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Determination of Mercury in Candidate Oyster Tissue CRM using Isotope Dilution-inductively Coupled Plasma Mass Spectrometry

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Isotope dilution-inductively coupled plasma mass spectrometry (ID-ICP/MS) was applied to determine mercury in oyster tissue. Microwave digestion method using $HNO_3/H_2O_2/HF$ media for the dissolution of solid sample was studied. The procedure for accurate determination of total mercury in oyster tissue sample by ID-ICP/MS is described. For the method validation, total Hg concentration in standard mussel tissue reference material (NIST SRM 2976) was determined by ID-ICP/MS after addition of 202Hg to CRM followed by acid decomposition of the spiked sample. This method was applied to the determination of Hg in candidate oyster CRM prepared by KRISS (Korea Research Institute of Standards and Science).

Key words: Hg, Isotope dilution-inductively coupled plasma mass spectrometry, NIST SRM 2976, Mussel tissue CRM.

1. Introduction

Mercury is a serious environmental toxicant and there are several reviews on different aspects of mercury toxicology.[1-3] Exposure to elemental mercury vapor may cause effects on the central nervous system, with a change of personality and tremor.[4] Also, mercury may affect the kidney, this may occur as tubular and/or glomerule malfunction.[5,6] To control the amount of mercury polluty of our environment, mercury has to be monitored in all areas of modern life. The main source of human intake of mercury contaminants originates from methyl-mercury in fish and fishery products. Fish and marine. mammals and birds preying on fish may contain considerable amounts of mercury.[7] The mercury in fish originates from mercury in water, which is methylated, and accumulates in the fish as such. Mercury in environmental and biological samples is commonly determined by atomic absorption spectrometry (AAS) or inductively coupled plasma mass spectrometry (ICP-MS). These analytical techniques are suitable for routine analysis because of their high sensitivity and reasonable accuracy and precision.

In general, it is well known that isotope dilution-inductively coupled plasma/mass spectrometry (ID-ICP/MS) has a high potential for routine analysis of trace elements if the accuracy of results is of predominant analytical importance. Therefore, ID-ICP/MS has frequently been employed in the certification of element contents in certified reference materials (CRMs).[8-11] Although ID-ICP/MS method has been widely used for the determination of trace element in various matrices, only a few applications have been reported for the determination of Hg. The problem in Hg analysis is known that the memory effect increases the blank counts and worsens the analytical performance of ICP-MS.[12] Another problem is the possibility of Hg loss during sample decomposition procedure due to its volatility.

This paper describes a method to determine Hg by IDMS using quadrupole-inductively coupled plasma mass spectrometry. Microwave digestion with $HNO_3/H_2O_2/HF$ media was applied to the dissolution of solid sample. For the method validation, total Hg concen-

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tration in mussel tissue SRM (NIST SRM 2976) was determined by ID-ICP/MS after addition of ^{202}Hg to samples followed by the acid decomposition of the spiked samples. Finally the present method was applied to the determination of Hg in candidate oyster CRM prepared by KRISS.

2. Experimental

2.1. Instrument

ICP-MS (ELAN 6100 DRC-ICP-MS (Perkin-Elemer SCIEX, Concord, ON, Canada)) equipped with was used for the study. Fig. 1 shows PFA spray chamber used in this experiment. This spray chamber and nebulizer were designed and made by our laboratory. All data was acquired in normal mode conditions without reaction cell gas. The material of sampling cone and skimmer is platinum. Typical operating conditions are summarized in Table 1.

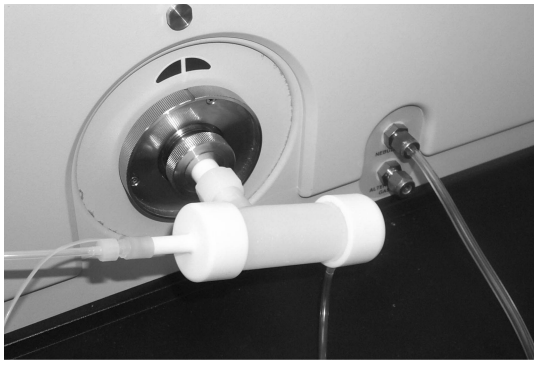

Fig. 1. PFA Spray Chamber and Polyethylene Nebulizer this experiment for HF media sample (This was r KRISS Lab).

