

METHOD 3650A

ACID-BASE PARTITION CLEANUP1.0 SCOPE AND APPLICATION

1.1 Method 3650 was formerly Method 3530 in the second edition of this manual.

1.2 Method 3650 is a liquid-liquid partitioning cleanup method to separate acid analytes, e.g. organic acids and phenols, from base/neutral analytes, e.g. amines, aromatic hydrocarbons, and halogenated organic compounds, using pH adjustment. It may be used for cleanup of petroleum waste prior to analysis or further cleanup (e.g., alumina cleanup). The following compounds can be separated by this method:

Compound Name	CAS No. ^a	Fraction
Benz(a)anthracene	56-55-3	Base-neutral
Benzo(a)pyrene	50-32-8	Base-neutral
Benzo(b)fluoranthene	205-99-2	Base-neutral
Chlordane	57-74-9	Base-neutral
Chlorinated dibenzodioxins		Base-neutral
2-Chlorophenol	95-57-8	Acid
Chrysene	218-01-9	Base-neutral
Creosote	8001-58-9	Base-neutral and Acid
Cresol(s)		Acid
Dichlorobenzene(s)		Base-neutral
Dichlorophenoxyacetic acid	94-75-7	Acid
2,4-Dimethylphenol	105-67-9	Acid
Dinitrobenzene	25154-54-5	Base-neutral
4,6-Dinitro-o-cresol	534-52-1	Acid
2,4-Dinitrotoluene	121-14-2	Base-neutral
Heptachlor	76-44-8	Base-neutral
Hexachlorobenzene	118-74-1	Base-neutral
Hexachlorobutadiene	87-68-3	Base-neutral
Hexachloroethane	67-72-1	Base-neutral
Hexachlorocyclopentadiene	77-47-4	Base-neutral
Naphthalene	91-20-3	Base-neutral
Nitrobenzene	98-95-3	Base-neutral
4-Nitrophenol	100-02-7	Acid
Pentachlorophenol	87-86-5	Acid
Phenol	108-95-2	Acid
Phorate	298-02-2	Base-neutral
2-Picoline	109-06-8	Base-neutral
Pyridine	110-86-1	Base-neutral
Tetrachlorobenzene(s)		Base-neutral
Tetrachlorophenol(s)		Acid
Toxaphene	8001-35-2	Base-neutral
Trichlorophenol(s)		Acid
2,4,5-TP (Silvex)	93-72-1	Acid

^a Chemical Abstract Services Registry Number.

2.0 SUMMARY OF METHOD

2.1 The solvent extract from a prior solvent extraction method is shaken with water that is strongly basic. The acid analytes partition into the aqueous layer, whereas, the basic and neutral compounds stay in the organic solvent. The base/neutral fraction is concentrated and is then ready for further cleanup, if necessary, or analysis. The aqueous layer is acidified and extracted with an organic solvent. This extract is concentrated (if necessary) and is then ready for analysis of the acid analytes.

3.0 INTERFERENCES

3.1 More extensive procedures than those outlined in this method may be necessary for reagent purification.

3.2 A method blank must be run for the compounds of interest prior to use of the method. The interferences must be below the method detection limit before this method is applied to actual samples.

4.0 APPARATUS AND MATERIALS

4.1 Drying column - 20 mm ID Pyrex chromatographic column with Pyrex glass wool at bottom, or equivalent.

NOTE: Fritted glass discs are difficult to clean after highly contaminated extracts have been passed through them. Columns without frits are recommended. 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.

4.2 Kuderna-Danish (K-D) apparatus

4.2.1 Concentrator tube - 10 mL graduated (Kontes K570050-1025 or equivalent). A ground glass stopper is used to prevent evaporation of the extracts.

4.2.2 Evaporation flask - 500 mL (K-570001-0500 or equivalent). Attach to concentrator tube with springs, clamps, or equivalent.

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

4.2.4 Snyder column - Two ball micro (Kontes K569001-0219 or equivalent).

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

4.3 Vials - Glass, 2 mL capacity with Teflon lined screw-caps or crimp tops.

4.4 Water bath - Heated, concentric ring cover, temperature control of $\pm 2^{\circ}\text{C}$. Use this bath in a hood.

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

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

4.7 Separatory funnel - 125 mL.

4.8 Erlenmeyer flask - 125 mL.

5.0 REAGENTS

5.1 Reagent grade inorganic chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all inorganic 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.

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, NaOH, (10N) - Dissolve 40 g of sodium hydroxide in 100 mL of organic-free reagent water.

5.4 Sulfuric acid, H₂SO₄, (1:1 v/v in water) - Slowly add 50 mL H₂SO₄ to 50 mL of organic-free reagent water.

5.5 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.

