Analysis of Total Mercury in Sediments, Soils, and Plants by Cold Vapor Atomic Fluorescence Detection with the Brooks-Rand "MERX" Automated Mercury Analytical System

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Scope and Application

The following standard operating procedure (SOP) is used by the U.S. Geological Survey's Mercury Research Laboratory (MRL) to determine total mercury (HgT) concentrations in sediments, soils, and plants. This SOP describes the preparation of the sample and subsequent analysis. Lyophilized and homogenized solid sample is weighed into Teflon bombs. The sample is first digested in concentrated aqua regia and then diluted up to 30 ml with a solution of 5% Bromine Monochloride (BrCl). Immediately prior to analysis, the BrCl is neutralized by the addition of Hydroxylamine Hydrochloride ($NH_2OH*HCl$). Following neutralization, Stannous Chloride ($SnCl_2$) is added to the sample to reduce the mercury from Hg^{2+} to Hg^0. The volatile Hg^0 is purged from the sample and captured on a gold sand trap, desorbed, and detected by cold vapor atomic fluorescence spectrometry (CVAFS). Sample analysis is conducted by the Brooks-Rand "MERX" automated mercury analytical system. Quality assurance and control protocols are employed throughout sample preparation and analysis, including: laboratory practices to prevent sample contamination, method and analytical blanks, method and analytical replication, analytical matrix spikes, and analysis of certified reference material (CRM).

Laboratory Safety

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

Multiple safety concerns are present in the conduct of this method; detailed information is included for each reagent specific to the method later in this SOP, and additional safety information can be found in the safety data sheets (SDS) located in the lab. Mercury is a toxic metal and caution should be exercised to limit exposure during daily operations. While samples and working standards are relatively low in concentration, concentrated stock solutions containing elevated HgT levels are occasionally encountered. Concentrated HgT stock solutions should only be handled by experienced lab personnel. Additionally, other hazardous chemicals used in this method include concentrated strong acids, $SnCl_2$, and BrCl (a strong oxidizer). Finally, during analysis the automated sample introduction system may begin moving without warning and presents a mechanical hazard.

Equipment

Trace level mercury analyses of samples at parts per billion concentrations are susceptible to contamination. Equipment that comes into contact with samples or reagents should be free of residual mercury and can consist of (but not be limited to) Teflon, glass, and polycarbonate containers. Brand new and previously used Teflon equipment should be washed in acid before use. The equipment is first rinsed with tap water, and then cleaned by immersing in 4 N HCl heated to 65°C for at least 12 hours (48 hours for new Teflon equipment). Immediately following removal from the bath, equipment is completely immersed in reagent-grade water and then additionally triple-rinsed in reagent-grade water. After rinsing, each container is air dried under a mercury-free class 100 laminar flow hood. Dry equipment is stored double bagged in zip-type bags.

Reagents and Standards

Reagents

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

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

Hydrochloric Acid (HCl): EM Science Omni Pure HCl (containing less than 5 ng/L Hg) or equivalent.

Nitric Acid (HNO_3): EM Science Omni Pure HNO3 (containing less than 5 ng/L Hg) or equivalent.

Bromine Monochloride (BrCl): Dissolve 27.0 g of reagent grade Potassium Bromide (KBr) in a new 2.5 L bottle of concentrated HCl. Place a Teflon coated stir bar into the bottle and stir for 1 hour or until dissolved. Slowly add 38.0 g reagent grade Potassium Bromate ($KBrO_3$) to the bottle while stirring. CAUTION: This needs to be done slowly and in a fume hood because large quantities of free halogens are produced. Addition of $KBrO_3$ to the solution should produce a color change from orange to red to yellow. Cap bottle loosely, stir for an additional hour, and remove stir bar. BrCl is stored in the original acid container in the acid cabinet. Replace the original acid label with a preprinted BrCl label and record your initials and the date made.

