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1 Revision Log:

Revision: 06	**Effective Date:** This version	
Section	**Justification**	**Changes**
Cover	Required change	Changed company name from Frontier Global Sciences to Eurofins Frontier Global Sciences.
All	Formatting requirement per LOM SOP-LAB-201	Reformatted document to new corporate specifications.
8.1 - 8.17	Required	Updated Definitions
12	Required	Added section on Sample Collection , Pres., and Handling
13.1	Required	Changed distillation rate
15.11	Required	Changed approximate distillation time
17.3	Required	Replaced MDL with LOD
18.2 – 18.8	Required	Added QC samples and acceptance limits

2 Reference:

2.1 Bloom, N.S. Determination of Picogram Levels of Methyl Mercury by Aqueous Phase Ethylation, Followed by Cryogenic Gas Chromatography with Cold Vapour Atomic Fluorescence Detection. Can. J. Fish. Aq. Sci. 1989, 46, 1131.

2.2 Bloom, N.S.; Fitzgerald, W.F. Determination of Volatile Mercury Species at the Picogram Level by Low-Temperature Gas Chromatography with Cold-Vapor Atomic Fluorescence Detection. Anal. Chem. Acta. 1988, 208, 151.

2.3 Horvat, M.; Bloom, N.S.; Liang, L. A Comparison of Distillation with Other Current Isolation Methods for the Determination of Methyl Mercury Compounds in Low Level Environmental Samples Part 2, Water. Anal. Chem. Acta. 1993, 282, 153.

2.4 Chemical Hygiene Plan, Eurofins Frontier Global Sciences, current version.

2.5 EPA Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry, 2002.

2.6 National Environmental Laboratory Accreditation Conference, NELAC Standard September 8, 2009.

2.7 Department of Defense Quality Systems Manual for Environmental Laboratories, prepared by DoD Environmental Quality Workgroup, Final Version 4.2, October 2010

3 Cross Reference:

Document	Document Title
SOP FGS-003	Pipette Verification, Calibration and Maintenance
SOP FGS-008	Ultra Clean Aqueous Sample Collection
SOP FGS-038	Data Review and Validation
SOP FGS-094, App F	Standard Operating Procedure Training Record
SOP FGS-099	Waste Disposal Procedure for Client Sample Waste
SOP FGS-070	Determination of Methyl Mercury in Various Matrices by Cold Vapor-Gas Chromatography- Atomic Fluorescence Spectrometry (EPA Method 1630)
SOP FGS-155	Calibration of Volumetric Dispensers

4 Purpose:

4.1 The purpose of this Standard Operating Procedure (SOP) is to describe the method for the distillation of aqueous samples prior to analysis by CV-GC-AFS for methyl mercury.

5 Scope:

5.1 This method is for the distillation of aqueous samples (natural waters, precipitation, pore water, industrial and municipal effluents) for the analysis of monomethyl mercury (MMHg) at concentrations as low as 0.05 ng/L. In general, using clean handling and reagents, method detection limits (MDLs) in the range of 0.005 - 0.030 ng/L are routinely attainable.

5.2 Methyl mercury as defined by this method means all chloride-distillable methyl mercury forms and species found in aqueous solution and on aqueous suspended matter. This includes, but is not limited to, CH_3Hg^+, CH_3HgCl, CH_3HgOH, and CH_3HgS-R.

6 Basic Principles:

6.1 Samples are collected using ultra-clean sample handling protocols into rigorously cleaned or tested clean glass bottles.

6.2 The samples must be acid-preserved within 48 hrs of sampling. Acid-preserved samples are stable for at least six months, if kept dark and cool (EPA 1630). Samples are preserved with hydrochloric acid (HCl) and stored dark, in a refrigerator at a temperature between 0-4°C until distillation.

6.3 Before analysis, the MMHg in an aliquot of the sample is co-distilled into pure water. The distillates are then analyzed for MMHg by aqueous phase ethylation and Cold Vapor-Gas Chromatography-Atomic Fluorescence Spectroscopy (CV-GC-AFS) as described in SOP FGS-070 “Methyl Mercury Calibration and Analysis.”

