

Appendix V: USGS SOPs

USGS Standard Operating Procedures			
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B	Water TOC Analysis Using the Shimadzu TOC-VCSN and ASI-V Autosampler	METH011.00 (Appendix V B)	July 2013
C	Analysis of Methylmercury in Water by Distillation, Gas Chromatography Separation, and Speciated Isotope Dilution Mass Spectrometry		
D	Solids, volatile-on-ignition, total-in-bottom-material, gravimetric	Techniques of water-Resources Investigations of the United States Geological Survey, Third Edition, Book 5 Laboratory Analysis, p 451	1989
E	Digestion of Sediments and Biological Tissues for Analysis by Isotope Dilution Hydride Generation Inductively Coupled Mass Spectrometry (ID HGICP-MS)		May 2014
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Appendix V A: Sample Processing (USGS-FRESC)

Sample Processing Standard Operating Procedures



Forest and Rangeland Ecosystem Science Center

**CONTAMINANT ECOLOGY
RESEARCH PROGRAM**

Collin Eagles-Smith
Revised 12/27/2011



Quick View Processing SOP

1. Clean work space, tools, analytical equipment.
2. Thaw samples.
3. Prepare data sheets.
4. Generate unique tissue CERP ID Codes (if dissecting tissues from sample).
5. Generate tough tags for tissue samples.
6. Update google docs.
7. Weigh whole body sample (wet weight).
8. Dissect (this process differs by project and sample type and often includes additional steps not identified in this SOP. (For birds, refer to *S:\Projects\Eagles-SmithLab\SOPs\Birds\Egg dissection*).
9. Dry samples.
10. Weigh dry sample.
11. Grind samples.
12. Enter data.
 - a. Use *DataEntryTemplates_EasyAccesssTemplate* found in *S:\Projects\Eagles-SmithLab\Data*.
 - b. Save data in *S:\Projects\Eagles-SmithLab\Active Projects*.
13. Verify (proof) data.
14. Scan data sheets.
 - a. Save scans in *S:\Projects\Eagles-SmithLab\Scanned datasheets*
15. Store original data sheets in data repository filing cabinet.
16. Update google docs.
17. Once all data are verified, move folder into the “*Data*” folder (*S:\Projects\Eagles-SmithLab\Data*) indicating data are ready to be uploaded to database.

USGS FRESO Contaminant Ecology Research Program
Standard Operating Procedure (SOP)
Fish Sample Processing
12/20/2011

The sample processing component is the step in the workflow immediately after cataloging, in which samples are dissected (when applicable), dried, and homogenized prior to chemical analyses. This step often occurs over several days as samples are dried, then homogenized. Thus, special care must be taken to ensure that datasheets are carefully tracked.

In some cases, this step is merged with the cataloging step and both are completed simultaneously. Be sure to check with project leader(s) to verify the most appropriate approach for each sample set.

1. Laboratory conditions and equipment cleanliness

- 1.1. Tape clean lab bench paper or aluminum foil to the lab bench.
- 1.2. Prior to sample processing, the surfaces of all processing locations and tools shall be cleaned and thoroughly rinsed with DI water. All processing tools shall also be rinsed with DI water and wiped with a clean KimWipe between each sample.
- 1.3. Gently wipe the analytical balance (using care not to apply pressure to the weigh pan) and ensure that it is calibrated daily, prior to use.

2. Initial sample tracking and cataloging

- 2.1. From the freezer, remove only the number of samples that can be initially processed (cleaned, weighed, and inserted into drying oven) in the allotted time for the given day. These samples should not be thawed for ~1 hour before processing.
- 2.2. Prepare data sheets. Use the processing datasheet template Excel file in the "*Data sheet templates*" folder and add or remove columns as necessary. (See the *CERP_DATA_database* or previous project templates for reference).
- 2.3. Save this file as: "*ProjectID_Proc_MATRIX_##_mmddyy.xls*" (where: *ProjectID* is the unique ID for that project (refer to *tbl CERP_Project List* in the database: *S:\Projects\Eagles-SmithLab\Databases*), (where *MATRIX* is the type of sample being analyzed fish/bird eggs/invertebrates/etc...) and *##* is a unique number for that datasheet template). Put a copy of these datasheets in the *lab datasheet templates* subfolder within the *datasheet templates* folder on the share drive.
- 2.4. Open the Google Docs file entitled "*Lab project tracking*" and enter the page numbers you will be using under the Drying and grinding data sheet page numbers column.
- 2.5. Print datasheets and make sure each datasheet has the current date and page numbers in the header.

- 2.6. Prepare tough tags with appropriate *CERP ID Code* (refer to the *Master Sample ID codes ToughTAGs* (*S:\Projects\Eagles-SmithLab\Sample ID Codes ToughTags*) Excel sheet and the Access database to identify a list of unique codes. Note: all tissue samples have different codes (refer to the '*tbl TissueCode*' in the database (*S:\Projects\Eagles-SmithLab\Databases*). See *ID Code SOP* for more detail.
 - 2.7. Store all data sheets in the respective binder until all samples have been processed.
3. **Sample cleaning and wet weight – This step may seem redundant with the weighing involved in the cataloging step. However, recording the exact wet weight of a sample just prior to drying is critical for data integrity. We often assess these values in comparison to the catalog weights to evaluate desiccation in the freezer due to sublimation.**
- 3.1. Wearing powderless nitrile gloves remove sample from container and rinse the surface of each thawed sample with DI water and pat dry with clean KimWipe.
 - 3.2. Obtain one drying vessel (e.g. aluminum or plastic weigh boat, sample vial, etc.) for each sample and write the appropriate *CERP ID Code* on each weigh boat.
 - 3.3. With the balance empty, press the tare button to zero the balance. Place each weigh boat on the balance and record the weight in the “**Drying vessel weight**” cell on the hard copy data sheet.
 - 3.4. With a clean, dry KimWipe, pat the surface of the sample dry and place in weigh boat. Obtain a sample wet weight (be sure that the weight of the drying vessel is included in this – **i.e. DON'T TARE THE WEIGHT BOAT!**) and hand record in the “**Lab wet weight + drying vessel**” cell on the hard copy data sheet.
4. **Dissection – The dissection process varies by project. The following are general procedures outlining the most basic dissection steps. In most cases either 1) the whole organism will be processed and thus no dissection process is warranted, or 2) tissue samples will be extracted and processed for later analyses.**
- 4.1. With the balance empty, press the tare button to zero the balance.
 - 4.2. Label each drying weigh boat with *CERP ID Code*.
 - 4.3. Place each weigh boat on the balance and record the weight in the “Drying vessel weight” cell on the data sheet.
 - 4.4. Dissect out appropriate tissue(s). Record *CERP ID* with respective tissue code in appropriate column(s).
 - 4.5. For samples in which a muscle tissue is extracted for processing instead of the whole organism, dissect the dorsal portion of muscle between the head and dorsal fin, along the side of the spine (see Figure 1). Remove at least **1 gram** of muscle (both sides of the fish can be pooled if necessary; when applicable make note on data sheet).
 - 4.6. Obtain a sample wet weight for each tissue (be sure that the weight of the drying vessel is included in this – **i.e. DON'T TARE THE WEIGHT BOAT!**) in the “**tissue wet weight + drying vessel**” cell on the data sheet. Be sure to indicate the tissue type on the data sheet or that the appropriate column exists for the tissue (e.g. IDMuscleAxial, IDKidney, etc.).

4.7. Place tissue in drying oven.

5. Sample drying

- 5.1. Turn on drying oven and set to 50 degrees C.
- 5.2. Load samples onto a plastic or aluminum tray, and place the tray in oven.
- 5.3. Log the sample on the drying oven log sheet (on the front of the oven).
- 5.4. Record the dry start date and drying temperature on the processing data sheet.
- 5.5. Allow sample to dry for 48 hours or until a constant mass is achieved (change in mass in 8 hours is <1%).
- 5.6. Open the Google Doc file "*Lab_project_tracking*" and fill out the appropriate fields with your initials and % completed: (e.g. NB 50%)
 - 5.6.1. Samples dried (N) [N=number]

6. Sample dry weight

- 6.1. After samples have dried, remove them from the oven (while still warm) and place into large dessicator to cool (~ 20 minutes).
- 6.2. Log sample removal on drying oven log sheet.
- 6.3. Record total dry time in the appropriate cell on processing data sheet.
- 6.4. Remove 3-4 samples from dessicator (NOT the whole tray), place a dried sample AND its associated weigh boat onto a calibrated balance and record mass (in grams) in the "**Dry weight + drying vessel**" column on the data sheet.
- 6.5. Place sample in clean glass vial and label with a ToughTag that has *Project ID* and *CERP ID* code on it. Tissue may need to be broken into pieces to fit into vials.

7. Sample grinding

- 7.1. Clean grinding apparatus (Wiley Mill, Cryogrinder, IKA mill, or mortar and pestle). The specific apparatus will vary with sample type and many samples can be ground using a variety of methods (discuss with the project leader(s)). If the sample mass is very small, use mortar and pestle with wax paper, as it reduces sample loss during the grinding process.
- 7.2. Grind sample to a uniform consistency (fine powder) and record grind method and date on data sheet.
- 7.3. Carefully pour ground sample back into glass vial, using care not to lose material in the transfer, or contaminate other samples.
- 7.4. Clean grinding apparatus between samples. Mortar and pestle: clean with DI water and dry with KimWipe; Wiley Mill: clean with compressed air and brush; Cryogrinder and IKA mill: clean with either DI water and KimWipe or compressed air.

