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

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1. SCOPE AND APPLICATION

This standard operating procedure (SOP) describes the analysis of water samples for total mercury content utilizing cold vapor-atomic absorption (CVAA) spectroscopy. The procedure is based on EPA Method 245.1 and SW-846 Method 7470A. It is applicable to the determination of mercury in surface, saline, effluent, and waste waters. It is also used for the analysis of TCLP and SPLP extracts. This SOP is applicable to the analysis of samples with Test Identifiers (Test ID) W-HG-H, W-HG-H-F, W-HG-H-R, TCLP-HG-H and SPLP-HG-H. The method detection limit (MDL) is 0.10 µg/L and the practical quantitation limit is 0.30 µg/L.

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 sodium chloride-hydroxylammonium chloride 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 nitrogen gas or air. The mercury present is measured as a change in absorption in a photomultiplier tube. Mercury in water is measured and reported as µg/L mercury.

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 Pipettes:

- 3.2.1 100 – 1000 µL Eppendorf pipette
- 3.2.2 10 – 100 µL Eppendorf pipette
- 3.2.3 Bottle-top dispensers

3.3 Denver Instrument MXX-123 top-loading balance. 120 g capacity.

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 placed on the bottle of reagent in the laboratory. If a balance is used to prepare a reagent,

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document the balance ID in the LIMS standard prep tracker module. The reagents used in analysis are documented on the MT-HG-RGNT form located on the Division of Environmental Assessment and Restoration (DEAR) internet site. Update the form for each analysis and place a copy in the lab report. See DEP SOP MT-069 for reagent 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 and the expiration date is provided by the manufacturer.

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

4.4 50 % (v/v) Sulfuric Acid - Add 250 mL of concentrated H_2SO_4 to 500 mL volumetric flask half filled with 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.

4.5 12 % Sodium chloride-hydroxylammonium chloride solution ($NH_2OH{\cdot}HCl$) - Add 60 g of hydroxylamine hydrochloride and 60 g sodium chloride to a 500 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.

4.6 10 % Stannous Chloride Solution ($SnCl_2{\cdot}2H_2O$) - Working in the fume hood, slowly add with gentle mixing 7 mL of H_2SO_4 to a 500 mL Teflon bottle half full with DI water. Weigh 50 g of $SnCl_2$ di-hydrate ($SnCl_2{\cdot}2H_2O$) 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.

4.7 5 % Potassium Permanganate ($KMnO_4$) Solution - Add 25 g of $KMnO_4$ crystals to a 500 mL Teflon bottle half full with DI water. Dilute to volume with DI water and mix well. The expiration date for this prepared reagent is one day after preparation.

4.8 5 % Potassium Persulfate ($K_2S_2O_8$) Solution - Add 12.5 g of $K_2S_2O_8$ crystals to 250 mL Teflon bottle half full with DI water and dilute to volume with DI water. Mix well. The expiration date for this prepared reagent is one day after preparation.

5. CALIBRATION STANDARDS

Preparation of all Hg standards must be documented in the LIMS standard prep tracker. A label must be printed from the standard prep tracker that shows the identity of the standard, preparation date, expiration date, and serial number. The label must be placed on the bottle of standard in the laboratory. See DEP SOP MT-069 for standard documentation requirements and expiration dates. See DEP SOP HG-012 for expiration dates and additional information about preparing mercury standards.

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Support equipment used during calibration standard preparation includes the Ohaus top loading balance 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\Pipette Calibration\High Level Hg pipette calibration and in the 'Denver MXX-123 Balance Calibration Logbook'.'.

Document the intermediate standards used for each analysis on the MT-HGINT-STD form located on the DEAR internet site. Update the form for each analysis and print the form to include in each laboratory report.

5.1 Commercial Stock Standard 1000 µg/mL - Commercially available inorganic mercury stock from SPEX, Accustandard, Baker, Fisher, VSR, etc.

5.2 WTHG-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 about 50 mL of DI water. Add 5 mL of HNO_3. Dilute to volume with DI water and mix well.

5.3 WTHG-2 (500 µg/L) Instrument Calibration Intermediate Solution - Pipette 5.0 mL of WTHG-1 into a 1 L volumetric flask containing about 500 mL of DI water. Add 50 mL of HNO_3. Dilute to volume with DI water and mix well.

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

5.4.1 Prepare a set of calibration standards using clean, dry and pre-labeled Teflon bottles. Add DI water using a calibrated bottle-top dispenser and use a calibrated pipette for the WTHG-2 addition.

5.4.2 Using Table 1 as a guide, pipette the appropriate aliquots of WTHG-2 into each container. The concentrations are 0.30, 1.0, 2.0, 5.0 and 10.0 µg/L. W-STD3 is both a calibration standard and the continuing calibration verification (CCV) check standard.

