

EXTRACTION OF BIOLOGICAL TISSUES FOR AROMATIC AND CHLORINATED HYDROCARBONS

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ABSTRACT

Determining organic contaminant levels in tissues requires extraction, isolation and concentration from the matrix. Tissue extracts require extensive clean-up procedures in order to remove lipids that may cause severe analytical interferences. Bivalves are shucked and homogenized. An aliquot of homogenized sample is chemically dried using Hydromatrix® and extracted in dichloromethane using an automated a Dionex ASE200 Accelerated Extractor. The extracts are concentrated and then purified using alumina/silica gel chromatography columns. The resultant eluent is concentrated to 4 mL and further purified using a gel permeation column coupled to a high performance liquid chromatograph. The resultant eluent is concentrated to a final volume of 0.5 mL and submitted for the determination of aromatic and chlorinated hydrocarbons by gas chromatography/mass spectrometry (GC/MS) and gas chromatography/electron capture detection.

1.0 INTRODUCTION

The procedure described is capable of extracting, isolating, purifying and concentrating aromatic and chlorinated hydrocarbon contaminants at the parts per billion or parts per trillion level in lipid rich biological tissues. Mollusk samples are processed so that the soft tissue is removed from the shell. Numerous organisms are processed as one sample in order to have sufficient sample to complete the analyses. Tissue samples are typically homogenized using a stainless steel blender outfitted with titanium blades. Once the samples are thoroughly homogenized, an aliquot of 1-2 g is removed for % moisture determination. Aliquots of approximately 15 g of wet tissue are chemically dried with Hydromatrix®. These dry samples are extracted with 100% dichloromethane using a Dionex ASE200 Accelerated Solvent Extractor operated at 100°C and 2,000 psi. The extracts are concentrated to 3 mL by evaporative solvent reduction in a 55-60°C water bath. A 100 µL aliquot is removed and weighed to determine percent lipid weight. The remaining sample portion is purified using alumina/silica gel column chromatography and gel permeation column (GPC)/high performance liquid chromatography (HPLC). After HPLC, the extracts are concentrated to 0.5 mL and submitted for instrumental analyses.

2.0 APPARATUS AND MATERIALS

2.1 EQUIPMENT

- Dionex, ASE200 Accelerated Solvent Extractor (ASE) with 33 mL extraction cells
- Water bath, capable of maintaining a temperature of 55-60°C
- Balance, top loading, tare capacity to 300 g, capable of weighing to 1 mg
- Microbalance, capable of weight 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
- Conditioning oven, electric, gravity convection, capable of maintaining a stable temperature of up to 200°C
- Tumbler, Lortone rock tumbler or equivalent
- HPLC system, Water Model 590 programmable solvent delivery module HPLC pump, Waters 717 plus autosampler, Waters UV absorbance detector, Waters 746 data module, Waters Fraction Collector, Phenogel 10µ GPC 100A size exclusion columns and Phenogel 100A guard column.
- Glass fiber filter circles, 2.4 cm diameter
- Collection vials, 60 mL certified pre-cleaned with open screw caps and Teflon lined VOA septa
- Micropipettors, calibrated, 1% accuracy, disposable tips
- Zymark®, 50 mL concentration tubes
- 250 mL flat bottom, boiling flasks
- Borosilicate glass chromatography columns, 300 mm x 19 mm, with Teflon stopcock
- Kurderna-Danish (K-D) tubes, 25 mL, slow dry concentrator tubes
- Synder columns, 3 ball
- Boiling chips, Teflon
- Glass wool

2.2 REAGENTS

- Water, gas chromatography/HPLC grade or equivalent purity
- Acetone, pesticide grade or equivalent purity
- Dichloromethane, pesticide grade or equivalent
- Hexane, pesticide grade or equivalent
- Pentane, pesticide grade or equivalent
- Hydromatrix®, conditioned by combustion at 400°C for at least 4 hours and stored at 120°C

- Sodium sulfate, purified by combusting at 400°C for at least 4 hours and stored at 120°C.
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- Alumina, 80-325 mesh, basic, purified by combusting at 400°C for at least 4 hours and stored at 120°C
- Silica gel, grade 923, 100-200 mesh, purified in an oven at 170°C for at least 16 hours and store at 170°C
- Nitrogen, 99.8% purity

