Simple and selective determination of arsenite and arsenate by electrospray ionization mass spectrometry

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ABSTRACT

Arsenic pollution of public water supplies has been reported in various regions of the world. Recently, some cancer patients are treated with arsenite (As^{III}); most Japanese people consume seafoods containing large amounts of negligibly toxic arsenic compounds. Some of these arsenic species are metabolized, but some remain intact. For the determination of toxic As^{III}, a simple, rapid and sensitive method has been developed using electrospray ionization mass spectrometry (ESI-MS). As^{III} was reacted with a chelating agent, pyrrolidinedithiocarbamate (PDC, $C_4H_8NCSS^-$) and tripyrrolidinedithiocarbamate-arsine, $As(PDC)_3$, extracted with methyl isobutyl ketone (MIBK). A 1-μL aliquot of MIBK layer was directly injected into ESI-MS instrument without chromatographic separation, and was detected within 1 min. Arsenate (As^V) was reduced to As^{III} with thiosulfate, and then the total inorganic As was quantified as As^{III}. This method was validated for the analysis of urine samples. The limit of detection of As was 0.22 μgL^{-1} using 10 μL of sample solution, and it is far

below the permissible limit of As in drinking water, 10 μgL^{-1}, recommended by the WHO. Results were obtained in <10 min with a linear calibration range of 1–100 μgL^{-1}. Several organic arsenic compounds in urine did not interfere with As^{III} detection, and the inorganic As in the reference materials SRM 2670a and 1643e were quantified after the reduction of As^{V} to As^{III}.

Key words: Arsenite, Arsenate, Mass spectrometry, Electrospray ionization, Pyrrolidinedithiocarbamate

1. Intoduction

There is considerable interest in establishing methods for determining the concentration of individual species of arsenic (As) because toxicity depends on its molecular form. Toxicity increases in the approximate order: metallic As < organoarsenic compounds < arsenate (As^{V}) < arsenite (As^{III}) < arsine (AsH_3), although some methylated As^{III} compounds are reported to be more toxic than As^{III} [1]. Many analytical techniques have been employed for various samples to separate and quantify these As species: atomic absorption spectrometry [2], gas–liquid chromatography [3], liquid chromatography (LC)-hydride generation-atomic fluorescence spectrometry [4,5] and LC-inductively coupled plasma (ICP) mass spectrometry (MS) [1,6–11]. Although these techniques (particularly LC-ICP-MS) offer the advantages of high selectivity in determining its atomic mass m/z = 75 and high sensitivity with a limit of detection (LOD) of 0.14–0.33 μgL^{-1} [1, 6–8], the identification of chemical species is based entirely on agreement of the chromatographic retention time with that of the

reference compound. In some chromatographic separations, highly toxic As^{III} elutes with negligibly toxic arsenobetaine (AsB; derived from seafoods) [10] or monomethylarsonic acid (MMA; metabolite of As^{III} excreted into urine) [1,10]. Detection of ^{75}As, *m/z* = 75 is readily interfered with by isobars such as FeOH [11] and ArCl resulting from the combination of Ar (from plasma gas) and Cl (from sample solution) [1,11].

Electrospray ionization (ESI)-MS provides a unique opportunity for the analysis of molecular forms of species [6,12–15], and the LODs for several organoarsenic compounds on LC-ESI-MS/MS, 1 $\mu g(kg)^{-1}$ [15], is comparable to that on LC-ICP-MS [1,6–8]. Inorganic As^{III} and As^{V}, however, could not be quantified on ESI-MS (/MS) sensitively, and only qualitative data have been provided in reports [6,12–15]. Instruments of ICP-MS and ESI-MS (/MS) were therefore required for the selective and sensitive quantification of inorganic and organic As species. We intended to quantify inorganic As sensitively by ESI-MS. Inorganic As^{III} was reacted with a chelating agent, pyrrolidinedithiocarbamate (PDC; $C_4H_8NCSS^-$), and tripyrrolidinedithiocarbamate-arsine, $As(PDC)_3$, was extracted with methyl isobutyl ketone (MIBK) [16,17]. An aliquot (1 μL) of the MIBK layer was directly injected into the ESI-MS instrument without chromatographic separation, although chromatographic separation was adopted in other studies [1,3–11,13]. As^{V} does not react with PDC [16,17], so As^{V} was reduced to As^{III} with 0.01 M $Na_2S_2O_3$ in 0.1 M HCl for 1–5 min and the total inorganic As quantified as As^{III}. The present method could afford a sensitive determination of As. LOD was 0.22 μgL^{-1} with a quantification range of 1–100 μgL^{-1} using 10 μL of sample solution. LOD was far below the

permissible limit of As in drinking water, 10 μgL^{-1}, recommended by the WHO [2].