8. Post processing

- 8.1. Enter all data from hard copy datasheet into the appropriate electronic file and save as "*ProjectID_Proc_MATRIX_data_ddmmyy.xls*" (Where **MATRIX** is the type of sample being analyzed fish/eggs/inverts/etc...). Use the *DataEntryTemplate_Easy AccessUpload* excel file as a template for data entry. This file contains the correct column headings and additional information used in the database but not recorded in the laboratory during dissecting/processing samples. Note: column headings used on laboratory data sheets are identified in row 2 (under the headings used in access). Appropriate column headings may be found on different worksheets for database purposes (i.e., data collected during the "cataloging" process in the lab may actually be found in the "morphology/dissection" worksheet in the DateEntryTemplate excel file). Add and delete columns as necessary for each particular project. All data can be entered on one excel worksheet. For further description of column headings see the access database design view for the respective table
- 8.2. Proof (verify) all entered data.
- 8.3. Initial the data sheets following data entry and proofing data.
- 8.4. Scan hard copy datasheets and save the file as "*ProjectID_Proc_MATRIX_scandata_ddmmyy.xls*" (Where **MATRIX** is the type of sample being analyzed fish/eggs/inverts/etc...) Place PDF of datasheets into the Scanned datasheets folder on the share drive.
- 8.5. File the hard copy of the original datasheets in the current year's data folder in the data repository filing cabinet.
- 8.6. Data are ready for uploading to the database, so move excel files to data folder on the share drive (*S:\Projects\Eagles-SmithLab\Data*).
- 8.7. Open the Google Docs file entitled "*Lab_project tracking*" and update the processing fields with your initials and necessary information. The following fields **must** be completed on the Google Docs project tracking datasheet before moving on to processing:
 - 8.7.1. Samples dried (N) N= number of samples
 - 8.7.2. Samples ground (N) N=number of samples
 - 8.7.3. Drying and grinding data entered
 - 8.7.4. Drying and grinding data sheet file names and locations
 - 8.7.5. Drying and grinding data sheet page numbers
 - 8.7.6. Drying and grinding data sheet file names and locations
 - 8.7.7. Processing data proofed
 - 8.7.8. Drying data sheets scanned and filed

Appendix V B: Water TOC Analysis Using the Shimadzu TOC-VCSN and ASI-V Autosampler

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Water TOC Analysis Using the Shimadzu TOC-Vcsn and ASI-V Autosampler

KEY WORDS

TOC-V, total carbon, inorganic carbon, oxygen, NPOC, HCl, calibration curve

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STANDARD OPERATING PROCEDURE

Water TOC Analysis Using the Shimadzu TOC-Vcsn and ASI-V Autosampler

1.0 INTRODUCTION

1.1 Purpose

The Shimadzu Total Organic Carbon (TOC) Analyzer in combination with the ASI-V automated sampler is used to measure TOC in water samples. The amount of TOC in a water sample can be an indicator of the amount of pollution in a sample.

2.0 MATERIALS

2.1 TOC-V CSH/CSN component (herein after referred to as TOC-V)

2.2 ASI-V autosampler

2.3 2M HCl

2.4 DI water

2.5 Low TOC water (certified < 100 ppb TOC)

2.6 40 ml vials

2.7 Parafilm®M (cut into 1 inch squares)

2.8 Calibration standard(s)

2.9 Alconox® or Liquinox®

2.10 Potassium hydrogen phthalate (KHP [$\text{KHC}_8\text{H}_4\text{O}_4$])

3.0 PROCEDURES

3.1 Equipment Checks

3.1.1 Open the front door of the TOC-V to check the DRAIN VESSEL volume (back of machine). If needed, fill with DI water to the level indicated by the drain discharge tube on the side of the container. Use DI wash bottle to fill the container through the slits in the top of the black cap *in situ*. Do not remove the cap or unclip the bottle from its current location.

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- 3.1.2 With the door open, check the HUMIDIFIER water container level in the front of the TOC-V; fill if needed. Add low TOC water using a wash bottle to fill the container to the 'Hi' level mark. Squirt water through the water supply port opening in the top of the container. Close the door of the TOC-V.
- 3.1.3 Fill the RINSE BOTTLE with DI water to the 2000 ml mark (outside the ASI-V).
- 3.1.4 Fill the DILUTION WATER BOTTLE (located between the ASI-V and TOC-V) with low TOC water. Verify that the intake tubing nearly reaches to the bottom.
- 3.1.5 Check the ACID BOTTLE located between ASI-V and TOC-V. Ensure acid tube is near the bottom of the acid bottle and that the bottle is at least 50% full. If not, fill with 2M HCl. Recap bottle at the end of the day (or seal with Parafilm®M). If making up 2M HCl, always wear gloves and safety glasses with side shields (or goggles) and prepare under the hood. Always add concentrated HCl to the low TOC water (acid to water). If you spill some acid on your skin, IMMEDIATELY wash it off with copious amounts of running cold water and seek medical attention.
- 3.1.6 Monitor the PRINTER PAPER by removing the cover to the paper roll. Replace the paper roll if necessary (to run a full tray of 68 vials will take approximately 10 feet of printer paper).



Figure 1. TOC-V machine.

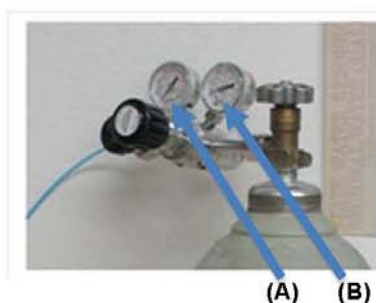


Figure 2. Oxygen tank; gas pressure (A) and tank volume (B).

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3.2 Turning on the TOC-V

- 3.2.1 Press the "Power" button located in the lower right hand corner to power on the TOC-V (Figure 1). It will take 20 to 30 min to reach the operating temperature. The TOC-V is at the correct operating temperature when the green light below the READY signage on the keypad is steady green (not flashing).
- 3.2.2 Immediately after turning the machine on, supply oxygen by turning the knob on the oxygen tank counter-clockwise. Use the two gauges on the top of the tank to regulate the amount of oxygen available to the TOC-V. The right gauge measures the amount of O₂ gas in the tank whereas the left gauge regulates the pressure of the gas entering the TOC-V (Figure 2). The needle of the left gauge should be between 44-87 psi; 60 psi is the optimum operating pressure. If the needle of the right gauge is below 400 psi, there may not be enough O₂ to properly conduct an analysis and the oxygen tank should be replaced.
- 3.2.3 Open the front door of the machine and adjust the two carrier gas regulators (Figure 3). The round CARRIER GAS PRESSURE REGULATOR is located on the left hand side of the TOC-V. Use the knob below the gauge to adjust the carrier gas pressure to 200 KPa. The long rectangular CARRIER GAS FLOW METER is located on the right hand side. Adjust the carrier flow using the carrier gas knob to the left of the gauge. The bottom of the ball should be level to the 150 mL/min increment in the scale. Close door.

3.3 Preparing Samples for Analysis

- 3.3.1 As the TOC-V comes up to operating temperature, prepare the water samples. Using a Sharpie®, label the vials according to your protocol. Fill the glass vials with approximately 35 mL of water with the meniscus just below the shoulders of the vial and cap or cover with a 1-inch square of parafilm®M. Do not let the parafilm®M hang below the threads on the vial.

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Figure 3. Setting the carrier gas pressure and flow.

3.3.2 Set vials into the sampling rack. To ensure the correct QA/QC for the analysis it is recommended that for every 20 vials analyzed, include one sample of each: 1) low TOC water; 2) lab duplicate; and 3) concentration standard. Repeat this pattern for every 20 samples. Check with project lead to confirm. Set rack into the ASI-V Autosampler.

3.3.3 No visible sediment or organic matter should be included in the water samples as this could clog the uptake needle.

3.4. Programming Conditions

3.4.1 In the INITIAL DISPLAY screen, press the F4 key on the keypad to access the CONDITIONS screen. Set the parameters for conducting a water TOC analysis (Figure 4). To change any of the parameters use the arrow keys to scroll down and highlight the selected measurement. Press the SELECT key and scroll through the options until the desired setting is highlighted. Press the ENTER key to change the parameter.

3.4.2 Next, press the F4 key again to access the MEASUREMENT CONDITIONS screen. Ensure that the parameters are set correctly

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(Figure 5). Use the arrow keys to change any parameters in the same manner used in 3.4.1.

3.4.3 Press the F1 (RETURN) key to return to the Initial Display screen.

Conditions	
Basic Unit	Printer
Furnace Power :ON	Routine Report :ON
Temp :680 °C	Peak Print :OFF
Catalyst Type :Regular	Cal.Grp.Print :ON
TN	ASI
Power	Vial :40ml
SSM	Needle Set :Sampling and Sparge
SSM Measurement :OFF	Needle Rinse :ON
SSM-TC Furnace :OFF	Needle Rinse after acid add :ON
SSM-IC Furnace :OFF	Stirrer :ON
Cell Length :Long	Flowline wash A :1
Other Units	Flowline wash B :2
ESU :Disable	
SP Kit :Disable	
Manual Inj: :Disable	

Figure 4. Parameters on the Conditions screen (F4) for conducting a TOC water analysis.

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Conditions	
Meas.	Output
# Of Syringe Wash :3	Unit -Liquid Sample :ppm
# of Syringe Wash(sp) :1	Solid Smpl (Cal) :%
Auto Inject Vol. Change :ON	Solid Sample :%
Auto Dil Change :ON	Name of Convert Val :CNV
Multiple Injection :OFF	Object for Conversion :NONE
Auto IC Regenerate :OFF	Equation(y=Ax+B) A:1.000
Min.Mes.Span [TC/NPOC] :0 sec	B:0.000
Min.Mes.Span [IC/POC] :0 sec	
Name of Method Group	
F2: TC	
F3: ASI-V	
F4:Group-3	
F5:Group-4	
F6:Group-5	
Misc.	
Date: :2(FEB)-10-2010 14:59	
Buzzer :ON	
Scrn off: :ON	

Figure 5. Default parameters on the Measurement Conditions screen (F4, then F4 again) for conducting a TOC water analysis.

ASI Measurement Settings				Cal. Curves 7 / 25					
TC #	Conc	DL	InjVol	Status	TC #	Conc	DL	InjVol	Status
0					5				
1					6				
2					7				
3					8				
4					9				
Sample Name: 11FB2012A					User defines these parameters; press ENTER key after entry.				
Vial # : 1-68									
[0- 9]	TC				NPOC				
Calib. (1 st)	***				***				
(2 nd)	---				---				
(3 rd)	---				---				
Inj. Volume	50 µL				50 µL				
Inj #	3				3				
Max # Inj	6				6				
SD	0.10				0.10				
CV%	2.00				2.00				
Dil. Factor	1				1				
Acid Ratio	0.0 %				1.5 %				
Spurge Time	--- min				1.5 min				

Figure 6. Recommended settings on the ASI Measuring Setting screen. Most of the parameters are user defined.

