METHOD 3620C

FLORISIL CLEANUP

1.0 SCOPE AND APPLICATION

1.1 Florisil, a registered trade name of U. S. Silica Co., is a magnesium silicate with basic properties. It is used to separate analytes from interfering compounds prior to sample analysis by a chromatographic method.

1.2 Florisil has been used for the 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. Additionally, Florisil is considered good for separations with steroids, esters, ketones, glycerides, alkaloids, and some carbohydrates.

1.3 Florisil cleanup may be accomplished using a glass chromatographic column packed with Florisil or using solid-phase extraction cartridges containing Florisil.

1.4 This method includes procedures for cleanup of sample extracts containing the following analyte groups:

Phthalate esters	Chlorinated hydrocarbons
Nitrosamines	Organochlorine pesticides
Nitroaromatics	Organophosphates
Haloethers	Organophosphorus pesticides
Aniline and aniline derivatives	PCBs

1.5 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 Florisil cleanup of solvent extracts of environmental samples. It provides the option of using either traditional column chromatography techniques to 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 required 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 protocol uses solid-phase extraction cartridges containing 40 µm particles of Florisil (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 required to obtain reproducible results. The eluate may be further concentrated prior to gas chromatographic analysis.

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. However, during the evaluation of the cartridge cleanup procedure, phthalate esters were detected in the Florisil cartridge method blanks at concentrations up to 400 ng per cartridge. Therefore, complete removal of the phthalate esters from Florisil cartridges may not be possible.

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 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 - Appropriate sizes.

4.3 Reagent bottle - Appropriate sizes.

4.4 Muffle furnace - Capable of maintaining 400°C.

4.5 Vials - Glass, 10-mL and 25-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 required for use of the cartridge cleanup protocol.

4.7 Top-loading balance - Capable of weighing to 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 Granular Florisil - For column cleanup procedure. Florisil is produced in four grades, two of which are appropriate for this procedure. The differences between grades are primarily a function of the activation temperature, resulting in somewhat different chemical characteristics among the grades. Florisil PR is activated at 675°C and is most useful for pesticide residue analyses. Florisil A is activated at 650°C and is generally used for other analytes. Whichever grade is used, store Florisil in glass containers with ground-glass stoppers or foil-liner screw caps.

5.3 Lauric acid - Reagent grade. Used for the standardization of the Florisil activity. Weigh 10.00 g of lauric acid in a 500-mL volumetric flask. Add 50 mL of hexane to the flask to dissolve the lauric acid. Swirl the flask gently until the lauric acid is dissolved, then dilute the solution in the flask to 500 mL with additional hexane.

5.4 Phenolphthalein Indicator - Dissolve 1 g of phenolphthalein in ethanol and dilute to 100 mL in a 100-mL volumetric flask.

5.5 Sodium hydroxide - Weigh out 20 g of NaOH (pellets, reagent grade) in a 500-mL volumetric flask. Dissolve in organic-free reagent water and dilute to 500 mL to make a 1 N solution. Dilute 25 mL of the 1 N NaOH to 500 mL with water in a second 500-mL volumetric flask, yielding a 0.05N solution. The NaOH solution must be standardized against lauric acid, as follows.

5.5.1 Weigh 100 - 200 mg of lauric acid to the nearest 1 mg in a 125-mL Erlenmeyer flask. Add 50 mL of ethanol to the flask and swirl to dissolve the lauric acid.

5.5.2 Add 3 drops of phenolphthalein indicator to the flask, and titrate with the 0.05 N NaOH solution to a permanent endpoint (i.e., the indicator color does not disappear when the solution is allowed to stand for 1 min).

5.5.3 Calculate the "strength" of the NaOH solution as the mg of lauric acid neutralized per mL of NaOH solution.

5.6 Deactivation/activation of Florisil

5.6.1 Deactivation of Florisil - for cleanup of phthalate esters. To prepare for use, place 100 ± 10 g of Florisil into a 500-mL beaker and heat to 140°C for approximately 16 h. After heating, transfer to a 500-mL reagent bottle. Tightly seal and cool to room temperature. When cool, add 3 ± 0.1 mL of organic-free reagent water. Mix thoroughly by shaking or rolling for 10 min and let stand for at least 2 h. Keep the bottle sealed tightly.

5.6.2 Activation of Florisil - for all cleanups other than phthalate esters. It is advisable to treat both Florisil A and Florisil PR prior to use to drive off any moisture adsorbed during storage and handling. Heat the Florisil in a glass container loosely covered with aluminum foil in an oven at 130°C overnight. Cool the Florisil in a dessicator before use.

5.6.3 Florisil from different batches or sources may vary in adsorptive capacity. To standardize the amount of Florisil which is used, use the lauric acid value, described below. The procedure determines the adsorption from a hexane solution of lauric acid (mg) per g of Florisil.

