

# **Analysis of Methylmercury in Biota 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 methylmercury (MeHg) concentrations in biota. This SOP describes the preparation of the sample and subsequent analysis. Biological sample is weighed into Teflon vials and digested in 4.5 M Nitric Acid at 60°C for 8 hours. Sample extract is added to reagent water in 42 ml glass vials, titrated with an equivalent volume of 5 M Potassium Hydroxide, and buffered with Sodium Acetate/Acetic Acid to a pH of 4.5 – 5.0. Sodium tetraethylborate (NaTEB) is added to the sample resulting in ethylation of the oxidized mercury species ( $\text{Hg}^{2+}$  and  $\text{MeHg}^+$ ). The volatile ethylated species, as well as elemental mercury, are purged from the sample with argon gas, retained on Tenex traps, thermally desorbed back into the sample stream, and separated by mass with a gas chromatography column. The elemental and ethylated mercury species are released from the column *en masse* into the sample stream, thermally oxidized to elemental mercury, and detected by cold vapor atomic fluorescent 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, sample replication, and analysis of certified reference materials (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 as that chemicals are present in the laboratory and often in use by other analysts. Hazardous chemicals should only be handled by adequately trained personnel under a high volume fume hood with extreme caution.

Multiple safety concerns are present in the conduct of this method; detailed information is included for each reagent specific to the method later in this SOP, and additional information can be found in the safety data sheets (SDS) located in the lab. MeHg is an extremely toxic organic metal and caution should be exercised to limit exposure during daily operations. While samples and working standards are relatively low in concentration, concentrated stock solutions containing elevated MeHg levels are occasionally encountered. Concentrated MeHg stock solutions should only be handled by experienced lab personnel. Additionally, reagents used in this method include strong acid and an organometallic ethylating compound. 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): Ultrapure reagent grade water containing less than 0.1 ng/L Hg with a resistance greater than 18 M $\Omega$ -cm. The water is delivered through a 0.2  $\mu$ m 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.

4.5 M Nitric Acid (HNO<sub>3</sub>): EM Science Omni Pure HNO<sub>3</sub> (containing less than 5 ng/L Hg) or equivalent. Add 500 ml of reagent water to a 1 L volumetric flask. Add 285 ml of concentrated HNO<sub>3</sub>, and bring up to volume with reagent water. Transfer solution to the appropriate repipette container and label with your initials and date mixed.

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/Acetic Acid/Antifoaming Reagent Buffer: To make a stock solution of Sodium Acetate buffer, measure approximately 50 ml reagent grade water, 47.2 ml Glacial Acetic Acid, and 108.8 g Sodium Acetate into a 500 ml Teflon bottle. Bring

up to 400 ml volume and shake until all solids dissolve. Transfer 100 ml of the stock buffer solution to a 125 ml Teflon bottle and add 1 drop of silicon antifoam B reagent. Use this as a working solution.

5 M Potassium Hydroxide (KOH): In a 100 ml volumetric flask, add 25.2 g of KOH, bring up to volume with reagent grade water and mix until all KOH dissolves. Transfer to a 125 ml Teflon bottle.

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 effects of NaTEB exposure is unknown, it should be assumed that repeated exposure may have adverse health effects. All use of NaTEB should take place inside a high-volume fume hood, and special consideration for equipment exposed to NaTEB in the fume hood (i.e. gloves, wipes, pipette tips, containers, etc...) must be made.

Pure solid NaTEB is purchased in 1 gram sealed glass vials. To dilute NaTEB to a 1% working solution, dissolve 2 g of KOH in 100 mL of reagent water in a 125 ml Teflon vial and chill to sub-freezing temperatures. Check the condition of the solution often. Once the KOH solution begins freezing, remove the KOH solution and the vial of NaBEt<sub>4</sub> from the freezer. It is best to work quickly at this point as to keep the pure NaTEB cold and to limit its exposure. Open and immediately dump the pure NaTEB into the 2% KOH solution and gently swirl to dissolve. Rinse the NaTEB vial with the KOH solution if any significant amount of NaTEB remains in the vial. When the NaTEB solution is almost entirely melted, homogenize, and pour equally into 18 clean chilled 5 mL Teflon vials. Cap the vials, store in a sealed bag, and record the date prepared. This solution should be kept frozen and made fresh every 4 weeks. Never use NaBEt<sub>4</sub> 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.

