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Quantification of arsenic compounds using derivatization, solvent extraction and liquid chromatography electrospray ionization tandem mass spectrometry

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ABSTRACT

The difficulty in detecting inorganic arsenic compounds using conventional settings of mass chromatography has led to the use of derivatizing agents to aid in their detection. A recent study indicated that 2,3-dimercaptopropanol (BAL) could be used to derivatize arsenic compounds to make them detectable by LC coupled to UV detector. A speciation analysis method was then developed for arsenic compounds after derivatization using the LC–MS/MS with the aim to improve the sensitivity and specificity. The arsenic compounds were derivatized with BAL before solvent extraction was carried out. The resultant extract was analyzed using the LC–MS/MS. However, our finding showed that BAL, being a thiol, reduced the pentavalent arsenic compounds, As(V) to a trivalent state, As(III). The arsenic metabolites, monomethyl arsonate (MMA) and dimethyl arsenic acid (DMA) could also be reduced by BAL to As(V)– and As(III)–BAL adducts. Despite this, the assay could be used to quantify the total arsenic concentration as a summation of all these adducts, when speciation of individual arsenic species is not required. The developed LC–MS/MS assay was subsequently applied to detect arsenic compounds in rat urine samples after oral administration of arsenic trioxide.

1. Introduction

Speciation is defined as the "distribution of an element amongst defined chemical species in a system" while speciation analysis is defined as "analytical activity of identifying and/or measuring the quantities of one or more individual chemical species in a sample" [1]. Arsenic exists in different forms, inorganic and organic and also in various valencies. It is an environmental hazard [2] capable of causing acute toxicity in humans in minute amounts. Its toxicity differs from one form to the other; inorganic arsenic compounds are much more toxic compared to organic arsenic compounds, with inorganic trivalent species being the most toxic and organic arsenobetaine being the least. In general, the trivalent arsenic species are also more toxic compared to the pentavalent species [3]. Due to its toxicity, the limit of total arsenic in potable water has been established by World Health Organization (WHO) to be 0.010 mg/l [http://www.who.int/water_sanitation_health/naturalhazards/en/index1.html].

Interestingly, arsenic trioxide is a drug approved by Food and Drug Administration (FDA) for the treatment of acute promyelotic leukaemia (APL) [4]. Identification and quantification of arsenic compounds is important for pharmacokinetic studies after administration, and for ensuring that its levels are kept within limits in the environment [5]. With such low limits, a highly sensitive method for speciation analysis of arsenic compounds is needed.

Currently, the most sensitive and effective analytical method for arsenic is to employ high performance liquid chromatography linked to inductively coupled plasma mass spectrometry (HPLC–ICP-MS). There are many advantages of using the ICP-MS as a detector; namely its high specificity, a high linear range, multi-element detection and its ability to remove signals of interference from those of interest [6]. However, ICP-MS requires rather large sample sizes that are not applicable to small biological samples [7]. Moreover, ICP-MS is a highly specific elemental detector that is not commonly found in many laboratories. Other disadvantages of the ICP-MS include the restraints placed on the selection of the mobile phase for use in the HPLC [6] and its high cost [2].

Recent studies indicate that arsenic could complex with certain derivatizing agents, rendering them detectable by ultraviolet detector [2]. However, the wavelength of 190 nm employed for detection is expected to detect a lot of interference from endogenous compounds in biological samples. Another recent study demonstrates that derivatized platinum could be made detectable by the common LC–MS/MS [7]. As arsenic and platinum are both

Abbreviations: As(III), arsenite; As(V), arsenate; BAL, British Anti Lewisite; CE, collision energy; CXP, collision exit potential; DP, declustering potential; DMA, dimethyl arsenic acid; DCM, dichloromethane; EPI, enhanced product ion; ER, enhanced resolution; EP, entrance potential; MMA, monomethyl arsenic acid.

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inorganic molecules, it is possible that derivatized arsenic can also be detected by LC–MS/MS. The need for derivatizing arsenic arises from the fact that it has been proven difficult to separate un-derivatized arsenic species using conventional reversed phase liquid chromatography and almost impossible to detect them by spectrophotometry or mass spectrometry [2]. The use of LC–MS/MS in arsenic speciation after derivatization could overcome these difficulties by increasing their retention in liquid chromatography and ionization in mass spectrometry.

The aim of this study was to develop a speciation analysis method for arsenic compounds using the LC–MS/MS. 2,3-Dimercaptopropanol, better known as British Anti Lewisite (BAL) was experimented as derivatizing agents. In this study, arsenic compounds would be derivatized with BAL, followed by solvent extraction and analysis by the LC–MS/MS.

2. Experimental

2.1. Materials

Potassium arsenate (KH_2AsO_4, As(V)), dimethyl arsenic acid ($(CH_3)_2AsO(OH)$, DMA), arsenic trioxide (As_2O_3), BAL and hexane were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium monomethyl arsonate ($CH_4AsNaO_3 \cdot (3/2)H_2O$, MMA) was purchased from Chem Service (West Chester, PA). Sodium hydroxide, hydrochloric acid (fuming 37% extra pure) and dichloromethane were purchased from Merck KGaA Co. (Darmstadt, Germany). Ethanol was purchased from Aik Moh Paints & Chemicals Pte Ltd. (Singapore). Methanol was obtained from Tedia (USA). Deionized water was produced by a Milli-pore purification system and used throughout the experiment.

2.2. Instrumentation

The method for the analysis of arsenic compounds was developed for the QTRAP™ 2000 LC–MS/MS system (Applied Biosystems/MDS Sciex, Concord, Ontario, Canada) coupled to an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA). The QTRAP™ analyzer combines a fully functional triple-quadrupole and linear ion trap mass spectrometer within the same platform. Scan types used for the MS/MS included two triple-quadrupole scan types, multiple reaction monitoring (MRM) and Q1 scan types as well as two linear ion trap scan type, enhanced product ion (EPI) and enhanced resolution (ER) scan types. The analyses were performed using a turbo ion spray source in a negative mode. Nitrogen served as nebulizer gas and collision gas in this mode. The mass spectrometer, the HPLC system, data acquisition and processing were controlled by Analyst 1.4.1 software from Applied Biosystems/MDS Sciex.

2.3. Direct infusion by MS only

2.3.1. Preparation of stock solution

The aqueous stock solutions of As(V), MMA and DMA each with a concentration of 1000 ± 5 ppm were prepared. Sodium arsenite (As(III)) stock solution (1000 ± 5 ppm) was made by first dissolving 5.82 mg As_2O_3 in 10 ml 0.1 M NaOH and then being neutralized with concentrated HCl to pH 7.0 ± 0.2.

2.3.2. Arsenic compound for direct infusion without derivatization

A 1 ppm diluted stock solution was prepared by diluting the stock solution with methanol. The diluted sample was then analyzed by direct infusion. The operation conditions for the analysis in negative ionization mode were the following: ion spray voltage, −5500 V; curtain gas (CUR), 10 (arbitrary units); GS1 and GS2, 20 and 20 psi, respectively; probe temperature, 300 °C.

