Soxhlet Extraction

Reference Method: **EPA Method 3540C** SW-846, Test Methods for Evaluating Solid Waste: Physical/Chemical Methods, EPA SW-846, Update III, 1997.

Method 8081B, Reference: SW-846, Test Methods for Evaluating Solid Waste: Physical/Chemical Methods, EPA SW-846, Update IV, Feb 2007.

Method 8082A, Reference: SW-846, Test Methods for Evaluating Solid Waste: Physical/Chemical Methods, EPA SW-846, Update IV, Feb 2007.

Method 8270D, Reference: SW-846, Test Methods for Evaluating Solid Waste: Physical/Chemical Methods, EPA SW-846, Update IV, Feb 2007.

EPA 8015B (Modified), SW-846, Test Methods for Evaluating Solid Waste: Physical/Chemical Methods, EPA SW-846, Update III, December 1996

1. Scope and Application

Matrices: This method is applicable to solids, sediments, soils, and sludges.

Definitions: Refer to Alpha Analytical Quality Systems Manual.

This method is applicable to the extraction of semivolatile organic compounds from solids such as soils, sediments, sludges, and wastes. The Soxhlet extraction procedure ensures intimate contact of the sample matrix with the extraction solvent.

This method is applicable to the extraction of a variety of semivolatile organic compounds, which are then to be analyzed by the appropriate chromatographic procedure(s).

The data report packages present the documentation of any method modification related to the samples tested. Depending upon the nature of the modification and the extent of intended use, the laboratory may be required to demonstrate that the modifications will produce equivalent results for the matrix. Approval of all method modifications is by one or more of the following laboratory personnel before performing the modification: Area Supervisor, Department Supervisor, Laboratory Director, or Quality Assurance Officer.

This method is restricted to use by or under the supervision of trained analysts. Each analyst must demonstrate the ability to generate acceptable results with this method by performing an initial demonstration of capability and completing the record of training.

After initial demonstration, ongoing demonstration is based on acceptable laboratory performance of at least a quarterly laboratory control sample or acceptable performance from an annual proficiency test sample. A major modification to this procedure requires demonstration of performance. The identification of major method modification requiring performance demonstration is directed by the Quality Assurance Officer and/or Laboratory Director on a case-by-case basis.

2. Summary of Method

Weighed samples, typically 5-30g grams, are prepared for extraction by mixing the sample with anhydrous sodium sulfate until the sample is free flowing. The sample is then placed in an extraction body and extracted using the appropriate solvent in a Soxhlet extractor. The extract is allowed to cool prior to proceeding with additional extract preparation steps.

Any water is removed from the sample extract by filtering through a powder funnel containing approximately 10-30g of anhydrous sodium sulfate. The extract is then concentrated and, as needed, exchanged into a solvent compatible with the cleanup (Table 1) or determinative step being employed.

2.1 Method Modifications from Reference

None.

3. Reporting Limits

The laboratory follows the procedure found in 40CFR Part 136 to determine the MDLs for the various determinative methods per NELAC or DoD requirements. Details on MDL levels and frequency are in the SOPs for the determinative methods. The method detection limits determined by the laboratory are on file for review according to SOP/08-05.

4. Interferences

4.1 The most common cause of contamination is from improperly cleaned glassware and lab supplies. All glassware and re-useable extraction equipment (i.e. spatulas) must be scrupulously cleaned, following the glassware cleaning SOP (G-002).

4.2 Impurities in solvents and reagents may also yield artifacts and/or interferences that may compromise the results of sample analysis. All of these materials must be demonstrated to free from interferences under the conditions of extract preparation and analysis by preparing method blanks with each extraction batch. The same solvents and reagents are used for the method blank and the associated samples.

4.3 Phthalate esters contaminate many types of products used in the laboratory. Plastic materials must not contact the samples or extracts, as phthalates could be easily leached from the plastic.

4.4 Additional specific interference or contamination concerns are addressed in the various analytical SOPs

5. Health and Safety

The toxicity or carcinogenicity of each reagent and standard used in this method is not fully established; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. A reference file of material safety data sheets is available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available in the Chemical Hygiene Plan.

All personnel handling environmental samples known to contain or to have been in contact with municipal waste must follow safety practices for handling known disease causative agents.

5.1 Lab coats, safety glasses, and gloves must be worn when handling samples, extracts, standards or solvents and when washing glassware.

5.2 All extract concentration steps must be performed in the extraction hoods. All solvent and extract transfers must also be handled in the hood.

5.3 All expired stock standards, working standards, and spent sample extracts must be placed into the waste bucket in the lab, for future disposal by the Hazardous Waste Manager. The container must be properly labeled with hazard warning labels indicating the container contents.

