TELEDYNE LEEMAN LABS
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APPLICATION NOTE:	AN2102
TECHNOLOGY:	Hg
INDUSTRY:	ENV

Selective Extraction with Combustion Atomic Absorption (CAA) Detection for Methyl Mercury Using the Hydra II_C CAA Combustion Mercury Analyzer

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Introduction

Total inorganic and methylmercury (MeHg) determination is an important tool for monitoring mercury uptake and bioaccumulation in ecosystem flora and/or fauna. By quantifying environmental mercury contamination, the risk of human exposure can be adequately assessed.

The heavy metal mercury (both organic and inorganic) is known to have toxic effects on human nervous, digestive and immune systems, and is a threat to fetal and early childhood development, resulting in impaired neurological development.[1]

Quantitative and/or qualitative research of mercury species (both organic or inorganic) in environmental samples is a valuable resource that can be used to validate modeling of mercury transport and fate in the global environment. In some cases, knowing the origin species can aid in mitigating and/or preventing harmful exposure to the methyl mercury produced by bioaccumulation.

This application note will demonstrate the capability of the Teledyne Leeman Labs Hydra II_C mercury analyzer for methyl mercury determination by selective extraction with combustion atomic absorption (CAA) detection.

A novel method developed by the Ministry of Food and Drug Safety of Korea, "*Notice No. 2018-98: Methyl Mercury Test Method 9.1.9.2 - Law 2*", specifies selective extraction with CAA detection for the determination of MeHg, and was used for this study.[2] This method applies to all foods in Korea where the standard "specification of MeHg" has been set.

The analytical principles of the method consist of pretreatment of a sample of known weight with HCl (3:1 HCl/ DI water), NaCl (25% w/v DI water) and toluene, which is then subjected to centrifugation at 3000 RPMs. The MeHg is then selectively extracted from the toluene fraction into an L-cysteine solution and the L-cysteine solution combusted. Post combustion, mercury is collected using gold amalgamation, and then liberated as elemental mercury (Hg^0) using a desorption process. Lastly, quantification of the collected mercury is performed using atomic absorption and the quantity of MeHg calculated using mass conversion of the analytical results on a dry matter basis.

Certified reference material (CRM) BCR®-463 Tuna Fish was used for samples in this study and has a reported MeHg concentration at CH_3Hg^+ 3.04 µg/g ±0.16. A diverse set of standard reference materials (SRMs) was also used for calibration verification to test the analytical stability of the analyzer.

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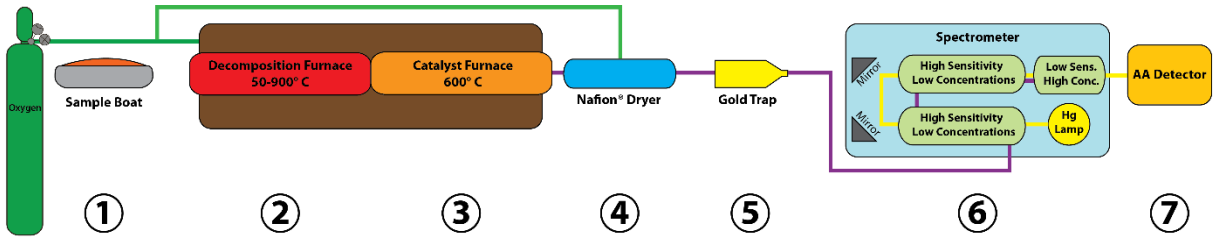
Instrumentation

The Hydra IIC combustion atomic absorption (CAA) mercury analyzer is an independent, stand-alone analyzer that uses atomic absorbance (AA) spectroscopy to obtain reliable quantitative data from simple to complex sample matrices. By using direct combustion combined with a proprietary catalyst, interfering compounds such as sulfur and nitrogen oxides are removed. The combustion process entails a continuous linear flow of carrier gas through the entire system, including both the decomposition and catalyst furnaces.

Figure 1 shows the system components and processes of the Hydra IIC CAA mercury analyzer. First a weighed sample (1) is introduced to a decomposition furnace (2) with oxygen (or air) flowing over the sample. The furnace temperature is then raised in two stages to first dry the sample, then to combust or decompose the sample.

The gases resulting from the decomposition are carried through a catalyst furnace to remove halogens, nitrogen oxides, and sulfur oxides (3). The remaining combustion products, including elemental mercury (Hg^0), are swept through a Nafion® dryer (4) and then to a gold trap (5) that captures the mercury while letting the other gases pass through. The gold trap is then heated to release the Hg^0 into a carrier gas which transports it to the spectrometer (6). In the spectrometer, the gas is directed through two 5" high-sensitivity (for low concentrations) optical cells, and then one 1" low-sensitivity (for high concentrations) optical cell. The light from the Hg lamp travels through the cells using a system of reflective mirrors. The transient signal is measured by the AA Detector (7). 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 a detection limit provided by a single optical cell path length. Waste gases exiting the system are chemically scrubbed with an activated carbon mercury trap or exhausted out of the lab at the end of the process.

Figure 1 Teledyne Leeman Labs Hydra IIC Process Diagram



The Hydra IIC has an analytical working range from 0.001 ng to >1500 ng. This dynamic quantitative range allows mercury concentrations to be determined in a broad range of sample matrices without dilution or preconcentration. The analyzer was equipped with an autosampler for hands-free sample batch analysis.

Moisture Removal

For this application, the Hydra IIC CAA mercury analyzer was equipped with an external, in-line drying system as demonstrated in Application Note – *AN1701 "Enhanced Moisture Control for USEPA Method 7473 Using the Hydra IIC, CAA Mercury Analyzer*" (viewable Here) to remove excess water vapor prior to the detector cells.[3] The drying system consisted of an "off the shelf" Teledyne Leeman Labs chemical drying tube (SP5653) filled with magnesium perchlorate ($Mg(ClO_4)_2$) sieved on a #20 sieve (pore size 0.850 mm). The filled tube was easily installed between the gold amalgamation trap/Nafion® dryer and the detector cells due to the Hydra IIC's modular design. The use of this external drying system improves peak shape due to modulation of the Hg evolving from the gold amalgamation trap.

