METHOD 3510B

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 Sec. 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.

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. 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 Under basic extraction conditions required to separate analytes for the packed columns of Method 8250, the decomposition of some analytes has been demonstrated. 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. Methods 3520/8270, 3510/8270, and 3510/8250, respectively, are preferred over Method 3520/8250 for the analysis of these classes of compounds.

4.0 APPARATUS AND MATERIALS

4.1 Separatory funnel - 2 liter, with Teflon stopcock.

4.2 Drying column - 20 mm ID Pyrex chromatographic column with Pyrex glass wool at bottom and a Teflon 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).

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

4.5 Water bath - Heated, with concentric ring cover, capable of temperature control ($\pm 5^{\circ}C$). The bath should be used in a hood.

4.6 Vials - 2 mL, glass with Teflon lined screw-caps or crimp tops.

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

4.8 Erlenmeyer flask - 250 mL.

4.9 Syringe - 5 mL.

4.10 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 (10N), NaOH. Dissolve 40 g NaOH in organic-free reagent water and dilute to 100 mL.

5.4 Sodium sulfate (granular, anhydrous), Na_2SO_4. Purify by heating at $400^{\circ}C$ 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.

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

5.6.1 Methylene chloride, CH_2Cl_2 - Pesticide quality or equivalent.

5.6.2 Hexane, C_6H_{14} - Pesticide quality or equivalent.

5.6.3 2-Propanol, $CH_3CH(OH)CH_3$ - Pesticide quality or equivalent.

5.6.4 Cyclohexane, C_6H_{12} - Pesticide quality or equivalent.

5.6.5 Acetonitrile, CH_3CN - Pesticide quality or equivalent.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

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

7.0 PROCEDURE

7.1 Using a 1 liter graduated cylinder, measure 1 liter (nominal) of sample and transfer it quantitatively to the separatory funnel. If high concentrations are anticipated, a smaller volume may be used and then diluted with organic-free reagent water to 1 liter. Add 1.0 mL of the surrogate standards to all samples, spikes, and blanks (see Method 3500 and the determinative method to be used, for details on the surrogate standard solution and the matrix spike solution). For the sample in each analytical batch selected for spiking, add 1.0 mL of the matrix spiking standard. For base/neutral-acid analysis, the amount added of the surrogates and matrix spiking compounds should result in a final concentration of 100 ng/µL of each base/neutral analyte and 200 ng/µL of each acid analyte in the extract to be analyzed (assuming a 1 µL injection). If Method 3640, Gel-Permeation Cleanup, is to be used, add twice the volume of surrogates and matrix spiking compounds since half the extract is lost due to loading of the GPC column.

7.2 Check the pH of the sample with wide-range pH paper and, if necessary, adjust the pH to that indicated in Table 1 for the specific determinative method that will be used to analyze the extract.

7.3 Add 60 mL of methylene chloride to the separatory funnel.

7.4 Seal and shake the separatory funnel vigorously for 1-2 minutes with periodic venting to release excess pressure.

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. Venting of the separatory funnel should be into a hood to avoid needless exposure of the analyst to solvent vapors.

7.5 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.

7.6 Repeat the extraction two more times using fresh portions of solvent (Secs. 7.3 through 7.5). Combine the three solvent extracts.

7.7 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. 7.3 through 7.5. Collect and combine the extracts and label the combined extract appropriately.

7.8 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).

7.9 Perform the concentration (if necessary) using the Kuderna-Danish (K-D) Technique (Secs. 7.10.1 through 7.10.4).

7.10 K-D Technique

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

7.10.2 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.

7.10.3 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. Concentrate the extract, as described in Sec. 7.11, raising the temperature of the water bath, if necessary, to maintain proper distillation.

7.10.4 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. 7.11 or adjusted to 10.0 mL with the solvent last used.

7.11 If further concentration is indicated in Table 1, either the micro-Snyder column technique (7.11.1) or nitrogen blowdown technique (7.11.2) is used to adjust the extract to the final volume required.

7.11.1 Micro-Snyder Column Technique

7.11.1.1 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 and rinse the flask and its lower joints into the concentrator tube with 0.2 mL of extraction solvent. Adjust the final volume to 1.0-2.0 mL, as indicated in Table 1, with solvent.

7.11.2 Nitrogen Blowdown Technique

7.11.2.1 Place the concentrator tube in a warm bath (35°C) and evaporate the solvent volume to 0.5 mL using a gentle stream of clean, dry nitrogen (filtered through a column of activated carbon).

<u>CAUTION</u>: New plastic tubing must not be used between the carbon trap and the sample, since it may introduce interferences.

7.11.2.2 The internal wall of the tube must be rinsed down several times with methylene chloride or appropriate solvent during the operation. During evaporation, the tube solvent level must be positioned to avoid water condensation. Under normal procedures, the extract must not be allowed to become dry.

<u>CAUTION</u>: When the volume of solvent is reduced below 1 ml, semivolatile analytes may be lost.

7.12 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 Teflon lined screw-cap or crimp top, and labeled appropriately.

8.0 QUALITY CONTROL

8.1 Any reagent blanks or matrix spike 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

9.1 Refer to the determinative methods for performance data.

10.0 REFERENCES

1. U.S. EPA 40 CFR Part 136, "Guidelines Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act; Final Rule and Interim Final Rule and Proposed Rule," October 26, 1984.

