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Speciation of Methyl Mercury and Mercury in Honey using High Performance Liquid Chromatography hyphenated with Inductively Coupled Plasma Mass Spectrometry

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1. Overview

In the present work, we performed the analysis of honey for its methyl mercury and mercury content using hyphenation of liquid chromatography and inductively coupled plasma mass spectrometry.

2. Introduction

Mercury is a major environmental pollutant on a global scale. Its toxicity is further enhanced by bio-methylation carried out by microorganisms in air, water and soil. The resultant product of bio-methylation is methyl mercury. Due to environmental pollution, it can enter human food items like honey. Food Safety and Standards Authority of India (FSSAI) has laid down maximum permissible limits for methyl mercury and mercury in honey (Table 1)

Table 1. FSSAI maximum permissible limits

Compound	Limit in µg/g
Methyl mercury	0.25
Mercury	1.0

The analytical techniques that are frequently employed for mercury speciation involve a chromatographic separation system and an element selective detection system. High performance liquid chromatography, gas chromatography and capillary electrophoresis are the prevailing separation techniques and have been widely used for mercury speciation analysis in various matrices. High-performance liquid chromatography offers several advantages, including comparatively simple sample pretreatment, ease of interface to atomic spectrometers, and the capability to analyse organic and inorganic species. ICPMS is used most frequently for elemental detection due to its ultra-trace level detection capabilities. In the present work, a method involving aqueous extraction of mercury species with subsequent separation on HPLC followed by ICPMS detection was developed to determine methyl mercury and mercury in honey.

3. Method

3-1. Standards preparation

1000 ppm methyl mercury stock was prepared from Methylmercury (II) chloride reagent (Aldrich). 1000 ppm NIST traceable standard of mercury was used. Calibration standards were prepared in the range of 0.1 to 10 ppb. All the standards were prepared in mobile phase. The mobile phase was 0.1 % L-cysteine (pH adjusted to 2.5). As a QC check, 0.5 ppb standard was measured periodically (Initial calibration verification (ICVs) and continuous calibration verification checks (CCVs))

3-2. Sample Pretreatment

About 0.5 g of honey sample was weighed into a 10 mL volumetric flask. The content was diluted up to the mark with the mobile phase. The solutions were shaken using a vortex.

The samples were then heated in a water bath at 60 ºC for 2 hrs. The samples were cooled to room temperature. After cooling, the samples were transferred to an HPLC vial with filtration through a 0.45 µm nylon filter..

3-3. Analytical conditions

The analysis was performed using Shimadzu Inert LC coupled with ICPMS 2030 (Figure 1). The chromatographic conditions were as per Table 2. The ICPMS parameters were as per Table 3.

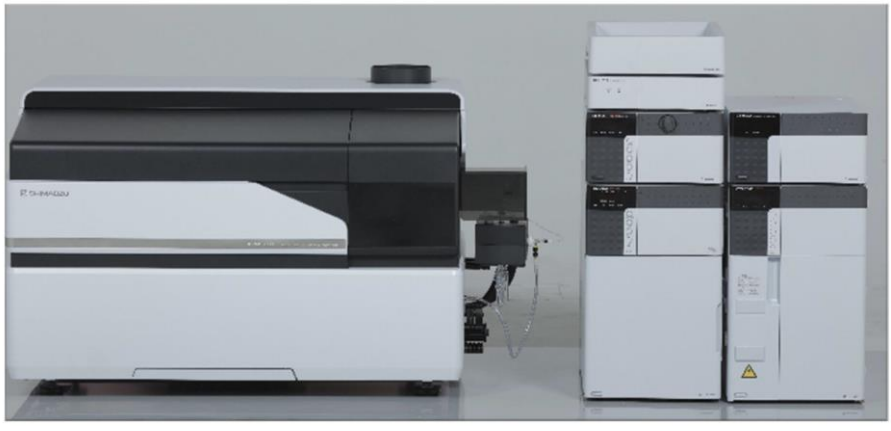

Figure 1. Shimadzu Inert LC with ICPMS 2030

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Table 2. LC Parameters

Parameter	Value
Mobile phase	0.1 % L-cysteine pH adjusted to 2.5 with dil HCl
Flow rate	1.2 mL/min
Injection volume	100 µl
Run time	5 min
Column temperature	25 °C
Column	Phenomenox Synergi Hydro-RP (C-18) 150 X 4.6 mm , 4 µm particle size (P/N: 00F-4375-E0)

Table 3. ICPMS Parameters

Parameter	Value
RF Power	1.2 kW
Plasma gas flow	8.0 L/min
Carrier gas flow	0.7 L/min
Sampling depth	5 mm
Chamber temperature	5 °C
Cell gas flow	6 mL
Mass	202 amu

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4. Results

The calibration standards showed good linearity (Figure 2) with a correlation coefficient ≥ 0.999. The peaks of methyl mercury and mercury were well resolved.

