MICROWAVE DIGESTION OF TISSUE SAMPLES

CONTENTS

1.0 SCOPE AND APPLICATION

2.0 METHOD SUMMARY

3.0 SAMPLE PRESERVATION, CONTAINERS, HANDLING, AND STORAGE

4.0 INTERFERENCES AND POTENTIAL PROBLEMS

5.0 EQUIPMENT/APPARATUS

6.0 REAGENTS

- 6.1 Type I Water
- 6.2 Concentrated Nitric Acid
- 6.3 1:1 Nitric Acid Solution

7.0 PROCEDURES

8.0 CALCULATIONS

9.0 QUALITY ASSURANCE/QUALITY CONTROL

- 9.1 Method Blank
- 9.2 Blank Spike
- 9.3 Matrix Spike/ Matrix Spike Duplicate
- 9.4 Reference Tissue

10.0 DATA VALIDATION

11.0 HEALTH AND SAFETY

12.0 REFERENCES

13.0 APPENDICES

Complete Rewrite, Revision 0.0, December 2002

SUPERCEDES: SOP #1823; Revision 0.0;11/25/94; U.S. EPA Contract EP-W-09-031.

MICROWAVE DIGESTION OF TISSUE SAMPLES

1.0 SCOPE AND APPLICATION

This method is applicable to the microwave-assisted acid digestion of animal and/or plant tissue samples. The tissue sample is digested and metal concentrations are determined by Graphite Furnace Atomic Absorption (GFAA) and/or Inductively Coupled Plasma (ICP) spectroscopy techniques.

2.0 METHOD SUMMARY

Tissue samples are to be collected and homogenized in accordance with applicable Scientific, Engineering, Response and Analytical Services (SERAS) standard operating procedures (SOPs). A representative portion of the sample is carefully weighed out into a digestion vessel. All samples are partially digested in 10 milliliters (mL) of 1:1 nitric acid (HNO_3) using a hot plate, and then completely digested using a suitable laboratory microwave system. Upon completion of the digestion process, the digestate is diluted to 50 mL using Type I reagent water in a volumetric flask. Sample digestates contain approximately 10 percent (%) HNO_3 and may vary slightly depending on sample matrix.

3.0 SAMPLE PRESERVATION, CONTAINERS, HANDLING, AND STORAGE

Sample holding times, suggested collection amounts, preservative, and type of containers are as follows:

Matrix	Collection Amount Required	Containers	Type of Preservative	Holding Time Metals
Tissue	5 - 200 g	G	0 - -10°C	6 months

G - glass, g - grams

Homogenized samples are received frozen and allowed to thaw to ensure that a properly homogenized portion of sample is weighed out for analysis. Once all of the samples have been weighed, the samples are returned to the sample freezer, in a timely manner, for proper storage. Additional information regarding the proper storage and handling of tissue samples is provided in SERAS SOP#1820, *Tissue Homogenization Procedure*.

4.0 INTERFERENCES AND POTENTIAL PROBLEMS

Due to the complex nature of tissue samples, the strength of acid mixture added to the sample may be adjusted to ensure complete digestion. Information regarding sample interferences and/or other analytical issues related to GFAA and/or ICP analysis are discussed in SERAS SOP #1818, *Determination of Metals by Graphite Furnace Atomic Absorption (GFAA) Method* and SERAS SOP #1811, *Determination of Metals by Inductively Coupled Plasma (ICP) Methods* respectively.

5.0 EQUIPMENT/APPARATUS

- Microwave Digestion System, CEM MDS-2100 or equivalent, for controlled digestion of tissue samples with rotary turntable
- Hot Plate, for pre-digestion of all tissue samples

- Digestion Vessels, for the complete digestion of all tissue samples
- Pipets, Class A, 10 mL
- Volumetric Flasks, Class A, 50 mL
- Balance, capable of accurately weighing all tissue samples to ± 0.01g, for digestion and percent solids determination.

  NOTE: An analytical balance is recommended for weighing percent solid portions to the nearest 0.001g when small sample amounts are provided.
- Nalgene Bottles, for storing digested samples
- Disposable syringes with filters, for filtering digestate prior to analysis
- Polypropylene funnel

6.0 REAGENTS

6.1 Type I Water

Use Type I water [American Society for Testing and Materials (ASTM) D1193]for the preparation of all reagents and as dilution water.

6.2 Concentrated Nitric Acid

Use trace metal grade HNO_3 acid certified for GFAA use.

6.3 Nitric Acid Solution (1:1)

Prepare a 1:1 dilution by adding concentrated HNO_3 to an equal volume of Type I water.

