

CALTEST STANDARD OPERATING PROCEDURE

SOXHLET EXTRACTION METHOD

EPA 3540C

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This SOP outlines the exact procedure to be followed by all staff of Caltest Laboratory who are performing the indicated method. It is the responsibility of any individual performing the procedure to follow these instructions outlined in this document. Any significant modifications to this method require a revision to this SOP. Any deviations from this SOP require prior authorization from the departmental Coordinator/Manager and the QAO. In addition, all deviations from the written procedure require complete documentation in the appropriate logbook.

Amendment	Summary of Change	Initials		Date
		Pages	Coord/ LD /QAO	
#1	_____	_____	_____	____/____/____
#2	_____	_____	_____	____/____/____
#3	_____	_____	_____	____/____/____
#4	_____	_____	_____	____/____/____
#5	_____	_____	_____	____/____/____

A maximum of 5 amendments can be added to each SOP, at which point the entire SOP warrants revision.

METHOD 3540C SOXHLET EXTRACTION

1.0 SCOPE AND APPLICATION

- 1.1 Method 3540 is a procedure for extracting nonvolatile and semivolatile organic compounds from solids such as soils, sludges, and wastes. The Soxhlet extraction process ensures intimate contact of the sample matrix with the extraction solvent.
- 1.2 This method is applicable to the isolation and concentration of water-insoluble and slightly water soluble organics in preparation for a variety of chromatographic procedures.
- 1.3 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.

2.0 SUMMARY OF METHOD

- 2.1 The solid sample is mixed with anhydrous sodium sulfate, placed in an extraction thimble, and extracted using an appropriate solvent in a Soxhlet extractor.
- 2.2 The extract is then dried, concentrated (if necessary), and, as necessary, exchanged into a solvent compatible with the cleanup or determinative step being employed.
- 2.3 This method is applicable to the extraction of nonvolatile and semivolatile organic compounds from solids such as soils, relatively dry sludges, and solid wastes. Soxhlet extraction uses relatively inexpensive glassware, once loaded requires no hands-on manipulation, provides efficient extraction, but is rather lengthy (16 to 20 hours) and uses fairly large volumes of solvent. It is considered a rugged extraction method because there are very few variables that can adversely affect extraction efficiency.

3.0 DEFINITIONS

- 3.1 **METHOD BLANK:** An analyte-free matrix to which all reagents are added in the same volumes or proportions as used in sample processing. The method blank should be carried through the complete sample preparation and analytical procedure. The method blank is used to document contamination resulting from the analytical process.
- 3.2 **LABORATORY CONTROL SAMPLE:** A known matrix spiked with compound(s) representative of the target analytes. This is used to document laboratory performance.

- 3.3 **MATRIX:** The component or substrate (e.g., surface water, drinking water) which contains the analyte of interest.
- 3.4 **MATRIX DUPLICATE:** An intra-laboratory split sample which is used to document the precision of a method in a given sample matrix.
- 3.5 **MATRIX SPIKE:** An aliquot of sample spiked with a known concentration of target analyte(s). The spiking occurs prior to sample preparation and analysis. A matrix spike is used to document the bias of a method in a given sample matrix.
- 3.6 **MATRIX SPIKE DUPLICATES:** Intra-laboratory split samples spiked with identical concentrations of target analyte(s). The spiking occurs prior to sample preparation and analysis. They are used to document the precision and bias of a method in a given sample matrix.
- 3.7 **FIELD DUPLICATES:** Independent samples which are collected as close as possible to the same point in space and time. They are two separate samples taken from the same source, stored in separate containers, and analyzed independently. These duplicates are useful in documenting the precision of the sampling process.
- 3.8 **BATCH:** A group of samples which behave similarly with respect to the sampling or the testing procedures being employed and which are processed as a unit. For QC purposes, if the number of samples in a group is greater than 20, then each group of 20 samples or less will all be handled as a separate batch.
- 3.9 **SURROGATE:** An organic compound which is similar to the target analyte(s) in chemical composition and behavior in the analytical process, but which is not normally found in environmental samples.

4.0 INTERFERENCES

- 4.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences to sample analysis. All these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be necessary. Refer to each method for specific guidance on quality control procedures and to the SOP Organic Glassware Washing for guidance on the cleaning of glassware.
- 4.2 Interferences co-extracted from the samples will vary considerably from source to source. If analysis of an extracted sample is prevented due to interferences, further cleanup of the sample extract may be necessary.

- 4.3 Phthalate esters contaminate many types of products commonly found in the laboratory. Plastics, in particular, must be avoided because phthalates are commonly used as plasticizers and are easily extracted from plastic materials. Serious phthalate contamination may result at any time if consistent quality control is not practiced.
- 4.4 Soap residue (e.g. sodium dodecyl sulfate), which results in a basic pH on glassware surfaces, may cause degradation of certain analytes. Specifically, Aldrin, Heptachlor, and most organophosphorus pesticides will degrade in this situation. This problem is especially pronounced with glassware that may be difficult to rinse (e.g., 500-mL K-D flask). These items should be hand-rinsed very carefully to avoid this problem. See the glassware cleaning SOP.

