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Abstract A rapid method was developed to identify and quantify the azide ion (N_3^-) in gastric fluid and urine. N_3^- in diluted biological fluids was reacted with $NaAuCl_4$ to produce $Au(N_3)_2^-$, which was extracted with octanol. Five microliters of the extract were flow-injected into an electrospray ionization tandem mass spectrometric instrument. Quantification of N_3^- was performed by selected reaction monitoring of the product ion $Au(N)(N_3)^-$ at *m/z* 253 which was derived from the precursor ion $Au(N_3)_2^-$ at *m/z* 281, using 50 μL of aqueous solution within 10 min. This method was found to be linear up to 10^{-5} M, to have a limit of quantification of 10^{-7} M, a limit of detection of 3.0×10^{-8} M, and a coefficient of variation of ≦ 10 % at 10^{-7} M. In case of urine, 50 μL of urine were spiked with N_3^-, this was diluted to10-fold and passed through 1 mL of a resin and finally diluted to 100-fold of the original. This method was linear up to 10^{-3} M, had a limit of quantification of 10^{-5} M, a limit of detection of 3.0×10^{-6} M, and coefficient of variation ≦ 8.8 % for an original urine concentration of 10^{-5} M. The practical applicability of this method was checked by diluting 1 μL of a suspected suicide victim's gastric fluid 20,000-fold and 1 μL of the victim's urine 5,000-fold and then measuring the N_3^- levels. These levels were found to be $(7.5 \pm 1.0) \times 10^{-2}$ M and $(3.2 \pm 0.4) \times 10^{-3}$ M, respectively.

Key words: Azide・Gold・Electrospray ionization・Tandem mass spectrometry・Gastric fluid・Urine

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Introduction

Sodium azide (NaN_3) is used to provide the nitrogen gas for the inflation of automobile "air" bags and as a preservative for laboratory reagents. NaN_3 is a highly toxic substance inhibiting cytochrome oxidase like cyanide (CN^-) and it exerts a hypotensive action too, but its toxic mechanism is not well known yet [1,2]. The oral LD_{50} in white mice was 27 mg/kg [3,4]. Several non-fatal and fatal cases of human NaN_3 poisoning were reported: The maximum non-fatal amount ingested was 0.15 g [3] or 1 g with an immediate aggressive supportive treatment [2]; the minimum fatal amount ingested was 0.7 g [5]; and the

other data indicating fatal amounts varied from 1 g to 55 g [1-6], and the intoxication involved mostly people working in laboratories and factories using this chemical [2,3].

A rapid determination of N_3^- is required in the diagnosis of suspected poisoning. Several methods were reported for the determination of N_3^-. Potentiometry [7], spectrophotometry [8,9], gas chromatography (GC) [2], liquid chromatography (LC) [4-6] and ion chromatography (IC) [10] were used, but the determination of N_3^- by those methods lacked specificity as well as sensitivity. The specificity of mass spectrometry (MS) is much better than that of the other methods mentioned above since MS can determine the analyte in mass to charge ratio. Since N_3^- is a non-volatile molecule, GC-MS was introduced after the derivatization of N_3^- to a volatile pentafluorobenzyl (PFB)- N_3 [11,12]. Determination of N_3^- by the GC-MS methods provided the best specificity as well as sensitivity with the limit of detection (LOD) of 2.4×10^{-7} M [12] among the methods mentioned above. However, it has some drawbacks to be improved. Firstly, these methods take more than 30 min for the derivatization and further15 min for GC separation. Secondly, the single MS without accurate mass measurement cannot distinguish isobars such as PFB-N_3 and PFB-OCN at *m/z* = 223, respectively, and N_3^- and OCN^- at *m/z* = 42, respectively. Here, OCN^- is an oxidation product of CN^- that is a highly toxic substance and thiocyanate that is found abundantly in biological fluids [13].

To eliminate drawbacks mentioned above, we have developed a flow-injection (FI) electrospray ionization (ESI) tandem mass spectrometric (MS/MS) method for a rapid determination of N_3^-. Fragment ions in MS/MS contain a large amount of compound-specific information and can discriminate isobars. In the present determination isobars, N_3^- and OCN^-, have been discriminated by their product ion spectra although the precursor ions $Au(N_3)_2^-$ and $Au(OCN)_2^-$ have the same *m/z* 281 in a single MS. That is, the main product ion from the precursor ion $Au(N_3)_2^-$ was $Au(N)(N_3)^-$ at *m/z* 253, whereas no product ion was detected at *m/z* 253 from the precursor ion $Au(OCN)_2^-$. The precursor ions $Au(N_3)_2^-$ were formed and extracted with octanol within a few minutes, and the time was much shorter than 30 min that was required for the derivatization of N_3^- [11,12]. The extraction with octanol contributed to the elimination of biological substances dissolved in water, and MS/MS could eliminate more interfering ions than a single MS by selecting *m/z* values twice. FI-MS/MS has been adopted because under suitable conditions it offers a fast and cheaper alternative to chromatography assisted MS. The present FI-ESI-MS/MS took 1 min for

detection and 1 min for resetting, and the lowest LOD of N_3^-, i.e., 3.0×10^{-8} M, has been achieved.

Materials and methods

Materials

Chemicals of analytical grade were obtained from Wako Pure Chemicals, Osaka, Japan. $Na^{15}N^{14}N_2$ (1-^{15}N 98 %+) was obtained from Cambridge Isotope Laboratories, Inc., Andover, MA, USA. Pure water with a specific resistance of 18 MΩcm was used (Millipore, Bedford, MA, USA). The control urine samples were obtained from our laboratory staffs (n = 6) under permission, placed in new tubes and stored at – 20 ℃, respectively.

