

**Total Mercury Concentrations in Eggs of Female Sea Lamprey Collected from Rivers in
Wisconsin and Michigan During Summer 2014**

by

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Introduction

Eggs from female sea lamprey (*Petromyzon marinus*) captured during the summer of 2014 from rivers in the 1842 ceded territory of Wisconsin and Michigan were analyzed for total mercury (Hg) content at the University of Wisconsin-Superior's Lake Superior Research Institute (LSRI). Twenty-four egg samples were collected from female sea lamprey, which were collected from three rivers: Misery River, Bad River, and Middle River, and delivered to LSRI for mercury analysis.

Methods

Adult, parasitic phase sea lamprey (approximately 5-10 years old) returning to spawning tributaries of Lake Superior were captured by trained GLIFWC staff using portable assessment traps. At the time of capture the sex of each lamprey was determined (egg expression in females or presence of swollen dorsal ridge in males) and its total length measured. Lamprey were assigned a unique number (i.e. a fish identification number) and placed in a Ziploc freezer bag labeled with the fish identification number. The samples were frozen within 4 hours of capture. At the GLIFWC laboratory, females were thawed and eggs were harvested through an incision made along the ventral surface from the gill slits to the anus. The egg samples were labeled with the same unique identification number as the adult they were harvested from and were sealed in Ziploc snack bags within a second Ziploc bag. Sea lamprey egg samples and associated chain-of-custody forms were transferred to the GLIFWC laboratory freezers until they were delivered to LSRI on September 4, 2014.

Processing and analysis of sea lamprey eggs was not covered under any existing Quality Assurance Project Plan (QAPP), but proceeded with only minor modifications of the QAPP entitled *Great Lakes Indian Fish and Wildlife Commission Mercury Testing and Updating Tribal-Walleye Consumption Advice* approved in June 2011. Deviations to the protocols outlined in the QAPP were implementation of an alternate grinding procedure (SOP SA/38 v.2) due to the fact that the egg sample was homogenized (rather than a fillet) and the fact that an LSRI QA audit was not performed. An LSRI QA audit was recently performed during the testing of the GLIFWC walleye, northern pike, and musky samples on August 11 and 12, 2014. That QA report was provided to GLIFWC as an appendix to the report entitled 'Total Mercury Concentrations in Muscle Tissue from Walleye, Northern Pike, and Muskellunge Collected from Inland Lakes during Spring 2014' and dated October 31, 2014.

Before processing the sea lamprey egg samples, all glassware, utensils, and blenders were cleaned according to the appropriate methods (LSRI SOP SA/8 v.7). The egg samples to be processed were removed from the freezer just prior to processing so that they remained frozen. The samples were further frozen with liquid nitrogen (LSRI SOP SA/38 v.2). The sample was then placed into the blender and was ground into a fine powder. A sub-sample of the processed tissue was placed into a certified clean glass vial and stored in a freezer until mercury analysis was conducted. After each lamprey egg sample was processed, the blender cup was disassembled and washed according to the blender cleaning procedure (SOP SA/8 v.7).

Commercial canned tuna fish (*Thunnus sp.*) was used as a procedural blank for this project. This

procedural blank consisted of one aliquot from a can of tuna that was transferred into a sample bottle after the packing liquid was removed and the tuna was mixed thoroughly to produce a homogeneous sample. The second portion was ground in the same manner as the lamprey egg samples. This check was made to ensure that no contamination or loss of mercury was occurring during the processing. One procedural blank was prepared during this project. It was prepared on the same day that the lamprey eggs were processed.

Lamprey eggs were weighed for mercury analysis following standard laboratory procedure (SOP SA/11 v.7). Mercury solutions for making tissue spikes and preparing analytical standards were prepared following the procedures in SOP SA/42 v.2. Selected samples were spiked with 500 µL of 500 µg/L mercury sub-stock solution. Mercury analyses were performed using cold vapor mercury analysis techniques on a Perkin Elmer FIMS 100 mercury analysis system (SOP SA/49 v.3 draft). Sample analysis yielded triplicate absorbance readings whose mean value was used to calculate the concentration of each sample. If the relative standard deviation (RSD) of the three measurements was greater than 5%, additional aliquots of the digested sample were analyzed in an attempt to obtain an RSD of less than 5%. Mercury concentrations and quality assurance calculations were done in Microsoft Excel according to SOP SA/37 v.1. The biota method detection limit was 0.0027 µg Hg/g for an average sample mass of 0.23 g (Appendix A). This limit of detection was determined using a ground tuna sample (8-2-13) containing a low concentration of mercury (SOP SA/35 v.1).

Moisture content of tissue was calculated using the wet and dried tissue weights (SOP SA/51 v.4). A portion (1-5 grams) of ground tissue was placed into a dried and weighed aluminum pan immediately following tissue grinding. The pan and wet tissue were immediately weighed and placed into an oven (60°C) and dried for approximately 24 hours. Nine of the lamprey egg samples analyzed for mercury had moisture content determined. One of these samples was analyzed in duplicate.

Data Quality Assessment

Data quality was assessed using four data quality indicators: analysis of similar fish tissues (Commercial canned tuna; *Thunnus* sp.) before and after the tissue grinding process (procedural blanks) to measure laboratory bias; analysis of dogfish shark (*Squalus acanthias*) from the Canadian government (certified reference material from National Research Council Canada, Ottawa, Ontario, Canada) that has a certified concentration of mercury to measure analytical accuracy; duplicate analysis of lamprey eggs from the same individual to measure analytical precision; and analysis of eggs with known additions of mercury to determine spike recovery and possible analytical interferences. Two sets of analytical standards with known amounts of mercury were analyzed with the group of lamprey egg samples. The concentrations of the mercury standards analyzed with each set of samples were 0, 100, 500, 1000, 5000, and 10,000 ng Hg/L. Standards were prepared from a purchased 1000 ± 10 ppm mercury (prepared from mercuric nitrate) reference standard solution (Fisher Scientific, Pittsburgh, PA). A summary table of the mercury calibration curve data is provided (Appendix A).

Results for the quality assurance samples were considered acceptable when the value determined for a quality assurance sample fell within the limits established in the Quality Assurance Project Plan (QAPP) approved in June 2011. Results for the procedural blanks were considered

acceptable when the relative percent difference was < 50%. Duplicate agreement values were acceptable when having a relative percent difference < 25%. The acceptable range for the daily mean value for the DORM standard reference material was 75 to 125% of certified value. Prior to digestion, tissues from 12.5 percent of the lamprey egg samples were spiked, in duplicate, with a known quantity of mercury and analyzed for recovery of the spiked mercury. Spike recovery was considered acceptable when the calculated daily mean recovery was 70 to 130% of the spike.

Results of Lamprey Egg Analyses

Quality Assurance – One tuna procedural blank was processed coincident with the processing of lamprey eggs collected for the project. The procedural blank was digested with the set of mercury samples resulting in a 20.5 percent difference between the ground and unground portions of tuna (Table 1).

Analysis of dogfish shark tissue DORM-4 was conducted concurrently with lamprey egg analysis (Table 2). The certified mercury concentration for the dogfish tissue was $0.410 \pm 0.053 \mu\text{g Hg/g}$. The individual recovery values ranged from 78.7 to 92.8% with the mean and standard deviation of the recoveries being 87.5 ± 7.7 percent of the certified value. The DORM-4 reference sample daily mean value was within the acceptance range.

Lamprey eggs were analyzed for mercury in duplicate three times. Two portions of the same tissue were digested and analyzed independently. The relative percent difference between duplicate analyses of the same tissue ranged from 0.7 to 8.2% with the average and standard deviation of the differences being $4.8 \pm 3.8\%$ (Table 3).

Samples of eggs were spiked in duplicate with known concentrations of mercury prior to digestion. Mean recovery for the three spiked samples was 82.7 ± 10.1 percent with the reported individual average recovery values ranging from 72.4 to 98.0% (Table 4).

Mercury Analysis – Samples from homogenized eggs of 24 sea lamprey collected from a total of three rivers in Wisconsin and Michigan were analyzed for total mercury concentration. Total mercury concentrations on a wet weight basis (Table 5) ranged from 0.486 to $1.55 \mu\text{g Hg/g}$ (parts per million).

Tissue Moisture Analysis – Percent moisture was measured in nine of the 24 lamprey egg samples. Moisture analysis took place immediately following processing. The data obtained through drying and weighing the samples twice indicates that drying for 24 hours was sufficient to remove the moisture from the samples used for moisture determination. Lamprey egg samples had a mean moisture value of 61.7 ± 5.8 percent (Table 6). Two samples were dried a minimum of an additional 24 hours and reweighed to ensure dryness, yielding an average relative percent difference of 0.0 percent.

Table 1. Relative Percent Difference of Total Mercury for Procedural Blank Sample (Before and After Grinding). Data quality indicator for laboratory bias is <50% relative percent difference.

Analysis Date	Grinding Date	Before Grinding $\mu\text{g Hg/g}$	After Grinding $\mu\text{g Hg/g}$	Mean $\mu\text{g Hg/g}$	Relative Percent Difference
11/20/2014	11/18/2014	0.057	0.070	0.064	20.5
				Mean \pm Std. Dev.	20.5

Table 2. Mercury Concentrations of Dogfish Shark Tissue (Standard Reference Material DORM-4) Analyzed during Lamprey Egg Analysis. The Standard Reference Material has a Certified Mercury Concentration of $0.410 \pm 0.053 \mu\text{g Hg/g}$ Tissue. Data quality indicator for accuracy is 75.0 to 125% agreement between the certified concentration and the daily mean value for the reference standard.

Date of Analysis	DORM 4-1		DORM 4-2		DORM 4-3		Mean	
	$\mu\text{g Hg/g}$	% of Certified Value	$\mu\text{g Hg/g}$	% of Certified Value	$\mu\text{g Hg/g}$	% of Certified Value		
11/20/2014	0.373	90.9	0.381	92.8	0.323	78.7	87.5	
							Mean \pm Std. Dev.	87.5 \pm 7.7

Table 3. Relative Percent Difference for Duplicate Analysis of Total Mercury Content in Lamprey Eggs. Data quality indicator for precision is <25% relative percent difference.

Date of Analysis	Sample Location and Tag Number	$\mu\text{g Hg/g}$	Duplicate $\mu\text{g Hg/g}$	Mean $\mu\text{g Hg/g}$	Relative Percent Difference	
11/20/2014	Misery River MIS-07	0.914	0.965	0.940	5.4	
11/20/2014	Bad River BAD-06	1.510	1.520	1.515	0.7	
11/20/2014	Middle River MID-07	1.010	0.930	0.970	8.2	
					Mean \pm Std. Dev.	4.8 \pm 3.8

Table 4. Percent of Mercury Recovered from Lamprey Eggs Spiked with a Known Concentration of Mercury. Data quality indicator for accuracy is a mean spike recovery of 70 to 130%.

Date of Analysis	Sample Location and Tag Number	Spike #1	Spike #2	Mean Spike Recovery	Std. Dev.
11/20/2014	Misery River MIS-07	91.5	72.4	81.9	13.5
11/20/2014	Bad River BAD-06	98.0	81.9	90.0	11.4
11/20/2014	Middle River MID-07	74.7	77.8	76.3	2.2
				Mean \pm Std. Dev.	82.7 \pm 10.1

Table 5. Total Mercury Concentration (Wet Weight) of Eggs from Lamprey Captured during the Summer of 2014.

Analysis Date	Sample Location	Tag Number	County	Fresh Length (in)	Female Mass (g)	Egg Mass (g)	µg Hg/g
11/20/2014	Misery River	MIS-02	Ontonagon	40.0	167.2	35.5	0.953
11/20/2014	Misery River	MIS-03	Ontonagon	40.0	173.3	50.7	0.781
11/20/2014	Misery River	MIS-04	Ontonagon	30.7	128.3	17.8	1.00
11/20/2014	Misery River	MIS-05	Ontonagon	42.0	236.3	54.9	0.763
11/20/2014	Misery River	MIS-06	Ontonagon	42.3	194.3	46.4	1.04
11/20/2014	Misery River	MIS-07	Ontonagon	45.5	234.5	55.6	0.940
11/20/2014	Misery River	MIS-09	Ontonagon	39.3	153.7	50.9	0.697
11/20/2014	Misery River	MIS-10	Ontonagon	38.3	159.9	34.3	0.748
11/20/2014	Bad River	BAD-01	Ashland	41.0	175.0	44.3	1.34
11/20/2014	Bad River	BAD-02	Ashland	39.2	130.0	21.9	0.967
11/20/2014	Bad River	BAD-03	Ashland	47.5	260.0	53.2	0.486
11/20/2014	Bad River	BAD-04	Ashland	45.0	193.0	43.1	0.700
11/20/2014	Bad River	BAD-05	Ashland	42.6	210.0	54.9	0.983
11/20/2014	Bad River	BAD-06	Ashland	42.0	199.0	51.1	1.52
11/20/2014	Bad River	BAD-07	Ashland	47.5	260.0	55.7	1.00
11/20/2014	Bad River	BAD-09	Ashland	42.5	180.0	31.9	1.11
11/20/2014	Middle River	MID-01	Douglas	38.0	157.8	29.5	0.532
11/20/2014	Middle River	MID-02	Douglas	36.5	146.8	39.9	0.883
11/20/2014	Middle River	MID-03	Douglas	35.0	114.0	39.6	0.744
11/20/2014	Middle River	MID-04	Douglas	37.0	157.7	40.4	0.745
11/20/2014	Middle River	MID-05	Douglas	39.0	178.0	38.1	1.55
11/20/2014	Middle River	MID-06	Douglas	41.5	213.8	43.9	1.33
11/20/2014	Middle River	MID-07	Douglas	40.0	184.8	39.3	0.970
11/20/2014	Middle River	MID-08	Douglas	45.0	240.7	48.2	0.830

Table 6. Percent Moisture in Lamprey Eggs (Measured Immediately after Grinding).

