Forensic Toxicol (2009) 27:37–40
DOI 10.1007/s11419-008-0064-8

SHORT COMMUNICATION

Sensitive determination of arsenite and arsenate in plasma by electrospray ionization tandem mass spectrometry after chelate formation

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Received: 10 November 2008 / Accepted: 25 December 2008 / Published online: 31 January 2009
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Abstract Inorganic arsenite (As^{3+}) and arsenate (As^{5+}) are well-known poisons, and the toxicity of As^{3+} is about ten times that of As^{5+}. In this study, a simple, rapid, and sensitive method was developed for As^{3+} in plasma using electrospray ionization (ESI) tandem mass spectrometry (MS-MS). After washing plasma with trichloroethylene (TCE), As^{3+} in the aqueous layer was reacted with pyrrolidinedithiocarbamate (PDC, $C_4H_8NCSS^-$), and the produced $As(PDC)_3$ was extracted with methyl isobutyl ketone (MIBK); a 1-μl aliquot of the MIBK layer containing $As(PDC)_3$ was introduced into the MS-MS instrument in the direct-flow injection mode. Other arsenic compounds such as As^{5+}, monomethyl arsonic acid, dimethyl arsinic acid, arsenobetaine, arsenocholine, and tetramethyl arsonium did not produce $As(PDC)_3$. Therefore, without liquid chromatographic separation, As^{3+} alone could be detected after washing with TCE followed by solvent extraction of $As(PDC)_3$ with MIBK. Thus, inorganic As^{5+} was reduced to As^{3+} with thiosulfate, and then the total inorganic As was quantified as As^{3+}; As^{5+}could be calculated by subtracting $As^{3+}$from the total inorganic As. The MS-MS quantification was performed by selected reaction monitoring using a peak at *m/z* 114 of a product ion $(C_4H_8NCS)^+$ formed by collision-induced dissociation from the precursor ion $As(PDC)_2^+$ at *m/z* 367. The mass spectral identification on MS-MS spectrum was possible even at 1 ng As^{3+}/ml plasma. The calibration curve for As^{3+} showed linearity from 0.5 to 100 ng/ml plasma. The limits of detection by selected reaction monitoring were 0.3 ng/ml in water and 0.2 ng/ml in plasma. The analysis could be completed in less than 15 min, because chromatographic separation was not necessary before the MS-MS detection.

Keywords Arsenite · Arsenate · MS-MS · Direct-flow injection · Pyrrolidinedithiocarbamate · Plasma

Introduction

The major poisons being dealt with in forensic toxicology are organic compounds. However, incidents of poisoning by inorganic compounds sometimes take place, and thus are not insignificant. The typical inorganic poisons are arsenic compounds [1]. Various techniques have been reported to analyze arsenic species; atomic absorption spectroscopy [2], gas chromatography [3], liquid chromatography (LC)-hydride generation-atomic fluorescence spectroscopy [4,5], and LC-inductively coupled plasma (ICP) mass spectrometry (MS) [1,6–11]. Very recently, we reported a new simple and sensitive method for analysis of inorganic arsenite (As^{3+}) and arsenate (As^{5+}) in water or urine by electrospray ionization (ESI)-single MS [12]. In this study, we have extended this line of experiments to analysis of As^{3+} and As^{5+} in human plasma by tandem MS (MS-MS).

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Materials and methods

Materials and their preparation

Atomic absorption standard solutions (AASS) of As^{3+} and atomic-absorption-grade methyl isobutyl ketone (MIBK), ammonium pyrrolidinedithiocarbamate (PDC), and analytical-grade $NaAsO_2$ (As^{3+}), $Na_2HAsO_4{\cdot}7H_2O$ (As^{5+}), sodium disulfite ($Na_2S_2O_5$), sodium thiosulfate ($Na_2S_2O_3$), and other reagents were obtained from Wako, Osaka, Japan. Pure water having a specific resistance of 18 MΩ cm was used. Plasma was obtained from healthy volunteers. AASS of As^{3+} at 1 mg/ml was used as stock solution. Other stock solutions at 1 mg/ml of As were prepared by dissolving $NaAsO_2$ (As^{3+}) or $Na_2HAsO_4{\cdot}7H_2O$ (As^{5+}) in water, and were used for 1 week. Calibration standard solutions and quality control solutions were prepared just prior to each use by spiking stock solutions at concentrations of 0, 0.5, 1, 10, and 100 ng arsenic per milliliter into plasma containing $Na_2S_2O_5$ at 3 mM for As^{3+} or As^{5+}.

