

STANDARD OPERATING PROCEDURE

For

Modified EPA Method 3052

Multiwave Microwave Digestion of Fish/Biota Tissue

SOP #: Modified EPA 3052

REVISION #: 0

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Page 1 of 10

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TABLE OF CONTENTS

	Page
LIST OF REVISIONS	3
LIST OF TABLES	4
1.0 SCOPE AND APPLICATION	5
2.0 SUMMARY OF METHOD	5
3.0 DEFINITIONS.....	5
4.0 INTERFERENCES	6
5.0 SAFETY	6
6.0 EQUIPMENT AND SUPPLIES	6
7.0 REAGENTS AND STANDARDS	6
8.0 SAMPLE PRESERVATION AND STORAGE.....	7
9.0 QUALITY CONTROL.....	7
10.0 CALIBRATION AND STANDARDIZATION.....	7
11.0 PROCEDURE	7
12.0 DATA ANALYSIS AND CALCULATIONS.....	8
13.0 METHOD PERFORMANCE	8
14.0 POLLUTION PREVENTION	8
15.0 WASTE MANAGEMENT	8
16.0 REFERENCES.....	8
17.0 TABLES AND VALIDATION DATA.....	9



LIST OF REVISIONS

Rev. #	Date	Description of Revision	Page #
0	March 2003	None	



LIST OF TABLES

	Page
TABLE 1. QUALITY CONTROL TESTS AND ACCEPTANCE LIMITS FOR THE MODIFIED EPA METHOD 3052 – MULTIWAVE MICROWAVE DIGESTION OF FISH/BIOTA TISSUE	9
TABLE 2. QUALITY CONTROL ELEMENTS AND ACCEPTANCE LIMITS FOR THE MODIFIED EPA METHOD 3052 – MULTIWAVE MICROWAVE DIGESTION OF FISH/BIOTA TISSUE	10



1.0 SCOPE AND APPLICATION

- 1.1 Method for digestion of fish/biota tissue for analysis by Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) for selenium (Se) and arsenic (As), and for analysis by Inductively Coupled Argon Plasma Optical Emission Spectroscopy (ICP-OES) for cadmium (Cd), chromium (Cr), copper (Cu), and lead (Pb):

Analyte	Chemical Abstract Services Registry Number (CASRN)
Arsenic (As)	7440-38-2
Cadmium (Cd)	7440-43-9
Chromium (Cr)	7440-47-3
Copper (Cu)	7440-50-8
Lead (Pb)	7439-92-1
Selenium (Se)	7631-86-9

2.0 SUMMARY OF METHOD

- 2.1 A representative sample of 0.5 to 5.0 g is digested in 5 mL of concentrated nitric acid. Sample and reagent are placed in appropriate 50-mL TFM (modified PTFE Fluoropolymer) liner. Maximum volume of 25 mL (1/2 the vessel volume) is placed in a high-pressure vessel. The sample is then digested in a PAAR Multiwave Microwave Digester using method "PAAR001M" or sample "fish". After digestion is complete, the sample is then placed in a labeled skirted centrifuge tubes (50 mL) and brought up to a volume of 20 to 40 mL using ASTM Type I reagent water.

3.0 DEFINITIONS

- 3.1 QCS – Quality Control Sample: A 0.2-g aliquot of freeze-dried oyster tissue is digested in the exact manner as a field sample. Analyte recovery of this sample should be in the range of 75-125%.
- 3.2 LFM – Laboratory Fortified Sample Matrix: An aliquot of an environmental (field) 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.
- 3.3 LRB – Laboratory Reagent Blank: 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.
- 3.4 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 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.



- 3.5 LFB – Laboratory Fortified Blank: 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.

- 3.6 Stock/Spike: A solution containing one or more method analytes.

4.0 INTERFERENCES

- 4.1 Samples that have high lipid content (> 2%) have the potential to cause the digestion vessel to heat rapidly and rupture the sealed “closed vessel system”. In these instances, digestion must be repeated using a smaller sample aliquot.

5.0 SAFETY

- 5.1 Material Safety Data Sheets (MSDS) for all chemical reagents are available and must be understood by all personnel using this method.
- 5.2 Personnel using this method must be trained to operate the microwave digester, and must read the instruction manual and have experience with sample digestion.
- 5.3 Acidification of samples must be done in a fume hood.
- 5.4 Nitric acid is toxic and extremely irritating to skin and mucus membranes; if eye or skin contact occurs, flush with large volumes of water.
- 5.5 Always wear safety glasses or a shield for eye protection and protective clothing.
- 5.6 Avoid soaking ceramic support vessel during cleaning – thoroughly dry before use in microwave digester.

