

Elemental Analysis Manual

for Food and Related Products

Archive Notes

This method has been placed in archive status only because it is no longer used at FDA laboratories. It remains the most current version and is still considered a valid analysis option.

4.5 Cold Vapor Atomic Absorption Spectrometric Determination of Total Mercury in Seafood Using Microwave Assisted Digestion

Version 1.0 (June 2008)

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GLOSSARY

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4.5.1 SCOPE AND APPLICATION

This method describes procedures for using cold-vapor atomic absorption spectroscopy (CVAAS) and microwave assisted digestion to determine total mercury in seafood. Other matrices may be analyzed by these procedures if performance is demonstrated in the matrix of interest, at the concentration levels of interest. The limits listed in 4.5 Table 1 are intended as a guide and actual limits are dependent on the sample matrix, instrumentation and selected operating conditions.

4.5 Table 1. Analytical Limits

Element	Symbol	ASDL ^a (µg/L)	LOD ^b (µg/kg)	LOQ ^b (µg/kg)
Mercury	Hg	0.0085	0.86	6.7
^a Based on method blanks.				
^b Based on 0.5 g analytical portion.				

This method should be used by analysts experienced in the use of cold vapor atomic absorption spectrometry, including the interpretation of spectral and matrix interferences and procedures for their correction; and should be used only by personnel thoroughly trained in the handling and

analysis of samples for determination of trace elements in food products.

4.5.2 SUMMARY OF METHOD

An analytical portion (0.5 g) is digested with nitric acid in the presence of sodium chloride using multi-step high-pressure microwave heating in a closed-vessel with a feedback program to monitor temperature and pressure. A 50 mL analytical solution containing hydrochloric acid is prepared from the digest. Total mercury is determined in the analytical solution by CVAAS using stannous chloride to reduce mercury (II) ion to mercury (0).

4.5.3 EQUIPMENT AND SUPPLIES

Disclaimer: The use of trade names in this method constitutes neither endorsement nor recommendation by the U. S. Food and Drug Administration. Equivalent performance may be achievable using apparatus and materials other than those cited here.

- (1) Mercury analyzer—A self contained unit or assembled apparatus capable of mixing stannous chloride solution with analytical solutions, separating mercury (0) vapor that forms upon mixing from the liquid stream, and sweeping mercury (0) vapor into the path of an atomic absorption detector; consisting of a multichannel peristaltic pump for controlling liquid flow, liquid mixing tee, device for regulating carrier gas flow, gas-liquid separator, device for drying carrier gas, thermally stable double beam optics (temperature controlled sample and reference cells), and ultra stable mercury vapor lamp and detector capable of measuring atomic absorbance at 253.7 nm. Analyzer must have computerized control of operating conditions, an autosampler, and software for calculating mercury concentration in solutions. Analyzer should be capable of determining concentrations in solution ≥ 0.05 $\mu\text{g/L}$ with precision $\leq 2\%$ relative standard deviation during a period of 2 to 3 hours. Analyzer with capability to determine 0.0005 $\mu\text{g/L}$ with precision $\leq 2\%$ relative standard deviation during a period of 2 to 3 hours is preferable.

Note: Drying carrier gas by diffusing water vapor through a selective membrane such as Nafion[®] into argon sheath-gas is the preferred method of removing water vapor. Chemical desiccants are not recommended.

- (2) Microwave digestion system—Temperature control to 200 °C, pressure control to at least 600 psi, power 0-100% full power (minimum 630 watts, 900 watts for 12 position carousel), programmable in 1% increments. Digestion vessels must be TEM Teflon[®] lined. In this method, directions on use of microwave digestion equipment are specific to CEM Corporation brand. Use of method with other brands of equipment may require procedural modifications. Vessels designed to vent and reseal can be used provided they vent at pressures >300 psi.

Safety Note: Microwave digestion systems can be potentially dangerous. Vessels contain concentrated nitric acid at high temperatures and pressures. Analyst must be familiar with manufacturer's recommended safety precautions including connection of the system to an appropriate exhaust system.

- (3) Gas supply—Ultra-high purity (99.999%) argon. Use 2-stage regulator to deliver argon at

pressure and flow rate recommended by manufacturer of mercury analyzer. Use an additional tank equipped with 2-stage regulator and capillary Teflon® tubing to purge stannous chloride reducing solution with ultra high purity argon flowing at <50 mL/min and <5 psi.

- (4) Polypropylene centrifuge tubes for preparation and holding of standard and analytical solutions—50 mL capacity with screw caps.

Note: Accuracy of tubes should be periodically checked by randomly selecting several tubes from each case, filling to 50 mark with reagent water of known temperature, and weighing. Calculate volume as $V=W/d$ where V is volume (mL), W is weight (g) of water added to mark, and d is density (g/mL or g/cc) of water at the temperature used. Do not use tubes with volume markings that are more than 1% in error.

- (5) Miscellaneous laboratory ware—Use plastic and Teflon® laboratory items and containers whenever possible.