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3.5 Conducting a TOC water analysis using the ASI-V autosampler

- 3.5.1 In the Initial Display screen, press the ASI key to select the ASI SETTING screen.
- 3.5.2 Move the cursor to an empty row in the ASI Schedule Setting screen. Press the MEASURE SAMPLE key and the ASI Measurement Setting screen will appear.
- 3.5.3 In the ASI Measurement Setting screen, enter a unique sample name and the total number vials that will be processed (1 ~ n; n = total number of vials). Select the SHIFT key to toggle between numbers/alphabet (Figure 6).
- 3.5.4 Press the F5 key to select NPOC. Most analyses will be acidified with 2M HCl to convert inorganic carbon into CO₂, then purged with oxygen to remove the CO₂. Hence most analyses will be processed using Non Purgeable Organic Carbon (NPOC) method. Using the Total Carbon (TC) method instead of the NPOC method will analyze CO₂ as part of the TC.
- 3.5.5 Set other parameters in ASI Measurement Setting screen, including calibration curve and number of injections (Figure 6). More than one calibration curve may be selected. Only select acid ratio and sparge time if samples have not already been acidified with HCl.
- 3.5.6 Press the NEXT key to complete the setup. The schedule has been stored and the display will return to the ASI Schedule Settings screen. The sample name and analysis mode of the schedule will be displayed with the message "Not Measured".
- 3.5.7 Using arrow keys highlight the desired row for analysis.
- 3.5.8 Press the NEXT key
- 3.5.9 Select which option is to be performed when analysis is completed: 1) POWER OFF [F3], powers off 30 minutes after completion and carrier gas flow (oxygen) is halted; 2) SLEEP [F4], temporarily stopped, and will start again at a specified time and date; or 3) WAITING [F5], will remain on, ready to run another analysis. For

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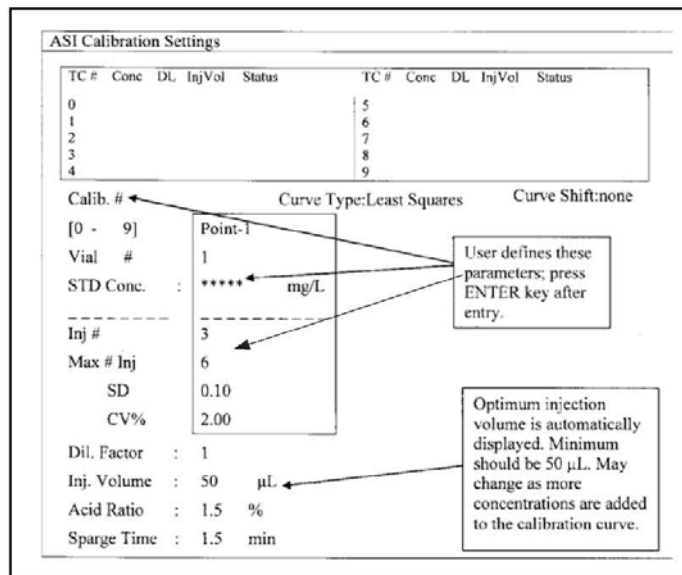


Figure 7. ASI Calibration Setting screen.

- 3.6.6 Additional concentration points may be added in the same manner once the parameters box for the first calibration point has been completed. With "Point-1" highlighted, press the right facing arrow key to display the parameters box for the second concentration point.
- 3.6.7 If more than five concentration points are required use the arrow keys to toggle between parameter boxes. To delete a point, highlight the parameters box for that point and press the F6 [DELETE] key.
- 3.6.8 When all desired points are added to the curve, press the NEXT key. This returns the user to the ASI Schedule Setting.
- 3.6.9 Check that all of the vials are in their correct position in the sample rack. Highlight the row for analysis. Once verified, press the NEXT key to run the analysis.

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most analyses, including ones to be conducted over night, select POWER OFF [F3].

3.5.10 Ensure that the vials are in the sample rack in the correct order.

3.5.11 Press the flashing START key to begin the analysis.

3.5.12 If POWER OFF is not selected, return to ASI Settings Screen. Highlight your analysis (message should display "Measure End") and select Delete (F4), Edit (F5), or Reset Schedule (F6). Press F6 (Yes) to complete.

3.5.13 Deselect the ASI key to return to the Initial Display screen.

3.6 Creating a Calibration Curve using the ASI-V

3.6.1 While in the Initial Display screen press the ASI key.

3.6.2 Move the cursor to an empty row in the ASI Schedule Setting screen.

3.6.3 Press the CAL key; this will display the ASI Calibration Settings screen (Figure 7). A list of previously generated calibration curves will appear at the top of the screen.

3.6.4 Press the F2 (TC) key to create a new calibration curve.

3.6.5 Enter the user defined parameters (press ENTER after each entry):

3.6.5.1 calibration curve number not previously used

3.6.5.2 vial number

3.6.5.3 calibration standard concentration(s)

3.6.5.4 number of injections

3.6.5.5 maximum number of injections

3.6.5.6 injection volume.

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the F6 [YES] key to shut down the machine. You will get the message "It is a termination".

3.7.3.3 It will take the TOC-V about 30–40 minutes to cool. DO NOT turn off the oxygen until the TOC-V has completely cooled and the furnace has shut down (the green light on the Power Button is off).

3.7.4 Calibration standards and stock solutions. Standard solutions can be purchased or prepared from potassium hydrogen phthalate (KHP [$\text{KHC}_8\text{H}_4\text{O}_4$]). Directions to make a 1000 mg carbon/L (1000 ppm carbon) stock solution:

3.7.4.1 Dry KHP at 105-120°C for 1 hr, then cool to room temperature in a desiccator.

3.7.4.2 Weigh out 1.0625 g KHP and add to a 500 ml volumetric flask.

3.7.4.3 Bring volumetric flask up to volume with low TOC water; ensure that the KHP is completely dissolved.

3.7.4.4 To make up 100 mL of a 10 ppm standard, add 1 ml of the 1000 ppm stock solution to a 100 mL volumetric flask. Add 99 ml of low TOC water to bring up to volume.

3.7.4.5 Confirm concentration with known standard.

3.7.4.6 Store solution in refrigerator; discard after 2-3 weeks.

4.0 CALCULATIONS

4.1 TOC units can be expressed in %, mg/L, μL , and ppm. For TOC water, ppm units are recommended. The TOC machine will print out the result of TC or NPOC, the mean (MN), the standard deviation (SD) and the coefficient of variation (CV). No calculations are necessary by the user.

4.2 Accuracy and precision calculations. Accuracy can be measured against the results of a known TOC standard included in the analysis. Precision can be measured by comparing the results of a lab duplicate to the

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original sample. Both accuracy and precision can be quantified by calculating the relative percent differences (RPD):

$$\text{RPD} = \frac{|C_1 - C_2| \times 100\%}{(C_1 + C_2)/2}$$

For Accuracy calculations: C_1 = the results of the standard, and C_2 = the know value of the standard.

For Precision calculations: C_1 = the result of the original sample; C_2 = the result of the lab duplicate

5.0 REMEDIAL ACTION IN CASE OF FAILURE AND USER HELP

5.1 See DPR video presentations of this SOP:

http://www.youtube.com/watch?v=bx7eky_bABc&feature=related (Part I),
<http://www.youtube.com/watch?v=o9Ln8nCCNfY&feature=related> (Part II),
and
http://www.youtube.com/watch?v=AGW3kTqrDKU&list=UUqC2ZGkVe7XKFBRrKL_3J7g&index=22&feature=plcp (Part III).

5.2 For minor problems, visit Shimadzu's TOC Advisor at
<https://tocvva.ssi.shimadzu.com/>.

5.3 For additional help contact a Shimadzu sales rep at
http://www.ssi.shimadzu.com/about/aboutssi_id8.cfm.

6.0 SAFETY

6.1 Wear gloves for personal protection and to prevent sample contamination.

6.2 Wear closed toe shoes when conducting TOC analysis.

6.3 Wear safety glasses with side shields or goggles for eye protection, especially when using HCl.

6.4 Ensure the oxygen tanks are secured to the wall.

6.5 Do not touch hot components or open up housing when unit is in use.

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- 6.6** Keep hands away from moving components (i.e., syringe injector). Never remove the sample cover to the automated sampler (ASI-V) when an analysis is in process.
- 6.7** Ensure that the external drain tubing does not touch the surface of the liquid in the waste container.

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- 3.6.10 When instructed, press the flashing START key. A calibration curve will be generated at the completion of the analysis. The curve will be stored and may be used in future TOC sample analyses.
- 3.6.11 When the calibration curve is completed, return to the ASI Schedule Settings Screen. Highlight your analysis (message should display "Measure End"). Select Delete (F4), Edit (F5), or Reset Schedule (F6).
- 3.6.12 Press F6 (Yes) to complete selection.
- 3.6.13 Deselect the ASI key to return to the Initial Display screen.

3.7 Housekeeping

- 3.7.1 Cleanup: remove all vials from sample rack, erase labeling, and wash vials with Alconox® or Liquinox® and triple rinse with DI water.
- 3.7.2 Deleting data. Data is stored internally in the TOC machine. Delete your data after confirming the printout of the data is complete.
 - 3.7.2.1 In the Initial Display, press the F6 [Data Report] key.
 - 3.7.2.2 The Data Report – Measurement Log screen is displayed.
 - 3.7.2.3 Press the F4 [Clear All] key.
 - 3.7.2.4 Press the F6 [Yes] key to delete data.
 - 3.7.2.5 Press F1 key to return to the Initial Display.
- 3.7.3 Manual shutdown. Prior to turning off the TOC-V manually, follow the sequence below to ensure the furnace cools without damage.
 - 3.7.3.1 Press the Press F1 [STANDBY OPTION] key in the Initial Display screen.
 - 3.7.3.2 Press F5 [POWER OFF], then F6 [Execute]. After the message "Do you want to stop this process?" press

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

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

Ogorek, J.

Scope and Application

The following standard operating procedure (SOP) describes the (1) preparation of water samples by distillation, and (2) subsequent analysis for methylmercury (MeHg). Analysis is facilitated by coupling the Brooks-Rand "MERX" Automated Methylmercury Analytical System with the Elan Inductively Coupled Plasma-Mass Spectrometer (ICPMS). Quantification of MeHg is by isotope dilution. This method is used by the Wisconsin Mercury Research Laboratory (WMRL) to determine MeHg concentrations in filtered or unfiltered water samples. Quality assurance and control protocols are employed throughout sample distillation and analysis, including: laboratory practices to prevent sample contamination, method blanks, and matrix spikes.

Distillation is required to remove components in natural waters that interfere with methylmercury (MeHg) analysis. In Teflon distillation vials, approximately 50 ml of acidified environmental samples (0.5% HCl) are spiked with isotopically enriched MeHg, amended with copper sulfate, heated in an aluminum block (121° C), and purged with argon gas. The resulting vapor is carried to and condensed in chilled Teflon receiving vials. Distillation is stopped when approximately 25% of the water remains in the distillation vessels.

