

# Application Note - Tissue Digestion

## **Objective:**

To digest various types of tissue samples for mineral determination.

## **Sample Type:**

Plant

Sample weight: 1.0 g

## **Scope:**

Fresh, frozen, preserved or freeze-dried tissues are digested in acid in order to obtain a liquid sample, which can be used on an atomic absorption spectrometer to analyze for various types of metals.

## **Principle:**

In order to analyze samples by atomic absorption, the tissues are digested by nitric acid and heat. The resulting clear liquid is diluted with distilled deionized water.

## **Apparatus and Reagents:**

- 1) 48 position *Digi***PREP MS**
- 2) 24 position rack-lock rack
- 3) 50 ml polypropylene digestion tubes and caps - *Digi***TUBES**
- 4) External Temperature Monitoring Device *Digi***PROBE**
- 5) Disposable ribbed watch glasses
- 6) Fume Scrubbing System *Digi***VAC**
- 7) Nitric acid, *Plasma***PURE** grade
- 8) Large weigh boats
- 9) Small weigh boats
- 10) Balance
- 11) Scalpel
- 12) Spatula

## **Safety Precautions:**

The analyst must be read all MSDS sheets pertaining to the chemicals used in this procedure each calendar year before performing this method. Ensure that all weigh dishes and any other material that is considered waste or comes into contact with biological tissue be disposed of in a biohazard bag. Keep open tubes under fumehood at all times when using nitric acid.

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## Procedure:

- 1) The evening before the digestion, take the samples out of the freezer and place in fridge for the samples to thaw overnight.
- 2) On the day of digestion, tune the *DigiPREP* with the *DigiPROBE* in the control sample and set to 100°C
- 3) Prepare a worksheet for each metal that is to be analyzed
- 4) Each 5<sup>th</sup> tissue sample is analyzed in duplicate (if there is enough sample). A blank sample is also included in each run. The blank will include all materials except tissue (deionized water, nitric acid and peroxide).
- 5) Label each *DigiTUBE*. Tare each empty *DigiTUBE* prior to adding tissue or QC material.
- 6) Cut portions of tissues to obtain a representative sample of each tissue, and place into tared *DigiTUBE*, then weigh on balance. Sample should be 1.000g – 1.090g.
- 7) For the QC samples, 0.100 – 0.120g is weighed out of tissue to get the actual weight of QC sample placed in the *DigiTUBE*. Write the date and final weight of each QC sample on the tube. This information makes it possible for QC samples to be re-used to check future runs.
- 8) 1 ml of deionized, distilled water and 2.5 ml of concentrated nitric acid (or 4 ml for feed samples) are added to each digestion tube and then placed in the *DigiPREP*. Ribbed watch glasses are placed on the tubes to prevent the samples from evaporating to dryness. The *DigiPROBE* is inserted into the control sample. The Hood of the *DigiVAC* is closed (Note: Ensure the Blower is turned on).
- 9) Use the ramp rate of 1 degree/minute on *DigiPREP* to reach temperature. Once the *DigiPREP* reaches 100 degrees the samples are left for 3 to 3.5 hours or until the volume is low but not completely evaporated.
- 10) The samples should be cooled for 20 minutes then add 1.5 ml of H<sub>2</sub>O<sub>2</sub> (hydrogen peroxide) to each digestion tube. The *DigiPREP* is turned down to 95°C and left at the temperature for another 40-60 minutes.
- 11) The samples are then removed and diluted with deionized water to a final volume of 10 ml then capped and vortexed.

## Critical Control Points:

Weight of samples, weight of QC's, volume of nitric acid and dilution volume.

## Stability:

Quality control substances in their dry state are stable for several years and when digested in acid they are stable for several months.

## Quality Control:

The following is a list of the Quality Controls and the corresponding metal they are run for:

- |            |                |
|------------|----------------|
| • Lead     | • Pine Needles |
| • Copper   | • Bovine Liver |
| • Zinc     | • Bovine Liver |
| • Selenium | • Tort-2       |
| • Cadmium  | • Tort-2       |
| • Iron     | • Bovine Liver |

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DigiPREP

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## **CLEANUP:**

- 1) The ribbed watch glasses are rinsed well, allowed to air dry, then disposed of in a waste container.
- 2) Kimwipes, gloves and anything else that comes into contact with tissue are disposed of into a biohazard bag.
- 3) Once the sample is no longer needed, it is washed down the sink with copious amounts of water, the digestion tubes and caps are rinsed well, air dried then disposed of in a waste container.

## **CALCULATIONS:**

See appropriate SOP for metal procedure.

## **DATA ARCHIVING:**

Upon completion of the digestion, the worksheet is sent with the samples to be run on the AA. Once the analysis is completed the results are recorded and stapled together and kept.

## **REFERENCES:**

- 1) EPA Method 200.2 Revision 2.8
- 2) Digestion of Tissue for Atomic Absorption Analysis, Darlene Mahar and Orysia Dawydiak, Atlantic Veterinary College University of Prince Edward Island, 2000.

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