Standard Operating Procedure for Analysis of Sediment for Total Mercury Using the Cold Vapor Technique with the Leeman Labs, Inc. Automated Mercury System

Theresa Uscinowicz, A & O Chemical Company[1]
and
Ronald Rossmann, USEPA
Large Lakes Research Station
9311 Groh Road
Grosse Ile, MI 48138

Mid-Continent Ecology Division - Duluth
National Health and Environmental Effects Research Laboratory

October 9, 1996

Revision 1

[1] Current affiliation is SoBran, Inc./Pathology Associates International.

Standard Operating Procedure for Analysis of Sediment for Total Mercury Using the Cold Vapor Technique with the Leeman Labs, Inc. Automated Mercury System

1.0 Introduction

Elemental concentrations of mercury in sediment and water are determined by the PS200 system, and its operation is based upon cold vapor AAAS. The prepared sample enters the system in the divalent phase, and is mixed with stannous chloride to form elemental mercury vapor. This mixture moves to the liquid gas separator, and argon carries the mercury vapor through a drying tube for vapor removal. The vapor enters one path of the cell optimized. The mercury lamp emits light at 254 nm, and absorbance is measured by the detector.

2.0 Materials Required

2.1 Chemicals

Reagents needed include the following ultra pure grade chemicals:

2.1.1 Leeman Labs 100ppm Mercury Standard

2.1.2 Leeman Labs Hydrochloric, Nitric Acids

2.1.3 Leeman Labs Ultra Pure Water

2.1.4 Liquinox

2.1.5 J.T. Baker Brand Hydrochloric, Nitric Acids

2.1.6 J.T. Baker Brand Stannous Chloride or Leeman Labs Stannous Chloride

2.1.7 Hydroxylamine Hydrochloride

2.1.8 Magnesium Perchlorate 10-20 size mesh

2.1.9 Potassium Permanganate

2.2 Equipment and Supplies

2.2.1 Supplies Needed for analyses include:

2.2.1.1 PS200 Automated Mercury Analyzer with autosampler, with

associated data acquisition system

2.2.1.2 AP200 Automated Preparation System with associated data acquisition system

2.2.1.3 Analytical Balance in Biology Lab, Mettler 2100T

2.2.1.4 EDP pipettors and associated disposable tips

2.2.1.5 Associated pump tubing for sample drainage, tin chloride

2.2.1.6 Polyethylene tubes 12 mL capacity, and caps

2.2.1.7 Polyethylene tubes 45mL capacity

2.2.1.8 Teflon or polyethylene beakers

2.2.1.9 Teflon wash bottles

2.2.1.10 Teflon bottles (60 mL)

2.2.1.11 Electronic balance

2.2.1.12 PVC gloves

2.2.1.13 Paper towels, clean wipes

2.2.1.14 Lubricating oil for autosampler

2.2.1.15 Quartz wool and quartz glass drying tubes

2.2.1.16 Teflon spatula

2.2.2 Supplies needed before sample analyses if not using automated preparation system:

2.2.2.1 CEM microwave digester with associated Teflon PFA vessels

2.2.2.2 Low density 30-mL polyethylene bottles for sample storage

2.3 Reference Documents

The user of this method must be familiar with the following established standard operation procedures:

LLRS-MET-SOP-001 Standard Operating Procedures for the Preparation of Materials used for Ultra-low Trace Element Analyses

LLRS-MET-SOP-003	Standard Operating Procedures for the Maintenance of the LLRS Trace Metal Laboratories
LLRS-MET-SOP-010	Standard Operating Procedures for Analysis of Total Mercury in Tissue and Sediment using the Cold Vapor Technique with the Perkin-Elmer Model MHS-20 Gold Amalgam System
LLRS-QA-001	Minimum Analytical Quality Assurance Objectives for U. S. EPA Large Lakes Research Station
LLRS-QA-SOP-001	Standard Operating Procedures for the Release of Data
LLRS-QA-SOP-002	Standard Operating Procedures for the Routine Review of Data Quality and Quantity

3.0 Reagent Preparation

3.1 5% Nitric Acid Solution

This acid solution is used for rinsing materials used for cleaning materials used in the analysis of samples.

3.4.1 Rinse a pre-cleaned graduated cylinder (100 mL) with three rinses of MSQ.

3.4.2 To the graduated cylinder, add 95 mL of MSQ.

3.4.3 Carefully add 5 mL of concentrated reagent grade nitric acid.

3.4.4 Transfer the solution to a squirt bottle.

3.4.5 Repeat steps 3.4.1 through 3.4.3 but add five milliliters of reagent grade acid. (For preparation of 5% nitric acid rinses).

3.4.6 Repeat step 3.4.4 but add to separate precleaned teflon bottle.

3.2 10% Hydrochloric Acid Rinse Solution

Use reagent grade J.T. Baker or Leeman Labs hydrochloric acid. The amount needed for a one day run is 300 mL. Approximately 2 L will be needed to be prepared weekly.

WARNING

Hydrochloric acid is highly corrosive and incompatible with metals, hydroxides, amines, and alkalis. Handle it while wearing personal protection gear. A full face shield is recommended. Always use the concentrated acid under a fume hood. Store it

in an appropriate place. Store concentrated acid in a corrosives cabinet.

3.2.1 Use a precleaned 1000 mL polyethylene graduated cylinder. Carefully rinse the inside of the cylinder with 5% reagent grade nitric acid using a Teflon (precleaned) squirt bottle. Follow this rinse with three rinses of Millipore Super-Q water (MSQ).