6.0 EQUIPMENT AND SUPPLIES

- 6.1 Multiwave Microwave Digester (Anton Paar), with built-in computer control, PTFE reaction vessels surrounded by a bomb jacket, infrared temperature sensor, and cooling airflow to quickly reduce temperature after digestion is completed.
- 6.2 Pressure vessels & liners HF50)
- 6.3 Analytical balance – capable of accurately weighing to ± 0.01 g
- 6.4 Fume hood
- 6.5 Skirted centrifuge tubes (50-mL capacity)
- 6.6 Wash bottle
- 6.7 Eppendorf pipettes
- 6.8 Hand food processor
- 6.9 Spatula
- 6.10 Knives
- 6.11 Plastic containers for storage of tissue samples

7.0 REAGENTS AND STANDARDS

- 7.1 Reagent-grade chemicals must be used in all tests. The reagent blank must be less than the MDL in order to be used. All reagents used must conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society.
- 7.2 Reagent water (ASTM Type I)
- 7.3 Nitric acid (concentrated) – HNO_3



- 7.4 Selenium stock standard (10 mg/L)

8.0 SAMPLE PRESERVATION AND STORAGE

- 8.1 Refer to the WES Fish Processing SOP (i.e., Processing Fish & Other Biota Tissue Samples Intended for Contaminant Analysis).
- 8.2 Samples are stored at -10° to -20°C prior to digestion.
- 8.3 Filleted fish tissue samples are stored in plastic sample cups with snap-on-lids (cups are demonstrated to be contaminant free).

9.0 QUALITY CONTROL

- 9.1 A single QCS, LFB, and LRB must be digested along with samples on a daily basis.
- 9.2 A Laboratory duplicate and LFM must be run with every batch of 10 or fewer samples.
- 9.3 See SOP for EPA Methods 200.7 and 200.9 for quality control elements.

10.0 CALIBRATION AND STANDARDIZATION

- 10.1 Analytical balance is calibrated at the beginning of each digestion run with a calibrated weight set consisting of 0.1-, 0.2-, 0.5g-, and 1.0-g weights. Balance readings are recorded in the Balance Log Book.

11.0 PROCEDURE

- 11.1 Each sample is homogenized thoroughly using a hand food processor. Processor container, lid, and blade must be acid washed between each sample. After homogenization, the sample is placed back in the container and into the freezer until ready for digestion.
- 11.2 Weigh 0.5 to 5.0 g (\pm 0.05 g wet) of tissue sample, using an analytical balance, into a TFM liner being careful not to scrape liner edges with the metal spatula. Clean the spatula between samples using 30% HNO₃ and reagent water.
- 11.3 Add 5 mL of HNO₃ (concentrated) to each liner. Make sure each sample is floating. If any tissue is touching the edges, use a wooden cotton-tipped applicator to push the sample down into the acid (**Note:** Stock solution/spike should be added to appropriate sample at this point.)
- 11.4 Liners are then placed inside ceramic supporting vessels and bomb jacket. The titanium plug and safety disk are then placed on top of the liner. The seal must be expanded to ensure a proper pressure seal. Use the seal-forming tool (reamer) to carefully expand the seal lip. If done correctly, the plug should be slightly raised off the liner, and should "bounce" back when pushed. With the liner correctly sealed, the screw cap is attached to the bomb jacket (hand tightened).
- 11.5 When loading the rotor with the pressure vessels, make sure to take care of the proper sequence (each vessel and location is numbered 1-6). If a full set of six samples is not applicable, arrangements of 2, 3, 4, and 6 are possible – this ensures that the rotor's upper plate will rest in a horizontal position. Levers put the protection jacket over the rotor, and then tighten the three other screws, so the levers are swiveled over the protection jacket, thus fixing it (**Note:** Just hand tighten the fastening screws – never use the ring wrench supplied with the instrument to tighten).
- 11.6 Turn the Multiwave Microwave Digester on – the program takes about 30 seconds to start running. Make sure exhaust hose is secured inside the fume hood. Place loaded rotor into the cavity and push back until rotor locks into the turnstile. Make sure #1 on rotor is facing forward. Carefully close the safety door of the Multiwave (interior illumination and ventilation will turn off).
- 11.7 Selecting decomposition program from main menu, select **SAMPLE** using the keypad. Under sample, select **FISH** and when prompted, push the start button to activate program. If at anytime the program must be aborted, push the stop button (**Note:** If the temperature and pressure are too high, a security cooling program will be automatically activated).