4.5.4 REAGENTS AND STANDARDS

Use reagents with sufficiently high purity and low mercury contamination to ensure that results are accurate and that quality control criteria can be met. Mercury analyzers with higher sensitivity require use of more highly purified reagents than with less sensitive analyzers. Reagents should be checked for contamination before use. Prepare reagents as close in time as possible to the day of use and no longer than 5 days before use. Hold solutions in tightly sealed containers.

Safety Note: Reagents should be regarded as potential health hazards and exposure to these materials should be minimized as much as possible.

- (1) Nitric acid (HNO₃)—Concentrated, sp gr 1.41, preferably ultra-high purity grade.
- (2) Hydrochloric acid (HCl)—Concentrated, sp gr 1.18, preferably ultra-high purity grade.
- (3) Reagent water—Water that meets specifications for ASTM Type I water¹.
- (4) Sodium chloride solution, 1%, (w/v)—Dissolve 0.5 g sodium chloride crystals in 50 mL reagent water in polypropylene tube.
- (5) Stannous chloride reducing solution, 10% (w/v) in 7% (v/v) HCl—Mix 800 mL reagent water, 60 mL HCl, and 86 g stannous chloride dihydrate crystals (SnCl₂•2H₂O) in an acid-cleaned, glass container dedicated for stannous chloride use. Solution must be colorless and particle-free. Discard solutions that indicate presence of stannic ion (are turbid or yellow) or contain particles. Purge with argon and spinning magnetic stir-bar for 3 to 24 hours immediately before use to purge mercury contamination from solution. Left-over solution may be used up to 5 days after preparation if held in tightly sealed container away from light (wrapped in aluminum foil).
- (6) Diluent, 10% (v/v) HNO₃, 7% (v/v) HCl, 0.02% (w/v) NaCl—Mix approximately 1700 mL reagent water, 200 mL HNO₃, 140 mL HCl, and 0.4 g sodium chloride crystals in acid-cleaned, 2-L that has been marked on the outside at the 2-L level. Mix, cool to room temperature, and dilute to 2 L.
- (7) Autosampler rinse solution, 1% (v/v) HNO₃, 1% (v/v) HCl—Mix 1960 mL reagent water, 20 mL HNO₃ and 20 mL HCl in acid-cleaned, 2-L container. Use this solution when determining mercury concentrations ≤1 µg/L.

- (8) Alternative autosampler rinse solution, 2% (v/v) HNO₃, 2% (v/v) HCl—Mix 1920 mL reagent water, 40 mL HNO₃, and 40 mL HCl in acid-cleaned, 2-L container. Use this solution when determining mercury concentrations >1 µg/L.
- (9) Hydrochloric acid solution, 7% (v/v) HCl—Mix 93 mL reagent water and 7 mL HCl in acid-cleaned, 100-mL container. Use this solution to prepare intermediate standard solution.
- (10) Standard blank—Diluent or 0 concentration mercury standard solution.
- (11) Mercury stock standard solution, 1000 mg/L in 2–10% (v/v) HNO₃—Use commercially prepared, single-element solution prepared specifically for spectrometric analysis. A second stock standard solution obtained from a different source (or starting material) should be used to prepare the independent check solution (see below).
- (12) Intermediate mercury standard solution, 5 mg/L Hg in 7% (v/v) HCl—Prepare by diluting 250 µL mercury stock standard solution to 50.0 mL with 7% (v/v) HCl. Use 100 µL of this solution to fortify analytical portions for percent recovery determination and various volumes to prepare standard solutions.
- (13) Mercury standard solutions (various concentrations in 10% (v/v) HNO₃, 7% (v/v) HCl, 0.02% (w/v) NaCl—Prepare standard solutions with concentrations that are appropriate for the sensitivity of the analyzer in 2 steps. (a) Prepare a secondary intermediate solution with appropriate concentration by diluting intermediate mercury standard solution with diluent. (b) Prepare standard blank and at least 4 standard solutions by gravimetrically diluting secondary intermediate solution with diluent.

Example A (for mercury analyzers with limited sensitivity): Mercury standard solutions: 0, 0.5, 1, 2 and 5 µg/L—Prepare secondary intermediate solution with mercury concentration of 50 µg/L by diluting 500 µL of intermediate mercury standard solution (5 mg/L) to 50.0 mL with diluent. Prepare mercury standard solutions by weighing 0.5, 1, 2 and 5 g portions of 50 µg/L secondary intermediate solution in polypropylene tubes and diluting to 50 g with diluent. Record weights to 4 significant figures.

Example B (for analyzers with high sensitivity): Mercury standard solutions: 0, 0.05, 0.1, 0.2 and 0.5 µg/L—Prepare secondary intermediate solution with mercury concentration of 5 µg/L by diluting 50 µL of intermediate mercury standard solution (5 mg/L) to 50.0 mL with diluent. Prepare mercury standard solutions by weighing 0.5, 1, 2 and 5 g portions of 5 µg/L solution in polypropylene tubes and diluting to 50 g with diluent. Record weights to 4 significant figures.

