Analysis of Methylmercury in Water by Distillation, Gas Chromatography Separation, and Speciated Isotope Dilution Mass Spectrometry

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Scope and Application

The following standard operating procedure (SOP) is used by the U.S. Geological Survey's Mercury Research Laboratory (MRL), and describes the preparation of water samples by distillation and subsequent analysis for methylmercury (MeHg). Quality assurance and control protocols are employed throughout sample distillation and analysis, including: laboratory practices to prevent sample contamination, method blanks, MeHg additions to asses sample matrix interference, and isotopic MeHg additions for quantification by isotope dilution (ID).

Prior to analysis, distillation is required to disassociate MeHg from the sample matrix and reduce matrix interference during analysis. In Teflon distillation vials, approximately 40 ml of acidified water samples are spiked with isotopically enriched MeHg and amended with 1 ml of a 25% Copper Sulfate solution. The samples are heated to 121°C and purged with nitrogen gas. The resulting water vapor carries the disassociated MeHg from the sample and is condensed in chilled Teflon receiving vials (distillate). Distillation is stopped when approximately 25% of the sample remains in the distillation vessels.

Analysis is conducted via the Brooks-Rand "MERX" automated MeHg analytical system coupled to the Elan inductively coupled plasma-mass spectrometer (ICPMS). Distillate is added to 42 ml glass vials and buffered with Sodium Acetate/Acetic Acid to a pH of 4.5 – 5.0. Sodium Tetraethylborate (NaTEB) is added to the sample resulting in ethylation of the oxidized mercury species (Hg^{2+} and $MeHg^{+}$). The volatile ethylated species, as well as elemental mercury, are purged from the distillate with argon gas, retained on Tenex traps, thermally desorbed back into the sample stream, and separated by mass with a gas chromatography column. The elemental and ethylated mercury species are released from the column *en masse* into the sample stream, thermally oxidized to elemental mercury, and introduced to the ICPMS for detection. Quantification of MeHg concentrations in the samples are calculated using isotope dilution.

Laboratory Safety

Analysts who use the MRL must have read, understood, and signed the Chemical Hygiene Plan for the MRL prior to potential exposure to any chemicals. The analyst must have a thorough understanding of the required safety protocols for the lab chemicals prior to their use of the lab. Adequate personal protection equipment such as safety glasses, gloves, and chemical resistant clothing must be worn when exposure to hazardous chemicals are possible. Caution should always be exercised as that chemicals are present in the laboratory and often in use by other analysts. Hazardous chemicals should only be handled by adequately trained personnel under a high volume fume hood with extreme caution.

Multiple safety concerns are present in the conduct of this method; detailed information is included for each reagent specific to the method later in this SOP, and additional information can be found in the safety data sheets (SDS) located in the lab. MeHg is an extremely toxic organic metal and caution should be exercised to limit exposure during daily operations. While samples and working standards are relatively low in concentration, concentrated stock solutions containing elevated MeHg levels are occasionally encountered. Concentrated MeHg stock solutions should only be handled by experienced lab personnel. Additionally, reagents used in this method include strong acid, strong base, and an organometallic ethylating compound. During analysis the automated sample introduction system may begin moving without warning and presents a mechanical hazard. Finally, equipment and sample in excess of 100°C will be encountered and presents a burn hazard.

Equipment

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 polycarbonate 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.

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 record the chemical contents and concentration, and initial and date the reagent container. Reagents and manufacture instructions follow below.

Reagent Water (Milli-Q water): Ultrapure 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.

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.

Nitrogen (N_2): Nitrogen gas is provided by a Peak Scientific nitrogen generator (model NM32LA) and is scrubbed of gaseous mercury by passing through a gold bead trap.

Sodium Acetate/Acetic Acid 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 and shake until all solids dissolve. Transfer to a 125 ml Teflon bottle for use as a working solution.

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 effects of NaTEB exposure is unknown, it should be assumed that repeated exposure may have adverse health effects. 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. 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. Once the KOH solution begins freezing, remove the KOH solution and the vial of $NaBEt_4$ from the freezer. It is best to work quickly at this point as to keep the pure NaTEB cold and to limit its exposure. Open and immediately dump the pure NaTEB into the 2% KOH solution and gently swirl to dissolve. Rinse the NaTEB vial with the KOH solution if any significant amount of NaTEB remains in the vial. When the NaTEB solution is almost entirely melted, homogenize, and pour equally into 18 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 4 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.

1 M Potassium Hydroxide (KOH) solution: In a 1.5 L Teflon bottle, add 56 g of KOH to 250 ml of reagent water and bring up to 1L.

1 M Hydrochloric Acid (HCl): In a 1.5 L Teflon bottle, add 100 ml of concentrated HCl to approximately 100 ml of reagent water and bring up to 1 L.

25% Copper Sulfate ($CuSO_4$) solution: In a 500 ml Teflon bottle, add 125 g $CuSO_4$ and bring up to 500 ml volume with reagent water. Shake well until all solids dissolve.

