Mercury Determination in Tuna, CRM 463 by USDA Method CLG-MERC1.01 Using the QuickTrace® M-7600 CVAAS Mercury Analyzer

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Introduction

Throughout the world, elevated levels of mercury in freshwater and marine species of fish have been a well-documented environmental problem for many years.

The process of mercury accumulation in fish tissue begins with natural and anthropogenic sources of mercury in the bodies of water in which the fish inhabit. Microorganisms that form the base of the aquatic food chain convert elemental mercury to organic methylmercury. The methylmercury then binds tightly to the proteins in fish tissue and increases as it moves up the food chain with progressively larger fish consuming smaller fish.[1] Through the process of bio-magnification, the mercury levels of top predatory fish can increase approximately one million times in comparison to the surrounding water.[2]

Consumption of fish provides important nutrients like omega-3 fatty acids, and is a substantial source of protein,[3] but is also is typically the main route of mercury exposure for humans.[2] While mercury is a known toxin that can be damaging to the cardiovascular, immune, respiratory, gastrointestinal, and reproductive systems of humans, it is especially detrimental during nervous system development in children.[3]

Instrumentation

The QuickTrace® M-7600 is an independent stand-alone mercury analyzer from Teledyne Leeman Labs that utilizes CVAA spectrometry to produce reliable quantitative data in both simple and complex matrices. The working range of the QuickTrace® M-7600 Mercury Analyzer is < 0.5 ng/L to > 500 µg/L without the need for any physical changes to the system's configuration.

Pairing an autosampler with the QuickTrace® M-7600 allows for completely automated sample batch analysis. The twelve-roller, four-channel peristaltic pump ensures consistent sample delivery to the analyzer providing consistent online sample/reagent mixing and mercury reduction in the analyzer's closed system architecture. The reduced (elemental) sample flows over the post of the non-foaming Gas Liquid Separator (GLS) as it is continuously purged with argon or nitrogen. The elemental mercury vapor evolved from the liquid sample then passes through a Perma Pure® drying cartridge and finally to the sample cell where its absorbance is measured at a wavelength of 253.7 nm. The QuickTrace® software's control of method parameters includes gas flow control, pump control, uptake settings, a customizable smart rinse feature, over-range protection, and other options. Optimization of these parameters allows the analyst to tailor sample introduction and handling for increased or decreased sensitivity, providing the best performance at various analyte concentrations.

