Analysis of Total Mercury in Waters, Soils, and Sediments with the Tekran 2600 by Cold Vapor Atomic Fluorescence Spectrometry

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Method Summaries

The following standard operating procedure (SOP) describes the analysis of aqueous, particulate, and solid samples for total mercury (HgT) with a Tekran automatic mercury analysis system. The instrument functions as three interconnected Tekran subunits, and includes a detector (Tekran 2600, cold vapor atomic fluorescence spectrometer (CVAFS)), a peristaltic pump (Tekran 2610), and an autosampler (Tekran 2620).

Analyses of different sample matrices will vary slightly depending on type. Prior to analysis, samples require strong acid and bromine monochloride (BrCl) treatment, and is described elsewhere. Following preparation, the aqueous-phase of the sample is first pre-reduced with hydroxylamine hydrochloride (to neutralize BrCl) and then introduced to the instrument directly or as a diluted aliquot. Oxidized mercury (Hg^{II}) in the sample is reduced to gaseous elemental mercury, stripped from the aqueous sample by Argon gas via phase separator, and detected by CVAFS. Quality assurance and control protocols are employed throughout sample analysis, and include laboratory practices to prevent sample contamination, as well as the analysis of analytical blanks, sample replicates, and standard reference materials (SRM).

Laboratory Safety

Persons involved in this method must have read, understood, and signed the Chemical Hygiene Plan for the Wisconsin Mercury Research Laboratory (WMRL) prior to potential exposure to any chemicals. Specific safety concerns for most chemicals are addressed in the Material Safety Data Sheets(MSDS) which are located within the laboratory. Not included in the MSDS is BrCl; a strong oxidizer which should be treated as an extremely hazardous chemical. Additional hazardous chemicals used in this method include mercury (a potent neurotoxic agent), concentrated strong acids (HCl, H_2NO_3, and H_2SO_4) which are extremely corrosive and have irritating vapors, and $SnCl_2$. Adequate personal protection equipment such as safety glasses, gloves, and chemical resistant clothing must be worn when exposure to hazardous chemicals are possible. Hazardous chemicals should only be handled by adequately trained personnel under a high volume fume hood with extreme caution.

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

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

Reagent water: Ultra pure reagent grade water containing less than 0.1 ng/L Hg with a resistance greater than 18 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: EM Science Omni Pure HCl (containing less than 5 ng/L Hg) or equivalent.

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

Sulfuric Acid: EM Science Omni Pure H_2SO_4 (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 yellow to red to orange. Cap bottle loosely, stir for an additional hour, and remove stir bar.

Hydroxylamine hydrochloride ($NH_2OH*HCl$): Dissolve 120 g of $NH_2OH*HCl$ in a Teflon bottle containing 400 mL of reagent grade water. Add 50 µL $SnCl_2$ to the solution and purge with Argon at 30 mL/min for 1 hour. Store the $NH_2OH*HCl$ solution in the refrigerator when not in use and prepare fresh monthly.

Stannous chloride ($SnCl_2$): Add 60 g $SnCl_2$ to 20 mL concentrated HCl in a dark 2.5 L glass bottle by rinsing the dry chemical out of the weigh boat with reagent water. Once dissolved, bring the solution up to 2 L with reagent water. Purge with Argon at 30 mL/min during initial start up and during analysis. Store the $SnCl_2$ solution in the refrigerator when not in use and prepare fresh monthly.

Rinse water (1% HCl): Add 50 ml concentrated HCl to 5 L reagent water. Prepare daily.

1M KOH rinse solution: Add 28 g of KOH to 250 ml of reagent water and bring up to 500 ml.

Aqua Regia rinse: Add 25 and 75 ml of concentrated HNO_3 and HCl (respectively) to 100 ml of reagent water and bring up to 500 ml.

Soda lime: Purchased from Spectrum Chemicals, 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.

Sample Preparation

Soils, Sediments, and Waters

1. Weigh approximately 100 mg of sample into a Teflon bomb

2. Record sample information, such as sample ID, bomb ID, and sample mass into the appropriate Excel setup sheet. A minimum of one triplicate, three blanks, and three SRM's should be included in each run, with an additional blank, SRM, and triplicate for every additional ten samples.

3. Working under a fume hood, dispense concentrated acids into the Teflon bombs to digest samples.

    a. For soils and sediments, add 6 and 2 ml of HCl and HNO_3 (respectively) to the digestion bombs, cap loosely for one hour, wrench-tighten, and store under the fume hood at room temperature for 12 hours.
    b. For waters, upon receiving, ~6mL of BrCl is added to each sample. The samples are then heated at 50 º C for 5 days.