3.2.2 Add 900 mL of MSQ to the graduated cylinder.

3.2.3 Carefully add 100 mL of reagent grade hydrochloric acid to the cylinder.

3.2.4 Transfer the mixture to a high density polyethylene bottle from Leeman Labs.

3.3 1:1 Nitric Acid Solution

As recommended by the manufacturer, polyethylene autosampler cups must soak in 1:1 nitric acid before running to ensure acceptable results. Soak the cups for at least two hours. Use J.T. Baker trace metal grade nitric acid for preparation. This solution can be recycled, provided the autosampler cups are adequately rinsed with MSQ a minimum of ten times before and after use. Dispose of 1:1 nitric after one month to eliminate the possibility of any residual contamination.

WARNING

Nitric acid is corrosive and incompatible with combustible materials, metallic powders, hydrogen sulfide, carbides, and alcohols. Handle it with personal protection. A full face shield is recommended for the concentrated acid. Always use the acid under a fume hood. Store the concentrated acid in a corrosives cabinet.

3.3.1 Use an appropriate pre-cleaned container. An empty Suprapure hydrochloric acid bottle has been used. Rinse the container at least three times with MSQ before addition of nitric acid.

3.3.2 Using the graduations on the glass bottle, add 400 mL MSQ.

3.3.3 Carefully add 400mL of J.T. Baker nitric acid to the glass bottle.

3.3.4 Swirl the contents of the bottle and , if necessary, label.

3.4 10%(w/v) Stannous Chloride Solution

The volume of tin chloride solution required to run is dependent upon the daily run time. To run for six hours approximately 250 mL are required.

WARNING

Stannous chloride should be handled with care. Avoid contact with eyes, skin, and clothing. Avoid breathing its dust. Handle solid chemical under a fume hood. Handle while wearing personal protection gear for eyes and skin.

3.4.1 Rinse a precleaned wide mouth teflon bottle three times with quartz distilled water.

3.4.2 Tare the bottle and add 25 g of tin chloride to the bottle.

3.4.3 Rinse a precleaned graduated cylinder with 5% reagent grade nitric acid solution followed by three rinses of MSQ.

3.4.4 Carefully add 25 mL of reagent grade hydrochloric acid to the graduated cylinder. Carefully pour the cylinder's contents into the bottle containing the stannous chloride.

3.4.5 Swirl the contents of the bottle vigorously to dissolve the stannous chloride.

3.4.6 After the stannous chloride has been dissolved, add 200 mL of MSQ using the same graduated cylinder.

3.4.7 Replace the cover and vigorously shake the bottle to ensure all of the tin chloride is dissolved.

3.5 10% Nitric Acid Solution

This acid solution is used for preparation of standards and for dilution of samples. Historically, this solution is used for samples that have undergone microwave digestion.

3.5.1. Rinse a pre-cleaned graduated cylinder (100 mL) with 5% reagent grade nitric acid solution followed by three rinses of MSQ.

3.5.2 To the graduated cylinder, add 90 mL of MSQ.

3.5.3 Carefully add 10 mL of concentrated Seastar or reagent grade nitric acid (whichever matches the matrix of samples).

3.5.4 Transfer the solution to a teflon bottle.

3.6 Working Mercury Standard Solution

Commercial 100 ppm Leeman Labs mercury standard is used to prepare the working standard. The working standard is prepared at a concentration of 100 ppb or 0.1 µg Hg/mL and is made fresh weekly.

WARNING

Mercury is a poison. Handle it, its compounds, and its solutions with personal protection. Mercury can form a vapor and be inhaled. It also is absorbed through the skin. Use this material in a fume hood. Always wear personal protection gear.

3.6.1 Rinse a pre-cleaned teflon bottle (LLRS-MET-SOP-001) three times with MSQ and air dry.

3.6.2 Using a precleaned graduated cylinder, rinse three times with 5% nitric and three times with MSQ.

3.6.3 Using two EDP separate automatic pipettors, rinse 2-1000 mL tips three times with a 5% reagent grade nitric solution followed by three rinses with MSQ.

3.6.4 Tare a dry bottle on the electronic scale.

3.6.5 Using an additional EDP pipettor, rinse a 100 µL tip in the same manner as 3.6.2

3.6.6 Using the cleaned 100 µL tip, carefully add 50 µL of the commercial 100 ppm Leeman Labs mercury standard solution to the bottle.

3.6.7 Transfer 45mLs of the 10% nitric acid solution using the cleaned graduated cylinder.

3.6.8 Using an EDP pipettor, with a 1000 µL precleaned tip, add 10% nitric acid solution to the bottle until the weight is 50 g.

3.7 Preparation of Recommended Range of Calibration Standards

The calibration standards are prepared fresh semiweekly.

3.7.1 Microwave Digestion Standard Range

The following range of standards has been used for analyses of Green Bay Sediment samples and bracket the samples well. 0.250 ppb, 0.500 ppb, 1.00 ppb 2.00 ppb, 5.00 ppb are used (0.00025 µg/mL, 0.00050 µg/mL, 0.001 µg/mL, 0.002 µg/mL, 0.005 µg/mL). It may be possible to go below 0.250 ppb depending upon instrument performance. The lowest concentration above background noise

is approximately 0.087 ppb (0.000087 µg/ mL). 60 mL of each calibration standard will last for two daily runs. Prepare double of the desired standard that will be run as a check standard. The autosampler cups must be filled to at least 40 milliliters, (60 mL total capacity).