Analytical Standards

Upon receipt at the laboratory or on the day of preparation, standards should be labeled with the content and concentration, and 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% acetic acid and 0.2% HCl concentration. This solution should be transferred to a Teflon bottle designated specifically for mercury standards, stored in an amber bag at 4°C, and remade every 6 months. All standards must be assigned a unique letter-number-letter identification code and be entered into the laboratory database system. Isotopically enriched MeHg standard concentrations are determined by ICPMS analysis as part of instrument calibration. Working solutions of non-isotopic MeHg standards are determined against a NIST certified total mercury standard. 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°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^{2+}

6. Subtract the average blank mercury mass and the $SnCl_2$ reducible fraction of Hg^{2+} from the total mercury mass determined MeHg working standard to determine the actual MeHg mass in the vials and subsequent concentration.

Sample Preparation by Distillation

Analysis of waters for MeHg requires distillation to remove matrix interference. A typical distillation contains 30 samples, four method blanks, and three duplicate spikes (one per 10 samples). In small sample sets (<10), at least four method blanks and one duplicate spike should still be included in each run.

1. Open a template of the Excel data sheet by navigating to the "ID Tracer Template" folder (HG4 → hg4data→Isotope methyl data→ID Tracer Templates). Make sure to open the template specific to water analysis.

2. Select "Save As" and save the file in the folder named with the current year (HG4→ hg4data→Isotope methyl data→2016). Use the following naming convention for the analytical files:

    - "M" for ambient or "I" for isotopic samples
    - Run date (DDMMYY)
    - "ID"
    - Brief description of samples
    - Example: "M080613 ID ENP, GL INPUTS.xls"

3. Before beginning sample setup, turn on the aluminum block heater so that it can reach temperature (approximately 120°C) while samples are being prepared.

4. Arrange an adequate number of clean 60 ml Teflon vials into the wire racks. Each position (sample, method blank, and duplicate spike) to be distilled requires 2 vials; one vial for heating (distillation vial) and one chilled vial to receive the water vapor (receiving vial).

5. See the figure below for the run position of the distillation vials:

1	2	3	4
5	6	7	8
9	10	11	12
13	14	15	16
17	18	19	20

21	22	23	24
25	26	27	28
29	30	31	32
33	34	35	36
37	38	39	40

6. See the figure below for the run position of the receiving vials:

4	3	2	1
8	7	6	5
12	11	10	9
16	15	14	13
20	19	18	17

24	23	22	21
28	27	26	25
32	31	30	29
36	35	34	33
40	39	38	37

7. Select 30 samples for a full distillation.

8. Choose three of the samples to analyze as duplicate spikes. Do not set up field blank samples as a duplicate spike. Distribute the duplicate spike samples evenly across the sample set.

9. Every distillation requires four method blanks, which is the distillation of acidified reagent water (1% HCl). A method blank should be set up in every tenth position, and occur in the position that is one greater from the previous distillation. For example, if method blanks were run in positions 1, 11, 21, and 31 for Monday's distillation, they should be located in positions 2, 12, 22, and 32 for Tuesday's distillation.

10. In the setup worksheet (see Appendix 1 for reference), record the sample identification code, sample information, distillation/receiving vial identification code's (each vial has a unique code etched onto the bottom), and the vial tare weights. Be sure that you enter the distillation and receiving vial codes so that they are correctly matched up by their respective run position (note in the above figures that the run order for the receiving vials are different from the distillation vials). Enter method blanks into designated part of the setup sheet.

    - Sample identification code in the in the "Bottle ID" field
    - Sample information in the "SAMPLE" field
    - Distillation vial identification codes in the "FROM VIAL" field
    - Receiving vial identification codes in the "TO VIAL" field
    - Vial tare weights in the "TARE WT." fields

11. Weigh approximately 40 grams of sample (or reagent water for method blanks) into each of the distillation vials. Homogenize the sample by inverting the sample bottle several times before dispensing to the distillation vial. Samples suspected to be high in MeHg (>2 ng/L) should be diluted to reduce exposure to distillation equipment. Setup three samples in triplicate for the duplicate spike analyses. Be sure that you record the mass of the samples or blanks (recorded as the combined mass of water and distillation vial) into the appropriate field in the setup sheet.

12. Acidify the method blanks to 1% HCl. If samples have been diluted, adjust acidity to 1% HCl by adding the appropriate amount of acid.

13. Add 5 pg of the isotopically enriched working solution to each of the distillation vials. If working with isotopically enriched samples, be sure that the spiked isotope fraction has not been amended in the sample.

14. Add 50 pg of the ambient MeHg from the working standard to the duplicate spike samples.

15. Add 1 ml of 25% $CuSO_4$ to each of the distillation vials.

16. Fit each distillation vial with a combined distillation cap/transfer tube assembly corresponding to its position (each cap has been engraved with a number between 1 and 40).

17. To each of the receiving vials, add 10 ml of reagent water. Fit each receiving vial with a cap corresponding to its position in the rack (each cap has been engraved with a number between 1 and 40) and place in the cooler.

