

Laboratory Procedure Manual

Analyte: **Mercury**

Matrix: **Hair**

Method: **Cold Vapor Atomic Fluorescence
Spectrometry**

Method No.:

Revised:

as performed by: *Research Triangle Institute
Research Triangle Park, NC*

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Important Information for Users

The Research Triangle Institute periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for NHANES 1999–2000 data.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label
lab22	HRQ010	Permanent, Straightened, or Dyed
	HRXHG	Total Hair Mercury (ppm)
	HRDHGLC	Total Hair Mercury Comment Code
	HRDHG	
	HRDHGLC	

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The purpose of this protocol is to provide guidelines for the analysis of hair samples for total mercury (Hg) by cold vapor atomic fluorescence (CVAFS) spectrometry. This protocol describes the methodology and all other analytical aspects involved in the analysis of hair samples for mercury (Hg) by cold vapor atomic fluorescence spectrometry (CVAFS).

This method involves the extraction of the analyte from hair samples using 30/70 sulfuric/nitric acid, and subsequent analysis by cold vapor atomic fluorescence spectrometry. The analyte is identified by the presence of fluorescence signal from a mercury-specific detector. Quantitation of the analyte is carried out using a standard calibration curve. Recovery of the analyte is monitored using samples that are spiked with standards prior to the extraction process.

2. SAFETY

Since the toxicity of the chemicals used in this method is not clearly defined, they should be treated as potential health hazards at all times. All laboratory personnel are advised to take full precautions (wearing gloves, eye protection etc.) when handling these chemicals and personal exposure to these chemicals should be minimized. Safety issues in the ACS clean lab facility are discussed in detail in the ACS/SOP-174-006, "Standard Operating Procedures for Personnel Safety in the ACS Inorganic Clean Lab Facility."

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

A. Processing of Data Output

Instrument software is capable of processing the raw data (fluorescence intensities) and generating a printed output. The output contains information on sample I.D., analyte tested, fluorescence, concentration of the analyte in the sample, and statistical data. This type of output helps in visual examination of data for any unusual behavior and/or any inadequate method performance.

B. Data Storage

All raw data acquired during the analysis are stored on the hard drive of the computer dedicated to the instrument, along with the processed data. At the end of each day of analysis all data are transferred to floppy disks. These floppy disks are labeled and stored in a central storage area along with the printed outputs.

C. Data Transmission

Once reviewed and accepted by the laboratory manager, raw and processed data generated by the instrument are converted to ASCII format and handed over to the database manager for further processing. Example of an ASCII data file generated by CVAFS instrument software is given in Table 3 below.

TABLE 3. Example of an ASCII data file generated by the CVAFS software

No.	Tag	Sample	Ref	El	Concentration	Runs	SD	Output		Time	Date
2	Std	1	0.000	Hg	0.000 ppb	2	0.034	O/P	0.034	17:15	20Dec94
Posn = 5	Runs: 1 = 0.000	Runs: 2 = 0.068									
3	Std	2	0.050	Hg	0.050 ppb	2	9.723	O/P	14.56	17:22	20Dec94
Posn = 6	Runs: 1 = 4.843	Runs: 2 = 24.29									
4	Std	3	0.100	Hg	0.100 ppb	2	0.125	O/P	10.20	17:27	20Dec94
Posn = 7	Runs: 1 = 10.33	Runs: 2 = 10.08									

