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Abstract A rapid and sensitive mass spectrometric method was developed for the simultaneous determination of coinage metals such as copper (Cu), silver (Ag) and gold (Au). The metals in a wet ashed tissue solution were complexed with diethyldithiocarbamate (DDC; $C_4H_{10}NCS_2$) and were extracted together with isoamyl alcohol. After the acidification of the extract with oxalic acid, metals were quantified using their product ions, $Cu(DDCH)^+$, $Ag(DDCH)^+$ and $Au(DDCH)^+$ that derived from the precursor ions $Cu(DDC)_2^+$, $Ag(DDC)_2^+$ and $Au(DDC)_2^+$, respectively, by electrospray ionization tandem mass spectrometry. The limits of detection were 0.6, 0.3 and 1 $\mu g l^{-1}$ for Cu, Ag and Au and the quantification ranges were 2 – 100, 1 – 100 and 3 – 100 $\mu g l^{-1}$ for Cu, Ag and Au, respectively. Cu levels in spontaneously hypertensive osteogenic disorder rats at 6 weeks old and at 30 weeks old were found to be 1.8 times and 5.1 times those of the normotensive osteogenic disorder rats, respectively, when using wet-ashed kidney solutions diluted to 1,000 fold.

Key words Copper • Silver • Gold • Electrospray ionization • Tandem mass spectrometry • Diethyldithiocarbamate

Introduction

The determination of metals is indispensable to clarify the cause of poisonings, the relationship between metals and diseases and the effect of medicines containing metals [1]. That is, the cause of unnatural death in 48 cases occurred during 4 years in Japan was attributed to the intake of metal ions by victims [2]. Copper (Cu) levels were reported to be significantly higher in plasma of essentially hypertensive patients [3] and in plasma and tissues of spontaneously hypertensive rats (SHR) [4,5] than those of the control, respectively, although the contribution of Cu to hypertension is difficult to be understood at present, since hypertension may conversely contribute to the redistribution of Cu and some other elements. Cu levels in blood and tissues were easily affected with diseases [3-5] and toxicological substances [6]. Silver (Ag) nitrate known already in ancient Egypt and other Ag compounds including Ag nanoparticles are used as antibacterial agents [7]. Gold (Au) compounds are one of the most effective medicaments available for rheumatoid arthritis [8], and after its treatment the elevation of not only Au but also Cu in renal metallothionein was reported [8].

Above three metals, Cu, Ag and Au, have similar chemical properties and are termed as coinage metals. Therefore, we tried to develop a mass spectrometric (MS) method that can determine them simultaneously. Simultaneous determination of metals is currently performed by inductively coupled plasma (ICP) MS [6,9]. It is a highly sensitive method but several isobaric interferences occurred from artificial products coming from various samples and carrier gas argon (Ar) because several chemical reactions are easily occurred at around 7,000 K of the atomization temperature. Signals of $^{31}\text{P}^{16}\text{O}_2$, $^{23}\text{Na}^{40}\text{Ca}$, $^{23}\text{Na}^{40}\text{Ar}$ and $^{25}\text{Mg}^{40}\text{Ar}$ coincide with those of ^{63}Cu and ^{65}Cu [10], $^{91}\text{Zr}^{16}\text{O}$, $^{92}\text{Zr}^{16}\text{O}^1\text{H}$, $^{93}\text{Nb}^{16}\text{O}$ and $^{92}\text{Mo}^{16}\text{O}^1\text{H}$ coincide with ^{107}Ag and ^{109}Ag [11,12] and $^{181}\text{Ta}^{16}\text{O}$ and

$^{180}\text{Hf}^{16}\text{O}^1\text{H}$ coincide with ^{197}Au [13], respectively. Furthermore, the mass range of these metals is lower than m/z 200 and belongs to the chemical noise region in MS [14].

Electrospray ionization (ESI) MS is used mainly for the determination of the molecular structure of organic molecules, and its application to the quantification of metal ions such as Cu, Ag and Au was only limited to the following two works. ESI-MS detection of porphyrin-chelated Cu, Ag and Au [15], however, was not applicable to the quantification of them because the porphyrin reacted with Cu quite slowly, it required five times Ag to form a complex with Ag and it produced not a complex but only colloidal substance with Au, respectively. In another ESI-MS work, an EDTA complex of Cu was reported but it could not be used practically because the limit of detection (LOD) of Cu was $1\text{ }\mu\text{M}$, i.e., $63\text{ }\mu\text{g l}^{-1}$, and that was equal to one tenth of normal serum Cu level [16], although the chelate complex contributed to increase m/z value in MS detection from 63 of Cu to 354 of $\text{Cu}(\text{H}_3\text{EDTA})^+$.

