Analysis of Methylmercury in Plant, Sediment, and Soil Samples by Cold Vapor Atomic Fluorescence Detection with the Brooks-Rand “MERX” Automated Methylmercury Analytical System

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Scope and Application

The following standard operating procedure (SOP) describes the (1) preparation of plant, soil, and sediment samples and (2) subsequent analysis for methylmercury (MeHg) by Cold Vapor Atomic Fluorescence Detection with the Brooks-Rand "MERX" Automated Methylmercury Analytical System. Samples are weighed into polypropylene digestion tubes and digested in 2 ml of 25% KOH/Methanol at 60º C for 4 hours. Following the digestion, sample extract is diluted up to a 10 ml volume with reagent water. Quality assurance and control protocols are employed throughout sample setup and analysis, including: laboratory practices to prevent sample contamination, method blanks, triplicates, matrix spikes, and certified reference materials (CRM) when available.

The MERX is a closed analytical system comprised of four interconnected modules (autosampler, purge and trap module, GC/Pyrolytic module, and detector). In 42 ml glass vials, diluted extract is buffered to a pH of 4.5 – 5.0 and treated with Sodium Tetraethylborate (NaTEB), resulting in ethylation of oxidized mercury species. These volatile ethylated species (as well as elemental mercury) are stripped from the liquid phase with Argon gas, retained on Tenex traps, desorbed back into the sample stream, and separated with a gas chromatography column. Each ethylated mercury species is released from the column *en masse* into the sample stream, thermally oxidized to elemental mercury, and then detected by cold vapor atomic fluorescence spectrometry. This method is designed to quantify methylmercury in environmental samples per the United States Environmental Protection Agency draft method 1630.

This document is intended as an additional SOP designed to guide the user through MeHg analysis specific to the Wisconsin Mercury Research Laboratory (WMRL). A condensed version can also be found following the detailed SOP (Appendix 1), and is intended as a quick reference bench guide for the analyst. However, the analyst is required to be familiar with the detailed SOP as well as the original user's manuals provided by Brooks-Rand which will be referred to when appropriate.

Safety Concerns

Multiple safety concerns are present in the conduct of this method. Persons involved must have read, understood, and signed the Chemical Hygiene Plan for the WMRL prior to potential exposure to any chemicals. Although MeHg is an extremely toxic organic metal, concentrations encountered in samples and working standards on this instrument are generally low. However, caution should still be exercised to limit chronic exposure during daily operations. Concentrated stock solutions containing elevated MeHg levels are occasionally encountered, and should only be handled by experienced lab personnel. Reagents used in this method include strong acid, strong base, and an organometallic ethylating compound. The analyst must have a thorough understanding of these

chemicals, including their required safety protocols, prior to their use. More detailed information is included for each reagent later in this SOP, and additional information can be found in the attached material data safety sheet. Finally, during analysis the automated sample introduction system may begin moving without warning and presents a mechanical hazard.

Sample Preparation

Unless otherwise specified, solid samples are lyophilized ("freeze-dried" under vacuum while frozen) to a consistent weight and homogenized (via ball mill, coffee grinder/food processor, or mortar/pestle) prior to digestion. The samples should appear to be well pulverized and mixed to a consistent composition before subsampling.

A typical plant/soil/sediment extraction contains up to 30 samples, one triplicate, one duplicate spike, three blanks, and three CRM's. In small sample sets (< 15), one triplicate, one duplicate spike, three blanks, and three CRM's should be included in each run.

1. Arrange an adequate number of new polypropylene digestion tubes into a sample rack and number the caps for reference.

2. Weigh 25 – 250 mg of sample into the tubes. Be sure that you record the sample ID and sample mass into the appropriate Excel setup sheet.

3. Print off the sample identification code with the barcode printer and label each tube with the sample it contains.

4. Working under a fume hood, dispense 2 ml of the 25% KOH in methanol solution into each digestion tube and firmly cap. Completely homogenize the sample, leaving a minimum amount of sample out of the solution on the wall of the tube.