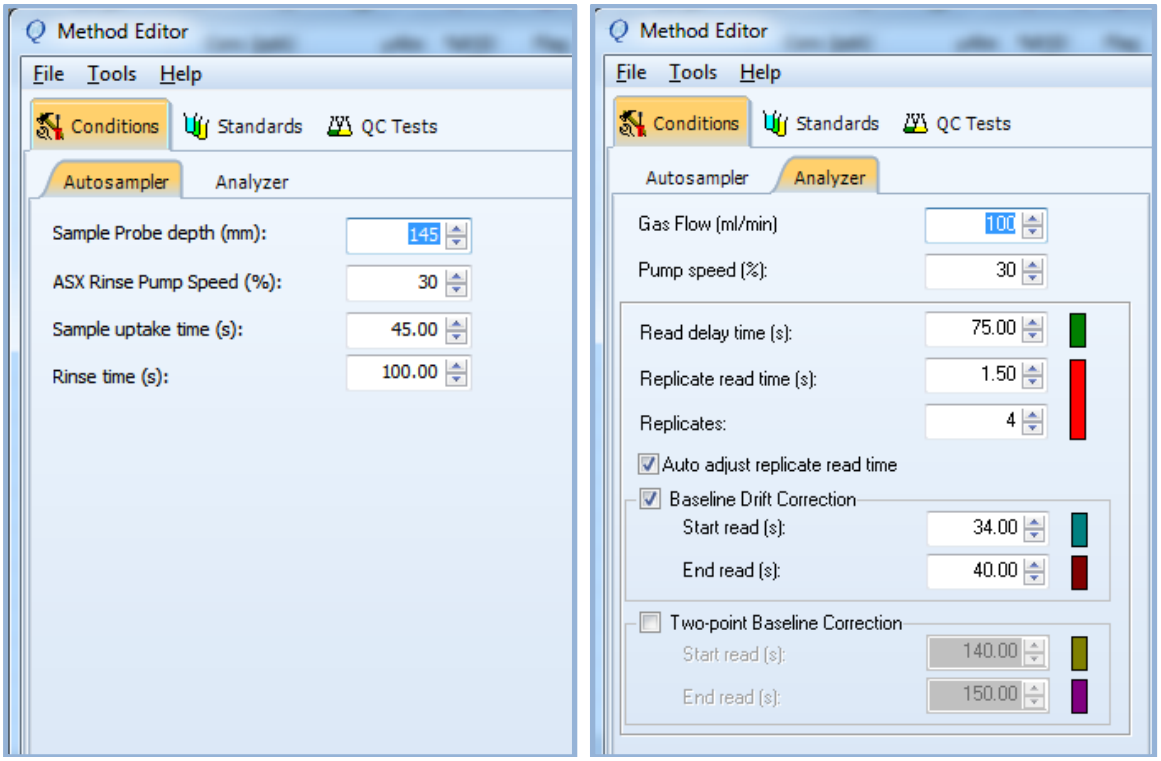
Experimental

The goal of this application note is to optimize the Teledyne Leeman Labs QuickTrace® M-7600 Mercury Analyzer instrument operating conditions for quantification of mercury at the µg/L level in Tuna, CRM 463 by USDA Method CLG-MERC1.01.

Tuna samples were digested from Certified Reference Material (CRM) 463, purchased from the European Commission Joint Research Centre Environment Institute. In their final report on the production of the CRM they stated, "The two candidate reference materials were collected in the Adriatic Sea; they were produced from tuna fishes that were rejected from the normal trade because their total mercury content exceeded 0.8 Mg/g. 302 kg and 322 kg of tuna fish, respectively for CRM 463 and CRM 464, were sliced, frozen (-25 °C) and transported to Ecoconsult in Gavirate(I). The dorsal fish muscles of each material were minced using a Quick Mill 2300 mincer with tungsten carbide blades. After mincing, the material obtained was stored frozen in high density polyethylene containers. The material was then freeze-dried until reaching a moisture mass fraction below 2.5 %. The resulting material (ca. 36 kg of each candidate CRM) was immediately frozen. The freeze-dried materials were sent to the Joint Research Centre of Ispra where they were ground using a mill equipped with zirconium dioxide balls. The ground materials were sieved using a vibrating stainless steel sieve. The fractions with particles larger than 125 /xm were discarded and the remaining materials were stored in polyethylene boxes in an argon atmosphere. The two materials were then homogenized in a mixing drum for 16 days and bottled in brown borosilicate glass bottles. A total of 1000 bottles each containing ca. 15 g of material was produced for each candidate reference material. Both materials were stored at 4 °C."[4]

As instructed by the CRM documentation, the reference material was shaken for approximately five minutes to re-homogenize the CRM prior to sample weighing. The samples were digested and analyzed in 50 mL hot-block digestion tubes. Weighed amounts of the CRM were placed in the digestion tubes and digested first with a 1:4 HNO_3/H_2SO_4 solution, followed by an additional digestion period using Potassium Permanganate and Potassium Persulfate solutions. Once cooled, the samples were reduced using 12% hydroxylamine hydrochloride, brought to a final volume of 50 mL, and re-homogenized.[5] To assure matrix-matching, the Standards and QC solutions were prepared using the same reagents and digest conditions as the samples. Appropriate aliquots of a 100 µg/L Intermediate Standard were used to prepare the calibration curve that consisted of one blank and five non-zero Standards that ranged from 0.2 µg/L to 10.0 µg/L. Online reduction of the inorganic mercury to elemental mercury was carried out by an excess of 10% stannous chloride in 7% hydrochloric acid. Once calibrated, seven sample replicates were analyzed along with the appropriate Quality Control (QC) checks which validated the instrument performance after the calibration and again after the sample replicates. Total analysis time was approximately 54 minutes, with each sample analysis lasting approximately 145 seconds (Uptake Time plus Rinse Time). Due to the relatively high concentration of the CRM, only 3.5 mL of sample was used per analysis by optimizing conditions (Figure 1). CRM 463 for total mercury is certified at 2.85 mg/kg with an uncertainty of ± 0.16 mg/kg.