### **Analytical Standards**

Upon receipt at the laboratory or on the day of preparation, standards should be labeled with the content and concentration, and the date received or made and the initials of the person preparing them. Highly concentrated stock solutions should be stored away from the main working areas to prevent contamination of the clean lab.

Working standards and (if necessary) subsequent sub-stock dilutions should be made in a class A volumetric flask in a matrix of reagent grade water at a 2% acetic acid and 0.2% HCl concentration. This solution should be transferred to a Teflon bottle designated specifically for mercury standards, stored in an amber bag at 4° C, and remade every 6 months. All standards must be assigned a unique letter-number-letter identification code and be entered into the laboratory database system. The concentrations of MeHg working solutions are determined against a NIST certified total mercury standard. After making the MeHg standard, allow it to equilibrate for at least 24 hours and then determine the concentration using the method described below:

1. Mass of mercury in the MeHg standard: To four 15 ml Teflon vials, add 8.0 ml of reagent grade water, 1.000 ml of the MeHg working standard, and 1.0 ml of BrCl.
2. Blank contribution of mercury: To four 15 ml Teflon vials, add 9.0 ml of reagent grade water and 1.0 ml of BrCl.
3. Store the vials in a rack, seal in a bag, and heat in an oven to 50° C for eight hours.
4. Analyze the contents of the eight Teflon vials by EPA method 1631.
5. Analyze four 1.000 ml additions of the MeHg working standard to determine the SnCl<sub>2</sub> reducible fraction of Hg<sup>II</sup>
6. Subtract the average blank mercury mass and the SnCl<sub>2</sub> reducible fraction of Hg<sup>II</sup> from the total mercury mass determined MeHg working standard to determine the actual MeHg mass in the vials and subsequent concentration.

## **Sample Preparation**

Analysis of biological material for MeHg requires a 4.5 M HNO<sub>3</sub> extraction to break down the matrix and liberate the MeHg from the sample. Unless otherwise specified, biological 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 extraction. The samples should appear to be well pulverized and mixed to a consistent composition before subsampling.

A typical biological extraction contains 20 samples, three blanks, and three CRM's. One sample should be weighed in triplicate for every 10 analyzed (two triplicate analyses for a typical 20 sample extraction). In smaller sample sets, one triplicate, three blanks, and two CRM's should still be included in each setup. An example of a typical set up sheet is shown in Appendix 1.

1. Arrange an adequate number of clean Teflon extraction vials into a sample rack.

2. Open a solid setup template Excel file (SCUData→SOLIDS→2016) and save as HNO3 MMDDYY.xls.
3. Weigh 10 – 30 mg of sample into the vials. Be sure that you complete the following fields in the sample setup sheet: Sample Bottle ID, Extraction Vial ID, Sample Weight, and Extractant Volume.
4. Working under a fume hood, dispense 2-5 ml of the 4.5 M HNO<sub>3</sub> solution into each extraction vial and cap. As a general rule, dispense 1 ml of HNO<sub>3</sub> solution for every 5 mg of sample. For high level samples (fish), you may need to reduce the mass added and/or increase the volume of extractant. Shake each vial, leaving the minimum amount of sample on the vial wall.
5. Place the vials in a rack, enclose in a large bag, and heat at 60°C for 8 hrs.
6. The extract should be stored in the lab refrigerator for short periods of time (1-2 days) or be frozen for longer periods.
7. Prior to analysis, centrifuge the vials at 500 RCF for 30 seconds to concentrate the extractant to the bottom of the vial.