18. Place the distillation vials in the bore holes of the preheated aluminum block in the same order that they are in the rack. Attach a nitrogen gas line (flow rate approximately 70 ml/min) to each of the distillation caps and verify that gas flows through the sample.

19. In an ordered manner, thread the transfer tubes through the Teflon ports in the side of the cooler.

20. In the cooler, attach each transfer tube to the corresponding receiving vial. Verify that gas flows in the reagent water of the receiving vial.

21. Throughout the distillation, check the temperature of the heating block often. Adjust to maintain a temperature of 121°C (± 5).

22. Check the distillation vials regularly. Samples should distill at about 6-8 ml/hour and are finished when approximately 25% of the original sample is left.

23. Once a sample is finished, disconnect the transfer tube from the receiving vial and the gas supply line from the distillation cap. Cover the gas inlet port of the distillation cap quickly with a gloved finger to keep the sample in the vial from discharging.

24. Thoroughly rinse the combined distillation cap/transfer tube assembly with reagent water. Use a cotton swab and your fingers to remove organic material from the stem. Place the caps in the laminar flow hood to dry.

25. Once the entire distillation is finished, remove the receiving vials from the cooler and remove the distillation caps. Record the mass of the receiving vial in the data sheet and attach a standard cap.

26. Rinse the caps from the receiving vials with reagent water.

27. Distillates should be analyzed within 48 hours of distillation.

Instrument Operation

Analysis is conducted via the Brooks-Rand “MERX” automated MeHg analytical system coupled to the Elan inductively coupled plasma-mass spectrometer (ICPMS). An accessory program, Chromera, is also used to process the data output from Elan. Both the instruments and the Chromera software run simultaneously and must be prepared to operate before analysis can begin. Figures below identify important features of Elan and Chromera.

The MERX instrument consists of four interconnected modules: the autosampler, purge and trap unit, the gas chromatography column, and the detector. The instrument is designed to operate on a specific mixture of reagents that are prepared in septa sealed 42 ml 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. A typical analytical run is shown in Appendix 2.

Brooks-Rand Start Up

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

2. Launch the Merx software with the icon on the desktop. Open an analytical template file for the planned analysis. From the “File” dropdown menu, select “Open” and navigate to the template.brt file (D:drive→HG1Data). Save the file as data (from the “File” dropdown menu) in the data folder from the current

year found in the MERX RUNS folder. Name the new run file by date (MMDDYY.brd).

3. 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. The communication status at the top of the screen will indicate connection with each module (see figures below for reference).

4. 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.

Elan/Chromera Start Up

1. Start the Elan software from the desktop shortcut.

2. Open the methylmercury w/204 workspace with the icon on the left of the display.

3. Click the “R” button in the upper left corner of the workspace and verify that the Default.dac file has been loaded.

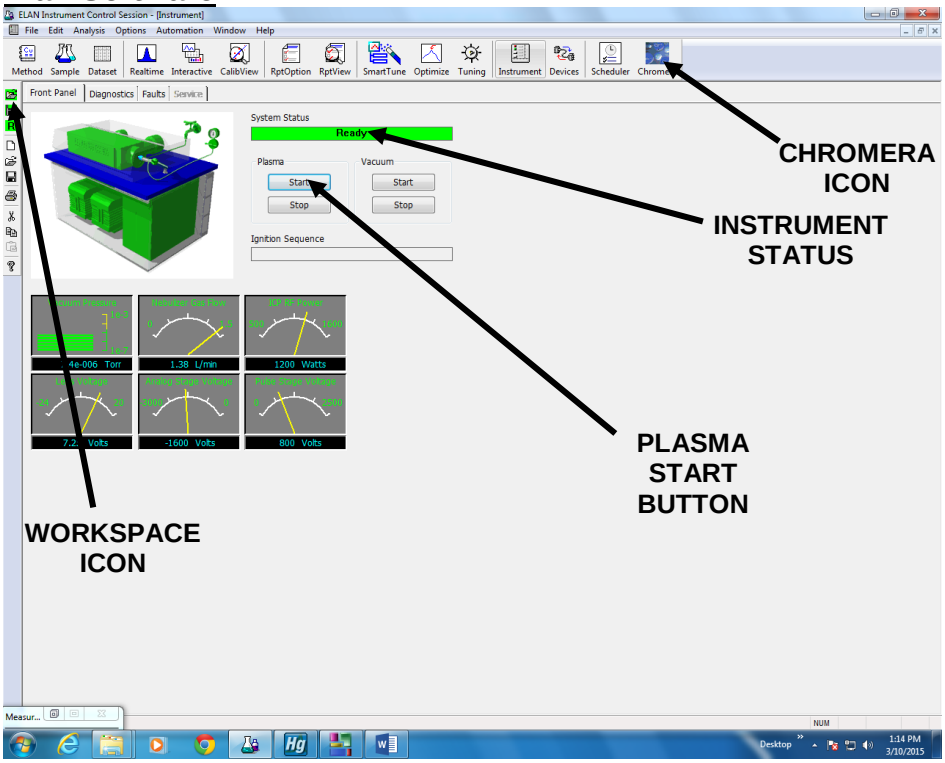
4. Click the “instrument” icon to display the ICPMS status. Turn on the coolant pump with the switch near the door and turn the vent fan on with the switch above the desk.

