Available online at www.sciencedirect.com
ScienceDirect
Analytica Chimica Acta 578 (2006) 186–194
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Arsenic speciation analysis of human urine using ion exchange chromatography coupled to inductively coupled plasma mass spectrometry

Ruimin Xie [a,b], Willie Johnson [a], Steve Spayd [c], Gene S. Hall [b], Brian Buckley [a,*]

[a] *Environmental and Occupational Health Sciences Institute, Rutgers, The State University of New Jersey, 170 Frelinghuysen Road, Piscataway, NJ 08854, USA*
[b] *Department of Chemistry and Chemical Biology, Rutgers, The State University of New Jersey, 610 Taylor Road, Piscataway, NJ 08854, USA*
[c] *New Jersey Department of Environmental Protection, P.O. Box 427, Trenton, NJ 08625-0427, USA*

Received 8 May 2006; received in revised form 28 June 2006; accepted 29 June 2006
Available online 7 July 2006

Abstract

A sensitive and robust method for the determination of seven inorganic and organic arsenic species was developed using ion exchange chromatography combined with inductively coupled plasma mass spectrometry (IC-ICP-MS). Both anion and cation exchange columns were used in a complementary fashion. Arsenite (As(III)), arsenate (As(V)), monomethylarsonic acid (MMA(V)) and dimethylarsinic acid (DMA(V)) were selectively separated by an anion exchange column using sodium hydroxide (NaOH) gradient elution, while monomethylarsonous acid (MMA(III)), dimethylarsinous acid (DMA(III)) and arsenobetaine (AsB) were separated by a cation exchange column using 70 mM nitric acid as the mobile phase. Baseline separation, high repeatability and low detection limits (0.10–0.75 ng mL^{-1}) were achieved. The spiked urine samples were analyzed with this method to evaluate the matrix effect on the method. The results suggest 1–10 dilutions should be made to urine samples before sample injection for the anion exchange analysis to minimize the matrix effect. To validate the method, a new standard reference material (NIST SRM-2670a) was also analyzed. The arsenic species in NIST SRM-2670a were determined by this method, and the sum of their concentrations agreed well with the total arsenic content certified for NIST SRM-2670a. Moreover, this method was applied to measure arsenic species in urine samples from one subject living in New Jersey who drank well water contaminated with arsenic. By this method, two key arsenic metabolites, MMA(III) and DMA(III), were found to be present in these urine samples, which has previously been rarely reported.

Keywords: Arsenic; Speciation; Urine; Ion chromatography–inductively coupled plasma mass spectrometry

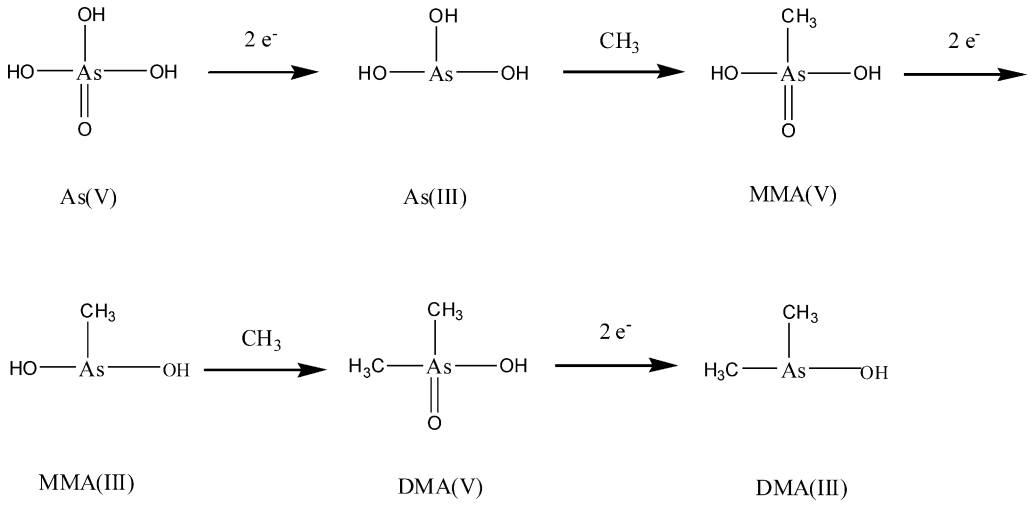
1. Introduction

Arsenic is the twentieth most abundant element in the earth's crust. In certain of its compounds, it is carcinogenic for men, affecting millions of people worldwide. Exposure to arsenic is considered to be a significant world-wide public health problem. Chronic exposure to arsenic in drinking water at levels of several hundred micrograms per liter has been associated with skin, bladder and lung cancers, as well as hypertension and cardiovascular effects [1–6].

