Food Safety

Quantification of Methylmercury in Shrimp and Sugar Samples using IC-ICP-MS

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Keywords

Speciation, Methylmercury, iCAP RQ ICP-MS, ICS-6000, ICP-MS, IC, KED, Qtegra ISDS, ChromControl, Shrimp, Sugar.

Goal

The objective of this application note is to demonstrate the capabilities of Thermo Scientific™ iCAP™ RQ ICP-MS, hyphenated with Thermo Scientific™ Dionex™ ICS-6000 ion chromatography (IC), for trace level quantification of methylmercury in shrimp and sugar samples. This analysis is conducted in accordance with the Food Safety and Standards Authority of India (FSSAI) Maximum Residue Level (MRL)[1] and the United States Food and Drug Administration (USFDA) Elemental Analysis Manual[2] (EAM 4.8).

Introduction

Mercury pollution in the environment arises from both natural phenomena such as the presence of the naturally occurring mineral cinnabar as well as anthropogenic activities, such as mining and processing of primary mercury, and the use of mercury in industry and products, for example artisanal and small-scale gold mining, coal combustion, as well as improper management of mercury waste. The toxicity of mercury diers among its dierent chemical forms or species, with methylmercury being regarded as more hazardous than its inorganic form. Methylmercury is an organometallic compound, considered as a neurotoxin and most common form of organic mercury. It is generated through the biotic conversion of mercury in oceanic waters, and subsequently bioaccumulates in seafood, particularly in predatory fish. As a result, contaminated seafood such as shrimp is a major source of methylmercury exposure for humans[3]. Methylmercury can also occur as a contamination in water and soils as it can also undergo methylation by microbes. [4,5]. In this way, methylmercury could also find its way into crops used for sugar production, and hence ultimately enter a wide range of manufactured food products. India is the second largest sugar producer with average annual production of 30 million tons and one of the largest consumers with average annual consumption of 26 million tons. Sugar is consumed directly or as an ingredient in many food products. Methylmercury accumulation in sugar and its pathway is unclear, however, in food safety perspective, sugar is included in this study considering its regular consumption worldwide. Due to high toxicity of methylmercury, but also high mobility in living beings, it is essential to discriminate methylmercury from inorganic mercury or other alkylated forms. Whereas ICP-MS is a powerful and element selective detector, it is not able to retrieve any information related to the chemical form of an analyte (i.e., the species), unless it is coupled to an appropriate separation device. For the analysis of methylmercury, ion chromatography is a well-suited option for speciation analysis. To ensure public health and food safety in India, the FSSAI has set the highest permissible limit of methylmercury in all food products at 0.25 mg·kg^{-1} (Table 1). In this study ICP-MS technology, hyphenated with IC, was used to analyze methylmercury in shrimp and sugar samples.

Table 1: Target analyte with FSSAI MRL value in mg·kg^{-1}.

Analyte	FSSAI Limit
Methylmercury	0.25

Experimental

Equipment and apparatus

- iCAP RQ ICP-MS
- Dionex ICS-6000 HPIC system with Thermo Scientific™ Dionex™ AS-AP Auto sampler
- Precision balance (Aczet, CY205C, San Diego, CA)
- Vortex mixer (Thermo Scientific, P/N 88880017TS)
- Sonicator with temperature control (Fisherbrand, FB11207)
- Variable volume micropipettes (Thermo Scientific)
- Mixer grinder, (Crompton, Kurla west, Mumbai)

Chemicals and reagents

- Deionized water (18.20 MΩ·cm), Thermo Scientific™ Barnstead™ MicroPure™ Water Purification System
- L-Cysteine Hydrochloride Monohydrate, (Fisher Chemical, Q10104)
- Sodium Perchlorate, (Fisher Chemical, S/5966/50)
- Acetic Acid, (Fisher Chemical, A507-P1)
- Methylmercury (II) chloride (Sigma Aldrich,442534-5G-A)
- Potassium Hydroxide solution (Sigma Aldrich,417661-500ML)

Eluent Preparation

0.8782 g of L-cysteine hydrochloride monohydrate was weighed in a pre-cleaned 1000 mL volumetric flask. 0.14 g of sodium perchlorate and 60 µL of acetic acid were added to the volumetric flask, dissolved, and made up to the volume using deionized water. The solution pH was adjusted to 4 using 45% potassium hydroxide solution[7,8].

