METHOD 3620A

FLORISIL COLUMN CLEANUP

1.0 SCOPE AND APPLICATION

1.1 Florisil, a registered trade name of the Floridin Co., is a magnesium silicate with acidic properties. It is used for general column chromatography as a cleanup procedure prior to sample analysis by gas chromatography.

1.2 General applications: Cleanup of pesticide residues and other chlorinated hydrocarbons; the separation of nitrogen compounds from hydrocarbons; the separation of aromatic compounds from aliphatic-aromatic mixtures; and similar applications for use with fats, oils, and waxes (Floridin). Additionally, Florisil is considered good for separations with steroids, esters, ketones, glycerides, alkaloids, and some carbohydrates (Gordon and Ford).

1.3 Specific applications: This method includes guidance for cleanup of sample extracts containing the following analyte groups: phthalate esters; nitrosamines; organochlorine pesticides; nitroaromatics; haloethers; chlorinated hydrocarbons; and organophosphorus pesticides.

2.0 SUMMARY OF METHOD

2.1 The column is packed with the required adsorbent, topped with a water adsorbent, and then loaded with the sample to be analyzed. Elution 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 Beaker - 500 mL.

4.2 Chromatographic column - 300 mm long x 10 mm ID or 400 mm long x 20 mm ID, as specified in Section 7.0; 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). Ground-glass stopper is used to prevent evaporation of extracts.

4.3.2 Evaporation flask - 500 mL (Kontes K-570001-0500 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 Muffle furnace.

4.5 Reagent bottle - 500 mL.

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

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

4.8 Erlenmeyer flasks - 50 and 250 mL.

4.9 Top-loading balance - 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 Florisil - Pesticide residue (PR) grade (60/100 mesh); purchase activated at 1250°F (677 °C), stored in glass containers with ground-glass stoppers or foil-lined screw caps.

5.2.1 Deactivation of Florisil - for cleanup of phthalate esters. To prepare for use, place 100 g of Florisil into a 500 mL beaker and heat for approximately 16 hr at 40°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 stand for at least 2 hr. Keep the bottle sealed tightly.

5.2.2 Activation of Florisil - for cleanup of nitrosamines, organochlorine pesticides and PCBs, nitroaromatics, haloethers, chlorinated hydrocarbons, and organophosphorus pesticides. Just before use, activate each batch at least 16 hr at 130°C in a glass container loosely covered with aluminum foil. Alternatively, store the Florisil in an oven at 130°C. Cool the Florisil before use in a desiccator. (Florisil from different batches or sources may vary in adsorptive capacity. To standardize the amount of Florisil which is used, the use of lauric acid value is suggested. The referenced procedure determines the adsorption from hexane solution of lauric acid (mg) per g of Florisil. The amount of Florisil to be used for each column is calculated by dividing 110 by this ratio and multiplying by 20 g (Mills).

5.3 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.

5.4 Eluting solvents

5.4.1 Diethyl ether, $C_2H_5OC_2H_5$ - Pesticide quality or equivalent. Must be free of peroxides, as indicated by test strips (EM Quant or equivalent). Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL ethyl alcohol preservative must be added to each liter of ether.

5.4.2 Acetone, CH_3COCH_3 - Pesticide quality or equivalent.

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

5.4.4 Methylene chloride, CH_2Cl_2 - Pesticide quality or equivalent.

5.4.5 Pentane, $CH_3(CH_2)_3CH_3$ - Pesticide quality or equivalent.

5.4.6 Petroleum ether (boiling range 30-60°C) - Pesticide quality or equivalent.

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 deactivated Florisil (Section 5.1.1) into a 10 mm ID chromatographic column. Tap the column to settle

the Florisil and add approximately 1 cm of anhydrous sodium sulfate to the top.

7.1.3 Preelute 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 40 mL of hexane and continue the elution of the column. Discard this hexane eluate.

7.1.4 Next, elute the column with 100 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 as needed. No solvent exchange is necessary. Adjust the volume of the cleaned-up extract to whatever volume is required (10 mL for Method 8060) and analyze by gas chromatography. Compounds that elute in this fraction are:

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 volume to 2 mL prior to cleanup.