The toxicity and bioavailability of arsenic depend on its chemical form. Most chronic arsenic poisoning cases are caused by the consumption of inorganic arsenic, arsenite (As(III)) and arsenate (As(V)), in well water [1,2]. As(III) and As(V) may undergo metabolism to form methylated species. According to the metabolic pathway postulated by Cullen et al. [7,8], methylation of arsenic involves a two-electron reduction of the pentavalent arsenic species to the trivalent arsenic species followed by oxidative addition of a methyl group to arsenic, shown in Scheme 1. During the metabolism, glutathione can act as reducing agent, and *S*-adenosylmethione (SAM) is the methyl donor [9,10]. The metabolites, monomethylarsonic acid (MMA(V)) and dimethylarsinic acid (DMA(V)), have been reported to be present in human urine, which provides direct evidence in support of the biomethylation pathway [11,12]. Pre-

* Corresponding author. Tel.: +1 732 445 0204; fax: +1 732 445 0131.
E-mail address: bbuckley@eohsi.rutgers.edu (B. Buckley).

0003-2670/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2006.06.076



Scheme 1. Possible pathway for the metabolism of inorganic arsenic in the human body.

viously, methylation of arsenic has been considered a detoxification process, because the primary metabolites, MMA(V) (LD_{50} 800–1600 mg kg^{-1} via oral exposure) and DMA(V) (LD_{50} 800–2600 mg kg^{-1}) are less acutely toxic than the inorganic arsenic species (LD_{50} 10–20 mg kg^{-1}) [2]. Recently, however, it has been shown that the proposed essential intermediates, monomethylarsonous acid (MMA(III)) and dimethylarsinous acid (DMA(III)), may be more toxic than inorganic arsenic and may induce oxidative DNA damage [13–17]. The presence and concentration of MMA(III) and DMA(III) in urine may be closely related to human health risk when exposed to inorganic arsenic. Therefore, to evaluate people's exposure to arsenicals and the metabolism of arsenic in human body, it is necessary to develop a sensitive method to identify and quantify the key metabolites, As(III), As(V)), MMA(III), DMA(III), MMA(V) and DMA(V)), in human urine. In urine, there may also be another major arsenic species, arsenobetaine (AsB), which comes from eating sea food. AsB is mainly present in sea food at concentrations as high as the ppm level and is not metabolized in the human body [2]. Although AsB is believed to be non-toxic, it may interfere with the determination of inorganic and methylated arsenic if it co-elutes with other arsenic species during speciation analysis. Therefore, AsB needs to be separated from other metabolites in the speciation assay.

Many separation techniques, such as ion-exchange [18–24], ion-pairing with reversed phase HPLC [25–27], ion exclusion [28,29] and detection methods (atomic absorption, atomic emission and ICP-MS) have been used for arsenic speciation. Few methods have been successfully used to determine the seven major arsenic species in urine with good resolution, especially MMA(III) and DMA(III). Le et al. [26,27] used ion pair chromatography coupled to hydride generation and atomic fluorescence spectrometer to determine As(III), As(V), MMA(III), DMA(III), MMA(V) and DMA(V) in urine, and since AsB usually does not form a hydride, it did not interfere with the detection of other species in this method. But the overlap of some peaks limited the sensitivity of this method, and the complicated hydride interface may introduce some errors. Suzuki and coworkers [30] reported the separation of the seven species using an anion exchange column coupled to ICP-MS, but the peaks in the chromatograms were not baseline resolved. Meanwhile, the identification of species in Suzuki's method may be partially in error, because they prepared MMA(III) and DMA(III) standards based on the reduction of MMA(V) and DMA(V) by metabisulfite and thiosulfate [31]. The products of the reductive reaction are believed to be sulfur-containing arsenicals, dimethylarsinothioic acid, instead of DMA(III) [32,33].

To accurately determine the arsenic species in urine, we have developed an arsenic speciation method, using anion and cation exchange columns in a complementary fashion to selectively separate seven major arsenic species in human urine, As(III), As(V), MMA(III), DMA(III), MMA(V), DMA(V) and AsB, with baseline peak separation, using inductively coupled plasma mass spectrometry (ICP-MS) as the online detector producing a low detection limit.

2. Experimental

2.1. Instrumentation

For ion chromatographic (IC) separations, a Dionex GS50 (Dionex Co., Sunnyvale, CA, USA) gradient pump with degasser was used for eluent delivery. Two kinds of ion exchange columns were used in these experiments: a polymer-based IonPac AS11 analytical column (4 mm × 250 mm) along with an IonPac AG11 guard column (4 mm × 50 mm, Dionex Co.) were used for anion exchange separatioin; and a polymer-based PCX-500 (4 mm × 250 mm) along with guard column (4 mm × 50 mm, Dionex Co.) were used for cation exchange separation. Columns were switched between each other in off-line mode. A PlasmaQuad 3 (VG Elemental, USA) ICP-MS was used as an online chromatographic detector. The outlet of the IC system was coupled directly to the inlet of the concentric nebulizer (Precision Glassblowing, Englewood, CO, USA). A water-cooled conical spray chamber (Precision Glassblowing) was employed. The signal at *m/z* 75 was used to monitor