Hair Mercury
NHANES 1999–2000

5	Std	4	0.300	Hg	0.300 ppb	2	0.558	O/P	35.90	17:31	20Dec94
Posn = 8	Runs: 1 = 36.46		Runs: 2 = 35.34								
6	Std	5	0.700	Hg	0.700 ppb	2	0.334	O/P	86.67	17:36	20Dec94
Posn = 9	Runs: 1 = 87.00		Runs: 2 = 86.33								
7	Std	6	1.000	Hg	1.000 ppb	2	60.34	O/P	60.34	17:40	20Dec94
Posn = 10	Runs: 1 = 120.6		Runs: 2 = 0.000								
8	Blank	BLANK		Hg	0.022 ppb	1	0.000	ppb	2.563	18:38	20Dec94
Posn = 5	Runs: 1 = 2.563										
Least Sq To 1.000 ppb RSF = 1.000 Slope = 139.8 Corr Coeff = 0.999 Y intercept = -0.57											
9	AutoQC	0.300		Hg	0.289 ppb	1	0.000	ppb	39.88	18:41	20Dec94
Posn = 8	Runs: 1 = 39.88										
11	112294	RB1		Hg	0.012 ppb	1	0.000	ppb	1.126	18:45	20Dec94
Posn = 11	Runs: 1 = 1.126										
12	112294	RB2		Hg	0.011 ppb	1	0.000	ppb	1.097	18:47	20Dec94
Posn = 12	Runs: 1 = 1.097										
13	112294	LWW-BLK1		Hg	0.244 ppb	1	0.000	ppb	33.59	18:50	20Dec94
Posn = 13	Runs: 1 = 33.59										
14	112294	LWW-BLK2		Hg	0.253 ppb	1	0.000	ppb	34.91	18:52	20Dec94
Posn = 14	Runs: 1 = 34.91										
15	112294	LWW-BLK3		Hg	0.251 ppb	1	0.000	ppb	34.58	18:54	20Dec94
Posn = 15	Runs: 1 = 34.58										
16	112294	LWW-003		Hg	0.008 ppb	1	0.000	ppb	0.546	18:56	20Dec94
Posn = 16	Runs: 1 = 0.546										
19	112294	LWW-005		Hg	0.194 ppb	1	0.000	ppb	26.67	19:08	20Dec94
Posn = 17	Runs: 1 = 26.67										
20	112294	LWW-007		Hg	0.400 ppb	1	0.000	ppb	55.42	19:10	20Dec94
Posn = 18	Runs: 1 = 55.42										
22	112294	LWW-009		Hg	0.093 ppb	1	0.000	ppb	12.56	19:15	20Dec94
Posn = 19	Runs: 1 = 12.56										
23	Blank	BLANK		Hg	0.041 ppb	1	0.000	ppb	5.185	19:18	20Dec94
Posn = 4	Runs: 1 = 5.185										
24	AutoQC	0.300		Hg	0.300 ppb	1	0.000	ppb	41.45	19:20	20Dec94
Pos = 7	Run:1 = 41.45										

4. Specimen Collection, Storage, and Handling Procedures; Criteria for Specimen Rejection
5. Procedures for Microscopic Examinations; Criteria for Rejection of Inadequately Prepared Slides
Not applicable for this procedure
6. Preparation of Reagents, Calibration (Standards), Controls, and All Other Materials; Equipment and Instrumentation
 - A. Laboratory Equipment

- (1) Beakers, Teflon and glass
- (2) Volumetric flasks, glass
- (3) Pipette tips, plastic
- (4) Scintillation vials
- (5) Teflon liners and silicone/Teflon septa, 22 mm diam. for scintillation vial caps
- (6) Storage bottles, glass or Teflon
- (7) Hot plates
- (8) Muffle oven
- (9) Automatic pipettors, fixed and variable volume
- (10) Refrigerator
- (11) Freezer
- (12) Analytical balance with 0.01 mg to 205 g weighing range, capable of 0.01 mg readability
- (13) Hydrochloric acid ("Trace Metal" grade or better)
- (14) Nitric acid ("Trace Metal" grade or better)
- (15) Tin (II) chloride, dihydrate (ACS reagent grade)
- (16) Hydroxylamine hydrochloride (ACS reagent grade)
- (17) Potassium bromide (ACS reagent grade)
- (18) Potassium bromate (ACS reagent grade)
- (19) Sulfuric acid (Trace Metal grade or better)
- (20) Deionized water (Hydro Pico System, 18 megaohm quality, better-than or equivalent to ASTM Type I grade.)
- (21) Commercially available, spectrometric grade, certified mercury reference solution (NIST traceable) at 1000 mg/L is used as the stock solution.

