Characterization of Mercury Species using HPLC-ICP-MS

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1 Introduction

The methylation of mercury takes place naturally in the receiving environment as a result of microbial activity and once methylated is biomagnified. Since fish are at a higher trophic level than other seafood commodities and are eaten in vast quantities, it is increasing important to accurately measure methylmercury in fish.

GC-ICP-MS has historically been the method of choice for the characterization of the chemical species of mercury. However, GC-ICP-MS methods can be tedious, often involving complex sample preparation and derivatization steps. HPLC-ICP-MS, in contrast, can be performed with relatively little sample preparation, dramatically improving the speed of analysis.

Since stainless steel is a known scavenger of mercury, stainless steel HPLC systems can give low-biased results when it comes to the characterization of mercury species using HPLC. For this reason, metal-free and inert systems are often preferred.

In this poster we shall present a method for the characterization and accurate quantification of MeHg in fish using the NexSAR™ Speciation Solution which is comprised of an inert and metal-free HPLC.

2 Instrumentation

All analyses were performed on a NexSAR Speciation Solution (PerkinElmer, Inc., Figure 1) comprised of a NexSAR Inert HPLC system coupled to a NexION® ICP-MS.

The NexSAR HPLC system consists of the following components, all with inert and metal-free fluid paths:

- Dual-piston binary pump – providing accurate, pulse-free delivery of the mobile phase
- Peltier-cooled autosampler – providing options for sample cooling and µL pick-up, partial loop-fill and full-loop fill injection modes
- Peltier-cooled column oven – improving analysis reproducibility and reducing mass transfer co-efficient
- Vacuum degasser – to eliminate bubbles from the mobile phase, ensuring accurate flow rates
- Post-column switching valve – to allow the optimization of the NexION ICP-MS while equilibrating the column and to support post-analysis washout and shutdown procedures.

Figure 1: NexSAR Speciation Solution

Table 1 shows the NexSAR and NexION instrumental parameters for mercury speciation.

Table 1: Instrumental and method parameters

Component/Parameter	Description/Value
Nebulizer	Glass Meinhard Concentric
Spray Chamber	Glass Baffled Cyclonic
Injector/Torch	2.0 mm Quartz injector torch
RF Power	1600 W
Nebulizer Flow	Optimized for < 2% oxides
Column	C18
Mobile Phase/pH	L-Cysteine HCl + methanol, pH 2.1
Flow Rate	1.5 mL/min
Separation Scheme	Isocratic
Injection Mode/Volume	Partial Loop / 50 µL
Column Temperature	35 °C
Analyte	^{202}Hg

3 Methodology

The procedure for analyzing fish samples is summarized in Figure 2. Here, freeze-dried CRM samples (BCR-463 , 0.1 g) were accurately weighed and extracted with L-cysteine.HCl buffer solution at 60 °C in a hot water bath via agitation for 30 minutes. The samples were then centrifuged, and the supernatants further diluted to within calibration range for analysis.

All measurements were made against external standards prepared in the L-cysteine.HCl buffer. Mixed calibration standards of 0.5, 1.0, 2.0, and 5.0 mg/L were prepared from the serial dilution of a 10 ppm inorganic mercury stock standard (PerkinElmer, Inc.) and from 0.1 g of methylmercury chloride (Fluka) dissolved in deionized water with an appropriate amount of 2-mercaptoethanol (Sigma-Aldrich).

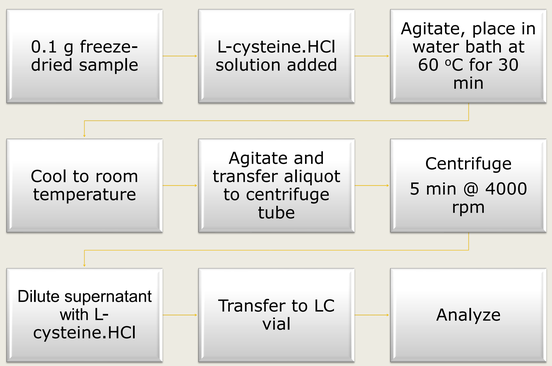

Figure 2: Sample preparation process for determination of mercury species in freeze-dried fish sample

Accuracy was ensured through the analysis of 5 replicates of a CRM sample for methylmercury and a spike recovery check was performed on inorganic mercury.

