ANALYSIS OF TOTAL MERCURY IN SEDIMENTS AND WASTES BY COLD VAPOR ATOMIC ABSORPTION (CVAA) SPECTROSCOPY

1. SCOPE AND APPLICATION

This standard operating procedure (SOP) describes the analysis of sediment and waste samples for total mercury content utilizing cold vapor-atomic absorption (CVAA) spectroscopy techniques. The procedure is a combination of EPA Method 245.5 and SW846 method 7471B and is applicable to the determination of mercury in soil, sediments, sludge, and waste samples. This SOP is applicable to samples having the Test Identifiers (Test ID) S-HG-H and WASTE-HG-H.

2. SUMMARY OF THE METHOD

Samples are digested using a combination of acids and strong oxidizers which converts all mercury present in the sample to Hg^{+2}. The samples are then treated with a hydroxylamine hydrochloride solution to remove excess oxidizing reagents. Each sample digestate is introduced into a gas-liquid separator via an autosampler and peristaltic pump delivery system. The mercuric ions are reduced to atomic mercury vapor by stannous chloride in the gas-liquid separator and purged into the spectrometer's absorption cell by a stream of air. Mercury in sediment is measured as µg/L and reported as mg/kg of dry sediment.

3. APPARATUS AND EQUIPMENT

3.1 Nippon RA-3320 Reduced Vaporization Mercury Analyzer; Serial No. 06400552. Software: RA3Win, version 1.2.2 (2000):

- 3.1.1 Nippon SC-3 autosampler: Serial No.06410266. Autosampler with a 10 standard sample and 50 test sample rack.
- 3.1.2 Nippon RD-3 reagent dispenser: Serial No. 06420390.
- 3.1.3 Nippon sample test tubes.

3.2 Class A volumetric flasks

3.3 Pipettes:

- 3.3.1 Eppendorf Reference 100 – 1000 uL adjustable
- 3.3.2 Eppendorf Reference 10 – 100 uL adjustable

3.4 Denver S-403 top-loading balance, S/N 257509421, max capacity 400 g.

3.5 Sartorius A 120 S analytical balance, S/N 3908007, max capacity 121 g.

3.6 Denver MXX-123 top-loading balance, S/N 25955867, max capacity 120 g.

3.7 Bottle-top dispensers

4. REAGENTS AND CHEMICALS

Reagents used for analysis must be logged in to the LIMS standard prep tracker to generate a serial number. The label printed from the LIMS standard prep tracker must be

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placed on the bottle of reagent in the laboratory. The reagents used in analysis are documented on the 'MT-HG-RGNT' form located on the Department's computer network, \\FLDEP1\DEAR\Labs\Lab5\Chemistry\InorganicPrep\Mercury\S-HG-H\. Update the log for each analysis and place a copy in the lab report.

Save all certificates of analysis for reagents as pdf files in the network location, \\FLDEP1\DEAR\Labs\Lab5\Chemistry\InorganicPrep\Certificates of Analysis for Reagents and Standards\.

See DEP SOP MT-069 for reagent and standard documentation requirements and expiration dates.

4.1 Deionized (DI) water.

4.2 Trace Metal Grade Sulfuric Acid (H_2SO_4) - The purchased acid is stored in the fume hood cabinet at room temperature and the expiration date is provided by the manufacturer.

4.3 Trace Metal Grade Hydrochloric Acid (HCl) - The purchased acid is stored in the fume hood cabinet at room temperature and the expiration date is provided by the manufacturer.

4.4 Trace Metal Grade Nitric Acid (HNO_3) - The purchased acid is stored in the fume hood cabinet at room temperature and the expiration date is provided by the manufacturer.

4.5 50 % (v/v) Sulfuric Acid - Add 250 mL of concentrated H_2SO_4 to 500 mL volumetric flask containing 200 mL of DI water. Dilute to volume and mix well. Store this reagent in a 1 L Teflon bottle. The expiration date given for this prepared reagent is the same as the manufacturer's expiration date for the concentrated acid.

4.6 20 % (w/v) Hydroxylamine Hydrochloride Solution - Add 50 g of hydroxylamine hydrochloride to a 250 mL Teflon bottle. Dilute to volume with DI water and mix well. The expiration date for this prepared reagent is 1 year from the preparation date.

