

STANDARD OPERATING PROCEDURE
For
USEPA METHOD 245.1, Rev. 3.0
Determination Of Mercury In Water
By Cold Vapor Atomic Absorption Spectrometry

SOP #: EPA 245.1

SOP REVISION #: 2.1

DATE: December 2012

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LIST OF REVISIONS

Rev. #	Date	Description of Revision	Page #
0	April 1999	None	
1.0	December 2000	Section 3.6, 3.13, 3.16 and 3.19 deleted. Section renumbered Section 5.4 deleted Section 9.3.5 deleted Section 14.0 and 15.0 changed Section 17.0 Table 3, 4, & 5 updated Assorted minor typos etc. corrected	6-7 7 12 16 18-19 Throughout document
1.1	December 2001	New Table 1	16
1.2	November 2003	Table 5 – updated MDL	21
1.3	April 2004	Section 6.2 – Instrument general maintenance added Section 7.11 – HCl preparation procedure added Section 11.2 – Instrument daily maintenance procedures added Section 11.2.9 – Analytical sequence added Table 1 – QC elements and acceptance criteria updated	9 10 15 16 19
1.4	November 2006	Replaced old DEP Logo with state seal + MassDEP Tables 3 – 5 updated with 2006 accuracy, precision, & MDL data	Title page & header 19 – 20
1.4	January 2007	Section 7.9 – 50 g of hydroxylamine changed to 120 g of hydroxylamine. Method has not been changed; it has always been done this way but was entered in the SOP incorrectly.	9
1.5	April 2008	Section 7.7: Typo 57 should be 5% Section 7.8: Typo 47 should be 5%; and 40 g of $K_2S_2O_8$ changed to 50 g Section 11.1.3: Included pH test at time samples are poured. Section 11.1.7: 2 mL of 4% changed to 1.6 mL of 5% (Method has not been changed; solution is chemically equivalent with volume and % w/v change). Section 11.2.10: Analytical sequence updated Table 1: Updated – MRL check standard acceptance criterion typo – should be 20% not 5%; other updates Minor edits	9 9 13 13 15 18 Throughout document



Rev. #	Date	Description of Revision	Page #
2.0	October 2010	Section 7.9 – Corrected name of solution Section 10 – Added number of points needed for calibration curve Section 11.1.13 – Changed the amount of solution added; corrected typo on name of solution Section 11.2.5 – Added time frame for read time and cleanout on FIMS Section 11.2.7 – Added part number of filter for gas-liquid separator Section 11.2.10, Sequence #5 – Clarified the use of standards in the calibration curve	
2.1	December 2012	Section 9.2.4 – Added the mercury wavelength Tables 2, 3, 4 and 5 – Updated	



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1.0 SCOPE AND APPLICATION

- 1.1 This procedure measures total mercury (organic + inorganic) in drinking, surface, ground, sea, and brackish water, and in industrial and domestic wastewater.

<u>Analyte</u>	<u>Chemical Abstracts Service Registry Number (CASRN)</u>
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Mercury	7439-97-6
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- 1.2 The range of the method is 0.1 to 20 µg Hg/L. The range may be extended above or below the normal range by increasing or decreasing sample size. However, the actual method detection limit and linear working range will be dependent on the sample matrix, type of instrumentation configuration, and selected operating conditions (See Sect. 11 Procedure).
- 1.3 Reduced volume or semi-automated versions of this method that use the same reagents and molar ratios are acceptable provided they meet the quality control and performance requirements stated in the method (Sect. 9.0).
- 1.4 For reference where this method is approved for use in compliance monitoring programs [e.g., Clean Water Act (NPDES) or Safe Drinking Water Act (SDWA)], consult both the appropriate sections of the Code of Federal Regulation (40 CFR Part 136 Table 1B for NDPES, and Part 141 § 141.23 for drinking water), and the latest Federal Register announcements.

2.0 SUMMARY OF METHOD

A 20-mL portion of water sample is transferred to a 50-mL metal free centrifuge tube. It is digested in diluted potassium permanganate-potassium persulfate solutions and oxidized for 2 h at 95°C. Mercury in the digested water sample is reduced with stannous chloride to elemental mercury and measured by the conventional cold vapor atomic absorption technique.

3.0 DEFINITIONS

- 3.1 Calibration Blank - A volume of reagent water acidified with the same acid matrix as in the calibration standards. The calibration blank is a zero standard and is used to calibrate the Flow Injection Mercury System (FIMS).
- 3.2 Calibration Standard (CAL) - A solution prepared from the dilution of stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.
- 3.3 Field Reagent Blank (FRB) - An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to the sample site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.
- 3.4 Instrument Detection Limit (IDL) - The concentration equivalent to the analyte signal that is equal to three times the standard deviation of a series of ten replicate measurements of the calibration blank signal at the same wavelength.



