

METHOD 3610A

ALUMINA COLUMN CLEANUP

1.0 SCOPE AND APPLICATION

1.1 Scope: Alumina is a highly porous and granular form of aluminum oxide. It is available in three pH ranges (basic, neutral, and acidic) for use in column chromatography. It is used to separate analytes from interfering compounds of a different chemical polarity.

1.2 General Applications (Gordon and Ford):

1.2.1 **Basic (B)** pH (9-10): USES: Basic and neutral compounds stable to alkali, alcohols, hydrocarbons, steroids, alkaloids, natural pigments. DISADVANTAGES: Can cause polymerization, condensation, and dehydration reactions; cannot use acetone or ethyl acetate as eluants.

1.2.2 **Neutral (N)**: USES: Aldehydes, ketones, quinones, esters, lactones, glycoside. DISADVANTAGES: Considerably less active than the basic form.

1.2.3 **Acidic (A)** pH (4-5): USES: Acidic pigments (natural and synthetic), strong acids (that otherwise chemisorb to neutral and basic alumina).

1.2.4 **Activity grades**: Acidic, basic, or neutral alumina can be prepared in various activity grades (I to V), according to the Brockmann scale, by addition of water to Grade 1 (prepared by heating at 400-450°C until no more water is lost). The Brockmann scale (Gordon and Ford, p. 374) is reproduced below:

Water added (wt. %):	0	3	6	10	15
Activity grade:	I	II	III	IV	V
RF (p-aminoazobenzene):	0.0	0.13	0.25	0.45	0.55

1.3 Specific applications: This method includes guidance for cleanup of sample extracts containing phthalate esters and nitrosamines. For alumina column cleanup of petroleum wastes, see Method 3611, Alumina Column Cleanup of Petroleum Wastes.

2.0 SUMMARY OF METHOD

2.1 The column is packed with the required amount of adsorbent, topped with a water adsorbent, and then loaded with the sample to be analyzed. Elution of the analytes is effected with a suitable solvent(s), leaving the interfering compounds on the column. The eluate is then concentrated (if necessary).

3.0 INTERFERENCES

3.1 A reagent blank should be performed for the compounds of interest

prior to the use of this method. The level of interferences must be below the method detection limit before this method is performed on actual samples.

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

4.0 APPARATUS AND MATERIALS

4.1 Chromatography column: 300 mm x 10 mm ID, 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.

4.2 Beakers: 500 mL.

4.3 Reagent bottle: 500 mL.

4.4 Muffle furnace.

4.5 Kuderna-Danish (K-D) apparatus:

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

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

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

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

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

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

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

4.8 Vials: Glass, 2 mL capacity, with Teflon lined screw caps or crimp tops.

4.9 Erlenmeyer flasks: 50 and 250 mL

5.0 REAGENTS

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

5.2 Sodium sulfate: Sodium sulfate (granular, anhydrous), Na_2SO_4 . 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.

5.3 Eluting solvents:

5.3.1 Diethyl Ether, $\text{C}_2\text{H}_5\text{OC}_2\text{H}_5$. 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.

5.3.2 Methanol, CH_3OH - Pesticide quality or equivalent.

5.3.3 Pentane, $\text{CH}_3(\text{CH}_2)_3\text{CH}_3$ - Pesticide quality or equivalent.

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

5.3.5 Methylene chloride, CH_2Cl_2 - Pesticide quality or equivalent.

5.4 Alumina:

5.4.1 For cleanup of phthalate extracts: Alumina-Neutral, activity Super I, W200 series (ICN Life Sciences Group, No. 404583, or equivalent). To prepare for use, place 100 g of alumina into a 500 mL beaker and heat for approximately 16 hr at 400°C . After heating, transfer to a 500 mL reagent bottle. Tightly seal and cool to room temperature. When cool, add 3 mL of organic-free reagent water. Mix thoroughly by shaking or rolling for 10 min and let it stand for at least 2 hr. Keep the bottle sealed tightly.

