METHOD 3510C

SEPARATORY FUNNEL LIQUID-LIQUID EXTRACTION

1.0 SCOPE AND APPLICATION

1.1 This method describes a procedure for isolating organic compounds from aqueous samples. The method also describes concentration techniques suitable for preparing the extract for the appropriate determinative methods described in Section 4.3 of Chapter Four.

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 A measured volume of sample, usually 1 liter, at a specified pH (see Table 1), is serially extracted with methylene chloride using a separatory funnel.

2.2 The extract is dried, concentrated (if necessary), and, as necessary, exchanged into a solvent compatible with the cleanup or determinative method to be used (see Table 1 for appropriate exchange solvents).

3.0 INTERFERENCES

3.1 Refer to Method 3500.

3.2 The decomposition of some analytes has been demonstrated under basic extraction conditions. Organochlorine pesticides may dechlorinate, phthalate esters may exchange, and phenols may react to form tannates. These reactions increase with increasing pH, and are decreased by the shorter reaction times available in Method 3510. Method 3510 is preferred over Method 3520 for the analysis of these classes of compounds. However, the recovery of phenols may be optimized by using Method 3520, and performing the initial extraction at the acid pH.

4.0 APPARATUS AND MATERIALS

4.1 Separatory funnel - 2-liter, with polytetrafluoroethylene (PTFE) stopcock.

4.2 Drying column - 20 mm ID Pyrex® chromatographic column with Pyrex® glass wool at bottom and a PTFE stopcock.

NOTE: Fritted glass discs are difficult to decontaminate after highly contaminated extracts have been passed through. Columns without frits may be purchased. Use a small pad of Pyrex® glass wool to retain the adsorbent. Prewash the glass wool pad with 50 mL of acetone followed by 50 mL of elution solvent prior to packing the column with adsorbent.

4.3 Kuderna-Danish (K-D) apparatus.

4.3.1 Concentrator tube - 10-mL, graduated (Kontes K-570050-1025 or equivalent). A ground-glass stopper is used to prevent evaporation of extracts.

4.3.2 Evaporation flask - 500-mL (Kontes K-570001-500 or equivalent). Attach to concentrator tube with springs, clamps, or equivalent.

4.3.3 Snyder column - Three-ball macro (Kontes K-503000-0121 or equivalent).

4.3.4 Snyder column - Two-ball micro (Kontes K-569001-0219 or equivalent).

4.3.5 Springs - 1/2 inch (Kontes K-662750 or equivalent).

NOTE: The following glassware is recommended for the purpose of solvent recovery during the concentration procedures requiring the use of Kuderna-Danish evaporative concentrators. Incorporation of this apparatus may be required by State or local municipality regulations that govern air emissions of volatile organics. EPA recommends the incorporation of this type of reclamation system as a method to implement an emissions reduction program. Solvent recovery is a means to conform with waste minimization and pollution prevention initiatives.

4.4 Solvent vapor recovery system (Kontes K-545000-1006 or K-547300-0000, Ace Glass 6614-30, or equivalent).

4.5 Boiling chips - Solvent-extracted, approximately 10/40 mesh (silicon carbide or equivalent).

4.6 Water bath - Heated, with concentric ring cover, capable of temperature control (± 5°C). The bath should be used in a hood.

4.7 Vials - 2-mL, glass with PTFE-lined screw-caps or crimp tops.

4.8 pH indicator paper - pH range including the desired extraction pH.

4.9 Erlenmeyer flask - 250-mL.

4.10 Syringe - 5-mL.

4.11 Graduated cylinder - 1-liter.

5.0 REAGENTS

5.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that 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 may be 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. Reagents should be stored in glass to prevent the leaching of contaminants from plastic containers.

5.2 Organic-free reagent water - All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

5.3 Sodium hydroxide solution (10 N), NaOH. Dissolve 40 g NaOH in organic-free reagent water and dilute to 100 mL. Other concentrations of hydroxide solutions may be used to adjust sample pH, provided that the volume added does not appreciably change (e.g., <1%) the total sample volume.

