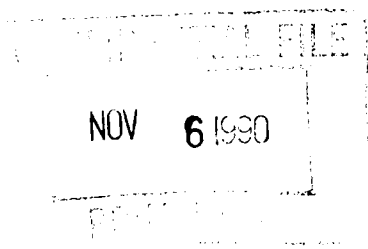




PENDIMETHALIN-03



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RECOMMENDED METHOD OF ANALYSIS

Herbicide, pendimethalin (CL 92,553): Determination of CL 92,553 and CL 202,347 (Metabolite) Residues in Walnut and Pecan Nut Meat

A. Principle

CL 92,553 and CL 202,347 are extracted from walnut and pecan nut meat with 1:3 isopropanol:hexane or from the other commodities with 1:1 methanol:water. Both compounds are partitioned into hexane. The hexane solution is cleaned up by passing an aliquot through a GPC column. The CL 92,553 and CL 202,347 eluates are cleaned up further with SPE LC-FLORISIL[®] using an ethyl acetate:hexane elution system. Both compounds are determined by fused silica capillary gas chromatography equipped with an electron capture detector. The analyses are accomplished using external standardization. A method sensitivity of 0.05 ppm is achieved for both compounds.

B. Apparatus (Items from other manufacturers may be used provided they are functionally equivalent).

1. Gas Chromatograph: An instrument equipped with an inlet system for a capillary column and with a standard Nickel-63 electron capture detector is suitable. The Tracor Model 565 gas chromatograph equipped with a packed column system can be adapted to fit the capillary column.

2. Capillary Adaptors (if required): Adaptors for fused silica capillary column (0.53 mm nominal I.D.)
 - a. Inlet: Adaptor with 1/4" O.D. x 90 mm long stem (SGE Part Number 1034610, Scientific Glass Engineering, Incorporated).
 - b. Detector Connector: connector with make-up gas of 1/16" O.D. line (SGE Part Number 103462).
3. Fused Silica Capillary Column: Fused silica column, 15 M long x 0.53 mm nominal I.D. SPB-1 bonded phase with film thickness of 0.5 μ (Supelco Cat. NO. 2-5314).
4. Ultrasonic Extractor: POLYTRON[®] Homogenizer, Model PT10ST (Brinkmann Instruments, Incorporated).
5. Filtration Apparatus: A 500-mL suction flask fitted with a 600-mL Buchner porcelain funnel by means of a rubber adapter.
6. Filter Paper: 9 cm (Whatman Number 40).
7. Rotary Evaporator: Buchler Flash-Evaporator (Buchler Instruments, Fort Lee, New Jersey), or equivalent, equipped with a water bath at about 40°C.
8. Evaporation Flasks: Round bottom, with T 24/40 joint, 250-mL, 500-mL capacity and pear-shaped with T 24/40 joint, 100-mL capacity (Kontes Glass Company, Vineland, New Jersey).
9. Solid Phase Extraction Cartridges: SPE LC-FLORISIL[®], 1000 mg (6-mL) (Supelco Cat. No. 5-7057).
10. Laboratory Glassware: Assorted graduated cylinders, volumetric flasks, pipets and 250-mL separatory funnels.
11. GPC Column: 2.5 cm I.D. x 62 cm glass column (ABC Laboratories).
12. Metering Pump approximately 5 mL/min: Eldex Model No. E-120-S (Cat. No. ELD-1001).

