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Ultrasonic Extraction by EPA Method 3550B

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1 Scope and Application

1.1 This procedure is for the extraction of pesticides and PCBs from biological tissue and the extraction of chlorinated herbicides from soil and waste.. This process ensures intimate contact of the sample matrix with the extraction solvent.

2 Definitions –

2.1 Refer to Chapter 3 of the Georgia EPD Laboratory Quality Assurance Manual for Quality Control Definitions.

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3 Interferences

3.1 All glassware is to be meticulously cleaned and solvent rinsed before it is to be used for this procedure. Refer to SOP “Glassware Maintenance”.

4 Safety –

4.1 Refer to Laboratory Chemical Hygiene Plan, online revision.

5 Apparatus and Equipment

5.1 Ultrasonic disrupter: minimum power wattage of 300 watts, with pulsing capability.
5.1.1 Horn: a ½” horn for low concentration method and a ¾” horn for medium/high concentration method.
5.2 Pasteur pipets: 1ml, disposable
5.3 Beakers: 250ml and 400ml
5.4 Buchner funnel: 126mm diam
5.5 Filtration Flask: 500ml, with vacuum side arm
5.6 Glass fiber filter: Whatman No. 934AH or equivalent, 11cm in diameter
5.7 Kuderna-Danish apparatus:
5.7.1 Concentrator tube: 10ml, jacketed
5.7.2 Evaporation flask: 500ml
5.7.3 Snyder column: 250mm, three-ball macro
5.8 Solvent recovery system
5.8.1 Inverted Hopkins condenser, 375mm
5.8.2 Flat bottom flask: 500ml

5.9 Boiling chips
5.10 Water bath: capable of temperature control. Used inside a safety hood.
5.11 High capacity balance: capable of accurately weighing 0-180g ±0.01 grams
5.12 Analytical balance: capable of accurately weighing to the nearest 0.001 grams
5.13 Test tubes-13mm and 16mm, with PTFE-lined screw caps
5.14 Spatula: stainless steel or PTFE
5.15 Nitrogen blow-down rapid evaporation system, such as RapidVap or equivalent (optional, in lieu of K-D apparatus)

6 Reagents

6.1 Methylene chloride: pesticide grade

7 Sample Collection –

7.1 Refer to Chapter 5 of the Georgia EPD Laboratory Quality Assurance Manual for Sample Container, Sample Preservation, and Sample Holding Times.

8 Calibration

8.1 Analytical balances are serviced and calibrated once per year by qualified, independent technician.. Daily readings with certified weights are taken early each morning to insure calibration. A daily log is maintained with this information.

9 Quality Control

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9.1 Refer to Table 13.5.1 for Reporting Limits (RLs), Table 13.5.2 for Quality Control Acceptance Criteria, and Table 13.5.3 of the Georgia EPD Laboratory Quality Assurance Manual for Quality Control Procedures associated with this method.
9.2 Proper performance of the extraction is validated when quality control criteria for laboratory control samples (LCS/LCSD) are met for the analytical method being used.
9.3 The analyst should perform an Initial Demonstration of Capability before applying this method to actual samples.

10 Procedure

10.1 Ultrasonic extraction is not as rigorous a method as others. Thus, it is critical that the method is followed explicitly to achieve maximum efficiency. The use of this technique requires that:
10.1.1 The extraction device must have a minimum of 300 watts of power and equipped with the proper size horns.
10.1.2 The horn must be properly maintained.
10.1.3 Samples must be properly mixed with sodium sulfate so that it forms a free-flowing powder before the addition of solvent.
10.1.4 Extractions are performed with methylene chloride in the appropriate pulse mode (50%) and with the horn tip positioned just below the surface of the solvent yet above the sample.
10.1.5 Very active mixing of the sample and the solvent must occur when the ultrasonic pulse is activated. The analyst must observe such mixing during the extraction process.
10.2 Sample Handling:
10.2.1 Follow SOP “Biological Tissue Preparation” for the handling and preparation of

samples prior to extraction.