30% w/v Hydroxylamine hydrochloride ($NH_2OH*HCl$): Dissolve 120 g of $NH_2OH*HCl$ in a Teflon bottle containing 400 mL of reagent grade water. Hydroxylamine hydrochloride typically contains significant amounts of HgT and needs to be treated to reduce the contamination to acceptable levels (< 0.02 ng/ml). Add 50 µL $SnCl_2$ to the solution and purge with Argon gas (300 mL/min for 1 hour). After the solution has been purged, analyze 0.1 ml in reagent water. If necessary, repeat the $SnCl_2$ addition and purging steps until the solution is below the acceptable level. Store the $NH_2OH*HCl$ solution in the refrigerator when not in use.

20% w/v Stannous chloride ($SnCl_2$): Dissolve 200 g of $SnCl_2$ in 100 mL concentrated HCl in a 1 L Teflon bottle. Add 900 mL reagent water. Purge for 1 hour with Nitrogen gas at 300 mL/min. Store the $SnCl_2$ solution in the refrigerator when not in use.

Soda lime: Purchased from Alpha Aesar, 4-8 mesh.

Argon (Ar): Grade 5.0 (ultra high purity) Argon gas that is scrubbed of gaseous mercury by passing through a gold bead trap.

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

Analytical and Quality Control Standards

Upon receipt at the laboratory and on the day of preparation, mercury standard solutions should be labeled with the concentration, date received/prepared, and analyst initials. All standards must also be assigned a unique letter-number-letter identification code and must be entered into the laboratory database system. Concentrated (> 10 ng/ml) standard solutions should be stored outside of the main laboratory area to avoid contamination of the lab. Dispose of the working and concentrated mercury solutions in the appropriate waste container when expired (>6 months old for working solutions) or when the solution no longer contains BrCl (yellow color has faded to clear). Two standard solutions from different sources are required for analysis: an “analytical standard” which is used to calibrate the instrument and for matrix spikes, and a “quality control standard” which is purchased from a separate source and used to validate instrument performance throughout analyses.

Stock mercury standard solutions: The stock mercury standard solutions are commercially available mercury standards verified against a NIST standard reference material.

Working mercury standard solutions: The working mercury solutions are used in the daily operation of the instrument and are prepared from the stock solutions. The analytical standard and quality control standard (QCS) are prepared at 1 ng/ml

(subsequent dilutions of the stock solutions may be necessary and should follow a similar preparation). Dispense approximately 50 mL of reagent grade water and 3 mL of BrCl into a 100 mL mercury clean class A volumetric flask. Pipette the appropriate volume of stock solution (or diluted stock solution) and bring to volume with reagent water. Store the standard in a designated Teflon mercury standard bottle, and label with concentration, bottle identification code, date prepared, and analyst initials. Enter the working standard solutions into the lab database. This working standard must be compared to the previous working standard and agree within ± 5%. Prepare fresh every 6 months.

Sample Preparation

Analysis of sediments, soils, and plant material for HgT requires aqua regia digestion in Teflon bombs followed by dilution with a 5% BrCl solution. Unless otherwise specified, solid samples are lyophilized ("freeze-dried" under vacuum while frozen) to a consistent weight and homogenized (via ball mill, coffee grinder/food processor, or mortar/pestle) prior to digestion. The samples should appear to be well pulverized and mixed to a consistent composition before subsampling.

A typical extraction batch contains 22 samples, four blanks, and two CRM's. One sample should be weighed in triplicate for every 11 analyzed (two triplicate analyses for a typical 22 sample extraction). In smaller sample sets, one triplicate, three blanks, and two CRM's should still be included in each setup.

1. Arrange an adequate number of clean Teflon bombs onto lab trays.

2. Open the "template for bomb set up" Excel file in the current year's data folder (SCUData→SOLIDS→2015) and save as MMDDYY.xls.