5.4 Sodium sulfate (granular, anhydrous), Na_2SO_4. Purify by heating to 400°C for 4 hours in a shallow tray, or by precleaning the sodium sulfate with methylene chloride. If the sodium sulfate is precleaned with methylene chloride, a method blank must be analyzed, demonstrating that there is no interference from the sodium sulfate. Other concentrations of acid solutions may be used to adjust sample pH, provided that the volume added does not appreciably change (e.g., <1%) the total sample volume.

5.5 Sulfuric acid solution (1:1 v/v), H_2SO_4. Slowly add 50 mL of H_2SO_4 (sp. gr. 1.84) to 50 mL of organic-free reagent water.

5.6 Extraction/exchange solvents - All solvents must be pesticide quality or equivalent.

5.6.1 Methylene chloride, CH_2Cl_2, boiling point 39°C.

5.6.2 Hexane, C_6H_{14}, boiling point 68.7°C.

5.6.3 2-Propanol, $CH_3CH(OH)CH_3$, boiling point 82.3°C.

5.6.4 Cyclohexane, C_6H_{12}, boiling point 80.7°C.

5.6.5 Acetonitrile, CH_3CN, boiling point 81.6°C.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

See the introductory material to this chapter, Organic Analytes, Sect. 4.1.

7.0 PROCEDURE

7.1 Using a 1-liter graduated cylinder, measure 1 liter (nominal) of sample. Alternatively, if the entire contents of the sample bottle are to be extracted, mark the level of sample on the outside of the bottle. If high analyte concentrations are anticipated, a smaller sample volume may be taken and diluted to 1-L with organic-free reagent water, or samples may be collected in smaller sample bottles and the whole sample used.

7.2 Pipet 1.0 mL of the surrogate spiking solution into each sample in the graduated cylinder (or sample bottle) and mix well. (See Method 3500 and the determinative method to be used for details on the surrogate standard solution and matrix spiking solution).

7.2.1 For the sample in each batch (see Chapter One) selected for use as a matrix spike sample, add 1.0 mL of the matrix spiking standard.

7.2.2 If Method 3640, Gel-Permeation Cleanup, is to be employed, add twice the volume of the surrogate spiking solution and the matrix spiking standard, since half of the extract is not recovered from the GPC apparatus. (Alternatively, use 1.0 mL of the spiking solutions and concentrate the final extract to half the normal volume, e.g., 0.5 mL instead of 1.0 mL).

7.3 Check the pH of the sample with wide-range pH paper and adjust the pH, if necessary, to the pH indicated in Table 1, using 1:1 (v/v) sulfuric acid or 10 N sodium hydroxide. Lesser strengths of acid or base solution may be employed, provided that they do not result in a significant change (<1%) in the volume of sample extracted (see Secs. 5.3 and 5.5).

7.4 Quantitatively transfer the sample from the graduated cylinder (or sample bottle) to the separatory funnel. Use 60 mL of methylene chloride to rinse the cylinder (or bottle) and transfer this rinse solvent to the separatory funnel. If the sample was transferred directly from the sample bottle, refill the bottle to the mark made in Sec. 7.1 with water and then measure the volume of sample that was in the bottle.

7.5 Seal and shake the separatory funnel vigorously for 1 - 2 minutes with periodic venting to release excess pressure. Alternatively, pour the exchange solvent into the top of the Snyder column while the concentrator remains on the water bath in Sec. 7.11.4.

NOTE: Methylene chloride creates excessive pressure very rapidly; therefore, initial venting should be done immediately after the separatory funnel has been sealed and shaken once. The separatory funnel should be vented into a hood to avoid exposure of the analyst to solvent vapors.