Assay procedure

For measurements of As^{3+}, 10 µl of 6 mM $Na_2S_2O_5$ and 20 µl of trichloroethylene (TCE) were added to 10 µl of plasma in a polypropylene tube with a cap (Eppendorf AG, Hamburg, Germany), and mixed with a vortex mixer for 60 s and centrifuged at 5000 *g* for 60 s. A 10-µl aliquot of the upper aqueous layer was transferred to another tube, and 1 µl of 1 M PDC was added. After 30 s, 15 µl of MIBK was added and vortex-mixed for 30 s, and centrifuged at 5000 *g* for 30 s. The MIBK layer was used for MS detection of As^{3+} [12–14].

For measurements of As^{5+}, 10 µl of 0.2 M HCl and 1 µl of 0.2 M $Na_2S_2O_3$ solution were added to 10 µl of plasma. The mixture was left to stand for 5 min at room temperature to reduce As^{5+} to As^{3+} completely. Then the pH of the solution was adjusted to between 3 and 5 using 1 M NaOH solution, and 20 µl of TCE was added, mixed with a vortex mixer for 60 s, and centrifuged at 5000 *g* for 60 s. A 10-µl aliquot of aqueous layer was transferred to another tube and 1 µl of 1 M PDC was added. After 30 s, 15 µl of MIBK was added and mixed with a vortex mixer for 30 s, and centrifuged at 5000 *g* for 30 s; the MIBK layer was used for MS detection. Under these treatments, As^{3+} in the sample remained unchanged and total amounts of As^{5+} plus As^{3+} were determined in the form of As^{3+}. The amount of As^{5+} was calculated by subtracting the amount of initially determined As^{3+} from the total amount of As determined secondly.

Conditions for ESI-MS-MS

ESI-MS-MS was performed on a TSQ 7000 LC-quadrupole mass spectrometer (ThermoFisher, San Jose, CA, USA) in the positive ion mode. A 1-µl aliquot of the MIBK layer was manually injected into the MS instrument in the direct-flow injection mode. A characteristic spectrum appeared 30 s after sample injection, and a sample could be injected every 60 s. Methanol was used as the mobile phase at 200 µl/min and the capillary temperature was set at 250°C as the optimal temperature. The electrospray voltage was set at 4.5 kV, multiplier voltage at 1.3 kV, and collision voltage at 20 V. Nitrogen was used as sheath gas (469 kPa) and also as auxiliary gas (8 units), and argon was used as collision gas (134 kPa). MS-MS data were collected in the range of *m*/*z* 30–380. Quantification was performed by integration of the selected reaction monitoring (SRM) peak of a product ion $C_4H_8NCS^+$ at *m*/*z* 113.7 ± 0.2 derived from the precursor ion $As(PDC)_2^+$ at *m*/*z* 366.7 ± 0.3, using a calibration curve constructed from spiked samples at different concentrations.

Results and discussion

When we applied our previous procedure [12] to human plasma without modification, the results were unsuccessful; when As^{3+} was spiked into 100% plasma at 100 ng/ml, the SRM peak area at *m*/*z* 114 extracted from plasma was only 2% of that extracted from water, and then decreased to zero within 10 min. When the concentration of PDC in plasma was increased tenfold, the SRM peak area at *m*/*z* 114 was about 50% of that extracted from water, but decreased to 40% after 30 min. Therefore, we assumed the presence of unknown interfering substance(s) in plasma. To eliminate such substance(s) in plasma, 10 µl of plasma sample was diluted with 10 µl of 6 mM $Na_2S_2O_5$ solution and mixed with each of various organic solvents heavier than water, such as TCE, chloroform, tetrachloroethylene, tetrachloromethane, and *o*-dichlorobenzene. After such treatment, As^{3+} in 10 µl of aqueous layer was reacted with 1 µl of 1 M PDC, and the produced $As(PDC)_3$ was extracted with 15 µl of MIBK. As results, the SRM peak areas at *m*/*z* 114 derived from $As(PDC)_3$ in plasma were highest with TCE or chloroform, followed by tetrachloroethylene, tetrachloromethane, and *o*-dichlorobenzene. The peak areas derived from blank plasma were lower with TCE than with chloroform. Therefore, TCE was chosen to eliminate the interfering substances. Under the conditions, the peak area at *m*/*z* 114 derived from $As(PDC)_3$ in plasma was nearly 80% of that in water, and the decay

