ANALYSIS OF MERCURY IN SOLIDS AND SOLUTIONS BY THERMAL DECOMPOSITION, AMALGAMATION, AND ATOMIC ABSORPTION SPECTROPHOTOMETRY

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1. SCOPE AND APPLICATION

This standard operating procedure (SOP) details the analysis of mercury in sediment, waste, and biological tissue samples by thermal decomposition, amalgamation, and atomic absorption (TDA/AAS). The calibration range is 0.05 ng – 600 ng. The method detection limit (MDL) for the tissue matrix is 0.1 ng. The practical quantitation limit (PQL) for the tissue matrix is 0.3 ng. The MDL for the sediment and waste matrices is 0.05 ng. The PQL for the sediment and waste matrices is 0.15 ng. Laboratory tests covered by this SOP are T-HG-TDA, S-HG-TDA, and WAS-HG-TDA.

2. SUMMARY OF THE METHOD

2.1 This SOP is based on EPA method 7473, Revision 0.

2.2 Controlled heating in an oxygenated decomposition furnace is used to liberate mercury from solid and aqueous samples in the Milestone DMA-80 instrument. The sample is weighed into a quartz boat and placed on the autosampler. The sample is dried and then thermally and chemically decomposed within the decomposition furnace. The decomposition products are carried by flowing air to the catalytic section of the furnace. Oxidation is completed and halogens and nitrogen/sulfur oxides are trapped. The remaining decomposition products are

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then carried to an amalgam trap that selectively traps mercury. The analytical system is flushed with flowing air to remove the remaining gases and decomposition products, followed by the rapid heating of the amalgam trap, releasing the mercury vapor. The mercury vapor is carried by flowing air through absorption cells positioned in the light path of a single wavelength atomic absorption spectrophotometer. Absorbance is measured at 253.7 nm as a function of mercury concentration.

2.3 The Tricell DMA-80 contains three absorption cells with different path lengths for measuring varying concentrations of mercury in the sample. The DMA-80 software automatically chooses which peak to use for sample measurement based on the absorbance. Cell 0 is used for absorbance results of up to 0.5. Cell 1 is used for absorbance of up to 0.85. Cell 2 is used for absorbance of up to 1.0.

2.4 Co-absorbing gases, which are common interferences, such as free chlorine and certain organics, are purged from the analytical system after release by the decomposition furnace and the mercury vapor is selectively trapped on the amalgam trap.

2.5 Memory effects: Mercury vapor may remain in the decomposition tube, amalgam trap, or absorbance cells and be released in a subsequent analysis resulting in a positive bias. For example, this may occur when a low concentration sample is analyzed after a sample of high mercury content. To prevent memory effects, the DMA-80 automatically runs a cleaning cycle after samples that contain more than 30 ng of mercury in the tissue matrix and more than 9 ng in the sediment/waste matrix.

3. APPARATUS AND EQUIPMENT

3.1 Milestone DMA-80 instrument, serial number 14041581.

3.2 Milestone DMA-80 instrument, serial number 15081813.

3.2 Analytical balance.

3.3 Quartz Boats, part number, DMA8347.

3.4 Eppendorf pipettes - 100 µL fixed volume and 100 – 1000 µL adjustable volume.

3.5 Finnpipette, 2 – 10 mL adjustable volume.

4. REAGENTS AND CHEMICALS

4.1 Deionized (DI) water - Available from plastic spigots in the laboratory.

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

4.3 Standards - Preparation of all Hg standards must be documented in the LIMS standard prep tracker. A label should be printed from the standard prep tracker that shows the identity of the standard, preparation date, expiration date, and serial number. See DEP SOP MT-069 for standard documentation requirements and expiration dates.

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4.3.1 Primary Commercial Stock Standard 1000 mg/L - Commercially available inorganic mercury stock from SPEX, Accustandard, Baker, Baxter, Fisher, VSR, etc. The expiration date is assigned by the manufacturer and the standard is stored at room temperature. This primary stock standard is used to prepare the calibration standards.