5. When the status of the instrument displays “Ready”, click the “Start” button to initiate the plasma field. You will hear the vacuum pumps start in the ICPMS and can monitor the progress on the “Ignition Sequence” status bar.

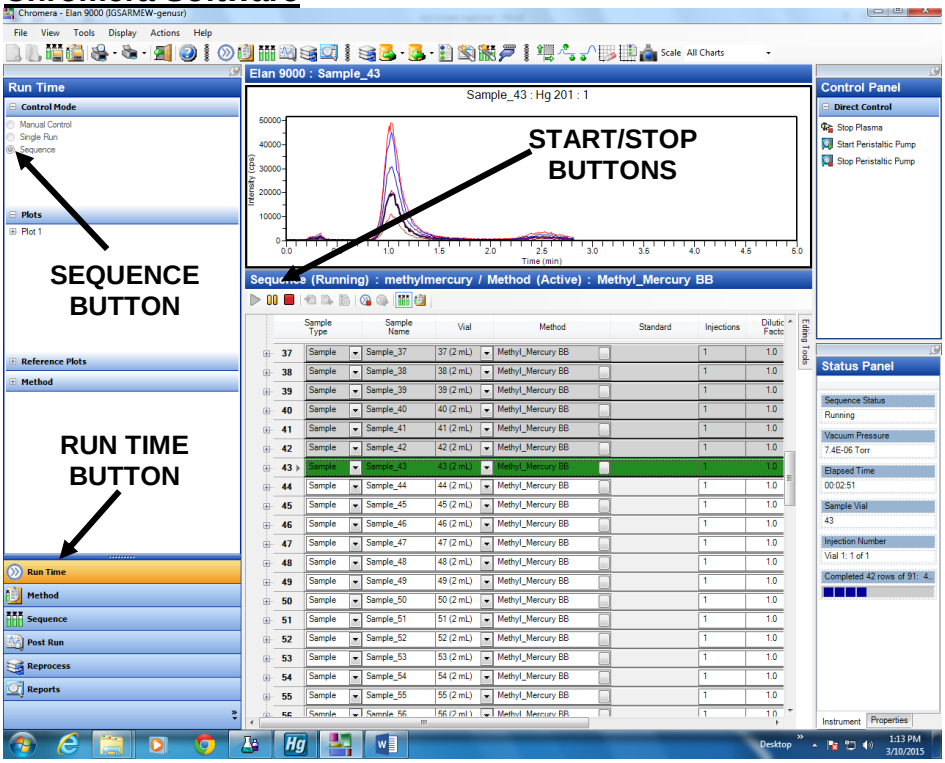
6. Launch the Chromera software with the icon in the Elan software.

7. Activate the Run Time window and select the Sequence radio button. Open the “methylmercury” sequence if necessary.

Elan Software



Chromera Software



Preparation of Vial for Analysis

The following instructions should be used to prepare vials for the analysis of standards, samples, and blanks.

1. Place the clean vials in the sample rack. Add approximately 35 ml of Milli-Q to vials that will be used for blanks or standards.

2. Add the MeHg source (standard or sample) to the appropriate vials.

3. Add 200 μL of the Sodium Acetate Buffer to all vials to adjust the pH of the mixture to 4.5 – 5.0.

4. Add 50 μL of 1% NaTEB to all vials.

    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 Milli-Q 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.

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

Instrument Calibration

Analysis by ID requires the addition of a known quantity of an isotope to the sample prior to sample preparation, and the analysis of the added isotope and a non-amended isotope by ICPMS. Sample concentration is calculated by adjusting the recovery of the non-amended isotope based upon the recovery of the known addition. Isotope dilution requires two separate calibrations; a mass bias curve and a reverse ID calibration curve. See Appendix 2 for an example of a typical analytical run.

1. Set up vials 1-3 with 50 pg of MeHg standard. These vials are not part of the calibration, but are used to condition the traps and give you advanced indication of instrument performance

2. Set up vials 4-11 as blanks (reagent water, buffer, and NaTEB). Vials 4-8 are instrument blanks, 9-11 are calibration blanks.

3. Create a mass bias curve in vials 12-16. Add ambient MeHg standard to the vials (as you would for a typical calibration curve) that spans the expected masses of the samples (2.5 – 50 pg is sufficient for most samples).

4. Create a reverse ID calibration curve in vials 17-21. Add 12.5 pg of ambient MeHg standard to each vial. Add 5 pg of isotopically enriched MeHg standard to each vial.

5. As previously described, add the buffer and NaTEB, fill the vial with Milli-Q, cap, and put the sample rack into the autosampler.

