

Colorado Department of Public Health and Environment



**Laboratory Services Division
Inorganic Chemistry**

Preparation-Acid Digestion, Mercury Analysis, Fish Tissues

Revision 1

DECEMBER 2004

TITLE

Preparation-Acid Digestion, Mercury Analysis, Fish Tissue

REFERENCE

Methods for Determination of Metals in Environmental Samples, EPA/600/R-94/111, May 1994

METHOD

Acid digestion of fish tissue for mercury analysis

PRINCIPLE

A pre-weighed portion of fish tissue is placed in a combination of nitric and sulfuric acid and heated to 58°C for at least one hour to solubilize the tissue. Subsequently the sample is oxidized overnight in a combination of potassium permanganate and potassium persulfate.

SAMPLE

0.2 to 0.5 gram sample of fish tissue that has been dissected and preserved in accordance with EPA method 200.3

SAFETY

Read all MSDS sheets before handling unfamiliar reagents. Use precautions found in the Chemical Hygiene Plan (Appendix I – Safety Manual) when working in the laboratory

EQUIPMENT

1. 300 mL BOD bottles with ground glass stoppers. Bottles must be soaked in 5% Nitric acid for a minimum of 4 hours then rinsed with Reagent water and allowed to dry.
2. Pipettes capable of 80-1000 µL, 1-5mL, 100-500 µL
3. Blender
4. Analytical balance capable of weighing 0.001 grams
5. Volumetric flasks, 100-ml, 1-L, and 2-L
6. Water bath

REAGENTS

Reagent grade chemicals shall be used in all tests unless otherwise indicated. It is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficient high purity to permit its use without lessening the accuracy of the determination.

1. Certified mercury in fish tissue reference material, NIST traceable
2. Hydrochloric Acid (HCL), 12M, concentrated
3. Mercury Chloride (HgCl₂)
4. Nitric acid (HNO₃), 16M, concentrated
5. Potassium Permanganate (KMnO₄)
6. Potassium Persulfate (K₂S₂O₈)
7. Reagent Water, ASTM Type II
8. Sulfuric acid (H₂SO₄), 18M, concentrated

Reagent Preparation

Note: All reagents must be “Suitable for Mercury Determination”, and used only for mercury analysis. These reagents must be stored separately from other reagents.

Note: Label all solutions appropriately with analyst initials, date of preparation, solutions description and concentration, special storage instructions and hazards as appropriate.

1. Nitric Acid, 15%. To a 2 L volumetric flask containing approximately 1500 mL reagent water slowly add 300 mL concentrated nitric acid. Place the solution on a stir plate and stir until solution cools. Dilute to the mark and transfer to a storage bottle.

Caution: Solution gets very hot.

2. Nitric Acid, 0.15%. To a 2 L volumetric flask containing approximately 1000 mL reagent water slowly add 20 mL of the 15% nitric acid solution. Dilute to the mark with additional reagent water.
3. Potassium Permanganate, 5% (w/v). Mix 100g KMnO₄ in 2L reagent water for at least 1 hr using labeled plastic container set aside for this solution. Assure complete dissolution before

using. Store in labeled KMnO_4 reagent bottle. This solution is stable for six months.

4. Potassium Persulfate, 5% (w/v). Mix 100g $\text{K}_2\text{S}_2\text{O}_8$ in 2L reagent water using plastic container set aside for this solution. Prepare well in advance to allow complete dissolution. Store in $\text{K}_2\text{S}_2\text{O}_8$ reagent bottle . This solution is stable for six months.

Standards Preparation

1. Mercury Stock Standard, (1000mg/L). To a 100 mL volumetric flask add 0.1354g Mercury Chloride (HgCl_2) . Dilute to the mark with **15% HNO_3** . Refrigerate after use. Solution is stable for six months.
2. Intermediate Mercury Standard, (1mg/L). To a 1 L volumetric flask add 500 mL 0.15% HNO_3 . Using a Class A volumetric pipette, add 1 mL of Mercury Stock standard that has been allowed to warm to room temperature. Dilute to the mark with additional 0.15% HNO_3 . Refrigerate after use. This solution is stable for one month.
3. Working Mercury Standard, (100 ug/L). To a 100 mL volumetric flask add 50 mL 0.15% HNO_3 . Using a Class A volumetric pipette, add 10 mL of Intermediate Mercury Standard that has been allowed to warm to room temperature. Dilute to the mark with additional 0.15% HNO_3 . This solution is stable for one month.