4.7 10 % (w/v) Stannous Chloride ($SnCl_2$) Solution - In a fume hood, slowly add and mix 25 mL of H_2SO_4 to a 500 mL Teflon bottle containing 250 mL of DI water. Weigh 50 g of anhydrous $SnCl_2$ crystals or 60 g of $SnCl_2$ di-hydrate ($SnCl_2 \bullet 2H_2O$) and add to the acidic solution. Dilute to volume and mix well. The expiration date for this prepared reagent is 2 days after the preparation date.

4.8 6 % (w/v) Potassium Permanganate ($KMnO_4$) Solution - Add 30 g of $KMnO_4$ crystals to a 500 mL Teflon bottle containing 250 mL of DI water. Dilute to volume with DI water and mix well. The expiration date for this prepared reagent is one day after preparation.

4.9 6 % (w/v) Potassium Persulfate ($K_2S_2O_8$) Solution - Add 15 g of $K_2S_2O_8$ crystals to a 250 mL Teflon bottle containing 125 mL of DI water. Dilute to volume with DI water and mix well. The expiration date for this prepared reagent is one day after preparation.

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4.10 Potassium Bromate ($KBrO_3$, 0.05 M)/Potassium Bromide (KBr, 0.1 M) Solution - Place 8.4 g of $KBrO_3$ and 11.9 g of KBr in a 1 L Teflon bottle and dissolve with 1 L of DI water. The expiration date for this reagent is one year from preparation and it is stored at room temperature.

4.11 1 M Sodium Hydroxide Solution - Dissolve 40 g of NaOH in 1 L of DI water. This solution is used to clean stannous chloride residue from the Nippon autosampler tubes.

5. STANDARDS

The calibration standards used for the analysis must be logged in to the LIMS standard prep tracker to generate a serial number and a label, which must be placed on the bottle of calibration standard in the laboratory. The calibration standards used for analysis are documented on the 'MT-HGCAL-STD' form located on the Department's computer network, \\FLDEP1\DEAR\Labs\Lab5\Chemistry\InorganicPrep\Mercury\S-HG-H\. Update the form for each analysis and print a copy to place in the lab report.

The stock and intermediate standards must be logged in to the LIMS standard prep tracker to generate a serial number and a label, which must be placed on the bottle of stock or intermediate standard in the laboratory. Stock/Intermediate standards are documented on the 'MT-HGINT-STD' form, located on the Department's computer network, \\FLDEP1\DEAR\Labs\Lab5\Chemistry\InorganicPrep\Mercury\S-HG-H\. Print a copy to include in each lab report.

See DEP SOP MT-069 for reagent and standard documentation requirements and expiration dates.

See DEP SOP HG-012 for additional information about preparing mercury standards.

Support equipment used during calibration standard preparation includes various balances as well as various Eppendorf pipettes. Prior to use, verify the balance calibration has been checked and is within acceptable tolerance and verify the pipette calibration has been checked within the last month. Calibration documentation can be found on the network, \\FLDEP1\DEAR\Labs\Lab5\Chemistry\InorganicPrep\Mercury\Prep\Pipette Calibration and in the balance calibration logbooks.

Save all certificates of analysis for standards and standard reference materials as pdf files in the network location, \\FLDEP1\DEAR\Labs\Lab5\Chemistry\InorganicPrep\Certificates of Analysis for Reagents and Standards\.

5.1 Commercial Stock Standard 1000 µg/mL - Commercially available inorganic mercury stock from SPEX, Accustandard, Baker, Baxter, Fisher, VSR, etc. The expiration date is assigned by the manufacturer. Stored at room temperature.

5.2 THG-1 (100 mg/L) Instrument Calibration Stock Solution - Pipette 10.0 mL of commercial 1000 µg/mL stock standard into a 100 mL volumetric flask containing 50 mL of DI water. Add 5 mL of HNO_3, 3 mL of HCl, and 3 mL of the bromate/bromide solution (4.10). Dilute to volume with DI water and mix well. The expiration date for this prepared standard is the same as the commercial stock from which it was made.

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5.3 THG-2 (500 µg/L) Instrument Calibration Intermediate Solution - Pipette 5 mL of THG-1 into a 1 L volumetric flask containing 500 mL of DI water. Add 50 mL of HNO_3, 30 mL of HCl, and 30 mL of the bromide/bromate solution (4.10). Dilute to volume with DI water and mix well. The expiration date for this prepared standard is the same as the commercial stock from which it was made.

5.4 PACS-3 (3.0 ± 0.5 mg/kg) Marine Sediment Certified Reference Material – Purchased from the National Research Council Canada. This standard is stored at room temperature in a desiccator and the expiration date is assigned by the manufacturer.