5. Place the samples in a rack, enclose in a large bag, and heat at 60º C for 4 hrs.

6. Following the digestion, add 8 ml of reagent water to each vial, homogenize, and store at –20º C until analysis. Although analysis the following day is preferred, frozen sample extractant remains viable for MeHg up to one week.

7. On the day of analysis, allow the frozen sample to thaw, homogenize, and centrifuge at 3000 RPM for 20 minutes.

Instrument Operation

Start Up

1. Check that all modules of the instrument have power and the Argon gas supply is turned on. Empty the waste receptacle in the cabinet below.

2. If necessary, open the Mercury Guru4 software with the shortcut on the desktop.

3. Open a new analytical template for the planned analysis. From the "File" dropdown menu, select "Open", navigate to the "TEMPLATE" folder (D:drive→HG4Data→MERX), and select the calibration appropriate to the samples to be analyzed (high/low cal). Save the file as data (from the "File" dropdown menu) in the data folder from the current year found in the GURU RUNS folder. Name the new run file by date and sample description (MMDDYY_*sample name*.brd).

4. From the "Instrument" dropdown menu, select "Connect", prompting a popup window displaying three communication ports. Select the appropriate ports (CVAFS = COM4, Purge and Trap = COM5, and Autosampler = COM6) and click "Accept". The communication status at the top of the screen will turn green indicating connection with each module.

5. Finally, adjust the sensitivity of the detector so that the baseline offset is approximately 50,000 by changing the PMT value using the up/down arrows on the front of the detector. When the PMT value is changed, the offset value will go blank, and the new offset value will temporarily appear in the signal field. Press the autozero button when the signal value is approximately 50,000 (± 1000). Once the offset value stabilizes (2-3 minutes), measure the instrument noise (found in the "File" dropdown menu). Record the new offset, PMT, and noise values in the lab notebook

Preparation of Vials for Analysis

The MERX instrument is designed to quantify MeHg in a specific mixture of reagents that are prepared in sealed 42 ml amber glass vials. The autosampler holds three removable 24 vial sample racks, each consisting of 3 rows of 8 vials. Vial number one is the upper right position, with vial position descending from right to left-then top to bottom. Once prepared, the vials are sealed to the atmosphere and remain viable for analysis up to 48 hours.

1. Place the clean vials in the sample rack and add approximately 35 ml of reagent water to each vial.

2. Add the MeHg source (standard or sample extract) to appropriate vials. Sample extract volumes are dependant on mass of MeHg expected for the sample and can vary from 10 to 150 μL (try to keep the mass within the range of the standard curve). Method blank volumes should be 300 μL.

3. Adjust the pH of the mixture to 4.5 – 5.0 by adding 300 μL of the sodium acetate buffer/antifoaming reagent to every vial.

4. Add 50 μL of 1% NaTEB to every vial.

    a. NaTEB is an unstable reagent and must always remain at freezing temperatures to slow degradation. Begin thawing several minutes before use but always make sure that some frozen NaTEB remains in the vial. Promptly cap and return the vial of NaTEB to the freezer after use.
    b. NaTEB is toxic and spontaneously combustible in air. Only open vials and dispense NaTEB under a fume hood. Add NaTEB directly to the sample mixture (not to the glass surface inside the vial) to reduce volatilization.

5. Fill the vials with reagent water using a squirt bottle until a reverse meniscus forms (convex water surface). Seal the vial carefully (without headspace or spilling) with a new clean cap and septa assembly. Vigorously shake the vial, check for any air bubbles in the vial, and refill if necessary.

6. Place the full rack on the autosampler tray, making sure that the rack is properly positioned and oriented. Under the Automation tab, select the number of vials to be analyzed, the starting position, and choose the “Start Run” button.

Initial Instrument Calibration

Begin calibration with several instrument blanks consisting of reagent water, buffer, and NaTEB to clear the sample train of residual MeHg. Create a six point calibration curve with mercury masses that span levels expected in the samples. Typically, plants/soils/sediments span 1 – 40 pg. End the calibration curve with three instrument blanks to ensure that carryover is not occurring.