3.0 PROCEDURE

All glassware used to extract tissues is washed, solvent rinsed or combusted at 400°C for at least 4 hours. All other items that come in contact with samples are washed and solvent rinsed with acetone and dichloromethane. Tissue samples are thawed and re-homogenized. A portion is removed for percent moisture determination (see Dry Weight Determination of Tissues). Approximately 15 g of tissue is thoroughly mixed and ground with a sufficient quantity of prepared Hydromatrix® (approximately 40 g) 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 automated extraction device ASE200. Tissue/Hydromatrix® mixtures are loaded into 33 mL ASE extraction cells. Appropriate surrogate and spikes are added on top of the samples. Quality Control samples (e.g., blanks, duplicates, matrix spikes and standard reference materials) are prepared in the same manner as samples. The ASE extractor tubes are sealed and place on 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 concentrated 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 concentrated to 3 mL in a 55-60°C water bath. A 100 µL aliquot is removed and weighed to determine lipid content (see Determination of Percent Lipid in Tissue).

Extracts are initially purified using alumina/silica gel chromatography columns. Borosilicate glass columns (300 mm x 19 mm) are packed with conditioned sodium sulfate, alumina and silica gel. Combusted and cooled alumina is deactivated by adding 1% (w/w) reagent water and tumbled for at least 1 hour using a Lortone rock tumbler. Combusted and cooled silica gel is deactivated by adding 5% (w/w) reagent water and tumbling for at least 1 hour using a Lortone rock tumbler. The columns are packed in dichloromethane with glass wool, 1-2 g of cooled sodium sulfate, 10 g of deactivated alumina, 20 g of deactivated silica gel and another 1-2 g of sodium sulfate. The

dichloromethane is drained to the top of the column and replaced with 50 mL of pentane. The pentane is drained to the top of the upper sodium sulfate layer and discarded. The sample extract (approximately 3 mL) is loaded on top of the column and flushed with 200 mL of a 50:50 mixture of pentane and dichloromethane at a flow rate of 1 mL/min. The eluent is collected in a 250 mL flat-bottom flask. The eluent is concentrated to approximately 10 mL in a 55-60°C water bath. The extract is transferred to 25 mL K-D tubes and concentrated to 1-2 mL. The concentrate is transferred to 4 mL amber HPLC vials and brought up to 4 mL with dichloromethane.

The extract is further purified using HPLC. The extract is injected using a Waters, Model 717 plus, autosampler and eluted through two Phenogel 10 μ GPC 100A size exclusion columns with 100% dichloromethane, at a flow rate of 7 mL per minute. Elution times for compounds of interest are monitored using standards and an UV absorbance detector (254 nm). The appropriate fraction is collected using a Waters Fraction Collector. The sample is collected in 50 mL Zymark tubes and concentrated to 10 mL in a 50-60°C water bath. The extract is transfer to K-D tubes and concentrated. The dichloromethane is exchanged to hexane and concentrated to a final volume of 0.5 mL. The concentrate is transferred to 2 mL amber vials and stored at -20°C until analysis. Figure 1 shows a flow chart of the extraction and purification procedure.

