Ancillary Methods of the National Status and Trends Program: Update 2000-2006

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Cover photograph of oyster bed Sapelo Island, GA.

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Ancillary Methods of the National Status and Trends Program: Update 2000-2006

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CHAPTER 1. DRY WEIGHT DETERMINATION OF SEDIMENT

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ABSTRACT

Aliquots of field collected wet sediments are oven dried in the laboratory to a constant weight. Dry weights are determined so that sediment contaminant levels can be reported as a concentration per gram dry weight. The following method describes the procedure by which percent moisture in sediments is determined.

1.0 INTRODUCTION

An aliquot of approximately 1 to 2 g of homogenized wet sediment is dried at 105°C to a constant weight. Percent moisture is determined by calculating the amount of mass lost during the drying procedure for each aliquot.

2.0 APPARATUS AND MATERIALS

2.1 EQUIPMENT

- Balance, top loading, tare capacity to 300 g, capable of weighing to 1 mg
- Calibrated weights, certified
- Oven, electric convection, capable of maintaining stable temperatures of at least 200ºC
- Desiccator, cabinet style
- Spatulas, stainless steel
- Beakers, glass 10 mL

2.2 REAGENTS

- Water (CAS 7732-18-5), deionized and activated-carbon filtered, organic free
- Acetone (CAS 67-64-11), pesticide grade or equivalent purity
- Methanol (CAS 67-56-1), pesticide grade or equivalent purity

- Dichloromethane (CAS 75-09-2), pesticide grade or equivalent purity

3.0 PROCEDURE

Frozen samples are thawed to room temperature. Large-sized extraneous material (i.e., leaves, twigs, rocks, shells) are removed prior to homogenization. Sediment samples are homogenized in pre-cleaned, glass collection jars by stirring vigorously with stainless steel spatulas. Spatulas are cleaned with Micro® soap and solvent rinsed to remove all traces of organic materials.

Dry weight is determined by placing sample aliquots in pre-weighted, aluminum weighing pans. Weighing pans are pre-dried in an oven at 105ºC and cooled in a desiccator. Once the pans have cooled for a minimum of 30 minutes, the weight of the empty pan is recorded to the nearest 1 mg. Approximately 1 to 2 g of thoroughly homogenized sample is placed into a labeled weighing pan using a solvent rinsed stainless steel spatula. The weight of the pan plus wet sample is recorded to 1 mg. Sediment samples are dried in a 105ºC oven for at least 24 hours followed by cooling in a desiccator for at least 30 minutes prior to weighing. Samples and pans are weighed to the nearest 1 mg. Samples are placed back into the oven and dried for another 24 hours at 105°C, cooled in a desiccator, and weighed. This cycle is repeated until successive weight differences are less than 4%.

4.0 QUALITY CONTROL (QC)

Solvents are verified contaminant free by lot tests prior to use. All equipment and glassware used are thoroughly cleaned by solvent rinsing or combustion at 400°C. The calibration and accuracy of balances and thermometers are checked daily using certified weights and thermometers with calibrations traced to the National Institute of Standards and Technology. The calibration and accuracy of balances and weights are verified yearly by an independent source. A sample is analyzed in duplicate with each batch of 20 samples or fewer. The following quality control is used to evaluate the precision of dry weight data:

Duplicate

- The relative percent difference (RPD) between the duplicate and original sample is ≤ 25%.

- If this criterion is not met after re-weighing, corrective action may result in re-processing all samples in the QC batch.

5.0 CALCULATIONS

5.1 PERCENT DRY WEIGHT

$$\text{Dry Wt. \%} = \frac{(\text{Beaker Wt.} + \text{Dry Wt.}_{\text{sample}}) - (\text{Beaker Wt.})}{(\text{Beaker Wt.} + \text{Wet Wt.}_{\text{sample}}) - (\text{Beaker Wt.})} \times 100$$

5.2 DUPLICATE SAMPLE ANALYSES

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

CHAPTER 2. DRY WEIGHT DETERMINATION OF TISSUE

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ABSTRACT

Aliquots of tissues are dried in the laboratory to a constant weight. Dry weights are determined so that tissue contaminant levels can be reported as a concentration per dry weight. The following method describes the procedure by which percent moisture in tissues is determined.

