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ABSTRACT

Inorganic arsenic species are metabolized to monomethylarsonic acid (MMA^V) and dimethylarsinic acid (DMA^V) and excreted into urine. A simple, rapid and sensitive method has been developed using electrospray ionization tandem mass spectrometry (ESI–MS–MS) for the simultaneous determination of MMA^V and DMA^V. MMA^V and DMA^V in a sample were allowed to react with citric acid (CiA). Adduct compounds were extracted together with isoamyl alcohol (IAA). An aliquot (1-μL) of the IAA layer was directly injected into the ESI–MS–MS instrument, and was detected within 1 min. Quantification was done using selected reaction monitoring for MMA^V and DMA^V as follows.

$[MMAH + 2CiA - 3H_2O]^+ \rightarrow [MMAH + CiA - 2H_2O]^+$

$[DMAH + CiA + MeOH - 2H_2O]^+ \rightarrow [DMAH + MeOH - H_2O]^+$

Where, MMAH and DMAH denote the protonated forms of MMA^V and DMA^V, and MeOH denotes methanol (carrier liquid in ESI–MS–MS). This method was validated for the analysis of urine samples. The limit of detection of As was 0.3 μgL^{-1} for MMA^V and 0.6 μgL^{-1} for DMA^V using 10 μL of sample solution. Results were obtained in <10 min with a linear calibration range of 3–100 μgL^{-1}. Inorganic arsenic compounds (and other organic arsenic compounds) found in urine did not interfere with the detection of MMA^V and DMA^V. Concentrations of MMA^V and DMA^V in the reference urine SRM 2670a were estimated after partial purification, and those in urine of a patient treated with As_2O_3 were measured after dilution.

Key words: Monomethylarsonic acid, Dimethylarsinic acid, Tandem mass spectrometry, Electrospray ionization, Citric acid, Adduct

1. Introduction

Inorganic arsenic species such as arsenite (As^{III}) and arsenate (As^V) in drinking water at levels of several hundred micrograms per liter are known to induce cancers of skin, liver, lung, bladder and kidney as well as disorders of circulatory and nervous systems in human body [1,2]. On the other hand, As_2O_3 is administered intravenously to acute promyelocytic leukemia patients at a daily dose of 0.15 $mgkg^{-1}$ body weight in some medical treatment [3]. The absorbed As is metabolized by consecutive reduction and oxidative methylation mainly to monomethylarsonic acid (MMA^V), monomethylarsonous acid (MMA^{III}), dimethylarsinic acid (DMA^V), and

dimethylarsinous acid (DMA^{III}) [1-5]. Here, MMA^{III} and DMA^{III} are more toxic whereas MMA^{V} and DMA^{V} are less toxic than inorganic arsenic species, respectively [1-5]. They are then excreted into urine mostly in the form of MMA^{V} and DMA^{V} since MMA^{III} and DMA^{III} are chemically labile compounds. Other organic As compounds such as arsenobataine (AsB), arsenocholin (AsC) and arsenosugars are contained abundantly (usually in the $mgkg^{-1}$ range) in fish, mussel and algae [6], but their toxicity is low. For example, AsB is excreted into urine quickly and unchanged.

Many analytical techniques have been employed to separate and quantify these various arsenic compounds having different toxicities. Liquid chromatography–inductively-coupled plasma–mass spectrometry (LC–ICP–MS) produced a high selectivity in determining atomic mass $m/z = 75$ of As and high sensitivity having a limit of detection (LOD) of 0.14–0.33 μgL^{-1} [5]. LC–ICP–MS offers a great advantage in quantification of atoms, but identification of chemical species containing certain atoms is based entirely on agreement of the chromatographic retention time with that of available reference chemical species. In some chromatographic separations, highly toxic inorganic As^{III} elutes together with MMA^{V} [5,7] or negligibly toxic AsB [6,7], and in other separation, the peak of dimethylthioarsenical was mis-assigned as that of DMA^{III} [2]. The atomic mass in the detection of ^{75}As, $m/z = 75$, is easily interfered with by isobars such as FeOH [8] and ArCl resulting from the combination of Ar from plasma gas and Cl from the sample solution [5,8].

Electrospray ionization (ESI) mass spectrometry (MS) provides a unique opportunity for the analysis of molecular forms of species [4,9-11]. Before 1997,

MMA^V (and sometimes DMA^V) could not be quantified on ESI–MS, and only qualitative data were provided in reports [9,11] because they are quite soluble in water and are difficult to ionize. Subsequent studies succeeded in the quantification, but LODs for MMA^V and DMA^V remained unsatisfactory, i.e., 305 pg and 72 pg in 1997 [10], and 200 pg and 78 pg in 1999 [4], respectively. To accelerate ESI of MMA^V and DMA^V, we added several acids to aqueous samples and extracted analytes with polar organic solvents (e.g., isoamyl alcohol (IAA)). In these studies, we found that MMA^V and DMA^V formed adduct ions with citric acid (CiA), having an intensity of 20-fold that of the protonated molecular ions. Signal enhancement due to adducts was also reported in anabolic steroids [12]. Adducts of MMA^V and DMA^V with CiA have not been reported, although adducts with methanol (MeOH) and ethanol used for the carrier liquid have [9]. The present study adopted tandem mass spectrometry (MS–MS) after ESI in the quantification of adducts of MMA^V and DMA^V to further increase detection sensitivity.

