FSIS **MER**

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DETERMINATION OF MERCURY BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

Contents

DETERMINATIVE METHOD

A. INTRODUCTION

Theory

The sample is digested with sulfuric acid-potassium permanganate solution to free organic mercury compounds as ionic mercury. Excess permanganate is destroyed with hydroxylamine and further reduction with stannous chloride to metallic mercury makes possible the measurement of the mercury vapor.

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B. EQUIPMENT

Apparatus

a. Reaction flasks, pear-shaped, 100 mL, 14/20 standard taper (Kontes Glass Company, Vineland, NJ, K-294250 or equivalent).

b. Continuous flow spectrophotometric cell, cylindrical, 100 -150 mm, with tube on each end with push backs. (Optical Cell Company, Inc., Beltsville, MD, No. 4-435 or equivalent).

c. The aeration equipment is illustrated in Figure 1. If continuum background correction is unavailable, concentrated sulfuric acid can be put in the bottom of the water trap to within 5 mm of the end of the tube. Any number of compounds, such as hydrocarbons of water, display molecular absorption in this region.

d. Aeration tube should have an extra coarse frit (Kontes Glass Co., or equivalent).

e. Water trap (Kontes Glass Co., or equivalent).

f. Modified 3-way stopcock: Figure 2 shows a full-sized view. (Kontes Glass Co., or equivalent.)

g. Flowmeter: Should have a range of 0-1.7 L/min—Brooks Sho-Rate with a R-2-15A tube using a steel ball (A. H. Thomas Company, Philadelphia, PA, 5545-B [easel] and 5545 [tube]), or equivalent.

2. Instrumentation

Atomic absorption spectrophotometric equipment capable of continuum background correction is used in the analysis. A mercury electodeless discharge lamp (EDL) is used as a resonance source. With the EDL, background correction is difficult to achieve; therefore, a trap of concentrated sulfuric acid is used. (Refer to section G.2.c.)

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B. EQUIPMENT (Continued)

