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Florisil Cleanup-EPA Method 3620B

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1 Scope and Application

Method 3620B is a cleanup method used to separate analytes from interfering compounds prior to sample analysis by a chromatographic method. This method includes procedures for the cleanup of organochlorine pesticides, PCBs, and herbicides. Florisil cleanup is accomplished by using a chromatographic column packed with Florisil and eluted with solvent mixtures of varying polarity.

2 Definitions

Refer to Chapter 3 of the Georgia EPD Laboratory Quality Assurance Manual for Quality Control Definitions.

3 Interferences

All glassware is to be meticulously cleaned and solvent rinsed before it is to be used for this procedure. Refer to SOP "Glassware Maintenance".

4 Safety

Safety- Refer to the Laboratory Chemical Hygiene Plan, online revision

5 Apparatus and Equipment

5.1 Chromatographic column: 280-300mm long x 18-20mm ID, with a PTFE stopcock.
5.2 Beakers: 250ml
5.3 Reagent bottles: 4L size
5.4 Boiling chips
5.5 Kadurna-Danish (K-D) sample concentration apparatus: (Optional, to be used instead of TurboVap Nitrogen blow-down water bath)
- 5.5.1 Concentrator tube: 10-ml, jacketed
- 5.5.2 K-D evaporation flask: 500-ml, attached with clamps
- 5.5.3 Snyder column: three ball macro

5.6 Funnel: glass, for powders
5.7 Precision Oven
5.8 Graduated cylinders: 1000ml, 100ml, 10ml
5.9 Glass stirring rod
5.10 Aluminum weighing boats
5.11 Volumetric pipet: 1ml, Class A
5.12 Test tubes: 13mm with PTFE screw caps
5.13 Volumetric flasks: 100ml, 500ml
5.14 Erlenmeyer flasks: 300ml, 25ml w/glass stopper
5.15 TurboVap or equivalent blow-down water bath (Optional, to be used instead of K-D

concentration apparatus)

6 Reagents

6.1 Granular Florisil (magnesia-silica gel): Pesticide Residue (PR) grade, 60-100 mesh, pre-activated @ 675ºC.

6.1.1 Heat the Florisil at 130-150ºC in a glass container overnight.

6.1.2 Cool the Florisil before use.

6.1.3 Florisil from different batches may vary in adsorptive capacity. The following procedure determines the lauric acid value and the amount of florisil from a particular batch to be used for each sample extract:

6.1.3.1 Weigh 2.00gm of Florisil in a 25ml Erlenmeyer flask w/stopper.

6.1.3.2 Cover with aluminum foil and heat overnight at 130-150EC.

6.1.3.3 Stopper the flask and cool to room temperature.

6.1.3.4 Add 20ml of the lauric acid solution to the flask.

6.1.3.5 Stopper the flask and shake occasionally for 15 minutes.

6.1.3.6 Let the Florisil settle and using a volumetric pipet, transfer 10ml of the liquid into a 125ml Erlenmeyer flask. Avoid getting any Florisil.

6.1.3.7 Add 60-ml of ethanol and 3 drops of phenolphthalein indicator solution to the flask.

6.1.3.8 Titrate the solution in the flask with the 0.05N NaOH solution until a permanent end point is reached (when the indicator color is maintained after the solution is allowed to stand for 1 minute).

6.1.3.9 Record the volume of NaOH solution needed to reach end point.

6.1.3.10 Lauric Acid Value (LAV) is calculated as follows:

(LAV) = 200- (titration volume in ml from Sec. 6.1.3.9) (Strength of NaOH from Sec. 6.6.3.4),

6.1.3.11 Use the following equation to obtain the required amount of Florisil for sample cleanup (this amount is unique per Florisil batch):

$$F = \frac{(LAV)}{110} \times 20 \text{ gm} = \text{required amount of Florisil}$$

6.2 Sodium Sulfate: granular, anhydrous, pesticide grade

6.3 Solvents: All solvents must be pesticide quality or equivalent

6.3.1 Ethyl ether (Ether)

6.3.2 Hexane

6.3.3 Methylene chloride

6.3.4 Ethanol

6.3.5 6% Ether in Hexane (for pesticides)

6.3.5.1 Add 60 ml of diethyl ether to 940 ml of hexane in a graduated cylinder and mix with a glass stirring rod.

6.3.5.2 Transfer to a properly labeled jug.

6.3.6 15% Ether in Hexane (for pesticides)

6.3.6.1 Add 150 ml of diethyl ether to 850 ml of hexane in a graduated cylinder and mix with a glass stirring rod.

6.3.6.2. Transfer to a properly labeled jug.

6.3.7 50% Ether in Hexane (for pesticides)

6.3.7.1 Add 500 ml of diethyl ether to 500 ml of hexane in a graduated cylinder and mix with a glass stirring rod.

6.3.7.2 Transfer to a properly labeled jug.

6.3.8 20% Methylene Chloride in Hexane (for phenoxy acid herbicides)

6.3.8.1 Add 200ml of methylene chloride to 800ml of hexane in a graduated cylinder and mix with a glass stirring rod.

6.3.8.2 Transfer to a properly labeled jug.

6.3.9 50% Methylene Chloride in Hexane (for phenoxy acid herbicides)

6.3.9.1 Add 500ml of methylene chloride to 497ml of hexane and 3ml of acetonitrile in a graduated cylinder and mix with a glass stirring rod. *Note: use other graduated cylinders as needed to measure these amounts.*

