

Extraction Method for Etridiazole in Cotton

Step 1 Extraction

Weigh 10 g of sample in a 250-mL Teflon® flask. Fortify the two spike samples used to determine extraction method recoveries for the set of samples. Add 200 mL 50:50 v/v acetone:hexane and shake on a wrist action shaker for 20 min. Filter the sample into a 250-mL filter flask. Rinse the Teflon® flask with 50 mL of 50:50 acetone:hexane. Pour filtrate into 500-mL separatory funnel.

Step 2 Partition

Add 150 mL Type I water to separatory funnel and gently invert 8-10 times. Drain the water layer into a filter flask. Add another 50 mL of the water to the solvent layer in the separatory funnel and gently invert 8-10 times. Drain the water layer into a 150-mL beaker. Pour the solvent layer into a 500-mL round-bottom flask. Re-extract the water layers with 50 mL hexane, gently inverting 8-10 times. Drain and discard the water layer and add the remaining hexane layer to the round-bottom flask. Roto-evaporate the sample down to approximately 10 mL.

Step 3 Florisil Clean-up

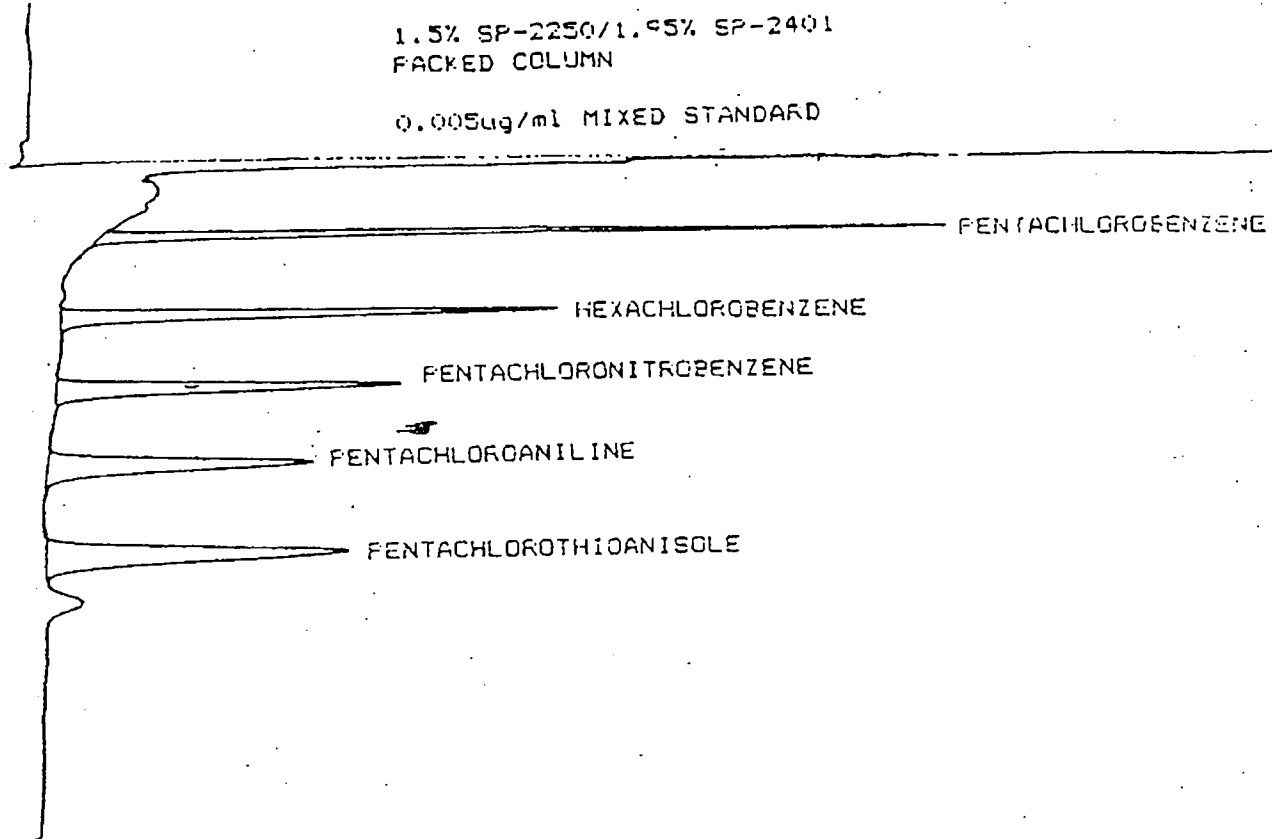
Prepare a wet packed Florisil column by first adding about 50 mL of hexane to a 300 mm x 20 mm column. Fill with Florisil to about 5 inches, when settled (approximately 15 g). Cover the Florisil with about 1 cm of anhydrous sodium sulfate. Drain and discard the excess hexane in the column, only to the point where the florisil is still fully covered and wet with hexane. Do not allow the column to become dry at any time. Pour the sample onto the column. Collect the sample into a round-bottom flask by opening the stop cock enough for a slow but constant drip rate. Just as the sample goes fully into the florisil, begin eluting with 100 mL of 5% ether:hexane.

