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Automated Soxhlet Extraction – Method SW846-3541

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1 Scope and Application

1.1 Method SW846-3541 is used for the extraction of organic analytes in soil, sediment, sludges and wastes for methods SW846-8081A, SW846-8082, SW846-8081B and SW846-8270C. The samples are extracted in a 1:1 (v/v) solvent mix of acetone and methylene chloride. Pesticide and PCB samples are further exchanged into hexane solvent after extraction. DRO and BNA samples remain in methylene chloride.

1.2 This method is restricted to analysts who have completed the requirements of the Initial Demonstration SOP. (SOP Reference 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. (SOP Reference 13.15)

2.2 Refer to GA EPD Laboratory SOP – Organics Data Validation, SOP 1-052, online revision.(SOP Reference 13.17)

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. (SOP Reference 13.16)

5 Apparatus and Equipment

5.1 Sample container: 250mL clear, wide-mouthed jar with lid

5.2 Vials: auto-sampler vials, clear and amber, screw top, 2mL, caps with septa and 300µL inserts

5.3 Glass culture tubes: 5 & 10mL with caps

5.3 Micro-syringes: various sizes

5.4 Syringes: various sizes

5.5 Spatulas: stainless steel or aluminum

5.6 Beakers: 250mL

5.7 Volumetric flasks (Class A): various sizes

5.8 Sample extract vials: minimum 10mL culture tubes with caps

5.9 Disposable pipettes and bulbs

5.10 Detergent: Steris Labklenz or equivalent

5.11 Brushes: various sizes

5.12 Volumetric Pipet, (Class A): 1mL & 2mL with squeeze bulb

5.13 Balance: Top loading, capable of accurately weighing to the nearest 0.01g

5.14 Balance: Analytical, capable of accurately weighing to the nearest 0.0001g

5.15 Aluminum Foil

5.16 Aluminum weigh boats

5.17 Automated Soxhlet system: Gerdhardt or equivalent

5.18 Soxhlet extraction beaker, 200mL with 6-hole rack

5.19 Cellulose thimbles: Gerdhardt 33x80mm or equivalent

5.19.1 Cellulose thimbles must be solvent rinsed before use.

5.19.1.1 Place thimbles in a tumbler extraction bottle.

5.19.1.2 Cover the thimbles with a solvent mix of 1:1 v/v methylene chloride and acetone.

5.19.1.3 Cap the bottle and allow the thimbles to soak in the solvent mix for 1 hour.

5.19.1.4 After 1 hour, drain the solvent mix off and refill the tumbler extraction bottle again with the 1:1 v/v methylene chloride and acetone solvent mix and soak again for 1 hour a second time.

5.19.1.5 After 1 hour, drain the solvent mix off of the cellulose thimbles and allow the thimbles to dry on aluminum foil completely inside a fume hood, typically overnight.

5.19.1.6 Once the cellulose thimbles are dry, they are ready for use and may be re-boxed for storage until needed.

5.20 Thimble clamps and wire thimble holders

5.21 Boiling chips

5.22 Oven: Fisher Isotemp, 105°C ± 2°C

5.23 GPC instrument (Gel Permeation Chromatography): Optional

5.23.1 *Note: If GPC cleanup is used, MDL studies must be in place with the use of the GPC instrument. If the GPC is not use, MDL studies must be in place without the use of the GPC instrument. MDL studies with and without the use of the GPC instrument may be maintained concurrently. Whichever option is used most frequently must be maintained with on-going MDLs. The alternate, least used option, either with or without GPC, may have MDLs maintained at baseline level. If either one of the options is not used, MDLs are not required for that option.*

5.24 Luer-Lock syringe: 10mL

5.25 Syringe filter: Whatman 0.45µm PTFE w/GMF or equivalent

5.26 TurboVap or similar concentrator with nitrogen blow down and controlled heating capabilities

5.27 TurboVap or similar concentration tubes with at least 50mL volume

5.28 RapidVap or similar concentrator with nitrogen blow down and controlled heating capabilities

5.29 RapidVap or similar concentration tubes with at least 300mL volume

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

6.6 Sand: purified, baked at 450°C for 4 hours

7 Sample Collection

7.1 Refer to SW846-8000B, SW846-8081A, SW846-8082, SW846-8015B and SW846-8270C for sample collection procedures.

8 Calibration

8.1 Analytical balances are serviced and calibrated once per year by an independent technician. Daily readings with certified weights are taken each morning to ensure

calibration. A daily log is maintained with this information. All precision oven temperatures are measured with NIST approved thermometers and these measurements are recorded every morning in the daily temperature log.