Experimental

The Hydra IIC CAA mercury analyzer is operated by the Envoy software, which provides method specific control of the system. Parameter optimization allows for the quantitation of mercury in the trace ng range. The goal of this application is to determine optimized instrument and extraction parameters for the determination of total mercury in aqueous extractions and demonstrate those settings to be effective. Once the total mercury has been determined, a mass conversion is used to determine the sample's MeHg content.

Laboratory Apparatus

1. 40 mL clear volatile organic analysis (VOA) vial (16 vials were used for this application)
2. Vial rack
3. Centrifuge capable of 3000 RPM rotation at 20 °C
4. Disposable, single use, plastic transfer pipettes
5. Volume dependent air displacement pipettes and tips, 10 mL, 5 mL and 0.1 mL
6. 25 mL glass graduated cylinder
7. Top loading balance, 1 mg readability
8. Small spatula
9. ARM SHAKER, Model ASH3; FINEPCR®, 28-9, Heungan-daero 27 Beon-gil, Gunpo-si, Gyeonggi-do, 15809, Korea (http://www.finepcr.com)
10. Custom shaker rack for 40 mL vial (PN R-407); FINEPCR®

Reagents

1. A source of either distilled or deionized (DI) water; DI water should be >18.18 MΩ-cm resistivity
2. ACS Grade toluene
3. ACS Grade sodium chloride crystals
4. ACS Grade sodium acetate trihydrate
5. ACS Grade sodium sulfate anhydrous
6. Hydrochloric acid, 99.99% trace, metal-grade
7. L-Cysteine hydrochloride monohydrate 99% (F.W 175.64)

Solution Preparation

1. HCl (3:1 HCl/ DI water)
2. NaCl 25% (w/v DI water)
3. L-Cysteine solution:
    1. L-cysteine hydrochloride monohydrate, 1.0 g
    2. Sodium acetate trihydrate, 0.8 g
    3. Sodium sulfate anhydrous, 12.5 g
    4. 100 mL DI or distilled water (add each chemical with gentle swirling, after final addition, cap and shake to dissolve)

Sample Preparation

1. Weigh sample into 40 mL, clear VOA vial, 0.25 to 1 g (record weight). For convenience:
    1. Label VOA vials, E1, E2, etc. for “extractions”
    2. Label VOA vials SX1, SX2, etc. for “final solution”
2. Add 10 mL of 25% NaCl solution
    1. Cap and shake for approximately 2 minutes
3. Add 4 mL of HCl solution
4. Add 15 mL of toluene
    1. Use a glass graduated cylinder
    2. Cap and shake for approximately 2 minutes

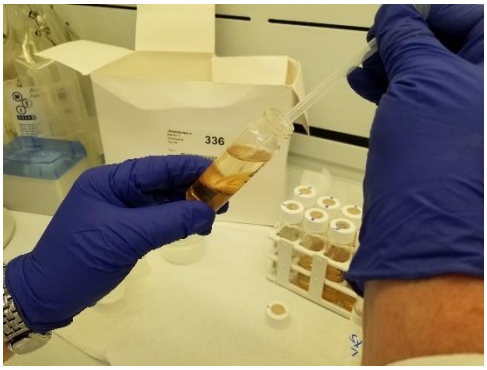
Note: Solution fractions will begin to present

5. Place capped solution in centrifuge and process at 3,000 RPM for 20 minutes

Note: Hold temperature between 20 to 25 °C

6. Carefully remove the vials from the centrifuge without disturbing solution layers
7. Transfer top layer (toluene) from “extraction” vial to the corresponding 40 mL, clear VOA “final solution” vial (e.g. vial E1 to vial SX1)

Note: Toluene transfer works best with the VOA vial slightly tilted. Disposable single-use plastic transfer pipettes work well for this task.



Note: If the pipette pulls a small amount of the aqueous portion at the solution interface when extracting the toluene layer, allow separation to take place in transfer pipette. Discard the bottom layer and add the top layer to vial.

8. Add 5 mL of L-cysteine to each vial of extracted toluene
    1. Cap and shake vigorously for 10 minutes
    2. Let extractions separate for ~10 minutes
9. The analytical portion of the sample is the L-cysteine portion (bottom layer)

Sample Percent Moisture Determination

All solid and semi-solid samples require a percent moisture determination in order to calculate final results.

Sample Boat Cleaning/Conditioning

To ensure the cleanliness of each sample boat prior to calibration and sample analysis, the vessels were subjected to a fast-analytical protocol consisting primarily of a combustion and gold trap elution stages.

Calibration and Instrument Parameters

Calibration standards consisted of 0.001, 0.01, 0.1, 1.0 and 10.0 mg/L prepared by serial dilution (1 to 10) in ultra-pure 1% HNO_3. The ultra-pure 1% HNO_3 was prepared using 18.2 MΩ DI water and double-distilled, concentrated HNO_3. The serial dilutions began with a 1 to 10 dilution of a 1000 mg/L certified mercury standard preserved in 12% HNO_3. A 1 to 10 dilution of the previously made dilution followed and subsequent dilutions continued until a final concentration of 0.001 mg/L was achieved (Table I). The instrument calibration sequence was prepared by varied weights of each standard (Table II). Optimized instrument parameters are shown in Table III The final mass of standards was 0.1, 0.5, 1, 5, 10, 20, 50, 100, 200, 400, 600, 800 and 1000 ng of Hg. (Figure 2 and Figure 3).