- (14) Check solution—Use a mercury standard solution approximately mid-range of the standard curve for the check solution.
- (15) Independent check solution (ICS)—Prepare a mercury independent check solution with diluent from a different stock solution than that used to prepare the calibration standard solutions. Mercury concentration of ICS should be approximately midpoint of the standard curve.

4.5.5 DIGESTION PROCEDURE

The following operations should be performed in a clean environment to reduce contamination. An exhausting hood must be used when working with nitric acid.

Note: To assist homogenization of the analytical sample, reagent water $\leq 20\%$ of the mass of seafood may be added if its addition provides a more visually homogenous and easier-to-manipulate material. If reagent water is added to assist homogenization, record to 4 significant figures the weights of edible portion and reagent water that are combined to prepare the analytical sample and apply mass correction factor (MCF) in calculation of concentration of analyte in analytical portion. Reserve a portion of reagent water used for homogenization to prepare method blanks.

- (1) Weigh analytical portion into clean vessel liner and determine mass of analytical portion. Generally, weigh 0.5 ± 0.1 g analytical sample. Use 0.5 g reagent water for method blanks (MBKs). Use 0.1 ± 0.01 g for reference materials (RMs).
- (2) Pipet 1.0 mL 1% (w/v) sodium chloride solution and 5.0 mL concentrated HNO_3 into vessel liner.
- (3) Seal vessels, tighten pressure relief nuts and perform the digestion using the program outlined in 4.5 Table 2.
- (4) After vessels have cooled to less than 50°C remove them to an exhausting hood and vent excess pressure slowly.
- (5) Place approximately 10 mL reagent water and 3.5 ± 0.1 mL HCl in 50 mL capacity polypropylene tube. Quantitatively transfer digestion solution to the 50 mL tube. Seal tube, shake vigorously and remove cap for approximately 5 minutes to release trapped gas from the warm solution. Dilute to slightly less than 50 mL with reagent water. Reseal container and cool to room temperature. Dilute to 50.0 ± 0.5 mL with reagent water, reseal, and mix. Analyze analytical solutions within 3 days.

Note: Analytical solutions that are clear and colorless to faintly yellow indicate that digestion is acceptably complete. If turbidity, deep color, or charred particles are present indicating incomplete digestion, discard analytical solution and prepare another analytical portion. Do not add hydrogen peroxide to decolorize solutions because it interferes with measurement of mercury.

4.5 Table 2. Microwave Digestion Program^a

<i>Digestion Program for CEM MARS 5 with 12-Position Carousel: 800 psi Maximum Pressure with Ramp to Temperature Feature</i>		
Stage:	1	2
Maximum Power (Watts)	300	1200
Control Pressure (psi)	800	800
Ramp Time (min)	5	20
Hold Time (min)	0	3
Control Temperature ($^\circ\text{C}$)	130	200
^a For each stage, power is applied for the Ramp Time minutes or until Control Pressure or Control Temperature is met. If Control Pressure or Control Temperature are met before end of Ramp Time then program proceeds to Hold Time prior to proceeding to next stage. If Ramp Time is met then program proceeds to next stage.		

The determination procedure was developed using a CETAC Technologies Quick Trace mercury Analyzer, Model M-75000. 4.5 Table 3 is an example of operating conditions used with this instrument. The optimum conditions must be determined for the equipment used.

Conditions for CETAC Quick Trace Mercury Analyzer M-75000			
Instrument Conditions		Instrumental Zero	
Gas flow (mL/m)	125	Before first sample	no
Sample Uptake (s)	25	Periodically (before each calibration)	yes
Rinse (s)	65		
Read replicates (number)	3	Baseline Correction	
Read time (s)	1	#1 Start time (s)	15
Pump speed (% of full)	80	#1 End time (s)	20
Wavelength (nm)	253.7	#2 Start time (s)	not used
Absorption cell temperature		#2 End time (s)	not used
heater set point (°C)	60	Calibration Settings	
		Algorithm	linear
		Through blank	no
		Weighted fit	no
		Recalibration rate	0
		Reslope rate	0
		Reslope standard	N/A

- (1) Setup cold vapor atomic absorption spectrometer according to manufacturer's recommendations and with the following conditions:
 - Set number of read (integration) replicates to 3 per sample uptake.
 - Set the absorption cell heater block set point to a temperature at least 10 °C above ambient.
 - Allow adequate time to warm up instrument and Hg lamp before analyzing solutions.
- (2) Optimize operating conditions.
 - Start gas and liquid flows and ensure that liquid flow through uptake tubing, gas-liquid separator, and drain tubing is continuous and pulse free.
 - Analyze a standard solution and follow manufacturer's recommendations for adjusting operating conditions to obtain ideal peak profile.
 - When proper operating conditions are achieved, zero the instrument, then immediately analyze a standard blank, a standard solution with high concentration 3 or more times, and standard blank again. Visually inspect instrument peak profiles and calculate instrument sensitivity (see §3.2.1) and relative standard deviation of the high concentration standard solution. Verify absence of carry-over from high concentration standard to standard blank. Adjust operating conditions and repeat procedure if necessary to meet instrument performance requirements described below (3).
 - Determine baseline correction start and end times from the peak profile. Use a flat region of baseline immediately before start of the mercury signal for the correction.