5.6.3.1 Weigh 2.000 g of Florisil in a 25-mL glass-stoppered Erlenmeyer flask. Cover loosely with aluminum foil and heat overnight at 130°C. Stopper the flask and cool to room temperature.

5.6.3.2 Add 20.0 mL of the lauric acid solution to the flask, stopper, and shake occasionally for 15 min.

5.6.3.3 Let the Florisil settle and using a volumetric pipet, transfer 10.0 mL of supernatant liquid into a 125-mL Erlenmeyer flask. Avoid inclusion of any Florisil.

5.6.3.4 Add 60 mL of ethanol and 3 drops of the phenolphthalein indicator solution to the flask.

5.6.3.5 Titrate the solution in the flask with the 0.05N NaOH solution until a permanent end point is reached (i.e., the indicator color does not disappear when the solution is allowed to stand for 1 min).

5.6.3.6 The lauric acid value is calculated as follows:

$$\text{Lauric acid value} = 200 - (\text{titration volume in mL of NaOH})\,(\text{strength of NaOH})$$

where the strength of the NaOH is measured in Sec. 7.5.3 as the mg of lauric acid neutralized per mL of NaOH solution.

5.6.3.7 Use the following equation to obtain an equivalent quantity of any batch of Florisil.

$$\frac{110}{\text{lauric acid value}} \times 20\ \text{g} = \text{Required weight of Florisil}$$

NOTE: This equation was written incorrectly in the previous version of this method.

5.7 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.

5.8 Florisil cartridges - 40 µm particles, 60 Å pores. The cartridges from which this method were developed consist of 6-mL serological- grade polypropylene tubes, with the 1 g of Florisil held between two polyethylene or stainless steel frits with 20 µm pores. Cartridges

containing 0.5 g and 2.0 g of Florisil are available, however, the compound elution patterns must be verified when cartridges containing other than 1 g of Florisil are used.

5.9 Eluting solvents - All solvents must be pesticide quality or equivalent.

5.9.1 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.

5.9.2 Pentane, $CH_3(CH_2)_3CH_3$

5.9.3 Hexane, C_6H_{14}

5.9.4 Methylene chloride, CH_2Cl_2

5.9.5 Acetone, CH_3COCH_3

5.9.6 Petroleum ether (boiling range 30-60°C)

5.9.7 Toluene, $C_6H_5CH_3$

5.9.8 2-Propanol, $(CH_3)_2CHOH$

5.10 Florisil cartridge phenol check solution (for the organochlorine pesticide technique) - Prepare a solution of 2,4,5-trichlorophenol in acetone at a concentration of 0.1 mg/L.

5.11 Florisil cartridge pesticide check solution - Prepare a solution containing the following analytes in hexane:

α-BHC	5 mg/L
Heptachlor	5 mg/L
γ-BHC	5 mg/L
Endosulfan I	5 mg/L
Dieldrin	10 mg/L
Endrin	10 mg/L
4,4'-DDD	10 mg/L
4,4'-DDT	10 mg/L
Methoxychlor	50 mg/L
Tetrachloro-*m*-xylene	20 mg/L
Decachlorobiphenyl	20 mg/L

5.12 Chlorophenoxy acid herbicide check solution - Prepare a solution containing 2,4,5-T methyl ester at 100 mg/L, pentachlorophenyl methyl ester at 50 mg/L, and Picloram methyl ester at 200 mg/L.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

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

7.0 PROCEDURE

Sec. 7.1 describes the procedures for assembling and conditioning the Florisil cartridges. Sec. 7.2 describes general procedures for handling sample extracts prior to cleanup. Secs. 7.3 - 7.13 describe the column and cartridge procedures for phthalate esters; nitrosamines; organochlorine pesticides, haloethers, and organophosphorus pesticides; nitroaromatics and isophorone; chlorinated hydrocarbons; aniline and aniline derivatives; organophosphates; and derivatized chlorophenoxy acid herbicides.

The column chromatography procedures employ a larger amount of Florisil 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.

If the interference is caused by high boiling materials, then Method 3640 should be employed prior to Florisil cleanup. If the interference is caused by relatively polar compounds in the same boiling range as the analytes of interest, then multiple column or cartridge cleanups may be required. For additional cleanup of organochlorine pesticides and PCBs, see Method 3665. If crystals of sulfur are present in the extract, then Method 3660 should be employed prior to Florisil cleanup.

Whenever Florisil is used to fractionate groups of target compounds (rather than to simply remove potential interferents) it is critical that the specific fractionation scheme be validated using spiked solutions or spiked sample extracts that contain most or all of the analytes of interest. This may be particularly important when the Florisil cartridge techniques are employed, as the differences between the various cartridge formats and manufacturers may affect the fractionation patterns. In addition, it may be useful to archive any fractions not originally intended for analysis, in the event that the fractionation scheme chosen does not yield the intended results. Once the determinative analysis has been performed and demonstrates that the fractionation has been successful, such archived fractions may be disposed of in an appropriate manner. However, if the fractionation did not perform as intended, the analytes of interest may be contained in the archived fractions which may be able to be analyzed or combined with the other fraction(s) for reanalysis.