7.2.2 Add a weight of activated Florisil (nominally 22 g) predetermined by calibration (Section 5.1.2) into a 20 mm ID chromatographic column. Tap the column to settle the Florisil and add about 5 mm of anhydrous sodium sulfate to the top.

7.2.3 Pre-elute the column with 40 mL of ethyl ether/pentane (15:85) (v/v). 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 pentane to complete the transfer.

7.2.4 Elute the column with 90 mL of ethyl ether/pentane (15:85) (v/v) and discard the eluate. This fraction will contain the diphenylamine, if it is present in the extract.

7.2.5 Next, elute the column with 100 mL of acetone/ethyl ether (5:95) (v/v) into a 500 mL K-D flask equipped with a 10 mL concentrator tube. This fraction will contain all of the nitrosamines listed in the scope of the method.

7.2.6 Add 15 mL of methanol to the collected fraction, concentrate as needed using pentane to prewet the K-D column and set the water bath at 70 to 75°C. When the apparatus is cool, remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of pentane.

7.3 Organochlorine pesticides, haloethers, and organophosphorus pesticides (see Tables 1 and 2 for fractionation patterns of compounds tested)

7.3.1 Reduce the sample extract volume to 10 mL prior to cleanup. The extract solvent must be hexane.

7.3.2 Add a weight of activated Florisil (nominally 20 g), predetermined by calibration (Section 5.1.2), to a 20 mm ID chromatographic column. Settle the Florisil by tapping the column. Add anhydrous sodium sulfate to the top of the Florisil to form a layer 1 to 2 cm deep. Add 60 mL of hexane to wet and rinse the sodium sulfate and Florisil. Just prior to exposure of the sodium sulfate to air, stop the elution of the hexane by closing the stopcock on the chromatographic column. Discard the eluate.

7.3.3 Adjust the sample extract volume to 10 mL with hexane and transfer it from the K-D concentrator tube to the Florisil column. Rinse the tube twice with 1-2 mL hexane, adding each rinse to the column.

7.3.4 Place a 500 mL K-D flask and clean concentrator tube under the chromatographic column. Drain the column into the flask until the sodium sulfate layer is nearly exposed. Elute the column with 200 mL of 6% ethyl ether in hexane (v/v) (Fraction 1) using a drip rate of about 5 mL/min. All of the haloethers are in this fraction. Remove the K-D flask and set aside for later concentration. Elute the column again, using 200 mL of 15% ethyl ether in hexane (v/v) (Fraction 2), into a second K-D flask. Perform a third elution using 200 mL of 50% ethyl ether in hexane (v/v) (Fraction 3), and a final elution with 200 mL of 100% ethyl ether (Fraction 4), into separate K-D flasks.

7.3.5 If necessary, concentrate the eluates by standard K-D techniques using the water bath at about 85°C (75 °C for Fraction 4). Adjust the final volume to whatever volume is required (1-10 mL).

7.4 Nitroaromatics and isophorone

7.4.1 Reduce the sample extract volume to 2 mL prior to cleanup.

7.4.2 Add a weight of activated Florisil (nominally 10 g) predetermined by calibration (Section 5.1.2) into a 10 mm ID chromatographic column. Tap the column to settle the Florisil and add about 1 cm of anhydrous sodium sulfate to the top.

7.4.3 Pre-elute the column with methylene chloride/hexane (1:9) (v/v) at about 2 mL/min. Discard the eluate and, just prior to exposure of the sodium sulfate layer to the air, quantitatively transfer the 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 30 mL of methylene chloride/hexane (1:9) (v/v) and continue the elution of the column. Discard the eluate.

7.4.4 Elute the column with 90 mL of ethyl ether/pentane (15:85) (v/v) and discard the eluate. This fraction will contain the diphenylamine, if it is present in the extract.

7.4.5 Next, elute the column with 100 mL of acetone/ethyl ether (5:95) (v/v) into a 500 mL K-D flask equipped with a 10 mL concentrator tube. This fraction will contain all of the nitrosamines listed in the scope of the method.