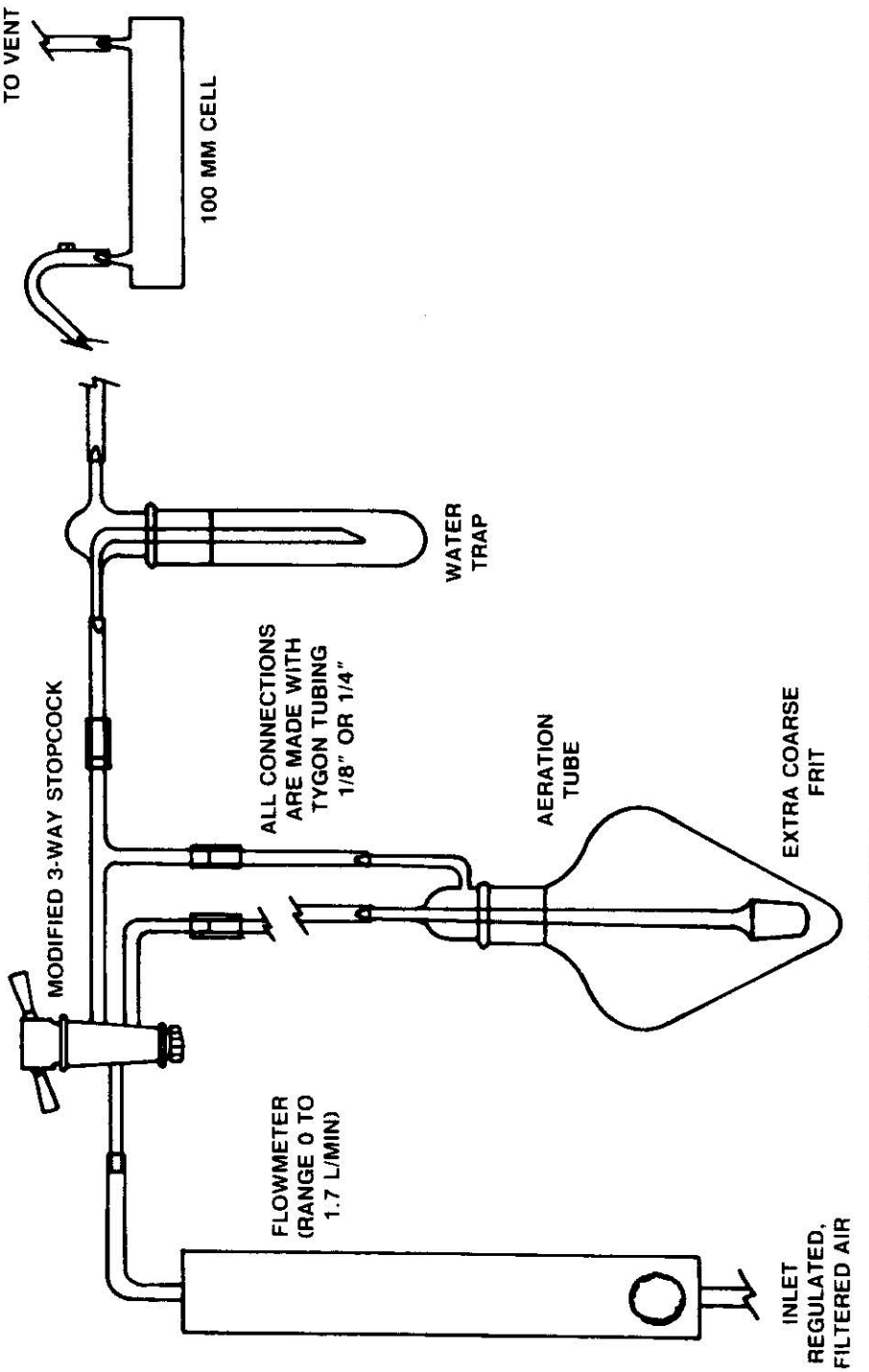

FIGURE 1—FLOW DIAGRAM FOR MERCURY ANALYSIS

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B. EQUIPMENT (Continued)