6. In the Chromera software, select the green start button. The first line of the sequence should turn green. It is necessary to start the analytical sequence in the Chromera software prior to initiating the Brooks Rand for sample analysis.

7. Activate the Brooks Rand software. Under the Automation tab, select the number of vials to be analyzed for the entire run (72 for a full distillation) and the starting position. Click the start button to activate the Brooks Rand modules and subsequent detection by the ICPMS.

8. After approximately 15 minutes, chromatograms will start appearing in the Chromera software. The initial three chromatograms will be Tenex trap desorption blanks, and the following are the chromatograms from the analytical vials.

9. Review the results from the first three 50 pg MeHg standards. In a typical analysis, there is not enough time to wait for the instrument calibration to finish before setting up the samples. If the peak heights are relatively

consistent to each other and reasonably close to expected peak heights, proceed with the analysis of sample distillate.

Sample Analysis

Prepare sample distillate as you would a standard (to the analytical vial, add distillate, 200 μL of Sodium Acetate Buffer, 50 μL NaTEB, and fill to capacity with reagent water). Throughout the run assess for sample carryover and instrumental drift by the regular analysis of instrument blanks and reverse ID check standards. Five bubbler blanks should be spread throughout an analysis. Reverse ID check standards should be analyzed regularly throughout the analysis and span the expected masses of the samples. Analyze check standards at least once every 8 samples and at least four per sample set. See Appendix 2 for an example of a typical analytical run.

Data Processing and Capture

1. Open the analytical Excel spread sheet that was initially filled out when the samples were distilled.

2. Add the following information in the “Isotope Info” worksheet (see Appendix 3)

    - Header information
    - Ambient and ID standard information
    - Matrix and ID spike volumes
    - Select the isotopes used for ambient and ID spike
    - For isotopically enriched samples, select the batch of each isotope used to amend the sample

3. In the “Peak Area-Curve” worksheet, record the volume of standard used for the mass bias curve in cells A2-A6 and the volume of ambient standard used for reverse ID check standards in cells A27-A32 and A35-A40.

4. As the run progresses, the report files for the integration of the chromatograms will be sent to the “Chromera Report” folder. The Chromera run files are named numerically, and appear in the order in which they are analyzed. Chromera does not sort the isotope data in any particular order. To sort the data by isotope, copy the isotope number and peak height data from the report file and paste in the designated place in the “Peak Area-Curve” worksheet of the Excel spread. Then copy the output and paste the sorted data into the appropriate place in the Peak Area-Curve worksheet.

5. Final sample concentrations, recovery of the duplicate spikes, and recovery of the reverse ID check standards can be seen in the “Summary” worksheet.

6. The mass bias value and relative standard deviation, as well as the results of the reverse ID calibration curve can be seen in the “ID calculations” work sheet.

Shutdown

Analyze two vials of Milli-Q water at the end of the run. The purpose of these vials is to flush the sample line, purge vessel, and the dumping valve in the Merx. It is not necessary to wait for the ICP analysis of these blanks; as soon as the Milli-Q has been flushed through the MERX, the run can be ended.

Exit the MERX software and do not save changes if prompted.

In Chormera, click the red stop button and close the software.

In Elan, turn off the plasma and exit. Do not save changes to the method file when prompted. Turn the coolant pump and vent fan off.

In the Chromera Report folder, create a new folder in the “old” folder. The naming convention is Mxxxxxx, where xxxxxx represents the six digit date MMDDYY. Cut all of the data output files from the Chromera Report folder (except the “old” folder) and paste them into the new folder within the old folder.

Quality Assurance and Control Objectives

During the analytical run, the analyst must evaluate the calibration data, instrument blank values, duplicate spike recoveries, and reverse ID check standard recoveries to ensure acceptance criteria are being met.

Instrument Calibration

Mass Bias Calibration Curve: Mass bias is the measured isotopic fractionation of ambient MeHg compared to published values and is used to determine the mass bias correction. The mass of ambient MeHg used for the measurement of the mass bias should span values expected in the samples. Create a five point calibration curve with the MeHg working standard. For most samples, MeHg mass spanning 2.5 – 50 pg will be sufficient.

Reverse ID Calibration Curve: The reverse ID calibration curve is used to determine the concentration of the isotopic MeHg standard used for the isotopic spike. The reverse ID calibration curve is created by adding both the MeHg working standard and the isotopic MeHg spike used during the distillation. For most samples, add 12.5 pg of MeHg working standard to 5 analytical vials and 5 pg of isotopically enriched MeHg spike to these same vials.

Reverse ID Check Standard: The ID calibration of the instrument should be verified throughout the run with reverse ID check standards. Reverse ID check standards are prepared similar to the reverse ID calibration curve. Add various masses of MeHg in the check standards. Also add to these vials the same mass of isotopic MeHg spike that was added to the reverse ID Calibration Curve. At the minimum, a reverse ID check standard should be analyzed to verify instrument calibration in every eighth position, and have a measured mass within 80 – 120% of its true value.