7 Reference Modifications:

7.1 No significant modifications were made to this method

8 Definitions:

8.1 Batch – no more than 20 client samples grouped for preparation. 3 Preparation Blanks, *1 CRM* or 1 LCS/LCSD (or BS/BSD) set and 1 MD are prepared per every 20 samples; 1 MS/MSD set is prepared for *every 10 samples*.

8.2 CDOC - Continuing Demonstration of Capability.

8.3 Certified Reference Material (CRM) – a standard of known composition that is certified by a recognized authority and representing a sample matrix. It is used to verify the accuracy of a method.

8.4 IDOC - Initial Demonstration of Capability.

8.5 Laboratory Control Sample (LCS) and Laboratory Control Sample Duplicate (LCSD), *reagent water is spiked with a secondary source at 1.0 ng/L* and is taken through the entire preparation and analysis process in the same manner as the samples to monitor

complete method performance. A Certified Reference Material (CRM) is preferred as the LCS, but a blank spiked sample also meets the requirement.

8.6 LIMS: Laboratory Information Management System. Computer software used for managing samples, standards, and other laboratory functions.

8.7 May: This action, activity or procedure is optional

8.8 May Not: This action, activity or procedure is prohibited

8.9 Method or Preparation Blank (BLK) – Method blanks consist of the same reagents used to digest the samples, in the same volume or proportion, and are carried through the complete sample preparation and analytical procedure.

8.10 Matrix Duplicate (MD) – a representative sample is selected and digested in the same manner. This QC sample will indicate sample homogeneity on the analytes of interest.

8.11 Matrix Spike (MS) and Matrix Spike Duplicate (MSD) – *a representative sample is selected and spiked with a secondary source at 1.0 ng/L*. These QC samples will indicate sample matrix effects on the analytes of interest.

8.12 May: This action, activity or procedure is optional.

8.13 May Not: This action, activity or procedure is prohibited.

8.14 Method Detection Limit (MDL) – the limit derived from an exercise as described in 40 CFR, Part 136, Appendix B. The exercise produces a defined value that is the minimum concentration that can be measured and reported with 99% confidence that the analyte concentration is greater than zero from a given matrix.

8.15 MMHg - monomethyl mercury

8.16 Shall: This action, activity or procedure is required.

8.17 Should: This action, activity or procedure is suggested, but is not required.

9 Interferences:

9.1 The use of the distillation procedure eliminates most method interferences from organic matter, particulate, and sulfides, all of which can affect the recovery of methyl mercury.

9.2 The low detection limit of this method depends on the stringent cleaning of equipment used for sample collection and storage.

10 Safety Precautions, Pollution Prevention and Waste Handling:

10.1 Personnel will don appropriate laboratory attire according to the Chemical Hygiene Plan. This includes, but is not limited to, laboratory coat, safety goggles, and nitrile gloves under clean gloves.

10.2 The toxicity or carcinogenicity of reagents used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Chemists should refer to the MSDS (Material Safety Data Sheets) for each chemical they are working with.

10.3 All personnel handling environmental samples known to contain or to have been in contact with human waste should be immunized against known disease-causative agents. Eurofins Frontier will reimburse the expense of Hepatitis A and B immunizations for any laboratory staff member who desires this protection.

10.4 Hydrochloric Acid (HCl) is hazardous. Always work in fume hood wearing safety glasses and gloves while using this chemical. In case of skin contact (corrosive, irritant, permeator), of eye contact (irritant, corrosive), of ingestion. Slightly hazardous in case of inhalation (lung sensitizer). Non-corrosive for lungs. Liquid or spray mist may produce tissue damage particularly on mucous membranes of eyes, mouth and respiratory tract. Skin contact may produce burns. Inhalation of the spray mist may produce severe irritation of respiratory tract, characterized by coughing, choking, or shortness of breath. Severe over-exposure can result in death. Inflammation of the eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering.

10.4.1 Eye Contact: Check for and remove any contact lenses (contact lenses shall never be worn in the laboratory). In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. Get medical attention immediately.

10.4.2 Skin Contact: In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Cover the irritated skin with an emollient. Cold water may be used. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention immediately.

10.4.3 Serious Skin Contact: Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek immediate medical attention.

10.4.4 Inhalation: If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention immediately.

10.4.5 Serious Inhalation: Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. WARNING: It may be hazardous to the person providing aid to give mouth-to-mouth resuscitation when the inhaled material is toxic, infectious or corrosive. Seek immediate medical attention.

