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Title: Determination of Mercury by Atomic Absorption Spectroscopy		
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Contents

A. INTRODUCTION

1. Summary of Procedure

A portion of homogenized tissue sample is digested with concentrated sulfuric and nitric acids at 80 °C followed by oxidation with potassium permanganate and potassium persulfate at 30 °C. The sample is diluted to a known volume; an aliquot is removed and analyzed by reducing any contained mercury (Hg) with stannous chloride to elemental mercury. The elemental mercury is measured by a conventional Atomic Absorption Spectrometer or stand alone cold vapor Flow Injection Mercury System.

2. Applicability

This method is suitable for the determination of total mercury (organic + inorganic) in tissues at levels ≥ 0.2 ppm.

B. EQUIPMENT

Note: Equivalent equipment may be substituted.

1. Apparatus

a. Biochemical Oxygen Demand (BOD) bottle - 300 mL with a ground glass stopper.

b. Centrifuge tubes - 15 mL, polypropylene.

c. Water bath - Complete with a covered top and the ability to maintain water at a depth of 2 - 3 in. at 95 ± 5 °C.

d. Volumetric flask - 1 L.

e. Graduated cylinders - 100 mL, 500 mL, and 1 L

2. Instrumentation

Perkin/Elmer, cold vapor, Flow Injection Mercury System (FIMS) which includes a computer system with software capable of controlling the instrumentation and calculating the final concentrations of Hg in samples. Follow manufacturers suggested instrument settings.

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents / solutions may be substituted. The stability time frame of the solution is dependent on the expiration date of the components used or the listed expiration date, whichever is soonest.

1. Reagents

   a. Hydroxylamine Hydrochloride ($NH_2OH{\cdot}HCl$) - CAS 5470-11-1, Cat. No. 255580, Sigma-Aldrich.

   b. Hydroxylamine Sulfate $(NH_2OH)_2{\cdot}H_2SO_4$ - CAS 10039-54-0, Sigma-Aldrich, Cat. No. 210250 - Optional. May be used as a one-to-one direct substitute for hydroxylamine hydrochloride.

   c. Nitric Acid, Concentrated (HNO_3) - CAS 7697-37-2, Cat. No. 258113, Sigma-Aldrich.

   d. Potassium Permanganate ($KMnO_4$) - CAS 7722-64-7, Cat. No. 399124, Sigma-Aldrich.

   e. Potassium Persulfate ($K_2S_2O_8$) - CAS 7727-21-1, Cat. No. 216224 Sigma-Aldrich.

   f. Reagent grade Water - ASTM type I or Type II.

   g. Sodium Chloride (NaCl) - CAS 7647-14-5, Cat. No. S7653, Sigma-Aldrich.

   h. Stannous Chloride ($SnCl_2$) - CAS 10025-69-1, Cat. No. 31669, Sigma-Aldrich.

   i. Stannous Sulfate ($SnSO_4$) - CAS 7488-55-3, Cat. No. 244635, Sigma-Aldrich - Optional. May be used as a one-to-one direct substitute for stannous chloride.

   j. Sulfuric Acid, Concentrated (H_2SO_4) - CAS 7664-93-9, Cat. No. 320501, Sigma-Aldrich.

2. Solutions

   a. 12% (w/v) Hydroxylamine Solution:

      Dissolve 12 g of sodium chloride and 12 g of hydroxylamine hydrochloride (or 12 g of hydroxylamine sulfate) in 70 mL of water in a 100 mL graduated cylinder. Bring to 100 mL with water

   b. 5% (w/v) Potassium Permanganate Solution:

      Dissolve 5 g of potassium permanganate in 100 mL of water in a graduated cylinder.

   c. 5% (w/v) Potassium Persulfate Solution:

      Dissolve 5 g of potassium persulfate in 100 mL of water in a graduated cylinder.

   d. 10% (w/v) Stannous Chloride Solution:

      To 300 mL of reagent grade water, in a graduated cylinder, add 5.6 mL of

concentrated sulfuric acid, and 40 g of stannous chloride ($SnCl_2$). Dilute to 400 mL with reagent grade water, mix well and stopper.