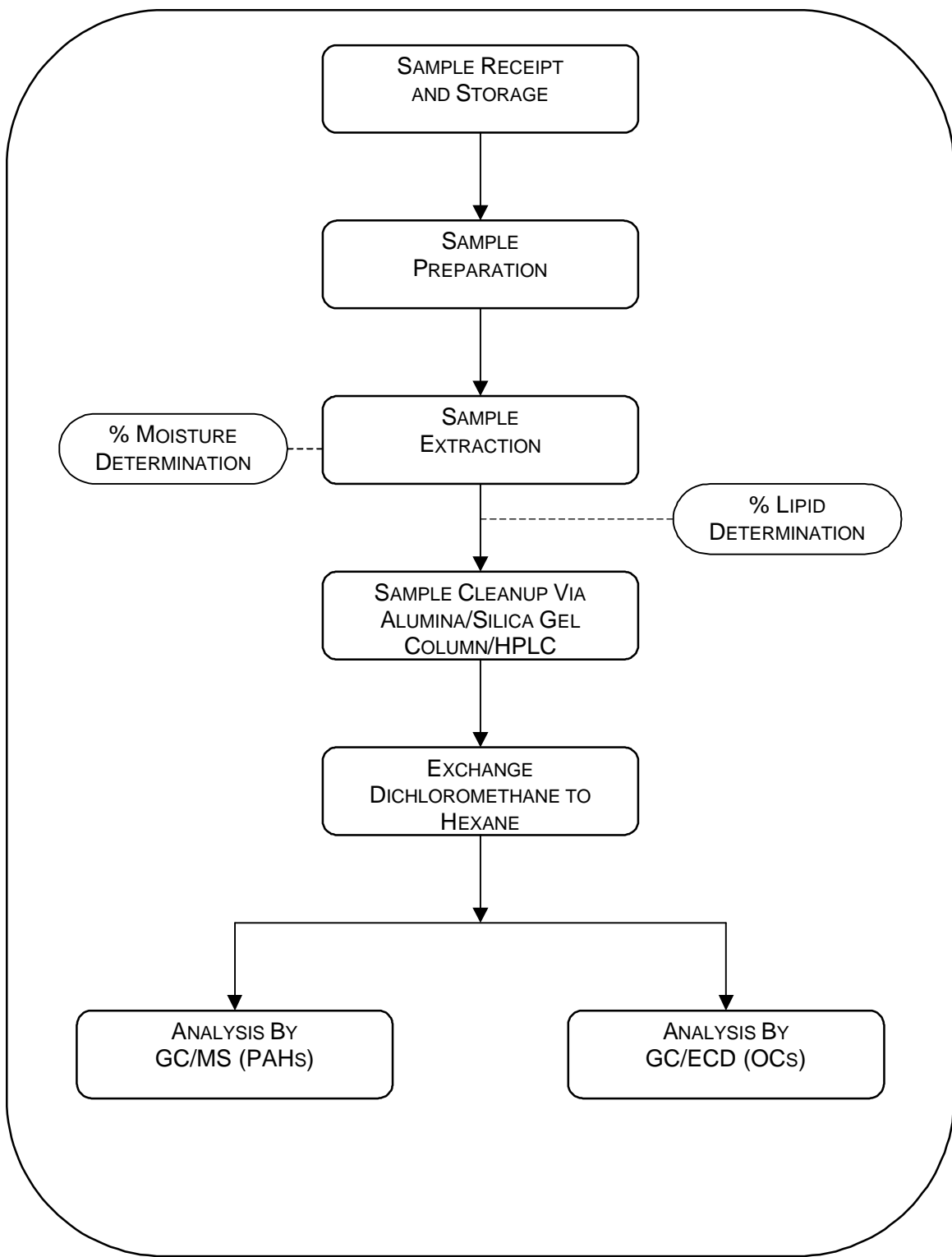


Figure 1. Methodology for Extraction, Isolation and Quantification of Tissue Samples for Aromatic and Chlorinated Hydrocarbons.

4.0 QUALITY CONTROL (QC)

All reagents used are verified to be contaminant free. 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. The calibration and accuracy of balances, weight, pipettors and thermometers are verified yearly by an independent source. All samples are shipped and received under chain-of-custody. 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 tissue data.

- **Surrogates.** Solutions containing analytes that do not interfere with the analytes of interest are prepared at concentrations approximately 5 to 10 times the method detection limit (MDL). Specified surrogates are added to each sample extracted, including QC samples, at a specified volume (typically 100 µL) immediately prior to extraction.
- **Method Blank.** Method blanks are extraction of all support material used for extraction of samples, with the exception of tissue. A method blank is analyzed with each extraction batch of 20 or fewer samples. The method blank is extracted and analyzed in a manner identical to samples.
- **Matrix Spike.** Matrix spikes are extractions of sample matrix fortified with spikes of selected target analytes. A matrix spike and matrix spike duplicate are analyzed with each extraction batch of 20 or fewer samples. Matrix spikes are extracted and analyzed in a manner identical to samples.
- **Laboratory Duplicates.** A sample is analyzed in duplicate with each extraction batch of 20 or fewer samples.
- **Standard Reference Material.** A standard reference material from the National Institute of Standards and Technology (NIST) is analyzed with each extraction batch of 20 or fewer samples.