2.3.3. Arsenic compounds derivatized with BAL in water

The derivatization and extraction process was based on a recent study by Cathum et al. [2]. A 2% BAL solution was prepared by diluting the BAL with ethanol. Arsenic stock solutions were diluted to 1 ppm using deionized water. 50 μl of the BAL was added to 1 ml of the diluted arsenic solutions and ultrasonicated for 30 min. Either a 1 ml 1:1 hexane/DCM mixture or pure DCM was added to the arsenic–derivative solution and shaken vigorously for 20 s before left to equilibrate for 1 h. The organic layers were extracted and dried using compressed air before dissolving the residue in methanol for analysis by direct infusion. The operation conditions for the analysis were the same as described in Section 2.3.2.

2.3.4. Inorganic arsenic compounds derivatized with BAL in rat urine

Three male Sprague–Dawley (SD) rats (7–8 weeks of age, average 250 g) were purchased from Animal Holding Unit, National University of Singapore. They were housed in metabolic cages and provided with a standard diet and water ad libitum. The room was kept on a 12/12-h light/dark cycle at a temperature of 23 ± 1 °C and relative humidity of 50 ± 10%. Urine blanks were collected at the end of interval of 0–24 h after a 24-h fasting period. The urine blanks were pooled and centrifuged at $3000 \times g$ for 15 min to remove particulate materials. A 2% BAL solution was prepared by diluting the BAL with ethanol. Inorganic arsenic stock solutions, namely As(III) and As(V), were diluted to 0.01, 0.1 and 1 ppm using rat blank urine, respectively. 50 μl of the BAL was subsequently added to 1 ml of rat urine with the spiked inorganic arsenic and ultrasonicated for 30 min. 1 ml DCM was added and shaken vigorously for 20 s before left to equilibrate for 1 h. The bottom organic layers were extracted and dried using compressed air before dissolving the residue in methanol for analysis by direct infusion under the same operation conditions as described in Section 2.3.2. The preparation of urine negative controls was the same as described above except the replacement of the spiked As(III) and As(V) with water.

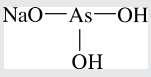
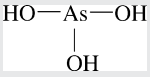
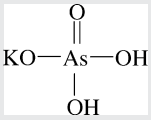
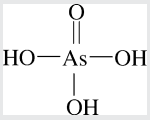
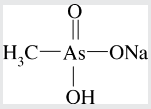
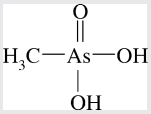
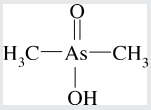
2.4. Rat urine analysis by LC–MS/MS after administration of As_2O_3

As_2O_3 solution was prepared as described in Section 2.3.1. One healthy male Sprague–Dawley rat (7–8 weeks of age, around 250 g) purchased from Animal Holding Unit was housed in the metabolic cage under the same condition as described in Section 2.3.4. At least one week of acclimatization period was allowed for the rat prior to drug administration. Urine blank was collected at the end of interval of 0–24 h after a 24-h fasting period. The rat subsequently received As_2O_3 solution by gavage administration with a single dosage of 10 mg/kg body weight. Every 24 h urine outputs were collected for consecutive 3 days and stored frozen at −80 °C until analysis.

The urine blank and sample were centrifuged at $3000 \times g$ for 15 min to remove particulate materials. A 2% BAL solution was prepared by diluting the BAL with ethanol. 50 μl of the BAL was subsequently added to 1 ml of rat urine and ultrasonicated for 30 min. 1 ml DCM was added and shaken vigorously for 20 s before left to equilibrate for 1 h. The bottom organic layers were extracted and dried using compressed air before dissolving the residue in methanol for analysis by LC–MS/MS.

Separations were accomplished on a Luna C18 column (50 mm × 1.0 mm, 3 μm) (Phenomenex, Torrance, CA, USA) with a guard cartridge at ambient temperature of about 22 °C. The injection volume was 5 μl. The isocratic mobile phase consisted of 90% solvent A (0.1% formic acid in water) and 10% solvent B (acetonitrile)

Table 1
Names, structures, pK_a, molecular weights and optimized parameters for the QTRAP analysis of four arsenic compounds.

Names	Structures		pK_a of salt	Molecular weights of free acid	DP (V)	CE (eV)	EP (V)	CXP (V)
	Salt	Free acid						
As(III)			9.2 13.5	126	NA	NA	NA	NA
As(V)			2.3 6.9	142	−40	−30	−4	−3
MMA			11.4 3.6	140	−35	−20	−4	−3
DMA	–		8.2 9.3	138	−30	−30	−4	−3

NA – not applicable, as As(III) cannot be detected by LC–MS/MS as an inorganic compound without derivatization.

and was delivered at a flow rate of 0.1 ml/min. The total run time was 10 min. The mass spectrometer was operated in the negative ion mode with a TurboIonSpray source. Mass spectrometry optimization was achieved by adjustments of both the compound dependent parameters (declustering potential (DP), entrance potential (EP), collision energy (CE)) and source dependent parameters (curtain gas, collision gas, ion spray voltage, source temperature, ion source gas 1 (GS1), ion source gas 2 (GS2)). Multiple reaction monitoring was utilized to obtain quantitative information of the arsenic compounds.