2. Materials and methods

Atomic absorption standard solutions (AASS) of As^{III} and other 20 metals, atomic absorption grade of MIBK, PDC, sodium diethyldithiocarbamate (DDC), diethylammonium DDC and ammonium DDC, and analytical grade of $NaAsO_2$ (As^{III}), $Na_2HAsO_4.7H_2O$ (As^V), sodium disulfite ($Na_2S_2O_5$), sodium thiosulfate ($Na_2S_2O_3$), sodium hydrosulfite ($Na_2S_2O_4$), dimethylarsinic acid (DMA), AsB, arsenocholine (AsC) bromide and other reagents were obtained from Wako Pure Chemicals, Osaka, Japan. Analytical-grade MMA was obtained from Tri Chemical Laboratories Incorporated, Yamanashi, Japan. Standard reference materials (SRM 2670a and 1643 e) were purchased from the National Institute of Standards and Technology, Gaithersburg, MD, USA. Pure water having a specific resistance of 18 MΩ cm was used. AASS of As^{III} at 1 gL^{-1} was the stock solution. Other stock solutions at 1 gL^{-1} As were prepared by dissolving $NaAsO_2$ (As^{III}) and $Na_2HAsO_4.7H_2O$ (As^V) in water, and used for one week. Calibration standard solutions and quality control solutions were freshly prepared before use by spiking stock solutions at As concentrations of 0, 1, 10 and 100 $\mu g\ L^{-1}$ to water and urine that contained $Na_2S_2O_5$ at 0.003 M in the case of As^{III}, and to water and urine in the case of As^V, respectively. pH of the sample and standard solutions was adjusted to 3–5 using 1 M HCl solution and 1 M NaOH solution using pH test paper in assay for As^{III} or As^V, if necessary.

As^{III} was assayed as follows. To 10 μL of sample or standard solution in a polypropylene tube with cap (Eppendorf AG, Germany), 1 μL of 0.03 M $Na_2S_2O_5$ and 1

μL of 0.1 M PDC were added. After 30 s, 10 μL of MIBK was added, mixed with a vortex mixer for 30 s, and centrifuged at 5000 × g for 30 s. The MIBK layer was used for the detection of As^{III} [16,17].

As^{V} was assayed as follows. To 10 μL of sample or standard solution, 1 μL of 1 M HCl solution and 1 μL of 0.1 M $Na_2S_2O_3$ solution were added. The reaction was allowed to proceed for 1–5 min at room temperature to reduce As^{V} to As^{III} completely. pH of the solution was adjusted to 3–5 using 1 M NaOH solution, and 1 μL of 0.1 M PDC added. After 30 s, 10 μL of MIBK was added, mixed with a vortex mixer for 30 s, and centrifuged at 5000 × g for 30 s. As^{III} in the sample remained unchanged, and total As^{V} and As^{III} were determined as As^{III}. The amount of As^{V} was calculated by subtracting the amount of As^{III} determined initially from the total amount of As^{v} and As^{III} determined subsequently.

ESI-MS was done on a TSQ 7000 LC-quadrupole mass spectrometer (Thermo Quest, Japan) in positive ion mode. One microliter of the MIBK layer was injected manually in direct mode. The characteristic spectrum appeared 30 s after sample injection, and a sample could be injected every 60 s. Methanol was used as a mobile phase at 200 $\mu L min^{-1}$ and the capillary temperature was set at 250°C. The electrospray voltage was set at 4.5 kV and multiplier voltage at 1.3 kV. Nitrogen was used as a sheath gas (469 kPa) and as an auxiliary gas (8 units). MS data were collected at *m/z* 340–390. The quantification in ESI-MS was done by the integration of the peak area of $As(PDC)_2^+$ at *m/z* 367 ± 0.5 in selected ion monitoring using a calibration curve comprising spiked matrix samples at different concentrations.