7.6 Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the size of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample and may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the solvent extract in an Erlenmeyer flask. If the emulsion cannot be broken (recovery of < 80% of the methylene chloride, corrected for the water solubility of methylene chloride), transfer the sample, solvent, and emulsion into the extraction chamber of a continuous extractor and proceed as described in Method 3520, Continuous Liquid-Liquid Extraction.

7.7 Repeat the extraction two more times using fresh portions of solvent (Secs. 7.2 through 7.5). Combine the three solvent extracts.

7.8 If further pH adjustment and extraction is required, adjust the pH of the aqueous phase to the desired pH indicated in Table 1. Serially extract three times with 60 mL of methylene chloride, as outlined in Secs. 7.2 through 7.5. Collect and combine the extracts and label the combined extract appropriately.

7.9 If performing GC/MS analysis (Method 8270), the acid/neutral and base extracts may be combined prior to concentration. However, in some situations, separate concentration and analysis of the acid/neutral and base extracts may be preferable (e.g. if for regulatory purposes the presence or absence of specific acid/neutral or base compounds at low concentrations must be determined, separate extract analyses may be warranted).

7.10 Perform the concentration (if necessary) using the Kuderna-Danish Technique (Secs. 7.11.1 through 7.11.6).

7.11 K-D technique

7.11.1 Assemble a Kuderna-Danish (K-D) concentrator (Sec. 4.3) by attaching a 10-mL concentrator tube to a 500-mL evaporation flask.

7.11.2 Attach the solvent vapor recovery glassware (condenser and collection device) (Sec. 4.4) to the Snyder column of the K-D apparatus following manufacturer's instructions.

7.11.3 Dry the extract by passing it through a drying column containing about 10 cm of anhydrous sodium sulfate. Collect the dried extract in a K-D concentrator. Rinse the Erlenmeyer flask, which contained the solvent extract, with 20 - 30 mL of methylene chloride and add it to the column to complete the quantitative transfer.

7.11.4 Add one or two clean boiling chips to the flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL of methylene chloride to the top of the column. Place the K-D apparatus on a hot water bath (15 - 20°C above the boiling point of the solvent) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 10 - 20 minutes. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes.

7.11.5 If a solvent exchange is required (as indicated in Table 1), momentarily remove the Snyder column, add 50 mL of the exchange solvent, a new boiling chip, and reattach the Snyder column. Alternatively, pour the exchange solvent into the top of the Snyder column while the concentrator remains on the water bath in Sec. 7.11.4. Concentrate the extract, as described in Sec. 7.11.4, raising the temperature of the water bath, if necessary, to maintain proper distillation.

7.11.6 Remove the Snyder column and rinse the flask and its lower joints into the concentrator tube with 1 - 2 mL of methylene chloride or exchange solvent. If sulfur crystals are a problem, proceed to Method 3660 for cleanup. The extract may be further concentrated by using the technique outlined in Sec. 7.12 or adjusted to 10.0 mL with the solvent last used.

7.12 If further concentration is indicated in Table 1, either the micro-Snyder column technique (7.12.1) or nitrogen blowdown technique (7.12.2) is used to adjust the extract to the final volume required.

7.12.1 Micro-Snyder column technique

If further concentration is indicated in Table 1, add another clean boiling chip to the concentrator tube and attach a two-ball micro-Snyder column. Prewet the column by adding 0.5 mL of methylene chloride or exchange solvent to the top of the column. Place the K-D apparatus in a hot water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature, as required, to complete the concentration in 5 - 10 minutes. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes. Remove the Snyder column, rinse the flask and its lower joints into the concentrator tube with 0.2 mL of methylene chloride or the exchange solvent, and adjust the final volume as indicated in Table 1, with solvent.

7.12.2 Nitrogen blowdown technique

7.12.2.1 Place the concentrator tube in a warm bath (35°C) and evaporate the solvent to the final volume indicated in Table 1, using a gentle stream of clean, dry nitrogen (filtered through a column of activated carbon).