The MERX consists of three interconnected modules (autosampler, purge and trap module, GC/Pyrolytic module), and is coupled to the ICPMS for detection. In 42 ml glass vials, distillate 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 introduced to the ICPMS. Elemental mercury in the sample stream is ionized by a radio-frequency generated plasma field, and detected using Speciated Isotope Dilution Mass Spectrometry (SIDMS).

This document is intended as an SOP designed to guide the user through MeHg analysis specific to the WMRL. Additional details helpful to the analyst can also be found following the SOP (Appendices 1-3), and is intended as a quick reference bench guide. However, the analyst is required to be familiar with the detailed SOP as well as the original user's manuals provided by Brooks-Rand and Elan 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 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. 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.

Distillation Procedure

A typical distillation contains 32 samples, four blanks, and two duplicate spikes. In small sample sets (< 16), four method blanks, and one duplicate spike should be included in each run.

1. Throughout the distillation process, it is important for the analyst to develop and maintain a structured and organized system. Each distillation and receiving vessel has a unique identification code etched onto the wall. The corresponding vials must be appropriately linked to each other, as well as back to the original sample. Good record keeping must occur not only throughout the distillation process, but must be similarly well documented in the Excel data sheet (See Appendix 1 for more details). A template of the Excel data sheet can be found in the "ID TRACER TEMPLATE" folder (HG4→ hg4data→Isotope methyl data).
2. Before beginning sample setup, turn on the aluminum block heater so that it can reach temperature while samples are being prepared.
3. Arrange an adequate number of clean Teflon distillation and receiving vials into four wire racks.
4. Weigh approximately 50 grams of sample (or reagent water for method blanks) into each of the distillation vials. Be sure to homogenize the sample by inverting the sample bottle several times before dispensing to the distillation vial. Setup two samples in triplicate for the duplicate spike analysis. Be sure that you record the sample ID, distillation vial ID, distillation vial tare weight, and the sample mass into the appropriate places in the Excel data sheet.
5. To each of the distillation vials, add 1 ml of 25% CuSO₄ solution. The method blanks should be acidified with 500 µl of concentrated HCl.

6. Add 100 μl of the working standard to each of the duplicate spike samples.
7. Add 50 μl of the isotopically enriched (Me^{199}Hg) working solution. If working with isotopically enriched samples, be sure that the spiked isotope fraction has not been amended in the sample.
8. Fit each distillation vial with a combined distillation cap/transfer tube assembly corresponding to the block position to be occupied by that vial (each cap has been engraved with a number between 1 and 40).
9. To each of the receiving vials, add 10 ml of reagent water. Be sure that you record the receiving vial ID and receiving vial tare weight.
10. Fit each receiving vial with a distillation cap corresponding to the rack position to be occupied by that vial (each cap has been engraved with a number between 1 and 40).
11. Place the distillation vials in the bore holes of the preheated (approximately 120°C) aluminum block. Attach an argon gas line to each of the distillation caps and ensure that gas flows through the sample.
12. In an ordered manner, thread the transfer tubes through the Teflon ports in the side of the cooler.
13. In the cooler, attach each transfer tube to the corresponding receiving vial. Ensure that gas flows in the reagent water of the receiving vial.
14. Throughout the distillation, check the temperature of the heating block often. Adjust to maintain a temperature of 121°C (± 5).
15. Check the distillation vials regularly. Samples are finished distilling when approximately 25% of the original sample is left.
16. Once a sample is finished, disconnect the transfer tube from the receiving vial and the gas 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.
17. Thoroughly rinse the combined distillation cap/transfer tube assembly with copious amounts of reagent water. Place the caps in the laminar flow hood to dry.
18. 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 Excel data sheet and attach a standard cap.

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

Brooks-Rand 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 located on the floor.
2. If necessary, open the Mercury Guru4 software with the shortcut on the desktop.
3. Open the analytical file BR549.brd (found on HG4) and save the file as data (from the "File" dropdown menu).
4. From the "Instrument" dropdown menu, select "Connect", prompting a popup window displaying three communication ports. Select the appropriate ports (CVAFS = ICPMS, Purge and Trap = COM5, and Autosampler = COM4) and click "Accept". The communication status at the top of the screen will turn green indicating connection with each module.

Preparation of Vials for Analysis

The MERX instrument is designed to operate on 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. A typical analytical run is shown in Appendix 2.

1. Place the clean vials in the sample rack and add approximately 40 ml of sample distillate or reagent water (for instrument blanks and MeHg standard additions) to each vial.
2. Record the identity of the sample, analytical vial number, date, and standard information on a bench sheet (Appendix 3).
3. Adjust the pH of the mixture to 4.5 – 5.0 by adding 200 μL of the sodium acetate buffer 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

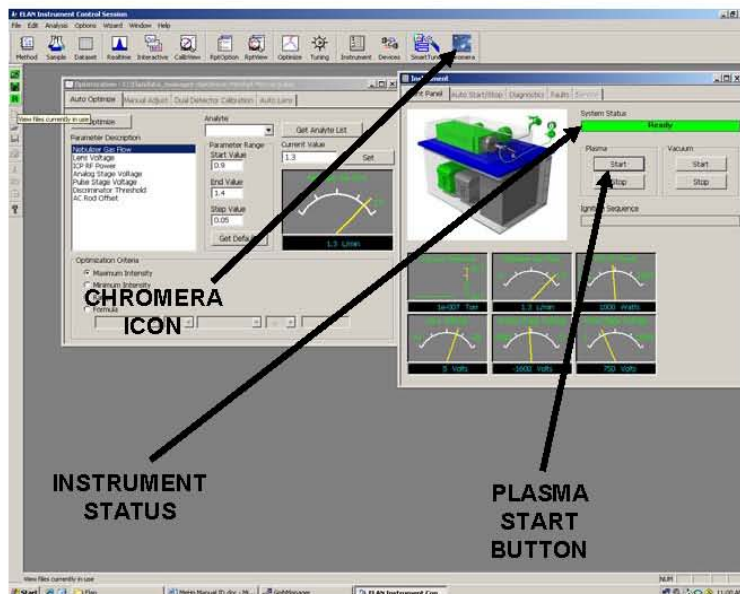
before use but always make sure that some frozen NaTEB remains in the vial. Promptly cap and return the vial of NaTEB to the freezer after use.

- 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.

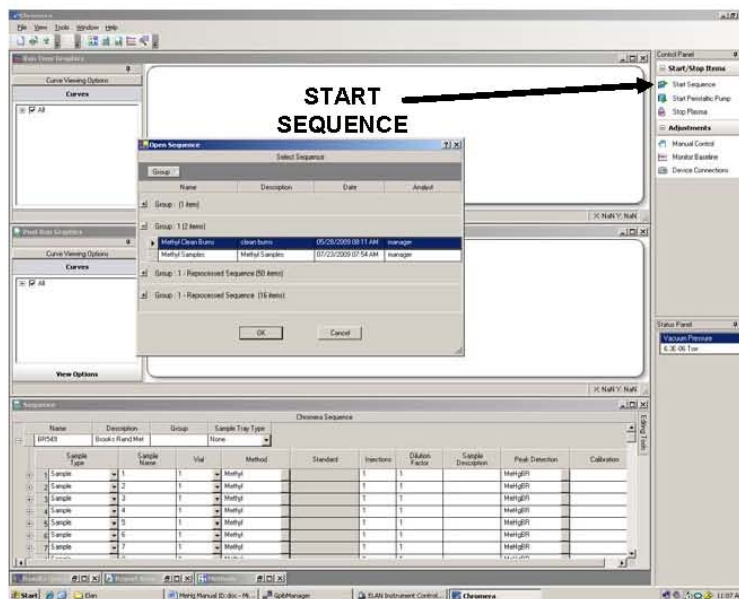
Elan ICPMS Operation

Start up (see figures below for reference)

1. Start the Elan software from the desktop shortcut.
2. Turn on the coolant pump with the switch near the door and check that the external mass flow controller displays 30.0 ml/minute.
3. Click the “**R**” button in the upper left corner of the workspace and verify that the Methyl Mercury.dac file has been loaded.
4. Click the “instrument” icon to display the ICPMS status.
5. When the status of the instrument displays “Ready” click the “Start” button to initiate the plasma field.

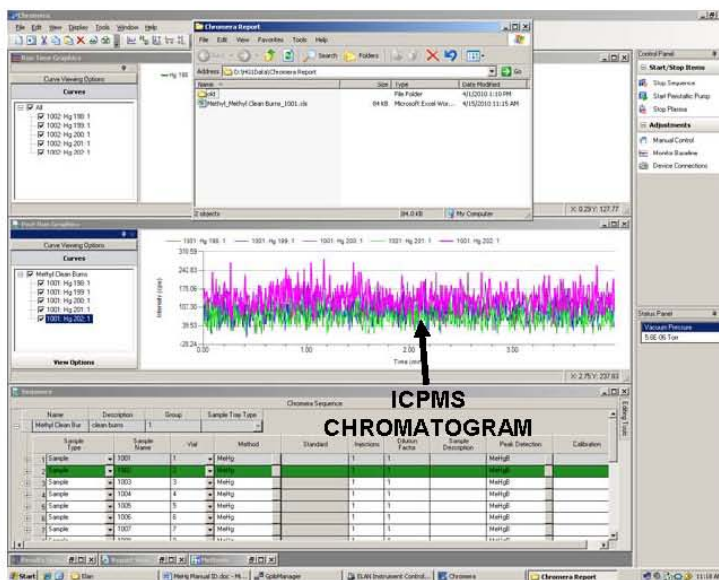


6. Launch the Chromera software by clicking the icon at the far right of the toolbar.



7. Click on the sequence window to make it the active application and choose "Open Sequence" from the File menu. The group "BR549" should have 75 preprogrammed analytical sequences.

8. In the Control Panel, select "Start Sequence". The first line of the sequence should turn green. It is necessary to start the analytical sequence in the Chromera software (to "prepare" the ICPMS detector to detect the sample) prior to initiating the Brooks Rand for sample analysis.
9. Activate the Brooks Rand software. Under the Automation tab, select the number of vials to be analyzed and the starting position. Click the start button to activate the Brooks Rand modules and subsequent detection by the ICPMS.
10. 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.



