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Application News

Inductively Coupled Plasma Mass Spectrometer ICPMS-2050
High Performance Liquid Chromatograph Nexera™ Series

Speciation Analysis of Mercury in Seafood by LC-ICP-MS and Introduction of Autosampler Automatic Dilution Function

Kosuke Naka, Yujing Jiang, Natsuki Iwata

User Benefits

- Speciation analysis of mercury in seafood by LC-ICP-MS system can be performed accurately.
- Using a method package for analyzing mercury species eliminates the need to register analytical conditions, etc.
- The autosampler's auto-dilution function eliminates any manual dilution process, saving time and effort.

■ Introduction

Mercury tends to accumulate through the food chain, and some foods, such as seafood, may contain high levels of mercury. However, mercury exists in various forms, and the toxicity differs depending on the form. Generally, methyl mercury is more toxic than inorganic mercury and other organic mercury. Evaluating the toxicity in food requires not only analysis as total mercury but also by the form of mercury. LC-ICP-MS, which separates mercury by species using HPLC and detects mercury with high sensitivity using ICP-MS, is an analytical method suitable for the speciation analysis of mercury.

In this Application News, speciation analysis of methyl mercury and total mercury in seafood was performed using an LC-ICP-MS system, which connected ICPMS-2040/2050 with Nexera XS inert. Furthermore, we also evaluated the automatic dilution function of the Nexera series autosampler for the speciation analysis of mercury.

■ Sample Preparation

Commercially available tuna seafood was used as the sample.

Pretreatment was performed according to the U.S. Food and Drug Administration (FDA) Elemental Analysis Manual (EAM) 4.8[1)].

(1) Weigh approx. 0.25 g edible portion of tuna seafood. The sample is homogenized for 30 seconds using a bead crushing device to improve the extraction efficiency. (The mercury standard was added to the sample for spike recovery test at this time.)

(2) Add 25 mL extraction solution (aqueous 1 % (w/v) L-cysteine•HCl•H2O) to extraction vials, cap tightly, and shake vigorously by hand.

(3) Heat the extraction vials for 120 min in a water bath at 60 °C. Shake each vial vigorously by hand after 60 minutes of heating and again after 120 minutes.

(4) Remove extraction vials from the water bath and allow cooling to room temperature.

(5) Filter a portion of the extract through a 0.45 µm filter directly into the HPLC autosampler vial.

■ Calibration Standards and Mobile Phase

Diluent

Diluent for standards was prepared by mixing aqueous 1 % (w/v) L-cysteine•HCl•H2O with 8 % (v/v) methanol.

Stock standard solution

1 mg/L inorganic mercury solution was prepared by diluting 1000 mg/L inorganic mercury (commercially available) with aqueous 1 % (w/v) L-cysteine•HCl•H2O. 1 mg/L alkyl mercury solution was prepared by diluting 2 alkylmercury mixture standard solution (Each 10 mg/L for methylmercury and ethyl mercury in toluene solution) (commercially available) with methanol.

The 1 mg/L inorganic mercury solution and the 1 mg/L alkyl mercury solution were mixed and diluted to make each 50 µg/L.

Calibration standards

The calibration standards were prepared by diluting stock standard solution. The concentration of each form of mercury in the calibration standard is 0.5 to 20 µg/L.

Internal standard solution

The internal standard solution was prepared by diluting commercially available Tl standard solution with 1 % (v/v) HNO_3. The concentration of Tl in the internal standard solution was 100 µg/L.

Mobile phase

Methanol and 0.01 mol/L ammonium acetate solution (containing 0.12 % L-cysteine with ammonia solution adjusted to pH 7.5) (8 : 92)

■ Instrument Configuration and Analytical Conditions

Samples were analyzed with the LC-ICP-MS system, which consisted of the ICPMS-2040/2050 connected to a Nexera XS inert (Fig. 1). LabSolutions™ ICPMS TRM software can control the ICPMS-2040/2050 system and Shimadzu LC units. This enables everything from sample injection to chromatogram analysis to be performed via a single software program. The analytical conditions used for analysis were those included in the LC-ICP-MS Method Package for Mercury Speciation Analysis.