Sample Location	Species	Tag Number		Percent Moisture	Relative Percent Difference
Misery River	Lamprey (Eggs)	MIS-02		56.3	
Misery River	Lamprey (Eggs)	MIS-03		57.7	
Misery River	Lamprey (Eggs)	MIS-05		68.8	
Bad River	Lamprey (Eggs)	BAD-01		62.4	
Bad River	Lamprey (Eggs)	BAD-01	DUP	63.7	2.0
Bad River	Lamprey (Eggs)	BAD-02		55.9	
Bad River	Lamprey (Eggs)	BAD-03		56.6	
Middle River	Lamprey (Eggs)	MID-01		72.3	
Middle River	Lamprey (Eggs)	MID-02		58.0	
Middle River	Lamprey (Eggs)	MID-03		65.8	
Mean ± Std. Dev.				61.7 ± 5.8	

Appendix A

Determination of 2014 Limit of Detection (LOD) and Limit of Quantitation (LOQ) using a ground tuna sample from August 2, 2013

Sample	Tissue Type	ng/L	ng Hg	g sample	ug Hg/g
Tuna 2 August 2013 -1	ground tuna	44.3	2.22	0.233	0.010
Tuna 2 August 2013 -2	ground tuna	44.3	2.22	0.240	0.009
Tuna 2 August 2013 -3	ground tuna	37.3	1.86	0.231	0.008
Tuna 2 August 2013 -4	ground tuna	40.8	2.04	0.241	0.009
Tuna 2 August 2013 -5	ground tuna	40.8	2.04	0.225	0.009
Tuna 2 August 2013 -6	ground tuna	40.8	2.04	0.238	0.009
Tuna 2 August 2013 -7	ground tuna	33.8	1.69	0.247	0.007
Tuna 2 August 2013 -8	ground tuna	33.8	1.69	0.219	0.008
				Mean	0.0086
				Std. Dev.	0.00092

2014 LOD = Std. Dev. x t = 0.00092 x 2.998 = 0.0028

2014 LOQ = 10/3 x LOD = 0.0093

LOD= 0.00275

LOQ= 0.00916

June 11, 2014	Hg LOD = 0.0028 µg/g	LOQ = 0.0093 µg/g
May 1, 2013	Hg LOD = 0.0073 µg/g	LOQ = 0.0245 µg/g
May 31, 2012	Hg LOD = 0.0030 µg/g	LOQ = 0.0099 µg/g
2011	Hg LOD = 0.0017µg/g	LOQ = 0.0057µg/g
2010	Hg LOD = 0.0046 µg/g	LOQ = 0.0153 µg/g
2009	Hg LOD = 0.0066 µg/g	LOQ = 0.0220 µg/g
2008	Hg LOD = 0.0126 µg/g	LOQ = 0.0421 µg/g
2007	Hg LOD = 0.0047 µg/g	LOQ = 0.0157 µg/g
2006	Hg LOD = 0.0042 µg/g	LOQ = 0.0141 µg/g
2005	Hg LOD = 0.0113 µg/g	LOQ = 0.0368 µg/g
2004	Hg LOD = 0.0013 µg/g	LOQ = 0.0042 µg/g

Appendix B

Calibration Curve Data Generated during the Analysis of GLIFWC's 2014 Lamprey Eggs. Indicators for Calibration Curves include a Slope of $2.0-3.0 \times 10^{-5}$ and a Coefficient of Determination of >0.995 .

Analysis Date	Standard Conc. ng Hg/L	Blank Corrected Abs. 1	Blank Corrected Abs. 2	Blank Corrected Mean	Standard Deviation	Slope	Y-Intercept	Corr.
11/20/2014	0	0.0001	0.0001	0.0000	0.0000	2.8677×10^{-5}	0.001493	0.9999
11/20/2014	100	0.0028	0.0034	0.0031	0.0004			
11/20/2014	500	0.0159	0.0155	0.0157	0.0003			
11/20/2014	1000	0.0352	0.0311	0.0332	0.0029			
11/20/2014	5000	0.1536	0.1369	0.1453	0.0118			
11/20/2014	10,000	0.2844	0.2912	0.2878	0.0048			

Appendix C

Standard Operating Procedures (SOPs) Used During Project

ROUTINE LABWARE CLEANING FOR METALS ANALYSIS

INTRODUCTION

This standard operating procedure (SOP) describes the process used for the routine cleaning of labware and tissue grinding equipment used for metals analysis. The equipment used for tissue grinding (e.g., grinder attachment for KitchenAid™ Stand Mixer, blender, bowls, fillet knife, etc.) must be prepared by following the entire cleaning procedure before the initial use of the equipment if it has not been used for more than one week, as well as, after each use of the equipment. Labware is typically in contact with higher metal concentrations than the equipment used for tissue grinding and, therefore, must be cleaned using a different procedure (i.e., 10% (v/v) nitric acid) than the tissue grinding equipment. In addition, the stronger acid concentration used to clean the labware will cause damage to the tissue grinding equipment. The proper personal protective equipment must be worn during the entire cleaning procedure. This includes gloves, safety glasses or goggles, and lab coat.

DEFINITIONS

Labware: For metals analysis, this refers to all glassware or plasticware used in the preparation of samples, analytical standards, and spikes; as well as, all equipment used for weighing tissue samples (e.g., spatulas).

EQUIPMENT LIST

- ◆ Aluminum Foil
- ◆ Ammonium Hydroxide, Concentrated (Approximately 30%)
- ◆ Deionized Water
- ◆ Dish Pan
- ◆ Fillet Knife
- ◆ Gloves
- ◆ KitchenAid™ Food Grinder Attachment
- ◆ Hydrochloric Acid, Concentrated (Approximately 37%)
- ◆ Lab Coat
- ◆ Labware to be Washed
- ◆ Liquinox® Detergent
- ◆ Nalgene® 10-L Carboy, Marked with 1-L Graduations
- ◆ Nitric Acid, Concentrated (Approximately 70%)
- ◆ pH Indicator Strips
- ◆ Plastic Bottles
- ◆ Plastic Dish Rack
- ◆ Safety Glasses or Goggles
- ◆ Sodium Bicarbonate (Baking Soda)
- ◆ Spatula (Stainless Steel)
- ◆ Stainless Steel Bowls

- ◆ Various Labware
- ◆ Volumetric Flasks
- ◆ Volumetric Pipets
- ◆ Wash Bottle
- ◆ Washing Brushes

PROCEDURE

Cleaning Equipment used for Tissue Grinding (e.g., Grinder Attachment, Blender, Stainless Steel Bowls, Fillet Knife, Spatula)

Note: Equipment should be processed through this entire cleaning procedure before the initial use if it has not been used for more than one week, as well as, after each use.

Preparing 0.1 M Hydrochloric Acid (HCl) for Cleaning Tissue Grinding Equipment

1. Fill a 10-L carboy to the 10-L mark with deionized water. Add 83 mL concentrated hydrochloric acid. Cover the solution and mix. The 0.1 M hydrochloric acid is now ready to be used to soak the grinding equipment (i.e., for a minimum of 30 seconds). Used acid should not be returned to the 10-L carboy. Remake the 0.1 M hydrochloric solution every six months or when the supply has been depleted. Unused acid should be stored in a tightly sealed carboy labeled with the contents of the bottle, the date of preparation, and initials of the preparer.
2. Neutralize used or expired acid prior to disposal in a laboratory sink. Neutralize the acid with ammonium hydroxide or sodium bicarbonate until a pH of between 6 and 9 is achieved. Measure the pH with pH indicator strips.
3. Pour the neutralized acid down the drain while running cold water. Record the disposal of neutralized acid on the appropriate disposal form or lab notebook.

Cleaning Tissue Grinding Equipment

4. Dismantle the KitchenAid™ food grinder attachment before washing.
5. Scrub all grinding equipment in hot¹ water containing Liquinox® detergent. Replace soapy water as needed during washing process when the water becomes contaminated with fish tissue.
6. Rinse equipment with tap water until there is no presence of soap.
7. Rinse equipment once with deionized water.

¹ In the event that hot water is unavailable (i.e., during UWS Steam Plant shutdown; usually in August), an attempt should be made to obtain hot water from the dechlorinated lab water supply for at least the scrubbing portion of the cleaning. Rinsing can be done with cold water when hot water is unavailable.

8. Soak equipment in 0.1 M hydrochloric acid for a minimum of 30 seconds (be sure acid comes in contact with all surfaces of equipment).
9. Rinse equipment three times with deionized water.
10. Upon drying, cover equipment with aluminum foil to store until used. Note that the filet knife and can opener rust quickly and should be dried by hand after completing the cleaning procedure, covered with aluminum foil, and stored in a drawer.

Cleaning Labware (e.g., Volumetric Flasks, Beakers, Spatulas used for Weighing)

Note: This procedure should only be used to clean glassware or plastic labware and to clean spatulas used to weigh tissue samples. It should not be used to clean tissue grinding equipment.

Preparing 10% (v/v) Nitric Acid (HNO₃) for Labware Cleaning

11. Prepare the acid by adding concentrated nitric acid to deionized water in the ratio of 1 volume of acid per 9 volumes of deionized water. The acid solution can be made in a carboy. Given the corrosive nature of the nitric acid fumes, the minimal amount of 10% nitric acid required should be prepared.
12. Store unused acid in a tightly-sealed carboy labeled with the contents of the bottle, the date of preparation, and initials of the preparer.
13. After use, neutralize the acid prior to disposal in a laboratory sink. Neutralize the acid with ammonium hydroxide or sodium bicarbonate until a pH of between 6 and 9 is achieved. Measure the pH with pH indicator strips.
14. Pour the neutralized acid down the drain while running cold water. Record the disposal of neutralized acid on the appropriate disposal form or lab notebook.

Labware Cleaning

15. Scrub the labware thoroughly in hot¹ water containing Liquinox® detergent.
16. Rinse the labware with hot¹ water until there is no presence of soap.
17. Rinse the labware once with deionized water.
18. Fill a container with 10% nitric acid (place spatulas in a beaker of 10% nitric acid being sure to use only the side that has been submerged for weighing samples). Be sure the portion of the labware that comes into contact with the sample or standard is completely covered and filled with acid (e.g., fill volumetric flasks with acid). Allow the labware to soak for a minimum of 1 minute.
19. Empty the acid from the container back into the acid storage carboy.

20. Rinse the labware a minimum of three times with deionized water.
21. Place the clean labware in a plastic rack to air dry. When the labware is dry, cover the labware with a lid, stopper, or aluminum foil. Place the labware in a proper storage location until used.

SAMPLE WEIGHING FOR METALS ANALYSIS

INTRODUCTION

This standard operating procedure (SOP) describes the method used to weigh processed biological tissue samples, typically fish tissue samples, for mercury or other metals analysis. The tissue samples should be processed according to *LSRI/SOP/SA/10 - Sample Grinding for Metals Analysis* (issued 1992) or *LSRI/SOP/SA/38 - Preparation of Tissues for Analytical Determinations Using Liquid Nitrogen* (issued 1999). All labware used in this procedure should be cleaned according to *LSRI/SOP/SA/08 - Routine Labware Cleaning for Metals Analysis* (issued 1992). The proper personal protective equipment must be worn during this entire procedure. This includes gloves, safety glasses/goggles, and lab coat.

REFERENCES

Lake Superior Research Institute. 1995. LSRI/SOP/GLM/12 - Procedure for Verifying Calibration of Laboratory Balances.

Lake Superior Research Institute. 1992. LSRI/SOP/SA/08 – Routine Labware Cleaning for Metals Analysis.

Lake Superior Research Institute. 1992. LSRI/SOP/SA/10 – Sample Grinding for Metals Analysis.

Lake Superior Research Institute. 1999. LSRI/SOP/SA/38 – Preparation of Tissues for Analytical Determinations using Liquid Nitrogen.

EQUIPMENT LIST

- ◆ Beaker (2; 1 for rinsing and 1 for nitric acid)
- ◆ Balance Verification Datasheet
- ◆ Deionized Water
- ◆ Digestion Vessel Racks
- ◆ Gloves
- ◆ Ground/Processed Samples
- ◆ KimWipes®
- ◆ Lab Coat
- ◆ Permanent Marker
- ◆ Polypropylene Digestion Vessels (from a commercial supplier such as Environmental Express)
- ◆ Safety Glasses/Goggles
- ◆ Sample Weighing Datasheet (see Appendix 1) and/or Project-Specific Laboratory Notebook
- ◆ Spatula

- ◆ Top-Loading or Analytical Balance (must be capable of reading to at least 0.001 g)

REAGENTS

- ◆ **Nitric Acid (10% v/v):** Add 100 mL of concentrated nitric acid to 900 mL of deionized water. This solution should be prepared in a laboratory hood. The preparer must wear a lab coat, gloves and safety glasses/goggles.

PROCEDURE

1. Remove the sample(s) to be analyzed from the freezer and allow the sample(s) to thaw until able to be mixed with a spatula.
2. Label clean, polypropylene digestion vessels with the appropriate sample number and collection site name.
3. Check the level of the balance and adjust if necessary. Clean the balance pan by removing any foreign materials with a soft brush. Record the balance ID number on the appropriate datasheet (see Appendix 1 for example) or in a project-specific laboratory notebook.
4. Zero the balance with the zero adjustment. If balance calibration check has not been previously performed on the day of sample weighing, the balance calibration must be verified following *LSRI/SOP/GLM/12 - Procedure for Verifying Calibration of Laboratory Balances* (issued 1995).
5. Place several spatulas in a beaker containing 10% (v/v) nitric acid and allow them to soak for at least one minute.
6. Place a clean, labeled sample digestion vessel on the pan of the balance and tare the balance.
7. Remove a spatula from the 10% (v/v) nitric acid, rinse with deionized water, and wipe with a KimWipe® prior to using.
8. With a spatula, stir the sample to ensure homogeneity. Weigh the appropriate quantity (i.e., approximately 0.2-0.3 g for mercury analyses and 1.0 g for other metals analyses) of tissue into the sample container. Be sure that none of the tissue adheres to the upper sides of the sample container.
9. Record the weight of the sample on the appropriate datasheet (see Appendix 1 for example) or in a project-specific laboratory notebook. The date and initials of the individual performing the procedure must also be recorded.

10. Rinse the used spatula with deionized water to remove the majority of the fish tissue from the spatula and place it back into the beaker with 10% (v/v) nitric acid for a minimum of one minute.
11. Remove the spatula from the 10% nitric acid, rinse with deionized water and wipe with a KimWipe® prior to using the spatula on another sample.
12. Repeat Steps 6 to 11 for all tissue samples to be weighed.