Step 4 Change Solvent and Bring to Final Volume

Roto-evaporate the sample down to about 5 mL. Bring the final volume to 10 mL with hexane. The sample is now ready for analysis on the GC.

Figure 1

Copy of A Typical Chromatogram of 0.005 ug/mL Mixed
Standard On A 1.5% SP-2250/1.95% SP-2401 Column

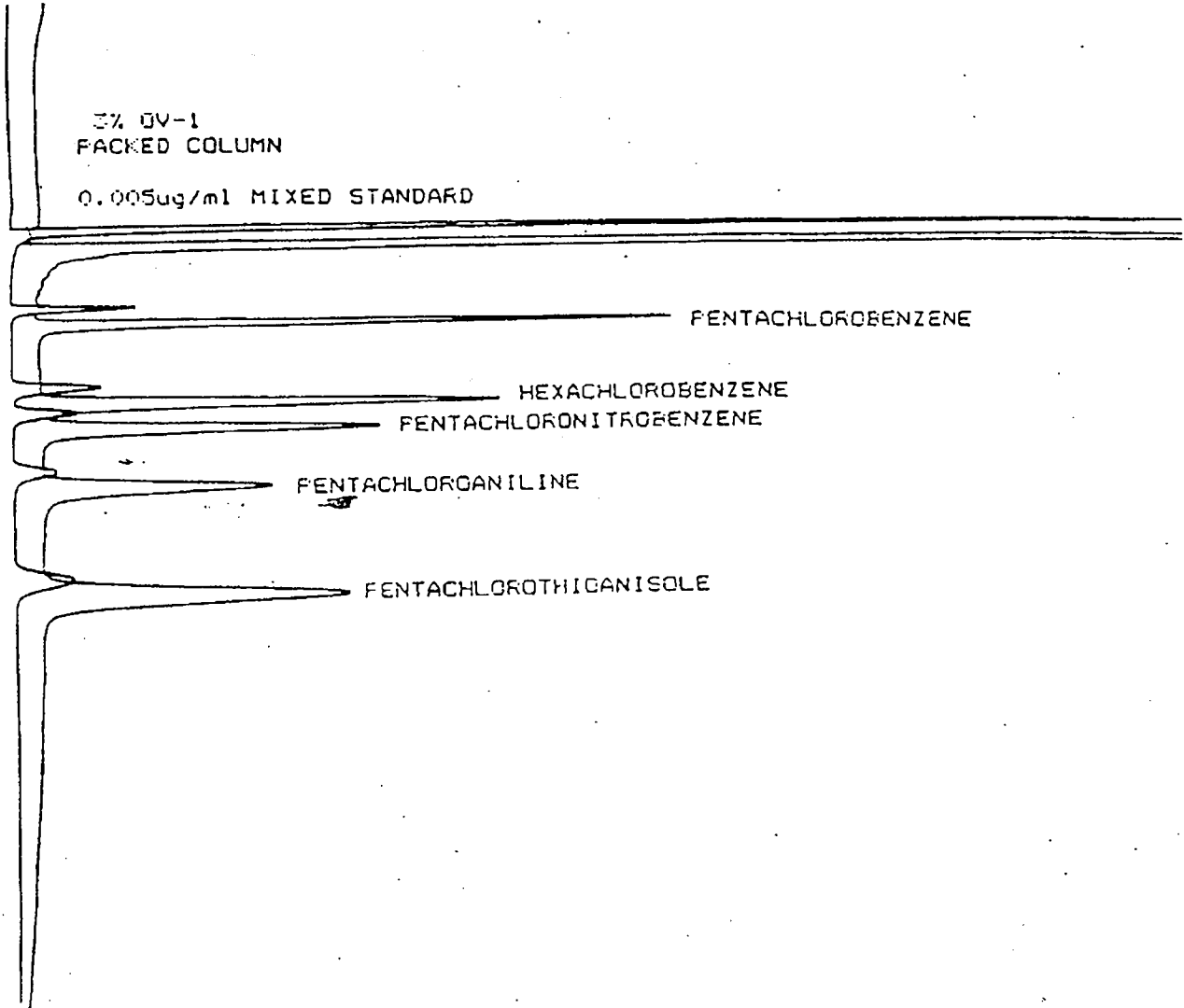


Retention Times (RT)

<u>Compound</u>	<u>RT</u> <u>(Minutes)</u>
Pentachlorobenzene	1.1
Hexachloronitrobenze	2.3
Pentachloronitrobenzene	3.4
Pentachloroaniline	4.5
Pentachloroanisole	5.8

Figure 2

Copy of A Typical Chromatogram of 0.005 ug/mL Mixed
Standard On A 3% OV-1 Column



Retention Times (RT)

<u>Compound</u>	<u>RT</u> <u>(Minutes)</u>
Pentachlorobenzene	1.3
Hexachloronitrobenzene	2.6
Pentachloronitrobenzene	3.0
Pentachloroaniline	3.9
Pentachloroanisoole	5.7

METHOD MODIFICATIONS

The following method modifications had no effect on the study.

- o The gas chromatograph oven temperature was lowered from 190°C to 150°C for the 3% OV-1 column.
- o Results were generated on a Hewlett-Packard A400 Laboratory Automation System (LAS) computer instead of on a recorder.
- o The calculations shown on page 13 of this report are different than those stated in the method. The difference is that the method indicates a volume ratio factor that is not included in the report and a sample weight factor is shown in the report, but not in the method.