10.4.6 Ingestion: If swallowed, do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention immediately.

10.5 See Eurofins Frontier Global Sciences Chemical Hygiene Plan (CHP) for general information regarding employee safety, waste management, and pollution prevention.

10.6 Pollution prevention information can be found in the current Eurofins Frontier Global Sciences Chemical Hygiene Plan (CHP), which details and tracks various waste streams and disposal procedures.

10.7 All laboratory waste is accumulated, managed, and disposed of in accordance with all federal, state, and local laws and regulations. Any waste generated by this procedure should be disposed of according to SOP FGS-099 "Waste Disposal Procedure for Client Sample Waste," which provides instruction on dealing with laboratory and client waste.

11 Personnel Training and Qualifications:

11.1 An analyst must perform an initial demonstration of capability (IDOC) that includes four replicates of a secondary source before being qualified to analyze samples without supervision. Continuing DOC will be maintained and monitored via performance on CRMs and other QC samples, as well as obtaining acceptable results on proficiency testing exercises.

11.2 The analyst/laboratory technician must have read this SOP and other relevant SOPs and have the training documented on the applicable form(s). The analyst may be questioned on SOP by supervisor(s) and/or trainers.

11.3 Training is documented by the employee and supervisor, and is kept on file in the QA Office. The employee must read, understand, and by signing the training document, agree to perform the procedures as stated in all Standard Operating Procedures (SOPs) related to this method.

11.4 Reading of the SOP must be documented on the correct form such as "Standard Operating Procedure Training Record," Appendix F in FGS-094, the last page of this SOP, Appendix A "Standard Operating Procedure Training Record" or a similar document."

11.5 All employees must also, on a yearly basis, read the Quality Manual (QM), and complete the yearly Ethics training.

11.6 All training documents including IDOCs, CDOCs, SOP reading, Initial QA orientation, and Ethics training are stored by the Quality Assurance Manager in the employees training file for ten years after the employee is no longer working for Eurofins Frontier Global Sciences.

11.7 Chemical Safety Training, Compressed Gas Training, Chemical Hygiene Plan documentation, and Shipping of Hazardous goods, are stored by the Health and Safety Officer for ten years after the employee is no longer working for Eurofins Frontier Global Sciences.

12 Sample Collection, Preservation, and Handling:

12.1 Samples are collected using ultra-clean sample handling protocols into rigorously cleaned or tested clean glass bottles.

12.2 Aqueous sample preservation - The samples must be acid-preserved within 48 hrs of sampling. Samples are preserved with hydrochloric acid (HCl) and stored dark, in a refrigerator at a temperature between 0-4°C until distillation.

12.3 Acid-preserved samples are stable for at least six months, if kept dark and cool (EPA 1630).

13 Apparatus and Equipment:

13.1 *LIMS; Computer – Windows XP, 7 or 8*

13.2 Teflon Still.

13.3 Sampling Bottles.

13.4 *Pipettors: All-plastic, pneumatic, fixed volume and variable pipettes in the range of 5 µL to 10 mL. Pipettes are to be calibrated weekly according to SOP FGS-003 and FGS-155.*

13.5 Clean hood.

13.6 Analytical Balance: A laboratory analytical balance that measures accurately to 0.01mg, with documented calibration.

14 Reagents and Standards:

14.1 Reagent Water: 18 MΩ ultra-pure deionized water starting from a pre-purified (distilled, R.O., etc.) source. As a final mercury and organic removal step, the activated carbon cartridge on the 18-MΩ system is placed between the final ion exchange bed and the 0.2 µm filter.

14.2 Hydrochloric Acid: Trace metal purified reagent-grade HCl is pre-analyzed and lot sequestered. Several brands (Baker, Fisher, Omnitrace) have been found to have lots with acceptably low levels of trace metals. This reagent should be from a lot number that has been previously tested to be low for the analytes of interest. This reagent shall be entered into LIMS and the expiration date is set to the same as the manufacturer's expiration date.

14.3 APDC Solution.

14.4 Nitrogen: Grade 4.5 (standard laboratory grade) nitrogen that has been further purified by using a gold-coated sand trap to remove any residual Hg.