APPENDIX 1

EXAMPLE SAMPLE TISSUE WEIGHING DATASHEET

PROCEDURE FOR VERIFICATION OF LABORATORY BALANCES

INTRODUCTION

This standard operating procedure (SOP) describes the method used for routine maintenance and verification of laboratory balances. This SOP applies to those precision or analytical balances used for accurate weighing. Examples include preparation of reconstituted water for culturing, *S. capricornutum* media, analytical standards and samples, stock solutions, and balances used for weighing test organisms and weighing filters for the determination of total suspended solids concentrations. This SOP does not apply to those balances that are used for non-accurate weighing, such as during the preparation of various diets for test organisms reared at the Lake Superior Research Institute, weighing whole fish collected for metals analysis, or weighing large quantities of salt for large-volume salt water preparation. Balance verification checks must be conducted each day before the first use of a balance, or when weighing outside the range of standard weights used to verify the balance on that day. If the results fall outside of the designated acceptance limits for the balance (see Appendix 2), the balance should not be used until it has been calibrated by an LSRI senior staff member (or a service technician, if necessary) and meets the calibration limits.

Laboratory balances are verified using ANSI/ASTM Class 1 weights. The Class 1 weights are *never* to be touched with the hands. Always use the forceps supplied with the weights. Care should be taken to avoid scratching or getting dirt, oil, or moisture on the weights. Improper use or care of the weights may affect the calibration and could result in declassification of the weights.

The frequency with which precision/analytical balances must be serviced by an outside vendor is project-specific, and will be specified in the project planning documentation (i.e., Quality Assurance Project Plan). In addition, recertification frequency of ANSI/ASTM Class 1 weights used to verify balance accuracy is dependent upon the project and will be specified in the project planning documentation.

DEFINITIONS

Accuracy: How closely an instrument measures the true or actual value of the variable being measured.

ANSI/ASTM Class 1 Weights: Weights that can be used as a reference standard in calibrating other weights and that are appropriate for calibrating high precision analytical balances with a readability as low as 0.1 mg to 0.01 mg.

Calibration: An *adjustment* of an instrument based on comparison to materials with known or certified values.

Verification: A *check* of instrument accuracy with an external known source.

REFERENCES

Mettler Toledo Operating Instructions Manual for B-S Line of Balances. April 2001. Mettler-Toledo GmbH, Laboratory and Weighing Technologies, CH-8606 Greifensee, Switzerland.

National Institute of Standards and Technology Handbook 44, 2007 Edition. Specifications, Tolerances, and Other Technical Requirements for Weighing and Measuring Devices. National Institute of Standards and Technology, Weights and Measures Division, Gaithersburg, MD.

EQUIPMENT LIST

- ◆ ANSI/ASTM Class 1 Weights (usually Troemner)
- ◆ Balance Accuracy Tolerances for NIST Class I Balances (Appendix 2)
- ◆ Balance Routine Maintenance and Verification Datasheet (Appendix 1)
- ◆ Forceps for Weights
- ◆ Gloves
- ◆ Laboratory Balance Log Book and/or Three-Ring Binder (may use one log book/binder for labs with multiple balances)
- ◆ Laboratory Precision or Analytical Balance (usually Mettler Toledo)

PROCEDURE

Before beginning to use balance, check to see that it has been serviced by an outside vendor within the time frame specified in the project planning documentation. This can be done by checking the sticker applied to the balance by the servicing company. Also, confirm that the Class 1 weights have been certified within the time frame specified in the project planning documentation. The date of most recent certification will be noted on the box containing the weights.

1. Ensure that the balance is located in a stable, vibration-free position that is free from direct sunlight, excessive temperature fluctuations, and drafts. If the balance is not located in an area where the greatest accuracy of weighing can be achieved, move the balance to a stable bench in an area that is protected against drafts and is as far away as possible from doors, windows, radiators, or air conditioning units. Alternatively, a marble slab or similar device may be used to reduce vibration on laboratory benches.
2. Clean the draft shield, weighing pan, and bottom plate ***before verification and after using the balance:***
 - 2.1. Use a soft-fiber (hair) brush or dry Kimwipe™ to sweep away loose debris.
 - 2.2. If necessary, **remove** the weighing pan from the balance and clean with a damp Kimwipe™ or wash the weighing pan.
 - 2.3. If using a moist Kimwipe™ for cleaning, be sure balance pan is dry before using.
3. Ensure that the balance is level by checking the level gauge/spirit (usually located at the back of the balance). Level the balance if needed using the adjustable leveling feet (located either on the front or back of the balance); the balance is level when the air bubble is in the middle

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of the level gauge/spirit.

4. Remove any load from the weighing pan and turn on the balance. Check the zero on the balance and, if necessary, adjust to read zero (using the "Tare" or "Zero" key) with no load.
5. Select three ANSI/ATSM Class 1 weights, which bracket the weight being determined. The Class 1 weights typically present in LSRI laboratories are: 20 mg, 200 mg, 2 g, and 20 g, therefore, if the sample weight is about 0.5 g, use the 20 mg, 200 mg, and 2 g weights.
6. Place the lowest-mass weight on the pan using the forceps. When the display stabilizes, read and record the mass (as grams) of the weight on the "Balance Routine Maintenance and Verification Datasheet" (Appendix 1). The verification datasheets should be kept in a three-ring binder located in an easily accessible area of the laboratory.
7. Repeat Step 6 with the middle- and highest-mass weights. Record the verification check date, results (pass or fail according to Step 8 below), and your initials in the laboratory notebook/three-ring binder for the balance(s) used.
8. Compare the values obtained with the actual value for the weights. If the difference is larger than the balance accuracy tolerance (see acceptable accuracy tolerances in Appendix 2), do not use the balance. Report the problem to the lab supervisor. The supervisor should recheck the balance and recalibrate, if necessary. If the balance is unable to be calibrated, a professional service and calibration should be scheduled. If the balance is found to be out of specification, a note should be attached to warn others not to use it. Record any maintenance (cleaning, etc.) and calibration activities in the laboratory notebook/three-ring binder.
9. Weigh test materials, samples, etc. following the appropriate SOP, if one exists. For example, follow LSRI/SOP/SA/11 - *Sample Weighing for Metals Analysis* (issued 1992) if weighing biological tissue samples for metals analysis. When applicable, record the mass of the material(s) weighed in the balance laboratory notebook or on a project-specific datasheet or notebook.
10. When weighing is completed, turn balance off. Clean the balance housing, weighing pan, and bottom plate after using a laboratory balance according to Step 2 of this procedure. In addition, clean the counter around the balance.
11. Technical specifications for each balance can be found in the operator's manual for that particular balance. The original copy of operator's manuals for every balance at LSRI should be kept on file by the LSRI Quality Assurance Manager. If needed, a copy of the operator's manual can be made and kept next to the balance (i.e., in the three-ring binder).

APPENDIX 1

BALANCE MAINTENANCE AND VERIFICATION DATASHEET

APPENDIX 2

BALANCE ACCURACY TOLERANCE RANGES

LSRI Balance Accuracy Tolerances* for NIST Class I Balances**

VERIFICATION MASS VALUE	BALANCE READABILITY:		
	0.001 g (1 mg)	0.0001 g (0.1 mg)	0.00001 g (0.01 mg)
	BALANCE TOLERANCE RANGE (g)	BALANCE TOLERANCE RANGE (g)	BALANCE TOLERANCE RANGE (g)
300 g	299.925-300.075	299.9250-300.0750	299.92500-300.07500
200 g	199.950-200.050	199.9500-200.0500	199.95000-200.05000
100 g	99.975-100.025	99.9750-100.0250	99.97500-100.02500
50 g	49.987-50.013	49.9875-50.0125	49.98750-50.01250
30 g	29.992-30.008	29.9925-30.0075	29.99250-30.00750
20 g	19.995-20.005	19.9950-20.0050	19.99500-20.00500
10 g	9.997-10.003	9.9975-10.0025	9.99750-10.00250
5 g	4.997-5.003	4.9987-5.0013	4.99875-5.00125
3 g	2.997-3.003	2.9992-3.0008	2.99925-3.00075
2 g	1.997-2.003	1.9995-2.0005	1.99950-2.00050
1 g	0.997-1.003	0.9997-1.0003	0.99975-1.00025
500 mg	0.497-0.503	0.4997-0.5003	0.49987-0.50013
300 mg	0.297-0.303	0.2997-0.3003	0.29992-0.30008
200 mg	0.197-0.203	0.1997-0.2003	0.19995-0.20005
100 mg	0.097-0.103	0.0997-0.1003	0.09997-0.10003
50 mg	0.047-0.053	0.0497-0.0503	0.04997-0.05003
30 mg	0.027-0.033	0.0297-0.0303	0.02997-0.03003
20 mg	^a Below Min. Capacity	0.0197-0.0203	0.01997-0.02003
10 mg	^a Below Min. Capacity	0.0097-0.0103	0.00997-0.01003
5 mg	^a Below Min. Capacity	0.0047-0.0053	0.00497-0.00503
3 mg	^a Below Min. Capacity	0.0027-0.0033	0.00297-0.00303
2 mg	^a Below Min. Capacity	^a Below Min. Capacity	0.00197-0.00203
1 mg	^a Below Min. Capacity	^a Below Min. Capacity	0.00097-0.00103

*Balance tolerances are ¼ of the 0.1% tolerance as stated in the United States Pharmacopeia (USP) code or 3 times the balance readability, whichever is larger.

**Class I accuracy as determined by NIST Handbook 44-2007 Tables 3 and 8.

^aMinimum balance capacity is the mass below which the acceptable error is greater than ±10%.

PROCEDURE FOR DETERMINATION OF METHOD DETECTION LIMIT AND LIMIT OF QUANTIFICATION

INTRODUCTION

Method detection limits (MDL) and limit of quantification (LOQ) should be determined using the following procedure for each analyte and analytical method of interest, for those analytical methods utilizing a calibration curve. Examples of instruments that would provide data used to generate calibration curves are: gas chromatograph, organic carbon analyzer, high pressure liquid chromatograph, atomic absorption spectrophotometer, and specific ion electrodes.

DEFINITIONS

Method Detection Limit (MDL): The constituent concentration that, when processed through the complete method, produces a signal with a 99% probability that is different from the blank (Eaton et al. 2005)

Limit of Quantification (LOQ): The constituent concentration that produces a signal sufficiently greater than the blank that it can be detected within specified levels during routine conditions (Eaton et al. 2005). Typically, it is the concentration that produces a signal 10/3 that of the method detection limit.

EQUIPMENT

- ◆ Calculator capable of doing standard deviations (or MS Excel spreadsheet)
- ◆ Standard or sample estimated to be within 5-10 times the expected detection limit
- ◆ Student's *t*-distribution chart

PROCEDURE

1. Select a low-level standard or sample that is estimated to be within 5-10 times the method detection limit for the analyte and analytical method.
2. If the analysis method involves sample preparation before analysis, the standard or sample should be carried through the entire preparation method before instrumental analysis is conducted. A minimum of seven aliquots/replicates of the standard or sample are carried through the entire preparation and analysis.
3. Determine a mean and standard deviation, $SD_{(n-1)}$, for the calculated concentration of each of the seven or more replicates.
4. Calculate the method detection limit by multiplying the standard deviation of the concentrations by the Student's *t* value (Appendix 1) for the number of replicates ($n-1$):

$$MDL = SD \times t_{(n-1)}$$

5. Compare the detection limit to the mean concentration. If the mean concentration is greater than 5-10 times the calculated detection limit, repeat steps 1-4 using a lower concentration for the replicates.
6. Once the MDL has been determined, the limit of quantification is calculated by multiplying the MDL by 10/3.

$$LOQ = MDL \times \frac{10}{3}$$

REFERENCES

Eaton, AD, Clesceri, LS, Rice, EW, and AE Greenberg, Eds. (2005). Standard Methods for the Examination of Water and Wastewater, 21st Edition. American Public Health Association, Washington, DC.

US Environmental Protection Agency, Electronic Code of Federal Regulations. Definition and Procedure for the Determination of the Method Detection Limit (revision 1.11). Title 40, Part 136, Appendix B. Accessed from:
http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&tpl=/ecfrbrowse/Title40/40cfr136_main_02.tpl
November 2009.

APPENDIX 1. STUDENT'S *t*-DISTRIBUTION CHART

Note: Chart displays only the 99% probability values for values of $t_{(n-1)}$ up to 21.

DF = $n-1$	0.01
1	31.82052
2	6.96456
3	4.54070
4	3.74695
5	3.36493
6	3.14267
7	2.99795
8	2.89646
9	2.82144
10	2.76377
11	2.71808
12	2.68100
13	2.65031
14	2.62449
15	2.60248
16	2.58349
17	2.56693
18	2.55238
19	2.53948
20	2.52798
21	2.51765

Accessed from StatSoft, Inc. (<http://www.statsoft.com/textbook/sttable.html>) 11/04/2009.

PROCEDURES FOR CALCULATING MERCURY CONCENTRATIONS USING COLD VAPOR MERCURY ANALYSIS

INTRODUCTION

This standard operating procedure (SOP) describes the process used to calculate mercury concentrations at various stages during the analysis of mercury using the cold-vapor atomic absorption method. The following equations are used in calculating mercury concentrations in stock solutions, sub-stock solutions, and in biological tissue samples.

EQUIPMENT

- ◆ Calculator (or MS Excel Spreadsheet)
- ◆ Certified Mercury Standard Solution (i.e., to be used as a stock)
- ◆ Study-Specific Laboratory Notebook/Three-Ring Binder

PROCEDURE

1. Use a purchased a mercury stock solution with a certified concentration of mercury
Note: $\mu\text{g/mL} = \text{mg/L} = \text{ppm}$.