## **Instrument Operation**

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

### **Start Up**

1. Check that all modules of the instrument have power and the Argon gas supply is turned on. Empty the waste receptacle.
2. If necessary, open the Mercury Guru4 software with the shortcut on the desktop.
3. Launch the Merx software with the icon on the desktop. Open an analytical template file for the planned analysis. From the “File” dropdown menu, select “Open”, navigate to the “TEMPLATE” folder (D:drive→HG4Data→MERX), and select the calibration appropriate to the samples to be analyzed (high/low cal). Save the file as data (from the “File” dropdown menu) in the data folder from the

current year found in the GURU RUNS folder. Name the new run file by date and sample description (MMDDYY\_ *sample name*.brd).

4. From the “Instrument” dropdown menu, select “Connect”, prompting a popup window displaying three communication ports. Select the appropriate ports (CVAFS = COM4, Purge and Trap = COM5, and Autosampler = COM6) and click “Accept”. The communication status at the top of the screen will turn green indicating connection with each module.
5. Adjust the sensitivity of the detector so that the baseline offset is approximately 50,000 by changing the PMT value using the up/down arrows on the front of the detector. When the PMT value is changed, the offset value will go blank, and the new offset value will temporarily appear in the signal field. Press the autozero button when the signal value is approximately 50,000 ( $\pm 1000$ ). Once the offset value stabilizes (2-3 minutes), measure the instrument noise (found in the “File” dropdown menu). Record the new offset, PMT, and noise values in the lab notebook.

### **Preparation of Vials for Analysis**

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

1. Place the clean vials in the sample rack and add approximately 35 ml of Milli-Q to each vial.
2. Add the MeHg source (standard or sample extract) to the appropriate vials.
3. Titrate the vials that have had extractant added to them with an equivalent volume of 5 M KOH solution.
4. Add 300  $\mu\text{L}$  of the sodium acetate buffer/antifoaming reagent to all vials to adjust the pH of the mixture to 4.5 – 5.0.
5. Add 50  $\mu\text{L}$  of 1% NaTEB to all vials.
  - a. NaTEB is an unstable reagent and must always remain at freezing temperatures to slow degradation. Begin thawing several minutes before use but always make sure that some frozen NaTEB remains in the vial. Promptly cap and return the vial of NaTEB to the freezer after use.
  - b. NaTEB is toxic and spontaneously combustible in air. Only open vials and dispense NaTEB under a fume hood. Add NaTEB directly to the sample mixture (not to the glass surface inside the vial) to reduce volatilization.

6. Fill the vials with Milli-Q using a squirt bottle until a reverse meniscus forms (convex water surface). Seal the vial carefully (without headspace or spilling) with a new clean cap and septa assembly. Vigorously shake the vial.
7. Place the full rack on the autosampler tray, making sure that the rack is properly positioned and orientated. Under the Automation tab, select the number of vials to be analyzed, the starting position, and choose the “Start Run” button.

### **Initial Instrument Calibration**

Calibrate the instrument in the following order: (1) three 50 pg standards, (2) three instrument blanks, (3) a 6-8 point calibration curve, and (4) three instrument blanks. The three initial standards and instrument blanks are used to condition the traps and are not part of the instrument calibration. Create a calibration curve that spans the expected levels of MeHg in the samples. Typically, plankton and invertebrates span 0.5 – 40 pg and fish span 5 – 300 pg. See Appendix 2 for an example of a typical analytical run.

### **Sample Analysis**

Proceed with sample setup after the instrument has been successfully calibrated. Due to matrix interference, do not add more than 300  $\mu\text{L}$  of sample extract to an analytical vial. Throughout the run assess for sample carryover and instrument drift by the regular analysis of instrument blanks and check standards. Five bubbler blanks should be spread throughout an analysis. Check standards should be analyzed regularly throughout the analysis and span the expected masses of the samples. Analyze check standards at least once every 8 samples and at least four per sample set. See Appendix 2 for an example of a typical analytical run.