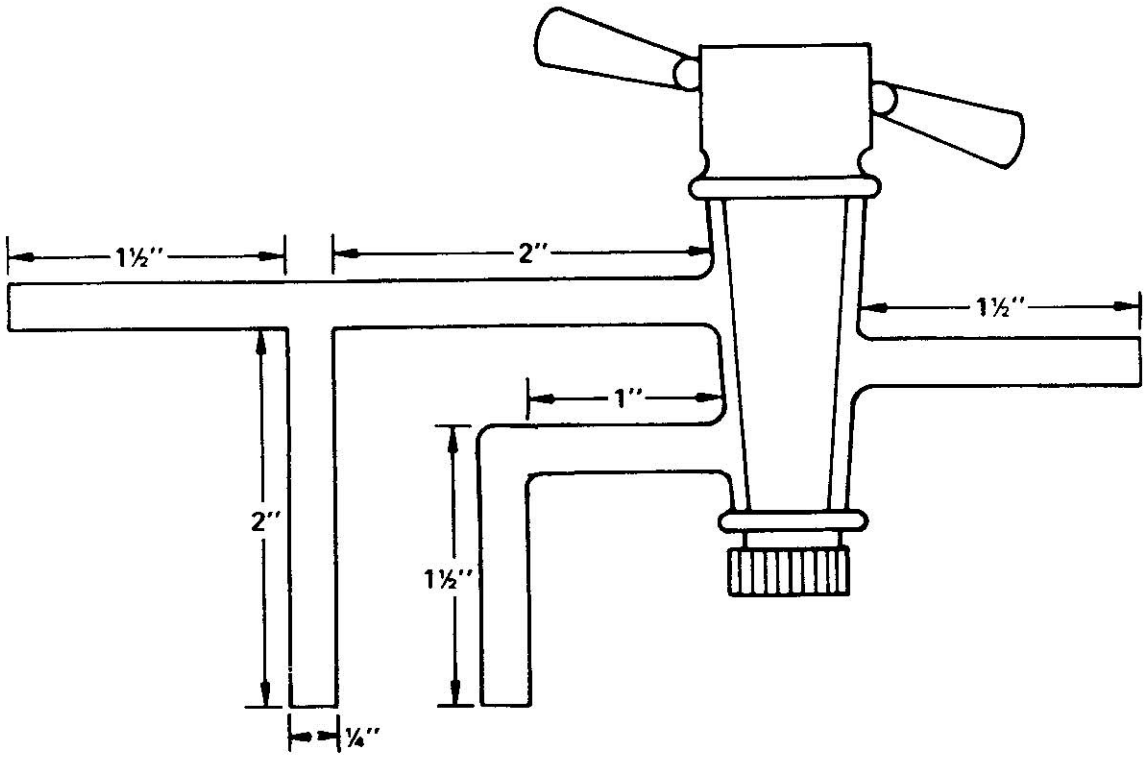

FIGURE 2—MODIFIED 3-WAY STOPCOCK

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C. REAGENTS AND SOLUTIONS

Reagent and Solutions List

a. Concentrated reagent-grade nitric acid, 1 + 1 with water (v/v).

b. Hydroxylamine hydrochloride (NH_2OH-HCl) solution,10% (w/v): Dissolve *25* g of reagent-grade NH_2OH-HCl in about 200 ml of distilled water. Transfer to a 250 mL volumetric flask, dilute to volume with distilled water, and mix well.

c. Potassium permanganate ($KMnO_4$) solution, 6% (w/v): Completely dissolve 60 g of reagent-grade $KMnO_4$, in about 800 mL of distilled water in a 1 L beaker using a heated magnetic stirrer. Transfer the solution quantitatively to a 1 L volumetric flask, cool, dilute to volume with distilled water, and mix well.

d. Stannous chloride ($SnCl_2$) solution, 10% (w/v). Prepare fresh every week. Dissolve 20 g of reagent-grade $SnCl_2 \cdot 2H_2O$ in 40 mL of warm concentrated hydrochloric acid. When all the stannous chloride has dissolved, add 160 mL of distilled water. Mix well and store in a 250 mL reagent bottle.

e. Concentrated Sulfuric acid (H_2SO_4): reagent grade.

NOTE: Check each lot of compounds b through e. The amount of mercury found in them may vary markedly.

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D. STANDARDS

1. Source

a. Inorganic mercury standard, 1000 µg/mL (Fisher Scientific Co., Pittsburg, PA; catalog No. SO-M-114 or equivalent).

b. Organic mercury standard, 1000 µg/mL: (Alfa Products, P.O. Box 299, 152 Andorf Street, Danvers, MA; catalog No. 88036, or equivalent).

2. Preparation of Standards

a. Mercury standard solution: 10 µg Hg/mL. (NOTE: 100 µg/mL standard may be prepared as an intermediate step if desired.) Pipet 1.0 mL of standard 1.a into a 100 mL volumetric flask containing 2 mL of redistilled nitric acid. Dilute to volume with distilled water and mix well.

b. Preparation of standard curve. Make fresh daily.

mL reagent	*final volume with Distilled water*	*µg Mercury in 20 µL*
1	10 mL	0.02 (1 µg/mL)
3	10 mL	0.06 (3 µg/mL)
5	10 mL	0.10 (5 µg/mL)

c. Methyl mercury standard solution: 10 µg/mL. Pipet 1.0 mL of standard 1 .b into a 100 mL volumetric flask, add 2.0 mL redistilled concentrated nitric acid and dilute to volume with distilled water.

d. 5.0 µg/mL fortification standard. Pipet 5.0 mL of standard solution 2.c into a 10 mL volumetric flask and dilute to volume with distilled water.

NOTE: For samples with higher amount of Hg, the standard curve (refer to step 2.b) may be extended using 0.2, 0.6 and 1.0 µg Hg in 20 µL standard solutions.