15 Procedure:

15.1 Samples are collected and handled according to SOP FGS-008, "Ultra Clean Aqueous Sample Collection."

15.1.1 MDN samples are collected and handled according to MDN-001 "MDN Glass Sample Train Collection and Deployment- Trace Metal Clean Sample Handling.

15.2 Obtain pairs of clean sample and receiving distillation vials. Empty the contents of the vials into a large beaker with sodium bicarbonate. Rinse each vial three times with reagent water, making sure to rinse the lids and tubes thoroughly.

15.3 Tape the receiving vials, distinguished by colored bands around the caps, so that the bottom of the tape is just touching the top of the 40.0-mL engraved mark. Pipette 5 mL of reagent water into each of the receiving vials, so that the bottom of the tubing is covered.

15.4 Retrieve the samples to be distilled from the walk-in refrigerator. Organize the samples in the same order as they are listed on the LIMs bench sheet, and make sure that all samples are accounted for.

15.4.1 MDN Samples: Organize the samples in the same order as they are listed on the MDN MMHg Accounting Sheet, and make sure that all samples are accounted for. Record the sample IDs and aliquot size in the "Sample Digestion Logbook" along with the spiking levels for any applicable quality control samples. Spiking levels are 1.0 ng/L for all quality control samples. The LIMS number of the spike and volume used is recorded in the logbook.

15.5 Each sample vial is placed on the balance and tarred before adding an aliquot of sample. A smaller aliquot volume may be used if samples are suspected to be high in MMHg, or if there is a limited amount of sample available. These smaller aliquots are diluted with HCl. After all samples are prepared, spike standard into the digested matrix spike samples.

Note: For samples that are suspected to have densities that are not 1.00 ± 0.02 g/mL, the density must be determined and the aliquot size must be adjusted to accommodate this difference between mass and volume.

15.6 Prepare three blanks, one blank spike, and one blank spike duplicate using HCl as the diluent.

15.7 To each sample, with a pipette add APDC before capping the vial. The sample vial is then placed next to its corresponding receiving vial in a holding rack. Record the LIMS ID of the APDC solution in the logbook.

15.8 After all samples are prepared, spike the blank spike and matrix spike samples for a true value. The LIMS ID of the standard used for spiking must be added to LIMS bench sheet.

15.8.1 Due to the lack of a CRM for methyl mercury in water, at least one blank spike/blank spike duplicate must be analyzed per analytical batch.

15.9 Turn on the heating blocks in the distillation room after verifying to make sure that they are set at 120°C. Fill the ice-water baths with ice after about half of the water has been poured out of the container. Turn on the nitrogen gas lines, assuring the flow rates are equivalent..

15.10 Place the receiving vessel into the ice/water bath and its sample vial counterpart into the corresponding heating block hole. After all vials are in a block, the aluminum cover piece is placed over the sample vials.

15.11 A distillation rate should be maintained, until a total volume of 40 ± 1 mL is achieved in a receiving vial. The receiving vial is then disconnected from the sample vial.

15.12 The pH is measured of all the distillation sets and is recorded on the logbook.

15.13 The optimum pH for the distillate is 3.5-7. For pH <3.0, the sample will be analyzed, but should be re-distilled if sufficient sample volume remains. This shall be entered as a comment in the MMO notes in LIMS.

15.14 The distillate may then be analyzed using aqueous phase ethylation, GC separation, and CVAFS detection. For water samples, the entire sample will usually be analyzed. Calculation of MMHg content is based upon the actual volume of sample distilled corrected by the empirically derived distillation efficiency factor. For samples determined to be possibly high in MMHg, a fraction of the total distillate may be analyzed.

16 Calculations:

16.1 This preparation procedure does not involve calculations.

17 Statistical Information/Method Performance:

17.1 Method Detection Limit (MDL) and Practical Quantitation Limit (PQL) studies are based on 40 CFR 136, Appendix B. The MDL and PQL must be performed for each analyte/matrix/preparation combination.

17.2 The Practical Quantitation Limit (PQL) is the reporting limit for this method and is included as the lowest calibration point (2003 NELAC regulation 5.5.5.2.2.1.h.3). The PQL is determined by running ten replicate samples with a concentration that will produce a recovery of 70-130% for most analytes, but the recovery requirements are analyte dependent. The PQL is referred to as the Method Reporting Limit (MRL) in LIMS.