Typically samples prepared for MeHg analysis will be carried on to secondary analysis for total mercury. It is important to record the extractant volume that is consumed for MeHg analysis in the original setup sheet.

### **Data Management**

1. Data created on the Merx should be recorded in an Excel analytical spread sheet. Open the template for the biological sample analysis (Merx→GURU XL results→2016→”TEMPLATE\_HNO3 XL DATA SHEET.xls”). Save the Excel file in the same folder with a new name that includes “HNO3”, the date, and sample description (ex “HNO3 122516 ELF TISSUE.xls”).
2. In the Excel spread sheet, fill out the header information and the volume of mercury standard used to create the calibration curve and check standards.

3. Copy the Extraction vial ID, Sample Bottle ID, Sample ID, Sample Weight, and Extractant Volume from the sample setup spreadsheet and paste them into the table on the analysis sheet.
4. Record the volume analyzed in the analysis sheet.
5. Enter the barcode or other identification of the samples being analyzed in the Name/ID field in the spread sheet found in the “Run Information” tab on the Guru software.
6. As the analysis progresses, data will appear in the “results” page of the Guru software. Open the “Peak” page and check each chromatogram to make sure that the integration software selected the correct peak. A typical chromatogram displays three distinct peaks, with the retention time for MeHg at 1-2 minutes. If the selected data is not the MeHg peak, click the peak select button and change it to the correct peak. Scroll through the rest of the chromatograms checking that the correct peak was selected.
7. 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→GURU XL results. Save the report and name by date and sample description (MMDDYY\_sample name.csv) in the current year folder.
8. Copy the peak areas from the report file generated by the Guru software and paste into the Peak Area column of the spread sheet. See Appendix 3 for an example of a completed Excel file.

## **Quality Assurance and Control Protocols**

**Certified Reference Material:** A CRM should be analyzed once every ten samples, and be within a recovery of 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 407 (fish homogenate) as a CRM for low level fish.

DORM 2 (fish homogenate) as a CRM for high level fish.

IAEA 452 (mussel homogenate) as a CRM for invertebrate and plankton.

**Sample Precision:** One sample should be weighed out in triplicate for every 10 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.

Matrix Interference: Check for peak areas that are considerably less than blank values, and for wide variations in concentrations for sample precision. These may indicate matrix interference. Reanalyze the questionable sample to rule out operator error. If a repeated analysis results in poor response, reanalyze the sample at a lower volume and notify the quality assurance officer.

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

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

Instrument Calibration: The instrument should be calibrated with a 6-8 point calibration curve prior to sample analysis and regular checks of instrument calibration should be conducted throughout the run. A standard curve should be created with levels of MeHg similar to that of the samples, and have an  $r^2$  value greater than 0.995. At the minimum, a check standard should be analyzed to verify instrument calibration in every eighth position, and have a measured mass within 80 – 120% of its true value. The failure of subsequent check standards is an indication of instrumental drift which may require recalibration of the instrument.

## **Additional Instructions**

### **MERX Maintenance**

The MERX system requires some short- and long-term maintenance. Empty the waste receptacle daily to prevent the overflow of spent sample medium. The three analytical Tenex traps will last for approximately 2000 desorption's each before they need replacing (see pages 29-30 of the MERX user's guide for trap replacement).

The purge vessel will require monthly cleaning under regular use or sooner as evidenced by elevated bubbler blanks or excessive deposits. Clean the purge vessel by soaking it in the following reagents: 1 M KOH (8 hrs), reagent water rinse, 10% HCl (8 hrs), and reagent water rinse. The sample line may also need to be occasionally replaced if elevated bubbler blanks persist.