Sample Analysis

Proceed with sample setup after the instrument has been successfully calibrated. Throughout the run assess for sample carryover and instrumental drift by the regular analysis of instrument blanks and check standards. Five instrument blanks should be spread throughout a regular run. Check standards should be

performed in every eighth position and as the last position of the run. An example of a typical 40 sample analysis is shown in Appendix 2.

Enter the information associated with the vials into the spreadsheet found in the "Run Information" tab. Complete the following fields:

Type: Select the correct menu choice for the type of sample you are analyzing

- Equipment Blank: Any non-analytical instrument blank performed throughout the run
- Calibration/Instrument Blank: Instrument calibration and ongoing instrument blanks
- Method blanks: Blanks carried through the extraction procedure
- Sample: Samples being analyzed
- Ongoing precision and recovery: Check standards throughout the run

Name/ID: The barcode or other identification of the vial being analyzed
Sample Weight: The weight of the sample (in milligrams) being analyzed
Dilution Volume: The final volume of extracting reagent(s) added to solid samples
Analytical Volume: The volume of extractant analyzed in the 42ml glass vial

Data Processing and Capture

Following an analytical run, examine the dataset for suspicious results.

1. Check each chromatogram to make sure that the integration software selected the correct peak. The typical chromatogram displays three distinct peaks, with the retention time for MeHg at 1-2 minutes. Open the Peak tab on the run sheet and select the first line of data. The corresponding chromatogram will appear. If the selected peak in the line of data does not match the MeHg peak, click the peak select button and change it to the correct peak.

2. Check for sample peak heights that are considerably less than blank values (typically 1-100 peak height units). These may be the result of a non-sealing analytical vial, the omission of a reagent, or matrix interference (see section "Quality Assurance and Control Objectives" below), and requires repeated analysis.

Once the run is determined as successful the data may be exported to an external file. Open the Report tab on the run sheet, select Save Report, and set the file format as a .CSV file. Name the file so that it reflects the type of extraction, date, and samples analyzed (ie. KOH_010210_indiana leaves) and save the file in the folder named for the current year in the GURU RUNS folder (D:Drive→HG4Data→MERX→GURU RUNS→2010).

Reagents and Standards

Reagents

All reagents and/or dry chemicals used to make reagents must be of the highest purity available from the vendor and shown to be low in mercury. Upon receipt at the laboratory, containers will be marked with the date of receipt and stored in the appropriate areas. When reagents are mixed for use in this method, the person who mixes them will initial and date the reagent container. Reagents and manufacture instructions follow below.

Reagent Water: Ultra pure reagent grade water containing less than 0.1 ng/L Hg with a resistance greater than 18 MΩ-cm. The water is delivered through a 0.2 µm filter, as obtained from a Millipore Academic water-purification system or equivalent.

25% KOH in Methanol: To make 900 ml of solution, fill an amber glass 1 L bottle with 666 g of methanol and slowly add 225 g of KOH. Carefully monitor the temperature of the solution as that the dissolution of KOH generates heat and methanol has a relatively low boiling point. Mix hourly until all the KOH is dissolved.

Argon: Ultra high purity grade 5.0 Argon is used as the carrier gas in the analytical system. The Argon is first passed through a gold bead trap to remove any Hg.

Antifoaming Agent: Silicone Emulsion Solution purchased from J.T. Baker (PN B531-07).

Sodium Acetate Buffer: To make a stock solution of sodium acetate buffer, measure approximately 50 ml reagent grade water, 47.2 ml glacial acetic acid, and 108.8 g sodium acetate into a 500 ml Teflon bottle. Bring up to 400 ml volume, shake until all solids dissolve, and expose to ultraviolet light for 1 week (acetate buffer may be contaminated with MeHg). For a working solution, fill a 125 ml Teflon bottle with stock buffer, add 0.1 ml of antifoaming agent, and shake well before use.