Note: This solution tends to precipitate out Sn(OH)Cl with prolonged exposure to air. It should be prepared fresh daily, if possible. If this solution needs to be made up ahead of time, it should be stored in a container that can be closed against atmospheric gases. Precipitation of solid materials can often be controlled by adding a small amount of elemental tin to the bottom of the container.

e. 0.5 N Sulfuric Acid Solution:

Slowly add 14 mL of concentrated sulfuric acid to 900 mL of water, mix and dilute to 1000 mL with water in a 1 L volumetric flask.

D. STANDARD(S)

Note: Equivalent standards / solutions may be substituted. Purity and counterions are to be taken into account when calculating standard concentrations. The stability time frame of the solution is dependant on the expiration date of the components used or the listed expiration date, whichever ends sooner.

1. Standard Information

Primary Mercury Standard, Catalog Number MSHG-10PPM, Inorganic Ventures, 300 Technology Drive, Christiansburg, VA 24073. Alternative concentrations and manufacturers are permissible with proper documentation.

2. Preparation of Standard Solution(s)

Stock Standard/ Fortification Standard (1.00 µg/mL):

Pipet 5.00 ± 0.01 mL of the 10 µg/mL primary standard into a 50 mL volumetric flask, add 5 mL concentrated HNO3, and bring to volume with reagent grade water.

The stock standard expires one year from the date of production when it's been stored refrigerated at 2 - 8 °C.

3. Preparation of Calibration Curve

Prepare Calibration Solutions by pipetting 0, 150, 300 and 600 µL of the Fortification Standard into four separate 300 mL BOD bottles. These four solutions are equivalent to 0, 0.5, 1.0, and 2.0 ppm, respectively. Continue with F.2.b.

E. SAMPLE PREPARATION

Process tissue until homogeneous. All samples are stored refrigerated or frozen until analyzed.

F. ANALYTICAL PROCEDURE

1. Preparation of Controls and Samples

   a. Tissue Blank - Weigh 0.30 ± 0.10 g of homogenized, known blank tissue into a 300 mL BOD bottle and stopper until use.

   b. Recovery Sample - Weigh 0.30 ± 0.10 g of homogenized sample into a 300 mL BOD bottle. Fortify the sample with 0.30 µg of Hg in solution (equivalent to 1.0 ppm). If using the 1.00 µg/mL Hg Fortification Standard, spike with 300 µL.

   c. Internal Check Sample - Weigh 0.30 ± 0.10 g of homogenized sample into a 300 mL BOD bottle. Fortify the sample with 0.00 µg of Hg or any concentration between 0.15 - 0.60 µg (0.5 - 2.0 ppm) of Hg in solution.

   d. Reagent blank

2. Extraction Procedure

   a. Weigh 0.30 ± 0.10 g of homogenized sample into a 300 mL BOD bottle and stopper until use.

   b. To the BOD bottles, add 4 mL of conc. H_2SO_4 and 1 mL of conc. HNO_3. Mix the contents thoroughly.

   c. Place the BOD bottles in a water bath set at 80 ± 5 °C for 30 mins. (Until samples completely dissolve.)

   d. Remove the BOD bottles from the hot water bath and allow the contents to cool to room temperature. This process may be speeded by the use of a cold water bath.

   e. **Note: This next step adds solutions that are mostly water to the very acidic solutions contained in the BOD bottles. Caution should be exercised.** Cautiously and slowly add 5 mL of 5% potassium permanganate solution to the BOD bottles, stopper and swirl to mix. Further add an additional 10 mL of 5% potassium permanganate followed by 8 mL of 5% potassium persulfate to the BOD bottles. Stopper and swirl to mix.

   f. Place the BOD bottles in a water bath set at 30 ± 5 °C for 90 mins.

   g. Remove the BOD bottles from the water bath and cool to room temperature, and add 50 mL of water to each BOD bottle. Swirl to mix the contents thoroughly.

