# **RESEARCH ARTICLE**

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# Quantitation and speciation of inorganic arsenic in a biological sample by capillary ion chromatography combined with inductively coupled plasma mass spectrometry



Seon-Jin Yang, Yonghoon Lee\* and Sang-Ho Nam\*

#### **Abstract**

The toxicity and biological activity of arsenic depend on its chemical form. In particular, inorganic arsenics are more toxic than organic ones. Apart from the determination of total arsenics, their accurate speciation is important for toxicity assessment. To separate arsenic species using a cation or an anion separation column, at least 0.5–1.0 mL of sample is required because conventional ion chromatography columns use a sample loop of  $100-200~\mu$ L. It is thus difficult to analyze samples with small volumes, such as clinical and biological samples. In this study, a method for separating arsenic species using a 5- $\mu$ L sample loop combined with a capillary ion exchange column has been developed for analyzing small volume of samples. The separated arsenics were determined by inductively coupled plasma mass spectrometry. By oxidizing As(III) to As(V) prior to analysis, the total inorganic arsenics, As(III) and As(V), could be well separated from the organic ones. Linear calibration curves (0.5–50  $\mu$ g/kg) were obtained for total inorganic arsenics dissolved in water. Sub-picogram-level detection limit was obtained. The analytical capability of this method was successfully validated for certified reference materials, namely water and human urine, with total inorganic arsenic recovery efficiencies of 100% and 121%, respectively. Our method requires less than ~ 10  $\mu$ L of sample and will be very useful to analyze valuable samples available in limited amounts.

**Keywords:** Arsenic speciation, Human urine, Ion chromatography, Capillary column, Inductively coupled plasma mass spectrometry

# Introduction

Arsenic, the twentieth most abundant element in the Earth's crust, is a non-metallic element known to be toxic to human. It is ubiquitously distributed in the soil, ocean, and air. Its accumulation in our body through food and drinking water can cause severe diseases, such as liver, bladder, lung, and skin cancers. Arsenic was designated as one of the ten chemicals harmful to public health by the World Health Organization (WHO) (Zhao and Wang

2020; World Health Organization. Arsenic 2018; Taylor et al. 2017; Pasias et al. 2013; Srivastava 2020).

The toxicity and bioavailability of arsenic compounds depend on their chemical forms (Llorente-Mirandes et al. 2017). Investigations regarding the different chemical forms of arsenic species have been performed (Cornelis et al. 2005). Arsenic compounds are mainly categorized into two groups: (i) inorganic arsenics, such as arsenite (As(III)) and arsenate (As(V)), and (ii) organic ones, namely monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenocholine (AsC), and arsenobetaine (AsB). The Environmental Protection Agency (EPA) has set the toxicity index,  $LD_{50}$ , 50% of lethal dosage, of the main arsenic species, and it shows that inorganic arsenics

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are much more toxic than organic ones (Cornelis et al. 2005; Reid et al. 2020) The amount of inorganic arsenics in food and drinking water is thus of critical concern. The regulation limit has been set up for the safety of food in many countries (Fontcuberta et al. 2011; Llorente-Mirandes et al. 2012; Hamano-Nagaoka et al. 2008). Many researchers have developed analytical methodologies for the determination of total arsenic and also different arsenic species in various samples. For the separation of arsenic species, ion exchange (Lee et al. 2019; Chen et al. 2007), high-performance liquid chromatography (HPLC) (Jia et al. 2016; Carlin et al. 2016), and ion exclusion (Schriewer et al. 2017) chromatography have been used. The separated species were detected by inductively coupled plasma mass spectrometry (ICP-MS) (Fitzpatrick et al. 2002; Komorowicz et al. 2019), inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Cui et al. 2013), atomic fluorescence spectroscopy (AFS) (Guo et al. 2017), and atomic absorption spectroscopy (AAS) (Santos et al. 2017; Viitak and Volynsky 2006).