Following Florisil cleanup, extracts may require further concentration and/or solvent exchange. Consult the appropriate determinative method and 3500 Series extraction method for details.

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

Most sample extracts will have to be concentrated to a smaller volume prior to the use of Florisil cleanup. The extract volume is a function of the analytical sensitivity necessary to meet the project objectives. The extract volume will also affect the ability of the Florisil to separate target analytes from potential interferences, particularly for the cartridge procedures, where applying large extract volumes to the cartridges may cause poor results. As noted in Sec. 7.0, consult the appropriate extraction and determinative methods for the details on final extract volumes, extract concentration techniques, and solvent exchange procedures.

7.2.1 Reduce the sample extract volume to 2 mL prior to cleanup for:

Phthalate esters	Chlorinated hydrocarbons
Nitrosamines	Chlorophenoxy acid herbicides
Nitroaromatics and isophorone	Aniline and aniline derivatives

The extract solvent should be hexane for the phthalate esters, nitroaromatics, chlorinated hydrocarbons, and chlorophenoxy acid herbicides, and methylene chloride for the nitrosamines and aniline and aniline derivatives.

7.2.2 Reduce the sample extract volume to 10 mL prior to cleanup for:

Organochlorine pesticides	Organophosphates
Haloethers	PCBs
Organophosphorus pesticides	

The extract solvent should be hexane for these analytes. In most cases, given the sensitivity of the determinative methods, only 1 mL of the 10 mL extract needs to be subjected to the Florisil cleanup procedure. The remaining 9 mL should be archived for later use, if needed.

7.2.3 Allow the 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 deactivated Florisil (Sec. 5.2.1) 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.

7.3.2 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 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 40 mL of hexane and continue the elution of the column. Discard this hexane eluate.

7.3.4 Elute the column with 100 mL of ethyl ether/hexane (20/80, v/v) and collect this fraction in a flask (e.g., a 500 mL K-D flask equipped with a clean 10 mL concentrator tube). Concentrate the eluate to the volume listed in the determinative method, 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

7.4.1 Using 1-g Florisil 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 cartridges, but before the cartridge becomes dry, rinse the sample vials with an additional 0.5 mL of solvent, and add the rinse to the cartridges 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 If the sample is suspected to contain organochlorine pesticides, elute the cartridge with methylene chloride/hexane (20/80, v/v). Turn on the vacuum pump and adjust the pump pressure to 10 inches (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 (this fraction contains the organochlorine pesticides, and should be discarded).

7.4.7 Close the cartridge valve, replace collection vials, and add 10 mL of acetone/hexane (10/90, v/v) to the cartridge. Slowly open the cartridge valve and collect the eluate into the collection vial. This fraction contains the phthalate esters, and should be retained for analysis.

7.4.8 Concentrate the eluate 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 Add a weight of activated Florisil (nominally 22 g) predetermined by calibration (Sec. 5.6.3.7) 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.

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

7.5.3 Just prior to the exposure of the sodium sulfate layer to the air, elute the column with 90 mL of ethyl ether/pentane (15/85, v/v). Discard the eluate. This fraction will contain and diphenylamine present in the extract.

7.5.4 Elute the column with 100 mL of acetone/ethyl ether (5/95, v/v), collecting the eluate in a flask (e.g., a 500 mL K-D flask equipped with a clean 10 mL concentrator tube). This fraction will contain all of the nitrosamines listed in the scope of the method.

7.5.5 Add 15 mL of methanol to the collected fraction, and concentrate this fraction to the volume listed in the determinative method, using the techniques described in the appropriate 3500 series method.

7.6 Column procedure for organochlorine pesticides, haloethers, and organophosphorus pesticides (see Table 2 for fractionation patterns of organophosphorus pesticides)

7.6.1 Add a weight of activated Florisil (nominally 20 g), predetermined by calibration (Sec. 5.6.3.7), 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.

7.6.2 Pre-elute the column with 60 mL of hexane and discard the eluate. Just prior to exposure of the sodium sulfate to air, quantitatively transfer the 10-mL sample

extract (Sec. 7.2) onto the column, completing the transfer with two 1-2 mL rinses with hexane.

7.6.3 Place a flask (e.g., a 500 mL K-D flask equipped with a 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 ethyl ether/hexane (6/94, v/v) using a drip rate of about 5 mL/min. This is Fraction 1, and all of the haloethers are in this fraction. Remove the flask and set aside for later concentration.

7.6.4 Elute the column again, using 200 mL of ethyl ether/hexane (15/85, v/v), into a second flask. This is Fraction 2.

7.6.5 Perform a third elution using 200 mL of diethyl ether/hexane (50/50, v/v), collecting the eluate in a third flask. This is Fraction 3.