17.3 The current values for MMHg in aqueous samples are 0.05 ng/L for the LOD and 0.05 ng/L for the PQL.

17.4 Current LODs and PQLs are stored at: \General and Admin\Quality Assurance\MDLs & PQLs.

18 Quality Assurance/Quality Control:

18.1 MDN precipitation samples are limited in volume. MDN samples should be distilled by senior lab assistants. MDN samples may not be distilled by personnel in training.

18.2 Maximum Sample Batch Size: 20 samples.

18.3 Preparation Blanks: Minimum of three per batch. Each preparation blank must be less than one-half the PQL for the method.

18.3.1 The preparation blanks are prepared using the HCl diluents; these are put through the same preparation process as the samples.

18.4 Certified Reference Material (CRM, representing the sample matrix when commercially available); a Laboratory Control Spike (LCS) and Laboratory Control Spike Duplicate (LCSD) is used when a suitable CRM is not available: One per batch in duplicate. The control limits are 70-130% recovery.

18.5 Matrix Duplicate (MD) Sample: One per batch. The control limit for the RPD is ≤ 35%.

18.6 Matrix Spike/Matrix Spike Duplicate (MS/MSD) Samples: One set per 10 samples. The control limits are 64-130% recoveries and an RPD of ≤ 35%.

18.7 Follow the flow charts in SOP FGS-038 “Data Review and Validation” to determine if any QC falling outside the established control limits can be qualified.

18.8 All of the quality control limits for the analysis method are included on the "Data Review Checklist.

18.8.1 The data review checklists are located at: \\cuprum\General and Admin\Quality Assurance\Data Review\Current Data Review Checklists.

19 Corrective Action:

19.1 Limiting the source of contamination/error in the preparatory stage can decrease QC problems during analysis. Limiting such contamination/error sources may include: cleaning all digestion tools in a 10% HCl solution, ensuring all samples are thoroughly homogenized, changing gloves whenever appropriate, flushing repipettors at least three times before dispensing into vials and, in general, following ultra-clean procedures.

19.2 A failing QC point does not necessary fail the entire dataset. If upon analysis a QC sample is out of control, some investigation must be performed to assess if the difficulties are related to matrix effects. The cause and method of determining the set's failure must be documented on the checklist and in the MMO notes, and the Group Supervisor shall be informed. See SOP FGS-038 "Data Review and Validation" for flow charts regarding analytical issues.

19.3 If a matrix spike sample does not show recovery within the control limits, it is possible that the sample was not spiked properly. The analyst preparing the samples should review the spiking level for accuracy. Also, proper pipetting techniques should be reviewed.

19.4 If contamination is suspected to be a problem, the vials may need to be deep cleaned using BrCl. Additionally, reagents should be checked for contamination or remade in clean bottles.

19.5 If samples render abnormal results, several other variables should be investigated to discover the source of error. The Senior Analyst, and/or the Group Supervisor should be consulted to discuss possible areas for trouble-shooting.

19.6 If the distillation process is determined to be the cause of the analytical problems, then all samples with sufficient volume must be re-distilled. Samples without sufficient volume will be flagged during the review process and then narrated in the case narrative by the Project Manager.

20 List of Attachments

Appendix A: Example - Standard Operating Procedure Training Record

Appendix A: Example - Standard Operating Procedure Training Record

By signing this document, I the employee, certifies to have read, understood and agreed to follow the test method and quality procedure as described in this procedure.

Reading of SOP EFGS-013.06:
Distillation of Aqueous Samples for Methyl Mercury Analysis.

SOP name and Revision number

Employee name (print)

Employee name (sign) Date:

Supervisor name (sign) Date:

Initial SOP Training (leave blank if not applicable)

Initial reading of method and training	Initials	Date	Supervisor
1. Read method			
2. Observe the method			
3. Detailed review of method and associated literature			
4. Supervised practice of method with trainer			
5. Unsupervised practice of the method with trainer			
6. Review of work with trainer and/or peer-review			
7. IDOC to determine precision and accuracy			
8. Determination of blanks			