5.4 Bottles containing flammable solvents must be stored in the flammables cabinet or in the vented cabinets found under the hoods

5.5 All waste solvents must be transferred to the satellite waste storage containers located in the extraction lab. Separate containers are provided for chlorinated and non-chlorinated solvents and must be used accordingly. Under no circumstances are solvents to be poured down the sink drains.

5.6 Inspect all glassware prior to use. Do not use any glassware that is chipped, cracked or etched if it could present a safety hazard. Damaged glassware is put aside for repair, otherwise discard the piece.

6. Sample Collection, Preservation, Shipping and Handling

6.1 Sample Collection

Sample collection and preservation requirements are described in the various analytical method SOPs.

6.2 Sample Preservation

None.

6.3 Sample Shipping

No specific requirements.

6.4 Sample Handling

All soil/sediment samples are stored, refrigerated or frozen, in the Custody Sample Bank. Samples are taken by the analyst immediately prior to sample extraction. The analyst must take custody of the samples by signing them out utilizing the LIMS.

All samples must be fully homogenized prior to taking the sample aliquot, as described in Section 9.1. After the sample aliquot is removed, the samples are returned to the Sample Bank and placed in the sample return refrigerator. Custody of the samples is transferred back by signing them back in utilizing the LIMS.

After the extraction is performed, the sample extract is stored in a stoppered KD tube before being transferred to a crimp-top or screw cap vial for analysis and long-term storage.

7. Equipment and Supplies

7.1 Small Soxhlet Extractor: 45/50, with 250mL or 300mL round bottom flask.

7.2 Large Soxhlet Extractor: 55/50, with 500mL round bottom flask.

7.3 Refrigerated Water Recirculator: Allows solvents to cool in the condenser while extracting.

7.4 Analytical Balance: Capable of weighing to 0.1g.

7.5 Heating Mantle: Rheostat controlled.

7.6 Spatulas: Stainless steel and Teflon.

7.7 Beakers: 250mL, 400mL.

7.8 Mortar and Pestle: Capable of reducing particle size to <1mm.

7.9 Kuderna-Danish (K-D) apparatus:

7.9.1 Concentrator tube: 10mL, graduated and calibrated. A ground-glass stopper is used to prevent evaporation of extracts.

7.9.2 Evaporation flask: 250 and 500mL. Attach to concentrator tube with springs, clamps, or equivalent.

7.9.3 Snyder column: Three-ball macro.

7.9.4 Plastic clamps

7.10 Water Bath: Heated, with concentric ring cover, capable of temperature control (± 5°C). The bath must be used in a hood.

7.11 Boiling Chips: Solvent-extracted, approximately 10/40 mesh (silicon carbide).

7.12 Vials: Glass, 2mL capacity, with PTFE-lined screw or crimp top.

7.13 Syringes: 100, 250, 500, 1000µL

7.14 Brady labeling system: Thermal label generator

7.15 Powder Funnel: Stainless Steel

7.16 Glass wool: SUPELCO, silane treated, baked at 400 C for 1 hour.

7.17 Graduated Cylinder: 25 and 50mL.

7.18 N-EVAP: Organomation; utilized for micro blow down.

7.19 S-EVAP: Organomation; utilized for hexane blow downs

8. Reagents and Standards

Reagent grade inorganic chemicals are used in all tests. All reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades are used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

8.1 Reagent Water: All references to water in this method refer to reagent water from Alpha's DI water treatment system.

8.2 Sodium Sulfate (Na_2SO_4): Granular anhydrous; purified by baking at 400ºC for 4 hours in a shallow tray. Store in closed glass containers. All references to sodium sulfate in this method refer to this prepared reagent.

8.3 Methylene Chloride: Pesticide quality or equivalent.

8.4 Hexane: Pesticide quality or equivalent.

8.5 Acetone: Pesticide quality or equivalent.

8.6 Nitrogen Gas: Reagent grade, used to purge and pressurize the extraction cell and as the blow-down gas in the Turbovap II auto-concentrator units and the N-EVAP.

8.7 Spiking Solutions: The preparation of these solutions is described in the analytical SOPs.

8.8 Copper: Granular (20-30 mesh)

9. Quality Control

The laboratory must maintain records to document the quality of data that is generated. Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

Each extraction batch contains various QC samples used to ensure the validity of the sample results. The particular QC elements performed for a given extraction batch are determined by the requirements of the determinative method. The purpose and definition of the QC samples performed are listed below.