7.6.6 Perform a final elution with 200 mL of 100% ethyl ether, collecting the eluate in a fourth flask. This is Fraction 4.

7.6.7 Concentrate the four eluates to the volume listed in the determinative method, using the techniques described in the appropriate 3500 series method.

7.7 Cartridge procedure for organochlorine pesticides and PCBs

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

7.7.2 Transfer the 1 mL (or other appropriate volume) of 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.

7.7.3 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 hexane, and add the rinse to the cartridge to complete the quantitative transfer.

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

7.7.5 Place a 10-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 with the collection vial.

7.7.6 If there is no need to separate the organochlorine pesticides from the PCBs, then add 9 mL of acetone/hexane (10/90, v/v) to the cartridge. Turn on the vacuum pump and adjust the pump pressure to 10 inches (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. Go directly to Sec. 7.7.8.

7.7.7 The following procedures are used to separate the organochlorine pesticides from the PCBs.

7.7.7.1 Add 3 mL of hexane to the cartridge. Turn on the vacuum pump and adjust the pump pressure to 10 inches (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. This is Fraction 1 and it will contain the PCBs and a few of the organochlorine pesticides (see Table 5).

7.7.7.2 Close the cartridge valve, replace the collection vial, and add 5 mL of methylene chloride/hexane (26/74, v/v) to the cartridge. Slowly open the cartridge valve and collect the eluate into the collection vial. This is Fraction 2 and it will contain most of the pesticides.

7.7.7.3 Close the cartridge valve, replace collection vials, and add 5 mL of acetone/hexane (10/90, v/v) to the cartridge. Slowly open the cartridge valve and collect the eluate into the collection vial. This is Fraction 3 and it will contain the remaining pesticides.

7.7.8 As needed, perform a solvent exchange and 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.

7.8 Column procedure for nitroaromatics and isophorone

7.8.1 Add a weight of activated Florisil (nominally 10 g) predetermined by calibration (Sec. 5.6.3.7) 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.

7.8.2 Pre-elute the column with methylene chloride/hexane (10/90, 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 2-mL sample extract (Sec. 7.2) onto the column using an additional 2 mL of hexane to complete the transfer.

7.8.3 Just prior to exposure of the sodium sulfate layer to the air, add 30 mL of methylene chloride/hexane (10/90, v/v) and continue the elution of the column. Discard the eluate.

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

7.8.5 Elute the column with 100 mL of acetone/ethyl ether (5/95, v/v), and collect the eluate in a flask (e.g., 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.

7.8.6 Add 15 mL of methanol to the collected fraction, and concentrate to the volume listed in the determinative method, using the techniques described in the appropriate 3500 series method.

7.8.7 Elute the column with 30 mL of acetone/methylene chloride (10/90, v/v), and collect the eluate in a flask (e.g., a 500-mL K-D flask equipped with a 10-mL concentrator tube). Concentrate the collected fraction to the volume listed in the determinative method, using the techniques described in the appropriate 3500 series method, and exchanging the solvent to hexane. Compounds that elute in this fraction are:

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

7.9 Column procedure for chlorinated hydrocarbons

7.9.1 Add a weight of activated Florisil (nominally 12 g) predetermined by calibration (Sec. 5.6.3.7) 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.

7.9.2 Pre-elute 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 (Sec. 7.2) to the column by decantation and subsequent petroleum ether washings. Discard the eluate.

7.9.3 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 flask (e.g., a 500-mL K-D flask equipped with a 10-mL concentrator tube). This fraction should contain the following chlorinated hydrocarbons:

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

7.9.4 Concentrate the collected fraction to the volume listed in the determinative method, using the techniques described in the appropriate 3500 series method.

7.10 Cartridge procedure for chlorinated hydrocarbons

7.10.1 Using 1-g Florisil cartridges, condition the cartridges with 5 mL of acetone/hexane (10/90, v/v) as described in Sec. 7.1.

7.10.2 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.

7.10.3 When the entire extract has passed through the cartridges, but before the cartridge becomes dry, rinse the sample vial with an additional 0.5 mL of acetone/hexane (10/90), and add the rinse to the cartridges to complete the quantitative transfer.

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

7.10.5 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.

7.10.6 Add 10 mL of acetone/hexane (10/90, v/v) to the cartridge. Turn on the vacuum pump and adjust the pump pressure to 10 inches (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.

7.10.7 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.

7.11 Column procedure for aniline and aniline derivatives (see Table 4 for elution patterns)

7.11.1 Add a weight of activated Florisil predetermined by calibration (Sec. 5.6.3.7) into a 20 mm ID chromatographic column. Tap the column to settle the Florisil.

7.11.2 Pre-elute the column with 100 mL of 2-propanol/methylene chloride (5/95, v/v), followed by 100 mL of hexane/methylene chloride (50/50, v/v), followed by 100 mL of hexane. Discard the eluate and leave a column of about 5 cm of hexane above the Florisil.