2. Materials and methods

IAA suitable for nucleic acid purification was obtained from Sigma-Aldrich, USA. Analytical grade MMA^V was obtained from Tri Chemical Laboratories Incorporated (Yamanashi, Japan). Analytical grade DMA^V, tetramethylarsonium (TeMA) iodide, AsB, AsC bromide, CiA, $NaAsO_2$ (As^{III}), $Na_2HAsO_4 .7H_2O$ (As^V) and other reagents were obtained from Wako Pure Chemicals (Osaka, Japan). Pure water having a specific resistance of 18 MΩ cm was used.

Control urine was obtained from healthy volunteers. Standard reference urine

(SRM 2670a) was purchased from the National Institute of Standards and Technology (Gaithersburg, MD, USA). We used SRM 2670a because a few reports on MMA^V and DMA^V were published (although it is a standard material for 29 inorganic ions).

Patient urine was obtained from a woman in her late seventies diagnosed as acute promyelocytic leukemia, who was administered with As_2O_3 intravenously daily over 2 h at a dose of $0.15 mg kg^{-1}$ for 25 consecutive days during induction therapy [3]. Void urine samples were collected on the last day of the therapy (day 1), 4 days after (day 5) and 7 days after (day 8), respectively, and were stored at - 20 °C for < 24 days before the assay.

MMA^V and DMA^V (1 g As L^{-1} in water) were the stock solutions. Calibration standard solutions and quality-control solutions were prepared by spiking stock solutions at 0, 3, 6, 10 and 100 μg As L^{-1} to water and urine that contained 1 M CiA.

MMA^V and DMA^V were assayed as follows. To 10 μL of sample or standard solution in a polypropylene tube with cap (Eppendorf AG, Germany), CiA as a solid (2 mg) was added to make a solution of 1 M CiA. CiA was not added to standard solutions if they contained CiA. After 60 s, 10 μL of IAA was added, vortex-mixed for 60 s, and centrifuged at 5000 × g for 30 s. The layer of IAA was used for detection of adducts of MMA^V and DMA^V.

When the concentrations of MMA^V and DMA^V in urine were <10 μg As L^{-1}, urine (or spiked urine) was concentrated to tenfold as follows. One milliliter of urine was passed through a cartridge for 1-mL sample (Waters Oasis Max 1cc 30 mg Extraction Cartridge consisted of a copolymer designed to have a hydophilic-liphophilic balance, WAT094225, Waters Company, Milford, MA, USA) that had been conditioned with 1

mL of MeOH and 1 mL of water in advance. Then 2 mL of water was flushed through the cartridge to wash-out analytes thoroughly. That is, MMA^V and DMA^V did not but some interfering substances did retain on the cartridge. Three milliliters of aqueous eluate was evaporated to dryness at 100°C under air flow in a hood chamber, and the analytes extracted twice with 50 μL of MeOH. One-hundred microliters of MeOH solution was evaporated to dryness at 45°C, and the analytes extracted twice with 10 μL of MeOH. To 20 μL of MeOH, 80 μL of 1.25 M CiA solution was added, and 10 μL of the solution was used for analyte assay. The treatments for urine containing MMA^V and DMA^V >10 μg As L^{-1} were described in section 3.3.

ESI–MS–MS was carried out on a TSQ 7000 LC-quadrupole mass spectrometer (Thermo Quest, Japan) in positive-ion mode. One microliter of the IAA layer was injected using a syringe (1701 PTFE-coated; Hamilton Company, Reno, NV, USA) manually in direct infusion. A characteristic spectrum appeared 30 s after sample injection; a sample could be injected every 60 s. MeOH was used as a mobile phase at 200 $\mu L min^{-1}$, and the capillary temperature set at 280°C. The electrospray voltage was 4.5 kV, the multiplier voltage was 1.3 kV, and the collision voltage was 10–50 V. Nitrogen was used as a sheath gas (469 kPa) and also as an auxiliary gas (8 units). Argon was a collision gas (134 kPa). Data on MS and MS–MS were collected at *m/z* 100–480 and *m/z* 50–500, respectively. Quantification in ESI–MS–MS was done by integration of the peak area of product ions using a calibration curve comprising spiked matrix samples at different concentrations.

3. Results and discussion

3.1 Production and extraction of adducts with CiA

The ionization efficiency of MMA^V and DMA^V is quite low, and their quantification was not done before 1997 [9,11]. Only qualitative studies were carried out on protonated molecular ions ($MMAH^+$ and $DMAH^+$) or adduct ions of MMA^V and DMA^V with MeOH used for the carrier liquid in ESI [9,11]. We therefore added various acids to the solutions of MMA^V and DMA^V because acidic environments enhance ionization in positive mode. When CiA was added, we found not only the ions reported previously [9,11] but also new ions such as $[MMAH + 2CiA - 3H_2O]^+$ and $[DMAH + CiA + MeOH - 2H_2O]^+$ derived from adducts of MMA^V and DMA^V with CiA. Solutions added with 1 M of HCl, H_2SO_4, HNO_3, acetic acid, oxalic acid, oxalacetic acid, tartaric acid, phenol or ammonium formate exhibited molecular ions as well as ions derived from adducts with MeOH and IAA. When 1 M of *cis*-aconitic acid or *trans*-aconitic acid was added to MMA^V and DMA^V, $MMAH^+$ and $DMAH^+$ were produced mainly but $[MMAH + 2 (aconitic\ acid) - H_2O]^+$ was not produced at all, and the production of $[DMAH + aconitic\ acid + MeOH - H_2O]^+$ from either of the two aconitic acids was only 10 % of the production of $[DMAH + CiA + MeOH - 2H_2O]^+$ from CiA. Therefore, the OH group attached at the central carbon of CiA may have played an important role in the production of $[MMAH + 2CiA - 3H_2O]^+$ and $[DMAH + CiA + MeOH - 2H_2O]^+$.

