Trace Level Total Mercury Analysis in Tissue By Cold Vapor Atomic Fluorescence (CVAF) Spectroscopy

TABLE OF CONTENTS

1. SCOPE AND APPLICATION 2
2. SUMMARY OF METHOD 2
3. APPARATUS AND EQUIPMENT 2
4. CONTAINER PREPARATION 3
5. REAGENTS AND CHEMICALS 3
6. SAMPLE COLLECTION, PRESERVATION, AND HANDLING 6
7. SAMPLE PREPARATION 6
8. CALIBRATION STANDARD PREPARATION 8
9. INSTRUMENT SETUP 8
10. INSTRUMENT PARAMETERS 9
11. INSTRUMENT OPERATION 10
12. CALCULATIONS 12
13. QUALITY CONTROL 12
14. INTERFERENCES 13
15. CORRECTIVE ACTIONS FOR COMMON PROBLEMS 13
16. SAFETY/HAZARDOUS WASTE MANAGEMENT 14
17. REFERENCES 14
APPENDIX OF SIGNIFICANT CHANGES 14

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

1. SCOPE AND APPLICATION

This standard operating procedure (SOP) describes the procedure for determining trace levels of total mercury in tissue samples using cold vapor atomic fluorescence detection (CVAF). This method is applicable to the determination of total mercury in animal tissue, plant tissue, and blood. This SOP is used for the analysis of samples with test identifier (Test ID) T-HG-AF. The linear dynamic range of this instrument for digested samples is 1.0 – 30 ng/L. The method detection limit (MDL) is 1.0 ng/L (0.00050 mg/kg), and the practical quantitation limit (PQL) is 3.0 ng/L (0.0015 mg/kg).

2. SUMMARY OF METHOD

This method is a modification of the method detailed in DEP SOP HG-001. Modifications include exposing the tissue samples to UV light which decomposes organomercurial compounds. By using CVAF instead of cold vapor atomic absorption detection (CVAA) (EPA 245.1), total mercury can be detected with an MDL of 0.00050 mg/kg. Calibration standards and blanks are prepared using Solution 2000™ purified deionized (DI) water. The linear reporting range for this method and instrument is 1.0 – 30 ng/L. Digested samples having concentrations greater than 30 ng/L must be diluted to bring their concentrations within range.

3. APPARATUS AND EQUIPMENT

3.1. Tekran Model 2600 Mercury Analyzer, Serial No. 001, Software: Tekran – MDS, Version 2.5.1.610 with Tekran Model 2620 autosampler.

3.2. Tekran Model 2600 Mercury Analyzer, Serial No. 0154, Software: Tekran – MDS, Version 2.5.1.610 with Tekran Model 2621 autosampler.

3.3. Solution 2000 Type I Reagent Grade Water Purification System.

3.4. 125 mL Teflon wide mouth bottles.

3.5. 60 mL Teflon narrow mouth bottles.

3.6. 500 mL cleaned glass I-Chem bottles with Teflon lined caps.

3.7. Cabinet with 4, 15 W mercury vapor lamps with 254 nm output.

3.8. Pipettes:

3.8.1. 100 µL and 1000 µL non-adjustable Eppendorf pipette.

3.8.2. 10 – 100 µL adjustable Eppendorf pipette.

3.8.3. 2 – 10 mL Finnpipette.

3.9. Class A volumetric flasks: 1 L, 250 mL, and 100 mL.

3.10. Glass vials (28 x 95 mm) for the Tekran Model 2621 autosampler and 2620 autosampler

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

3.11. Metter Toledo top loading balance, model MS1602S, S/N B336753389, max capacity 1600 g.

3.12. Sartorius Practum513-1S Analytical Balance, S/N 0031950031, max capacity 250 g.

4. CONTAINER PREPARATION

4.1. All containers used to store reagents are cleaned thoroughly with solutions of 5 % aqua regia and DI water before use. All containers are rinsed twice with this acid solution, followed by rinsing with DI water at least six times. All beakers, measuring cylinders, volumetric flasks, and plastic pipette tips (Eppendorf) are also subject to the same cleaning procedure before use.