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- 3.5 Instrument Performance Check (IPC) Solution - A solution of method analytes, used to evaluate the performance of the instrument system with respect to a defined set of method criteria.
- 3.6 Laboratory Duplicates (LD1 and LD2) - Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analysis of LD1 and LD2 indicates precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.
- 3.7 Laboratory Fortified Blank (LFB) - An aliquot of LRB to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements.
- 3.8 Laboratory Fortified Sample Matrix (LFM) - An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.
- 3.9 Laboratory Reagent Blank (LRB) - An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, and internal standards that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or apparatus.
- 3.10 Linear Dynamic Range (LDR) - The concentration range over which the instrument response to an analyte is linear.
- 3.11 Method Detection Limit (MDL) - The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero.
- 3.12 Quality Control Sample (QCS) - A solution of method analytes of known concentration that is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check either laboratory or instrument performance.
- 3.13 Solid Sample - For the purpose of this method, a sample taken from material classified as soil, sediment, or sludge.
- 3.14 Standard Addition - The addition of a known amount of analyte to the sample in order to determine the relative response of the detector to an analyte within the sample matrix. The relative response is then used to assess either an operative matrix effect or the sample analyte concentration.
- 3.15 Stock Standard Solution - A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.
- 3.16 Water Sample - For the purpose of this method, a sample taken from one of the following sources: drinking, surface, ground, storm runoff, industrial or domestic wastewater.



4.0 INTERFERENCES

- 4.1 Interferences have been reported for waters containing sulfide, chloride, copper and tellurium. Organic compounds that have broadband UV absorbance (around 253.7 nm) are confirmed interferences. The concentration levels for interferants are difficult to define. This suggests that quality control procedures (Sect.9) must be strictly followed.
- 4.2 Volatile materials (e.g., chlorine) that absorb at 253.7 nm will cause a positive interference. In order to remove any interfering volatile materials, the sample is allowed to degas in the fume hood after the hydroxylamine hydrochloride solution is added and mixed with the sample. The sample is then mixed with the stannous chloride solution by the auto sampler in the mixing block of the FIMS.
- 4.3 Low level mercury sample preparation, digestion, and analysis may be subject to environmental contamination if performed in areas with high ambient backgrounds where mercury was previously employed as an analytical reagent in analyses such as total Kjeldahl nitrogen (TKN) or chemical oxygen demand (COD).

5.0 SAFETY

- 5.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. All laboratory personnel are trained on the laboratory safety procedures applicable to, and the OSHA and other regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets is available to all personnel involved in the chemical analysis. Specifically, concentrated nitric and hydrochloric acids present various hazards and are moderately toxic and extremely irritating to skin and mucus membranes. These reagents are used in a fume hood and if skin or eye contact occurs, large volumes of water are applied to flush the area of contact. An emergency shower and eyewash station are located in the laboratory. Safety glasses are used for eye protection, and protective clothing is worn.
- 5.2 The acidification of samples containing reactive materials may result in the release of toxic gases, such as cyanides or sulfides. Acidification of samples is performed in a fume hood.
- 5.3 All personnel handling potentially infectious environmental samples are immunized against known disease causative agents.
- 5.4 All laboratory personnel fully comply with all relevant federal, state, and local waste management and disposal regulations. (Sect 14.0 and 15.0)
- 5.5 Mercury compounds are highly toxic if swallowed, inhaled, or absorbed through the skin. All analyses are conducted in a laboratory exhaust hood. The user of this method wears chemical resistant gloves when handling concentrated mercury standards.

6.0 EQUIPMENT AND SUPPLIES

- 6.1 Flow Injection Mercury System (FIMS) – Atomic Absorption Cold Vapor System (Perkin-Elmer FIMS Serial Number 61911). Includes:
- 6.1.1 Absorption Cell: Approximately 21-cm long, with quartz windows; heated to remove condensation.



- 6.1.2 Peristaltic Pump: Uses manifold tubing with appropriate I.D. to introduce reagents and samples into system.
- 6.1.3 Mixing Block: Unit where sample, stannous chloride, and argon gas are mixed.
- 6.1.4 Gas-Liquid Separator: Hg vapor is generated and excess liquid is removed as waste.
- 6.1.5 Hollow-Cathode Mercury Lamp
- 6.1.6 Atomic Absorption Detector: set at 253.7 nm
- 6.1.7 Auto sampler: AS-90. Computer controlled sample introduction system.
- 6.1.8 Computer: Windows 95 software compatible with P.E. Winlabs Software Version 2.5
- 6.1.9 Hot block: Temperature controlled Environmental Express Hot Block, model SC154 set at 95 degrees Celsius.
- 6.1.10 Compressed Argon: 99% pure Argon, carrier gas.

