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Application News

Inductively Coupled Plasma Mass Spectrometry

Elemental Analysis with Mercury Speciation in Honey Using LC-ICP-MS

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User Benefits

- Simultaneous analysis of essential elements for normal metabolism and harmful toxic elements.
- LC-ICP-MS system enables speciation analysis of mercury.
- TRM software enables centralized analysis of LC-ICP-MS systems.

■ Introduction

Honey is the natural sweet substance, produced by Honeybees from the nectar of plants. Honey has a superior nutritional value and contains a variety of minerals that are minor constituents. Elements such as Cu, Sn, and Zn are essential for normal metabolism in human and elements such as Pb, Cd, As and Hg are considered as toxic and harmful to human metabolism. Mercury (Hg) is considered as an element of concern for major public health by the World Health Organization (WHO)[1]. Methylmercury (CH_3Hg) is the most common and toxic organic form of mercury. The source of these toxic elements could be from air pollution, pesticides, industrial waste or natural disaster which goes from ground water to soil and then from plants nectar to Honeybees.

The potential for mercury contamination has become serious concern to human and environmental health. The contamination of mercury and its conversion to methylmercury is largely depending on the location in which it arrives. If the location is favorable, then the conversion of inorganic mercury to the significantly more toxic organic form of methylmercury happens.

The Food Safety and Standards Authority of India (FSSAI) has laid down limits of 1.0 mg/kg for total mercury and 0.25 mg/kg CH_3Hg in food products[2]. To meet these limits ICP-MS is widely used as an accurate and reliable technique for quantification. In the present study, one honey sample was analyzed using Shimadzu ICPMS-2030 for quantitation of total and inorganic mercury along with quantification of methyl mercury by coupling ICPMS-2030 to HPLC as a speciation technique.

■ Analysis of As, Cd, Cu, Hg, Pb, Sn and Zn

❑ Sample preparation

The sample of Honey was purchased from local market. About 0.5 g of sample weighed accurately in PTFE vessels. 5 mL of suprapure nitric acid and 1 ml of suprapure hydrogen peroxide were added to it. The samples were then kept standing for 10 min to allow some pre-digestion.

The samples were digested in microwave digestion system using temperature program as per below Table 1. After the digestion was complete, the content was quantitatively transferred to 50 mL volumetric flask and diluted up to mark with distilled water. The samples were prepared in triplicates.

Table 1: Microwave digestion method

Ramp (Minute)	Temperature (°C)	Hold (Minute)
10	120	3
10	200	15

❑ Preparation of additive recovery test solution

The samples were spiked at concentration levels of 10 % and 20 % of maximum permissible limits for recovery study. The maximum permissible limits are given in Table 2[2),3)]. The samples were prepared in triplicates.

❑ Standard preparation

NIST traceable elemental standards were used for preparation of intermediate standard stock which were then further diluted to prepare final standard solution with concentrations as in Table 3.

Table 2. FSSAI maximum permissible limits in µg/g

Elements	FSSAI limits (µg/g)
Arsenic (As)	1.1
Cadmium (Cd)	1.5
Copper (Cu)	30
Mercury (Hg)	1
Lead (Pb)	2.5
Tin (Sn)	250
Zinc (Zn)	50
Methylmercury (CH_3Hg)	0.25

Table 3. The concentration of calibration standards in µg/L used in microwave digestion study

Element	STD 1	STD 2	STD 3	STD 4	STD 5	STD 6	STD 7
As	0.55	1.1	2.2	5.5	11	16.5	22
Cd	0.75	1.5	3.0	7.5	15	22.5	30
Cu	15	30	60	150	300	450	600
Hg	0.5	1	2	5	10	15	20
Pb	1.25	2.5	5	12.5	25	37.5	50
Sn	12.5	25	50	125	250	375	500
Zn	25	50	100	250	500	750	1,000

❑ Instrument and Analytical Conditions

The Shimadzu ICPMS-2030 inductively coupled plasma mass spectrometer with auto sampler AS-10 is shown Figure 1. Analytical condition of ICPMS-2030 are shown in Table 4. In addition to providing high sensitivity, ICPMS-2030 is equipped with a collision system using helium gas, which reduces interferences from argon and chlorine significantly.

