

Metals in Animal Tissue and Vegetation (Biota) - Prescriptive

Parameter Metals in Tissue and Vegetation (Biota).

Analytical Method Nitric acid, Hydrochloric acid, and Hydrogen peroxide digestion (followed by appropriate instrumental analysis).

Introduction This method was prepared for BC MOE by the BCELTAC to provide improved interlaboratory consistency of results for metals in tissue and vegetation. All definitive elements of the method have been prescribed to minimize inter-laboratory variability.

This method uses a mixture of nitric acid, hydrochloric acid, and hydrogen peroxide, with a standardized digestion time and temperature. Laboratories are allowed some flexibility regarding apparatus and heating methods, but variations in acid mixture composition, digestion time, or digestion temperature are not permitted.

Method Summary Samples are homogenized and digested with a mixture of nitric acid, hydrochloric acid, and hydrogen peroxide. Instrumental analysis of sample extracts can be performed by a variety of analytical methods.

This method provides the sample preparation procedure for the analysis of Total Metals, but does not imply a complete dissolution of some materials, as would occur with digestions using perchloric and hydrofluoric acids. The strong acid leach prescribed by this method is intended to provide a conservative estimate of the concentrations of metals that may be bio-available (for example, through mammalian digestion processes). The method achieves near complete recoveries of most toxicologically important metals, but some elements associated with recalcitrant minerals (e.g. aluminosilicates) are only partially recovered, especially those that originate from dust, soil, or sand particles that can be present in some types of biota samples.

This method is prescriptive. It must be followed exactly as described. Where minor deviations are permitted, this is indicated in the text.

Method Limitations This method does not dissolve all materials and may result in a partial extraction, depending on the sample matrix, for some metals, including, but not limited to aluminum, chromium, iron, nickel, strontium, tin, titanium, uranium, and vanadium, especially when these elements are present in refractory mineral forms.

Analytes and EMS Codes This method is applicable to the following parameters:

Analyte	EMS Code	Analyte	EMS Code	Analyte	EMS Code
Aluminum	AL-T	Iron	FE-T	Silver	AG-T
Antimony	SB-T	Lead	PB-T	Sodium	NA-T
Arsenic	AS-T	Lithium	LI-T	Strontium	SR-T
Barium	BA-T	Magnesium	MG-T	Sulfur	S-T
Beryllium	BE-T	Manganese	MN-T	Thallium	TL-T
Boron	B-T	Mercury	HG-T	Thorium	TH-T
Cadmium	CD-T	Molybdenum	MO-T	Tin	SN-T
Calcium	CA-T	Nickel	NI-T	Titanium	TI-T
Chromium	CR-T	Phosphorus	P-T	Uranium	U-T
Cobalt	CO-T	Potassium	K-T	Vanadium	V-T
Copper	CU-T	Selenium	SE-T	Zinc	ZN-T

Other metals may be analyzed by this method if acceptable performance is demonstrated and validated. This method is not suitable for the determination of silica or silicon.

Reported results must be clearly indicated as being in either dry weight and/or wet weight concentration units, depending on the application, as requested by the data-user. The default Ministry preference is for animal tissues to be reported on a wet weight basis (e.g.

"mg/kg wwt"), and for vegetation to be reported on a dry weight basis (e.g. "mg/kg" or "mg/kg dwt"). Biota samples with excessively high moisture contents (i.e. >90% moisture, which may occur in periphyton, macrophyte, benthic invertebrates, eggs, etc.) should be dried at $\leq 60^\circ$ or freeze dried and weighed prior to digestion to minimize the error associated with moisture corrections.

Matrix

Tissue and Vegetation (Biota)

Interferences and Precautions

The interferences encountered will differ depending on the instrumental method used to analyze the sample extracts. Applicable interferences should be clearly identified and controlled in the analysis procedure. High concentrations of acids may cause physical interferences with some instrumental techniques.

Equipment used for sample homogenization, including various types of homogenizer probes, grinders, food processors, knife blades, may have the potential for the contamination of metals including, but not limited to aluminum, chromium, iron, manganese, molybdenum, nickel, tin, and vanadium. Such equipment must be tested and validated to ensure it does not introduce significant metallic contaminants to samples.