7.0 PROCEDURES

1. Allow frozen homogenized samples to thaw prior to weighing so that a representative portion of the sample is used.
2. Carefully weigh out 0.5 - 1.2 g of the tissue sample into a pre-cleaned digestion vessel.
3. Return all excess samples to the freezer for storage.
4. Add 10 mL of 1:1 HNO_3 to all sample vessels. (Some tissue samples may require addition of concentrated nitric acid to ensure proper digestion)
5. Loosely cap all digestion vessels and heat on a hotplate at 80 degrees Centigrade (^oC) for approximately 1

hour.

NOTE: Heating times may vary according to type of tissue.

6. Heat until the sample appears to be partially digested by the acid, and carefully vent all fumes into the fume hood prior to continuing with complete digestion.

7. Completely close all digestion vessels and attach pressure and temperature sensing lines to the monitoring vessel. Distribute vessels evenly in the carousel, and digest using the TISSUE program.

Stage 1	100% Power for 5 minutes with a maximum temperature of 140 ^{o}C.
Stage 2	50 % Power for 5 minutes with a maximum temperature of 160 ^{o}C.
Stage 3	50 % Power for 10 minutes with a maximum temperature of 160 ^{o}C.

(General tissue program parameters; actual program may vary according to type of tissue)

8. Once the TISSUE program is complete, allow all digestion vessels to cool. Monitor by using temperature and pressure gauges on microwave light-emitting diode (LED). Close the pressure monitoring valve on the line leading to the monitoring vessel prior to disconnecting the pressure sensing line to ensure no sample loss and safe removal from the microwave. Finally, remove the entire carousel from the microwave.

9. Carefully vent all fumes in the fume hood and transfer the digestate using a polypropylene funnel, into a clean acid rinsed 50-mL volumetric flask. Dilute to final volume using Type I water.

10. Allow all samples to settle overnight or filter samples to remove suspended particulates.

11. Samples are now ready for analysis using GFAA and/or ICP. Refer to SERAS SOP #1818, *Determination of Metals by Graphite Furnace Atomic Absorption (GFAA) Method* and SOP #1811, *Determination of Metals by Inductively Coupled Plasma (ICP) Methods* for detailed instructions.

8.0 CALCULATIONS

All digestion data will be used to calculate and report final results on a dry weight basis (mg/kg) unless otherwise noted. Refer to SERAS SOP #1811, *Determination of Metals by Inductively Coupled Plasma (ICP) Methods* and SERAS SOP #1818, *Determination of Metals by Graphite Furnace Atomic Absorption (GFAA) Method* for specific details.

9.0 QUALITY CONTROL

All quality control data should be maintained and available for easy reference or inspection.

9.1 Method Blank

A method blank must be prepared for each analytical batch of samples (not to exceed 20 samples) using Type I water.

9.2 Blank Spike

A Blank Spike (BS) sample must be prepared for each analytical batch of samples (not to exceed 20 samples). A BS is prepared by spiking a known concentration of analytes into Type I water. The spiking level should be at or near mid-range of the calibration.

9.3 Matrix Spike/Matrix Spike Duplicate

At least one matrix spike/matrix spike duplicate (MS/MSD) must be digested with every 10 samples, or with each tissue type to verify the accuracy of the method. The spike level should be at or near mid-range of the calibration. In the event there is insufficient sample available in the batch to run a MS/MSD, a blank spike/blank spike duplicate (BS/BSD) will be run.

9.4 Reference Tissue

A reference (control) tissue may be provided by the SERAS Task Leader for a specific project. This sample will be prepared and analyzed in conjunction with the tissue samples.

10.0 DATA VALIDATION

Tissue preparation documentation in conjunction with the analytical data will be assessed by the Data Validation and Report Writing Group using the most current version of SERAS SOP #1017, *Data Validation Procedure for Routine Inorganic Analysis*. However, data is considered satisfactory for submission purposes when all of the requirements listed in the method are met.

11.0 HEALTH AND SAFETY

The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. However, each chemical compound should be treated as a potential health hazard. The laboratory is responsible for following the chemical hygiene plan and laboratory safety program regarding the safe handling of the chemicals specified in this method.

When working with potentially hazardous materials, refer to U.S. EPA, Occupational Safety and Health Administration (OSHA) and corporate health and safety practices. More specifically, refer to SERAS SOP #3013, *SERAS Laboratory Safety Program*. Any waste generated must be disposed of in accordance with SERAS SOP#1501, *Hazardous Waste Management*.

12.0 REFERENCES

U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response. 1996. *Test Methods for Evaluating Solid Waste*, SW-846, 3rd ed. Update III. Method 3052.

CEM Corporation, Operation Manual. *Microwave Sample Preparation System, MDS-2100*. 1994. Rev. 1