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Regulatory Method A Gas Chromotography - Mass Spectrometry Method for the Determination of Residues of the Tefluthrin Metabolite PP890 in Crops

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Regulatory Method
A Gas Chromatography - Mass Spectrometry Method for the Determination of Residues of the Tefluthrin Metabolite PP890 in Crops

Author: Mr. R. D. Fitzpatrick
Date: February 2, 1988

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Date: 8 Feb 88

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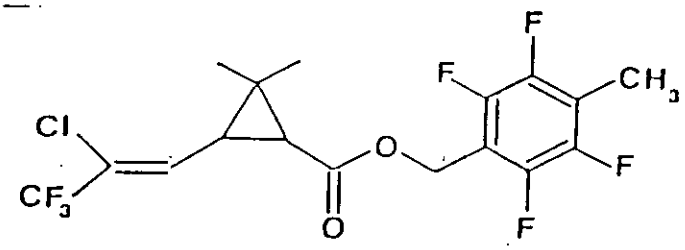
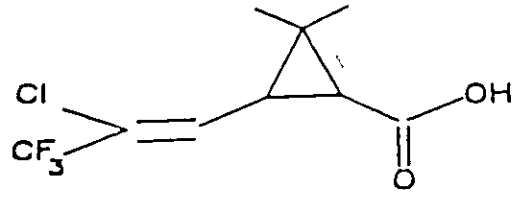
A Gas Chromatography - Mass Spectrometry Regulatory Method for the Determination of Residues of the Tefluthrin Metabolite PP890 in Crops

1. SCOPE

The method is suitable for the determination of the conguga-ted residues of the Tefluthrin metabolite; (1RS)-cis-3-(ZE-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcycloprane-carboxylic acid (PP890) in crops. The limit of determination is 0.02 ppm.



Tefluthrin (PP993): 2,3,5,6-tetrafluoro-4-methylbenzyl(1RS)-cis-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-cyclopropanecarboxylate. M.W. = 418.7, Molecular Formula $C_{17}H_{14}ClF_7O_2$.



PP890: (1RS)-cis-3-(ZE-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylic acid

2. SUMMARY

Crop samples are extracted by homogenization with acetoni-trile:water followed by methanol:water to extract the conjugated residues of PP890. Any parent pyrethroid extracted is partitioned out using a C^{18} disposable extrac-tion column. The remaining conjugated residues are subjected to 2N HCL acid hydrolysis for 2 hours which cleaves the con-jugated metabolite. The free metabolite is purified with gel permeation chromatography using Bio-Beads SX-3 and dichloro-methane:hexanes as the eluant. After evaporation the metabolite is derivatized by extractive alkyation with pentafluorobenzyl bromide (PFBBr) using tetrabutylammonium-hydrogen sulfate as a phase transfer catalyst. The penta-fluorobenzyl ester derivative is quantitatively determined using gas chromatography - mass spectrometry (GCMS) operated in the selected ion (SIM) mode.

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3. REAGENTS

a. Solvents: hexanes, dichloromethane, acetonitrile, toluene, diethyl ether (stabilized with 2% ethanol) - pesticide quality.

b. Sodium Sulfate, granular anhydrous, ACS reagent - Heat in oven at 600°C for 15 hours to remove contaminates.

c. Disposable extraction columns.

- silica gel, 3 ml, J. T. Baker Chemical Co. (Cat. No. 7086-3).

- C^{18}, 6-ml HC - High Capacity, J. T. Baker Chemical Co. (Cat. No. 7020-7).

f. Pentafluorobenzyl bromide (PFBBr), Regis Chemical Co. (Cat. No. 270587).

g. Tetrabutylammoniumhydrogen sulfate, Regis Chemical Co. (Cat. No. 680500).

h. Bio Beads SX-3, 200-400 mesh, Bio Rad Laboratories.

i. Hydrochloric acid, concentrated, ACS reagent.

j. Glass wool, fiber, Pyrex.

k. Sodium Hydroxide, ACS reagent.

l. Analytical standards of PP890.