(3) Check instrument performance

- Verify sensitivity is within 80-120% of manufacturer specifications.
- Verify short term precision is less than 5% relative standard deviation.
- Verify absence of instrument carry-over.

Determination of Analyte Concentration Using Standard Curve

- (1) Zero the detector and immediately standardize the instrument using standard blank and standard solutions. Use a linear, least squares calculated intercept curve fit algorithm.
- (2) Check standardization performance
 - Correlation coefficient (r) of linear regression (absorbance verses concentration) is ≥ 0.998 .

Note: Optionally, verify that standards have been prepared correctly and are in the linear range by calculating percent relative difference of known concentrations and concentrations calculated from slope, intercept and instrument response of standards. Calculated concentrations that differ $\leq 2\%$ relative difference from actual concentrations indicates that preparation of standard solutions is acceptable and concentrations are in the linear range. If relative difference is $> 2\%$, prepare new standard solutions and re-standardize the instrument.

- Analyze ICS and standard blank immediately following instrument standardization. Acceptance criteria: ICS recovery within $100 \pm 5\%$, standard blank $< \text{ASDL}$.
- (3) Analyze analytical and quality control solutions. Dilute analytical solutions with diluent if concentration is above the highest standard. Interpolate analyte concentration in analytical solution from standard curve using least squares linear regression. A typical sequence for an analytical run is listed in 4.5 Table 4.

4.5 Table 4. Typical Analytical Sequence

Auto-Sampler Tube #	Solution	Purpose	QC Criteria
	standard blank	standardize instrument	Check for contamination
	standard solution #1		$r \geq 0.998$
	standard solution #2		
	standard solution #3		
	standard solution #4		
1	ICS	verify standardization	95-105% of expected
2	standard blank	verify absence of carry-over	$< \text{ASDL}$
3	MBK #1	verify absence of contamination	$\leq \text{MBK}_C$
4	MBK #2		$\leq \text{MBK}_C$
5	sample #1	determine mercury	$\leq 5\%$ RSD read (integration) replicates if concentration $\geq \text{ASQL}$
6	sample #2		
7	sample #3		
8	sample #4		
9	sample #4 FAP	spike recovery	80-120% recovery
10	RM	accuracy	80-120% recovery
11	check solution	verify standardization	90-110% of expected
12	standard blank	verify absence of carry-over	$< \text{ASDL}$

(4) Check instrument measurement performance

- RSD of analytical solution read (integration) replicates is 5% or less for concentrations \geq ASQL.
- Check solution (mid-level standard) analyzed at a frequency of 10% and at end of the analytical run has a recovery of $100 \pm 10\%$ (continuing calibration verification).
- Standard blank analyzed following each check solution analysis is $<$ ASDL (to verify absence of carry-over).
- Measurements are below concentration of highest standard. Dilute analytical solution with diluent if necessary to comply with criteria.
- Peak profile of analytical solution is comparable to standard solution.

Determination of Analyte Concentration Using Standard Additions

- (1) Analyze analytical solutions and quality control solutions using minimum of 2 additional portions of solution with added amounts of analyte at approximately 2 and 5 times, respectively, of the amount of analyte in solution but not less than ASQL. Extrapolate analyte concentration from x-intercept of linear regression curve.
- (2) Check standard additions performance
 - Check solution analyzed at a frequency of 10% and at the end of the analytical run has a recovery of $100 \pm 10\%$.
 - Measurements are below upper end of linear working range. Dilute analytical solution with diluent if necessary to comply with criteria.
 - Correlation coefficient (r) of standard additions curve (absorbance verses concentration added) is ≥ 0.995 .
 - Slope of standard additions curve for analytical solution is $\pm 50\%$ of the slope of standard additions curve for a standard blank (or a standard solution without any matrix effect such as the ICS).
 - Peak profile of analytical solution is comparable to standard solution.

4.5.7 CALCULATIONS

Calculate the concentration (mass fraction) of the analyte in the analytical portion according to the formula

$$\text{Concentration } (\mu\text{g/kg}) = \left[(S \times \text{DF}) - \text{MBK}_L \right] \times \frac{V}{m \times \text{MCF}}$$

where

S = concentration of analyte in analytical solution (or diluted analytical solution) ($\mu\text{g/L}$)

MBK_L = laboratory MBK ($\mu\text{g/L}$)

V = volume (L) of analytical solution (0.050 L)

m = mass of analytical portion (kg)

DF = dilution factor (1 if analytical solution not diluted)

MCF = mass correction factor (1 if no water or other solvent was added to aid homogenization)

Round calculated concentration to at most 3 significant figures. Concentration may be converted

to other convenient units (e.g., mg/kg, ng/kg).