The production rate of the adducts of MMA^V and DMA^V with CiA increased with concentration up to 1 M of CiA without increasing the signal of blank solution at the *m/z* in the detection for both adducts. CiA solution at 1 M was therefore used in the quantification. Efficiencies of the extraction of adducts with several organic solvents were then compared. Extraction efficiency decreased in the order, IAA > hexanol > octanol > cyclohexanol >> methylisobutyl ketone, and the intensity of the

blank decreased in the order, cyclohexanol > octanol > hexanol > IAA, for both adducts. IAA was therefore selected as the extraction solvent.

3.2 MS and MS–MS spectra

MMA^{V}

The mass spectrum of the IAA layer extracted from 200 ngL^{-1} MMA^{V} in 1 M CiA solution is shown in Fig. 1 (a). It shows the molecular ion $MMAH^{+}$ at *m/z* 141, $[MMAH + MeOH - H_2O]^{+}$ at *m/z* 155, $[MMAH + 2MeOH - 2H_2O]^{+}$ at *m/z* 169, $[MMAH + CiA - 2H_2O]^{+}$ at *m/z* 297, $[MMAH + CiA + MeOH - 3H_2O]^{+}$ at *m/z* 311, $[MMAH + CiA + MeOH - 2H_2O]^{+}$ at *m/z* 329, $[MMAH + CiA + 2MeOH - 3H_2O]^{+}$ at *m/z* 343, $[MMAH + 2CiA - 4H_2O]^{+}$ at *m/z* 453 and $[MMAH + 2CiA - 3H_2O]^{+}$ at *m/z* 471. Other ions having an abundance of >5 % in Fig. 1 (a) were derived from CiA. The intensity of an adduct ion $[MMAH + 2CiA - 3H_2O]^{+}$ at *m/z* 471 was about 20-fold that of the molecular ion $MMAH^{+}$ at *m/z* 141 used for the quantification in other reports [4,10]. The adduct ion at *m/z* 471 was therefore selected as the precursor ion in the present MS–MS. Fig. 1 (b) shows the product ion spectrum with the collision voltage at 17 V. The product ions shown there were $[MMAH + 2CiA - 4H_2O]^{+}$ at *m/z* 453, $[MMAH + CiA - 2H_2O]^{+}$ at *m/z* 297, $[MMAH + CiA - 3H_2O]^{+}$ at *m/z* 279, and $MMAH^{+}$ at *m/z* 141. The peak at *m/z* 297 was the main peak at the collision voltages from 10 to 30 V showing the highest intensity of 3600 in normalized level (NL) at 17 V, whereas the peak at *m/z* 141 was the main peak at the collision voltage from 30 to 50 V showing the highest intensity of 620 in NL at 35 V. Therefore, the peak at *m/z* 297 with the collision voltage at 17 V was used for quantification.

DMA^V

The mass spectrum of the IAA layer extracted from 200 ngL^{-1} DMA^V in 1 M CiA solution is shown in Fig. 2 (a). It shows the molecular ion $DMAH^+$ at *m/z* 139, $[DMAH + MeOH - H_2O]^+$ at *m/z* 153, $[DMAH + CiA - 2H_2O]^+$ at *m/z* 295 and $[DMAH + CiA + MeOH - 2H_2O]^+$ at *m/z* 327. Other ions having an abundance of >5% in Fig. 2 (a) were derived from CiA. The intensity of an adduct ion $[DMAH + CiA + MeOH - 2H_2O]^+$ at *m/z* 327 was about 20-fold that of the molecular ion $DMAH^+$ at *m/z* 139 used for quantification in other studies [4,10]. The adduct ion at *m/z* 327 was therefore selected as the precursor ions in MS–MS analyses. Fig. 2 (b) shows the product ion spectrum with the collision voltage at 18 V. The product ions shown there were $[DMAH + CiA + MeOH - 3H_2O]^+$ at *m/z* 309, $[DMAH + CiA - 2H_2O]^+$ at *m/z* 295, $[DMAH + MeOH - H_2O]^+$ at *m/z* 153 and $DMAH^+$ at *m/z* 139. The peak at *m/z* 153 was the main peak at the collision voltages from 10 to 35 V showing the highest intensity of 9300 in NL at 26 V, whereas the peak at *m/z* 121 was the main peak at the collision voltage from 35 to 50 V showing the highest intensity of 3300 in NL at 40 V. Therefore, the peak at *m/z* 153 with the collision voltage at 26 V was used for quantification.

CiA adducts extracted with IAA were quite stable. In the present determination, 90% of the complex remained after 24 h in the IAA extract from aqueous solution and under room light at 25°C.

