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Separatory Funnel Liquid-Liquid Extraction – Method SW846-3510C

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1 Scope and Application

1.1 Method SW846-3510C is used to determine the concentrations of various chlorinated hydrocarbon pesticides and PCBs for EPA Methods SW846-8081A, SW846-8082 and EPA 608.3 in aqueous samples and diesel range organics for SW846-8015B. The pesticide and PCB samples are extracted at neutral pH with methylene chloride then solvent exchanged with hexane. The diesel range organics remain in methylene chloride.

1.2 This method is restricted to analysts who have completed the requirements of the Initial Demonstration SOP. Refer to Section 13.1.

2 Definitions

2.1 Refer to Section 3 and Section 4 of the Georgia EPD Laboratory Quality Assurance Manual for Quality Control definitions.

2.2 Refer to GA EPD Laboratory SOP – Organics Data Validation, SOP 1-052, Rev. 0 or later.

3 Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baselines in chromatograms.

3.2 Glassware must be scrupulously cleaned with hot water and detergent followed by de-ionized water then rinsed with methanol followed by acetone.

3.3 The use of high purity reagents and solvents is absolutely necessary to minimize interference problems.

3.4 Interfering contamination may occur when a sample containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes.

3.5 Matrix interferences may be caused by containments that are co-extracted from the sample.

4 Safety

4.1 Refer to Georgia EPD Laboratory Chemical Hygiene Plan, online revision.

5 Apparatus and Equipment

5.1 Sample container: 1 Liter amber bottle with Teflon-lined caps
5.2 Vials: auto-sampler vials, clear, screw top, 2mL, caps with septa and 300µL inserts
5.3 Micro-syringes: various sizes
5.4 Syringes: various sizes
5.5 Drying column: 20-22mm ID, heavy wall glass column
5.6 Separatory Funnel: 2 Liters with PTFE or glass stopcock
5.7 Separatory Funnel shaker
5.8 Graduated cylinders (Class A): 1000mL and 100mL
5.9 Erlenmeyer flasks: 250mL – 300mL
5.10 Beakers: various sizes
5.11 Volumetric flasks (Class A): various sizes
5.12 pH indicator paper: pH 0-14
5.13 RapidVap or similar concentrator with nitrogen blow down and controlled heating capabilities
5.14 RapidVap or similar concentration tubes with at least 300mL volume
5.15 TurboVap or similar concentrator with nitrogen blow down and controlled heating capabilities
5.16 TurboVap or similar concentration tubes with at least 50mL volume
5.17 Sample extract vials: minimum 5mL & 10mL culture tubes with caps
5.18 Disposable pipettes and bulbs
5.19 Detergent: Steris Labklenz or equivalent
5.20 Glass wool: baked in muffle furnace at 450°C for 4 hours
5.21 Brushes: various sizes
5.22 Volumetric Pipet, (Class A): 1mL with squeeze bulb
5.23 Balance: Top loading, capable of accurately weighing to the nearest 0.01g
5.24 Balance: Analytical, capable of accurately weighing to the nearest 0.0001g
5.25 Clamp Stand with support rod
5.26 Clamps: ring clamps and three prong extension clamps
5.27 Aluminum foil

6 Reagents and Standards

6.1 Methylene chloride: pesticide grade or equivalent
6.2 Hexane: pesticide grade or equivalent

6.3 Acetone: pesticide grade or equivalent

6.4 Methanol: pesticide grade or equivalent

6.5 Reagent water: Purified water which does not contain any measureable quantities of target analytes or interfering compounds for each compound of interest (deionized, HPLC, Milli-Q or equivalent). Milli-Q water has a resistivity of 18 MΩ·cm or greater at 25°C and a TOC of 50µg/L or less.

6.6 Sodium sulfate: granular, anhydrous, certified ACS grade suitable for pesticide residue analysis or equivalent, baked at 450°C for 4 hours

6.7 pH adjustment solutions

6.7.1 Sulfuric Acid Solution (H_2SO_4 1:1 v/v)

6.7.1.1 Slowly add 250mL of concentrated sulfuric acid to 250mL of reagent water or DI water.

6.7.1.2 Allow to cool and transfer to a solvent rinsed, glass bottle.

6.7.1.3 Alternatively, measure 4mL reagent or DI water in a glass container and slowly add 4mL reagent grade concentrated sulfuric acid using reagent pump and mix thoroughly and allow the solution to cool.

