 mM Na_2S. Experimental parameters were the same as those described in the legend of Fig. 1.

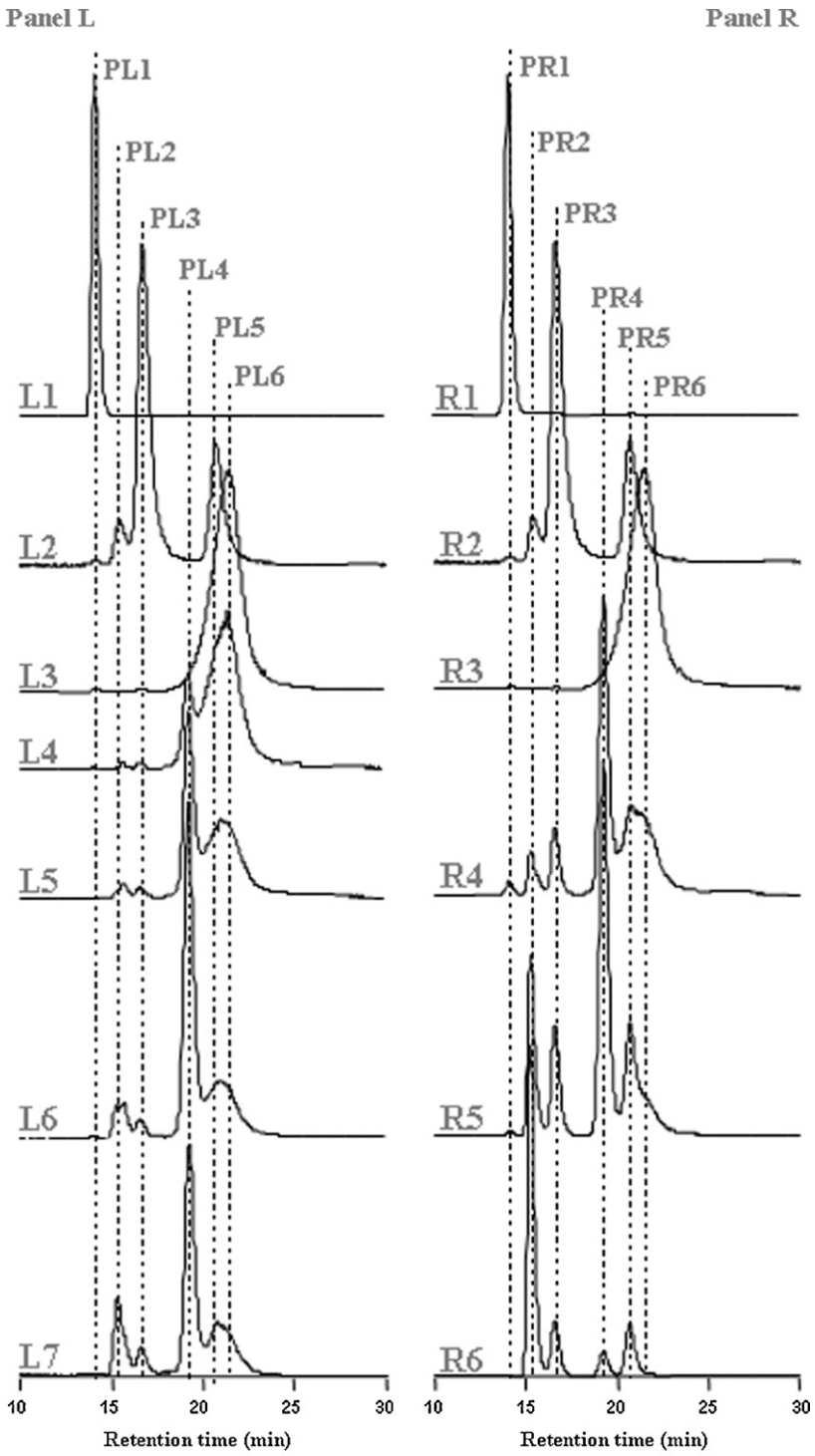


Fig. 3. Effects of Na_2S on MeAs^{III}-Cys. In Panel (L): (L1) 2.0 mM Na_2S was added to 1.0 mM MeAs^{V} and eluted by a size exclusion column after 30 min; (L2) MeAs^{V} reduced by 150 mM Na_2S and 100 mM H_2SO_4; (L3) MeAs^{V} reduced by 150 mM cysteine; (L4) 1.0 mM MeAs^{III}-Cys was mixed with 0.5 mM Na_2S; (L5) 1.0 mM MeAs^{III}-Cys was mixed with 1.0 mM Na_2S; (L6) 1.0 mM MeAs^{III}-Cys was mixed with 2.0 mM Na_2S; (L7) 1.0 mM MeAs^{III}-Cys was mixed with 3.0 mM Na_2S and in Panel (R) all conditions were same as those in Panel (L), but analysis was carried out after 12 h (R1–R6). Experimental parameters were the same as those described in the legend of Fig. 1.

of $DMeAs^{V}$ with cysteine were analyzed by a polymer-based SEC, only one peak appeared at the retention time of 30 min (Fig. 1R2 and R4). Similarly, when the authentic sample prepared by hydrolysis of $MeAs^{III}$-I_2 in distilled water under nitrogen atmosphere or reduction products of $MeAs^{V}$ with cysteine were analyzed, only one peak appeared at the retention time of 22 min (Fig. 1R1 and R3).

In addition, the reaction product of $DMeAs^{V}$ (prepared by hydrogen sulfide gas (H_2S) or sodium sulfide (Na_2S) and sulfuric acid (H_2SO_4) or Reay and Asher method) was separated into two peaks (DMeAs-B and DMeAs-A) (at 19.8 and 25.1 min, respectively) (Fig. 2R4 and R5) while the reduction product of $MeAs^{V}$ (prepared by hydrogen sulfide gas (H_2S) or sodium sulfide (Na_2S) and sulfuric acid (H_2SO_4) or Reay and Asher method) was separated into three peaks (MeAs-C, MeAs-A and MeAs-B) (at 15.2, 17 and 20.2 min, respectively) (Fig. 2R1–R3).