4.2. Narrow mouth 60 mL Teflon bottles with leak proof Tefzel caps are used to digest the samples. The bottles are cleaned with 5 % aqua regia solution before use. Fill all the Teflon digestion bottles to the brim with this solution, and cap them firmly. The bottles may be stored in this manner indefinitely until needed. The minimum amount of time the bottles should soak with aqua regia is 24 hours. The bottles are emptied and rinsed thoroughly with DI water (at least six times) before use. The Tefzel caps are also treated with the same degree of thoroughness as the bottles themselves. Care should be taken to ensure that the threads on the bottles and their caps are washed with acid and DI water.

4.3. After use any remaining solution in the digestion bottles is transferred into a mercury waste disposal container. The bottles are then rinsed once with tap water and filled to the brim with 5 % aqua regia. They are capped firmly and stored until needed.

4.4. The glass vials (28 x 95 mm) used during analyses are immersed and stored in 5 % aqua regia solution in a large covered beaker overnight (or at least two hours) and rinsed with DI water six times prior to use.

5. REAGENTS AND CHEMICALS

Reagents used for analysis must be logged in to the LIMS standard prep tracker to generate a serial number. The label printed from the LIMS standard prep tracker must be placed on the bottle of reagent in the laboratory. The reagents used in analysis are documented on the ‘MT-HG-RGNT’ form located on the network at \\FLDEP1\DEAR\Labs\Lab5\Chemistry\InorganicPrep\Mercury\T-HG-AF\. The form is printed and included with each analysis laboratory report.

Preparation of all intermediate Hg standards must be documented in the LIMS standard prep tracker. A label, to be placed on the prepared standard container, should be printed from standard prep tracker that shows the identity of the standard, preparation date, expiration date, and serial number. The reagents used during the preparation of the intermediate Hg standards are documented on the ‘MT-HGINT-STD’ form located on the

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

network at \\FLDEP1\DEAR\Labs\Lab5\Chemistry\InorganicPrep\Mercury\T-HG-AF\. This form is printed and included with the analysis laboratory report.

The working calibration standards should have their preparation documented on the 'MT-HGCAL-STD' form located on the network at \\FLDEP1\DEAR\Labs\Lab5\Chemistry\InorganicPrep\Mercury\T-HG-AF\. This form is printed and included with the analysis laboratory report. The working calibration standards must also be logged in to the LIMS standard prep tracker to generate a serial number and a label, which is placed on each bottle.

See DEP SOP MT-069 for reagent and standard documentation requirements and expiration dates.

5.1. Deionized (DI) Water - All references to DI water within this SOP refer to DI water that has been purified with the Solution 2000™ Type I Reagent Grade Water Purification System.

5.2. Trace Metal Grade Hydrochloric acid (HCl) - In this procedure, HCl is used for the preparation of stannous chloride, preparation of aqua regia, preparation of standards, and digestion of samples. The expiration date is given by the manufacturer on the bottle. Stored at room temperature in the fume hood cabinet.

5.3. Trace Metal Grade Nitric acid (HNO_3) - In this procedure, HNO_3 is used for the preparation of aqua regia, preparation of standards, and digestion of samples. The expiration date is given by the manufacturer on the bottle. Stored at room temperature in the fume hood cabinet.

5.4. Industrial Grade 1 argon (99.997 %) is supplied to the instrument from the laboratory's Microbulk argon delivery system. Argon is used as the carrier gas for the mercury vapor from the gas liquid separator to the detector.

5.5. Industrial grade 1 nitrogen (99.998 %) is supplied from the laboratory's Microbulk nitrogen delivery system. Nitrogen is used to purge reagents before use.

5.6. Potassium Bromate ($KBrO_3$, 0.1 M) / Potassium Bromide (KBr, 0.2 M) Solution - Add 8.4 g of $KBrO_3$ and 11.9 g of KBr to an acid cleaned 1 L Teflon bottle. Dilute and dissolve both salts with DI water to 1 L. The solution is prepared as needed and is stored at room temperature. Expiration date for this reagent is 1 year. Purge the solution for at least two hours with a stream of nitrogen before use.