4.3.2 TDA-1, 10 mg/L standard - Pipette 1.0 mL of the 1000 mg/L purchased primary standard into a 100 mL volumetric flask containing 50 mL of DI water. Add 5.0 mL of HCl. Dilute to volume with DI water and mix well. The expiration date for this prepared standard is 60 days from preparation and it is stored at room temperature.

4.3.3 TDA-2, 1.0 mg/L standard - Pipette 0.1 mL of the 1000 mg/L purchased primary standard into a 100 mL volumetric flask containing 50 mL of DI water. Add 5.0 mL of HCl. Dilute to volume with DI water and mix well. The expiration date for this prepared standard is 60 days from preparation and it is stored at room temperature.

4.3.4 TDA-3, 100 µg/L standard - Pipette 1.0 mL of TDA-1 (10 mg/L) into a 100 mL volumetric flask containing 50 mL of DI water. Add 5.0 mL of HCl. Dilute to volume with DI water and mix well. The expiration date for this prepared standard is 60 days from preparation and it is stored at room temperature.

4.3.5 TDA-4, 10 µg/L standard - Pipette 0.1 mL of TDA-1 (10 mg/L) into a 100 mL volumetric flask containing 50 mL of DI water. Add 5.0 mL of HCl. Dilute to volume with DI water and mix well. The expiration date for this prepared standard is 60 days from preparation and it is stored at room temperature.

4.3.6 TDA-5, 5 µg/L standard - Pipette 2.5 mL of TDA-3 (100 µg/L) into a 50 mL volumetric flask containing 25 mL of DI water. Add 2.5 mL of HCl. Dilute to volume with DI water and mix well. The expiration date for this prepared standard is 60 days from preparation and it is stored at room temperature.

4.3.7 Calibration Standards - The calibration standards are prepared from standards TDA-5, TDA-4, TDA-3, TDA-2, and TDA-1. See section 6 for preparation details.

Note: The absorption cell used for sample measurement is automatically chosen by the DMA-80 software based on the absorbance. Cell 0 is used for absorbance of up to 0.5. Cell 1 is used for absorbance of up to 0.85. Cell 2 is for absorbance of up to 1.0.

4.3.7.1 Cell 0 Calibration Standards:

STD1 (0.1 ng)

STD2 (0.25 ng)

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STD3 (0.5 ng)

STD4 (1 ng)

STD5 (2 ng)

STD6 (3 ng)

4.3.7.2 Cell 1 Calibration Standards:

STD1 (0.1 ng)

STD2 (0.25 ng)

STD3 (0.5 ng)

STD4 (1 ng)

STD5 (2 ng)

STD6 (3 ng)

STD7 (4 ng)

STD8 (5 ng)

STD9 (10 ng)

STD10 (15 ng)

4.3.7.3 Cell 2 Calibration Standards:

STD11 (20 ng)

STD12 (30 ng)

STD13 (50 ng)

STD14 (100 ng)

STD15 (200 ng)

STD16 (400 ng)

STD17 (600 ng)

4.3.8 Second Source Standard (1000 mg/L) - Commercially available inorganic mercury stock from SPEX, Accustandard, Baker, Baxter, Fisher, VSR, etc. The expiration date is assigned by the manufacturer and the standard is stored at room temperature. This purchased standard must be purchased from a different vendor than the primary standard used to prepare the calibration standards.

4.3.8.1 TDA-1C, 10 mg/L standard - Pipette 1.0 mL of the 1000 mg/L purchased second source standard into a 100 mL volumetric flask containing 50 mL of DI water. Add 5.0 mL of HCl. Dilute to

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volume and mix well. The expiration date for this prepared standard is 60 days from preparation and it is stored at room temperature.

4.3.8.2 TDA-2C, 1.0 mg/L standard - Pipette 0.1 mL of the 1000 mg/L purchased second source standard into a 100 mL volumetric flask containing 50 mL of DI water. Add 5.0 mL of HCl. Dilute to volume and mix well. The expiration date for this prepared standard is 60 days from preparation and it is stored at room temperature.