3.2.2. *Effects of Na_2S on synthesized $MeAs^{III}$-Cys and $DMeAs^{III}$-Cys*

When 2 mM sodium sulfide was added to 1 mM $MeAs^{V}$ at room temperature and the reaction mixture was analyzed after 30 min, it showed a peak at 13.5 min (Fig. 3L1), which was $MeAs^{V}$ itself. But three peaks (at 15.2, 17 and 20.2 min, respectively) as shown in Figs. 2R1 and 3L2, appeared with 2 mM Na_2S and 100 mM H_2SO_4. And $MeAs^{III}$-Cys appeared at 22 min (Fig. 3L3). To check the effect of sodium sulfide on $MeAs^{III}$-Cys different concentrations of sodium sulfide were added to 1 mM $MeAs^{III}$-Cys and analyses were carried out after 30 min. A new peak (MeAs-D) appeared at 19 min with 0.50 mM sodium sulfide (Fig. 3L4), whereas other three peaks (MeAs-A, MeAs-B and MeAs-C) appeared along with MeAs-D with increase of sodium sulfide concentrations (1–3 mM) (Fig. 3L5–L7).

Similarly, when 2 mM sodium sulfide was added to 1 mM $DMeAs^{V}$ at room temperature and the reaction mixture was analyzed after 30 min, it showed a peak at 15 min (Fig. 4C1), which was $DMeAs^{V}$ itself. But two peaks (at 19.8 and 25.1 min, respectively) as shown in Fig. 4C2, appeared with 2 mM Na_2S and 100 mM H_2SO_4. And $DMeAs^{III}$-Cys appeared at 30.2 min (Fig. 4C3). To check the effect of sodium sulfide on $DMeAs^{III}$-Cys different concentrations of sodium sulfide were added to 1 mM $DMeAs^{III}$-Cys and analyses were carried out after 30 min. A new peak (DMeAs-C) appeared at 16.8 min with 0.50 mM sodium sulfide (Fig. 4C4) along with other two peaks (DMeAs-A and DMeAs-B) with increase of sodium sulfide concentrations (1–2 mM) (Fig. 4C5 and C6).

3.2.3. *Stability of $MeAs^{III}$-Cys and $DMeAs^{III}$-Cys with time in the presence of Na_2S*

When $MeAs^{III}$-Cys, which appeared at 22 min (Fig. 3L3 and R3), was mixed with different concentrations of sodium sulfide (1–3 mM), different monomethylated thioarsenicals (MeAs-A, MeAs-B, MeAs-C and MeAs-D) appeared (Fig. 3L4–L7 and R4–R6). But after 12 h of addition of sodium sulfide all the initial samples were reanalyzed to check their stability with time of storage and Fig. 3R1–R6 showed their HPLC-ICP MS chromatograms.

MeAs-A was extracted in chloroform, freeze-dried and then the residue was dissolved in Milli-Q water under nitrogen atmosphere. The solution that was kept at room temperature was analyzed repeatedly at 0, 30 and 60 days as shown in Fig. 5R1–R3, respectively. Similarly, DMeAs-A was extracted in diethyl ether, freeze-dried and then the residue was dissolved in Milli-Q water under nitrogen atmosphere. The solution that was kept at room temperature was analyzed repeatedly at 0, 30, 60 and 90 days as shown in Fig. 5L1–L3, respectively.

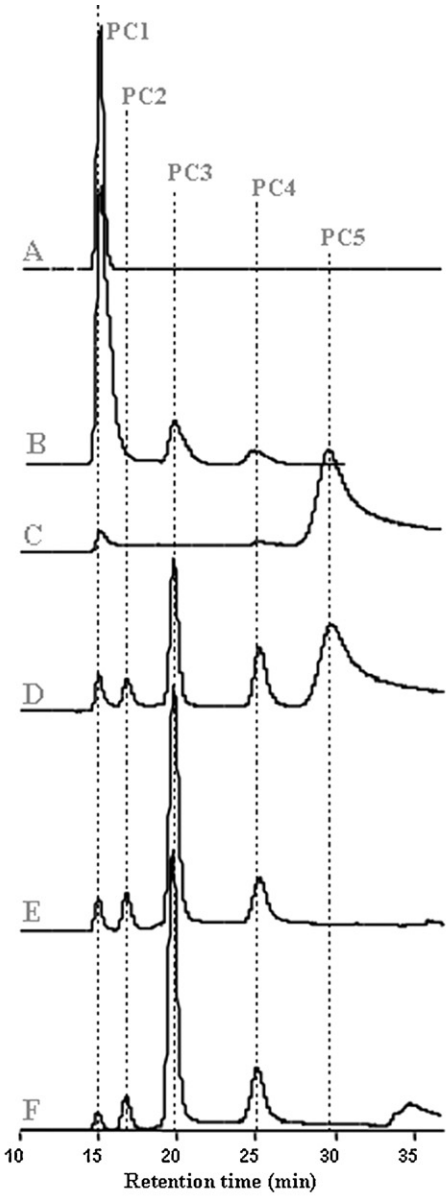


Fig. 4. Effects of Na_2S on $DMeAs^{III}$-Cys: 2.0 mM Na_2S was added to 1.0 mM $DMeAs^{V}$ and eluted by a size exclusion column (4C1); $DMeAs^{V}$ reduced by 150 mM Na_2S and 100 mM H_2SO_4 (4C2); $DMeAs^{V}$ reduced by 150 mM cysteine (4C3); 1.0 mM $DMeAs^{III}$-Cys was mixed with 0.5 mM Na_2S (4C4); 1.0 mM $DMeAs^{III}$-Cys was mixed with 1.0 mM Na_2S (4C5); 1.0 mM $DMeAs^{III}$-Cys was mixed with 2.0 mM Na_2S (4C6). In all cases analyses were carried out after 30 min of the reaction initiation. Experimental parameters were the same as those described in the legend of Fig. 1.

3.3. *Characterization of thioarsenicals by ESI-MS and ESI-TOF MS*

Mass spectrometric data were obtained for DMeAs-A and DMeAs-B by ESI MS with positive and negative ion modes, respectively (experimental details are given in Section 2.3). Molecular masses for DMeAs-A and DMeAs-B were $m/z = 138$ (in positive ion mode, 139) (Fig. 6) and $m/z = 170$ (in negative ion mode, 169) (Fig. 7).