Conversion from $\mu\text{g/mL}$ to ng/mL

$$\frac{\mu\text{g}}{\text{mL}} \times 10^3 \frac{\text{ng}}{\mu\text{g}} = \frac{\text{ng}}{\text{mL}}$$

Concentration of Mercury Sub-Stocks

$$C_1 \times V_1 = C_2 \times V_2$$

Where, C_1 = Concentration of Mercury Stock Solution (see above)
 C_2 = Desired Concentration of Mercury Sub-Stock/Diluted Solution
 V_1 = Volume of Stock Solution Needed
 V_2 = Desired Volume of Mercury Sub-Stock/Diluted Solution

Amount of Mercury in each Standard Solution

$$\text{ng of Hg} = \text{Concentration of Hg Sub Stock} \left(\frac{\text{ng}}{\text{mL}} \right) \times \text{Volume of Sub Stock Used (mL)}$$

2. Determine the concentration of mercury in each prepared sample using the calibration curve generated from the mercury standard solutions prepared in step 1. Plot the amount of mercury in each standard solution (x) vs. the mean blank-corrected peak height for each sample of interest (y), and use the resulting linear regression line's slope and intercept to calculate sample mercury concentration:

Amount of Mercury in each Sample

$$y = mx + b$$

Where, m = Slope of Linear Regression Line
 b = Intercept of Linear Regression Line

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y = Mean Blank-Corrected Peak Height for Sample of Interest
 x = Amount (ng) of Mercury in Sample of Interest

3. Multiply the resulting amount of mercury in each sample by “1 $\mu\text{g}/1000 \text{ ng}$ ” to convert to amount of mercury in μg .
4. Calculate the concentration of mercury in each tissue sample by dividing the amount of mercury in each sample by the mass of the tissue analyzed:

$$\text{Concentration of Mercury in each Biological Tissue Sample} \\ \frac{\text{Amount of Hg in Sample } (\mu\text{g})}{\text{Mass of Tissue Sample } (\text{g})}$$

HOMOGENIZATION OF TISSUES FOR METALS ANALYSIS USING LIQUID NITROGEN

INTRODUCTION

This standard operating procedure (SOP) describes the method for blending tissue samples into homogenous samples, which is based on the procedure used by EnChem, Inc. (1997). This SOP is applicable to preparation of tissue samples (i.e. clams, snails, fish fatty tissue, fish skin, fish muscle plugs, insects, plants) and other samples that are too small to be homogenized using a meat grinder. Liquid nitrogen is used to freeze the tissue sample, which is then processed in a blender to obtain a more homogenous sample than is obtained with a meat grinder. The blender and labware used in this procedure must be cleaned following the procedure outlined in *LSRI SOP SA/08 - Routine Labware Cleaning for Metals Analysis*. Sample vials for storage of homogenized samples are ordered certified pre-cleaned. The proper safety equipment must be worn during the entire grinding procedure; this includes gloves, safety glasses and lab coats.

REFERENCES

EnChem, Incorporated. 1997. Preparation of Tissues for Analytical Determination in the Laboratory. Madison, WI.

Lake Superior Research Institute. 1992. SA/08 – Routine Labware Cleaning for Metals Analysis.

EQUIPMENT LIST

- ◆ Can Opener
- ◆ Certified Pre-Cleaned Glass Vials
- ◆ Dry Wash Cloths (to protect skin against the cold metal bowls/pitcher/spatulas)
- ◆ Fillet Knives
- ◆ Glass Cutting Board
- ◆ Gloves
- ◆ Industrial Strength Blender (two speeds with a stainless steel pitcher)
- ◆ KimWipes
- ◆ Liquid Nitrogen
- ◆ Liquid Nitrogen Dewars
- ◆ Safety Glasses
- ◆ Samples
- ◆ Spatula
- ◆ Stainless Steel Bowls
- ◆ Tuna Fish, Canned (typically packed in water)

SAMPLE HANDLING REQUIREMENTS

1. After samples are received, they must be stored in a freezer at $< -10^{\circ}\text{C}$. After homogenization, samples must be stored in a freezer at $< -10^{\circ}\text{C}$ until digestion.
2. Care must be taken when using liquid nitrogen. Liquid nitrogen is -196°C and causes rapid freezing on contact with living tissue.

PROCEDURE

Preparing the Procedural Blank

1. The frequency of processing procedural blanks, as well as, acceptance criteria and corrective actions are specified in the Quality Assurance Project Plan or other project planning documentation.
2. Open a can of tuna (typically packed in water) and drain the liquid from the can. Homogenize the tissue with a spatula and transfer a portion to a certified-clean sample container labeled "Tuna before Grinding" and include the date of processing and your initials. The unground tuna blank is included with an analysis set.
3. Place the remainder of the tuna sample in a stainless steel bowl. Homogenize the tuna sample by following Steps 5 and 6 below. Place the tuna in a certified-clean sample vial using a spatula and label the tuna fish as "Tuna after Grinding" and include the date of processing and your initials. The ground tuna blank is included with the same analysis set as the unground tuna blank prepared on the same day.

Homogenizing the Samples

4. Remove the sample from the freezer. Larger samples should be cut into approximately $\frac{1}{4}$ -inch cubes on a clean, glass cutting board using a sharp fillet knife. If the sample has skin, such as musky muscle plugs, remove and discard the skin (unless the skin is to be analyzed separately) prior to cubing the sample. The smaller the cubes are, the more quickly and thoroughly the sample will freeze. Smaller samples can be placed directly into the stainless steel bowl and frozen with liquid nitrogen.
5. Place the sample into a stainless steel bowl. Pour liquid nitrogen over the sample until the sample is frozen solid. When this occurs, the sample typically breaks free from the bowl easily.
6. Pre-cool the blender pitcher just prior to adding the frozen tissue sample to be homogenized. The pitcher is cooled by the addition of a small volume of liquid nitrogen ($< 100\text{ mL}$). Transfer the frozen sample into a pre-cooled blender pitcher and pulse the blender until the sample is broken into very small pieces of approximately uniform size. Place the homogenized sample into an appropriately labeled certified-clean sample vial. Place the sample in the freezer ($< -10^{\circ}\text{C}$) until digestion.
7. Wash the blender pitcher parts, stainless steel bowls and spatulas following SOP SA/08 –

Routine Labware Cleaning for Metals Analysis before homogenizing the next sample. When reassembling the blender pitcher for the next sample, it is important to dry all parts using a KimWipe to prevent the moving parts from freezing when liquid nitrogen is added to the pitcher.

8. Continue to homogenize samples following Steps 4-7.

STOCK, STANDARD, AND SPIKE PREPARATION FOR MERCURY ANALYSIS

INTRODUCTION

This standard operating procedure (SOP) is used for the preparation of the stock, analytical standards, blanks, and spikes for mercury analysis. The fish/tissue used for the spikes should be weighed according to *LSRI/SOP/SA/11 - Sample Weighing for Metals Analysis* (issued 1992). The labware used in this procedure should be cleaned following the method described in *LSRI/SOP/SA/08 - Routine Labware Cleaning for Metals Analysis* (issued 1992).

REFERENCES

Lake Superior Research Institute. 1992. LSRI/SOP/SA/08 – Routine Labware Cleaning for Metals Analysis.

Lake Superior Research Institute. 1992. LSRI/SOP/SA/11 – Sample Weighing for Metals Analysis.

EQUIPMENT LIST

- ◆ Adjustable-Volume Micropipettes (ranging from 10-100 μ L and 100-1000 μ L) and Tips
- ◆ Adjustable-Volume Pipettes (ranging from 1-5 mL) and Tips
- ◆ Concentrated Hydrochloric Acid (HCl), Trace Metal Grade
- ◆ Deionized Water
- ◆ Ground Fish/Tissue Samples for Spikes
- ◆ Mercury (Hg) Stock/Reference Solution, (i.e., 1000 mg/L from mercuric nitrate)
- ◆ Mercury Waste Container and Hazardous Waste Container Inventory Form
- ◆ Polypropylene Digestion Vessels (from commercial supplier, such as Environmental Express)
- ◆ Potassium Permanganate (KMnO₄), 5% (w/v)
- ◆ Volumetric Flasks (100 mL)

PROCEDURE

Mercury (Hg) Sub-Stock Preparation: 10.0 mg/L Hg Sub-Stock

1. Add ~60 mL deionized (DI) water to a 100-mL volumetric flask.
2. Into the flask, add the following:
 - 1.00 mL (i.e., using an adjustable-volume, 100-1000 μ L pipette) of a 1000 mg/L mercury stock/reference solution
 - 1 mL trace metal grade concentrated HCl
 - 100 μ L 5% (w/v) KMnO₄

3. Dilute to 100 mL with deionized water and mix thoroughly by inverting flask to prepare the 10.0 mg/L Hg sub-stock.
4. Label this solution with the concentration, date prepared, initials, and date of expiration as it must be remade **once a month**. The stock solution is stored at room temperature.

Mercury (Hg) Sub-Stock Preparation: 500 µg/L Hg Sub-Stock

5. Add ~60 mL of deionized water to a 100-mL volumetric flask.
6. Into the flask, add the following:
 - 5.00 mL (i.e., using an adjustable-volume, 1-5 mL pipette) of the 10.0 mg/L Hg substock solution prepared in Steps 1 - 4
 - 0.5 mL trace metal grade concentrated HCl
 - 100 µL 5% (w/v) KMnO₄
7. Dilute to 100 mL with deionized water and mix thoroughly by inverting flask to prepare a 500 µg/L Hg sub-stock.
8. Label this solution with the concentration, date prepared, initials, and expiration date as it must be remade **once a week**. The stock solution is stored at room temperature.

Mercury Standards Preparation

9. Label digestion cups with the appropriate Hg concentrations (concentrations are listed in Table 1).
10. Pipet the volumes of deionized water and 500 µg/L Hg sub-stock into digestion vessels according to the table below (Table 1). Mercury concentrations of standards are based on the final volume (50 mL) of standard at the time of analysis.
11. Use an adjustable-volume, 10-100 µL or 100-1000 µL micropipette to deliver all water volumes and 500 µg/L Hg sub-stock Hg volumes less than 1 mL.
12. Each blank and standard should be prepared in duplicate.

Table 1. Mercury (Hg) Standard Preparation Volumes for Standards Ranging from 0 ng/L to 10,000 ng/L Hg.

Hg Standard Concentration (ng/L)	Volume of 500 µg/L Hg Sub-Stock	Volume of DI Water
Blank	0	1.00 mL
100	10 µL	990 µL
500	50 µL	950 µL
1000	100 µL	900 µL
5000	500 µL	500 µL
10,000	1.00 mL	0 mL

Mercury Spike Preparation

13. Spike a minimum of 10% of samples analyzed for mercury in duplicate.

14. Prepare each mercury spike by using an adjustable-volume micropipette to deliver 500 μL of 500 $\mu\text{g/L}$ Hg sub-stock into a digestion vessel containing a known weight of fish/tissue (i.e., weighed following the procedure outlined in *LSRI/SOP/SA/11*).

Waste Disposal

15. All mercury waste from rinsing pipettes, beakers, etc. should be disposed of in a mercury waste container. Volume and concentration placed in waste container should be recorded on the Hazardous Waste Container Inventory Form for that bottle.

COLD VAPOR MERCURY DETERMINATION IN BIOLOGICAL TISSUES USING THE FIMS-100

INTRODUCTION

This standard operating procedure (SOP) describes the operation of the FIMS-100 and S10 Autosampler (PerkinElmer Life and Analytical Sciences, Shelton, CT) to determine total mercury (organic and inorganic) concentrations in fish, hair, and other biological tissue samples. Do not use this procedure for analyzing human blood.

In this method, pre-weighed tissue samples are digested with sulfuric acid and nitric acid and oxidized overnight with potassium permanganate and potassium persulfate. Mercury in the digested samples is reduced with stannous chloride to elemental mercury and measured using flow-injection technique with atomic absorption (AA) detection (Lobring and Potter 1991). Note that the abbreviation 'FIMS' used in this procedure stands for 'Flow-Injection Mercury System', and the abbreviation 'FIAS' stands for 'Flow-Injection Analysis System'.

REFERENCES

- Lake Superior Research Institute. 1992. LSRI/SOP/SA/10 – Sample Grinding for Metals Analysis.
- Lake Superior Research Institute. 1992. LSRI/SOP/SA/11 – Sample Weighing for Metals Analysis.
- Lake Superior Research Institute. 1999. LSRI/SOP/SA/38 – Preparation of Tissues for Analytical Determinations using Liquid Nitrogen.
- Lake Superior Research Institute. 2002. LSRI/SOP/SA/42 – Stock, Standard, and Spike Preparation for Mercury Analysis.
- Lake Superior Research Institute. 2005. LSRI/SOP/SA/46 – Processing Several Large Fish into one Homogenous Fish Composite.
- Lake Superior Research Institute. 2007. LSRI/SOP/SA/50 – Routine Maintenance for FIMS-100.
- Lobring, L.B. and Potter, B.B. 1991. Method 245.6, Revision 2.3: *Determination of Mercury in Tissues by Cold Vapor Atomic Absorption Spectrometry*. Method from US Environmental Protection Agency, Office of Research and Development, Environmental Monitoring Systems Laboratory.
- PerkinElmer. 2007. S10 Autosampler For Atomic Spectrometry User's Guide.
- PerkinElmer. Various Dates. FIMS Flow Injection Mercury System Manuals. (Installation Maintenance System Description and Setting Up and Performing Analyses).

EQUIPMENT LIST

- ◆ 10 mg/L Mercuric Nitrate Sub-Stock for FIMS-100 Analysis (see *LSRI/SOP/SA/42*)
- ◆ 1000 µg/mL Purchased Mercuric Nitrate Stock
- ◆ 500 µg/L Mercuric Nitrate Sub-Stock for FIMS-100 Analysis (see *LSRI/SOP/SA/42*)
- ◆ Autosampler Rinse Bottle
- ◆ Autosampler Waste Container
- ◆ Balance, Top Loading or Analytical (must be capable of reading to 0.001 g)
- ◆ Beakers
- ◆ Certified Reference Material for Trace Metals (i.e., DORM-3)
- ◆ Deionized Water
- ◆ FIMS-100 (PerkinElmer) Mercury Analyzer
- ◆ FIMS-100 Record Notebook
- ◆ FIMS-100 Waste Container
- ◆ HotBlock™ (Environmental Express) and HotBlock™ Racks
- ◆ Hydrochloric Acid, Trace Metals Grade
- ◆ Hydroxylamine Hydrochloride, Reagent Suitable for Mercury Determination
- ◆ Kimwipes®
- ◆ Lab Coat
- ◆ Nitric Acid, Trace Metals Grade
- ◆ Pipets/Pipettors
- ◆ Polypropylene Digestion Cups and Covers
- ◆ Potassium Permanganate, Certified ACS
- ◆ Potassium Persulfate, Reagent Suitable for Mercury Determination
- ◆ Procedural Blanks
- ◆ Repipet Dispensers, 10 mL and 1-5 mL
- ◆ S10 Autosampler and Autosampler Racks
- ◆ Safety Glasses and Goggles
- ◆ Samples (prepared following *LSRI/SOP/SA/10*, *LSRI/SOP/SA/38*, or *LSRI/SOP/SA/46*)
- ◆ Sample Waste Container
- ◆ Silicon Defoaming Agent
- ◆ Sodium Chloride, Certified ACS
- ◆ Spatulas
- ◆ Stannous Chloride, Analytical Reagent
- ◆ Sulfuric Acid, Certified ACS, Reagent Suitable for Mercury Determination
- ◆ WinLab32™ for AA Software (PerkinElmer)

Reagents

- ◆ **10% (w/v) Hydroxylamine Hydrochloride with 10% (w/v) Sodium Chloride:**
Dissolve 200 g of hydroxylamine hydrochloride and 200 g of sodium chloride in 2 L of deionized water. Prepare solution as needed; expiration is six months from the date of preparation. Store solution at room temperature.
- ◆ **3% (v/v) Hydrochloric Acid (Carrier Solution):** Add 300 mL of trace metal grade hydrochloric acid to 10 L of deionized water. Prepare solution as needed; expiration is six months from the date of preparation. Store solution at room temperature.