Figure 1 QuickTrace® M-7600 Mercury Analyzer Operational Conditions



Calibration Standardization

Laboratory 18.2 Mohm-cm resistivity DI water was used to prepare a 3% HCl solution for Intermediate Standards dilution. A 10,000 µg/L Intermediate Standard was made from the primary commercial Standard by performing a 100x dilution using the prepared 3% HCl solution. A 100 µg/L Intermediate Standard was then made by performing a 100x serial dilution using the prepared 3% HCl diluent solution (Table I).

Table I Serial Dilutions for Intermediate Standards

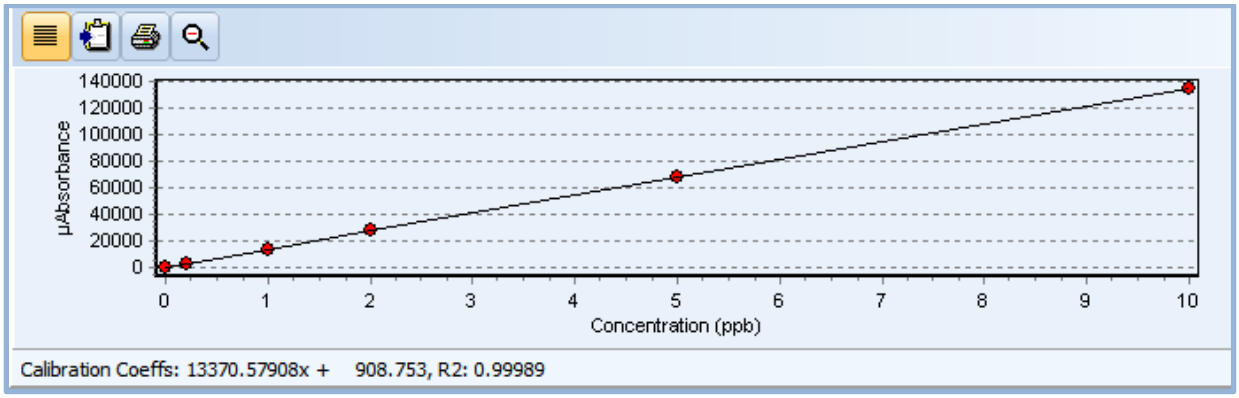
	Primary Standard	100x Dilution	100x Dilution
Hg Concentration	1,000,000 µg/L	10,000 µg/L	100 µg/L
HCl Concentration		3 %	3 %

3% HCl diluent solution was used for the analyzer calibration blank. Calibration Standards were prepared with aliquots from the 100 µg/L Intermediate Standard. The calibration blank and non-zero Standards were prepared using a final volume of 50 mL. Aliquot volumes of 0.1 mL, 0.5 mL, 1.0 mL, 2.5 mL, and 5.0 mL of the primary 100 µg/L Intermediate Standard were added to digest tubes containing the same amounts of the digest reagents used for the samples. Calibration Standard concentrations were 0.2, 1.0, 2.0, 5.0, and 10.0 µg/L in solution (Table II).

Table II Prepared Analyzer Calibration Standards	
Calibration Point	**Aliquot of Primary Intermediate Standard (100µg/L)**
Blank	0.00 mL
0.2 µg/L	0.10 mL
1.0 µg/L	0.50 mL
2.0 µg/L	1.00 mL
5.0 µg/L	2.50 mL
10.0 µg/L	5.00 mL

The Standards were introduced into the system beginning with the calibration blank and then preceded from lowest concentration Standard to the highest concentration Standard using the operating conditions shown in Figure 1. Each peak was integrated for a total of 6 seconds and a linear-fit calibration curve of absorbance versus concentration was created covering a range of 0.0 - 10 µg/L mercury as shown in Figure 2.