Case history of a suspected suicide victim

A man around 50 was found dead in a forest. A suicide note and an empty plastic bottle for mineral water were found near the scene but the plastic bottle was not preserved since it was empty. The autopsy was performed at our department by the authors on 4.5 days after someone saw him alive finally. External examination revealed no particular abnormalities. Internal examination showed erythema of gastric lining and bilateral pulmonary edema and congestion but no other cause of death. The gastric fluid, urine and blood were obtained from the victim at autopsy, placed in new tubes and stored at – 20 ℃ by the authors.

The routine drug screening of urine was performed using The Triage Drugs of Abuse Panel Plus TCA kit (Biosite Inc., San Diego, CA, USA), and all eight kinds of drugs were negative. Ethanol levels in the blood and the urine were determined using GC, and were 0.45 and 0.9 mg/mL, respectively, indicating a weak intoxication and/or postmortem production of alcohol. The CN^- level in the gastric fluid was only 4.5×10^{-7} M indicating its non-lethality. Arsenic compounds such as As^{3+}, As^{5+}, monomethylarsonic acid and dimethylarsinic acid were not detected in the gastric fluid and urine.

Standard solutions

The 1 M N_3^- stock solution was prepared by dissolving NaN_3 into water and stored at – 20 ℃. The 1 M stock citrate buffer solution at pH 5 was prepared by mixing of 1 M citric acid and 1 M tri-sodium citrate. The 0.1 M Au stock solution was prepared by dissolving $NaAuCl_4$ into water. The 1 M stock citrate buffer solution and 0.1 M Au stock solution were stored at ambient temperature and used for 1 month, respectively.

The standard solutions of N_3^- were prepared prior to use by diluting N_3^- stock solution with water. The 2×10^{-3} M Au working solution was prepared prior to use by diluting Au stock solution with water. The 0.02 M citrate buffer solution at pH 5 was prepared prior to use and was abbreviated as CB hereafter. The 2×10^{-3} M ascorbic acid (AsA) solution was prepared prior to use.

Calibration standard samples of N_3^- were prepared by spiking standard solutions into water at 0, 10^{-7}, 10^{-6} and 10^{-5} M (n=3) and into control urine at 0, 10^{-5}, 10^{-4} and 10^{-3} M (n=3), respectively. Precision and accuracy were assessed by analyses of water spiked N_3^- at 10^{-7}, 10^{-6} and 10^{-5} M and control urine samples spiked N_3^- at 10^{-5}, 10^{-4} and 10^{-3} M, respectively. These samples were analyzed six times a day, as well as on six different days.

The recovery of N_3^- from a sample solution was defined as the value (A-B)/(C-D), where A, B, C and D were the peak area of a sample solution with N_3^- added, the peak area of the sample solution without N_3^- added, the peak area of water with N_3^- added, and the peak area of water without N_3^- added, respectively.

Assay procedure for aqueous solutions

To 50 μL of sample solution in a tube (Eppendorf AG, Hamburg, Germany), 1 μL of 1 M citrate buffer solution of pH 5 and 1 μL of AsA at 2×10^{-3} M were added. That is, 0.02 M citrate buffer solution of pH 5 (i.e., CB) containing AsA at 4×10^{-5} M was selected for the following chelate complex formation, but 1 μL of 1 M citrate buffer was not added when the sample contained it already. Less than 1 μL of N_3^- standard solution was taken using PIPETMAN P2 obtained from Gilson SAS, Villiers-le-Bel, France, and

added to the sample solution if necessary. Then 1 μL of Au working solution and 75 μL of octanol were added and mixed mildly for 15 s using a vortex mixer before centrifugation at 1,000 × g maintained for 10 s. Here, 1 μL of Au working solution per assay costs only < 10^{-4} dollars. The upper octanol extract was placed into another tube for auto-sampler FI. When the N_3^- levels in gastric fluid and urine are high enough and the detections after ≧1,000-fold dilution with CB are possible, the diluted solutions can be assayed in the same way as that of aqueous solution.

Assay procedure for urine

To 50 μL of urine, N_3^- standard solution was added if necessary and diluted to 0.5 mL. A cation exchange resin DOWEX™ 50W × 8 100-200 mesh obtained from Wako Pure Chemicals, Osaka, Japan was activated according to the instruction, and 0.5 mL of 10-fold diluted urine was passed through 1 mL of the resin. The pH of the urine was adjusted to 5 with 1 M tri-sodium citrate solution, and the urine was diluted finally to 100-fold of the original urine. To 50 μL of thus treated urine, 1 μL of 1 M citrate buffer solution and 3 μL of AsA at 2×10^{-3} M were added and assayed in the same way as that of aqueous solution.

Instruments

ESI-MS/MS was performed using a TSQ 7000 LC-triple stage quadrupole mass spectrometer equipped with flow-injector (ThermoQuest, Japan) in the negative ion mode. Methanol was flowed as the mobile phase at 300 mL/min and the capillary temperature was set at 250 ℃. The electrospray voltage was set at 4.5 kV, multiplier voltage at 1.3 kV, and collision voltage at 18 V. Nitrogen was used as the sheath gas (469 kPa) and also as an auxiliary gas (8 units) and argon was used as the collision gas (134 kPa). Five μL of the octanol extract was flow-injected using an auto-sampler into ESI-MS/MS apparatus with the time interval of 2 min (i.e., 1 min for detection and 1 min for resetting). The quantification in ESI-MS/MS was performed by the integration of the peak area of the product ion at *m/z* 253.0±0.2 that was derived from the precursor ion at *m/z* 281.0±0.3, using a calibration curve made up with spiked matrix samples at

different concentrations.

Results and discussion

Suitable pH, solvent, reducing agent and working solution of Au ion

The signal intensities of $Au(N_3)_2^-$ produced in 0.02 M citrate buffer solutions at pH 3.5, 4 – 6 and 7 were 60, 100 and 70 %, respectively, using octanol as the extractor, and the same pH dependence of the signal intensity was observed when 0.02 M oxalate buffer solution was used. When 0.02 M phosphate buffer solution was used, the best pH range was the same but the signal intensity was only 20 % of that of citrate buffer solution. The signal intensities extracted from citrate buffer solution at 0.005 M, 0.01-0.03 M and 0.04 M were 85, 100 and 90 %, respectively. Therefore, 0.02 M citrate buffer solution at pH 5, i.e., CB, was selected.