Standard preparation

A methylmercury solution of 1000 µg·mL^{-1} concentration was prepared from methylmercury chloride (Sigma Aldrich). A stock solution of 10 µg·mL^{-1} and 100 µg·L^{-1} were prepared from 1000 µg·mL^{-1} standard. The calibration solutions of 0.05 to 10 µg·L^{-1} were prepared from these stock solutions by diluting with the eluent solution. Since methylmercury is extremely carcinogenic, throughout the analysis, personal protective equipment such as mask, impermeable gloves, long sleeve apron, and safety goggles were used.

Sample Preparation

Shrimp and sugar samples were purchased from a local market and homogenized using an electric grinder. Aliquots of 0.5 g ± 0.05 g of the sample were weighed into 50 mL polypropylene centrifuge tubes. Spiking was performed at this stage followed by the addition of 10 mL of eluent and vortex well. Shrimp and sugar samples were spiked at four dierent levels, ranging from 0.02-0.25 mg·kg^{-1} using methylmercury stock solutions. Next, the sample volumes were made up to 50 mL each with the eluent and vortexed again. Shrimp samples were then incubated in a water bath at 60°C for 120 minutes. After cooling the sample solutions to room temperature, shrimp and sugar samples were filtered using a 0.45 µm PTFE syringe filter. The first 2 mL of the filtrates was discarded, and the remaining sample solutions were collected in vials for injection by ion chromatography.

IC-ICP-MS Analysis

Given its metal-free solvent pathway, the ICS-6000 system is perfectly suited for elemental speciation. For simple hardware connection, the PFA-LC nebulizer directly connects to the column outlet of the ICS-6000 system. To separate methylmercury, a Thermo Scientific™ Dionex™ IonPac™ CS5A cation exchange column was used, which allowed an isocratic elution of methylmercury in less than 4 minutes, using the parameters described in Table 2a & 2b. For the detection of mercury, the iCAP RQ ICP-MS was operated using kinetic energy discrimination (KED) mode. Although the appearance of polyatomic interferences is highly unlikely under this conditions, KED mode was still preferred as it allowed for an additional increase of sensitivity due to collisional focusing of the ion beam inside the QCell collision/reaction cell (CRC).

Table 2a. IC operating parameters

Parameter	Value
Column	IonPacCS5A 2 x 250 mm
Eluent	5mM L-Cysteine Hydrochloride Monohydrate, 1 mM Acetic Acid, 1 mM Sodium Perchlorate
Flow rate	0.4 mL·min^{-1}
Injection Volume	20 µL
Run time	5 min

Table 2b. ICP-MS instrument operating conditions

Parameter	Value
Plasma power (RF)	1550 W
Nebulizer gas	1.02 L·min^{-1}
Auxiliary gas	0.8 L·min^{-1}
Cool gas flow (Argon)	14.0 L·min^{-1}
CCT gas flow (He gas)	5.2 mL·min^{-1}
Dwell time	0.1 s

Data acquisition and processing

The Thermo Scientific™ ChromControl plug-in for Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) software was used for data acquisition and processing in this study. The software controls the ICS-6000 and iCAP RQ ICP-MS operations over a single user interface and simplifies the user experience throughout the complete process of speciation analysis including method setup, data interpretation and report generation. Additionally, the software enables the creation of an instrument method for ion chromatography in the LabBook file format, which can be easily integrated with the ICP-MS method, thus further simplifying the overall method setup. Data evaluation was performed using the tQuant virtual evaluation module in Qtegra software. This virtual evaluation allows to acquire transient signals, automatically recognizes, and integrates chromatographic peaks, and generates compound specific calibration curves.

Results & Discussion

Method performance:

This methodology for methylmercury analysis in shrimp and sugar samples was verified for its performance in terms of linearity, selectivity, practical limits of quantification, accuracy, and precision at four dierent concentration levels. The quality control checks, and performance parameters were verified in accordance with USFDA EAM 4.8.