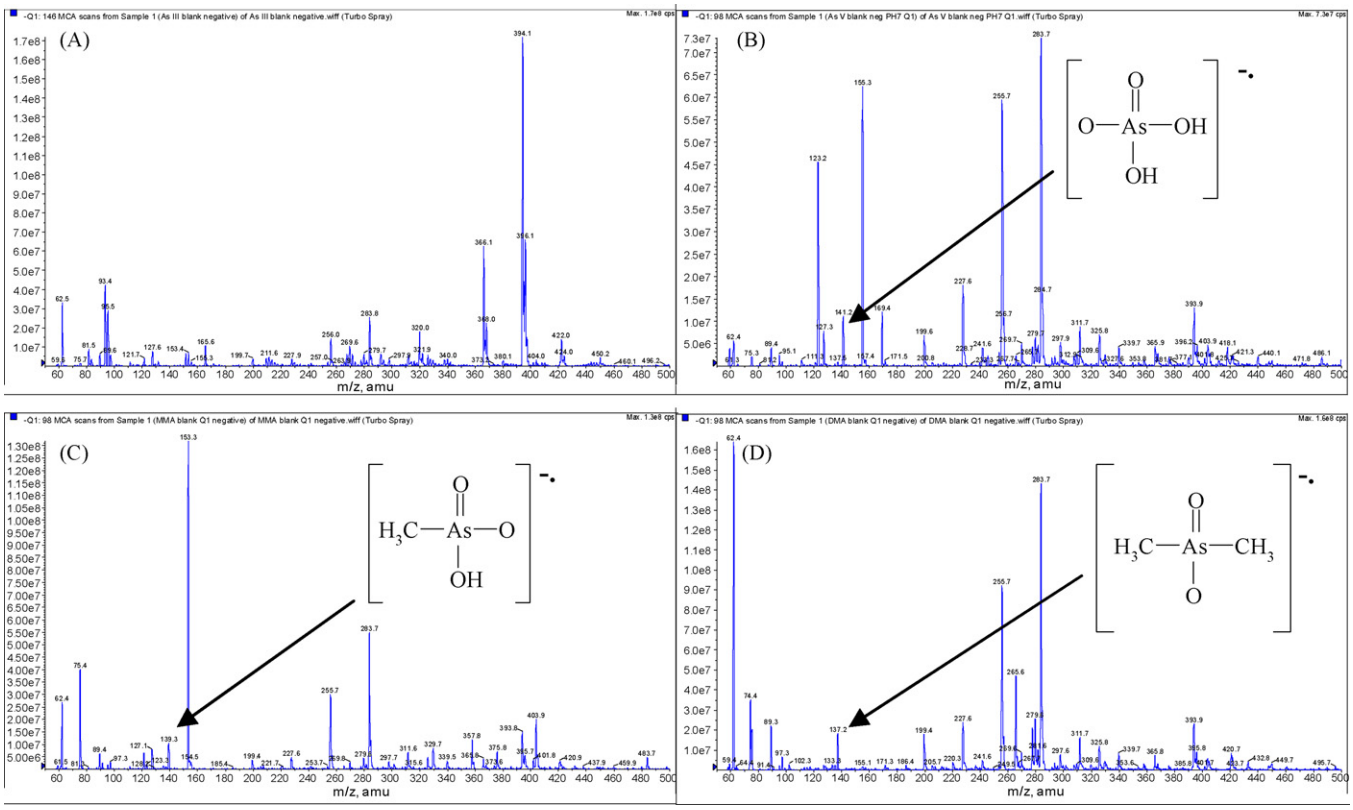


Fig. 1. Mass spectra of As(III) (A), As(V) (B), MMA (C) and DMA (D). Peak of As(III) (m/z = 125) was not observed, whereas peaks of As(V) (m/z = 141), MMA (m/z = 139) and DMA (m/z = 137) were observed in the spectra after direct infusion into the mass spectrometer.

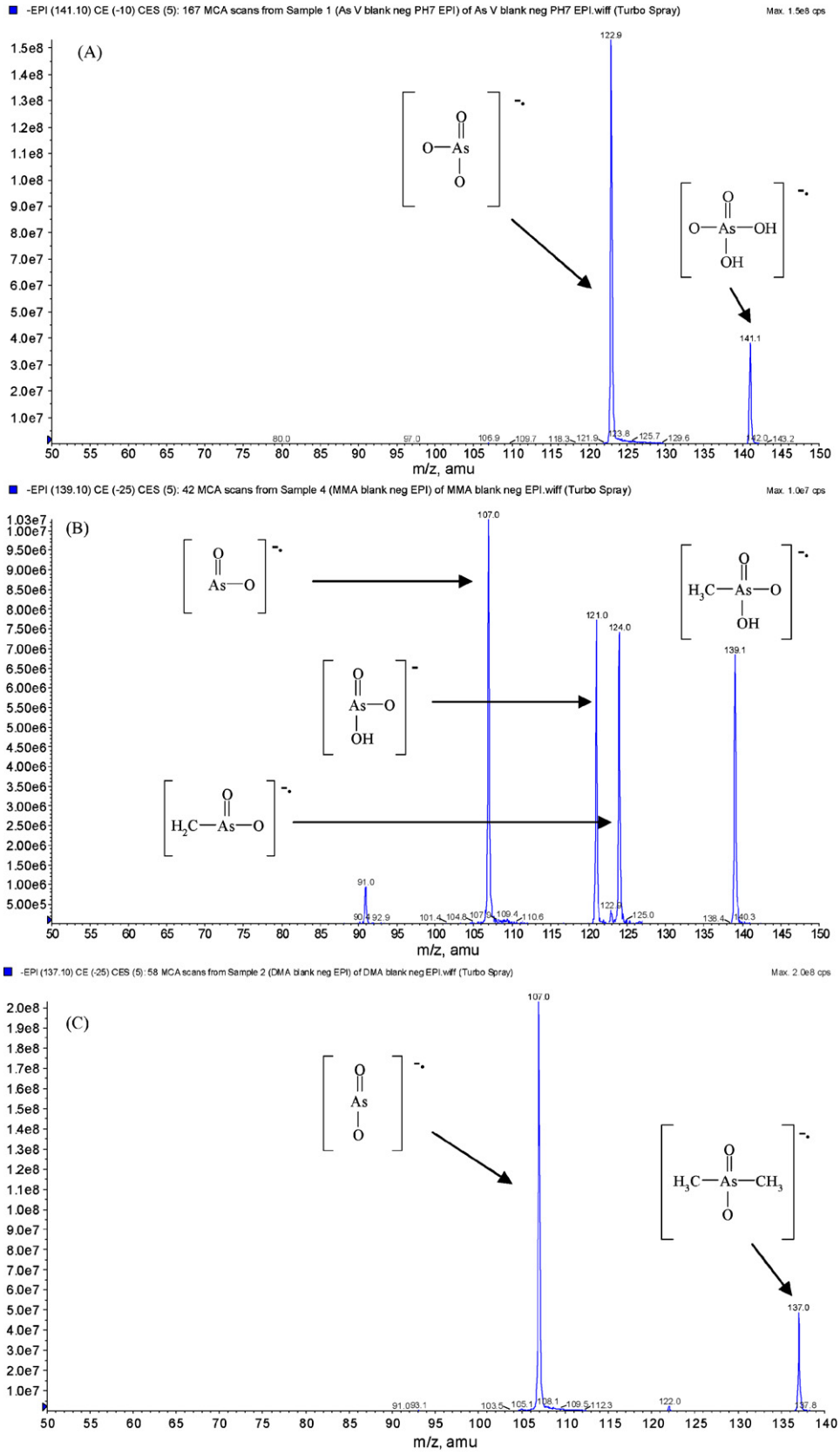


Fig. 2. Enhanced product ion (EPI) spectra of As(V) (A), MMA (B) and DMA (C). (A) The precursor ion showed *m*/*z* 141.1 while that of the product ion was *m*/*z* 122.9, which corresponded to the fragment $[As(V)–H–H_2O]^{-\bullet}$ (B) The precursor ion showed *m*/*z* 139.1 and those of the product ions were *m*/*z* 124.0, 121.0 and 107.0. The *m*/*z* 124.0 corresponded to the fragment $[MMA–H–CH_3]^{-\bullet}$; *m*/*z* 121.0 to fragment $[MMA–H–H_2O]^{-\bullet}$ and *m*/*z* 107.0 to $[MMA–H–CH_3OH]^{-\bullet}$. (C) The precursor ion showed *m*/*z* 137 while that of the product ion was *m*/*z* 107.0.

