Application Note

Food

Fast and Accurate Speciation of Mercury in Milk and Honey Food Products by HPLC-ICP-MS

Method compliant with FSSAI regulations using an Agilent 7850 ICP-MS with HPLC and integrated chromatographic data analysis

Authors

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Introduction

Mercury (Hg) is considered one of the top 10 chemicals of concern for public health by the World Health Organization (WHO) (*1*). Hg occurs naturally in the environment and can also be released through man's activities such as burning coal, mining, industrial processes, and incinerating waste. Hg exists in several different chemical forms or species, including elemental mercury (Hg^0), inorganic mercury (Hg^{2+}), and various organic forms. Methylmercury (MeHg) is the most common organic form and is also the most toxic Hg compound (*2, 3*), with most being derived from ingesting fish or shellfish.

Given the potential health risks, many countries set limits for the intake of mercury and its compounds in foods. In 2020, the Food Safety and Standards Authority of India (FSSAI) published maximum permissible limits of 1.0 mg/kg for total mercury and 0.25 mg/kg MeHg in milk and honey (and other foods) (*4*). In the European Union, the tolerable weekly intake (TWI) is 4.0 µg/kg body weight (bw) for inorganic Hg and 1.6 µg/kg bw for MeHg (*5*).

Milk, honey, and related food-products are consumed in almost every part of the world by people of all ages. According to the Food and Agriculture Organization (FAO) of the United Nations, India is the world's largest producer of milk (*6*), and in 2019, India was the seventh largest global producer of honey (*7*). Milk can be contaminated with heavy metals, including Hg, if animals graze on grass grown on polluted land. Honey can be similarly affected if bees forage on flowering plants that are grown in contaminated soils. Land can be polluted if untreated industrial effluents are discharged into water courses that are used for irrigation or if untreated effluents are used directly on agricultural land.

To meet the concentration limits for total Hg and MeHg in foods set by regulatory bodies such as the FSSAI, analysts need accurate and reliable methods for the determination of total Hg and Hg species. ICP-MS is widely used to quantify a wide range of elements, including total Hg, in foods (*8*). However, total elemental concentrations do not give adequate information for food safety assessment where a metal's toxicity depends on its chemical form. For this type of assessment, ICP-MS can provide speciation analysis of individual Hg compounds, including Hg^{2+} and MeHg, by coupling the ICP-MS to a high-performance liquid chromatography (HPLC) system (*9*).

Agilent ICP-MS instruments can easily be connected to Agilent HPLC systems through optimized interfaces and integrated software control. The coupled systems are controlled from the Agilent ICP-MS MassHunter software, simplifying the analytical workflow from method development, data acquisition, and data reporting.

An Agilent 1260 HPLC coupled to the 7850 ICP-MS gives a cost-effective method for the separation and detection of Hg species in food samples.

ICP-MS and ICP-MS MassHunter software

The 7850 is suited to routine ICP-MS applications such as the multi-element analysis of environmental and food samples, including high matrix samples (*10*). The 7850 uses auto-optimization routines, preset methods, intuitive software, built-in report templates, and pre- and post-analysis performance checks. These ease-of-use features make method setup faster, and operation and maintenance easier—especially for nonexpert users. The 7850 uses a robust plasma with UHMI aerosol dilution technology, and a helium (He) collision cell with kinetic energy discrimination (KED) for effective control of common polyatomic interferences.

The 7850 enables routine laboratories to deliver fast and reliable analysis while ensuring accurate data and avoiding the need to remeasure samples.

Speciation analysis is increasingly important in routine applications, particularly for samples such as food, where safety is of concern. The 7850 can be used to detect the different chemical forms of an element after chromatographic separation, as well as providing conventional total metals concentrations.