5.5 Working calibration standards are prepared according to the following procedure:

5.5.1 Prepare a set of calibration standards using clean, dry and pre-labeled Teflon bottles. Add DI water with a calibrated bottle-top dispenser or verify the volume of DI water added with a calibrated balance and use a calibrated pipette for addition of the THG-2 standard.

5.5.2 Using Table 1 as a guide, pipette the appropriate aliquots of THG-2 into each container. The concentrations are 0.625, 1.25, 3.75, 6.25 and 10.0 µg/L. S-STD3 is both a calibration standard and the continuing calibration verification (CCV) check standard and S-STD4 is both a calibration standard and the initial calibration verification (ICV). The volume of DI water added includes an extra 4 mL in order to compensate for the volume of H_2O_2 used (added) in the digestion of sediments and wastes.

TABLE 1.

Calibration Standards Preparation

Level	Concentration (µg/L)	Volume of THG-2 (µL)	Volume of DI water (mL)
S-STD1	0.625	100	84.0
S-STD2	1.25	200	84.0
S-STD3 (CCV)	3.75	600	83.5
S-STD4 (ICV)	6.25	1000	83.0
S-STD5	10.0	1600	82.5

5.5.3 Add 10 mL of HNO_3 to all standard vessels. Gently swirl digestion vessels to mix. Solution may become hot. Allow to cool if necessary.

5.5.4 Add 20 mL of 6 % $KMnO_4$ to all standard vessels. Add 8 mL of 6 % $K_2S_2O_8$ to all standard vessels. Tighten cap and invert standard vessels to mix. Loosen caps and allow to cool.

5.5.5 The expiration date for the working standards (S-STD1 – S-STD5) is 7 days from the preparation date.

5.5.6 The working calibration standards must be documented in the LIMS standard prep tracker and a label printed from the LIMS standard prep tracker placed on each bottle.

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5.5.7 Prepare the calibration blank by pouring DI water into the autosampler vial. The DI water is also used as the continuing calibration blank (CCB).

5.5.8 Add 8 mL of the 20 % hydroxylamine hydrochloride to each bottle of calibration standard. This reagent reduces the excess oxidant in the standard. Invert the bottle several times to mix and periodically loosen the cap to release pressure. The purple color of the standard should disappear leaving behind a clear colorless to pale yellow liquid.

6. SAMPLE COLLECTION, PRESERVATION AND HANDLING

Sediment or waste samples are collected and stored in 250 mL wide-mouth glass containers with screw caps. The samples are preserved by refrigerating the containers to a temperature of 4 °C. Prior to digestion, the samples are allowed to warm to room temperature before weighting.

7. SAMPLE PREPARATION PROCEDURE

7.1 Refer to DEP SOP HG-020 for sample preparation details.

7.2 Prepare the samples for analysis by adding 4 mL of the 20 % hydroxylamine hydrochloride to each sample. This reagent reduces the excess oxidant in the digested sample. Tighten the digestion vessel caps and gently swirl and invert the vessels to mix the reagents. Loosen the cap to release pressure. Make sure that all visible particulates are mixed into the liquid. The purple color of the digestates should disappear leaving behind a clear colorless to pale yellow liquid.

7.3 If the purple color persists, add additional hydroxylamine to the sample in 1 mL increments and mix until the solution turns colorless. Record the amount of additional hydroxylamine added to the sample in the sample comments column of the prep sheet in the LIMS Prep Worksheet Manager. Notify supervisor if the LIMS prep sheet has already been completed. This information will be used later to compensate for changes in volume during calculations.

8. INSTRUMENT SETUP AND OPERATION

8.1 Start the instrument by turning on the switches at the rear of the RA-3 analyzer and at the front of the SC-3 autosampler. A standby screen will appear on the instrument display for 20 minutes while the system is warming up and stabilizing.

Note: The instrument can be left on overnight. However, if it will not be used on consecutive days it should be shutdown following the procedure in section 8.12.

8.2 While the instrument is warming up prepare a 50 % H_2SO_4 solution and a 10 % $SnCl_2$ solution. Reagent preparation instructions can be found in section 4.

8.3 Fill the bottles on the RD-3. The large bottle should be filled with DI water. The middle bottle should be filled with 50 mL of 50 % H_2SO_4 solution and the front bottle should be filled with 50 mL of 10 % $SnCl_2$ solution. Connect the reagent tube from each peristaltic pump to its respective bottle.