Table 1. Operating conditions for ICP-MS

ICP-MS instrument	Perkin-Elmer SCIEX Elan 6100DRC
Nebulizer Gas Flow	1.20 L/min
Auxiliary Gas Flow	1.20 L/min
Plasma Gas Flow	17.00 L/min
Lens Voltage	10 V
ICP RF Power	1350 W
Analog Stage Voltage	-2550 V
Pulse Stage Voltage	1400 V
Discriminator Threshold	80.00
Dwell Time	100 ms
Sweeps	30
Replicates	5

2.2. Reagents

A stock standard solution of Hg was prepared by dissolution of pure metals (99.999995%, Alfa, Catalogue # 10634). Working primary standard solutions were prepared by serial dilutions of the stock standard solution. Electronic grade HNO_3 (Dong Woo Pure Chemicals, IkSan, Korea) was used after further purification by sub-boiling distillation. The ^{202}Hg enriched spike isotope (IRMM 640) and isotopic standards for mass bias correction (IRMM 639) were obtained from IRMM (Retieseweg, B-2440 GEEL, Belgium). These isotopes were dissolved in dilute HNO_3 and stored in clean Teflon FEP bottles. Low-density polyethylene (LDPE) containers were utilized. These bottles were cleaned by immersing the vessels in 20% HNO_3 for 2 days, and washed successively with de-ionized water. The composition of enriched isotope (IRMM 640) and isotopic standard (IRMM 639), and potential interferences of Hg isotopes in ICP-MS are shown in Table 2.

2.3. Sample decomposition procedure by microwave digestion method

The oyster sample, typically 500 mg, was decomposed by microwave digestion method, after adding an appropriate amount of the ^{202}Hg spike solution. On the basis of preliminary investigations and the certified Hg contents of the reference material NIST 2976 and KRISS candidate oyster CRM, the optimum blend ratio was calculated as a compromise between lowest error magnification factor and sufficient counting rate.[13] In this case, the calculated optimum $^{200}Hg/^{202}Hg$ ratio was in the range 0.06-0.3, with a corresponding error magnification factor of approximately 1.1-1.6. The sample was weighted accurately into the Teflon PTFE microwave vessel together with the ^{202}Hg spike solution. The spike addition was varied with regard to an optimized value of 0.06-0.3 for the amount of mercury from sample to spike in the isotope diluted sample. Concentrated HNO_3 (5 mL) and H_2O_2 (1 mL)

Table 2. Potential interferences of Hg isotopes and isotopic compositions of natural mercury, isotopic standard and 202Hg enriched isotope used for IDMS method

Isotope	Natural abundances (%)[a)]	Amount fraction of ^{202}Hg enriched isotope (IRMM 640)[b)]	Amount fraction of IRMM 640 Isotopic standard[b)]	Potential interferences
^{196}Hg	0.15	0.001767(37)	0.1489(13)	Pt, TaO, HfO, WO
^{198}Hg	9.97	0.0608(11)	9.900(52)	Pt, TaO, HfO, WO
^{199}Hg	16.87	0.1566(16)	16.826(64)	TaO, WO
^{200}Hg	23.10	0.5371(33)	23.073(58)	WO
^{201}Hg	13.18	1.3042(50)	13.213(25)	WO
^{202}Hg	29.86	97.6859(68)	29.944(53)	WO
^{204}Hg	6.87	0.2535(21)	6.895(30)	Pb, WO

[a)] : The relative isotopic abundances are from the table of Isotopic Compositions of the Elements 1989, J.R.De Laeter et al., Pure Appl. Chem, 63, 991 (1991).
[b)]: The values are taken from the certificates of supplier (IRMM, GEEL, Belgium). Numbers in parentheses indicate the uncertainty of the reported value.

were added as a sample decomposition media. It should be noted that overnight digestion at room temperature in the closed vessel was performed before pressurized microwave digestion to ensure enough isotope equilibrium. Microwave heating was then performed on samples as presented Fig. 2.