The sample loop volume for conventional chromatography column is generally more than 100–200 μL (Son et al. 2019; Rosa et al. 2019; Nogueira et al. 2018). The amount of sample required for analysis using this sample loop is typically 0.5-1.0 mL or more. However, this method will not be feasible with very limited amount of sample. In case of biological samples obtained from animals or humans are very limited, the conventional column might not be suitable. In this study, the volume of loop used for the capillary column was 5  $\mu$ L. With this tiny-volume chromatography system, a new method for the quantitation of total inorganic arsenics has been developed. Prior to the analysis, As(III) was oxidized to As(V), and the total inorganic arsenics (As(III) + As(V)) were separated from the organic ones by a capillary column. Then, the separated species were determined by ICP-MS. The developed method was validated by the determination of arsenic species in the standard reference materials (SRMs) of water and human urine. Our method which requires a sample volume of ~ 10 μL will be very helpful to analyze valuable samples available in limited amounts, such as clinical or forensic liquid specimens.

# Materials and methods

# Instrumentation

The ion chromatography (IC) device used in this work was assembled using a sample loop with a volume of 5  $\mu$ L, a capillary chromatography column (0.4 × 250 mm, Dionex IonPac AS-11 HC, Thermo Fisher Scientific, Waltham, USA), a guard column (0.4 × 50 mm, Dionex IonPac AG-11, Thermo Fisher Scientific), and a sample introduction pump (S 1130, Sykam GmbH, Eresing, Germany). The operating parameters of IC are summarized

**Table 1** Operating parameters of IC using the capillary column

Parameter	Setting
Pump	Sykam S 1130
Analytical column	Thermo Fisher AS-11 HC
Column dimensions	$0.4 \times 250 \text{ mm}$
Flow rate	0.03 mL/min
Sample loop	5 μL
Mobile phase	10 mM Ammonium phosphate (pH 6.0)

**Table 2** Operating parameters of ICP-MS

Parameter	Setting
RF Power	1600 W
Cool gas flow rate	15 L/min
Auxiliary gas flow rate	1.2 L/min
Nebulizer gas flow rate	1.08 L/min
Monitor m/z	75
DRC gas	Ammonia
DRC gas flow	0.3 mL/min
DRC rejection parameter a	0.00 V
DRC rejection parameter q	0.55 V

in Table 1. For sample injection, a 6-port 2-position injection valve (Rheodyne Model 7125, Bellefonte, USA) was used. All samples and standards were injected with a syringe (710 RN, Hamilton Company, Reno, NV, USA) through the sample loop. An ICP-MS instrument (PerkinElmer Life Sciences, Shelton, CT, USA) was used to detect separated arsenic species from IC. Argon chloride (ArCl) is a well-known polyatomic interferent for arsenic (Tan and Horlick 1986). The ICP-MS instrument has a reaction gas channel which supplied anhydrous ammonia to the direct reaction cell (DRC) mode to remove the interference of <sup>40</sup>Ar<sup>35</sup>Cl<sup>+</sup> to <sup>75</sup>As<sup>+</sup>. The operating parameters of ICP-MS are summarized in Table 2. The experiment using conventional column (PRP-X100, Hamilton, Reno, NV, USA) was also performed to compare the detection limits of the methods using the capillary and conventional columns. A sample loop with a volume of 100 µL was used for the analysis using the conventional column. The other experimental conditions for IC and ICP-MS were similar to those of the analysis using the capillary column.

# Reagents and standards

Deionized water (DIW, 18.2 M $\Omega$  cm) from a water purification system (PURESAB CLASSIC, CLASSIC UV MK2, ELGA, USA) was used for the preparation of standard solutions, eluents, and sample solutions. Stock solutions

(100 mg/kg) of arsenic species were prepared using the following reagents: sodium arsenate dibasic heptahydrate ( $\geq$  98%, Sigma-Aldrich) for As(V), sodium metaarsenite (98%, Sigma-Aldrich, Steinheim, Germany) for As(III), cacodylic acid ( $\geq$  98%, Sigma-Aldrich) for DMA, and disodium methyl arsonate hexahydrate (98%, Sigma-Aldrich) for MMA. The prepared stock solutions were stored in the dark at 4 °C. Working solutions were prepared daily. Calibration standards and samples were prepared using phosphate buffer solution (Sigma-Aldrich) at pH 6.0. Ammonium phosphate dibasic ( $\geq$  98%, Sigma-Aldrich) was used as the eluent for IC.