11. The report files for the integration of the chromatograms will be sent to the "Chromera Report" folder. Record the file number of the report file for each analytical event in the bench sheet.
12. Copy the peak areas out of the Chromera report files and paste them into the Excel data sheet. Orientate the data horizontally by selecting Paste Special-Transpose and paste into the appropriate conversion section located in the "peak area-curve" workbook. The data will be converted to non-comma delimited. Copy and Paste these non-comma data into the appropriate field of the workbook.

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 M Ω -cm. The water is delivered through a 0.2 μ m 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.

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). Transfer to a 125 ml Teflon bottle for use as a working solution.

Sodium Tetraethylborate (NaTEB): Sodium tetraethylborate 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 N₂ 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 NaBEt₄ 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 NaBEt₄ 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 HNO₃ and HCl (respectively) to approximately 100 ml of reagent water and bring up to 1000 ml.

25% CuSO₄ solution: In a 500 ml Teflon bottle, add 125 g CuSO₄ and bring up to 500 ml volume with reagent water. Shake well until all solids dissolve.

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° 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. For working solutions of native MeHg, 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 SnCl₂ reducible fraction of Hg^{II}.
6. Subtract the average blank mercury mass and the SnCl₂ 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

During the analytical run, the analyst must evaluate the calibration data, instrument blank values, duplicate spike recoveries, and check standard recoveries to ensure acceptance criteria are being met. The “summary” workbook in the Excel data sheet is where this information is displayed.

Matrix Interference

A sample should be run as a duplicate spike twice in every distillation/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 distillation. In the case of failure, repeat the duplicate spike (if possible) and bring 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 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 distillation set up and consist of a distillation vial with reagent water, 1 ml of 25% CuSO₄, isotopic MeHg spike, and 500 µl of HCl. Elevated method blanks indicate contamination in the distillation vials or reagents and are used to calculate the DDL (daily detection limit) for the extraction batch. The Absolute Detection Limit (three times the standard deviation of the method blank masses) must be less than or equal to 0.0024.

Instrument Calibration

Instrument calibration requires the measurement of mass bias (measured isotopic fractionation of native MeHg compared to published values) and the analysis of five isotopically spiked native MeHg standards (reverse ID). The native MeHg mass in the reverse ID calibration and check standards should be near that expected in the samples.

Reverse ID calibration: Add 25 – 100 µl of native MeHg standard to 5 analytical vials. Add 25 – 100 µl of isotopically enriched MeHg standard to these same vials. Continue with the setup of analytical vials as previously described. Enter the volume of native and isotopically enriched standards added to the appropriate fields of the “peak area-curve” workbook.

Reverse ID check standard: A reverse ID check standard is prepared similar to the reverse ID calibration vials. Vary the volumes of native MeHg standard throughout the check standards so that the mass brackets the native MeHg mass of the samples-with emphasis near the most common levels within the samples. 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. Enter the volumes of native and isotopically enriched standards added to the appropriate fields of the “peak area-curve” workbook. The failure of subsequent check standards is an indication of instrumental drift which may require recalibration of the instrument.

Mass Bias: The typical calibration curve (created with native MeHg) is used to determine the mass bias correction. Create a five point calibration curve with volumes of native MeHg standard spanning 10 – 200 µl. Mass bias is determined by dividing the measured ratio of the isotopes of interest (amended isotope/non-amended isotope) by published values (IUPAC) and must be within 5% of the expected value. The standard deviation for the ratio among the measured isotopes must be less than 10%.

Isotopic Fractionation of Enriched Standard: The enriched 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, the instrument must be calibrated to account for these contaminants. The isotopic fractionation of the enriched isotopes can be found in the "isotope info" workbook. See the quality control officer for further direction as to the appropriate source for which to reference.

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 ° C for 2 hrs. Inspect the vials prior to use for chips or cracks.

Shutdown: Turn off the plasma and exit Elan. Do not save changes to the method file when prompted. Turn the coolant pump off. Create a new folder in the "old" folder in the Chromera Report folder. The naming convention is Mxxxxxx, where xxxxxx represents the six digit date MMDDYY. Cut all of the reports from the Chromera Report folder (except the "old" folder) and paste them into the new folder within the old folder.

Appendix 1. Additional instructions for distillation data entry.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Bottle ID	Param	SAMPLE	FROM VIAL	TARE WT	FULL WT	TO VIAL	TARE WT	WATER W	FULL WT	BEFORE	AFTER	WT	PEAK	AREA
2									40.0						
3									40.0						
4									40.0						
5									40.0						
6									40.0						
7									40.0						
8									40.0						
9									40.0						
10									40.0						
11									40.0						
12									40.0						
13									40.0						
14									40.0						
15									40.0						
16									40.0						
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26									40.0						
27									40.0						
28									40.0						
29									40.0						
30									40.0						
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32									40.0						
33									40.0						
34									40.0						
35									40.0						
36									40.0						
37									40.0						
38		DST BLANK		1		VOLUME ADDED TO RECEIVING VESSEL			40.0						
39		DST BLANK 1							40.0						
40		DST BLANK 11							40.0						
41		DST BLANK 21							40.0						
42		DST BLANK 31							40.0						
43															
44															
45															
46	STND DATE	21-Oct-05				Date Run			Cap Set						
47	STND CONC.	0.53				Dist. type			DISTILLATION BY:						
48									ANALYSIS BY:						

Enter the following information into the appropriate fields: STND DATE, STND CONC., Date Run, DISTILLATION BY, and ANALYSIS BY.

Enter the 3 digit numeric codes (etched onto the wall of the vial) for the corresponding distillation and receiving vials into the columns titled "FROM VIAL" and "TO VIAL" (respectively). Be sure to enter the blanks into the appropriate area.

Enter the corresponding sample ID's into the column titled "BOTTLE ID". Duplicate spikes should be chosen randomly from the sample group.

Enter the full weight of the distillation vials after the sample has been added to the column "FULL WT".

Enter the tare weights for the distillation and receiving vials and the sample information using the database query tool found in *query_TAre_FieldSampleInfo.xls*. Follow the directions in each tab to query for the needed information and enter it into the spreadsheet.

Following the distillation, enter the final mass of the receiving vial into the column titled "FULL WT."

Following the analysis, enter the peak area of the analyzed samples into the column titled "PEAK AREA".

APPENDIX 2. Example of a typical analytical run.

Rack 1

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

Rack 2

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

Rack 3

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

Appendix 3. Analytical bench sheet for the ICPMS.

Date: _____ ID Std: _____ Native Std: _____ Initials: _____

ID	Sample ID	Vial ID	Chromera File #
1			
2			
3			
4			
5			
6			
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8			
9			
10			
11			
12			
13			
14			
15			
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ID	Sample ID	Vial ID	Chromera File #
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Appendix V D: Solids, volatile-on-ignition, total-in-bottom-material, gravimetric



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Techniques of Water-Resources Investigations of the United States Geological Survey



Chapter A1 **METHODS FOR DETERMINATION OF INORGANIC SUBSTANCES IN WATER AND FLUVIAL SEDIMENTS** By **Marvin J. Fishman and Linda C. Friedman, Editors**

First Edition 1970
Second Edition 1979
Third Edition 1989

Book 5
LABORATORY ANALYSIS

Solids, volatile-on-ignition, total-in-bottom-material, gravimetric

Parameter and Code:

Solids, volatile-on-ignition, total-in-bottom-material, dry wt, I-5753-85 (mg/kg): 00496

1. Application

This method may be used to analyze samples of bottom material. Usually, only the material that will pass a 2-mm sieve is taken for analysis.

2. Summary of method

A portion of well-mixed sample is dried at 105°C. A portion of dry, well-mixed sample is carefully weighed and then ignited at 550°C. The loss of weight on ignition represents the amount of volatile solids in the sample.

3. Interferences

None.

4. Apparatus

4.1 *Desiccator*, charged with indicating silica gel or other efficient desiccant.

4.2 *Muffle furnace*, 550°C.

4.3 *Oven*, 105°C, uniform temperature throughout.

4.4 *Platinum evaporating dishes*, 75- to 125-mL capacity, weighing less than 50 g. Platinum is recommended because the change in weight of glass or porcelain dishes may introduce appreciable error into the determination.

5. Reagents

None required.

6. Procedure

6.1 Spread approx 1 g of sample in the

bottom of a platinum evaporating dish. Place in an oven at 105°C and heat overnight.

6.2 Place the dish containing the dry sample in a desiccator, cool, and weigh to the nearest 0.1 mg.

6.3 Ignite the weighed dry residue at 550°C for 1 h, cool in a desiccator, and weigh to the nearest 0.1 mg.

7. Calculations

Solids, volatile-on-ignition (mg/kg) =

$$\frac{(DR-IR) \times 10^6}{DR}$$

where

IR = weight of ignited residue, milligrams, and

DR = weight of dry residue, milligrams.

8. Report

Report solids, volatile-on-ignition, total-in-bottom-material (00496), concentrations as follows: less than 1,000 mg/kg, whole numbers; 1,000 mg/kg and above, three significant figures.

9. Precision

Precision data are not available for this method.

Appendix V E: Digestion of Sediments and Biological Tissues for Analysis by Isotope Dilution Hydride Generation Inductively Coupled Mass Spectrometry (ID HGICP-MS)

Standard Operating Procedure (SOP) for Digestion of Sediments and Biological Tissues for Analysis by Isotope Dilution Hydride Generation Inductively Coupled Plasma Mass Spectrometry (ID HGICP-MS)

May 7, 2014

Originated by: Amy Kleckner, Evangelos Kakouros, Robin Stewart, & Kent Elrick

Revised by: Amy Kleckner, Evangelos Kakouros, & Robin Stewart

I. Introduction:

This method outlines digestion and analysis of sediments and biological tissues for selenium concentrations using a multi-step wet digestion with a mixture of nitric acid (HNO₃) and hydrogen peroxide (H₂O₂). An ⁸²Se enriched isotope spike is used to measure isotope dilution. Calibration of the enriched ⁸²Se spike is achieved by reverse spike isotope dilution. The digestates are mixed with concentrated hydrochloric acid to reduce the selenium to the most favorable valence for hydride generation. The solutions are then analyzed by inductively coupled plasma mass spectrometry coupled with hydride generation (ID HGICP-MS). Polyatomic and isobaric interferences are removed through the use of hydride generation and background correction using ⁸²Se enriched isotope spike.

