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Short communication

Determination of iodide in urine using electrospray ionization tandem mass spectrometry☆

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ABSTRACT

A rapid and sensitive electrospray ionization (ESI) tandem mass spectrometry (MS–MS) procedure was developed for the determination of iodide (I^-). A gold (Au) and I^- complex was formed immediately after the addition of the chelating agent $NaAuCl_4$ to I^- solution, and was extracted with methyl isobutyl ketone. One to five microliters of the extract were injected directly into an ESI–MS–MS instrument. I^- quantification was performed by selecting reaction monitoring of the product ion I^- at m/z 127 derived from the precursor ion $^{197}AuI_2^-$ at m/z 451. I^- concentration was measured in the quantification range from 10^{-7} to 10^{-5} M using 50 μL of solution within 10 min. Iodate was reduced to I^- with ascorbic acid and determined. I^- concentration in reference urine 2670a was measured after treatments.

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1. Introduction

Iodine is an essential nutrient required for the synthesis of thyroid hormones. Iodine deficiency induces enlargement of the thyroid gland (i.e., goiter), a wide range of mental, psychomotor and growth abnormalities, as well as increased infant mortality. In urine, >90% of total iodine is present as iodide (I^-), so the determination of I^- excreted into urine is considered to be a good parameter to estimate iodine availability and its daily average consumption [1]. According to the World Health Organization, the median urinary iodine concentration in iodine-sufficient populations should be >100 μg L^{-1} (ca. 10^{-6} M) [2].

Various methods based on different principles have been proposed to determine I^-. These include electrochemical detection [1], spectrophotometry [3], chemiluminescence [4], polarography, gas chromatography (GC) [5], liquid chromatography (LC) [6] and ion chromatography (IC) [7]. Although the sensitivity of some methods is high, these methods fail to provide decisive determinations of analytes in biological samples that give various types of matrix interferences. Mass spectrometry (MS) can determine the analyte in mass to charge ratio. Recently, inductively coupled plasma-mass spectrometry (ICP-MS) has been widely used for the determination of iodine in urine, water and seawater [2,8,9]. To separate iodide (I^-), iodine (I_2), iodate (IO_3^-) and organic iodine that all give a signal at m/z = 127 in ICP-MS, chromatographic separation has been done before ICP-MS, but the contamination of unknown iodine compounds cannot be eliminated from each known iodine species because ICP-MS provides only elemental information [8,9]. GC–MS [10] and electrospray ionization mass spectrometry (ESI–MS) can provide molecular information [11–13]. The ESI–MS method was applied in 1985 to the determination of I^-, IO_3^- and NO_3^- in glycerol solution where a long-chain cationic surfactant served to bind the anions to the surface of the solution [11]. The sensitivity of the method was low, and only the mass spectrum of I^- at 10^{-3} M was obtained. In recent ESI–MS studies, two or more cartridges such as the cartridge to remove Cl^- and SO_4^{2-} ions, that to remove Na^+ and K^+ ions and that to separate I^- from other anions were used for the sensitive detection of I^- [12,13]. In the present ESI–MS–MS method, methyl isobutyl ketone (MIBK) was used as the solvent to eliminate interferences contained in urine as well as the solvent to facilitate ESI more than that of the aqueous solution [14–17]. In the MS–MS analysis, the first MS selected a chelated complex of I^- with gold (Au), AuI_2^- at m/z = 451, and the second MS selected I^- at m/z = 127. After these repetitive selections, the present method can rapidly and accurately quantify I^- from 10^{-7} to 10^{-5} M.

2. Materials and method

2.1. Materials

MIBK of atomic absorption grade, metals of atomic absorption standard, and other chemicals of analytical grade were obtained

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from Wako Pure Chemicals (Osaka, Japan). Pure water with a specific resistance of 18 MΩ cm was used (Millipore, Bedford, MA, USA).

Urine samples were obtained from ten healthy non-smokers and a smoker after permission. Urine samples from non-smokers were used as control urines. Urine samples were also obtained at forensic autopsies from July 2009 to January 2010 from nine fire victims whose blood CN level was $>10^{-5}$ M by a preliminary investigation and a victim who had died after taking a NaCN solution. All urine samples were refrigerated at −20 °C until analysis. Standard reference urine (SRM 2670a, low-level urine) was purchased from the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA).

A stock solution of I^- (1 mM) was prepared by dissolving KI into water and freezing until use. Standard solutions and quality control solutions were prepared before measurement by diluting the stock solution with aqueous solution and urine.