3. Results and discussion

3.1. Preservation of valence states of As^{III} and As^{V}

We found that As^{III} at <10 μgL^{-1} in pure aqueous solution was undetectable when it reacted with PDC and was extracted with MIBK. This may be due to non-optimized pH of the solution, as well as the oxidation of As^{III} under our assay treatment, which involved violent mixing using a vortex mixer. $Na_2S_2O_5$, HCl [5], HNO_3 and EDTA disodium salt (2Na) [5] were tested to preserve the valence states of As^{III} and As^{V} and to sensitively detect only As^{III}. The peak area derived from As^{III} at 100 μgL^{-1} in 0.003 M $Na_2S_2O_5$ solution was taken as 100%, and the relative percentage values of the peak area derived from the other solutions were examined. Concentration of As^{III} or As^{V} was 0 in blank solution or 100 μgL^{-1} in test solution. The As–PDC complex was produced most efficiently at pH 3–5 in this study, although the suitable pH range reported previously was 1–6.8 [17]. $Na_2S_2O_5$ was chosen because it is a moderate reducing agent and its pH value is about 4 at 0.001–0.1 M solution. As^{III} at 100 μgL^{-1} in 0.001–0.02 M $Na_2S_2O_5$ solutions showed the peak of $As(PDC)_2^+$ corresponding to 100%, whereas As^{V} at 100 μgL^{-1} did not show the peak of $As(PDC)_2^+$, even in 0.1 M $Na_2S_2O_5$ solution. The peak area of the blank solution (0.001–0.02 M $Na_2S_2O_5$ solutions) was <1 % of that of As^{III} at 100 μgL^{-1}. Therefore, 0.001–0.02 M $Na_2S_2O_5$ solutions can be used for the selective determination of As^{III} without interference from As^{V}, although 0.01 M HCl solution and 0.01 M EDTA 2Na solution gave satisfactory results in other studies on As^{III} [5].

We confirmed that spiked As^{III} at 1–100 μgL^{-1} into urine could be detected completely when urine contained $Na_2S_2O_5$ at 0.001–0.02 M.

3.2 Suitable chelating agents and extracting solvents

Four chelating agents, sodium DDC, diethylammonium DDC, ammonium DDC and ammonium PDC, were compared. Three types of DDC compounds gave almost identical amounts of As–DDC complex. The amount of As–DDC complex produced from any of the three DDC compounds was only one-tenth that of the As–PDC complex produced from ammonium PDC: ammonium PDC was chosen as the chelating agent.

MIBK was chosen as the extraction solvent of the As–PDC complex. Firstly, the peaks of blank extracted with MIBK, isoamylalcohol, octanol and cyclohexanol corresponded to approximately 45, 80, 120 and 780%, respectively, of the peak of 1 pg As^{III} extracted with MIBK. Secondly, the peak of 100 pg As^{III} extracted with MIBK was highest among the peaks of 100 pg As^{III} extracted with these four solvents.

3.3 Reduction of As^{V} to As^{III}

Under the present treatments for the assay of As^{III}, As^{V} did not react with PDC [15,16], so a reduction step must be preceded to detect As^{V}. Na_2SO_3, $NaHSO_3$, $Na_2S_2O_5$, $Na_2S_2O_3$ and $Na_2S_2O_4$ were tested to reduce As^{V} to As^{III}. Of these, only ≥0.01 M $Na_2S_2O_4$ solution (pH 4) could reduce As^{V} to As^{III} completely after 5 min at 100°C; the other four solutions, even at 0.05 M, could not. $Na_2S_2O_4$ solution at ≥0.01 M could not efficiently reduce As^{V} to As^{III} in urine, so reduction in 0.1 M HCl [17] was attempted because As^{V} tends to be As^{III} in acid solution [5,17]. We found that 0.01 M $Na_2S_2O_3$ in 0.1 M HCl solution and 0.01 M $Na_2S_2O_4$ in 0.1 M HCl solution could completely reduce As^{V} to As^{III} in urine after 1–5 min at room temperature. Although this

reduction treatment is sufficient for further detection based on atomic absorption spectrometry [17], pH of the solution should be adjusted to 3–5 in our assay before the addition of the chelating agent (PDC). After these treatments, complete recovery was obtained in urine samples spiked with As^{V} and As^{III} at 1–100 μgL^{-1}, respectively.

The As–PDC complex extracted with MIBK was reported to be stable for 2 months [17]. We observed that ninety percent of the complex remained after 24 h in the MIBK extract from aqueous solution and, after 3 h in the extract from urine, respectively, under room light at 25°C.