4.3.8.3 TDA-3C, 100 µg/L standard - Pipette 1.0 mL of TDA-1C (10 mg/L) into a 100 mL volumetric flask containing 50 mL of DI water. Add 5.0 mL of HCl. Dilute to volume and mix well. The expiration date for this prepared standard is 60 days from preparation and it is stored at room temperature.

4.3.8.4 TDA-4C, 10 µg/L standard - Pipette 0.1 mL of TDA-1C (10 mg/L) into a 100 mL volumetric flask containing 50 mL of DI water. Add 5.0 mL of HCl. Dilute to volume and mix well. The expiration date for this prepared standard is 60 days from preparation and it is stored at room temperature.

4.3.8.5 The second source standard is analyzed at three different concentrations for each absorption cell.

4.3.8.5.1 TDA-CA (1.0 ng) - Pipette 100 µL of TDA-4C into a quartz boat.

4.3.8.5.2 TDA-CB (10 ng) - Pipette 100 µL of TDA-3C into a quartz boat.

4.3.8.5.3 TDA-CC (100 ng) - Pipette 100 µL of TDA-2C into a quartz boat.

4.4 DORM-4, 0.412 mg Hg/kg - Standard reference material (SRM) purchased from the National Research Council of Canada (NRCC). This standard is used as the laboratory control sample (LCS) for each batch of tissue samples analyzed. The expiration date is provided by the manufacturer.

4.5 PACS-3, 3.0 mg Hg/kg - SRM purchased from NRCC. This standard is used as the LCS for each batch of sediment samples analyzed. The expiration date is provided by the manufacturer.

4.6 T-PQL, 0.3 ng Hg – This standard is used as the practical quantitation level check standard for each batch of samples analyzed. Add 30 µL of TDA-4 (10 µg/L) to a quartz boat.

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4.7 S-PQL, 0.15 ng Hg – This standard is used as the practical quantitation level check standard for each batch of sediment samples. Add 30 µL of TDA-5 (5 µg/L) to a quartz boat.

5. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

5.1 Tissue samples are collected and stored in polyethylene or glass vials with airtight closures. They are preserved by storage in a freezer at -20 °C. The frozen containers are shipped in ice-packed coolers via overnight mail to the laboratory. The samples are then stored in a freezer at -20 °C. Samples are removed from the freezer and allowed to thaw to room temperature before analysis. The hold time for tissue samples is 1 year (Table FS 1000-10, DEP-SOP-001/01 FS 1000, General Sampling Procedures).

5.2 Sediment or waste samples are collected and stored in 250 mL wide-mouth glass containers with screw tops. The samples are preserved by refrigerating the containers at a temperature of 4 °C. Prior to sample analysis, the samples are allowed to warm to room temperature before weighing. The hold time for sediment and waste samples is 28 days.

6. CALIBRATION STANDARDS PREPARATION PROCEDURE

Using the table below as a guide, pipette the appropriate amounts of TDA-5 (5 µg/L), TDA-4 (10 µg/L), TDA-3 (100 µg/L), TDA-2 (1.0 mg/L), and TDA-1 (10 mg/L) into quartz boats.