Note: If contamination is suspected bake $KBrO_3$ at 250 ^oC overnight in a glass scintillation vial in a mercury free oven to remove all traces of Hg in the salt. Do the same with KBr in a clean glass vial.

Bromine monochloride is generated in situ by adding hydrochloric acid and the bromate/bromide mixture to the sample.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

5.7. Hydroxylamine Hydrochloride (10 %) - Weigh 10 g of hydroxylamine hydrochloride and place in 125 mL acid cleaned Teflon bottle. Add 90 mL DI water and swirl the solution until the salt dissolves completely. Purge the solution for at least two hours with a stream of nitrogen before use. The expiration date for this reagent is 1 year and it is stored at room temperature.

5.8. Stannous Chloride (3 %) - Weigh 60 g of stannous chloride ($Sn_2Cl{\cdot}2H_2O$) and place in a 2 L acid cleaned Teflon bottle. Add 100 mL of concentrated HCl and swirl the solution until the salt completely dissolves in the acid. Dilute the solution to 2 L. This solution is prepared as needed. A 2 L stock of stannous chloride solution typically lasts for 1 analytical run. Purge the solution with nitrogen for at least two hours before use to remove traces of mercury in the solution. This reagent expires 12 hours after preparation.

5.9. 5 % Aqua Regia - To a 20 L carboy containing 10 L of DI water, slowly add 750 mL of HCl and 250 mL of HNO_3 in the hood. Dilute to the 20 L mark and mix thoroughly. The expiration date for this cleaning solution is 1 year.

5.10. NIST 2976 Standard - This SRM consists of freeze-dried defatted low-level mussel tissue. It has a certified total mercury concentration of 0.061 mg/kg. 0.114 g of the tissue is weighed into a digestion vessel and digested like a regular tissue sample. This SRM expires January 31, 2018.

5.11. THG-7 (1 µg/L) - This solution is prepared by serial dilution of a 1000 mg/L mercuric chloride ($HgCl_2$) standard (SPEX Plasma Standard, VWR Scientific Products, or Exaxol.) in Class A volumetric flasks. First, 1 mL of the standard is diluted to 100 mL in a volumetric flask, yielding a stock with a concentration of 10 mg/L (THG-5). Next, 2 mL of this solution is further diluted to 100 mL in a second volumetric flask to yield a solution with a concentration of 200 µg/L (THG-6). Finally 1.25 mL of the 200 µg/L stock is diluted to 250 mL in a third volumetric flask resulting in the 1.0 µg/L Hg THG-7 standard. Bromine monochloride is used as the preservative for all of these standards. Add 2 mL of HCl, 5 ml of HNO_3, and 3 mL of the bromide/bromate solution (5.6) for every 100 mL of stock prepared. The final solution (1 µg/L) is used to prepare calibration standards. The expiration date for this working standard is 60 days from preparation and it is stored at room temperature.

5.12. MHG-6 (1 µg/L) - This standard is prepared by serial dilution of a 1000 mg/L methylmercury as Hg standard (MHG-1). MHG-1 is prepared by dissolving 0.1255 g of solid methylmercury chloride in optima grade isopropanol in a 100 mL Class A volumetric flask. First, 1 mL of the 1000 mg/L standard is diluted to 100 mL in a volumetric flask, yielding a standard with a concentration of 10 mg/L (MHG-4). Next, 1 mL of this solution is further diluted to 100 mL in a second volumetric flask to yield a standard with a concentration of 100 µg/L (MHG-5). Both these serial dilutions are performed using isopropanol as the solvent. The final dilution to prepare the 1 µg/L Hg standard is done in an aqueous medium. Add 2 mL of glacial acetic acid and 200 µL of HCl to a clean 100 mL volumetric

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

flask containing 50 mL of DI water. Pipette 1 mL of the 100 µg/L stock (MHG-5) into this flask. Bring the volume up to the mark with DI water. Cap and shake well. Transfer all standards to clean amber glass bottles. All standard prepared in isopropanol should be stored in a freezer at -10 to -18 ^{o}C. The MHG-6, 1 µg/L standard, prepared in an aqueous matrix, should be stored in a refrigerator at 2 -10 ^{o}C. The expiration date is 60 days from preparation.

