METHOD 3610B

ALUMINA CLEANUP

## 1.0 SCOPE AND APPLICATION

1.1 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 chromatographic cleanup procedures. It is used to separate analytes from interfering compounds of a different chemical polarity.

1.2 Each of the three pH ranges of alumina has different uses and disadvantages as a cleanup procedure.

1.2.1 Basic alumina has a pH of 9-10. It is used to separate basic and neutral compounds that are stable to alkali, alcohols, hydrocarbons, steroids, alkaloids, natural pigments. Its disadvantages are that it can cause polymerization, condensation, and dehydration reactions, and one cannot use acetone or ethyl acetate as eluants.

1.2.2 Neutral alumina has a pH of 6-8. It is used to separate aldehydes, ketones, quinones, esters, lactones, glycoside. Its disadvantage is that is it considerably less active than the basic form.

1.2.3 Acidic alumina has a pH of 4-5. It is used to separate acidic pigments (natural and synthetic), and strong acids (that otherwise chemisorb to neutral and basic alumina). This method does not address the use of acid alumina.

1.3 Basic, neutral, and acidic alumina can be prepared in various activity grades (I to V), based on the Brockmann scale reproduced below. Grade I is prepared by heating alumina until no more water is lost (typically overnight at 400-450°C, but other time-temperature relationships may be employed). The other grades (II-V) are prepared by adding water to Grade I to deactivate it.

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

where RF is the retention factor for p-aminoazobenzene.

1.4 Alumina cleanup may be accomplished using a glass chromatographic column packed with alumina or using solid-phase extraction cartridges containing alumina.

1.5 This method includes procedures for cleanup of sample extracts containing phthalate esters and nitrosamines. See Method 3611, Alumina Column Cleanup of Petroleum Wastes, for alumina cleanup of petroleum wastes.

1.6 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 This method describes procedures for alumina cleanup of solvent extracts of environmental samples. It provides the option of using either traditional column chromatography techniques or solid-phase extraction cartridges. Generally, the traditional column chromatography technique uses larger amounts of adsorbent and, therefore, has a greater cleanup capacity.

2.2 In the column cleanup protocol, the column is packed with the appropriate amount of adsorbent, topped with a water adsorbent, and then loaded with the sample extract. Elution of the analytes is effected with a suitable solvent(s), leaving the interfering compounds on the column. The eluate may be further concentrated prior to gas chromatographic analysis.

2.3 The cartridge cleanup procedure uses solid-phase extraction cartridges containing 40 µm particles of alumina (60 Å pores). Each cartridge is washed with solvent immediately prior to use. The sample extract is loaded onto the cartridge which is then eluted with suitable solvent(s). A vacuum manifold is needed to obtain reproducible results. The eluate may be further concentrated prior to gas chromatographic analysis.

2.4 The phthalate esters may be considered either the analytes of interest or the interferants, depending on which eluant fraction is analyzed.

## 3.0 INTERFERENCES

3.1 A reagent blank should be prepared and analyzed 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 The procedures for reagent purification outlined here should be considered to be the minimum requirements for use of this method. More extensive procedures may be necessary to achieve acceptable levels of interferences for some analytes.

## 4.0 APPARATUS AND MATERIALS

4.1 Chromatography column - 300 mm x 10 mm ID, with a polytetrafluoroethylene (PTFE) stopcock.

NOTE: Columns with fritted glass discs are difficult to clean once the column has been used to process highly contaminated extracts. Columns without frits may be purchased, and a small pad of Pyrex® glass wool may be used 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 - Appropriates sizes

4.3 Reagent bottle - Appropriate sizes

4.4 Muffle furnace - capable of maintaining 400°C.

4.5 Vials - Glass, 2-mL capacity, with PTFE-lined screw caps or crimp tops.

4.6 Vacuum manifold - VacElute Manifold SPS-24 (Analytichem International), Visiprep (Supelco, Inc.) or equivalent, consisting of glass vacuum basin, collection rack and funnel, collection vials, replaceable stainless steel delivery tips, built-in vacuum bleed valve and gauge. The system is connected to a vacuum pump or water aspirator through a vacuum trap made from a 500-mL sidearm flask fitted with a one-hole stopper and glass tubing. The manifold is needed for use of the cartridge cleanup protocol.

4.7 Top-loading balance - capable of weighing 0.01 g.

## 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. A method blank must be analyzed in order to demonstrate that there is no interference from the sodium sulfate.