Fractionation of the Isotopically Enriched MeHg Standard: The enriched MeHg isotopes used to create the reverse ID calibration/check standards and to amend environmental samples is contaminated with small amounts of other isotopes. Due to the sensitive nature of isotopic analysis, concentration calculations must be corrected to account for these contaminants. The identity of the enriched isotopes used for ID quantification and environmental additions must be specified in the “Isotope Info” worksheet of the Excel spread sheet so the appropriate correction can be made. See the quality control officer for further direction as to the appropriate source for which to reference.

Matrix Interference (Duplicate Spike)

One sample should be run as a duplicate spike for every 10 samples in a distillation/analysis, with a recovery 75 – 125% of the known addition and percent difference between the two recoveries less than 25%. A duplicate spike is set up

similar to a triplicate analysis, except that two of the three samples are spiked with 50 pg of MeHg prior to the distillation. In the case of failure bring to the attention of the quality assurance officer.

Instrumental Carryover

Background MeHg signal is assessed by the calibration blanks which are run previous to the mass bias curve and throughout the run. Calibration blanks are the analysis of reagent water with buffer and NaTEB. Additionally, 5 non-analytical instrument blanks are analyzed previous to calibration to clear the sample train of residual MeHg. Excessive instrument carryover indicates that the sample train or reagents have been contaminated with MeHg.

Method Blank

A method blank should be analyzed at least once every ten samples. Method blanks are part of the distillation set up and consist of a distillation vial with reagent water, 1 ml of 25% $CuSO_4$, isotopic MeHg spike, and 400 µl of HCl. Elevated method blanks indicate contamination in the distillation vials, equipment, or reagents.

Additional Instructions

MERX 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 three analytical Tenex traps will last for approximately 2000 desorption's each before they need replacing (see pages 29-30 of the MERX user's guide for trap replacement).

The purge vessel will require monthly cleaning under regular use or sooner as evidenced by elevated bubbler blanks or excessive deposits. Clean the purge vessel by soaking it in the following reagents: 1 M KOH (8 hrs), reagent water rinse, 1 M HCl (8 hrs), and reagent water rinse. The sample line may also need to be occasionally replaced if elevated bubbler blanks persist.