The extraction efficiencies of several organic solvents such as isoamyl alcohol, methylisobutyl ketone, cyclohexanol and chloroform were nearly 20, 20, 5 and 1 %, respectively, by taking the efficiency of octanol as 100 %. The blank signals of these solvents showed the same noise levels, and octanol was therefore selected as the extractor of the complex.

Under the above conditions, however, the concentration linearity of signals dropped off at lower than 10^{-5} M N_3^-. One of the reasons may be due to the oxidation of N_3^- to N_2, N_2O and/or NO by O_2 and/or Au^{3+}. Therefore, the effect of several reducing agents such as AsA, dithiothreitol, glutathione and sodium hydrosulfite were examined to improve the recovery. Among these reducing agents only AsA at $(2 – 6) \times 10^{-5}$ M in CB worked very well for the present determination. That is, the signal derived from N_3^- at 10^{-6} M in CB containing AsA at 4×10^{-5} M was 100 times that derived from N_3^- at 10^{-6} M in CB without AsA. Therefore CB containing AsA at 4×10^{-5} M was used as the solution in which the production of $Au(N_3)_2^-$ was taken place.

The suitable working solution of Au was examined using the solution of N_3^- at $10^{-7} – 10^{-5}$ M. When the concentration of $NaAuCl_4$ was 4×10^{-5} M in the reacting solution, the concentration linearity of the signals was held at $10^{-7} – 10^{-5}$ M N_3^-.

The observed concentration of $Au(N_3)_2^-$ in octanol extracted under the above condition was maintained to be > 90 % of the original concentration for 24 h at ambient temperature under visible light. Since the $Au(N_3)_2^-$ is an anion, it may be dissolved in octanol with other cations, such as H^+, Na^+ and Au^+. These cations could not be identified in the present work.

MS and MS/MS spectra of $Au(N_3)_2^-$ and $Au(OCN)_2^-$

The mass spectrum of $Au(N_3)_2^-$ consisted of two peaks at *m/z* 281 and 282 having relative abundance of 100 : 2 and this ratio agreed with the relative abundance of 100.00 : 2.23 : 0.02 for the peaks at *m/z* 281, 282 and 283, respectively, that was calculated theoretically using the Isotopic Distribution Calculator of Applied Biosystems Japan Limited. Choosing $Au(N_3)_2^-$ at *m/z* 281 as the precursor ion, selected reaction monitoring was performed by changing the collision voltage from 10 to 50 V. Under these conditions, the observable product ions were $Au(N)(N_3)^-$ at *m/z* 253, $Au(N)_2^-$ at *m/z* 225 and N_3^- at *m/z* 42 as shown in Fig. 1 **a**. $Au(N)(N_3)^-$ was the main product ion when the collision voltage was from 10 to 30 V, and its maximum was observed at 18 V with the peak height of 1.7×10^4 when the concentration of N_3^- was 10^{-5} M. On the other hand, N_3^- was the main product ion when the collision voltage was from 30 to 50 V, and its maximum was observed at 40 V with the height of 3.6×10^3 when the concentration of N_3^- was 10^{-5} M. The maximum of $Au(N)_2^-$ at *m/z* 225 was observed at 20 V with the height of 1.8×10^3 when the concentration of N_3^- was 10^{-5} M. Therefore, the product ion $Au(N)(N_3)^-$ at *m/z* 253 at the collision voltage of 18 V was the highest peak among three product ions derived from $Au(N_3)_2^-$ at collision voltage from 10 to 50 V and hence used for the quantification. The product ion spectrum at the collision voltage of 18 V shown in Fig. 1 **a** is most suitable for the identification of N_3^- because it shows the precursor ion and three product ions, and the relative peak heights of the three product ions at *m/z* 253, 225 and 42 were 100.0, 6.9 and 3.2, respectively.

When $^{15}N\,^{14}N_2^-$ was used as a source of N_3^-, the highest peak was observed at *m/z* 283 in the mass spectrum, and the highest peaks were observed at *m/z* 254 and *m/z* 43 at collision voltage at 18 V and 40 V, respectively, in the product ion spectra.

In the single MS the $Au(OCN)_2^-$ was observed at the same *m/z* 281 as that of $Au(N_3)_2^-$. Choosing

$Au(OCN)_2^-$ at *m/z* 281 as the precursor ion, selected reaction monitoring was performed by changing the collision voltage from 10 to 50 V. Under these conditions, the observable product ion was only OCN^- at *m/z* 42 as shown in Fig. 1 **b**, and its maximum was observed at 40 V with the height of 18 when the concentration of OCN^- was 10^{-5} M.

The peak height of $Au(N)(N_3)^-$ at collision voltage of even 10 V attained to 50 % of its maximum height at 18 V. This means that N_3^- decomposes quite easily to N^- and N_2, and hence this may be one of the reasons why N_3^- is used for the inflation of air bags. On the other hand OCN^- did not decompose further when Ar gas collided to $Au(OCN)_2^-$ at 10 – 50 V. Therefore, in the present MS/MS the interference from OCN^- was almost nothing when the product ion at *m/z* 253 was selected for the detection of N_3^-. If the product ion N_3^- at *m/z* 42 had been selected for the detection of N_3^-, however, the interference from the same amount of OCN^- would be about 0.5 % since the signal height of N_3^- at *m/z* 42 was 3.6×10^3 at 10^{-5} M N_3^- whereas that of OCN^- at *m/z* 42 was 18 at 10^{-5} M OCN^-.