5.3 Eluting solvents - all solvents must be pesticide quality or equivalent.

5.3.1 Diethyl Ether, $C_2H_5OC_2H_5$. 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, $CH_3OH$

5.3.3 Pentane, $CH_3(CH_2)_3CH_3$

5.3.4 Hexane, $C_6H_{14}$

5.3.5 Methylene chloride, $CH_2Cl_2$

5.3.6 Acetone, $CH_3COCH_3$

5.4 Granular alumina, for column cleanup procedure

5.4.1 Neutral alumina, for cleanup of phthalates, activity Super I, W200 series (ICN Life Sciences Group, No. 404583 or equivalent). To activate, 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 the bottle 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. The preparation should be homogeneous before use. Keep the bottle sealed tightly to ensure proper activity. Super I alumina cited above is a Grade I reagent with a very high binding capacity. The neutral alumina employed in this method may be prepared from reagents other than Super I, provided that adequate performance can be demonstrated.

5.4.2 Basic alumina, for cleanup of nitrosamines, activity Super I, W200 series (ICN Life Sciences Group, No. 404571, or equivalent). To activate, place 100 g of alumina into a 500-mL reagent bottle and add 2 mL of organic-free reagent water. Mix 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. Super I alumina cited above is a Grade I reagent with a very high binding capacity. The basic alumina employed in this method may be prepared from reagents other than Super I, provided that adequate performance can be demonstrated.

5.5 Alumina cartridges - 40 µm particles, 60 Å pores, for cleanup of phthalates. The cartridges from which this method were developed consist of 6-mL serological-grade polypropylene tubes, with the 1 g of alumina held between two polyethylene or stainless steel frits with 20 µm pores. Cartridges containing 0.5 g and 2.0 g of alumina are available, however, the compound elution patterns need to be verified when cartridges containing other than 1 g of alumina are used.

## 6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

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

## 7.0 PROCEDURE

The chromatographic separation procedures for the phthalate esters may be accomplished by either the column cleanup approach (Sec. 7.3) or the cartridge cleanup approach (Sec. 7.4). The procedure for the nitrosamines includes only the column cleanup approach (Sec. 7.5). Sec. 7.1 describes the procedures for assembling and conditioning the alumina cartridges. Sec. 7.2 describes general procedures for handling sample extracts prior to cleanup.

The column chromatography procedures employ a larger amount of alumina than the cartridge procedures and, therefore, have a greater cleanup capacity. Samples that exhibit greater degrees of interferences should be cleaned up using the column procedures. However, both techniques have limitations on the amount of interferences that they can remove.

### 7.1 Cartridge set-up and conditioning

7.1.1 Arrange the cartridges on the manifold in the closed-valve position.

7.1.2 Turn on the vacuum pump and set the vacuum to 10 in (254 mm) of Hg. Do not exceed the manufacturer's recommendation for manifold vacuum. Flow rates may be controlled by opening and closing cartridge valves.

7.1.3 Condition the cartridges by adding 4 mL of hexane to each cartridge. Slowly open the cartridge valves to allow hexane to pass through the sorbent beds to the lower frits. Allow a few drops per cartridge to pass through the manifold to remove all air bubbles. Close the valves and allow the solvent to soak the entire sorbent bed for 5 minutes. Do not turn off the vacuum.

7.1.4 Slowly open cartridge valves to allow the hexane to pass through the cartridges. Close the cartridge valves when there is still at least 1 mm of solvent above the sorbent bed. Do not allow cartridges to become dry. If cartridges go dry, repeat the conditioning step.

7.2 Handling sample extracts

7.2.1 Reduce the sample extract volume to 2 mL (per 3500 series methods) prior to cleanup. The extract solvent should be hexane for the phthalate esters and methylene chloride for the nitrosamines.

7.2.2 Allow extract to reach room temperature if it was in cold storage. Inspect the extract visually to ensure that there are no particulates or phase separations and that no evaporative loss has taken place. If crystals of sulfur are visible or if the presence of sulfur is suspected, proceed with Method 3660.

7.3 Column procedure for phthalate esters

7.3.1 Place approximately 10 g of neutral alumina (Sec. 5.4.1) 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.

7.3.2 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 (Sec. 7.2) onto the column using an additional 2 mL of hexane to complete the transfer.

7.3.3 Just prior to exposure of the sodium sulfate layer to the air, add 35 mL of hexane to the column and continue the elution of the column. Discard this hexane eluate.

7.3.4 Elute the column with 140 mL of ethyl ether/hexane (20/80, v/v) and collect this fraction in a flask for concentration.

7.3.5 Concentrate the collected fraction to the volume required by the determinative method (e.g., 2 mL for Method 8061), using the techniques described in the appropriate 3500 series method. No solvent exchange is necessary. Compounds that elute in this fraction are:

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

7.4 Cartridge procedure for phthalate esters

NOTE: If organochlorine pesticides are known to be present in the extract, Florisil cartridges (Method 3620) are recommended instead of Alumina cartridges.

7.4.1 Using 1-g alumina cartridges, condition the cartridges with hexane as described in Sec. 7.1.

7.4.2 Transfer the extract (Sec. 7.2) to the cartridge. Open the cartridge valve to allow the extract to pass through the cartridge bed at approximately 2 mL/minute.