For the quantification of MMA^V and DMA^V, mass chromatograms of selective-reaction monitoring at *m/z* 297 were taken for MMA^V (a), and at *m/z* 153 for DMA^V (b), respectively (Fig. 3). As indicated in Fig. 3, 3 pg of As could be quantified in MMA^V and DMA^V.

3.3 Interference

The signals of MMA^V and DMA^V adducts did not disturb each other. Both adducts in a sample could be quantified by changing the *m/z* values of the precursor ion and the product ion, as well as the collision voltage. Interferences from various compounds were examined using the solution containing MMA^V and DMA^V at 100 μgL^{-1}. Inorganic As (As^{III} and As^V) and metal ions such as Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Zr, Mo, Ru, Pd, Ag, Cd, W, Pt and Pb at 10 mgL^{-1} did not interfere with detection of MMA^V and DMA^V in the present assay. Effects of organic As compounds such as TeMA, AsB and AsC at 100 mgL^{-1} were also examined. They did not form adducts with CiA and did not interfere with the signal of MMA^V and DMA^V adducts in the present assay. Recovery of >98 % was observed in the following solutions: <50 mM solution of *cis*-aconitic acid, *trans*-aconitic acid, HCl, H_3PO_4, H_2SO_4, HNO_3, $MgSO_4$, KH_2PO_4 or urea, <15 mM solution of NaCl, KCl, Na_2SO_4, $NaNO_3$, $NaHCO_3$ EDTA or nitrilotriacetic acid (NTA) for MMA^V; and <50 mM solution of of *cis*-aconitic acid, *trans*-aconitic acid, HCl, H_2SO_4, HNO_3, $MgSO_4$, KH_2PO_4 or urea, <15 mM solution of NaCl, KCl, Na_2SO_4, EDTA or NTA for DMA^V. Solutions with higher concentrations than the ones mentioned above inhibited detection more strongly. Severe matrix effects of urine have been reported in the previous work [10], and MMA^V and DMA^V in urine were not detected there. Signals of MMA^V and DMA^V in some urine samples were only one-hundredth of those in water. On the contrary, these signals extracted from the urine diluted to 1/2 – 1/5 with water were more than twice those extracted from the undiluted urine. Urine samples

containing MMA^V and DMA^V >50 μg As L^{-1} could be quantified only by dilution, whereas those for 50 to 10 μg As L^{-1} could be quantified by passing the urine through an Oasis cartridge and then diluting the eluate, since the cartridge could eliminate the interfering substances satisfactorily.

3.4 Precision and accuracy

Calibration standard solutions of MMA^V and DMA^V were prepared by spiking stock solutions at 0, 3, 10 and 100 μg As L^{-1} to water. Concentrations determined from the peak area (y) were linear to the concentrations spiked (x) up to 100 μgL^{-1} (i.e., $y = 11.38 x + 1.34$ with a correlation coefficient of 0.9993 for MMA^V, and $y = 10.83 x + 28.77$, with a correlation coefficient of 0.9938 for DMA^V). Precision and accuracy were assessed by analyses of water spiked at 3, 10 and 100 μgL^{-1} (Table 1). These samples were analyzed three times a day, as well as on three different days. The coefficient of variation was <24 %, and accuracy was 83–105 % for intra-day and inter-day variations. The limit of quantification (LOQ) of the present method was therefore 3 μgL^{-1} for MMA^V or DMA^V in aqueous solutions that contained non-interfering substances certified in the *Interference* section. Blanks that were not spiked with MMA^V and DMA^V were measured six times, and their standard deviations (σ) were calculated in pg based on the calibration. LODs in the present assay were 0.3 μgL^{-1} for MMA^V and 0.6 μgL^{-1} for DMA^V because LOD was defined to be 3σ for blank signals [5].

In case of urine containing MMA^V and DMA^V <10 μg As L^{-1}, calibration standard solutions were prepared by spiking stock solutions at 0, 3, 6 and 10 μg As L^{-1} to urine

and were concentrated to tenfold as explained in *Materials and method*. The concentration was expressed as that in the original urine although the quantification was performed using urine concentrated to tenfold. Concentrations determined from the peak area (y) were linear to the concentrations spiked (x) up to 10 μgL^{-1} (i.e., y = 20.49 x + 34.51 with a correlation coefficient of 0.9927 for MMA^V, and y = 16.21 x + 51.65, with a correlation coefficient of 0.9857 for DMA^V). Precision and accuracy were listed in Table 2, and the coefficient of variation was <19 % and accuracy was 85–108 %. The LOQ was therefore 3 μgL^{-1} for MMA^V or DMA^V in urine. Urine samples not spiked with MMA^V and DMA^V were measured six times, and LODs were 0.40 μgL^{-1} for MMA^V and 0.41 μgL^{-1} for DMA^V [5].