Table 1 shows the analytical conditions for HPLC, and Table 2 shows the analytical conditions for ICP-MS.

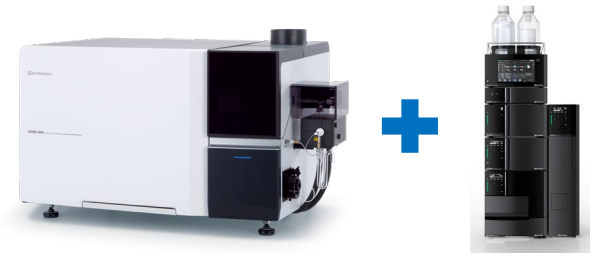

Fig. 1 LC-ICP-MS System

Table 1 Analysis Conditions of HPLC

System	:	Nexera XS inert
Column*1	:	Shim-pack Scepter™ C18-120 [Metal free column] (150 mm × 4.6 mm I.D., 5 µm)
Eluent	:	0.01 mol/L Ammonium acetate (pH 7.5) : Methanol = 92 : 8
Flowrate	:	1 mL/min
Temp. of Column Oven	:	40 °C
Injection Volume	:	50 µL
Rinse Solution	:	Water
Vial*2	:	Shimadzu Vial, LC, 1.5 mL, Polypropylene

*1: P/N : 227-31076-03
*2: P/N : GLC-IVS-100

Table 2 Analysis Conditions of ICP-MS

Instrument	: ICPMS-2040/2050
Nebulizer	: Nebulizer DC04
Torch	: Mini-torch
Chamber	: Cyclone Chamber (electronically cooled)
Sampling Cone	: Nickel
Skimmer Cone	: Nickel
RF Power	: 1.20 kW
Sampling Depth	: 7.0 mm
Flowrate of Plasma Gas	: 9.0 L/min
Flowrate of Auxiliary Gas	: 1.10 L/min
Flowrate of Carrier Gas	: 0.85 L/min
Flowrate of Dilution Gas	: 0 L/min
Collision Gas	: He
Flowrate of Cell Gas	: 6.0 mL/min
Cell Voltage	: -25 V
Energy Filter	: 7 V

■ Analysis of Calibration Standards

Fig. 2 shows the chromatogram of inorganic mercury, methyl mercury and ethyl mercury, each at a concentration of 0.5 µg/L. Inorganic mercury, methyl mercury and ethyl mercury were separated and detected successfully. The calibration curves are shown in Fig. 3. Good linearity with a correlation coefficient of over 0.999 was obtained. The 0.2 µg/L calibration standard was analyzed 10 times to calculate the low limit of detection (LOD). The LOD of each component is shown in Table 3. The formula is as follows:

LOD (3σ) = 3 x (Standard Deviation from 10 Measurements of 0.2 µg/L Solution) x Calibration Curve Slope