1.0 INTRODUCTION

An aliquot of approximately 1 to 2 g of homogenized wet tissue is dried at 105°C to a constant weight. Percent moisture is determined by calculating the amount of mass lost during the drying procedure for each aliquot. The percent moisture is used to calculate the dry weight of the corresponding weighed wet aliquot.

2.0 APPARATUS AND MATERIALS

2.1 EQUIPMENT

- Balance, top loading, tare capacity to 300 g, capable of weighing to 1 mg
- Calibrated weights, certified
- Oven, electric convection, capable of maintaining stable temperatures of at least 200ºC
- Desiccator, cabinet style
- Spatulas, stainless steel
- Beakers, glass 10 mL
- Waring industrial blender, titanium blades and Teflon rings

2.2 REAGENTS

- Water (CAS 7732-18-5), deionized and activated-carbon filtered, organic free
- Acetone (CAS 67-64-11), pesticide grade or equivalent purity

- Methanol (CAS 67-56-1), pesticide grade or equivalent purity

- Dichloromethane (CAS 67-56-1), pesticide grade or equivalent purity

3.0 PROCEDURE

Bivalves are shucked using clean, solvent rinsed stainless steel utensils and homogenized. The samples are homogenized in a Waring Industrial Blender that has been outfitted with titanium blades and Teflon rings. The samples are stored frozen in pre-cleaned glass jars. Frozen bivalve samples are thawed at room temperature. Samples are re-homogenized by stirring vigorously with stainless steel spatulas. All equipment used to process tissues is cleaned with Micro® soap and solvent rinsed to remove all traces of organic materials. Dry weight is determined by placing sample aliquots in pre-weighed, aluminum weighing pans. Weighing pans are pre-dried in an oven at 105ºC and cooled in a desiccator. Once the pans have cooled for a minimum of 30 minutes, the weight of the empty pan is recorded to the nearest 1 mg. Approximately 1 to 2 g of thawed, thoroughly homogenized sample is placed into a labeled weighing pan using a solvent rinsed stainless steel spatula. The weight of the pan plus wet sample is recorded to 1 mg. Tissue samples are dried in a 105ºC oven for at least 24 hours followed by cooling in a desiccator for at least 30 minutes prior to weighing. Samples and pans are weighed to the nearest 1 mg. Samples are placed back into the oven and dried for another 24 hours at 105°C, cooled in a desiccator and weighed. This cycle is repeated until successive weight differences are less than 4%.

4.0 QUALITY CONTROL (QC)

Solvents are verified to be contaminant free by lot tests prior to use. All equipment and glassware used are thoroughly cleaned by solvent rinsing or combustion at 400°C. The calibration and accuracy of balances and thermometers are checked daily using certified weights and thermometers with calibrations traced to the National Institute of Standards and Technology. The calibration and accuracy of balances and weights are verified yearly by an independent source. A sample is analyzed in duplicate with each batch of 20 samples or fewer. The following quality control is used to evaluate the precision of dry weight data:

Duplicate

- The relative percent difference (RPD) between the duplicate and original sample is ≤ 25%.

- If this criterion is not met after re-weighing, corrective action may result in re-processing all samples in the QC batch.