Calibration Standard Concentration	Amount of Standard to deliver to sample boat
STD1 0.10 ng (cell 0)	20 µL of TDA-5 (5 µg/L)
STD2 0.25 ng (cell 0)	25 µL of TDA-4 (10 µg/L)
STD3 0.50 ng (cell 0)	50 µL of TDA-4 (10 µg/L)
STD4 1.0 ng (cell 0)	100 µL of TDA-4 (10 µg/L)
STD5 2.0 ng (cell 0)	200 µL of TDA-4 (10 µg/L)
STD6 3.0 ng (cell 1)	30 µL of TDA-3 (100 µg/L)
STD7 4.0 ng (cell 1)	40 µL of TDA-3 (100 µg/L)
STD8 5.0 ng (cell 1)	50 µL of TDA-3 (100 µg/L)
STD9 10 ng (cell 1)	100 µL of TDA-3 (100 µg/L)
STD10 15 ng (cell 1)	150 µL of TDA-3 (100 µg/L)
STD11 20 ng (cell 1)	200 µL of TDA-3 (100 µg/L)
STD12 30 ng (cell 2)	30 µL of TDA-2 (1 mg/L)
STD13 50 ng (cell 2)	50 µL of TDA-2 (1 mg/L)
STD14 100 ng (cell 2)	100 µL of TDA-2 (1 mg/L)
STD15 200 ng (cell 2)	20 µL of TDA-1 (10 mg/L)
STD16 400 ng (cell 2)	40 µL of TDA-1 (10 mg/L)
STD17 600 ng (cell 2)	60 µL of TDA-1 (10 mg/L)

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7. SAMPLE ANALYSIS PROCEDURE

7.1 Open the air supply.

7.2 Turn the DMA-80 on with the switch on the front panel of the instrument.

7.3 Turn on laptop computer and open the DMA-80 software. Log in as an administrator with the password 123456, then press enter.

7.4 The instrument will require about 15 to 20 minutes of warm up time.

7.5 Calibration:

The calibration is performed initially and when any significant instrument maintenance is performed. For example, a new calibration should be performed after the decomposition tube or amalgam trap is replaced. A new calibration must also be performed if the daily calibration check does not pass (see section 7.6.5).

7.5.1 To create a new calibration curve, choose from "Method" Standard.m80 and go to "Calibration." Clear the old calibration (do not delete) using the blank page icon and save the new one in the calibration folder.

7.5.2 Go to the "Measurement Sample". Clear the old measurements using the blank page icon and create a new file for calibration analysis. Analyze each calibration standard twice. In the "Measurement Sample" window type 2 under "n" to create two standard measurements. Open the small balance icon and choose "calibration". The software will mark the small balance icon with letter "C."

7.5.3 To complete the program for calibration switch to the "Measurement Results" window. Enter the concentration for each standard used along with the amount in grams in the "Amount" column.

7.5.4 Save the file.

7.5.5 Push START to start analyzing calibration standards.

7.5.6 After measurements are finished, the instrument will stop. Save the results.

7.5.7 Go to "Calibration" and choose fit calibration in every cell. Save the results. The calibration curve is non-linear. Select the 'square' fit.

7.6 Sample Analysis:

7.6.1 Under 'Sample Measurement' create a new file for the analysis.

7.6.2 Select the appropriate calibration under "Measurement Links."

7.6.3 Select the method under 'Measurement/Links'.

7.6.4 For analyzing tissue samples, choose Fish Method.m80 and for analyzing sediment samples, choose Sediment.m80.

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7.6.5 Daily Calibration Check for tissue samples - Analyze the following standards at the beginning of every analysis. The results must be within 10 % of their true value.

7.6.5.1 Cell 0 daily calibration check - STD4 (0.5 ng) and STD6 (2.0 ng).

7.6.5.2 Cell 1 daily calibration check - STD9 (5.0 ng) and STD11 (15 ng).

7.6.5.3 Cell 2 daily calibration check - STD13 (30 ng) and STD17 (400 ng).

7.6.6 Daily Calibration Check for low level sediment samples – Analyze the following standards at the beginning of every analysis. The results must be within 10 % of their true value. Only sediment samples with mercury concentrations below 15 ng may be reported from the low level sediment analysis.

7.6.6.1 Cell 0 daily calibration check – STD4 (0.5 ng) and STD6 (2.0 ng).

7.6.6.2 Cell 1 daily calibration check – STD9 (5.0 ng) and STD11 (15 ng).

7.6.7 Daily Calibration Check for high level sediment samples – Analyze the following standards at the beginning of every analysis. The results must be within 10 % of their true value.

