

MODESTO METHOD SERIES

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SHELL DEVELOPMENT COMPANY
BIOLOGICAL SCIENCES RESEARCH CENTER
MODESTO, CALIFORNIA

DETERMINATION OF SD 43775 RESIDUES IN CROPS, ANIMAL TISSUES, SOIL AND WATER

Electron-Capture Gas Chromatographic Method

SCOPE

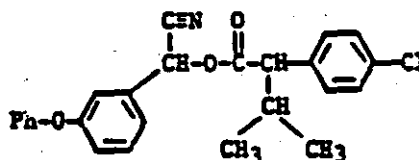
1. A GLC method, using the electron-capture (EC) detector, is described for determining residues of the pyrethroid insecticide SD 43775 in crops, animal tissues, soil and water. The minimum detectable concentration (MDC) is about 0.01 milligram per kilogram or 0.01 ppm for crops, animal tissues and soil, and about 0.05 micrograms per kilogram or 0.05 ppb for water.

ANALYTE

CHEMICAL NAME

STRUCTURE

SD 43775 Benzenecetic acid, 4-chloro-
-alpha-(1-methylethyl)-, cyano
(3-phenoxyphenyl)methyl ester



METHOD SUMMARY

2. Crops and animal tissues are extracted with hexane/isopropanol (3:1) and the isopropanol removed by water partitioning. The hexane extracts of animal tissues and oily crops are partitioned with acetonitrile to separate lipids from the SD 43775. The acetonitrile is exchanged back to hexane and cleaned up by liquid-solid chromatography through an activated Florisil column. Hexane extracts of non-oily crops are cleaned up directly without acetonitrile partitioning.

Soil samples are extracted by high frequency vibration in the presence of acetone/hexane (1:1), the solvent is exchanged to hexane, and an aliquot subjected to liquid-solid chromatography using Florisil adsorbent.

Water samples are extracted by partitioning with hexane. Clean-up is achieved by liquid-solid chromatography using Florisil adsorbent.

SD 43775 is quantitated by GLC-EC.

SPECIAL APPARATUS

3. (a) Steam table. A flat-topped steam bath with sufficient number of holes equipped with concentric rings.

(b) Air evaporation manifold, designed to direct streams of clean, dry air downward into vessels.

SPECIAL APPARATUS (Cont.)

- (c) Waring blenders (explosion-proof).
- (d) GLC fitted with a high temperature electron-capture detector.
- (e) Miniature chromatographic columns. Columns should be about 200 mm long with ca 10 mm inside diameter topped with a 50 ml solvent reservoir, and with a sintered glass plate (coarse porosity) and stopcock at the lower end.
- (f) Braun-Sonic 1510 ultrasonic generator.
- (g) Homogenizer - Polytron or tekmar Super Dispax.
- (h) Hobart food chopper, Model 8142.
- (i) Laboratory mill - Wiley Model No. 3 or equivalent.

REAGENTS

- 4. (a) Hexane, isopropanol, acetonitrile, acetone, ethyl acetate. Distilled in glass solvents from Burdick and Jackson Labs., Muskegon, Michigan (or equivalent).
- (b) 3% Dexsil 300 on 100/120 mesh Supelcoport. Supelco, Inc., Bellefonte, Pennsylvania.
- (c) Florisil, 60/100 mesh. Floridin Company, Tallahassee, Florida.
- (d) Analytical standard of SD 43775 (available from Shell Chemical Company, Houston, Texas).

SAMPLE REDUCTION

5. Homogenize bulk crops and green foliage in a Hobart food chopper prior to sampling for analysis. Grind hays and dried foliage in a Wiley lab mill and homogenize by tumbling in an oversized container. Grind difficult samples (such as whole ginned cotton seeds, animal tissues, etc.) in the presence of dry ice or liquid nitrogen in Waring blenders. Allow dry ice or liquid nitrogen to dissipate before sampling for analysis.

EXTRACTION

- 6. (a) Crops and animal tissues (except fat)

Weigh 10-50 grams of representative sample into Waring blender cups and add 200 ml of hexane/isopropanol (3:1). Blend at high speed for 1-3 minutes. For smaller samples or tough animal tissues, homogenize in the presence of the same solvent using a Polytron tissue reducer.

Filter watery crops and green foliage extracts into 250 ml separatory funnels. Centrifuge extracts of animal tissues and oily crops (seeds, etc.) and decant supernatant into 250 ml separatory funnels.