4. SAFETY COMMENTS

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in doubt, consult the appropriate material safety data sheet (MSDS) or safety manual containing recommendations and procedures for handling chemicals, and/or references such as CRC Handbook of Laboratory Safety by Norman V. Steel, CRC Press Inc.

a. Solvent Hazards

	Acetone	Acetonitrile	Dichloro methane	Diethyl ether	Hexane	Toluene
Harmful vapor	X	X	X	X	X	X
Highly flammable	X	X	-	X	X	X
Harmful by skin adsorption	-	X	X	-	-	X
TLV-TWA, ppm	1,000	40	300	400	100	100
-STEL, ppm	1,250	60	250	500	125	150

In all cases, avoid breathing vapor. Avoid contact with eyes/skin.

b. Concentrated Hydrochloric Acid (HCL)

Concentrated hydrochloric acid is highly corrosive and users must always wear adequate protective clothing. Concentrated acid must not be diluted by the addition of water but always by adding to water. All operations involving concentrated acid should be carried out in a fume hood.

c. Pentafluorobenzyl Bromide (PFBBr)

The full toxicological properties of PFBBr are unknown and therefore should be handled with great care. PFBBr is corrosive and a strong lachrymater and should always be handled in a fume hood. Avoid contact with skin, eyes, and clothing.

d. PP890 is a synthetic pyrethroid metabolite of unknown toxicity.

5. APPARATUS

a. Hobart VCM-40 or other apparatus suitable for grinding frozen plant tissue.

b. Sorvall Omni-Mixer or other suitable high speed blender.

c. Reflux Condensors and extraction assembly with unitized hot plates (Precision 65500).

d. Rotary Evaporation unit e.g. Buchi.

e. Vibrating mechanical shaker, Tekmar Model VXR or equivalent.

f. Fused Silica Capillary Column, Durabond DB-5 (5% diphenyl:95% dimethylpolysiloxane), 30 m x 0.25 mm i.d., 0.25 um film thickness, J & W Scientific, Inc. (Cat. No. 122-5032) or equivalent.

g. Evaporation Manifold, N-Evap Model 112, Organomation Assoc. Inc. or equivalent manifold for providing a gentle stream of nitrogen to evaporate small volumes of solvent.

h. Graduated glass centrifuge tubes of 10 ml capacity calibrated down to 1 ml in 0.1 ml units (accuracy of at least +/- 1%).

i. Finnegan model 5100 guadrapole GC/MS/DS with Finnegan MAT 9611 gas chromatograph, EI source, and Superincos data

system or other Gas Chromatography-Mass Spectrometer capable of operating in the selected ion mode (SIM).

j. Syringes for gas-liquid chromatography, e.g. Hamilton 10 µl.

NOTE: The use of a autosampler with GLC equipment, e.g. Varian 8000, is satisfactory provided (a) suitable precise injections are achieved i.e. reproducibility of >5%, (b) no cross contamination from consecutive injections is observed, and (c) that no contamination arises in the final sample due to the autosampler vials or vial caps.

k. Centrifuge, IEC Model K or similar instrument capable of centrifugation at 2,500 rpm.

l. Ultrasonic bath, e.g. Bransonic

m. Gel Permeation Chromatograph - GPC Autoprep 1001, Analytical Biochemistry Laboratories, Inc.

n. pH Meter or pH Indicator Sticks (colorpHast® EM - Reagents Cat. No. 9590)

6. Instrumental Operating Conditions

6.1 Gas-Liquid Chromatography

The precise conditions for the separation by GLC will depend on the equipment available. The operating manuals for the instrument should always be consulted to ensure safe and optimum use. The following conditions have been found to be satisfactory using the GC/MS instrumentation described in 5.i.

Column: 30 m x 0.25 mm i.d. Durabond DB-5, fused silica capillary column, 0.25 µm film thickness

Carrier Gas Linear Velocity: 25 cm/sec Helium

Column Temperature:

PP890-PFB ester: 100°C (1 min.) program at 6°C/min. to 170°C 10°/min to 230°C hold 3 min.

Grob type splitless injection with 1 minute purge delay (200 cm/sec).

Injector Temperature: 250°C.

Interface Temperature: 250°C.

Injector Volume: 2 ul splitless (1 min purge delay)

Retention data for the pentafluorobenzyl derivative when chromatographed using the above sets of conditions is 13.25 min.

6.2 Mass Spectrometry

Finnegan Model 5100

Electron impact mode; source pressure 1 x 10^6 Torr, electron energy 70 eV, electron multiplier -2000 V.

Calibration with perfluorotributylamine.

The mass spectra of the pentafluorobenzyl derivative of PP890 is shown in Figures 1. The relative abundance of the major ions are listed below:

PP890-PFB - m/z 387 (1.2%), m/z 197 (39.4%), m/z 181 (100%)

For quantitative analysis the m/z 197 ion is selected for the determination of the PP890 derivative.

6.3 Gel Permeation Chromatography

a. Instrument - GPC Autoprep 1001, Analytical Biochemistry Laboratories, Inc.

b. Column - 30 cm x 2.5 cm glass column packed with 35 grams of Bio-Beads SX-3, 200-400 mesh.

c. Solvent - 40% dichloromethane in hexane.

d. Flow Rate - 5.0 ml/min

e. Elution Scheme - Dump 60 ml (12 min), collect 100 ml (20 min), and wash 50 ml (10 min). (The exact program must be predetermined for each column.)