The chromatographic module (p/n G7205C) of ICP-MS MassHunter software provides fully functional, integrated control for Agilent chromatography-systems, and comprehensive chromatographic data analysis with advanced integration tools, including:

- Chromatographic and spectrum-based internal standard correction
- Chromatographic internal standards that correct for changes in both response and retention time
- Peak auto integration using global or user-selectable integration parameters per peak
- Interactive manual integration
- Snapshot data analysis of current sample during acquisition run-time
- Compound-independent calibration (CIC)
- Signal-to-noise calculation
- Automatic retention time updates

Experimental

Sample and standard preparation

Samples of milk (full-fat) and honey were bought in a local supermarket. All samples, mobile phase solution, and calibration standards were freshly prepared on the day of the analysis. The mobile phase (0.1% L-cysteine, pH adjusted to 2.6 using 1.0 M ultrapure grade hydrochloric acid) was used to prepare the calibration standards and food samples.

The sample preparation procedure (Figure 1) was based on a US FDA Elemental Analysis Manual (EAM) method for the determination of methylmercury and total mercury in seafood by HPLC-ICP-MS (*11*).

About 0.50 ± 0.1 g of sample was accurately weighed into a 10 mL vial. The samples were then made up to volume by adding the mobile phase, giving a total dilution factor of 20x. Six replicates of each sample were prepared. The solutions were shaken using a vortex mixer. The vials were heated in a water bath at 60 ± 2 °C. Each vial was shaken vigorously after 60 minutes of heating and again after 120 minutes of heating. The vials were allowed to cool to room temperature. After cooling, the honey samples were transferred to HPLC autosampler vials for analysis, while the milk samples were passed through a 0.45 µm syringe filter.

Mixed calibration standards for inorganic and methyl Hg were prepared from 10 mg/L single species standards. Calibration standards were prepared at levels of 0, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0 µg/L (ppb).

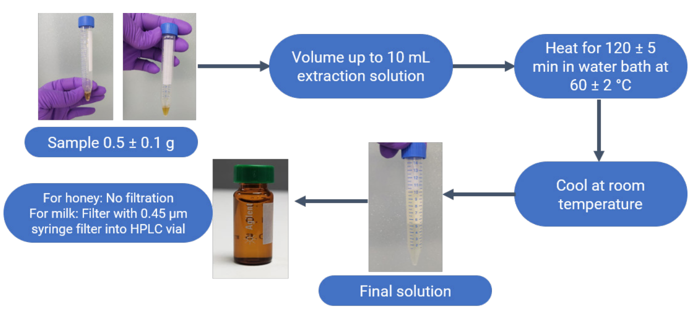

Figure 1. Sample preparation procedure for milk and honey samples.

Instrumentation

The Agilent 7850 ICP-MS was set up using the standard configuration consisting of a MicroMist nebulizer, quartz spray chamber and torch, and nickel cones. The 7850 ICP-MS was connected to an Agilent 1260 LC system using the Agilent LC connection kit (p/n G1833-65200). The 1260 LC, which can operate at a pressure up to 600 bar, was fitted with an Agilent ZORBAX SB C-18, 4.6 x 250 mm column, with a particle size of 3.5 microns (p/n 884950-567).

The 7850 ICP-MS was optimized using default autotune conditions that were set directly from the ICP-MS MassHunter software. No further optimization was necessary for the speciation study. The ICP-MS and HPLC operating conditions are presented in Table 1 and Table 2, respectively. Different mobile phase flow rates (1.0, 1.2, and 1.4 mL/min) and sample injection volumes (25 and 50 µL) were tested. The optimum elution (best peak shape and resolution) of Hg^{2+} and MeHg was observed at 1.2 mL/ min using a 50 µL injection.

Table 1. 7850 ICP-MS operating conditions.

Parameters	Value
RF Power (W)	1550
Sampling Depth (mm)	8.0
Nebulizer Gas Flow (L/min)	1.05
Nebulizer Pump Speed (rps)*	0.40
Spray Chamber Temperature (°C)	2.0
Cell Gas	Off

*The shaded parameters were set automatically. * Nebulizer pump was used for the spray chamber drain. Sample was delivered to the ICP-MS nebulizer by the HPLC pump.*

Table 2. Optimized HPLC operating conditions.