3.4 Interference

PDC is known to form di- and tripyrrolidinedithiocarbamates of various transition metals. We confirmed that the signal of As at *m/z* 367 was not interfered with by Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Zr, Mo, Ru, Pd, Ag, Cd, W, Pt, Au, Tl and Pb at 10 mgL^{-1} under the present assay treatment for As^{III} or As^{V}. The effects of organic As compounds such as MMA, DMA, AsB and AsC at 100 mgL^{-1} as As were examined, but none were converted into the same As–PDC complex derived from As^{III} under the present assay treatment for As^{III} or As^{V}. Interference by salts such as NaCl, $NaNO_3$ and Na_2SO_4 at levels up to 0.3 M solution and that by urea up to 10% solution, respectively, were not observed.

3.5 Mass spectra

Figure 1 (a) and (b) show the mass spectrum of $As(PDC)_2^+$ derived from 200 pg of As^{III}

in 0.003 M $Na_2S_2O_5$ solution, and that derived from10 pg of As^{III} in urine containing 0.003 M $Na_2S_2O_5$, respectively. Although As is a mono-isotopic element, the $As(PDC)_2^+$ ion shows several lines due to isotopes of H C, N, O and S; these patterns serve as the identification of the ion. The theoretical abundance ratio of lines at *m/z* 367: 368: 369: 370: 371 calculated using the Isotopic Distribution Calculator of Applied Biosystems Japan Limited was 100.0: 15.2: 18.8: 2.6: 1.4 and the ratio was held down to that of 10 pg As. That is, the ratio of 10 pg As extracted from urine was 100.0: 16.4: 19.8: 3.9: 0.0 (Figure 1 (b)), and the deviation of each value from the theoretical value was <1.4, where the value of signal observed at *m/z* = 367 was taken to be 100. The lines with higher intensity observed at *m/z* 346 - 352 in Figure 1 (b) were derived mainly from $Fe(PDC)_2^+$, and this figure indicated that common transition elements in urine did not interfere with the detection of As^{III}.

For the quantification of As^{III}, plots of selective ion monitoring at *m/z* 367 were taken for As^{III} in water (a) and urine (b) (Figure 2); 1 pg As^{III} could be quantified.

3.6 Precision and accuracy

Calibration standard solutions of As were prepared by spiking stock solutions at concentrations of 0, 1, 10 and 100 μgL^{-1} to water and urine. The concentrations of As^{III} determined from the peak area (y) were linear to the concentrations spiked (x) up to 100 μgL^{-1}, i.e., $y = 0.9996 x + 0.9763$ with a correlation coefficient of 0.9998 in water, and $y = 1.000 x + 0.9376$, with a correlation coefficient of 0.9996 in urine, respectively. Precision and accuracy were assessed by analysis of water and urine spiked at 1, 10 and 100 μgL^{-1}. These samples were analyzed thrice daily as well as

on three different days. The coefficient of variation was <10.5 %, and accuracy was 88–125% for intra-day and inter-day variations in any samples (even urine spiked at 1 μgL^{-1}).

The blanks (e.g., water and urine that were not spiked with As) were measured six times and their standard deviations (σ) calculated in pg based on the calibration. LOD was defined as 3σ for blank signals [1], so LODs were calculated to be 0.22 μgL^{-1} in water, and 0.25 μgL^{-1} in urine, respectively.

To check the recovery and validate the proposed procedure, standard reference materials, 2670a high-level urine and 1643e water, were examined. The high-level urine was diluted to one tenth, and the 1643e water was pH adjusted and diluted to one-tenth, respectively, to suit assay conditions. The concentrations of As^{III} and As^{V} in high-level urine were reported to be 59.7 ± 2.1 and 153 ± 3 [7], and 50.8 ± 2.7 and 166.3 ± 6.4 [8]; our values were 53.5 ± 4.8 and 156.9 ± 12.8, respectively (n = 6). The concentration of total As in 1643e water in the NIST list was 60.45 ± 0.72; our value was 60.7 ± 5.5 (n = 6).

3.7 Comparison with other MS methods

Solvent extraction after chelate complex formation is an effective way to concentrate transition metals from aqueous solution. We confirmed that the peak area of $As(PDC)_2^+$ increased linearly up to five times without increasing the peak area of blank solution in accordance with the decrease of relative volume of MIBK from 1 to 0.2, although enhancement factors up to 100 were reported in another study [17]. The present solvent extraction eliminates not only organic As compounds such as AsB and

MMA that interfere with the detection of As on ICP-MS, but also matrix effects due to water-soluble ions such as Na^+, Mg^{2+}, Ca^{2+}, K^+, Cl^-, NO_3^-, SO_4^{2-}, amino acids and proteins [1,10,11]. Although a solvent extraction is a simple way to eliminate interfering substances as well as matrix effects that reduce sensitivity in any kind of MS, it cannot be applicable to ICP-MS determination of several elements such as As and Cr because the carbon atom derived from organic solvents interferes with their detection especially [9]. LC separation was applied mostly for crude materials before the determination of arsenic compounds on ICP-MS and ESI-MS (/MS) [1,6-8,10,13,15]. Present method does not need to use LC separation for urine sample and can quantify inorganic As^{III} and As^{V} with the same highest sensitively as that on ICP-MS [1].