ETRIDIAZOLE

CAM-24-73

July 3, 1973

DETERMINATION OF TERRAZOLE[®] (5-ETHOXY-3-TRICHLOROMETHYL-1,2,4-THIADIAZOLE) AND TERRACLOR[®] (PENTACHLORONITROBENZENE) AND ALLIED METABOLITES IN PLANT TISSUES OR HARVEST SAMPLES

I. PRINCIPLE

A representative sample of plant tissue or harvest sample is extracted with hexane. The hexane is then analyzed for TERRAZOLE, TERRACLOR and allied metabolites using a chromatograph with an electron affinity detector.

II. SCOPE

The method has been used to determine residual TERRAZOLE, TERRACLOR and impurities in plant tissues or harvest samples with a reliable sensitivity down to 0.005 ppm. Recovery standards at this level are greater than 70 percent.

III. EQUIPMENT

A. Apparatus

1. Perkin Elmer 990 gas chromatograph equipped with nickel detector or equivalent.
2. Osterizer and 4 oz. jar with cutting assembly.
3. 250 ml Erlenmeyer flask with ground glass stopper.
4. Column

Five feet of stainless steel tubing (1/4" O.D. 1/64" wall) containing ~ 23.5 gm of 3 percent SE-30 on Chromosorb G. DMCS treated and acid washed, 80 - 100 mesh. Column conditioned at 300°C with N₂ flow of 100 cc per minute for about 24 hours in column conditioner.