Mass spectrometric data were obtained for MeAs-A and DMeAs-C by ESI MS with positive and negative ion modes, respectively (experimental details are given in Section 2.3). Molecular masses for DMeAs-A and DMeAs-B were $m/z = 122$ (in positive ion mode,

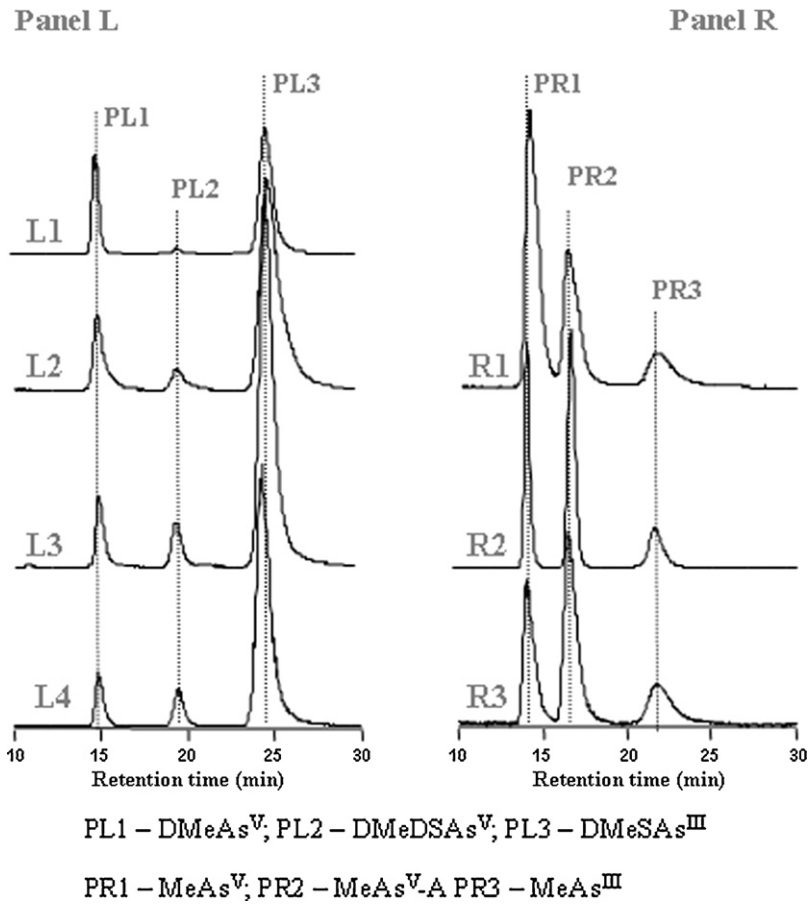


Fig. 5. Effects of Na_2S on MeAs^{III}-Cys. In Panel (L) DMeAs-A was extracted in diethylether, freeze-dried, dissolved in Milli-Q water and kept at room temperature. The sample was analyzed at 0 day (L1); at 30 days (L2); at 60 days (L3); at 90 days (L4) and in Panel (R) MeAs-A was extracted in chloroform, freeze-dried, dissolved in Milli-Q water and kept at room temperature. The sample was analyzed at 0 day (R1); at 30 days (R2); at 60 days (R3). Experimental parameters were the same as those described in the legend of Fig. 1.

123) (Fig. 8) and m/z = 188 (in negative ion mode, 187) (Fig. 9) while molecular mass of MeAs-C (by ESI-TOF) was m/z = 188 (in negative ion mode, 186.76295) (Fig. 10).

3.4. Analysis of human urine and nails

The same analytical technique was applied to analyze human urine and nail samples collected from the population of the As-affected areas of West Bengal, India. Afterwards both urine and nail samples were processed for analysis as detailed in Section 2.10. The analyses results of human urine and nails samples are presented in Fig. 12L1–L3 analyzed by an AEC and in Fig. 12R1–R3 analyzed by a SEC.

4. Discussion

Comparison between the results obtained by both anion exchange and size exclusion columns clearly indicated the superiority of SEC over AEC for separation of thioarsenicals.

4.1. Disadvantages and advantages of anion exchange column (AEC)

When the authentic sample prepared by hydrolysis of DMeAs^{III}-I or reduction products of DMeAs^{V} with cysteine was analyzed by a polymer-based SEC GS 220, only one peak appeared at the retention time of 30 min (Fig. 1R2 and R4). These results suggested that hydrolysis product of DMeAs^{III}-I in distilled water under nitrogen atmosphere and reduction product of DMeAs^{V} with cysteine was the same compound. Similarly, when the authentic sample prepared by hydrolysis of MeAs^{III}-I_2 in distilled water under nitrogen atmosphere or reduction products of MeAs^{V} with cysteine was analyzed by a polymer-based SEC, only one peak appeared at the retention time of 22 min (Fig. 1R1 and R3), which suggested that they were the same compound.

Similarly the reaction product of DMeAs^{V} detected as a single peak on an AEC, was separated into two peaks (DMeAs-B and DMeAs-A) (at 19.8 and 25.1 min, respectively) on SEC (Fig. 2R4 and R5) or the reaction product of MeAs^{V} detected as a single peak on an AEC, was separated into three peaks (MeAs-C, MeAs-A and MeAs-B) (at 15.2, 17 and 20.2 min, respectively) on SEC (Fig. 2R1–R3), clearly suggested that the present AEC was unable to separate all reaction products of DMeAs^{V} or MeAs^{V} and sulfur-containing reducing agents into separate components (Fig. 2L1–L5). Although non-thioarsenicals could be easily separated within 10 min by this AEC [18] thioarsenicals could not be separated out using this column.

4.2. Study on effects of Na_2S on MeAs^{III}-Cys and DMeAs^{III}-Cys

Results presented in Figs. 2 and 3 clearly suggest that initially MeAs^{III} was produced and then it was converted to mono-, di- and tri-thiolated monomethylarsenicals, i.e., MeSAs^{III}, MeDSAs^{V} and MeTSAs^{V} (Figs. 2 and 3) with the increasing sulfide concentrations.