7.4.6 Add 15 mL of methanol to the collected fraction, concentrate using pentane to prewet the K-D column, and set the water bath at 70 to 75°C. When the apparatus is cool, remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of pentane.

7.4.7 Next, elute the column with 30 mL of acetone/methylene chloride (1:9) (v/v) into a 500 mL K-D flask equipped with a 10 mL concentrator tube. Concentrate the collected fraction, while exchanging the solvent to hexane. To exchange the solvent, reduce the elution solvent to about 10 mL. Add 50 mL of hexane, a fresh boiling chip, and return the reassembled K-D apparatus to the hot water bath. Adjust the final volume of the cleaned-up extract to whatever volume is required (1-10 mL). Compounds that elute in this fraction are:

2,4-Dinitrotoluene
2,6-Dinitrotoluene
Isophorone
Nitrobenzene.

7.5 Chlorinated hydrocarbons

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

7.5.2 Add a weight of activated Florisil (nominally 12 g) predetermined by calibration (Section 5.1.2) into a 10 mm ID chromatographic column. Tap the column to settle the Florisil and add about 1 to 2 cm of anhydrous sodium sulfate to the top.

7.5.3 Preelute the column with 100 mL of petroleum ether. Discard the eluate and, just prior to exposure of the sodium sulfate layer to the air, quantitatively transfer the sample extract to the column by decantation and subsequent petroleum ether washings. Discard the eluate. Just prior to exposure of the sodium sulfate layer to the air, begin eluting the column with 200 mL of petroleum ether and collect the eluate in a 500 mL K-D flask equipped with a 10 mL concentrator tube. This fraction should contain all of the chlorinated hydrocarbons:

2-Chloronaphthalene
1,2-Dichlorobenzene
1,3-Dichlorobenzene
1,4-Dichlorobenzene
Hexachlorobenzene
Hexachlorobutadiene
Hexachlorocyclopentadiene
Hexachloroethane
1,2,4-Trichlorobenzene.

7.5.4 Concentrate the fraction, using hexane to prewet the column. When the apparatus is cool, remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with hexane. Adjust the final volume of the cleaned-up extract to whatever volume is required (1-10 mL).

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 should also be processed through this cleanup method.

9.0 METHOD PERFORMANCE

9.1 Table 1 indicates the distribution of chlorinated pesticides, PCB's, and haloethers in various Florisil column fractions.

9.2 Table 2 indicates the distribution of organophosphorus pesticides in various Florisil column fractions.

10.0 REFERENCES

1. Gordon, A.J. and R.A. Ford, The Chemist's Companion: A Handbook of Practical Data, Techniques, and References (New York: John Wiley & Sons, Inc.), pp. 372, 374, and 375, 1972.

2. Floridin of ITT System, Florisil: Properties, Application, Bibliography, Pittsburgh, Pennsylvania, 5M381DW.

3. Mills, P.A., "Variation of Florisil Activity; Simple Method for Measuring Absorbent Capacity and its use in Standardizing Florisil Columns," Journal of the Association of Official Analytical Chemists, 51, 29, 1968.

4. U.S. Food and Drug Association, Pesticides Analytical Manual (Volume 1), July 1985.

5. 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

DISTRIBUTION OF CHLORINATED PESTICIDES, PCBs, AND HALOETHERS INTO FLORISIL COLUMN FRACTIONS

Parameter	Percent Recovery by Fraction[a] 1	2	3
Aldrin	100		
α-BHC	100		
β-BHC	97		
γ-BHC	98		
δ-BHC	100		
Chlordane	100		
4,4'-DDD	99		
4,4'-DDE	98		
4,4'-DDT	100		
Dieldrin	0	100	
Endosulfan I	37	64	
Endosulfan II	0	7	91
Endosulfan sulfate	0	0	106
Endrin	4	96	
Endrin aldehyde	0	68	26
Haloethers	R		
Heptachlor	100		
Heptachlor epoxide	100		
Toxaphene	96		
PCB-1016	97		
PCB-1221	97		
PCB-1232	95	4	
PCB-1242	97		
PCB-1248	103		
PCB-1254	90		
PCB-1260	95		

[a] Eluant composition: Fraction 1 - 6% ethyl ether in hexane
Fraction 2 - 15% ethyl ether in hexane
Fraction 3 - 50% ethyl ether in hexane

R = Recovered (no percent recovery data presented).