   Stopping point: The contents of the BOD bottles are quite stable at this point and may be stored at room temperature for up to a week.

h. Swirl to mix the contents thoroughly and pipet 10 mL of the contents from each BOD bottle into separate, clean, 15 mL polypropylene centrifuge tubes.

Note: Care should be exercised to avoid transferring any bottom sediment or floating fatty material that may exist in the BOD bottles.

i. Add 750 µL of 12% hydroxylamine solution to each 15 mL centrifuge tube and mix the contents thoroughly.

Note: Any existing solution color should disappear. If solution color does not disappear, add 25 µL aliquots of 12% hydroxylamine solution until solution is colorless.

j. The 15 mL centrifuge tubes are transferred to the FIMS autosampler. A predetermined amount of solution is removed by the instrument and reduced with 10% stannous chloride solution. Any resulting elemental mercury is swept by carrier gas into the FIMS instrument for measurement and quantification.

3. Instrumental Settings

Note: The instrument parameters may be optimized to ensure system suitability.

Set up the AAS according to the manufacturer's instructions.

a. Operating parameters for Perkin-Elmer FIMS-400.

Lamp:	Hg
Wavelength:	253.7 nm

b. Pump Operating parameters for FIMS-400.

Step #	Time (s)	Pump I (rpm)	Pump 2 (rpm)	Valve	Read (s)
Prefill	15	100	120	Fill	
1	10	100	120	Fill	
2	15	0	120	Inject	25

Individual settings may be adjusted to obtain best results on any given day.

Note: It is also permissible to use an AA instrument that generates mercury hydride instead of elemental mercury generated in this example. Follow manufacturer's instructions when setting up such a system.

4. Injection sequence /Sample Set

Each sample set must contain one QA sample/20 samples:

a. Calibration Standard curve at 0.00, 0.15, 0.30 and 0.60 µg of Hg, (equivalent to 0, 0.5, 1.0, and 2.0 ppm in tissue).

b. Reagent Blank.

c. Tissue Blank.

d. Recovery.

e. Internal check (if needed).

f. Samples.

g. Reinjection of standard/recovery for system suitability.

G. CALCULATIONS / IDENTIFICATION

1. The instrument controlling software is capable of performing all of the calculations needed for this analysis.

2. Quantitation may be performed by following the steps outlined below. Either peak height or peak area may be used for calculations.

3. Construct a linear regression line for the calibration solutions. The µg of Hg known to be in the solution should be plotted against the instrument response for each calibration solution.

   The equation is $y = mx + b$

   Where:

   x = µg of Hg in the original calibration solution.

   y = Instrument response.

   m = calculated slope of the regression line.

   b = calculated y intercept of the regression line.

4. Calculate the percent recovery for any recovery (or internal check) samples according to the following formula…

$$\%\,\text{Recovery} = \left[\left\{ \left[\frac{(\text{Resp}_{\text{Rec}} - b)}{m} \Big/ \text{Wt}_{\text{Rec}} \right] - \left[\frac{(\text{Resp}_{\text{Mtrx Blk}} - b)}{m} \Big/ \text{Wt}_{\text{Mtrx Blk}} \right] \right\} \Big/ (\mu g/g)_{\text{Fortified Hg}} \right] * 100\%$$

   Where:

   Resp_{Rec} = Instrument response for a recovery (or internal check) sample.

   $\text{Resp}_{\text{Mtrx Blk}}$ = Instrument response for the matrix blank sample.

Wt_{Rec} = Original weight of recovery sample in grams.

Wt_{Blk} = Original weight of blank tissue in grams.

$(\mu g/g)_{Fortified\ Hg}$ = Original fortified level of Hg in a recovery (or internal check) sample assuming the original weight of the sample was 0.20 g.

5. Samples that have extracted solutions with total mercury concentrations in excess of 2.00 ppm of Hg in solution should be re-run with calibration standards that bracket the sample concentration. Calibration Solutions with levels of Hg as high as 5.00 ppm in solution are permissible without loss of linearity in the calibration curve. Should a sample have a level of Hg above 5.00 ppm in solution, dilution of the final sample extract is also a permissible means of keeping the final sample concentration within the scale of the calibration standards.