3.7.1.1 Rinse six pre-cleaned teflon bottles three times with MSQ and allow to air dry.

3.7.1.2 Prepare two separate EDP pipettors each with a precleaned 1000 µL tip. (Rinse each pipette tip three times with 5% nitric acid followed by three rinses with MSQ) .

3.7.1.3 Rinse a precleaned graduated cylinder with 5% nitric acid followed by three rinses of MSQ.

3.7.1.4 For each standard, tare each bottle individually**.**

3.7.1.5 For the 0.250 ppb standard, pipette 150 µL of the 100 ppb working standard to the bottle.

3.7.1.6 Using the graduated cylinder, bring the total volume up to 55 mL by the careful addition of prepared Seastar 10% nitric acid. The weight of the bottles contents should now be roughly 55 g.

3.7.1.7 Add prepared Seastar 10% nitric acid with the other EDP pipettor and precleaned tip until the total weight is 60 g.

3.7.1.8 Repeat steps 3.7.1.6 - 3.7.1.9 for the remaining standards by the addition of 300 µL of the 100 ppb standard for a 0.500 ppb calibration standard, 600 µL of the 100 ppb standard for the 1.00 ppb calibration standard, 1200 µL of the 100 ppb standard for the 2.00 ppb calibration standard, and 3000 µL of the 100 ppb standard for the 5.00 ppb calibration standard.

3.7.1.9 Do not recycle the standards remaining in the autosampler cups at the end of the day. Properly dispose of these daily. Attempts to recycle the standards from autosampler cups have given diminished intensities.

3.7.2 Automated Digester Standard Range

Use the following range of standards: 0.000 ppb, 0.125 ppb, 0.250 ppb, 0.500 ppb, 1.00 ppb, 2.00 ppb. Prepare in a 2% hydrochloric acid matrix in precleaned Teflon bottles. Use Leeman Labs or J.T. Baker hydrochloric acid. Prepare 50mL of each

standard weekly or as needed.

3.7.2.1 0 ppb Standard

3.7.2.1.1 Rinse a precleaned graduated cylinder with 5% nitric acid followed by three rinses of MSQ. Dispose of waste in an appropriate container.

3.7.2.1.2 Rinse a precleaned 500mL teflon bottle three times with MSQ.

3.7.2.1.3 Add 245 mL MSQ to the bottle.

3.7.2.1.4 Carefully add 5 mL of concentrated hydrochloric acid to the bottle.

3.7.2.1.5 Transfer the solution to a teflon bottle.

3.7.2.2 0.125 ppb Standard

3.7.2.2.1 Prepare one EDP pipettor with a precleaned 100 µL tip.

3.7.2.2.2 Prepare two separate EDP pipettors each with a precleaned 1000 µL tip.

3.7.2.2.3 Rinse a teflon bottle three times with MSQ that will be used for each standard.

3.7.2.2.4 Tare the bottle on the balance.

3.7.2.2.5 Pipette 62.5 µL of the 100 ppb working standard into the bottle

3.7.2.2.6 Use the rinsed graduated cylinder to transfer 45 mL of the 0 ppb standard to the bottle.

3.7.2.2.7 Slowly pipette 5 mL of 0 ppb standard to the bottle until the bottle contents weight 50 g.

3.7.2.3 0.250 ppb Standard

For the 0.250 ppb std, follow steps 3.7.2.2.1 through 3.7.2.2.4. In step 3.7.2.2.5, substitute 125 µL of the 100 ppb working standard using a new 1000 µL precleaned tip. Follow steps 3.7.2.2.6 through 3.7.2.2.7.

3.7.2.4 0.500 ppb Standard

For the 0.500 ppb std, follow steps 3.7.2.2.1 through 3.7.2.2.4. In step 3.7.2.2.5, substitute 250 µL of the 100 ppb working standard using a new 1000 µL precleaned tip. Follow steps 3.7.2.2.6 through 3.7.2.2.7.

3.7.2.5 1.00 ppb Standard

For the 1.00 ppb std, follow steps 3.7.2.2.1 through 3.7.2.2.4. In step 3.7.2.2.5, substitute 500 µL of the 100 ppb working standard using a new 1000 µL precleaned tip. Follow steps 3.7.2.2.6 through 3.7.2.2.7.

3.7.2.6 2.00 ppb Standard

For the 2.00 ppb std, follow steps 3.7.2.2.1 through 3.7.2.2.4. In step 3.7.2.2.5, substitute 1000 µL of the 100 ppb working standard using a new 1000 µL precleaned tip. Follow steps 3.7.2.2.6 through 3.7.2.2.7.

3.8 Reagents Needed for Preparation of Sediment Samples using Protocol PRP7471

3.8.1 50% Aqua Regia (3:1 Hydrochloric : Nitric)

3.8.1.1 Use the glass container that previously held the Suprapure hydrochloric acid, this is supplied with graduations. Rinse three times with MSQ to eliminate any residual contamination.

3.8.1.2 To this glass container add 400 mL of MSQ water.

3.8.1.3 To the glass container add 300 mL of Leeman Labs hydrochloric acid to the bottle.

3.8.1.4 Finally add to the bottle 100 mL of Leeman Labs nitric acid.

3.8.1.5 Carefully transfer solution to instrument bottle.

3.8.2 Potassium Permanganate Solution

3.8.2.1 Rinse a 100 mL precleaned finely graduated cylinder three times with 5% reagent grade nitric acid followed by three rinses of MSQ.

3.8.2.2 Dispose of the instrument bottle's contents if applicable and rinse three times with MSQ.

3.8.2.3 Add 40mLs of the Leeman Labs potassium permanganate

solution to the rinsed 100 mL graduated cylinder.