Hydra II_C (Calibration Preparation) - Intermediate Calibration Standard Preparation

Table I Intermediate "Working" Calibration Standard Preparation

Standard Concentration	Stock 1000 mg/L	100 mg/L	10 mg/L	1 mg/L	0.1 mg/L	0.01 mg/L	0.001 mg/L
1% HNO_3	-	9 mL	9 mL	9 mL	9 mL	9 mL	9 mL
Standard Added	-	1 mL Stock	1 mL 100 mg/L	1 mL 10 mg/L	1 mL 1 mg/L	1 mL 0.1 mg/L	1 mL 0.01 mg/L
Total Volume	-	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL
~% acid	12%	2.2%	1.22%	1.12%	1.1%	1.1%	1.1%

Hydra II_C (Calibration Preparation) - Calibration Standard Preparation in Quartz Boats

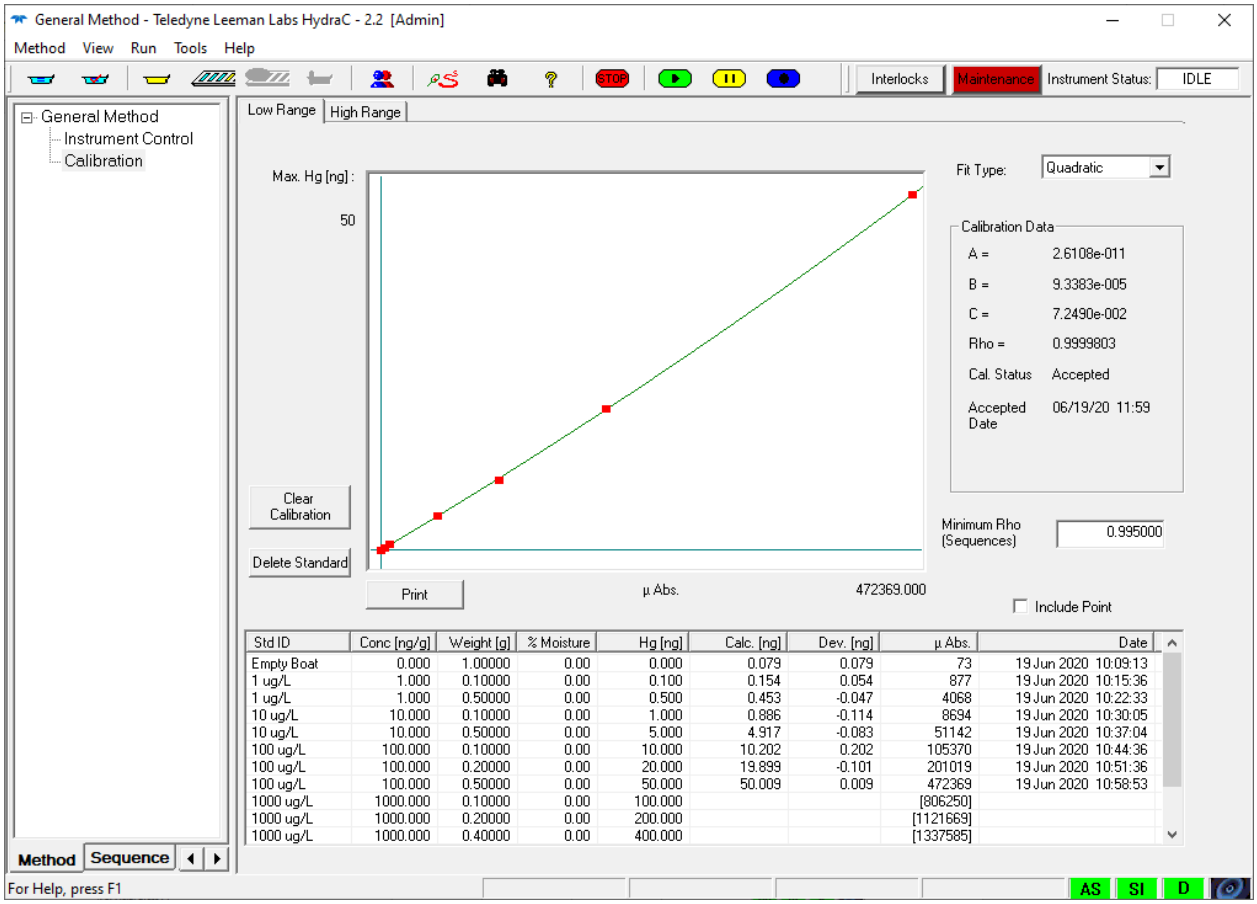
Calibration standards were prepared by varying the volume/weight of the appropriate intermediate standard as shown in Table II.

Table II Instrument Calibration Standards Preparation

Standard (ng Hg)	Inter. Standard/Volume	~Response Expected	Analytical Cell
Blank	Empty Quartz Boat	220	Low
0.1 ng	0.001 mg/L - 100 µL	1390	Low
0.5 ng	0.001 mg/L - 500 µL	5548	Low
1 ng	0.01 mg/L - 100 µL	13785	Low
5 ng	0.01 mg/L - 500 µL	68193	Low
10 ng	0.1 mg/L -100 µL	140309	Low
20 ng	0.1 mg/L - 200 µL	268815	Low
50 ng	0.1 mg/L - 500 µL	55416	High
100 ng	1 mg/L - 100 µL	111838	High
200 ng	1 mg/L - 200 µL	223741	High
400 ng	1 mg/L - 400 µL	428757	High
600 ng	10 mg/L - 60 µL	618840	High
800 ng	10 mg/L - 80 µL	759222	High
1000 ng	10 mg/L - 100 µL	868711	High

Table III Hydra II_C CAA Method Parameters and Instrument Settings			
Parameter	**°C**	**Seconds**	**Other**
Drying	200	60	
Catalyst	600	20	
Decomposition	800	150	
Oxygen Flow			350 mL/min
Integration		80	
Amalgamator	700	30	
Ramp Time*		60	TempRamp 60
Nafion Temp*	~70		NafionFurnaceTemp 600
Gold Trap Hold*	~175		EluteWarmTempOverride 175
µ Absorbance level cut-off*			LowPeakAbsLimit 380000
* This parameter revised in the Envoy software startup.ini file.			