B. Preparation of Labware

(1) General Labware, Teflon and Glass

All new labware is soaked for a minimum of 48 hours (two 8-hour days at 65-75°C and overnight as the bath cools) in a 4 N hydrochloric acid bath, rinsed five times with DI water, and dried under HEPA filtered air. An additional soak in 1% HCl for a minimum of 16 hours (one 8-hour day at 60-70°C and overnight as the bath cools) is required for sample storage vials. (After the initial cleaning, the labware is soaked for a minimum of six hours in a 4 N hydrochloric acid bath at 65-75°C.) Labware may be soaked in a solution of low metal, phosphate-free detergent (e.g., Alconox Detergent 8) and rinsed with DI water prior to acid soaking. If not used immediately, the clean labware is sealed in double plastic bags for storage in the Class 100 lab. Cleaning of labware in the ACS Inorganic Clean Lab Facility is described in ACS/SOP-174-002, "Standard Operating Procedures for Cleaning Labware in the ACS Inorganic Class 100/10,000 Clean Lab Facility."

(2) Pipette Tips

Plastic pipet tips are rinsed with DI water, dried under HEPA filtered air, and stored in sealed plastic bags or plastic storage boxes in the Class 100 lab.

(3) Autosampler Cups

Plastic autosampler cups are soaked in a 50% nitric acid bath for a minimum of sixteen hours, drained, rinsed five times with DI water, dried under HEPA filtered air, and stored in sealed double plastic bags in the Class 100 lab.

Autosampler cups are reused after cleaning. After use, cups are rinsed with DI water and soaked in water or water containing low metal, phosphate-free detergent, rinsed with DI water, and soaked in the 50 % nitric acid bath as described above.

(4) Capliners, Caps, and Septa

Teflon capliners are cleaned.

Caps for scintillation vials are processed as follows. If caps come with liners, the original foil liner is removed from the cap and traces of glue are removed with acetone. The caps are rinsed with DI water 3 times, then soaked in DI water for ≥ 1 hour. They are rinsed with DI water 3 times and dried under HEPA filtered air. Clean caps are stored in double sealed plastic bags. (Black plastic caps are used when the sealed vial will be heated.)

Teflon-faced silicone septa (22 mm diameter) are rinsed and soaked in DI water as described above for caps.

(5) Preparation of Reagent Solutions

(a) 10% (v/v) HCl

To approximately 200 mL of DI water in a 1-L volumetric flask add 100 mL of concentrated HCl. Add water to volume and mix well. Transfer to a glass or Teflon bottle with a Teflon-lined cap for storage in a refrigerator.

(b) Tin (II) Chloride, 2% (w/v) in 10% (v/v) HCl

Weigh a 20-g aliquot of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ in a tared glass beaker. Add approximately 60-80 mL of a 100-mL aliquot of concentrated HCl to the compound in the beaker. Allow the compound to completely dissolve in the acid, then transfer to a 1-L volumetric flask. Add the remaining HCl to the flask. Rinse the beaker several times with DI water and add the rinses to the flask. Add water to volume and mix well. Transfer to a glass or Teflon bottle with a Teflon-lined cap for storage in a refrigerator.

(c) 12% (w/v) Hydroxylamine HCl

Weigh a 12-g aliquot of $\text{NH}_2\text{OH} \cdot \text{HCl}$ into a tared 100-mL volumetric flask. Add DI water to the flask (to approximately the bottom of the neck) and allow the solid to dissolve completely. Add DI water to volume and mix well. If necessary, the solution may be filtered through a 0.2- μm pore size, 25-mm diameter Gelman Ion Chromatography Acrodisc filter. The solution is transferred to a Teflon or glass bottle with a Teflon-lined cap and stored in a refrigerator.

(d) KBr/ KBrO_3 Solution

Weigh separate aliquots of KBr (nominally 5.95 g) and KBrO_3 (nominally 1.39 g) into small glass beakers. Cover the beakers with watch glasses and place in a muffle oven set at 250°C for ≥ 8 hours. Cool the beakers in a desiccator, then transfer both aliquots to the same 500-mL volumetric flask. The beakers are rinsed with DI water several times with rinses added to the flask. Dilute to volume and mix well. Transfer the solution to a glass or Teflon bottle with a Teflon-lined cap for storage in a refrigerator.

(e) Blank Solution

The blank solution for the instrument should be prepared to match as much as possible the background of the samples, i.e., it should contain the same reagents at the same concentrations. The calibration standards are also diluted in this blank solution. Generally, 2-4 liters of blank solution are required for a mercury analysis. The solution is stored in a glass or Teflon bottle with a Teflon-lined cap in a refrigerator.