Interferences

Our examination revealed that N_3^- itself at 10^{-7} – 10^{-5} M was stable in 0.02 M citrate buffer with pH $\geqq$ 5 and N_3^- at 10^{-5} – 10^{-3} M in urine with pH $\geqq$ 5 when they were stored in closed tubes at least for two days at 25 ℃, respectively, as reported in another works on the stability of N_3^- [9,12]. When oxidizing agents were contained, however, N_3^- became unstable as described in the case of blood where N_3^- was converted to N_2, N_2O and/or NO, and the formation of the complex between N_3^- and methemoglobin was also demonstrated [1,2,12].

Metal ions such as Cr^{3+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Mo^{6+}, Pd^{2+}, Ag^+, Cd^{2+}, Pt^{3+}, Hg^{2+}, Tl^+ and Pb^{2+} at 10^{-4} M, respectively, did not interfere with the detection of N_3^- under the present condition. These ions, however, are well known to form chelate complexes with N_3^- in aqueous solution under suitable conditions [8,9]. When the amount of $AuCl_4^-$ was abundant enough to react with N_3^- completely, negative ions such as CN^-, OCN^-, Cl^-, ClO_3^-, NO_2^-, NO_3^-, SO_4^{2-}, CO_3^{2-}, PO_4^{3-}, oxalate and tartrate did not interfere with the detection of N_3^- under the present condition when they were added to the solution at 100 times that of N_3^-, respectively.

The recoveries of N_3^- spiked at 10^{-5} M into 100-fold diluted urine and 500-fold diluted urine were about 50 % and >95 % under assay procedure for aqueous solution with the suitable concentrations of AsA at 2.4×10^{-4} M and 1.2×10^{-4} M, respectively. We found that some of the interfering substances in urine were urea, creatinine and ammonia. Therefore, a cation exchange resin was used to eliminate these cationic substances as described in the section of assay procedure for urine. Then the recovery of N_3^- spiked at 10^{-5} – 10^{-3} M into control urine and assayed according to the procedure for urine was >95 % with the concentration of AsA at 1.2×10^{-4} M.

Matrix effects

The matrix effects, ion suppression and enhancement, are often observed in real samples. There are two ways to compensate the matrix effects on the quantification. One is to use a suitable internal standard and the other is to use a standard addition method. The isotope of an analyte is a suitable internal standard in most cases, and an isotope $^{15}N^{14}N_2^-$ is available commercially. When the isotope $^{15}N^{14}N_2^-$ was added to a biological sample solution consisted substantially of $^{14}N_3^-$, a mixed chelate complex $Au(^{15}N^{14}N_2)(^{14}N_3)^-$ was produced and it depressed the production of $Au(^{14}N_3)_2^-$ and $Au(^{15}N^{14}N_2)_2^-$. Therefore, a standard addition method was adopted for the quantification in the present determination.

Precision and accuracy in water and the treated urine

Calibration curve was calculated for water spiked and urine spiked and treated, respectively. Concentrations determined from the peak area (y) were linear to the spiked concentrations in water and in urine samples spiked and treated (x in 10^{-7} M), respectively. That is, $y = 1.008\ x + 0.082$ with the correlation coefficient (γ) of 0.999 for water, and the curves for six urine samples were given by $y = \alpha\ x + \beta$ with γ, where α ranged from 0.958 to 1.022, β ranged from -0.007 to 0.125 and γ ranged from 0.993 to 0.999, respectively. Precision and accuracy were assessed by analyses of water spiked (Table 1 a) and urine samples spiked and treated, respectively. Table 1 b showed the result for the urine sample having the worst γ value, i.e., 0.993. The coefficient of variation was $\leqq$ 10 %, and accuracy was 91–107 % for

intra-day and inter-day variations for water and six urine samples, respectively. The limit of quantification of the present method was therefore 10^{-7} M in water and 10^{-5} M in the original urine since urine was diluted to 100-fold as described in the assay procedure for urine. The LOD based on a concentration giving three times the back ground noise was 3.0×10^{-8} M in water and in the urine spiked and treated as shown in Fig. 2.

Determination of N_3^- in the samples of a suspected suicide victim

The victim's gastric fluid showing pH 8, and the urine showing pH 5, were centrifuged at $10,000 \times g$ for 3 min, and the upper layers of gastric fluid and urine were diluted with CB to 20,000-fold and 5,000-fold, respectively, since the concentrations of N_3^- in undiluted samples exceeded the range of quantification. The determination of N_3^- was performed using the diluted samples as shown in Figs. 2 and 3. Concentrations of N_3^- in gastric fluid and urine measured three times were found to be $(7.5 \pm 1.0) \times 10^{-2}$ M and $(3.2 \pm 0.4) \times 10^{-3}$ M, respectively. The total NaN_3 amounts were calculated to be 3.1 g in 637 mL of gastric fluid remained and 2.6 mg in 13 mL of urine remained, respectively.

Previous works reported that the concentrations of N_3^- in gastric fluid were from 1.2×10^{-2} to 9.1×10^{-1} M in four fatal cases [4,6] and those in urine were 2.1×10^{-6} and 3.2×10^{-6} M in two fatal cases [5,11]. Therefore, the concentration of N_3^- in the present gastric fluid was in the middle of the values reported whereas that in the present urine was quite higher than the levels reported, although the number of N_3^- poisoning cases was limited.

Comparison with other methods

LODs for N_3^- in referred works decreased in the following orders: 5×10^{-4} M [9] and 2.9×10^{-5} M [8] in colorimetry, 1.9×10^{-5} M [7] in potentiometry, 4.8×10^{-6} M [4] and 1.9×10^{-6} M [6] in LC, 1.2×10^{-6} M [10] in IC, 5×10^{-6} M [11] and 2.4×10^{-7} M [12] in GC-MS, 2.4×10^{-7} M [2] in GC and 3.0×10^{-8} M in the present FI-ESI-MS/MS, respectively, although their matrices were different. In these analyses

detections themselves such as UV and visible absorption in LC and colorimetry, potentiometry, NPD in GC, MS and MS/MS do not take substantial time but the formation of suitable compounds of N_3^- and the elimination of interfering substances take longer time. The organic derivatization took ca. 30 min in [4,6,11,12] and 5 min in [2], whereas the chelate complex formation took less than 1 min in [8,9] and in the present work. The evaporation of some derived products took 20 min in [10] and 5 min in [2] and the liquid-liquid extraction of the chelate complex took 1 min in the present work. The chromatographic separation took 8-30 min in [4,6,10,12] and 5 min in [2,12] for detection and another ca. 10 min for resetting, whereas the present FI-ESI-MS/MS took only 1 min for detection and another 1 min for resetting.

