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Determination of Trace Levels of Elements in Urine by Inductively Coupled Plasma Mass Spectrometry

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A biological specimen, urine, was prepared by the most simple sample preparation method, direct dilution, and determination of seven elements in the urine samples, Fe, Cu, Zn, Se, As, Cd, and Pb, was investigated by inductively coupled plasma mass spectrometry (ICP-MS). In addition, acid-decomposed samples were similarly investigated. Regarding the matrix effect of concomitant components, as the sodium hydrochloride concentration increased, the observed ion intensity of the measured element linearly decreased. Matrix interference by sodium hydrochloride could be fairly corrected by addition of internal standard elements excluding determination of Fe and Zn. On comparison of the measured values of samples prepared by direct dilution and acid-decomposition samples, the values were slightly lower in the samples prepared by direct dilution for many elements. For Zn and As, the analytical results of the human urine standard reference material were well consistent with the certified values.

Key words —— inductively coupled plasma mass spectrometry, urine, trace levels of elements, direct dilution, matrix effect

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INTRODUCTION

Trace elements have recently been attracting attention in wide scientific areas related to human health such as clinical analysis, nutritional diagnosis, and environmental analysis. In these areas related to human health, trace element analysis is the basis of all studies coping with problems of trace elements, and the importance of microanalysis of biological samples has been increasing.[1,2] For analytical methods necessary for analysis of trace elements in the body, it is important to measure many elements at the same time and obtain accurate analytical values. Since inductively coupled plasma mass spectrometry (ICP-MS) is able to determine many elements at high sensitivity and precision in a wide dynamic range, it is appropriate for determination of many elements present in sample matrices in a wide range of concentration.[3–5] However, in biological specimens for trace elemental analysis such as blood and urine, the concentrations of elements to be analyzed are generally low, while the concentrations of major component elements such as alkali metals, alkaline earth metals, carbon, nitrogen, and chlorine are high. As described above, ICP-MS is able to measure rapidly at high sensitivity, but many problems remain to be solved, for example, when samples containing salts at a high concentration are analyzed, the signal intensity of the elements of interest is suppressed, and correction by the internal standard method may not be effective depending on the concomitant salt concentration. In this study, a biological specimen, urine, was prepared by direct dilution, the most simple sample preparation method, and determination of seven elements, Fe, Cu, Zn, Se, As, Cd, and Pb, in the urine samples was investigated. In addition, determination in acid-decomposed samples was also investigated

MATERIALS AND METHODS

Instruments and Measurement Conditions —— ICP-MS used in the experiment was X7 of Thermo Electron Co. (Winsford, U.K.). The measured mass of each element was selected in consideration of sensitivity and mutual interference of concomitant elements after confirmation of the presence or absence of interference of the solution containing a single element, solution corresponding to the matrix blank test, and sample solution. The measurement conditions of ICP-MS are shown in Table 1.

Table 1. Instruments and Analytical Parameters for ICP-MS

Inductively coupled plasma	
ICP Source	1200 W, 27.12 MHz
Coolant gas	13 l/min
Aux. gas	0.7 l/min
Nebulizer gas	0.92 l/min
CCT gas	9 ml/min (He-H_2 8%)
Nebulizer	Micromist Nebulizer
Sample introduction	Peristaltic pump
Chamber	Peltier Cooled impact bead chamber
ICP-MS interface	
Sample uptake rate	1.0 ml/min
Sampling cone	Nickel, 1.0 mm orifice
Skimmer cone	Nickel, 0.7 mm orifice
Data acquisition parameters	
Dwell time	10 ms/ch
Channels/mass	3
Sweep	62

Since the instrument used for analysis is able to select the use of collision gas for each element, spike recovery tests of urine samples using the standard mode and CCT mode using collision gas were compared to determine the measurement mode for each element. Fe and Se were measured using the CCT mode because of the interference of many argon plasma-derived polyatomic ions. The mass numbers of the elements and internal standard elements in each mode are shown in Table 2. Microwave-heating digestion equipment for acid-decomposition was used a MARS5 of CEM Corporation (North Carolina, U.S.A.).