3. Results and discussions

3.1. Arsenic compound without derivatization through direct infusion

Both positive and negative scan modes were experimented in pilot study with negative scan mode having the more satisfactory results. It could be explained by the fact that arsenic compounds are acidic in nature and have deprotonatable functional groups to donate protons to the solvent.

Direct infusion of the arsenic compounds was experimented to determine if the compounds could be detected in the mass spectrometry without a derivatizing agent. Table 1 gives the name, structure, pK_a and molecular weight of the arsenic compounds. In solution, the arsenic salts dissociate partially to form free acids. Interestingly, the peaks of these free acids were observed in the spectra while that of the salt were not as the counter ion has been separated during the ionization process.

Fig. 1 shows the spectra of As(III), As(V), MMA and DMA, respectively, while Fig. 2 provides their EPI spectrum. EPI of As(III) could not be obtained as it could not be detected in the mass spectrum. From Fig. 1A, As(III) (m/z 125) could not be detected. This was predicted as As(III) is inorganic and the conventional settings of QTRAP™ 2000 mass spectrometry are not suited to detect inorganic compounds. Interestingly, As(V) could be detected in the spectrum (Fig. 1B). It is theorized that the presence of a double bond improved the detection of As(V). From Fig. 1C and D, MMA and DMA could be detected. The detection was expected as they are organometallic molecules.

Optimization of the mass spectra parameters was performed to improve the intensity of the peaks through direct infusion of each compound. Q1 was scanned across a specific mass range of 50–500. Identification of the m/z of interest was then carried out. ER was carried out to allow for high resolution mass spectrum of the ion of interest to be obtained. Q1MI was done and the optimized compound dependant parameters: declustering potential, entrance potential and collision exit potential (CXP) were attained. EPI was scanned to obtain the optimized collision energy value. Under EPI scan, the precursor ion is isolated in Q1, fragmented in the LINAC collision cell (Q2) and the product ions are trapped in the Q3, which operates as a linear ion trap. The product ions are then mass selectively scanned to the ion detector. From this, the specific m/z values of the product ions from its precursor ion can be achieved. Table 1 shows the values of the parameters optimized.

The EPI spectrum of As(V) is illustrated in Fig. 2A. The theoretical structures of the product ions are also represented in the diagram. The precursor ion has a m/z 141.1 while that of the product ion was determined to be m/z 122.9; which corresponded to the fragment $[As(V)–H–H_2O]^{-\bullet}$. A water molecule has been removed from the parent ion. Its fragmentation pattern is shown in Fig. 3A.

In Fig. 2B, EPI of MMA shows the precursor ion of m/z 139.1 and that of the product ion to be m/z 124.0, 121.0 and 107.0. The m/z 124.0 corresponds to the fragment $[MMA–H–CH_3]^-$, with the removal of a methyl radical from the parent ion (a); m/z 121.0 to fragment $[MMA–H–H_2O]^{-\bullet}$, the removal of a water molecule (b) and m/z 107.0 to $[MMA–H–CH_3OH]^{-\bullet}$, the leaving of a methanol group (c). The fragmentation patterns are illustrated in Fig. 3B.

In Fig. 2C, EPI of DMA shows the precursor ion of m/z 137 and the product ion of 107.0 corresponding to the fragment $[DMA–H–C_2H_6]^{-\bullet}$, where two methyl radicals are removed. Its fragmentation pattern is shown in Fig. 3C.

3.2. Arsenic compounds derivatized with BAL through direct infusion

3.2.1. Optimization of solvent extraction

Solvent extraction was done to extract the compound of interest from the water sample [8]. Water from the environment is

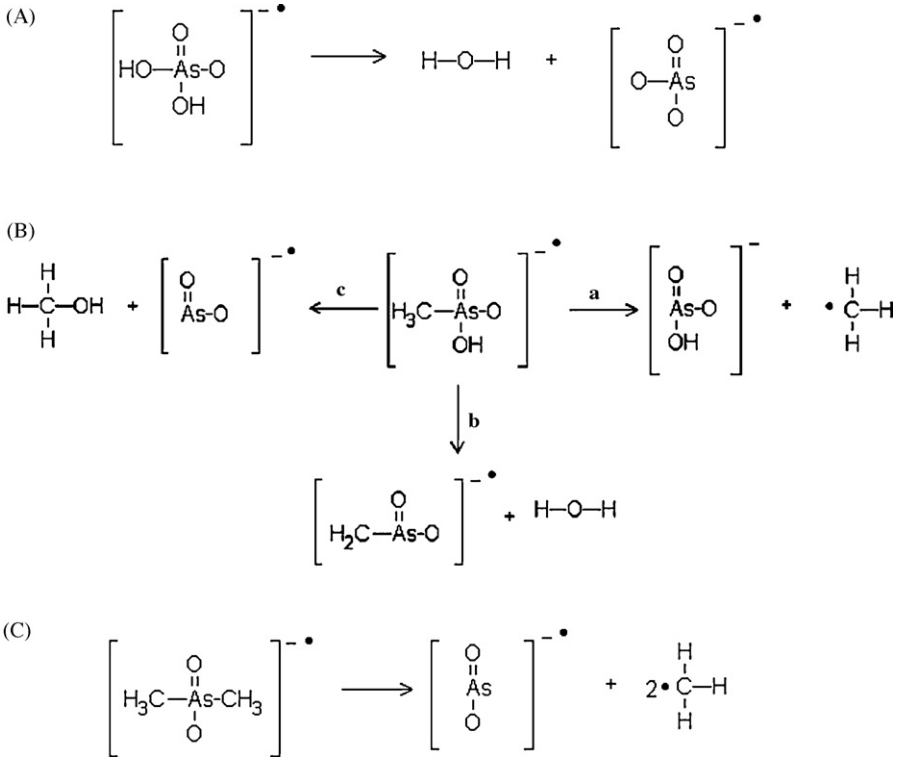


Fig. 3. Fragmentation patterns of As(V) (A), MMA (B) and DMA (C). (A) A water molecule has been removed from As(V). (B) Removal of methyl radical from MMA (a); removal of water molecule from MMA (b); removal of a methanol group (c). (C) Removal of two methyl radicals from DMA.

expected to contain contaminants, for example electrolytes, that may interfere with LC–MS/MS measurement. Extraction using organic solvents is able to eliminate these polar/charged contaminants from the sample.

The organic solvent had to be replaced after extraction. Neither hexane nor DCM were suitable solvents to be used in the LC–MS/MS as they are non-polar solvents and are not known to be able to give a stable spray or a satisfactory spectrum [9]. This could be due to their lack of conductivity as ESI-MS is essentially an electrochemical process. Methanol was chosen as it was reasonably volatile, to allow for the drying gas to remove it from the charged droplets formed during the ESI process [14] and polar, to allow for conduction necessary for the ionization process to take place.