3. Weigh 50-150 mg of sample into each bomb. Be sure that you record the sample identification, bomb identification, and sample mass into the setup sheet (see Appendix 1 for an example).

4. Working under a fume hood, pipette 6 ml concentrated HCl and 2 ml concentrated HNO_3 into each bomb to create the aqua regia solution. Initially gas will be produced by the addition of aqua regia. Loosely cap each bomb so that it can degas.

5. After an hour, firmly tighten each bomb with the bomb wrenches. Let the bombs sit under the fume hood for at least 8 hours.

6. Bring each bomb up to 30 ml total volume with a 5% BrCl solution. Tighten the bombs and heat to 50°C for at least 8 hours.

Instrument Operation

The MERX instrument consists of three interconnected modules: the autosampler, trap/desorption unit, and the detector. The instrument is designed to operate on a specific mixture of reagents that are prepared in septa sealed 42 ml glass vials. The autosampler holds three removable 24 vial sample racks, each consisting of 3 rows of 8 vials. Vial number one is the upper right position, with vial position descending from right to left, then top to bottom. Once prepared, the vials are sealed to the atmosphere and remain viable for analysis up to 48 hours. A typical analytical run is shown in Appendix 2.

Start Up

1. Check that all modules of the instrument have power and the Argon gas supply is turned on.

2. Open the Mercury Guru4 software with the shortcut on the desktop.

3. Open the analytical template file ".brt" for sediment analysis in the File drop down menu (File→Open→Merx→Template)

4. Save the file as data (File→"save file as data" →Merx→Runs→2016) in the data folder from the current year. Name the new run file by date and sample description (MMDDYY_sample name.brd).

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

6. In the automation page of the Guru software, click the Argon gas button to start gas flow to the detector. Adjust the sensitivity of the detector so the baseline offset is approximately 3000 by changing the PMT value (use the up/down arrows on the front of the detector). When the PMT value is changed, the offset value will go blank, and the new offset value will temporarily appear in the signal field. Press the autozero button when the signal value is 3000. Allow the signal to stabilize (2-3 minutes), and then measure the instrument noise (found in the "File" dropdown menu). Record the new offset, PMT, and noise values in the instrument log notebook.

7. Replace the Soda Lime in the trap on the front of the instrument.

Initial Instrument Calibration

1. Put 20 new 42 ml glass vials into a sample rack. Remove the caps and put into a clean bag.

2. Add 100 μl of $NH_2OH*HCl$ and 20 ml of reagent water to vials 5-20 (leave the first four vials empty).

3. Create a six point calibration curve in vials 9-14 using the analytical working standard (generally a 10-400 pg calibration curve is sufficient for most samples).

4. Add QCS checks in vials 19-20 that are near the low and high ends of the calibration curve.

5. Add 100 μl of $SnCl_2$ to vials 5-20, and seal all vials promptly.

6. Vials 7,8, 17, and 18 are used as blanks for instrument calibration. Vials 1-6 and 15-16 are instrument blanks and are not used for instrument calibration.

7. Install the sample rack in the autosampler, making sure that it is orientated properly.

8. Update the “Name/ID” field in the sample information page in the Guru software.

9. In the automation page, select the start vial number (1) and the number of vials to be analyzed (20), and click start analysis.

10. Enter the results into the appropriate Excel spread sheet (see “Data Management” section below). Review the calibration curve and QCS check standards in the Excel sheet; proceed with sample analysis only if all quality control parameters are met.

Sample Analysis

1. Select 10 bombs for analysis (see Appendix 2 to determine analysis order). Be careful not to use method blanks or CRM’s in the duplicate/spike position.

2. Put 16 new 42 ml glass vials into a sample rack. Remove the caps and put into a clean bag.

3. Add 100 μl of $NH_2OH*HCl$ to each vial.

4. Pipette 20 ml of reagent water into each of the vials. Do not exceed 25 ml of volume in the analytical vials as that may flood the instrument.