Figure 2 Calibration Curve



Procedure

After thoroughly shaking the CRM bottle before taking each aliquot, ~ 0.060 g of the CRM was added to each of 9 digestion tubes to create 7 sample replicates for the statistical analysis, and 2 replicates for matrix spike preparation. The exact weight of each tube was then recorded. The Standards, QC Checks and CRM samples were then digested, first with 2.5 mL of a 1:4 HNO_3/H_2SO_4 solution for 30 minutes at 80 °C, followed by an additional digestion period using 7 mL of a Potassium Permanganate solution (5%) and 4 mL of a Potassium Persulfate solution (5%) for 90 minutes at 30 °C. Once cooled, all solutions were reduced using 1.25 mL of a 12% hydroxylamine hydrochloride, brought to a final volume of 50 mL, re-homogenized and allowed to sit for a minimum of five minutes.[5] The digestion tubes were then organized in proper sequence in the test tube rack, placed in the autosampler and analyzed. Inorganic mercury in each tube, starting with the calibration and initial QC checks was reduced to elemental mercury with online excess addition of 10% stannous chloride in 7% hydrochloric acid. Peak height of the solution in each digest tube was integrated for 6 seconds.

After successful calibration and initial QC Checks (Initial Calibration Verification, Initial Calibration Blank, and the Laboratory Control Sample (LCS) prepared from a second source commercial Hg standard), the 7 reps of the CRM were similarly analyzed. Lastly, a Matrix Spike and Matrix Spike Duplicate were analyzed to identify any method inconsistencies and a Continuing Calibration Verification (CCV)/Continuing Calibration Blank pair was analyzed to verify the instrument's stability.

The ICV, CCV and LCS were prepared with a 1.0 mL aliquot of a 100 µg/L Intermediate Standard to yield concentrations of 2.0 µg/L. The Matrix Spike and Matrix Spike Duplicate were each prepared with 1.0 mL aliquots of the primary 100 µg/L Intermediate Standard to give spike concentrations of 2.0 µg/L (recoveries were MS = 100.9%; MSD = 103.5%).

Results

Using the Teledyne Leeman Labs QuickTrace® M-7600 Mercury Analyzer for measurement of mercury at µg/L levels was effective and obtained reliable quantitative data. By optimizing carrier gas flow, pump speed, sample uptake and rinse time in the QuickTrace® software, analysis of samples over a broad dynamic range was possible and total mercury in Tuna, CRM 463 was easily recovered.

Method development included calibration, quality controls, and spike recoveries. Seven replicates of the digested CRM were analyzed. The results were corrected for moisture content and mean concentration and standard deviation were calculated. An analytical result of 2.75 mg/kg ± 0.18 dry mass basis was obtained and is illustrated in Table III and Figure 3. A representative peak is shown in Figure 4.

Uncertainty values correspond to a level of confidence at 95% and were calculated for the seven replicates of the CRM analyzed. CRM 463 has a certified concentration of 2.85 mg/kg with an uncertainty of ±0.16 mg/kg.

Table III Tuna, CRM 463, 2.85 mg/Kg ± .16

Sample	Result
1	2.864 mg/Kg
2	2.592 mg/Kg
3	2.673 mg/Kg
4	2.752 mg/Kg
5	2.801 mg/Kg
6	2.801 mg/Kg
7	2.772 mg/Kg
Mean = 2.751	
Uncertainty = 0.178	
n = 7 Replicates	STDEV = 0.091 RSD% = 3.306