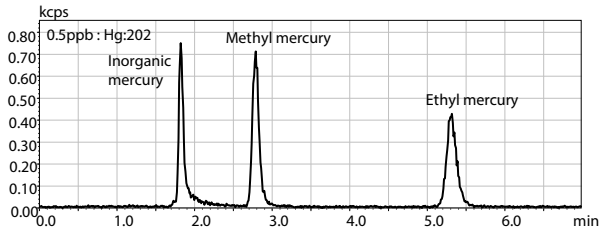

Fig. 2 Chromatogram of 0.5 µg/L Mercury

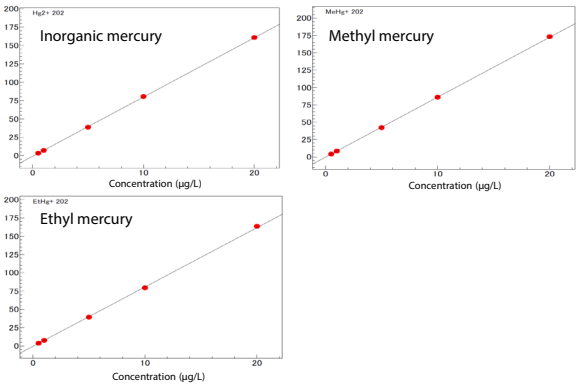

Fig. 3 Calibration Curves of Mercury

Table 3 Correlation Coefficient and LOD of Mercury

Component	Correlation coefficient	LOD (µg/L)
Inorganic mercury	0.99994	0.02
Methyl mercury	0.99996	0.01
Ethyl mercury	0.99988	0.03

■ Speciation Analysis of Mercury in Seafood

Fig. 4. shows a chromatogram of the seafood (tuna) extraction. Speciation analysis of methyl mercury and total mercury in the seafood was performed following FDA EAM 4.8. Hence, the total mercury was calculated as the sum concentration of inorganic mercury and methyl mercury. The results of the seafood analysis and spike recovery tests are shown in Table 4. Good recoveries were obtained for each component (Methyl mercury 102 %, total mercury 105 %).

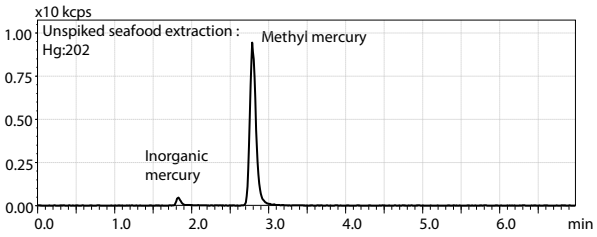

Fig. 4 Chromatogram of Seafood Extraction

Table 4 Analytical Results of Seafood

Species	Methyl mercury	Total mercury
Results in the solution (µg/L)		
Seafood (tuna)	6.16	6.48
Spike conc.	10	20
Recovery	102 %	105 %
Results in the tuna seafood (mg/kg)		
Seafood (tuna)	0.614	0.646
CODEX Standard [2)]	1.2	

■ Preparation of Calibration Standards Using Automatic Dilution Function

To analyze samples by LC-ICP-MS, it is common to first create a calibration curve using a standard sample and perform quantification. However, preparing calibration standards by diluting standard solutions is time-consuming. In recent years, there has been an increasing trend towards automated preparation to improve work efficiency and increase productivity.

The Nexera XS inert autosampler is equipped with an automatic dilution function. The conditions for dilution factors and mixing operations can be set using the LabSolutions ICPMS TRM software. An example of the autosampler's rack is shown in Fig. 5, and the setup window for the autosampler pretreatment program is shown in Fig. 6. A volume corresponding to the dilution ratio is aspirated from the vial containing mercury stock solution (Green) and dispensed together with the diluent into an empty mixing vial (Orange) that has been pre-set in the autosampler (final volume is 100 µL in this example). Some of the pretreatment program commands are shown in Table 5. To customize the vial position or the dilution factor, users should edit the commands highlighted in blue in Table 5.

The calibration standards from 0.5 µg/L to 20 µg/L were automatically prepared using this function. Table 6 shows the configuration and analytical conditions for HPLC when using the automatic dilution function.

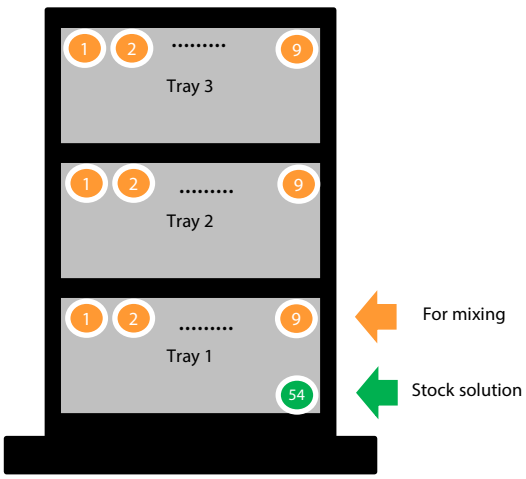

Fig. 5 An Example of the Rack of Autosampler
(Stock Solution Vial: Tray Number 1, Vial Number 54)