9.1 Blank

Blanks, or method blanks, are measured aliquots of clean matrix (typically sodium sulfate for soil extractions) that are treated identically to the associated samples. Surrogates are added, and the blanks are carried through all stages of the sample extraction, concentration, and cleanup procedures. Blanks serve to ensure that no systematic contamination exists. A blank is extracted with each batch or 20 or less samples.

9.2 Laboratory Control Sample / Laboratory Control Sample Duplicate (LCS/LCSD)

LCS/LCSD samples are measured aliquots of clean matrix (typically sodium sulfate for soil extractions) that are spiked with a solution containing known amounts of target compounds, in addition to the surrogate solution. The LCS/LCSD is carried through all stages of the sample extraction, concentration, and cleanup procedures. LCS/LCSD samples serve as batch specific quantitative checks of the extraction. An LCS/LCSD pair is extracted with each batch of 20 or less samples.

9.3 Initial Calibration Verification (ICV)

Not Applicable.

9.4 Continuing Calibration Verification (CCV)

Not Applicable.

9.5 Matrix Spike / Matrix Spike Duplicate (MS/MSD)

MS and MSDs are field samples spiked with a known quantity of the target analyte(s). They are prepared by taking additional sample aliquots, and adding the appropriate amounts of surrogate and spiking solutions. The MS/MSD are carried through all stages of the sample extraction, concentration, and cleanup procedures. MS samples serve as a measure of extraction accuracy, by allowing the comparison of the found amount(s) of target analyte(s) with the spiked amount(s). An MS/MSD set also allows for the calculation of the extraction precision, by comparing the results of the two samples. These are prepared per client request.

9.6 Laboratory Duplicate

Duplicates are laboratory selected replicate samples, prepared by taking an additional sample aliquot of a sample. The duplicate is carried through all stages of the sample extraction, concentration, and cleanup procedures. Duplicates serve as a measure of the extraction precision, by comparing the results of the sample and duplicate. These are prepared per client request.

9.7 Method-specific Quality Control Samples

9.7.1 Surrogate

Surrogates are compounds specified by the analytical method that are added to all samples and QC samples prior to beginning the extraction process. Surrogate recoveries are calculated and serve as a sample specific quantitative check of the extraction. The various spiking solutions are prepared according to the directions found in the analytical SOPs.

9.8 Method Sequence

See Section 10.

10. Procedure

10.1 Sample Preparation and Extraction

10.1.1 Before starting the extraction process, each soil or sediment sample should be visually inspected. If a sample contains a significant amount of free water, mix the water into the sample unless specified to decant based on Client instructions. Record this in the extraction logbook.

Any artifacts (rocks, leaves, sticks, or similar materials) are not typically considered part of the soil/sediment sample and should not be included. If necessary, transfer these artifacts to another container prior to homogenizing the sample. Note the presence of sample artifacts in the extraction logbook. Gummy, fibrous, or oily materials not amenable to grinding should be cut, shredded, or otherwise reduced in size to allow mixing and maximum exposure of the sample surfaces for the extraction. The addition of anhydrous sodium sulfate to the sample may make the mixture amenable to grinding. Sodium sulfate is added to dry the sample until the sample is free flowing. If this needs to be done, weigh the sample prior to addition of the sodium sulfate.

10.1.2 Clean the Soxhlet Extractor with Methylene chloride three times. Add glass wool to the extractor and approximately 5g of sodium sulfate. In addition add approximately 5g of Copper to any sediment samples. This is done to prevent the samples from passing

through the extractor. It only allows extraction solvent to move through. It also assists in the drying process.

10.1.3 Homogenize the sample well by mixing the entire contents of the sample container. In instances where the sample container is filled to the top and packed in tightly, the entire sample contents are emptied into a larger glass container (i.e., a 400mL glass beaker) and homogenized. If this is difficult due to sample matrix, describe the non-homogeneity in the extraction logbook. Weigh out the appropriate amount of sample, typically 5-30 grams, into weighing dish. If sample is wet use sodium sulfate to dry sample.

10.1.4 The analyst must demonstrate that all parts of the equipment in contact with the sample and reagents are interference-free. This is accomplished through the analysis of a solid matrix (Na_2SO_4) Method Blank. A Method Blank is extracted with each batch of 20 or less samples. The samples are transferred to the soxhlet extractor plugged with glass wool and sodium sulfate. The sample must be added so that the soxhlet will drain freely for the duration of the extraction period.

10.1.5 Pour approximately 200mL of the DCM into a 250mL or 300mL round bottom flask containing one or two clean boiling chips. Label the flask with label generated from the LIMS. Attach the flask to the extractor.