PROCEDURE

Initial Preparation

1. The Standard curve is prepared from commercially available fish tissue which has been tested and found to be low in mercury (<0.02 mg Hg/g tissue). The tissue is blended with a blender.
2. Label all BOD bottles needed for samples including the following Quality Control (QC) samples,
 - a) Laboratory Reagent Blank (LRB). A reagent blank must be analyzed immediately after instrument calibration, after every tenth sample, and at the end of the sample run.
 - b) Laboratory Fortified Blank (LFB). Add 1 mL of the working mercury standard to 70 mL of reagent water.
 - c) Quality Control Standard (QCS). A commercially available reference, or standard prepared from a source different from the calibration standards, must be run with each analytical batch. Dilute to a concentration within the range of the calibration curve.
 - d) Laboratory Fortified Matrix (LFM). Add 1.0 mL of the working mercury standard to

another 25-mL aliquot of sample. A laboratory fortified matrix must be run at a frequency of 10%.

- e) Laboratory Duplicate. Duplicates must be run at a frequency of 10%. The duplicates may be fortified.
 - f) Instrument Performance Check (IPC). A midrange standard must be analyzed after every ten samples and at the end of the analytical run.
 - g) A Quality Control Standard (QCS), Reference, must be analyzed with each analytical batch.
3. Weigh out 0.2-0.3 grams of the fish tissue from step a above and place into the labeled BOD bottles used for standards as well as the LFB. Cap these bottles with the ground glass stopper and set aside
 4. Weigh out 0.2-0.3 grams of unknown fish tissue sample and samples for spikes and place in the appropriate labeled BOD bottle. Record the amount of fish tissue used in grams. Cap with ground glass stopper and set aside.
 5. Weigh out 0.02 grams of fish reference material and place in the labeled reference BOD bottle. Record the amount used in grams. Cap with ground glass stopper and set aside.
 6. Carefully add 4 mL of concentrated sulfuric acid and 1 mL of concentrated nitric acid to each flask.

Caution: Toxic Fumes! Work under fume hood.

7. Recap each bottle after the addition and place into a 58°C water bath for two hours.

Preparation of Standard Curve

1. Prepare the following standards, presented in Table 1, in the appropriately labeled BOD bottle containing digested fish tissue. Place the BOD bottle into an ice bath before addition of either the mercury standard or the reagent water.

Caution: Solution gets very hot use gloves, eye protection and ice bath.

Table 1. Recommended Mercury Standards.

<i>Volume of Mercury Working Standard</i>	<i>Volume of Reagent Water (mL)</i>	<i>Standard Concentration (µg)</i>
None	70.0	None
400 µL	69.6	0.040
1.0 mL	69.0	0.10

<i>Volume of Mercury Working Standard</i>	<i>Volume of Reagent Water (mL)</i>	<i>Standard Concentration (µg)</i>
2.0 mL	68.0	0.20
3.0 mL	67.0	0.30
4.0 mL	66.0	0.40

Note: To obtain actual fish tissue standard concentrations divide the standard concentration from the chart by the initial wet weight of the fish tissue used in grams.