5.0 CALCULATIONS

5.1 PERCENT DRY WEIGHT

$$\text{Dry Wt. \%} = \frac{\left(\text{Beaker Wt.} + \text{Dry Wt.}_{\text{sample}}\right) - \left(\text{Beaker Wt.}\right)}{\left(\text{Beaker Wt.} + \text{Wet Wt.}_{\text{sample}}\right) - \left(\text{Beaker Wt.}\right)} \times 100$$

5.2 DUPLICATE SAMPLE ANALYSES

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

CHAPTER 3. DETERMINATION OF PARTICLE SIZE DISTRIBUTION (GRAVEL, SAND, SILT AND CLAY) IN SEDIMENT SAMPLES

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ABSTRACT

Particle size distribution is determined in marine sediments on the basis of the Wentworth scale method. The major size classes determined are: gravel (-2 phi to -5 phi), sand (+4 phi to -1 phi), silt (+5 phi to +7 phi), and clay (+8 phi and smaller). Determining particle size in sediments is important due to potential correlations with contaminant levels. Sediments are pre-treated with hydrogen peroxide to remove organic matter prior to particle size determination.

1.0 INTRODUCTION

High contaminant concentrations are often associated with finer-grained sediments. Consequently, correlations can be made between particle size distribution and contaminant concentrations. Particle size distribution is a cumulative frequency distribution, or a frequency distribution of relative amounts of particles in a sample within specified size ranges (phi scale is normally used). The size of a discrete particle is characterized as a linear dimension, and is designated as a particle diameter. The use of sieves and settling tubes, as described below, results in a sediment description based on particle size and density, reported as percentages of the total sample weight.

2.0 APPARATUS AND MATERIALS

2.1 EQUIPMENT

- Balance, analytical accurate to 0.1 mg
- Calibrated weights, certified
- Oven, electric convection, capable of maintaining stable temperatures of at least 200ºC
- Desiccator, cabinet style
- Spatulas, stainless steel

- Beakers, glass 50 mL and 150 mL
- Glass pipette, 25 mL
- Wide mouth jars, 32-ounce
- Wet sieve, 8-inch #230 (63 μm)
- 9-inch plastic funnel
- Metal ring stand
- Graduated cylinder, 1000 mL
- Plunging device
- Sieve shaker, Humboldt H-4325
- Testing sieves, 8-inch diameter, #10 (2000 μm, -1 phi), #230 (63 μm, +4 phi), sieve cover, sieve bottom pan

2.2 REAGENTS

- Acetone (CAS 67-64-1), reagent grade
- Dichloromethane (CAS 75-09-2), pesticide grade or equivalent
- Deflocculent solution (5 g/L sodium hexametaphosphate in deionized water), CAS 10124-56-8
- Hydrogen peroxide (CAS 7722-84-1), 30%

3.0 PROCEDURE

Sediments are collected in plastic bottles, plastic bags or glass bottles and stored refrigerated (4°C) until analysis. Samples are homogenized in the sample container using a stainless steel spatula. Approximately 45 to 50 g of sandy sediment or 20 to 25 g of sediment is weighed into a 32-ounce wide-mouth jar. Sufficient water is added to cover the sample. The jars are placed under a hood and small quantities of 30% hydrogen peroxide (H_2O_2) are carefully added until all reactions (bubbling) have ceased. Once the bubbling has stopped, the jars are placed into a 65°C water bath and small amounts of H_2O_2 continue to be added until no more reaction is observed. The jars are removed from the bath and cooled. Once the jars are cool, 20 mL of deflocculent solution and 30 mL of deionized water are added. The jars are sealed and shaken until the sample is disaggregated. The sample and deflocculent solution are poured through a 63 μm sieve into a 1,000 mL graduated cylinder using deionized water to rinse all material out of the jar. The sediment remaining on the sieve is thoroughly rinsed with deionized water to ensure that the particles smaller than 63 μm pass through the sieve. The volume in the