H. SAFETY INFORMATION AND PRECAUTIONS

1. Required Protective Equipment — Safety glasses, appropriate gloves, lab coat.

2. Hazards

Procedure Step	*Hazard*	*Recommended Safe Procedures*
Hydroxylamine Hydrochloride	Corrosive. Will cause burns to all body tissue. May be fatal if swallowed or inhaled. Possible skin sensitizer.	Isolate from sources of ignition and oxidizing materials
Hydroxylamine Sulfate	Corrosive. Causes burns. Harmful if swallowed. Dangerous for the environment. Avoid breathing in dust. Avoid contact with eyes, skin and clothing.	Prepare solutions in a fume hood.
Mercury Standard, 10 ppm in 10% HCl	Corrosive. Causes burns. Harmful if swallowed. Vapors may irritate to eyes, nose, throat and lungs. Avoid contact with eyes, skin and clothing.	Prepare solutions in a fume hood.
Nitric Acid, Sulfuric Acid	Will cause severe burns to all body tissue. May be fatal if swallowed or inhaled.	Prepare solutions in a fume hood. Store out of direct sunlight. Regulate contact with

		heat, water, and incompatible materials.
Potassium Persulfate	Oxidizer, contact with reducing agents or combustibles may cause ignition. Irritant to eyes, respiratory system and skin. Avoid breathing in dust. Avoid contact with eyes, skin and clothing.	Prepare solutions in a fume hood. Avoid contact with combustible materials.
Potassium Permanganate	Oxidizer, contact with reducing agents or combustibles may cause ignition or extremely violent combustion. Causes burns to all tissue. Toxic metal fumes may form when heated to decomposition.	Prepare solutions in a fume hood. Avoid contact with combustible materials.
Stannous Chloride	Corrosive. Causes burns. Harmful if ingested. Avoid breathing in dust. Avoid contact with eyes, skin and clothing.	Prepare solutions in a fume hood.
Stannous Sulfate	Irritant to eyes, respiratory system and skin. Avoid breathing in dust. Avoid contact with eyes, skin and clothing.	Prepare solutions in a fume hood.

3. Disposal Procedures

   Follow local, state and federal guidelines for disposal.

I. QUALITY ASSURANCE PLAN

1. Performance Standard

Analyte	*Analytical Range (ppm Hg)*	*Acceptable Recovery (%)*	*Acceptable Repeatability (% CV)*
Mercury	0.20 - 5.00	85 - 115	≤ 10

   a. Acceptability criteria:

      i. Correlation coefficient ≥ 0.995.

      ii. No false positive or false negative results.

2. Critical Control Points and Specifications

	Record	*Acceptable Control*
a.	Reagent Blank	Absorbance should produce a response ≤ 0.1 ppm, (equivalent concentration in tissue). If greater, check for cleanliness and contamination of glassware and reagents.
b.	Tissue dissolution (F.1.c)	The tissue must be observed to completely dissolve during the digestion phase of this method.

3. Intralaboratory Check Samples

   a. System, minimum contents.

      i. Frequency: One per week per analyst when samples analyzed.

      ii. Records are to be maintained.

   b. Acceptability criteria.

      Refer to I. 1.

      If unacceptable values are obtained, then:

      i. Investigate following established procedures.

      ii. Take corrective action as warranted.

4. Sample Condition upon Receipt - Cold

J. APPENDIX

1. References

   a. EPA Method 245.6, “Determination of Mercury in Tissues by Cold Vapor Atomic Absorption Spectrometry”, Revision 2.3, April 1991.

   Note: Matrices successfully verified using this method include catfish, bovine, swine, and chicken muscle; bovine, swine, and chicken liver; and processed products.

K. APPROVALS AND AUTHORITIES

1. Approvals on file.

2. Issuing Authority: Director, Laboratory Quality Assurance Division.