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Figure caption

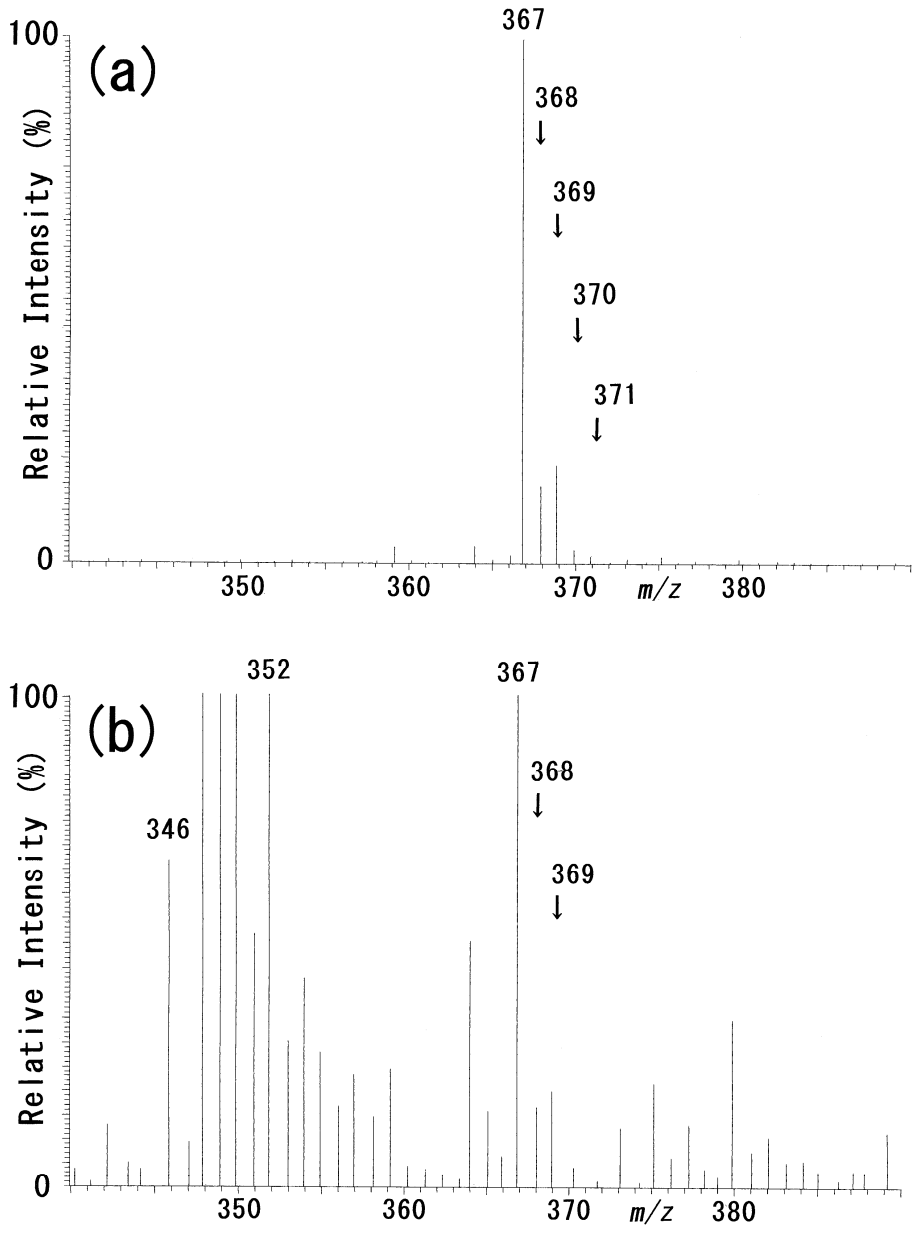

Figure 1: Mass spectrum of $As(PDC)_2^+$ derived from 200 pg of As^{III} in water (a) and that from 10 pg of As^{III} in urine (b), respectively. Although As is a mono-isotopic element, the $As(PDC)_2^+$ ion shows several lines due to isotopes of H C, N, O and S. The theoretical abundance ratio of lines at *m/z* 367: 368: 369: 340: 341 was 100.0: 15.2: 18.8: 2.6: 1.4, and the observed value in Figure 1 (b) was 100.0: 16.4: 19.8: 3.9: 0.0.