EXTRACTION (Cont.)

Add 100 ml of water to each separatory funnel, shake carefully for ca 1 minute, drain, and discard lower (aqueous) phase. Wash hexane with two additional 100 ml volumes of water to remove all isopropanol. The crop-to-solvent ratio after removal of isopropanol equals the weight of sample (grams) extracted divided by 150 (mls of hexane).

For watery crops and some green foliage proceed with "Clean-up by Liquid-Solid Chromatography" (Section 8). For all other samples proceed with "Clean-up by Liquid Partitioning" (Section 7).

(b) Animal fat

Weigh 10-20 grams of a representative sample into a Waring blender cup, add 200 ml of hexane, and 20 grams of sodium sulfate. Blend at high speed for 1 minute and decant into a 500 ml Erlenmeyer flask. Repeat the extraction with an additional 200 ml of hexane and combine extracts in flask. Add three or four boiling chips to the flask and concentrate on a steam table to 50-75 ml. Transfer hexane to a 100 ml graduated cylinder and adjust final volume to 100 ml using hexane to rinse flask and boiling chips. Proceed with "Clean-up by Liquid Partitioning" (Section 7).

(c) Soil ←

Pass 500 grams or more of representative soil through a No. 8 U.S. Standard sieve. Prior to screening, (1) break up the larger agglomerates into smaller particles, (2) spread soils that are excessively wet onto a flat surface and allow to dry sufficiently enough at room temperature to enable passage through the screen, and (3) discard rocks, plants, and other extraneous material. After screening and blending, determine the soil's water content.

Weigh 50 grams of a representative sample into a Kalgene centrifuge bottle. Add 150 ml of 1:1 acetone/hexane, immerse the probe tip of the Braun-Sonic 1510 about 0.50 to 0.75 inch beneath the surface of the solvent, and apply 300 watts or Peak Envelope Power (PEP) for two minutes. Rinse probe with acetone, cap, and centrifuge. Filter the solution into a 500 ml Erlenmeyer flask, retaining the sample matrix in the bottle. Add 100 ml of the 1:1 extraction solvent to the matrix, cap, shake thoroughly, centrifuge, and filter as before. Add three or four boiling chips and concentrate to 30-40 ml on a steam table. Add 200 ml of hexane and again concentrate to 30-40 ml. Transfer the extract to a 100 ml graduated cylinder and adjust volume to ca 80 ml using hexane. Transfer extract to a 250 ml separatory funnel, add 100 ml of water, and shake carefully for one minute. Discard lower (aqueous) phase and drain extract into 100 ml graduated cylinder. Adjust to a final volume of 100 ml using hexane. Proceed with "Clean-up by Liquid-Solid Chromatography" (Section 8).

(d) Water

SD 43775 is extremely hydrophobic and will quickly sorb onto particulates or containers from aqueous solutions. Small enough water samples should be taken such that the entire sample can be analyzed, since proper sub-sampling is difficult and results can be misleading. Eight or sixteen ounce glass sampling bottles with aluminum foil-lined caps are adequate. The following method assumes 8 oz glass sample bottles

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containing 200-250 grams of water for analysis. Glassware, solvent volumes, and technique should be adjusted according to actual sample size, using this method as a guide.

Weigh sample bottle and sample prior to analysis. Decant entire sample (usually 200-250 ml) into a 500 ml separatory funnel leaving as much sediment as possible in the bottle. Add 20 ml of acetone to the bottle, cap with original aluminum foil-lined cap, and shake vigorously. Add 50 ml of hexane to the bottle, cap, and shake again. Transfer the contents of the bottle to the separatory funnel containing the water sample and shake vigorously for at least two minutes. Reweigh the empty sample bottle to obtain sample weight. Drain and discard lower (aqueous) phase. Concentrate hexane extract to 1 or 2 ml. Proceed with "Clean-up by Liquid-Solid Chromatography" (Section 8).