B. Instrument Conditions

Injection Port	195°C
Detector	200°C
Column	185°C
Nitrogen	100 cc per minute
Attenuator	x 8
Amplifier Range	x 10
Polarity Recorder	+
Chart Speed	1/2" per minute

C. Reagents

1. n-hexane; distill reagent grade hexane (high purity) from sodium dispersion in mineral oil. Use ~1 ml of 50 percent dispersed sodium in mineral oil per liter of hexane.
2. Anhydrous Na₂SO₄ (granular).
3. TERRAZOLE, Technical Grade, Olin Chemical Corporation, minimum 95 percent assay.
4. Pentachlorobenzene (PCB); Olin Chemical Corp., obtained from PCNB process and recrystallized. Structure confirmed by infrared spectroscopy.
5. Hexachlorobenzene (HCB) obtained in similar manner as pentachlorobenzene.
6. PCNB; Olin Technical Grade - 98 percent PCNB.
7. Pentachloroaniline (PCA); prepared from PCNB by reduction with zinc in ethanol - HCl, and recrystallized from ethanol.
8. Methyl pentachlorophenyl sulfide; prepared from PCNB by reacting with Na₂S followed by CH₃I with subsequent recrystallization from ethanol; MP 95 - 96°C. Structure confirmed by mass spectroscopy.

IV. STANDARD SOLUTIONS

Suitable concentrations are PCB at 0.007 $\mu\text{g}/\text{ml}$, HCB, PCNB at 0.01 $\mu\text{g}/\text{ml}$, PCA, methyl pentachlorophenyl sulfide and TERRAZOLE at 0.02 $\mu\text{g}/\text{ml}$. These concentrations are in the linear range of peak heights versus μl injected when 3 - 8 μl are employed. Solutions of methyl pentachlorophenyl sulfide decompose with time; therefore, when the peak height begins to drop \sim 15 percent, then a new diluted standard should be prepared.

V. PROCEDURE

- A. 20 to 40 grams of tissue or harvest sample are weighed into a 4 oz. osterizing jar to which is added 50 grams of Na_2SO_4 and 100 ml distilled n-hexane.
- B. Osterize at a blending speed for 2 minutes.
- C. Decant hexane into 250 ml Erlenmeyer flask (Note 1).
- D. Analyze the hexane extract for residual fungicides via the Perkin Elmer 990 chromatograph.

VI. ANALYSIS OF THE EXTRACT

- A. Prepare the chromatograph for analysis (consult Perkin Elmer 990 Gas Chromatograph Instruction Manual for general guidance).
- B. Inject 3 - 8 μl of the hexane extract and compare versus standards run before each sample injection.
- C. Calculate the concentration of the unknown per the following equation.

$$C_u = \frac{V_S \times C_S}{R_S} \times \frac{R_u}{V_u} \times V_t \div W$$

Where C_u = Apparent ppm component in sample
 V_S = Microliters of standard injected
 C_S = Micrograms of component per ml in standard
 R_S = Peak height in millimeters for V_S
 R_u = Peak height in millimeters for unknown
 V_u = Microliters of unknown injected
 V_t = Volume of extracted solution
 W = Weight of sample extracted.

July 3, 1973

Corrected ppm component in treated sample: $CR = Cu \times \frac{100}{R.S.}$

Where CR = corrected ppm component in treated sample
Cu = ppm component in treated sample

R.S. = % Recovery of component added to untreated sample.

NOTE IN PROCEDURE

1. If extract is cloudy, it is well to centrifuge before chromatographing.

SCOPE:

This method has been used to determine residual pentachloronitrobenzene and impurities in plant tissues or harvest samples.

PRINCIPLE:

Plant tissues are dried with anhydrous sodium sulfate and extracted with hexane. The hexane extract is concentrated and cleaned up using Florisil® column chromatography. Samples high in lipid content, such as soybeans, are cleaned up using gel permeation chromatography before Florisil chromatography. The cleaned up extract is concentrated and injected intermittently with standards on a gas chromatograph equipped with an electron capture detector and a 1.5% SP-2250/1.95% SP-2401 or 3% OV-1 column.