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8.4 At the end of the stabilization period the instrument display will show two options, 'SC/RD' and 'Maintenance'. Select the 'SC/RD' by touching it on the screen. On the next screen select 'Measurement Preparation' and hit 'ENT'. Place a tube in the waste position of the autosampler and a rinse test tube in the cleaning holder. Push the start button. When reagent filling is complete, press esc to return to the main screen.

8.5 Double click on the 'RA3Win' icon on the desktop to launch the software.

8.6 In the Table window click on the 'STD' tab. Type in the standard value and check the 'Mes' cell for each line that is being used in the table. The first two standards run should be DI water blanks. Press start

8.7 After pressing start, the calibration sequence initiates. Five calibration standards of varying concentration are run plus a blank. The calibration curve is calculated by the instrument software using the equation $y = ax + 0$.

8.8 In the Table window click on the 'SMP' tab. Type in the sample IDs. Check the 'Mes' cell for each line that is being used in the table. Every 10 samples, a CCV and every 5 samples a CCB must be analyzed. In the 'SMP' table window, the CCV and CCB can be typed in the sequence every 10 and 5 samples.

8.9 Additional samples can be added to the run while samples are running by typing the sample information into the 'SMP' table and being sure to check the 'Mes' cell.

8.10 Measurement conditions can be found by selecting the Measurement Condition option in the Run Menu. Table 3 contains the values for the parameters for this method.

TABLE 3

Instrument Parameters for Nippon RA-3320

Parameter	**Value**
Common Tab	
Measurement Time (seconds)	120
Time axis (seconds)	180
Analog out range	0.05
Upper limit (Absorbance)	1.50000
Purge time (seconds)	180
Measurement	Continue
Control Tab	
Std (µg/L)	5
Max Dev (%)	10
Abnormal Conditions	stop
Calibration Curve	y = mx + 0
Peak/Integ	Integ

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Parameter	Value
Common Tab	
Unit	µg/L
Decimal Places	3
Drift Correction	No
Description of Minus	Minus as it is

8.11 When the run is complete select the 'SC/RD' screen on the instrument display. Select the 'Measurement Complete' option and follow the onscreen directions for rinsing the reagent tubes. When the 'SC/RD' screen returns the instrument can be shut down by turning off the switch on the back of the instrument and turning off the switch on the front side of the autosampler.

8.12 Nippon test tubes must be cleaned after each run. This is accomplished by rinsing the tubes with water followed by soaking them for a minimum of two hours in aqua regia. If the tubes contain a stannous chloride residue after cleaning with aqua regia, the tubes should be soaked overnight in a 1 M sodium hydroxide base bath.

9. DATA ARCHIVAL

The Nippon instrument software does not automatically save the results of an analytical run. After measurements are complete, click File>Save and save the instrument software file (RA3 Data File) on the hard drive of the instrument computer with the format N2014aa0216w1. Then, the worksheet needs to be saved as a comma delimited 'CSV' text file. This is done by selecting 'save as text file' under the edit menu. The text file is saved to \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Mercury\Nippon_AA\. A file with the name N2014aa0216s1 would be the first sediment run on February 16, 2014 using the Nippon RA-3320.

10. QUALITY CONTROL

10.1 Table 4 lists the quality control parameters and their acceptable limits for sediment, soil, and waste samples analyzed by this method (where MDL is the method detection limit, PQL is the practical quantitation level, and LFB is the laboratory fortified blank). Consult supervisor or see DEP SOP LB-027 for reporting data associated with out-of-control quality control measures.

TABLE 4.

QC for Sediments and Waste

CALIBRATION CURVE	$R \geq 0.995$		
MDL	0.15 µg/L		
CCB	-MDL < CONCENTRATION < MDL		
	CONCENTRATION	**RECOVERY**	**RPD**
PQL	0.6250 µg/L	70 % - 130 %	

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	CONCENTRATION	RECOVERY	RPD
PACS-3	3.0 mg/kg	80 % - 120 %	
DUP. SPIKE (sediment)	1.25 µg/L	75 % - 125 %	≤ 20 %
DUP. SPIKE (waste)	2.50 µg/L	75 % - 125 %	≤ 20 %
ICV	6.25 µg/L	85 % - 115 %	
CCV	3.75 µg/L	85 % - 115 %	
LFB	1.25 µg/L	75 % - 125 %	

10.2 Calculations for replicate analyses to evaluate precision, the recovery of laboratory control samples, and the recovery of matrix spikes are detailed in section 5.9 of the Laboratory Quality Manual.

