Mercury Determination in Fish and Shellfish Tissues, USEPA Method 7473, Using the Teledyne Leeman Labs Hydra II_C Combustion CVAAS

Bruce MacAllister, App. Chemist and John McQuatters, Product Specialist, Teledyne Leeman Labs, Hudson, NH

Introduction

Throughout the world, elevated levels of mercury in fish and shellfish has been a well-documented environmental problem for many years.

The process of mercury accumulation in marine animal tissue begins with natural and anthropogenic sources of mercury in the bodies of water in which the animals 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 the animals tissue and increases as it moves up the food chain with progressively larger predators consuming smaller prey.[1] Through the process of bio-magnification, the mercury levels of top predators can increase approximately one million times in comparison to the surrounding water.[2]

Consumption of fish and shellfish provides important nutrients like omega-3 fatty acids, and is a substantial source of protein,[3] but is also 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]

This application note will demonstrate the ability of the Teledyne Leeman Labs Hydra II_C Mercury Analyzer to determine total mercury by USEPA Method 7473 in a variety of marine fish and shellfish species, as well as aquatic plant tissue. Method 7473 is approved for both laboratory and field analysis for mercury in solids, semi-solids and solutions using thermal decomposition, amalgamation and Atomic Absorption (AA) spectroscopy.[4] Four Certified Reference Materials (CRMs) were analyzed and five unknown samples were evaluated on the instrument.

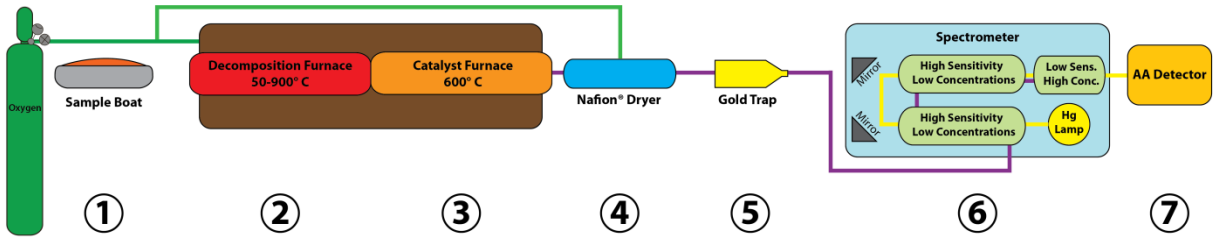
Instrumentation

The Teledyne Leeman Labs Hydra II_C is a fully automated mercury analyzer that measures mercury in diverse sample matrices directly with little to no sample preparation. Instead, it employs sample combustion (thermal decomposition), mercury concentration by gold amalgamation and detection by Cold Vapor Atomic Absorption Spectroscopy (CVAAS). The instrument operates using a universal power supply compatible with main power input 110/220 V, 50/60 Hz power outlet and oxygen supplied at 15 to 20 psig. All instrument operating parameters (including furnace/catalyst temperature, gas flows, and autosampler control) and sample cycle stages are computer controlled for ease-of-use. Through proper selection of the instrument's operational parameters, mercury determination can be performed on a diverse sample set across a dynamic range consisting of absolute per sample mass of mercury from 0.001 ng to 1500 ng. The Teledyne Leeman Labs *Hydra II_C Mercury Analyzer Operator's Manual* provides extensive guidance on parameter optimization.

Figure 1 depicts the analytical process with gas flowing from left to right. The Hydra II_C mercury analyzer employs combustion of a sample at high temperatures with oxygen. The gases resulting from this decomposition are carried through a heated catalyst to remove halogens, nitrogen oxides, and sulfur oxides. The remaining combustion products, including elemental mercury (Hg^0), are swept through a dryer and then to a gold amalgamation trap which captures the mercury while allowing the other gases to pass through. The amalgamator is then heated to release the accumulated Hg^0 into a carrier gas, which transports it into the Cold Vapor Atomic Absorption Spectrometer. The transient signal is measured in series by a high-sensitivity cell followed by a low-sensitivity cell.

The two peaks are integrated and reported against the best calibration of the two cells available. The use of two cells provides the best detection limit with a wider dynamic range than that provided by a single optical cell path length. Waste gases exiting the system are chemically "scrubbed" with a carbon trap or exhausted safely out of the lab at the end of the process.

Figure 1 Hydra IIC Mercury Analyzer Principle of Operation



Experimental

The Hydra IIC is operated by the Teledyne Leeman Labs Envoy software that provides sample specific control of the system. The software's parameters can be optimized for sample drying and decomposition (the drying step and the combustion step are both customizable for temperature and duration) for each individual sample to facilitate accurate analysis of mercury in various sample matrices. For this experiment, the system was calibrated up to 400 ng. The operating parameters for the Hydra IIC used for sample analyses are shown in Table I.

Note: CRM sample preparation was minimal. CRMs bottles were mixed initially and between each sample weighing to assure and maintain homogeneity.