9 Quality Control

9.1 8270C Samples are screened by GC/FID prior to transfer of custody to the GCMS laboratory. See GA EPD Laboratory SOP – Laboratory SOP for EPA Methods 625 and 8270C: Semi-Volatile Organic Compounds by Gas Chromatography/Mass Spectrophotometry (GC/MS): Capillary Column Technique, SOP 7-007, online revision for QC criteria.

10 Procedure

10.1 **Pesticide/PCB samples:** 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 sediment samples. *Note: A single Blank can be used with both batches if extracted on the same day.*

10.2 **DRO samples:** Create a batch consisting of a Blank/LCS/LCSD/MS/MSD and up to 20 matrix samples.

10.2.1 The Blank is defined as 20g of sand spiked with 1mL of surrogate solution.

10.2.2 The LCS/LCSD are each defined as 20g of sand spiked with 1mL appropriate QC spike containing surrogates.

10.2.3 The MS/MSD are each defined as 20g of sand spiked with 1mL of appropriate QC spike containing surrogates.

10.2.4 *Note: Waste samples for Pesticide/PCB/DRO analysis are weighed at 2g (± 0.1g) including waste QC samples.*

10.3 **BNA samples:** Create an extraction batch consisting of a Blank/LCS/LCSD/MS/MSD and up to 20 sediment samples.

10.3.1 The Blank is defined as 20g of sand spiked with 1mL of surrogate solution.

10.3.2 The LCS/LCSD are each defined as sand spiked with 1mL appropriate QC spike and 1mL surrogate solution.

10.3.3 The MS/MSD are each defined as 20g of sample matrix spiked with 1mL of appropriate QC spike and 1mL surrogate solution.

10.3.4 *Note: Waste samples for BNA analysis are weighed at 2g (± 0.1g) including waste QC samples.*

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

10.5 Decant any water present in the sample and discard any foreign objects such as sticks, leaves, rocks or living organisms if possible.

10.6 **For all soil samples:** Mix the sample well in the container jar using a spatula then weigh out 20g ± 0.1g of sample (or 2g ± 0.1g if waste) in a 250mL beaker including QC samples.

10.6.1 If the sample is wet (typically <85% solids), weigh out 15g ± 0.1g of sample.

10.6.2 If the sample is very wet (typically <70% solids), weigh out 10g ± 0.1g of sample.

10.7 Determine the percent solids in the samples. See SOP References 13.5.

10.8 Using the metal scoop, add ~25g of baked sodium sulfate to each of the samples, adding more if necessary, and mix well until the sample is granular and free flowing. The sample/sodium sulfate mixture must fit inside a single cellulose thimble.

10.9 In a fume hood, set up the extraction beakers in the 6-hole rack and label each beaker.

10.9.1 Place 1-2 boiling chips in each extraction beaker.

10.10 Carefully transfer each sample to a pre-rinsed cellulose thimble then secure in the corresponding extraction beaker with a thimble clamp or wire holder.

10.11 **Pesticide/PCB samples:** Add 1mL of QC spike containing surrogates to the appropriate QC samples then add 1mL of Surrogate Spike to the Blank and matrix samples. *Note: it is not necessary add surrogates to the QC samples as the surrogate is contained within the QC spike.*

10.12 **DRO samples:** Add 1mL QC spike to all appropriate QC samples followed by 1mL of Surrogate Spike to all samples including QC samples.

10.13 **BNA samples:** Add 1mL of QC spike to all appropriate QC samples followed by 1mL of Surrogate Spike to all samples including QC samples. Note: if GPC is going to be used, 2mL of QC spike and 2mL of Surrogate Spike should be added to each sample.

10.14 Note: a member of the GCMS lab should spike the BNA samples with QC and surrogate mixtures whenever possible.

10.14 **For all soil samples:** After spiking, carefully fill the extraction beaker with 1:1 (v:v) methylene chloride/acetone solvent mix up to the top indention of the extraction beaker taking care not to completely cover the top rim of the cellulose beaker with solvent. Allow the thimble to absorb solvent and add more if necessary.

10.15 Ensure that the temperature key on top of each soxhlet unit is securely in place.

10.16 Ensure that the instrument solvent waste reservoir is empty of solvent.

10.17 Ensure that boiling chips are in each extraction beaker.

10.18 Ensure that the water hose is connected and “On.”

10.19 Check that the regulator behind the soxhlets. It should show that there is nitrogen pressure being applied to the units.