2.4. Dry mass correction

The oyster and mussel tissue sample absorbs ambient moisture at typical laboratory temperature and humidity conditions. Therefore, the sample bottle was opened immediately before weighing aliquots for the IDMS blend preparation. For correction of measured values to dry mass, water content measurement was made on a separate portion of the same material with a mass of 0.5 g sample. The sample was dried before weighing for 7 days in a desiccators containing P_2O_5. In this experiment, the content of moisture of KISS candidate oyster CRM was 4.5 ± 0.05%. and the moisture content of NIST SRM 2976 was 5.27 ± 0.20%.

3. Results and Discussion

3.1. Analysis of mussel tissue SRM (NIST 2976)

Several kinds of solutions were prepare for the application of IDMS. These are a primary assay

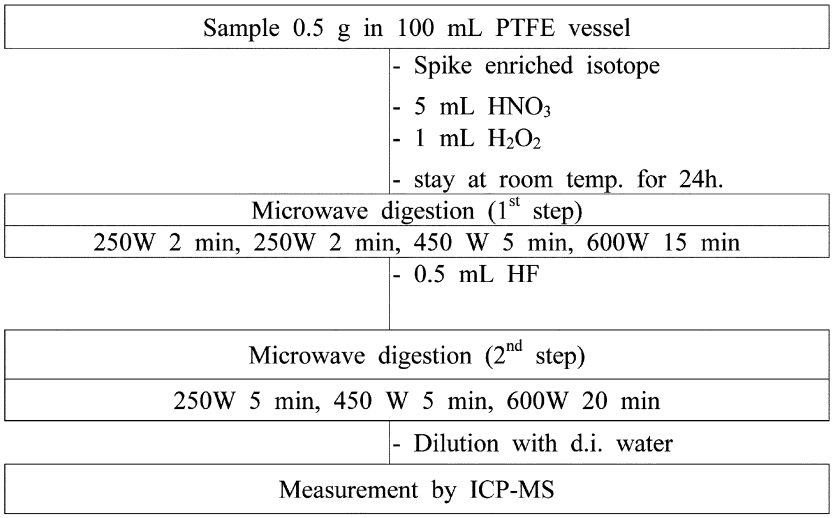

Fig. 2. Microwave digestion procedure for Hg in Oyster Tissue

Table 3. Typical value of intensities and ratios for isotopic standard, spike calibration solutions and sample blends in ICP-MS (The level of concentrations were about 2 μm/kg)

Solutions	isotope	Intensity (cps)	Ratios	RSD% for ratio
Isotopic standard (IRMM-639)	^{199}Hg	5077.5	0.5990	1.54
	^{200}Hg	6875.7	0.8112	1.06
	^{201}Hg	3828.7	0.4517	1.30
	^{202}Hg	8475.3	1	
Spike calibration solutions	^{199}Hg	1250.7	0.2492	1.35
	^{200}Hg	1750.9	0.3489	2.21
	^{201}Hg	951.70	0.1896	2.15
	^{202}Hg	5017.5	1	
Sample Blend (sample + ^{202}Hg)	^{199}Hg	1267.9	0.2642	1.69
	^{200}Hg	1787.7	0.3725	1.21
	^{201}Hg	983.8	0.2050	1.16
	^{202}Hg	4798.2	1	

standard solution, an enriched spike isotopic solution, a spike calibration solutions (the mixed solution of primary assay standard solution and an enriched spike isotopic solution) and sample blend (a mixed solution of sample and enriched spike isotope). The levels of concentration of Hg in sample blends, spike calibration solutions and isotopic standard solution for IDMS were about 2 μg/kg. The typical value of intensities and isotopic ratios for each solution in ICP-MS was shown in Table 3. The choice of reference isotope and enriched isotope considering possible interferences and natural abundances is important for the accurate determination by IDMS. In general, the isotope with the highest abundance in the sample was chosen as a reference isotope if there is no interference from another element.