Calibration Standardization

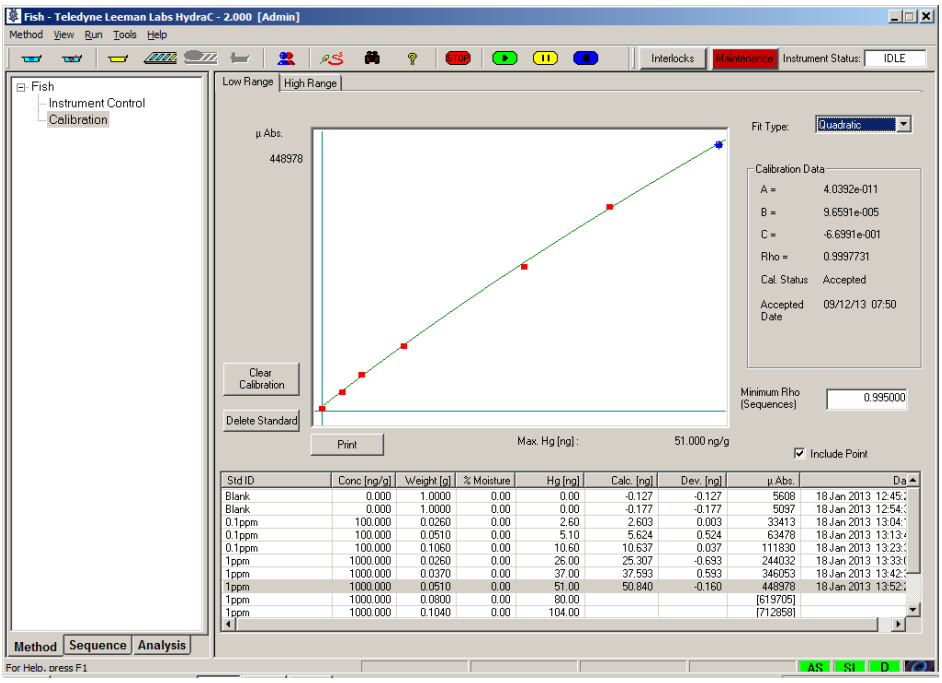
Nickel boats were cleaned just prior to calibration by running them through the same method created for the experiment, with any unnecessary dry time removed.

While the boats were being cleaned, intermediate standards were prepared by serial dilutions of a 1000 mg/L certified primary standard purchased from LabChem®. Laboratory 18 Mohm DI water and concentrated, reagent grade HNO_3 were used to create appropriate standard dilutions with a resulting nitric acid concentration of 1.0%.

Using the pre-cleaned nickel boats, aliquots from the mercury standards were introduced into the system to create quadratic-fit calibration curves in the low calibration (high sensitivity) cell and the high calibration (lower sensitivity) cell. The operating conditions for calibration and subsequent analyses are shown in Table I. Using these conditions, the system developed two curves covering a range of 0.0 to 400 ng of mercury. The Envoy software displays calibration plots as mass of mercury in nanograms versus micro absorbance of Hg. Results are displayed in both mass(ng) and concentration(ng/g) of Hg. The resulting calibration curves are presented in Figure 2 and Figure 3**.**

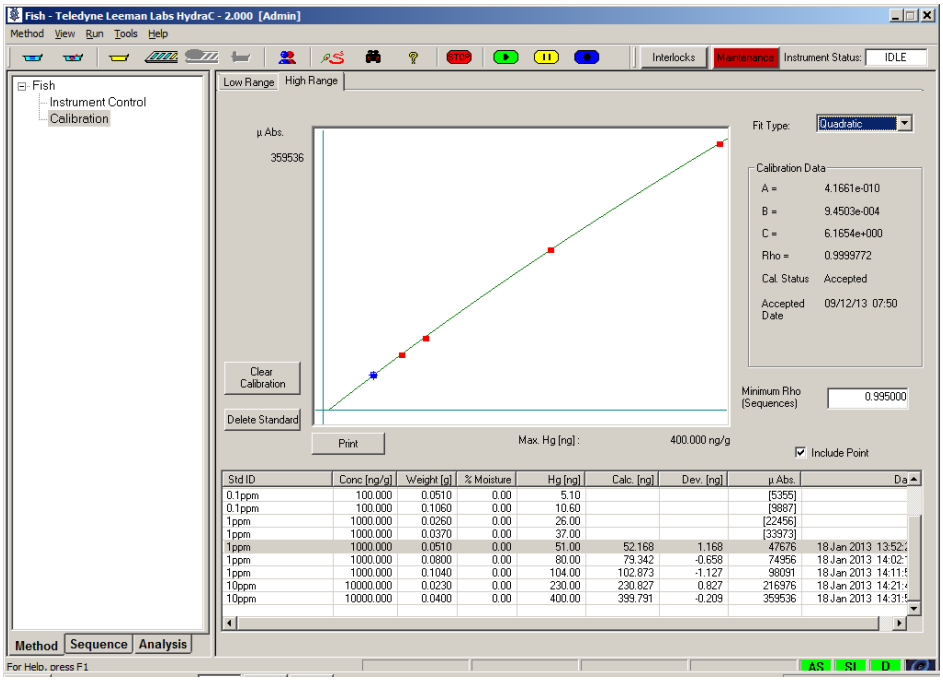
Table I System Parameters		
Sample	**Method Parameter**	**Parameter Value**
Various Fish and Shellfish Tissue	Drying Temperature (˚C)	300
	Drying Time (seconds)	70
	Decomposition Temperature (˚C)	800
	Decomposition Time (seconds)	250
	Catalyst Temperature (˚C)	600
	Wait Time (seconds)	60
	Amalgamator Temperature (˚C)	600
	Amalgamator Time (seconds)	30
	Integration Time (seconds)	100
	Oxygen Flow (ml/min)	250
Note: As a general rule, when calibrating with or analyzing liquids, a ratio of 70 seconds per 100 microliters (at the typical temperature setting of 300 degrees Celsius) is suggested for the Dry Time parameter.		