We have found that chelate complexes of Cu, Ag and Au with diethyldithiocarbamate, (DDC; $\text{C}_4\text{H}_{10}\text{NCS}_2$) extracted with isoamylalcohol (IAA) have showed only faint signals on ESI-MS (-MS) but the signals have increased to more than 1,00 times the originals after the acidification with oxalic acid, respectively. Cu, Ag and Au contained in a same solution could be determined simultaneously because their DDC complexes were extracted together with IAA, and their signals were detected at different m/z values on MS (-MS). The extraction with IAA, i.e., a polar organic solvent, could decrease the interferences from biological materials that were soluble in aqueous solution. Furthermore the ionization of chelate complexes in IAA was enhanced more than that in methanol-water mixture used in [16].

Materials and methods

Materials

Cu, Ag, Au and other metal standards of atomic absorption grade, HNO_3 of atomic absorption grade, and other chemicals of analytical grade were obtained from Wako Pure Chemical Ltd., Osaka, Japan. IAA suitable for nucleic acid purification was obtained from Sigma-Aldrich, USA. The Cu and Ag standards dissolved in 0.1 M NaNO_3 at 1 g l^{-1} each were used for the Cu and Ag stock solutions, respectively. The Au standard is dissolved in 1 M HCl solution, and hence Cl ion may precipitate Ag ion as AgCl to a certain extent even though they are mixed after high-fold dilution of them. Therefore, NaAuCl_4 dissolved in water at 1 g l^{-1} was used for the Au stock solution after its calibration with the Au standard. Pure water with a specific resistance of $18 \text{ M}\Omega\text{cm}$ was used (Millipore, Bedford, MA, USA). All glassware and plastics were soaked in 1 M and 0.3 M HNO_3 , respectively, overnight and rinsed more than 10 times with pure water.

Animal samples

SHR osteogenic disorder (SHR-od) rats were bred and raised by Horio et al. at the animal colony in Nagoya University, Nagoya, Japan [17]. Normotensive osteogenic disorder (ODS) rats were purchased from CLEA, Tokyo, Japan. At Nagoya University, male rats of the two strains were allowed to drink water and eat a commercial diet that had been supplemented with ascorbic acid (300 mg kg^{-1}), because both SHR-od and ODS rats cannot synthesize vitamin C like humans. Rats were killed at 6 weeks old ($n = 6$ for each strain) and 30 weeks old ($n = 4$ for each strain) by cardiac puncture under light Nembutal anesthesia. Kidneys

maintained at $-20\text{ }^{\circ}\text{C}$ were sent to Hamamatsu University School of Medicine and stored at $-80\text{ }^{\circ}\text{C}$ until analysis. Wet tissue (10 mg) spiked with coinage metals at $0 - 100\text{ }\mu\text{g}^{-1}$ was mixed with $10\text{ }\mu\text{L}$ of conc. HNO_3 and left overnight. Then the slurry solution was wet-ashed at $100\text{ }^{\circ}\text{C}$ for 8 h. The ratio between the weight of tissue and the volume of conc. HNO_3 was kept constant when another amount of tissue was used. Before the determination on ESI-MS (-MS), the pH of the wet-ashed tissue solution was raised to ~ 7 with NaOH solution, and the solution was diluted finally to 100 (v/w) of the original tissue for the validations of the present method, and diluted to 1,000 fold (v/w) of the original tissue for the quantification of Cu level in tissue.

Assay procedures

To $50\text{ }\mu\text{L}$ of sample solution, $1\text{ }\mu\text{L}$ of 0.1 M DDC was mixed. After 1 min, $75\text{ }\mu\text{L}$ of IAA was added and mixed for 30 sec using a vortex mixer, and separated by centrifugation at $1,000\text{ }g$ for 10 s. The IAA layer was transferred into another tube and mixed with $50\text{ }\mu\text{L}$ of 0.3 M oxalic acid for 10 s and centrifuged at $1000\text{ }g$ for 10 s. An aliquot of $1 - 5\text{ }\mu\text{L}$ IAA extract was subjected to ESI-MS (-MS). The peaks of Cu, Ag and Au appeared about 0.3 min after the injection. A standard addition method was adopted to compensate the matrix effects on the quantification.