7.11.3 Quantitatively transfer the 2-mL sample extract (Sec. 7.2) onto 2.0 g of activated Florisil in a 50-mL beaker, using a small volume of methylene chloride, and dry under a gentle stream of nitrogen.

7.11.4 Place the dried Florisil containing the sample extract onto the chromatographic column, and wash the beaker which contained the Florisil with 75 mL of hexane, adding this wash to the reservoir.

7.11.5 Elute the hexane from the column and discard. Stop the column flow just prior to the exposure of the Florisil to air.

7.11.6 Elute the column with 50 mL of methylene chloride/hexane (50/50, v/v), using a drip rate of about 5 mL/minute, and collect the eluate in a flask (e.g., a 500-mL K-D flask equipped with a 10-mL concentrator tube). This is Fraction 1.

7.11.7 Elute the column with 50 mL of 2-propanol/hexane (5/95, v/v), and collect the eluate in a second flask. This is Fraction 2.

7.11.8 Elute the column a third time using 50 mL of methanol/hexane (5/95, v/v). Collect the eluate in a third flask. This is Fraction 3. Frequently, it will prove useful to combine the three fractions prior to analysis. However, in some situations, analysis of each separate fraction may be required. Refer to Method 8131.

7.11.9 Concentrate the collected fractions to the volume listed in the determinative method, using the techniques described in the appropriate 3500 series method.

7.12 Column procedure for organophosphates

7.12.1 Add a weight of activated Florisil, predetermined by calibration (Sec. 5.6.3.7), 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.

7.12.2 Pre-elute the column with 50-60 mL of hexane. Discard the eluate and just prior to exposure of the sulfate layer to air, quantitatively transfer the 10-mL sample extract (Sec. 7.2) onto the column using a hexane wash to complete the transfer.

7.12.3 Just as the sample reaches the sodium sulfate, elute the column with 100 mL of diethyl ether/hexane (10/90, v/v). Discard the eluate.

7.12.4 Just prior to exposure of the sodium sulfate to air, elute the column with 200 mL of diethyl ether/hexane (30/70, v/v). This fraction contains all of the target analytes except for tris(2,3-dibromopropyl) phosphate.

7.12.5 Elute the column with 200 mL of diethyl ether/hexane (40/60, v/v). This fraction contains tris(2,3-dibromopropyl) phosphate.

7.12.6 Concentrate the collected fraction to the volume listed in the determinative method, using the techniques described in the appropriate 3500 series method.

7.13 Column procedure for derivatized chlorophenoxy acid herbicides

7.13.1 Add a weight of activated Florisil (nominally 4 g) predetermined by calibration (Sec. 5.6.3.7) into a 20 mm ID chromatographic column. Tap the column to settle the Florisil and add approximately 5 mm of anhydrous sodium sulfate to the top.

7.13.2 Pre-elute the column with 15 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 to 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.

7.13.3 Just prior to the exposure of the sodium sulfate layer to the air, elute the column with 35 mL of methylene chloride/hexane (20/80, v/v), collecting the eluate in a clean flask (e.g., a 500 mL K-D flask equipped with a concentrator tube). This is Fraction 1, and will contain any pentachlorophenyl methyl ester that is present.

7.13.4 Elute the column with 60 mL of methylene chloride/acetonitrile/hexane (50/0.035/49.65, v/v/v), collecting the eluate in a second flask. This is Fraction 2.

7.13.5 If picloram is to be determined, perform a third elution with the volume of diethyl ether determined from the Florisil check in Sec. 8.2.4, collecting this eluate in a third flask. This is Fraction 3, and will contain the Picloram.

7.13.6 The three fractions may be combined for analysis. Concentrate the combined fractions to the volume listed in the 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 being quantitatively recovered before applying this method to actual samples. This test applies to both the column cleanup and cartridge cleanup procedures. A recovery check must be performed using standards of the target analytes at known concentration.

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

8.2.2 The efficiency of each lot of the solid-phase extraction cartridges must 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.

8.2.3 Organochlorine pesticides - To check each new lot of Florisil cartridges before use, perform the following in duplicate:

8.2.3.1 Combine 0.5 mL of the 2,4,5-trichlorophenol solution in Sec. 5.10, 1.0 mL of the pesticide solution in Sec. 5.11, and 0.5 mL of hexane in a vial.

8.2.3.2 Condition the cartridge as described in Sec. 7.1 and then perform the cartridge cleanup starting with Sec. 7.7.

8.2.3.3 Elute the cartridge with 9 mL of acetone/hexane (10/90, v/v) only. Reduce the volume to 1.0 mL and analyze by Method 8081.

8.2.3.4 The lot of Florisil cartridges is acceptable if all pesticides are recovered at 80 to 110 %, if the recovery of trichlorophenol is less than 5 %, and if no peaks interfering with the target analytes are detected.

