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# Mercury in Biological Tissue

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## 1 Scope and Application

1.1 Method 245.6 is used to prepare and analyze samples for Mercury analysis by Cold Vapor Atomic Absorption Spectroscopy in biological tissues. Due to volume limitations of the digestion equipment, all digestion volumes are reduced to 50%.

| Compound | CAS No. |
|---|---|
| Mercury | 7439-97-6 |



1.2 Restricted Procedure
This procedure is restricted to use by an analyst experienced in the operation of atomic absorption spectrometers. Additionally, the analyst must complete the requirements of the GAEPD Initial Demonstration of Analyst Proficiency prior to the analysis of actual samples. Analysts are further warned that performance of this analysis involves the use of potentially hazardous chemicals; refer to the GAEPD Chemical Hygiene Plan for additional information regarding chemicals required by this method.

## 2 Definitions

Refer to Chapter 3 of the Georgia EPD Laboratory Quality Assurance Manual for Quality Control Definitions.

## 3 Interferences

3.1 Potassium Permanganate is added to the samples to eliminate possible interference from sulfide. Concentrations as high as 20 mg/L of sulfide as sodium sulfide do not interfere with the recovery of added inorganic mercury from reagent water.

3.2 Copper has also been reported to interfere, however, copper concentrations as high as 10 mg/L have had no effect on recovery of mercury from spiked samples.

3.3 Certain volatile organic materials that absorb at 253.7 nm may also interfere. Analyzing the sample without reagents shall validate a positive mercury result. The presence of mercury in the sample without reagents confirms the presence of interference and the concentration of mercury present in the treated sample is commented.

## 4 Safety

Refer to Laboratory Chemical Hygiene Plan

## 5 Apparatus and Equipment

5.1 Complete cold vapor mercury analyzer system and controller.
5.2 Autosampler tubes.
5.3 50 mL hot block digestion vessels with screw caps.
5.4 Hot block digestion system.
5.5 Assorted volume graduated cylinders.
5.6 Magnetic stirrer and magnetic stir bars.
5.7 Pipettors capable of delivering the required volumes of reagents.
5.8 Assorted high quality pipette tips.

## 6 Reagents and Standards

All reagents or standards that are prepared must be logged into the standard log notebook, the standard number must be written on the sample prep log, and the container must be labeled with the standard number, standard name, initials, and the expiration date.

6.1 Reagent water: 18MΩ water.
6.2 Concentrated reagent grade sulfuric acid.
6.3 Concentrated reagent grade nitric acid.
6.4 Stannous chloride: Add 11 g stannous chloride ($SnCl_2 \cdot 2H_2O$) to a graduated cylinder, add 3% (v/v) HCl to bring the final volume to 1000 mL. Place the graduated cylinder on a magnetic stirrer, add a magnetic stir bar to the cylinder, and stir continuously during use.
6.5 Sodium chloride-hydroxylamine hydrochloride solution: Dissolve 12 g of sodium chloride and 12 g hydroxylamine hydrochloride in 18MΩ water and dilute to 100 mL.
6.6 Potassium permanganate, mercury-free, 5% solution (w/v): Dissolve 5 g potassium permanganate in 100 mL of 18MΩ water.
6.7 Potassium persulfate, mercury-free, 5% (w/v): Dissolve 5 g potassium persulfate in 100 mL of 18MΩ water.
6.8 3% Hydrochloric Acid: Bring 30 mL concentrated hydrochloric acid to 1000 mL with 18MΩ water.



## 7 Sample Collection

Fish tissue samples are received in the labs as frozen composites wrapped in aluminum foil. Samples are held frozen until the composites are homogenized then refrozen until analysis.

## 8 Calibration

8.1 Calibration Curve
The Mercury analyzer is calibrated daily using a multipoint calibration curve. The concentrations of the calibration standards are (in mg/kg): 0.0, 0.0002, 0.0005, 0.001, 0.002, 0.003, and 0.006.
8.2 Calibration Verification
An ICV, CCC, and CCB are analyzed immediately after calibration. A CCC and CCB are analyzed after calibration, before each batch, after every ten samples, after each batch and as the last samples in an analytical sequence.

## 9 Quality Control

Refer to Table 13.1 for Reporting Limits (PQLs), Table 13.2 for Quality Control

Acceptance Criteria, and Table 13.3 for Quality Control Procedures associated with this method.

9.1 Record all reagents used, volumes, standard or lot numbers, time, temperature, and sample IDs on the digestion log. Fill out a run log with every use of the instrument. The run log must include all samples and standards analyzed in the order they were analyzed.

9.2 Verify the pipette calibration by weighing 100 ul and 500 ul of MillQ water. Acceptable weights are 0.100g ±0.019g for 100 ul and 0.500g ± 0.05g for 500 ul. Record the pipette number, the volumes and the weights on the digestion sheet.