SOURCE: U.S. EPA and FDA data.

TABLE 2

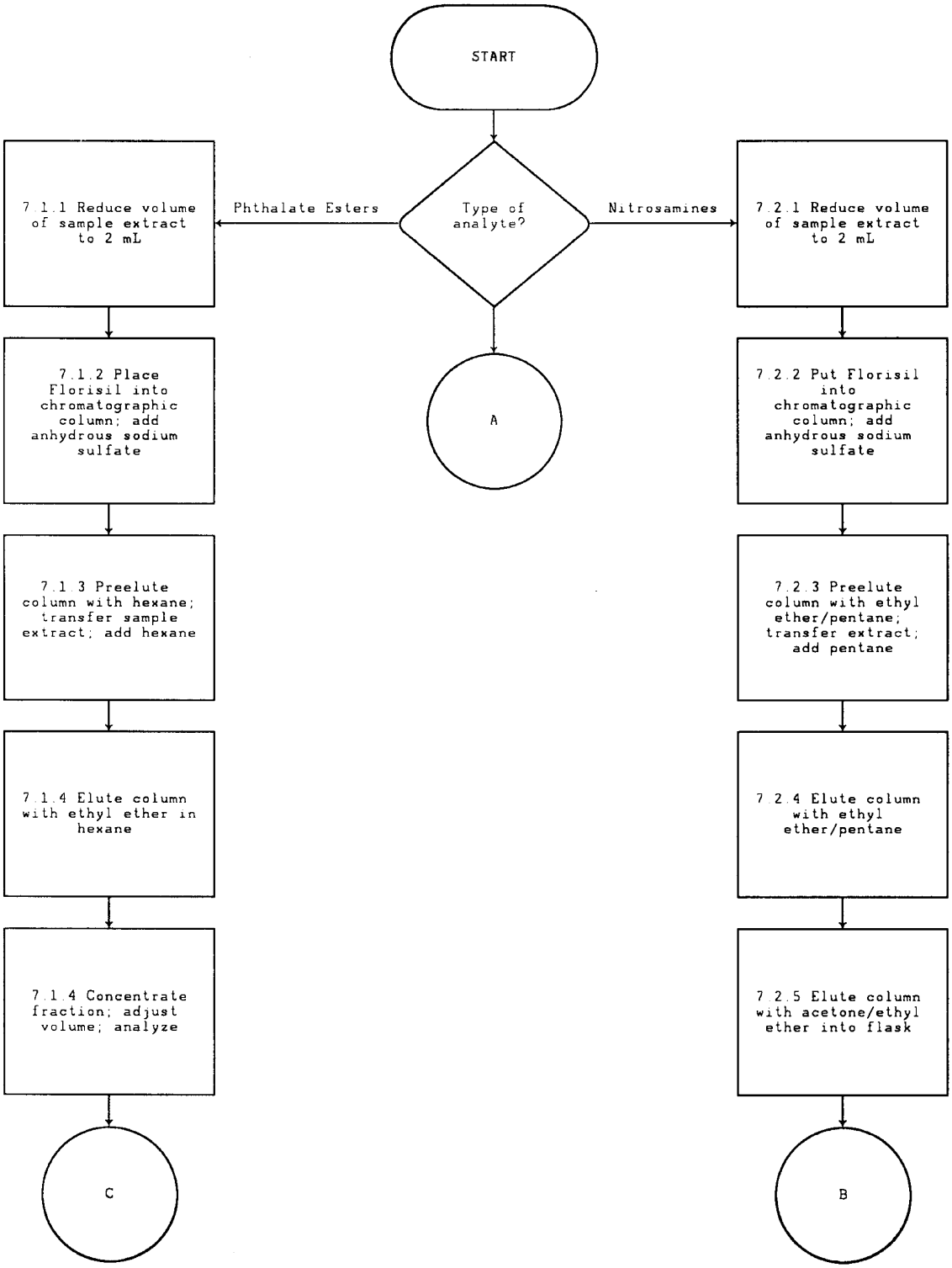
DISTRIBUTION OF ORGANOPHOSPHORUS PESTICIDES
INTO FLORISIL COLUMN FRACTIONS

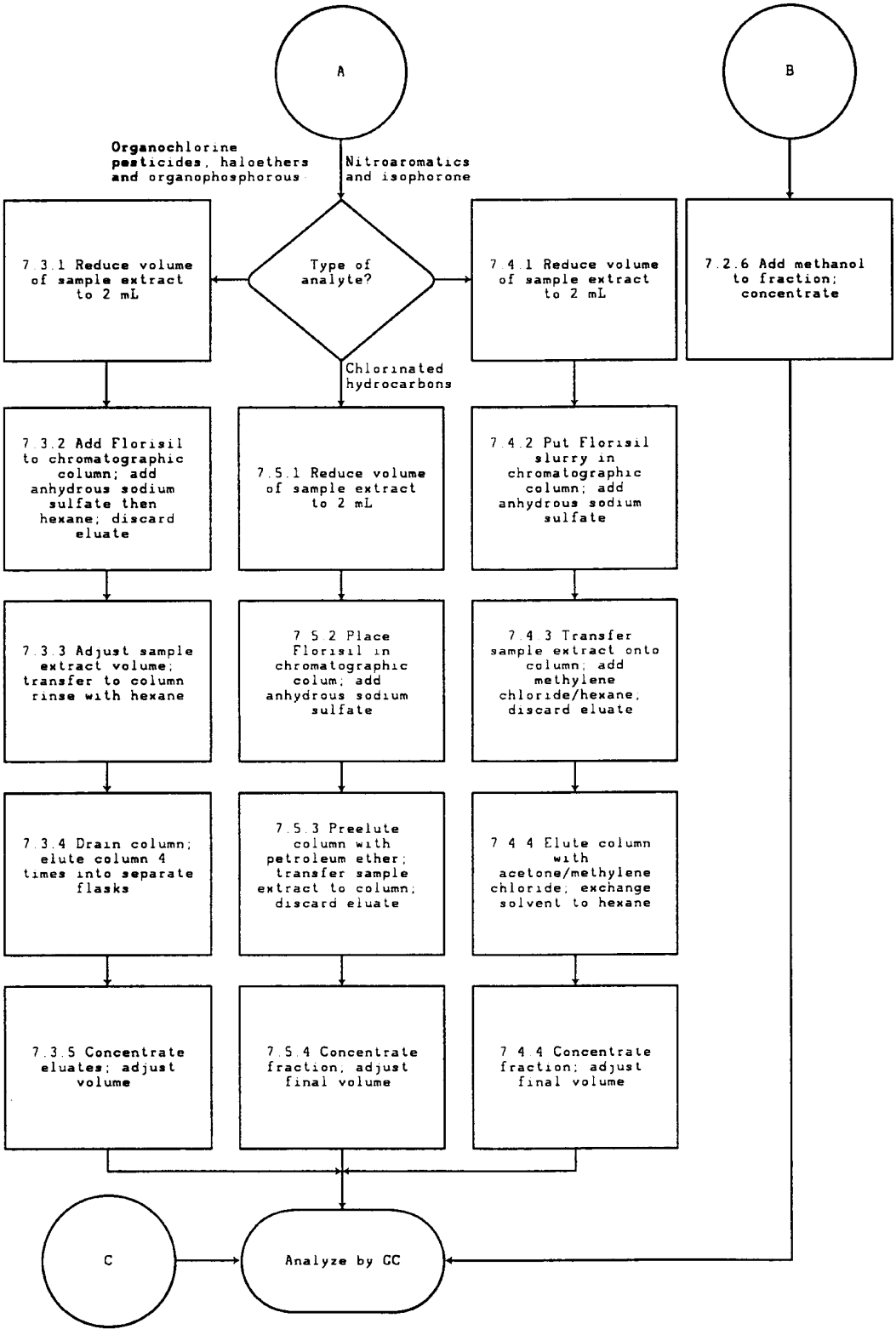
Parameter	Percent Recovery by Fraction[a]			
	1	2	3	4
Azinphos methyl			20	80
Bolstar (Sulprofos)	ND	ND	ND	ND
Chlorpyrifos	>80			
Coumaphos	NR	NR	NR	
Demeton	100			
Diazinon		100		
Dichlorvos	NR	NR	NR	
Dimethoate	ND	ND	ND	ND
Disulfoton	25-40			
EPN		>80		
Ethoprop	V	V	V	
Fensulfothion	ND	ND	ND	ND
Fenthion	R	R		
Malathion		5	95	
Merphos	V	V	V	
Mevinphos	ND	ND	ND	ND
Monochrotophos	ND	ND	ND	ND
Naled	NR	NR	NR	
Parathion		100		
Parathion methyl		100		
Phorate	0-62			
Ronnel	>80			
Stirophos (Tetrachlorvinphos)	ND	ND	ND	ND
Sulfotepp	V	V		
TEPP	ND	ND	ND	ND
Tokuthion (Prothiofos)	>80			
Trichloronate	>80			

