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 steps described in Sec. 4.3 of Chapter Four.

1.2 This method is applicable to the isolation and concentration of water-insoluble and slightly soluble organics in preparation for a variety of chromatographic procedures.

1.3 Method 3520 is designed for extraction solvents with greater density than the sample. Continuous extraction devices are available for extraction solvents that are less dense than the sample. The analyst must demonstrate the effectiveness of any such automatic extraction device before employing it in sample extraction.

2.0 SUMMARY OF METHOD

2.1 A measured volume of sample, usually 1 liter, is placed into a continuous liquid-liquid extractor, adjusted, if necessary, to a specific pH (see Table 1), and extracted with organic solvent for 18-24 hours. The extract is dried, concentrated (if necessary), and, as necessary, exchanged into a solvent compatible with the cleanup or determinative method being employed (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 Continuous liquid-liquid extractor - Equipped with Teflon or glass connecting joints and stopcocks requiring no lubrication (Kontes 584200-0000, 584500-0000, 583250-0000, or equivalent).
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°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 Heating mantle - Rheostat controlled.

4.9 Syringe - 5 mL.

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), Na₂SO₄. Purify by heating at 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.

5.5 Sulfuric acid solution (1:1 v/v), H₂SO₄. Slowly add 50 mL of H₂SO₄ (sp. gr. 1.84) to 50 mL of organic-free reagent water.

5.6 Extraction/exchange solvents
   5.6.1 Methylene chloride, CH₂Cl₂ - Pesticide quality or equivalent.
   5.6.2 Hexane, C₆H₁₄ - Pesticide quality or equivalent.
   5.6.3 2-Propanol, (CH₃)₂CHOH - Pesticide quality or equivalent.
   5.6.4 Cyclohexane, C₆H₁₂ - Pesticide quality or equivalent.
   5.6.5 Acetonitrile, CH₃CN - 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 out 1 liter (nominal) of sample and transfer it quantitatively to the continuous extractor. If high concentrations are anticipated, a smaller volume may be used and then diluted with organic-free reagent water to 1 liter. 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. Pipet 1.0 mL of the surrogate standard spiking solution into each sample into the extractor and mix well. (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 of the surrogates and matrix spiking compounds added to the sample 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 Add 300-500 mL of methylene chloride to the distilling flask. Add several boiling chips to the flask.

7.3 Add sufficient water to the extractor to ensure proper operation and extract for 18-24 hours.

7.4 Allow to cool; then detach the boiling flask. If extraction at a secondary pH is not required (see Table 1), the extract is dried and concentrated using one of the techniques referred to in Sec. 7.7.

7.5 Carefully, while stirring, adjust the pH of the aqueous phase to the second pH indicated in Table 1. Attach a clean distilling flask containing 500 mL of methylene chloride to the continuous extractor. Extract for 18-24 hours, allow to cool, and detach the distilling flask.

7.6 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 and base compounds at low concentrations must be determined, separate extract analyses may be warranted).

7.7 Perform concentration (if necessary) using the Kuderna-Danish (K-D) Technique (Secs. 7.8.1 through 7.8.4).

7.8 K-D Technique

7.8.1 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 flask which contained the solvent extract with 20-30 mL of methylene chloride and add it to the column to complete the quantitative transfer.

7.8.2 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. Remove the Snyder column and rinse the flask and its lower joints into the concentrator tube with 1-2 mL of extraction solvent.

7.8.3 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. 7.9, raising the temperature of the water bath, if necessary, to maintain proper distillation.

7.8.4 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 techniques outlined in Sec. 7.9 or adjusted to 10.0 mL with the solvent last used.

7.9 If further concentration is indicated in Table 1, either the micro-Snyder column technique (7.9.1) or nitrogen blowdown technique (7.9.2) is used to adjust the extract to the final volume required.

7.9.1 Micro-Snyder Column Technique

7.9.1.1 Add another one or two clean boiling chips 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 exchange solvent, and adjust the final volume to 1.0 to 2.0 mL, as indicated in Table 1, with solvent.

7.9.2 Nitrogen Blowdown Technique

7.9.2.1 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).

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

7.9.2.2 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.

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

7.10 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, matrix spike, 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

9.1 Refer to the determinative methods for performance data.

10.0 REFERENCES

### Table 1.
**Specific Extraction Conditions for Various Determinative Methods**

<table>
<thead>
<tr>
<th>Determinative method (mL)</th>
<th>Initial extraction pH</th>
<th>Secondary extraction pH</th>
<th>Exchange solvent required for analysis</th>
<th>Exchange solvent required for cleanup</th>
<th>Volume of extract required for cleanup (mL)</th>
<th>Final extract volume for analysis</th>
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<tr>
<td>8040</td>
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<td>2-propanol</td>
<td>hexane</td>
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<td>&lt;2</td>
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</tr>
</tbody>
</table>

---

**Notes:**

- **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** If further separation of major acid and neutral components is required, Method 3650, Acid-Base Partition Cleanup, is recommended. Reversal of the Method 8270 pH sequence is not recommended as analyte losses are more severe under the base first continuous extraction (see Sec. 3.2).
METHOD 3520B
CONTINUOUS LIQUID-LIQUID EXTRACTION

Start

7.1 Add appropriate surrogate and matrix spiking solutions.

7.2 Add methylene chloride to distilling flask.

7.3 Add reagent water to extractor; extract for 18-24 hours.

7.5 Adjust pH of aqueous phase; extract for 18-24 hours with clean flask.

7.6 Combine acid and base/neutral extracts prior to concentration.

7.7 - 7.8 Concentrate extract.

7.8.3 Is solvent exchange required?

Yes

7.8.3 Add exchange solvent; concentration extract.

No

7.9 Further concentrate extract if necessary; adjust final volume.

7.10 Analyze using organic techniques.

7.6 GC/MS analysis (Method 8270) performed?

Yes

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No