## 10 Procedure

10.1 Overnight digestion procedure

10.1.1 Weigh a 0.15 gram portion of an untreated (not dried) sample into a 50 mL hot block digestion vessel. If a sample exists with a mercury concentration <RL, weigh out 0.15 g of that tissue in various 50ml hot block digestion vessels for standards and QC samples, otherwise weigh out 0.15g 18MΩ water.

10.1.2 Prepare QC samples according to Table 10.1.

10.1.3 Add 2.0 mL concentrated sulfuric acid and 0.5 mL concentrated nitric acid to each vessel.

10.1.4 Place each vessel into the hot block and heat at 58$^{o}$C ± 5$^{0}$C until the tissue is completely dissolved, usually about 30 - 60 minutes. Heat the standards as well as the samples.

10.1.5 Remove the vessels from the hot block, cool in an ice bath to 4$^{o}$C, ± 2$^{0}$C remove from the ice bath and add 7.5 mL 5 % potassium permanganate in 1 mL aliquots. If necessary, add an additional 3 mL or more of 5% potassium permanganate to maintain oxidizing conditions (the purple color remains). Mix thoroughly. All samples and standards must have the same amount of potassium permanganate added.



10.1.6 Add 4.0 mL 5% potassium persulfate to each vessel, cap and mix well. Allow the samples to sit at room temperature overnight. Add 3.0 mL sodium chloride-hydroxylamine hydrochloride to each sample. Bring sample up to 40 mL with 18MΩ water.

10.1.7 Any sample with a Hg concentration > LDR must be diluted to bring the Hg concentration between the RL and LDR. If the sample was spiked, then both the spike and spike duplicate must be diluted by the same amount.

10.2 Alternate digestion procedure.

10.2.1 If a sample exists with a mercury concentration <RL, weigh out a 0.15 gram portion of an untreated (not dried) sample into a 50 mL hot block digestion vessel, otherwise weigh out 0.15g 18MΩ water.

10.2.2 Prepare QC samples:

LCS: Add 0.12 mL Hg intermediate standard to a digestion vessel, once per batch, final concentration: 0.003 mg/kg.

LCSD: Add 0.12 mL Hg intermediate standard to a digestion vessel, once per batch, final concentration: 0.003 mg/kg.

ICV: Add 0.12 mL Hg 1000 ug/kg stock to a digestion vessel, only after calibration, final concentration: 0.003 mg/kg.

CCC: Add 0.12 mL Hg intermediate standard to a digestion vessel, after calibration, after every ten samples, and at the end of the batch, final concentration: 0.003 mg/kg.

CCB: Add 0.12 mL 18MΩ water to a digestion vessel, after calibration, after every ten samples, and at the end of the batch.
Matrix blank: Add 0.12 mL 18MΩ water to a digestion vessel.

10.2.3 Add 2.0 mL concentrated sulfuric acid and 0.5 mL concentrated nitric acid to each vessel.

10.2.4 Place each vessel into the hot block and heat at $80^0$C $\pm5^0$C for 30 minutes.

10.2.5 Remove the vessels from the hot block, cool in an ice bath to 4°C $\pm2^0$, and add 7.5mL 5% potassium permanganate. Mix thoroughly. If necessary, add an additional 3 mL of 5% $KMnO_4$ to maintain oxidizing conditions (the purple color remains). Mix thoroughly. All samples and standards must have the same amount of $KMnO_4$ added.

10.2.6 Add 4.0 mL 5% potassium persulfate to each vessel, cap and mix well.

10.2.7 Place the vessels into the hot block and heat at 30°C ±5°C for 90 minutes.

10.2.8 Remove the vessels from the hot block and cool to room temperature, add 3.0 mL sodium chloride-hydroxylamine hydrochloride to each sample. Bring samples up to 40 mL with18MΩ water.

10.2.9 Any sample with a Hg concentration > LDR must be diluted to bring the Hg concentration between the RL and LDR. If the sample was spiked, then both the spike and spike duplicate must be diluted by the same amount.