Solvent extraction of the arsenic–BAL was done using a mixture of hexane and DCM as according to Cathum et al. [2]. After leaving the mixture to stand, three immiscible layers were observed. DCM has a relative density of 1.3 [http://www.pcl.ox.ac.uk/MSDS/DI/dichloromethane.html] (compared to 1 of water), while hexane has a relative density of 0.659 [http://www.pcl.ox.ac.uk/MSDS/HE/hexane.html]. The top layer is identified as hexane, the middle layer water and the bottom layer DCM.

The extraction efficiency of the two organic solvents and the efficiency of one with respect to another are unknown. As such, the percentage of arsenic that was extracted by hexane as compared to DCM cannot be calculated. Accurate quantification of the various arsenic compounds cannot be carried out without these values.

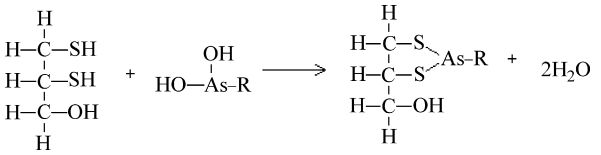
As a result, a single organic solvent was chosen to perform the extraction. DCM was chosen over hexane as it has a lower boiling point (40 °C compared to 69 °C) and would be easier to remove after extraction by drying. Although hexane was not experimented as an extractant, it is not eliminated as a choice for future studies.

3.2.2. *Inorganic and organic arsenic compounds derivatized with BAL in water*

The arsenic–BAL compounds were analyzed by direct infusion. Table 2 gives a summary of the structures and molecular weights of BAL and arsenic–BAL. It should be noted that intramolecular hydrogen bondings shown with dashed line were formed for both As(V)–BAL and MMA–BAL in Table 2.

As(III)–BAL (*m*/*z* 213) (Fig. 4A) could be observed in the spectrum, which proves that derivatization does occur under the conditions provided. Its methanol adduct (*m*/*z* 245) was also observed. As(V)–BAL (*m*/*z* 229) (Fig. 4B) was also observed. However, peaks at *m*/*z* 213 and 245 were also present in the spectrum. EPI done following this showed that those fragment ion peaks were not due to product ions of As(V)–BAL but due to the presence of As(III)–BAL. Peaks of MMA–BAL (*m*/*z* 227) (Fig. 4C) were not observed, but peaks of As(III)–BAL, As(III)–BAL methanol adduct and As(V)–BAL were observed in its spectrum. Peaks of DMA–BAL (Fig. 4D) could not be established, but peaks of As(III)–BAL, As(III)–BAL methanol adduct and As(V)–BAL were observed in its spectrum.

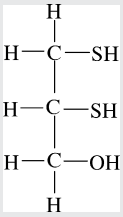
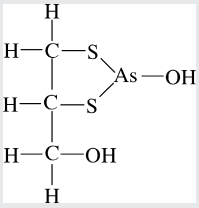
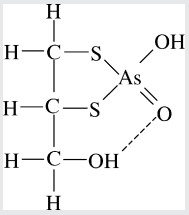
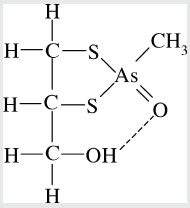
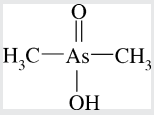
The reaction between BAL and arsenic is illustrated in Eq. (1) below.



(1)

Enhanced product ion spectra of As(III)–BAL, As(III)–BAL methanol adduct and As(V)–BAL were shown in Fig. 5. Based on the Eq. (1), the fragmentation mechanism of As(III)–BAL, As(III)–BAL methanol adduct and As(V)–BAL were investigated, respectively. The dashed arrows in Fig. 5 showed the positions of cleavage, respectively, which led to the generation of the most abundant fragment ions i.e. *m*/*z* 89, 155 and 105.

Table 2
Structures and molecular weights of BAL and arsenic–BAL.

Names	Structures	Molecular weight of species
BAL		124
As(III)–BAL		214
As(V)–BAL		230
MMA–BAL		228
DMA		138

pH adjustment was done as the peaks observed in the spectrum were not very strong in intensity. It is hypothesized that due to the conditions in which the experiment was carried out, the arsenic compounds remained largely in an ionized state. The organic DCM was unable to extract the highly polar compounds. Hence it was put forward that if the pH of the solution was adjusted such that the arsenic species were present in unionized forms, the extraction process will undertake more effectively. Concentrated HCL was used to adjust the pH of the arsenic solutions to pH 1. The pH was chosen after careful consideration of the pK_a of the arsenic molecules (Table 1). Analysis of these acidified arsenic–BAL solutions revealed that the intensity did not improve. Hence, pH being an important determinant of the intensity of the peaks was eliminated.

It was deduced that the instability of the BAL solution resulted in the production of peaks of low intensity [10]. The degradation of the BAL would lead to a decrease in the amount of derivatized products being formed and hence giving rise to a much smaller peak than expected. The arsenic–BAL compounds formed were not highly charged and this may also have affected the intensity as an important factor governing the production of sufficiently high