(f) Preparation of the Extracting Solvent (30/70 Sulfuric/Nitric Acids)

The following describes the preparation of 200 mL of the concentrated acid mixture. Other volumes may be prepared by combining appropriate volumes of the acids. The procedure should be performed in an exhaust hood under Class 100 conditions. To a Teflon or glass bottle in an ice bath add 140 mL of nitric acid with stirring. Slowly add 60 mL of sulfuric acid. Continue to stir the mixture in the ice bath uncapped to allow fumes to be exhausted.

WARNING! This mixture gets very hot and generates caustic fumes! Perform the preparation with caution in an exhaust hood in an ice bath with constant stirring to prevent exposure to hot concentrated acid and caustic fumes!

(6) Working Standard Solutions

Working standard solutions are prepared at 10 ng/mL in duplicate by diluting the stock standard solution using 10% HCl to stabilize the analyte. Working standard solutions are stored in acid

washed Teflon or glass containers and prepared fresh periodically (annually for ≥ 1 ppm and monthly for concentrations between 1 ppm and 10 ppb).

(7) Calibration Standards

A series of calibration standards is prepared on a daily basis by dilution of working standard solutions with the reagent blank solution [Sections 6.B.(5)(e) and (6)]. Two working standards are used alternately to prepare the calibration standards. At least five different concentration standards must be used to define the linear portion of the calibration curve, and a calibration blank should also be prepared and included in the calibration. The calibration blank should contain all the components in the calibration standards except the analyte. Calibration standards should cover the full range of analyte concentrations expected to be analyzed by the instrument. The concentrations of calibration standards are based on the concentrations found in typical hair samples, and are given in Table 1.

TABLE 1. SUGGESTED CALIBRATION SOLUTIONS

Analyte	Concentration of the Analyte (pg/mL)						
	#1	#2	#3	#4	#5	#6	#7
Hg	0.0	5	10	30	50	80	95

7. Calibration and Calibration Verification Procedures

Acceptable performance of the instrument must be demonstrated and documented as described in Section 2.3 of ACS/SOP-174-010, "Standard Operating Procedure for the Operation of PS Analytical Merlin Plus Atomic Fluorescence Spectrometer" prior to any sample analysis and the demonstrated acceptable level must be maintained throughout the analysis. A new calibration curve is constructed at the beginning of each day of analysis. Additional calibration checks are performed periodically during sample analysis as described in Section 11.0.

A. Initial Calibration

- (1) An instrument performance test is performed prior to the analysis of any samples as described in Section 2.3 of ACS/SOP-174-010, "Standard Operating Procedure for the Operation of PS Analytical Merlin Plus Atomic Fluorescence Spectrometer". If the performance test meets the acceptance criteria, defined in the SOP, proceed with the initial calibration (starting from Section 10.1.2).

If the performance test does not meet the acceptance criteria, necessary steps (Section 10.2.5) will be taken to bring the performance of the instrument to an acceptable level.

- (2) Acceptable performance of the instrument must be demonstrated and documented as described in Section 2.3 of ACS/SOP-174-010, "Standard Operating Procedure for the Operation of PS Analytical Merlin Plus Atomic Fluorescence Spectrometer" prior to any sample analysis and the demonstrated acceptable level must be maintained throughout the analysis. A new calibration curve is constructed at the beginning of each day of analysis
- (3) At the beginning of each day of analysis, a new calibration curve is constructed. Calibration blank and calibration standards are analyzed for mercury starting from the lowest concentration to the highest. Each solution (blank and standards) is analyzed singly.
- (4) The fluorescence intensity is measured for each calibration standard and a least square linear regression calibration curve is constructed as follows for each analyte by the instrument software:

$$y = a + bx$$

where:

$$y = \text{net fluorescence intensity}$$

a = y intercept
b = slope
x = analyte concentration (pg/mL)

- (5) Linearity of the calibration curve must be verified both visually and mathematically. The correlation coefficient (r) must be greater than 0.99.
- (6) Concentration of the analyte in the calibration standards calculated using the measured signal and the calibration curve must be $\pm 20\%$ of the nominal concentration for lowest calibration standard and $\pm 10\%$ for all other calibration standards.