TABLE 1.

Level	Concentration (µg/L)	Volume of WTHG-2 (µL)	Volume of DI water (mL)
W-STD1	0.30	60	100.0
W-STD2	1.00	200	100.0
W-STD3 (CCV)	2.00	400	99.6
W-STD4	5.00	1000	99.0
W-STD5	10.00	2000	98.0

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5.4.3 Add 5 mL of H_2SO_4 to all standard vessels. Add 2.5 mL HNO_3 to all standard vessels. Gently swirl digestion vessels to mix. The solution may become hot. Allow the solution to cool if necessary.

5.4.4 Add 15.0 mL 5 % $KMnO_4$ to all standard vessels. Add 8.0 mL 5 % $K_2S_2O_8$ to all standard vessels. Tighten cap and invert standard vessels to mix. Loosen caps and allow to cool.

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

5.4.6 Prepare the calibration blank. The calibration blank must contain all reagents in the same concentration and in the same volume as used in preparing the calibration standards. The calibration blank is also used as the continuing calibration blank (CCB).

5.4.7 Document the calibration standards used for analysis on the MT-HGCAL-STD form located on the DEAR internet site. Update the form for each analysis and print a copy of the form to include in each laboratory report folder.

6. SAMPLE COLLECTION, PRESERVATION AND HANDLING

Water samples are collected in 1 L polyethylene bottles with screw caps and preserved by acidifying them with nitric acid to a pH below 2. The preservation is done immediately after the samples are collected, prior to shipment to the laboratory. The samples are stored at room temperature.

7. SAMPLE PREPARATION PROCEDURE

7.1 Refer to DEP SOP HG-018 for sample preparation details. A copy of the LIMS Prep Worksheet Manager worksheet is included with the digested samples.

7.2 Prepare the digestates for analysis by adding 2.5 mL of the 12 % sodium chloride-hydroxylammonium chloride solution to each sample digestate. This reagent reduces the excess oxidant in the digested sample. Tighten the digestion vessel caps and gently swirl and invert the vessels several times to mix the reagents. Loosen the cap to release pressure. Make sure that you mix all visible particulates into the liquid. The purple color of the digestates should disappear leaving behind a clear colorless liquid.

7.3 If the purple color persists, add additional 12 % sodium chloride-hydroxylammonium chloride solution 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 comment section of the LIMS Prep Worksheet Manager worksheet. This information will be used later to compensate for changes in volume during calculations.

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8. INSTRUMENT SETUP AND OPERATION

8.1 Nippon RA-3320 - 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 8.1.11.

8.1.1 While the instrument is warming up prepare a 50 % H_2SO_4 solution and a 10 % $SnCl_2 \cdot H_2O$ solution. Reagent preparation instructions can be found in section 4.

8.1.2 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 % $SnCl_2 \cdot H_2O$ solution. Connect the reagent tube from each pump to its respective bottle.

8.1.3 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.

8.1.4 Double click on the ‘RA3Win’ icon on the desktop to launch the software.

8.1.5 The online profile window displays a peak profile of absorbance during measurement. At the same time, it also indicates an instantaneous absorbance value, a peak value, and an integrated value. The calibration window shows the created calibration curve.

8.1.6 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.

8.1.7 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 $y = mx + 0$.

8.1.8 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 respectively.

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Example Autosampler Sequence:
DI Water Blank
DI Water Blank
W-STD1
W-STD2
W-STD3
W-STD4
W-STD5
ICV (W-STD4)
CCB
CCV (W-STD3)
CCB
Method Blank (batch 1)
PQL
LFB
W-NIST3133
Sample 1
CCB
Samples 2 – 5
CCV (W-STD3)
CCB

8.1.9 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.

8.1.10 Measurement conditions can be found by selecting the 'Measurement Condition' option in the Run Menu. Table 2 contains these parameters.

TABLE 2

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

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Parameter	Value
Abnormal Conditions	Stop
Calibration Curve	y = mx + 0
Peak / Integ	Integ
Unit	µg/L
Decimal places	3
Drift Correction	No
Description of Minus	Minus as it is

8.1.11 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. Flip the switch on the back, right side of the instrument to off and flip the switch on the front side of the autosampler to off.

8.1.12 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. $SnCl_2$ build up appears as a haze on the autosampler tubes. The $SnCl_2$ residue can be removed by soaking the tubes in a base bath. Prepare a 1M NaOH base bath by dissolving 40 g of NaOH pellets to 1 L of water in a bucket. Soak the tubes in the base bath for at least 2 hours. Remove the tubes from the base bath and rinse with DI water three times and allow to dry.