Sodium Tetraethylborate (NaTEB): Sodium tetraethyborate is a toxic organometallic compound that is spontaneously combustible in the presence

of oxygen and other oxidizing chemicals (such as strong acids), and volatilizes toxic gases (triethyl boron). Sodium tetraethylborate has a distinctive “sweet” smell, and should be considered an indication of analyst exposure. Although the long-term health affects of NaTEB exposure is unknown, it should be assumed that repeated exposure may have adverse health affects. All use of NaTEB should take place inside a high-volume fume hood, and special consideration for equipment exposed to NaTEB in the fume hood (i.e. gloves, wipes, pipette tips, containers, etc...) must be made.

Pure solid NaTEB is purchased in 1 gram sealed glass vials (stored under N_2 gas) and kept in the freezer until use. To dilute NaTEB to a 1% working solution, dissolve 2 g of KOH in 100 mL of reagent water in a 125 ml Teflon vial and chill to sub-freezing temperatures. Check the condition of the solution often. As soon as the KOH solution begins freezing, remove the vial of $NaBEt_4$ from the freezer and score the neck of the bottle with a glass cutter or the back of a ceramic knife. Wrap the vial in a lab wipe and break the neck of the vial. It is best to work quickly at this point as to keep the pure NaTEB cold and to limit its exposure to oxygen to reduce the risk of combustion. Immediately dump the pure NaTEB into the 2% KOH solution and gently swirl to dissolve. Rinse the glass vial with the solution if any significant amount of NaTEB remains in the vial. When the NaTEB solution is almost entirely melted, homogenize, and pour equally into 20 clean chilled 5 mL Teflon vials. Cap the vials, store in a sealed bag, and record the date prepared. This solution should be kept frozen and made fresh every 2 weeks. Never use $NaBEt_4$ solid or solutions that are yellow in color. Following use, NaTEB should be stored in an appropriately labeled and sealed bag in the freezer until the solution can be disposed of properly.

To dispose of old or unused portions of the 1% NaTEB solutions, thaw the vials and pour into a beaker under a fume hood. Fill the beaker with an equivalent volume of 6M HCl (50% concentrated solution), place on a hotplate, boil down to half-volume, and then discard the remaining solution as an acid waste. Never dispose of concentrated NaTEB in this fashion, as that it will combust, but rather dilute to a 1% concentration with water and then process as previously described.

1M KOH rinse solution: In a 500 ml Teflon bottle, add 28 g of KOH to 250 ml of reagent water and bring up to 500 ml.

Aqua Regia rinse solution: In a 1000 ml Teflon bottle, add 25 and 75 ml of concentrated HNO_3 and HCl (respectively) to approximately 100 ml of reagent water and bring up to 1000 ml.

Standards

Upon receipt at the laboratory or on the day of preparation, standards should be labeled with the date received or made and the initials of the person preparing them. Highly concentrated stock solutions should be stored away from the main working areas to prevent contamination of the clean lab. Working standards and (if necessary) subsequent sub-stock dilutions should be made in a class A volumetric flask in a matrix of reagent grade water at a 2% and 0.2% concentration of glacial acetic acid and hydrochloric acid, respectively. This solution should be transferred to a Teflon bottle designated specifically for mercury standards, stored in an amber bag at 4^{o} C, and remade every 6 months. Methylmerucry working solutions are typically prepared at concentrations of 0.2 and 0.5 ng/ml for instrument calibration and at 15 ng/ml as a spiking solution. All standards must also be assigned a unique letter-number-letter identification code and must be entered into the laboratory database system. For working solutions, allow the solution to equilibrate for at least 24 hours and then determine the concentration by analysis via cold vapor atomic fluorescence spectrometry as follows:

1. Mass of mercury in the MeHg standard: To four 15 ml Teflon vials, add 8.0 ml of reagent grade water, 1.000 ml of the MeHg working standard, and 1.0 ml of BrCl.