7.6.5.1 Cell 0 daily calibration check - STD4 (0.5 ng) and STD6 (2.0 ng).

7.6.5.2 Cell 1 daily calibration check - STD9 (5.0 ng) and STD11 (15 ng).

7.6.5.3 Cell 2 daily calibration check - STD13 (30 ng) and STD17 (400 ng).

7.6.6 For tissue samples, use a clean wooden applicator stick to transfer the entire tissue sample (or a ~ 10 g sub-sample if the sample is inordinately large) to a disposable weigh boat. Use a clean scalpel to carefully chop the tissue into very small pieces to homogenize the sample. Carefully transfer 0.05 to 0.2 g of chopped tissue to the quartz boat. Record the exact weight transferred.

7.6.7 For sediment samples, thoroughly mix the sample by removing the lid of the sample container and stirring the sample with a clean spatula. If the sample cannot be thoroughly mixed within its container without spilling, empty the entire sample into a clean stainless steel tray. Do not decant any liquid or remove any objects from the sample. Contact the supervisor if any non-representative material is found in the sample. A mortar and pestle can be used if necessary. Any large objects should be cut up or crushed, but not removed from the sample. Return the homogenized sample to the sample jar. Weigh into a quartz boat 0.05 – 0.4 g of sample. Record the exact weight.

7.6.8 The sequence of samples and quality control samples to be analyzed, including the daily calibration check, are typed into the sample file. See Appendix A for an example sequence.

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8. MAINTENANCE

8.1 During routine maintenance of the DMA-80, both the instrument and laptop computer should be turned off and disconnected from the power supply. Turn off the air supply. Allow the instrument to cool to room temperature before beginning maintenance.

8.2 Refer to the DMA-80 Operator Manual for replacement of the catalyst and the amalgam trap.

9. DATA ARCHIVAL

The Milestone instrument software does not automatically save the results from the analytical run. To distinguish files between the two DMA-80 instruments, a suffix is used. The 'DMA' suffix is for S/N 14041581. The'DMA2' suffix is for S/N 15081813. See below for details.

9.1 DMA-80 (S/N 14041581): After measurements are complete, click File > Save and save the instrument software file on the hard drive of the instrument computer with the format 20160805t1-DMA.d80. The file name reflects the year, month, and day the samples were analyzed on. The letter following the date in the file name reflects the matrix of samples that were analyzed ('t' for tissue and 's' for sediment or waste). The number after the letter in the file name indicates the sequence of analyses performed on that day. If more than one analysis is performed on one day, then the second file name should have a '2' following the letter, and so on. Then, the file needs to be saved as a text file with 'DMA' appended to the file name, for example, 20160805t1-DMA.txt. The text file is saved to \\FLDEP1\dear\labs\lab3\Chem_Data\Inorganic\Mercury\DMA-80.

9.2 DMA-80 (S/N 15081813): After measurements are complete, click File > Save and save the instrument software file on the hard drive of the instrument computer with the format 20160805t1-DMA2.d80. The file name reflects the year, month, and day the samples were analyzed on. The letter following the date in the file name reflects the matrix of samples that were analyzed ('t' for tissue and 's' for sediment or waste). The number after the letter in the file name indicates the sequence of analyses performed on that day. If more than one analysis is performed on one day, then the second file name should have a '2' following the letter, and so on. Then, the file needs to be saved as a text file with 'DMA2' appended to the file name, for example, 20160805t1-DMA2.txt. The text file is saved to \\FLDEP1\dear\labs\lab3\Chem_Data\Inorganic\Mercury\DMA-80.

10. QUALITY CONTROL

See DEP SOP MT-058 for calculation of relative percent difference (RPD) and percent recovery. Contingencies for out-of-control data are listed in DEP SOP LB-027.

The calculation equation used for matrix spike percent recovery is:

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[[(Matrix Spike (ng)) – (QC Sample (ng) * Weight of Matrix Spike (g) / Weight of QC Sample (g))] / Spike Concentration (ng)] * 100

10.1 One method blank per batch of 20 samples. Prepare a solution of 5 % HCl in DI water. Weigh 0.1 mL of the 5 % HCl into a quartz boat. The result of the method blank must be below the MDL.