10.20 Both the water and nitrogen should already be “On.”

10.21 Carefully transfer the extraction beakers with samples and solvent from the fume hood and securely clamp them into the extraction positions on the soxhlet extraction unit.

10.22 If there are not enough samples to fill all positions, place and empty extraction beaker in the remaining extraction slots. All extraction positions must have an extraction beaker covering the soxhlet opening to contain methylene chloride fumes. If you remove a sample from the soxhlet, you must replace that beaker with an empty beaker.

10.23 Once the samples are secured on the soxhlet, press the arrow keys on the controller until the display reads "Start Analysis."

10.24 Press the button indicating the desired instrument to be started.

10.25 Select the Analysis on the controller and follow the prompts to start the soxhlet on the P1 program.

10.26 Select "Start" on the controller and follow the prompts.

10.27 Check the samples often as the soxhlet cycles through the extraction program (see Section 13, Table 13. Soxtherm Program Parameters) so that the samples do not dry out.

10.28 Once the extraction is finished, remove the beakers from the soxhlet when they are concentrated to about 5-10mL.

10.29 Carefully remove the thimbles and allow them to dry overnight on aluminum foil under a fume hood.

10.30 The samples should be leveled to 10mL.

10.31 For samples that are NOT cleaned by GPC:

10.31.1 **Pesticide and PCB samples**: The samples are drawn up into a 10mL luer-lock syringe and filtered into a 50mL TurboVap tube.

10.31.1.1 Place the TurboVap tube in a TurboVap at 38°C with nitrogen pressure at 3-4psi.

10.31.1.2 Allow the sample to blow down to ~5mL.

10.31.1.3 Add ~5mL of hexane and gently swirl to blend the solvents.

10.31.1.4 Allow the sample to reduce to ~5mL, checking the sample often to prevent total loss of sample and swirling each time.

10.31.1.5 Repeat Sections 10.30.1.3 and 10.30.1.4 two more times.

10.31.1.6 After the sample has been reduced to ~5ml in hexane three times, using a disposable pipette, gently transfer the sample to a 10mL culture tube.

10.31.1.7 Rinse the TurboVap tube with hexane and transfer the rinsate to the 10mL culture tube.

10.31.1.8 Bring the volume of sample in the culture tube up to 10mL with hexane using a premeasured 10mL model as comparison and securely cap the vial.

10.31.1.9 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.

10.32 **DRO samples:** The samples are drawn up into a 10mL luer-lock syringe and filtered into a 5mL culture tube and brought up to 5mL volume in methylene chloride using a premeasured 5mL model as comparison and securely cap the vial.

10.32.1 If the volume is greater than 5mL, the sample can be gently blown down with nitrogen directly in the 5mL vial.

10.32.2 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.

10.33 **BNA samples:** The samples are drawn up into a 10mL luer-lock syringe and filtered into a TurboVap tube.

10.33.1 Place the TurboVap tube in a TurboVap at 38°C with nitrogen pressure at 3-4psi.

10.33.2 Allow the sample to concentrate to less than 1mL, checking often to not lose the sample.

10.33.3 After the sample has concentrated to less than 1mL, remove it from the TurboVap and carefully transfer the sample to a 2mL, amber auto-sampler vial.

10.33.4 Rinse the TurboVap tube with methylene chloride and transfer the rinsate to the 2mL amber auto-sampler vial up to the 1mL mark and securely cap the vial. Properly discard any remaining rinsate.

10.33.5 The BNA samples are now ready for screening and transfer of custody to the GCMS laboratory.

10.34 <u>For sample that are cleaned by GPC (Optional):</u>

10.34.1 Follow the steps outlined in SOP 1-005 Rev. 6 or later to set up the GPC for prepping and cleaning samples by GPC. *Note: DRO samples do not go through GPC cleanup.*

10.35 **Pesticide and PCB samples:** Once GPC cleanup is complete, the samples must be concentrated and solvent exchanged in hexane.

10.35.1 If the sample extract is greater than 50mL, it will have to be concentrated in a RapidVap. If it is less than 50mL, proceed to Section 10.35.5 and follow the instructions for transferring the sample to a TurboVap tube omitting the steps for the RapidVap tube.