Instruments

ESI-MS (-MS) was performed using a TSQ 7000 LC-quadrupole mass spectrometer (Thermo Quest, Tokyo, Japan). Methanol was flowed as the mobile phase at $300\text{ }\mu\text{L min}^{-1}$. The spray voltage was set at $+4.5\text{ kV}$ in the positive ion

mode. The desolvation temperature of fused silica capillary was set to 280 °C because the three complexes showed the highest signals at the temperature, respectively. Nitrogen was used as the sheath gas (68 p.s.i.) and also as the auxiliary gas (8 units), and Ar was used as the collision gas (134 kPa). The electron multiplier was set at 1.3 kV and the scan time was set at 1.8 s between m/z 100 and 1000. An aliquot of 1 µl IAA was flow-injected to ESI-MS (-MS) apparatus manually with time interval of 1 min, and an aliquot of 5 µl IAA was flow-injected using an auto sampler with time interval of 2 min, respectively. The quantification in ESI-MS-MS was performed by integration of the peak area of the product ion at m/z 212 ± 0.2 , derived from the precursor ion at m/z 359 ± 0.3 for Cu, the product ion at m/z 256.0 ± 0.2 , derived from the precursor ion at m/z 403 ± 0.3 for Ag, the product ion at m/z 346 ± 0.2 , derived from the precursor ion at m/z 493 ± 0.3 for Au using a calibration curve made up with spiked matrix samples at different concentrations, respectively.

Results and discussion

ESI-MS and ESI-MS-MS spectra

Figure 1 shows an ESI-MS of 5 µl of IAA extracted from 0.1 M NaNO₃ solution containing Cu, Ag and Au at 100 µg l⁻¹ each and measured at the desolvation temperature of 280 °C. The relative peak heights for Cu(DDC)₂⁺ at m/z 359, 360, 361, 362, 363 observed in Fig. 1 were 100.0, 16.3, 62.8, 10.0, 11.6, 0.0 and they agreed well with the theoretical values, 100.0, 15.2, 63.4, 9.4, 9.8, 1.3, calculated for Cu(C₄H₁₀NCS₂)₂⁺ using the Isotope Distribution Calculator of Applied Biosystems, Japan, respectively. The shape of the cluster was helpful for the identification of the complex. The highest peak at m/z 359 corresponding to

$^{63}\text{Cu}(^{12}\text{C}_4^1\text{H}_{10}^{14}\text{N}^{12}\text{C}^{32}\text{S}_2)_2^+$ was selected as the precursor ion for quantification of Cu.

The relative peak heights for $\text{Ag}(\text{DDC})_2^+$ at m/z 403, 404, 405, 406, 407, 408, 409 observed in Fig. 1 were 88.7, 9.4, 100.0, 13.2, 15.6, 2.0, 1.3 and those calculated were 89.5, 13.6, 100.0, 15.0, 16.9, 2.3, 1.2, respectively. The secondly highest peak at m/z 403 corresponding to $^{107}\text{Ag}(^{12}\text{C}_4^1\text{H}_{10}^{14}\text{N}^{12}\text{C}^{32}\text{S}_2)_2^+$ was selected as the precursor ion because the highest peak at m/z 405 consisted of several isobars such as $^{109}\text{Ag}(^{12}\text{C}_4^1\text{H}_{10}^{14}\text{N}^{12}\text{C}^{32}\text{S}_2)_2^+$, $^{107}\text{Ag}(^{12}\text{C}_4^1\text{H}_9^2\text{H}^{14}\text{N}^{12}\text{C}^{32}\text{S}_2)_2^+$, $^{107}\text{Ag}(^{12}\text{C}_4^1\text{H}_{10}^{15}\text{N}^{12}\text{C}^{32}\text{S}_2)_2^+$, $^{107}\text{Ag}(^{12}\text{C}_4^1\text{H}_{10}^{14}\text{N}^{12}\text{C}^{32}\text{S}_2)(^{12}\text{C}_4^1\text{H}_{10}^{14}\text{N}^{12}\text{C}^{32}\text{S}^{34}\text{S})^+$ and others, and hence they gave several product ions containing Ag.