Linearity

A linear calibration curve for methylmercury was plotted using standard solutions with concentration levels ranging from 0.05 to 10 µg·L^{-1}. The methylmercury chromatogram and calibration curve that were obtained are shown in Figures 1 & 2. The chromatogram showed a single distinct peak, observed after a retention time of 160 seconds. The calibration curve showed excellent linearity as demonstrated by the R^2 value surpassing 0.999.

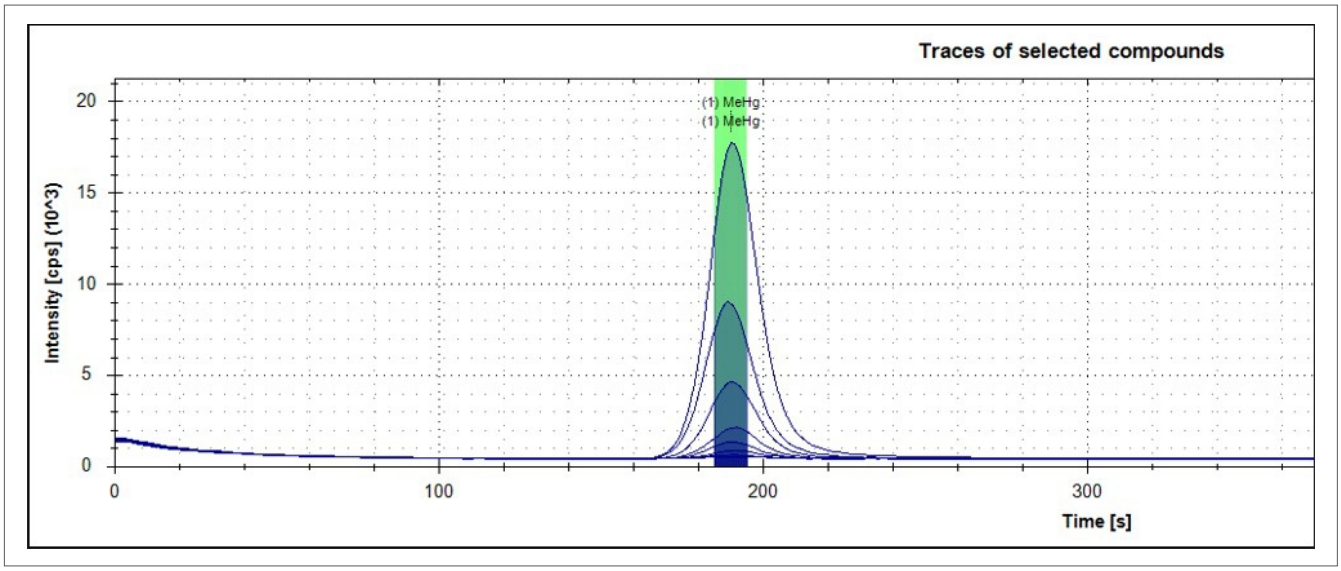

Figure 1. Methylmercury chromatogram obtained from Qtegra ISDS software over a range of 0.05 – 10 µg·L^{-1} (ppb).