7. DETERMINATION

7.1 Sample Preparation

Samples which are removed from the freezer having previously been homogenized, should be allowed to defrost for a minimum period only before breaking and weighing out. This ensures that no partition of the endogenous water content can occur prior to weighing out the sample.

7.2 Extraction and Hydrolysis

a. Weight a ground representative sample (10 g) into a blender cup or jar.

b. Blend with 50 ml of 1:1 (v/v) acetonitrile: pH 7 water for 5 minutes.

c. Decant the organic extract into a buchner funnel containing Whatman No. 1 or 4 filter paper and filter under vacuum into a 500 ml round bottom flask. (Do not dump solid crop material; save for another extraction.)

d. Repeat the extraction with a further 50 ml of 1:1 (v/v) acetonitrile:pH 7 water. Decant into the same funnel and flask.

e. Repeat the extraction with two 50 ml portions of 1:1 (v/v) methanol:pH 7 water filtering and combining the extracts as above and decanting both the extract and the residual crop material after the final extraction.

f. Rinse the blender cup or jar and blades with additional acetonitrile and decant rinsings through the filter paper. Rinse the solid crop material contained in the buchner funnel and collect the rinsings into the 500 ml round bottom flask.

g. Rotary evaporate at 45°C under vacuum to aqueous phase only.

h. Adjust the aqueous solution to pH 7 with 0.1N NaOH using either a pH meter or pH indicator sticks.

i. Measure the volume of the aqueous solution and adjust to 20% acetonitrile in water.

j. Prepare two Baker C_{18} high capacity solid phase extraction cartridges by conditioning the columns with acetonitrile (5 ml) followed by DI water (5 ml).

k. Elute by vacuum or force one-half of the adjusted aqueous solution (step h.) through a C_{18} cartridge and collect the eluant followed by 5 ml of 80:20 pH 7 water:acetonitrile into a 250 ml boiling flask.

l. Repeat step k. with a new C_{18} cartridge and the remaining solution from step i. Combine the eluants into the same 250 ml boiling flask.

m. Rotary evaporate the combined extracts to aqueous phase only at 45°C.

n. Adjust the aqueous solution to 2N HCL and gently reflux for 2 hours. Allow the hydrolysate solution to cool to room temperature and wash the reflux

condensor with approximately 10 ml 0.1N NaOH followed by approximately 10 ml of dichloromethane.

o. Transfer the hydrolyzate to a 250 ml separatory funnel and partition with the dichloromethane wash. Dry the dichloromethane extract by slowly passing through approximately grams of anhydrous sodium sulfate contained in a glass funnel plugged with glass wool into a 250 ml boiling flask.

p. Re-extract the hydrolyzate solution with two additional 50 ml portions of dichloromethane. Dry each partition through the sodium sulfate and combine into the 250 ml boiling flask.

q. Rotary evaporate the combined dichloromethane extracts to approximately 0.5 ml at a temperature not exceeding 30°C.

r. Redissolve the residue in 7 ml of 40% dichloromethane:hexane, swirl the sample in a ultrasonic bath, and transfer to a 10 ml glass culture tube. Centrifuge to remove any insolubles.

s. Inject 5.0 ml of solution into the gel permeation chromatograph operated under the conditions described in Section 6.3. Elute at the rate of 5.0 ml per minute and collect the 60 to 160 ml fraction in a 250 ml boiling flask. (NOTE: The exact volume must be predetermined for each gel permeation column.)

7.3 Derivatization of PP890 by Extractive Alkyation with Pentfluorobenzyl Bromide (PFBBr)

a. Rotary evaporate the fraction collected at a temperature not exceeding 30°C to 2-3 ml and quantitatively transfer to a 10 ml graduated culture tube with dichloromethane. Evaporate to near dryness with a gentle stream of nitrogen.

b. Redissolve the residues in 1.0 ml of dichloromethane and add 1 ml of 0.1M tetrabutylammonium hydrogen sulfate (TBA)/0.2M sodium hydroxide (NaOH).

c. Add 60 ul of PFBBr, tightly cap, and shake vigorously for 1 hour on a wrist action or vortex shaker.

NOTE: ADD REAGENTS IN A FUME HOOD!

d. Remove as much of the top aqueous layer as possible with a disposable pasteur pipet and discard.

e. Evaporate the contents to near dryness with a gentle stream of N_2 and redissolve in 1 ml of hexane.

f. Prepare a J. T. Baker silica gel solid phase disposable extraction column by adding approximately 1/4 inch of granular anhydrous sodium sulfate and rinsing with 5 ml hexane.