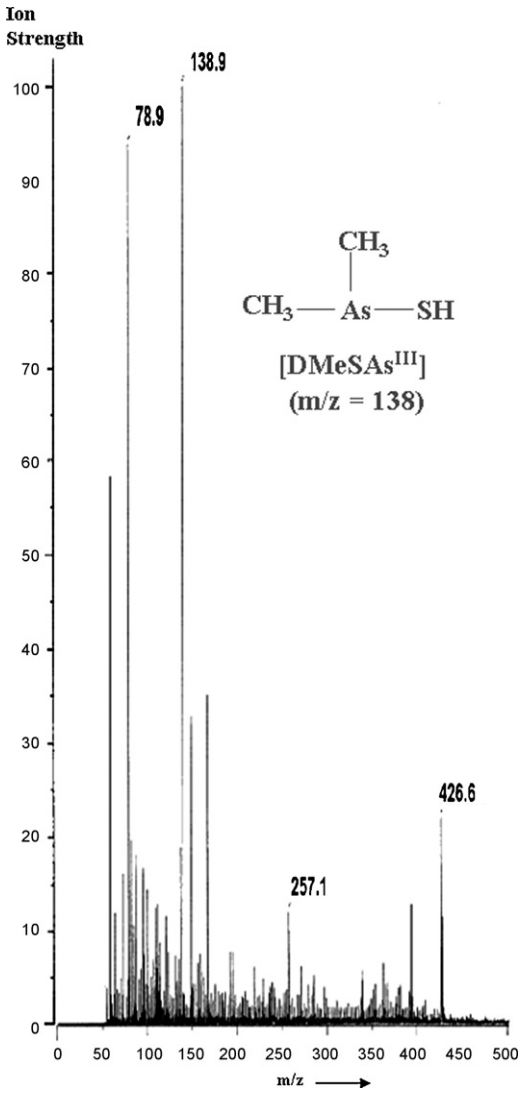


Fig. 6. Mass spectra of DMeAs-A on an electrospray mass spectrometer. DMeAs-A was prepared by the standard reaction conditions ($DMeA^{V}$:Na_2S:H_2SO_4 = 1:1.6:1.6) for 1 h followed by extraction with diethylether. DMeAs-A was subjected to electrospray mass spectrometry with positive ion mode.

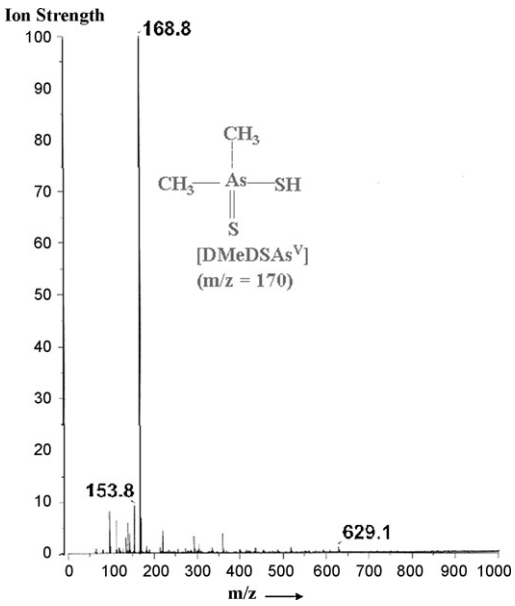


Fig. 7. Mass spectra of DMeAs-B on an electrospray mass spectrometer. DMeAs-B was prepared by bubbling H_2S gas overnight in an ethanol solution of $DMeA^{V}$. DMeAs-B was subjected to electrospray mass spectrometry with negative ion mode.

Similarly, when 2 mM sodium sulfide was added to 1 mM $DMeAs^{V}$ at room temperature and the reaction mixture was analyzed after 30 min, it showed a peak at 15 min on a SEC (Fig. 4C1), which was $DMeAs^{V}$ itself. But two peaks (at 19.8 and 25.1 min, respectively) as shown in Fig. 4C2, appeared with 2 mM Na_2S and 100 mM H_2SO_4 on SEC while $DMeAs^{III}$-Cys appeared at 30.2 min (Fig. 4C3). The peaks (at 19.8 and 25.1 min, respectively) were $DMeSAs^{III}$ (Fig. 6) and $DMeDSAs^{V}$ (Fig. 7), respectively.

4.3. Stability study of $MeAs^{III}$-Cys and $DMeAs^{III}$-Cys with time in the presence of Na_2S

When $MeAs^{III}$-Cys, which appeared at 22 min (Fig. 3L3 and R3) on SEC, was mixed with different concentrations of sodium sulfide (1–3 mM), different monomethylated thioarsenicals appeared (Fig. 3L4–L7 and R4–R6). But after 12 h of addition of sodium sulfide all methylated thioarsenicals (MeAs-A, MeAs-B and MeAs-D) got converted to MeAs-C (Fig. 3R6), which suggested that MeAs-C was the most stable form among the four thioarsenicals.

The effect of sodium sulfide on $DMeAs^{III}$-Cys were carried out mixing different concentrations of sodium sulfide with 1 mM $DMeAs^{III}$-Cys and the resulting solutions were analyzed after 30 min. A new peak (DMeAs-C) appeared at 16.8 min with 0.50 mM sodium sulfide (Fig. 4C4) along with other two peaks (DMeAs-A and DMeAs-B) with the increase of sodium sulfide concentrations (1–2 mM) (Fig. 4C5 and C6), which suggested that $DMeAs^{III}$ was produced first and then mono- and di-thiolated dimethylarsenicals formed with the increasing sulfide concentrations. Among them DMeAs-B was the most stable dimethylated thioarsenic form (Fig. 5L1–L4).

The results presented in Fig. 5R1–R3 showed that both MeAs-A and $MeAs^{III}$ slowly converted to $MeAs^{V}$ with time. Similarly, both DMeAs-A and DMeAs-B slowly converted to $DMeAs^{V}$ with time (Fig. 5L1–L4). So, none of MeAs-A, $MeAs^{III}$, DMeAs-A and DMeAs-B were stable with time at room temperature. Also, they were not stable for long time even at −28 °C (data not shown).