Reagents and Samples —— Pure water used for preparation of samples and reagents was purified using Mili Q (Millipore, Japan). For sample dilution and decomposition, nitric acid for precise analysis (Ultra Pure, Kanto Kagaku, Tokyo, Japan) and hydrogen peroxide for harmful metal analysis (Kanto Kagaku) were used. For the original standard solution of metal elements (1 mg/ml), standard metal solutions for atomic absorption spectrometry (Kanto Kagaku) were used. The standard multiple element solution for calibration curve was prepared by mixing the original standard solutions to adjust the concentrations of Fe, Cu, Zn, Se, As, Cd, and Pb to 100, 200, 400, 10, 10, 5, and 10 μg/ml, respectively, followed by 1000-, 2000-, 10000-, 20000- and 100000-fold dilution with 1% nitric acid. The urine samples used for analysis were prepared by filtration of urine collected for one day from adult males. For the samples prepared by direct dilution, the urine samples were 10-fold diluted with 1% nitric acid, and used as the measurement samples. For preparation of acid-decomposed samples, the microwave-heating digestion method was used. In decomposition, 4 ml of the urine sample, 4 ml of nitric acid, and 2 ml of hydrogen peroxide were added to a Teflon inner tube container, and this decomposition container was placed in a polypropylene outer tube container. The tube was capped tightly and set in the instrument, and subjected to microwave-heating digestion using the equipped program (Stage 1; Power 300 W, Ramp time 4 min, Temp. 140°C, Hold Time 2 min. Stage 2; Power 300 W, Ramp time 2 min, Temp. 180°C, Hold Time 2 min. Stage 3; Power 300 W, Ramp time 2 min, Temp. 200°C, Hold Time 4 min.) input with decomposition conditions for urine samples. After decomposition, the sample was diluted with 1% nitric acid, and adjusted to 50 ml.

RESULTS AND DISCUSSION

Calibration Curve

For calibration curves, the standard solution mixture was diluted step-wise with 1% nitric acid, and solutions for 4 points including the blank test solution were prepared. The concentration range of the Calibration curve of except for the blank solution is 1 to 10 ng/ml for Fe, 2 to 20 ng/ml for Cu, 4 to 40 ng/ml for Zn, 1 to 10 ng/ml for Se, As, Pb and 0.5 to 5 ng/ml for Cd. In preparation of the calibration curve, 1.8 sec integration was repeated three times at each concentration. Repeatability slightly varied, 0.05–2.7%, among the elements and its concentrations. When the linear expression was approximate within the concentration range, the correlation coefficient was not less than 0.999 in all elements, showing a good linear correlation.

Detection and Determination Limits

Table 2 shows the detection limit and determination limit of the elements. The detection limit was defined as the element concentration at which the signal intensity corresponds to 3-fold of the standard deviation of the blank test values obtained by 1.8 sec integration repeated 10 times using 1% nitric acid solution as the test solution. The determination limit was set to 10-fold of the standard deviation. The determination limit of Se was slightly high, 0.17 ng/ml, but those of the other elements

Table 2. Detection Limit and Determination Limit of Elements and Recoveries of Elements in Preparation added before and after Decomposition (%)