## Sample and sample preparation

The SRM of trace elements in water (NIST SRM 1643f, Trace Elements in Water, National Institute of Standards and Technology, Gaithersburg, MD, USA) and that of human urine (NIST SRM 2669, Arsenic Species in Frozen Human Urine, National Institute of Standards and Technology, Gaithersburg, MD, USA) was employed for the validation. Prior to IC-ICP-MS analysis, all the samples were filtered through a 0.2  $\mu m$  PVDF membrane filter (Whatman International Ltd., Kent, England).

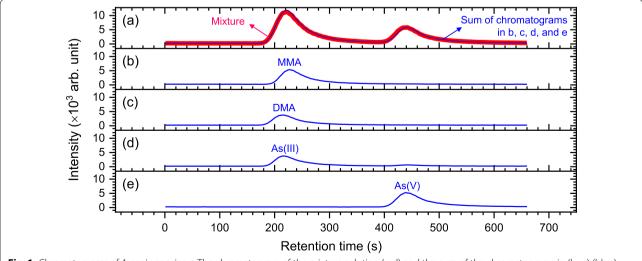
#### Results and discussion

#### Speciation by capillary ion chromatography

First, standard solutions of As(III), As(V), MMA, and DMA and their mixture were prepared. Each of the standard solutions contained 5  $\mu$ g/kg of arsenic. In the mixture solution, arsenic concentration from each species was set to 5  $\mu$ g/kg. The prepared standard solutions were injected into the capillary column. The separated

arsenic species were detected by ICP-MS. As shown in Fig. 1a, for the mixture solution, only two peaks were observed at around retention times 222 and 441 s. The two peaks could be identified by comparing the chromatogram with those of the standard solutions of MMA, DMA, As(III), and As(V) which are shown in Fig. 1b-e, respectively. In the chromatograms of the standard solutions, the peaks corresponding to MMA, DMA, As(III), and As(V) were observed at 227, 216, 215, and 440 s, respectively. This indicates that As(V) was well separated from the other species, but MMA, DMA, and As(III) were not resolved by the capillary column. Therefore, in the chromatogram of the mixture solution, the peak at 222 s was assigned to the mixture of As(III), MMA, and DMA, and that at 441 s was identified as the separated As(V). Also, it should be noted that the sensitivity was different among the four arsenic species (compare the intensities of the four peaks in Fig. 1b-e). The four chromatograms in Fig. 1b-e were added and plotted along with the chromatogram of the mixture solution in Fig. 1a. The two chromatograms agreed with each other. This indicates that the sensitivity difference was not due to experimental error but to the intrinsic properties of the different species.

In our previous works, the four arsenic species were completely separated by conventional column (Lee et al. 2019; Son et al. 2019; Nam et al. 2016). The lower resolving power of the capillary column used in this work could be attributed to its smaller inner diameter (0.4 mm) and particle size (4  $\mu$ m) as compared to the conventional column (inner diameter=4.6 mm and particle size=10  $\mu$ m). However, the aim of this work is

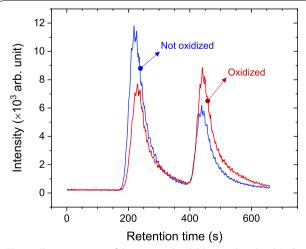


**Fig. 1** Chromatograms of Arsenic species. **a** The chromatogram of the mixture solution (red) and the sum of the chromatograms in (b–e) (blue), and the chromatograms of the standard solutions of **b** MMA, **c** DMA, **d** As(III), and **e** As(V). In the mixture solution, the concentrations of As from each species were equally set to 5 µg/kg. Each standard solution contained 5 µg/kg of arsenic

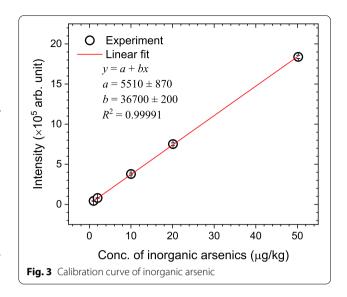
to determine the total inorganic arsenics (As(III) and As(V)) using capillary column since the toxicity of the organic ones is ignorable in comparison with that of the inorganics. In this regard, hydrogen peroxide was used to selectively oxidize As(III) to As(V). Then, the solution was injected into the capillary column to separate As(V). The detected intensity for As(V) resulted from the sum of As(V) and As(III) oxidized to As(V) and thus represented the total inorganic arsenics (As(III) + As(V)). The selective oxidation of As(III) to As(V) could be confirmed as explained thereafter. Figure 2 compares the chromatograms of the non-oxidized (blue) and oxidized (red) mixture solutions. As observed in the chromatogram of the oxidized mixture solution, the peak at 222 s decreased and that at 441 s increased in contrast to the non-oxidized solution. This can be attributed to the oxidation of As(III) to As(V). Thus, total inorganic arsenics could be separated and determined by the capillary column of ion chromatography coupled with ICP-MS.