5. Pipette 200 - 500 μl of extractant (1-2 ml for method blanks) into the vials. Add volumes of extractant so that HgT masses fall within the calibration curve.

6. Add the QCS check standards and the instrument spike to the appropriate vials.

7. Add 100 μl of $SnCl_2$ to each of the vials and promptly cap.

8. Install the sample rack in the autosampler, making sure that it is orientated properly.

9. In the automation page, select the start vial number and the number of vials to be analyzed, and click start analysis.

Data Management

1. Data created on the Merx should be recorded in an Excel analytical spread sheet. Open the template for the sediment sample analysis (Merx→XL runs→2016→"MERX bomb template.xls"). Save the Excel file in the same folder with a new name that includes the date, and sample description (MMDDYY_sample name.xls).

2. In the Excel spread sheet, fill out the header information and the amount of mercury used to create the calibration curve (as mass, in nanograms).

3. In the Excel spread sheet, fill out the sample barcodes, Teflon bomb ID's, sample masses, and volumes of extractant or QCS analyzed.

4. The analysis spread sheet has designated rows for duplicates, instrument spikes, equipment blanks, and QCS checks standards. These fall in the appropriate places in the run and should not be used for sample analyses.

5. As the analysis progresses, data will appear in the "results" page of the Guru software. To avoid typographical error, data should be copied from a report created by the Guru software. Create the report from the "reports" page of the Guru software (click "create a report", select the .csv output, and navigate to Merx→XL runs→2016). Save the report and name by date and sample description (MMDDYY_sample name.csv).

6. Copy the peak areas from the report file generated by the Guru software and paste into the Peak Area column of the spread sheet. Following the completion of the set of samples, update the method blanks in the summary sheet of the Excel file. See Appendix 3 for an example of a completed Excel file.

Quality Assurance and Control Protocols

Certified Reference Material: A certified reference material (CRM) should be analyzed once every 11 samples, and be within a recovery that is 80-120 % of its certified value. If available, the CRM should be of the same matrix as the samples and be reasonably similar to the expected concentrations. In the event of a failed CRM recovery, repeat the CRM analysis to rule out a spurious result and seek the guidance of the quality assurance officer.

Currently, MRL analyzes:

IAEA SL 1 (sediment homogenate)
NIST 1575a (pine needle homogenate)

Sample Precision: One sample should be weighed out in triplicate for every 11 samples. The relative standard deviation for the triplicate should be <15%. In the event of failure, repeat the analysis of the samples and bring to the attention of the quality assurance officer.

Analytical Precision: One sample extractant should be analyzed in duplicate for every 10 samples analyzed. The percent difference for duplicate samples should be less than 10%. In the case of failure, repeat the duplicate and bring to the attention of the quality assurance officer.

Matrix Interference: One sample extractant should be analyzed as an instrument spike for every 10 samples analyzed to assess for matrix interference. The sample that was analyzed in duplicate should be set up a third time and spiked with 100 pg of the analytical mercury standard. The recovery of the spike should be within 85 – 115 % of the known addition. In the case of failure, repeat the duplicate spike in the failed sample and in another sample from the set and bring to the attention of the quality assurance officer.