5.13. W-STD7 (30 ng/L) – This standard is used as the initial calibration verification. Prepare by adding 6.0 mL of THG-7, 20 mL of HNO_3, 174.0 mL of DI water, 4 mL of HCl, and 6.0 mL of the KBR/$KBrO_3$ solution in a 500 mL I-Chem bottle. Allow at least 2 hours for the generated BrCl to oxidize mercury, then add 0.4 mL of hydroxylamine hydrochloride.

5.14. W-STD5 (15 ng/L) – This standard is used as the continuous calibration verification (CCV) standard. Prepare by adding 3.0 mL of THG-7, 20 mL of HNO_3, 177.0 mL of DI water, 4 mL of HCl, and 6.0 mL of the KBr/$KBrO_3$ solution in a 500 mL I-Chem bottle. Allow at least 2 hours for the generated BrCl to oxidize mercury, then add 0.4 mL of hydroxylamine hydrochloride.

6. SAMPLE COLLECTION, PRESERVATION AND HANDLING

Tissue samples are collected and stored in polyethylene or glass vials with airtight closures. They are preserved by storage in a freezer at < -20 ^{o}C. The frozen containers are shipped in ice-packed (or blue ice) coolers via overnight mail to the lab. The samples are then stored in a freezer at < -20 ^{o}C. Samples are removed from the freezer and allowed to thaw to room temperature before preparation. The holding time from collection to analysis for tissue samples is 365 days from collection.

7. SAMPLE PREPARATION

Note: Support equipment used to measure volumes of reagents and samples include the Mettler Toledo top-loading balance, the Sartorius Practum513-1S analytical balance, and pipettes. Prior to use, verify the balance calibration has been checked and is within the acceptable tolerance and verify the pipette calibration has been checked within the last month. Calibration check documentation for pipettes can be found on the Division's computer network, \\FLDEP1\DEAR\Labs\Lab5\Chemistry\InorganicPrep\Mercury\Prep\Pipette Calibration. Calibration check documentation for the balances can be found in the 'Mettler Toledo Balance Calibration Logbook' and the 'Balance Calibration Logbook Sartorius Practum513-1S'.

7.1. Using the Backlog Report module of LIMS choose a batch of samples to digest. All samples in the batch should be of the same matrix. A batch can contain a maximum of 20 samples.

7.2. Gain custody of the samples in the receiving room and set aside in the laboratory to allow equilibration to room temperature.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

7.3. Label the 60 mL Teflon bottles with the sample IDs.

7.4. Weigh between 0.100 and 0.200 g of each sample into a clean 60 mL Teflon bottle. Enter the weight of each sample aliquot in the LIMS prep worksheet manager using three significant figures.

7.4.1. Choose one sample to spike.

7.4.2. Prepare two matrix spikes by weighing two more sample aliquots into 60 mL Teflon bottles. Using a calibrated pipette, add 4 mL of MHG-6 (1 µg/L) to each matrix spike bottle.

7.5. Add 5 mL of HNO_3 to each sample and matrix spike.

7.6. Prepare a NIST2976 mussel tissue SRM by weighing 0.114 g of the tissue into a clean 60 mL Teflon bottle and add 5 mL of HNO_3 to the bottle.

7.7. Prepare a method blank for the batch by adding 5 mL of HNO_3 and 45 mL of DI water to a 60 mL Teflon bottle.

7.8. Prepare a PQL standard by adding 0.15 mL of THG-7 to 44.85 mL of DI water in a 60 mL Teflon bottle. Add 5 mL of HNO_3 for a final volume of 50 mL.