6.3.9.2 Transfer to a properly labeled jug.

6.3.10 Acetone: pesticide grade or better

6.3.11 Water: organic free reagent water or equivalent

6.4 Lauric acid: reagent grade, used for standardization of the Florisil activity.

6.4.1 Weigh 10gm of lauric acid and quantitatively transfer to a 500ml vol. flask.

6.4.2 Add 50ml of hexane to the flask and swirl until lauric acid is dissolved.

6.4.3 Dilute the solution in the flask to 500ml with additional hexane.

6.5 Phenolphthalein indicator:

6.5.1 Weigh 1.0 gm of phenolphthalein in a 100ml volumetric flask

6.5.2 Dilute to 100ml with ethanol

6.6 Sodium hydroxide, 1N

6.6.1 Weigh out 20 grams NaOH in a 500-ml volumetric flask

6.6.2 Dissolve in organic-free reagent water and dilute to 500-ml to make a 1N solution.

6.6.3 Dilute 25ml of the 1 N NaOH to 500 ml with water in a second 500ml volumetric flask, yielding a 0.05N solution. This solution must be standardized against lauric acid as follows:

6.6.3.1 Weigh 100-150mg of lauric acid to the nearest 1mg in a 125ml Erlenmeyer flask.

6.6.3.2 Add 50ml of ethanol to the flask and swirl to dissolve the lauric acid.

6.6.3.3 Add 3 drops of phenolphthalein indicator to the flask, and titrate with 0.05N NaOH solution to a permanent endpoint.

6.6.3.4 Calculate the strength of the NaOH solution as the mg of lauric acid neutralized per ml of NaOH solution.

$$S = \text{Strength of NaOH soln.} = \frac{\text{mg of lauric acid}}{\text{ml of NaOH used}}$$

6.7 Glass wool: solvent cleaned

6.7.1 Cut 1-inch strips of the new glass wool with clean scissors.

6.7.2 Loosely fill 2 or 3 tumbler bottles with the cut glass wool.

6.7.3 Add 1 liter of methylene chloride:acetone (1:1) and tumble for 2-3 hours.

6.7.4 Discard the used solvent and repeat step 6.7.3.

6.7.5 Discard the used solvent and air-dry the washed glass wool under a hood.

7 Sample Collection

Refer to Chapter 5 of the Georgia EPD Laboratory Quality Assurance Manual for sample container, sample preservation, and sample holding times.

8 Calibration

Analytical balances are serviced and calibrated once per year. Daily readings with certified weights are taken early each morning to insure calibration. A daily log is maintained with this information. Oven temperatures are measured with NIST certified thermometers and the readings are recorded daily on a designated log.

9 Quality Control

Proper performance of the clean-up procedure is validated when quality control criteria for laboratory control samples (LCS/LCSD) are met for the analytical method being used.

10 Procedure

10.1 Take a chromatographic column with reservoir and stopcock, and stopper the bottom with approximately 2 inch of solvent cleaned glass wool.
10.2 Add the appropriate amount of activated and cooled Florisil that was pre-determined by the lauric acid value (approximately 20g for Pesticides/PCB and 4g for Herbicides).
10.3 Add sodium sulfate to the top of the Florisil to form a layer about 0.5-1cm thick.
10.4 Settle the Florisil and the sodium sulfate by tapping the column.
10.5 Add 60 ml of hexane to wet and rinse the sodium sulfate and the Florisil.
10.6 Start to elute the solvent into a waste beaker.
10.7 Just prior to exposure of the sodium sulfate to air, stop the elution of the solvent by closing the stopcock.
10.8 Discard the rinsing eluate.
10.9 Place K-D apparatus with a 10-ml concentrator tube or TurboVap concentrating beaker under the column to collect the eluted sample extract.
10.10 With a disposable pipet, carefully transfer the sample extract to the Florisil column.
10.11 Rinse the sample tube with hexane and transfer this to the Florisil column.
10.12 For Pesticide and PCB sample extracts (5ml volume):

- 10.12.1 Elute the column with 200ml of 6% ether/hexane at a dripping rate of 5 ml/min.
- 10.12.2 Drip the eluate into the K-D or TurboVap beaker until the sodium sulfate layer is nearly exposed. Do not permit exposure to air.
- 10.12.3 Elute the column now with 200ml of 15% ether/hexane into the same K-D or TurboVap beaker.
- 10.12.4 Follow with 200ml of 50% ether/hexane into an Erlenmeyer flask.
  Note: This fraction or elution may be combined with the previous 2 fractions.

10.13 For derivatized Herbicide sample extracts (2ml volume):

- 10.13.1 Elute the column with 35ml of 20% methylene chloride/hexane at a dripping rate of 2ml/min.
- 10.13.2 Drip the eluate into the K-D or TurboVap beaker until the sodium sulfate layer is nearly exposed. Do not permit exposure to air.
- 10.13.3 Elute the column again with 60ml of 50% methylene chloride/hexane into same K-D or TurboVap beaker.

10.14 Add a boiling chip to each K-D, and attach a three ball Snyder column.
10.15 Concentrate the eluates in a hot water bath to a final volume of 5ml in hexane for Pesticides/PCBs or to a final volume of 10ml in hexane for Herbicides, or, you may concentrate eluates using a TurboVap unit or equivalent.

11 Calculations

See Section 6.1.3.10 for Lauric Acid Value (LAV), Section 6.1.3.11 for Required Amount of Florisil (F), Section 6.6.3.4 for Strength of NaOH (S).

12 References - SW846 EPA Method 3620B.

13 Method Reference Tables

Table 13.1 Distribution of Organochlorine Pesticides and PCBs in Florisil Fractions

COMPOUND	% Recovery Frac 1	% Recovery Frac 2	% Recovery Frac 3
Aldrin	100		
a-BHC	100		
b-BHC	97		
d-BHC	98		
Lindane	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		
Aroclor 1242	97		
Aroclor 1248	103		
Aroclor 1254	90		
Aroclor 1260	95		