The relative peak heights for $\text{Au}(\text{DDC})_2^+$ at m/z 493, 494, 495, 496, 497 403, 404 observed in Fig. 1 b were 100.0, 16.7, 22.8, 3.4, 2.2 and those calculated were 100.0, 15.2, 18.8, 2.6, 1.4, respectively. The highest peak at m/z 493 corresponding to $^{197}\text{Au}(^{12}\text{C}_4^1\text{H}_{10}^{14}\text{N}^{12}\text{C}^{32}\text{S}_2)_2^+$ was selected as the precursor ion for quantification of Au.

Figures 2 a, b and c show the product ion spectra at collision voltage of 18 V from the precursor ions $^{63}\text{Cu}(^{12}\text{C}_4^1\text{H}_{10}^{14}\text{N}^{12}\text{C}^{32}\text{S}_2)_2$, $^{107}\text{Ag}(^{12}\text{C}_4^1\text{H}_{10}^{14}\text{N}^{12}\text{C}^{32}\text{S}_2)_2$ and $^{197}\text{Au}(^{12}\text{C}_4^1\text{H}_{10}^{14}\text{N}^{12}\text{C}^{32}\text{S}_2)_2$, respectively. Here, peaks at m/z 116, 88 and 72 were the degradation products from DDC with m/z 148. That is, the peaks at m/z 116, 88 and 72 corresponded to $\text{C}_4\text{H}_{10}\text{NCS}$, $\text{H}(\text{C}_2\text{H}_5)\text{NCS}$ and CH_2NCS , respectively. The product ions at m/z 212, m/z 256 and m/z 346 corresponded to $\text{Cu}(\text{DDCH})^+$ {i.e., $^{63}\text{Cu}^{12}\text{C}_4^1\text{H}_{11}^{14}\text{N}^{12}\text{C}^{32}\text{S}_2$ }, $\text{Ag}(\text{DDCH})^+$ {i.e., $^{107}\text{Ag}^{12}\text{C}_4^1\text{H}_{11}^{14}\text{N}^{12}\text{C}^{32}\text{S}_2$ } and $\text{Au}(\text{DDCH})^+$ {i.e., $^{197}\text{Au}^{12}\text{C}_4^1\text{H}_{11}^{14}\text{N}^{12}\text{C}^{32}\text{S}_2$ }, respectively. The collision voltage of 18 V was selected for the quantification of Cu, Ag and Au because all the three product ions containing Cu, Ag and Au gave the highest peak at the voltage, respectively.

In the negative ion mode Cu, Ag and Au did not show any peaks corresponding to their DDC complexes under the same treatment as in the positive ion mode.

Comparison of pH at complex formation, acids and solvents

The effect of pH on the DDC complex formation was examined at pH 1 – 10 shifted successively by 1 using 1 M HNO₃ and 1 M NaOH, and the maximum complex formation were obtained in the solutions at pH 2 – 9 for Cu, Ag and Au, respectively. Therefore the pH of the wet-ashed tissue solutions and standard solutions was adjusted to around 7.

The MS (-MS) signals of Cu, Ag and Au complexes extracted with IAA from 0.1 M NaNO₃ solutions were very low but they increased more than 100 times the originals after the acidification with 0.3 M oxalic acid. The MS-MS signals after the acidification with several acids at 0.3 M were compared using IAA as an extractor because the maximum signals were observed at 0.2 – 0.8 M in case of oxalic acid. The relative signal intensities of IAA extracts after the acidification with oxalic acid, citric acid, H₂SO₄, HCl and HNO₃ were 100, 70, 60, 10 and 5 for Cu, 100, 50, 60, 40 and 100 for Ag and 100, 80, 20, 80 and 100 for Au, respectively.

The extraction efficiencies of several solvents were compared after the acidification of the extracts with 0.3 M oxalic acid. The relative extraction efficiencies of IAA, octanol, cyclohexanol, chloroform and acetonitrile were 100, 80, 50, 0 and 0 for Cu, 100, 100, 100, 20 and 0 for Ag and 100, 100, 60, 100 and 2 for Au, respectively. Therefore, IAA and 0.3 M oxalic acid were selected.