7.9. Prepare an LFB by adding 0.25 mL of THG-7 to 44.75 mL of DI water in a 60 mL Teflon bottle. Add 5 mL of HNO_3 for a final volume of 50 mL.

7.10. Swirl each bottle (including QC samples) gently to ensure complete immersion of the sample in the acid. Place the caps of the bottles on loosely and place them in a water bath at 60 °C for 1 hour. The tissue will dissolve in the acid. Bring the total volume of the digestates and QC samples (except the LFB, PQL, and method blank) up to 50 mL by adding 45 mL of DI water to them with a calibrated bottle top dispenser.

7.11. After the tissue samples have dissolved in the HNO_3, cap each bottle and place them in the UV cabinet. Allow the samples to be exposed to the 254 nm UV radiation for 48 hours. Document the date and time the samples are placed in the UV cabinet as well as when the samples are removed from the UV cabinet in the Preservation and UV Box Logbook. At the end of this period, the digestate should be a clear, near-colorless solution. If the samples are not clear and near-colorless, the samples may be left in the UV cabinet for additional time. Document any extra UV time in the Preservation and UV Box Logbook. Plant samples may not be completely digested. Certain fibrous polymeric components of plant tissue such as lignin remain undigested.

7.12. Transfer the samples from the UV cabinet to the fume hood. Add 1 mL of HCl and 1.5 mL of the $KBr/KBrO_3$ solution to all samples and QC samples in the batch. Cap the bottles tightly and shake them a couple of times, to ensure good mixing. Allow the samples to react with the generated BrCl for at least 2 hours.

7.13. Add 0.1 mL hydroxylamine hydrochloride to all samples and QC samples to neutralize excess BrCl. The digestates are now ready to be poured into

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

autosampler tubes. The matrix spikes should be diluted by 4 and the NIST2976 should be diluted by 10 prior to analysis.

8. CALIBRATION STANDARD PREPARATION

8.1. Prepare the calibration standards by adding measured volume of THG-7 using an Eppendorf pipette to a glass 500 mL I-Chem bottle containing a measured (by weight) volume of DI water. See Table 1 below for concentrations and volumes of the calibration standards.

8.2. Prepare five calibration blanks and 1 CCB in 6 different 500 mL clean glass I-Chem bottles.

8.3. Add 20 ml of HNO3, 4 mL of HCl, and 6 mL of the $KBr/KBrO_3$ solution to each 500 mL bottle of calibration standard, calibration blank and CCB. Cap each bottle and invert 3 times to ensure adequate mixing. Allow the generated BrCl to react with the standards and calibration blanks for at least 2 hours prior to analysis.

8.4. Add 0.4 mL of hydroxylamine hydrochloride to each bottle of calibration standard and calibration blank (0.1 ml for every 50 ml).

TABLE 1.

Standard or Sample	Volume of THG-7 (1 µg/L)	Volume of Nitric acid (mL)	Volume of DI Water (mL)	Conc. of Hg^{+2} (ng/L)
STD30 / W-STD7	6.0 mL	20	174.0	30.0
STD20	4.0 mL	20	176.0	20.0
STD15 / W-STD5	3.0 mL	20	177.0	15.0
STD5	1.0 ml	20	179.0	5.0
STD3	600 µL	20	179.4	3.0

9. INSTRUMENT SETUP

9.1. The argon supplied to the instrument is generally left on in order to continuously purge the detector. The argon delivery pressure should be regulated to 30 psi. Before turning the instrument on, make sure the argon pressure is 30 psi by checking the regulator.

9.2. Prepare the stannous chloride solution. Make sure at least 2 L is available for the analytical run. Purge the solution for at least 2 hours using nitrogen prior to analysis. Check the stannous chloride solution for clarity, the solution should be clear and colorless. If the solution is cloudy, opaque, white, or yellow, discard the solution, clean the bottle thoroughly, and reprepare.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

9.3. Check the volume of rinse solution (DI water). There should be at least 20 L of rinse solution in the appropriately labeled 25 L carboy.