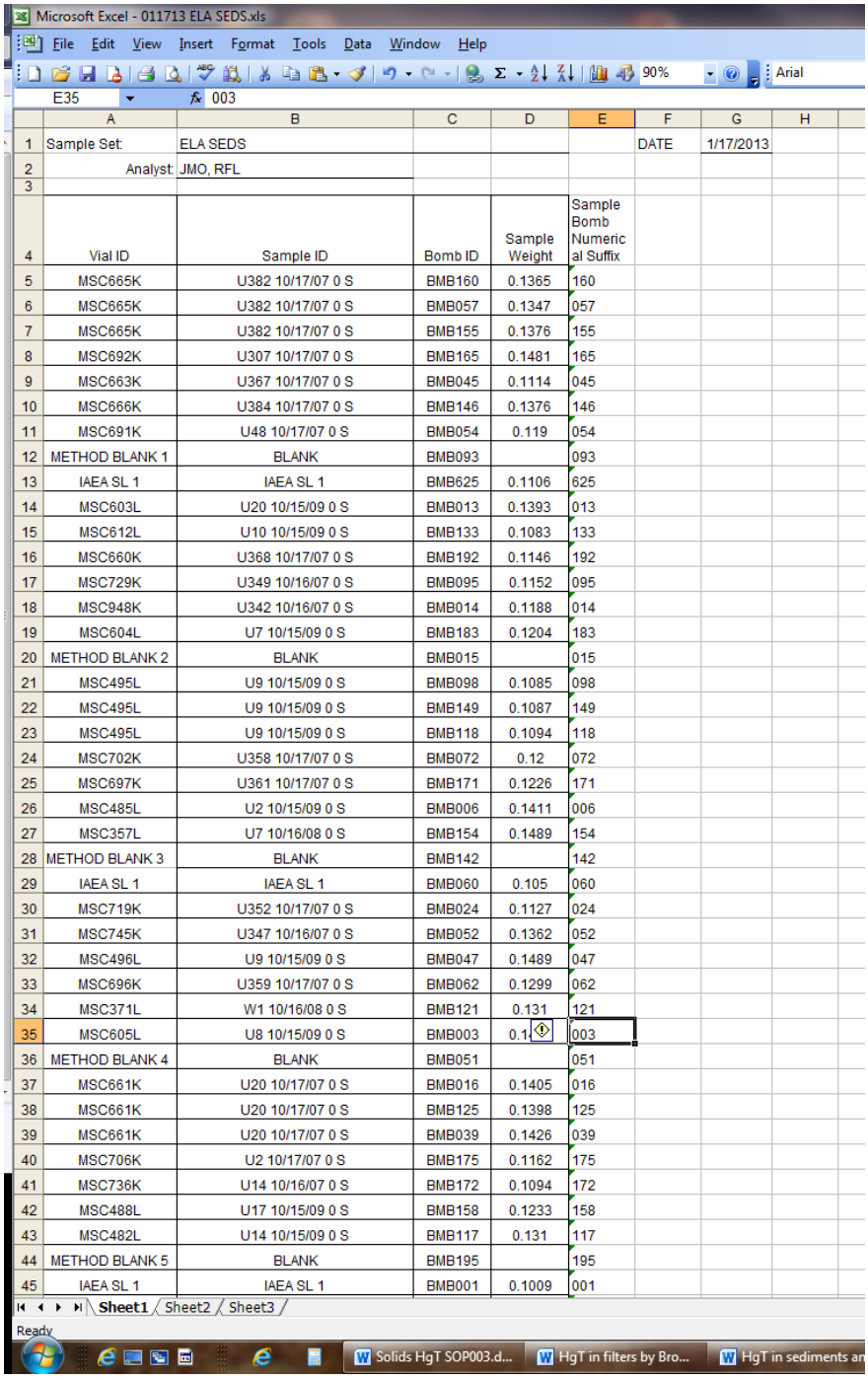
Instrumental Carryover: Instrumental carryover is assessed with instrument blank samples analyzed throughout the run. A blank sample is the analysis of reagent water (with NH_2OH*HCl and $SnCl_2$). Excessive instrument carryover (peak areas two times greater than the instrument calibration blanks) indicates instrumental contamination with HgT.

Method Blank: A method blank should be analyzed at least once every ten samples. Method blanks are part of the sample digestion set up and consist of a digestion bomb with reagents but no sample. Elevated method blanks indicate contamination in the bombs or reagents. If method blanks exceed 0.3 ng/bomb, the set up should be repeated or flagged.