Chromatographic separation reduces the matrix effects, i.e., ion suppression and interferences, but takes the cost for columns and the time for separation. As an alternative to the chromatographic separation, the extraction with octanol followed by MS/MS has been adopted in the present method. The solubility of octanol in water is only 0.06 wt %, and it means that octanol can eliminate a large amount of biological substances dissolved in aqueous layer and hence it reduces greatly the matrix effects. Electrospray ionization has been enhanced when the solvent was changed from water to octanol, a polar organic solvent. MS/MS detection can eliminate in a moment a large amount of false signals still contained in a single MS. That is, the peak height ratios of the precursor ion at *m/z* 281 versus the product ion at *m/z* 253 were 0.32, 0.86 and 4.3 at 10^{-5} M in Fig. 1 **a**, at 6.4×10^{-7} M in Fig. 3 **b** and at 10^{-7} M in Fig. 3 **a**, respectively, indicating that false signals at the first MS (i.e., at *m/z* of the precursor ion) relatively increased as the concentration of N_3^- decreased, and those false signals were eliminated at the second MS (i.e., at *m/z* of the product ion). Consequently, the determination of N_3^- with the lowest LOD could be achieved rapidly by the present FI-ESI-MS/MS. This method is applicable to analytes dissolved in water but the derived products from analytes are soluble in polar organic solvents and are detectable by MS/MS.

Conclusion

An MS/MS determination of N_3^- has not been reported yet. A single MS cannot distinguish between N_3^- and OCN^- since they have the same *m/z* 42, but present MS/MS can distinguish them by their MS/MS product ion spectra. Previous GC-MS methods needed >45 min for the derivatization of N_3^- and GC-MS, but present method needs <10 min for the chelate complex formation, its extraction and FI-ESI-MS/MS.

The method presented here is therefore suitable for a rapid diagnosis of N_3^- poisoning.

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Figure captions

Fig. 1a-b Product ion spectra at collision voltage of 18 V from the precursor ion $Au(N_3)_2^-$ at *m/z* 281 (**a**) and from the precursor ion $Au(OCN)_2^-$ at *m/z* 281 (**b**), which were extracted with octanol from 10^{-5} M N_3^- and 10^{-5} M OCN^- aqueous solutions, respectively.

Fig. 2a-f Amount of the $Au(N)(N_3)^-$ product ion at *m/z* 253 after flow-injection of octanol layer extracted from the blank of a treated control urine sample (**a**); urine spiked with N_3^- at 3.0×10^{-6} M and treated (i.e., 3.0×10^{-8} M in the solution) (**b**); the urine spiked with N_3^- at 10^{-5} M and treated (i.e., 10^{-7} M in the solution) (**c**); the victim's urine diluted 5,000-fold (**d**); the victim's gastric fluid diluted 20,000-fold (**e**); and 10^{-5} M N_3^- aqueous solution (**f**).

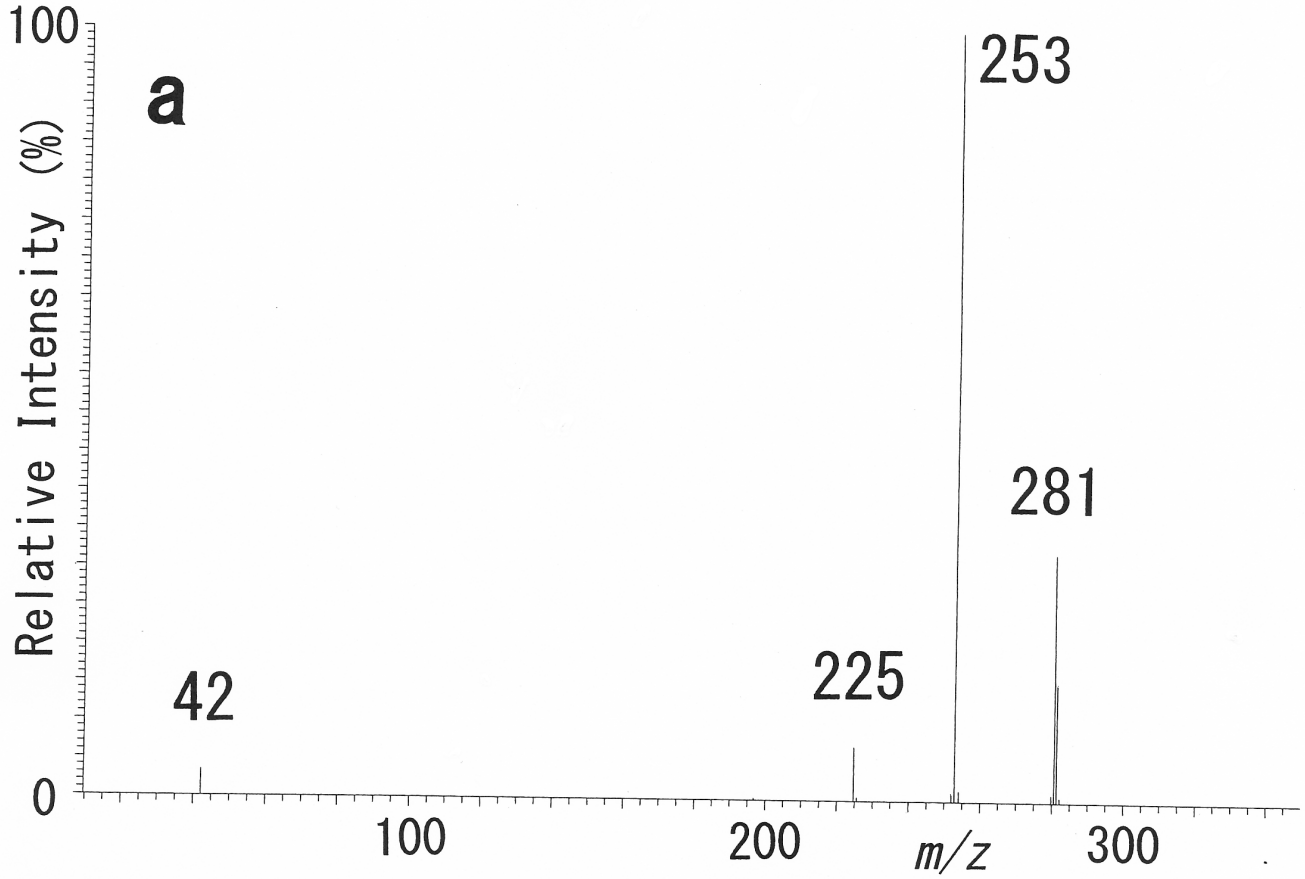
Fig. 3a-b Product ion spectra obtained at collision voltage of 18 V from the precursor ion $Au(N_3)_2^-$ at *m/z* 281 extracted from a control urine sample spiked with N_3^- at 10^{-5} M and treated (i.e., 10^{-7} M in the solution) (**a**); the victim's urine sample diluted 5,000-fold (**b**).