6.2. General Maintenance Procedures

- 6.2.1 Maintenance of instrument is performed on a daily basis by lead analyst (See Section 11.2). Major maintenance operations are performed by a Perkin-Elmer Customer Service Engineer. Service calls are placed to company only when lead analyst is not capable of performing the required maintenance. Service call reports are kept on file.
- 6.2.2 All glassware is washed with detergent, rinsed with reagent water, and then soaked overnight in 30% HNO₃.

7.0 REAGENTS AND STANDARDS

Note: All reagents and standards preparations must be recorded in a logbook so that each reagent and standard can be linked from the date of preparation to the sample's raw data sample run.

- 7.1 Reagents may contain elemental impurities that bias analytical results. All reagents are assayed by the chemical manufacturer for mercury and must meet ACS specifications. The assayed mercury level of all solid reagents used in this method is less than 0.005 ppm.
- 7.2 Reagent Water, ASTM Type I.
- 7.3 Nitric Acid (HNO₃) concentrated (sp.gr. 1.41) – Assayed mercury level is not to exceed 1 µg/L.
 - 7.3.1 Nitric acid (1+1) – Add 500 mL concentrated HNO₃ to 400 mL of reagent water and dilute to 1 L.
- 7.4 Sulfuric Acid (H₂SO₄) concentrated (sp.gr. 1.84) – Assayed mercury level not to exceed 1 µg/L.
 - 7.4.1 Sulfuric acid, (95-98%) w/w – trace metal grade
- 7.5 Mercury stock standard, 1 mL = 1000 µg Hg: Mercuric nitrate purchased from VWR Scientific Co.



- 7.6 Mercury calibration standard (CAL) – To each volumetric flask used for serial dilutions, acidify with (0.1 to 0.2% by volume) HNO_3 (Sect. 7.3). Using mercury stock standard (Sect. 7.5), make serial dilutions to obtain a concentration of 0.1 $\mu\text{g Hg/mL}$.
- 7.7 Potassium permanganate solution (5% w/v solution) – Dissolve 50 g of KMnO_4 in 1000 mL of reagent water.
- 7.8 Potassium persulfate solution (5% w/v solution) – Dissolve 50 g of $\text{K}_2\text{S}_2\text{O}_8$ in 1000 mL of reagent water.
- 7.9 Sodium chloride-hydroxylammonium chloride solution – Dissolve 120 g of NaCl and 120 g of hydroxylamine hydrochloride ($\text{NH}_2\text{OH}\cdot\text{HCl}$) in 1000 mL reagent water.
- 7.10 Stannous chloride solution – Add 11 g of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ to 30 mL of HCl and dilute to 1L with reagent water.
- 7.11 Hydrochloric Acid – Add 30 milliliters of Concentrated Hydrochloric Acid to 1 Liter of reagent water.
- 7.12 Blanks – Three types of blanks are required for the analysis. The calibration blank is used in establishing the analytical curve, the laboratory reagent blank is used to assess possible contamination from the sample preparation procedure, and the laboratory-fortified blank is used to assess routine laboratory performance.
- 7.12.1 Calibration blank contains all reagents in the same concentrations and in the same volume as used in preparing the calibration solutions. It is used to establish the baseline of the curve and it is used after calibration, after every 10 samples analyzed, and at the end of the analytical run.
- 7.12.2 Laboratory reagent blank (LRB) is prepared similarly as the calibration blank except the LRB must be carried through the entire sample preparation scheme.
- 7.12.3 The laboratory fortified blank (LFB) is prepared by fortifying a sample size volume of laboratory reagent blank solution with mercury to a suitable concentration of $> 10\times$ the MDL, but $<$ the midpoint concentration of the calibration curve; which is 2 $\mu\text{g Hg/L}$ for this method.
- 7.13 Instrument Performance Check (IPC) Solution – The IPC solution is used after calibration, after every 10 samples analyzed and at the end of the analytical run to verify instrument performance. It contains all reagents in the same concentration as the calibration solutions and mercury at an appropriate concentration to approximate the midpoint of the calibration curve, which is 3 $\mu\text{g Hg/L}$ for this method.
- 7.14 Quality Control Sample (QCS) – For initial and periodic verification of calibration standards and instrument performance, analysis of a QCS is required. The QCS is obtained from an outside source different from the standard stock solution, but prepared in the same manner as the calibration solutions. The concentration of the mercury in the QCS solution provides an absorbance reading near the midpoint of the calibration curve (3 $\mu\text{g Hg/L}$). The QCS is analyzed with every analytical run.