Figure 2 "Typical" High Sensitivity Calibration Curve



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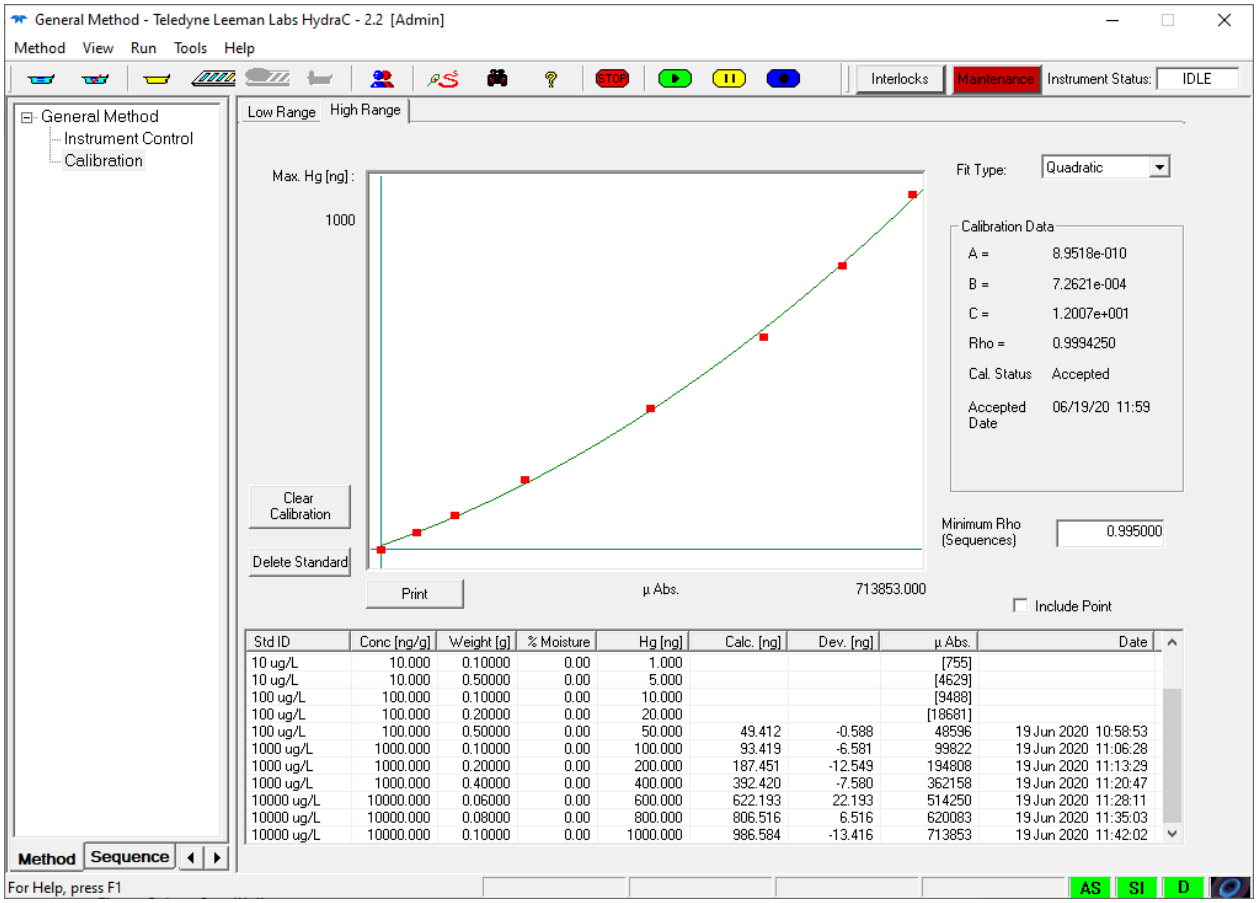
A single blank and 50 ng Hg standard was applied to both calibration fits.

Low Concentration	Blank, 0.1, 0.5, 1, 5, 10, 20, 50 ng
High Concentration	Blank, 50, 100, 200, 400, 600, 800, 1000 ng