- 11.8 Printing Graphs: When the program is complete and security cooling has stopped (vessels must be less than 65°C to open), a **PRESS ANY KEY** prompt will appear. When done, the main menu will reappear. Again under **SELECT** highlight **LAST RUN** and press **ENTER**. This will bring up a graph of the power and fan output as well as maximum temperature for each vessel from the last run. Press **F2** to print. When the printer is done with this graph, another screen will appear with the temperature layout for each vessel; again push **F2** to print screen. The computer will automatically return to the main menu when complete.
- 11.9 Remove rotor 6 from the Multiwave and place in adjacent vented fume hood. Loosen the fastening screws in the same manner as changing a tire. Loosen opposite screws at the same time being sure not to put too much pressure on one side. Remove the protection jacket and each of the pressure vessels. Put rotor aside until later use.
- 11.10 When loosening the caps on the pressure vessels, nitrous gas (brown gas) will escape rapidly. **(Be sure not to inhale)**. Put screw cap aside and remove plug from the liner rinse plug using reagent water and rinse bottle. Pour excess liquid into liner and place plug in holding rack. Remove TFL liner from ceramic supporting vessel and thoroughly rinse using reagent water bottle **(Be sure not to exceed 40 mL)**. Pour sample into skirted centrifuge tube (50 mL) and bring up to 40 mL. Repeat with each sample.
- 11.11 TFM liners should be rinsed completely before the next use. If time permits, or at least, once a week, run the pressure vessels through the cleaning program. Use about 10 mL of HNO₃ as a reagent. From main menu, select, **SAMPLE, <ENTER> CLEANING <ENTER>**.
- 11.12 Samples are now ready for analysis.

12.0 DATA ANALYSIS AND CALCULATIONS

Not Applicable

13.0 METHOD PERFORMANCE

- 13.1 WES successfully participated in the analysis of SW-846 metals in soil proficiency test sample (Lot No: BL040), which included the analytes described in Section 1.0 above.

14.0 POLLUTION PREVENTION

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

15.0 WASTE MANAGEMENT

- 15.1 Refer to the WES Environmental Management System (EMS) policy and SOPs regarding waste management. All waste products from this method are collected in the laboratory and then disposed of by a licensed hazardous waste management contractor.

16.0 REFERENCES

Not Applicable



17.0 TABLES AND VALIDATION DATA

TABLE 1. Quality Control Tests and Acceptance Limits for the Modified EPA Method 3052 – Multiwave Microwave Digestion of Fish/Biota Tissue

Accuracy			Precision		
QC Test	Acceptance Limits (% Recovery)	Frequency	QC Test	Acceptance Limits (RPD ^a)	Frequency
LFB ^b	85 – 115 ^e	≥ 10%	Duplicates	< 20 ^f	> 10%
LFM ^c	70 – 130 ^e	≥ 10%			
QCS ^d	90 – 110 ^e	> 10%			

^a RPD = relative percent difference among duplicates.

^b LFB = laboratory fortified blank sample.

^c LFM = laboratory fortified matrix sample

^d QCS = quality control sample from source outside of the laboratory.

^e Based on ± 3 standard deviations (SD) of the mean % recovery of a 30-sample set.

^f Based on ± 3 standard deviations (SD) of the mean RPD of the 30-sample set.



TABLE 2. Quality Control Elements and Acceptance Limits for the Modified EPA Method 3052 – Multiwave Microwave Digestion of Fish/Biota Tissue

QC Elements	Frequency	Acceptance Criteria	Corrective Action
Quality Control Sample (QCS)	After calibration and at the end of the run.	$\pm 20\%$ Recovery	Acceptable range must be met before continuing with sample analysis. Recalibrate and repeat.
Laboratory Reagent Blank (LRB)	One with each batch of 20 or less 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
Duplicates	Every 10 samples or less	Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures	
LFM	Every 10 samples or less	70 – 130% Note: Recovery calculation are not required if the concentration added is less than 35% of the unfortified sample concentration	Perform Method of Standard Additions
LFB	One with each batch of 20 samples	85 – 115%	The source of the problem must be identified and resolved before continuing analysis