graduated cylinder must remain under 1,000 mL. The coarse sediment remaining on the sieve is gently concentrated against the bottom lip of the sieve. The sediment on the sieve is rinsed with deionized water into a labeled 150 mL beaker. The coarse sediment in the beaker is oven dried at a temperature between 70 °C and 90 °C. Once the coarse fraction is dry, the material from the 150 mL beaker is transferred to the top sieve in a sieve stack that is arranged in descending order (i.e., -1 phi and +4 phi). A bottom collection pan and top cover are added to the sieve stack. The stack is shaken for 15 minutes on the shaker. The top sieve (-1 phi) is removed and emptied onto a large piece of clean paper, using a small nylon brush to remove as much sediment as possible. The sediment from the paper is poured into a tarred weighing boat and weighed to the nearest 0.0001 g. Next the bottom sieve (+4 phi) is inverted and brushed clean over the paper and added to the weighing boat. The total weight of the sediment from both sieves is recorded to obtain a cumulative weight. The difference in the values of the cumulative weight, and the weight of the gravel fraction alone, represents the weight of the sand fraction. Any remaining sediment in the bottom pan (<63µm) is added to a corresponding graduated cylinder for that sample to determine the silt/clay fraction. The silt/clay fraction is for each sample is determined by filling the corresponding graduated cylinder to 1,000 mL with deionized water. The cylinder is covered and maintained at 24 °C for approximately 24 hours. After 24 hours, the contents of the cylinder are mixed for 1 minute with the plunger. The contents of the cylinder are allowed to sit undisturbed for 20 seconds, after which a 25 mL aliquot is withdrawn with a calibrated pipette from a depth of 20 cm. The 25 mL aliquot is placed in a labeled pre-weighed 50 mL beaker. The pipette is washed with deionized water and the wash is added to the sample beaker. This first aliquot represents 1/40 of the total fine sediment (silt/clay) in the sample. In order to separately report the clay fraction (+8 phi and smaller) and the silt fraction (+5 phi to +7phi), a second 25 mL aliquot is withdrawn with a calibrated pipette from a depth of 10 cm at a time interval determined by the temperature of the liquid in the cylinders (typically around 2 hours). The aliquot is placed into a second labeled pre-weighed 50mL beaker. The pipette is washed with deionized water and the wash is added to the sample beaker. This second aliquot represents 1/40 of the total clay fraction in the sample. The difference in the dry weights of the first aliquot (silt/clay) and the second aliquot (clay) represents 1/40 of the total silt fraction in the sample. All beakers are placed into an oven at a temperature between 70 °C and 90 °C to dry. Once samples are dry they are transferred to a desiccator. Samples are allowed to cool and are then weighed.

4.0 CALCULATIONS

4.1 SAMPLE WEIGHT

The weight of each fraction is calculated using the following equation:

$$\text{Dry Wt.} = \left(\text{Vessel Wt.} + \text{Dry Wt.}_{\text{sample}}\right) - \left(\text{Vessel Wt.}\right)$$

4.2 PIPETTE CALIBRATION FACTOR

A pipette calibration factor is determined for each analyst to minimize weighing errors associated with imprecise pipetting of sample aliquots. A 25 mL volumetric pipette is

filled with deionized water, emptied into a clean pre-weighed 50 mL beaker and weighed, this represents 1/40 of the total volume in a 1,000 mL graduated cylinder. This process is repeated nine more times. The mean water weight is determined and the following equation is used to calculate the pipette calibration factor for each analyst.

$$\text{Pipette Calibration Factor} = (40)\frac{\text{Mean Water Wt.}}{25}$$

5.0 QUALITY CONTROL (QC)

A duplicate analysis is performed for every 20 samples analyzed with a quality control (QC) batch. A second aliquot of a sample is processed identical to the original sample. 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 the duplicate samples, corrective action is taken which may result in re-processing all samples in the QC batch.