II. Safety Issues:

Before beginning any of the procedures involved in this method, individuals must read and sign the Chemical Hygiene Plan developed for the lab. Specific safety concerns for each chemical can be found in the Material Safety Data Sheets (MSDS) for that chemical – all of which are located in the laboratory.

Strong acid solutions are employed in the cleaning of equipment, preparation of reagents and in sample preservation. Proper acid handling techniques should be employed whenever acids are being used. These techniques include the use of acid resistant clothing and the utilization of high volume fume hoods.

III. Materials

1. Freeze dried/ground or otherwise homogenized samples and certified reference materials.
2. Acid washed 6 mL screw cap Teflon vials. Vials are soaked for 24 hours in dilute micro solution, rinsed 3x with Milli-Q water, soaked for 3 days in a 15% HNO₃ bath, rinsed 3x with Milli-Q water, dried in a Laminar flow hood and stored in a zip lock bag until use.
3. 50 ppb enriched ⁸²Se standard solution.
4. Nitric Acid (HNO₃) (Trace metal grade).
5. 30% Hydrogen Peroxide (H₂O₂) (Ultrex II grade).

6. Hydrochloric acid (HCl) (Trace metal grade).
7. Hot plates.
8. Pressure steam sterilizer.
9. Milli-Q Water – 18 megohm ultrapure (<0.2 ng/L total Hg)
10. Selenium standard solution.
11. 2% hydrochloric acid solution.
12. Water bath heated to ~90 °C
13. KOH pellets
14. Sodium borohydride powder

IV. Selenium Standard and Reagent Preparations

Enriched ⁸²Se spike solution in 2% HCl:

Prepare enriched isotope stock by accurately weighing approximately 1.25 mL secondary ⁸²Se stock (4-Feb-2013) into a tared, empty, 50 mL Falcon tube or pre-cleaned PE plastic bottle. The solution is brought to 50 mL with 2% HCl. The resulting concentration should be near 50 ppb.

Selenium Standard solutions in 2% HCl:

Prepare standards according to the nominal concentrations listed for each standard. Selenium standard solutions are prepared by accurately weighing a suitable amount of Stock or Intermediate Stock solution (density approximately 1.011 g/mL) into a tared, empty, 50 mL Falcon tube or pre-cleaned PE plastic bottle. The solutions are brought to final volume with 2% HCl (density approximately 1.007 g/mL). RECORD the exact weight of each addition. Mix well and allow standards to equilibrate before using. Final concentrations are calculated by weight. Note: Secondary and tertiary stock solutions are made up in 2% HNO₃

~Conc. (µg/L, ppb)	Stock Se: ~1,000 mg/L, 1000 ppm (mL)	Secondary Stock Se: 10000 µg/L, 10000 ppb (mL)	Tertiary Stock Se: 100 µg/L, 100 ppb (mL)	2% HCl (mL) *2% HNO ₃	Final Volume (mL)
10000	0.5	--	--	49.5*	50
100	--	0.5	--	49.5*	50
0.2	--	--	0.1	49.9	50
1	--	--	0.5	49.5	50
2	--	--	1	49	50
4	--	--	2	48	50
10	--	0.05	--	49.95	50
20	--	0.1	--	49.9	50
40	--	0.2	--	49.8	50
60	--	0.3	--	49.7	50

Standard solutions can be stored at room temperature for several months but must be reduced prior to HGICP-MS analysis. Note that concentrations of reduced standards used for creating calibration curves for the analysis will be ½ their original concentrations.

RSID in 2% HCl:

Prepare the RSID by accurately weighing approximately 1 mL of the enriched ^{82}Se spike solution, and 2.5 mL of ambient 100 ppb Se stock into a tared, empty, 50 mL Falcon tube or pre-cleaned PE plastic bottle. The solution is brought to 50 mL with 2% HCl.

One day prior to ID HG-ICP-MS analysis three replicate (RSID) solutions are prepared by transferring an aliquot of the RSID in 2% HCl solution to a 15 mL Falcon tube and adding an equal amount of 12M HCl. (minimum total volume of 8 mL). The RSID is reduced along with all the ambient standards and samples.

*For ~45 lines/samples you will need approximately 1L NaBH_4 solution and 2.5L **50% HCl**.

NaBH_4 :

1. Fill a 1L volumetric flask with approximately 800mL Milli-Q water.
2. Add 18 g KOH to the volumetric flask and dissolve.
3. Add 6 g NaBH_4 to the volumetric flask and dissolve.
4. Bring to volume with Milli-Q water.
5. Store solution in an airtight container in the fridge. Will keep for ~ 1 month.

Calibration/Cleaning Blank:

Prepare a 1:1 2% HCl : 12M HCl solution to be sampled as a calibration blank and as a cleaning blank periodically during the run.

V. Digest Procedure

1. Weigh and transfer 10-20 mg of freeze dried/ homogenized sample to a labeled 6ml Teflon vial. Store dried samples in desiccator. For every ten samples digested one sample should be run in duplicate.
2. Weigh out appropriate reference standard materials, and use empty vials for blanks. (ex. TORT ~ 10 mg, PACS ~ 50 mg)
3. Two reagent blank samples will be run with each sample set. This is prepared with all the reagents and digested exactly like the actual samples.
4. Add 100uL of a solution of 50ppb ^{82}Se enriched isotope to each vial. Record weight.
5. Add 0.6 mL of concentrated HNO_3 (Trace metal grade) to each vial and cap tightly.
6. Place samples in pressure steam sterilizer and incubate for 3 hours at ~ 20psi and ~ 125C.
7. After 3 hours turn off pressure steam sterilizer and carefully remove samples. Allow the samples to come back to room temperature overnight.

8. Place a hotplate in a fume hood, place aluminum heating block on top of the hotplate, and adjust the hot plate to heat the block to a temperature between 170 & 190C.
9. Remove the lids and place them on a clean kimwipe with the label up.
10. Add 200µL H₂O₂ to each vial and allow it to react for ~15 minutes at room temperature.
11. Place all samples in the heating block.
12. Heat until the residue is nearly dry (<~100 µL or “lentil-sized” drop). They will need to be monitored continuously when they are almost evaporated to prevent “charring.” This step can take anywhere from 20-40 minutes.
13. Remove samples from the heating block and allow them to come to room temperature.
14. Bring samples up to volume with 5 mL 2% HCl solution. Samples can now be stored at room temperature until ready for analysis.

VI. Sample Reduction Procedure

1. One day prior to HG-ICP-MS analysis, transfer an aliquot (min. 4 mL) of each sample, 3 RSID, and each nominal standard solution to a 15mL Falcon tube and add an equal amount of 12M HCl. (minimum total volume of 8 mL).
2. Samples and standards need to be reduced one day prior to analysis. To do this place Falcon tubes in a water bath (temp ~90 °C) in a fume hood, and heat to “near boiling” for 30 minutes.
3. Allow samples to equilibrate and come to room temperature over night.

*For PACS-2 samples, pipette off supernatant. Do not allow for undigested sediments (sample) to go into ICP-MS.

Samples are now ready for analysis by hydride generation ICP-MS.

VII. Analysis procedure – HGICP-MS (Perkin Elmer SCIEX Elan DRC II)

1. Check that there is sufficient Argon gas and that the gauge reads ~ 80 psi.
2. Check the chiller/recirculator gauge is at ~70 psi and that water level is about 4/5ths full.
3. Check the gas gauge on the back of the ICP-MS is at ~55 psi.
4. Replace waste container.
5. Assemble tubing on FIAS.
6. Open gas valve to FIAS.
7. Turn on FIAS. Gas pressure on the front of the FIAS should be ~130 psi.
8. Make sure the ICP-MS “ready light” is ON.
9. Log onto computer: username: pki, password is found in the log book.
10. Start ELAN software.
11. Go into “Instrument” and “start plasma.” May come back as “failed”, just try again.
12. In log book, record start time etc.

13. If peristaltic pump on ICP-MS is running go to "Devices", stop peristaltic pump.
14. Allow ICP-MS to "warm up" for about 30-60 minutes.
15. Under "Method", check that you are using the correct files.
"File", "Open"
Method: C:\elandata_pki\Method\Se-total-FIASHGstdmode3.mth
Optimization: C:\elandata_pki\Optimize\Default.dac
Tuning: C:\elandata_pki\Tuning\Default.tun
16. In "Method", "Report", Report Filename, "Browse" create new file with today's date.
17. Send to printer? Check or don't check box for print outs of every sample. Check that there is sufficient paper in the printer.
18. Under "Report", Options template: seextract1.rop.
19. Under "Sample", create "Batch", with MQ in autosampler position 1. Always start with a MQ sample and MQ in place of acid and NaBH₄ to check for leaks and to make sure everything is running in the proper directions. If OK, then replace with acid and NaBH₄ solutions.
20. Fill autosampler probe cup with 10% HCl solution for rinsing the autosampler probe between samples.
21. Load autosampler.
22. Now create a sample "Batch" according to the run list starting with a couple of cleaning blanks and then the standard curve. After the highest standard and again after the RSID samples, insert a couple cleaning blanks. Every 4-5 samples insert a cleaning blank, standard to check for instrument drift, and another cleaning blank.
23. Highlight all lines then hit "Analyze Batch". ("Save changes?"- No)
24. At some point during the run, go under "Instrument" and record Int. temp., Box temp., Pressure, etc. in the log book.
25. Before shutting down the instrument, run a MQ sample and then air to clean out the FIAS tubing.
26. Turn off FIAS, close FIAS gas valve, release tubing, stop plasma, remove waste, in the log book record end time, and log off computer.