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E. EXTRACTION PROCEDURE

1. Sample Preparation

a. Muscle tissue—Eliminate as much fat from tissue as possible. Pass rapidly three times through food chopper with plate opening less than or equal to 1/8 inch, mixing thoroughly after each grinding.

b. Liver or Kidney—Eliminate as much fat and connective tissue as possible from both the kidney and liver. Place tissues in a separate blender jar and blend until well homogenized (Waring Blender should be used with extreme care when blending). Blend 1 min; permit blender to cool for not less than 1 min before blending again. Do not use variable transformers to control speed of blender. Freeze tissues until determinations are ready to be run on each.

c. Hair—Wash with plain tap water to remove extraneous material, rinse with distilled water, and then dry. Place in freezer until ready to start determinations.

2. Sample Extraction

a. Clean all glassware with concentrated nitric acid (refer to section C, Reagent and Solution List, item a) and rinse with distilled water just before each use.

NOTE: Avoid use of towels, which may contain mercury, and do not use detergents.

b. Place approximately 0.600-0.750 g of the homogenized tissue (muscle, liver, or kidney), or 0.5 g hair, into a tared 100 mL pear-shaped flask, taking care that all of the sample is deposited in the bottom of the flask and none is left in the neck. Reweigh the flask and obtain the sample weight to the nearest 0.01 g, by difference. Cap the flask with a clean 10 mL beaker inverted over the top of the flask. This beaker is left on the flask during all stages of the digestion procedure.

c. Pipet 5.0 mL of concentrated H_2SO_4 into the flask and place it on a steam bath to digest the sample (20-45 min is usually sufficient). Swirl flask during digestion to break up particles. The completed digested sample will form a highly colored solution with no pieces of undissolved matter, although the solution may be slightly cloudy.

NOTE: For complete digestion, the sample should be in solution and there should be no undissolved particles in the flask.

d. When the sample is digested, place the flask into an ice bath for 5-10 min. Then pipet 15.0 mL of 6% $KMnO_4$ solution into the flask and swirl the flask, gently at first and then vigorously, until sample is well mixed. Place sample in rack and continue until $KMnO_4$ has been added to all samples.

e. Swirl and place the flask on steam bath and allow the sample to digest further. Swirl the flask occasionally, and continue to heat until frothing ceases and all foam disappears (usually 15-20 min). Do not heat longer than is needed. Some foam may be present when reaction has stopped.

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E. EXTRACTION PROCEDURE (Continued)

f. Remove the flask from the steam bath and pipet a further 5.0 mL of 6% $KMnO_4$ (10.0 mL of 6% $KMnO_4$ for hair samples) solution into it.

g. Place the flask back on the steam bath for 15 min.

h. Cool the flask to room temperature and analyze the contents for mercury by atomic absorption.

3. Internal Standard for Checking Analytical Performance

Using a microliter syringe, add 20 µL (0.10 µg) of fortification standard (refer to section D.2.d to 0.75 g of homogenized tissue in 100 mL pear-shaped flask. Proceed as in steps section E.2.a-h.

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F. ANALYTICAL QUANTITATION

1. Preparation of a Calibration Curve

NOTE: To be carried out at least once per day.

a. Into each of eight clean 100 mL flasks, pipet 20 mL 6% KMnO4 solution (refer to section C, Reagent and Solution List, item c). Cap the flasks with clean 10 mL beakers inverted over the tops of the flasks,

b. Cool the flasks in an ice bath for a few minutes, then slowly and cautiously pipet 5.0 mL of concentrated sulfuric acid into each flask. Swirl gently and allow to cool.

c. Using a micro liter syringe, add 20 µL of each inorganic standard (1 µg, 3µg and 5 µg/mL) so that duplicate standards are obtained for each level. The levels will be 0 (none added), 0.02 µg, 0.06 µg, and 0.10 µg.

d. Cool the flasks to room temperature prior to the aeration and atomic absorption analysis.