Table 4. Analytical conditions

Instrument	: ICPMS-2030
Auto sampler	: AS-10
RF Power	: 1.2 kW
Plasma gas flowrate	: 8 L/min
Auxiliary gas flowrate	: 1.1 L/min
Carrier gas flow	: 0.7 L/min
Nebulizer	: Nebulizer 10
Chamber	: Cyclone chamber (electronically cooled)
Plasma torch:	: Mini torch
Skimmer Cone/ Sampling Cone	: Copper
Collision gas	: Helium

Fig.1 Inductively Coupled Plasma Mass Spectrometer ICPMS-2030 with Autosampler AS-10

❑ Analysis

The calibration curve method was used for quantitative analysis of As, Cd, Cu, Hg, Pb, Sn and Zn. The internal standards used were Bi, Ce, Ge and Sc.

❑ Results & Discussion

Correlation coefficient for As, Cd, Cu, Hg, Pb, Sn and Zn were more than 0.999. The analytical results along with instrument detection limit [IDL] are mentioned in Table 5. IDL's were determined using following procedure :The blank calibration curve sample solution was measured 10 times, and the concentration giving a signal 3 times blank standard deviation was calculated as the detection limit.

As there is no CRM available for honey matrix, to validate the developed method, spike recoveries were performed at 10 % and 20 % of the maximum permissible limit. The lowest calibration point corresponds to 5 % of the maximum permissible limit as per FSSAI guidelines. As per AOAC[4], the limit of quantification should be equal to or greater than lowest calibration point. In the present case, 10 % maximum permissible limit of FSSAI for individual elements can be considered as limit of quantification (LOQ). The LOQ values w.r.t sample are listed in Table 5. The recoveries for all elements at 10 % and 20 % maximum permissible limit concentration levels were between 90 to 120 %. The concentrations of all elements were below LOQ. The repeatability at concentration of 10 % and 20 % maximum permissible limit was good with RSD less than 3 %.

Table 5. LOQs established in the present work

Element	LOQ in ug/g
As	0.11
Cd	0.15
Cu	3
Hg	0.1
Pb	0.25
Sn	2.5
Zn	5

Table 6. The Results and Recovery test data at 10 % and 20 % of the maximum permissible limits (Average of 3 measurements)

Element	IDLs in (µg/L)	Sample Results in Honey (µg/g)	10 % Maximum permissible limit		20 % Maximum permissible limit	
			% Recovery	% RSD	% Recovery	% RSD
As	0.02	Below LOQ	105.9	2.3	102.0	0.8
Cd	0.003	Below LOQ	95.6	1.4	92.6	1.6
Cu	0.04	Below LOQ	117.9	0.4	111.1	2.0
Hg	0.02	Below LOQ	92.4	0.2	91.6	0.8
Pb	0.22	Below LOQ	109.1	1.0	105.0	2.1
Sn	0.17	Below LOQ	106.0	1.9	104.3	1.2
Zn	0.08	Below LOQ	109.0	1.4	106.5	2.2

* Note: In present work Spike recoveries for Sn were performed at 1% and 2% of FSSAI Maximum permissible limit.

■ Speciation Analysis of Mercury

Speciation analysis is defined by the International Union of Pure and Applied Chemistry (IUPAC) as an activity of identifying and measuring the quantities of one or more chemical species in a sample. In the present study, Inorganic mercury and methylmercury were analyzed using the LC-ICP-MS system as speciation analysis method.

❑ Sample preparation

About 0.5 g of sample was accurately weighed into 10 mL volumetric flask (Dilution factor 20x). The samples were then made up to the volume by adding the mobile phase. The solutions were shaken using vortex. The samples were heated in water bath at 60°C for 2 h. The samples were cooled to room temperature. After cooling the samples were transferred to HPLC vial with filtration through 0.45 µm Nylon filter. The samples were prepared in six replicates.

❑ The Standard preparation

.All seven Intermediate standard stock solutions were prepared by mixing 1000 ppm stock solutions of inorganic mercury and methylmercury. These solution were then further diluted with 0.1% L-Cysteine (pH 2.5) to get final concentration as in Table 7.