4.5.8 METHOD VERIFICATION

The following minimum number of quality control samples are analyzed with each batch of samples: 1 reference material (RM), 1 fortified analytical portion (FAP), and 2 method blanks (MBKs). Replicate analytical portions should be analyzed for each sample whenever analyte nonhomogeneity may be an issue.

A fortified method blank (FMB) checks the accuracy of the fortification procedure without any matrix effects and is an optional quality control sample. Use same fortification level as the FAP.

Reference Material

Control limits for RM Recovery are $100 \pm 20\%$ or within concentration uncertainty (converted to percent relative uncertainty) supplied on certificate, whichever is greater. The z-score procedure, which allows for greater deviation and is discussed in §3.5.3, may also be used, although it requires additional calculations. If three or more RMs are analyzed then only two-thirds of an element's RM recovery results must meet the control limit.

FAP Recovery

Control limit for FAP recovery is $100 \pm 20\%$.

Method Blanks (MBK)

Minimum of 2 MBKs analyzed and concentration of both MBKs are $\leq \text{MBK}_C$. If 3 or more MBKs are analyzed then at least two-thirds of MBKs are $\leq \text{MBK}_C$.

Relative Percent Difference (RPD) of Two Replicate Analytical Portions

Control limit for RPD is 10%.

FMB Recovery (optional)

Control limit for FMB recovery is $100 \pm 10\%$.

4.5.9 REPORT

Report results only when quality control criteria for a batch have been satisfactorily met. Report results that are $\geq \text{LOQ}$ as the mass fraction determined followed by the units of measurement. Report results that are $\geq \text{LOD}$ and $< \text{LOQ}$ as the mass fraction determined followed by the units of measurement and the qualifier that indicates analyte is present at a trace level that is below the limit of reliable quantification (TR). Report results that are $< \text{LOD}$ as 0 followed by the units of measurement and the qualifier that indicates analyte is below the level of reliable detection or is not detected (ND).

Example: $\text{LOQ} = 6.7 \mu\text{g/kg}$; $\text{LOD} = 0.86 \mu\text{g/kg}$. Levels found for three different samples were $7.5 \mu\text{g/kg}$, $2.1 \mu\text{g/kg}$ and $0.5 \mu\text{g/kg}$.

$7.5 \mu\text{g/kg}$ is $\geq \text{LOQ}$; report $7.5 \mu\text{g/kg}$

$2.1 \mu\text{g/kg}$ is $\geq \text{LOD}$ but also $< \text{LOQ}$; report $2.1 \mu\text{g/kg}$ (TR)

$0.5 \mu\text{g/kg}$ is $< \text{LOD}$; report 0 $\mu\text{g/kg}$ (ND)

4.5.10 METHOD VALIDATION

The application of microwave assisted digestion sample preparation to CVAAS determination of total mercury is well documented in scientific literature². Closed-vessel microwave digestion is fast and contamination is extremely low while modern mercury analyzers are precise and very sensitive.

In-house validation. The method was validated by determining total mercury in 5 reference materials, various seafood products, portions of seafood products fortified with inorganic and organic mercury, and method blanks fortified with inorganic and organic mercury³. Validation results are presented in Appendix A. The average RM recovery for reference materials was 99% with a range of 90 to 103%. The average fortification recovery for seafood products was 101% with a range of 90 to 115%. The average fortification recovery for method blanks was 100% with a range of 95 to 106%.

Uncertainty. A result above LOQ has an estimated combined uncertainty of 10%. Use of a coverage factor of 2 to give an expanded uncertainty at about 95% confidence corresponds with the RM Recovery control limit of $\pm 20\%$. A result above LOD but below LOQ is considered qualitative and is not reported with an uncertainty.

A detailed discussion of method uncertainty is presented in §3.3. This method conforms to the information contained in that discussion. Derivation of an estimated uncertainty specific to an analysis is discussed §3.3.2.

Interlaboratory trial. Interlaboratory performance of EAM Method 4.5 was estimated from analytical data and results from laboratory proficiency testing. Further details can be found in Appendix B. FDA laboratories using EAM Method 4.5 demonstrated satisfactory performance, individually and collectively, with an estimated interlaboratory precision of 5 to 12% relative standard deviation (HORRAT of 0.3 to 0.6) for seafood samples containing approximately 0.2 to 0.6 mg/kg total mercury.

REFERENCES

- (1) ASTM International (2006) ASTM D 1193-06, "Standard Specification for Reagent Water". [ASTM](#).
- (2) Clevenger, W. L., Smith, B. W., and Winefordner, J. D. (1997) Trace Determination of Mercury: A Review, *Crit. Rev. Anal. Chem.* **27**, 1-26.
- (3) Hight, S. C., and Cheng, J. (2005) Determination of Total Mercury in Seafood by Cold Vapor-Atomic Absorption Spectroscopy after Microwave Decomposition, *Food Chem.* **91**, 557-570.