The RPD for each duplicate is calculated using the following equation:

$$\text{RPD} = \frac{\left|\left(\text{Particle Size Wt.}_{\text{sample 1}} - \text{Particle Size Wt.}_{\text{sample 2}}\right)\right|}{\left(\text{Particle Size Wt.}_{\text{sample1}} + \text{Particle Size Wt.}_{\text{sample2}}\right)(0.5)} \text{x100}$$

CHAPTER 4. DETERMINATION OF TOTAL CARBON, TOTAL ORGANIC CARBON AND INORGANIC CARBON IN SEDIMENT

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ABSTRACT

Total carbon content is determined in dried sediments and total organic carbon is determined in dried and acidified sediments using a LECO CR-412 Carbon Analyzer. Sediment is combusted in an oxygen atmosphere and any carbon present is converted to CO_2. The sample gas flows into a non-dispersive infrared (NDIR) detection cell. The NDIR detection cells measures the mass of CO_2 present and the mass of the CO_2 is measured relative to a calibration curve. The mass is converted to percent carbon based on the dry sample weight. The total organic carbon content is subtracted from the total carbon content to determine the total inorganic carbon content of a given sample.

1.0 INTRODUCTION

Total carbon (TC) includes both organic and inorganic sample constituents. Total organic carbon (TOC) is determined by treating an aliquot of dried sample with sufficient phosphoric acid (1:1) to remove inorganic carbon prior to instrument analysis. Percent TOC and TC are determined in sediments dried at 105°C using a LECO CR-412 Carbon Analyzer. Prepared sediments are combusted at 1,350°C in an oxygen atmosphere using a LECO CR-412. Carbon is oxidized to form CO_2. The gaseous phase flows through two scrubber tubes. The first scrubber tube is packed with Drierite® ($CaSO_4$) and copper granules to trap water and chlorine gas; the second scrubber tube is packed with Anhydrone® ($Mg(ClO_4)_2$) to remove residual moisture. The gaseous phase then flows through a non-dispersive infrared (NDIR) detection cell tuned to selectively respond to CO_2. The integrated area under the signal detected is proportional to the amount of CO_2 passing through the NDIR cell. The weight-corrected result is percent carbon (% C).

2.0 APPARATUS AND MATERIALS

2.1 EQUIPMENT

- Conditioning oven, electric, gravity convection, capable of maintaining a stable temperature of up to 200°C

- Combustion furnace, electric, capable of combusting glassware at 400°C for at lease 4 hours

- LECO CR-412 Carbon Analyzer, IR detector and 36 position autosampler rack
- Glazed and unglazed combustion boats
- Analytical balance, capable of weighing to 1 mg
- Calibrated weights, certified
- Glass measuring scoop
- Mortar and pestle
- Aluminum weighing boats
- Forceps
- Glass wool

2.2 REAGENTS

- Phosphoric acid (CAS 7664-38-2), 1:1 v/v
- Anhydrone® (CAS 10034-81-8), magnesium perchlorate
- Drierite® (7778-18-19), calcium sulfate
- Granular copper (CAS 7440-50-8), 20-30 mesh
- Oxygen (CAS 7782-44-7), 99% purity

3.0 PROCEDURE

All glassware and ceramic ware used in sample processing are combusted at 400°C for at least 4 hours. Samples remain frozen at –20°C until processing. Sediment samples are thawed and homogenized. The sample is dried in an oven at 40°C. A portion of sample is removed, ground and homogenized using a mortar and pestle. An aliquot of dried, homogenized sample is placed in an aluminum-weighing pan and is re-dried at 105°C.

The LECO CR-412 Carbon Analyzer is calibrated prior to the analysis of samples. Different amounts of high purity calcium carbonate standard (99.95% purity, carbon content of 12.0%) are used to calibrate the instrument. The approximate amounts of calcium carbonate used for the six-point calibration are; 0.01 g, 0.05 g, 0.10 g, 0.25 g and 0.50 g. An empty carbon-free combustion boat is analyzed as a blank for the calibration curve. The calibration curve provides an analysis range of approximately 0.0 to 0.06 g total carbon. Each calibration standard must fall within 3% of the known percent carbon value to meet acceptance criteria. A continuing calibration check standard (mid-level standard) is analyzed every ten samples and must be within 5% of the known value of the standard.