4. For soils and sediments, following acid digestion, bring up to 30 ml with a 5% BrCl solution and heat to 50 º C for 12 hours.

Analytical and Quality Control Standards

Upon receipt at the laboratory and on the day of preparation standard solutions should be labeled with the mercury 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. The concentrated (> 10 ng/ml) standard "stock" solutions should be stored outside of the working laboratory area to avoid contamination. 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.

1000 ng/L standard solutions: Dispense approximately 500 mL of reagent grade water and 5 mL of BrCl into a 1 L mercury clean volumetric flask. Add the appropriate volume of stock solution that results in a final concentration of 1000 ng/L, and bring solution up to a 1L volume with reagent grade water. Analytical and quality control standards should be made from different mercury sources, with the quality control standard lines always further identified as "QCS". Mix standard solution well and store in an appropriately labeled (concentrations, analyst initials, and date) amber glass bottle for up to one year.

1-40 ng/L standard solutions: Standards analyzed by the instrument range from 1- 40 ng/L, spanning the expected sample mercury concentrations. To make these standards, dispense approximately 500 mL of reagent grade water and 5 mL of BrCl into a 1 L mercury clean volumetric flask. Add the appropriate volume of the 1000 ng/L solution that results in the desired standard concentration, and bring solution up to a 1L volume with reagent grade water. Analytical and quality control standards should be made from different mercury sources, with the quality control standards always further identified as "QCS". Mix standard solution well and store in an appropriately labeled (concentrations, analyst initials, and date) amber glass bottle for up to one year. Analytical standards at 1, 2, 5, 10, 20, and 40 ng/L are generally used for the instrument calibration, while quality control standards at 5, 10, and 30 ng/L are analyzed for quality control requirements.

Instrument Operation

This document is intended as an additional SOP designed to guide the user through mercury analysis specific to the WMRL. A condensed version is also

provided following the detailed SOP, and is intended as a quick reference bench guide for the analyst. However, the analyst is required to be familiar with the detailed SOP as well as the original user's manual provided by Tekran which will be referred to when appropriate.

Start up

1. Prepare the peristaltic pump for operation. Inspect the pump tubing, looking for excessive wear and fatigue at the cassette and kinking of the tubing. Replace tubing when worn. Check the Teflon lines up and down stream of the pump tubing for blockage. Engage the cassettes into the pump head by locking down the lever on the right side of each cassette.

2. Prepare the liquid reagents for operation. Prepare 5 L of acidified rinse water and an adequate volume of $SnCl_2$ solution.

3. Activate the peristaltic pump by toggling the switch to "local". Immerse the Teflon lines labeled "rinse" and "$SnCl_2$" into the rinse water and check for flow. Allow the system to rinse for the duration of setup (approximately 20 min.) Immerse the Teflon line labeled "purge" into the $SnCl_2$ solution.

4. Prepare the instrument for operation.

    a. Check that the lamp light located on the front of the detector is not on. If the lamp light is on, see the User Guide for instructions on adjustment/replacement.

    b. Adjust the gas regulator for the purge flow to 100 mL.min and the phase separator flow to 400 ml/min.

    c. Remove the soda lime trap from the sample train, empty, and refill with fresh reagent. Inspect for excessive build up of soda lime dust in the quartz fiber filter, the intake/outlet orifices, and the gasket surface. Clean with concentrated HCL as necessary.

    d. Visually inspect the phase separator for buildup of particulate matter, and that rinse water covers the entire surface but does not bridge to the outside of the apparatus.

5. Open the Tekran software (shortcut on the desktop of HG7).

6. Click on the "run" icon (the left-most icon) to bring up the method selection window.

    a. Select the appropriate method for the analyzed samples.

i. Method #6 for waters and filters (single dip)
ii. Method #2 for sediments and biological (double dip)

b. Click “new” to open a new worksheet.

i. Record date and sample description in the “Description” field
ii. Record your initials in the “Operator” field

7. Select “Expansion2” from the Options menu to populate the worksheet with the default starting sequence.

8. Select the “Init” button in the pop-up window, the autosampler will activate indicating communication.

a. In the pop-up window, enter the sampler model as “223” and each of the five racks as “112”, and close window.

9. Send the autosampler back to the wash station by clicking “A/S wash” button.

10. Switch the Teflon line for $SnCl_2$ from rinse water to the $SnCl_2$ reagent, allow to flush the remaining rinse from the line (5 minuntes), and then switch the toggle for the pump from local to remote.