Appendix A – Supplemental Information on In-house Method Validation

Version 1 (June 2008)

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The use of cold vapor atomic absorption spectrometry for mercury determination is well established and documented in literature¹. Microwave assisted nitric acid sample digestion in closed vessels is highly effective for decomposition of biological materials and contamination is low. EAM Method 4.5 was developed and validated in-house using a CEM Corporation MARS-5 microwave digestion system with XP-1500 Plus vessels and a CETAC Technologies Quick Trace model M-7500 mercury analyzer with an ASX-510 autosampler. Method performance was demonstrated by analyses of reference materials, seafood samples, portions of seafood samples fortified separately with inorganic and organic mercury, and method blanks fortified separately with inorganic and organic mercury².

4.5A.1 ANALYTICAL LIMITS

Analytical limits were estimated based on concentration measurements of unfortified method blanks (MBKs) from separate analytical batches. Results are summarized in 4.5A Table 1.

4.5A Table 1. Estimate of Analytical Limits

	Mercury
Number of MBKs	10
Average conc. Found (µg/L)	0.0013
Standard deviation (µg/L)	0.00222
ASDL (µg/L)	0.0085
ASQL (µg/L)	0.067
LOD (µg/kg) for 0.5 g anal. portion	0.86
LOQ (µg/kg) for 0.5 g anal. portion	6.7

4.5A.2 REFERENCE MATERIAL RESULTS

Four lyophilized seafood and one natural water reference materials with total mercury concentrations ranging from 0.0371 to 1.590 mg/kg were analyzed and the results presented in 4.5A Table 2. All results were from 1 analytical portion and were within the stated (certificate) concentration uncertainty except for Oyster Tissue (NIST 1566b) and Natural Water (NIST 1641d), which were in agreement with stated values within analytical uncertainty ($\pm 10\%$).

4.5A Table 2. Reference Material Results

Reference Material ^a	Total Hg		RM Recovery (%)
	Reference Value (mg/kg)	Found (mg/kg)	
NIST 1566b Oyster Tissue	0.0371 ± 0.0013	0.033	90
NIST 2976 Mussel Tissue	0.0610 ± 0.0036	0.062	102
NIST 2977 Mussel Tissue	0.101 ± 0.004	0.099	98
NIST RM 50 Albacore Tuna	0.95 ± 0.1	0.948	100
NIST 1641d Natural Water	1.590 ± 0.018	1.64	103
		mean:	99
		minimum:	90
		maximum:	103

^aNIST= National Institute of Standards and Technology; n=1.

4.5A.3 FORTIFICATION RECOVERY RESULTS

Eleven seafood products with total mercury concentrations of 0.014 to 1.85 mg/kg were analyzed unfortified and fortified separately with inorganic and organic mercury to assess recovery (4.5A Table 3). Relative fortification levels, expressed as the ratio of fortified to unfortified Hg concentration levels, ranged from 0.54 (Shark, fresh) to 71 (Trout, fresh). The combined average fortification recovery was 101% and the range was 90 to 115%.

4.5A Table 4 lists concentration measurements of duplicate method blanks and recoveries of method blanks fortified separately with inorganic and organic mercury in separate analytical batches. Fortification levels were 1, 2 and 10 µg/L. The combined average fortification recovery was 100% and the range was 95 to 106%.

4.5A Table 3. Fortification Recovery of Organic and Inorganic Mercury in Seafood Products^a

Product	Total Hg (mg/kg)	Fortification Recovery		Level Added	
		Inorganic Hg ^b (%)	Organic Hg ^c (%)	Hg (µg)	Hg (mg/kg)
Trout, fresh	0.014	99	95	0.5	1
Catfish, fresh	0.058	98	90	0.5	1
Tuna, canned	0.06	100	98	0.05	0.1
Tilapia, fresh	0.067	97	95	0.5	1
Orange roughy, fresh	0.149	102	99	0.5	1
Swordfish (A), fresh	0.199	109	103	0.25	0.5
Tuna, fresh	0.365	115	104	0.5	1
Bluefish, fresh	0.372	101	101	0.5	1
Grouper, fresh	0.472	105	100	0.5	1
Swordfish (B), fresh	1.77	114	109	0.5	1
Shark, fresh	1.85	90	not determined	0.5	1
	mean:	103	99		
	minimum:	90	90		
	maximum:	115	109		

^aValues are the result from 1 analytical portion.

^bThe source of inorganic mercury is inorganic mercury(II) ion in 7% (v/v) HCl solution.

^cThe source of organic mercury is methylmercury(II) chloride dissolved in 7% (v/v) HCl solution.

4.5A Table 4. Total Mercury and Fortification Recovery of Organic and Inorganic Mercury in Method Blanks

Batch	Total Hg (µg/L)		Fortification Recovery ^a		Level Added	
	MBK#1	MBK#2	Inorganic Hg ^b (%)	Organic Hg ^c (%)	Hg (µg)	Hg (µg/L)
1	0.0038	0.0033	99	95	0.5	10
2	0.0003	0.0002	103	99	0.5	10
3	-0.0020	-0.0019	106	102	0.5	10
4	0.0025	0.0039	101	100	0.1	2
5	0.0017	0.0009	101	96	0.05	1
mean:	0.0013		102	98		
minimum:	-0.0020		99	95		
maximum:	0.0039		106	101		

^aValues are the result from 1 fortified method blank.
^bThe source of inorganic mercury is inorganic mercury (II) ion in 7% (v/v) HCl solution.
^cThe source of organic mercury is methyl mercury (II) chloride dissolved in 7% (v/v) HCl solution.