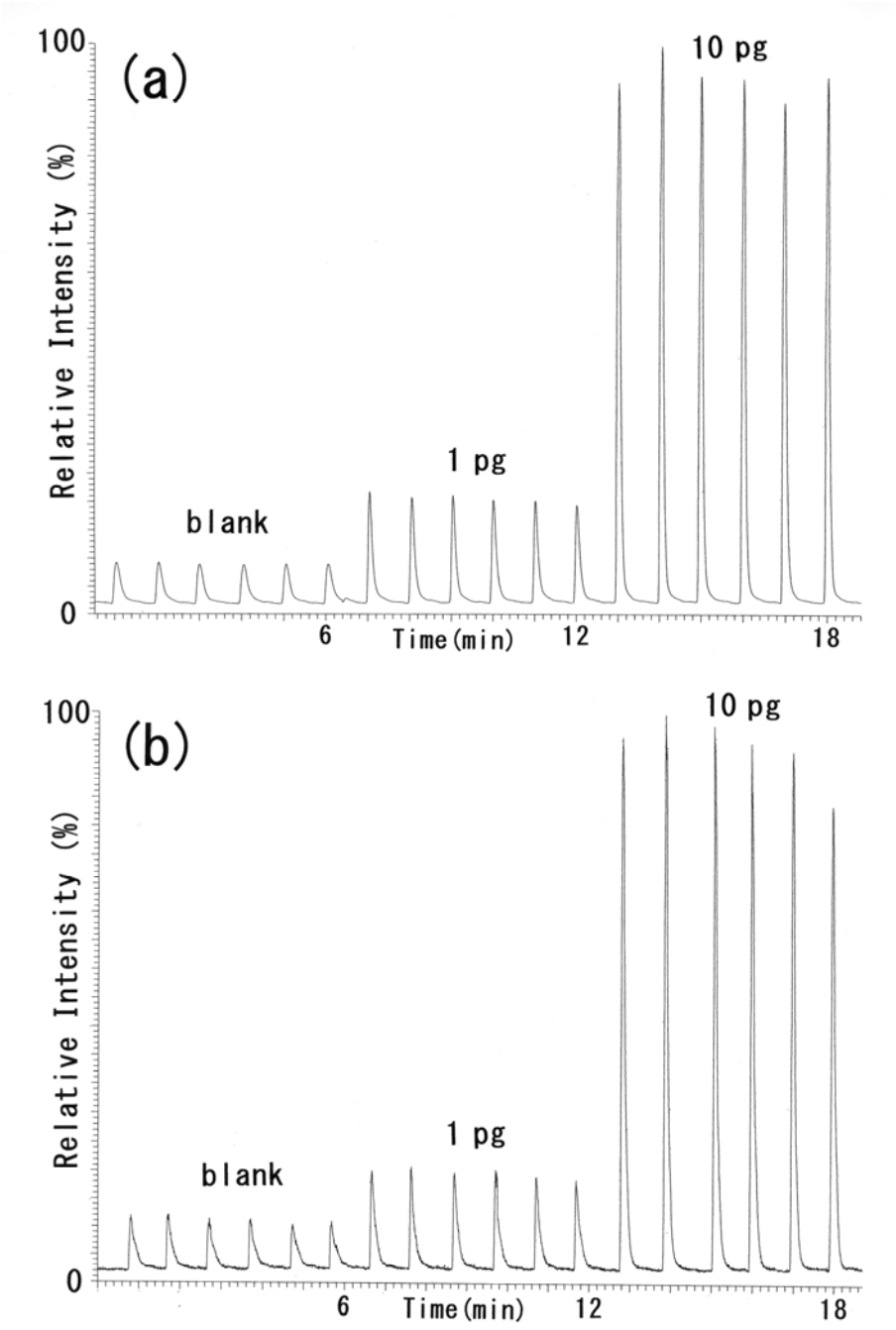

Figure 2: Plots of selected reaction monitoring at *m/z* 367 derived from water (a) and urine (b), respectively. Samples were injected six times for each amount of As^{III}.