8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

- 8.1 Because of the extreme sensitivity of the analytical procedure and the possible presence of mercury in the laboratory environment, care is taken to avoid extraneous contamination. Sampling devices, sample containers and plastic items are free of mercury; the sample is not exposed to any condition in the laboratory that would result in contamination from airborne mercury vapor.
- 8.2 For the determination of total mercury (inorganic + organic) in aqueous samples, samples are not filtered, but acidified with (1+1) nitric acid (Sect. 7.3.1) to pH < 2 (normally 3 mL of (1+1) acid per liter of sample is sufficient for most ambient and drinking water samples). Preservation is sometimes done at the time of collection, but, the pH of all samples is checked and if necessary, the samples are acidified in the laboratory. The preserved sample is analyzed within 28 days of collection.

9.0 QUALITY CONTROL

- 9.1 The laboratory conducts a formal quality control (QC) program. An initial demonstration of laboratory capability was performed before samples were analyzed and laboratory reagent blanks, fortified blanks and quality control samples are analyzed on a continual basis to check method performance. The laboratory maintains performance records that define the quality of the data generated.

9.2 Initial Demonstration of Performance

- 9.2.1 Initial demonstration of performance was conducted immediately after instrument installation. LDR, MDL, IDL were produced prior to any analysis of environmental samples.
- 9.2.2 Linear Dynamic Range (LDR) - Established for the wavelength utilized and determined from a linear calibration prepared in the normal manner using the established analytical operating procedure for the instrument. The LDR was determined by analyzing increasingly higher standard concentrations of the analyte until the observed analyte concentration was no more than 10% below the stated concentration of the standard. The LDR are documented and kept on file. Determined sample analyte concentrations that are greater than 90% of the determined upper LDR limit are diluted and reanalyzed. The LDR are verified annually or whenever a change in analytical or instrument performance occurs, which would then dictate that the LDRs be re-determined.
- 9.2.3 Quality control sample (QCS) - The QCS is analyzed with every analytical run to verify the calibration standards. To verify the calibration standards, the mean concentrations from the QCS must be within $\pm 10\%$ of the stated values. If the calibration standards are not verified, performance is unacceptable and the determination of analytes is not continued. The source of the problem is identified and corrected before proceeding on with any analyses.
- 9.2.4 Method detection limit (MDL) - MDL is established for the wavelength of 253.7 nm (see Table 5) using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit (see Table 1). To determine MDL values, seven replicate aliquots of the fortified reagent water are processed through the entire analytical method. Calculation of the MDL is as follows:

$$MDL = (t) \times (S)$$



Where: t = students' t value for a 99% confidence level and a standard deviation estimate with $n-1$ degrees of freedom [$t = 3.14$ for seven replicates].

The MDL determined for this method is sufficiently low to detect mercury at the required levels.

9.3 Assessing Laboratory Performance

9.3.1 Laboratory reagent blank (LRB) - The laboratory analyzes one LRB with every batch of 10 samples of the same matrix. LRB data is used to assess contamination from the laboratory environment. LRB values that exceed the MDL indicate laboratory or reagent contamination.

9.3.2 Laboratory fortified blank (LFB) - The laboratory analyzes one LFB with each batch of samples. The LFB accuracy is calculated as percent recovery using the following equation:

$$R = \frac{LFB - LRB}{S} \times 100$$

Where:

R = Percent recovery

LFB = Laboratory-fortified blank

LRB = Laboratory reagent blank.

S = Concentration equivalent of analyte added to fortify the LRB solution.

If the recovery of the analyte falls outside the required control limits of 85 - 115%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3 The LFB analyses data are used to assess laboratory performance against the required control limits of 85-115%. When sufficient internal performance data becomes available (usually a minimum of twenty to thirty analyses), optional control limits are developed from the mean percent recovery (\bar{x}) and the standard deviation (S) of the mean percent recovery. These data are used to establish the upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = \bar{x} + 3SD$$

$$\text{LOWER CONTROL LIMIT} = \bar{x} - 3SD$$

The optional control limits must be equal to or better than the required control limits of 85-115%. After each five to ten new recovery measurements, new control limits are calculated using the most recent twenty to thirty data points. The standard deviation (S) data is used to establish an on-going precision statement for the level of concentrations included in the LFB. This data is kept on file and available for review.

9.3.4 Instrument performance check (IPC) solution - Analyzed with every analytical run immediately after calibration, and at the end of the analytical run. The IPC and



calibration blank are analyzed to verify that the instrument is within $\pm 5\%$ of calibration. Subsequent analyses of the IPC solution must be within $\pm 10\%$ of calibration. If the calibration cannot be verified within the specified limits, the IPC and the calibration blank are reanalyzed. If the second analysis of the IPC solution or the calibration blank is outside the limits, sample analysis is discontinued, and the cause of the problem is determined, corrected and/or the instrument recalibrated. All samples following the last acceptable IPC solution are reanalyzed. The analysis data of the calibration blank and IPC solution is kept on file with the sample analyses data.