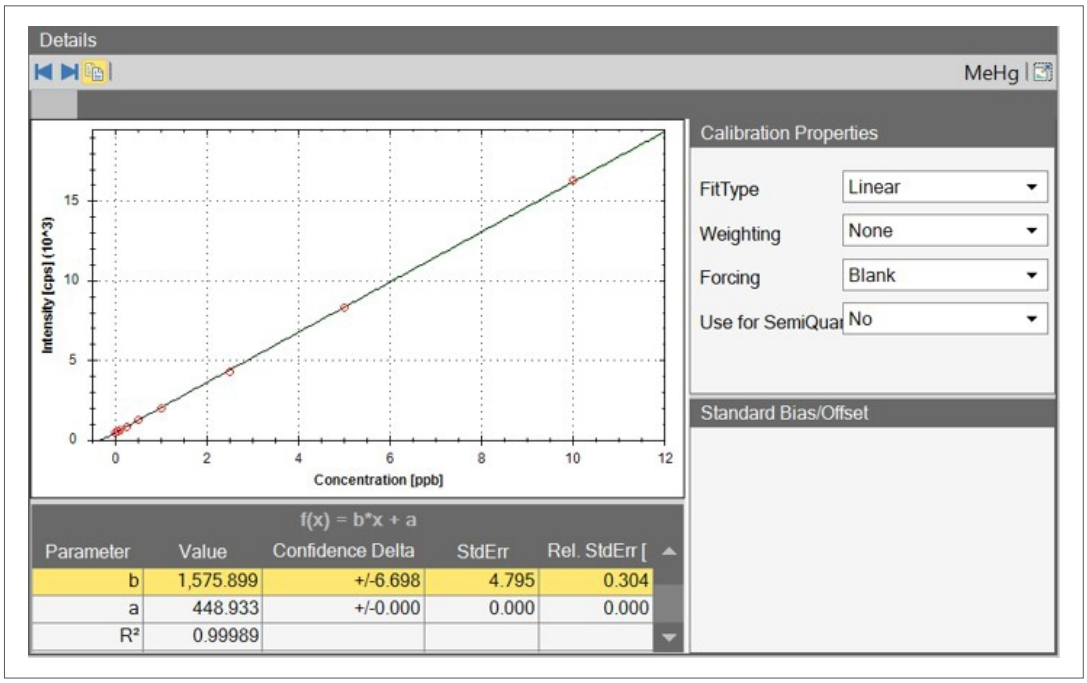

Figure 2. Calibration curve of Methylmercury obtained from Qtegra ISDS software over a range of 0.05 - 10 µg·L^{-1} (ppb).

Sample results

Shrimp and sugar samples were prepared and injected to check the inherent methylmercury. Inherent values of the shrimp and sugar samples were less than the quantification limit. To define the limit of quantification (LOQ), shrimp and sugar samples were spiked with concentrations in decreasing order. Practical LOQs were determined by verifying recoveries and selecting lower concentrations that exhibited recovery within 80-120% and RSD less than 10% RSD. Achieved LOQs were well below the target levels required for food analysis (refer level[1] of Table 3).

Accuracy and Precision:

To demonstrate the accuracy and precision of the method, a spike recovery experiment was conducted. Shrimp and sugar samples were spiked at four dierent levels, each level spiked samples were prepared and analyzed in triplicate. Percentage recoveries were calculated against the respective spiked concentrations, and the percentage relative standard deviations (% RSD) were calculated from the standard deviation of three replicates of each spiked sample. The known spiked concentration and the recovery results are tabulated below in Tables 3. The observed recoveries were within 88 -110% with RSD of <6 %. The results are in alignment with the QC check criteria of ± 20% recovery of USFDA EAM 4.8 method.

Table 3. Method performance data for shrimp and sugar samples: Spiked concentrations of methylmercury in mg·kg^{-1}, accuracy (% recovery) and precision (% RSD).

Shrimp spike level mg·kg^{-1}	Recovery (%)	Sugar spike level mg·kg^{-1}	Recovery (%)
0.025	90.9 ± 5.2	0.02	94 ± 3.5
0.050	89.9 ± 0.6	0.05	99 ± 4.9
0.100	88.2 ± 1.6	0.1	106.5 ± 1.1
0.250	92.0 ± 1.4	0.25	107.8 ± 0.44

To ensure the reliability of the generated data across the sample sequence, quality control checks using continuous calibration verification (CCV) standards were run for the shrimp and sugar samples sequence. A sequence containing both sugar and shrimp samples with a total of 67 injections (including 36 unknown samples) with a runtime of almost 6 hours was run with three CCV injections at 1 µg·L^{-1} level. The average recovery percentage of three CCV injections were 105.3 ± 4.5. The results were well within 90-110% showing that there was minimal drift between the batches of samples, eliminating the need for any sensitivity re-calibration during the analysis period.

Conclusion

- The iCAP RQ ICP-MS hyphenated with the Dionex ICS- 6000 provided an excellent solution for methylmercury quantification in shrimp and sugar samples.
- The ChromControl plugin integrated in the Qtegra ISDS software simplifies the analytical process with a single interface to control chromatography data acquisition and method setup of Chromeleon.
- Excellent linearity, repeatability and accuracy were achieved for the quantification of methylmercury, which demonstrates the capability of both instruments working in conjunction for the analysis of methylmercury in food samples.
- The method performance data are well within the criteria set by USFDA EAM 4.8.

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