NOTE: Reagent blanks should show absorbances which are equivalent to 0.02 µg or less of Hg. If reagent blanks are higher, check glassware cleanliness and reagent solutions. Some checking of various suppliers' reagents may be necessary to determine those most suited to this analysis.

2. Atomic Absorption Analysis

a. Set up the atomic absorption equipment according to the diagram in Figure 1. Set the air flow to give good sensitivity and low foaming (0.7-1.0 L/min).

b. Add 5.0 mL of 10% $NH_2OH \cdot HCl$ solution (10.0 mL in the analysis of hair) to the digestion flask and swirl to dissolve the manganese oxides. Add about 10 mL of distilled water to bring the total volume to 40 mL + 2.0 mL.

NOTE: This solution should not have any color or any particles suspended in it, but it may be slightly cloudy. See NOTE in section E.2.c.

c. Add 2 mL of 10% SnCI2.2H2O solution and immediately aerate the solution.

d. Discontinue the aeration after the recorder pen has settled back to within a few chart divisions (2 or 3) of its original baseline—usually 1 to 1½ min depending on the actual aeration rate.

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G. CALCULATIONS

1. Procedure

a. Calculate area of peak by measuring its height in mm and multiplying by the mm width at one-half its height (read to nearest 0.25 mrn). Standard curve is calculated using linear regression.

The linear regression formula is: $y = mx + b$

Where: y = µg Hg, x = area, m = slope, b = intercept

b. Calculate m and b. Calculate µg mercury in sample and divide by sample weight in grams to obtain ppm.

2. References

a. "Determination of Mercury in Fish (Atomic Absorption Spectrophotometric Method)," Method CAS-AM-70.10, June 11, 1970, Dow Chemical Company, Midland, MI.

b. Manning, D. C. "Compensation for Broad-Band Absorption Interference in the Flameless Atomic Absorption Determination of Mercury," *Atomic Absorption Newsletter,* Vol. 9, No. 5 (Sept-Oct 1970), 109.

c. Kothandaraman, P. and Dallmeyer, J. F., "Improved Desiccator for Mercury Cold Vapor Technique," Atomic Absorption Newsletter, Vol. 15, No. 5 (Sept-Oct 1976), 120-121.

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H. HAZARD ANALYSIS

1. Method Title

Determination of Mercury by Atomic Absorption Spectrophotometry.

2. Required Protective Equipment

Safety glasses, face shield, heat resistant gloves, plastic gloves, lab coat.

3. Procedure Steps

	Hazards	Recommended Safe *Procedures*
C. Reagents		
Conc. nitric acid Conc. sulfuric acid	Acid fumes and/or spattering can result in burns or irritation of skin, eyes, and respiratory system.	Work in efficient fume hood and wear protective equipment at all times.
E.2. Sample Extraction		
Clean all glassware with nitric acid	See above.	Exposure is greatly reduced by working in a hood with a distilled water supply and a sink.
Pipet 5.0 mL conc. H_2SO_4	See above.	Use repipettor or equivalent.
Pipet 15.0 of 6% $KMNO_4$	Acid spattering when mixed with this strong oxidizing agent.	Add the $KMNO_4$ slowly to the cold acid digest.
F.2. Atomic Absorption Analysis	Acid spattering and mercury vapors may produce cumulative toxic effects.	The reaction used to introduce mercury vapor to the A.A. is pressurized. Therefore, it is important to check for cracks in the glass components and to work behind some protective shield.

4. Disposal Procedures

Acid digest	See above.	May be diluted with water and flushed down a waste disposal sink.

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J. QUALITY ASSURANCE PLAN

1. Performance Standard

Element	*Analytical Range (ppm)*	*Acceptable Recovery (%)*	*Repeatability %CV*	*Reproducibility %CV*
Mercury	0.02 - 0.10†	80 -110 (inorganic) > 55 (organic)	≤ 10‡	≤ 15

† Upper limit depends on dilution volume.
‡ CV ≤ 15% (running average of last 10).