- ◆ **5% (w/v) Potassium Permanganate:** Dissolve 100 g of potassium permanganate in 2 L of deionized water. Prepare solution as needed; expiration is six months from the date of preparation. Store solution at room temperature.
- ◆ **5% (w/v) Potassium Persulfate:** Dissolve 100 g of potassium persulfate in 2 L of deionized water. Prepare solution as needed; expiration is six months from the date of preparation. Store solution at room temperature.
- ◆ **5% (w/v) Stannous Chloride in 3% (v/v) Hydrochloric Acid (Reductant Solution):** Dissolve 75 g of stannous chloride in 1.5 L of 3% (v/v) Hydrochloric Acid. **This solution must be prepared daily.** Dispose of any unused solution as acid/base waste at the end of mercury analysis.

PROCEDURE

Sample and Standard Preparation

1. Turn the HotBlock™ on. Verify the digestion solution temperature by placing a digestion tube containing 50 mL deionized water into the HotBlock™. Allow the tube to remain in the HotBlock™ for a minimum of 30 minutes after the unit has reached the set-point temperature (i.e., 115°C ±5°C). Record the location of the tube in the HotBlock™ and measure and record the temperature of the water in the digestion tube on the Microsoft Excel “Mercury Master Daily Analysis Form”. The temperature of the water in the digestion cup should be 90°C ±5°C. If not, adjust the temperature setting on the HotBlock™ until the temperature of the water is within the accepted range. A different location in the HotBlock™ should be chosen each time a digestion is performed.
2. Prepare samples for mercury digestion and analysis following the appropriate LSRI SOP (e.g., *LSRI/SOP/SA/10 – Sample Grinding for Metals Analysis*, *LSRI/SOP/SA/46 – Processing Several Large Fish into one Homogenous Fish Composite*, or *LSRI/SOP/SA/38 – Preparation of Tissues for Analytical Determinations using Liquid Nitrogen*).
3. Weigh samples, including a set of procedural blanks, using the procedure outlined in *LSRI/SOP/SA/11 – Sample Weighing for Metals Analysis*. A minimum of 10% of the samples must be weighed in duplicate for duplicate analysis.
4. Weigh an appropriate mass of Certified Reference Material (CRM) for Trace Metals (i.e., DORM) using the procedure outlined in *LSRI/SOP/SA/11 – Sample Weighing for Metals Analysis*. An appropriate mass is one in which the analyzed CRM will fall within the range of the standard curve. For a set of mercury samples, CRM samples should be prepared and analyzed in a ratio of one CRM sample per 15 tissue samples. Typically, one set contains up to 40 tissue samples.
5. Prepare standards and spikes for mercury digestion and analysis following *LSRI/SOP/SA/42 – Stock, Standard, and Spike Preparation for Mercury Analysis*. Two sets of standards should be prepared for each set of mercury samples. In addition, 10% of the samples should be spiked in duplicate.

Sample Digestion

Note: The addition of acids and digestion of samples must be conducted in a fume hood. Proper personal protective clothing (e.g., gloves, lab coat, and safety goggles) must also be worn.

6. Add 4.0 mL of concentrated sulfuric acid and 1.0 mL of concentrated nitric acid to each sample, standard, spike, duplicate, and blank to be analyzed.
7. Place the racks containing the sample digestion cups into the HotBlock™. Allow samples to digest for approximately 15 minutes or until all the tissue is dissolved.
8. Turn off the HotBlock™, remove the HotBlock™ rack containing the digestion cups from the HotBlock™, and allow contents to cool to room temperature in the fume hood.
9. Add 15.0 mL of 5% (w/v) potassium permanganate to each digestion cup in 5.0 mL increments. Gently swirl the HotBlock™ rack holding the digestion cups after 15.0 mL is added.
10. Ensure that the samples remain purple in color for at least 15 minutes. If not, add additional 5% (w/v) potassium permanganate solution (maximum of 5 mL) to the samples. If additional 5% (w/v) potassium permanganate is added to a sample, an equal amount should be added to one set of standards and a blank.
11. Add 8.0 mL of 5% (w/v) potassium persulfate to each digestion cup and gently swirl to mix. After removing your gloves (to avoid contamination of caps), place a threaded cap loosely on top of each digestion cup to cover samples.
12. Allow the digestion cups to react overnight at room temperature to oxidize organic mercury compounds to inorganic mercury ions.
13. The samples can be stored covered in the fume hood, and will remain stable for up to three days before analysis. Samples are typically analyzed the day following the digestion process.

Sample Analysis Preparation

14. Prepare the carrier and reductant solutions (see "Reagents" section):
 - 14.1. **Carrier Solution:** 3% (v/v) hydrochloric acid.
 - 14.2. **Reductant Solution:** 5% (w/v) stannous chloride in 3% (v/v) hydrochloric acid. The volume of 5% stannous chloride prepared will depend on the number of samples to be analyzed. For a full set of 40 samples, prepare 1500 mL of Reductant Solution. **This solution must be prepared daily.**
 - 14.3. If the samples appear to be producing excessive foam during analysis (not typical), 10 mL of Silicon Defoaming Agent may be added per liter reductant solution.
15. Turn on computer and printer.

16. Press Ctrl+Alt+Del on the computer keyboard and log in using your UWS username and password.
17. Turn on FIMS-100 Mercury Analyzer and allow it to warm up for a minimum of 30-45 minutes.
18. On the FIMS-100, turn pump magazine pressure adjustment levers so that they fit into the notch on the back of the pump magazine (Figure 1).
19. Check Gas/Liquid Separator to be sure the filter is in place (smooth side down) and the cover has been tightened (Figure 1).
20. Attach tubing from Gas/Liquid Separator to the FIMS-Absorbance [Quartz] Cell (Figure 1).

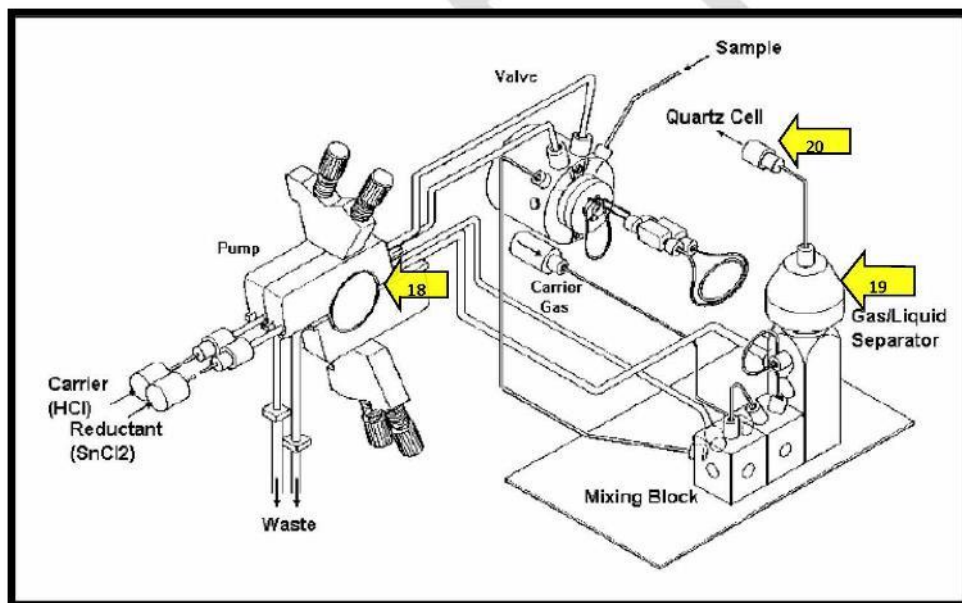


Figure 1. Diagram of the PerkinElmer FIMS-100. The yellow arrows indicate important areas of the instrument that need attention according to the referenced sections of this SOP.

21. Turn on Nitrogen (set pressure at 360 kPa or 52 psi).
22. If a Microsoft (MS) Excel file has been created for the project and stored on the "LSRItemp" Drive, access the file by clicking on the "LSRItemp" Drive shortcut on the desktop. After the FIMS-100 Mercury Analyzer has warmed up for a minimum of 10

minutes, double click on the **WinLab32 for AA** icon on the computer desktop.

23. Click on **Wrkspe** icon (Figure 2) and double-click on the **Auto.ffm** workspace when using the autosampler (normal operation) or the **Maunal.ffm** workspace when performing a manual analysis.
 - 23.1. Click on the Method button (Figure 2) and double click on **Hg extended RT5000**, which is the correct method for analysis. The method will then show in the **Automated Analysis Control** window.
24. In the **Automated Analysis Control** window near the Results Data Set Name click **open** and enter a new name or choose a file in the list (e.g., DateProject, see Figure 2). Be sure that the **save data** box is checked.

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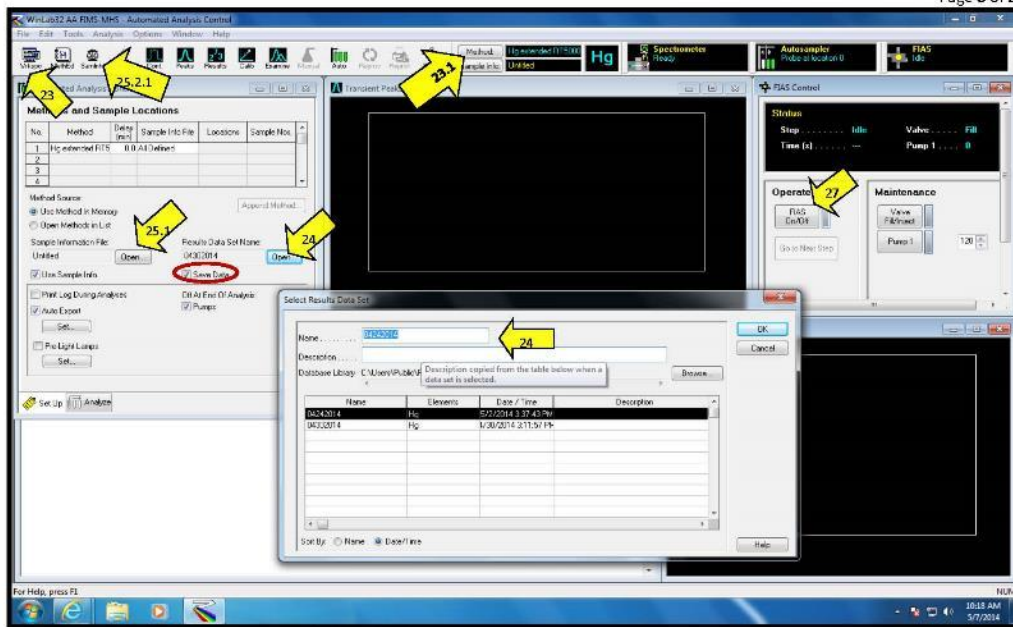


Figure 2. Screen shot of the control window of the WinLab32™ for AA Software. The yellow arrows indicate areas of importance and the corresponding steps referenced within this SOP.

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25. Choose or prepare the Sample Information File (SIF) using WinLab32™ for AA Software (Figure 3).
 - 25.1. If a sample set is to be run again, a previous SIF may be chosen by clicking on the **open** button near the **Sample Information File** field in the **Automated Analysis Control** window (Figure 2).
 - 25.2. To prepare a SIF with the same format as the MS Excel file (Figure 3):
 - 25.2.1. Click on the SamInfo button in the top left corner of the toolbar in the WinLab32™ for AA software (Figure 2). This will open the Sample Information Editor window where you can enter Autosampler (A/S) Location, Sample ID, Initial Sample Weight, and Sample Prep. Volume for each tissue sample and CRM sample (Figure 3).
 - 25.2.2. Highlight the rows of sample IDs and sample weights in the Excel file to be added to the Sample Information Editor (Figure 4) and copy (Ctrl+C) them. Paste (Ctrl+V) these rows into the Sample Information Editor. Note that Sample ID names must contain less than 25 characters. Also, be sure to leave room for where the blank-2, 100 ng/L-2 standard, and CRM samples and weights will be included in the Sample Information Editor. Note: The first CRM (e.g. DORM) sample is incorporated into the method to be analyzed immediately after the Calibration Curve, so this sample will not need to be included in the Sample Information Editor list.
 - 25.2.3. Samples should start in A/S location 12. A/S locations 1-11 are reserved for the first and second set of standards (excluding the second blank and 100 standard) and the first CRM sample. This can be altered if need be in the method (refer to Figure 6 under Method Modification Section below).
 - 25.2.4. After all sample information is entered, close the Sample Information Editor window.
 - 25.2.5. In the **Automated Analysis Control** window click on the open button near the Sample Information File field. A window will pop up prompting you to save changes to the sample information file (what you just entered into the Sample Information Editor). Click **yes** and save your new SIF under an appropriate name (e.g., Project Name and Date). You will then be prompted to choose a file to open.