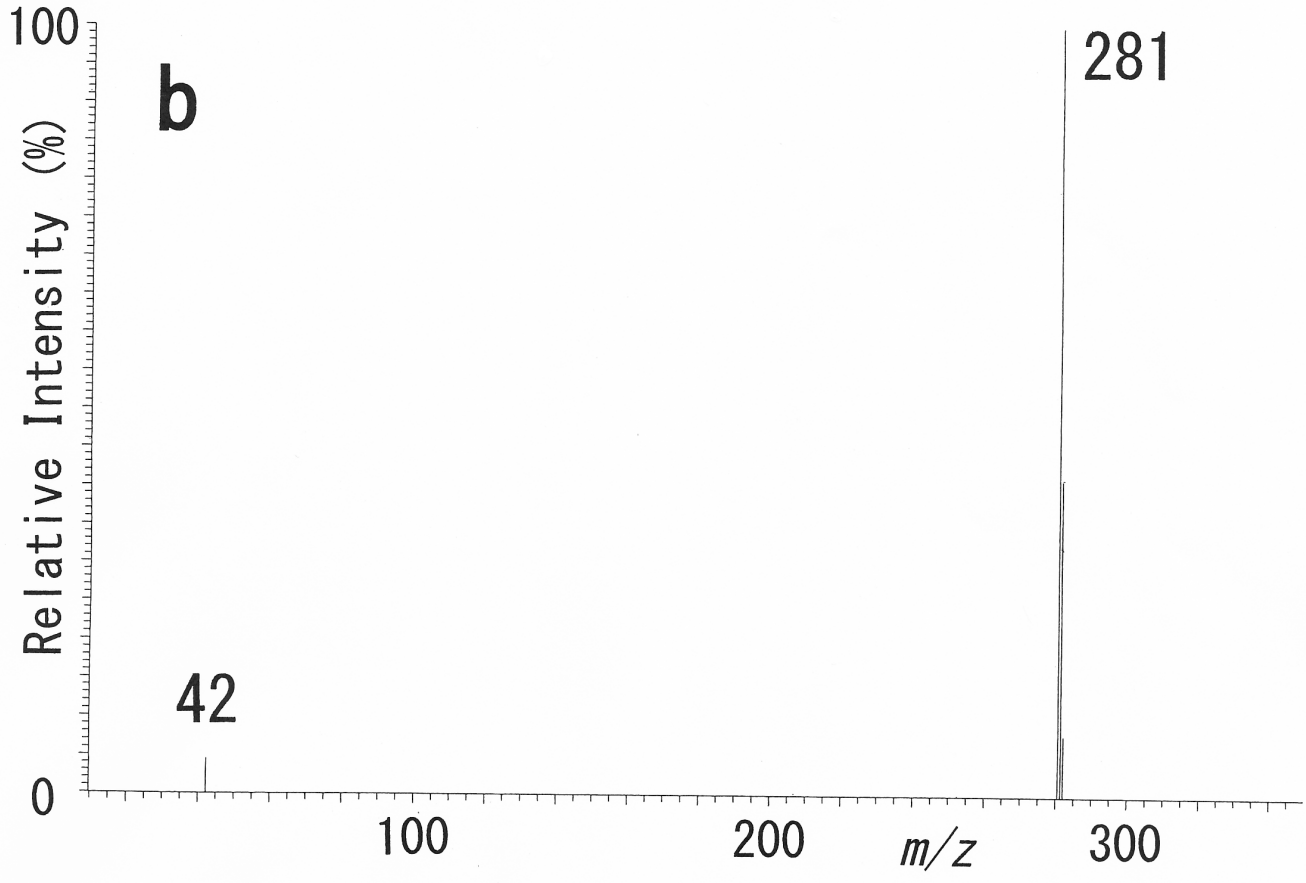
Table 1 Intraday (six times) and interday (six days) accuracy/precision for N_3^- spiked into water