WARNING

Potassium permanganate is a strong oxidizer. Keep it from contact with clothing or combustible materials. Avoid contact with eyes or skin. Avoid breathing the dust. Handle solid chemical under a fume hood. Handle it while wearing personal protection gear.

3.8.2.4 Transfer to the instrument bottle.

3.8.2.5 Add 760 mL of MSQ to the bottle.

3.8.2.6 Swirl the contents of the bottle to ensure a mixed solution.

3.8.2.7 Attach to the instrument.

3.8.3 Hydroxylamine Sulfate Solution

3.8.3.1 Rinse a 100 mL precleaned finely graduated cylinder three times with 5% reagent grade nitric acid followed by three rinses of MSQ.

3.8.3.2 Dispose of the instrument bottle's contents if applicable and rinse three times with MSQ.

WARNING

Hydroxylamine sulfate is an eye, skin, inhalation, and ingestion hazard. It will cause skin irritation and may be absorbed through the skin. Always wear eye and skin protection.

3.8.3.3 Add 96mL of the Leeman Labs Hydroxylamine Sulfate solution to the 100mL graduated cylinder.

3.8.3.4 Transfer the Hydroxylamine Sulfate to the instrument bottle and continue to add 704 mL of MSQ to the instrument bottle.

3.8.3.5 Swirl bottle to ensure a mixed solution.

3.8.3.6 Attach bottle to instrument.

NOTE

This is a modification of the protocol that calls for full strength concentrations of Potassium Permanganate and Hydroxylamine Sulfate. Full strength concentrations of these chemicals were attempted and the fumes generated were very strong. There was no difference in instrument performance in using some higher concentrations of reagents in the protocol. Both gave acceptable recoveries on the SRM material and similar instrumental intensities.

4.0 Microwave Sample Preparation

4.1 Preparation of Teflon Digester Vessels

Teflon digester vessels are cleaned following the procedure in section 4 of LLRS-MET-SOP-010. An alternate method proposed by the manufacturer to increase the life of the digestion vessels is under consideration. It may not be used until it is verified that blanks are equally low for the two methods. Upon verification, the following alternate steps may be substituted for steps 4.4 through 4.7 in LLRS-MET-SOP-010. Steps are taken directly from CEM manual with slight variations to fit constraints of laboratories (Gilman 1988).

4.1.1 Add 20 mL of concentrated nitric acid to the digestion flask. Place the safety disk on the vessel and tighten finger tight only. Place the vessel in the turntable, and attach a venting tube.

4.1.2 Repeat step until the turntable contains 12 vessels.

4.1.3 Turn the MDS-81D exhaust onto the maximum fan speed. Ensure the turntable is rotating.

4.1.4 Program the instrument for five minutes and 100% power. Depress the start key and allow the acid to heat.

4.1.5 Allow the acid to cool to room temperature and manually vent each vessel. Open vessels and pour the acid into an appropriate waste container.

4.1.6 Rinse the vessels three times with MSQ water and allow them to dry in a clean area.

4.2 Extraction of Samples

The maximum weight of samples extracted in CEM vessels without venting and still obtaining acceptable recoveries for all metals has been two grams. For mercury, an average weight of 0.300g has been used. The total volume is 25mL in 10% Seastar nitric acid. Follow steps 5.1.1 through 5.1.11 in LLRS-MET-SOP-010 for preparation of sediments

5.0 AUTOMATED DIGESTER (AP200) SAMPLE PREPARATION

5.1 Daily Instrument Setup

5.1.1 Clean autosampler rails with isopropyl alcohol.

5.1.2 Lubricate rails with oil daily

NOTE

Only if reagents are low or instrument was in shutdown will following steps need to be followed (5.1.3 through 5.)

5.1.3 Open cover and carefully disconnect each bottle separately from the interior of the instrument.

5.1.4 Rinse each bottle three times with MSQ

5.1.5 Fill bottles numbered 1, 5, 6 with MSQ up to the 800ml mark.

5.1.6 Prepare and fill bottle #3 with the Potassium Permanganate Solution.

5.1.7 Prepare and fill bottle #4 with Hydroxylamine Sulfate Solution

5.1.8 Carefully prepare and fill bottle #2 with the 3:1 Aquaregia solution

5.1.9 Check conditions of all fittings, caps and bottles

5.1.10 Close cover and pressurize system (turn gas on).

5.1.11 Set gas pressure = 20 psi

CAUTION

Do not exceed 25 psi

5.1.12 Turn instrument on

5.1.13 Turn computer and monitor on.

5.1.14 Turn printer on and press the online button.

5.1.15 After the computer has booted up, at the C: prompt, type apps.

5.1.16 Follow instructions on p4-4 of manual revision c.

5.2 Software and Instrument Setup

Follow the instruction in system startup section 4-3, What follows is a summary. The user should review the following sections prior to analyses.

References to AP200 Manual

Section 3- System Testing
Section 4- System Operation
Section 5- Routine Maintenance
Section 6- Troubleshooting

5.2.1 Select Protocal and Get PRP7471, defines method, (sec 4-4 of manual)

5.2.2 Check reagent pressure of system, ensure it is > then 5.5psi and within 6.5. (section 2-10 of manual)

5.2.3 Go to menu, F1, select **U**tility, select Diagnostics

5.2.4 Select Reagent Pressure

5.2.5 Run the change rinse solution macro, @CHRINS

5.2.6 Rinse the autosampler rinse tray 3-4 times with MSQ

5.2.7 Replace the rinse tray

.