13. Millex-SR Filter: (Millipore)

C. Reagents (Items from other manufacturers may be used provided they are functionally equivalent).

1. Analytical Standards: CL 92,553 and CL 202,347, analytical grade, known purity. Obtainable from American Cyanamid Company, Princeton, New Jersey 08543-0400.
2. Solvents: Residue analysis grade ("B & J" High Purity Solvent, American Burdick and Jackson, Muskegon, Michigan or equivalent).
 - a. Cyclohexane
 - b. Dichloromethane (methylene chloride)
 - c. Ethyl Acetate
 - d. Hexane
 - e. Isopropanol
 - f. Methanol
 - g. Mixed Solvents - Measure the required volume of each component separately, combine and mix well.
 1. Extraction Solvent for Seed - 25% isopropanol in hexane.
 2. Extraction Solvent for Other Commodities - 50% methanol in water.
 3. GPC Solvent - 15% dichloromethane in cyclohexane.
 4. SPE Solvent 1 - 10% ethyl acetate in hexane.
 5. SPE Solvent 2 - 20% ethyl acetate in hexane.
3. Bio-Beads: S-X3 (200/400 mesh), (Bio-Rad, Cat. No. 152-2750).

D. Preparation of Standard Solutions

Prepare separate standard solutions of CL 92,553 and CL 202,347 using the following procedures:

1. Stock Solutions

Accurately weigh about 100 milligrams (corrected for percent purity) of analytical standard into a small beaker. Dissolve the compound in ethyl acetate and quantitatively transfer the solution to a 100-mL volumetric flask. Dilute to the mark with ethyl acetate. This solution contains about 1.0 mg active ingredient/mL and is designated as the Standard Solution A from which other standard solutions may be prepared. This solution is stable for at least 5 months in room temperature if kept tightly stoppered.

2. Intermediate Standard Solution

Calculate the volume of Standard Solution A for both CL 92,553 and CL 202,347 which contains exactly 1.0 mg of the standard active ingredient and transfer that volume by pipet into a 100-mL volumetric flask; dilute to the mark with ethyl acetate. This solution contains 10 mcg/mL and is designated as the Standard Solution B.

3. Gas Chromatographic Working Standards

Pipet a 1-mL aliquot of Standard Solution B into a 10-mL volumetric flask and dilute to the mark with ethyl acetate. This solution contains 1 mcg standard/mL and is designated as Standard Solution C. Pipet a 5-mL aliquot of the Standard Solution C into a 10-mL volumetric flask and dilute to the mark with ethyl acetate. This solution contains 0.5 mcg standard/mL and is designated as Standard Solution D. Pipet a 5-mL aliquot of Standard Solution D into a 10-mL volumetric flask and dilute to the mark with ethyl acetate. This solution contains 0.25 mcg standard/mL and is designated as the Standard Solution E. Pipet a 5-mL aliquot of Standard Solution E into a 10-mL volumetric flask and dilute to the mark with ethyl acetate. This solution contains 0.125 mcg standard/mL and is designated as Standard Solution F. Pipet a 5-mL aliquot of Standard Solution F into a 10-mL volumetric flask and dilute to the mark with ethyl acetate. This solution contains 0.0625 mcg/mL and is designated as Standard Solution G. The working standards should be prepared fresh weekly to prevent solvent concentration.

4. Fortification and Linearity Standards

Utilize solutions for fortification of samples in recovery studies from the above solutions. The concentration of fortification solutions should be such that a 0.5-mL, 1-mL or 2-mL aliquot added to the sample will yield the desired fortification level.

Prepare standards for checking linearity of chromatographic response from Standard Solution C (i.e., the 1 mcg/mL standard solution).

E. Packing and Calibration of the Gel Permeation Chromatography Column

1. Packing

- a. Weigh 50 grams of Bio-Beads S-X3 into a 250-mL Erlenmeyer flask, add 150 mL of 15% dichloromethane in cyclohexane (DCM-CH), cover and soak overnight.
- b. Set up the GPC column for upward flow (sample injector valve at the bottom end) of DCM-CH solvent at a rate of about 5 mL/minute driven by the Eldex metering pump.
- c. Pour the Bio-Beads slurry into the column and drain the solvent; do not allow the bed to go dry at any time. Insert the upper piston into the column and adjust it so that its frit is just touching the top of the bed.
- d. Start the metering pump and pump the DCM-CH solvent through the column continuously for about 2 days to firm up the bed. The solvent may be recycled in this operation.
- e. Carefully adjust the lower piston so that its frit is in contact with the lower end of the bed. This step should be repeated as necessary to be sure that there are no gaps between the bed and the frits at both ends.