To check the recovery and validate the proposed procedure, standard reference urine, 2670a high-level urine, and low-level urine were examined. High-level and low-level urine indicate only the levels of inorganic ions. Reference urine, control urine and spiked urine were treated as described in the *Methods and materials* section because urine contains large amounts of interfering substances [10]. Figure 4 shows the results of urine samples. That is, blank, control urine, high-level urine (spiked at 0, 3 or 6 μgL^{-1}) and low-level urine (spiked at 0, 3 or 6 μgL^{-1}), respectively. The results indicated that MMA^V and DMA^V at 3 μgL^{-1} could be quantified in high-level urine and low-level urine, suggesting that the LOQ for MMA^V or DMA^V was 3 μgL^{-1} in urine. We tried to quantify the concentrations of MMA^V and DMA^V in the reference urine, although these were only estimated values. Concentrations of MMA^V and DMA^V in high-level urine were 0.39 ± 0.02 and 2.0 ± 0.1 μgL^{-1}, respectively, in [6]; 2.1 ± 0.4 μgL^{-1} and not detected in [2], respectively; and 0.5 ± 0.1 and 3.3 ± 0.1 μgL^{-1},

respectively, in this study. Concentrations of MMA^V and DMA^V in low-level urine were 0.34±0.02 and 1.2±0.1 μgL^{-1}, respectively in [6]; and 1.3±0.2 and 2.0±0.2 μgL^{-1}, respectively, in this study.

3.5 Determination of MMA^V and DMA^V in patient urine

Assay was made with 1 μL of each of the three void urine samples (day 1, 5 and 8) added to 9 μL of water. The concentrations of MMA^V on day 1, 5 and 8 were 226± 21, 154±3 and 80±2 μgL^{-1}, respectively (*n*=3), showing a significant decrease, whereas those of DMA^V were 833±44, 684±55 and 634±9 μgL^{-1}, respectively (*n*=3), retaining higher levels than those of MMA and showing a smaller decrease. Indeed, the concentrations of DMA^V on day 1, 5 and 8 were 3.9, 4.4 and 7.9 times those of MMA^V, respectively. Such higher concentrations of DMA^V than those of MMA^V were also reported on urines of inhabitants drinking well water polluted with As [1,5].

Compared with other ESI–MS–MS having an LOD of 72–305 pg in 1-μL injection [4,10], the present ESI–MS–MS has a much better LOQ (3 pg in 1-μL injection). Only by diluting urine with water, MMA^V and DMA^V can be assayed for urine collected from inhabitants in As-affected areas showing a high level of 56 μgL^{-1} and 161 μgL^{-1}, respectively [1], as well as urine from patients treated with As_2O_3 as in the present case. One of the reasons for much better sensitivity in the present method than that reported previously may be due to the 20-fold higher production of the CiA adduct ion than that of the molecular ion (Fig. 1 (a) and Fig. 2 (a), respectively).

Conclusion

Herein, we propose an ESI-MS-MS method for the simple, rapid and decisive determination of monomethylarsonic acid and dimethylarsinic acid after adduct formation with citric acid. The extraction of the adducts with isoamyl alcohol facilitated not only the ionization of the adducts but also the elimination of interfering substances in urine. Hence, it enabled a direct-flow-injection that gave the MS-MS data within 1 min. The isoamyl alcohol extract used for the determination of the adducts was available for the determination of AsB, AsC and TeMA only by changing the precursor ion and the product ion in the MS-MS, respectively.

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Table 1

Intra-day (3 times) and inter-day (3 days) variations of As values determined by the present method. The observed values and coefficient of variations (C.V.) for As spiked at 3 -100 μgL^{-1} in case of MMA (A) and DMA (B), respectively.

	Intra-day				Inter-day			
	A		B		A		B	
Spiked	Observed		Observed		Observed		Observed	
As	As	C.V.	As	C.V.	As	C.V.	As	C.V.
μgL^{-1}	μgL^{-1}	%	μgL^{-1}	%	μgL^{-1}	%	μgL^{-1}	%
3	2.67	8.4	2.66	23.9	2.86	9.7	2.49	10.0
10	9.94	4.4	10.13	4.3	9.26	6.9	10.84	22.4
100	99.7	2.6	95.1	10.2	100.1	3.3	104.8	3.8

Table 2

Intra-day (3 times) and inter-day (3 days) variations of As values in urine concentrated to tenfold and determined by the present method. The observed values and coefficient of variations (C.V.) for As spiked at 3, 6, 10 μgL^{-1} in case of MMA (A) and DMA (B), respectively.

	Intra-day				Inter-day			
	A		B		A		B	
Spiked	Observed		Observed		Observed		Observed	
As	As	C.V.	As	C.V.	As	C.V.	As	C.V.
μgL^{-1}	μgL^{-1}	%	μgL^{-1}	%	μgL^{-1}	%	μgL^{-1}	%
3	2.56	3.5	2.76	2.0	2.73	18.9	2.78	7.7
6	5.31	7.7	6.45	4.5	5.74	12.7	6.48	7.5
10	9.25	8.2	10.4	5.8	10.1	10.0	10.7	6.0