Table 7. The concentration of calibration standards of methyl mercury & inorganic mercury

Calibration standard	Inorganic Mercury (µg/L)	Methyl Mercury (µg/L)
STD 1	0.1	0.1
STD 2	0.2	0.2
STD 3	0.5	0.5
STD 4	1.0	1.0
STD 5	2.0	2.0
STD 6	5.0	5.0
STD 7	10.0	10.0

❑ Preparation of additive recovery test solution

The samples were spiked at concentration levels of 0.2 µg/L and 0.5 µg/L for recovery study. The samples were prepared in six replicates

❑ Instrument and Analytical Conditions

ICPMS-2030 and LC-20Ai were used as LC-ICP-MS systems (Shown in Figure 2). In LC-ICP-MS systems using inert LC and ICP-MS, LabSolutions ICPMS Time Resolved Measurement (TRM) software can perform LC control, ICP-MS measurements and chromatographic data analysis. The analysis conditions for each instrument are shown in Tables 8 and 9.

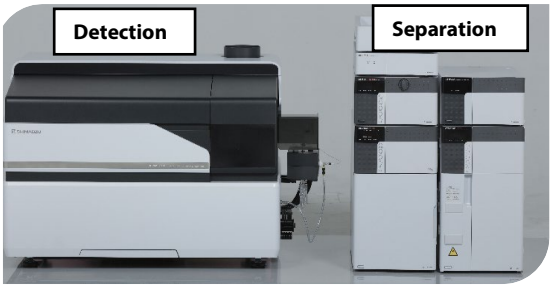

Fig. 2 Hyphenation of ICPMS-2030 (Left) with Inert LC-20Ai (right) using single LabSolution ICPMS software

Table 8. Chromatographic conditions

Instrument	: LC-20Ai inert LC
Column	: Synergi Hydro RP 150 x 4.6 mm, 4 µm.
Mobile phase	: 0.1 % L-Cysteine pH adjusted to 2.5
Flow rate	: 1.2 mL/min
Column temp.	: 25 °C
Injection volume	: 100 µL
Run time	: 5 min

Table 9. Analytical conditions of ICP-MS

Instrument	: ICPMS-2030
RF Power	: 1.2 kW
Plasma gas flowrate	: 8 L/min
Auxiliary gas flowrate	: 1.1 L/min
Carrier gas flow	: 0.7 L/min
Chamber	: Cyclone chamber (electronically cooled)
Plasma torch:	: Mini torch
Collision gas	: Helium

❑ Analysis

Inorganic mercury and methylmercury in the sample solution were quantitatively analyzed by the calibration curve method using the LC-ICP-MS system. In addition, for validation of analytical values, samples for additive recovery tests were used. Table 8 shows the analysis conditions for the chromograph, and Table 9 shows the analysis conditions for ICP-MS

❑ Results & Discussion

Chromatograms of inorganic mercury and methylmercury are shown in Figure 3. In this speciation analysis, one sample can be measured within five minutes, and the peaks of each species are well separated.

Figure 4 shows the calibration curve for each species of mercury. The calibration curve showed a correlation coefficient of more than 0.999.. As per AOAC[4], the limit of quantification should be equal to or greater than lowest calibration point. In the present work, 0.1 µg/L was the lowest calibration point. To establish LOQ, the samples were spiked at the concentration levels of 0.2 µg/L and 0.5 µg/L.

The results obtained along with LOQ are shown in Table 10. The 0.2 µg/L (4 µg/kg in honey sample) can be considered as limit of quantification (LOQ). The concentrations of both the species was below LOQ. The % recovery at 0.2 µg/L and 0.5 µg/L was between 90 to 120%, with good repeatability. The % RSD obtained for six measurements was less than 5. IDL's mentioned in Table 10 were determined using following procedure :The blank calibration curve sample solution was measured 10 times, and the concentration giving a signal 3 times blank standard deviation was calculated as the detection limit. The integration of blank signal was performed manually.