Total carbon (TC) is analyzed by placing approximately 0.35 g of dried, ground and homogenized sample into a clean, carbon-free combustion boat. The sample boat is placed on the autosampler rack assembly and loaded onto the LECO carbon analyzer.

Total organic carbon (TOC) is analyzed by placing approximately 0.35 g of dried, ground and homogenized sample into a clean, carbon-free combustion boat. Each sample boat is treated with phosphoric acid drop by drop until the sample stops "bubbling" and the sample is completely moist with acid. The sample is placed into an oven set at 40°C for 24 hours and then transferred to an oven set at 105°C. Once the sample is dry, the boat is placed on the autosampler rack assembly and loaded onto the LECO carbon analyzer.

4.0 QUALITY CONTROL (QC)

Lot tests are performed on all reagents used to verify that they are contaminant free. All equipment and glassware used to analyze samples are either purchased as certified clean or combusted at 400°C for a minimum of 4 hours. The calibration and accuracy of balances, weights, pipettors and thermometers are checked daily using NIST-certified calibration materials. The calibration and accuracy of balances, weight, pipettors and thermometers are verified yearly by an independent source. A series of quality control samples are processed with each batch of 20 samples or less. The following quality controls are used to ensure the accuracy and precision of data.

Method Blank. Method blanks are clean, carbon-free combustion boats. A method blank is analyzed with each batch of 20 or fewer samples. The method blank is analyzed in a manner identical to samples.

Laboratory Duplicates. A sample is analyzed in duplicate with each analytical batch of 20 or fewer samples.

Standard Reference Material (SRM). A SRM from the National Institute of Standards and Technology (NIST) containing a certified concentration of carbon (8704) is analyzed with each analytical batch of 20 or fewer samples. SRMs are analyzed in a manner identical to samples, except they are not acidified for TOC analysis. The SRM is only certified for TC and is not certified for TOC. However, an acidified SRM is analyzed in each TOC analytical batch and used as a laboratory control sample (LCS).

5.0 CALCULATIONS

5.1 CARBON CONTENT

$$\text{Carbon (g)} = (b)(A) + a$$

Where:

b = the slope of the linear calibration curve (g per unit area)

A = the area under the sample curve

a = the intercept of the calibration curve (g)

Note: When samples have been acidified, Organic Carbon (g), replaces Carbon (g) in the above equation.

5.2 PERCENT TOTAL CARBON (TC)

$$\text{TC (\%)} = \frac{\text{Carbon (g)}}{\text{W (g)}}$$

Where:

W (g) = dry sediment analysis weight (g)

5.3 PERCENT TOTAL ORGANIC CARBON CONTENT (TOC)

$$\text{TOC (\%)} = \frac{\text{Organic Carbon (g)}}{\text{W (g)}}$$

5.4 PERCENT TOTAL INORGANIC CARBON CONTENT (TIC)

$$\text{TIC (\%)} = \text{TC (\%)} - \text{TOC (\%)}$$

To express TIC as a percent calcium carbonate ($CaCO_3$), use the following equation.

$$CaCO_3\ (\%) = (\text{TC} - \text{TOC}) \times 8.33$$

5.5 DUPLICATE SAMPLE ANALYSES

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

CHAPTER 5. DETERMINATION OF PERCENT LIPID IN TISSUE

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ABSTRACT

Tissue percent lipid is determined by weighing an 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 µL 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 weight of the aliquot, extract volume, and sample weight.