Figure 3 Results with Uncertainties

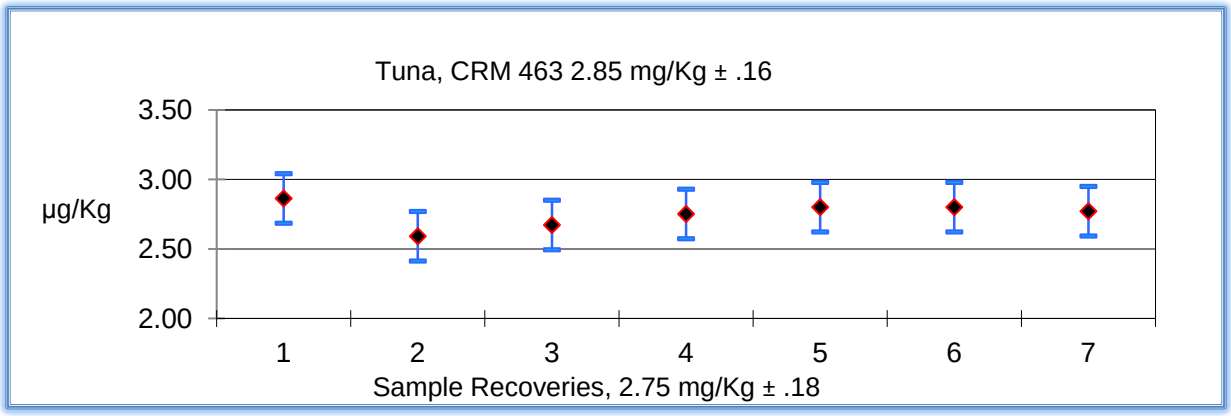
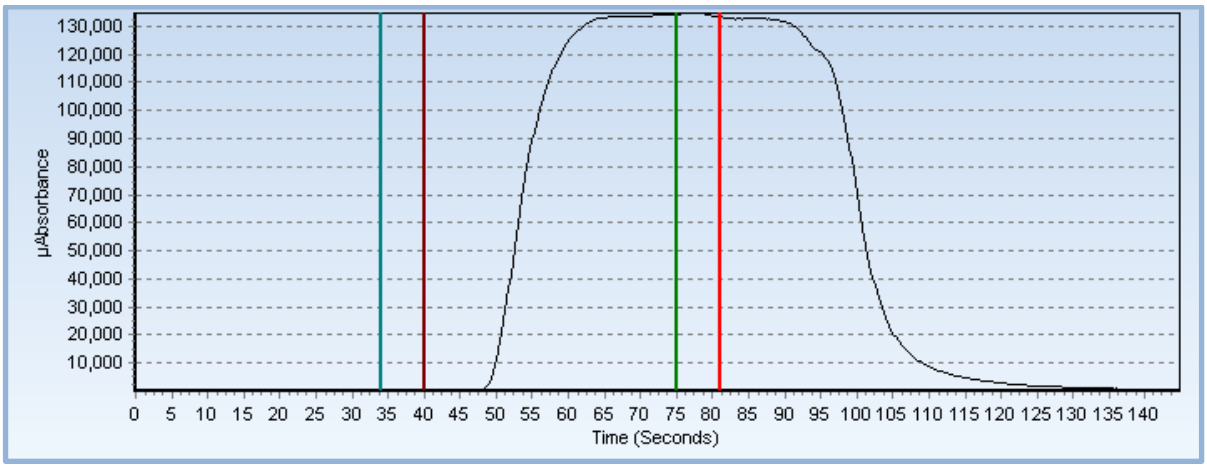


Figure 4 Representative Peak



The Quality Control (QC) Standards and Matrix Spikes are listed in Table IV with their recoveries. In addition to the ICV and CCV, an LCS, was prepared from a second-source commercial Hg Standard, and analyzed before the samples. After the initial seven replicates, the Matrix Spike and Matrix Spike Duplicate were also analyzed. The spikes were prepared by addition of 1.0 mL aliquots of the primary 100 µg/L Intermediate Standard.

Table IV Mercury Determination in Tuna, Quality Controls

Quality Control Standards		Resulting Concentration	Recovery
ICV - Pre-Sample	2.00 µg/L	2.02 µg/L	101.0 %
LCS - Pre-Sample*	2.00 µg/L	2.06 µg/L	103.0 %
CCV - Post-Sample	2.00 µg/L	1.98 µg/L	99.0 %
Matrix Spikes		**Resulting Concentration**	**Recovery**
Matrix Spike	2.00 µg/L	2.018 µg/L	100.9 %
Matrix Spike Duplicate	2.00 µg/L	2.070 µg/L	103.5 %

* Prepared from a 2nd source commercial Hg Standard.

Conclusion

Contamination from many sources can present problems and lead to inaccurate results. Because of this concern, careful attention was given to minimize contamination in reagents, acids, and the deionized water. Through method development, parameter optimization, and careful sample preparation, the QuickTrace® M-7600 mercury analyzer quantified total mercury in CRM 463 and produced reliable quantitative data.

The QuickTrace® M7600 was capable of analyzing and determining total elemental mercury (Hg^0) concentration in Tuna (CRM 463) following the guidance in USDA Method CLG-MERC1.01 and the operating conditions in Figure 1. Additionally, the autosampler permitted convenient, unattended analysis.

References

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