	^{56}Fe	^{65}Cu	^{66}Zn	^{75}As	^{78}Se	^{111}Cd	^{208}Pb
Internal Standard	Rh	Y	Y	Y	Rh	In	Tl
Mode	CCT	Standard	Standard	Standard	CCT	Standard	Standard
detection limits (ng/ml)	0.016	0.019	0.015	0.003	0.052	0.0003	0.007
determination limit (ng/ml)	0.052	0.062	0.049	0.011	0.17	0.001	0.023
Human urine (ng/ml)	24	15	560	110	51	0.59	1.6
added ng/4 ml	250	50	1000	25	25	12.5	25
Recoveries (%)							
dilution	94	94	87	124	89	93	93
add before decomposition	94	96	72	100	93	81	90
add after decomposition	113	111	89	134	123	81	100
(without internal standard correction)	57	70	55	73	40	51	42

were within a range of 0.001–0.06 ng/ml. The determination limit of Cd, which may be present at the lowest concentration in urine samples, was 0.001 ng/ml, and 50- to 100-fold dilution was possible when the matrix is absent.

Interference by Sodium Chloride

When samples with complex matrices such as urine samples are analyzed by ICP-MS, investigation of matrix-derived error factors is necessary.[6–8] Oliver *et al.*[9] and Tan *et al.*[10] reported the matrix effect in ICP-MS in detail, and it was found that errors resulted from the mutual relationship between the atomic numbers of analyzed and matrix elements and ionization potential. In urine samples, relatively light elements such as C, P, S, and Cl may serve as matrices. Since these elements have high ionization potentials, their matrix effects may not be strong. However, alkali metals and alkaline earth metals in urine samples may influence the analysis, and we investigated changes in the ion intensity caused by sodium chloride (NaCl) present in urine samples at the highest concentration. Since urinary NaCl is about 1%, 1% nitric acid solution containing 0.01%, 0.01%, or 0.1% NaCl (1/1000, 1/100, or 1/10 of the urinary NaCl concentration) was prepared, and 1 l was mixed with 10 μl mixed standard solution for calibration (Fe 100 μg/ml, Cu 200 μg/ml, Zn 400 μg/ml, Se 10 μg/ml, As 10 μg/ml, Cd 5 μg/ml, Pb 10 μg/ml). The influences of NaCl on the ionic intensity of each element were measured. As shown in Fig. 1A, 0.001% NaCl had no influence on the ion signal of most elements, but 0.1% and 0.01% NaCl caused marked linear decreases in the ion intensity, although the degree varied among the elements. To investigate the salt concentration that can be corrected by the internal standard method, the counts of the elements corrected by the internal standards are shown in Fig. 1B. The recovery was about 100% excluding all elements in 0.001% NaCl and Fe and As in 0.01% NaCl, showing that correction with the internal standard was effective. Even in the presence of 0.1% NaCl, 80–120% recovery could be obtained by correction for all elements.

Recovery

Twenty μl of the standard solution mixture for calibration curve was added to 4 ml of urine samples, and the solution was subjected to microwave-heating digestion/acid decomposition, followed by 5-fold dilution. Separately, acid-decomposed samples were combined with 20 μl of the standard solution mixture for calibration curve, followed by 5-fold dilution. The results are shown in Table 2. The recoveries of the elements in the above two preparations were investigated, and there was almost no difference between the recoveries of the elements added before and after decomposition, suggesting that the elements were not lost by the decomposition. In the samples to which the standards were added after decomposition, about 40% of sensitivity for Se and Pb was decreased without correction with the internal standard, and 51–73% of sensitivity was decreased for the other elements. However, when corrected with the internal standard, the recoveries were within the range of 89–113% excluding the recoveries of Cd, Se, and As, which were 81.1, 123, and 134%, respectively, suggesting that most elements can be measured after 10-fold direct dilution. Acid decomposition degrades protein, but inorganic salts