Figure 3 "Typical" Low Sensitivity Calibration Curve



Analytical Procedure

Instument Startup

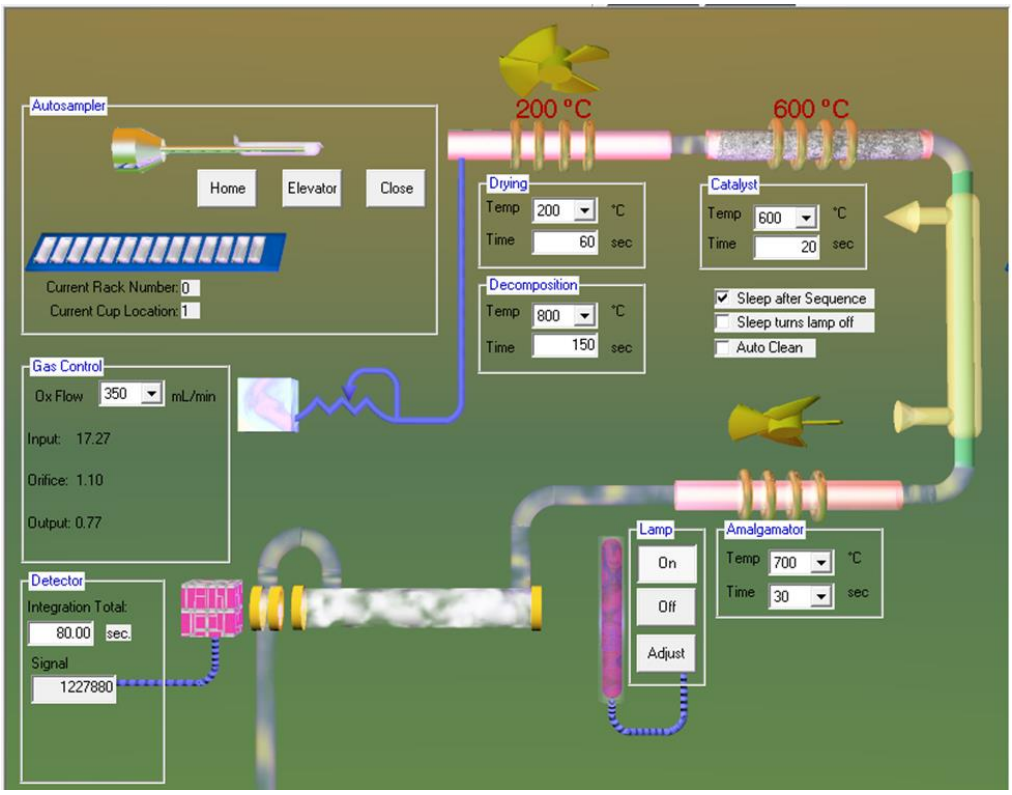
1. Start the Hydra II_C in accordance with the *Hydra II_C Operations Manual*.
2. Set the gas pressure to a value between 15 and 25 psi. Teledyne Leeman Labs recommends that the oxygen carrier gas be purified by an in-line mercury trap. For this application note, a Carrier Gas Purification Mercury Trap (P/N 15-2145-003) was used to ensure that the scrubbed carrier gas stream contributed a minimal amount of mercury contamination to the system.

15-2145-003	**Carrier Gas Purification Mercury Trap (M-8000)** - A pre-filter that uses a proprietary adsorbent to remove trace mercury vapor from the ultra-trace analytical carrier gas or ultra-trace solution purge gas.

Note: The Carrier Gas Purification Mercury Trap is typically used to purify argon gas in ultra-trace analysis using CVAF with a gold trap for preconcentration of mercury in the sample.

3. Create a new method by selecting Method “New” from the drop-down menu and name the method appropriately (e.g., General Method, EPA 7473 or similar). If a calibration with an appropriate calibration range exists, use the “Clone” option to automatically copy the calibration into a new method. Configure the method parameters according to those shown in Table III. These parameters were developed from the experimental findings of this study and determined to optimize analysis for aqueous samples using MeHg extraction.

Figure 4 Envoy Software Instrument Control Screen Showing Method Parameters



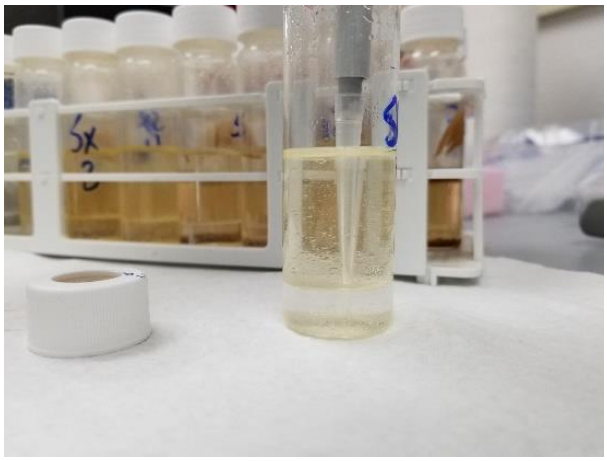
4. Select the “Start Up Button” to start the system, then allow 30 minutes for the instrument to stabilize at the method settings. Condition the catalyst before performing analysis.

Note: Each time the Hydra II_C is prepared for analysis it is important to condition the catalyst using the following sequence: a single boat with 100 mg of flour, a single boat with 100 µL of deionized water and an empty sequence position. Flour is composed of polysaccharides (starch) and has a general formula of $C_x(H_2O)_y$. The large amount of carbon atoms has a stabilizing effect on the catalyst.

5. Condition a sufficient quantity of nickel and/or quartz boats by rinsing with DI water and firing in a muffle furnace at 800 °C for 15 minutes with flow-through, inert gas. Boats may also be conditioned by running them as unknowns on an un-calibrated method. Repeat this process until the boats are clean with a consistent µabs response. Store conditioned boats in a sealed plastic bag until use. After analysis is complete, repeat the cleaning process and store boats until next use.
6. Prepare the standards according to Table I and Table II. It should be noted that the calibration is typically stable over the lifespan of the catalyst. Verify the calibration with a certified reference material (CRM) for each calibration range. This can be accomplished by either varying the weights of the CRM or using a CRM from a second source.
7. Once the system has stabilized, the analysis of either calibration standards or quality control (QC) checks, and samples may begin.

Analytical Sampling And Results Calculations

The lower layer in the VOA vial is the L-cysteine fraction containing the extracted organic mercury for analysis.



1. To sample the L-cysteine layer (lower), use an air displacement pipette set to 100 µL
2. Depress the pipette mechanism
3. Push the pipette tip through the toluene layer
4. Gently pull up the analytical portion of the solution into the pipette tip
5. Retract the pipette though the toluene layer
6. Dispense the sample into a conditioned sample boat
7. Record the sample name
8. Enter the sample weight as 0.1 g
9. Run the sample and acquire results

Note: Non-analytical samples require a % moisture determination for final calculated results on a dry basis.