Sample Preparation

1. Place each remaining BOD bottle one at a time into an ice bath and carefully add 70 mL of reagent water.
2. Prepare the following Quality Control Samples
 - a) Laboratory Reagent Blank (LRB). A reagent blank must be analyzed immediately after instrument calibration, after every tenth sample, and at the end of the sample run.
 - b) Laboratory Fortified Blank (LFB). Add 1 mL of the working mercury standard to 70 mL of reagent water.
 - c) Quality Control Standard (QCS). A commercially available reference, or standard prepared from a source different from the calibration standards, must be run with each analytical batch. Dilute to a concentration within the range of the calibration curve.
 - e) Laboratory Fortified Matrix (LFM). Add 1.0 mL of the working mercury standard to another 25-mL aliquot of sample. A laboratory fortified matrix must be run at a frequency of 10%.
 - e) Laboratory Duplicate. Duplicates must be run at a frequency of 10%. The duplicates may be fortified.
 - f) Instrument Performance Check (IPC). A midrange standard must be analyzed after every ten samples and at the end of the analytical run.
 - g) A Quality Control Standard (QCS), Reference, must be analyzed with each analytical batch.
3. Allow samples to set for at least 10 minutes after addition of the reagent water.
4. Wearing gloves, lab coat, eye protection and working under a fume hood, add the following reagents to all BOD bottles

- a) 15 mL 5% (w/v) KMnO_4
 - b) 10 mL 5% (w/v) $\text{K}_2\text{S}_2\text{O}_8$
5. Cap each BOD bottle with its ground glass stopper and gently mix.
 6. Place bottles under a fume hood and allow to stand overnight at room.
 7. Samples may now be analyzed according to current laboratory SOP for Mercury, Cold Vapor Atomic Absorption, (Hg).

Note: Confirmatory testing as described in the current laboratory SOP for Mercury, Cold Vapor Atomic Adsorption, (Hg) is not required since each fish tissue sample is spiked.

POLLUTION PREVENTION

1. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in a laboratory operation. The United States Environmental Protection Agency (US EPA) has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address waste generation. When wastes cannot feasibly be reduced at the source, the US EPA recommends recycling as the next best option.
2. The quantity of chemicals purchased should be based on expected usage during the shelf life and disposal cost of unused material. Actual reagent preparation volume should reflect anticipated usage and reagent stability.
3. For information about pollution prevention that may be applicable to laboratories, consult "Less is Better: Laboratory Chemical Management for Waste Reduction", available from the American Chemical Society's (ACS) Department of Governmental Regulations and Science Policy, 1155 16th Street N.W., Washington D.C. 20036, (202) 872-4477.

WASTE MANAGEMENT

1. The US EPA requires that laboratory waste management practice be consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any waste discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions.

2. For further information on waste management consult the “Waste Management Manual for Laboratory Personnel” available from the ACS at the address listed previously.
3. The appropriate disposal procedure for each laboratory chemical solution used in this analysis is summarized in Table 2.

Table 2. Chemical Solution Disposal Procedure

Solution	Disposal Method
Nitric Acid, 15%.	Neutralize to pH range of 5-9 with sodium bicarbonate. Dispose to Sanitary Sewer.
Nitric Acid, 0.15%.	Neutralize to pH range of 5-9 with sodium bicarbonate. Dispose to Sanitary Sewer.
Potassium Permanganate, 5% (w/v)	Acidify to pH 1 with phosphoric acid. Add a small amount of manganese II salt. Slowly add sodium bisulfite until the violet color becomes colorless. Neutralize with sodium bicarbonate to pH range of 5-9. Dispose to sanitary sewer. OR Dispose of as RCRA hazardous waste. Reactive (D003)
Potassium Persulfate, 5% (w/v).	< 20 mL to Sanitary Sewer. Greater than 20 mL Dispose of as RCRA hazardous waste. Reactive - (D003)
Mercury Stock Standard, (1000mg/L).	Dispose of as RCRA Hazardous Waste – (D009) Mercury
Intermediate Mercury Standard, (1mg/L).	Dispose of as RCRA Hazardous Waste – (D009) Mercury
Working Mercury Standard, (100 µg/L).	Neutralized with Sodium Bicarbonate to pH range of 5-9. Dispose to Sanitary Sewer.
Eluent from Mercury Analyzer	Neutralize. Dispose to sanitary sewer if mercury content is <200 µg/L.
Excess Samples	Neutralize. Dispose to sanitary sewer (liquid) if mercury content is <200 µg/L. Dispose to laboratory trash (solids) if mercury content is <200 µg/L

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