2. Calibration

- a. Mix CL 92,553 and CL 202,347 standard solutions containing 1 mg of each compound (1 mL of Solution A) in a evaporation flask. Evaporate to just dryness.
- b. Dissolve the residue in 10 mL of the DCM-CH solvent and load into the 5-mL sample loop. Drain the excess into a beaker.

- c. Place a 200-mL graduated cylinder below the column, start the flow of mobile phase and collect fractions.
- d. Observe the movement of CL 92,553 and CL 202,347 bands by their yellowish color.
- e. Cut the eluate fractions of both CL 92,553 and CL 202,347 in 100 mL graduate cylinders.
- f. Evaporate the eluates to dryness in a 100-mL round bottom boiling flask.
- g. Reconstitute both compounds in hexane using 100-mL volumetric flasks (expect 5 ng/ μ l)
- h. Dilute the solution 1:5 in ethyl acetate (expect 1 ng/ μ l) for analysis.
- i. For recovery assay, make up a final 1 ng/ μ l standard solution by pipetting Solution A, i.e., 1 mL of CL 92,553 and 1 mL of CL 202,347 into a 100-mL volumetric flask and dilute to 1:10 mL with ethyl acetate in a 10-mL volumetric flask.
- j. Regenerate the GPC column by washing the column with 200 mL mobile phase.
- k. Clean the sample loop thoroughly with DCM-CH.
- l. Check for cross contamination by collecting 100 mL effluent and evaporate to dryness.
- m. Reconstitute the residue in 1 mL ethyl acetate and analyze by gas chromatography.

F. Gas Chromatographic Conditions

The operating conditions described below are provided for use as guides in establishing actual operating conditions and should be adjusted as necessary to obtain peak shape and resolution from background peaks equivalent to or better than those shown on the attached figures.

1. Oven Temperature: Programming

Initial Temperature		150°C
Initial Time		2 min
Program Rate		10°C/min
Final Temperature		240°C
Final Time		20 min
Run Time		20 min
Retention Time:	CL 92,553	3.0 min
	CL 202,347	4.7 min
Attenuation		64

2. Inlet Temperature: 220°C

3. Injection Mode: On Column or Direct Injection

4. Carrier Gas Flow: 6 mL/min (5% methane in Argone).

5. Make-up Gas for EC Detector: 50 mL/min (5% methane in Argone).

G. Linearity Check

Check the instrument for linearity of chromatographic response every day it is used for the analysis of CL 92,553 and/or CL 202,347. Inject standard solutions of 0.0625, 0.125 and 0.25 mcg standard/mL (i.e., Standard Solutions G, F, E). Calculate the unit response (response/concentration) for each concentration and average the values. Departure at any concentration of more than 15% from the average indicates instrument malfunction or faulty standard preparation which must be corrected before proceeding with sample analysis.

H. Recovery Test

The ability of the analyst to perform these procedures satisfactorily must be demonstrated by recovery tests before analysis of unknown samples is attempted. In addition, at least one recovery sample must be run with each batch of unknown samples.

Weigh a 10-g portion of untreated sample into a 250-mL Erlenmeyer flask and add by pipet the volume of a fortification solution to yield the desired fortification level. A practical guide is provided by the following Table:

<u>Fortification Desired (ppm)</u>	<u>Standard Solution</u>	<u>Concentration of Standard Solution</u>	<u>Volume of Standard Standard Added</u>
0.05	C	1.0 mcg/mL	0.5 mL
0.10	C	1.0 mcg/mL	1.0 mL
0.50	B	10.0 mcg/mL	0.5 mL

I. Sample Handling Procedure

Freeze a quantity of the commodity sufficient to provide representative sampling, mix with powdered dry ice, and chop with a pre-chilled food chopper. Store the sample in a freezer until the dry ice has dissipated.