11. INTERFERENCES

11.1 Volatile compounds (benzene, toluene, acetone, carbon tetrachloride, etc.) that absorb at the same wavelength (253.7 nm) as mercury constitute a positive interferent for this method. These compounds are most likely volatilized during digestion. Volatile compounds may be removed by swirling the open digestion vessel after the addition of the stannous chloride solution or sonicating the open digestion vessel prior to transferring it to the autosampler rack.

11.2 Positive interference has been also observed in samples containing high levels of sulfides, copper, and chlorides probably due to the formation of free chlorine.

12. CORRECTIVE ACTIONS FOR COMMON PROBLEMS

Corrective actions for common QC failures and out-of-control data are listed in Table 5.

TABLE 5.

Corrective Action

Problem	Corrective Action
Calibration standard's response factor below expected value.	Check the instrument lamp voltage. Adjust voltage to 4.0 V if necessary, recalibrate. Check every tubing connection for possible gas leaks. Replace autosampler bubbler filter. Check pump tubing and if worn and flattened, replace.
Calibration curve correlation coefficient out of range.	Remake the standards and recalibrate the instrument.
Failure of CCB.	Check for stannous chloride residue in autosampler tubes. Soak tubes in NaOH base bath if residue observed, then recalibrate. Compare the blank absorption to previous calibrations.

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Problem	Corrective Action
Failure of CCV.	Check the detector for signal drift. Recalibrate if necessary.
Failure of second source standard, PQL, LFB, matrix spikes, or replicate RPD.	Redigest the sample and repeat the analysis or qualify results.

13. MAINTENANCE

13.1 Peristaltic pump tubing should be replaced once per year.

13.2 The filter on the autosampler bubbler should be changed at least every 3 months.

13.3 Light source lamp needs to be replaced if instrument display shows an alarm message of 'Lamp Life Exceeded' or 'Lamp Broken'. The lamp life error message will appear when the total operating time exceeds 20,000 hours.

13.4 The warning message 'Carbon Filter Full' appears when the activated carbon filter in the RA-3 unit has absorbed more than 1.5 g of mercury. Section 4.1.3 in the RA-3000 Maintenance Manual explains how to replace this filter.

13.5 If contamination or reduced sensitivity become a problem or the error message 'Cell Dark' appears the I joint, L joint, Teflon tubes, dehumidifier tube and joints, cell body, and quartz lenses need to be cleaned. Specific instructions are giving in section 5 of the RA-3000 Maintenance Manual.

14. SAFETY/HAZARDOUS WASTE MANAGEMENT

14.1 See DEP SOP HG-013 for correct waste disposal procedures.

14.2 Always wear safety glasses or protective eyewear and a laboratory coat while working in the laboratory. Powder-free latex gloves should be worn while handling chemicals. When disposing the method blank and PQL samples, carefully collect the glass beads for proper disposal. Concentrated acids should be handled in a well ventilated hood.

15. REFERENCES

15.1 Nippon Mercury/RA-3000 system measurement manual

15.2 Nippon Mercury/RA-3 Data Analysis software manual

15.3 Nippon Mercury/RA-3000 system maintenance manual

15.4 DEP SOP HG-012, Mercury Group Standards Preparation Procedures

15.5 DEP SOP HG-013, Mercury Waste Disposal

15.6 DEP SOP HG-020, Digestion of Sediment and Waste Samples for Total Mercury Analysis

15.7 DEP SOP MT-058, Procedure for Metals Data Review and Authorization

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15.8 DEP SOP MT-069, Metals Standard and Reagent Preparation Procedure

15.9 Quality Manual, DEP Laboratory.

15.10 Laboratory Health and Safety Plan, located on the Division's intranet page.

15.11 DEP SOP CM-011, Gravimetric Analysis: Analytical Weight and Balance Calibration

15.12 DEP SOP LB-027, Standard Operating Procedure for Reporting Qualified Data

Appendix of Significant Changes

07/09/2008 A couple of older sections were consolidated into Section 6. Other minor revisions to the text were made for format and clarity.

03/09/2009 The Cetac instrument was surplused. The Nippon RA-3320 sample analysis procedure was added (Section 8.2).

01/07/2015 The Varian SpectraAA-220 was surplused. Equipment and procedure related to the Varian AA instrument was removed from the SOP.

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