Instrument Calibration: Calibrate the instrument with a 6 point standard curve prior to sample analysis and conduct regular checks of instrument calibration throughout

the run (QCS check standards). A standard curve should be created with masses of HgT that span that of the samples, and have an r^2 value greater than 0.995. Two QCS check standards should be analyzed following every 10 samples to verify instrument calibration, and have concentrations of 90 – 110% of its true value. The failure of subsequent check standards is an indication of instrumental drift which may require recalibration of the instrument. If a sample exceeds the instrument calibration, either the sample should be repeated at lower volume or the instrument recalibrated with higher masses of HgT.

APPENDIX 1. Example of a completed bomb setup sheet.



Microsoft Excel - 011713 ELA SEDS.xls

File Edit View Insert Format Tools Data Window Help

E35 003

	A	B	C	D	E	F	G	H
1	Sample Set:	ELA SEDS				DATE	1/17/2013	
2	Analyst:	JMO, RFL						
3								
4	Vial ID	Sample ID	Bomb ID	Sample Weight	Sample Bomb Numerical Suffix			
5	MSC665K	U382 10/17/07 0 S	BMB160	0.1365	160			
6	MSC665K	U382 10/17/07 0 S	BMB057	0.1347	057			
7	MSC665K	U382 10/17/07 0 S	BMB155	0.1376	155			
8	MSC692K	U307 10/17/07 0 S	BMB165	0.1481	165			
9	MSC663K	U367 10/17/07 0 S	BMB045	0.1114	045			
10	MSC666K	U384 10/17/07 0 S	BMB146	0.1376	146			
11	MSC691K	U48 10/17/07 0 S	BMB054	0.119	054			
12	METHOD BLANK 1	BLANK	BMB093		093			
13	IAEA SL 1	IAEA SL 1	BMB625	0.1106	625			
14	MSC603L	U20 10/15/09 0 S	BMB013	0.1393	013			
15	MSC612L	U10 10/15/09 0 S	BMB133	0.1083	133			
16	MSC660K	U368 10/17/07 0 S	BMB192	0.1146	192			
17	MSC729K	U349 10/16/07 0 S	BMB095	0.1152	095			
18	MSC948K	U342 10/16/07 0 S	BMB014	0.1188	014			
19	MSC604L	U7 10/15/09 0 S	BMB183	0.1204	183			
20	METHOD BLANK 2	BLANK	BMB015		015			
21	MSC495L	U9 10/15/09 0 S	BMB098	0.1085	098			
22	MSC495L	U9 10/15/09 0 S	BMB149	0.1087	149			
23	MSC495L	U9 10/15/09 0 S	BMB118	0.1094	118			
24	MSC702K	U358 10/17/07 0 S	BMB072	0.12	072			
25	MSC697K	U361 10/17/07 0 S	BMB171	0.1226	171			
26	MSC485L	U2 10/15/09 0 S	BMB006	0.1411	006			
27	MSC357L	U7 10/16/08 0 S	BMB154	0.1489	154			
28	METHOD BLANK 3	BLANK	BMB142		142			
29	IAEA SL 1	IAEA SL 1	BMB060	0.105	060			
30	MSC719K	U352 10/17/07 0 S	BMB024	0.1127	024			
31	MSC745K	U347 10/16/07 0 S	BMB052	0.1362	052			
32	MSC496L	U9 10/15/09 0 S	BMB047	0.1489	047			
33	MSC696K	U359 10/17/07 0 S	BMB062	0.1299	062			
34	MSC371L	W1 10/16/08 0 S	BMB121	0.131	121			
35	MSC605L	U8 10/15/09 0 S	BMB003	0.1	003			
36	METHOD BLANK 4	BLANK	BMB051		051			
37	MSC661K	U20 10/17/07 0 S	BMB016	0.1405	016			
38	MSC661K	U20 10/17/07 0 S	BMB125	0.1398	125			
39	MSC661K	U20 10/17/07 0 S	BMB039	0.1426	039			
40	MSC706K	U2 10/17/07 0 S	BMB175	0.1162	175			
41	MSC736K	U14 10/16/07 0 S	BMB172	0.1094	172			
42	MSC488L	U17 10/15/09 0 S	BMB158	0.1233	158			
43	MSC482L	U14 10/15/09 0 S	BMB117	0.131	117			
44	METHOD BLANK 5	BLANK	BMB195		195			
45	IAEA SL 1	IAEA SL 1	BMB001	0.1009	001			