A quality control sample was measured at the end of analysis to validate the calibration curve and the stability of the method.

4 Result and Discussion

The overlayed chromatograms of the calibration standards (0.5, 1, 2 and 5 ppb) are shown in Figure 3. As can be seen, the two mercury species are completely baseline separated with extremely reproducible retention times, and a complete analysis time of 3 minutes, making this an attractive approach for high-throughput laboratories. The calibration standards were found to have a linear regression greater than 0.9999 as shown on calibration curves in Figure 4.

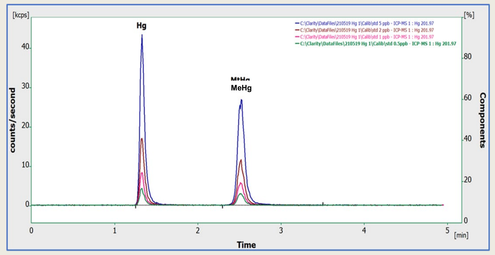

Figure 3: Chromatograms of calibration standards of mercury and methylmercury.

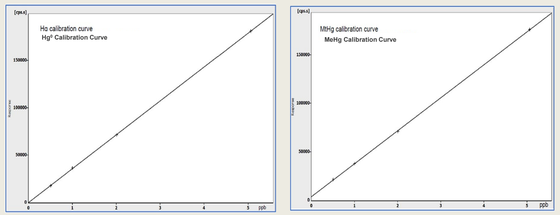

Figure 4: Calibration curve for 0.5 ppb, 1 ppb, 2 ppb and 5 ppb, demonstrating a R^2 value of 0.99998 and 0.99996 for Hg^0 and MeHg respectively.

The results in Table 2 demonstrate analyte recoveries within 10% of the true values for a freeze-dried CRM Tuna fish sample (BCR-463). The five replicate measurements demonstrate the reproducibility of the analysis with a %RSD of 2%.

Table 2: Five repeated analysis of CRM, BCR-463 Freeze-dried Tuna fish

Preparation	Certified (µg/g)	Experimental (µg/g)	% Recovery
1	3.04	2.92	96
2	3.04	2.94	97
3	3.04	2.91	96
4	3.04	3.07	101
5	3.04	3.04	100
%RSD		2%	

As previously discussed, spike recovery studies were performed by spiking 1 ppb of inorganic mercury into the CRM sample. Both the spiked and unspiked samples were measured five times, with recoveries within 10% for each measurement (Table 3).

Preparation	Experimental (µg/L)	% Recovery
1	0.97	97
2	0.96	96
3	0.99	99
4	1.02	102
5	1.01	101

Table 3: Spike of 1 ppb inorganic mercury in CRM Samples

To demonstrate the robustness of the method, a high-level spike of Hg^0 was added to a 1 ppb QC check solution. As can be seen in Figure 5, baseline resolution of Hg^0 and MeHg was maintained despite the high concentration of Hg^0. The accuracy at this concentration was verified by the >96% spike recovery.

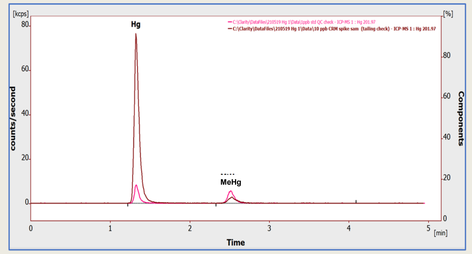

Figure 5: Overlay of high inorganic mercury spike sample with 1 ppb QC check.

5 Summary

The presented data clearly demonstrates that NexSAR Speciation Solution and the proposed method can achieve:

- The rapid and accurate measurement of both MeHg and Hg^0 in freeze dried fish within 3 minutes.
- Baseline separation of Hg^0 and MeHg
- Excellent robustness and repeatability