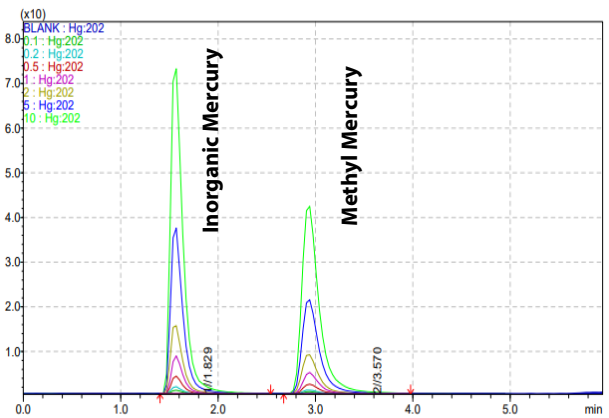

Fig. 3 Chromatogram obtained in the present study

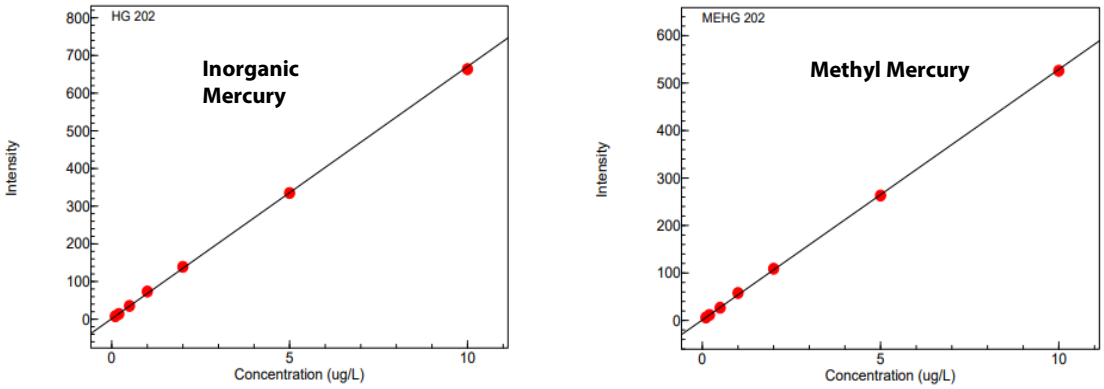

Fig. 4 Calibration curves of Inorganic mercury and Methyl mercury obtained in the present study

Table 10. Sample results and recovery data at 0.2 µg/L and 0.5 µg/L for inorganic mercury and methylmercury (Average of 6 measurements)

Analyte	IDL in (µg/L)	LOQ (µg/kg)	Sample Results in Honey (µg/kg)	Recovery at 0.2 µg/L		Recovery at 0.5 µg/L	
				% Recovery	% RSD	% Recovery	% RSD
Inorganic mercury	0.025	4	Below LOQ	94.3	3.9	96.4	2.3
Methyl mercury	0.016	4	Below LOQ	107.3	3.4	109.6	4.9

■ Conclusion

The present work demonstrates the suitability of the HPLC-ICP-MS method for the quantification of inorganic mercury and methylmercury in honey, in accordance with FSSAI regulations. The inorganic and methylmercury forms of Hg were measured accurately at low-ppb concentrations in honey sample using a Shimadzu Inert HPLC LC-20Ai coupled to a Shimadzu ICPMS-2030. The separation of both species was complete in less than five minutes. LabSolutions TRM software can perform LC and ICP-MS measurements. ICPMS-2030 enables quantitative analysis of elements essential for normal metabolism such as Cu, Sn and Zn and toxic trace elements such as Pb, Cd, As and Hg in honey. Excellent detection limits were obtained using Shimadzu ICPMS-2030 with unique mini-torch technology which uses lowest argon flow rates as compared to any other conventional ICPMS.

■ Note

This application is based on ICPMS-2030, but similar analysis can be performed with ICPMS-2040/2050.
In this case, the ICP-MS analysis conditions shown in Table 9 should be suitable for ICPMS-2040/2050.

■ Reference

1. World Health Organization, Mercury and Health, WHO fact sheet, 2017,https://www.who.int/newsroom/factsheets/detail/mercury-and-health

2. Food Safety and Standards Authority of India, Food Safety and Standards (Contaminants, Toxins and Residues) Regulations, 2011

3. FSSAI, Food Safety and Standards (Contaminants, Toxins and Residues), Regulations, 2006

4. Briscoe M., Determination of Heavy Metals in Food by Inductively Coupled Plasma-Mass Spectrometry: First Action 2015.01, J.AOAC Int. 2015, 98 (4), 1113-1120. 98(4), 1113–1120

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