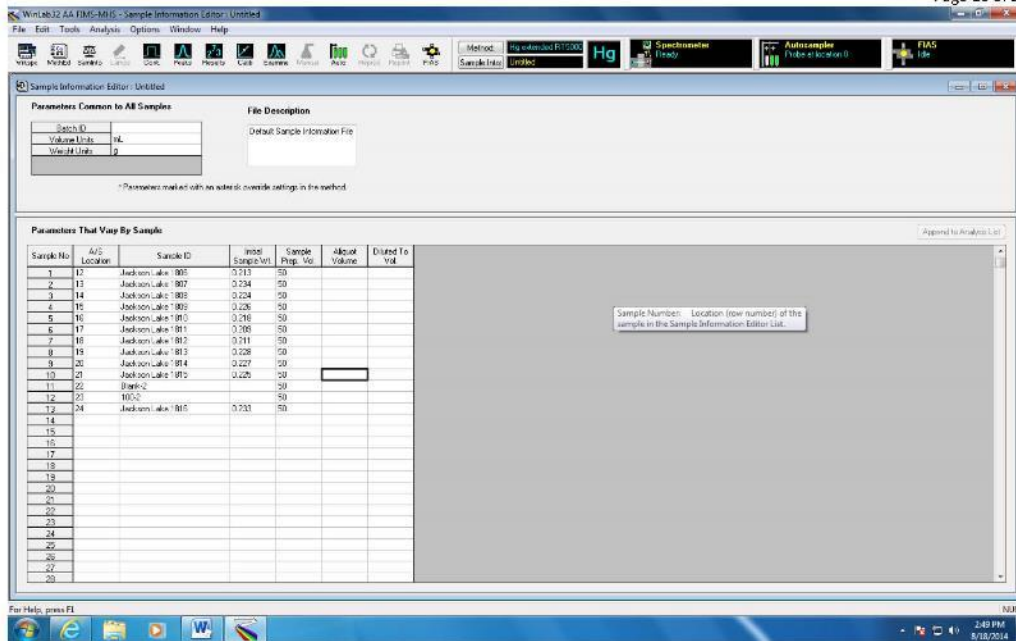


Figure 3. Preparation of a Sample Information File in WinLab32™ for AA Software from a Microsoft Excel File. Using Excel to create the Sample Information File is ideal if a project Excel file has been previously prepared.

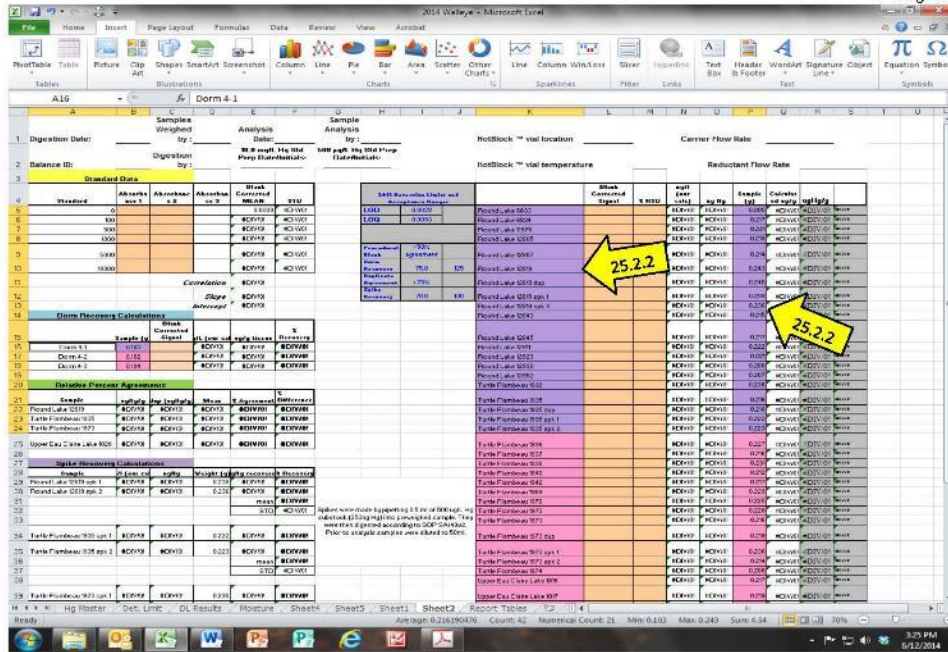


Figure 4. Example of a Microsoft Excel File that would be used to prepare a Sample Information File in WinLab32™ for AA Software

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26. The CRM concentration and minimum and maximum acceptable values need to be changed in the method prior to analysis. This must be changed for each analysis based on the weight in grams weighed out for the first CRM sample. Please refer to the **Method Modification** section below.
27. With all three collection tubes (sample, carrier, and reductant) in clean deionized water, run FIAS (Flow Injection Analysis System for Atomic Spectroscopy) once by clicking on the **FIAS on/off** button in the FIAS Control Window (Figure 2). Be sure that the waste tubing is in a waste collection container labeled "FIMS Waste".
28. Check the carrier and reductant flows. Place the carrier and reductant collection tubes into their appropriately labeled graduated cylinders and fill to 50 mL with deionized water. In the FIAS Control window, click **FIAS On/Off** under the **Operate** tab. Observe the volume withdrawn from each graduated cylinder over one minute.
 - 28.1. Once the time in the FIAS Control window reads **Idle**, remove the tubes from the water in the graduated cylinders and click FIAS On/Off again.
 - 28.2. When the **Pump 1** button in the FIAS Control window under the **Maintenance** tab becomes active (the small button to its right turns green), click on the **Pump 1** button to stop the pump. If this is not done then the pump will continue to draw up carrier and reductant and you will not get accurate flow rates. Place the tubes back into the graduated cylinders after Pump 1 has stopped to obtain accurate flow rate readings.
 - 28.3. Carrier volume should be between **9 and 11 mL/min** and reductant should be about half the carrier flow (**5 to 7 mL/min**). Record both the carrier flow and reductant flow in the project notebook and in the project MS Excel file. If needed, flow rates may be adjusted by turning the top knobs (clockwise to increase flow) on the pump magazine pressure adjustment levers.
 - 28.4. The waste flow rate should be set slightly higher than the flow rate into the gas/liquid separator. If it is not, liquid may get into the quartz cell. If the waste flow is higher than the flow into the gas/liquid separator, bubbles will appear in the waste outlet tube of the gas /liquid separator. The bottom knobs control the waste flow, and the presence of bubbles in the waste line must be verified each analysis day; the waste flow rate must be adjusted if no bubbles are observed in the waste line.
29. Place collection tubes into appropriate solution bottles (Red = Reductant solution, Yellow = Carrier Solution) and run FIAS one more time. Periodically check carrier and reductant volumes, so they do not deplete while running a sample set. After running the FIAS be sure to click the FIAS On/Off button again to stop the FIAS and click the **Pump 1** button, when it becomes active, to stop the pump from continually pumping carrier and reductant.
30. Just prior to analysis of blanks, standards, and samples, add 10 mL of 10% (w/v)

hydroxylamine hydrochloride with 10% (w/v) sodium chloride in two 5 mL aliquots, dilute accurately to 50 mL with deionized water using the correct line on the digestion vessel, cover with a screw cap and mix sample until no purple color remains and any brown precipitate dissolves. Be sure to loosen the cap periodically to vent the sample.

- 30.1. Continue to vigorously shake and vent the sample (even after color disappears and precipitate dissolves) until little to no bubbles are present in the sample. This will minimize off-gassing during analysis and prevent air bubbles from being taken up into the sample loop
- 30.2. The digestion vessels may appear brown due to staining from the chemical reagents. Safety glasses and gloves must be worn during this step.

Sample Analysis

31. Click on the **Analyze** tab at the bottom of the **Automated Analysis Control** window and then click **Calibrate**. This will begin the calibration curve starting with the blank. The autosampler will rinse after every standard. If at any time the calibration needs to be stopped, click on **Calibrate** again and choose from a list of options for stopping/pausing the calibration.
32. In the appropriate MS Excel file for the project, enter the Blank Corrected Signal values for the blanks and standards. Repeat this step for each of the five standards to be run in order of lowest to highest to develop the standard curve. Note: In the MS Excel file the Blank Corrected Mean value for the blank will always be 0.000.
33. Prior to analyzing samples check the following parameters:
 - 33.1. The slope of the calibration curve must fall between 2.0×10^{-5} to 3.0×10^{-5} and the correlation coefficient (r^2) must be greater than or equal to **0.995**.
 - 33.2. Review peak shape. The peak maximum should appear 5-10 seconds after the beginning of the read time and the signal should return to the baseline before the read time ends. If the peak is appearing too early, the carrier gas flow should be decreased. If the peak is appearing too late, the carrier gas flow should be increased. Generally, a flow in the range of **40-70 mL/min** is suitable.
 - 33.3. The 5000 ng/L standard must give a response between **0.12 and 0.17**.
 - 33.4. **If these checks do not fall in the acceptable range, check carrier and reductant flows, waste flows, and/or perform other maintenance as needed** (see *LSRI/SOP/SA/50 – Routine Maintenance for FIMS-100*).
34. After calibration is complete click on **Analyze Samples**. The method is set up to analyze the first CRM sample (e.g. DORM) as the first sample after the calibration curve. After this is complete then analysis of samples will begin in the order of autosampler location that was entered into the Sample Information File (starting with autosampler location 12 since 1-11 are reserved for two sets of standards and the first CRM sample).
 - 34.1. The autosampler racks have positions for up to 62 samples including the two sets of standards and the three CRM samples. If there are more than 45 samples in the set

(including duplicates and spikes) then you will need to start the autosampler location numbering over starting at 12 for the samples that exceed the 62nd position.

35. The autosampler probe will rinse after every sample. If at any time analysis needs to be stopped, click on **Analyze Samples** again and choose from a list of options for stopping/pausing the analysis.
36. Check that the sample ID, highlighted in green, in the Sample ID field of the Automated Analysis Control Window is correct.
37. Enter the mean Blank Corrected Signal and Percent Relative Standard Deviation (%RSD) values into the appropriate Excel file for that project. Repeat this step for each of the samples to be analyzed. Note that the **%RSD of the samples must be less than or equal to 5% for samples having concentration more than twice the limit of quantification (LOQ)** for that year. If the % RSD is greater than 5%, the sample must be re-analyzed.
38. The second set of standards (minus the blank and 100ng/L standard that will be run as samples) and CRM should be run as quality control (QC) checks, sometime in between samples, to check the precision and stability of the instrument. It is best to try to analyze the duplicates and spikes without interruption, so more or less than 10 samples may be analyzed between standards so that the samples can be kept together and in order. For example, if the sample set contains 50 samples, including duplicates and spikes, the set should be run in the following order:
 - First set of standards
 - Certified Reference Material
 - ~10 samples
 - Blank
 - Lowest standard (100 ng/L)
 - ~10 samples
 - Certified Reference Material
 - 500 ng/L standard
 - ~10 samples
 - 1000 ng/L standard
 - ~10 samples
 - 5000 ng/L standard
 - ~10 samples
 - Certified Reference Material
 - 10,000 ng/L standard

Note: Not all sample sets have the same amount of samples so the location of the second set of standards (minus the blank and 100 ng/L standard) may need to be modified in the method. Refer to Figure 5 under the Method Modification section below.

Completion of Analysis

39. Place autosampler probe and lines from reductant and carrier solutions into a clean beaker of deionized water.
40. Flush/clean probe and tubing with deionized water by running FIAS two times. This is accomplished by clicking the FIAS on/off button in the FIAS Control Window.
 - 40.1. After each time the FIAS has been run, the FIAS On/Off button must be clicked followed by the Pump 1 button. This will shut the pump off so it is not continually running.
41. Lift collection tubing and probe out of deionized water and run FIAS one more time to allow air to pass through all tubing and autosampler probe. When FIAS is finished running, return the autosample probe to its holder and place collection tubing back into beaker of DI water for storage.
42. Turn off the nitrogen gas.
43. Raise waste lines out of liquid in waste container so liquid does not back up.
44. Release the pump magazine pressure adjustment levers so that tubing is not compressed.
45. Unscrew line from FIMS-absorbance cell.
46. Unscrew the gas/liquid separator cover and, using forceps to handle filter, dry filter with a Kimwipe®. Replace filter and loosely put the cover back on.
47. Print report. Choose **File** → **Utilities** → **Data Manager** → Choose the data set for that day → Click **Report** → **Use Existing Design** and select **Browse** to choose **hg.rep** → **Open** → **Next** → Select all the samples for that date or choose **today only** → Choose **Preview**. If acceptable, print the report. If additional information or different settings are desired, **Next** may be chosen and the design may be modified.
48. Save the MS Excel file to the appropriate project folder.
49. Turn off FIMS instrument, computer, and printer.
50. Record the date, project, analyst, number of injections, and run time in FIMS-100 Record Notebook located in the laboratory with the instrument.
51. Any sample or standard remaining in the digestion tubes after the analysis has been completed should be collected in a container labeled "Waste Samples/Standards from Mercury Analysis" and disposed of in accordance with the rules and regulations of the UWS Environmental Health and Safety Office.

Method Modification (For Atypical Sample Sizes)

52. When analysis sets vary in size it must be determined where each standard in the second set of standards will be incorporated as a QC check. Click the **MethEd** button on the toolbar and select the **QC** tab in the Method Editor window and then click on the **Schedule QCs** side tab (Figure 5). Under each QC standard you can select when to run that standard, whether it be After Initial Calib, After Reclaibration, Periodically, or At End. If you select Periodically then you can also enter a number to indicate how frequently this standard will be analyzed (e.g. after **10** samples).

52.1. The Certified Reference Material should be analyzed **After Initial Calibration**.

The Blank and the 100 ng/L standard are analyzed with the sample set. The 500 ng/L standard is analyzed **Periodically** after 23 samples. The 1000 and 5000 ng/L standards are analyzed **Periodically** but their location depends on the size of the analysis set and also where duplicate and spike samples are located (it's best not to analyze a standard in the middle of a sample that has a duplicate and spikes). The 10,000 ng/L standard is analyzed **At End**.

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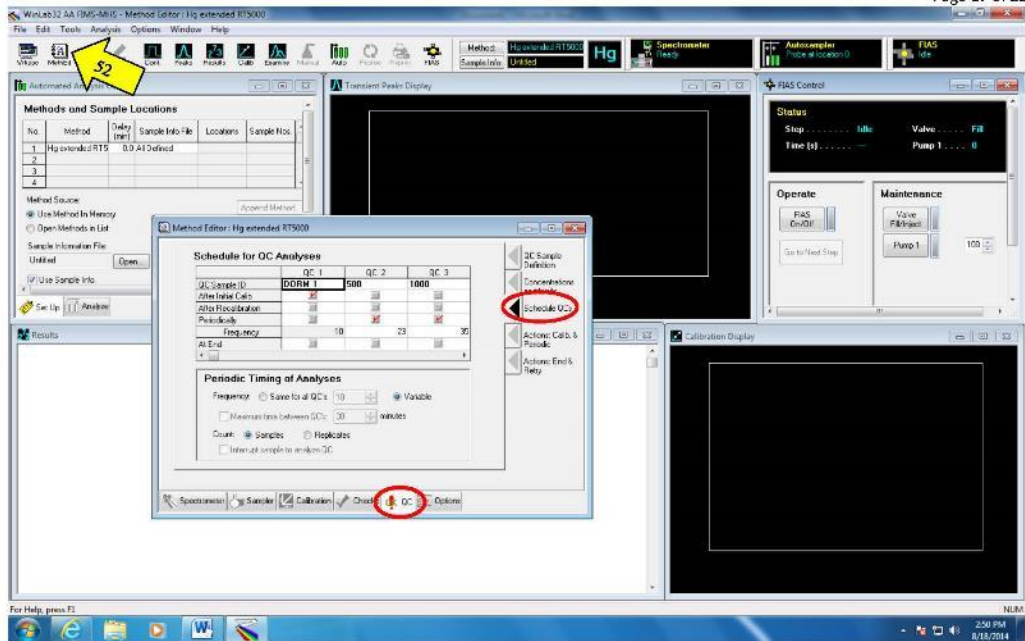


Figure 5. Screenshot of the Method Editor Window of the WinLab32™ for AA Software. The yellow arrow indicates an area of importance and the corresponding steps referenced within this SOP.