Figure captions

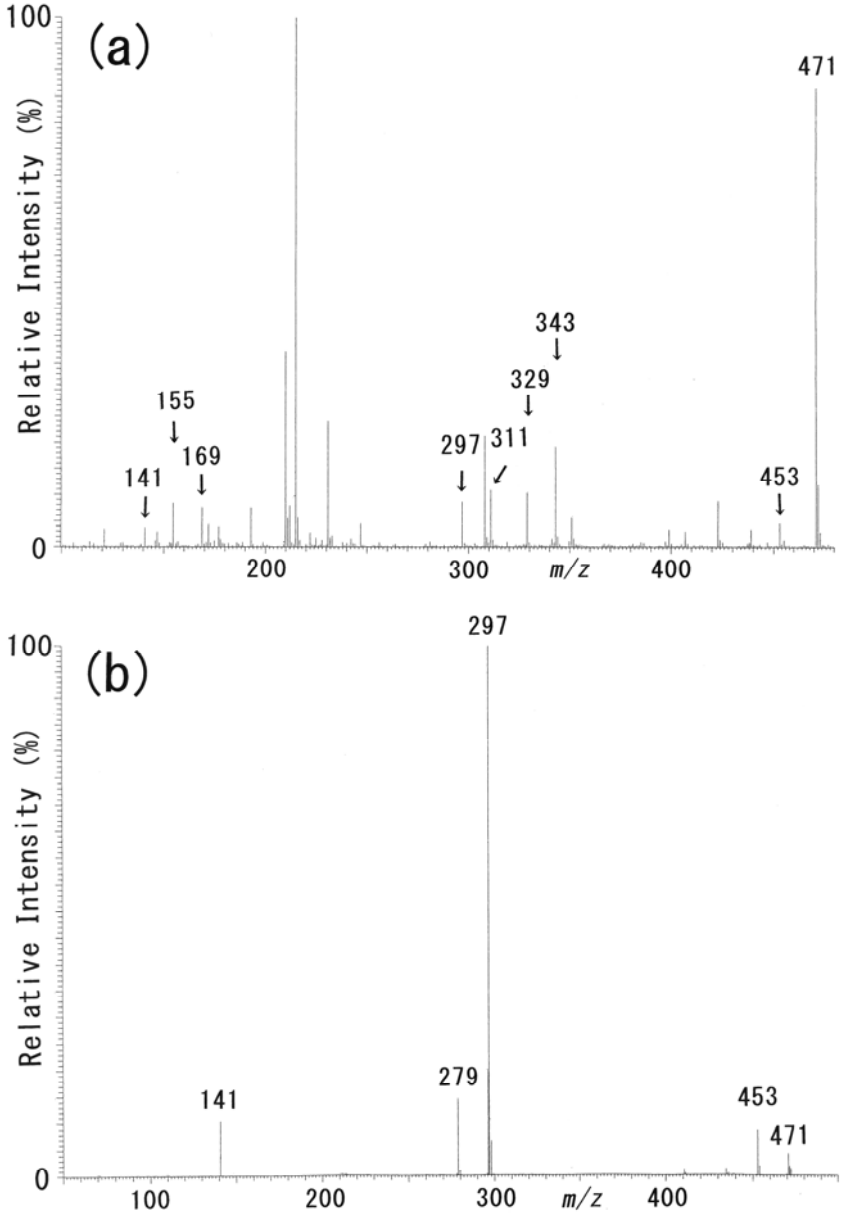

Figure 1: Mass spectrum of the layer of isoamyl alcohol extracted from 200 ngL^{-1} of MMA^{V} in 1 M citric acid (a) and the product ion spectrum at collision voltage of 17 V from the precursor ion $[MMAH + 2CiA - 3H_2O]^+$ at *m/z* 471 (b). The signal at *m/z* 141 is due to the molecular ion, and *m/z* values of ions related to MMA^{V} are denoted.