B. Initial Calibration Check

The performance of the initial calibration must be verified prior to any sample analysis as follows.

- (1) Analyze a predetermined QC check (see Section 8.C.) and compare the new results with the previous results.
- (2) Calculate the difference between the two values and verify that it has not changed by more than 10% from the initial calibration. If it has changed more than the above limits, adjustments must be made to restore system sensitivity and recalibration or resloping is required.

At the beginning of each day of analysis, a new calibration curve is constructed. Calibration blank and calibration standards are analyzed for mercury starting from the lowest concentration to the highest. Each solution (blank and standards) is analyzed singly.

- (3) The fluorescence intensity is measured for each calibration standard and a least square linear regression calibration curve is constructed as follows for each analyte by the instrument software.

$$y = a + bx$$

where:

y = net fluorescence intensity
a = y intercept
b = slope
x = analyte concentration (pg/mL)

- (4) Linearity of the calibration curve must be verified both visually and mathematically. The correlation coefficient (r) must be greater than 0.99.
- (5) Concentration of the analyte in the calibration standards calculated using the measured signal and the calibration curve must be $\pm 20\%$ of the nominal concentration for lowest calibration standard and $\pm 10\%$ for all other calibration standards.

8. Procedure Operating Instructions; Calculations; Interpretation of Results

A. Sample preparation

(1) Processing and Weighing

A 3-cm segment of hair from the scalp end will be selected for analysis. The hair sample is removed from the sealed plastic bag and a 3-cm segment is cut off with scissors and transferred to a cleaned glass vial. The vial is sealed with an airtight screw cap (lined with a Teflon-faced silicone septum), labeled, placed in double polyethylene bags (airtight), and stored in a freezer until use. The selected hair is transferred to a labeled, preweighed polyvial with a plastic cap, and rinsed with 0.5 mL of acetone, which is decanted from the vial. The hair is allowed to dry for ≥ 3 hrs (or until completely dry) with the cap off under HEPA filtered air. The next day the weight of the "sample + vial" is recorded in the lab notebook. The target sample weight is 5 mg. The hair is then transferred to a 20-mL glass scintillation vial with an airtight plastic screw cap (lined with a Teflon-faced silicone septum beneath a Teflon capliner). The polyvial is rinsed with 1 mL of DI water to transfer any remaining hair segments and added to the scintillation vial. Normal levels of Hg in hair (as cited in Reference 1) are 1.9 ± 0.9 ppm in persons eating fish 1-4 times per month.

(2) Sample Extraction

Extraction solvent (1 mL of 30/70 sulfuric/nitric acids) is added to the hair sample in the scintillation vial. The vial is sealed with an airtight cap [described in Section 6.B.(4)] and placed in an oven at 90°C for 6-8 hours. After cooling, 2.0 mL of DI water is added to each vial and mixed well. Two mL of solution is transferred to a 25-mL glass volumetric flask and 1 mL each of concentrated HCl and bromide/bromate solution is added to the flask, which is capped and allowed to sit overnight in class 100 hood at room temperature. Hydroxylamine hydrochloride solution (90–150 µL) is then added to decolorize the sample. The solution is then brought to volume with DI water. The sample is analyzed on the same day that hydroxylamine hydrochloride solution is added. The remaining portion (2 mL) of the original sample is transferred to a clean 15-mL low density polyethylene (LDPE) bottle, sealed with Teflon tape, and stored in the refrigerator.

B. Analysis procedure

(1) Analytical Conditions

The instrumental parameters for the analyte are given in Table 2.

TABLE 2. Operating parameters for the instrument

Cold vapor atomic fluorescence spectrometer (CVAFS)	
Experimental parameters:	
Element	Hg
Source	Hg lamp (254 nm)
Emission Filter	254 nm
Sample volume (mL)	10–12
Measurement mode	Peak Ht.
Sensitivity	Range: 1000 Fine Gain - 10.0
Analysis cycle (typical) :	
Delay (sec)	5
Rise (sec)	25
Analysis (min)	0.5
Memory (sec)	60
Reductant	2% SnCl ₂ in 10% HCl
Blank	matched to samples
Carrier gas flow (L/min)	0.4–0.5 (Ar)
Sheath gas flow (L/min)	0.3 L/min (Ar)
Dryer tube gas flow (L/min)	3.0 L/min (Ar)