Sheet1 Sheet2 Sheet3

Ready

Solids HgT SOP003.d... HgT in filters by Bro... HgT in sediments an

APPENDIX 2. Example of a typical analytical run.

Instrument Calibration

8 Calibration blank 2	7 Calibration blank 1	6 Instrument blank	5 Instrument blank	4 Empty vial	3 Empty vial	2 Empty vial	1 Empty vial
16 Instrument blank	15 Instrument blank	14 Calibration standard 6	13 Calibration standard 5	12 Calibration standard 4	11 Calibration standard 3	10 Calibration standard 2	9 Calibration standard 1
24	23	22	21	20 QCS standard	19 QCS standard	18 Calibration blank 4	17 Calibration blank 3

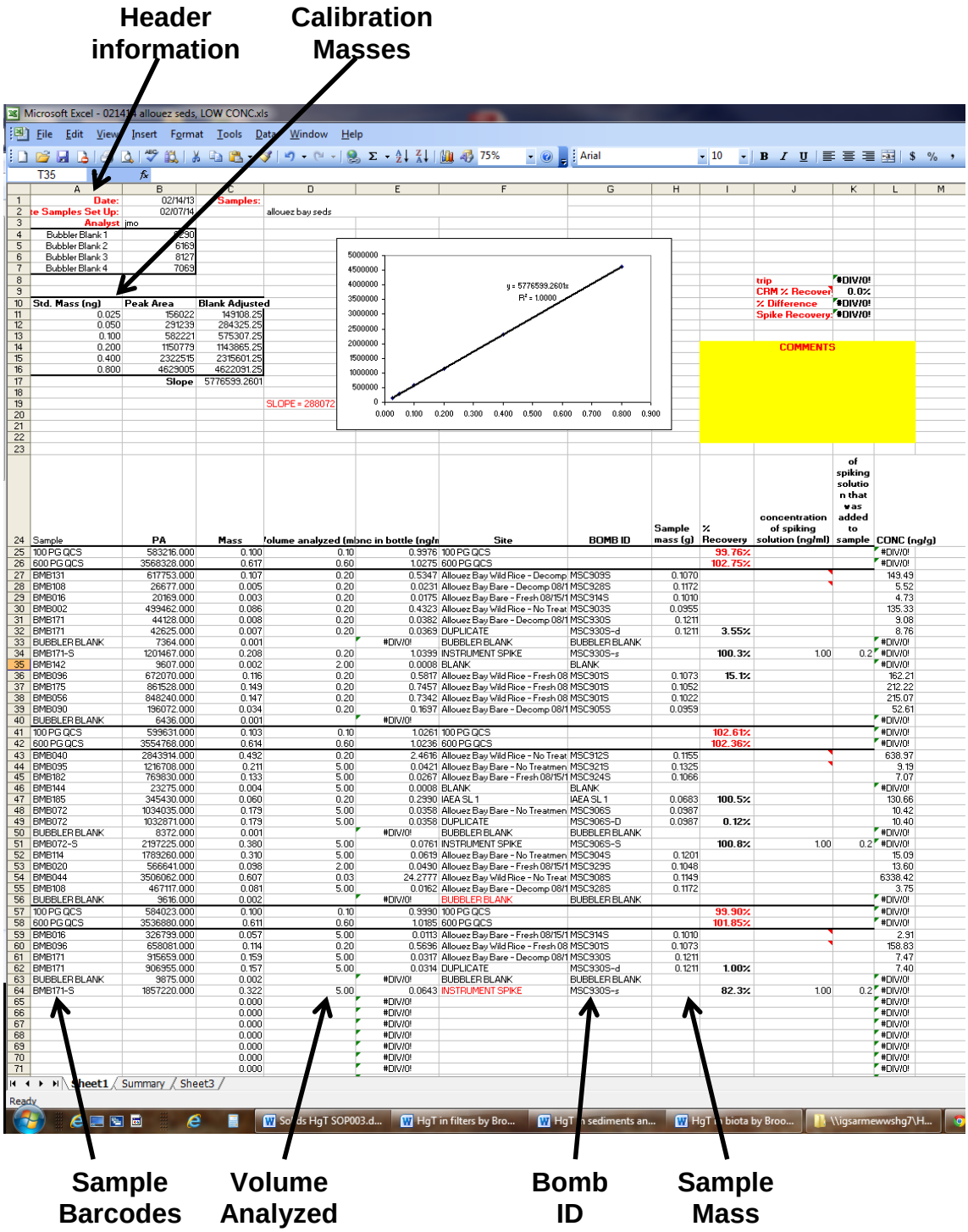
Sample Rack 1

32 Sample 5 matrix spike	31 Instrument blank	30 Sample 5 duplicate	29 Sample 5	28 Sample 4	27 Sample 3	26 Sample 2	25 Sample 1
40 QCS standard	39 QCS standard	38 Instrument blank	37 Sample 10	36 Sample 9	35 Sample 8	34 Sample 7	33 Sample 6
48 Sample 15 matrix spike	47 Instrument blank	46 Sample 15 duplicate	45 Sample 15	44 Sample 14	43 Sample 13	42 Sample 12	41 Sample 11

Sample Rack 2

56 QCS standard	55 QCS standard	54 Instrument blank	53 Sample 20	52 Sample 19	51 Sample 18	50 Sample 17	49 Sample 16
64	63	62	61	60	59	58	57
72	70	70	69	68	67	66	65

APPENDIX 3. Example of a completed Excel data sheet for sediment analysis.