2. Blank contribution of mercury: To four 15 ml Teflon vials, add 9.0 ml of reagent grade water and 1.0 ml of BrCl.

3. Store the vials in a rack, seal in a bag, and heat in an oven to 50^{o} C for eight hours.

4. Analyze the contents of the eight Teflon vials by EPA method 1631.

5. Analyze four 1.000 ml additions of the MeHg working standard to determine the $SnCl_2$ reducible fraction of Hg^{II}

6. Subtract the average blank mercury mass and the $SnCl_2$ reducible fraction of Hg^{II} from the total mercury mass determined MeHg working standard to determine the actual MeHg mass in the vials and subsequent concentration.

Quality Assurance and Control Objectives

Certified Reference Material

A CRM should be analyzed at least once every ten samples, with a recovery within 75-125% of its certified value. If available, the CRM should be of the same matrix as the samples and be reasonably similar to the expected concentrations.

In the event of a failed CRM recovery repeat the CRM analysis (if possible) to rule out a spurious result and seek the guidance of the quality assurance officer. The WMRL analyzes IAEA SL 1 (lake sediment) as an internal reference material for MeHg analysis in plants/soils/sediments. Currently, there are no available reference materials certified for MeHg that are either at an appropriate concentration (sediment) or composed of a similar matrix (soils and plants).

Sample Precision

A sample should be run in triplicate at least once every analysis for plant/soil/sediment analysis, with a relative standard deviation less than 25%. In the case of failure, repeat the triplicate (if possible) and bring to the attention of the quality assurance officer.

Matrix Interference

A sample should be run as a duplicate spike at least once every plant/soil/sediment analysis, with a recovery within 75 – 125% of the known addition and the relative percent difference between the recoveries of less than 25%. A duplicate spike is set up similar to a triplicate analysis, except that two of the three samples are spiked with MeHg prior to the digestion. In the case of failure, repeat the duplicate spike (if possible) and bring to the attention of the quality assurance officer.

Check for sample peak heights that are considerably less than blank values (typically 1-100 peak height units). These may indicate matrix interference. Reanalyze the sample to rule out operator error. If a repeated analysis results in a low response, reanalyze the sample with a MeHg spike in the analytical vial. A low recovery of the MeHg spike (< 75%), is a reasonable indication of matrix interference and should be brought to the attention of the quality assurance officer.

Instrumental Carryover

Instrumental carryover is assessed by the instrument blanks, which is the analysis of reagent water (with buffer and NaTEB), and should be analyzed throughout the run. Excessive instrument carryover (peak areas > 50% of the lowest point of the standard curve) indicates that the sample train has been contaminated with MeHg and requires cleaning (see below).

Method Blank

A method blank should be analyzed at least once every ten samples. Method blanks are part of the sample extraction set up and consist of an extraction vial with extraction reagents but no sample. Elevated method blanks indicate

contamination in the extraction vials or reagents and are used to calculate the DDL (daily detection limit) for the extraction batch.

Instrument Calibration

The instrument requires calibration with a 6 point standard curve prior to sample analysis and regular checks of instrument calibration throughout the run. A standard curve should be created with levels of MeHg similar to that of the samples, and have an r^2 value greater than 0.995. At the minimum, a check standard should be analyzed to verify instrument calibration in every eighth position, and have a measured mass within 85 – 115% of its true value. The failure of subsequent check standards is an indication of instrumental drift which may require recalibration of the instrument.

Additional Instructions

Instrument Maintenance

The MERX system requires some short- and long-term maintenance. Empty the waste receptacle daily to prevent the overflow of spent sample medium. The purge vessel and sample lines should be cleaned monthly or sooner if necessary. The three analytical Tenex traps will last for approximately 2000 desorption's each before they need replacing, and the detector lamp life is approximately 4-6 months. See pages 29-30 of the MERX user's guide for detailed instructions for lamp and trap replacement.

Sample Line/Purge Vessel Cleaning

The purge vessel and sample line will require monthly cleaning under regular use or sooner as evidenced by elevated instrument blanks. Clean this equipment in the following order: 1 M KOH (8 hrs), reagent water rinse, 10% aqua regia (8 hrs), and reagent water rinse. Dry the purge vessel with Argon gas and double bag prior to storage.