9.4. Replace the soda lime if moisture is observed in the lines anywhere between the soda lime and detector.

9.5. If necessary, adjust the flow meter for the detector line (argon) to 70 mL/min and the flow meter for the phase separator line to 450 mL/min.

9.6. Set-up the peristaltic pump. The grey-grey tubing is used for stannous chloride, blue-blue tubing for the sample, and purple-purple and blue-blue tubing are used for waste.

10. INSTRUMENT PARAMETERS

The instrument control parameters which define a method are stored in a method file. The values for these parameters are listed in Table 2 below. The instrument and its fluorescence detector are left on 24 hours a day.

Detector carrier gas flow rate: 70 mL/min
Phase Separator flow rate: 450 mL/min
Pressure at the Detector purge inlet: 25 psi

TABLE 2.

Tekran 2600 Method Parameters

	Comment	Value	Start	Duration	Notes	End
1	**MFC**	**80.0 mL/min**	**2**	**600**	**MFC:80 mL/min for 600 sec**	**602**
2	**Pump**	**42.0 %**	**3**	**600**	**PUMP: ON for 600 sec**	**603**
3	**V3:gas**	**ON**	**3**	**630**	**V3: gas ON for 630 sec**	**633**
4	**V4:WashPmp**	**ON**	**4**	**600**	**WashPmp: ON for 600 sec**	**604**
5	**V1:Vent**	**ON**	**5**	**140**	**V1: Vent A**	**145**
6	**A/S Up**	**5**	**6**	**0**	**A/S UP**	**6**
7	**A/S Move**	**-2**	**8**	**2**	**A/S Move to sample tube**	**10**
8	**Opt2:Beep**	**ON**	**11**	**2**	**Opto-2:Signal Position Change**	**13**
9	**A/S Down**	**5**	**12**	**0**	**A/S Down 5 Speed**	**12**
10	**Pump**	**52.0 %**	**12**	**23**	**Pump 52.0 % for 18 sec**	**35**
11	**AD12 Scan**	**0**	**20**	**5**	**AD12 Scan & Store**	**25**
12	**Pump**	**42.0 %**	**29**	**574**	**Pump 42.0 % for 574 sec**	**603**

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

	Comment	Value	Start	Duration	Notes	End
13	**V2:Load-A**	**ON**	**45**	**70**	**V2:Load ON for 70 sec**	**115**
14	**A/S Up**	**5**	**115**	**2**	**A/S UP**	**117**
15	**A/S Move**	**-1**	**117**	**3**	**A/S Move to Rinse Tube**	**120**
16	**Opt2:Beep**	**ON**	**120**	**1**	**Opto-2:ON for 1 sec**	**121**
17	**A/S Down**	**5**	**121**	**4**	**A/S Down**	**125**
18	**Heater-A**	**100.0 %**	**135**	**32**	**Heat A 100 %**	**167**
19	**A/S Up**	**5**	**137**	**2**	**A/S UP**	**139**
20	**A/S Move**	**0**	**139**	**3**	**A/S Move to Wash Stn**	**142**
21	**Opt2:Beep**	**ON**	**142**	**1**	**Opto-2:ON for 1 sec**	**143**
22	**A/S Down**	**5**	**143**	**4**	**A/S Down**	**147**
23	**Fan-A**	**ON**	**167**	**40**	**Fan A ON**	**207**
24	**Heater-B**	**100.0 %**	**185**	**35**	**Heat B 100 % for 32 sec**	**220**
25	**Heater-A**	**0.0 %**	**186**	**80**	**Heater-A: 0 % for 80 sec**	**266**
26	**Ad24 Start**	**0**	**195**	**50**	**AD24 Start: 50 lines**	**245**
27	**Fan-B**	**ON**	**245**	**50**	**Fan-B:ON for 40 sec**	**285**
28	**Done**	**0**	**246**	**0**	**Done**	**246**

11. INSTRUMENT OPERATION

11.1. Start the Tekran 2600 program and go to the menu “worksheet” followed by “run” and “new”.

11.2. Fill in the worksheet starting with:

CLEAN
CLEAN
CALBLK (W-BLK)
CALBLK (W-BLK)
CALBLK (W-BLK)
CALBLK (W-BLK)
CALBLK (W-BLK)
MDL
CALIBRATION STANDARDS
W-STD7
RINSE
CCB
T-BLK (MBLK)

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

T-PQL
LFB
NIST 2976
Sample 1
Sample1_SPK 1
Sample1_SPK 2
SAMPLE
SAMPLE
W-STD5
RINSE
CCB
etc.