9.4 Assessing Analyte Recovery and Data Quality

9.4.1 Sample homogeneity and the chemical nature of the sample matrix can affect analyte recovery and the quality of the data. Separate aliquots from the sample are taken for replicate and fortified analyses to assess the effect. Laboratory fortified matrix (LFM) samples and duplicate samples are processed to assess matrix effects.

9.4.2 The laboratory adds a known amount of each analyte to a minimum of 10% of the routine samples. In each case the LFM aliquot is a duplicate of the aliquot used for sample analysis and for total recoverable determinations added prior to sample preparation. For water samples, the added analyte concentration is the same as that used in the laboratory-fortified blank (Sect. 9.3.2).

9.4.3 Percent recovery for each analyte is calculated using the following equation.

Where:

$$R = \frac{(C_x - C) \times 100}{S}$$

R = Percent recovery

C_s = Fortified-sample concentration

C = Sample background concentration

S = Concentration equivalent of analyte added to fortify the sample.

9.4.4 If the recovery of the analyte falls outside the designated LFM recovery range, and the laboratory performance for that analyte is shown to be in control (Sect. 9.3.3), the recovery problem encountered with the fortified sample is judged to be matrix related, not system related. In this case, the Method of Standard Additions will be employed (Sect. 9.5).

9.4.5 Reference materials are utilized for every analytical run. They are analyzed to provide additional performance data, and demonstrate the ability to perform the method on a particular matrix.

9.5 The Method of Standard Additions (MSA) can be performed on samples that demonstrate matrix interference. Directions for using MSA are given in Section 11.5.

9.5.1 Analyte addition test: An analyte(s) standard added to a portion of a prepared sample, or its dilution, should be recovered to within 85% to 115% of the know value. The analyte(s) addition should produce a minimum level of 20 times and a maximum of 100 times the method detection limit. If the analyte addition is $< 20\%$ of the sample analyte concentration, the following dilution is used if recovery of the analyte(s) is not within the



specified limits, a matrix effect should be suspected, and the associated data flagged accordingly. The method of additions is employed to provide for accurate data.

- 9.5.2 Dilution test: If the analyte concentration is sufficiently high (minimally, a factor of 50 above the instrument detection limit in the original solution but < 90% of the linear limit), an analysis of a 1+4 dilution should agree (after correction for the fivefold dilution) within $\pm 10\%$ of the original determination. If not, a chemical or physical interference effect is suspected and the associated data flagged accordingly. The Method of Standard Additions provides more accurate data for samples failing this test.

10.0 CALIBRATION AND STANDARDIZATION (See also Section 11.0 - Procedure) Calibration curve must have 5 points starting with the calibration blank.

- 10.1 Acid wash and rinse pipettes before use.

11.0 PROCEDURE

11.1 Sample Preparation (Aqueous)

Must work under a fume hood

- 11.1.1 Spike LFB and LFM samples with appropriate concentrations.
- 11.1.1.1 Add 500 μL of the 0.10-mg/L-mercury standard prepared as described in Table 2 into a metal free 50-mL centrifuge tube marked for LFB or LFM. If spike is not high enough for sample, adjust LFM and rerun on the FIMS.
- 11.1.1.2 LFB - QS LFB to 20 mL with ASTM Type 1 reagent water
- 11.1.1.3 LFM – QS LFM to 20 mL with sample
- 11.1.2 Collect LRB, QCS, IPC, and MDL and MRL check samples. Transfer 20 mL of each check sample into corresponding marked 50-mL centrifuge tubes
- 11.1.3 Transfer 20 mL of sample into a 50-mL centrifuge tube. Test pH of sample at time sample is poured into centrifuge tube.
- 11.1.4 Add 1 mL of concentrated sulfuric acid (H_2SO_4)
- 11.1.5 Add 1 mL of diluted 1:1 nitric acid (HNO_3)
- 11.1.6 Add 3 mL of 5% w/v potassium permanganate (KMnO_4)
- 11.1.7 Add 1.6 mL of 5% w/v potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$)
- 11.1.8 Close vessel with screw cap, mix carefully, and vent any gas if necessary.
- 11.1.9 Digest samples for two hours in a water bath set at 95°C.
- 11.1.10 Ensure that the permanganate (purple color) has persisted throughout the digestion process; if it hasn't, then add more permanganate to ensure complete oxidation of the sample (KMnO_4 must be in excess). Add permanganate when sample has cooled.



Note: Color change usually happens as sample cools.

11.1.12 Remove sample from bath and allow it to cool to room temperature.

11.1.13 Add 1.2 mL of hydroxylammonium chloride solution to samples, and recap vessel and shake vigorously (Must work under a fume hood and cover vessel with a Kimwipe towel when shaking)

11.2 Instrumentation – Operation and Daily Maintenance

11.2.1 Turn on Argon gas cylinder. Argon gas must be at pressure > 50 psi. Turn on FIAS, Computer, and Printer.

11.2.2 Click on the AA Winlab Analyst Icon in the upper right of the Windows Screen. AAWinlab Version 2.3 appears, and initializes the Autosampler.

