

EXTRACTION OF BIOLOGICAL TISSUES FOR BUTYLTINS

Juan A. Ramirez, Donell S Frank, Susanne J. McDonald, and James M. Brooks
TDI-Brooks International/B&B Laboratories Inc.
College Station, Texas 77845

ABSTRACT

Determining organic contaminant levels in tissues requires extraction, isolation and concentration from the matrix. Bivalves are shucked and homogenized. An aliquot of homogenized sample is chemically dried using Hydromatrix® and extracted in 0.1% tropolone/ hexane or 0.1% tropolone/dichloromethane mixture with a tissuemizer. The extracts are concentrated to 10 mL and pentylated using pentylmagnesium bromide (Grignard reagent), followed by neutralization with hydrochloric acid. The organic fraction is concentrated and then purified using silica gel/florisil chromatography columns. The resultant eluent is concentrated to a final volume of 10 mL and submitted for the determination of butyltins by gas chromatography/flame photometric detection.

1.0 INTRODUCTION

The procedure described is capable of extracting, isolating, purifying and concentrating butyltin contaminants at the parts per billion level from lipid rich biological tissues. Mollusk samples are processed so that the soft tissue is removed from the shell. Numerous organisms are processed for each sample in order to have sufficient sample to complete the analyses. Tissue samples are homogenized using a Tekmar® tissumizer. Once the samples are thoroughly homogenized, an aliquot of 1-2 g is removed for dry weight determination. Aliquotst of approximately 10 g of wet tissue are chemically died with Hydromatrix®. The samples are extracted in 60 mL of 0.1% tropolone in either hexane or dichloromethane using a tissumizer. The liquid is decanted and the extraction procedure is repeated twice more. The combined extracts are concentrated to approximately 10 mL. The samples are then pentylated using pentyl magnesium bromide (Grignard reagent) and shaken for 1 hour after which they are neutralized with hydrochloric acid (HCl). The organic layer is drawn off and concentrated to 2 – 4 mL. Samples are purified using silica gel/florisil columns and hexane. The eluent is concentrated to 10 mL, from which 2 mL is prepared for analysis by gas chromatography/flame photometric detection (GC/FPD).

2.0 APPARATUS AND MATERIALS

2.1 EQUIPMENT

- Solvent reduction apparatus, Zymark TurboVap LV concentration workstation
- Tisumizer, Tekmar®, with stainless steel probes
- 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
- Concentration tubes, Zymark® 60 mL borosilicate glass
- Concentration tubes, 60 mL certified pre-cleaned VOA tubes with open screw caps and teflon lined VOA septa.
- Micropipettors, calibrated to 100 µL, 1% accuracy, disposable tips
- Centrifuge bottles, 200 mL
- Erlenmeyer flasks, 250 mL
- 2 mL amber extract vials with teflon-lined screw caps
- Glass wool

2.2 REAGENTS

- Silica gel/Florisil columns, Resteck, 16 g florisil and 5 g silica gel
- Dichloromethane, pesticide grade or equivalent purity
- Hexane, pesticide grade or equivalent purity
- Hydrochloric acid, Tracepur® Plus or equivalent purity
- Hydromatrix®, conditioned by combustion at 400°C for at least 4 hours and stored at 120°C
- Copper, granular
- Hydrochloric acid
- Topolone, 98% purity
- Grignard reagent, pentylmagnesium bromide
- Hydrochloric acid
- 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 tissues 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 10 g of tissue is thoroughly mixed with a sufficient quantity of prepared Hydromatrix® (10 to 20 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 tissue and percent moisture in that tissue.

An aliquot of wet tissue and Hydromatrix® is weighed into a 200 mL centrifuge bottle. Appropriate surrogates and spikes are added to the centrifuge bottle prior to extraction. Quality control samples (e.g., blanks, duplicate, matrix spikes) are prepared in the same manner as samples. The samples are extracted in 60 mL of 0.1% tropolone, in hexane or dichloromethane, using a Tekmar® tissumizer. The samples are macerated for 2 min. The liquid is decanted into a 250 mL Erlenmeyer flask. The extraction is repeated two more times.

The combined extracts are concentrated to 10 mL using a Zymark TurboVap concentration station set at 40°C and 15 psi. An aliquot of extract (between 20 and 45 mL) is added to a 60 mL VOA tube and concentrated. Additional aliquots are added to the tube as the extract volume decreases and concentrates, until the entire sample extract has been concentrated to approximately 10 mL. Samples extracted in dichloromethane are back extracted into hexane. The concentrated extract is quantitatively transferred to a clean 60 mL concentration tube and reduced to 10 mL. The sample extracts are then pentylated by adding 1 mL of pentylmagnesium bromide (Grignard reagent). The sample headspace is purged using nitrogen and then the tubes are capped and shaken for 1 hour on a shaker table. After shaking, the Grignard reagent is neutralized by adding 40 mL of 10% HCl to each tube, in an ice bath. The tubes are shaken and the upper organic layer is transferred to a 50 mL Zymark concentration tube. The acid fraction is rinsed twice more using 10 mL of hexane, each time transferring the organic layer to the concentration tube. The extract is then concentrated to 2 – 4 mL using a Zymark TurboVap LV concentrator station set at 40°C and 15 psi.