11.3. Every 10 samples, analyze a CCV (W-STD5, 15 ng/L) to ensure that the system is still in calibration and a CCB to check for baseline drift.

11.4. Click on the "start" icon to start the run.

11.5. It is the responsibility of the analyst to ensure that QC limits are not exceeded in the course of the analysis and that appropriate actions are taken. Any changes in the sample sequence, instrument problems, and corrective actions should be recorded on the cover page of the laboratory report. Instrument problems should be documented in the instrument maintenance logbook.

11.6. After the run is completed, print the data and 'export file' to Tek Data. The file should be stored in the directory 'Tek Data'. The naming of the data file should follow the following convention: YYYYafMMDDw1_A. As an example, consider 2017af0210t1_A. This name indicates that the file contains data from the first analytical run on tissue (t1) samples conducted on February 10, 2017. The 'af' stands for atomic fluorescence and thereby identifies the type of instrument used to analyze the sample. If more than one run is conducted on a given day, then the numbers 1, 2 … may be used to number successive runs in the day. The –A or –C refers to which atomic fluorescence instrument the samples were analyzed on. Tekran A has the serial number 001. Tekran C has the serial number 0154. Check to make sure the file is saved correctly before exiting the program. Then, the worksheet needs to be saved as an Excel and text file. The Excel and text file are saved to \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Mercury\Tekran with the same naming format, YYYYafMMDDt1_A.

11.7. Disconnect the pump tubing from the stannous chloride. Rinse out the stannous chloride line for 5 – 10 minutes by connecting it to DI water. Also make sure the phase separator is thoroughly rinsed in the process. Make sure the rinse water is continuously pumped away from the bottom drain on the phase separator.

11.8. After rinsing the lines for 5 – 10 minutes, release the pump tubing from the peristaltic pump rollers and the rinse pump.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

12. CALCULATIONS

12.1. Blank Correction - The mean of the five calibration blanks is subtracted from all samples and QC. The concentration of the blank correction is found by dividing the mean of the five calibration blanks by the mean calibration factor:

$$\text{Blank Correction} = \frac{\text{Mean or average of 5 calibration blanks}}{\text{Mean Calibration Factor}}$$

12.2. Results of samples are calculated by subtracting the blank correction from the instrument output and then dividing by the mean calibration factor:

$$\text{Blank-Corrected Concentration} = \frac{\text{Instrument Output} - \text{Blank Correction}}{\text{Mean Calibration Factor}}$$

12.3. A calibration factor is determined by dividing the instrument output of the calibration standard by the concentration (ng/L) of the calibration standard. The mean calibration factor is determined by averaging all calibration factors from each calibration standard.

12.4. Calculations for replicate analyses to evaluate precision, the recovery of laboratory control samples, and the recovery of matrix spikes are detailed in the Laboratory Quality Manual.

13. QUALITY CONTROL

13.1. The relative standard deviation (RSD) of the calibration factors for all calibration points must be less than 15 % where,

$$RSD = \frac{Standard\ Deviation\ of\ Calibration\ Factors}{Mean\ Calibration\ Factor} \times 100$$

13.2. The estimated MDL for the run is calculated by multiplying the standard deviation of the five blanks, by a factor of 3.7. This value must be below 1.0 ng/L. If it is not consult a supervisor. This requirement only applies for samples from the South Florida Water Management District (SFWMD).

13.3. Acceptance criteria for quality control measures are listed below in Table 3. Consult supervisor or see DEP SOP LB-027 for reporting data associated with out-of-control quality control measures.