6.7.2 Sodium Hydroxide Solution (NaOH), 10N: reagent grade

6.7.2.1 Alternatively, weigh then dissolve 200g of sodium hydroxide in reagent or DI water and dilute to 500mL final volume and mix thoroughly.

7 Sample Collection

7.1 Refer to SW846-8000B, SW846-8081A, SW846-8082, SW846-8015B and EPA 608.3 for sample collection procedures.

8 Calibration

8.1 Not Applicable

9 Quality Control

9.1 Not Applicable

10 Procedure

10.1 **For pesticide and PCBs**, create a batch consisting of a Blank and a Pesticide LCS/LCSD/MS/MSD (alternating between pesticide mixtures) and a batch consisting of a Blank and a PCB LCS/LCSD/MS/MSD and up to 20 aqueous samples. *Note: A single Blank can be used with both batches if extracted on the same day. For 608.3, only a single Blank/LCS/LCSD/MS/MSD set is necessary, alternating between all the Pesticide and PCB mixtures.* **For DRO samples**, create a batch of a Blank/LCS/LCSD/MS/MSD and up to 20 matrix samples.

10.1.1 The Blank is defined as 1000mL of reagent or DI water spiked with 1mL of surrogate solution.

10.1.2 The LCS/LCSD are each defined as 1000mL of reagent or DI water spiked with 1mL appropriate QC spike containing surrogates.

10.1.3 The MS/MSD are each defined as 1000mL of sample matrix spiked with 1mL of appropriate QC spike containing surrogates.

10.2 Remove sample bottles and standards from cold storage and allow equilibrium to room temperature prior to sample preparation, typically two hours.

10.3 Invert the sample bottles a few times to homogenize the samples and then pour each sample into individual, glass, 1000mL, Class A graduated cylinders to the 1000mL mark. Properly discard any remaining sample volume and pour the 1000mL samples back into their original 1000mL amber bottles using glass funnels if necessary to prevent spillage.

10.4 Add 1mL QC Spike to the appropriate LCS/LCSD/MS/MSD samples.

10.5 **For pesticide and PCB samples**, add 1mL Surrogate Spike to the Blank and all matrix samples. *Note: the LCS/LCSD/MS/MSDs already contain surrogates from the QC Spike and do not require additional surrogate spiking.* **For DRO samples**, add 1mL surrogate to all samples including QC.

10.6 After QC and Surrogate spiking, check the pH of each sample using a disposable pipette. The pH must be neutral at 7 (range 6.5-7.5).

10.6.1 If the pH is less than 6.5, add ~0.5-1mL of the 10N sodium hydroxide solution, cap and invert the sample to mix then re-check the pH. Repeat until the pH is within range.

10.6.2 If the pH is greater than 7.5, add ~5-10 drops of the 1:1 sulfuric acid solution, cap and invert the sample to mix then re-check the pH. Repeat until the pH is within range.

10.7 Carefully transfer each sample to a clean, pre-rinsed, glass 2 Liter separatory funnel. *Note: each separatory funnel and corresponding Erlenmyer flask and graduated cylinder must be labeled appropriately.*

10.8 Rinse each graduated cylinder with 60mL methylene chloride. Pour this into the corresponding amber sample bottle to rinse the bottle. Finally, add this methylene chloride to the corresponding separatory funnel.

10.9 Transfer the separatory funnels to the shaker and shake the samples for two minutes and allow for venting ensuring complete mixing of solvent and sample without spilling.

10.10 Close the stopcocks and return the separatory funnels to their ring clamps and allow the methylene chloride to settle, typically 10 minutes, then collect the sample extract in a 250mL or larger Erlenmyer flask.

10.11 Add 60mL of methylene chloride to each sample and shake for two minutes and collect the extract two more times. It is unnecessary to rinse the 1000mL graduated cylinder and amber bottle again after the initial rinse.

10.12 If emulsions occur after shaking, use a rinsed glass rod to break up the emulsion and/or filter the extract through baked glass wool using a glass funnel when collecting in the Erlenmeyer flask.

10.13 **To concentrate pesticide and PCB samples**, pour the extracts through a sodium sulfate drying column pre-rinsed with methylene chloride and collect the extract in a RapidVap concentration tube or equivalent of at least 300mL volume capacity.