5.2.8 Run the wake up macro @WAKEUP (section 4-3 of manual)

5.2.9 Check centering of autosampler tip over cups, adjust if necessary (section 3-1)

5.2.10 Check precision of dispenser 1X/week (section 3-3), or if there is a pressure change of .2 psi in system at step 14.3.3

5.2.11 Set up autosampler sequence, and start finish sequence (section 4.6)

5.3 Preparation for Sample Digestion

5.3.1 Soak the 45mL standard cups and sample cups in 1:1 HNO_3 for at least 2.0 hours.

5.3.2 Recycle the acid from the cups back into the Suprapure Acid bottle.

5.3.3 Rinse each cup three to six times with MSQ and allow to air dry.

5.4 Preparation of Standards for Automated Digestion

` 5.4.1 Prepare an EDP pipettor with a precleaned 1000ul tip.

5.4.2 Using the electronic scale, tare the beaker, and then the standard cup within the beaker.

5.4.3 Record the weight of the empty cup on the extraction sheet.

5.4.4 Under the laboratory hood, pipette 5mls of the 0 ppb std to the cup.

5.4.6 Weigh the cup to confirm volume delivered is 5mls, and adjust accordingly with the EDP pipettor.

5.4.7 Repeat steps 16.2 - 16.5 to extract multiple 0 standards.

.

5.4.8 Repeat steps 16.1 to 16.5 for the 0.125ppb std, 0.250ppb std, 0.500ppbstd. 1.00ppb std, and 2.00ppb std. Each time use a new precleaned 1000ul tip.

5.4.9 Rinse polyethylene vapor covers 3x with MSQ and place over sample cups.

5.4.10 Snap in place aluminum guard over polyethylene vapor barrier.

5.5 Preparation of Sediment Samples for Automated Digestion

Note

Please read the instruction manual for the Mettler Analytical Balance before proceeding.

5.5.1 Rinse a precleaned teflon spatula with 5% nitric acid.

5.5.2 Rinse the spatula three times with MSQ.

5.5.3 Use the analytical balance in the Biology Lab, Mettler 2100T

5.5.4 Tare the 100mL polyethylene beaker provided.

5.5.5 Add the 45mL sample cup to the beaker and tare the scale again.

5.5.6 Record the weight of the sample cup on the digestion sheet.

5.5.7 Carefully open the Whirlpak bag of sediment.

5.5.8 Open the Whirlpak bag partially, so only a small circular opening exists (about the size of a nickel). Caution: if the bag is open all the way, dust from the bag tends to migrate upwards.

5.5.9 Using the precleaned teflon spatula, carefully scoop an aliquot of sample = 0.100g.

5.5.10 Transfer this aliquot to the sample cup. Note: sand like samples will require a very small aliquot, and silty samples will require a larger aliquot.

5.5.11 Record the weight of the sample in the most significant digits available on the extraction log sheet.

5.5.12 Place the cup in its designated slot in a sample rack.

5.5.13 Zero the scale with the polyethylene beaker on it.

5.5.14 Rinse the teflon spatula with MSQ water three times.

5.5.15 Wipe teflon spatula dry with a fresh clean wipe square between samples.

5.5.16 Repeat steps 16.6.5 till 16.6.15 for the desired amount of samples, usually fourteen.

5.5.17 Load dummy cups into any space not occupied by actual sample.

5.5.18 Rinse polyethylene vapor covers 3x with MSQ and place over sample cups.

5.5.19 Snap in place aluminum guard over polyethylene vapor barrier.

5.6 Initiation of the Digestion Procedure

5.6.1 Load autosampler racks into PS200

5.6.2 Confirm autosampler start to finish sequence is correct.

5.6.3 Go to Main Menu, F1, press MACRO key, and type in @PRP7471

5.6.4 Method will begin to run at this point.

5.6.5 User will be prompted the following: Wait before reducing with KMNO4, answer Y

CAUTION

User will be prompted near end of 5 hr procedure: Add Hydroxylamine Sulfate, answer Y. Method will not proceed without this input.

5.7 Shutdown of AP200

5.7.1 After protocol is completed, wipe down bath to remove any remaining water.

5.7.2 Run an @APNAP two or three times to clean the dispenser with water.

5.7.3 Remove chemicals and replace reagents with MSQ if it will not be run for an extended period, and run an @CLEAN twice. This cleans not only the dispenser, but all reagent lines.

6.0 Automated Analysis of Digested Extracts

6.1 Preparation of the Instrument

6.1.1 Preparation of Drying Tube

6.1.1.1 Rinse a quartz drying tube three times with MSQ, followed by a dilute rinse of Liquinox, if it previously contained perchlorate.

6.1.1.2 Rinse several times with MSQ to eliminate Liquinox residuals.

6.1.1.3 Allow tube to air dry.

6.1.1.4 Rinse the teflon spatula three times with MSQ and dry with a fresh clean wipe.

6.1.1.5 Gently place a small plug of quartz wool into one end of drying tube.

6.1.1.6 Carefully pour the Leeman Labs Magnesium Perchlorate into the plugged drying tube. Try to fill with coarse grained perchlorate. Do not overfill the drying tube. Overfilling will cause the drying tube to become blocked more easily once it becomes moistened by the gas stream. When

filled, the perchlorate should be able to move within the tube when gently moving the drying tube from side to side.

WARNING

Magnesium perchlorate is moderately toxic and a strong oxidizing material. It is a dangerous fire and explosion risk in contact with organic materials. It is an inhalation hazard, and contact with skin or eyes can cause irritation. Work with it in a fume hood while wearing skin and eye protection.