1. Extraction

a. Nut Meat

Weigh a 10.0-gram portion of the sample into a 250-mL Erlenmeyer flask. Add 200 mL of 25% isopropanol in hexane and blend the mixture with a POLYTRON[®] homogenizer for about 2 minutes. Place a Whatman No. 40 filter paper in a Buchner funnel and wash with 50 mL of extracting solvent.

Discard the solvent wash and filter the homogenate with vacuum into a 500-mL filtering flask. Return the filter cake to the blending flask, add 200 mL of 25% isopropanol in hexane and blend the mixture again for about 2 minutes. Filter the second homogenate through the same filter paper. Transfer the filtrate to a 500-mL stoppered graduated cylinder. Adjust the volume to 400-mL with 25% isopropanol in hexane. Stopper the cylinder and shake to mix the contents thoroughly. Transfer a 100-mL aliquot (2.5-gram sample size equivalent) to a 500-mL evaporation flask and evaporate to dryness.

2. Partition

a. Nut Meat

Wash the residue in the 500-mL evaporating flask successively with 50 mL hexane, 25 mL distilled water and transfer each solution to a 250-mL separatory funnel. Shake the contents in the separatory funnel vigorously for 40 seconds. Draw off the bottom (aqueous) layer into the original evaporating flask and transfer the top (hexane) layer to a clean 250-mL evaporation flask. Return the aqueous extract to the 250-mL separatory funnel. Wash the 500-mL evaporating flask again with 50 mL hexane and decant into the 250-mL separatory funnel. Shake the mixture vigorously for 40 seconds. Draw off the bottom layer into the 500-mL evaporating flask and transfer the hexane layer to combine with the first extract in the 250-mL evaporation flask. Repeat the above procedure one more time to complete the partition step.

3. Cleanup

a. Gel Permeation Chromatography (GPC)

Conditions:

Column: 2.5 cm I.D. x 62 cm glass column, packed with 50 g Bio-Beads S-X3 (Bio Rad) (200/400 mesh) to a bed length of 29 cm

Solvent System: Dichloromethane-cyclohexane 15:85 (DCM-CH)

Flow Rate: 5.0 mL/min (after firmly packed)

Dump Volume: First 130 mL (approximate) (Macromolecules such as chlorophyll and lipids)

CL 92,553 Elution Volume: 131 - 160 mL (approximate)

CL 202,347 Elution Volume: 216 - 269 mL (approximate)

Wash Volume: 100 mL

Procedure:

- 1) Load the nut meat residue (i.e., after the hexane extract was evaporated to dryness) by quantitatively transferring the residue into a 10 mL graduated centrifuge tube with DCM-CH and bring the volume to 10 mL and load into the 5 mL loop. (Use Millex-SR filter if sample has particulates).
- 2) Elute the column with DCM-CH solvent.
- 3) Collect the first eluate (1-130 mL) and discard.
- 4) Collect the CL 92,553 eluate (131-160 mL).
- 5) Collect the CL 202,347 eluate (216-269 mL).
- 6) Regenerate the column with 100 mL of DCM-CH solvent.
- 7) Load the next sample residue.

b. SPE LC-Florisil Cartridge Clean-up:

CL 92,553

- 1) Evaporate the GPC eluate just to dryness.
- 2) Wash the cartridge with 5 mL hexane.
- 3) Load the sample residue with 3 x 2 mL hexane.
- 4) Discard the hexane eluate.
- 5) Elute CL 92,553 with 5 mL of 10% ethyl acetate in hexane.

- 6) Evaporate to dryness.
- 7) Dissolve in 1 mL ethyl acetate for GC analysis.