(2) Analysis Batches

Sample preparation and analysis are carried out in batches of samples. A batch is defined as a fixed number of samples than can be handled conveniently and efficiently in the laboratory. A batch may include 25-30 samples, 4-5 matrix standards, 3 reagent blanks, and 1 method control. At least one performance evaluation (PE) sample will be prepared with each batch of samples and analyzed provided that a suitable PE sample is available (see Section 8.D.). Field blanks may also be included in the batch if available, but as they are unknown to the analyst they will be treated as samples. The order of analysis is:

- Calibration blank
- QC check standard
- Reagent blank
- Method control
- Performance evaluation sample
- Matrix standards
- Samples
- Periodic QC checks

Prior to any sample analysis, a set of calibration standards will be analyzed and a calibration curve constructed. A pre-determined calibration standard will be selected as the QC check standard and analyzed along with the calibration blank.

Reagent blanks will be analyzed with each batch of samples and subtracted from method controls/samples.

A method control and at least one performance evaluation sample will be run with each batch of samples and the recovery of the analyte will be monitored.

(3) Data Acquisition

Data acquisition is carried out by the instrument software. The fluorescence signal given out by the excited atoms is measured during data acquisition. Once the data acquisition is completed, the concentration of the analyte in the sample is calculated based on the standard calibration curve.

(4) Identification of the Analyte

Analyte is identified by the presence of fluorescence signal, from Hg-specific detector. The analyte is present in the sample if the concentration of the analyte is greater than its method detection limit.

(5) Cold Vapor Atomic Fluorescence Spectrometer (CVAFS)

- (a) The spectrometer must have the capability of automation to reduce personnel errors to a minimum.
- (b) Vapor generator must have the capability to generate vapor species in both continuous mode and flow injection mode. It should have an active pumping system to deliver the sample, blank and reagent to the mixing chamber with high reproducibility. The presence of an efficient separation technique to separate gaseous species from the solution is also important and finally there should be an effective transport system to transport the gaseous species to the atomizer cell.
- (c) An autosampler is required to deliver reproducible volumes of sample and reagents into the reaction chamber or mixing valve in a consistent manner (addition of reagents in proper order).
- (d) The data system must be capable of data acquisition, storage and processing including producing an output. Data acquisition software must allow the use of different integration periods and also be capable of acquiring data under both peak height and peak area modes. Data processing software must be capable of analyzing the raw data, calculating net fluorescence, calculating statistics (mean, standard deviation, relative standard deviation), constructing calibration curves and calculating analyte concentrations. It is also recommended that the data system be able to transfer its data from the instrument to peripheral computers for further processing.

C. Calculations

The concentration of the analyte in the sample is calculated during the analysis by the instrument software against the reference calibration curve using the measured fluorescence as follows:

$$C_x = \frac{(y-a)}{b}$$

where:

- C_x = Measured concentration of the analyte in pg/mL or parts-per-trillion (ppt).
- y = The measured fluorescence for the sample.
- a = y intercept of the reference calibration curve in fluorescence.
- b = Slope of the reference calibration curve in fluorescence intensity per pg mL⁻¹.

A plot of measured fluorescence intensity versus added mercury will be constructed for the matrix standards and a matrix correction factor (CF) will be calculated as the slope ratio of the reference calibration curve/matrix curve. The corrected concentration of mercury in the hair sample is then calculated by multiplying the measured concentration by the matrix correction factor.

Samples with concentrations below MDL/MQL will be expressed appropriately, whereas over-range samples will be diluted and re-analyzed.

Once the data are transmitted to the central database, calculations may be performed to express the final results in any of the following formats.

The following format may be used to calculate the final results in µg of analyte per gram of hair sampled:

$$C_{X2} = (C_x - C_{RB}) \times CF \times V_x \times DF \times \frac{1}{WT} \times 10^{-6}$$

where:

- C_{X2} = Corrected concentration of the analyte in µg/g of hair
- C_x = Measured concentration of the analyte in pg/mL or ppt.
- V_x = Final volume of the sample in mL.
- DF = Dilution factor.
- WT = Mass of the hair sample in g.
- C_{RB} = The average concentration of the analyte in reagent blanks in pg/mL or ppt.
- CF = Correction factor.
- 10^{-6} = Unit conversion factor, µg/pg.