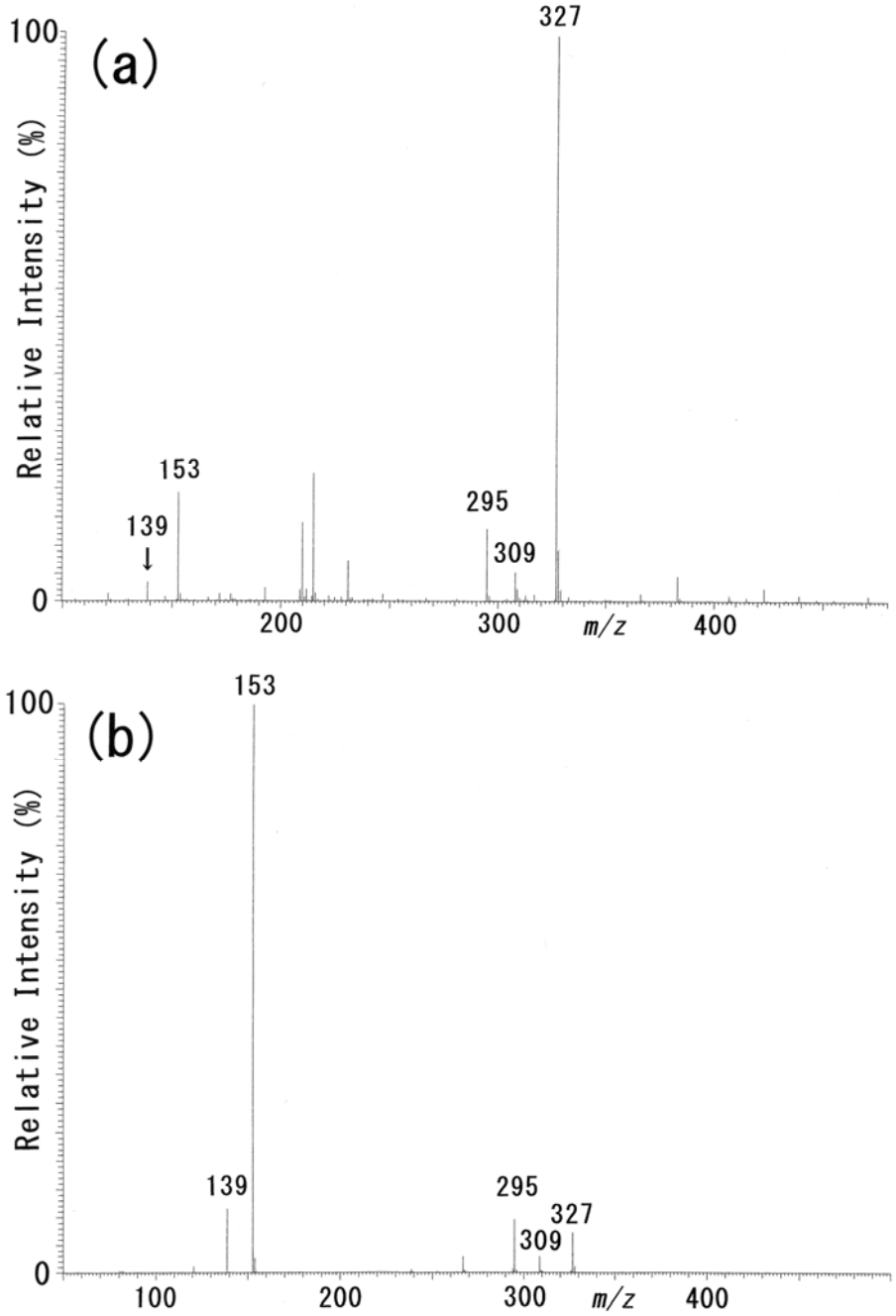

Figure 2: Mass spectrum of the layer of isoamyl alcohol extracted from 200 ngL^{-1} of DMA^{V} in 1 M citric acid (a) and the product ion spectrum at collision voltage of 18 V from the precursor ion $[DMAH + CiA + MeOH - 2H_2O]^+$ at *m/z* 327 (b). The signal at *m/z* 139 is due to the molecular ion, and *m/z* values of ions related to DMA^{V} are denoted.