11.2.3 Click on Workspace Icon found below the File Heading.

11.2.4 Open Auto.fms file, click on OK. The workspace, Auto, Method, Results, Curve windows appears

11.2.5 View all selections in the Method Editor. Several methods are on file. Choose one. (The S.O.P. Method has the most commonly used settings). (read time is 15 seconds; cleanout time between samples is 1 cycle, 90 seconds)

11.2.6 On the FIAS Pump, check that the argon gas pressure is between 50-75 mL/min on the Venturi tube on the right. Tubing on the FIAS-100 pump should be changed with each run. Each tube should be stretched 25 times before putting on the pump.

RED-RED	Reductant 1.1% Stannous Chloride	UPPER MAGAZINE
BLUE-YELLOW	Carrier solution 3.0% Hydrochloric Acid	UPPER MAGAZINE
BLUE-YELLOW	Sample/Waste	LOWER MAGAZINE
BLACK-WHITE	Waste solution	LOWER MAGAZINE

11.2.7 Change filter in gas-liquid separator for every run. PTFE-membrane smooth-side down (B050 – 8306 pack of 50)

11.2.8 To condition the system, the analyst should pump the solutions for at least 10 minutes. Check Sample Introduction System – check for tube flattening, abrasion, or stretching & change peristaltic pump tubes if necessary; check magazine fittings and adjust if necessary; check distilled water wash solution.

11.2.8.1 Clamp the tubes down over the rollers.

11.2.8.2 Open FIAS Control Screen and click on PUMP 1.

11.2.8.3 Allow solutions to pump through the system, and monitor the gas-liquid separator; check gas-liquid separator; check bubble pattern; check filter in gas-liquid separator; and check for leaks and replace O-rings if showing signs of wear.



11.2.8.4 Make sure that no liquid is entering the Quartz Cell. Check for vapor buildup in tubing and quartz tube. Any sign of condensation in the tubing beyond the gas liquid separator indicates that the tension on the pump magazine is not sufficient.

11.2.9 Enter sample identification numbers in Sample Information Editor. Include sample units here:

Volume is in milliliters

Weight is in grams

Sample Volume is 20 mL

Dilutions require a factor for automatic calculation.

11.2.10 Analytical Sequence

Sequence	Sample ID
1	Calibration Blank
2	0.50 µg/L Calibration Standard Solution – if curve goes to 5.00 µg/L
3	1.00 µg/L Calibration Standard Solution
4	3.00 µg/L Calibration Standard Solution
5	5.00 µg/L Calibration Standard Solution
6	10.00 µg/L Calibration Standard Solution (Optional – use if sample concentration may be in the range of 5 to 10 µg/L)
7	Instrument Performance Check (IPC) A (± 5%) (3 µg/L - at approx. Midpoint Calibration)
8	Continuing Calibration Blank (CCB)
9	Method Detection Limit (MDL) Check Standard (0.2 µg/L) (± 20%)
10	Laboratory Reagent Blank (LRB)
11	Laboratory-Fortified Blank (LFB) (range between 2.0 to 3.0 µg/L) (± 15%)
12	Quality Control Sample (QCS) (± 10%)
13	MRL Check Standard (0.6 µg/L) (± 20%)
14	Sample 1
15	Sample 2
16	Sample 3
17	Sample 4
18	Sample 5
19	IPC B (3.0 µg/L) (±10%)
20	CCB
21	Sample 6
22	Sample 7
23	Sample 8



Sequence Sample ID

24	Sample 8 Duplicate (RPD \leq 20%)
25	Sample 8 Laboratory-Fortified Matrix (LFM, 2.5 $\mu\text{g/L}$) (\pm 30%)
26	Sample 8 LFM Duplicate (2.5 $\mu\text{g/L}$) (Optional) (RPD \leq 20%) (\pm 30% recovery)
27	QCS (\pm 10%)
28	IPC B (3.0 $\mu\text{g/L}$) (\pm 10%)
29	CCB

Note: Once the standards are run and the curve correlation coefficient has been calculated, the next two analyses run must be an IPC and a CCB. Beginning to count after this first CCB, 10 analyses are run and then another IPC & CCB are entered into the sample sequence. Run all samples in this 10-analyses set & then IPC & CCB order ending with QCS, IPC & CCB even if there are not 10 analyses in the last set of analyses. Sample duplicates, LFMs, and LFM duplicates count as samples in the sequence.