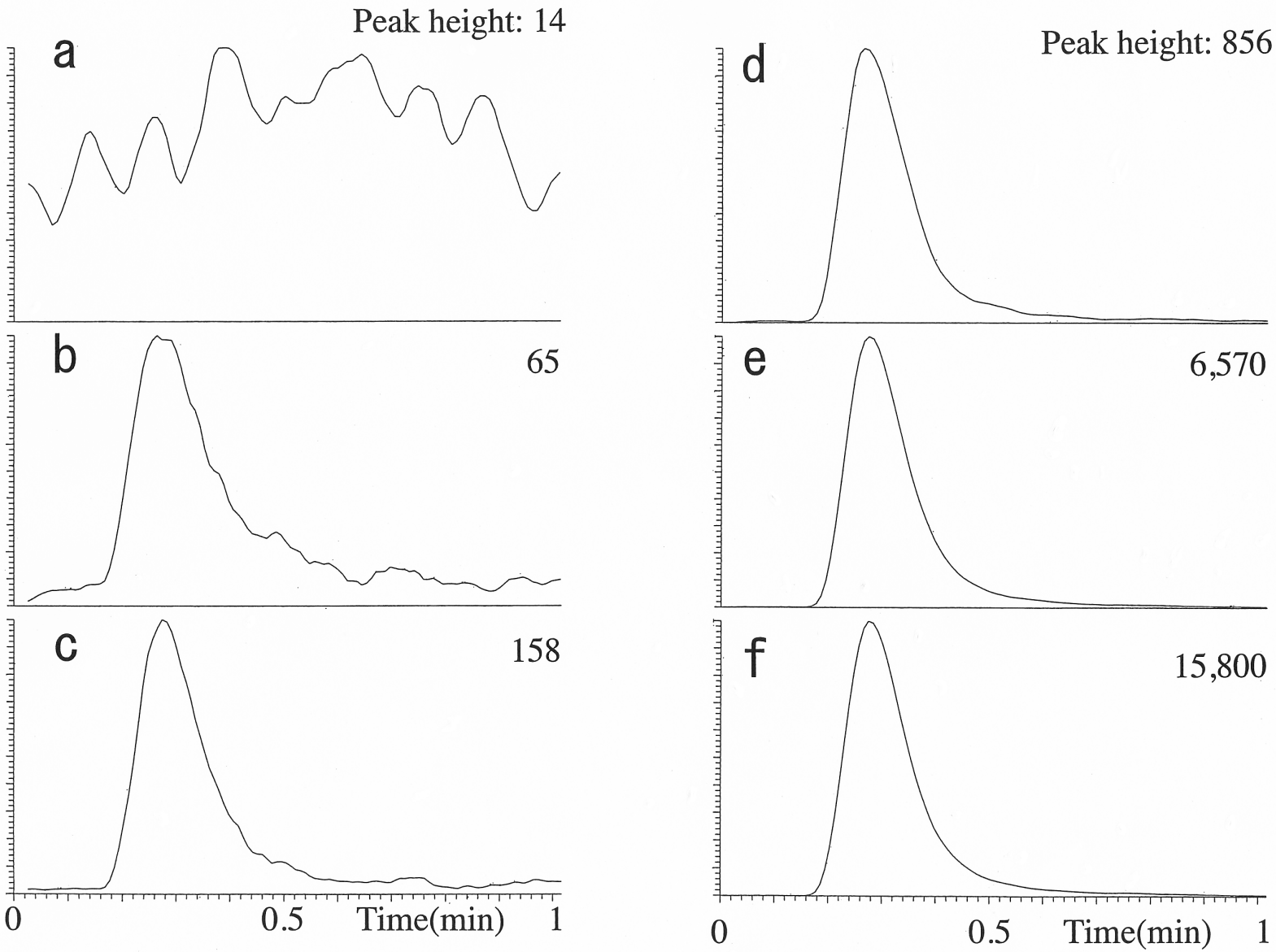
	Intraday		Interday	
N_3^- Spiked (10^{-7} M)	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
1.00	103.4	5.0	91.2	10.0
10.0	96.3	5.3	104.1	7.8
100.	101.8	5.9	102.9	8.6

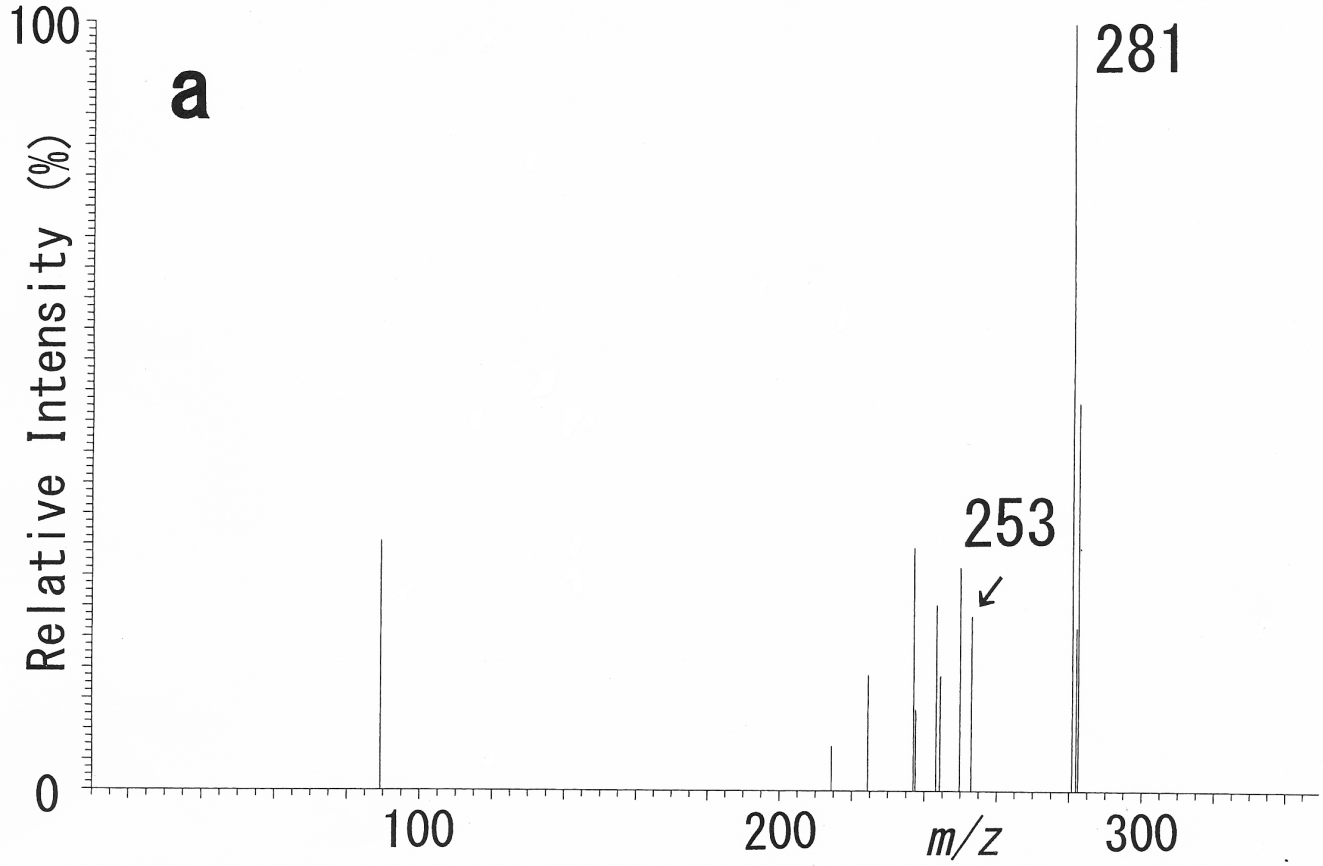
Table 2 Intraday (six times) and interday (six days) accuracy/precision for N_3^- spiked into urine