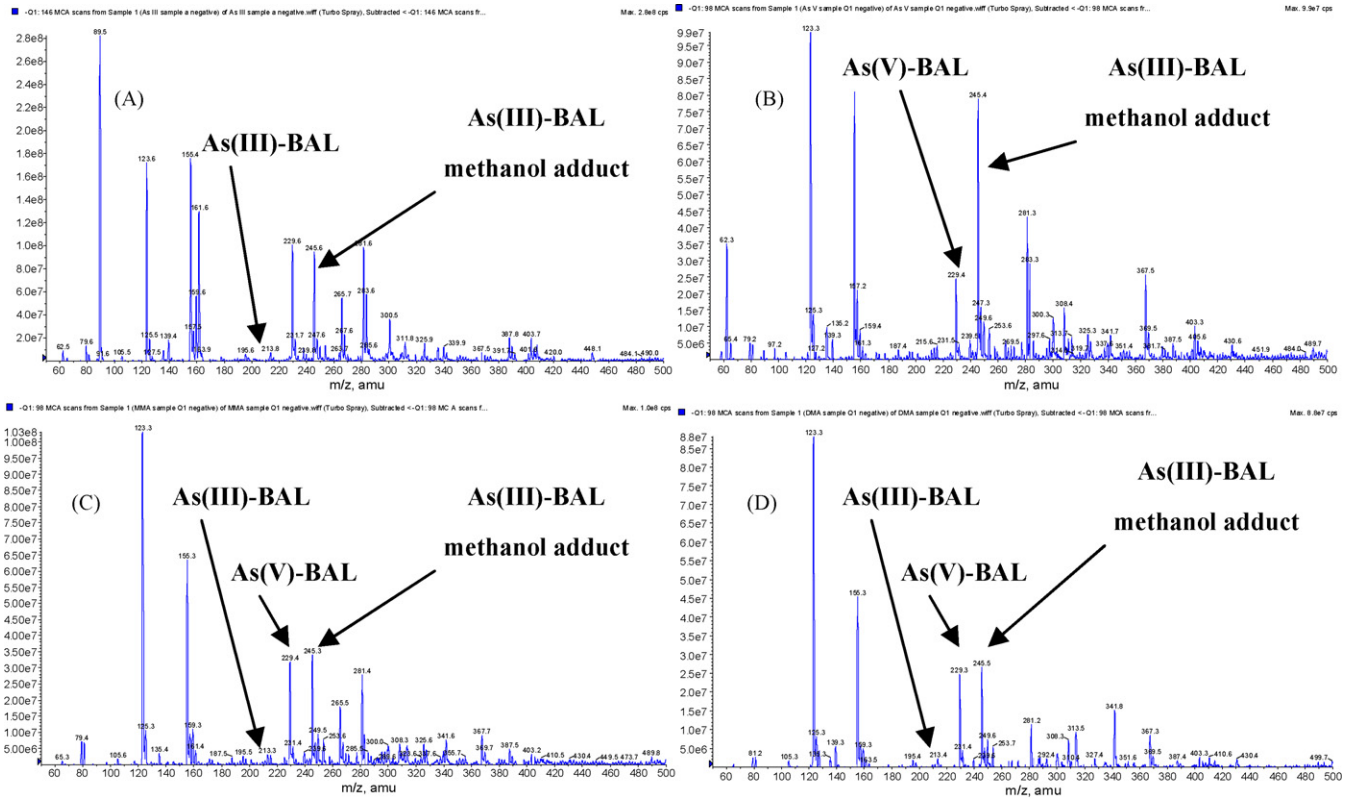


Fig. 4. Mass spectra of As(III)–BAL (A), As(V)–BAL (B), MMA–BAL (C) and DMA–BAL (D). (A) As(III)–BAL (m/z 213) could be observed in the spectrum. Its methanol adduct (m/z 245) was also observed. (B) As(V)–BAL (m/z 229) was observed. Peaks at m/z 213 and 245 were also present in the spectrum due to the presence of As(III). (C) Peaks of MMA–BAL (m/z 227) were not observed, but peaks of As(III)–BAL, As(III)–BAL methanol adduct and As(V)–BAL were observed. (D) Peaks of DMA–BAL could not be established, but peaks of As(III)–BAL, As(III)–BAL methanol adduct and As(V)–BAL were observed.

and sensitive peaks is the creation of preformed ions in solution [15].

It has been reported in many papers that thiol groups, BAL including, have reducing properties [10–13,16]. Presence of peaks of the trivalent arsenic compounds in the spectra of the pentavalent compounds further proves this. Thiol groups consist of a sulphur atom bonded to a hydrogen atom. It is a highly nucleophilic group [17] and is able to function as a reducing agent by donating electrons. Pentavalent arsenic compounds are electrophilic and would be able to accept the electrons, leading to its reduction to a trivalent state.

Hence, the accurate identification and quantification of the various arsenic compounds cannot be carried out using BAL as a derivatizing agent. It then comes to the question of the reliability of the relevant results obtained by Cathum et al. [2]. Arsenic was also derivatized by BAL in their study. From their results, all the arsenic species responded to the chelation and were detected by the HPLC with a diode array detector. The amount of arsenic species was also quantitated through multiple channel wavelengths [2]. However, considering BAL is able to reduce the pentavalent arsenic species into trivalent arsenic species based on our findings, those relevant results reported by Cathum would not be reliable and needs further verification.

3.2.3. Inorganic arsenic compounds derivatized with BAL in rat urine

It has been reported that urinary excretion is the major pathway of arsenic elimination from system circulation [18]. Hence, pooled rat urine was chosen to be the biological matrix to spike inorganic arsenic compounds, namely As(III) and As(V). Organic arsenic compounds such as MMA and DMA in rat urine were not evaluated herein since they could be detected easily even without derivatization with BAL.

Fig. 6 showed the reconstructed mass spectra of As(III)–BAL and As(V)–BAL in rat urine after subtraction of urine negative controls, respectively, with spiked As(III) of 0.01 ppm (A) and spiked As(V) of 0.01 ppm (B). As(III)–BAL methanol adduct (m/z 245) (Fig. 6A) could be observed in the mass spectra, which proved that derivatization with BAL did occur under the conditions provided. However, As(V)–BAL (m/z 229) was not observed in Fig. 6B. Only the peaks at m/z 245 were present in the spectrum. EPI done following this showed that those fragment ion peaks were not due to product ions of As(V)–BAL but due to the presence of As(III)–BAL methanol adduct. These results suggested that the total As(V) had been reduced to As(III) completely in the matrix of rat urine. Since this phenomenon was not observed in the matrix of water, it is possible to speculate that synergistic effect of certain reductive components in rat urine and BAL led to the enhancement of reduction from As(V) to As(III). Although further investigation need to be done to verify the postulation, this interesting finding gave a hint to individually quantify the As(III) and As(V) in the matrix of rat urine.

3.3. Rat urine analysis by LC–MS/MS after oral administration of As_2O_3

Most of the inorganic arsenic species i.e. As(III) and As(V) are metabolized in humans and many mammalians to methylated arsenic species including MMA and DMA, which are more readily excreted into the urine than the inorganic arsenic species [19]. Based on the results of Figs. 2 and 5, a list of MRM transitions, including 245.1 → 155.0 for As(III)–BAL methanol adduct, 229.4 → 105.0 for As(V)–BAL, 141.1 → 123.0 for As(V), 139.1 → 107.0 for MMA,