8.2.4 Chlorophenoxy acid herbicides - To check each new lot of granular Florisil perform the following:

8.2.4.1 Add 5 mL of the chlorophenoxy acid herbicide check solution (Sec. 5.12) to a Florisil column packed and washed as in Sec. 7.13.2.

8.2.4.2 Elute Fractions 1 and 2 as described in Secs. 7.13.3 and 7.13.4, collecting each in a separate flask.

8.2.4.3 Elute the column with approximately 100 mL diethyl ether and collect ten separate 10-mL fractions.

8.2.4.4 Concentrate Fraction 1 and Fraction separately and concentration each of the ten 10-mL diethyl ether fractions to 5 mL.

8.2.4.5 Analyze each of the 12 eluates by GC/ECD and calculate the recovery of each analyte. Pentachlorophenyl methyl ether should be found in Fraction 1. 2,4,5-T methyl ester (and the methyl esters of the other chlorophenoxy acids) should be found in Fraction 2. Determine the volume of diethyl ether that is required to elute picloram methyl ester.

8.2.4.6 The lot of Florisil is acceptable if the target analytes are quantitatively recovered and if the recovery of trichlorophenol is less than 5%. No interferences should be detected in any of these eluates.

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

9.0 METHOD PERFORMANCE

9.1 Table 1 provides recoveries of phthalate esters obtained from the Florisil column procedure.

9.2 Table 2 provides recoveries of phthalate esters obtained from the Florisil cartridge procedure.

9.3 Table 3 provides the distribution of organochlorine pesticides and PCBs from the Florisil column procedure.

9.4 Table 4 provides recoveries of Aroclors from the Florisil cartridge procedure.

9.5 Table 5 provides the distribution of organochlorine pesticides from the Florisil cartridge procedure, using 1-g cartridges.

9.6 Table 6 provides the distribution of organophosphorus pesticides from the Florisil column procedure.

9.7 Table 7 provides recoveries of chlorinated hydrocarbons obtained from the Florisil cartridge procedure.

9.8 Table 8 provides the elution patterns for aniline compounds from the Florisil column procedure.

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.

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6. US EPA "Evaluation of Sample Extract Cleanup Using Solid-Phase Extraction Cartridges," Project Report, December 1989.

7. US EPA Method 650, Aniline and Selected Substituted Derivatives.

8. Beckert, W.F., and Lopez-Avila, V., "Evaluation of SW-46 Method 8060 for Phthalate Esters," Proceedings of the Fifth Annual Waste Testing and Quality Assurance Symposium, 1989, pp. 144-156.

9. US EPA Method 608, Organochlorine Pesticides and PCBs, 40 CFR 136, October 26, 1984.

TABLE 1

AVERAGE RECOVERIES OF 16 PHTHALATE ESTERS FROM THE FLORISIL COLUMN PROCEDURE[a]

Compound	Average Recovery (%)
Dimethyl phthalate	40
Diethyl phthalate	57
Diisobutyl phthalate	80
Di-*n*-butyl phthalate	85
Bis(4-methyl-2-pentyl) phthalate	84
Bis(2-methoxyethyl) phthalate	0
Diamyl phthalate	82
Bis(2-ethoxyethyl) phthalate	0
Hexyl 2-ethylhexyl phthalate	105
Dihexyl phthalate	74
Benzyl butyl phthalate	90
Bis(2-*n*-butoxyethyl) phthalate	0
Bis(2-ethylhexyl) phthalate	82
Dicyclohexyl phthalate	84
Di-*n*-octyl phthalate	115
Dinonyl phthalate	72

[a] Average recovery from two determinations, data from Reference 8.

TABLE 2

AVERAGE RECOVERIES OF 16 PHTHALATE ESTERS FROM FLORISIL CARTRIDGES[a]

Compound	Average Recovery (%)
Dimethyl phthalate	89
Diethyl phthalate	97
Diisobutyl phthalate	92
Di-*n*-butyl phthalate	102
Bis(4-methyl-2-pentyl) phthalate	105
Bis(2-methoxyethyl) phthalate	78
Diamyl phthalate	94
Bis(2-ethoxyethyl) phthalate	94
Hexyl 2-ethylhexyl phthalate	96
Dihexyl phthalate	97
Benzyl butyl phthalate	99
Bis(2-*n*-butoxyethyl) phthalate	92
Bis(2-ethylhexyl) phthalate	98
Dicyclohexyl phthalate	90
Di-*n*-octyl phthalate	97
Dinonyl phthalate	105

[a] Average recovery from two determinations, data from Reference 6.

TABLE 3

DISTRIBUTION OF ORGANOCHLORINE PESTICIDES AND PCBs
IN FLORISIL COLUMN FRACTIONS

	Percent Recovery by Fraction[a]		
Compound	Fraction 1	Fraction 2	Fraction 3
Aldrin	100		
"-BHC	100		
$-BHC	97		
*-BHC	98		
(-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
Heptachlor	100		
Heptachlor epoxide	100		
Toxaphene	96		
Aroclor 1016	97		
Aroclor 1221	97		
Aroclor 1232	95	4	
Aroclor 1242	97		
Aroclor 1248	103		
Aroclor 1254	90		
Aroclor 1260	95		

[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

Data from Reference 9.