2.2. Assay procedure for aqueous solution

To 50 μL of aqueous solution containing $I^- > 10^{-7}$ M in a tube (Eppendorf AG, Hamburg, Germany), 5 μL of 0.3 M tetramethylammonium hydroxide (TMAH) solution was added to adjust the pH of the solution to 9–11. One microliter of 2×10^{-3} M $NaAuCl_4$ was added to achieve a 4×10^{-5} M concentration of Au ions and 50 μL of MIBK were added and vortexed for 20 s, then centrifuged at $500 \times g$ for 10 s. The upper MIBK layer was used for the MS–MS analysis. The injection volume was 1 μL.

2.3. Assay procedure for urine

An Oasis HLB cartridge for 1 mL (Waters, Milford, MA, USA) was activated with methanol and water, and 0.1 mL of urine diluted with water to 1 mL was passed through the cartridge. Hereafter this is referred as to "the treated urine". The treated urine (50 μL) was assayed as in the case of aqueous sample by adding 5 μL of 0.3 M TMAH, 1 μL of 2×10^{-2} M $NaAuCl_4$ and 50 μL of MIBK. Here, the concentration of Au ion was increased because the treated urine contains not only I^- but also other substances that react with Au ion but do not interfere with the assay. Each urine sample was quantified through the method of standard additions. That is, 0.1 mL each of the original urine was spiked with I^- at several suitable concentrations from 0 to 10^{-5} M, diluted to tenfold, passed through the cartridge and measured, respectively. The injection volume of the MIBK layer for the MS–MS analysis was 5 μL.

2.4. Assay procedure for IO_3^-

A 50 μL aliquot of aqueous solution or treated urine was added with 1 μL of 1 M HCl and 1 μL of 0.1 M ascorbic acid solution. After 10 min at ambient temperature, 3 μL of 1 M TMAH was added to finish the reduction and to raise the pH of the solution to 9–11, and assayed as in the case of aqueous solution or treated urine.

2.5. Instrumentation

ESI–MS–MS was carried out using a TSQ 7000 LC-quadrupole mass spectrometer (ThermoQuest, Tokyo, Japan) in negative ion mode. Methanol was the mobile phase and had a flow rate of 200 μL min^{-1}. The capillary temperature was set at 200 °C. The electrospray voltage was set at −4.5 kV, the multiplier voltage at 1.3 kV, and the collision voltage, at 50 V. Nitrogen was used as a sheath gas (469 kPa) and also as an auxiliary gas (8 units); argon was used as a collision gas (134 kPa). A 20 μL sample loop was used, and sample was injected manually or using autosampler into the ESI–MS–MS apparatus directly. The suitable sample volume was from 1 to 5 μL for both injections, and the suitable time interval was 1 min for the manual injection and 2 min for the auto sampler injection, respectively. Quantification in ESI–MS–MS was performed by integration of the peak area of the product ion at m/z 126.8 ± 0.2 derived from the precursor ion at m/z 450.8 ± 0.3 using a calibration curve comprising spiked matrix samples at different concentrations.

3. Results and discussion

3.1. Suitable conditions for the production and the extraction of AuI_2^- in aqueous solution

The production of AuI_2^- in 30 mM citric acid/30 mM TMAH solution of pH 8, 9–11 and 12 was nearly 90%, 100% and 90%, respectively. The signal peak areas derived from organic solvents such as pentanol, hexanol, isoamyl alcohol, octanol and cyclohexanol that were used for the extraction as well as for ESI were 30%, 40%, 70%, 50% and 20%, respectively, by taking the peak area of MIBK as 100%. The highest signal was observed by vortex-mixing the solutions of I^-, $NaAuCl_4$ and MIBK for 15–30 s; longer mixing decreased the signal. The production of the complex in 30 mM TMAH solution and the extraction of it with MIBK for 20 s were therefore selected. In the I^- solution at 10^{-5} M, complete production of AuI_2^- was observed when the concentration of $NaAuCl_4$ was $>4 \times 10^{-5}$ M. $NaAuCl_4$ at 4×10^{-5} M was therefore used for subsequent detections.

Under the condition stated above, the observed amounts of AuI_2^- in MIBK were >95% of the starting amounts during 3 h at ambient temperature under room lighting.