53. To change the concentration and acceptable range of the first CRM sample click the **MethEd** button on the toolbar and then select the **QC** tab in the Method Editor window and then click on the **Concentrations and Limits** side tab (Figure 6). Select DORM 1 in the drop down menu and determine and set the concentration and limits using the following equation:

$$\left[\frac{\text{Weight of CRM (g)} \times \text{Concentration of the CRM stock (ug/g)}}{\text{Sample Prep volume (L)}} \right] \times 1000$$

$$\pm 25\% \text{ e.g.: } \left[\frac{0.104\text{g} \times 0.410\text{ug/g}}{0.05\text{L}} \right] \times 1000 = 853 - 25\% = 640$$
$$853 + 25\% = 1066$$

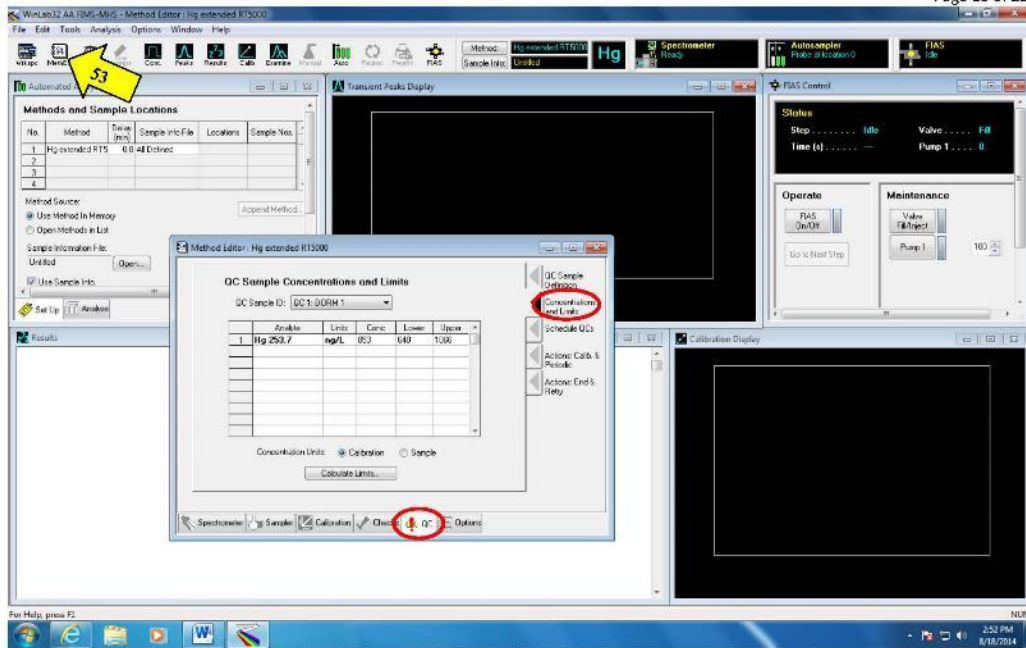


Figure 6. Screenshot of the Method Editor Window of the WinLab32™ for AA Software. Alterations must be made to the first CRM sample concentration and lower and upper limits depending on the weight of CRM sample weighed out.

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54. To change autosampler locations for QC samples click the **MethEd** button on the toolbar and then select the **QC** tab in the Method Editor window and then click on the **QC Sample Definition** side tab (Figure 7). Locations and names of the QC samples and standards can be altered in this window.

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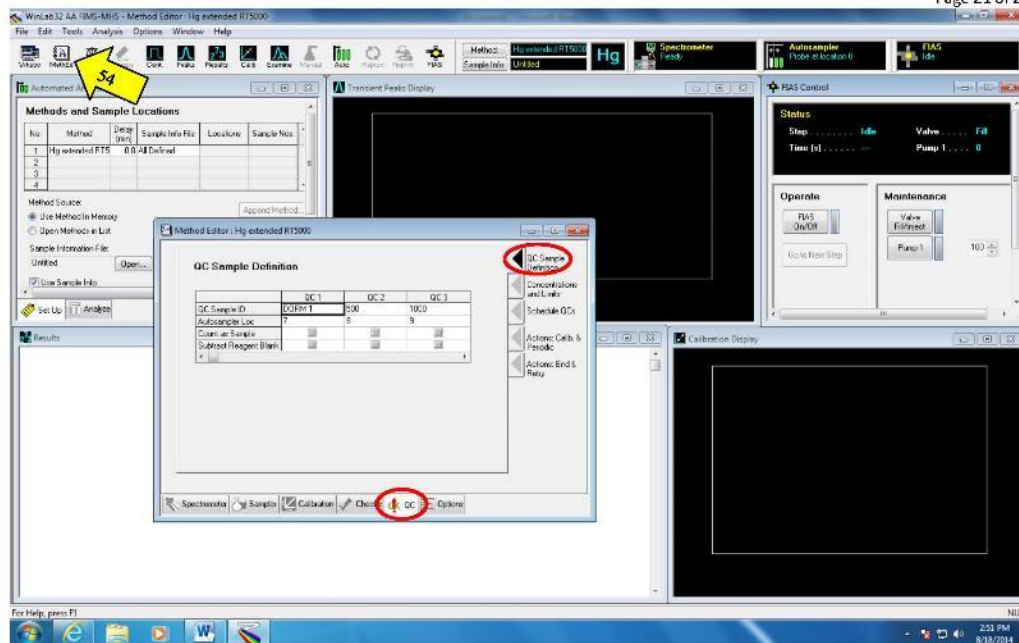


Figure 7. Screenshot of the Method Editor Window of the WinLab32™ for AA Software. Alterations can be made to the quality control sample names and locations in the Method Editor Window.

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ROUTINE MAINTENANCE FOR THE FIMS-100 MERCURY ANALYSIS SYSTEM

INTRODUCTION

This procedure is used for the routine maintenance of the PerkinElmer Flow Injection Mercury System Model 100 (FIMS-100; Waltham, MA USA) to ensure optimal performance of the instrument. The proper safety equipment must be worn during the entire cleaning procedure. This includes gloves, safety glasses, and lab coat.

REFERENCES

PerkinElmer Instruments. 1994. FIMS Flow Injection Mercury System- Installation, Maintenance and System Description Manual.

PerkinElmer Instruments. 1994. FIMS Flow Injection Mercury System – Setting Up and Performing Analyses Manual.

EQUIPMENT LIST

- ◆ FIMS-100
- ◆ Lab Coat
- ◆ Gloves
- ◆ Safety Glasses
- ◆ FIMS-100 Record Book
- ◆ PerkinElmer *FIMS-100 Installation, Maintenance and System Description Manual*
- ◆ PerkinElmer *FIMS Flow Injection Mercury System – Setting Up and Performing Analyses Manual*
- ◆ Spare Parts for FIMS-100
- ◆ 7 mm Wrench
- ◆ Hex/Allen Wrench
- ◆ Small Flathead Screwdriver
- ◆ Valve Dismantling Tool
- ◆ Silicone Vacuum Grease
- ◆ Silicone Spray Lubricant
- ◆ Kimwipes®
- ◆ Methanol
- ◆ Forceps
- ◆ 2- 50 mL Graduated Cylinders
- ◆ Deionized Water

PROCEDURE

General Preventative Maintenance

1. Wipe up spills immediately for safety reasons and to avoid contaminating new samples.
2. After each use, wipe over the instrument's outer surfaces with a clean cloth moistened with a dilute solution of laboratory detergent.
3. Record daily usage in FIMS-100 Record Book, including date, project, analyst, number of injections, and hours of use.
4. Record any routine and non-routine maintenance performed in the FIMS-100 Record Book.
5. Install a new air filter yearly or more often in a dusty environment. Refer to the *Installation, Maintenance and System Description Manual*, page 2-19:
 - a. Turn off the FIMS-100.
 - b. Remove the filter cover and filter (Figure 1).
 - c. Insert a new filter (Part Number B050-2706).
 - d. Firmly press the filter cover back in place.
 - e. Place a piece of tape on the filter cover stating the date the filter was replaced.
 - f. Record in the FIMS-100 Record Book the date that the filter was replaced.



Figure 1. Photograph showing the location of the air filter on the back of the FIMS-100 Mercury Analyzer.

6. Install a new mercury absorber cartridge yearly, by following the method below:
 - a. The mercury absorber cartridge is located at the end of a piece of silicon tubing that is attached to the left-hand nipple on the FIMS-cell (Figure 2).

- b. Remove the old mercury absorber cartridge by pulling it off of the end of the silicon tube.
- c. Push the new mercury filter on to the end of the silicon tube.
- d. Place a piece of tape on the mercury filter stating the date the mercury filter was replaced.
- e. Record in the FIMS-100 Record Book the date that the mercury filter was replaced.



Figure 2. Photograph showing the mercury filter attached to the silicon tube.

7. To perform the remaining routine maintenance tasks in this SOP, turn on the computer. Press Ctrl+Alt+Del on the computer keyboard and enter “Barstow 9B” for the username and “fims100” for the password, while “Bars 9B-9061” shows in the LOG ONTO window.
8. Turn on the nitrogen gas (set pressure at 400 kPa) and the FIMS-100 Mercury Analysis System. Allow the system to warm up for a minimum of 10 minutes.
9. On the desktop, double click on the icon named “WinLab 32 for AA” to open the WinLab application.

Carrier Gas System Maintenance

10. Periodically check the non-return valve located under the FIAS (Flow Injection Atomic Spectroscopy) valve (Figure 3A and 3B). If the rubber sleeve shows signs of deterioration, fit a new one (see page 2-18 of the *Installation, Maintenance, and System Description Manual*). The rubber sleeve should just cover the holes on the valve and not extend beyond the valve tip as this may alter the gas flow.

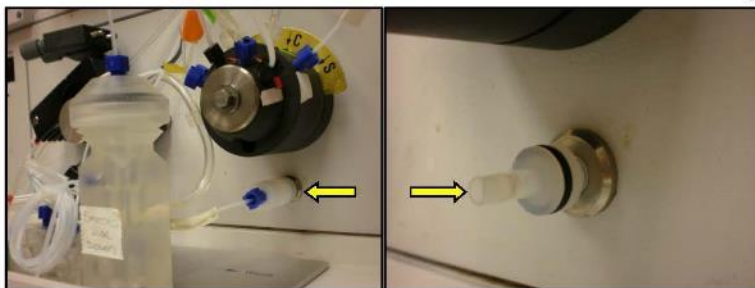


Figure 3A and 3B. Photograph showing the location of the non-return valve (3A) and the rubber sleeve covering the holes on the valve insert.

11. Carrier gas flow should read 40-70 mL/min on the Carrier Gas Flow Gauge while the FIAS pump is running (Figure 4). When the FIAS pump is not running, the carrier gas flow should rest between 70-100 mL/min. If the flow seems to fluctuate outside of this range it can be adjusted using the carrier gas flow regulator (refer to the *Installation, Maintenance and System Description Manual* page 1-25). If this does not work then the flow meter may need to be cleaned.

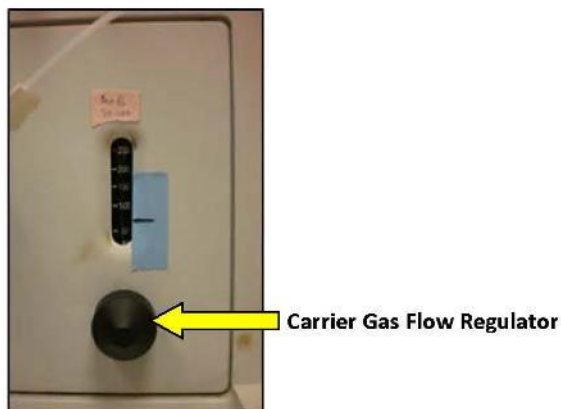


Figure 4. Photograph showing the Carrier Gas Flow Gauge and Carrier Gas Flow Regulator.

- a. The flow meter can be cleaned by removing it from the FIMS-100 (this must be done while the instrument is off and unplugged) and soaking the flow tube and ball in methanol. These parts must then be allowed to dry. Silicone high vacuum grease must be applied to O-rings on each end of the flow tube to prevent leaks. The flow meter must then be reassembled and reinstalled in the FIMS-100.

Carrier and Reductant Solution Flows

12. Prior to analysis, the carrier and reductant solution flows should be checked and flows recorded in the FIMS-100 Record Book.

13. Adjust the carrier and reductant flows to produce a ratio of carrier flow to reductant flow of approximately 2:1 with a carrier flow between 9 and 11 mL/min and the reductant flow between 5 and 7 mL/min.
 - a. Place the carrier tube inlet in the graduated cylinder labeled “carrier” and the reductant tube inlet in the graduated cylinder labeled “reductant.” Bring the deionized water level in the graduated cylinders to the 50 mL mark. In the **FIAS Control Window**, click on the **FIAS On/Off** under the **Operate** tab to start the FIAS (Figure 5). After running the FIAS for one minute, note the decrease in volume. The flow should be 9-11 mL/minute for the carrier tube and 5-7 mL/minute for the reductant tube.

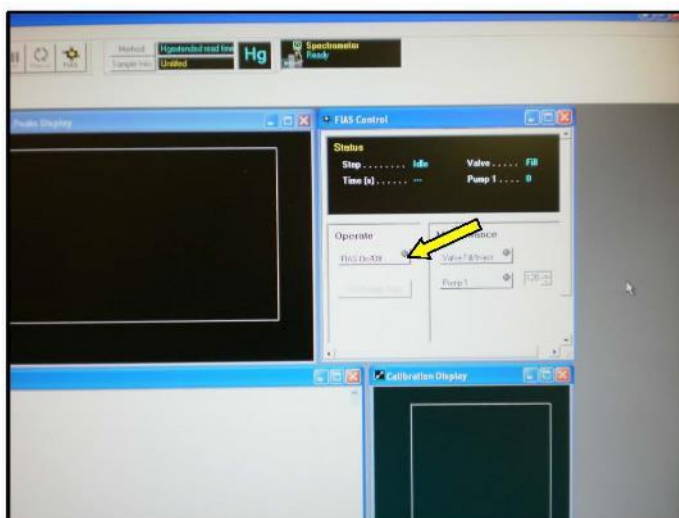


Figure 5. Photograph showing the screen image for initiating the FIAS to check carrier and reductant flow rates.