11.3 Automatic Analysis

- 11.3.1 **Setup.** Open Automatic Analysis Window and Enter Method Editor File and Sample Information File. Name a Result Data File
- 11.3.2 **Analyze.** Analyze All, or Calibrate. Sample run will start. Monitor Calibration Blank and standard absorbance. Check calibration curve and QCS recovery.
- 11.3.3 Analysis can be stopped at anytime during the run if you are not satisfied with instrument performance. Click on Analyze All. A window will appear asking what action you want to take. Stop immediately can be chosen to end analysis.

11.4 Report Utility. (To create a report)

- 11.4.1 Click on File Heading, then down to Utilities.
- 11.4.2 Report File. Report File opens.
- 11.4.3 Choose a design
- 11.4.4 Type in which result data file you want to print
- 11.4.5 Go to File, then print

12.0 DATA ANALYSIS AND CALCULATIONS

- 12.1 From the prepared calibration curve (Sect. 11.2.4), compute sample values by comparing response with the standard curve.
- 12.2 Calculate the mercury concentration in the sample by the formula:



$$\mu\text{g Hg/L} = (\mu\text{g Hg in aliquot}) \times \frac{(1,000)}{\text{mL of aliquot}}$$

12.3 Mercury concentrations are reported to the proper significant figures in mg/L or $\mu\text{g/L}$.

13.0 METHOD PERFORMANCE

13.1 Quality control samples and reference materials are used with every analytical run to demonstrate method performance.

14.0 POLLUTION PREVENTION

14.1 Refer to the WES Environmental Management System (EMS) policy and SOPs regarding pollution prevention.

14.2 The quantity of chemicals purchased should be based on expected usage during its shelf life. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

15.0 WASTE MANAGEMENT

15.1 WES laboratories fully comply with all applicable federal, state, and local environmental regulations. WES is also committed to protecting the air, water, and land by minimizing and controlling all chemical releases from fume hoods, biological safety cabinets, and bench operations. Refer to the WES EMS policy and SOPs regarding waste management.

15.2 All waste chemicals are collected in sealed waste containers. Once the waste containers reach capacity, they are transferred to the WES hazardous waste storage room where they are emptied into a waste drum (organic or inorganic). Within 180-days of waste accumulation, the waste drum is transported off the premises by a licensed hazardous waste management contractor. Under the WES EMS, a chemical inventory database has been developed to track purchases and use of chemicals and other hazardous materials, and the waste generated by the use of these chemicals.

16.0 REFERENCES

1. Kopp, J.F., Longbottom, M.C., and Lobring, L.B. 1972. Cold Vapor Method for Determining Mercury. *J. Am. Water Works Assoc.* 64(1).
2. American Chemical Society (ACS). 1979. *Safety in Academic Chemistry Laboratories*. 3rd Edition. ACS Committee on Chemical Safety.
3. Occupational Safety and Health Administration (OSHA). 1976. *OSHA Safety and Health Standards, General Industry*, 29 CFR 1910, OSHA 2206, revised January 1976.
4. Occupational Safety and Health Administration. 1986. Proposed OSHA Safety and Health Standards, Laboratories. *Federal Register*, July 24, 1986.
5. ASTM. 1990. Specification for Reagent Water, D1193. *Annual Book of ASTM Standards*, Vol. 11.01.
6. Code of Federal Regulations 40, Ch. 1, Pt. 136 Appendix B.



17.0 TABLES AND VALIDATION DATA

TABLE 1. Quality Control Elements and Acceptance Limits for the Analysis of Mercury In Water By Cold Vapor Atomic Absorption Spectrometry

QC Elements	Frequency	Acceptance Criteria	Corrective Action
Linear Dynamic Range (LDR)	Every year	At least three standards, one of which is close to the upper limit of the LDR (observed concentration is within 10% of standard).	Check/service instrument.
Instrument Stability	30-minute warm-up	RSD < 5% for a mid-point calibration standard.	Determine and correct the cause, recalibrate before analyzing samples
Initial Calibration	Every run	$r^2 > 0.995$	Recalibrate with new standards
Instrument Performance Check Standard, IPC A & IPC B	Immediately following each calibration, after every tenth sample, and at the end of the run	IPC A (a midpoint calibration standard; first IPC after running the curve; $\pm 5\%$) & IPC B (after every tenth sample and at the end of the run; $\pm 10\%$)	Reanalyze IPC, if outside range, recalibrate and re-analyze sample, or discontinue & recalibrate instrument if necessary.
Calibration Blank (CCB)	Immediately following each calibration, after every tenth sample and at the end of the run	< MDL but > a negative signal in concentration units equal to the MDL	Reanalyze. Determine cause or recalibrate instrument.
Quality Control Sample (QCS)	After calibration and at the end of the run.	$\pm 10\%$	Acceptable range must be met before continuing with sample analysis. Recalibrate and repeat.
Laboratory Reagent Blank (LRB)	One with each batch of 20 or fewer samples	< 2.2 times the analyte MDL or < 10% of the analyte level measured in the sample	Determine and eliminate the source of contamination and then repeat sample analysis
Duplicate	Every 10 samples or less	Relative Percent Difference (RPD) less than or equal to 20%	Repeat using fresh sample if possible. If the sample is non-homogenous, qualify the sample result.
LFM	Every 10 samples or less	70 – 130% Note: Recovery calculation is not required if the concentration spiked is less than 25% of the unfortified sample concentration.	Qualify the sample result as matrix interference or perform Method of Standard Additions (rarely done).
LFB	One with each batch of 20 samples	85 – 115%	Source of the problem must be identified and resolved before continuing analysis
MRL Check Standard	At the beginning of every analytical run	$\pm 20\%$	Acceptable range must be met before reporting data. If not acceptable, then recalibrate and repeat.
MDL Check Standard	At the beginning of every analytical run	$\pm 20\%$	Acceptable range must be met before reporting data. If not acceptable, then recalibrate and repeat.
MDL determination (USEPA, 1997)	Annually	Target analyte concentration spiked into the blank matrix must not exceed 10 times (approximately) the experimentally determined MDL	Repeat MDL study spiking the blank matrix with lower concentration of the target analyte