D. Method validation

The extraction method from Section 8.A.(2) will be validated prior to any sample analysis. Six different matrix standards will be prepared by spiking 5 mg aliquots of a suitable composite hair sample with mercury reference standards, with triplicate preparations of both the lowest and highest concentrations and single preparations of concentrations at the middle levels. Three unspiked hair samples will also be prepared. These samples will be carried through the extraction procedures above and analyzed with aqueous Hg calibration standards prepared at the same six concentrations (accounting for sample dilution) as well as a calibration blank. Linearity, accuracy, and recovery will be assessed from the analytical results.

Additionally, if a suitable certified reference material, such as NIES-13, is available, it will be analyzed to further demonstrate method performance.

(1) Minimum Detection Limit (MDL)

Minimum detection limit (MDL) is defined as 3× the standard deviation of reagent blanks. Minimum

detection limit is calculated for the analyte using the following equation:

$$\text{MDL} = 3 \times \text{SD}_{\text{bl}}$$

MDL = Minimum detection limit, pg/mL or ppt.

SD_{bl} = Standard deviation of reagent blanks, pg/mL or ppt.

(2) Minimum Quantitation Limit (MQL)

Minimum quantitation limit (MQL) is defined as 10× the standard deviation of reagent blanks.

Minimum quantitation limit is calculated for the analyte using the following equation;

$$\text{MQL} = 10 \times \text{SD}_{\text{bl}}$$

MQL = Minimum quantitation limit, pg/mL or ppt.

SD_{bl} = Standard deviation of reagent blanks, pg/mL or ppt.

9. Reportable Range of Results

The reportable range is based on the linear region of the calibration curve.

10. Quality Control (QC) Procedures

A. Reagent Blanks

Reagent blanks are extracting solvent carried throughout the sample preparation procedure along with each batch of samples analyzed. Analysis of these will provide information on contamination that result from sample extraction and preparation steps.

B. Method Controls

Method controls are extracting solvents spiked with a certified mercury reference solution (NIST traceable) and treated the same as reagent blanks. Analysis of these will provide information on recoveries of the target analyte from sample extraction and preparation steps.

C. QC Check Standard

A mid-level calibration standard will be selected as the QC check standard and analyzed every 10 samples during the analysis.

D. Performance Evaluation Samples

Once acceptable performance of the method is demonstrated for the analyte by method controls, a performance evaluation sample is analyzed to further evaluate the overall performance of the method. The performance evaluation sample must contain the target analyte with the level certified by an approved material certification agency, in a matrix almost identical to the sample matrix. An example of this type of material is, NIES-13, a Japanese certified human hair standard (National Institute for Environmental Studies, Japan). The mercury concentration in this material is $4.42 \pm 0.20 \mu\text{g/g}$.

E. Replicate Analysis

A subset of samples (approximately 10%) will be prepared and analyzed in duplicate, provided that sufficient amount of sample is available. These samples will be identified by an additional character, "R."

F. Quality Control

A method control will be run with each batch of samples and the recovery of the analyte will be monitored. If the calculated recoveries are outside the range 90–110% from the certified value or from one of the value limits, a second aliquot will be analyzed. Questionable results will be reviewed with the laboratory manager or facility supervisor and the QA officer and a final decision is made at that time.

A performance evaluation sample (NIES-13) is analyzed with each batch of samples and the recovery of the analyte monitored. If the calculated recovery is not within $\pm 10\%$ of the certified value or one of the value limits, a second aliquot is analyzed. Questionable results are reviewed with the laboratory manager or facility supervisor and the QA officer and a final decision made at that time.

The selected QC check standard is analyzed along with the calibration blank immediately after the calibration, after every 10 samples and at the end of the analysis. All QC check standards must be within 10% of their nominal value or initial value for the data to be accepted. Samples for which the QC checks differ by more than 10% of their nominal and initial values will be flagged and/or re-analyzed (if sufficient sample remains to do so).