5.4.2 For cleanup of nitrosamine extracts: Alumina-Basic, activity Super I, W200 series (ICN Life Sciences Group, No. 404571, or equivalent). To prepare for use, place 100 g of alumina into a 500 mL reagent bottle and add 2 mL of organic-free reagent water. Mix the alumina preparation thoroughly by shaking or rolling for 10 min and let it stand for at least 2 hr. The preparation should be homogeneous before use. Keep the bottle sealed tightly to ensure proper activity.

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 Phthalate esters:

7.1.1 Reduce the sample extract volume to 2 mL prior to cleanup. The extract solvent must be hexane.

7.1.2 Place approximately 10 g of alumina into a 10 mm ID chromatographic column. Tap the column to settle the alumina and add 1-2 cm of anhydrous sodium sulfate to the top.

7.1.3 Pre-elute the column with 40 mL of hexane. The rate for all elutions should be about 2 mL/min. Discard the eluate and, just prior to exposure of the sodium sulfate layer to the air, quantitatively transfer the 2 mL sample extract onto the column using an additional 2 mL of hexane to complete the transfer. Just prior to exposure of the sodium sulfate layer to the air, add 35 mL of hexane and continue the elution of the column. Discard this hexane eluate.

7.1.4 Next, elute the column with 140 mL of 20% ethyl ether in hexane (v/v) into a 500 mL K-D flask equipped with a 10 mL concentrator tube. Concentrate the collected fraction using the Kuderna-Danish technique. No solvent exchange is necessary. Adjust the volume of the cleaned up extract to whatever volume is required (10.0 mL for Method 8060) and analyze. Compounds that elute in this fraction are as follows:

- Bis(2-ethylhexyl) phthalate
- Butyl benzyl phthalate
- Di-n-butyl phthalate
- Diethyl phthalate
- Dimethyl phthalate
- Di-n-octyl phthalate.

7.2 Nitrosamines:

7.2.1 Reduce the sample extract to 2 mL prior to cleanup.

7.2.2 Diphenylamine, if present in the original sample extract, must be separated from the nitrosamines if N-nitrosodiphenylamine is to be determined by this method.

7.2.3 Place approximately 12 g of the alumina preparation into a 10 mm ID chromatographic column. Tap the column to settle the alumina and add 1-2 cm of anhydrous sodium sulfate to the top.

7.2.4 Pre-elute the column with 10 mL of ethyl ether/pentane (3:7)(v/v). Discard the eluate (about 2 mL) and, just prior to exposure of the sodium sulfate layer to the air, quantitatively transfer the 2 mL sample extract onto the column using an additional 2 mL of pentane to complete the transfer.

7.2.5 Just prior to exposure of the sodium sulfate layer to the air, add 70 mL of ethyl ether/pentane (3:7)(v/v). Discard the first 10 mL of

eluate. Collect the remainder of the eluate in a 500 mL K-D flask equipped with a 10 mL concentrator tube. This fraction contains N-nitroso-di-n-propylamine.

7.2.6 Next, elute the column with 60 mL of ethyl ether/pentane (1:1)(v/v), collecting the eluate in a second 500 mL K-D flask equipped with a 10 mL concentrator tube. Add 15 mL of methanol to the K-D flask. This fraction will contain N-nitrosodimethylamine, most of the N-nitroso-di-n-propylamine, and any diphenylamine that is present.

7.2.7 Concentrate both fractions using the Kuderna-Danish Technique (if necessary), using pentane to prewet the Snyder column. When the apparatus is cool, remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1-2 mL of pentane. Adjust the final volume to whatever is required in the appropriate determinative method (Section 4.3 of this chapter). Analyze the fractions.

8.0 QUALITY CONTROL

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

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

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

9.0 METHOD PERFORMANCE

9.1 Performance data are not available.

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.

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