3.2. MS and MS–MS spectra

The mass spectrum of AuI_2^- consisted of one peak at m/z 491 (Fig. 1(a)) because Au and I are mono-isotopic elements. Choosing AuI_2^- at m/z 491 as the precursor ion, the best condition for selected reaction monitoring was examined by changing the capillary temperature from 190 to 280 °C and the collision voltage from 10 to 50 V. A capillary temperature of 200 °C and a collision voltage of 50 V were used in the subsequent detection because the highest peak of I^- at m/z 127 was observed. Under these conditions, the observable product ion was only I^- at m/z 127 (Fig. 1(b)).

3.3. Interferences from cations and anions in aqueous solution

Metal ions such as Ti^{4+}, V^{5+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, As^{3+}, Mo^{6+}, Pd^{2+}, Cd^{2+}, W^{6+}, Pt^{3+}, Hg^{2+}, Tl^+, and Pb^{2+} at 10^{-4} M did not decrease the peak of I^- under the present condition (although Ag^+ at 10^{-4} M appreciably decreased the peak of I^-). When the amount of Au was sufficiently abundant to react completely with I^-, CO_3^{2-}, N_3^-, NO_2^-, NO_3^-, PO_4^{3-}, OCN^-, SCN^-, SO_3^{2-}, SO_4^{2-}, Cl^-, ClO_3^-, Br^-, IO_3^-, citrate and oxalate neither decreased the signals of AuI_2^- nor gave signals at the signals of AuI_2^- under the present condition when they were present at ten times that of I^-.

3.4. Recovery from urine

The determination of I^- at $<10^{-5}$ M in untreated urine was difficult when the assay procedure for aqueous solution was used. Therefore the elimination of interfering substances in urine was made using an Oasis HLB cartridge. The signal intensity of I^- in the treated urine that passed through the cartridge was nearly 100 times that of I^- in untreated urine, although not only I^- but also most inorganic ions only passed through the cartridge. The recovery of I^- spiked into a control urine at 10^{-7} M was >85%.

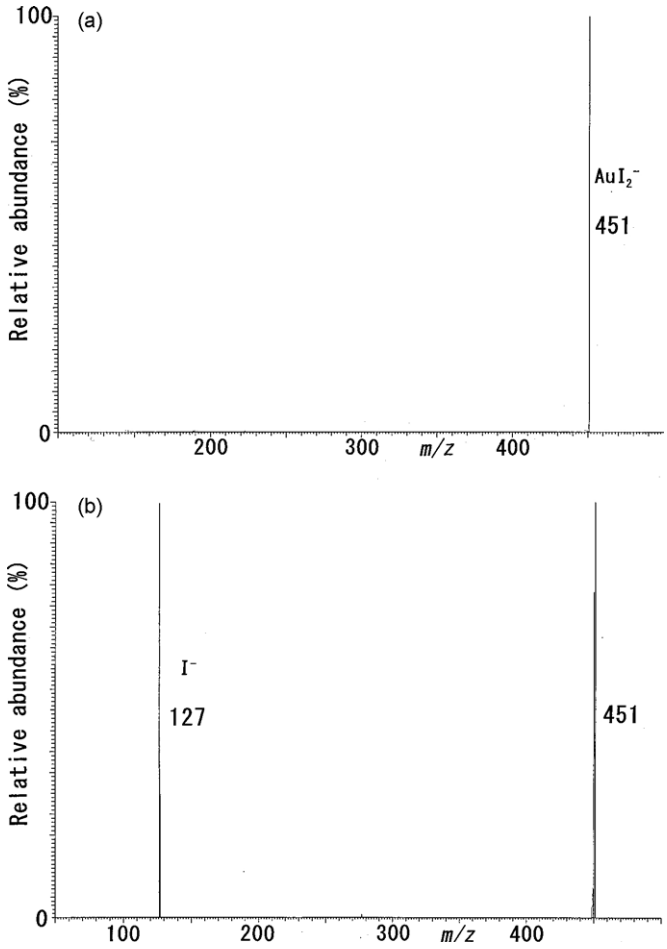

Fig. 1. Mass spectrum of the methyl isobutyl ketone layer extracted from 10^{-5} M I^- in water (a), and its product ion spectrum at a collision voltage of 50 V from the precursor ion at m/z = 491 (b). The injection volume was 1 μL in (a) and (b), respectively.