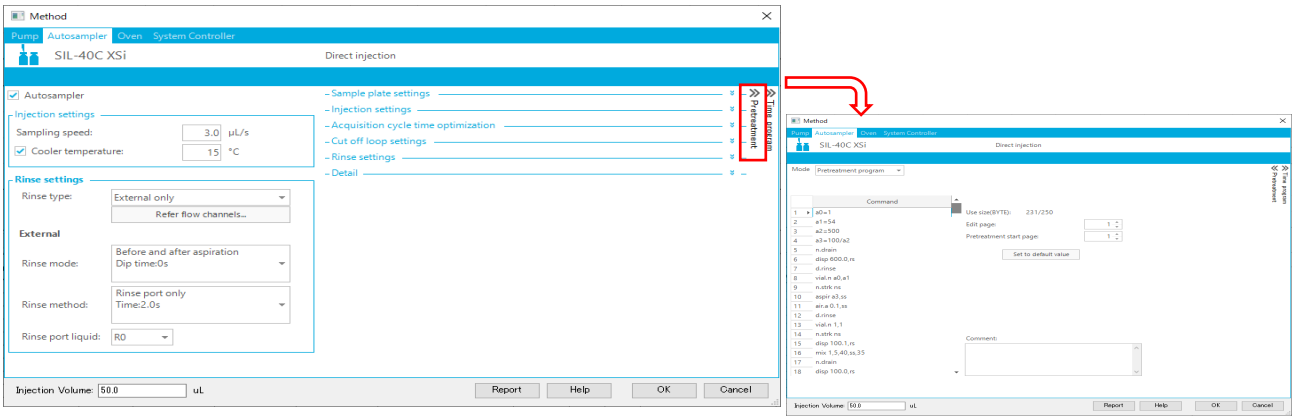

Fig. 5 Setup Window for Autosampler Pretreatment Program

Table 5 Pretreatment Program

Line	Command	Interpretation
1	a0=1	Tray number of stock solution
2	a1=54	Vial number of stock solution
3	a2=100	Dilution factor of the first sample
4	a3=100/a2	Calculation of aspiration volume of stock solution (Volume after dilution: 100 µL)
5	n.drain	The exchange of rinse solution to needle and needle loop
6	disp 600.0,rs	
7	d.rinse	Wash of the tip of needle
8	vial.n a0,a1	a3 µL aspiration from stock solution
9	n.strk ns	
10	aspir a3,ss	
11	air.a 0.1,ss	Air aspiration (0.1 µL)
12	d.rinse	Wash of the tip of needle
13	vial.n 1,1	100.1 µL injection to mixing vial (tray number 1, vial number 1), then mixing ● 100.1 µL = Volume after dilution + air
14	n.strk ns	
15	disp 100.1,rs	
16	mix 1,5,40,ss,35	
17	n.drain	The exchange of rinse solution to needle and needle loop
18	disp 100.0,rs	
19	d.rinse	Wash of the tip of needle
20	a2=50	Dilution factor of the second sample
21	a3=100/a2	Calculation of aspiration volume of stock solution (Volume after dilution: 100 µL)
...	...	...
49	inj.p	Move to injection port
50	v.inj	Injection
51	wait 2.0	2 min waiting (High pressure wash by the pump)
52	start	Start signal
53	end	The end of pretreatment program

Table 6 Configuration of HPLC and Analytical Conditions (Automatic Dilution)

System	:	Nexera XS inert
Column*1	:	Shim-pack Scepter C18-120 [Metal free column] (150 mm × 4.6 mm I.D., 5 µm)
Eluent	:	0.01 mol/L Ammonium acetate (pH 7.5) : Methanol = 92 : 8
Flowrate of Eluent	:	1 mL/min
Temp. of Column Oven	:	40 °C
Injection Volume	:	50 µL
Needle Stroke	:	45 mm
Rinse Solution (R0)	:	Diluent (1 % (w/v) L-cysteine•HCl•H2O with 8 % (v/v) methanol)
Mixing Vial*2	:	Shimadzu Vial, LC, 1 mL, Polypropylene
Vial for Stock Solution*3	:	Shimadzu Vial, LC, 1.5 mL, Polypropylene