6.1.2 Autosampler Tray Rinse with 10% Hydrochloric Acid

6.1.2.1 Rinse the autosampler rinse tray three times with MSQ.

6.1.2.2 Fill the tray with the 10% hydrochloric rinse prepared in step 3.1.

6.1.3 Tin Chloride Rinse

6.1.3.1 Rinse the tin chloride bottle out three times with MSQ.

6.1.3.2 Fill it with the 10% tin chloride prepared in step 3.3.

6.1.4 Check Tubing Condition and Adjust to Appropriate Tension

6.1.4.1 Check condition of tubing for flattening, abrasion or other signs of wear. If flattened, replace it.

6.1.4.2 Adjust tension on clamps to a halfway point. The sample line should be halfway minus one notch.

CAUTION

Do not over tighten clamps. Too much tension will cause tube flattening and a decrease in overall sensitivity.

6.1.5 Clean and Oil Autosampler Rails

6.1.5.1 Using a clean wipe or clean a paper towel, wipe the autosampler rails with isopropyl alcohol.

6.1.5.2 Place a small amount of oil on bottom of each rail.

6.1.5.3 Complete a high stress maintenance cleaning monthly.

6.1.6 Warmup Period

6.1.6.1 Allow the instrument to warmup for at least one hour time before analyses. If the instrument was in SHUTDOWN mode for an extended period allow it to warm up for several hours.

6.1.6.2 Software commands for warmstrt or coldstrt are found on page 16 of the manual.

6.1.6.3 Perform a COLDSTRT or WARMSTRT

6.1.7 Optimize Optics with an Aperture Test

6.1.7.1 Go to Main Menu, F1

6.1.7.2 Select Diagnostics, Select Aperture Test

6.1.7.3 Unscrew screw which is furthest out until the minimum absorbance is obtained. An acceptable value is 0-100.

6.1.7.4 Select Test Optics from Diagnostics menu, and confirm gain, or intensities are in the range of 500000-1100000 (= to voltage on lamp).

6.1.7.5 Difference between the two beams must be less than 100,000.

6.2 Software Setup for Routine Analysis

Consult Leeman Labs Automated Mercury Analyzer Manual pp 17-29 for guidance on the software setup. What follows are the software and instrumental parameters used to date. Complete the following steps before analyses.

6.2.1 Establishing a Protocol = Method file that Contains All Instrumental Parameters

6.2.1.1 From the Main Menu, select Protocol and then select **G**et.

6.2.1.2 To create a new Protocol, enter its name.

6.2.1.3 Suggested protocol naming is as follows: YYMMDD (Year, Year, Month, Month, Day, Day. Example 960916F = September 16, 1996.

Note
Limitation is 8 Characters

6.2.1.4 Computer will guide you through prompts (PS200 Manual pp. 18-19).

6.2.2 Creating a Folder=Data File

6.2.2.1 Press F1, to be at the Main Menu.

6.2.2.2 Select Data output and select Open Folder.

6.2.2.3 Type in a folder name, suggested name = same name as protocol to avoid confusion.

6.2.2.4 Follow instructions on page 19 of the manual.

6.2.3 Entering Instrumental Parameters

6.2.3.1 Follow instructions in PS200 instrument manual on pp 20-21.

6.2.3.2 The following are parameters used to date:

Integrations:	1
Uptake Time:	10
Weight:	Y
Dilution:	Y
Percent Recovery:	N
On/Off:	10 (higher integration time generates a nonlinear curve)
Flow Rate:	0.30 L/min

6.2.4 Standard Concentration Calculations

For those standards that are prepared with the automated digestion system, the appropriate concentration to be used for keying in standard concentration data must be calculated. Do not do this for microwave digested samples.

6.2.4.1 Tare a 100ml polyethylene beaker on the electronic scale.

6.2.4.2 Weigh a sample cup.

6.2.4.3 Record the weight on the extraction log, and zero the scale.

6.2.4.4 Repeat 6.1.1 through 6.1.3 for all sample cups.

6.2.4.5 Apply the following formula to calculate the final concentration of the extracted standards:

$$C_2 = C_1 * V_1/V_2 ;$$

where,

C_1 is the prepared standard concentration,
C_2 is the final concentration in the standard,
V_1 is the volume of prepared standard used,
and V_2 is the total volume of the extract

For example if 5 mL of 0.500 ppb standard was added to the digestion tube and the final volume of the extract is 43 mL, the resulting standard concentration is as follows:

$$C_2 = 0.500 \text{ ppb} * 5 \text{ mL}/43 \text{ mL} = 0.058 \text{ ppb}.$$

6.2.4.6 Record this concentration on the extraction log.

6.2.5 Entering Standard Concentrations

6.2.5.1 Follow instructions on p. 21 of the manual. Enter units in ppb**,** not ppm (i.e. 0.500 not 0.00050).

6.2.5.2 Calculations are only carried out to three decimal points, 0.00050 will be truncated to 0.000.

6.2.5.3 Do not enter terms of units, i.e. ppb, ppm. The final calculation will be in ug/g.

6.2.5.4 The following are ranges of standards used for analysis. These ranges have been successful in bracketing low level samples.

Microwave Digestion Standards (ppb)	Automated Prepared Standards (ppb) (Dependent upon total volume of extract. See section 6.2.4)
0.000	0.0000
0.250	~0.0140
0.500	~0.0280
1.000	~0.0570
2.000	~0.1140
4.000	~0.2280

6.2.6 Reset Calibration Intensity Data

Follow instructions on page 22 of manual.