Table 10.1 Standard Preparation.

| Reagent | Preparation | Final concentration (mg/kg) |
|---|---|---|
| Hg intermediate standard | Dilute 0.1mL 1000 ppm stock solution to 100 mL with 1% nitric acid. | 1.0 |
| Hg calibration blank | 18 MΩ water | <RL |
| Hg calibration standard 1 | Add 0.008 mL Hg intermediate standard to digestion tube. | 0.0002 |
| Hg calibration standard 2 | Add 0.02 mL Hg intermediate standard to digestion tube. | 0.0005 |
| Hg calibration standard 3 | Add 0.04 mL Hg intermediate standard to digestion tube. | 0.001 |
| Hg calibration standard 4 | Add 0.08 mL Hg intermediate standard to digestion tube. | 0.002 |
| Hg calibration standard 5 | Add 0.12 mL Hg intermediate standard to digestion tube. | 0.003 |
| Hg calibration standard 6 | Add 0.24 mL Hg intermediate standard to digestion tube. | 0.006 |
| HgICV | Add 0.12 mL of Hg 1 mg/L second source intermediate standard to hot block tube. Continue with step 10.1.3 | 0.003 |
| HgLCS/LCSD | Add 0.12 mL Hg intermediate stock to hot block tube. Continue with step 10.1.3. . | 0.003 |
| HgCCC | Add 0.12 mL of intermediate Hg standard to a digestion vessel. Continue with step 10.1.3 | 0.003 |
| CCB | Add 0.12 mL of 1% nitric acid to hot block tube, | <RL |

Table 10.1 Standard Preparation.

| Reagent | Preparation | Final concentration (mg/kg) |
|---|---|---|
| | after calibration, after every 10 samples, and at the end of the batch. Continue with step 10.1.3 | |
| HgMS/HgMSD | Add 0.15 grams of the sample and 0.12 mL of a 1 mg/L intermediate Hg standard to hot block tube. Continue with step 10.1.3 | 0.003 |

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Table 10.2 Reagent Preparation

| Reagent | Preparation |
|---|---|
| Stannous chloride | Mix 11g stannous chloride, bring to 1000 mL in 3% hydrochloric acid, mix well during use. |
| Sodium chloride-hydroxylamine hydrochloride | Mix 12g sodium chloride and 12g hydroxylamine hydrochloride together and bring to 100 mL in 18MΩ water. |
| 5% (w/v) Potassium permanganate | Add 5g potassium permanganate and bring to 100 mL in 18MΩ water. |
| 1% Nitric Acid | Bring 10 mL concentrated nitric acid to 1000 mL with 18MΩ water. |
| 3% Hydrochloric Acid | Bring 30 mL concentrated hydrochloric acid to 100 mL with 18MΩ water. |



Table 10.3 Procedure Summary.

| Step | Reagent | Amount |
|---|---|---|
| 10.1.1 | Sample | 0.15 g |
| 10.1.2 | Concentrated Sulfuric Acid | 2.0 mL |
| 10.1.2 | Concentrated Nitric Acid | 0.5 mL |
| 10.1.3 | 58ºC hot block | 30-60 minutes |
| 10.1.4 | 5% (w/v) potassium permanganate | 7.5mL |
| 10.1.5 | 5%(W/V) Potassium Persulfate | 4.0 mL |
| 10.1.6 | Allow samples to sit overnight at room temperature | |
| 10.1.6 | Sodium chloride-hydroxylamine hydrochloride | 3.0 mL |
| 10.1.6 | 18MΩ water | Bring to 40 mL with 18MΩ water |

10.3 Calibration and QC standard preparation: Prepare calibration standards according to tables. All standards and intermediate dilutions must be logged into the standard log book.

10.4 FIMS 400 Operating Parameters

| **FIMS 400 Parameter** | **FIMS 400 Setting** |
|---|---|
| Carrier gas | Argon |
| Wavelength | 253.7nm |
| Carrier solution | 3.0% (v/v) HCl |

| | |
|---|---|
| Sample diluent | 3.0% (v/v) HCl |
| Reductant | 1.1% $SnCl_2$ in 3.0% (v/v) HCl |
| Carrier gas flow rate | 50mL/min |
| Sample Volume | 0.5mL |
| Reaction coil | 110mm length, 1.0mm i.d. |
| Pump #1 speed | 100 |
| Pump # 2 speed | 120 |

## 11 Evaluation of the Linearity of the Initial Calibration

11.1 Print the calibration curve, the FIMS 400 software to calculate and print the linear correlation coefficient. The minimum acceptable linear correlation coefficient is 0.995.

11.2 Sample Concentration

The sample concentrations are computed from the following formula and are reported in mg/kg wet weight.

Solids Concentration Calculation

$$\text{Concentration} = \frac{CVD_F}{WW}$$

Where $C$= concentration from instrument in mg/L
$V$ = final digestion volume in L.
$D_f$= dilution factor
$WW$ = wet weight of sample.



Fish tissue samples reported for mercury trend monitoring will be reported with a quantitation limit of 0.01 mg/Kg. The reporting limit will be "J" flagged for concentrations between 0.01 mg/Kg and 0.10 mg/Kg with the analysis comment "J-Estimated value. The results below the State of Georgia EPD Laboratory reporting limit for this analyte."