Daily performance test results will be plotted against time (in days) for the CVAFS instrument. The sensitivity (Pk.Ht. per pg mL⁻¹) will be used as the performance parameter. This plot will be used as a control chart to continuously monitor the performance of the instrument over time. The operator of the instrument is responsible for maintaining the chart. The chart must be presented to the laboratory manager or to the task leader for his/her review at least once a month. The control chart and data must be stored along with the sample analysis data.

11. Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria

Possible remedial action are described in the "Standard Operating Procedure for the Calibration and Maintenance of PS Analytical Merlin Plus Atomic Fluorescence Spectrometer" (ACS/SOP-174-011) and in the "Standard Operating Procedure for the Operation of the PS Analytical Cold vapor Atomic Fluorescence Spectrometer" (ACS/SOP-174-010).

12. Limitations of Method; Interfering Substances and Conditions

- A. Contaminants in reagents, solvents, labware (glassware and plasticware) and other components of sample processing apparatus can cause interferences which may lead to increased analyte and/or background signals. These sources of contamination are monitored by the analysis of both reagent blanks and method controls as described in Sections 10.A. and B.
- B. Reduction of fluorescence response may be due to quenching of the signal by water vapor in the gas stream. An in-line dryer tube removes water vapor from the carrier stream before it reaches the detector. A consistent decrease in response over time may indicate that the dryer tube should be changed or regenerated. It is also important to make sure that the inner tube of the dryer tube does not become twisted when installing it. Holding the fitting to which the sealing nut/ferrule is being attached will prevent this twisting of the inner tube which renders the dryer ineffective.
- C. Another source of reduction of mercury response is shared use of the sample delivery system for hydride generation applications which use KI as a reagent. The formation of HgI complexes reduces the amount of Hg vapor formed from the samples. The symptom of this problem is a steady increase in the fluorescence signal from a Hg standard analyzed intermittently during an analysis. The increase in response is due to the washing out of residual iodide from the system. A thorough flush of the system (starting with the autosampler probes) with DI water prior to analysis will prevent this drift.
- D. Memory effects are a common source of contamination in CVAFS. A proper cleaning cycle (i.e., Memory time setting) must be employed to ensure the complete removal of any traces of analytes from the sample transport system and the detector.
- E. Once samples are brought to the analytical lab, all sample handling is performed in a clean laboratory environment under HEPA filtered air. All sample preparation steps must be carried out in the Class 100 clean lab environment to eliminate contamination that may result from the environment. If samples need to be carried out from the Class 100 clean lab at any stage of the sample processing, they should be tightly capped before taken out from the clean lab and never be opened to outside environment. All sample analyses must be performed in the Class 10,000 clean lab to eliminate any contamination that may occur during analysis. Proper procedures for the use of ACS clean lab facility are described in ACS/SOP-174-001, "Standard Operating Procedures for the ACS Inorganic Class 100/10,000 Clean Lab Facility."

13. Reference Ranges (Normal Values)

There are no reference ranges for mercury in hair.

14. Critical Call Results (Panic Values)

Results > 30 ppm are faxed to the NCHS Medical Officer.

15. Specimen Storage and Handling During Testing

All samples are placed in appropriate airtight containers and are stored in a freezer prior to sample preparation. All prepared samples are stored in glass or Teflon containers with air tight screw caps, in a refrigerator

16. Alternate Methods for Performing Test of Storing Specimens if Test System Fails

There are no acceptable alternative methods of analysis for

17. Test Result Reporting System; Protocol for Reporting Critical Calls (If Applicable)

Results > 30 ppm are faxed to the NCHS Medical Officer.

18. Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking

Samples are accompanied by their chain-of-custody sheets when received in the laboratory. All operations including sample receipt, extraction, analysis, and transmittal are noted, signed and dated on the chain-of-custody sheets by the analyst.

References

1. Lasorsa, B K, Citterman, RJ. Segmental analysis of mercury in hair in 80 women of Nome, Alaska Report (1991), PNL-7880, OCS/MMS-91-0065; Order No. DE92003656, 294 pp. Avail. NTIS.
2. Saouter, E. Analyses of organic and inorganic mercury by atomic fluorescence spectrometry using a semiautomatic analytical system. Anal Chem. 1994;66:2031–2037.