Sample Handling and Preservation

Sampling should be done by qualified personnel. Samples must be collected and stored such that degradation or alteration of the sample is minimized. Collect the sample in a clean plastic or glass container, appropriately sealed. Paper bags are suitable collection containers for vegetation where samples will be dried prior to digestion and where test results will be reported on a dry weight basis.

Where practical, submission of a minimum of 20 wet grams of animal tissue or 5 grams of low moisture vegetation is recommended.

The decision as to which components of animal tissue samples (e.g. organs, muscle tissue, skin, whole sample, etc.) is critical to this analysis. Specific instructions for the preparation of vegetation samples can also be critical (e.g. whether to include or exclude dirt residues, etc.). Ensure that clients have been adequately consulted for specific instructions.

Stability

Preservation: none

Holding time:

Metals (except Mercury): 2 years if frozen at $\leq -18^\circ\text{C}$ (Reference: Puget Sound Protocols). Freezing is optional for freeze dried tissue samples and for vegetation that is dried prior to digestion and reported on a dry weight basis; in these cases, samples may be stored up to 6 months at ambient temperature (based on BC MOE soil guidelines).

Mercury: 1 year if frozen at $\leq -18^\circ\text{C}$ (Reference: US EPA 1631E Appendix). Freezing is optional for freeze dried tissue samples and for vegetation that is dried prior to digestion and reported on a dry weight basis; in these cases, samples may be stored up to 28 days at ambient temperature (based on BC MOE soil guidelines).

Results reported for samples digested beyond holding times must be qualified.

Storage: Samples must be kept cool ($\leq 10^\circ\text{C}$) during transport. Biota samples where freezing is required (e.g. animal tissues, high moisture vegetation like berries, etc.) should be frozen as soon as possible, preferably within 48 hours of sampling, or digestion should be initiated within 96 hours of sampling.

Where practical, it is recommended for tissue dissections to be conducted prior to freezing, unless the whole sample will be homogenized.

Equipment and Supplies

1. Heating source (e.g. block digester, hotplate, water bath) capable of maintaining a sample extract temperature of $95 \pm 5^\circ\text{C}$
2. Balance (capable of weighing to at least 3 significant figures)
3. Drying oven (may not be required)
4. Freezer (may not be required)

5. Freeze Dryer (may not be required)
6. Acid dispensers
7. Vapour refluxing cover to fit digestion vessel (e.g. reflux cap, watch glass etc.)
8. Digestion Vessels (e.g. block digester tube, beaker, flask, etc.)
9. Gloves
10. Spatula
11. Equipment for sample homogenization

Reagents

1. Nitric acid (HNO₃), concentrated (67 - 70%), reagent grade minimum
2. Hydrochloric acid (HCl), concentrated (34 - 37%), reagent grade minimum
3. Hydrogen peroxide (H₂O₂) 30%, reagent grade minimum
4. Water, de-ionized (ASTM Type I or equivalent recommended)

Safety

Nitric and hydrochloric acid must not be premixed; they should be added individually to each sample vessel. Mixtures of nitric and hydrochloric acid must not be stored in closed containers. When mixing concentrated acids with water, slowly and carefully add the acids to water. Adding water to concentrated acids is a hazard and can cause a violent exothermic reaction.

Wear appropriate PPE (Personal Protective Equipment) including lab coat, gloves, and safety glasses. Add reagents to samples and perform digestions under a fume hood.

Procedure**Sample Preparation – Drying and Homogenization**

1. Depending on project requirements, samples may require drying prior to homogenization. Where drying is required, dry the sample at a temperature of ≤ 60°C. Freeze drying is acceptable.

Note: Sample drying temperature must not exceed 60°C to minimize evaporative loss of volatile analytes such as mercury (Reference: Puget Sound Protocols).

2. Homogenize the dried or as-received sample to a paste-like consistency either manually or mechanically using suitable equipment.

Sample Preparation - Digestion

The following procedure is based on the digestion of approximately 5 grams of wet sample or 1 gram of dry sample. The amount of sample digested can be scaled up or down as long as the acid ratios are maintained (9 parts HNO₃ : 1 part HCl). The default minimum total amount of reagents listed below may be used for sample weights up to 7.5 wet grams or 1.5 dry grams. For sample weights larger than this, increase the total amount of reagents.