In the present studies, IDMS was applied to obtain the content of Hg in mussel tissue SRM (NIST 2976) according to the variation of reference isotope shown in Table 4. As shown in this table, the content of Hg in mussel tissue SRM (NIST 2976) was found to be 57.7 ± 4.3 μg/kg, 59.6± 3.3 μg/kg and 59.8± 5.3 μg/kg when the measured isotope ratio was ^{199}Hg/^{202}Hg, ^{200}Hg/^{202}Hg and ^{201}Hg/^{202}Hg, respectively for applying IDMS. The certified value of Hg in this CRM was 61.0 ± 3.6 μg/kg. There is no meaningful difference between the measured and certified values. The results also indicate that the variation of reference isotope used for the determination of Hg does influence the analytical result. These results make it clear that the natural abundance of reference isotope lower, the uncertainty of measurement was higher. We may, therefore, reasonably conclude that it is desirable to choose ^{200}Hg as a reference isotope condisering the highest natural abundance. If the sample contains tungsten (W) component, there is possibility of formation of WO that is interfering molecular ion on the Hg isopotes as shown in Table 2. Howere, in the preliminary test tungsten was not detected in the present sample.

Table 4. Summary of analytical results for Hg in NIST SRM 2976 according to the variation of reference isotope

Reference Isotope/ Enriched isotope	Measured Concentration, μg/kg	Certified value and its uncertainty*, μg/kg
^{199}Hg/^{202}Hg	57.7±4.3	61.0±3.6
^{200}Hg/^{202}Hg	59.6±3.3	
^{201}Hg/^{202}Hg	59.7±5.2	

*Note: This uncertainty value was expressed as a half-width of the 95% confidence interval of the mean.

3.2. Analysis of KRISS candidate oyster reference material

Oyster candidate reference material prepared and certified by Inorganic Analysis Group of KRISS on April 2001.14 This oyster CRM was a freeze-dried and

Table 5. Individual data for Hg mass fraction in KRISS Oyster tissue CRM

No	Sample No	Analytical results (μg/kg)	Standard uncertainty due to the systematic effects (μg/kg)	Degree of freedom (ν)
1	o-5	185.6	2.3	6
2	o-35	182.6	2.3	6
3	o-70	184.4	2.3	6
4	o-100	184.2	2.3	6
5	o-130	180.7	2.3	6
6	o-171	181.1	2.3	6
7	o-210	183.0	2.3	6
8	o-250	181.5	2.3	6
9	o-290	179.4	2.3	6
10	o-327	181.0	2.3	6
Pooled standard deviation and degree of freedom due to the systematic effects			2.3	60
Mean Result		182.4		
Standard deviation of the mean (rel. stdev)		2.0(1.1%)		
Combined standard (u_c, μg/kg)		3.02 (Effective DoF : 40)		
Coverage factor (k)		2.02		
Expanded Uncertainty (U) at 95% confidence limit (μg/kg)		6.1		

ground oyster powder in amber glass vials (polyethylene insert and plastic screw cap; 120 mL). Each contains about 15 g of oyster powder. Among total 330 bottles, 10 bottles were chosen for certification of Hg. The present method described in this paper was applied to the determination of Hg in candidate oyster CRM. The certified value and uncertainty can be found in Table 5. The measured Hg concentration in KRISS oyster CRM was 182.4 ± 6.1 μg/kg. The uncertainty in our value was evaluated according to the ISO/EURACHEM guides.[15,16] The combined relative standard uncertainty (u_c) was found to be 1.66%. When multiplied by the coverage factor k=2, this gives an expanded uncertainty ($U = k \times u_c$) of 3.32%.

4. Conclusions

Despite its possibility of a loss by volatilization and its high memory effect, the content of Hg in a oyster reference material (NIST SRM 2976) and KRISS oyster CRM could be accurately and precisely determined by ID-ICP/MS using quadrupole-type ICP-MS. The HNO_3/H_2O_2/HF mixture was found to be a suitable medium for sample decomposition using a microwave digestion system. As is apparent from the results, mercury concentration in KRISS oyster CRM could be analyzed with a total expanded uncertainty of about 3.3%.

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