10.35.2 If not already in a RapidVap tube, transfer the sample to a RapidVap tube, rinsing the original container with methylene chloride or hexane and adding the rinsate to the RapidVap tube.

10.35.3 Place the RapidVap tube in a RapidVap at 38°C with nitrogen pressure at 4psi and shaking at 30RPMs.

10.35.4 Allow the sample to concentrate to ~5mL.

10.35.5 After the sample has concentrated to ~5mL, remove the RapidVap tube from the RapidVap and carefully transfer the sample to a TurboVap tube. Rinse the

RapidVap tube (or original container if other than a RapidVap tube) with hexane and add that rinsate to the TurboVap tube.

10.35.6 Place the TurboVap tube in a TurboVap at 38°C with nitrogen pressure at 3-4psi.

10.35.7 Add ~5mL of hexane and gently swirl to blend the solvents.

10.35.8 Allow the sample to reduce to ~5mL, checking the sample often to prevent total loss of sample and swirling each time.

10.35.9 Repeat Sections 10.30.6.7 and 10.30.6.8 two more times.

10.35.10 After the sample has been reduced to ~5ml in hexane three times, using a disposable pipette, gently transfer the sample to a 10mL culture tube.

10.35.11 Rinse the TurboVap tube with hexane and transfer the rinsate to the 10mL culture tube.

10.35.12 Bring the volume of sample in the culture tube up to 10mL with hexane using a premeasured 10mL model as comparison and securely cap the vial.

10.35.13 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.

10.36 **BNA Samples:** Once GPC cleanup is complete, if the sample extract is greater than 50mL, it will have to be concentrated in a RapidVap. If it is less than 50mL, proceed to Section 10.36.4 and follow the instructions for transferring the sample to a TurboVap tube omitting the steps for the RapidVap tube.

10.36.1 If not already in a RapidVap tube, transfer the sample to a RapidVap tube, rinsing the original container with methylene chloride adding the rinsate to the RapidVap tube.

10.36.2 Place the RapidVap tube in a RapidVap at 38°C with nitrogen pressure at 4psi and shaking at 30RPMs.

10.36.3 Allow the sample to concentrate to ~5-10mL.

10.36.4 After the sample has concentrated to ~5-10mL, remove the RapidVap tube from the RapidVap and carefully transfer the sample to a TurboVap tube. Rinse the RapidVap tube (or original container if other than a RapidVap tube) with methylene chloride and add that rinsate to the TurboVap tube.

10.36.5 Place the TurboVap tube in a TurboVap at 38°C with nitrogen pressure at 3-4psi.

10.36.6 Allow the sample to concentrate to less than 1mL, checking often to not lose the sample.

10.36.7 After the sample has concentrated to less than 1mL, remove it from the TurboVap and carefully transfer the sample to a 2mL, amber auto-sampler vial.

10.36.8 Rinse the TurboVap tube with methylene chloride and transfer the rinsate to the 2mL amber auto-sampler vial up to the 1mL mark and securely cap the vial. Properly discard any remaining rinsate.

10.36.9 The BNA samples are now ready for screening and transfer of custody to the GCMS laboratory.

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. (SOP Reference 13.14)

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 revision.

13.5 GA EPD Laboratory SOP – Percent Solids Determination – Method SW846-3541, SOP 1-042, online revision.

13.6 GA EPD Laboratory SOP – Gel Permeation Chromatography (GPC) Cleanup – Method 3640A, SOP 1-005, online revision.

13.7 EPA Method SW846-3541 – Automated Soxhlet Extraction, Rev. 0, September 1994.

13.8 EPA Method SW846-3640C – Gel Permeation Cleanup, Rev. 1, September 1994.

13.9 EPA Method SW846-8000B – Determinative Chromatographic Separations, Rev. 2, December 1996.

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

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

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

13.13 EPA Method SW846-8270C – Semivolatile Organic Compounds By Gas Chromatography/Mass Spectrometry (GC/MS), Rev. 3, December 1996.

13.14 GA EPD Laboratory SOP – EPD Laboratory Waste Management SOP, SOP 6-015, online revision.

13.15 GA EPD Laboratory Quality Assurance Plan, online revision.

13.16 GA EPD Laboratory Safety/Chemical Hygiene Plan & Fire Safety Plan, online revision.

13.17 GA EPD Laboratory SOP – Organics Data Validation, SOP 1-052, online revision.

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

14.1 Not Applicable

15 Associated Labworks Test Codes

15.1 Not Applicable

Updates: Updated for online revision.