CLEAN-UP BY LIQUID PARTITIONING

7. Transfer an aliquot of hexane extract, equivalent to 2 grams of sample (1 gram of animal fat or vegetable oil), to a 250 ml separatory funnel and add hexane to make a total volume of 50 ml. Add 100 ml of hexane saturated acetonitrile and shake vigorously for ca 1 minute. Drain the acetonitrile into a clean 250 ml separatory funnel and discard the hexane (upper phase). Add 50 ml of fresh hexane to the acetonitrile, shake vigorously, and allow phases to separate. Drain acetonitrile into a 250 ml Erlenmeyer flask containing a few boiling chips and concentrate to 20-30 ml on a steam table. Add 100 ml aliquots of hexane and concentrate until no acetonitrile remains. Concentrate hexane to ca 3 ml in tapered glass tube using a gentle air jet. Proceed with "Clean-up by Liquid-Solid Chromatography" (Section 8).

CLEAN-UP BY LIQUID-SOLID CHROMATOGRAPHY *← 50 ml / 1/20 ml*

8. Activate some Florisil by heating overnight at 145°C. Store the activated Florisil in air-tight containers at room temperature until used.

Fill the tube portion of a miniature chromatographic column (see "Special Apparatus") with hexane. Weigh out 6.0 grams of activated Florisil into a small beaker and cover with hexane. Mix and transfer the slurry to the chromatographic column using additional hexane to effect a complete transfer. With the hexane flow on, aid settling by tapping the sides of the column with a solid object. Add a layer of sodium sulfate (anhydrous) to the top of the column packing ca 1 cm deep. Allow the excess hexane to drain until ^{ca} 1 ml of hexane remains on top of the column packing.

Transfer an aliquot of the extract of Section 6 or 7, equivalent to 1.0 or 2.0 grams (200-250 grams for water samples), onto the prepared Florisil column. Drain the hexane extract into the column packing and wash with additional hexane (ca 5 ml). Add 50 ml of hexane to the column and drain at the rate of ca 2 drops/second. Discard the hexane fraction. Add 50 ml of 5% ethyl acetate in hexane to the column, drain at the same rate, and save for SD 43775 determination. The volumes of solvent required for the column chromatography should be confirmed by running a column "profile" of a fortified sample. Concentrate the final eluate to a crop to solvent ratio suitable for GLC analysis.

GLC ANALYSIS

9. GLC Operating Conditions. GLC columns and operating parameters are chosen which achieve optimum balance between sensitivity and degree of resolution with symmetrical peaks emerging at reasonable retention times. Retention times are relative since variation will occur in packings and uniformity in packed columns. Due to the number of variables involved, the operating conditions listed below should serve only as a guide.

Instrument	:	Packard, Model 7300
Detector	:	EC-N1 63 (Tracor)
Glass Column	:	4' x 2 mm ID (no glass wool at inlet)
Solid support	:	Supelcoport
Mesh size	:	100/120
Liquid phase	:	Dexsil 300
Percent weight	:	3.0
Temperatures, °C		
Column	:	280
Inlet	:	280
Detector	:	300
Carrier Gas	:	Argon-methane
Flow rate	:	20 ml/minute
Typical recorder response for 0.15 nanograms of SD 43775		
		<u>1st Peak</u> <u>2nd Peak</u>
Percent of full scale	:	44 40
Noise level	:	< 1 < 1
Retention time, min.	:	4.38 4.75

NOTE: SD 43775 is separated into two pairs of diastereoisomers which appear as two separate GLC peaks using this column.

SAMPLE ANALYSIS

10. (a) Prepare standards for GLC analysis in the range of 0.02 to 0.1 µg/ml SD 43775 in hexane. Inject 3 µl aliquots of these in order to construct a calibration curve. Inject 3 µl aliquots of the sample solutions with frequent intermittent (every 3-4 samples) injections of standards in order to monitor instrument sensitivity.

(b) In addition to non-treated check samples and recovery samples (spiked non-treated check samples), each series of samples should include a "reagent blank" which is also carried through from extraction to the final analysis.

FILE ANALYSIS (Cont.)

(c) Calculate the SD 43775 content of the sample by means of the following equation:

$$C = \frac{W}{S}$$

where:

C = concentration of the compound in milligrams/
kilograms of sample (ppm)