Equipment Cleaning

Trace level mercury analyses of samples at parts per billion concentrations are susceptible to contamination. Equipment that comes into contact with samples or reagents should be free of residual mercury and can consist of (but not be limited to) Teflon, glass, and polypropylene containers. Brand new and previously used Teflon equipment should be washed in acid before use. The equipment is first rinsed with tap water, and then cleaned by immersing in 4 N HCl heated to 65°C for at least 12 hours (48 hours for new Teflon equipment). Immediately following removal from the bath, equipment is completely immersed in reagent-grade water and then additionally triple-rinsed in reagent-grade water.

After rinsing, each container is air dried under a mercury-free class 100 laminar flow hood. Dry equipment is stored double bagged in zip-type bags.

Analytical Vial Cleaning

Clean the amber glass vials with the following method. Wash the vials with lab detergent and rinse with reagent water. Once dry, wrap the vials in aluminum foil and heat at 550 º C for 2 hrs. Inspect the vials prior to use for chips or cracks.

APPENDIX 1. Quick Reference Guide for MeHg analysis in soil and sediment samples with the Brooks-Rand MERX Automated Methyl Mercury Analytical System

- Make sure that the instrument has power and gas, empty the waste receptacle, and adjust the offset to 50,000 by changing the PMT
- Start the software, open the appropriate template, save as data (MMDDYY_*sample name*.brd), connect to the instrument, and measure noise
- Prepare vials for analysis, beginning with instrument calibration
  - Fill with ~ 35 ml of reagent water, add standard/sample (if necessary), add 300 µl sodium acetate buffer, and 50 µl NaTEB
  - Fill the vial to a reverse meniscus with reagent water, and seal without headspace with a new cap/septa assembly
  - Make sure the vials are correctly oriented in the sample rack and the sample rack is correctly oriented in the autosampler,
  - Enter the number of vials analyzed, starting position, and choose Start Run
- Once the initial calibration is successful, proceed to the sample run, following the vial preparation procedure described above
  - Add 10 – 150 µl of sample extract,
  - Analyze instrument blanks throughout the run (at least 5)
  - Analyze a check standard every eighth position
- As sample analysis progresses, check that the following QA/QC objectives are being met:

TYPE	QA/QC CRITERIA
Standard Curve	$r^2 > 0.995$
Samples	Mass within confines of standard curve
Triplicate	RSD < 25%
Duplicate Spike	Recovery range 75 – 125% Relative difference < 25%
CRM	Recovery range 75 – 125%

- Examine the dataset for suspicious analytical results
- Export the data to a .CSV file, and name it such that the file reflects the type of extraction, date, and samples analyzed,
  (ie. KOH_010210_indiana leaves) and save the file in the folder named for the current year in the GURU RUNS folder
  (D:Drive→HG4Data→MERX→GURU RUNS→2010)

APPENDIX 2. Example of a typical analytical run.

Rack 1

8 Check Std.	7 Instrument Blank	6 Sample 6	5 Sample 5	4 Sample 4	3 Sample 3	2 Sample 2	1 Sample 1
16 Check Std.	15 Instrument Blank	14 Sample 12	13 Sample 11	12 Sample 10	11 Sample 9	10 Sample 8	9 Sample 7
24 Check Std.	23 Sample 19	22 Sample 18	21 Sample 17	20 Sample 16	19 Sample 15	18 Sample 14	17 Sample 13

Rack 2

32 Check Std.	31 Instrument Blank	30 Sample 25	29 Sample 24	28 Sample 23	27 Sample 22	26 Sample 21	25 Sample 20
40 Check Std.	39 Instrument Blank	38 Sample 31	37 Sample 30	36 Sample 29	35 Sample 28	34 Sample 27	33 Sample 26
48 Check Std.	47 Sample 38	46 Sample 37	45 Sample 36	44 Sample 35	43 Sample 34	42 Sample 33	41 Sample 32

Rack 3

56	55	54	53	52 Check Std.	51 Instrument Blank	50 Sample 40	49 Sample 39
64	63	62	61	60	59	58	57
72	70	70	69	68	67	66	65