10. Perform the methylmercury (MeHg) mass conversion using the following formula:

([A X B]/C) X 1.075 = MeHg µg/g (ppm)

Note: Hydra II_C results in ng/g (ppb) are a direct conversion in units to µg/L (ppb)

Where:

- Results µg/L - Extraction Blank µg/L: **A**
- Extraction volume in liters at 0.005: **B**
- Sample weight (dry basis): **C**

Note: Total Mercury (THg) to MeHg Conversion **1.075** (Hg atomic weight conversion factor)

Analytical Experimental Validation Variables

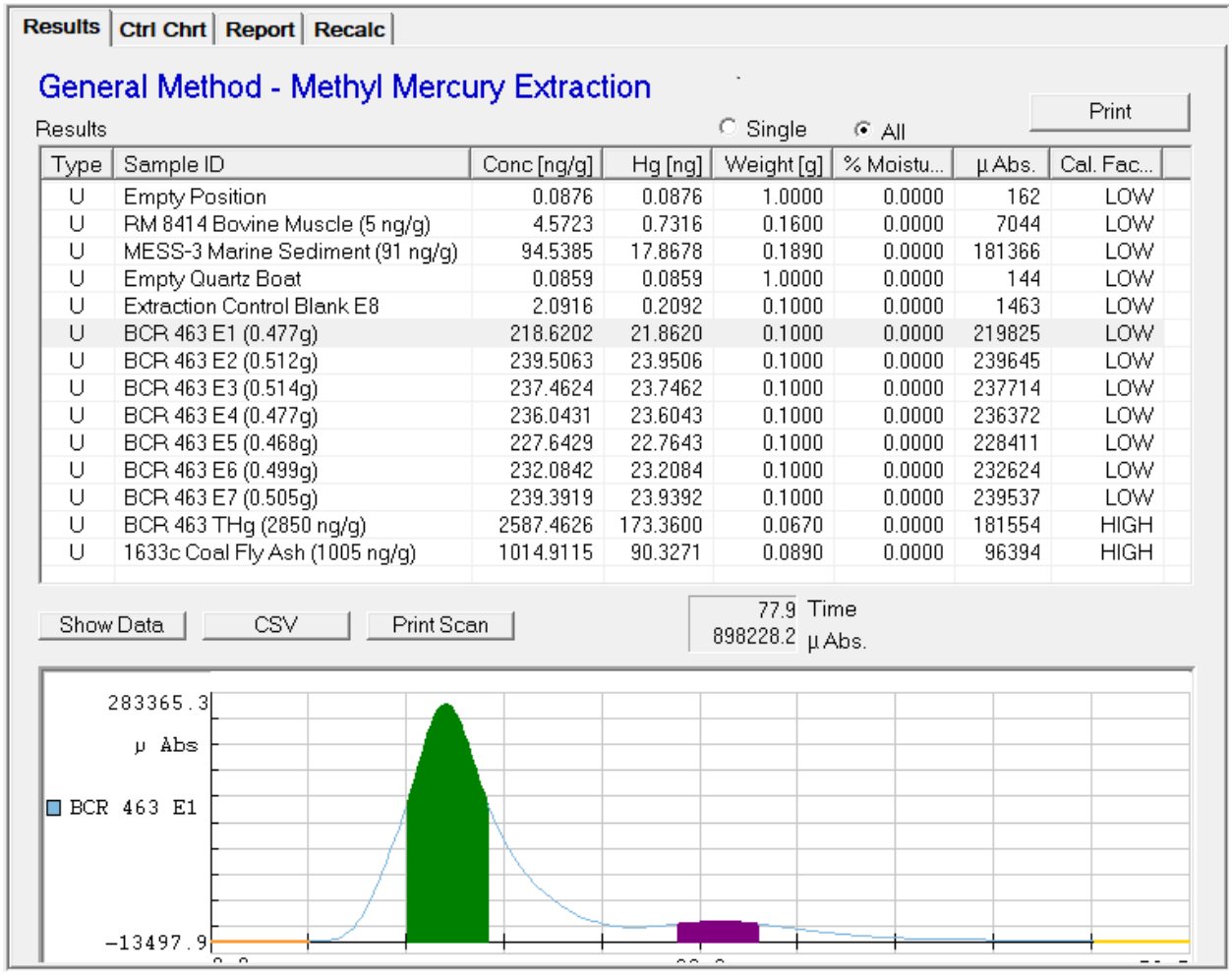
For this application, it was prudent to empirically test the method's recovery and precision by altering two variables: sample extraction weight (solid sample placed in the VOA vial) and shaking method (hand versus mechanical). Three different tests were performed differing these variables, while using the same sample:

- Test 1: Hand shaking with consistent sample extraction weights of ~0.25 g
- Test 2: Mechanical shaking with inconsistent sample extraction weights
- Test 3: Mechanical shaking with consistent sample extraction weights of ~0.5 g

Results are shown in Figure 5, Figure 6, Figure 7, Figure 8 and Table IV.

Results

Figure 5 Raw Values for Test 3 - 0.5 g Sample Extraction Weights With Mechanical Shaking



Type	Sample ID	Conc [ng/g]	Hg [ng]	Weight [g]	% Moistu...	µ Abs.	Cal. Fac...
U	Empty Position	0.0876	0.0876	1.0000	0.0000	162	LOW
U	RM 8414 Bovine Muscle (5 ng/g)	4.5723	0.7316	0.1600	0.0000	7044	LOW
U	MESS-3 Marine Sediment (91 ng/g)	94.5385	17.8678	0.1890	0.0000	181366	LOW
U	Empty Quartz Boat	0.0859	0.0859	1.0000	0.0000	144	LOW
U	Extraction Control Blank E8	2.0916	0.2092	0.1000	0.0000	1463	LOW
U	BCR 463 E1 (0.477g)	218.6202	21.8620	0.1000	0.0000	219825	LOW
U	BCR 463 E2 (0.512g)	239.5063	23.9506	0.1000	0.0000	239645	LOW
U	BCR 463 E3 (0.514g)	237.4624	23.7462	0.1000	0.0000	237714	LOW
U	BCR 463 E4 (0.477g)	236.0431	23.6043	0.1000	0.0000	236372	LOW
U	BCR 463 E5 (0.468g)	227.6429	22.7643	0.1000	0.0000	228411	LOW
U	BCR 463 E6 (0.499g)	232.0842	23.2084	0.1000	0.0000	232624	LOW
U	BCR 463 E7 (0.505g)	239.3919	23.9392	0.1000	0.0000	239537	LOW
U	BCR 463 THg (2850 ng/g)	2587.4626	173.3600	0.0670	0.0000	181554	HIGH
U	1633c Coal Fly Ash (1005 ng/g)	1014.9115	90.3271	0.0890	0.0000	96394	HIGH