TABLE 4

AVERAGE RECOVERIES OF AROCLORS FROM FLORISIL CARTRIDGES

Compound	Average Recovery (%)
Aroclor 1016	105
Aroclor 1221	76
Aroclor 1232	90
Aroclor 1242	94
Aroclor 1248	97
Aroclor 1254	95
Aroclor 1260	90

TABLE 5

ELUTION PATTERNS AND RECOVERIES OF ORGANOCHLORINE PESTICIDES FROM FLORISIL CARTRIDGES[a]

	Fraction 1		Fraction 2		Fraction 3	
Compound	% Rec.	RSD	% Rec.	RSD	% Rec.	RSD
"-BHC	-	-	111	8.3	-	-
$-BHC	-	-	109	7.8	-	-
(-BHC	-	-	110	8.5	-	-
*-BHC	-	-	106	9.3	-	-
Heptachlor	98	11	-	-	-	-
Aldrin	97	10	-	-	-	-
Heptachlor epoxide	-	-	109	7.9	-	-
Chlordane	-	-	105	3.5	-	-
Endosulfan I	-	-	111	6.2	-	-
4,4'-DDE	104	5.7	-	-	-	-
Dieldrin	-	-	110	7.8	-	-
4,4'-DDD	-	-	111	6.2	-	-
Endosulfan II	-	-	-	-	111	2.3
Endrin aldehyde	-	-	49	14	48	12
4,4'-DDT[b]	40	2.6	17	24	63	3.2
Endosulfan sulfate[b]	-	-	-	-	-	-
Methoxychlor	-	-	85	2.2	37	29

[a]1-g Florisil cartridges spiked with 0.5 µg of each compound.

[b]These two compounds coelute on the DB-5 capillary column.

Eluant composition: Fraction 1 - 3 mL of hexane
Fraction 2 - 5 mL of methylene chloride/hexane (26/74, v/v)
Fraction 3 - 5 mL of acetone/hexane (10/90, v/v)

TABLE 6

DISTRIBUTION OF ORGANOPHOSPHORUS PESTICIDES
IN FLORISIL CLEANUP FRACTIONS

	Percent Recovery by Fraction			
Compound	Fraction 1	Fraction 2	Fraction 3	Fraction 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 data provided by U.S. FDA)
NR = Not recovered (U. S. FDA)
V = Variable recovery (U. S. FDA)
ND = Not determined

Pre-release version - This method has NOT been released by OSW as part of Update IV

TABLE 7

PERCENT RECOVERIES AND ELUTION PATTERNS FOR 22 CHLORINATED HYDROCARBONS FROM 1-g FLORISIL CARTRIDGES[a]

	Fraction 2	
Compound	Average Recovery (%)	RSD
Hexachloroethane	95	2.0
1,3-Dichlorobenzene	101	2.3
1,4-Dichlorobenzene	100	2.3
1,2-Dichlorobenzene	102	1.6
Benzyl chloride	101	1.5
1,3,5-Trichlorobenzene	98	2.2
Hexachlorobutadiene	95	2.0
Benzal chloride	99	0.8
1,2,4-Trichlorobenzene	99	0.8
Benzotrichloride	90	6.5
1,2,3-Trichlorobenzene	97	2.0
Hexachlorocyclopentadiene	103	3.3
1,2,4,5-Tetrachlorobenzene	98	2.3
1,2,3,5-Tetrachlorobenzene	98	2.3
1,2,3,4-Tetrachlorobenzene	99	1.3
2-Chloronaphthalene	95	1.4
Pentachlorobenzene	104	1.5
Hexachlorobenzene	78	1.1
"-BHC	100	0.4
(-BHC	99	0.7
$-BHC	95	1.8
*-BHC	97	2.7

[a]Florisil cartridges (Supelco, Inc.) were conditioned with 4 mL of hexane. Five replicate experiments were performed.

The cartridges were spiked with 1.0 µg per cartridge for hexachloroethane, hexachlorobutadiene, hexachloropentadiene, pentachlorobenzne, and hexachlorobenzene. The trichlorobenzenes, tetrachlorobenzenes, benzal chloride, benzotrichloride, and the BHCs were spiked at 10 µg per cartridge. The dichlorobenzenes and benzyl chloride were spiked at 100 µg per cartridge, and 2-chloronaphthalene was spiked at 200 µg per cartridge.

The cartridges were eluted with 5 mL of acetone/hexane (10/90, v/v).