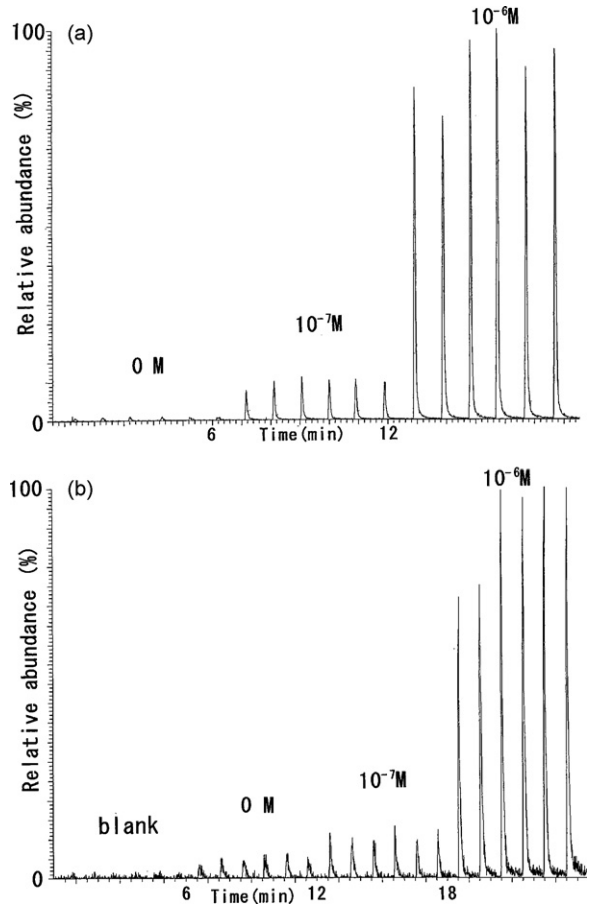

Fig. 2. Mass chromatograms of selected reaction monitoring at m/z = 127 derived from the precursor ion at m/z = 491 for the quantification of I^- in water (a) and in a control urine (b). The injection volume was 1 μL for each peak in (a) and 5 μL for each peak in (b), respectively.

3.5. Precision and accuracy

Calibration standard solutions of I^- were prepared by spiking stock solutions to water and a control urine at 0, 10^{-7}, 10^{-6} and 10^{-5} M three times for each concentration, respectively. The solutions that did not contain any added Au ion were considered as blanks. In case of water, blanks and the solutions that were not spiked with I^- (i.e., 0 M) but added Au ion, gave the same signal. Fig. 2 shows mass chromatograms of I^- in water (a) and in a control urine (b) at m/z 127 in selected reaction monitoring. The concentrations of I^- determined from the peak areas (y) were linear to the concentrations spiked (x) up to 10^{-5} M, i.e., $y = 21.74x + 2.82$ with a correlation coefficient of 0.9999 in water and $y = 22.69x + 10.65$ with a correlation coefficient of 0.9898 in the control urine. Data analysis was performed using KaleidaGraph (version 3.0.5) statistical software.

Precision and accuracy were assessed by the analysis of water and the control urine spiked at 10^{-7}, 10^{-6} and 10^{-5} M, respectively (Table 1). These samples were analyzed six times daily as well as on 6 different days. The coefficient of variation was <17% and accuracy was 72–117%. The signals at 10^{-7} M in water and those in the control urine spiked at 10^{-7} M were measured six times and their standard deviations (σ) were calculated. When the limit of detection (LOD) was defined to be 3σ for signals at 10^{-7} M, the lowest quantification, LODs were calculated to be 3.4×10^{-8} M in water and 5.0×10^{-8} M in the control urine.

To check the recovery and the proof of principle, the standard reference material 2670a low-level urine was used. The concentration of I^- reported in the Certificate Issue published from NIST was $6.94 \pm 0.09 \times 10^{-7}$ M; our value was $6.5 \pm 0.8 \times 10^{-7}$ M in six determinations after Oasis HLB cartridge treatment.

3.6. Determination of I^- in urines of healthy volunteers, victims of cyanide and/or fire

The transport of I^- by sodium iodide symporter is impaired by some anions such as thiocyanate, perchlorate and nitrate [13]. Cyanide is metabolized to thiocyanate by mitochondrial rhodanese [18,19]. To examine the influence of these anions, it may be necessary to detect I^- in solutions where the concentration of these interfering ions can be altered. Cyanide poisoning has recently been increased in fire victims due to the increased hydrogen cyanide production in fires from nitrogen-containing organic materials [20]. Therefore, as a preliminary study, urines of smoker [18], fire victim and victim poisoned with cyanide, denoted as group 1, was compared with urines of non-smoker, denoted as group 2, to see if the present method is applicable to those samples. Since levels in casual urine of Japanese showed a very large difference due to high iodine intakes [21], the level of each subjects was listed as follows: a smoker, 8.5×10^{-6} M; 8 fire victims (3, 4, 10, 11, 12, 15, 20, 31) $\times 10^{-7}$, respectively; a victim poisoned with cyanide, 6.0×10^{-5} M; 10 non-smokers (4, 5, 11, 12, 15, 20, 46, 56, 105, 310) $\times 10^{-7}$, respectively. The values of group 1 were from 3×10^{-7} to 6.0×10^{-5} M and those of group 2 were from 4×10^{-7}

Table 1
Intra-day (six times) and inter-day (6 days) variations of I^- values determined by the present method. The observed values and coefficient of variations (C.V.) for I^- spiked at 10^{-7} to 10^{-5} M in water and urine, respectively.

