

DETERMINATION OF PERCENT LIPID IN TISSUES

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ABSTRACT

Tissue percent lipid is determined by weighing a known aliquot of dichloromethane extractable material. Tissues are extracted using a Dionex ASE200 Accelerated Solvent Extractor. The extract is concentrated to 3 mL and a 100 μ L aliquot is removed and weighed to the nearest 0.001 mg on a pre-dried, tared glass fiber filter. Percent lipid is calculated based on extract volume and sample weight.

1.0 INTRODUCTION

Approximately 15 g of wet tissue is chemically dried with Hydromatix®. Samples are extracted with 100% dichloromethane using an automated extraction apparatus (Dionex ASE200 Accelerated Solvent Extractor) operated at 100°C and 2,000 psi. Extracts are concentrated to 3 mL by evaporative solvent reduction in a water bath at 55 - 60°C. An aliquot of 100 μ L is removed and weighed to the nearest 0.001 mg on a dried, tared glass fiber filter.

2.0 APPARATUS AND MATERIALS

2.1 EQUIPMENT

- Dionex, ASE200 Accelerated Solvent Extractor (ASE) with 33 mL extraction cells
- Balance, top loading, tare capacity to 300 g, capable of weighing to 1 mg
- Microbalance, capable of weight to 1 μ g
- Calibrated weights, certified
- Combustion furnace, electric, capable of combusting glassware at 400°C for at least 4 hours
- Oven, capable of 40°C temperature maintenance
- Water bath, capable of maintaining a temperature of 55-60°C
- Glass fiber filter circles, 2.4 cm diameter
- Collection vials, 60 mL certified pre-cleaned with open screw caps and Teflon lined VOA septa

- Kurderna-Danish (K-D) tubes, 25 mL, slow dry concentrator tubes
- Synder columns, 3 ball
- Boiling chips, Teflon
- Micropipettors, calibrated to 100 μ L, 1% accuracy, disposable tips

2.2 REAGENTS

- Acetone, pesticide grade or equivalent purity
- Dichloromethane, pesticide grade or equivalent
- Hydromatrix®, conditioned by combustion at 400°C for at least 4 hours and stored at 120°C
- Nitrogen, 99.8% purity

3.0 PROCEDURE

Tissues are extracted with dichloromethane using an ASE200 (see tissue extraction method for details). The extracts are concentrated to approximately 10 mL in 60 mL collection vials. Extracts are then quantitatively transferred to Kurderna-Danish (K-D) tubes and concentrated to 3 mL. A 100 μ L aliquot is removed and placed on a pre-dried, tared 2.4 cm glass fiber filter. The filter with the sample is dried in a 40°C oven to a constant weight (approximately 2 minutes). The filter and sample are weighed to the nearest 0.001 mg.

4.0 QUALITY CONTROL (QC)

All reagents are verified to be contaminant free. All equipment and glassware used are thoroughly cleaned by solvent rinsing or combustion at 400°C. The calibration and accuracy of balances, weights, and thermometers are checked daily. The calibration and accuracy of balances, weights are verified yearly by an independent source. All samples are shipped and received under chain-of-custody. A sample is analyzed in duplicate with each batch of 20 samples or fewer. The following quality controls are used to ensure the accuracy and precision of lipid data.

- Laboratory Duplicates. A sample is analyzed in duplicate with each extraction batch of 20 or fewer samples. The relative percent difference (RPD) is calculated for all duplicate samples and should be no more than 25%. If this criterion is not met after re-weighing, corrective action may result in re-processing all sample in the QC batch.

5.0 CALCULATIONS

5.1 PERCENT LIPID WEIGHT

$$\% \text{ Lipid} = \frac{\left(\frac{(W_r)(V_f)}{(W_s)(V_a)} \right) \times \left(\frac{1 \text{ g}}{1,000 \text{ mg}} \right) \times 100}{}$$

Where:

W_r = residual weight of the aliquot for lipid determination (mg)

W_s = sample weight (g)

V_f = final volume of sample extract

V_a = volume of extract aliquot used for lipid determination

5.2 DUPLICATE SAMPLE ANALYSES

$$\text{RPD} = \frac{\left| (\text{Lipid Wt.}_{\text{sample1}} - \text{Lipid Wt.}_{\text{sample2}}) \right|}{\left(\frac{(\text{Lipid Wt.}_{\text{sample1}} + \text{Lipid Wt.}_{\text{sample2}})}{2} \right)} \times 100$$