[a] Eluant composition:
Fraction 1 - 200 mL of 6% ethyl ether in hexane
Fraction 2 - 200 mL of 15% ethyl ether in hexane
Fraction 3 - 200 mL of 50% ethyl ether in hexane
Fraction 4 - 200 mL of 100% ethyl ether

R = Recovered (no percent recovery information presented) (U.S. FDA).
NR = Not recovered (U.S. FDA).
V = Variable recovery (U.S. FDA).
ND = Not determined.

SOURCE: U.S. EPA and FDA data.


START
Type of analyte?
Phthalate Esters
Nitrosamines
7.1.1 Reduce volume of sample extract to 2 mL
7.1.2 Place Florisil into chromatographic column; add anhydrous sodium sulfate
7.1.3 Preelute column with hexane; transfer sample extract; add hexane
7.1.4 Elute column with ethyl ether in hexane
7.1.4 Concentrate fraction; adjust volume; analyze
C
A
7.2.1 Reduce volume of sample extract to 2 mL
7.2.2 Put Florisil into chromatographic column; add anhydrous sodium sulfate
7.2.3 Preelute column with ethyl ether/pentane; transfer extract; add pentane
7.2.4 Elute column with ethyl ether/pentane
7.2.5 Elute column with acetone/ethyl ether into flask
B


A
B
Organochlorine pesticides, haloethers and organophosphorous
Nitroaromatics and isophorone
7.3.1 Reduce volume of sample extract to 2 mL
Type of analyte?
7.4.1 Reduce volume of sample extract to 2 mL
7.2.6 Add methanol to fraction; concentrate
Chlorinated hydrocarbons
7.3.2 Add Florisil to chromatographic column; add anhydrous sodium sulfate then hexane; discard eluate
7.5.1 Reduce volume of sample extract to 2 mL
7.4.2 Put Florisil slurry in chromatographic column; add anhydrous sodium sulfate
7.3.3 Adjust sample extract volume; transfer to column rinse with hexane
7.5.2 Place Florisil in chromatographic colum; add anhydrous sodium sulfate
7.4.3 Transfer sample extract onto column; add methylene chloride/hexane; discard eluate
7.3.4 Drain column; elute column 4 times into separate flasks
7.5.3 Preelute column with petroleum ether; transfer sample extract to column; discard eluate
7.4.4 Elute column with acetone/methylene chloride; exchange solvent to hexane
7.3.5 Concentrate eluates; adjust volume
7.5.4 Concentrate fraction; adjust final volume
7.4.4 Concentrate fraction; adjust final volume
C
Analyze by GC