1. Weigh approximately 5 grams of wet sample or 1 gram of dry sample into a digestion vessel, which must be fitted with a reflux cap and which must be capable of supporting reflux action. Examples of digestion vessels fitted with a reflux cap include a beaker, Erlenmeyer flask, or digestion tube fitted with a watch glass. Include Method Blanks, Lab Duplicates, and Reference Materials with each batch of samples. Weights must be recorded using a balance capable of weighing to at least 3 significant figures.
2. Add a minimum of 9 mL concentrated HNO₃, and a minimum of 1 mL concentrated HCl (per ~5 grams wet sample or ~1 gram dry sample). Optionally, 4 ± 0.5 mL of de-ionized water can be added to dried CRMs to be representative of the typical water content of wet tissue samples. Adding water to the CRMs may prevent excessive reactivity after acid addition.
3. Add a reflux cap to the top of the digestion vessel. Swirl acid and sample to mix gently. To reduce the reactivity of samples during initial heating, it is recommended to allow samples to equilibrate with the acid mixture at room temperature for a minimum of 1 hour prior to heating.
4. Digest samples for 2 hours ± 15 minutes at a temperature of 95 ± 5°C at atmospheric pressure. A hotblock or waterbath are the preferred methods of heating. The heat for digestion must maintain the sample extract temperature at 95 ± 5°C. This refers to the

temperature of the sample extract in a digestion vessel covered with a reflux cap, not the temperature setting on the heating source, and not the temperature of an uncovered digestion vessel. It is recommended that the sample extract temperature be monitored and recorded with each batch.

5. Allow the sample to cool and add a minimum of 4 mL 30% H₂O₂. It is acceptable to add the H₂O₂ in multiple additions.
6. Digest samples for a minimum of 1 hour at a temperature of 95 ± 5°C.
7. Dilute the entire sample with de-ionized water to the volume required for the analysis. The dilution volume will depend on the analytical method and objectives. Typical final dilution volumes for most instrumental techniques range from 25 mL to 100 mL. Include all undigested solid material as part of the final diluted volume.
8. Analyze the final extract using appropriate analytical methods and report the results on a dry weight and/or wet weight basis, depending on the application, as requested by the data-user. Refer to *Analytes and EMS Codes* section for Ministry preferences on reporting units for biota.
9. Report any anomalies observed during the digestion and analysis.

Quality Control

Summary of QC Requirements		
Method QC Component	Minimum Frequency	Minimum Data Quality Objectives*
Method Blank	1 per batch (max 20 samples)	Less than reported DL
Reference Material	1 per batch (max 20 samples)	70 – 130% of the laboratory's long term mean value or of certified targets for the method (if available)
Lab Duplicates	1 per batch (max 20 samples) where sufficient sample is available	≤ 40% RPD for all metals except those indicated below ≤ 60% RPD (for Ca & Sr)
Field Duplicates	Recommended	None Specified
* Minimum DQOs apply to individual QC samples at levels above 10x MDL. Report qualified data when DQOs are not met.		
Reference Material requirements: Any suitable RM (including in-house RMs) may be utilized for this method for routine Quality Control purposes.		

Method Validation Requirements

If metallic instruments or equipment are used to process or homogenize test samples, validation studies must be conducted to ensure that significant metallic contaminants are not introduced to test samples.

Specific method validation requirements for accuracy and precision may be established after interlaboratory consensus targets for appropriate Reference Materials have been established for this method by interlaboratory study or round robin.

References

US EPA Method 200.3, Sample Preparation Procedure for Spectrochemical Determination of Total Recoverable Elements in Biological Tissues, United States Environmental Protection Agency, 1996.

Recommended Guidelines for Measuring Metals in Puget Sound Marine Water, Sediment and Tissue Samples, Prepared for the US EPA, Puget Sound Water Quality Action Team, Olympia Washington, 1997.

US EPA Appendix to Method 1631 Total Mercury in Tissue, Sludge, Sediment, and Soil by Acid Digestion and BrCl Oxidation, United States Environmental Protection Agency, 1991.

Revision History

Aug 15, 2014 New prescriptive method added to BC Lab Manual to improve interlaboratory consistency. Effective date of this method is Jan 1, 2015.