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 N2017aa0216w1. Then, the worksheet needs to be saved as a comma delimited 'CSV' text file. This is done by selecting 'save as text file' in the edit menu. The text file is saved to \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Mercury\Nippon_AA. The text file naming convention includes an 'N' for Nippon, the year, the month, the day, and the run number. A file with the name N2017aa0216w1 would be the first water run on February 16, 2017 using the Nippon RA-3320.

10. QUALITY CONTROL

Table 3 shown below lists the quality control parameters and their acceptable limits for any water or TCLP extract analysis using this SOP. Consult supervisor or see DEP SOP LB-027 for reporting data associated with out-of-control quality control parameters.

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MDL is the method detection limit, PQL is the practical quantitation level, and LFB is the laboratory fortified blank.

TABLE 3.

CALIBRATION	R ≥ 0.995		
MDL	0.10 µg/L		
CCB	-MDL < CONCENTRATION < MDL		
	LEVEL	**RECOVERY**	**RPD**
PQL	0.30 µg/L	70 % - 130 %	
W-NIST3133	3.0 µg/L	90 % - 110 %	
DUP. SPIKE	1.0 µg/L	85 % - 115 %	≤ 20 %
ICV	5.0 µg/L	95 % - 105 %	
CCV	3.0 µg/L	90 % - 110 %	
LFB	1.0 µg/L	85 % – 115 %	

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 that absorb at the same wavelength (253.7 nm) as mercury constitute a positive interferent for this method.

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.

11.3 Addition of H_2SO_4 may induce the formation of a heavy precipitate. If this is present, the affected sample cannot be analyzed by this method.

12. CORRECTIVE ACTIONS FOR COMMON PROBLEMS

Corrective actions for common QC failures and out-of-control data are listed in Table 4 below.

TABLE 4.

Problem	Corrective Action
Calibration standard's response factor below expected value.	Check the instrument lamp and its alignment. Check absorption cell and its alignment. Check every tubing connection for possible gas leaks.
Calibration curve correlation coefficient out of range.	Remake the standards and recalibrate the instrument.

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Problem	Corrective Action
Failure of CCB.	Determine if the failure is caused by contamination or instrument drift by analyzing another blank after re-cleaning a new autosampler tube. If instrument drift is suspected, re-calibrate the instrument. If contamination is suspected, re-clean all tubes.
Failure of CCV.	Check the detector for signal drift. Recalibrate if necessary.
Failure of second source standard, PQL, LFB, matrix spikes, or matrix spike RPD.	Redigest the samples and repeat the analysis.

13. MAINTENANCE

13.1 Nippon RA-3320:

13.1.1 Pump Tubing should be replaced once per year

13.1.2 Filter on the autosampler bubbler should be changed every 3 months

13.1.3 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.

13.1.4 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.

13.1.5 If contamination or reduced sensitivity becomes 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 lab coat while working in the laboratory. Powder-free latex gloves should be worn while handling chemicals. Concentrated acids should be handled in a well ventilated hood.

15. REFERENCES

15.1 EPA Method 245.1, Rev. 3.0, Determination of Mercury in Water by Cold Vapor Atomic Absorption Spectrometry

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15.2 EPA Method 7470A, Mercury in Liquid Waste (Manual Cold Vapor Technique)

15.3 Nippon Mercury/RA-3000 system measurement manual

15.4 Nippon Mercury/RA-3 Data Analysis software manual

15.5 Nippon Mercury/RA-3000 system maintenance manual

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

15.7 DEP SOP HG-013, Mercury Waste Disposal

15.8 DEP SOP HG-018, Digestion of Water Samples for Total Mercury Analysis

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

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

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

15.12 Laboratory Quality Manual, located on the DEAR internet site.

15.13 Health and Safety Manual, located on the DEAR internet site.

Appendix of Significant Changes

07/10/2008 A couple of older sections were consolidated into Section 6. Other minor revisions to the text were made for format and clarity.

09/08/2008 Minor edits were made in Sections 1 and 14 to include the addition of test W-HG-H-R to the SOP.

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

08/04/2014 EPA Method 245.1 was re-validated.

- 12 % Sodium chloride-hydroxylammonium chloride replaces the 12 % hydroxylamine hydrochloride reagent.
- 10 % $SnCl_2 \cdot H_2O$ in 0.5 N H_2SO_4 now used for both instruments instead of 10 % $SnCl_2$ in 5 % H_2SO_4 for Nippon and 12 % $SnCl_2$ in 6 % HCl for Varian.
- The concentrations of the $KMnO_4$ and $K_2S_2O_8$ were decreased from 6 % to 5 %.
- HCl and the bromate/bromide reagent were removed from the intermediate standards.

07/13/2016 All references to Varian instrument removed.

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