5.0 SAFETY

- 5.1 This method is used to analyze potentially hazardous samples, and uses potentially hazardous reagents. Use of a respirator is advised if a hood is not adequate or feasible. Splash guard goggles should be used when acid rinsing, solvent rinsing, transferring samples, or when working with solvents under pressure.
- 5.2 Prior to performing this analysis, review the MSDS for all standards and reagents to be used. Observe the recommended safety precautions. Protective clothing and safety glasses should be worn when handling samples or reagents, and all manipulations should be done in a hood.
- 5.3 Maintain a clean and uncluttered workspace. Return all chemicals, reagents and resultant wastes to their designated storage area at the completion of the test.

6.0 APPARATUS AND MATERIALS

- 6.1 Soxhlet extractor - 40 mm ID
- 6.2 Drying column - 20 mm ID Pyrex®
- 6.3 Kuderna-Danish (K-D) apparatus
- 6.3.1 K-D flask and jacket - 500-mL (Kontes K-570001-500 or equivalent). Attach together with clamps. Aluminum foil is used to prevent evaporation of extracts.
- 6.3.2 Snyder column - Three-ball macro (Kontes K-503000-0121 or equivalent).
- 6.3.3 Snyder column - Two-ball micro (Kontes K-569001-0219 or equivalent).

6.3.4 Plastic clamps.

- 6.4 Water bath - Heated, with concentric ring cover, capable of temperature control ($\pm 5^{\circ}\text{C}$). The bath should be used in a hood.
- 6.5 Vials - Glass, 2-mL capacity, with polytetrafluoroethylene (PTFE)-lined screw or crimp top.
- 6.8 Glass or paper thimble or glass wool - Contaminant-free, bake for 4 hours, solvent rinsed. *DO NOT TOUCH!!*
- 6.9 Analytical balance - capable of weighing to 0.0001 g.
- 6.10 TurboVap apparatus

7.0 REAGENTS AND STANDARDS

- 7.1 Reagent grade methylene chloride.
- 7.2 Organic-free reagent water.
- 7.3 Sodium sulfate (granular, anhydrous), Na_2SO_4 . Purify by baking at 400°C for 4 hours, cooling and rinsing the sodium sulfate with methylene chloride, . A method blank must be analyzed, demonstrating that there is no interference from the sodium sulfate.
- 7.4 Extraction solvents - All solvents must be pesticide quality or equivalent.
- 7.4.1 Soil/sediment and aqueous sludge samples shall be extracted using either of the following solvent systems:
- 7.4.1.1 Methylene chloride, CH_2Cl_2 .
- 7.5 Exchange solvents - All solvents must be pesticide quality or equivalent.
- 7.5.1 Hexane, C_6H_{14} .
- 7.5.2 Acetonitrile, CH_3CN .

8.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

8.1 The containers used for sampling and storage should be glass or Teflon, and have screw caps with Teflon-lined septa. All sample extracts should be stored at 4°C.

8.2 All extracts must be analyzed within 40 days of extraction

9.0 QUALITY CONTROL

9.1 Any reagent blanks, matrix spikes, or replicate samples should be subjected to exactly the same procedures as those used on actual samples.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Not Applicable.

11.0 PROCEDURE

11.1 Sample Handling

11.1.1 Sediment/soil samples - Decant and discard any water layer on a sediment sample. Mix sample thoroughly, especially composited samples. Discard any foreign objects such as sticks, leaves, and rocks.

11.1.2 Waste samples - Samples consisting of multiple phases must be prepared by the phase separation method before extraction. This extraction procedure is for solids only.

11.1.3 Dry waste samples amenable to grinding - Grind or otherwise subdivide the waste so that it is reduced in size to allow mixing and maximum exposure of the sample surfaces for the extraction. Introduce sufficient sample into the grinding apparatus to yield at least 10 g after grinding.

11.1.4 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 (1:1) may make the mixture amenable to grinding.

11.2 GCMS and GC Soil extractions.

8270
 PAH
 Diesel
 Motor Oil
 8081
 8141
 8082

- Samples for 8081 and 8141 analyses can be co-extracted.
- QC's for 8081 & 8141 can be co-extracted.
- Whenever we receive a sample for 8081 or 8141, the LCS & LCSD should be spiked with both OC Pest spk & 8141 Spk.
- 8082 & Diesel need separate LCS & LCSD.

	SPIKE	SURROGATE
8270/PAH	BNA	BNA
Diesel/ Motor Oil	Diesel	O-Terphenyl
8081- GC 8081 -GCMS	OC Pest OC Pest	OC Pest B/N
8141-GC 8141 GCMS	614/8141 614/8141	OP Pest B/N
8082 -GC 8082 -GCMS	PCB PCB	OC Pest B/N
Pyrethroid-EI-MS Pyrethroid-CI-MS	Pyrethroid Pyrethroid	BN CI-PYR

11.3 Blend 30 g of the solid sample with enough anhydrous sodium sulfate to dry the sample (if needed) and place in an extraction thimble. The extraction thimble must drain freely for the duration of the extraction period. A glass wool plug above and below the sample in the Soxhlet extractor is an acceptable alternative for the thimble.