10.1.6 Make sure all glassware associated with the soxhlets are rinsed clean with DCM which includes the condensers, extractors and round bottoms. Clean the condensers from inside and around the frosted areas with DCM before putting apparatus up with the sample.

10.1.7 Add the appropriate volume(s) of surrogate and spike solution(s) to the samples and QC samples. See Organic Prep Lab Spiking Samples (Form No.: 102-19 and Form No.: 102-35) for specifics. A second analyst must verify spike and surrogate addition. Note this in the extraction logbook. As the first analyst spikes the samples and QC the second analyst rinses the inside of the Soxhlet down with the proper extraction solvent.

10.1.8 Extract the sample for 16 – 24 hours at 4-6 cycles/hour. Turn on the condenser for the entire extraction and cooling time. Use the extraction solvent between the connection of the condenser and the extractors to make a tight connection between the two pieces of glassware.

10.1.9 Allow the extract to cool after the extraction is complete.

10.1.10 After the extract has cooled, drain the solvent into the round bottom flask. Tip the Soxhlet to empty, the sample is collected in waste containers. Rinse the Soxhlet with solvent if any residual sample is present. Place the waste containers and Soxhlets in the hood to allow solvent to evaporate. Once dry, the samples are disposed of in the hazardous waste containers. Prior to next use, Chem Solve, wash, dry, and bake the Soxhlet extractors in a drying kiln for 4 hours at 400 degrees C, and thoroughly rinse with DCM.

10.2 Initial Concentration: KD Technique

10.2.1 Assemble the Kuderna-Danish (KD) apparatus (Section 7.10) by attaching a 10mL KD tube to a 250 or 500mL KD flask. Rinse set up completely with DCM. Add 5mL DCM to the assembled apparatus to check for leaks or cracks prior to transferring the sample. If the apparatus in leak free, discard the DCM.

10.2.2 Place a DCM rinsed filter funnel containing glass wool and sodium sulfate on top of the KD apparatus. Pour the extract through the funnel and into the KD flask, labeled with the sample I.D. Rinse the round bottom with approximately 10mL of methylene chloride

three times, pouring the rinseate into the funnel to complete the transfer. Add 1-2 boiling chips.

10.2.3 Attach a three-ball Snyder column to the top of the flask. Place the KD apparatus on a hot water bath (heated to approximately 80°C ±5°C for samples extracted in Methylene Chloride so that the concentrator tube is partially immersed in the hot water, and so that the entire lower rounded surface of the flask is bathed in hot water vapor. Adjust the position of the apparatus as required to complete the concentration in 30 to 40 minutes. At the proper rate of distillation, the balls in the column will actively chatter, but the chambers will not flood with solvent. Periodically rinse the internal walls of the concentrator tubes with the appropriate solvent.

10.2.4 If the extract appears extremely viscous and reduces in volume very slowly, a final volume of greater than 10mL may be used to ensure that there is no loss of surrogates or the compounds of interest. See the Section Supervisor or Laboratory Director for additional guidance on troublesome matrices.

10.2.5 Remove the KD apparatus from the water bath and let cool for at least 10 minutes. Disassemble the KD apparatus. Transcribe sample I.D. onto the KD tube. Rinse the Snyder column and KD flask with methylene chloride and set aside.

10.2.6 Place the KD concentrator tube on the N-EVAP. The N-EVAP is set at 37°C for samples extracted in Methylene Chloride with a gentle stream of nitrogen. The extract must be concentrated under a *gentle* steady stream of nitrogen. The internal walls of the concentrator tube must be rinsed periodically with the appropriate solvent during evaporation. The solvent level of the sample must be positioned to prevent water from condensing into the sample (*i.e.,* the solvent level should be below the level of the water in the bath). If the sample requires solvent exchange, add 50mL of the exchange solvent to the KD and mix well, letting it boil on water bath, then place it on the S-EVAP to boil down to around 10-15mls. Place concentrator tube on the N-EVAP set at 69 C for samples that have been exchanged with Hexane. Concentrate the solvent exchanged sample to the appropriate volume. See Organic Prep Lab Final Volumes (Form No.: 102-20) for additional guidance on sample final volumes.

10.2.7 If the extract appears extremely viscous and reduces in volume very slowly then a final volume of 5-10 mL should be used to ensure that there is no loss of surrogates or the compounds of interest. Note: Micro-concentration may not be needed depending upon the determinative analytical method. Pesticide/PCB samples are typically a 10mL final extract volume and semivolatile samples are typically a 1mL final extract volume. Client specifications may be different than this SOP. Always see the Section Supervisor for additional guidance when needed.