## 12 References

12.1 *Test Methods for Evaluating Solid Waste Physical/Chemical Methods* (SW846) Third Edition, Update IIB, March 1995.
*Method 245.6, Determination of Mercury in Tissues by Cold Vapor Atomic Absorption Spectrometry,* Revision 2.3, 1991.
FIMS Flow Injection Mercury System Software Guide, Perkin Elmer, release 1.0, July 1994.

## 13 Practical Quantitation Limits (PQLs), Precision and Accuracy Criteria, and Quality Control Approach

**Table 13.1 RL for Method 245.6**

| Parameter/Method | Analyte | Matrix Fish | |
|---|---|---|---|
| | | RL | Unit |
| 245.6 Mercury by Cold Vapor Atomic Absorption Spectrometry | Mercury | 0.1 | mg/Kg |

**Table 13.2 Acceptance Criteria for Method EPA 245.6**

| Method | Analyte | LCS Accuracy (%R) | LCSD Precision (%RPD) | MS Accuracy (%R) | MSD Precision (%RPD) |
|---|---|---|---|---|---|
| 245.6 Mercury by Cold Vapor Atomic Absorption Spectrometry | Mercury | 85-115 | ≤15 | 85-115 | ≤15 |



**Table 13.3 Summary of Calibration and QC Procedures for Method EPA 245.6**

| Method | Applicable Parameter | QC Check | Minimum Frequency | Acceptance criteria | Corrective Action | Flagging Criteria |
|---|---|---|---|---|---|---|
| 245.6 | Mercury | Analyst Initial Demonstration. | Once per analyst | Two clean Blanks, Average of 4 LCS recoveries between 85-115%. Recovery of unknown sample between 85-115%. | Recalculate results, correct problem, and then rerun the initial demonstration for those analytes that did not meet criteria. | |
| | | Continuing Demonstration | Every 6 Months | Average of 4 LCS recoveries between 85%-115%, Method Blank <RL, Unknown or PE. | Correct the problem. | |
| | | MDL study. | Once per year. | Analyte MDL must be < RL. | Correct the problem. | |
| | | Linear Dynamic Range (LDR) | Once per year. | | | |
| | | Analysis of PE sample. | Once per year. | Analyte results acceptable per the auditing agency. | Correct the problem. | |
| | | Initial Calibration. using 4 standards. | Daily initial calibration prior to sample analysis. | Correlation coefficient ≥ 0.995. | Correct the problem and recalibrate. | |
| | | Low Standard | During calibration. | Low standard concentration < RL. | | |
| | | Initial Calibration Verification (ICV) | Daily after calibration. | Analyte recovery between 90%-110%. | Correct the problem and recalibrate. | |

**Table 13.3 Summary of Calibration and QC Procedures for Method EPA 245.6**

| Method | Applicable Parameter | QC Check | Minimum Frequency | Acceptance criteria | Corrective Action | Flagging Criteria |
|---|---|---|---|---|---|---|
| 245.6 | Mercury | Continuing Calibration Blank (CCB). | Daily after calibration, before each batch, after every 10 samples, after each batch and at end of analysis sequence. | Analyte concentration <RL. | Correct the problem, recalibrate, and reanalyze all samples since the last acceptable CCB. | |
| | | Continuing Calibration Check (CCC). | Daily after calibration, after every 10 samples, and at end of analysis sequence. | Initial CCC recovery between 90%-110%, subsequent recoveries between 80%-120%. | Correct the problem, recalibrate, and reanalyze all samples since the last acceptable CCC. | |
| | | Laboratory Control Sample (LCS). | Once per batch. | Analyte recovery between 85-115%. | Correct the problem, recalibrate, and reanalyze all samples in the batch. | |
| | | Laboratory Control Sample Duplicate (LCSD). | Once per batch. | ≤ 15 RPD. | Correct the problem, recalibrate, and reanalyze all samples in the batch. | |
| | | Laboratory Control Sample Duplicate Recovery. | Once per batch. | Recovery between 85-115% | Comment report. | |
| | | Matrix Blank | Once per batch. | Analyte concentration < RL. | Correct the problem, recalibrate, and reanalyze all samples in the batch. | |
| | | Matrix Spike | Every 10 samples. | Analyte recovery between 70-130%. | All samples must be analyzed by method of standard additions. | |
| | | Matrix Spike Duplicate. | Every 10 samples. | ≤ 15 RPD. | None. | None |
| | | Matrix Spike Duplicate Recovery. | Every 10 samples. | Analyte recovery between 70-130%. | Comment report. | |
| | | Mercury Trend Monitoring Reporting Limit | As required. | 0.01 mg/Kg | Results reported between 0.01 mg/Kg and 0.10 mg/Kg | J |