Appendix 4. Definition of equations.

$$\text{Sample Hg Concentration} = \frac{\left(\begin{pmatrix}\text{Hg Concentration}\\ \text{In Analyzed Aliquot}\end{pmatrix}\begin{pmatrix}\text{Original Extract}\\ \text{Volume}\end{pmatrix}\right) - \begin{pmatrix}\text{Method Blank}\\ \text{Hg Mass}\end{pmatrix}}{(\text{Sample mass})}$$

$$\text{Hg Concentration In Analyzed Aliquot} = \frac{(\text{Analytical Hg Mass})}{(\text{Volume Analyzed})}$$

$$\text{Analytical Hg mass} = \frac{(\text{Sample Peak Area})}{(\text{Slope of Calibration})}$$

$$\text{Percent Relative Standard Deviation} = \frac{\begin{pmatrix}\text{Standard Deviation of}\\ \text{Triplicate Hg Concentrations}\\ \text{In Sample}\end{pmatrix}}{\begin{pmatrix}\text{Mean of Triplicate}\\ \text{Hg Concentrations}\\ \text{In Sample}\end{pmatrix}} \times 100$$

$$\text{Duplicate Percent Difference} = \frac{(\text{Hg Concentration of Sample}) - (\text{Hg Concentration of Duplicate})}{(\text{Hg Concentration of Sample})} \text{ X } 100$$

$$\text{Hg Spike Percent Recovery} = \frac{\begin{pmatrix}\text{Analytical Hg Mass}\\ \text{of Spiked Sample}\end{pmatrix} - \begin{pmatrix}\text{Analytical Hg Mass}\\ \text{of Unspiked Sample}\end{pmatrix}}{(\text{Spike Mass})} \text{ X } 100$$

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