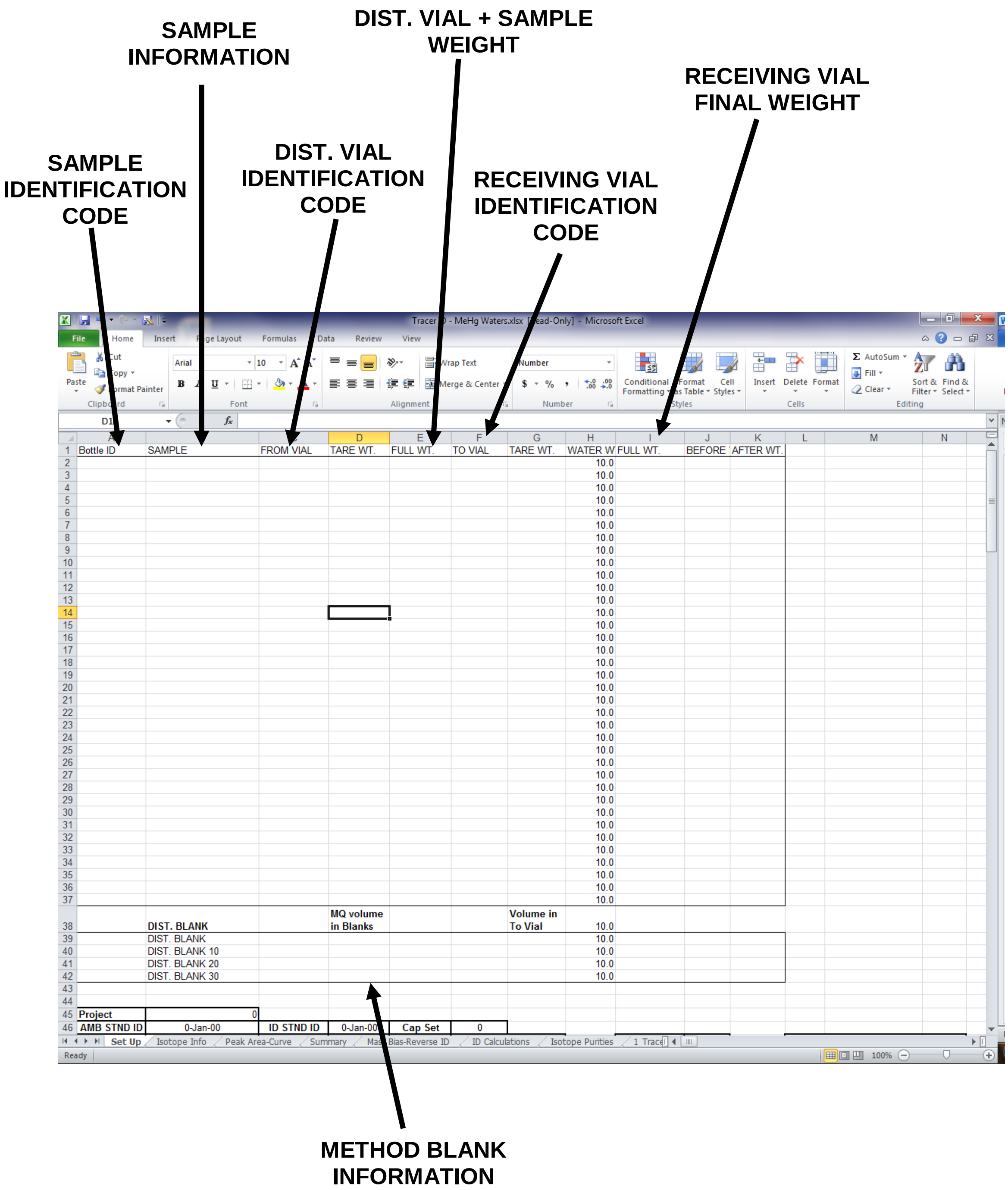
ICPMS Maintenance

Regular maintenance on the ICPMS should only be attempted by an experienced operator. Guidelines for regular maintenance can be found in the ELAN instruction manual, or by consulting the lab manager. Vent filters and vacuum pump oil should be inspected monthly on the ICPMS, and changed quarterly or sooner as necessary. Vacuum the exterior and interior of the ICPMS to remove dust monthly. Inspect the torch, RF coil, sampler cone, and skimmer cone weekly for excessive wear and clean/replace as necessary.

Analytical Vials

Analytical vials are purchased certified trace metal clean, and can be used without cleaning. Although it is not cost effective for the MRL to reuse analytical vials, they can be reused if properly cleaned. Wash the vials with lab detergent and rinse with reagent water. Once dry, wrap the vials in aluminum foil and heat at 550°C for 2 hrs. Inspect the vials prior to use for chips or cracks.

Appendix 1. Distillation setup data sheet.



APPENDIX 2. Example of a typical analytical run.

Rack 1

8 Instrument Blank	7 Instrument Blank	6 Instrument Blank	5 Instrument Blank	4 Instrument Blank	3 50 pg Working Std	2 50 pg Working Std	1 50 pg Working Std
16 Mass Bias Calibration Curve	15 Mass Bias Calibration Curve	14 Mass Bias Calibration Curve	13 Mass Bias Calibration Curve	12 Mass Bias Calibration Curve	11 Calibration Blank	10 Calibration Blank	9 Calibration Blank
24 Method Blank 3	23 Method Blank 2	22 Method Blank 1	21 Reverse ID Calibration Curve	20 Reverse ID Calibration Curve	19 Reverse ID Calibration Curve	18 Reverse ID Calibration Curve	17 Reverse ID Calibration Curve