Figure 6 Test 1 Results - 0.25 g Sample Extraction Weights with Hand Shaking (2.65 ±0.19 at 95% Confidence)

October 2019 Extraction Hand Shaken/Mixed	Sample Extraction Weight (g)	(10.76 %) Moisture Corrected Weight (g)	Raw Value ng/g	Control Blank Corrected Value (ng/g)	Moisture Corrected Value ng/g	MeHg (mg/Kg) 3.04 ± 0.16	% Recovery
Extraction Control Blank (E1)	0.1 g ES		3.036				
BCR 463 (E2) Rep 1	0.251	0.224	100.2929	97.26		2.33	76.6
BCR 463 (E3) Rep 2	0.256	0.228	103.491	100.46		2.37	78.0
BCR 463 (E4) Rep 3	0.250	0.223	113.2234	110.19		2.66	87.5
BCR 463 (E5) Rep 4	0.249	0.222	115.6785	112.64		2.73	89.8
BCR 463 (E6) Rep 5	0.251	0.224	129.494	126.46		3.03	99.7
BCR 463 (E7) Rep 6	0.252	0.225	110.8109	107.77		2.57	84.5
BCR 463 (E8) Rep 7	0.254	0.227	124.2773	121.24		2.87	94.4
STDEV						0.25	8.4
RSD %						9.43	9.6
Average						2.65	87.2

Figure 7 Test 2 Results - Varying Sample Extraction Weights with Mechanical Shaking (2.59 ±0.11 at 95% Confidence)

May 2020 Extraction Automated Shaken/Mixed	Sample Extraction Weight (g)	(10.76 %) Moisture Corrected Weight (g)	Raw Value ng/g	Control Blank Corrected Value (ng/g)	Moisture Corrected Value ng/g	MeHg (mg/Kg) 3.04 ± 0.16	% Recovery
RM 8414 (5 ng/g)			5.5975				112.0
Mess-3 (91 ng/g)			98.5918				108.3
Extraction Control Blank (E1)	0.1 g ES		2.2496				
BCR 463 (E2) Rep 1	0.203	0.181	93.9313	91.68		2.72	89.5
BCR 463 (E3) Rep 2	0.099	0.088	40.6405	38.39		2.34	77.0
BCR 463 (E4) Rep 3	0.148	0.132	69.0703	66.82		2.72	89.5
BCR 463 (E5) Rep 4	0.125	0.112	58.4486	56.2		2.70	88.8
BCR 463 (E6) Rep 5	0.097	0.087	42.7265	40.48		2.50	82.2
BCR 463 (E7) Rep 6	0.246	0.22	110.7613	108.51		2.65	87.2
BCR 463 (E8) Rep 7	0.09	0.08	39.5253	37.28		2.50	82.2
STDEV						0.15	4.8
RSD %						5.79	5.6
Average						2.59	85.2
BCR 463 THg (2850 ng/g ± 160)			2687.7791		3012		105.7
1633c Coal Fly Ash (1005 ng/g ± 22)			1030.4341				102.5

Figure 8 Test 3 Results - 0.5 g Sample Extraction Weights with Mechanical Shaking (2.81 ±0.06 at 95% Confidence)

February 2021 Extraction Automated Shaken/Mixed	Sample Extraction Weight (g)	(10.76 %) Moisture Corrected Weight (g)	Raw Value ng/g	Control Blank Corrected Value (ng/g)	Moisture Corrected Value ng/g	MeHg (mg/Kg) 3.04 ± 0.16	% Recovery
RM 8414 (5 ng/g)			4.5723				91.4
Mess-3 (91 ng/g)			94.5385				103.9
Extraction Control Blank (E8)	0.1 g ES		2.0916				
BCR 463 (E1) Rep 1	0.477	0.426	218.6202	216.53		2.73	89.8
BCR 463 (E2) Rep 2	0.512	0.457	239.5063	236.31		2.78	91.4
BCR 463 (E3) Rep 3	0.514	0.459	237.4624	234.26		2.74	90.1
BCR 463 (E4) Rep 4	0.477	0.426	236.0431	232.84		2.94	96.7
BCR 463 (E5) Rep 5	0.468	0.418	227.6429	224.44		2.89	95.1
BCR 463 (E6) Rep 6	0.499	0.445	232.0842	228.88		2.76	90.8
BCR 463 (E7) Rep 7	0.505	0.451	239.3919	236.19		2.81	92.4
STDEV						0.08	2.6
RSD %						2.85	2.8
Average						2.81	92.4
BCR 463 THg (2850 ng/g ± 160)			2587.4626		2899		101.7
1633c Coal Fly Ash (1005 ng/g ± 22)			1014.9115				101.0

Table IV Experimental Statistical Values of Merit

	Test 1	Test 2	Test 3
Sample Extraction Weight	0.25 g	Varied	0.5 g
Means of Shaking	Hand	Mechanical	Mechanical
Average W/Uncertainties @ 95%	2.65 ±0.19 (mg/Kg)	2.59 ±0.11 (mg/Kg)	2.81 ±0.06 (mg/Kg)
STDEV MeHg	0.25	0.15	0.08
%RSD MeHg	9.43	5.79	2.85
Average % Recovery	87.2	85.2	92.4

Conclusions

The Ministry of Food and Drug Safety of Korea *Notice No. 2018-98: Methyl Mercury Test Method 9.1.9.2 - Law 2* was not a difficult method. Both manual or automated extraction can be completed, start to finish, in single working day. The addition of the mechanical arm shaker from FINEPCR was a labor saving addition to the laboratory and removed a majority of analytical errors associated with manual shaking. It is estimated that with the assistance of automation, it is possible for a single lab chemist to complete analysis of three to four batches of seven samples, totaling 21 to 28 samples in a single, 8-hour shift.