Validations of the present method

LOD and limit of quantification (LOQ) of Ag and Au were determined using tissue spiked with their standard solutions, wet-ashed and diluted to 100-fold (v/w), respectively. That is, the blanks are shown in Figs. 3 d and g; LODs giving the peak three times the blank are shown in e and h; and LOQs giving the peak ten times the blank are shown in f and i, respectively. Determination of LOD and LOQ of Cu in tissue wet-ashed and diluted to 100-fold was difficult because kidney contains a large amount of Cu and hence the wet-ashed solution should be diluted to 1,000-fold (v/w) with 0.1 M NaNO₃ for the quantification. Cu standard solutions in 0.1 M NaNO₃ were used for the determination of the blank, LOD and LOQ as shown in Figs. 3 a, b and c, respectively, because blank, LOD and LOQ of Ag and Au standard solutions in 0.1 M NaNO₃ were the same within 5 % error as those in Figs. 3 d – i, respectively. The LODs for Cu, Ag and Au were 50, 30 and 100 ng g⁻¹ in tissue, and that corresponded to 0.5, 0.3 and 1 µg l⁻¹ in 0.1 M NaNO₃ solution and in wet-ashed tissue solution diluted to 100-fold, respectively. The LOQs for Cu, Ag and Au were 0.2, 0.1 and 0.3 µg g⁻¹ in tissue that corresponded to 2, 1 and 3 µg l⁻¹ in 0.1 M NaNO₃ solution and in wet-ashed tissue solution diluted to 100-fold, respectively.

The calibration curve for Cu at 0, 2, 10 and 100 µg l⁻¹ ($n = 3$ for each concentration) in 0.1 M NaNO₃ solution was $y = 999.1 x + 238$ ($\gamma = 0.999$). Here, y is the integrated peak area of the product ion of Cu-complex and x is the concentration of Cu (in µg l⁻¹). The calibration curve for Ag at 0, 1, 10 and 100 µg l⁻¹ ($n = 3$) in wet-ashed tissue solution was $y = 401.6 x + 58.8$ ($\gamma = 0.999$), and the curve for Au at 0, 3, 10 and 100 µg l⁻¹ ($n = 3$) in wet-ashed tissue solution was $y = 150.0 x + 46.8$ ($\gamma = 0.998$), respectively.

Intra-day and inter-day variations were examined for Cu in 0.1 M NaNO₃ and for Ag and Au in wet-ashed tissue solution at the respective LOQ (i.e., 2, 1 or 3

$\mu\text{g l}^{-1}$), 10 and 100 $\mu\text{g l}^{-1}$ ($n = 3$) as listed in Table 1 a, b and c, respectively.

Coefficients of variation and deviations from the nominal value were lower than 11 % in all solutions.

Cu levels in rat kidneys

Kidney is one of the most important organs in hypertension as evidenced by the fact that the blood pressure of the recipient of kidney followed that of the donor in humans and rats [18]. In the present work Cu level in the kidney of SHR-od [17] was compared with that of normotensive ODS since these rats of the two strains cannot synthesize ascorbic acid like humans [17], and hence SHR-od is more suitable as a model animal than the other SHR that can synthesize ascorbic acid [4,5].

Figure 4 shows a product ion after flow-injection of 1 μl IAA extracted from kidneys of six ODS rats and six SHR-od rats at 6 weeks old (a) and those of four ODS rats and four SHR-od rats at 30 weeks old (b). Although tissues contained several metals at high concentrations such as Fe and Zn that reacted with DDC, the signals of them did not interfere with those of Cu, Ag and Au. This fact was also confirmed by the observation that the complexes of metal standards such as Fe, Zn, Mn, Ti, V, Cr, Cd and Zr did not show any signals at those of Cu, Ag and Au, respectively. Cu levels in the kidneys of ODS and SHR-od rats at 6 weeks old were 10.2 ± 1.7 and $18.4 \pm 1.2 \mu\text{g g}^{-1}$, respectively, indicating the increase by 80 %, and those of ODS and SHR-od rats at 30 weeks old were 12.4 ± 0.9 and $62.8 \pm 5.4 \mu\text{g g}^{-1}$, respectively, indicating the increase by 406 %. The Cu levels in the kidney of SHR of Okamoto strain was reported to increase by only 29 % at 12 weeks old [5]. Therefore, age and strain may influence the accumulation of Cu in kidney. Cu is known to act as a catalyst in the formation of reactive oxygen species, leading to

oxidative stress and destructive lipid peroxidation damage. On the contrary, ascorbic acid is a typical reducing agent in biological systems, and an ascorbic acid treatment was reported to lower the blood pressure of hypertensive patients [7]. Most rats including SHR of Okamoto strain are inappropriate when the effects of oxidative stress on human hypertension are studied because these rats can synthesize ascorbic acid.