#### Calibration and detection limit

For the calibration curve of inorganic arsenic, 1.0, 2.0, 10, 20, and 50  $\mu$ g/kg inorganic arsenic standard solutions were prepared from As(III) and As(V) stock solutions (100 mg/kg). Hydrogen peroxide was added to oxidize As(III) to As(V). A linear calibration curve was obtained using the capillary column of IC coupled with ICP-MS as shown in Fig. 3. The correlation coefficient was 0.9999. The linear dynamic range was more than two orders of magnitude. The detection limit was estimated as the concentration giving a signal equivalent to three times the noise, the standard deviation of three repetitive



**Fig. 2** Chromatograms of the non-oxidized (blue) and oxidized (red) mixture solutions. In both mixture solutions, the concentrations of As from each species were equally set to 5 µg/kg



measurements of the background intensity. The detection limit of the inorganic arsenic was 0.13 µg/kg. In order to compare the value obtained using the capillary column with that of the conventional column, a separate experiment was performed. The volume of sample loop combined with the conventional column was 100 µL, which is 20 times larger than that (5 µL) using the capillary column. The detection limit, 0.033 µg/kg, from the experiment using the conventional column was much lower than that obtained using the capillary column. However, it might be unfair to compare the detection limits of the methods using capillary and conventional columns with different amounts of samples. Thus, instead of using concentrations, masses (=amount of sample × detection limit in concentration) were considered:

Capillary: 
$$5.0 \times 10^{-6} \text{ kg} \times \frac{0.13 \text{ } \mu\text{g}}{1 \text{ kg}} = 0.65 \times 10^{-6} \text{ } \mu\text{g}$$
 (1)

Conventional: 
$$100 \times 10^{-6} \text{ kg} \times \frac{0.033 \text{ } \mu\text{g}}{1 \text{ kg}} = 3.33 \times 10^{-6} \text{ } \mu\text{g}$$
 (2)

As a result, the capillary-column method was found to show even better detection-limit performance than the conventional column method.

# Quantitation of total inorganic arsenic in water and human urine

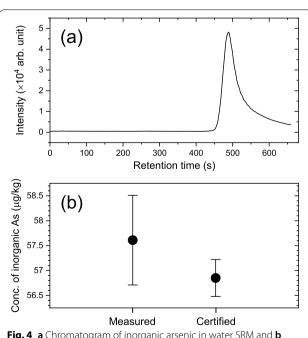
For the validation of the developed analytical method, the determination of inorganic arsenic species in the standard reference material of water was performed. A 2.706 mL of the water SRM was mixed with 0.800 mL of hydrogen peroxide to oxidize As(III) to As(V), and

the solution was diluted to 8.119 mL with the eluent. This process led to the dilution factor of 3. The resulting solution was analyzed by IC with the capillary column coupled with ICP-MS. As shown in Fig. 4a, the arsenic species present in the water SRM was identified as inorganic ones as compared to Fig. 1. The measured concentration of inorganic arsenic,  $57.61\pm0.90~\mu g/kg$ , was in agreement with the certified concentration of As,  $56.85\pm0.37~\mu g/kg$  (Fig. 4) in the SRM. The recovery efficiency was almost 101.3%. The result was obtained based on three analyses.