4.5A.4 CONCLUSION

EAM Method 4.5 is applicable for determination of total mercury in seafood. Following prescribed procedures and using specified equipment operated under recommended conditions, the method is capable of determining total mercury ≥ 6.7 µg/kg in seafood. Method accuracy was demonstrated by successful analyses of reference materials and acceptable fortification recovery results in seafood and method blanks.

4.4.1 REFERENCES

- (1) Clevenger, W. L., Smith, B. W., and Winefordner, J. D. (1997) Trace Determination of Mercury: A Review, *Crit. Rev. Anal. Chem.* **27**, 1-26.
- (2) Hight, S. C., and Cheng, J. (2005) Determination of Total Mercury in Seafood by Cold Vapor-Atomic Absorption Spectroscopy after Microwave Decomposition, *Food Chem.* **91**, 557-570.

Appendix B – Supplemental Information on Method Performance

Version 1 (June 2008)

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Interlaboratory performance of EAM Method 4.5 was estimated from analytical data and results from laboratory proficiency testing. Although the design and purpose of proficiency testing is different than for method performance studies, the fundamental requirements and conditions for reproducibility were met and the data and results were suitable for determining interlaboratory method performance.

4.5B.1 MERCURY QUALITY ASSURANCE PROGRAM (MQAP)

The MQAP is a proficiency testing program organized and managed by the Canadian Food Inspection Agency for assessing laboratory performance. Participation in the program is voluntary, free of charge, open to all organizations, and participant information is held confidential. Each dispatched round consists of several test materials that are sent to enrolled labs for analysis. Analytical instructions are provided along with a report form for reporting results. An extensive report summarizing results, descriptions of analytical methodologies used, and performance scores (participants are identified by code only) is distributed to all participants upon conclusion.

In 2004, four FDA laboratories participated in MQAP series 352-355. MQAP series 352-355 consisted of 4 canned seafood samples to be analyzed for total mercury. Each can was identified only by a sample number with no additional information. The accompanied paperwork instructed participants to analyze each sample in triplicate on 2 different days and to report all six total mercury values. All FDA labs followed procedures in EAM Method 4.5 draft A using identical mercury analyzers (CETAC Technologies Quick Trace model M-7500) and microwave digestion systems from CEM Corporation. FDA participation was coordinated through the Division of Field Science (DFS). A copy of the MQAP 352-355 final report¹ and the 4 laboratory codes used to identify FDA participants in the report was provided by DFS.

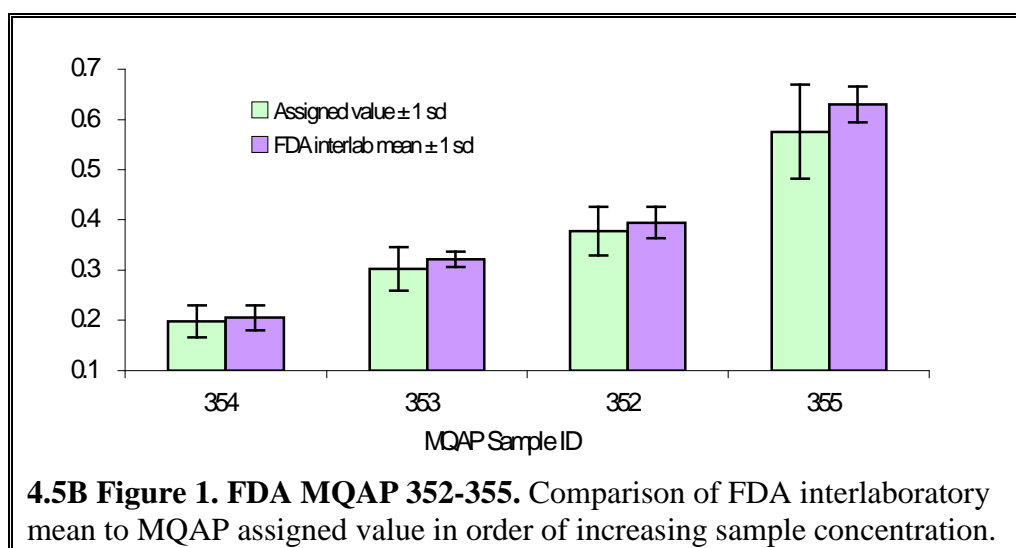
4.5B.2 PROFICIENCY RESULTS

To compare interlaboratory results, the MQAP used assigned values based on consensus, *i.e.*, the mean, corrected for outliers, of all reported results for each sample¹. FDA results, individually and collectively, were in good agreement with assigned values (4.5B Table 1). Individual FDA lab results and the interlaboratory mean were within 1 standard deviation of the assigned values for all 4 samples. However, the ratio of FDA mean to assigned value was slightly greater than 100% for all samples. This apparent difference is likely due to a lowering influence on the consensus value from participants that used open beaker acid digestion during sample preparation, a process that is susceptible to mercury loss due to volatilization. Also, assigned values in comparison studies are not intended to be used in the same context as true or certified values (reference materials) so a small difference is not a good indicator of error. A graphical comparison of means and variances (4.5B Figure 1) between FDA interlaboratory mean and the MQAP assigned value does not appear statistically or significantly different.