Intra-day					Inter-day			
Spiked			Observed		Observed			
Water			Urine		Water		Urine	
I^- [M]	I^- [M]	C.V. (%)	I^- [M]	C.V. (%)	I^- [M]	C.V. (%)	I^- [M]	C.V. (%)
$1(\times10^{-7})$	0.96	11.4	0.72	16.6	0.91	11.1	0.96	11.6
$1(\times10^{-6})$	1.01	8.6	1.02	15.0	1.17	11.0	0.89	14.0
$1(\times10^{-5})$	1.01	9.6	1.03	9.8	0.97	5.5	0.96	5.7

to 3.1×10^{-5} M. Therefore no appreciable difference was observed between two groups.

3.7. Determination of IO_3^-

IO_3^- did not interfere the detection of I^- at all, as listed in Section 3.3. The possibility of the determination of IO_3^- was examined, since IO_3^- could be reduced to I^- with reducing agents such as ascorbic acid [10] and $NaHSO_3$ [3], although IO_3^- did not form the complex $Au(IO_3)_2^-$. KIO_3 at 10^{-6} to 10^{-5} M in aqueous solution as well as in treated urine could be detected as AuI_2^- within 10% error, according to the assay procedure written in Section 2.4. However, the procedure mentioned was not applicable to urine samples at present. Firstly, I^- in urine should be removed using AgCl column or some other method [10], since >90% of total iodine is present as I^- whereas IO_3^- is a minor component in urine [1]. Secondly, IO_3^- should be concentrated to attain rather high quantification range since the complete reduction was possible only in treated urine that was diluted to tenfold from the original urine.

3.8. Comparison with other ESI–MS(–MS) methods

In recent ESI–MS(–MS) studies, sensitive detections of I^- were reported. That is, the LOD was 7.9×10^{-10} M under $S/N = 3$ definition in IC-MS [12] and 4.5×10^{-9} M under the 3σ definition in IC-MS–MS [13]. Our LODs were 3.4×10^{-8} M in water and 5.0×10^{-8} M in urine under the 3σ definition at 10^{-7} M, the lowest quantification.

In [12], I^- was reacted with a di-cation 1,2-bis(trimethylammonium)dodecane^{2+} and the resulting cationic ion pair was detected at $m/z = 413$ in MS [12]. The disadvantages of the method are that the di-cation should be synthesized and some impurities may be contained in the signal at $m/z = 413$ because it is only one MS selection. Even though MS–MS analysis would be applied to the ion pair at $m/z = 413$, the product ion may not be derived from I^- but be derived from the di-cation because a positive ion mode was adopted there. In [13], MS–MS analysis was introduced but the same $m/z = 127$ was used at the first MS selection as well as at the second MS selection. Because $m/z = 127$ belongs to a low-mass region that is abundant with naturally occurring low-mass ions, some impurities may be contained in the signal. In the present MS–MS, the first MS selected AuI_2^- at $m/z = 451$ that belongs to rather a high-mass region, and the second MS selected I^- itself at $m/z = 127$.

4. Conclusion

Herein, we propose an ESI–MS–MS method for the simple, rapid and decisive determination of I^-. The method (i.e., instantaneous chelate complex formation of a target small ion, the elimination of interfering substances with organic solvent, and the detection of the complex in organic solvent by ESI–MS (ESI–MS–MS)) was applied previously for small cations such as Pt^{4+}, Cr^{6+}, Co^{3+} and As^{3+} [14–17], but it has been applied to a small anion, I^-, for the first time. Except single-atomic anions such as I^- and Br^-, ICP-MS cannot detect small anions that consist of more than two atoms. In GC–MS analysis, most small anions should be derivatized to volatile compounds. The present method may be applicable for the detection of other small anions that are resistant to soft ionization (ESI) after choosing suitable conditions such as pH, cationic species, and the organic solvent to eliminate interferences and organic solvent to electrospray ionization.

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