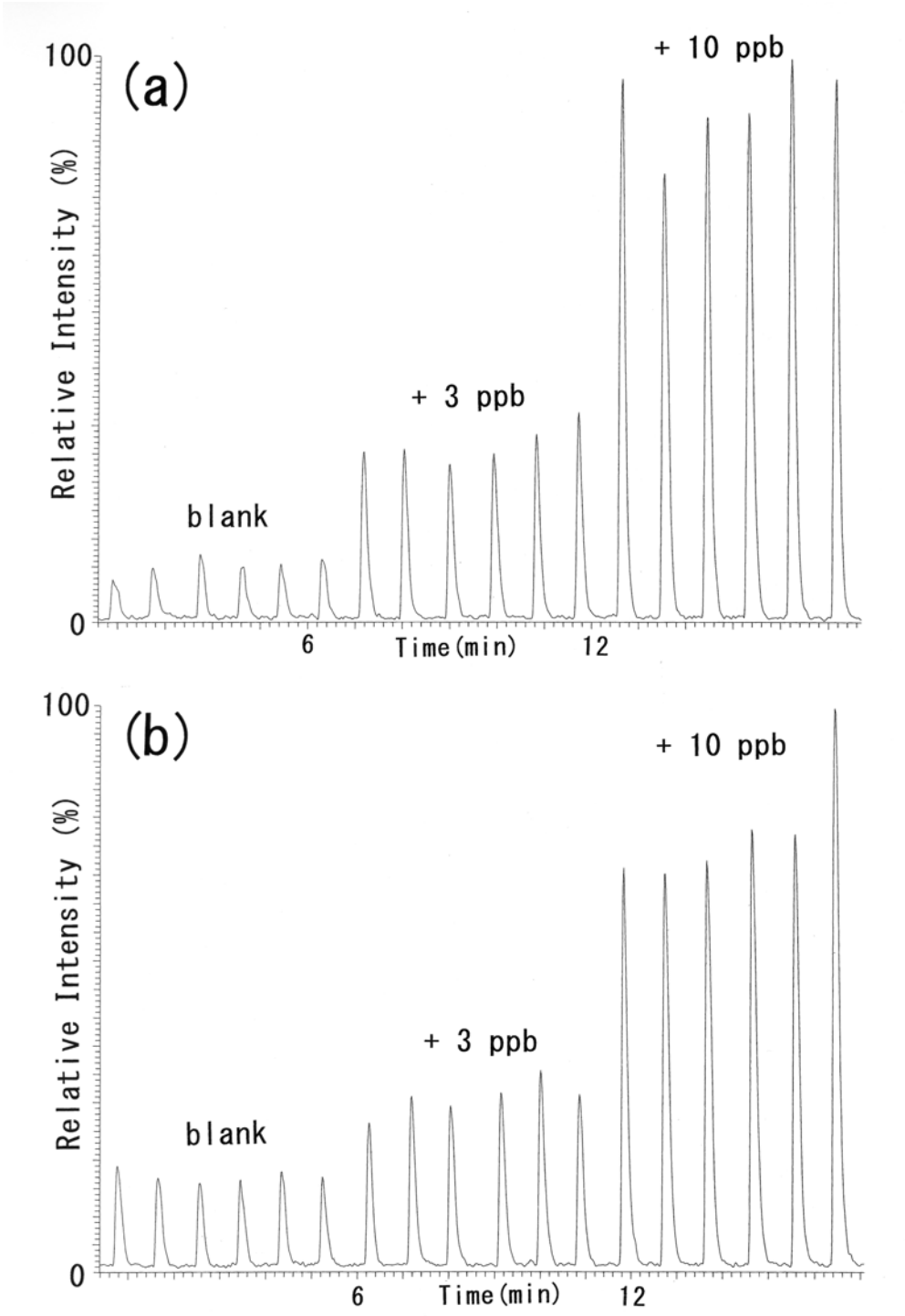

Figure 3: Mass chromatograms of selected reaction monitoring at *m/z* 297 derived from the precursor ion at *m/z* 471 for the quantification of MMA^{V} in water (a), and those at *m/z* 153 derived from the precursor ion at *m/z* 327 for the quantification of DMA^{V} in water (b). Samples were injected six times for each As concentration.

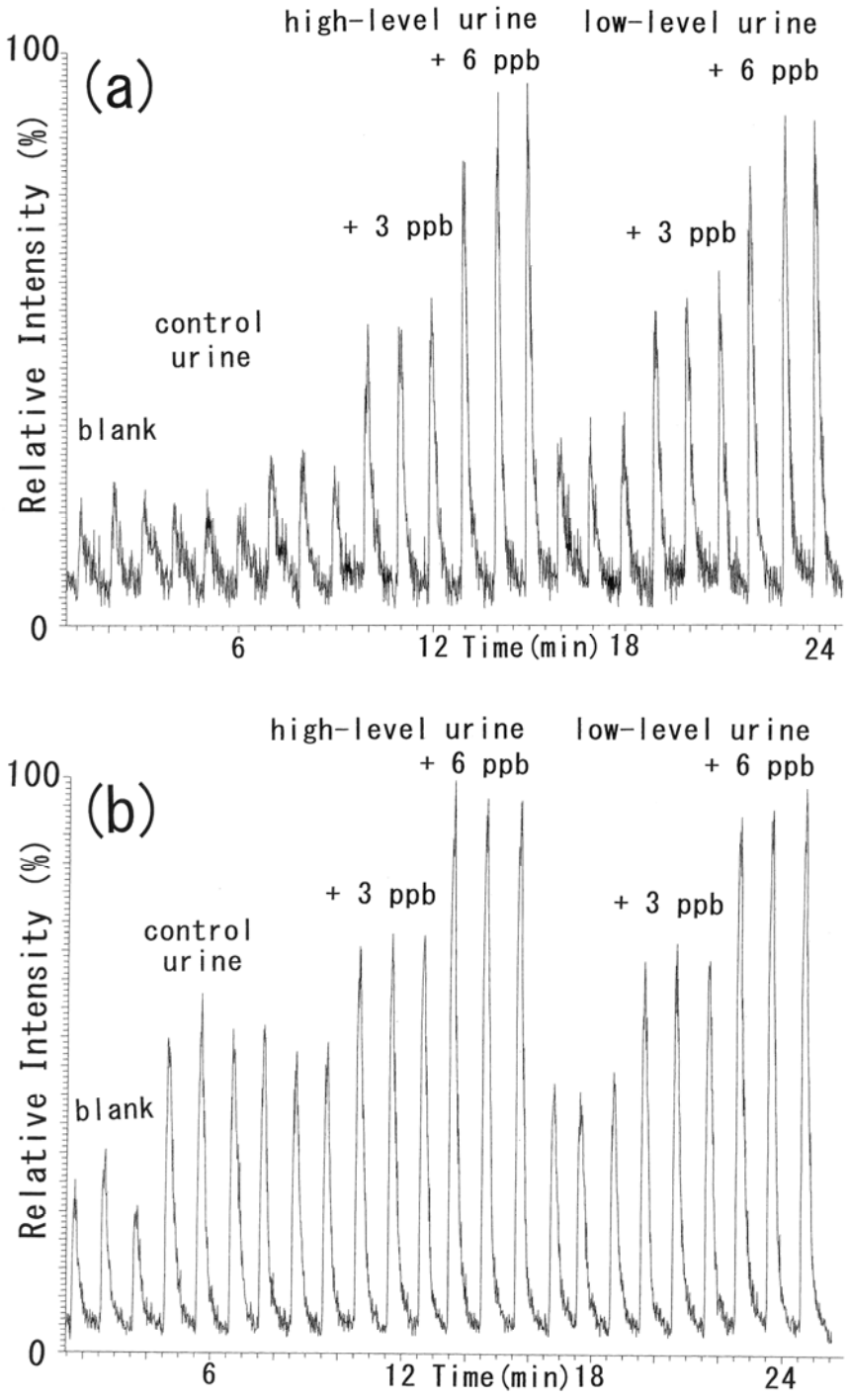

Figure 4: Mass chromatograms of selected reaction monitoring at *m/z* 297 derived from the precursor ion at *m/z* 471 for the quantification of MMA^{V} in treated urine (a), and those at *m/z* 153 derived from precursor ion at *m/z* 327 for the quantification of DMA^{V} in treated urine (b). Samples were injected three times for each As concentration.