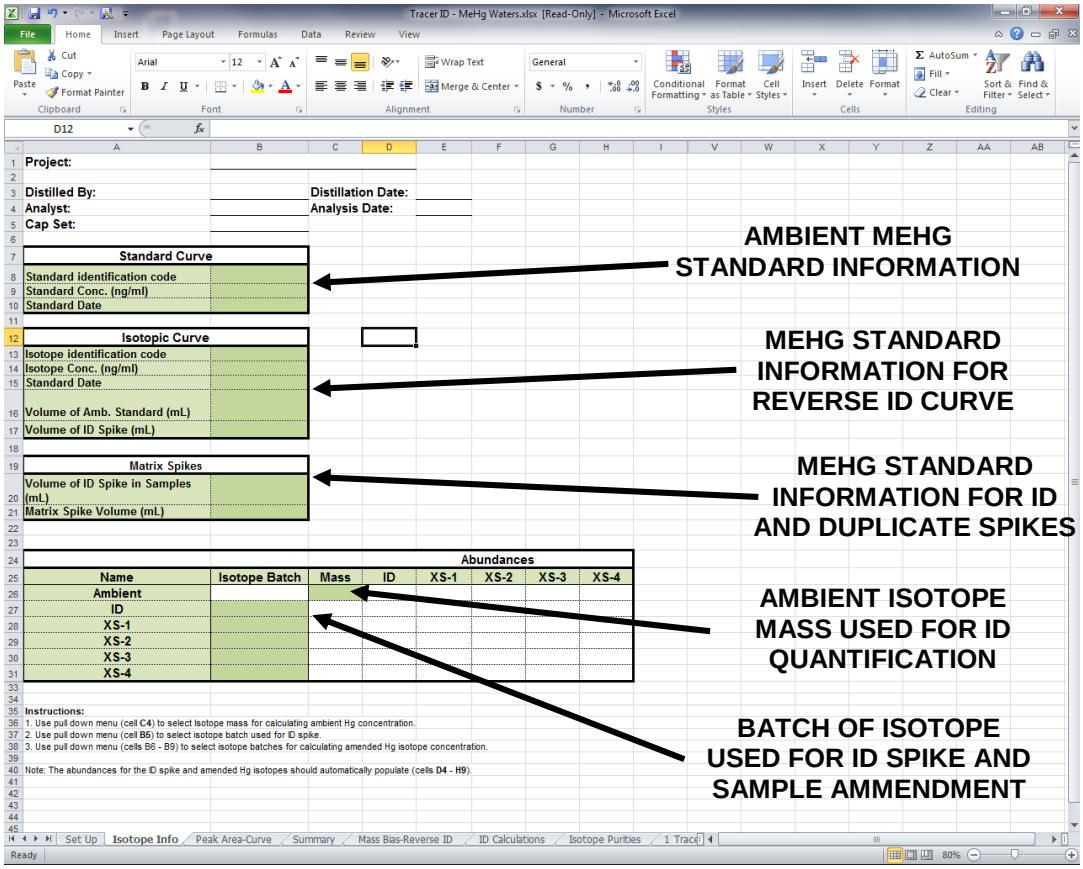
Rack 2

32 Reverse ID Check Standard	31 Sample 6	30 Sample 5	29 Sample 4	28 Sample 3	27 Sample 2	26 Sample 1	25 Method Blank 4
40 Reverse ID Check Standard	39 Sample 12	38 Sample 11	37 Sample 10	36 Sample 9	35 Sample 8	34 Sample 7	33 Calibration Blank
48 Reverse ID Check Standard	47 Sample 18	46 Sample 17	45 Sample 16	44 Sample 15	43 Sample 14	42 Sample 13	41 Calibration Blank

Rack 3

56 Reverse ID Check Standard	55 Sample 24	54 Sample 23	53 Sample 22	52 Sample 21	51 Sample 20	50 Sample 19	49 Calibration Blank
64 Reverse ID Check Standard	63 Sample 30	62 Sample 29	61 Sample 28	60 Sample 27	59 Sample 26	58 Sample 25	57 Calibration Blank
72 Reverse ID Check Standard	71 Sample 36	70 Sample 35	69 Sample 34	68 Sample 33	67 Sample 32	66 Sample 31	65 Calibration Blank

APPENDIX 3. Isotope info worksheet.



Appendix 4. Definition of equations.

$$\text{Sample MeHg Concentration} = \frac{\left(\text{MeHg Mass per Distillate}\right) - \left(\text{Blank MeHg Mass}\right)}{\text{Sample Volume}}$$

$$\text{MeHg Mass Per Distillate} = \frac{\left(\left(\left(\text{Unamended Isotope Peak Height}\right) - \left(\text{Unamended Isotope Contribution From ID Spike}\right)\right) / \left(\text{Excess Amended Isotope From ID Spike}\right)\right) * \left(\text{Mass of ID Spike}\right) * \left(\text{Instrument Mass Bias}\right)}{\text{Native Fraction of Unammended Isotope}}$$

$$\text{Excess Amended Isotope From ID Spike} = \left(\text{Amended isotope Peak Height}\right) - \left(\text{Amended Isotope Contribution From Mercury in Sample}\right)$$

$$\text{Instrument Mass Bias} = \left(\frac{\left(\text{IUPAC Fraction of Unamended Isotope}\right)}{\left(\text{IUPAC Fraction of Amended Isotope}\right)}\right) \Big/ \left(\frac{\left(\text{Unamended Isotope Peak Height}\right)}{\left(\text{Amended Isotope Peak Height}\right)}\right)$$

The amended isotope is the isotope we enrich prior to the distillation. In a typical analysis we add $Me^{199}Hg$ as the amended isotope.

The unamended isotope is not directly enriched with the ID spike. In a typical analysis we use $Me^{202}Hg$ as the unamended isotope.

The unamended isotope contribution from the ID spike is the trace amount of the unamended isotope present in the ID spike that needs to be subtracted from the unamended isotope peak height.

The excess amended isotope from the ID spike is the portion of the amended isotope peak height that is from the isotopic spike. The naturally occurring portion of the amended isotope is mathematically calculated from the unamended isotope and subtracted from the amended isotope peak height.

The instrument mass bias is the comparison of ratios of the unamended and amended isotopes as measured on the instrument relative to IUPAC accepted values.

$$\text{Percent Recovery} = \frac{(\text{Analyzed Concentration})}{(\text{Known Concentration})} \times 100$$

$$\text{Spike Recovery} = \begin{pmatrix}\text{MeHg Mass Per Distillate Of Spiked Sample}\end{pmatrix} - \left(\frac{\left(\begin{pmatrix}\text{MeHg Mass Per Distillate of Unspiked Sample}\end{pmatrix} * \begin{pmatrix}\text{Volume of Spiked Sample}\end{pmatrix} \Big/ \begin{pmatrix}\text{Volume of Unspiked Sample}\end{pmatrix} \right)}{\begin{pmatrix}\text{Spike Mass}\end{pmatrix}} \right)$$