CAUTION: New plastic tubing must not be used between the carbon trap and the sample, since it may introduce contaminants.

7.12.2.2 The internal wall of the tube must be rinsed several times with methylene chloride or appropriate solvent during the operation. During evaporation, the tube must be positioned to avoid water condensation (i.e., the solvent level should be below the level of the water bath). Under normal procedures, the extract must not be allowed to become dry.

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

7.13 The extract may now be analyzed for the target analytes using the appropriate determinative technique(s) (see Sec. 4.3 of this Chapter). If analysis of the extract will not be performed immediately, stopper the concentrator tube and store refrigerated. If the extract will be stored longer than 2 days it should be transferred to a vial with a PTFE-lined screw-cap or crimp top, and labeled appropriately.

8.0 QUALITY CONTROL

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

8.2 Refer to Chapter One for specific quality control procedures and Method 3500 for extraction and sample preparation procedures.

9.0 METHOD PERFORMANCE

Refer to the determinative methods for performance data.

10.0 REFERENCES

None.

TABLE 1
SPECIFIC EXTRACTION CONDITIONS FOR VARIOUS DETERMINATIVE METHODS

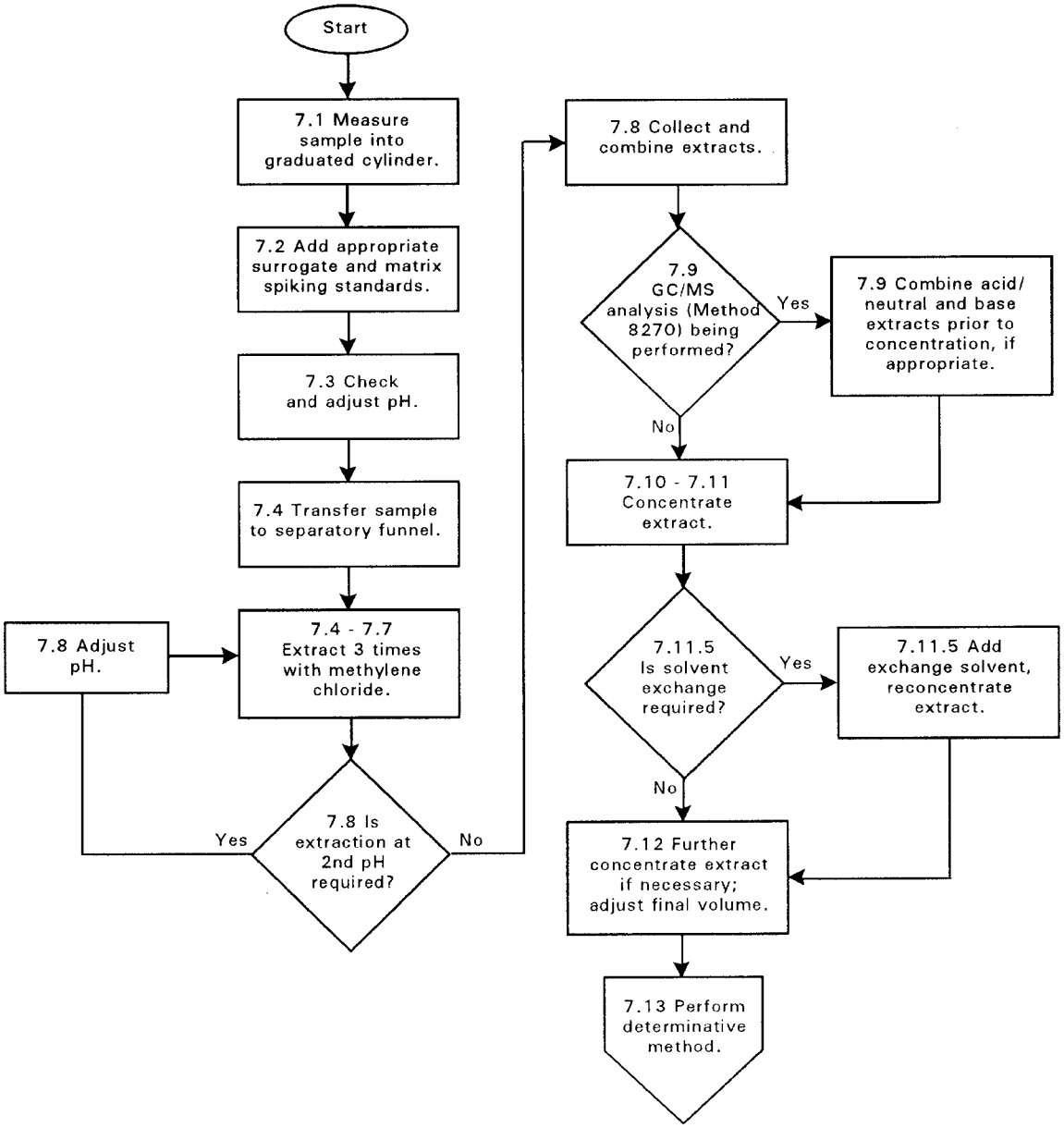
Determinative method	Initial extraction pH	Secondary extraction pH	Exchange solvent required for analysis	Exchange solvent required for cleanup	Volume of extract required for cleanup (mL)	Final extract volume for analysis (mL)[a]
8041	≤2	none	2-propanol	hexane	1.0	1.0, 0.5[b]
8061	5-7	none	hexane	hexane	2.0	10.0
8070	as received	none	methanol	methylene chloride	2.0	10.0
8081	5-9	none	hexane	hexane	10.0	10.0
8082	5-9	none	hexane	hexane	10.0	10.0
8091	5-9	none	hexane	hexane	2.0	1.0
8100	as received	none	none	cyclohexane	2.0	1.0
8111	as received	none	hexane	hexane	2.0	10.0
8121	as received	none	hexane	hexane	2.0	1.0
8141	as received	none	hexane	hexane	10.0	10.0
8270[c,d]	<2	>11	none	-	-	1.0
8310	as received	none	acetonitrile	-	-	1.0
8321	as received	none	methanol	-	-	1.0
8325	7.0	none	methanol	-	-	1.0
8410	as received	none	methylene chloride	methylene chloride	10.0	0.0 (dry)