CL 202,347

- 1) Evaporate the GPC eluate just to dryness.
- 2) Wash the cartridge with 5 mL 10% ethyl acetate in hexane.
- 3) Load the sample residue with 3 x 2 mL 10% ethyl acetate in hexane.
- 4) Discard the 10% ethyl acetate in hexane eluate.
- 5) Elute CL 202,347 with 10 mL of 20% ethyl acetate in hexane.
- 6) Evaporate to dryness.
- 7) Dissolve in 1 mL ethyl acetate for GC analysis.

J. Gas Chromatography

Inject standard and sample solutions alternately as follows: standard, sample in duplicate, standard, sample in duplicate, etc., allowing late-eluting peaks to clear between each injection. If the sample response exceeds full-scale pen deflection at the sensitivity used, make proper dilutions of the sample solution to keep the response on scale and record the dilution factor. Measure peak heights with a millimeter ruler or with an electronic integrator. If the duplicate injections for a given sample differ by more than 15% reinject the "standard, sample, sample, standard" set. In similar fashion, if the preceding and following standards, do not agree to within 15%, reinject the set.

K. Calculations

For purposes of calculations, use the average peak height of the duplicate injections for each samples and the average peak height for the standard immediately preceding and immediately following that sample. Calculate the apparent residues as follows:

$$\text{Apparent residues (ppm)} = \frac{R(\text{SAMP}) * V1 * V3 * V5 * C(\text{STD}) * DF}{R(\text{STD}) * W * V2 * V4}$$

Where:

R(SAMP) = Average sample response (mm or integrator units)

R(STD) = Average standard response (mm or integrator units)

C(STD) = Concentration of Working Standard (mcg/mL)

V1 = Initial volume of sample extract (400 mL)

V2 = Volume of equivalent sample aliquot used for analysis (50 mL)

V3 = Final volume of sample solution for GLC (1.0 mL)

V4 = Volume of sample solution injected (1.0 mL)

V5 = Volume of standard solution injected (1.0 mL)

W = Sample weight (10 grams)

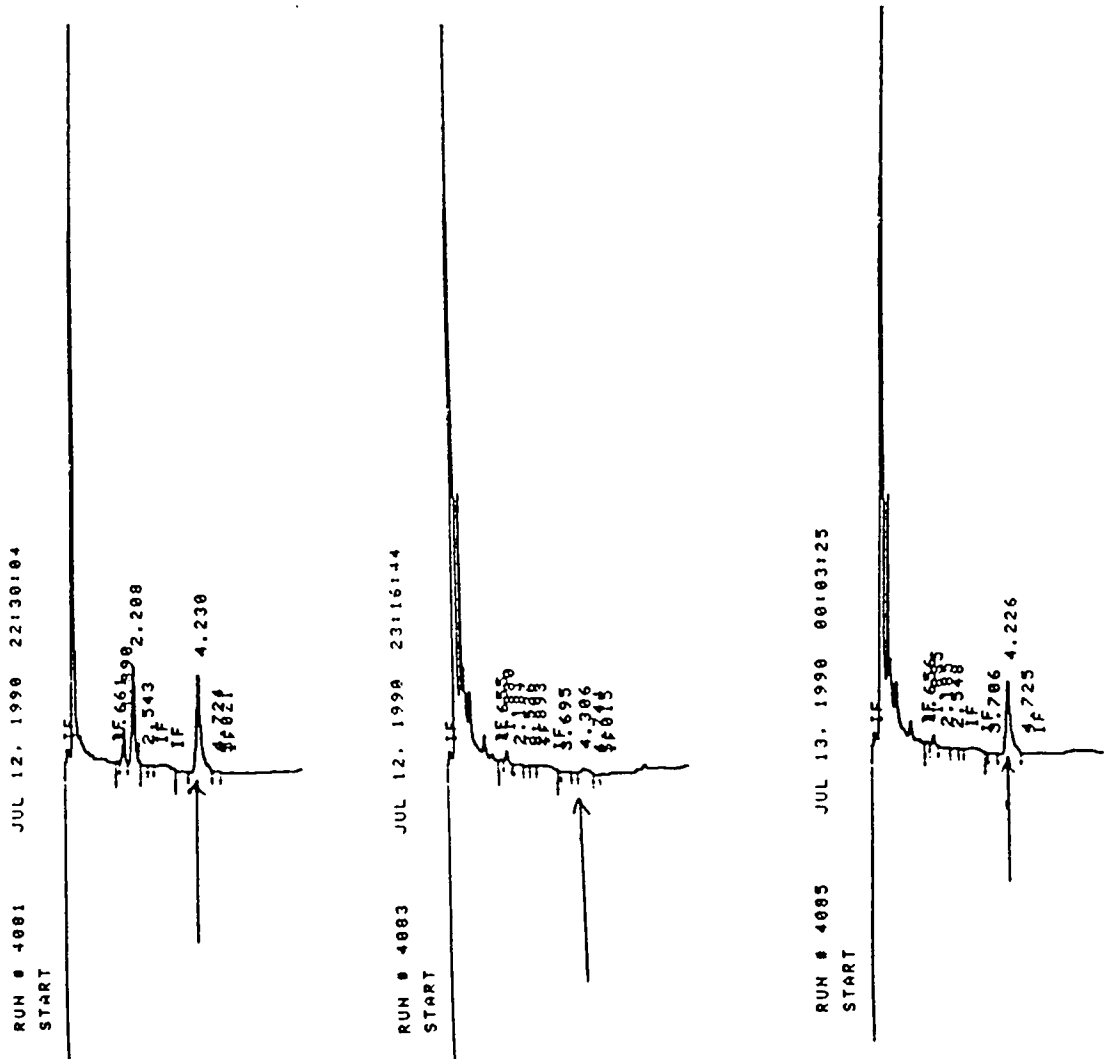
DF = Dilution Factor (1)