The LOD of Cu in tissue was 8 ng g^{-1} by ICP-MS [6] and that was 50 ng g^{-1} by the present ESI-MS-MS. Although the ICP-MS is a very sensitive method, only single MS can be performed by the method because it detects atomic ion whereas the present method can confirm the analyte twice by MS-MS. Although quantification of Cu by ESI-MS was reported previously using Cu-EDTA complex [16], the method was not sensitive as indicated by its LOD of $63 \text{ } \mu\text{g l}^{-1}$ in aqueous solution whereas LOD of the present ESI-MS-MS was $0.5 \text{ } \mu\text{g l}^{-1}$ in 0.1 M NaNO_3 solution. This may be due to the low efficiency of ESI in aqueous solution compared with the high efficiency of ESI in a polar organic solvent [14].

Conclusions

A method was proposed for the rapid and decisive determination of coinage metals by ESI-MS (-MS). The method was applied to the quantification of Cu in the kidneys of spontaneously hypertensive osteogenic disorder rats. Without ICP-MS instruments, present method enables the determination of some metal ions simultaneously when ESI-MS (-MS) instruments are available.

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Figure captions

Fig. 1 Mass spectrum of isoamyl alcohol that was extracted from 0.1 M NaNO₃ solution containing Cu, Ag and Au at 100 µg l⁻¹ each.

Fig. 2 Product ion spectra at collision voltage of 18 V from the precursor ions ⁶³Cu(¹²C₄¹H₁₀¹⁴N¹²C³²S₂)₂ shown in (a), ¹⁰⁷Ag(¹²C₄¹H₁₀¹⁴N¹²C³²S₂)₂ shown in (b) and ¹⁹⁷Au(¹²C₄¹H₁₀¹⁴N¹²C³²S₂)₂ shown in (c), respectively. The concentrations of Cu, Ag and Au were 100 µg l⁻¹, respectively.

Fig. 3 Product ion monitored at *m/z* 212 that was extracted from 0.1 M NaNO₃ solution (a), from 0.1 M NaNO₃ solution containing Cu at 0.5 µg l⁻¹ (b), and from 0.1 M NaNO₃ solution containing Cu at 2 µg l⁻¹ (c), respectively; product ion monitored at *m/z* 256 that was extracted from wet-ashed and diluted kidney solution (d), from wet-ashed and diluted kidney solution containing Ag at 0.3 µg l⁻¹ (e), and from wet-ashed and diluted kidney solution containing Ag at 1 µg l⁻¹ (f), respectively; and product ion monitored at *m/z* 345 that was extracted from wet-ashed and diluted kidney solution (h), from wet-ashed and diluted kidney solution containing Au at 1 µg l⁻¹ (i), and from wet-ashed and diluted kidney solution containing Au at 3 µg l⁻¹ (j), respectively.

Fig. 4 Product ion monitored at *m/z* 212 derived from six ODS rats and six

SHR-od rats at 6 weeks old, respectively, from left to right (a), and that derived from four ODS rats and four SHR-od rats at 30 weeks old, respectively, from left to right in duplicate (b).

Table 1 Intraday and interday accuracy and precision for Cu ($n = 3$)

Cu spiked ($\mu\text{g/l}$)	Intraday		Interday	
	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
2.00	92.0	6.0	99.0	7.0
10.0	102.0	5.4	102.9	8.7
100.	106.5	10.9	104.1	8.0

Table 2 Intraday and interday accuracy and precision for Ag ($n = 3$)

Ag spiked ($\mu\text{g/l}$)	Intraday		Interday	
	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
1.00	109.0	6.7	110.0	10.7
10.0	93.2	3.3	105.3	3.6
100.	97.8	4.7	102.8	6.1

Table 3 Intraday and interday accuracy and precision for Au ($n = 3$)

Au spiked ($\mu\text{g/l}$)	Intraday		Interday	
	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
3.00	106.0	7.3	92.7	4.5
10.0	91.9	6.8	104.4	3.8
100.	105.7	0.3	93.0	5.0

Relative intensity