10.13.1 Rinse the Erlenmyer flask with methylene chloride and pour through the drying column followed by an aliquot of methylene chloride to rinse any remaining sample extract out of the drying column.

10.14 Concentrate the extracts with nitrogen in a RapidVap to ~5mL at 38°C with nitrogen pressure at 4psi and shaking rate of 30 RPMs.

10.15 To exchange the methylene chloride to hexane, add ~5mL of hexane to the RapidVap tube, swirl gently to mix the solvents then concentrate again to ~5mL.

10.15.1 Repeat section 10.15 two more times, adding ~5mL of hexane and swirling each time.

10.16 Alternatively, solvent exchange may be done using a TurboVap concentrator.

10.16.1 To use the TurboVap, transfer the 5mL of the initial sample concentrate from the RapidVap tube to a TurboVap tube of 50mL capacity or equivalent. Rinse the RapidVap tube with either methylene chloride or hexane and add to the TurboVap tube.

10.16.2 Add ~5mL of hexane to the TurboVap tube and swirl gently to mix the solvents.

10.16.3 Place the TurboVap tube in a TurboVap at 38°C with nitrogen pressure at 3-4psi then concentrate to ~5mL. Manually check the solvent volume often to prevent loss of the sample extract, swirling each time.

10.16.4 Repeat sections 10.16.2 and 10.16.3 two more times, adding ~5mL of hexane and swirling each time.

10.17 After the third exchange, allow the extract to concentrate to ~5mL then transfer to a culture tube of at least 10mL capacity.

10.18 Rinse the RapidVap tube, or the TurboVap tube if used, and transfer the rinsate to the 10mL culture tube then bring the final volume up to 10mL using a pre-measured 10mL hexane culture tube model for comparison then cap the vial securely.

10.19 **To concentrate DRO samples**, follow section 10.14, concentrating to less than 5mL. The samples will remain in methylene chloride; do not solvent exchange in hexane.

10.20 After the sample has been concentrated to less than 5mL, carefully transfer the sample to a 5mL culture tube. Rinse the RapidVap tube and add the rinsate to the 5mL culture tube then bring the final volume up to 5mL using a pre-measured 5mL hexane culture tube model for comparison then cap the vial securely.

10.21 The sample extracts are now ready for dilutions, if necessary, and vialing for GC analysis. Store extracts in a refrigerator at ≤6°C, not frozen, and protected from light until ready for analysis.

11 Calculations

11.1 Not Applicable

12 Waste Management

12.1 See GA EPD Laboratory SOP-EPD Laboratory Waste Management Standard Operating Procedures, SOP6-015, online revision.

13 References

13.1 GA EPD Laboratory SOP's - Initial Demonstration of Capability SOP 6-001, online revision and/or Continuing Demonstration of Capability SOP 6-002, online revision.

13.2 GA EPD Laboratory SOP – Spike Witnessing in the Organics Laboratory, SOP 1-044, online revision.

13.3 GA EPD Laboratory SOP – SOP for Muffle Furnace Baking of Sodium Sulfate, Glass Wool, Sodium Chloride and Sand, SOP 1-051, online revision.

13.4 GA EPD Laboratory SOP – Glassware Maintenance, SOP 1-015, online revison.

13.5 EPA Method SW846-8000B – Determinative Chromatographic Separation, Rev. 2, December 1996.

13.6 EPA Method SW846-8015B – Nonhalogenated Organics Using GC/FID, Rev. 2, December 1996.

13.7 EPA Method SW846-8081A – Organochlorine Pesticides by Gas Chromatography, Rev. 1, December 1996.

13.8 EPA Method SW846-8082 – Polychlorinated Biphenyls (PCBs) By Gas Chromatography, Rev. 0, December 1996.

13.9 EPA Method 608.3 – Organochlorine Pesticides and PCBs by GC/HSD, December 2014.

13.10 EPA Method 3510C – Separatory Funnel Liquid-Liquid Extraction, Rev. 3, December 1996.

14 Reporting Limits (RLs), Precision and Accuracy Criteria, and Quality Control Approach

14.1 Refer to analytical method SOPs.

15 Associated Labworks Test Codes

15.1 Not Applicable

Updates: Updated for online revision.