6.2.7 Autosampler Rinse Time

6.2.7.1 Follow instructions on page 23 of manual.

6.2.7.2 Use a rinse time of 60, not 50 seconds. This is the rinse time between samples in the analyses mode.

6.2.8 Autosampler Rack Entry

6.2.8.1 For basic entry information, refer to page 25 of the manual. For extended information on macros and advanced command see reference section A-B-7.

6.2.8.2 What follows is an example of an autosampler rack file. It was used for analyses of Green Bay sediment. Prepare the file before analyses.

NOTE

Actual sample weight must be multiplied by 1000 to obtain results in ug/g.
Total volume = extraction volume * dilution factor.

1 0PPB	1.000		
2 GII25F102SQ*	323.00	250.00	SRM diluted 10x extracted in 25mls
3 GB88-71	280.00	125.00	Sample diluted 5x extracted in 25mls
4 GB89-73	241.00	125.00	
5 GB89-74	5.700	125.00	

A microwave digested SRM will need to be diluted 10 to 20 times depending upon the weight of the sample to be within range of standards. Certified value for SRM2704 = 1.47 ug/g. For a sample that has a extraction weight of 0.250g in 25mL, (1.47*.250)/25=0.018375 ppm=18.37 ppb. This is diluted 20X = .918 ppb

6.3 Analysis of Extracts

6.3.1 Filling Autosampler Cups

6.3.1.1 Recycle the 50% acid rinse used in the autosampler cups. Place it in the

glass SUPRAPURE acid bottle.

6.3.1.2 Rinse each cup 5-10 times with MSQ.

6.3.1.3 Allow cups to air dry

.

6.3.1.4 Fill each standard cup with its designated standard.

6.3.1.5 Using two separate EDP pipettors, prepare a clean 10mL tip and 1000uL tip. Rinse each tip three times with 5% nitric acid followed by three rinses of MSQ.

6.3.1.6 For samples and SRMS requiring dilution, dilute to at least half their capacity of the autosampler tips (6mL). First add the required volume of diluent with the 10mL tip and then the required amount of the sample. An SRM will need to be diluted 10 to 20 times depending on weight of sample. For dilutions of auto-digested samples use 0 ppb standard that has undergone digestion. For microwave digested samples use 10% Seastar nitric acid.

6.3.1.7 Mix the sample 3-5 times with the 1000uL tip.

6.3.1.8 Use a new precleaned 1000uL tip for each sample.

6.3.2 Calibrate the Instrument

Calibrate the instrument using the Macro CAL245 (p. 26 in PS200 instrument manual). Use a 5-point calibration curve that includes a zero standard. If an acceptable correlation coefficient is obtained (0.995) and a standard's intensity is within a the range expected, continue with SRM analyses. See Appendix A for historic performance of the instrument

6.3.3 Analyze the SRM

Analyze the SRM. Refer to page 25 in manual for autosampler start to finish sequence and reference section A-B 7.

6.3.4 Check Standards

6.3.4.1 Run check standards every 10 samples to ensure the instrument has not drifted from its calibration range.

6.3.4.2 Acceptable Check Standard Ranges are:

Microwave Digestion	Automated Digestion
0.250ppb = 15%	~0.014ppb = 15-20%
0.500ppb=10%	~0.028 ppb = 15%
1.00ppb=10%	~0.058ppb = 10%
2.00ppb=10%	~0.115 ppb = 10%

6.3.4.3 Refer to reference section c-11 for more information.

CAUTION

If check standards fail, recalibrate the instrument. Do not use update slope or intercept.

6.4 Data File Preparation

Refer to the reference sections D-1, E-1-5, R-3, and R-5 for preparation of post-run data and computer files in the AP200 Manual. These data references apply to digested and samples prepared on the Automated Digester.

6.5 Instrument Shutdown

6.5.1 Dispose of 10% Hydrochloric acid rinse in an appropriate container.

6.5.2 Rinse the tray out three times with MSQ.

6.5.3 Transfer remaining tin chloride to teflon bottle in which it was earlier prepared.

6.5.4 Rinse out tin chloride bottle three times with MSQ.

6.5.5 Fill autosampler tray and tin chloride bottle with MSQ, flush for ten minutes.

6.5.6 Use OVERNITE, or SHUTDOWN modes to shutdown the instrument.

6.5.7 If using OVERNITE MODE, check condition of drying tube, to ensure it is not saturated with moisture.

6.5.8 Repack a new drying tube if necessary.

7.0 Suggestions for Successful Analyses

7.1 Allow the autosampler cups and standard cups to rest in 50% nitric acid for at least two hours before analyses.

7.2 For best results prepare tin chloride and standards as described.

7.3 Prepare a loosely packed drying tube daily. After use, dispose of perchlorate in an appropriate container. Rinse a drying tube with MSQ and flush lightly with Liquinox. Rinse several times to eliminate any residual Liquinox.

7.4 Periodically check tin chloride line and liquid gas separator for any blockage.

7.5 Check at seals of teflon tubing of drying tube connection for gas leaks.

7.6 Change tin chloride line and sample line weekly or after four days of continuous use.

7.7 Change drain tubing every two weeks as needed.

7.8 Clean autosampler rails with isopropyl alcohol weekly and oil rails daily. If not sufficiently lubricated, the autosampler arm will encounter snags or stops.

7.9 Biweekly calibrate the autosampler tip to ensure it is picking up more than three milliliters.

8.0 Literature Cited

Gilman, L. B., 1988. *General Guidelines for Microwave Sample Preparation.* Revision of July 1988. CEM Corporation, Matthews, NC.