TABLE 2. Preparation of Mercury Standards

Concentration of Standard	Dilute	Function
100-mg/L standard	10 mL of 1000 mg/L q.s. to 100 mL	
10-mg/L standard	10 mL of 100 mg/L q.s. to 100 mL	
1.0- mg/L standard	10 mL of 10 mg/L q.s. to 100 mL	
100-µg/L (0.10-mg/L) standard	10 mL of 1.0 mg/L q.s. to 100 mL	Spiking Solution for LFM, LFB, IPC
20-µg/L (0.02-mg/L) standard	20 mL of 0.10 mg/L q.s. to 100 mL	Calibration Standard
10-µg/L (0.01-mg/L) standard	10 mL of 0.10 mg/L q.s. to 100 mL	Calibration Standard
**5-µg/L (0.005-mg/L) standard	5 mL of 0.10 mg/L q.s. to 100 mL	Calibration Standard
**3-µg/L (0.003-mg/L) standard	3 mL of 0.10 mg/L q.s. to 100 mL	Calibration Standard
2-µg/L (0.002-mg/L) standard	2 mL of 0.10 mg/L q.s. to 100 mL	Laboratory Fortified Blank
**1-µg/L (0.001-mg/L) standard	10 mL of 0.01 mg/L q.s. to 100 mL	Calibration Standard
0.6-µg/L (0.0006-mg/L) standard	6 mL of 0.001 mg/L q.s. to 100 mL	MRL Check Standard
0.2-µg/L (0.0002-mg/L) standard	2 mL of 0.001 mg/L q.s. to 100 mL	MDL Study & MDL Check Standard
** Signifies Normal Working Calibration Standards		

See Bench Prep Sheets for EPA 245.1:

[Bench Data Collection Forms\Mercury Std Prep for EPA 245.1.doc](#)

[Bench Data Collection Forms\Mercury QCS Std Prep for EPA 245.1.doc](#)

[Bench Data Collection Forms\Reagents Prep for EPA 245-1.doc](#)



TABLE 3. Accuracy of Mercury Analysis in Reagent Water by EPA Method 245.1

Date	Accuracy (% Recovery) ^a					
	Mean	SD ^b	Warning Limits (± 2 SD)		Control Limits (± 3 SD)	
			Upper (UWL)	Lower (LWL)	Upper (UCL)	Lower (LCL)
02-14-12 to 11-28-12	101	1.6	104	97	106	96

^a Based on analysis of 11 readings from mid-curve standards.

^b SD = standard deviation.



TABLE 4. Precision of Mercury Analysis in Reagent Water by EPA Method 245.1

Date	Precision ^a Relative Percent Difference (RPD)					
	Mean	SD ^b	Warning Limits (± 2 SD)		Control Limits (± 3 SD)	
			Upper (UWL)	Lower (LWL)	Upper (UCL)	Lower (LCL)
02-14-12 to 02-15-12	3.1	4.7	13	0	17	0
^a SD = Based on the analysis of 3 sample duplicates ^b SD = Standard deviation						

TABLE 5. Method Detection Limit (MDL) for Mercury Analysis in Reagent Water by EPA Method 245.1

Date of Study	No. of Samples Spiked (n)	Spiked Concentration (mg/L)	Accuracy (Mean % Recovery ^a)	Precision (SD ^b in mg/L)	MDL (mg/L)
02-14-12, 02-15-12 & 11-28-12	7	0.0004	101	0.000027	0.000084
^a Recovery of spiked concentration ^b SD = standard deviation of mean concentration measured					