- b. If the flows are not within the acceptable range, adjust the pressure on the appropriate pump tube by turning the top knobs (clockwise to increase flow) on the pump magazine pressure adjustment levers until the flow is within the range (Figure 6).
- c. If the desired flow is not attained by adjusting the pressure on the pump tubes, it suggests that there is an obstruction in a delivery tube. The tube will need to be removed and flushed with deionized water or replaced.

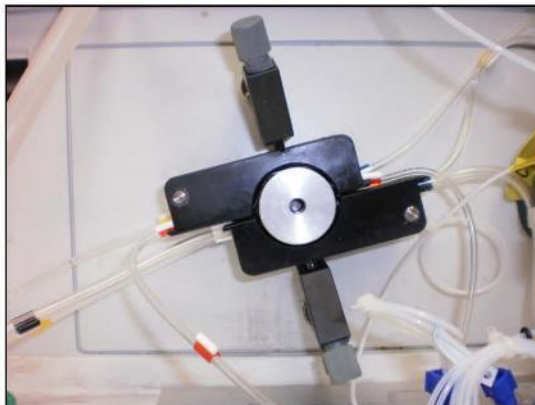


Figure 6. Photograph showing the location of the knobs which can be adjusted to change the flow rate of the pump.

14. During analysis, if the peak shape is abnormal, does not return to baseline, or the 5000 ng/l Hg standard gives an absorbance that is not between 0.12 and 0.17, the carrier and reductant flows should be checked again and flows recorded in the FIMS-100 Record Book.

Gas/Liquid Separator Maintenance

15. Prior to analysis, unscrew the gas/liquid separator cover and place a clean, dry polytetrafluoroethylene (Teflon, PTFE) membrane filter (smooth side down) on the separator block (Figures 7A and 7B), then replace the separator cover (refer to the *Installation, Maintenance and System Description Manual* page 1-22).



Figure 7A and 7B. Photograph showing the gas/liquid separator and the filter in placement in the gas/liquid separator.

- a. During analysis, monitor the liquid bubbling in the separator block below the filter. If the bubbling is excessive and it appears that the bubbles are reaching the filter this will cause the filter to become saturated. The filter must remain clean and dry for successful analyses.
- b. If the filter is saturated, remove the filter using a forceps and dry it with a Kimwipe®. Dry out the separator block and cover. Place a dry filter on the separator block and replace the cover.
- c. If the separator cover becomes saturated, verify that the sample transfer tubing connecting the separator cover to the FIMS cell does not contain moisture.
- d. If there is moisture in the tubing (part number: 198-097) replace the tubing and verify that the cell and cell windows are clean and dry.
- e. If moisture is visible in the cell or on the cell windows refer to “FIMS-Cell Maintenance” section below for proper cleaning techniques.

Spectrophotometer Maintenance

16. Measure and record the absorbance of the FIMS-cell window in the FIMS-100 Record Book regularly.
 - a. In the WinLab32 application, open the Continuous Graphics window (‘Cont’ on toolbar).
 - b. Remove the FIMS-cell from the cell compartment.
 - c. Click on **Autozero** in the **Continuous Graphics** window (Figure 8).

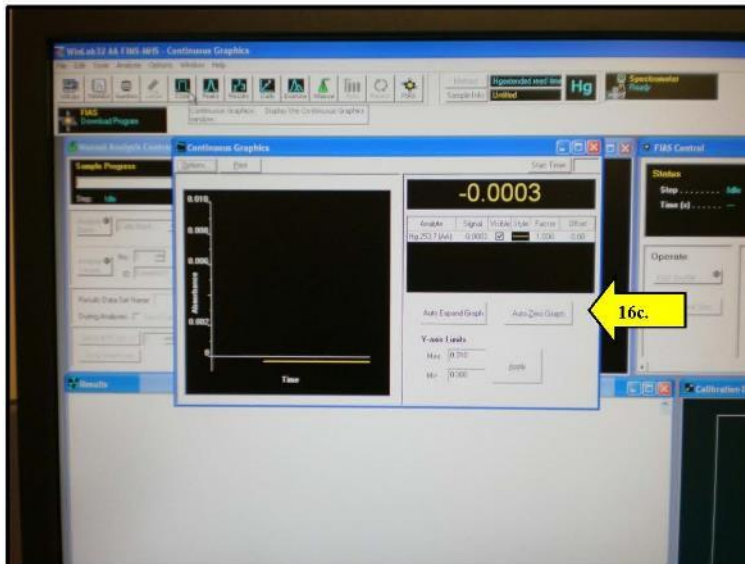


Figure 8. Photograph showing the screen image for measuring the absorbance of the FIMS-cell window.

- d. Install the FIMS-cell back in the cell compartment.
- e. The absorbance reading in the **Continuous Graphics** window is the absorbance of the FIMS-cell window. Clean windows should have an absorbance between 0.03 and 0.07. If the absorbance is greater than this, the windows should be cleaned. Refer to the Installation, Maintenance, and System Description manual page 2-10.

FIMS-Cell Maintenance

17. If there is a decrease in sensitivity (not attributable to factors such as unsuitable analytical parameters or instrument settings, or incorrectly prepared or contaminated solutions) or if moisture is visible in the cell or on the cell windows the cell and/or cell windows must be removed and cleaned (Figure 9).

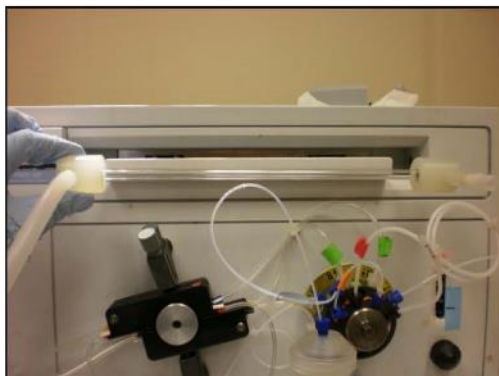


Figure 9. Photograph showing the FIMS-cell after it has been removed from the instrument.

- a. Be sure that there is no analysis in progress, remove the FIMS-cell. Pull and simultaneously twist the cell window assemblies off the ends of the FIMS-cell.
- b. Carefully use a small screwdriver to remove the outer O-ring that surrounds the window and carefully remove the cell window. Clean the windows with deionized water and dry with a Kimwipe[®] (refer to the *Installation, Maintenance and System Description Manual* page 2-9). If moisture has entered the cell, attempt to dry it with a Kimwipe[®]. If this is not feasible, the cell may need to air dry and analysis will temporarily be suspended.
- c. After cleaning and drying the cell and/or cell window, carefully fit the cell windows and window assemblies to the FIMS-cell. Re-install the FIMS-cell in the Spectrometer (refer to the *Installation, Maintenance and System Description Manual* page 2-8 and 2-9).
- d. Once the FIMS-cell is installed in the Spectrometer, measure the absorbance of the windows. If the absorbance is not within the acceptable range the cell/cell windows may need to be cleaned more thoroughly using a soft, lint-free cloth moistened with spectroscopic grade alcohol (see page 2-10 of *Installation, Maintenance and System Description Manual*). If the cleaning process still fails to produce an acceptable

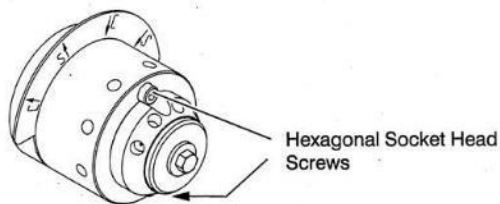
absorbance the cell windows may need to be replaced.

Fluid System Maintenance

18. To reduce wear on pump tubes, spray a small amount of silicone lubricant on the part of the tube in contact with the pump rollers prior to analysis of samples and standards (Figure 6).
19. Following analysis, rinse the fluid system with deionized water. This is done by placing the sample, carrier, and reductant tubes in deionized water and running the FIAS. The fluid system should be rinsed twice while in deionized water and once while being held out of the water so that air is allowed to pass through the system. After the system is flushed with air, the sample, carrier and reductant tubes should be placed back into deionized water until the next use.
20. Release tension on the pump tubes when analysis and tube rinsing is completed.
21. Wipe pump rollers with a dry lint-free cloth.
22. Inspect all fluid tubes daily during periods of instrument usage for damage such as kinks, leaks or clogs. Install new tubes as necessary.

FIAS-Valve Maintenance

23. Observe the FIAS valve prior to and during analysis for any clogs that may be present. A clog may be indicated by uncharacteristic absorbance results.
 - a. Pause the analysis if during analysis it is thought that there might be a clog in the valve or valve tubing. Disconnect all the tubes from the valve and pump deionized water through each channel of the valve and through all tubing that is connected to the valve.
 - b. If this is not sufficient in removing the clog then remove the valve from the pump unit and dismantle the valve (refer to the *Installation, Maintenance and System Description Manual* pages 2-14 through 2-16; Figure 10). Once the valve is dismantled, clean each individual part and again pump water through the valve channels and components.



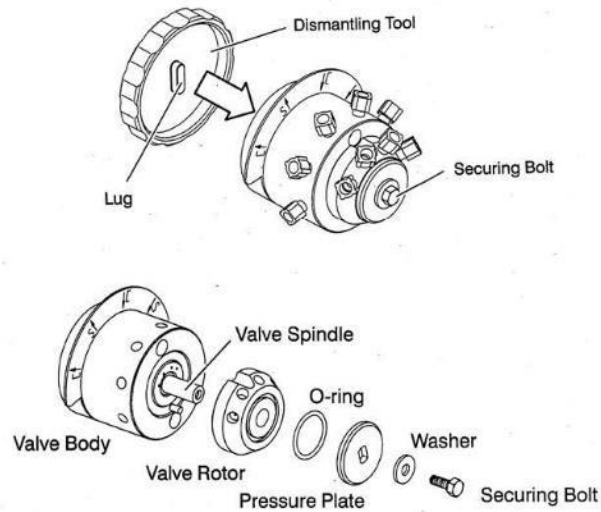


Figure 10. This series of diagrams shows how to remove the FIAS-valve from the motor mount, dismantle the FIAS-valve and separate the FIAS-valve components.

- c. To reassemble the valve and reattach the valve to the pump unit refer to the *Installation, Maintenance and System Description Manual* pages 2-14 and 2-17. Apply silicone vacuum grease to the O-ring located in the valve rotor prior to reassembly to prevent leakage.

PROCEDURE FOR DETERMINING PERCENT MOISTURE IN TISSUE SAMPLES

INTRODUCTION

This standard operating procedure (SOP) describes the method used in determining the percent moisture content in biological tissue samples. This is a gravimetric method that requires careful weighing techniques. Once the aluminum weigh pans have been dried, they must only be handled with forceps to avoid addition of oils/moisture from the researchers' hands. The addition of oils/moisture will cause an error in the pan weight.

DEFINITIONS

Gravimetric: Of or pertaining to measurement by weight.

REFERENCES

Lake Superior Research Institute. 1995. LSRI/SOP/GLM/12 – Procedure for Verifying Calibration of Laboratory Balances.

EQUIPMENT LIST

- ◆ Aluminum Weigh Pans
- ◆ Analytical Balance (i.e., capable of weighing to 0.001 g)
- ◆ ASTM/ANSI Class 1 Weights
- ◆ Balance Brush
- ◆ Desiccation Container with Dry Desiccant
- ◆ Drying Oven (60°C ± 10°C)
- ◆ Forceps
- ◆ Laboratory Notebook and/or Datasheet (see Appendix 1)
- ◆ Spatula

PROCEDURE

1. Label the aluminum weigh pans and dry at 60°C (±10°C) for a minimum of two hours. Record the date and time that the pans were placed into and removed from the oven in the appropriate laboratory notebook or on the "Tissue Moisture Determination" datasheet (Appendix 1).
2. Using forceps, place dried weighing pans in desiccator until cool (i.e., to approximately room temperature), which should take approximately 3-5 minutes.
3. Check analytical balance calibration using Class 1 weights according to *LSRI/SOP/GLM/12 – Procedure for Verifying Calibration of Laboratory Balances* (issued 1995). Weigh the dried

and cooled weighing pans on balance to the 0.001 g and record weight in the appropriate laboratory notebook or datasheet (Appendix 1).

4. Add tissue (i.e., 1.0 g – 5.0 g) to the labeled weighing pan.
5. Weigh the pan and the tissue on balance to the nearest 0.001 g and record weight in the appropriate laboratory notebook or datasheet (Appendix 1).
6. Dry pan and tissue in drying oven at 60°C (±10°C) for a minimum of 16 hours or until constant dry weight is achieved. Record the date and time that the pans were placed in the oven in the appropriate laboratory notebook or datasheet (Appendix 1).
7. Remove dried pans and tissue from the oven and place in a desiccator until cool. Record the date and time that the pans were removed from the oven in the appropriate laboratory notebook or datasheet (Appendix 1).
8. Weigh the pan with the dried tissue on a balance to the nearest 0.001 g and record weight in the appropriate laboratory notebook or datasheet (Appendix 1). It may be necessary to dry the pan and tissue a second time when the tissue is a large mass. In addition, a minimum of 10% of the samples must be dried a second time. Dry a second time, desiccate, and re-weigh to prove that constant dry weight (i.e., the weight change is less than 4% of the first dry weight) has been achieved. Record the date and time that the pans were weighed a second time, as well as, the second dry weight in the appropriate laboratory notebook or datasheet (Appendix 1).
9. Calculations:

$$\text{Wet Weight of Tissue (g)} = (\text{Weight of Pan} + \text{Wet Tissue}) - (\text{Weight Dry Pan})$$

Percent Moisture of Tissue

$$= \left(\frac{(\text{Weight Pan} + \text{Wet Tissue}) - (\text{Weight Pan} + \text{Dry Tissue})}{\text{Wet Tissue Weight}} \right) \times 100\%$$

APPENDIX 1

TISSUE MOISTURE DETERMINATION DATASHEET