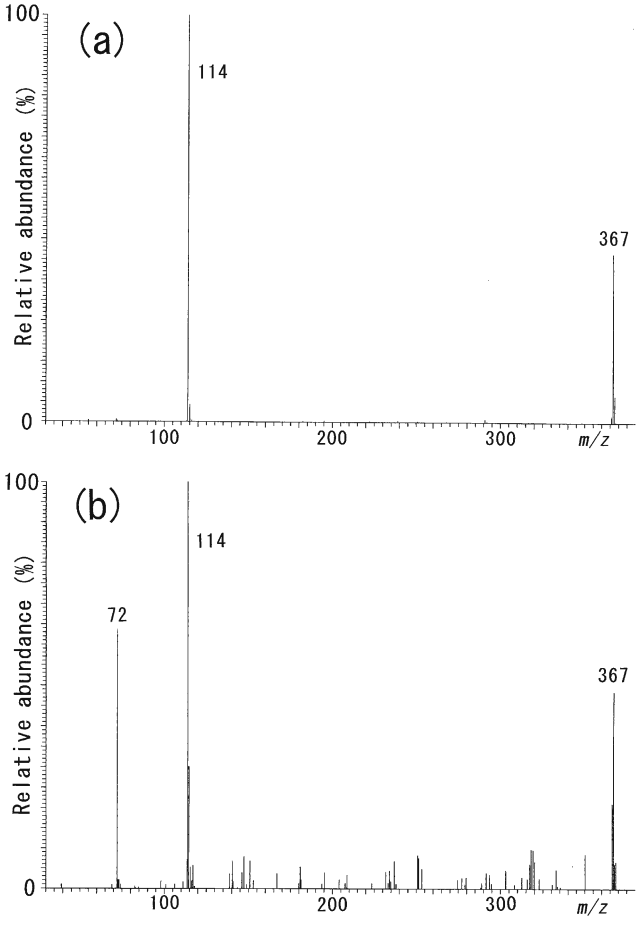

Fig. 1a,b Product ion mass spectra of the methyl isobutyl ketone layer extracted from **a** aqueous solution of As^{3+} at 100 ng/ml and **b** extracted from plasma containing As^{3+} at 1 ng/ml