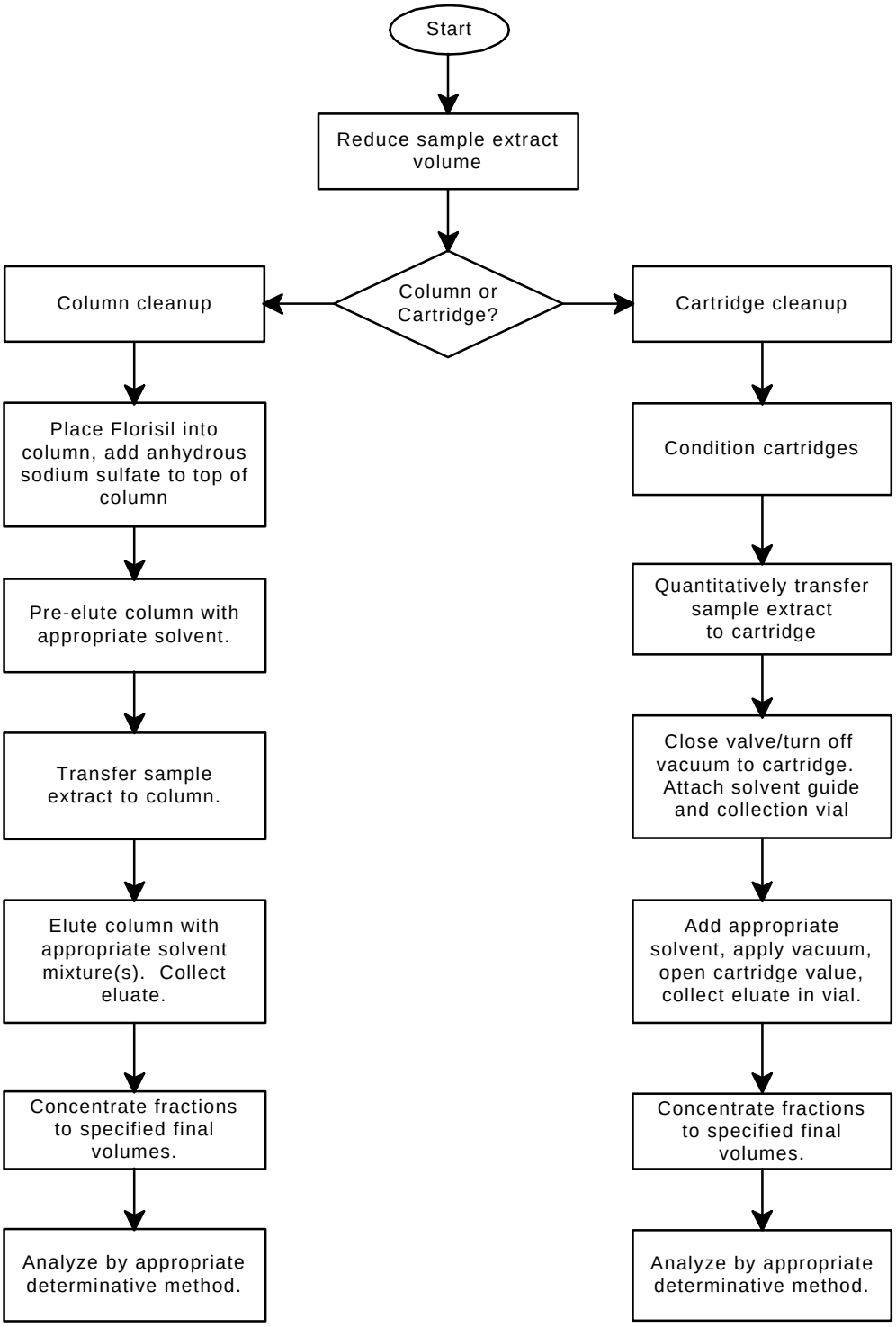
TABLE 8

DISTRIBUTION OF ANILINES IN FLORISIL CLEANUP FRACTIONS

	Percent Recovery by Fraction[a]		
Compound	Fraction 1	Fraction 2	Fraction 3
Aniline		41	52
2-Chloroaniline		71	10
3-Chloroaniline		78	4
4-Chloroaniline	7	56	13
4-Bromoaniline		71	10
3,4-Dichloroaniline		83	1
2,4,6-Trichloroaniline	70	14	
2,4,5-Trichloroaniline	35	53	
2-Nitroaniline		91	9
3-Nitroaniline		89	11
4-Nitroaniline		67	30
2,4-Dinitroaniline			75
4-Chloro-2-nitroaniline		84	
2-Chloro-4-nitroaniline		71	10
2,6-Dichloro-4-nitroaniline		89	9
2,6-Dibromo-4-nitroaniline		89	9
2-Bromo-6-chloro-4-nitroaniline		88	16
2-Chloro-4,6-dinitroaniline			76
2-Bromo-4,6-dinitroaniline			100

Eluant composition: Fraction 1 - 50% methylene chloride in hexane
Fraction 2 - 5% 2-propanol in hexane
Fraction 3 - 5% methanol in hexane

METHOD 3620C
FLORISIL CLEANUP



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