4.5B Table 1. FDA Mercury Results for MQAP Samples 352-355^a.

FDA Lab	Hg (mg/kg)			
	352	353	354	355
1	0.368 ± 0.027	0.316 ± 0.009	0.202 ± 0.013	0.610 ± 0.012
2	0.407 ± 0.012	0.333 ± 0.008	0.222 ± 0.004	0.654 ± 0.018
3	0.402 ± 0.041	0.317 ± 0.023	0.190 ± 0.039	0.638 ± 0.047
4	0.398 ± 0.007	0.316 ± 0.006	0.206 ± 0.006	0.616 ± 0.027
Mean	0.394	0.321	0.205	0.63
Assigned value ^b	0.377 ± 0.048 (61)	0.302 ± 0.043 (62)	0.198 ± 0.032 (62)	0.576 ± 0.094 (62)
<u>FDA Mean</u> assigned value	104%	106%	104%	109%

^aFDA results are mean ± 1 standard deviation; n=6.
^bCorrected mean ± 1 standard deviation of all MQAP participants in mg/kg. Number of data points is in parenthesis.



4.5B Table 2 shows the MQAP series 352-355 z-scores for FDA participants. Individual z-scores were calculated as follows:

$$Z = \frac{X_m - X_c}{\sigma}$$

where

x_m = laboratory mean

x_c = MQAP assigned value

σ = standard deviation of assigned value.

All FDA z-scores were acceptable, *i.e.*, $|z| \leq 2$, indicating satisfactory performance.

4.5B Table 2. FDA z-scores for MQAP 352-355.

FDA Lab	Sample ID			
	352	353	354	355
1	-0.188	0.336	0.133	0.365
2	0.619	0.732	0.749	0.831
3	0.509	0.359	-0.233	0.661
4	0.421	0.342	0.256	0.427

4.5B.3 INTERLABORATORY PRECISION AND HORRAT

Interlaboratory precision was estimated by adding the intra-laboratory variance and variance of the interlaboratory mean. The intra-laboratory variance was estimated from the pooled intra-laboratory standard deviations (average variance). The observed interlaboratory precision ranged 5 to 12% relative standard deviation and the HORRAT ranged 0.3 to 0.6 (4.5B Table 3).

4.5B Table 3. FDA interlaboratory precision for MQAP series 352-355. Mean and stdev (n = 6) in mg/kg.

Sample #352				Sample #353		
FDA Lab	mean	stdev	pooled stdev ^a	mean	stdev	pooled stdev ^a
1	0.368	0.027	0.0255	0.316	0.009	0.0133
2	0.407	0.012		0.333	0.008	
3	0.402	0.041		0.317	0.023	
4	0.398	0.007		0.316	0.006	
FDA interlab	0.394	0.018		0.321	0.008	
Interlab precision						
stdev ^b	0.031			0.0157		
% RSD	7.9			4.9		
HORRAT ^c	0.4			0.3		
Sample #354				Sample #355		
FDA Lab	mean	stdev	pooled stdev ^a	mean	stdev	pooled stdev ^a
1	0.202	0.013	0.0209	0.61	0.012	0.0292
2	0.222	0.004		0.654	0.018	
3	0.19	0.039		0.638	0.047	
4	0.206	0.006		0.616	0.027	
FDA interlab	0.205	0.013		0.63	0.02	
Interlab precision						
stdev ^b	0.0247			0.0355		
% RSD	12			5.6		
HORRAT ^c	0.6			0.3		
^a $\sqrt{\sum_{k=1}^4 s_k^2}$ where s_k = standard deviation of mean for laboratory k (k = 1 to 4)						
^b $\sqrt{s_1^2 + s_2^2 + s_3^2 + s_4^2}$ where s_i = pooled intra-laboratory standard deviation and s_d = standard deviation of interlaboratory mean						
^c Reference 2						

4.5B.4 CONCLUSION

The results from MQAP proficiency testing indicate satisfactory performance among the FDA laboratories using EAM Method 4.5 draft A. FDA interlaboratory results are in good agreement, within statistical uncertainty, with overall consensus values. The estimated interlaboratory precision ranged from 5 to 12% relative standard deviation for seafood samples containing approximately 0.2 to 0.6 mg/kg total mercury.

REFERENCES

- (1) Mercury Quality Assurance Program Series 352-355 Final Report, December 27, 2004. Canadian Food Inspection Agency.
- (2) Official Methods of Analysis of AOAC INTERNATIONAL (2005) 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis. [AOAC](#).