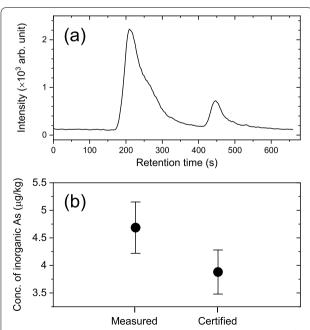
Due to the toxicity of arsenics (particularly inorganic arsenics), human exposure to arsenic needs to be investigated by an appropriate method capable of determining the quantity and the species of arsenic. In this regard, arsenics in food and drinking water have been analyzed (Nam et al. 2016; Lai et al. 2004). Ingested arsenics undergo various metabolisms in the human body and are finally excreted. Thus, analysis of arsenics in human urine is known to be the most reliable medical diagnosis to check if a person has been exposed to arsenics (Lai et al. 2004; Scheer et al. 2012). The method developed in this work was also applied to analyze total inorganic arsenic in human urine. A 0.498 mL of the human urine SRM was mixed with 0.500 mL of hydrogen peroxide to oxidize As(III) to As(V), and the solution was diluted to 1.448 mL with the eluent. This process led to dilution factor of 3. The resulting solution was analyzed by IC with the capillary column coupled with ICP-MS. As shown in Fig. 5a, the inorganic arsenic species were well separated from the organic ones in the human urine. The measured concentration of total inorganic arsenic was  $4.69 \pm 0.47$  µg/kg, which agreed with the certified value,  $3.88 \pm 0.40 \,\mu g/kg$ , within the experimental uncertainties (Fig. 5b). The result was obtained based on three analyses. The recovery efficiency was 121% and inferior to that obtained in the analysis of the water SRM. This could be attributed to the more complex matrix of human urine than that of water. The matrix effect on the recovery efficiency needs further detailed investigations. However, the bias (= measured concentration - certified concentration) values are very close to each other. The bias of the inorganic arsenic analysis in the human urine SRM is +0.81 (= 4.69-3.88) µg/kg, and that in the water SRM is +0.76 (= 57.61–56.85) µg/kg. Thus, the larger recovery efficiency observed in the analysis of the human urine SRM could be an accuracy issue raised by the instrument and not the sample matrix. The advantages of the method developed compared to other conventional methods including HPLC-ICP-MS were the improved detection capability, low sample volume, and fast analysis time.

#### Conclusion

An analytical method based on the IC-ICP-MS using a capillary chromatography column was developed for the determination of total inorganic arsenics in small-volume



**Fig. 4** a Chromatogram of inorganic arsenic in water SRM and **b** the measured concentration of inorganic arsenic and the certified concentration of As in the SRM



**Fig. 5** a Chromatogram of arsenic species in human urine SRM and **b** the measured and certified concentrations of inorganic arsenic in human urine SRM

samples. This method consumed 5 µL of the sample which is much smaller than that (100  $\mu$ L) for the IC-ICP-MS analysis using a conventional column. Although the chromatographic resolution was not high enough to separate the four arsenic species (DMA, MMA, As(III), and As(V)), the capillary column could be used to separately quantitate total inorganic arsenics (As(III) + As(V)) by oxidizing As(III) to As(V) using hydrogen peroxide prior to IC. The detection limit was 0.13 µg/kg for inorganic arsenics dissolved in water, which was lower than that of the conventional column IC-ICP-MS analysis (0.033 µg/kg). However, in terms of masses, the detection limit of the capillary column method was found to be at the level of sub-picogram that is even better than that of the conventional column method. A linear calibration curve could be obtained for inorganic arsenics dissolved in water. The developed method was successfully validated using the water SRM and showed excellent recovery efficiency. Finally, the developed method was applied to analyze inorganic arsenics in the human urine SRM with  $3.88 \pm 0.40$  µg/kg. In spite of the low concentration and the different matrix, the recovery efficiency was found to be 121%. Our method of analyzing total inorganic arsenics minimized sample consumption and thus would be particularly helpful for medical diagnosis and forensic investigations with limited amount of biological samples.

#### Acknowledgements

This work was supported by the Korea Basic Science Institute (KBSI) National Research Facilities & Equipment Center (NFEC) grant funded by the Korea government (Ministry of Education) (No. 2019R1A6C1010005) and the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2019R1A2C1003069). The authors thank Prof. Lee Wah Lim, Gifu University in Japan, for her technical support.

#### **Author contributions**

SHN designed the study and directed all experiments. YL advised on technical support and measurement. The experiment was executed by SJY. All authors read and approved the final manuscript.

#### **Funding**

No applicable.

## Availability of data and materials

All data generated and analyzed in this study have been provided in the manuscript.

#### **Declarations**

#### **Competing interests**

The authors declare that they have no competing interests.

Received: 8 July 2022 Accepted: 16 November 2022 Published online: 28 November 2022

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