4.4. Characterization of thioarsenicals by ESI-MS and ESI-TOF MS

The reduction products of $DMeAs^{V}$ by different reducing agents were supposed to be dimethylated thioarsenicals (Figs. 1L8 and R8 and 2L4–L5 and R4–R5), because the results of simultaneous determination of S (at m/z = 32) and As (at m/z = 75) by HPLC-ICP MS using SEC showed that DMeAs-A and DMeAs-B contained one and two S molar atoms, respectively (data not shown). Moreover, DMeAs-A was hygroscopic when it was extracted into diethyl ether and evaporated to dryness under nitrogen atmosphere. Hence elemental analysis was not carried out to know the presence or absence of oxygen atoms. Mass spectrometric data were obtained for DMeAs-A and DMeAs-B by ESI MS with positive and negative ion modes, respectively (experimental details are given in Section 2.3). Molecular masses for DMeAs-A and DMeAs-B were m/z = 138 (in positive ion mode, 139) (Fig. 6) and m/z = 170 (in negative ion mode, 169) (Fig. 7). So, DMeAs-A was established to be $DMeSAs^{III}$ [$(CH_3)_2AsSH$], while DMeAs-B was $DMeDSAs^{V}$ [$(CH_3)_2As(S)SH$]. In addition, Suzuki et al. [25] also reported identical findings. DMeAs-C was a transient species and the authors tried to extract it, but could not. It opts more research on synthesis and separation

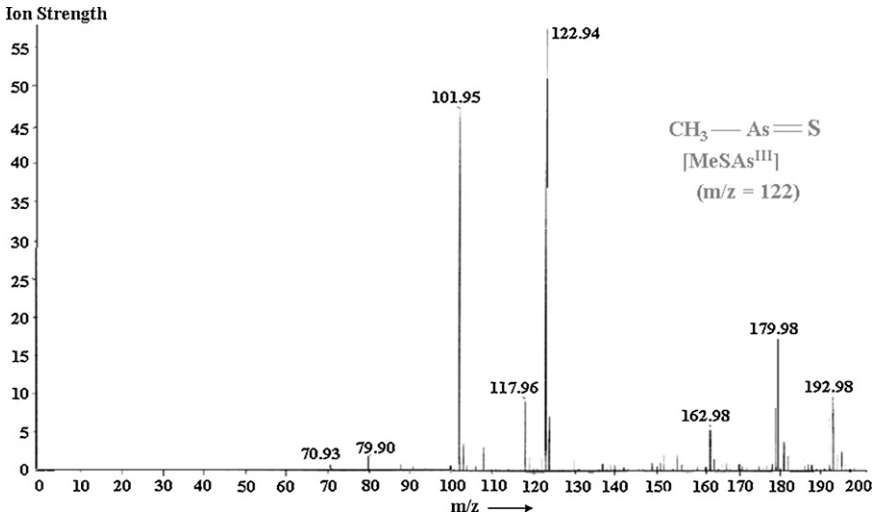


Fig. 8. Mass spectra of MeAs-A on an electrospray mass spectrometer. MeAs-A was prepared by adding $MeAs^V$, Na_2S and H_2SO_4 in the molar ratio of 1:1.5:5 at room temperature and kept for 1 h followed by extraction with chloroform. MeAs-A was subjected to electrospray mass spectrometry with positive ion mode.

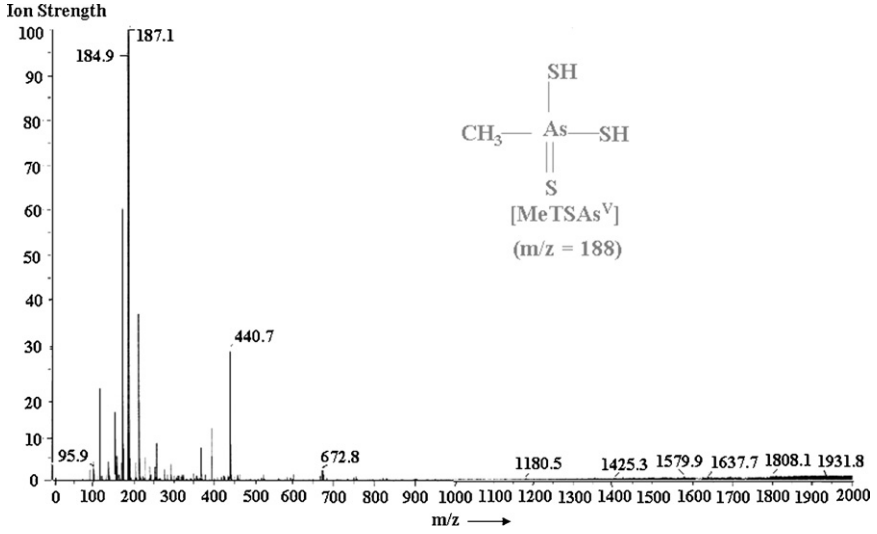


Fig. 9. Mass spectra of MeAs-C on an electrospray mass spectrometer. MeAs-C was prepared by adding $MeAs^V$, Na_2S and H_2SO_4 in the molar ratio of 1:7.5:5 at room temperature and kept for 1 day. The solution was freeze-dried, redissolved in Milli-Q water and filtered. MeAs-C was subjected to electrospray mass spectrometry with negative ion mode.

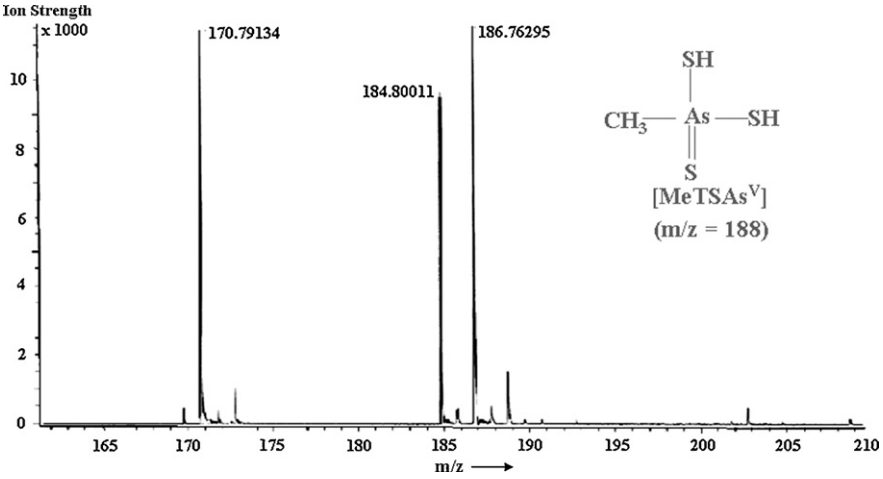


Fig. 10. Mass spectra of MeAs-C on an electrospray-time of flight-mass spectrometer. MeAs-C was prepared by adding $MeAs^V$, Na_2S and H_2SO_4 in the molar ratio of 1:7.5:5 at room temperature and kept for 1 day. The solution was freeze-dried, redissolved in Milli-Q water and filtered. MeAs-C was subjected to electrospray-time of flight-mass spectrometry with negative ion mode.