SENSITIVITY:

The sensitivity of this method is 0.005 ppm for all test compounds.

PRECISION AND ACCURACY:

The precision and accuracy of this method is shown in Table A.

REFERENCES:

1. "Determination of Terrazole® (5-Ethoxy-3-Trichloro-Methyl-1,2,4-Thiadiazole) and Terraclor® (Pentachloronitrobenzene) and Allied Metabolites in Plant Tissues or Harvest Samples," Uniroyal Method CAM-24-73, July 3, 1973.
2. FDA Pesticide Analytical Manual, 1: Section 210, Rockville, Maryland.

SAFETY PRECAUTIONS:

Observe all standard laboratory safety procedures as outlined in the Hazleton Laboratories America, Inc., Safety Training Manual.

QUALITY ASSURANCE:

Analysis sets of approximately 10 to 15 samples will include one reagent blank, one duplicate sample chosen at random, a fortified control, and a control sample.

APPARATUS:

1. Gel permeation chromatograph (GPC) Auto-Prep, Model 1001, Analytical Bio-Chemistry Laboratories, Columbia, Missouri, equipped with a 600-mm x 25-mm column packed with 62 g of Bio Beads S-X3. Operate according to proper Instrument Operating Procedure.
2. Blender, Waring, explosion proof with 1-qt jars
3. Chromatography column, 300 mm x 20 mm, equipped with a 250-mL reservoir on top and sintered glass/frit on the bottom
4. Round-bottom flasks, assorted sizes
5. Gas chromatograph column, 6 ft x 4 mm i.d., borosilicate glass
6. Funnel, powder, 100-mm opening
7. Volumetric flasks, assorted sizes
8. Gas chromatograph, Hewlett-Packard 5710A, equipped with a 7671A autosampler

Chromatographic Conditions for Pentachloronitrobenzene and Allied Metabolites

Primary Conditions:

Instrument: Model 5710A, Hewlett-Packard gas chromatograph
Column: 6 ft x 4 mm i.d., packed with 1.5% SP2250/1.95% SP2401 on 100/120 mesh Supelcoport
Detector: Ni⁶³ electron capture
Carrier gas: Argon:methane (95:5) at 48 mL/minute
Temperatures:
 Column: 200°C
 Injector: 250°C
 Detector: 300°C
Chart speed: 10 mm/minute
Attenuation: Variable
Injection volume: 4 µL

Gas chromatographic conditions may be modified as necessary to obtain satisfactory chromatography.

Secondary Conditions:

Instrument: Model 5710A, Hewlett-Packard gas chromatograph
Column: 6 ft x 4 mm i.d., packed with 3% OV-1 on 80/100 mesh Gas-Chrom Q
Detector: Ni⁶³ electron capture
Carrier gas: Argon:methane (95:5) at 51 mL/minute
Temperatures:
 Column: 190°C
 Injector: 250°C
 Detector: 300°C
Chart speed: 10 mm/minute
Attenuation: Variable
Injection volume: 4 µL

Gas chromatographic conditions may be modified as necessary to obtain satisfactory chromatography.

REAGENTS:

1. Hexane, pesticide residue (PR) grade, glass distilled
2. Florisil: 60/100 mesh, PR grade
Reactivation at 130°C overnight may be required
Standardization to greater than 90% recovery obtained by varying elution volumes
3. Sodium sulfate, analytical reagent (AR) grade, anhydrous, granular
4. Cyclohexane, PR grade, glass distilled
5. Methylene chloride, PR grade, glass distilled
6. Petroleum ether, PR grade, glass distilled
7. Ethyl ether, PR grade, glass distilled.
8. Ethyl ether (3%) in petroleum ether solution (v/v)
9. Bio Beads S-X3, 200-400 mesh, Bio Rad Laboratories, Richmond, California
10. Isooctane, PR grade, glass distilled
11. GPC chromatographic elution solvent, cyclohexane:methylene chloride (50:50). Prepare by adding 2 L of cyclohexane to 2 L of methylene chloride in a 4-L solvent bottle. Mix by inverting several times.
12. Column packings
 - 1.5% SP2250/1.95% SP2401 on 100/120 Supelcoport, Supelco, Inc., Bellefonte, Pennsylvania
 - 3% OV-1 on 80/100 Gas Chrom Q, Applied Science Laboratories, Inc., State College, Pennsylvania

Standards:

The analytical standards for the parent compound and its primary metabolite were provided by Uniroyal Chemical Company, Inc., Bethany, Connecticut.