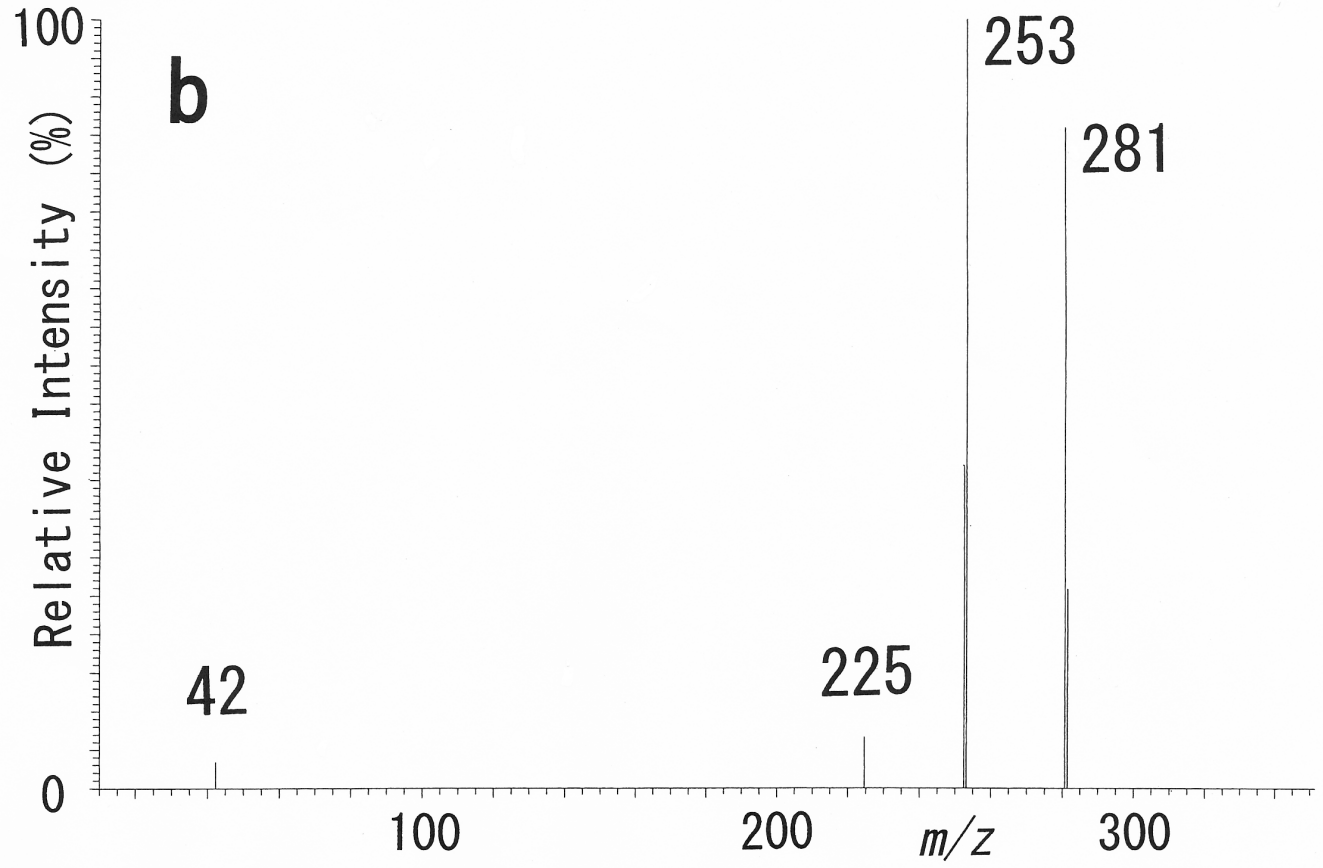
	Intraday		Interday	
N_3^- Spiked (10^{-5} M)	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
1.00	93.7	9.9	92.0	8.5
10.0	104.7	9.8	106.6	3.9
100.	103.0	7.2	100.0	9.9


a
100
Relative Intensity (%)
0
253
281
225
42
100
200
m/z
300


b
100
Relative Intensity (%)
0
281
42
100
200
m/z
300


Peak height: 14
a
b
65
c
158
0
0.5
Time(min)
1
Peak height: 856
d
e
6,570
f
15,800
0
0.5
Time(min)
1


a
100
0
Relative Intensity (%)
281
253
100
200
m/z
300


b
100
0
Relative Intensity (%)
253
281
42
225
100
200
m/z
300