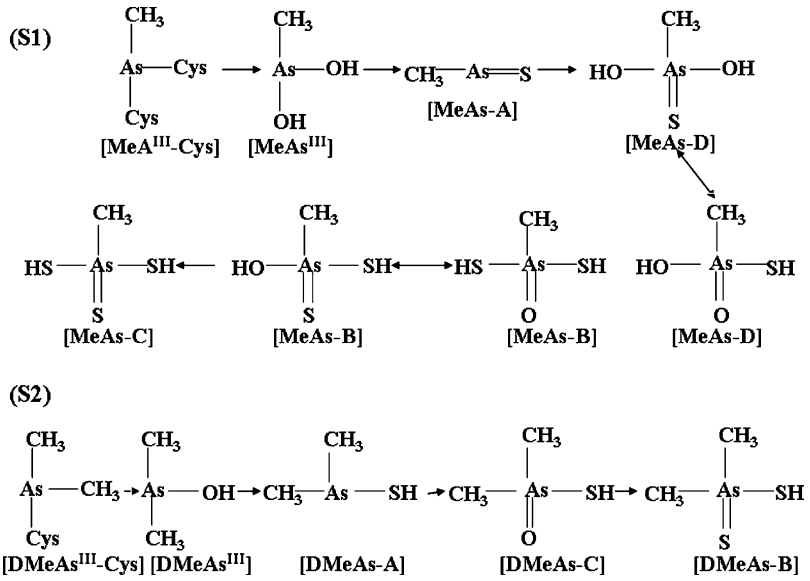


Fig. 11. Proposed reaction pathway for $MeAs^V$ (11S1) and $DMeAs^V$ (11S2) under sulfide reducing environment.

to characterize it accurately. Although reverse phase liquid chromatography (RP-LC) was found very hard to separate arsenicals (data not shown) researchers could make an effort to use RP-LC for better separation of thioarsenicals if possible.

The reduction products of $MeAs^V$ by different reducing agents were considered to be monomethylated thioarsenicals (Figs. 1L5–L6 and 2L1–L3 and R1–R3), because the results of simultaneous determination of S (at $m/z = 32$) and As (at $m/z = 75$) by HPLC-ICP MS using SEC showed that MeAs-A, MeAs-B and MeAs-C contained one, two and three S molar atoms, respectively (data not shown). Moreover, MeAs-A was hygroscopic (when extracted into chloroform and dried), and hence elemental analysis was not carried out to know the presence or absence of oxygen atoms. Mass spectrometric data were obtained for MeAs-A and MeAs-C by ESI MS with positive and negative ion modes, respectively (experimental details are given in Section 2.3). Molecular masses for MeAs-A and MeAs-C were $m/z = 122$ (in positive ion mode, 123) (Fig. 8) and $m/z = 188$ (in negative ion mode, 187) (Fig. 9), respectively. So, MeAs-A was proposed to be $MeSAs^{III}$ [CH_3AsS] while MeAs-C was structured to be $MeTSAs^V$ [$CH_3AsS(SH)_2$]. MeAs-D was a transient species like DMeAs-C and the authors tried to extract it, but could not. Also, the authors did not succeed to get pure MeAs-B for mass spectrometric analysis. The authors were unable to optimize the conditions where $MeAs^V$ was converted completely to MeAs-B. In addition, molecular mass of MeAs-C (by ESI-TOF) was $m/z = 188$ (in negative ion mode, 186.76295) (Fig. 10). Theoretical mass (up to five decimal places) of MeAs-C as [$CH_3AsS(SH)_2$] was calculated to be 187.87693 and it's mass should be in negative ion mode as 186.87693 which is almost identical (accuracy, 100.06%) to the measured value of 186.76295 (data not shown). This result clearly confirmed the formula of MeAs-C as [$CH_3AsS(SH)_2$]. The authors tried to get ESI-TOF MS spectrum for $DMeSAs^{III}$, $DMeDSAs^V$ and $MeSAs^{III}$ but instrument did not sense them under all plausible trials. This might be due to low molecular masses or lack of their standard reference materials (SRM).

So more research is needed to characterize DMeAs-C, MeAs-B and MeAs-D. On the basis of the above discussion the probable chemical formula of DMeAs-C might be dimethylthioarsinic acid ($DMeSAs^V$), i.e., $(CH_3)_2As(=O)SH$ or] $(CH_3)_2As(=S)OH$ (Fig. 11S2). Also, the probable chemical formula of MeAs-B might be methyldithioarsonic acid ($MeDSAs^V$), i.e., $(CH_3)As(=S)(SH)(OH)$ or $(CH_3)As(=O)(SH)_2$ and that of MeAs-D be methylthioarsonic acid ($MeSAs^V$), i.e., $(CH_3)As(=O)(SH)(OH)$ or $(CH_3)As(=S)(OH)_2$ (Fig. 11S1). But more research is desired to confirm the probable structures of the unknown thioarsenicals mentioned above. Also, metabolic as well toxicological studies of these monomethylated thioarsenicals might be able to answer the species-specific toxicity of As towards target organs in humans and animals.

4.5. Analysis of human urine and nails

The same analytical technique was applied to analyze human urine and nails samples collected from the population of the As-affected areas of West Bengal, India. Both $MeSAs^{III}$ and $DMeDSAs^V$ were found in human urine (Fig. 12R3). Mandal et al. [17] reported the presence of $DMeAs^{III}$ in human nails analyzed by HPLC-ICP MS using an AEC. But both $DMeSAs^{III}$ and $DMeDSAs^V$ were detected in human nails (Fig. 12R1–R2) by HPLC-ICP MS using a SEC. This result clearly highlights the drawback of AEC over SEC. Although SEC is superior to AEC for the separation of thioarsenicals, arsenate (iAs^V) and $MeAs^V$ coelute at a RT of 13.5 min (Fig. 12R1–R2) which limits the use of SEC in the presence of both iAs^V and $MeAs^V$ in the sample.

In analytical separation techniques, such as the chromatographies, the measurement of noise is not trivial and often subjective. It is obvious that low detection and quantification limits require S/Ns consistent with the method precision and accuracy requirements. Thus, one can confirm S/N quickly during a system suitability test without running many samples. Hence large S/Ns are needed for the 1–2% precision and accuracy of any analytical methods. Normally limit of detection (LOD) is the smallest concentration of the analyte that gives measurable response where signal to noise ratio (SNR) would be at least 3 and which would be 10 for limit of quantification (LOQ). Although estimation of absolute concentrations of arsenicals in the samples concerned was not attempted in the present study, signal to noise ratio was determined. The values of SNR were in between 68 and 72 (data not shown). So, accurate quantification of arsenicals would be possible by this hyphenated HPLC-ICP MS technique in the presence of commercially available SRMs of thioarsenicals. This result suggests that AEC ES-502N 7C was unable to separate dimethylated thioarsenicals from $DMeAs^{III}$.