10.2.8 If the sample in the 10mL concentrator tube is dark and viscous, an “auto-vial”, pre-fitted with a filtration disk, can be employed to remove particulate material. This is particularly evident in heavily contaminated petroleum samples. Reduce the extract to just less than 10mL. Remove it from the concentrator tube with an appropriate size syringe. Pass it through the auto-vial, and back into the concentrator tube. Rinse the syringe, tube and auto-vial as needed to ensure a thorough transfer. The extract may now concentrate more easily with the majority of the particulate matter removed. However, do not force the concentration as this may jeopardize the surrogate and the compounds of interest recoveries.

10.2.9 In some heavily contaminated petroleum extracts, it is possible to perform an extra step of hexane exchange to remove the asphaltene material that precipitates out in hexane. This decision must be made with the Section Supervisor approval. This may preserve the integrity of surrogates and the compounds of interest. This extract can also be auto-

vialed to further remove any unwanted particulate materials. Micro-concentration may then continue.

10.2.10 The extract is transferred to an autosampler vial, and is now ready for cleanup (if required) or transfer to the analytical laboratory.

10.3 Instrument Maintenance

10.3.1 Refrigeration Re-circulator

The Refrigeration Re-circulator should be checked periodically to insure that it is running correctly and that the level of reagent water is constant with manufactures recommendation.

The re-circulator tubing should be inspected for leaks, cracks, and crimps prior to each use.

10.3.2 Water Bath

10.3.2.1 The water bath should be kept full at all times. Add reagent water as necessary.

10.3.2.2 Keep unit clean. Avoid solvent spills on or around unit. Clean periodically with a damp cloth.

10.3.3 Analytical Balance

10.3.3.1 All balances are calibrated and serviced every six months by an instrument service company. All service records are kept on file.

10.3.3.2 Keep balances clean. Brush off any sample spills immediately. Keep the balance doors closed and the balance turned off when not in use.

10.3.4 Soxhlet Solvent Cycle Rate Check

10.3.4.1 Quarterly check on heating mantels to insure proper cycling of solvent though-out the soxhlet apparatus.

11. Data Evaluation, Calculations and Reporting

Not Applicable.

12. Contingencies for Handling Out-of-Control Data or Unacceptable Data

Holding time exceedence and improper preservation are noted on the nonconformance report form.

When analysis of samples indicates possible extraction problems, such as poor surrogate recoveries, poor LCS/MS/MSD recoveries, or suspected contamination in blanks or samples, re-extractions are required. Depending on the particular failure, the re-extraction may be of a specific sample or the entire extraction batch.

The analyst that determines the need for re-extraction must fill out a sample re-extract request form. This form notes the reason for the re-extraction request along with any special requirements, and the date and time that the re-extract is needed. Re-extraction request forms are maintained on

file to help track the cause for re-extractions, and to be used as a tool in improving systems to minimize the need for re-extractions.

Depending on the results of the re-extraction, the first, second, or both sets of results may be reported to the client, along with a narrative report detailing the problems encountered and the resolution.

13. Method Performance

13.1 Method Detection Limit Study (MDL) / Limit of Detection Study (LOD) / Limit of Quantitation (LOQ)

The laboratory follows the procedure to determine the MDL, LOD, and/or LOQ as outlined in Alpha SOP/08-05. These studies performed by the laboratory are maintained on file for review.

13.2 Demonstration of Capability Studies

Refer to Alpha SOP/08-12 for further information regarding IDC/DOC Generation.

13.2.1 Initial (IDC)

The analyst must make an initial, one-time, demonstration of the ability to generate acceptable accuracy and precision with this method, prior to the processing of any samples.

13.2.2 Continuing (DOC)

The analyst must make a continuing, annual, demonstration of the ability to generate acceptable accuracy and precision with this method.

14. Pollution Prevention and Waste Management

Refer to Alpha's Chemical Hygiene Plan and Waste Management and Disposal SOP for further pollution prevention and waste management information.

15. Referenced Documents

Chemical Hygiene Plan

SOP/08-05 MDL/LOD/LOQ Generation

SOP/08-12 IDC/DOC Generation

SOP/14-01 Waste Management and Disposal

Form/102-19 Organic Prep Lab Spiking Samples

Form /102-20 Organic Prep Lab Final Volumes

SOP/G-002 Glassware Cleaning

Soxhlet Solvent Cycle Rate Check for Mansfield

16. Attachments

Table 1: Solvent Exchange per Method

Table 1

Solvent Exchange per Method

Method	Exchange Solvent
8081A	HEXANE
8081A-low	HEXANE
8082	HEXANE
8082-low	HEXANE
Congener	HEXANE
Homolog	HEXANE
209 PCB	HEXANE
8270C	NONE
PAH-SIM	NONE