11.3.1 Add 200 μL of the surrogate standard spiking solution onto the sample (refer to the analytical method for which you are prepping these samples for details on the surrogate standard and matrix spiking solutions).

11.3.2 For the sample in each analytical batch selected for spiking, add 200 μL of the matrix spiking standard (the analytical sample used for matrix spike is selected at random) to the sample after it is placed in the thimble.

11.4 Assemble a Kuderna-Danish (K-D) apparatus (Sec. 6.3), if necessary, by attaching a 25-mL K-D jacket to a 500-mL K-D flask. Place approximately 225 mL of the methylene chloride into the K-D apparatus with the Soxhlet apparatus on top and the condenser attached to the top of the Soxhlet. Set up the manifold and glass apparatus, then attach the apparatus to the manifold and extract the sample for 16 - 20 hours at 20 cycles/hour (4 hours at 20 cycles/hour for oil and grease extractions). Make sure that there is enough water in the manifold. Turn the water re-circulator on, and test it to be sure it works. Then attach to the apparatus and turn on to chill.

11.5 Nitrogen blow-down technique (Turbo-Vap)

11.5.1 Place the concentrator tube in a warm water bath (approximately 35°C) and evaporate the solvent volume to the required level using a gentle stream of clean, dry nitrogen (filtered through a column of activated carbon).

CAUTION: *Do not use plasticized tubing between the carbon trap and the sample, since it may introduce contaminants.*

11.5.2 The internal wall of the tube must be rinsed several times with the appropriate solvent during the operation. During evaporation, the solvent level in the tube must be positioned to prevent water from condensing into the sample (i.e., the solvent level should be below the level of the water bath). Under normal operating conditions, the extract should not be allowed to become dry.

11.5.3 TurboVap Nitrogen Blow-down Technique

1. Nitrogen must be on.
2. Turn instrument to sensor.

3. Set appropriate temperature for solvents (Methylene Chloride at 35°C, Hexane at 50°C, Acetonitrile at 50°C); wait till temperature is reached.
4. Rinse tubes with methylene chloride.
5. Label tubes.
- 6.
7. Fill **only** to 200 mL mark, and **NO** further.
8. Enter information and data into Turbo Vap Logbook.
9. Place samples in order with the lab book, i.e. cell one, cell two, etc.
10. Close lid, then turn cells on.
11. Start with a pressure of 4 or 5.
12. As volume decreases, increase the pressure to 8 or 9. When it reaches the top of the nipple, it can be increased to 14.

11.5.4 Solvent Exchange

1. Concentrate to 30 mL for Hexane exchange/ 20 mL for ACN exchange
2. Add 20 mL hexane / 10 mL ACN and swirl
3. Reduce to 1 mL.
4. Place under the hood, and pipette the TurboVapped remainder into 2 mL vials (labelled with ID #, method, volume, and initials). Rinse the tubes 3 times with ACN and add the rinsate to the vials.
5. Add 2 mL ACN to a separate vial for a comparison vial, and bring sample vial to a 2 mL final volume.

CAUTION: *When the volume of solvent is reduced below 1 mL, semivolatile analytes may be lost.*

- 11.6 The extracts obtained may now be analyzed for the target analytes using the appropriate organic technique(s). If analysis of the extract will not be performed immediately, it should be transferred to a vial with a Teflon-lined screw cap, and labeled appropriately.

12. CALCULATIONS

- 12.1 See Sec 11.2.

13.0 POLLUTION PREVENTION

13.1 The solvents used in this method pose a threat to the environment and must be managed properly. Limit the volumes used whenever possible, and institute a solvent recovery program. For further information see Section 17.

13.2 Standards should be prepared in volumes consistent with laboratory use to minimize the volume of expired standards to be disposed

14. DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QC MEASURES

14.1 See Internal QC Summary

15. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

15.1 See Internal Quality Control Summary

16. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

16.1 See SOP # Q-CORRECT

17. WASTE MANAGEMENT

17.1 Methylene chloride — Methylene chloride waste drum.

17.2 Acetone — Flammable solvent waste drum.

17.3 All other solvents, standards, and reagents to their appropriate waste container.

17.4 For any further questions, see your coordinator or the Caltest Waste Management Coordinator.

18.0 METHOD PERFORMANCE

18.1 Refer to the analytical methods for performance data.

19.0 REFERENCES

- 19.1 Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, SW-846, 3rd Edition. Revision 1, July 1992. Method 3500A, Revision 1, July 1992
- 19.2 Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, SW-846, 3rd Edition. Revision 3, December 1996. Method 3540C, Revision 3, December 1996