10.2 One laboratory fortified blank (LFB) per batch of 20 samples. Standard reference material is used as the LFB. Weigh approximately 0.025 g of DORM-4 for tissue batches. Weigh approximately 0.010 g of PACS-3 for sediment batches (for low-level sediment analyses, 0.0050 g of the PACS-3 SRM is weighed in order to bring the concentration into cell 0 or 1). The acceptable recovery limits for the LFB is 80 – 120 %.

10.3 Matrix spikes - Duplicate matrix spikes are prepared by weighing two aliquots of the same sample and spiking with 50 µL of TDA-2. The acceptable recovery limits for the matrix spikes is 80 – 120 %. The RPD should be below 20 %.

10.3.1 Low Level Sediment Matrix Spikes – Duplicate matrix spikes are prepared by weighing two aliquots of the same sample and spiking with 50 µL of TDA-3. The acceptable recovery limits for the matrix spikes is 80 – 120 %. The RPD should be below 20 %.

10.4 Continuous Calibration Verification (CCV) - Every 10 samples, standards STD4, STD10, and STD15 are analyzed to verify the calibration. The STD4CCV is used for cell 0. The STD10CCV is used for cell 1. The STD15CCV is used for cell 2. If any of the CCV standards are outside of the acceptable recovery limits, data cannot be reported from the absorption cell affected without appropriate qualification. The acceptable recovery limits for CCVs is 80 – 120 %.

10.5 Continuous Calibration Blank (CCB) - An instrument blank (5 % HCl) is analyzed every 10 samples to verify no contamination is present and that no drift has occurred. Acceptable CCB results are between -0.02 ng and 0.02 ng.

10.6 Second Source Standard - The second source standard (see section 4.3.8) is used to verify the calibration. This standard is analyzed on every analytical run and the acceptable recovery limits are 90 – 110 %.

10.7 The correlation coefficient of the calibration curve must be ≥ 0.995.

10.8 Practical Quantitation Level Check Standard (S-PQL or T-PQL). Each batch of samples requires a PQL level check standard. See sections 4.6 and 4.7 for preparation.

11. SAFETY/HAZARDOUS WASTE MANAGEMENT

11.1 Always wear a lab coat and safety glasses or protective eyewear while working in the lab. Nitrile gloves and a lab coat should be worn while working in the laboratory. Concentrated acids should be handled in a fume hood.

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11.2 Many mercury compounds are highly toxic if swallowed, inhaled, or absorbed through the skin. Extreme care must be exercised in the handling of concentrated mercury standards.

11.3 Samples with a high organic content (e.g. oil saturated soil) should be analyzed with caution. The sample size should be scaled down as too large of a sample size may result in ignition of the sample within the decomposition tube.

12. REFERENCES

12.1 Milestone operator's manual MA122.

12.2 EPA Method 7473, Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation and Atomic Absorption Spectrophotometry.

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

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

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

12.6 Quality Manual for State of Florida Department of Environmental Protection Bureau of Laboratories.

12.7 DEP Bureau of Laboratories Health and Safety Manual.

12.8 DEP-SOP-001/01, FS 1000, General Sampling Procedures.

APPENDIX A

Example Sample Sequence

STD4
TDA-CA
STD6
STD9
TDA-CB
STD11
STD13 (Not required for low level sediment analyses)
TDA-CC (Not required for low level sediment analyses)
STD17 (Not required for low level sediment analyses)
Method Blank (batch 1)
SRM
Sample
Matrix spike
Matrix spike duplicate
Sample
Sample
Sample
Sample
Sample

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STD15CCV (Not required for low level sediment analyses)
STD10CCV
STD4CCV
CCB
etc.

Appendix of Significant Changes

03/03/2016: Separated the procedure for the analysis of sediment samples into a high concentration procedure and low concentration procedure.

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