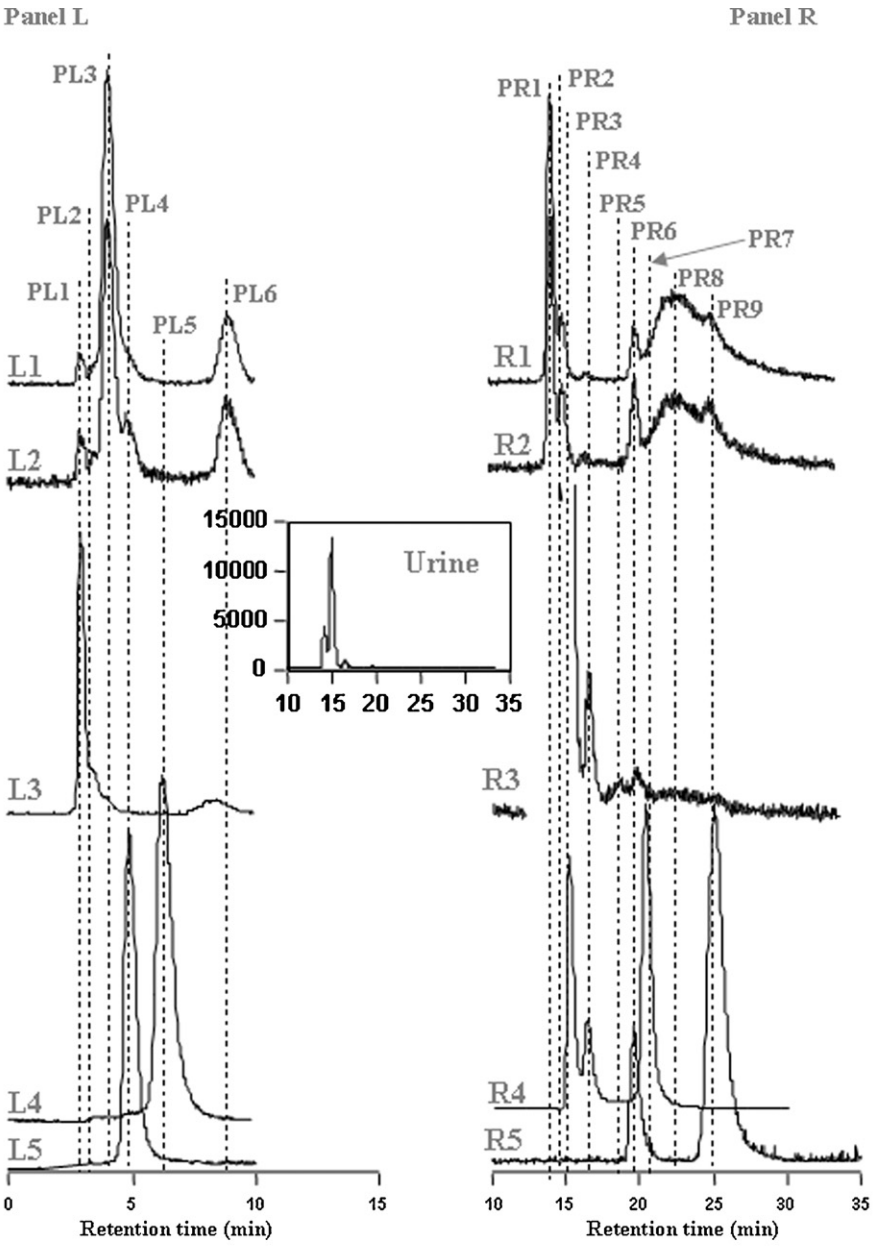


Fig. 12. In Panel (L) the human urine and nails were prepared as per Section 2.10 and analyzed by an anion exchange column as follows: Nail 1 (L1); Nail 2 (L2); urine (L3); std. MeAs^{III} prepared by Ref. [32] (L4) and std. DMeAs^{III} prepared by Ref. [32] (L5). In Panel (R) the human urine and nails were prepared as per Section 2.10 and analyzed by a size exclusion column as follows: Nail 1 (R1); Nail 2 (R2); urine (R3); std. MeAs^{III} prepared by Ref. [32] (R4) and std. DMeAs^{III} prepared by Ref. [32] (R5). Experimental parameters were the same as those described in the legend of Fig. 1.

In addition, due to scarcity of adequate urine and nails samples the authors analyzed about 20 samples each and found peaks for MeSAs^{III} and DMeDSAs^{V} in human urine while DMeSAs^{III} and DMeDSAs^{V} in human nails. The quantification of these thioarsenicals was not carried out because standards of these compounds were not quantified accurately after extraction from their reaction mixtures or SRM of them were not available commercially. Quantification of these thioarsenicals would be possible using this technique if their standards are readily available.

5. Conclusion

The results of the present study suggested that proper selection of an analytical column as well as a detector was the determining factor for the identification of methylated thioarsenicals. Mandal et

al. [17,18] reported the presence of $MeAs^{III}$ and $DMeAs^{III}$ in human urines and $DMeAs^{III}$ in human nails collected from As victims using this anion exchange column. Their results should be rechecked using a SEC for thioarsenicals. Because dimethylthioarsinic acid ($DMeSAs^{V}$) and dimethyldithioarsinic acid ($DMeDSAs^{V}$) were the most recent As metabolites discovered in the urine of man and animals [19–22]. Also, the present study identified $DMeSAs^{III}$ and $DMeDSAs^{V}$ in human nails and $MeSAs^{III}$ and $DMeDSAs^{V}$ in human urine. Another important conclusion could be made from this study is that ICP MS alone was not sufficient to identify and characterize the compounds after matching retention times without their mass data. Although this study has synthesized and characterized $MeSAs^{III}$ and $MeTSAs^{V}$, more research work is necessary for characterization of DMeAs-C, MeAs-B and MeAs-D, which will solve the problems to find out alternative trivalent As compounds because $MeAs^{III}$ and $DMeAs^{III}$ are unstable in aqueous solutions [33]. Finally, this technique could be applicable to identify sulfur-containing biomolecules in the biological samples.