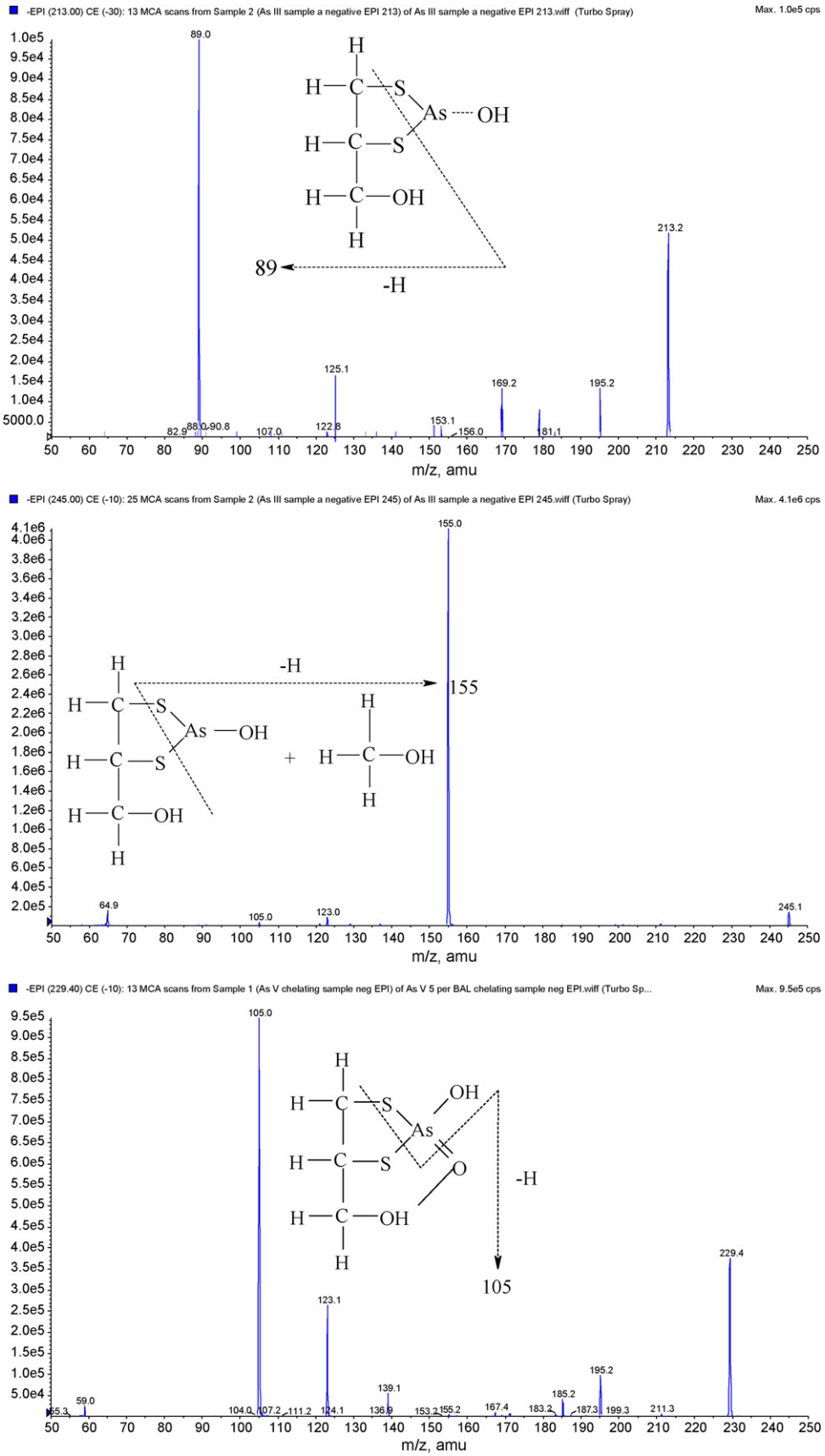


Fig. 5. Enhanced product ion (EPI) spectra of As(III)–BAL (A), As(III)–BAL methanol adduct (B) and As(V)–BAL (C).

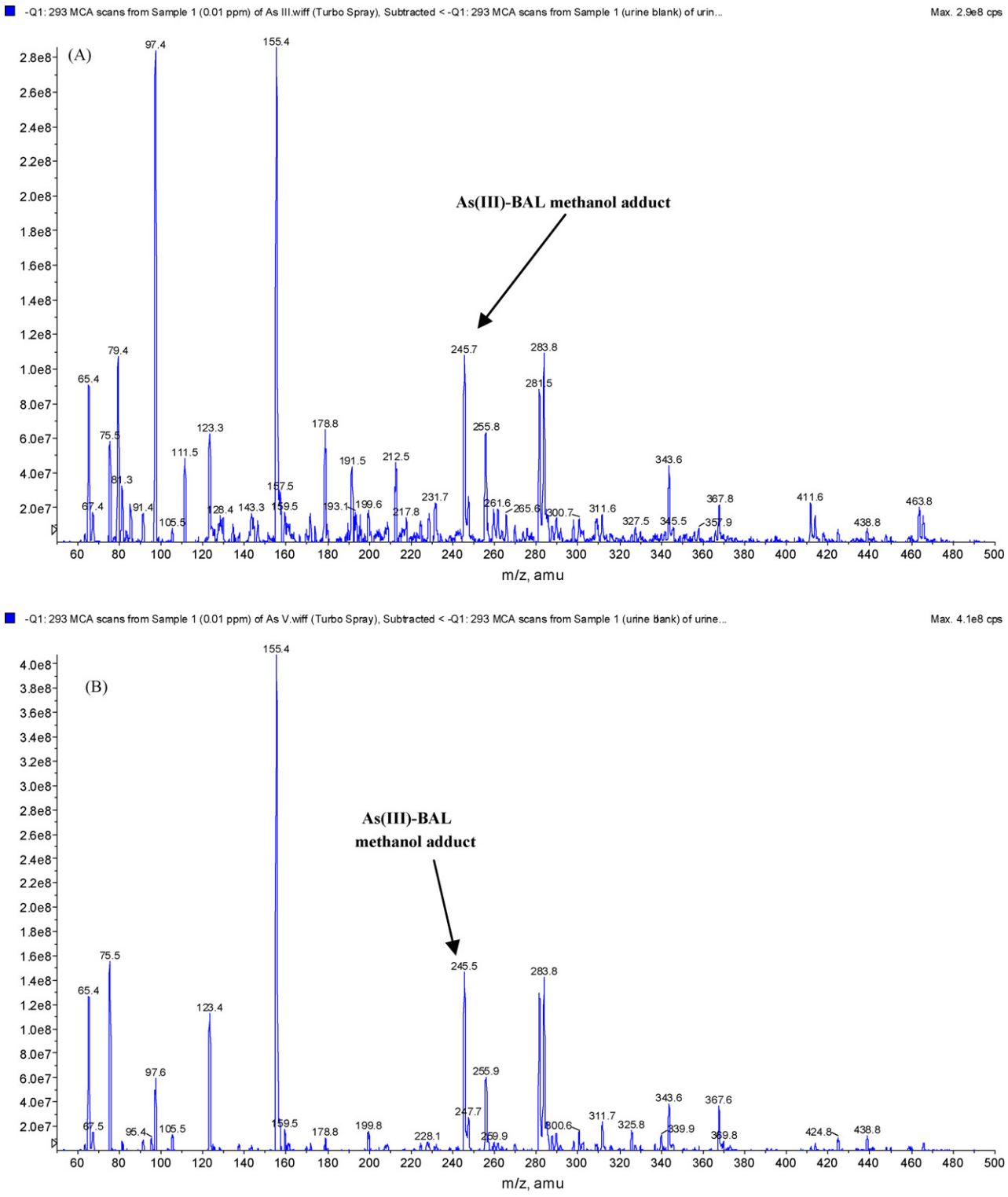


Fig. 6. Mass spectra of As(III)–BAL and As(V)–BAL in rat urine with spiked As(III) of 0.01 ppm (A) and As(V) of 0.01 ppm (B). (A) Only As(III)–BAL methanol adduct (m/z 245) could be observed in the spectrum. (B) Only As(III)–BAL methanol adduct (m/z 245) could be observed in the spectrum.