Parent Material

Chemical name:	Pentachloronitrobenzene
Common name:	Terraclor or PCNB
Chemical abstracts registry number:	82-68-8

Lot number: 874-142-WHH
Purity: 99.2%
Empirical formula: C₆Cl₅NO₂
Molecular weight: 295.26

Allied Metabolites

Chemical name: Pentachlorobenzene
Common name: PCB
Chemical abstracts
registry number: 608-93-5
Lot number: 062627
Purity: 97.4%
Empirical formula: C₆HCl₅
Molecular weight: 250.3

Chemical name: Pentachloroanisole
Common name: MPCPS
Chemical abstracts
registry number: 1825-19-9
Lot number: AC-885-9
Purity: 99.9%
Empirical formula: C₇H₃SCl₅
Molecular weight: 154.61

Chemical name: Hexachlorobenzene
Common name: HCB
Chemical abstracts
registry number: 118-74-1
Lot number: 052247
Purity: 97.0%
Empirical formula: C₆Cl₆
Molecular weight: 284.8

Chemical name: Pentachloroaniline
Common name: PCA
Chemical abstracts
registry number: 527-20-8
Lot number: AC-885-12
Purity: 97.4%
Empirical formula: C₆H₂NCl₅
Molecular weight: 265.35

Standard Preparation:

Weigh 0.01 g of each compound and transfer to separate 100-mL volumetric flasks using acetone or methanol. Dilute to volume and mix by inverting several times. Resulting concentration is 100 µg/mL.

Working standards are prepared by diluting in an appropriate solvent (acetone or methanol for fortification solutions, isooctane for mix standard solutions). Examples of standard preparations follow:

<u>Initial Concentration ($\mu\text{g}/\text{mL}$)</u>	<u>Aliquot (mL)</u>	<u>Final Volume (mL)</u>	<u>Resulting Concentration ($\mu\text{g}/\text{mL}$)</u>
100	10	100	10.0
100	1	100	1.00
1.00	10	100	0.10
0.100	5	100	0.005
0.100	3	100	0.003
0.100	2	100	0.002
0.100	1	100	0.001
0.100	0.5	100	0.0005

PROCEDURE:

1. Extraction

- 1.1 Weigh a 10-g sample into a blender jar. Repeat as necessary to obtain the correct number of samples. Fortify recoveries at this point.
- 1.2 Add 50 to 100 g of anhydrous sodium sulfate and 100 to 200 mL of hexane to the sample.
- 1.3 Blend at moderate speed for 2 to 3 minutes.
- 1.4 Decant hexane through a glass funnel lined with a plug of glass wool into a 1,000-mL round-bottom flask.
- 1.5 Repeat blending and filtration twice; combine the extracts.
- 1.6 After decanting the final extract, transfer solids to funnel and rinse into the 1,000-mL round-bottom flask with 50 mL of hexane.
- 1.7 Evaporate the hexane extract to approximately 5 mL on a rotary vacuum evaporator set at less than or equal to 40°C.
- 1.8 Transfer the extract to a 10-mL volumetric flask using hexane, dilute to volume, and mix.

- 2.10 After all the solvent has drained from the column, evaporate the solution to approximately 5 mL on a roto-evaporator at less than or equal to 40°C.
- 2.11 Add 5- to 10-mL isooctane and repeat evaporation to approximately 5 mL.
- 2.12 Transfer cleaned-up extract to a 10-mL volumetric flask using isooctane rinses, dilute to volume, and mix by inverting several times.
- 2.13 Make the final determination by injecting aliquots of cleaned-up sample extracts and standard mixtures on a gas chromatograph with the following conditions.