Figure 2 Low Range Calibration



Sales/Support: 800-634-9942 • **Main**: 603-886-8400
110 Lowell Rd, Hudson, NH 03051
www.teledyneleemanlabs.com

Figure 3 High Range Calibration



Procedure

The same procedure used to clean the calibration standard boats was used to clean sufficient empty nickel boats for CRM/unknown sample analysis. With thorough mixing of the CRM bottles between each sampling, ~0.050 to 0.100 grams of CRM was transferred into the pre-cleaned nickel boats. Exact weights for each individual CRM or sample were recorded and entered into the Envoy software. A total of three aliquots per CRM/sample were prepared in this manner and then loaded onto the boat shuttles for unattended analysis. The integrated cover over the shuttles was closed to prevent airborne particulates from contaminating the samples while they waited for analysis.

It is important to note that an Envoy time-saving feature can be employed during sample loading. Once sufficient samples have been weighed and the weights entered, the analyzer can begin the analytical run while the remaining samples are weighted and added to the end of the sequence.

Alternatively, samples can be analyzed individually by loading the weighed sample boat directly onto the injector and entering the weight when prompted by the Envoy software.

Results

Using the Teledyne Leeman Labs Hydra II_C Mercury Analyzer for measurement of mercury in these reference materials resulted in successful correlations with the certified values. Three replicates of each CRM and Unknown sample were analyzed using the instrument operating conditions shown in Table I. The mean concentrations (in ng/g), standard deviations and recoveries for the CRM's were calculated and are listed in Table II. The results for the five unknown samples are presented in Table III with the average weight, mean result and replicate standard deviation.

Table II Mercury Determination in Various Fish and Plant Tissues - CRM's					
Certified Reference Material (CRM)		Mean Conc. (ng/g)	Std. Dev.	Certified Conc. (ng/g)	Recovery (%)
BCR060	Aquatic Plant	320	2.8	340	94.1
BCR463	Tuna	2841	22.1	2850	99.7
DOLT3	Dogfish Liver	3398	23.2	3370	100.8
DORM2	Dogfish Liver	4311	36.7	4640	92.9

Table III Mercury Determination in Various Fish Tissues - Unknowns			
Sample	Average Weight (g)	Mean Conc. (ng/g)	Std. Dev
Bonito Flakes	0.106	101.0	7.3
Cod	0.124	59.24	5.7
Clam	0.100	41.98	1.3
Trout	0.120	23.16	1.0
Tuna	0.109	390.0	7.3

Conclusions

The Hydra IIc Combustion CVAAS Mercury Analyzer is capable of analyzing and determining total elemental mercury (Hg^0) concentrations in a varied set of fish and plant tissue using the guidance in EPA Method 7473 using the method parameters in Table I. Additionally, the integrated autosampler provides a fast, simple and convenient approach for the analysis of mercury. The use of the combustion technique (decomposition) virtually eliminates sample preparation as well as the production of hazardous chemical wastes resulting in reduced technician time and operating expenses.

References

1. Wooltorton, Eric. Facts on Mercury and Fish Consumption. *Canadian Medical Association Journal*. 2002, 167 (8), p 897. [Online] http://www.cmaj.ca/content/167/8/897.full (accessed April 04, 2017)
2. Gandhi, Nilima; Tang, Rex W. K.; Bhavsar, Satyendra P; and Arhonditsis, George B. *Fish Mercury Levels Appear to Be Increasing Lately: A Report from 40 Years of Monitoring in the Province of Ontario, Canada.* Environmental Science & Technology, 2014, 48 (10), 5404-5414 DOI: 10.1021/es403651x. [Online] http://pubs.acs.org/doi/abs/10.1021/es403651x (accessed April 04, 2017)
3. United Nations Environmental Programme (UNEP): DTIE Chemicals Branch and World Health Organization (WHO): Department of Food Safety, Zoonoses and Foodborne Diseases; Inter-Organizational Programme for the Sound Management of Chemicals (IOMC). *Guidance for Identifying Populations at Risk from Mercury Exposure*. Geneva; Switzerland, August, 2008. [Online] http://www.who.int/foodsafety/publications/chem/mercuryexposure.pdf (accessed April 04, 2017)
4. United States Environmental Protection Agency (USEPA). Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry - EPA Method 7473-2007 - Revision 0. [Online] http://www.epa.gov/wastes/hazard/testmethods/sw846/pdfs/7473.pdf (accessed April 04, 2017)