The method's recovery and precision were studied by altering sample extraction weights and means of sample shaking. Not only did the mechanical shaker increase the rate at which a sample set could be completed, but it also greatly improved the precision of the results as shown in Table IV, "Experimental Statistical Values of Merit". As noted, the MeHg %RSD improved from 9.43 to 2.85. Because both Test 1 and Test 3 used a consistent sample extraction weight, the observed improvement in precision can be attributed to the use of the mechanical shaker. Test 2, which consisted of varied sample extraction weights, also showed a 38.6% improvement in MeHg %RSD over Test 1 which used manual shaking.

A variety of standard reference materials (SRM) materials was used to test the analytical stability of the analyzer including: RM8414 (Bovine Muscle Powder), Mess-3 (Marine Sediment), BCR-463 (Total and Methylmercury in Tuna Fish), and NIST 1633c (Trace Elements in Coal Fly Ash). Responses fell in both the upper and lower sensitivity ranges and recoveries were ~91 to 112%. By subjecting the analyzer to such a diverse set of reference materials for calibration verification, the analyzer's ability to consistently maintain analytical control was demonstrated.

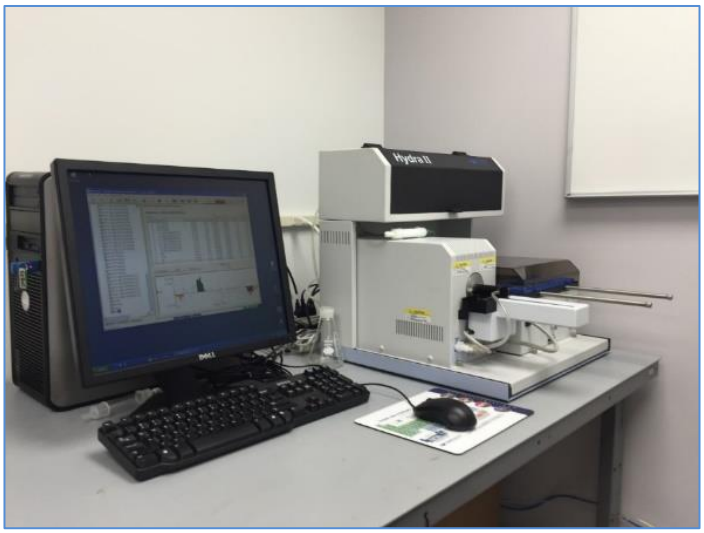
The MeHg % recoveries averaged 92.4% at 2.81 mg/Kg for Test 3, falling slightly out of the recovery range of 2.88 - 3.20 mg/Kg. It should also be noted, the lower sample weights tend to have lower recoveries, which in turn lowered the Test 2 average (although precision was improved by using the mechanical shaker).

Method Summary (Test 3)

- The extraction method yielded a final average result of 2.81 ±0.06 µg/g (mg/Kg), 92.4% recovery for CRM BCR®-463, Tuna Fish: CH_3Hg+ 3.04 µg/g ± 0.16.
- Method configuration for the L-cysteine extraction solutions was routine and did not entail any additional complexities to accomplish.
- Performing the method was relatively straight forward, but sample shaking automation was determined to be desirable both for expediting the preparation of daily extractions, as well as yielding improved precision and % recovery.
- The Hydra II_C is an ideal analytical tool for the Ministry of Food and Drug Safety of Korea *Notice No. 2018-98: Methyl Mercury Test Method 9.1.9.2 - Law 2* and/or similar MeHg extraction methods.

The Hydra II_C, CAA combustion mercury analyzer demonstrated that it is an effective analytical tool capable of obtaining reliable quantitative data in virtually any sample matrix, including an L-cysteine extraction solution. The addition of an external, in-line drying system filled with magnesium perchlorate $Mg(ClO_4)_2$, in conjunction with optimization of carrier gas flow, drying, decomposition, integration and amalgamation temperature/time, permitted analysis of calibration standards, quality control (QC) checks, and extraction samples without issue. The sample analysis time for direct combustion of MeHg extraction solution was ~6.5 minutes from loading the boat onto the sample injector forks to returning it post-analysis to the autosampler rack.

Figure 9 Hydra II_C Combustion Atomic Absorption (CAA) Mercury Analyzer in a Typical Laboratory Setting



References

1. World Health Organization (WHO). *Mercury and Health Fact Sheet*. January 2016 [Online] http://www.who.int/mediacentre/factsheets/fs361/en/ (accessed April 04, 2021).
2. Ministry of Food and Drug Safety of Korea. *Notice No. 2018-98:Methyl Mercury Test Method 9.1.9.2-Law 2*. November 29 [Online] https://www.foodsafetykorea.go.kr/foodcode/01_03.jsp?idx=12046 (translated) (accessed April 04, 2021).
3. United States Environmental Protection Agency. *USEPA Method 7473: Mercury In Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry*. Revision 0, February 2007 [Online] https://www.epa.gov/sites/production/files/2015-12/documents/7473.pdf (accessed April 04, 2021).