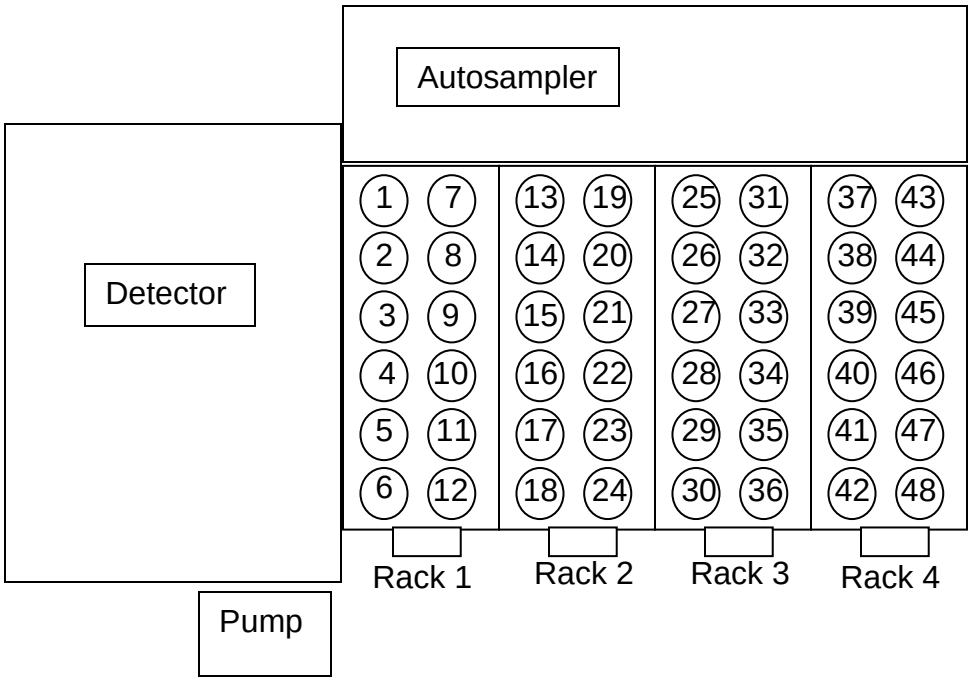

Figure 1. Overhead view of the Tekran automated mercury analyzer system.

Initial Calibration

1. Fill Rack 1 with twelve clean 60 ml autosampler vials.

2. Add 50 μl (for waters and solid samples) or 100 μl (for filters) of NH_2OH*HCl solution to vials in positions 1-9 to neutralize remaining BrCl.

3. Fill the autosampler vials with 45-50 ml of standards or other reagents, as described below. Rack 1 will continue to be utilized for QA/QC throughout sample analysis.

Vial Position	Standard or reagent
1	Reagent water
2	1 ng/L
3	2 ng/L
4	5 ng/L
5	10 ng/L
6	20 ng/L
7	40 ng/L
8	QCS
9	QCS
10	Reagent water
11	Aqua Regia rinse
12	Aqua Regia rinse

4. In the pop-up window (instrument control pad) on the sample worksheet select “Configure run”; a second window will appear (run configuration window).

    a) In the run configuration window, make sure the first and last samples are listed as worksheet lines 1 and 13 (respectively), and that the “clean” field is set to “Y”. Click “OK”.

5. Once the software loads the configured run list and the instrument is ready to analyze, a message will appear (“start run”) in the activity table window and the “Start run” button on the instrument control pad will become active.

6. Click “Start run” on the instrument control pad to begin analysis.

Sample analysis

1. Fill racks 2 or 3 (do not use Rack 4) with twelve clean 60 ml autosampler vials. Add 50 μl NH_2OH*HCl solution (100 μl for filters) to each sample vial as well as the QCS vials (8 and 9).

2. Pipette solid sample digestate (typically 0.1 – 1 ml), or pour waters and filter digestate samples directly (45 – 50 ml) into autosampler vials. Bring solid sample digestates up to a known volume (45 – 50 ml), and homogenize if necessary.

3. Refill the QCS vials with the appropriate standards, 5 ng/L and 10 ng/L for waters and filters, and 10 ng/L and 30 ng/L for solid samples.

4. Enter the sample information, followed by QCS analysis into the run list. Enter a single analytical event from each vial for waters and filter samples (single dip method), and two events from each vial for solid samples (double dip method). In the run list, you also need to specify sample identification, respective tube positions (sample positions 13-36, followed by 8 and 9 for QCS), and alternating rinse tube position (11 or 12).

5. Click "Configure run" in the instrument control pad and enter the appropriate tube positions for the first and last samples for that round of analysis, click OK, and start run.