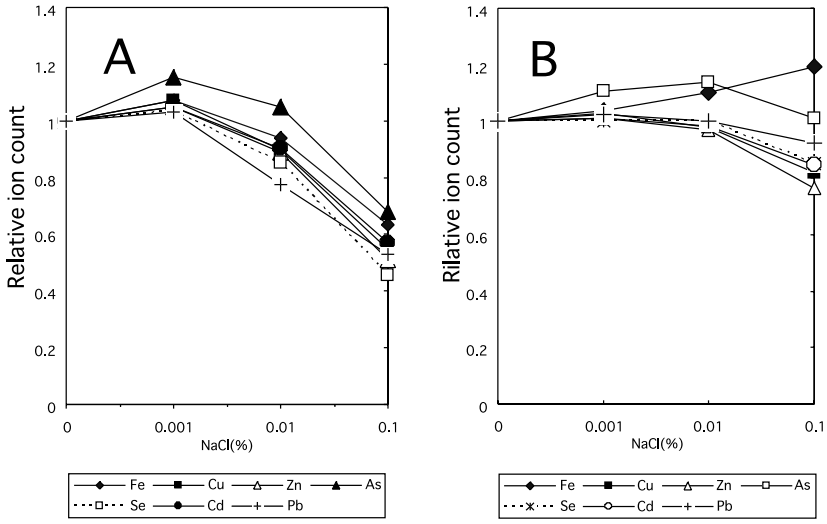

Fig. 1. The Effect of NaCl Concentration on each Analyte Signal Intensity [without Internal Standard Correction (A) and after Internal Standard Correction(B)]

Table 3. Analysis of Human Urine Standard Reference Materials

(ng/ml)

	^{56}Fe	^{65}Cu	^{66}Zn	^{75}As	^{78}Se	^{111}Cd	^{208}Pb
Mode	CCT	Standard	Standard	Standard	CCT	Standard	Standard
CRM 1							
dilution	11.5	10.8	482	147	95.2	0.845	1.37
decomposition	34.4	10.3	402	118	67.0	0.827	1.96
Analytical values		10	620 ± 50	137 ± 11	59 ± 5		1.1
CRM 2							
dilution	15.6	27.3	236	87.4	36.3	0.202	0.807
decomposition	27.7	27.8	206	69.5	29.2	0.187	1.70
Analytical values				83	14.4	0.11	0.65
				76–90	12.1–16.7	0.10–0.12	0.53–0.77

were present at the same concentrations as in the 10-fold diluted urine. In addition, it was difficult to completely match the final nitric acid concentration remaining in the digested solution with the concentration in the standard solution.

Analysis of Human Urine Standard Reference Materials

Using the human urine standard reference materials, accuracy of the measurements of the directly diluted and acid-decomposed samples was investigated. The results are shown in Table 3. The human urine standard reference materials used were NIES CRM. No. 18 Human Urine (CRM1) and Seronorm Trace Elements Urine LOTNO2524 (CRM2, Blank) by National Institute of Environmental Science. In CRM1, the values of Zn, As, and Se were certified, and the values of Cu and Pb were references. The value of Zn was about 80% of the certified value, and those of Se and As were about 90%. As for CRM2, the values of As, Se, Cd, and Pd were certified. The value of As was close to the certified value, but the values of Se, Cd, and Pb were 203, 170, and 124% of the certified values, respectively. Since the concentrations in CRM2 were 1/2–1/5 of those in CRM1, and the concentration of the main component of the matrix, NaCl, was about 0.1%, considering the lower limit of determination of the instrument, precision may have been lower for CRM2 than CRM1. On comparison of analytical values of the human urine standard reference materials 10-fold diluted with 1% nitric acid solution and the analytical values of acid-decomposed samples, the measured values of Fe and Pb tended to be about 2-fold higher in acid-decomposed samples. However, the values of the other elements were slightly lower in

acid-decomposed samples. This tendency was also observed in the recovery test, suggesting that the differences were caused by changes in matrix composition after acid decomposition. Based on these findings, the analytical values can be rapidly and accurately obtained by ICP-MS using the simple pretreatment of direct dilution of urine and internal standard, although investigation of the internal standard is necessary for some elements. ICP-MS may be very useful for trace elemental analysis of other biological samples in addition to urine because measurement sensitivity is very high and many elements can be analyzed simultaneously.

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