2. Critical Control Points and Specifications

Record	*Acceptable Control*
a. Background absorbance, date, signature (unless background is electronically removed).	
b. Reagent, lot no., date checked, findings, signature.	Combined reagents should show response ≤ 0.01 µg Hg.
c. Standard, lot no., source, date made, solvent, signature.	10 µg std. should be made monthly; all others daily. Solvent for 10 µg/mL dilute HNO_3; solvent for all others distilled H_2O. Samples should be homogeneous and show very little fat content. Glassware should be very clean with no leftover liver or grease present. Samples above 0.05 ppm Hg should be rerun in duplicate to check for any contamination. Blank should be ≤ 0.01 µg Hg.
d. Weight of sample (on worksheet).	Weigh 0.600 to 0.750 g of liver, muscle, and kidney, or 0.500 g hair in bottom of flask. Record wt. to nearest 0.001 g. If sample remains on side, discard and reweigh.
e. Time placed on steam bath and time removed (treat all samples the same).	After first 15 min, swirl to help dissolve tissue and check for undissolved tissue.
f. Time placed on steam bath and time removed.	Place all samples on and take all samples off at same time. Heat only until reaction stops: 15-20 min.

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J. QUALITY ASSURANCE PLAN (Continued)

Record	*Acceptable Control*
g. Time placed on and time removed.	15 min maximum,
h. % recovery, date, analyst.	Should range between 80 and 110% (inorganic Hg), > 55% (organic Hg) at 0.1 µg level.
i. Record by strip chart recorder.	With given setting for our instrument, 0.1 µg std should give approximately 50-60% deflection.
j. Yes or no (on worksheet).	Total digestion shows no visible dark material. Some fat may be present if a fatty sample (e.g., bologna) is being run.
k. Coefficient of correlation.	0.998 -1.000

3. Readiness To Perform

a. Familiarization.

i. Phase I: Standards—4 levels, 3 replicates each.

(a) 0.00 ppm.

(b) 0.02 ppm.

(c) 0.06 ppm.

(d) 0.10 ppm.

ii. Phase II: Fortified samples—4 levels, 3 acceptable replicates at same nominal level as above over a minimum of 3 separate days.

NOTE: Phase I and II may be performed concurrently.

iii. Phase III: Check samples for analyst accreditation.

(a) 14 samples provided by supervisor.

(b) Report analytical findings to Laboratory Quality Assurance Manager (QAM).

Notification from QAM is required to commence official analysis.

b. Acceptability criteria.

See section J.1 above.

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J. QUALITY ASSURANCE PLAN (Continued)

4. Intralaboratory Check Samples

a. System, minimum contents.

i. Frequency—initially, minimum of 1 per set, reduced to 1 per week per analyst. This sample is an internal check sample. It is a pooled sample analyzed at least 10 times to obtain a "running" average.

ii. A recovery (or recoveries) is analyzed with each set of samples. Also, a "blank" tissue is analyzed, and a running average is maintained for both the blank and recovery. Blank tissue matrix should not be from a single tissue source. Each species should be tested separately. Records are maintained by the analyst and reviewed by the supervisor and Laboratory QA Officer.

b. Acceptability criteria.

If unacceptable values are obtained, then:

i. Stop all official analyses for that analyst.

ii. Investigate and identify probable cause.

iii. Take corrective action,.

iv. Repeat Phase III of section J.3 above if cause was analyst-related.

5. Sample Acceptability and Stability

a. Matrices: Liver, kidney, muscle and hair.

b. Sample receipt size: Varied; enough to obtain matrix for all required quantitative tests and reserve sample.

c. Condition upon receipt: Not spoiled or rancid.

d. Sample storage:

i. Time: Indefinite.

ii. Condition: Frozen.

6. Sample Set

a. Reagent blank.

b. Tissue blank.

c. Fortified tissue at level of interest.

d. Sample(s).

7. Sensitivity

a. Lowest reliable quantitation (LRQ): 0.02 ppm.

b. Minimum proficiency level (MPL): 0.02 ppm.