*1: P/N : 227-31076-03
*2: P/N : 228-31600-91
*3: P/N : GLC-IVS-100

■ Evaluation of Calibration Curves Created by Automatic Dilution Function

The calibration curves created using the automatic dilution function were evaluated. For precision confirmation, a 1 µg/L calibration standard containing inorganic mercury, methyl mercury and ethyl mercury, which was prepared manually, was quantified using the calibration curve created by the automatic dilution function. Furthermore, the automatic dilution function was used to prepare a 1 µg/L standard 6 times to confirm accuracy. The correlation coefficients of the calibration curve and the accuracy and repeatability results are shown in Fig. 7 and Table 7, respectively.

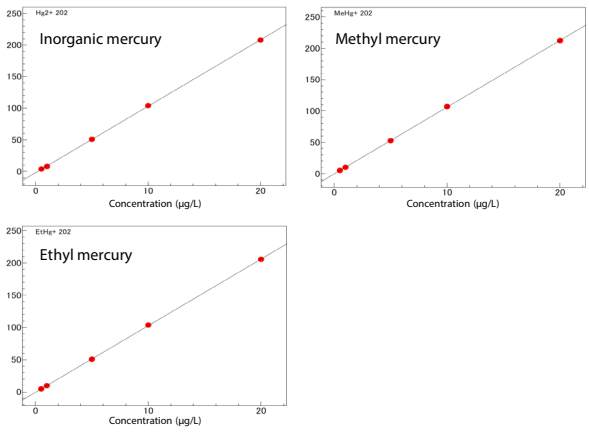

Fig. 7 Calibration Curves of Mercury by Automatic Dilution Function

Table 7 Evaluation of Automatic Dilution Function

Species	Correlation coefficient	Accuracy % (Result of 1 µg/L standard)	Repeatability % RSD
Inorganic mercury	0.99976	1.02	4.0%
Methyl mercury	0.99997	1.05	1.6%
Ethyl mercury	0.99997	1.05	2.8%

■ Conclusion

In this Application News, speciation analysis of methyl mercury and total mercury in seafood was performed using an LC-ICP-MS system that connected an ICPMS-2040/2050 to a Nexera XS inert according to the conditions in the "LC-ICP-MS Method Package for Mercury Speciation Analysis." The separation of inorganic mercury, methyl mercury, and ethyl mercury was confirmed by analyzing the calibration standards. Moreover, analysis of methyl mercury and total mercury in seafood was performed. Good spike recoveries indicated that the analysis of methyl mercury and total mercury in seafood can be performed accurately using this system.

The automatic dilution function of the Nexera XS inert autosampler was used to create calibration curves for each mercury species. In the validation test, the results for both accuracy and precision were good. This function simplifies the preparation of calibration standards.

Please contact us for more information about pretreatment program.

<References>

1) U.S. Food and Drug Administration Elemental Analysis Manual 4.8 High Performance Liquid Chromatographic-Inductively Coupled Plasma-Mass Spectrometric Determination of Methylmercury and Total Mercury, Version 1.0 (June 2008)
2) CODEX GENERAL STANDARD FOR CONTAMINANTS AND TOXINS IN FOOD AND FEED (CXS 193-1995)

<Related Applications>

1. Simple Labor-Saving Calibration Curve Creation Using Autosampler Automatic Dilution Function
Application News No.01-00717
2. Simple Labor-Saving Calibration Curve Creation Using Autosampler Automatic Dilution Function Part 2
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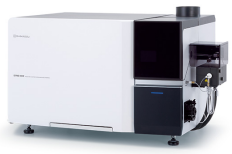

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