Analytical Vial Cleaning: Although it is not cost effective for the MRL to reuse analytical vials, they can be reused if properly cleaned. Wash the vials with lab

detergent and rinse with reagent water. Once dry, wrap the vials in aluminum foil and heat at 550°C for 2 hrs. Inspect the vials prior to use for chips or cracks.

**APPENDIX 1. Example of a completed biological sample setup sheet.**

Extraction Vial ID	Sample Bottle ID	Sample	Sample Weight (g)	Extractant Volume (ml)	Extraction Vial Numerical	Sum of Extractant for MeHg	Extractant used (ml) for Analysis #1	Analysis #2	Analysis #3	Analysis #4	Analysis #5	Analysis #6	Analysis #7	Analysis #8
HNO121	IAEA-407	IAEA-407	0.0156	5.0	121	0.100	0.1000							
HNO136	METHOD BLANK 1	METHOD BLANK 1		5.0	136	0.200	0.2000							
HNO157	WSV174CBK	SALMON A 05/27/15 1700 B	0.0120	5.0	157	0.100	0.1000							
HNO144	WSV174CBK	SALMON A 05/27/15 1700 B	0.0112	5.0	144	0.100	0.1000							
HNO006	WSV174CBK	SALMON A 05/27/15 1700 B	0.0120	5.0	006	0.100	0.1000							
HNO068	WSV142BOR	PETERSON B 04/18/15 1330 B	0.0112	5.0	068	0.100	0.1000							
HNO030	WSV211BXX	SHRINE B 05/02/15 0801 B	0.0144	5.0	030	0.100	0.1000							
HNO023	WSV220CDB	SALMON A 10/10/15 1500 B	0.0133	5.0	023	0.100	0.1000							
HNO111	WSV212BXX	BCC B 05/02/15 1000 B	0.0122	5.0	111	0.100	0.1000							
HNO116	WSV505BXX	BCC A 05/04/15 1101 B	0.0105	5.0	116	0.100	0.1000							
HNO147	WSV213BXB	SALMON A 05/07/15 1701 B	0.0129	5.0	147	0.100	0.1000							
HNO139	WSV221BXX	SHRINE A 04/29/15 1800 B	0.0127	5.0	139	0.100	0.1000							
HNO053	WSV225BXX	SHRINE A 04/29/15 1801 B	0.0111	5.0	053	0.100	0.1000							
HNO114	WSV150BXX	SALMON A 05/07/15 1700 B	0.0118	5.0	114	0.100	0.1000							
HNO093	IAEA-407	IAEA-407	0.0153	5.0	093	0.100	0.1000							
HNO105	METHOD BLANK 2	METHOD BLANK 2		5.0	105	0.200	0.2000							
HNO150	WSV165BXX	PETERSON A 04/18/15 1031 B	0.0114	5.0	150	0.100	0.1000							
HNO035	WSV165BXX	PETERSON A 04/18/15 1031 B	0.0112	5.0	035	0.100	0.1000							
HNO123	WSV165BXX	PETERSON A 04/18/15 1031 B	0.0114	5.0	123	0.100	0.1000							
HNO020	WSV202CCG	FISH B 05/09/15 0900 B	0.0114	5.0	020	0.100	0.1000							
HNO070	JPH012BXB	BCC A 05/04/15 1100 B	0.0115	5.0	070	0.100	0.1000							
HNO010	WSV186BXB	PETERSON B 04/18/15 1331 B	0.0135	5.0	010	0.100	0.1000							
HNO129	WSV197BXX	SHRINE B 05/02/15 0800 B	0.0129	5.0	129	0.100	0.1000							
HNO110	WSV134CBK	FISH B 05/09/15 0901 B	0.0122	5.0	110	0.100	0.1000							
HNO108	WSV155CBK	FISH A 05/09/15 1100 B	0.0126	5.0	108	0.100	0.1000							
HNO055	JPH035BOR	PETERSON A 04/18/15 1030 B	0.0127	5.0	055	0.100	0.1000							
HNO103	WSV168CBK	FISH A 05/09/15 1101 B	0.0127	5.0	103	0.100	0.1000							
HNO120	WSV138BCF	BCC B 05/02/15 1001 B	0.0107	5.0	120	0.100	0.1000							
HNO112	IAEA-407	IAEA-407	0.0143	5.0	112	0.100	0.1000							
HNO081	METHOD BLANK 3	METHOD BLANK 3		5.0	081	0.200	0.2000							
HNO						0.000								
HNO						0.000								