137.1 → 107.0 for DMA was used to simultaneously detect the arsenic compounds which were possible to be present in rat urine after administration of As_2O_3. The MRM transition 213.1 → 89.0 for As(III)–BAL was excluded due to its lower abundance as compared to that of 245.1 → 155.0 for As(III)–BAL methanol adduct. The mass parameter conditions were optimized as follows: curtain gas, 20 (arbitrary units); ion source gas 1, 50 (arbitrary units); ion source gas 2, 60 (arbitrary units); source temperature (TEM), 400 °C; declustering potential, −30 V; collision energy, −30 eV; entrance potential, −10 V. The dwell time of each MRM transition was 150 ms.

The LC–MS/MS results showed that both As(III)–BAL methanol adduct and As(V) were absent in rat urine throughout 3 days after administration of As_2O_3. As(V)–BAL was detected with trace

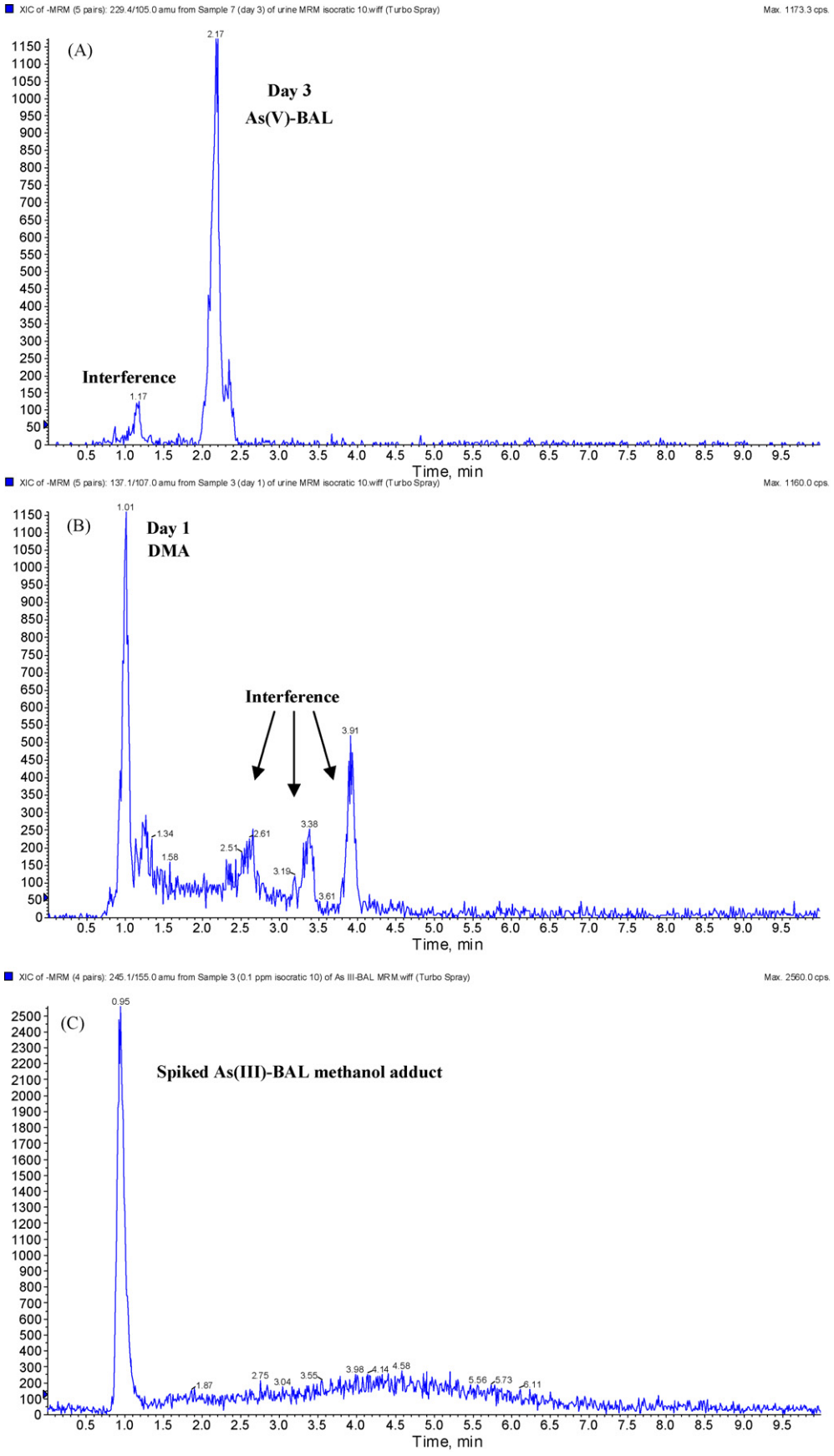


Fig. 7. Representative extracted ion chromatograms of MRM transitions in rat urine: 229.4 → 105.0 for As(V)–BAL at day 3 after administration (A), 137.1 → 107.0 for DMA at day 1 after administration (B), and 245.1 → 155.0 for As(III)–BAL methanol adduct after spiking 0.1 ppm As(III) (C).

amount at day 2 and with significant amount at day 3. The representative MRM chromatogram of As(V)–BAL in rat urine at day 3 was shown in Fig. 7A. In addition, DMA was significantly present in the rat urine at day 1 after administration of As_2O_3 while absent at day 2 and day 3. The representative MRM chromatogram of DMA in rat urine at day 1 was shown in Fig. 7B. Interference peaks in Fig. 7 were identified due to their presence in the rat urine blank before administration. Furthermore, MMA was detected with trace amount at day 1 while absent at day 2 and day 3. The reasons why As(III)–BAL methanol adduct could not be detected in the *in vivo* biological samples are unknown. After oral administration of arsenic compounds, the urine samples could contain various arsenic species. As(V)–BAL can be formed from As(V) itself and also from both MMA and DMA as shown in Fig. 4C and D. The results indicated the complication in interpretation of the mass spectra in determining the concentrations of the individual arsenic species. The proposed method could only be used to quantify the total arsenic concentration as the summation of concentrations of all the arsenic–BAL adducts.

In order to verify whether As(III)–BAL methanol adduct could be detected under the LC–MS/MS conditions described above, 0.1 ppm As(III) was spiked into rat urine blank and underwent the procedures of sample preparation in Section 2.3.4. The MRM chromatogram of As(III)–BAL methanol adduct in rat urine showed a significant peak without any interference peaks (Fig. 7C), suggesting As(III)–BAL methanol adduct was detectable by LC–MS/MS. Future work could involve the collection of rat urine with higher level of As(III) after repeated administration of As_2O_3 to further confirm this method.

4. Conclusion

Derivatization of arsenic species with BAL to render them suitable for speciation by the LC–MS/MS has not been completely successful. More optimization and experimentation would have to be carried out in order to develop a method of arsenic speciation using the LC–MS/MS. It must be noted that derivatizing agent with thiol groups, such as BAL is not recommended due to its reducing properties. Despite this, the assay could be used as an alternative to atomic absorption spectrometry to quantify the total arsenic concentration, when speciation of individual arsenic species is not required.

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