5.6 Solvents:

5.6.1 Methylene chloride, CH₂Cl₂ - Pesticide quality or equivalent.

5.6.2 Acetone, CH₃COCH₃ - Pesticide quality or equivalent.

5.6.3 Methanol, CH₃OH - Pesticide quality or equivalent.

5.6.4 Diethyl Ether, C₂H₅OC₂H₅ - Pesticide quality or equivalent. Must be free of peroxides as indicated by test strips (EM Quant, or equivalent). Procedures for removal of peroxides are provided with the test strips. After cleanup, 20 mL of ethyl alcohol preservative must be added to each liter of ether.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

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

7.0 PROCEDURE

7.1 Place 10 mL of the solvent extract from a prior extraction procedure into a 125 mL separatory funnel.

7.2 Add 20 mL of methylene chloride to the separatory funnel.

7.3 Slowly add 20 mL of prechilled organic-free reagent water which has been previously adjusted to a pH of 12-13 with 10N sodium hydroxide.

7.4 Seal and shake the separatory funnel for at least 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. The separatory funnel should be vented into a hood to prevent unnecessary exposure of the analyst to the organic vapor.

7.5 Allow the organic layer to separate from the aqueous 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.

7.6 Separate the aqueous phase and transfer it to a 125 mL Erlenmeyer flask. Repeat the extraction two more times using 20 mL aliquots of dilute sodium hydroxide (pH 12-13). Combine the aqueous extracts.

7.7 Water soluble organic acids and phenols will be primarily in the aqueous phase. Base/neutral analytes will be in the methylene chloride. If the analytes of interest are only in the aqueous phase, discard the methylene chloride and proceed to Section 7.8. If the analytes of interest are only in the methylene chloride, discard the aqueous phase and proceed to Section 7.10.

7.8 Externally cool the 125 mL Erlenmeyer flask with ice while adjusting the aqueous phase to a pH of 1-2 with sulfuric acid (1:1). Quantitatively transfer the cool aqueous phase to a clean 125 mL separatory funnel. Add 20 mL of methylene chloride to the separatory funnel and shake for at least 2 minutes. Allow the methylene chloride to separate from the aqueous phase and collect the methylene chloride in an Erlenmeyer flask.

7.9 Add 20 mL of methylene chloride to the separatory funnel and extract at pH 1-2 a second time. Perform a third extraction in the same manner combining the extracts in the Erlenmeyer flask.

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

7.11 Dry both acid and base/neutral fractions by passing them through a drying column containing about 10 cm of anhydrous sodium sulfate. Collect the dried fractions in K-D concentrators. Rinse the Erlenmeyer flasks which contained the solvents and the columns with 20 mL of methylene chloride to complete the quantitative transfer.

7.12 Concentrate both acid and base/neutral fractions as follows: Add one or two boiling chips to the flask and attach a three ball macro-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 (80-90°C) so that the concentrator tube is partially immersed in the warm water. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15-20 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 cool. Remove the Snyder column and rinse the flask and its lower joints into the concentrator tube with 1-2 mL of methylene chloride. Concentrate the extract to the final volume using either the micro-Snyder column technique (7.12.1) or nitrogen blowdown technique (7.12.2).

7.12.1 Micro-Snyder Column Technique

7.12.1.1 Add another one or two 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 to the top of the column. Place the K-D apparatus in a hot water bath (80-90°C) 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 the liquid reaches 0.5 mL, remove the K-D apparatus and allow it to cool. Remove the Snyder column and rinse the flask and its lower joints into the concentrator tube with 0.2 mL of methylene chloride. Adjust the final volume to 1 mL with methylene chloride.

7.12.2 Nitrogen Blowdown Technique

7.12.2.1 Place the concentrator tube in a warm water bath (35°C) and evaporate the solvent volume to 1.0-2.0 mL 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.

7.12.2.2 The internal wall of the concentrator tube must be rinsed down several times with the appropriate solvent during the operation. During evaporation, the tube solvent level must be positioned to avoid condensation water. 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 acid fraction is now ready for analysis. If the base/neutral fraction requires further cleanup by the alumina column cleanup for petroleum waste (Method 3611), the solvent may have to be changed to hexane. If a solvent exchange is required, momentarily remove the Snyder column, add approximately 5 mL of the exchange solvent and a new boiling chip, and reattach the Snyder column. Concentrate the extract as described in Section 7.12.1.1, raising the temperature of the water bath, if necessary, to maintain proper distillation. When the apparent volume again reaches 1 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes. Repeat the exchange 2 more times. If no further cleanup of the base/neutral extract is required, it is also ready for analysis.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One for general quality control procedures and Method 3600 for cleanup procedures.

8.2 The analyst must demonstrate that the compounds of interest are being quantitatively recovered before applying this method to actual samples.

8.3 For samples that are cleaned using this method, the associated quality control samples must be processed through this cleanup method.

9.0 METHOD PERFORMANCE

9.1 Refer to the determinative methods for performance data.

10.0 REFERENCES

1. Test Methods: Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater; U.S. Environmental Protection Agency. Office of Research and Development. Environmental Monitoring and Support Laboratory. ORD Publication Offices of Center for Environmental Research Information: Cincinnati, OH, 1982; EPA-600/4-82-057.

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