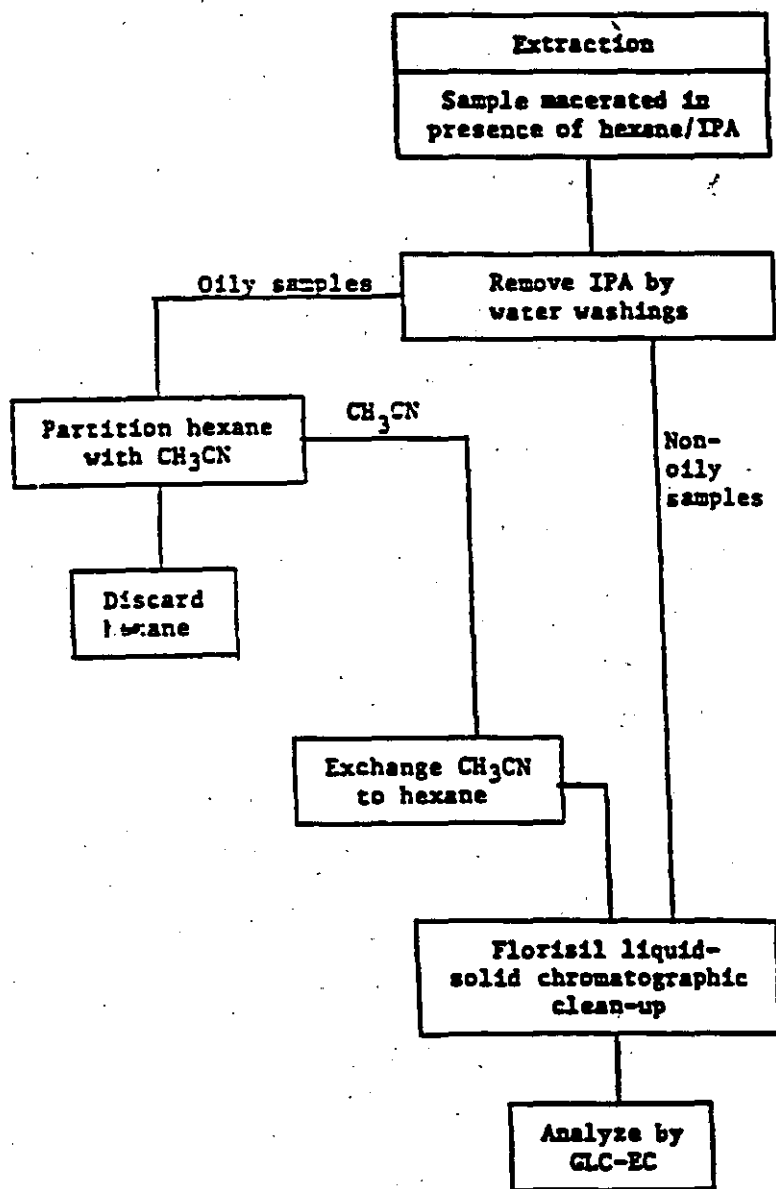
W = weight of SD 43775 in nanograms found in the
aliquot of sample injected

S = amount of the sample in milligrams represented
by the aliquot injected

NOTE: Either GLC peak may be used to calculate SD 43775 residues when the isomer peak ratios are the same for standards and samples. However, if the isomer ratio is significantly different between samples and standards, both peaks should be used for final determination of SD 43775. This can be done by quantifying each peak separately and combining results.

METHOD FLOWCHARTS

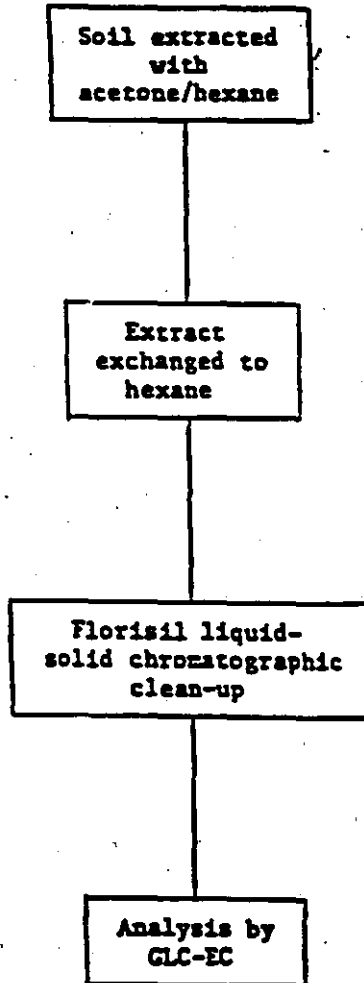
12. (a) Residue Determination of SD 43775 in Crops and Animal Tissues



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METHOD FLOWCHARTS (Cont.)

(b) Residue Determination of SD 43775 in Soil



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