Acknowledgements

Dr. Badal Kumar Mandal acknowledges the help of JSPS, Japan, for the financial support. Also, BKM acknowledges the help of Graduate School of Pharmaceutical Sciences, Chiba University as well as Redox Regulation Research Group, National Institute of Radiological Sciences, Chiba, Japan, for the uses of their all facilities to do this research work. This study was supported by Grants-in-Aid of Ministry of Education, Science, Sports and Culture (Nos. 12000236 and 12470509).

References

[1] NRC (National Research Council), Arsenic in Drinking Water, National Academy Press, Washington, DC, 1999, p. 330.
[2] W.R. Cullen, K.J. Reimer, Chem. Rev. 89 (1989) 713.
[3] WHO, Arsenic and Arsenic Compounds, Environmental Health Criteria No. 224, 2nd ed., World Health Organization, Geneva, 2001, p. 521.
[4] M. Styblo, L.M. Del Razo, L. Vega, D.R. Germolec, E.L. LeCluyse, G.A. Hamilton, W. Reed, C. Wang, W.R. Cullen, D.J. Thomas, Arch. Toxicol. 74 (2000) 289.
[5] D.J. Thomas, M. Styblo, S. Lin, Toxicol. Appl. Pharmacol. 176 (2001) 127.
[6] J.S. Petrick, F. Ayala-Fierro, W.R. Cullen, D.E. Carter, H.V. Aposhian, Toxicol. Appl. Pharmacol. 163 (2001) 203.
[7] M. Styblo, Z. Drobna, I. Jaspers, S. Lin, D.J. Thomas, Environ. Health Perspect. 110 (Suppl. 5) (2002) 767.
[8] L. Vega, M. Styblo, R. Patterson, W.R. Cullen, C. Wang, D. Germolec, Toxicol. Appl. Pharmacol. 172 (2001) 225.
[9] M. Styblo, D.J. Thomas, Toxicol. Appl. Pharmacol. 147 (1997) 1.
[10] S. Lin, L.M. Del Razo, M. Styblo, C. Wang, W.R. Cullen, D.J. Thomas, Chem. Res. Toxicol. 14 (2001) 305.
[11] J.S. Petrick, B. Jagadish, E.A. Mash, H.V. Aposhian, Chem. Res. Toxicol. 14 (2001) 651.
[12] X.C. Le, X. Lu, X.-F. Li, Anal. Chem. (2004) 27A.
[13] T. Sakurai, C. Kojima, M. Ochiai, T. Ohta, M.H. Sakurai, M.P. Waalkes, K. Fujiwara, Appl. Pharmacol. 195 (2004) 129.
[14] J. Pi, Y. Kumagai, G. Sun, H. Yamauchi, T. Yoshida, H. Iso, A. Endo, L. Yu, K. Yuki, T. Miyauchi, N. Shimojo, Free Radic. Biol. Med. 28 (2000) 1137.
[15] S. Hirano, Y. Kobayashi, X. Cui, S. Kanno, T. Hayakawa, A. Shraim, Toxicol. Appl. Pharmacol. 198 (2004) 458.
[16] S.V. Kala, M.W. Neely, G. Kala, C.I. Prater, D.W. Atwood, J.S. Rice, M.W. Lieberman, J. Biol. Chem. 275 (2000) 33404.
[17] B.K. Mandal, Y. Ogra, K.T. Suzuki, Toxicol. Appl. Pharmacol. 189 (2003) 73.
[18] B.K. Mandal, Y. Ogra, K.T. Suzuki, Chem. Res. Toxicol. 14 (2001) 371.
[19] H.R. Hansen, A. Raab, M. Jaspars, B.F. Milne, J. Feldmann, Chem. Res. Toxicol. 17 (2004) 1086.
[20] R. Raml, W. Goessler, P. Traar, T. Ochi, K.A. Francesconi, Chem. Res. Toxicol. 18 (2005) 1444.
[21] K. Yoshida, K. Kuroda, Y. Inoue, H. Chen, Y. Date, H. Wanibuchi, S. Fukushima, G. Endo, Appl. Organomet. Chem. 15 (2001) 539.
[22] K. Yoshida, K. Kuroda, X. Zhou, Y. Inoue, Y. Date, H. Wanibuchi, S. Fukushima, G. Endo, Chem. Res. Toxicol. 16 (2003) 1124.
[23] M.W. Fricke, M. Zeller, H. Sun, V.W.-M. Lai, W.R. Cullen, J.A. Shoemaker, M.R. Witkowski, J.T. Creed, Chem. Res. Toxicol. 18 (2005) 1821.
[24] H. Naranmandura, N. Suzuki, K.T. Suzuki, Chem. Res. Toxicol. 19 (2006) 1010.
[25] K.T. Suzuki, B.K. Mandal, A. Katagiri, Y. Sakuma, A. Kawakami, Y. Ogra, K. Yamaguchi, Y. Sei, K. Yamanaka, K. Anzai, M. Ohmichi, H. Takayama, N. Aimi, Chem. Res. Toxicol. 17 (2004) 914.
[26] H.R. Hansen, M. Jaspars, J. Feldmann, Analyst 129 (2004) 1058.
[27] E. Schmeisser, R. Raml, K.A. Francesconi, D. Kuehnelt, A.-L. Lindberg, C. Sörös, W. Goessler, Chem. Commun. 10 (2003) 1824.
[28] J. Meier, N. Kienzl, W. Goessler, K.A. Francesconi, Environ. Chem. 2 (2005) 304; M. Kahn, R. Raml, E. Schmeisser, B. Vallant, K.A. Francesconi, W. Goessler, Environ. Chem. 2 (2005) 171.
[29] K.T. Suzuki, K. Iwata, H. Naranmandura, N. Suzuki, Toxicol. Appl. Pharmacol. 218 (2007) 166.
[30] K. Kuroda, K. Yoshida, M. Yoshimura, Y. Endo, H. Wanibuchi, S. Fukushima, G. Endo, Toxicol. Appl. Pharmacol. 198 (2003) 345.
[31] K. Yamaguchi, J. Mass Spectrom. 38 (2003) 473.
[32] P.F. Reay, C.J. Asher, Anal. Biochem. 78 (1977) 557.
[33] Z. Gong, X. Lu, W.R. Cullen, X.C. Le, J. Anal. Atom. Spectrom. 16 (2001) 1409.