7.4.3 When the entire extract has passed through the cartridge, but before the cartridge becomes dry, rinse the sample vial with an additional 0.5 mL of solvent, and add the rinse to the cartridge to complete the quantitative transfer.

7.4.4 Close the cartridge valve and turn off the vacuum after the solvent has passed through, ensuring that the cartridge never gets dry.

7.4.5 Place a 5-mL vial or volumetric flask into the sample rack corresponding to the cartridge position. Attach a solvent-rinsed stainless steel solvent guide to the manifold cover and align it with the collection vial.

7.4.6 Add 10 mL of acetone/hexane (20/80, v/v) to the cartridge. Turn on the vacuum pump and adjust the pump pressure to 10 in (254 mm) of Hg. Allow the solvent to soak the sorbent bed for 1 minute or less. Slowly open the cartridge valve and collect the eluate into the collection vial.

7.4.7 Adjust the final volume of the eluant to the volume listed in the determinative method, using the techniques described in the appropriate 3500 series method.

7.5 Column procedure for nitrosamines

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

7.5.2 Place approximately 12 g of basic alumina (Sec. 5.4.2) 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.

7.5.3 Pre-elute the column with 10 mL of ethyl ether/pentane (30/70, 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 (Sec. 7.2, in methylene chloride) onto the column using an additional 2 mL of pentane to complete the transfer.

7.5.4 Just prior to exposure of the sodium sulfate layer to the air, add 70 mL of ethyl ether/pentane (30/70, v/v). Discard the first 10 mL of eluate. Collect the remainder of the eluate in a flask for concentration.

This fraction contains some N-nitroso-di-n-propylamine, if any is present in the sample extract.

7.5.5 Elute the column with 60 mL of ethyl ether/pentane (50/50, v/v), collecting the eluate in a second flask for concentration. Add 15 mL of methanol to the flask.

This fraction will contain N-nitrosodimethylamine, most of the N-nitroso-di-n-propylamine, and any diphenylamine that is present.

7.5.6 Concentrate both fractions to the final volumes listed in the appropriate determinative method, using the techniques described in the appropriate 3500 series method.

## 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 must demonstrate that the compounds of interest are quantitatively (70-130%) recovered before applying this method to actual samples. This test applies to both the column cleanup and cartridge cleanup procedures. A recovery check needs to be performed using standards of the target analytes at a known concentration near the regulatory limit or action level for the target analyte.

8.2.1 This test should be conducted on each batch of alumina following its activation (Sec. 5.4).

8.2.2 The efficiency of each lot of the solid-phase extraction cartridges needs to be verified. Only lots of cartridges from which the spiked analytes are quantitatively recovered may be used to process the samples. A check should also be performed at least once on each individual lot of cartridges and at least once for every 300 cartridges of a particular lot, whichever frequency is greater.

8.3 The quality control samples associated with sample extracts that are cleaned up using this method, should also be processed through this cleanup method.

## 9.0 METHOD PERFORMANCE

Table 1 provides data for the recoveries of phthalate esters obtained from 1-g alumina cartridges.

## 10.0 REFERENCES

1. U.S. EPA, "Evaluation of Sample Extract Cleanup Using Solid-Phase Extraction Cartridges", Project Report, December 1989.

TABLE 1

PERCENT RECOVERIES AND ELUTION PATTERNS FOR
16 PHTHALATE ESTERS FROM ALUMINA CARTRIDGES[a]

| Compound | Average % Recovery | Average RSD |
|---|---|---|
| Dimethyl phthalate | 108 | 4.6 |
| Diethyl phthalate | 129 | 6.6 |
| Diisobutyl phthalate | 92.6 | 7.3 |
| Di-n-butyl phthalate | 107 | 5.6 |
| Bis(4-methyl-2-pentyl) phthalate | 88.3 | 9.8 |
| Bis(2-methoxyethyl) phthalate | 92.2 | 5.0 |
| Diamyl phthalate | 100 | 6.4 |
| Bis(2-ethoxyethyl) phthalate | 101 | 6.3 |
| Hexyl 2-ethylhexyl phthalate | 93.2 | 13 |
| Dihexyl phthalate | 113 | 5.4 |
| Benzyl butyl phthalate | 104 | 3.9 |
| Bis(2-n-butoxyethyl) phthalate | 99.5 | 4.7 |
| Bis(2-ethylhexyl) phthalate | 101 | 6.1 |
| Dicyclohexyl phthalate | 97.2 | 6.2 |
| Di-n-octyl phthalate | 103 | 7.5 |
| Dinonyl phthalate | 110 | 5.2 |

[a] Alumina cartridges (J.T. Baker) were conditioned with 4 mL of hexane. Each experiment was performed in duplicate at three spiking concentrations (40 µg, 80 µg, and 120 µg per compound, per cartridge). The cartridges were eluted with 5 mL of acetone/hexane (20/80, v/v).

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