Samples and reverse ID samples are run on a same day to obtain ratios (mass bias corrected) which are then used to obtain final analyte concentration using Eq1.

$$\text{RSID: } C_y = C_z \cdot \frac{m_z}{m'_y} \cdot \frac{B_z \cdot R'_n - A_z}{A_y - B_y \cdot R'_n} \cdot \frac{AW_y}{AW_z} \quad (1)$$

where:

C_z is the concentration of analyte in the primary standard (mg/kg);

m_z is the mass of primary standard used (g);

m'_y is the mass of spike used to prepare the mixture of spike and primary standard (g);

A_y is the abundance of the reference isotope in the spike;

B_y is the abundance of the spike isotope in the spike;

A_z is the abundance of the reference isotope in the primary standard;

B_z is the abundance of the spike isotope in the primary standard;
 R'_n is the measured reference/spike isotope ratio (mass bias corrected) in the mixture solution of spike and primary standard;
 AW_y is the atomic weight of the analyte element in the spike (g/mol);
 AW_z is the atomic weight of the analyte element in primary standard(g/mol);

$$\text{ID: } C_x = C_y \cdot \frac{m_y}{w \cdot m_x} \cdot \frac{A_y - B_y \cdot R_n}{B_x \cdot R_n - A_x} \cdot \frac{AW_x}{AW_y} \quad (2)$$

where:

C_x is the concentration of analyte in the sample (mg/kg);
 C_y is the concentration of analyte in the enriched spike (mg/kg);
 m_y is the mass of spike solution used to prepare the mixture of sample and spike (g);
 m_x is the mass of sample used (g);
 w is the dry weight correction factor;
 A_y is the abundance of the reference isotope in the spike;
 B_y is the abundance of the spike isotope in the spike;
 A_x is the abundance of the reference isotope in the sample;
 B_x is the abundance of the spike isotope in the sample;
 R_n is the measured reference/spike isotope ratio (mass bias corrected) in the mixture solution of sample and spike;
 AW_x is the atomic weight of the analyte element in the sample (g/mol).

VIII. Quality Assurance

1. Sample Batch Size: Approximately 25 samples can be analyzed over a 5-day period (digested and run).
2. Certified Reference Material: Use an appropriate matrix and prepare at least 2 per batch. Digest and analyze with the same method as the samples. Recovery Limit = 80-120%.
3. Reagent Blanks: Prepare 2 sample blanks per batch. Digest and analyze with the same method as the samples.
4. Laboratory duplicate: At least 2 samples per batch should be run in duplicate. RPD limit = 15%
5. For a 10mg sample MDL = 0.026 ug/g MRL = 0.1 ug/g. (as of 4/15/14)

IX. References

Liber, K. 2011. Cold Digestion of Invertebrates for the Selenium Project. Method developed C.W. and E.F. 2007. Revised Mar 2011 MK. Water Quality Laboratory, Toxicology Center, University of Saskatchewan.

Elrick, K.A., and Horowitz, A.J., 1985. Analysis of rocks and sediments for arsenic, antimony, and selenium, by wet digestion and hydride generation atomic absorption: U.S. Geological Survey Open-File Report 85-497, p. 14.

X. Appendix

Appendix V F: Measurement of Dissolved Organic Matter Fluorescence and Absorbance

Updated: March 17, 2014
Version 1.3
Pete L. Lenaker

Procedure for the Measurement of Dissolved Organic Matter Fluorescence and Absorbance
using the Aqualog Spectrofluorometer at the Wisconsin Water Science Center, Middleton, WI

Standard Operating Procedure

1. Initial preparations


- a. Always wear gloves (nitrile or polypropylene) when handling cuvettes and any samples
- b. Computer login information
 - i. Username: plenaker
 - ii. Password: USGS1234
- c. Plug USB cable from Aqualog into USB port in back left corner of Dell laptop
- d. Plug USB dongle into USB port located on left side of Dell laptop
- e. Plug Passport external hard drive into remaining USB port on right side of Dell laptop
- f. Turn on the Aqualog and let it warm up (at least 30 minutes)
- g. Remove cuvettes from 2 M HNO₃ solutions and soak in 3 consecutive 18.2 MΩ MilliQ baths for 10 minutes at a minimum. Further rinse 10 times with MilliQ to insure they are clean of any remaining acid.
- h. Remove samples to be run from refrigerator and let equilibrate to room temperature prior to analysis
- i. Open Aqualog on desktop

2. Reagents and Standard Solutions

- a. 1% Lipton Unsweetened Tea Standard - **Daily**
 - i. Add 2.5 ml of Lipton unsweetened concentrate tea to a clean 250 ml volumetric flask containing 175 ml 18.2 MΩ Milli Q water. Bring up to 250 ml volume
 - ii. Pour 10 ml into a 250 ml baked amber bottle to rinse, then transfer remaining solution to the 250 ml baked amber bottle.
- b. 2 M HNO₃ cuvette bath solution - **Monthly**
 - i. Add 12.7 ml HNO₃ to a clean 100 ml volumetric flask containing 25 ml 18.2 MΩ Milli Q water. Bring up to 100 ml volume
 - ii. Transfer solution to Teflon container and mark with date prepared

3. Record lamp hours

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- a. Click on the Windows start menu 
 - i. Then select All Programs
 - ii. Jobin Yvon
 - iii. Utilities
 - iv. Lamp reset
 - 1. Record Lamp On time in hours in the Aqualog lab book (do NOT reset the lamp or filter, just close window when finished)

4. Create Project File for the Day

- a. Click on File and select Save Project As
- b. Create a folder (YYYYMMDD) in the following directory to save the project file in
 - i. Computer/OS(C:)/Users/Public/Public Documents/Jobin Yvon/Data/Projects/YYYYMMDD
 - ii. Save project file in above directory as YYYYMMDD (file will be given a .opj extension)

5. Create Data Folder for the Day

- a. Create a folder (YYYYMMDD) in the following directory to save the Aqualog data files in
 - i. Computer/OS(C:)/Users/Public/Public Documents/Jobin Yvon/Data/Output Files/YYYYMMDD
- b. Create an additional folder called "output" in the above data folder (YYYYMMDD). This will be used to export the absorbance and EEM python script data

6. Create Sample Log file


- a. Open previous day/sample run Microsoft excel sample log file and save in
 - i. Computer/OS(C:)/Users/Public/Public Documents/Jobin Yvon/Data/Output Files/YYYYMMDD/Sample Log_YYYYMMDD.xlsx
 - ii. Follow the format provided in Table 1. If more than 10 samples are to be run, continue "Group 2" format by making a "Group 3". You do NOT need to run "Group 1" again
 - iii. Dilution factor should stay 1, otherwise enter the dilution factor used
 - iv. "Project ID" should represent samples you are running, i.e. GLRI, MMSD, GMIA, Lake MI....

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Sample Type	Field ID	Aqualog ID	Dilution	Aqualog Sample #	Aqualog Experiment #
Initial Baseline	Baseline	Group001Baseline	1		1
Blank	blank	Group001"ProjectID"001	1	1	2
Blank	blank	Group001"ProjectID"002	1	2	3
Blank	blank	Group001"ProjectID"003	1	3	4
1% Tea Std.	1% Tea Std.	Group001"ProjectID"004	1	4	5
1% Tea Std.	1% Tea Std.	Group001"ProjectID"005	1	5	6
Initial Baseline	Baseline	Group002Baseline	1		1
Sample 1	BK ###	Group002 "ProjectID"001	1	1	2
Sample 2	CG ###	Group002 "ProjectID"002	1	2	3
Sample 3	MC ###	Group002 "ProjectID"003	1	3	4
Sample 4	UW ###	Group002 "ProjectID"004	1	4	5
Sample 5	MW ###	Group002 "ProjectID"005	1	5	6
Sample 6	RK ###	Group002 "ProjectID"006	1	6	7
Sample 7	DG ###	Group002 "ProjectID"007	1	7	8
Sample 8	TR ###	Group002 "ProjectID"008	1	8	9
Sample 9	PO ###	Group002 "ProjectID"009	1	9	10
Sample 10	MA ###	Group002 "ProjectID"010	1	10	11
1% Tea Std.	1% Tea Std.	Group002 "ProjectID"011	1	11	12
1% Tea Std.	1% Tea Std.	Group002 "ProjectID"012	1	12	13
Sample Replicate	MC ###-R	Group002 "ProjectID"013	1	13	14
Blank	blank	Group002 "ProjectID"014	1	14	15
Blank	blank	Group002 "ProjectID"015	1	15	16

Table 1. An example of your Microsoft excel sample log (YYYYMMDD_Sample Log.xls) and a typical days sample run using Sample Q. Group 001 is only run once per day, so additional samples would following the Group 002 sequence.

Water Raman Test

- b. Use the certified Raman Reference cell from Starna Cells for the water Raman test.
 - i. Remove sealed cuvette from Starna container and wipe clean w/ lens paper ensuring the cuvette is clear of all fine debris
 - ii. Place standard in position 1 of sample chamber and make sure the etched side of the cuvette faces the center of the sample carousel
- c. Click on Raman Scattering Area Unit Tool icon 
- d. **Data Description**
 - i. Change the data identifier to RSYYYYMMDD (e.g. RS20131217)
- e. **Aqualog Experiment Options**
 - i. Integration time = 10
 - ii. Accumulation = 1
 - iii. Excitation and emission do not change
 - iv. Increment or "bin" (nm) = 2.33 nm (4 pixel)
 - v. CCD gain = Medium

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NOTE: These settings, except the integration time, should match the settings you plan to use. The normalization factor can be adjusted for different integration times used when analyzing samples.

- f. **Blank/Sample Setup**
 - i. "Sample only" should be selected
- g. **Sample Selection**
 - i. "Position 1" should be selected
- h. Click **RUN** when ready
- i. When instrument is finished, click on the "Raman Area Graph" tab at bottom to see the intensity vs. wavelength graph
 - i. Visually inspect the water raman graph, should be relatively stable and provide a nice peak around 398
 - ii. Make sure the baseline is flat
 - iii. Record the Peak, Height and Area in the Aqualog lab book
 - iv. Height and Area, at a minimum, should be within the 3% range in Table 2


Settings: Integration time: 10 bin: 2 gain: medium			Settings: Integration time: 10 bin: 4 gain: medium			Settings: Integration time: 10 bin: 8 gain: medium		
%	Variable	Range	%	Variable	Range	%	Variable	Range
1	Height	971.42 – 991.05	1	Height	1910.17 – 1948.76	1	Height	3718.36 – 3793.47
1	Area	11581.58 – 11815.55	1	Area	23088.56 – 23555.00	1	Area	46492.57 – 47431.82
3	Height	951.80 – 1010.67	3	Height	1871.58 – 1987.34	3	Height	3643.24 – 3868.59
3	Area	11347.61 – 12049.52	3	Area	22622.12 – 24021.43	3	Area	45553.33 – 48371.06

Table 2. Area and Height ranges depending on which bin setting is chosen for the sealed Water Raman Standard.