(Values in parentheses are nominal values if the procedure is carried out exactly as described.)

L. Notes on the Procedure

1. All elution operations with SPE cartridges are accomplished by gravity, i.e., no vacuum or pressure used.
2. Before use, each lot of SPE LC-FLORISIL[®] cartridges must be checked for potential interferences. Prior to use, elute the SPE LC-FLORISIL[®] cartridge with 5 mL of ethyl acetate, evaporate the ethyl acetate and analyze for interference; if interferences are observed, notify the manufacturer (Supelco guarantee for replacement). SPE LC-FLORISIL[®] cartridges showing minor interferences may be used, but must be cleaned before use by washing them with 5 mL ethyl acetate followed by 5 mL hexane. The sample may then be loaded immediately.
3. Hexane may be used as an alternate gas chromatography injection solvent for standards and sample extracts provided that all the criteria set in the method can be met.

Figure 2: Gas Chromatograms of Extracts of Control and Fortified Walnut Nut Meat Analyzed for CL 202,347 by the SOP M2024

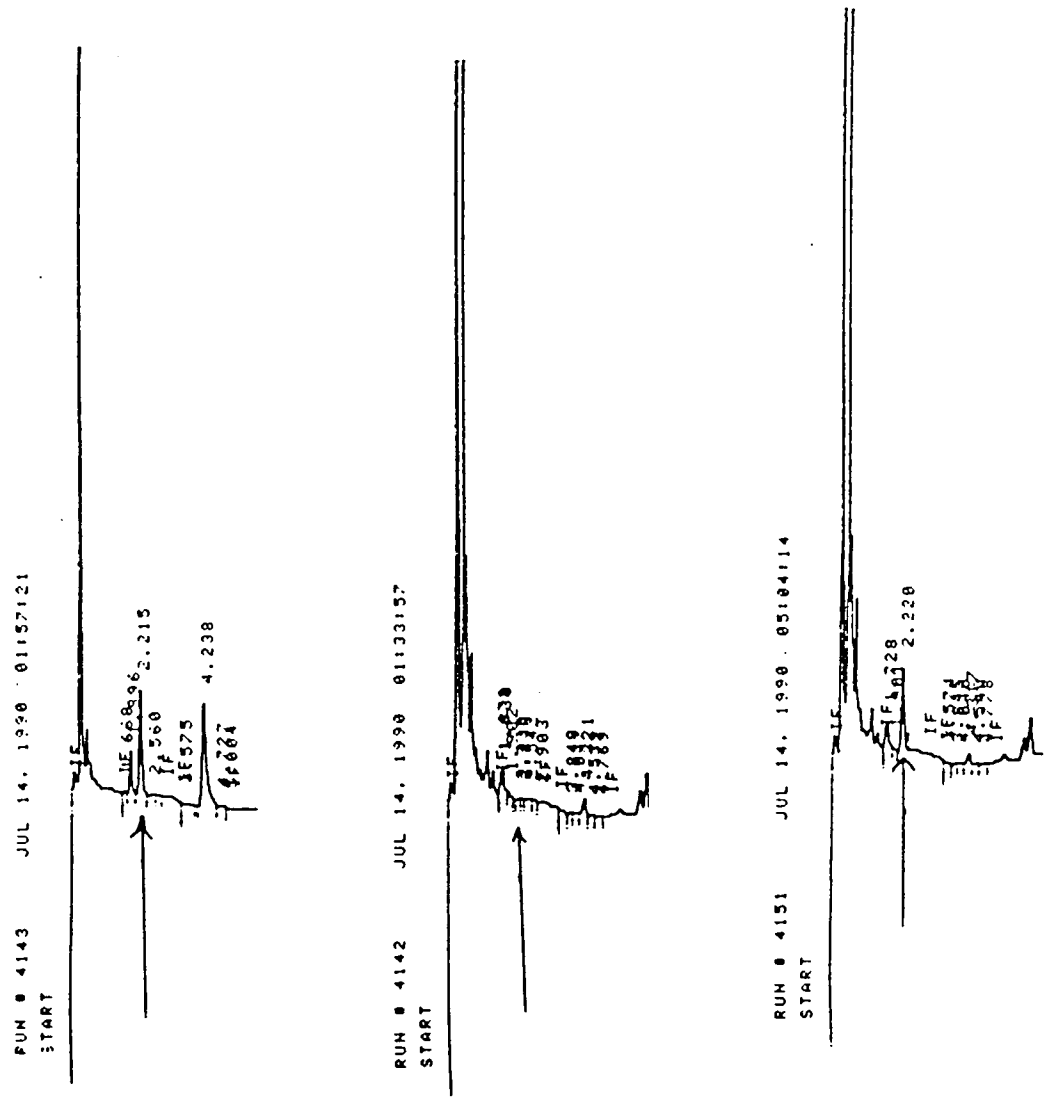


CL 202,347 Standard
0.0625 mcg/mL (1 mL)

Walnut Nut Meat,
Control Dilution
Factor = 1
1.25 mg Injection

Walnut Nut Meat,
Fortified at 0.05 ppm
Dilution Factor = 1
1.25 mg Injection

Figure 3: Gas Chromatograms of Extracts of Control and Fortified Pecan Nut Meat Analyzed for CL 92,553 by the SOP M2024