1.0 INTRODUCTION

Aliquots of approximately 15 g of wet tissue are dried with Hydromatix®. The tissue/ Hydromatix® mixtures are extracted with 100% dichloromethane using a Dionex Accelerated Solvent Extractor (ASE200) operated at 100°C and 2,000 psi. Extracts are reduced to 3 mL by evaporative solvent reduction in a water bath at 55 - 58°C. A 100 µL aliquot 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 weighing to 1 µg
- 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 pre-cleaned vials with open screw caps and Teflon-lined septa
- Kurderna-Danish (K-D) tubes, 25 mL, slow dry concentrator tubes
- Synder columns, 3 ball
- Boiling chips, Teflon
- Micropipettors, calibrated to 100 µL, 1% accuracy, disposable tips

2.2 REAGENTS

- Acetone (CAS 67-64-11), pesticide grade or equivalent purity
- Dichloromethane (CAS 75-09-2), pesticide grade or equivalent purity
- Hydromatrix® (CAS 68855-54-9/14464-46-1), conditioned by combustion at 400°C for at least 4 hours and stored at 120°C
- Nitrogen (7727-37-9), 99.8% purity

3.0 PROCEDURE

Bivalves are shucked and the soft tissue homogenized using a stainless steel Waring® blender. The samples are homogenized in a Waring Industrial Blender. Homogenized tissue samples are frozen at –20°C until extraction. Prior to extraction, tissue samples are thawed and re-homogenized using a stainless steel spatula (see Extraction of Biological Tissues for Aromatic and Chlorinated Hydrocarbons and Polybrominated Flame Retardants).

Approximately 15 g of tissue are thoroughly mixed and ground with a sufficient quantity (approximately 40 g) of prepared (combusted) Hydromatrix® to “dry” the sample. The tissue samples must be thoroughly dry to optimize the extraction efficiency. Hydromatrix® chemically dries samples by binding moisture. The amount of Hydromatrix® necessary to dry a sample depends upon the amount of sample and the percent moisture in that sample.

Tissues are extracted with dichloromethane using an ASE200. The tissue/Hydromatix® mixture is loaded into 33 mL ASE extraction cells. Appropriate surrogate and spikes are added to the top of the samples. The ASE extractor tubes are sealed and place in the ASE cell carousel. The ASE conditions are: 100% dichloromethane as the extraction solvent; 2,000-psi solvent pressure; 100°C cell temperature; and 2 static cycles for 2 minutes each. Extracts are collected in 60 mL collection vials. The extracts are reduced to approximately 10 mL in the 60 mL collection vials in a 55-60°C water bath. Extracts are then quantitatively transferred to Kurderna-Danish (K-D) tubes and the volume reduced to 3 mL in a 55-60°C water bath. A 100 µL aliquot is removed and weighed on a pre-

dried, tared 2.4 cm glass-fiber filter to determine lipid content. The filter and sample are dried in a 40°C oven to a constant weight. The filter and sample were weighed to the nearest 0.001 mg. Quality control (QC) samples (e.g., blanks and duplicates) are prepared and extracted in the same manner as samples.

4.0 QUALITY CONTROL (QC)

Solvents are verified to be contaminant-free by lot tests prior to use. All equipment and glassware used to extract samples are thoroughly cleaned by solvent rinsing or combustion at 400°C. The calibration and accuracy of balances, weights, pipettors and thermometers are checked daily using certified weights and thermometers with calibrations traced to the National Institute of Standards and Testing Technology (NIST). The calibration and accuracy of balances, weight, pipettors and thermometers are verified yearly by an independent source. A series of QC samples are processed with each batch of 20 samples or less. The following quality controls are used to ensure the accuracy and precision of tissue data.

Duplicate

- The RPD between the duplicate and original sample is ≤ 25%.
- If this criterion is not met after re-weighing, corrective action may result in re-processing all samples in the QC batch.

5.0 CALCULATIONS

5.1 PERCENT LIPID WEIGHT

$$\%\,\text{Lipid} = \left(\frac{(Wr)(Vf)}{(Ws)(Va)}\right) x \left(\frac{1g}{1{,}000\,mg}\right) x\,100$$

Where:

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

Ws = sample weight (g)

Vf = final volume of sample extract

Va = volume of extract aliquot used for lipid determination

United States Department of Commerce

Carlos M. Gutierrez
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