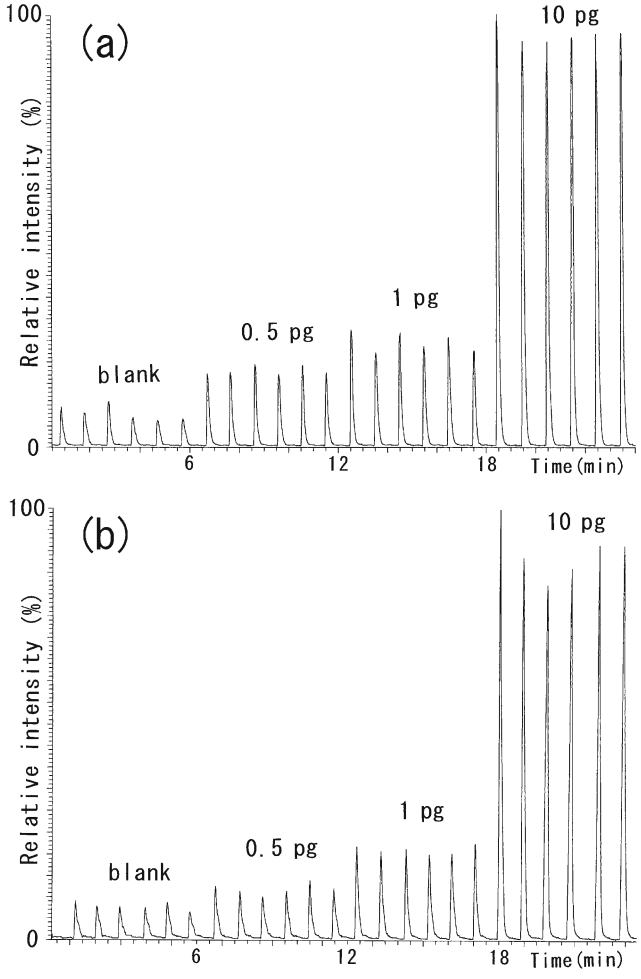

Fig. 2a,b Selected reaction monitoring chromatograms measured at *m/z* 114 obtained from **a** spiked aqueous solutions and **b** from spiked plasma. Samples were injected six times for each amount of As^{3+}. The amounts 0.5 pg to 10 pg shown in these figures are those contained in 1 µl of either aqueous or plasma solution at the initial step

was less than 5% of its initial value after 3 h. Ten microliters of blood hemolyzed with 30 µl of 4 mM $Na_2S_2O_5$ solution was treated in the same way as that for plasma. The peak area at *m/z* 114 derived from $As(PDC)_3$ in the hemolyzed blood was nearly 80%, but decreased to 40% after 30 min. Therefore, the present method cannot be used for blood samples, but can be used for fresh plasma samples.

Figure 1 shows product ion mass spectra at collision voltage 20 V obtained from precursor ion at *m/z* 367. The base peak at *m/z* 114 $[C_4H_8NCS]^+$ and the molecular peak at *m/z* 367 are observable as shown in Fig. 1. In this way, the mass spectra can serve in the identification of As in the present method, whereas only one peak is available for identification of As by ICP-MS because As is a monoisotopic element [5–11,15].

For quantification, the SRM chromatograms at *m/z* 114 were obtained for As^{3+} in water (Fig. 2a) and in plasma (Fig. 2b). As seen in these figures, 0.5 pg As^{3+} extracted from plasma could be quantified.

Calibration standard solutions of As^{3+} were prepared by spiking stock solutions at 0, 0.5, 1, 10, and 100 ng As^{3+}/ml into water or plasma. A linear relation was observed between peak area (y) and the As^{3+} concentration spiked (x) from 0.5 to 100 ng/ml ($y = 10.09x + 8.11$ with a correlation coefficient of 0.998 for water, and $y = 10.11x + 5.41$ with a correlation coefficient of 0.999 for plasma).

Precision and accuracy were assessed by analyzing As^{3+} in water or plasma spiked at 0.5, 1, 10, and100 ng/ml (Table 1). The coefficient of variation for precision was less than 17%, and the accuracy was 82%–123% for intraday and interday measurements. The limit of quantification was 0.5 ng/ml for water or plasma. Blanks that were not spiked with As^{3+} were measured six times, and the standard deviation (σ) was calculated in picograms

Table 1 Intraday and interday accuracy and precision for As^{3+} spiked into water and plasma

As^{3+} spiked (ng/ml)	Intraday[a]				Interday[a]			
	Water		Plasma		Water		Plasma	
	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
0.5	100	7.2	84	16.8	112	7.4	82	16.4
1	120	11.2	115	4.3	123	7.0	114	11.2
10	94	15.6	101	8.4	95	6.9	105	5.4
100	95	1.8	101	7.6	100	5.0	99	9.1

[a] $n = 3$

based on the calibration. The limit of detection (LOD) was 0.3 ng/ml in water or 0.2 ng/ml in plasma because LOD was defined as 3σ of blank signals [15].

To our knowledge, this is the first report dealing with LC-MS-MS analysis of inorganic As after chelate formation. This is the most sensitive method for analyzing As^{3+} and/or As^{5+} so far reported. The LOD is far below the permissible level of As in drinking water recommended by the World Health Organization [2]. In addition, the product ion mass spectrum seems to give the most reliable identification data for As^{3+}.

Acknowledgments This work was supported by a Grant-in-Aid for Scientific Research (No. 18590631) from the Ministry of Education, Science, Sports, and Culture of Japan.

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