- j. Click on the "RSU_Adjust" tab at bottom of workbook
 - i. Integration time = 1 (column C(Y), row 1)
 - 1. Integration time can be modified to reflect integration time used for running samples
 - ii. Right click on the Area value (column D(Y), row 2) and copy it. Also, record value in Aqualog lab notebook
 - 1. This will be used later when setting up your sample Q to normalize the EEMs data to Raman Standard units (RSU)

7. Quinine Sulfate Unit (QSU) Normalization Factor Test

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- a. Use the certified Quinine Sulfate Reference Standard from Starna Cells to collect and calculate the QSU normalization factor
 - i. Remove Quinine Sulfate Blank cuvette from container, wipe clean with lens paper
 1. Place blank cuvette in position 1 and make sure the etched side of the cuvette faces the center of the sample carousel
 - ii. Remove Quinine Sulfate cuvette from container, wipe clean with lens paper
 1. Place QS cuvette in position 2 and make sure the etched side of the cuvette faces the center of the sample carousel
- b. Click on Quinine Sulfate Normalization Factor Tool icon 
- c. **Data Description**
 - i. change the data identifier to QSYYYMMDD (e.g. QS20131217)
- d. **Aqualog Experiment Options**
 - i. Integration time = .05
 - ii. Accumulation = 1
 - iii. Excitation and emission do not change
 - iv. Increment or "bin" (nm) = 2.33 nm (4 pixel)
 - v. CCD gain = Medium

NOTE: These settings, except the integration time, should match the settings you plan to use. The normalization factor can be adjusted for different integration times used when analyzing samples.
- e. **Blank/Sample Setup**
 - i. "Sample and Blank" should be selected along with "Collect Blank"
- f. **Sample Selection**
 - i. The QS Blank should be "Position 1" and the QS Sample should be "Position 2"
- g. Click **RUN** when ready
- h. When instrument is finished, click on the "Emission Spectrum Graph" tab at bottom to see the intensity vs. wavelength graph
 - i. Visually inspect the QS emission graph, should be relatively stable and provide a nice peak around 450 nm
 - ii. Record the Excitation wavelength (EX WL), Absorbance (AU) and Percent Transmittance (%T) in the Aqualog lab book
- i. Click on the "QSU Calculation Worksheet" tab

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- i. Record the "QSU Adjust" value (column G(Y), row 1) in the Aqualog lab notebook.
 1. If planning to normalize the EEMs data to Quinine Sulfate units (QSU), this will be used later when setting up your sample Q

8. Run "Group 1" a Baseline, Set of Three Blanks and Two 1% Tea Standards

- a. Click on Aqualog SampleQ Tool icon  to set up initial baseline and blank evaluation
- b. **Sample Q File**
 - i. Click "Save As"
 - ii. Open Projects folder and select Project folder (YYYYMMDD) created for today's run
 - iii. Change file name to "AqualogSampleQ_YYYYMMDD.aqu"
 - iv. Make sure the proper folder was selected to save the file in:
C:\Users\Public\Documents\JobinYvon\Data\Projects\YYYYMMDD\AqualogSampleQ_YYYYMMDD.aqu
- c. **Experimental File** click "Create"
 - i. Change "Experiment File" name to DfltAqualogSampleQ_YYYYMMDD.xml.
 1. Click "Save As" to save the experiment file in the Project File established for today's run.
 - ii. Set up **Aqualog Experiment Options**
 1. Integration time = 1
 2. Excitation wavelength 800 – 240 with increment (nm) = 3
 3. Emission does not change
 4. Increment or "bin" (nm) = 2.33 nm (4 pixel)
 5. CCD gain = Medium.
 - iii. Click "Save" to save your experimental file settings
 - iv. Click OK when ready
- d. **Output File Names**
 - i. Leave the Blank Group Prefix as "Group"
 - ii. Change the Blank Base to "Baseline"
 - iii. Change Sample Prefix from "Sample" to "Project ID" representing the samples
 - iv. Set Output Folder location by clicking "Browse"

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1. Scroll down to output folder created in section 5, part a
C:\Users\Public\Documents\Jobin Yvon\Data\Output
Files\YYYYMMDD
2. Select the New Days Folder (YYYYMMDD) and click ok
3. Verify the Output Folder is correct. This is where all the data you
collect for the day will be exported to

e. Sample Setup

- i. Number of Blank Groups should be 1
- ii. Blank Group Start # should be 1
- iii. Samples per Blank should be 5 (we plan to run 3 blanks and 2 tea stds).
You should notice that the Total Samples should equal 6 (initial baseline
followed by 3 blanks and 2 teas)

f. Post Processing Options

- i. Check IFE
- ii. Check Normalize and Normalization Factor
- iii. Enter the RSU or QSU normalization factor
- iv. Check Rayleigh Masking and select 1st and 2nd order
- v. Change the Sum of slit widths from 10 to 12.

g. Export Options

- i. Check "Save Raw Data in Workbook Format (*.ogw)"
- ii. Check "Save worksheets as ASCII (*.dat)"
- iii. Select all data files you would like to save (Most common below)
 1. ABS
 2. Blank XYY
 3. Sample-Blank Raw (XYY) and (XYZ)
 4. Sample-Blank Processed (XYY) and (XYZ)

h. Sample Q File

- i. Click "Save" to make sure all settings above are saved
- i. Click **Run** when ready
 - i. A pop up box will appear telling you to insert the samples
 - ii. Fill your 4 cuvette's with MilliQ, wipe cuvette's clean with lens paper,
check for air bubbles (tap cuvette if air bubbles are present to get air
bubbles to move to top of cuvette) and load them into Sample Changer
 - iii. Click "Continue" when samples are loaded and ready
- j. When Sample run is complete open a second Aqualog window and open the
"baseline/initial Blank" and the 3 BLANK workbooks to visually evaluate
baseline/Blank and BLANKS.

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- i. Click “folder” icon at top to open baseline and blanks workbooks. Navigate to the day’s output folder you created earlier to find the four workbooks.
- ii. Visually inspect the initial baseline/Blank, all you should see are Rayleigh lines near the bottom left corner of the EEM
- iii. Visually inspect the 3 BLANK EEMs. They should be free of any high intensity peaks and have only signal noise
- iv. Visually inspect the Absorbance of the sample by clicking on the “Abs Spectra Graphs” to view Absorbance and percent transmittance. Should have very low absorbance readings if any.
- v. Run the raw blank data through the python/R script to evaluate the baseline and blanks
- vi. If baseline and blank samples pass the evaluation, then begin running samples


9. QA/QC Check the Baseline and Blank

- a. Open Windows command window
 - i. Change working directory by typing the following:
cd C:\Users\Public\Documents\EEM_scripts
 - ii. Type cd and hit enter to confirm change in working directory
- b. Open Aqualog_input.py file using Notepad or wordpad
 - i. Change the #date of analysis, #working directory, #output directory, #sample log file, #Raman normalization factor. This change will be updating the dates in all the above categories and entering the day’s normalization factor. .
 - ii. Save the Aqualog_input.py changes you just made
- c. Execute absorbance_01.py and eem_03.py scripts in Windows command window
 - i. Type: python absorbance_01.py and hit enter
 - ii. Type: python eem_03.py
 - iii. Open output file within the days run data folder and check to see that the script ran. You should see an absorbance and EEM plot for each of your blanks and tea standards. You should also see a absorbance and EEM summary text documents
 - iv. Inspect absorbance .png files and insure there are no trends in absorbance blank data. Should be a flat, but noisy line
 1. If trends do appear, do additional MilliQ rinses on cuvettes showing the trend and re-run them with new baseline.

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- v. Inspect EEM .png files and look for indications of contamination in the EEM
- vi. Evaluate the absorbance summary text file. Look for high values in any of the absorbance parameters, evaluate DDL for various absorbance parameters
- vii. Evaluate the EEM summary text file. Look at blanks for high values in EEM parameters (Peak_B and Peak_T tend to have high values, relative to other peaks), evaluate DDL for various EEM parameters

10. Running Samples and setting up Sample Queue

- a. Click on Aqualog SampleQ Tool icon  to set up sample Q for samples, blanks and standards
 - i. Refer to Table 1 for an idealized run of samples
- b. Check the **post processing** and **export** options
 - i. Confirm normalization factor is correct
 - ii. Confirm all data files desired are checked for export
- c. **Sample Setup**
 - i. Number of Blank Groups should be 1 (this will be the baseline for this group of samples)
 - ii. Blank Group Start # should change to 2
 - iii. Samples per Blank should be equal to the number of samples you plan to run (Table 1).

NOTE: This does not include the initial blank. You should notice that the Total Samples should equal 1 more than the samples per blank
 - iv. Sample start number should be 1
- d. **Output File Names**
 - i. Leave the Blank Group Prefix ID
 - ii. Leave the Blank Base as "Baseline"
 - iii. Leave the Sample Prefix "Project ID" representing the samples
 - iv. Verify the Output Folder is referring to the correct Output folder.
 - 1. C:\Users\Public\Documents\Jobin Yvon\Data\Output Files\YYYYMMDD
- e. **Experimental File**
 - i. click "Open"

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- ii. Confirm your experimental settings have not changed and they are set how you want them.
 - iii. If changes are made to experimental file, click "Save," then click OK
- f. Sample Q File**
- i. Click "Save"
- g.** Click **Run** when ready
- h.** A pop up box will show up telling you to insert the samples
- i.** Prepare cuvettes for running a sample
- i. Rinse cuvette 3x with MilliQ water
 - ii. Gently shake sample bottle to mix
 - iii. Rinse cuvette with sample water 3x
 - iv. Pour sample slowly into cuvette
 - v. Dry cuvette and clean with lens paper to ensure cuvette is free of spots, streaks, lint, etc.
 - vi. Check cuvettes prior to hitting "Continue" to make sure no air bubbles are present on cuvette, if so, tap with finger until they rise out of solution.
- j.** Upon completion of first four samples a pop up box will tell you to insert the next four samples prior to hitting continue.
- k.** When Sample run is complete open a second Aqualog window and open the workbooks to visually evaluate your initial baseline/blank, samples, standards, replicate and blanks.
- i. Click "folder" icon at top to open workbooks. Navigate to the day's output folder you created earlier to find the four workbooks.
 - ii. Visually inspect the initial baseline, all you should see are Rayleigh lines near the bottom left corner of the EEM
 - iii. Visually inspect the 2 Tea standard samples EEMs. They should have a very noticeable signal at EX 248-275 and EM 315-355
 - iv. Visually compare the replicate samples EEM and Absorbance plots
 - v. Visually inspect the 2 blanks by looking at EEM and Absorbance plots
 - vi. Visually inspect the EEM and Absorbance plots of the sample
 - vii. Run the blank data through the python/R script to evaluate them compared to the DDL
 - viii. If blank samples, replicates and tea standards pass the evaluation, then sample data is good and you can continue running more samples