TABLE 1.
SPECIFIC EXTRACTION CONDITIONS FOR VARIOUS DETERMINATIVE METHODS

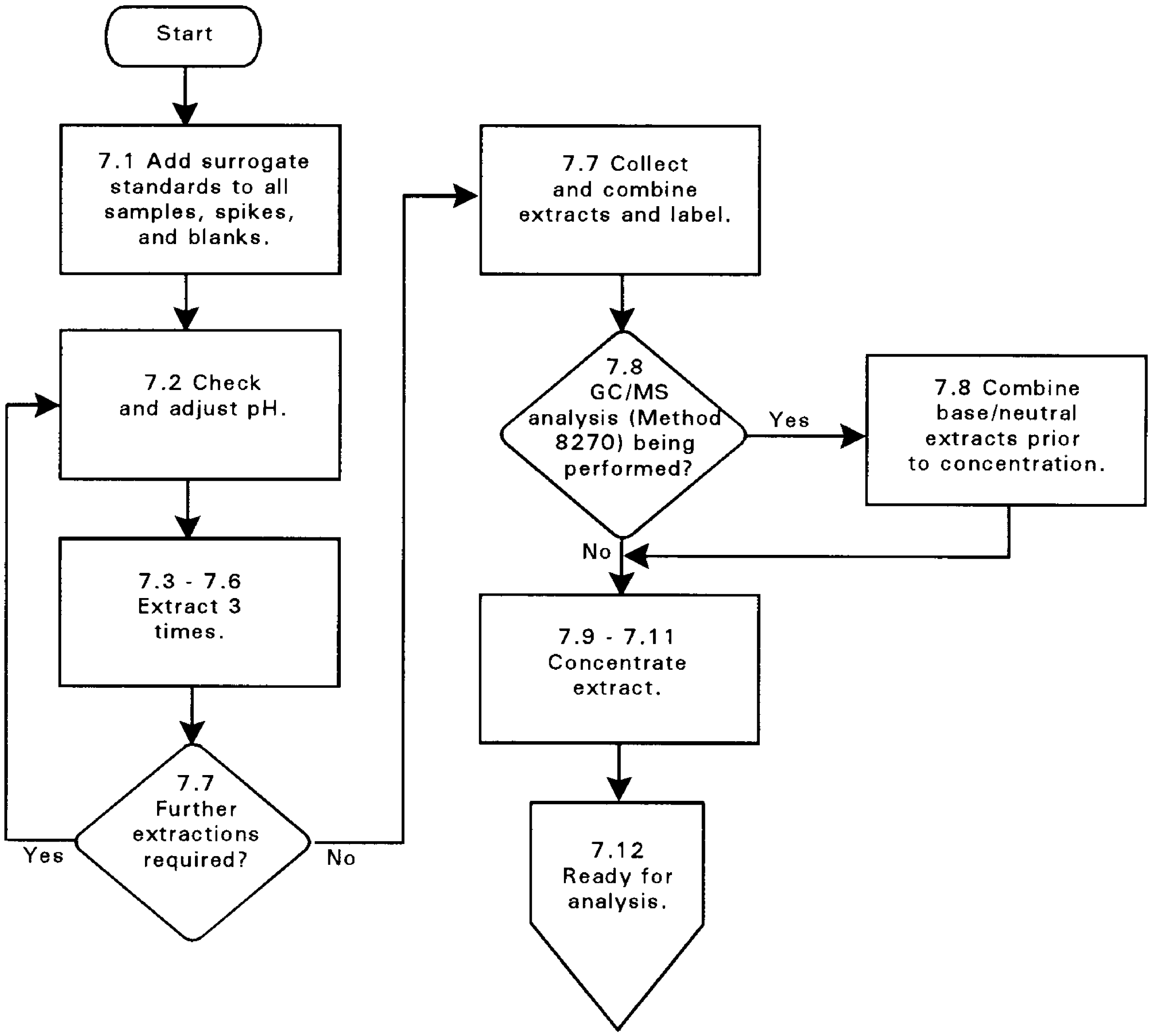
Determinative method	Initial extraction pH	Secondary extraction pH	Exchange solvent required for analysis	Exchange solvent required for cleanup	Volume of extract required for cleanup (mL)	Final extract volume for analysis (mL)
8040	≤2	none	2-propanol	hexane	1.0	1.0, 10.0[a]
8060	as received	none	hexane	hexane	2.0	10.0
8061	as received	none	hexane	hexane	2.0	10.0
8070	as received	none	methanol	methylene chloride	2.0	10.0
8080	5-9	none	hexane	hexane	10.0	10.0
8081	5-9	none	hexane	hexane	10.0	10.0
8090	5-9	none	hexane	hexane	2.0	1.0
8100	as received	none	none	cyclohexane	2.0	1.0
8110	as received	none	hexane	hexane	2.0	10.0
8120	as received	none	hexane	hexane	2.0	1.0
8121	as received	none	hexane	hexane	2.0	1.0
8140	6-8	none	hexane	hexane	10.0	10.0
8141	as received	none	hexane	hexane	10.0	10.0
8250[bc]	>11	<2	none	-	-	1.0
8270[bd]	<2	>11	none	-	-	1.0
8310	as received	none	acetonitrile	-	-	1.0
8321	as received	none	methanol	-	-	1.0
8410	as received	none	methylene chloride	methylene chloride	10.0	0.0 (dry)

a Phenols may be analyzed, by Method 8040, using a 1.0 mL 2-propanol extract by GC/FID. Method 8040 also contains an optional derivatization procedure for phenols which results in a 10 mL hexane extract to be analyzed by GC/ECD.

b 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.

c Loss of phthalate esters, organochlorine pesticides and phenols can occur under these extraction conditions (see Sec. 3.2).

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 Add surrogate standards to all samples, spikes, and blanks.
7.2 Check and adjust pH.
7.3 - 7.6 Extract 3 times.
7.7 Further extractions required?
Yes
No
7.7 Collect and combine extracts and label.
7.8 GC/MS analysis (Method 8270) being performed?
Yes
7.8 Combine base/neutral extracts prior to concentration.
No
7.9 - 7.11 Concentrate extract.
7.12 Ready for analysis.