Chromatographic Conditions for Pentachloronitrobenzene and Allied Metabolites

Primary Conditions:

Instrument: Model 5710A, Hewlett-Packard gas chromatograph
Column: 6 ft x 4 mm i.d., packed with 1.5% SP2250/1.95% SP2401 on 100/120 mesh Supelcoport
Detector: Ni⁶³ electron capture
Carrier gas: Argon:methane (95:5) at 48 mL/minute
Temperatures:
 Column: 200°C
 Injector: 250°C
 Detector: 300°C
Chart speed: 10 mm/minute
Attenuation: Variable
Injection volume: 4 µL

Gas chromatographic conditions may be modified as necessary to obtain satisfactory chromatography.

Secondary Conditions:

Instrument: Model 5710A, Hewlett-Packard gas chromatograph
Column: 6 ft x 4 mm i.d., packed with 3% OV-1 on 80/100 mesh Gas Chrom Q
Detector: Ni⁶³ electron capture
Carrier gas: Argon:methane (95:5) at 51 mL/minute
Temperatures:
 Column: 190°C
 Injector: 250°C
 Detector: 300°C
Chart speed: 10 mm/minute
Attenuation: Variable
Injection volume: 4 µL

2. Cleanup

- 2.1 If the sample is high in lipids, clean up using GPC. If the sample is not high in lipid, proceed directly to Step 2.5.
- 2.2 For GPC clean-up, transfer a 4-mL aliquot of the sample to a 500-mL round-bottom flask, add 20 to 25 mL of 50:50 methylene chloride:cyclohexane, evaporate on a rotary evaporator set at less than or equal to 40°C to approximately 2 mL, and dilute to 10 mL with 50:50 methylene chloride:cyclohexane.
- 2.3 Five-milliliter sample extracts are cleaned-up using a GPC auto-prep, Model 1001, Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri, with the following conditions:
 - Column: 600 mm x 25 mm i.d. packed with 62 g Bio Beads SX-3, Bio-Rod Laboratories, Richmond, California
 - Flow rate: 4.7 mL/minute
 - Solvent: 50% cyclohexane - methylene chloride
 - Dump time: 27 minutes
 - Collect time: 25 minutes
 - Rinse time: 5 minutesCollect eluate in 250- or 500-mL round-bottom flasks.
- 2.4 Roto-evaporate the cleaned-up sample to approximately 5 mL at less than or equal to 40°C. Add 20 mL of petroleum ether and evaporate to approximately 5 mL with roto-evaporation.
- 2.5 Pack a 300-mm x 20-mm Florisil clean-up column to the 150 mm (6 in.) mark with settled Florisil (approximately 21 g).
- 2.6 Cover the Florisil with approximately 1 cm of anhydrous sodium sulfate.
- 2.7 Pre-wet the column with approximately 100 mL of petroleum ether. Collect the eluate in a waste container.
- 2.8 As the petroleum ether layer reaches the top of the column, place a 500-mL round-bottom flask under the column and transfer the sample extract from Step 1.8 (2-mL aliquot) or Step 2.4 (whole sample) to the column with two 2 to 5 mL rinses of petroleum ether. Do not allow column to become dry at any time.
- 2.9 After the last rinse enters the column, elute the test compounds with 160 mL of 3% ethyl ether/petroleum ether.

Gas chromatographic conditions may be modified as necessary to obtain satisfactory chromatography.

Example chromatograms are in Figures 1 and 2.

CALCULATIONS:

To calculate ppm, a standard curve is generated using linear regression analysis of standard response versus concentration. Sample responses were subsequently compared to the curve and concentrations were generated.

$$\text{ppm} = U \times C \times D$$

where:

- U = concentration determined from curve
- C = ratio of final sample volume to original sample volume
- D = dilution factor

To calculate percent recovery, the following formula was used:

$$\text{Percent recovery} = \frac{\text{ppm found}}{\text{ppm added}} \times 100$$