**APPENDIX 2. Example of a typical analytical run.**

**Instrument Calibration**

<u>8</u> 1 pg calibration standard	<u>7</u> 0.5 pg calibration standard	<u>6</u> Equipment blank	<u>5</u> Equipment blank	<u>4</u> Equipment blank	<u>3</u> 50 pg standard	<u>2</u> 50 pg standard	<u>1</u> 50 pg standard
<u>16</u> Equipment blank	<u>15</u> Equipment blank	<u>14</u> Equipment blank	<u>13</u> 40 pg calibration standard	<u>12</u> 20 pg calibration standard	<u>11</u> 10 pg calibration standard	<u>10</u> 5 pg calibration standard	<u>9</u> 2.5 pg calibration standard
<u>24</u>	<u>23</u>	<u>22</u>	<u>21</u>	<u>20</u>	<u>19</u>	<u>18</u>	<u>17</u>

**Sample Rack 1**

<u>32</u> 10 pg check standard	<u>31</u> Bubbler blank	<u>30</u> Sample 2	<u>29</u> Sample 1	<u>28</u> Sample 1	<u>27</u> Sample 1	<u>26</u> CRM 1	<u>25</u> Method blank 1
<u>40</u> 2.5 pg check standard	<u>39</u> Bubbler Blank	<u>38</u> Sample 8	<u>37</u> Sample 7	<u>36</u> Sample 6	<u>35</u> Sample 5	<u>34</u> Sample 4	<u>33</u> Sample 3
<u>48</u> 10 pg check standard	<u>47</u> Bubbler Blank	<u>46</u> Sample 11	<u>45</u> Sample 11	<u>44</u> CRM 2	<u>43</u> Method blank 2	<u>42</u> Sample 10	<u>41</u> Sample 9

**Sample Rack 2**

<u>56</u> 2.5 pg check standard	<u>55</u> Bubbler blank	<u>54</u> Sample 16	<u>53</u> Sample 15	<u>52</u> Sample 14	<u>51</u> Sample 13	<u>50</u> Sample 12	<u>49</u> Sample 11
<u>64</u> 10 pg Check standard	<u>63</u> Bubbler blank	<u>62</u> CRM 3	<u>61</u> Method blank3	<u>60</u> Sample 20	<u>59</u> Sample 19	<u>58</u> Sample 18	<u>57</u> Sample 17
<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 4. Definition of equations.**

$$\text{Sample Hg Concentration} = \frac{\left( \frac{\text{MeHg Mass In Analyzed Aliquot}}{\text{Percent of Extract Analyzed}} \right) - \left( \frac{\text{Blank MeHg Mass}}{\text{Percent of Extract Analyzed}} \right)}{\text{Sample Mass}}$$

$$\text{Percent of Extract Analyzed} = \frac{\text{Volume of Analyzed Aliquot}}{\text{Total Extract Volume}}$$

$$\text{MeHg Mass In Analyzed Aliquot} = \frac{(\text{Sample Peak Area})}{(\text{Slope of Calibration})}$$

$$\text{Percent Relative Standard Deviation} = \frac{\left( \frac{\text{Standard Deviation of Triplicate Hg Concentrations In Sample}}{\left( \frac{\text{Mean of Triplicate Hg Concentrations In Sample}}{\right)} \right)}{\left( \frac{\text{Mean of Triplicate Hg Concentrations In Sample}}{\right)} \times 100$$