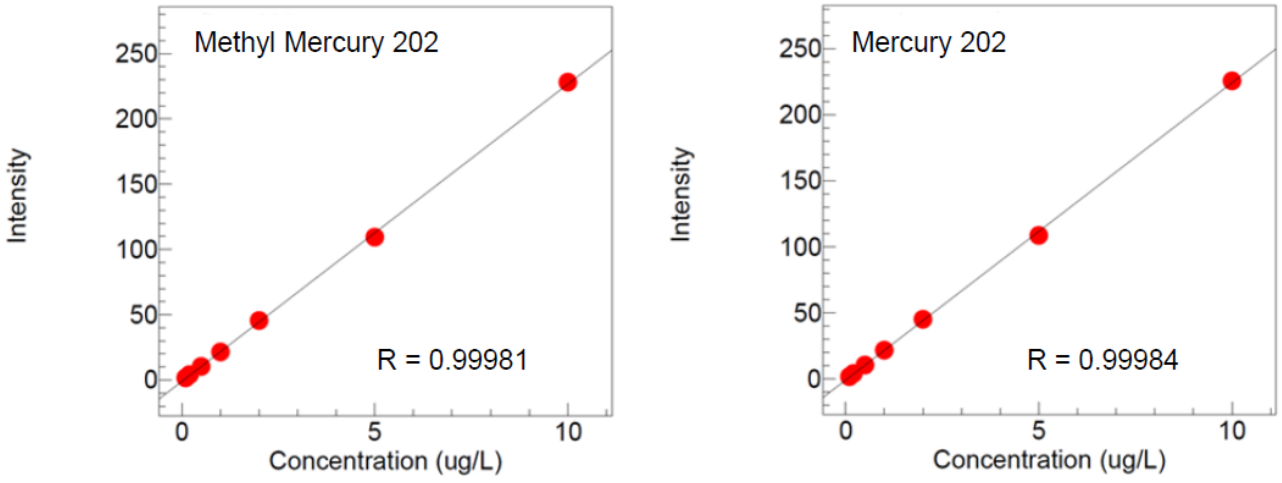

Figure 2. Typical linearity obtained in the present work

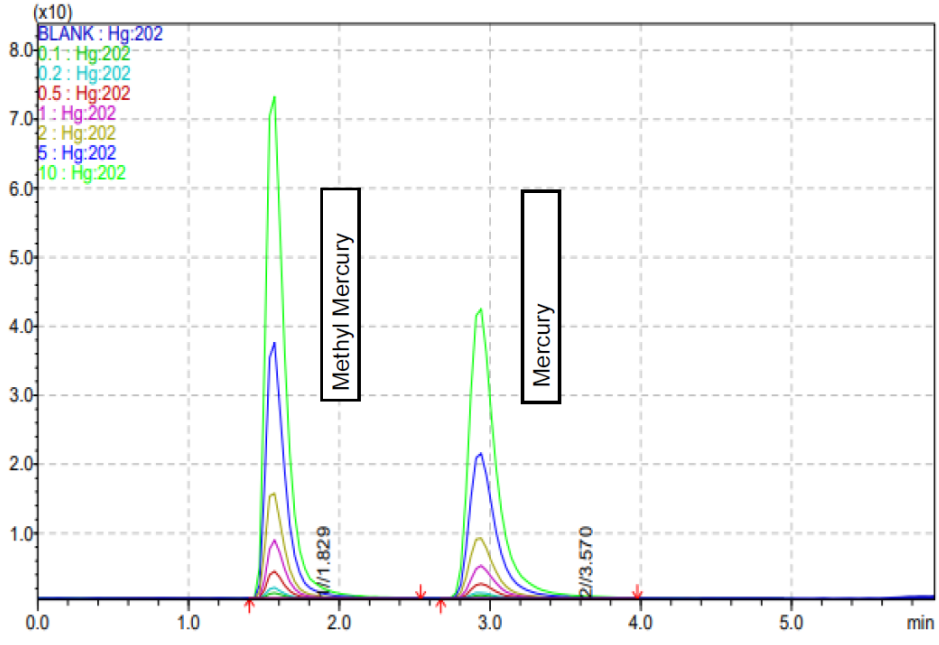

Figure 3. The chromatogram obtained in the present work

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Table 4 shows results obtained for % accuracies of calibration checks. The accuracies were between 90 to 100, showing the robustness of the plasma of Shimadzu ICPMS 2030 over a longer period while analyzing honey samples. The accuracies of ICV and CCVs indicate a complete washout of mercury species between the two sample measurements. Table 5 shows % recoveries at 0.2 ppb, 0.5 ppb and 1.0 ppb (w.r.t sample 4.0 ppb, 10.0 ppb and 20.0 ppb respectively) level. All recoveries were between 80 to 120 %. Excellent spike recoveries were obtained with RSD of less than 5 % showing the efficiency of the extraction procedure.

Table 4. Accuracies of ICV and CCVs

CCVs	% Accuracy
ICV	97.6
CCV 1	99.8
CCV 2	92.0
CCV 3	97.8
CCV 4	103.8

Table 5. Average % recoveries at 0.2 ppb. 0.5 ppb and 1.0 ppb (n =6)

Compounds	0.2 ppb		0.5 ppb		1.0 ppb	
	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Methyl mercury	94.3	3.9	96.4	2.3	93.7	2.2
Mercury	107.3	3.4	109.6	4.9	103.6	2.1

5. Conclusions

The study proves the applicability of the developed speciation method for the determination of methyl mercury and mercury in honey samples as per FSSAI guidelines. Accurate quantification of both forms of mercury was performed at low-ppb concentration levels using the Shimadzu Inert LC system coupled to ICPMS 2030. Complete separation of both species was achieved in less than five minutes. The content of the methyl mercury and mercury in the sample was less than 4 ppb. The excellent recoveries at lower concentrations demonstrated the accuracy and precision of the method.

The proposed method can be used for routine monitoring of toxic forms of mercury in a widely consumed product like honey.

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