Leeman Labs, Inc., 1991. *PS200 Automated Mercury Analyzer Set-up and Operating Manual.* Version of November 1991.

Leeman Labs, Inc., 1993. *AP200 Automated Mercury Preparation System Manual.* Revision C (11/20/93).

Rossmann, R. And K. A. Rygwelski, 1996. *Standard Operating Procedures for the Release of Data.* Revision 1 (1/18/96) LLRS-QA-SOP-001.

Rossmann. R., 1992. *Standard Operating Procedures for the Routine Review of Data Quality and Quantity.* Revision 0 (12/2/92) LLRS-QA-SOP-002.

Rossmann, R., 1992. *Minimum Analytical Quality Assurance Objectives for U. S. EPA Large Lakes Research Station.* Revision 1 (7/28/92) LLRS-QA-001.

Rossmann, R., 1993. *Standard Operating Procedures for the Preparation of Materials used for Ultra-low Trace Element Analyses.* Revision 1 (8/9/93) LLRS-MET-SOP-001

Rossmann, R., 1994. *Standard Operating Procedures for the Maintenance of the LLRS Trace Metal Laboratories*. Revision 1 (5/4/94) LLRS-MET-SOP-003.

Rossmann, R. and T. Uscinowicz, 1994. *Standard Operating Procedures for Analysis of Total Mercury in Tissue and Sediment using the Cold Vapor Technique with the Perkin-Elmer Model MHS-20 Gold Amalgam System*. Revision 1 (5/6/94) LLRS-MET-SOP-010.

APPENDIX: HISTORIC PERFORMANCE FOR STANDARDS ON PS200.

Daily Standard Intensities and Percent Drift for Standards. Italicized dates are check standards.

Date of Analysis	Standard Concentration (ppb) 0.250	0.500	1.000	2.000	5.000
01/17/96		30807	62450	128462	319499
01/18/96	11567	24577	52846	112763	289297
01/24/96	15405	27507	62220	127897	309450
01/25/96	14521	25177	55494	117531	279308
01/29/96	12899	30207	54795	108733	283705
01/31/96	12153	25133	47097	110427	280385
02/01/96	13360	26287	52552	113649	286800
02/05/96		28271	55116	107236	262538
02/05/96		28294	57208	116260	287149
02/06/96		21563	43415	95356	258884
2/6/96		25966	47569	103997	263870
2/6/96		24807	49621	102217	259967
2/6/96		23963	48835	99624	251241
02/08/96	15429	31433	62122	124122	312746
2/8/96		32989	62558	121403	316603
2/8/96		31222	60391	120489	312282
02/13/9	13614	27169	59876	121738	299635
2/13/96		30254	63307	125174	304689
2/13/96b	13675	26879	58308	121548	297642
2/13/96b		28977	60081	122166	278297
02/15/96	13628	24557	57516	118139	286801
2/15/96			54759	111736	268327
2/15/96b	12642	21928	53348	112076	270167
Begin New Lamp					
03/04/96	32237	44465	127713	233412	620712
03/04/96	31507	46352	130143	226759	626601
03/04/96	29918	41993	119879	217858	598765
03/05/96	22484	65754	124625	264984	628674
03/05/96		64815	114069	259107	609113
03/06/96	22151	45823	90596	180965	422997

APPENDIX: Continued.

Daily Standard Intensities and Percent Drift for Standards. Italicized dates are check standards.

Date of Analysis	Standard Concentration (ppb) 0.250	0.500	1.000	2.000	5.000
03/07/96	21132	44232	95743	194749	478754
03/07/96	19282	39037	79923	202894	
03/12/96		37940	79106	166230	411516
03/14/96	23058	46864	96048	192849	457716
03/14/96	24152	47468	95645	188087	469761
03/14/96	24206	47189	96993	188049	454648
03/14/96	24008	47491	94850	187538	453447
03/15/96	25333	48936	98592	195526	485709
03/15/96	23274	48272	98146	197376	473520
03/19/96		35727	81247	178190	469406
03/22/96	23430	43189	90404	194519	493374
03/26/96b	22930	45156	84083	188764	465947
03/28/96[1]	23663	44270	79330	184093	457756
03/28/96[2]	27619	53606	109573	212636	523307
03/29/96	22912	47100	96970	194573	483750
03/29/96				191256	474460
03/29/96	21975	44362	90593	196257	
03/29/96		44504			
04/03/96	20843	42173	85748	167910	429912
04/09/96	20494	41875	83552	169354	419310
04/09/96	42012			167949	
04/09/96	40964		81646	160894	401227
04/11/96	21043	42323	89779	178299	444557

[1] Old Tubing
[2] New Tubing

APPENDIX: Continued.

Daily Standard Intensities and Percent Drift for Standards Prepared with Automated Digestion System. Italicized dates are check standards.

Date of Analysis	Standard Concentration (ppb) ~0.014	~0.028	~0.056	~0.114	~0.228
06/11/96			4278	8331	
06/13/96			4275	8689	17993
06/18/96			4138	8979	17995
07/03/96			5043	8057	15743
07/09/96				8369	18117
07/12/96a		1937	4098	8088	17318
07/12/96b		2162		8118	17009
07/16/96		1867	4308	8488	17285
07/17/96	927	1973	4457	8454	16962
07/24/96	1175	2124	4146	8043	16190
07/25/96	980	1923	4492	7546	
08/01/96	600	1728	3989	7908	17072
08/15/96	895	1678	3910	7977	15964
08/16/96	1069		4189	8468	
09/18/96	816	1819	360	7382	15077