Parameters	Value
Mobile Phase Flow Rate (mL/min)	1.2
Injection Volume (µL)	50.0
Run Time*	7.00
Column Temperature	Ambient
Autosampler Temperature	Ambient
Mobile Phase	0.1% (w/v) L-cysteine adjusted to pH 2.6 using ultrapure HCl

** Although MeHg elutes at R.T.3.871 minutes, the run time was set at 7.00 minutes to make sure that no other compounds eluted and to flush the sample solution path.*

Results and discussion

The results of the linearity studies for Hg^{2+} and MeHg are shown in Figure 2. Both calibrations were linear (R >0.9999) over the concentration range, with background equivalent concentrations (BECs) around 0.001 ppb. The low BECs demonstrate the suitability of the 7850 ICP-MS with HPLC for the determination of trace concentrations of Hg species.

The chromatographic separation of Hg^{2+} and MeHg was achieved successfully in less than four minutes without compromising the resolution of either of the peaks, and while maintaining an excellent signal-to-noise (S/N) ratio.

Limits of detection (LOD) for the Hg species were calculated as three times the chromatographic peak-to-peak S/N ratio. The LODs were 0.0035 and 0.0038 ppb for Hg^{2+} and MeHg, respectively, as shown in Table 3. Figure 3 shows the overlaid chromatograms from a set of calibration standards from 0 to 10 ppb. The data from the 0.05 ppb standard was used for the S/N and LOD calculation. Figure 3 shows the excellent peak resolution of both the Hg species at all the levels from 0.05 to 10.0 ppb.

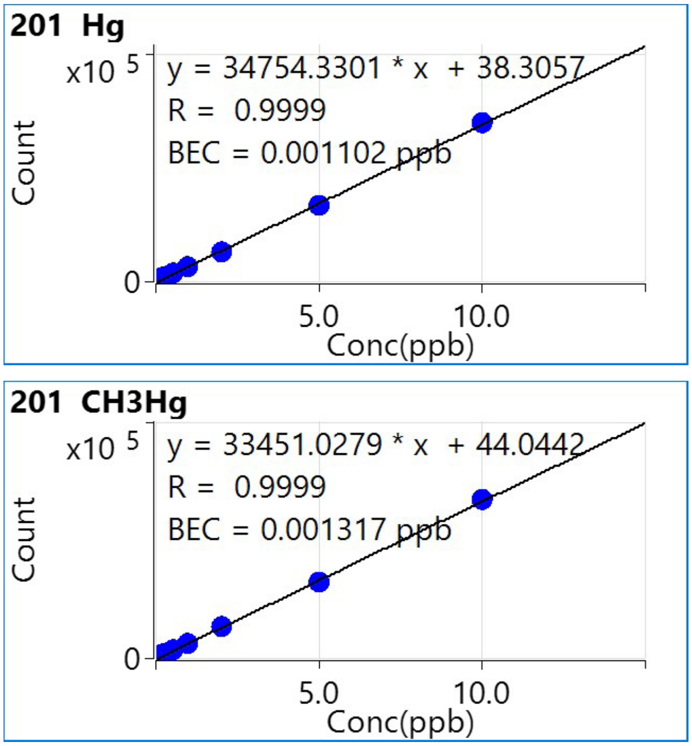

Figure 2. Linear calibrations for inorganic and methyl mercury by LC-ICP-MS.

Table 3. Chromatogram details of 0.05 ppb standard and 3x S/N LODs.

Analyte	RT, minute	Area of 0.05 ppb Standard	Noise	S/N Ratio	LOD (ppb)
201 Hg^{2+}	2.203	1654	38	43	0.0035
201 MeHg	3.871	1728	44	39	0.0038

Spike recoveries and evaluation of matrix effects

The milk and honey samples were analyzed by HPLC-ICP-MS. No Hg species were detected above the LOD in any of the samples. Therefore, to test accuracy and precision of the method, a spike recovery study was carried out by spiking replicate samples with Hg^{2+} and MeHg during the sample preparation. One mL of the spike solution containing 10 ppb of each Hg compound was added prior to sample dilution. The samples were prepared according to the procedure shown in Figure 1, giving a spike level of 1 ppb in the diluted samples as analyzed.