TABLE 3.
Tekran 2600 Method QC Limits

	LEVEL	**RECOVERY**	**RPD**
PQL	3.0 ng/L	70 % - 130 %	

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

	LEVEL	RECOVERY	RPD
ICV (W-STD7)	30 ng/L	80 % - 120 %	
LFB	5.0 ng/L	80 % - 120 %	
NIST 2976	0.061 mg/kg	85 % - 115 %	
DUP. SPIKE	80 ng/L	80 % - 120 %	≤ 20 %
CCV (W-STD5)	15 ng/L	85 % - 115 %	

14. INTERFERENCES

14.1. Potential negative interferences for this method include oxygen and water vapor. Oxygen quenches the fluorescence signal, while the presence of water vapor scatters the fluorescence signal, thereby lowering the detector's response. Oxygen is largely eliminated from the system by the use of argon as the carrier gas and by using an oxygen trap to capture trace quantities of the gas. The soda-lime trap eliminates moisture in the carrier stream before it reached the gold traps.

14.2. Compounds which fluoresce at the same wavelength as mercury constitute a positive interference for this method. Aromatic hydrocarbons are serious interferents, since these compounds fluoresce strongly at 254 nm. Again this interference is eliminated by the use of argon carrier gas and by the use of a hydrocarbon trap in the argon and nitrogen gas streams.

15. CORRECTIVE ACTIONS FOR COMMON PROBLEMS

Corrective actions for common QC failures and out-of-control data are listed in Table 4 below.

TABLE 4.
Corrective Actions

Problem	Corrective Action
Calibration standard's response factor below expected value.	Check the detector lamp and its alignment.
Calibration curve correlation coefficient out of range.	Remake the standards and recalibrate the instrument.
Failure of instrument blank.	Check vials for contamination and the detector for drift. Change soda-lime, and clean the phase separator. If these measures fail to fix the problem, change the pump tubing.
Failure of CCV.	Check the detector for drift.
Failure of external SRM (NIST2976), PQL, matrix spikes, or replicate RPD.	Redigest the samples and repeat the analysis.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

16. SAFETY/HAZARDOUS WASTE MANAGEMENT

16.1. Refer to DEP SOP HG-013 for correct waste disposal procedures.

16.2. Always wear safety glasses or protective eyewear while working in the lab. Powder free latex gloves should be worn while handling chemicals. Concentrated acids should be handled in a ventilated hood. Since this method is conducted in a clean room, appropriate clean room garb, including a *Tyvek* coat, *Tyvek* shoe covers, and a hair net should be worn at all times in the clean room.

17. REFERENCES

17.1. Tekran 2600 instrument manual.

17.2. EPA Method 1631, RevE, "Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry", U.S. EPA (1996).

17.3. DEP SOP HG-001, Trace-Level Total Mercury in Water by Purge and Trap (Dual Gold Traps) and Cold Vapor Atomic Fluorescence Detection (CVAFD)

17.4. DEP SOP HG-012, Mercury Group Standards Preparation Procedures

17.5. DEP SOP HG-013, Mercury Waste Disposal

17.6. DEP SOP MT-058, Procedure for Metals Data Review and Authorization

17.7. DEP SOP MT-069, Metals Standard and Reagent Preparation Procedure

17.8. DEP SOP LB-027, Standard Operating Procedure for Reporting Qualified Data

17.9. DEP Bureau of Laboratories Health and Safety Manual located on the Bureau's intranet site.

17.10. DEP Laboratory Quality Manual.

17.11 EPA-821-R-01-013, Appendix to Method 1631 for Solids (holding time).

17.12 DEP-SOP-001/01 FS 1000 General Sampling Procedure (preservation).

Appendix of Significant Changes

07/22/2008 A couple of sections were consolidated into Section 10. Other minor revisions to the text were made for format and clarity.

07/22/2009 Added expiration dates and storage information to section 4.
Clarified MDL check requirement for the SFWMD.

04/27/2016 Preservation requirements and holding time added to section 6. References to the preservation and holding added to section 17.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.