10.3 Extraction procedure:

10.3.1 Using a high capacity balance, weigh out 30 grams of the sample composite in a 250ml beaker (this composite represents 10gm of tissue and 20gm of sodium sulfate). Record the weight to the nearest 0.1 gram.

10.3.2 Add the appropriate surrogates to all samples, Blank, LCS/LCSD and MS/MSD.

10.3.2.1 For the Blank, weigh out 30gm of Sodium Sulfate powder. Spike with surrogate spike.

10.3.2.2 For the sample selected for the MS/MSD, spike it with the appropriate amount of matrix spike solution.

10.3.2.3 For LCS/LCS Duplicate, weigh out 30gm of Sodium Sulfate powder and add the appropriate amount of matrix spike solution.

10.3.3 Add 100 ml of methylene chloride to the beaker with sample.

10.3.4 Place the bottom surface of the tip ½-1 inch below the surface of the solvent, but above the sample layer. *Note: The tip must not come in contact with the sample matrix.*

10.3.5 Extract for 3 minutes at 50% pulsation.

10.3.6 Filter the extract through the glass fiber filter in a Buchner funnel that is attached to a clean 500ml filtration flask.

10.3.7 Repeat the extraction two more times with two additional 100ml portions of methylene chloride, filtering the extract after each sonication.

10.3.8 On the final extraction, pour the entire sample into the Buchner funnel and rinse with extraction solvent.

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10.3.8.1 Rinse the probe with methylene chloride to clean the probe between each sample, to avoid contamination.

10.3.8.2 Discard the extracted sample and keep the extract.

10.3.9 Concentrate extracts by K-D apparatus (optional, if not using Nitrogen blow-down rapid evaporation system

10.3.9.1 Assemble K-D apparatus by attaching a 10-ml jacketed concentrator tube to a 500-ml evaporator flask.

10.3.9.2 Transfer filtered extract to the K-D apparatus.

10.3.9.3 Add boiling chips.

10.3.9.4 Attach a three-ball macro snyder column.

10.3.9.5 Place the K-D on a hot water bath (70-80EC) and concentrate to a final volume of 10ml in methylene chloride, transferring to a 16mm test tube. If needed, adjust the volume with methylene chloride.

10.3.10 Concentrate extracts by Nitrogen blow-down rapid evaporation system (optional, if not using K-D apparatus), to a final volume of 10 ml.

10.3.11 Pipet out 1ml of sample extract into a previously weighed 13mm test tube to perform %Lipids calculation (see Sec. 11.1).

10.3.12 Perform GPC cleanup to 8ml of the sample extract. Follow EPA method 3640A for GPC cleanup.

10.3.13 Concentrate GPC extracts to 2-5ml and transfer to 13mm test tubes for Florisil cleanup. Follow EPA method 3620B for Florisil cleanup.

11 Calculations

11.1 Percent Lipids Calculation: for biological tissue only

11.1.1 After sample extraction and before GPC cleanup, transfer 1ml of your sample

extract into a pre-weighed 13mm test tube and record its weight (sample extract + test tube) to the nearest 0.01gm using an analytical balance.
Note: All % Lipids related weights must be entered in the log book designated "Percent Lipids".

11.1.2 Label the test tube using the proper sample number.
11.1.3 Air dry overnight under a hood.
11.1.4 Re-weigh each test tube the next day and record this weight.
11.1.5 Calculate and record % Lipids per sample:

11.1.5.1 Equation: $\% \text{ Lipids} = \frac{(\text{weight of dry sample - test tube})}{(\text{weight of wet sample - test tube})} \times 100$

11.2 Total Lipids Calculation:
11.2.1 Calculate Total Lipids in the sample.
11.2.1.1 Equation: $\text{TL Lipids} = \frac{(\text{weight of dry sample - test tube})}{(\text{weight of wet sample - test tube})} \times 10$

12 References

EPA Methods SW846-3550B, SW846-8081A & SW846-8082

13 Practical Quantitation Limits (RLs), Precision and Accuracy Criteria, and Quality Control Approach

Does not apply to this SOP.

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