6. Subsequent samples may be set up in the unused rack and analyzed provided the previous QCS samples were within 10% of the expected concentration.

Instrument Shutdown

1. Once analysis is complete the instrument must be properly cleaned in preparation for shut down.

2. Remove, empty, and rinse used vials. It is helpful to put them directly into an acid washing basket.

3. Cap the reagents and store the $SnCl_2$ in the refrigerator.

4. Place the $SnCl_2$, Purge, and Rinse lines into the first of a series of vials containing 1M KOH, MQ water, 10% Aqua Regia, and 1% HCl rinse solution (respectively) and switch the autosampler to "local". After all of the liquid has run through the system, the pump can be switched off.

5. Once the pump is off, unclamp all of the pumphead tubing.

Quality Assurance and Control Each analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This includes the ability to reproduce standards, produce acceptable relative percent differences between quality control samples and real environmental samples, and produce spike recoveries that meet acceptance criteria.

Blank: A blank is prepared by adding 50 mL of reagent water and 100 µL of $NH_2OH*HCl$ to a 60 mL autosampler vial. Blanks are critical to the reliable determination of Hg at low levels.

Standards: A standard curve is created by analyzing a series of analytical standards (1, 2, 5, 10, 20, and 40 ng/L). By plotting response vs.

concentration, a correlation coefficient is calculated. The standard curve must have a correlation coefficient greater than or equal to 0.995. All sample concentrations must fall within the calibration curve. If the sample concentration exceeds the upper limit of the standard curve, the sample must be diluted and reanalyzed. If the correlation coefficient fails to meet the above criteria, then an additional set of standards must be analyzed to rule out operator error. If the second set of standards fails, the analyst must isolate and correct the problem before continuing analysis.

Quality control sample: The quality control sample or QCS must be made from a mercury source different from that used for calibration standards. Each QCS must be analyzed prior to sample analysis. A 5 ng/L and 10 ng/L QCS is also analyzed after every rack of samples and at the end of the day. The recovery of the QCS must be between 90 and 110% (4.5 and 5.5 ng/L or 9.0 and 11.0 ng/L) of the expected value. If either of the initial QCS or any QCS analyzed prior to or subsequent to a batch of samples fails to meet the acceptance criteria, an additional QCS must be run. If the second QCS still does not meet acceptance criteria, the instrument is recalibrated and the QCS is analyzed until statistical control has been reestablished. After control has been reestablished, all samples analyzed since the last acceptable QCS measurement are reanalyzed. If insufficient sample volume remains to reanalyze the samples, they must be flagged appropriately.

Method Blanks: For every ten samples, a method blank must be processed. A minimum of 3 method blanks must be processed for a preparation batch. The preparation batch is all the samples that were prepared that day. A method blank includes an ashed QFF, 95 mL of reagent grade water, and 5 mL of BrCl. The method blank is used to correct for background levels of Hg found in reagents. All method blanks should have a mass of less than 0.20 ng. If a method blank has a mass greater than 0.20 ng, the batch will be evaluated and flagged.

Certified Reference Materials (CRM): A CRM that best represents the sample matrix is selected, processed, and analyzed every ten samples. The recovery of the CRM must be between 80 and 120% of the expected value. If a CRM falls outside the acceptable criteria, the batch needs to be evaluated and flagged.

Matrix Spike: A matrix spike is prepared by adding a known mass of Hg standard to an environmental sample. A matrix spike must be analyzed in every rack of samples. Therefore, the unspiked aliquot of the spiked sample in the second rack was analyzed in the first rack, for the third rack in the second, etc. Percent recovery for a matrix spike must fall between 85 and 115%. The percent recovery is calculated as follows:

$$\% \text{ Recovery} = ((|C_s - C_{us}|) / D) / ((M / V) / 1000) \times 100$$

C_s = Concentration of unspiked sample
C_{us} = Concentration of spiked sample
D = Dilution factor (sample aliquot volume in mL/50)
M = Mass of spike
V = Total volume of sample (mL)

If the percent recovery falls beyond the range of 90 and 110%, the environmental sample should be re-spiked, volume permitting, and another environmental sample should be spiked, to rule out any matrix interference. If the percent recoveries for the new spikes fall beyond the range of 90 and 110%, the sample set represented by the spiked samples are flagged.

Batch Detection Limit (BDL): A BDL is determined from the method blanks. The BDL is a function of the Hg detected in the reagents.

$$\text{BDL} = \sigma * 3$$

BDL = Batch Detection Limit
σ = Standard deviation from the mass of Hg detected in method blanks

If the BDL exceeds 0.059 ng, the run will be evaluated and the samples may be flagged.