[a] For methods where the suggested final extract volume is 10.0 mL, the volume may be reduced to as low as 1.0 mL to achieve lower detection limits.

[b] Phenols may be analyzed, by Method 8041, using a 1.0 mL 2-propanol extract by GC/FID. Method 8041 also contains an optional derivatization procedure for phenols which results in a 0.5 mL hexane extract to be analyzed by GC/ECD.

[c] The specificity of GC/MS may make cleanup of the extracts unnecessary. Refer to Method 3600 for guidance on the cleanup procedures available if required.

[d] Extraction pH sequence may be reversed to better separate acid and neutral waste components. Excessive pH adjustments may result in the loss of some analytes (see Sec. 3.2).


Start
7.1 Measure sample into graduated cylinder.
7.2 Add appropriate surrogate and matrix spiking standards.
7.3 Check and adjust pH.
7.4 Transfer sample to separatory funnel.
7.4 - 7.7 Extract 3 times with methylene chloride.
7.8 Adjust pH.
7.8 Is extraction at 2nd pH required?
Yes
No
7.8 Collect and combine extracts.
7.9 GC/MS analysis (Method 8270) being performed?
Yes
7.9 Combine acid/ neutral and base extracts prior to concentration, if appropriate.
No
7.10 - 7.11 Concentrate extract.
7.11.5 Is solvent exchange required?
Yes
7.11.5 Add exchange solvent, reconcentrate extract.
No
7.12 Further concentrate extract if necessary; adjust final volume.
7.13 Perform determinative method.