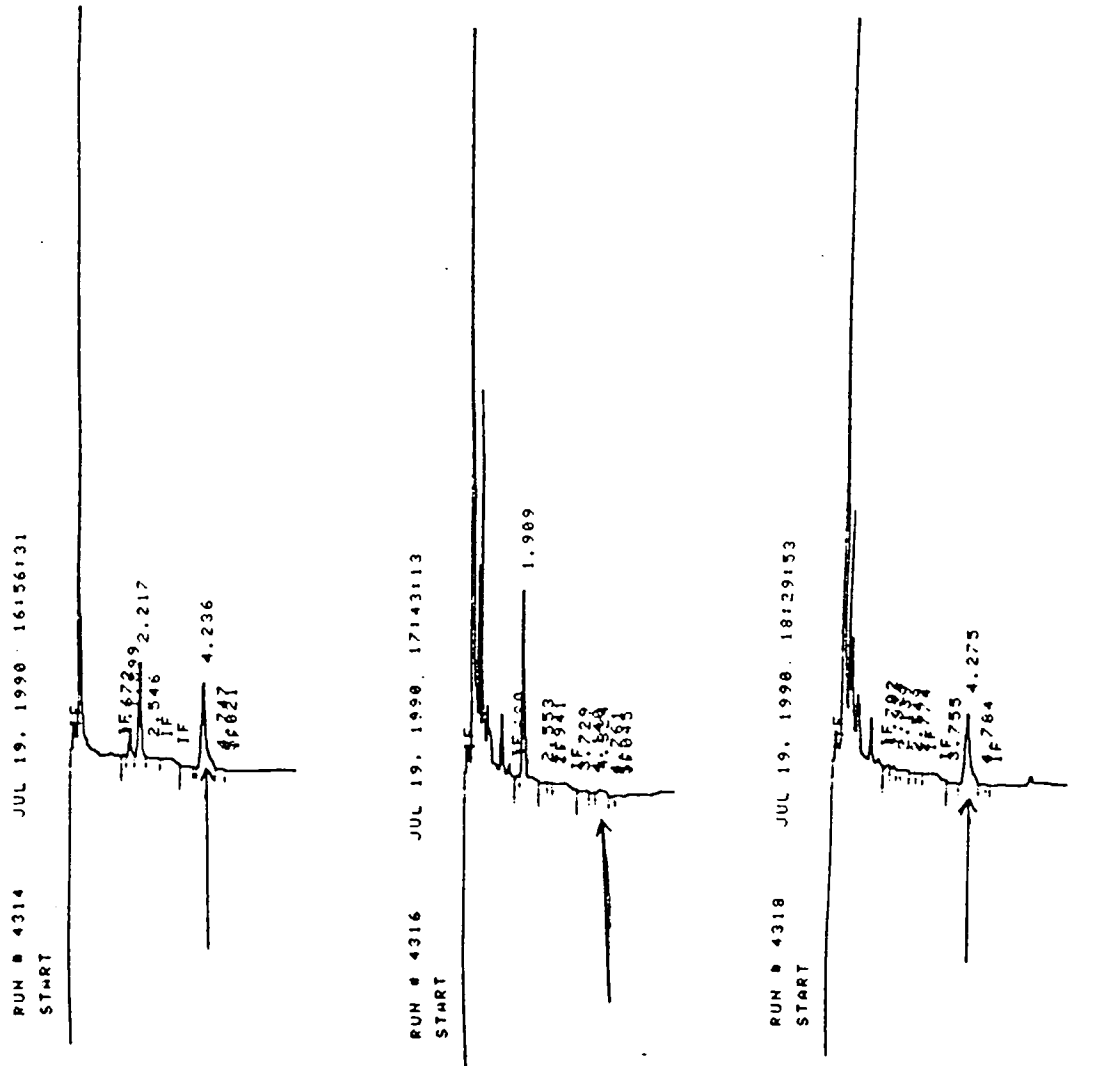


CL 92,553 Standard
0.0625 mcg/mL (1 mL)

Pecan Nut Meat,
Control Dilution
Factor = 1
1.25 mg Injection

Pecan Nut Meat,
Fortified at 0.05 ppm
Dilution Factor = 1
1.25 mg Injection

Figure 4: Gas Chromatograms of Extracts of Control and Fortified Pecan Nut Meat Analyzed for CL 202,347 by the SOP M2024



CL 202,347 Standard
0.0625 mcg/mL (1 mL)

Pecan Nut Meat,
Control Dilution
Factor = 1
1.25 mg Injection

Pecan Nut Meat,
Fortified at 0.05 ppm
Dilution Factor = 1
1.25 mg Injection