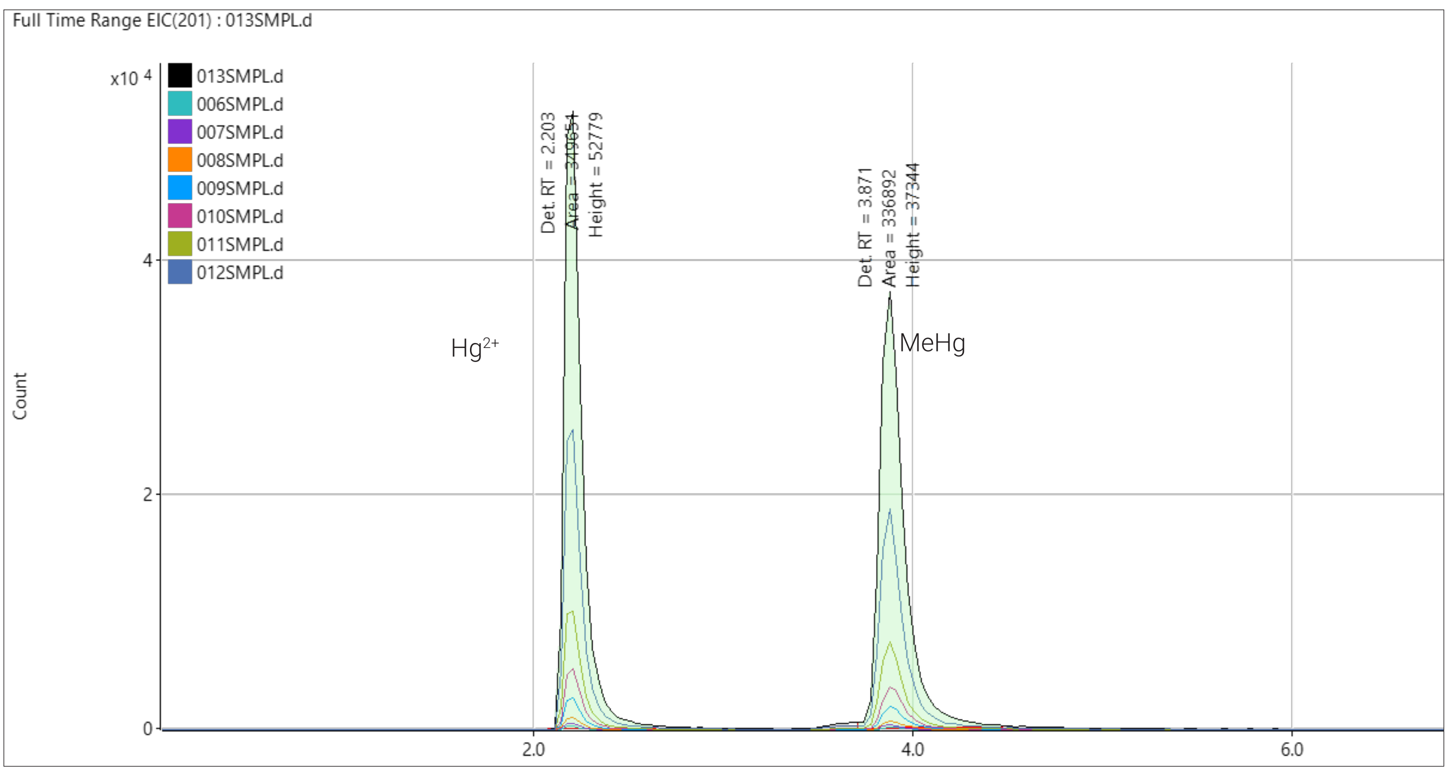

Figure 3. Overlaid chromatograms for a set of calibration standards of inorganic Hg and MeHg from 0.05 to 10 ppb, showing good sensitivity and peak separation.

The results in Table 4 show the spike recovery accuracy and precision for nine separate honey samples. Spike recoveries (n=9) for honey were within 100 ± 5% with %RSD less than 2%, indicating that both Hg species were unaffected by any matrix effects arising from the honey samples. Figure 4 shows a chromatogram of inorganic Hg and MeHg spike at 1.0 ppb in the diluted honey.

Milk samples were spiked at 0.1, 0.5, and 2.0 ppb with Hg^{2+} and MeHg (n=3 at each concentration level). The spike recovery results in Table 5 show excellent recoveries within 100 ± 5% and precision (%RSD) of 3% or less for the three replicate measurements at all the concentration levels.