The concentrated extract is purified using silica gel/florisil chromatography columns. The chromatography columns contain 16 g of florisil and 5 g silica gel and are conditioned by flushing with 30 mL of hexane. The sample and solvent rinses are drained to the top of column and 125 mL of hexane is added and eluted until the column is dry. The eluent is collected in 250 mL Erlenmeyer flasks. The eluent is quantitatively transferred to a Zymark concentration tube and concentrated to a final volume of 10 mL. Approximately

2 mL of each extract is transferred to a clean 2 mL amber extract vial to which the appropriate internal standard is added in preparation for instrument analysis.

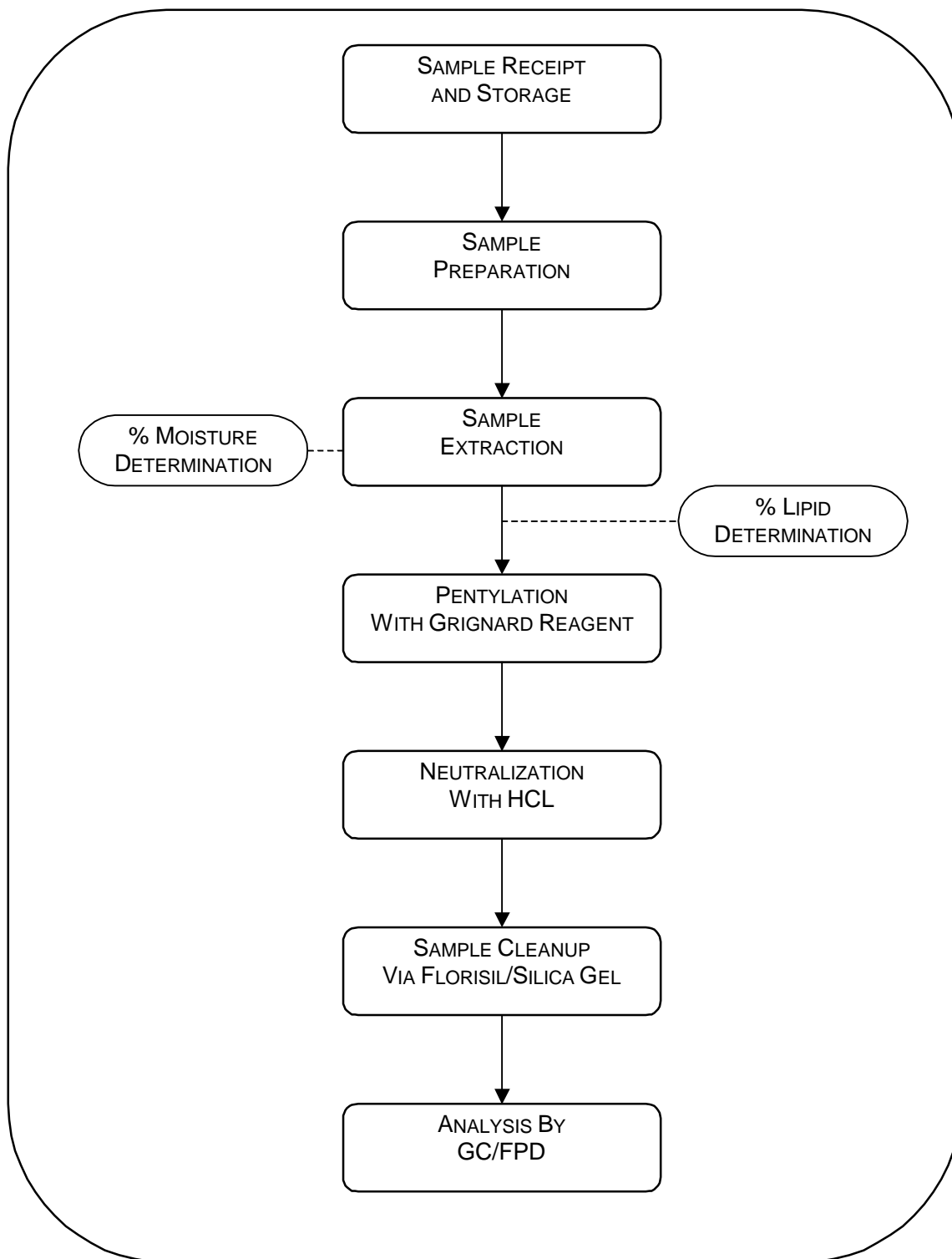


Figure 1. Methodology for Extraction, Isolation and Quantification of Tissue Samples for Butyltins.

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 extractions 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.