Table 4. Accuracy and precision of spike recovery of 1 ppb Hg^{2+} and MeHg spikes in 20x diluted honey sample by HPLC-ICP-MS.

Replicates	Recovery of Inorganic Hg Spike (%)	Recovery of MeHg Spike (%)
I	98	98
II	100	99
III	97	99
IV	100	99
V	101	101
VI	102	103
VII	97	97
VIII	98	100
IX	100	98
Average (n=9)	99	99
SD	1.80	1.79
%RSD	1.81	1.79

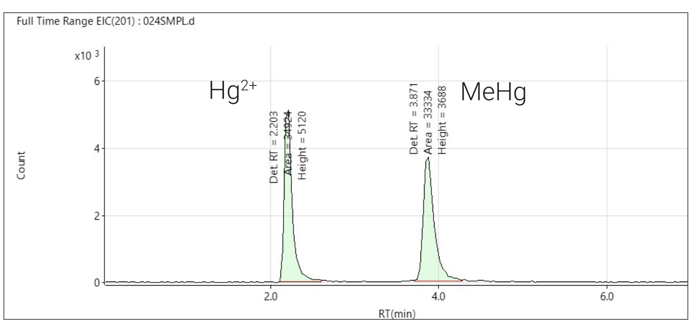

Figure 4. Chromatogram of a honey sample spiked with 1.0 ppb Hg species.

Table 5. Spike recoveries and precision of measurements of Hg^{2+} and MeHg spiked at 0.1, 0.5, and 2.0 ppb in milk by HPLC-ICP-MS.

Replicates	Recovery of 0.1 ppb Spike (%)		Recovery of 0.5 ppb Spike (%)		Recovery of 2.0 ppb Spike (%)	
	Inorganic Hg	MeHg	Inorganic Hg	MeHg	Inorganic Hg	MeHg
I	98	100	100	99	95	95
II	100	99	100	102	95	99
III	96	96	101	96	96	98
Average	98	98	100	99	95	97
SD	1.89	2.24	0.34	2.99	0.66	1.82
%RSD	1.92	2.27	0.33	3.01	0.69	1.85

Quality Control (QC)

As a QC check, the 0.05 and 0.5 ppb standards containing both inorganic Hg and MeHg were analyzed periodically throughout the run during a total analysis time that spanned 7 hours. The precision (%RSD) of the measurements was less than 4% at the 0.05 ppb level and less than 2.5% at the 0.5 ppb level, demonstrating the stability of the coupled HPLC-ICP-MS system (Table 6).

Table 6. QC check at 0.05 and 0.5 ppb.

Replicates	QC at 0.05 ppb Level		QC at 0.5 ppb Level	
	Inorganic Hg	MeHg	Inorganic Hg	MeHg
I	0.047	0.047	0.506	0.506
II	0.050	0.047	0.504	0.506
III	0.047	0.046	0.483	0.516
Average	0.048	0.047	0.498	0.509
SD	0.002	0.001	0.012	0.006
%RSD	3.365	1.689	2.493	1.118

Conclusion

The study has shown the suitability of the HPLC-ICP-MS method for the quantification of inorganic mercury (Hg^{2+}) and methylmercury (MeHg) in foods, in compliance with the Food Safety Standard Authority of India requirements. Producers, manufacturers, and importers of food into India must now test their products for levels of toxic mercury species, as well as total mercury.

The inorganic and methylmercury forms of Hg were measured accurately at low-ppb concentrations in milk and honey matrices using an Agilent 1260 Infinity HPLC coupled to an Agilent 7850 ICP-MS. Complete separation of both species was achieved in less than four minutes using a routinely available C18 column and simple mobile phase conditions.

The excellent recovery results of Hg^{2+} and MeHg spiked into milk and honey samples at different concentrations demonstrated the accuracy and precision of the method. None of the samples analyzed in this study contained Hg^{2+} or MeHg above the LODs of 0.0035 and 0.0038 ppb, respectively.

The method provides valuable information on the safety of foods such as milk and honey that are widely consumed by millions of people around the world.

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DE44344.1142361111.

Printed in the USA, June 15, 2021
5994-3628EN

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