Note: Prior to use, each batch of disposable silica gel extraction columns must be calibrated as follows:

Derivitize a known amount (i.e. 1 ug ml^{-1}) of PP890 as described in Section 7.4.

Transfer to the top of the column which has been prewashed with hexane. Elute the derivatized standard through the column with 20% diethyl ether/hexane and collect 1 ml fractions. Analyze each fraction by GCMS (Sections 6.1 and 6.2) to determine the proper elution volume.

g. Transfer the derivatized PP890 from step e. to the silica gel cleanup column and elute with 3 ml of 20% diethyl ether/hexane.

h. Analyze the derivatized PP890-PFB ester by GC-MS.

7.4 Preparation of Analytical Standards

Prepare individual stock solutions of PP890 at 1000 µg ml^{-1} as follows:

Accurately weigh 0.1 g of the metabolite using a analytical balance (4 or 5 figure) and dissolve in acetone (100 ml) in a volumetric flask. Make serial dilutions of each stock solution to give 100 µg ml^{-1} 10 µg ml^{-1}, 1 µg ml^{-1} and 0.1 g μl^{-1} standard solutions in acetone. These solutions may be used for fortification and the preparation of derivatized standards for GLC reference.

When not in use always store standard solutions in a refrigerator at <5°C to prevent evaporation of the solvent. Analytical standards are to be freshly prepared every six months.

Derivatized standards should be prepared with every analytical run as follows:

Evaporate a suitable volume of the appropriate solution to dryness with a gentle stream of nitrogen at <30°C. Derivatize as described in steps 7.3 b-g.

7.5 GCMS Determination

a. Properly tune the GCMS (zero the electrometer, adjust the resolution, ion energy, ion program, lens and extractor) to achieve the baseline resolution, good gaussian peak shape and calibrate the GC/MS using the reference compound FC43.

b. Inject 1-2 µl of a strong standard solution, e.g. 1.0 µg ml^{-1} of the derivatized PP890 into the GCMS operated under the conditions described in 6.1 and 6.2 to condition the column.

c. Optimize the response of the GCMS in order that the sensitivity is sufficient to give a measureable response at the limit of determination for each m/z.

d. Make repeated injections of 1-2 µl of a derivatized standard PP890 solution, i.e. 0.125 µg ml^{-1} into the GCMS system operated under the conditions described in 6.1 and 6.2 until a constant response is obtained. Measure the peak area corresponding to the metabolite.

e. Make an injection of each sample solution and measure the peak areas of the peaks corresponding to the metabolite retention time.

f. Re-inject the standard solution after a maximum of eight injections of sample solutions.

g. Calculate the PP890 residue in the sample, expressed as ppm, by proportionation of the PP890 response to the mean standard response from the injections bracketing the samples;

$$\frac{RS}{RA} \times \frac{VA}{VS} \times \frac{CA}{CS} = \text{measured residue (ppm)}$$

Where:

RS = Response (peak area) of the sample

RA = Response (peak area) of analytical standard solution

VS = Volume (µl) of sample injected

VA = Volume (µl) of analytical standard injected

CS = Concentration (g ml^{-1}) of final sample solution

CA = Concentration (µg ml^{-1}) of analytical standard solution

h. Correct the measured residue value calculated for each sample by the mean percent recovery obtained for the recovery experiments carried out simultaneously with the samples, e.g. for a mean 75% recovery, the corrected residue is equal to the measured residue x 100/75.

7.6 Limit of Determination

A true assessment of the limit of determination of the method may be determined by fortifying untreated samples with low concentrations of PP890 and subjecting them to the complete analytical procedure. The chromatographic response obtained for these recoveries at the retention time of the derivatized PP890 should exceed the background signal noise by at least a factor of 4 in order to be considered an acceptable quantitative limit of determination. In addition the precision of the measurement at this level should not exceed a relative standard deviation of 20%.

In these laboratories, the limit of determination for PP890 has been set at 0.02 ppm.

7.7 Accuracy and Precision

Recovery experiments in which untreated control samples are accurately fortified just prior to hydrolysis with a known amount of PP890 must be analyzed alongside each batch of samples. The fortification level should be in the range of the residue concentration expected in the treated samples. If no measureable residues are expected in the sample, then the fortification level should be at or near the limit of determination. In addition untreated control samples must also be analyzed in order to demonstrate that endogenous substances present in the sample do not interfere with the final determination of the derivatized PP890.

This method has been successfully applied to the analyses of PP890 in field corn fodder, forage and grain. The recovery values from fortified samples are summarized in Table 1. Appendix 1 display representative GCMS spectra from the procedure.

Table 1: PP890 External Recovery Data From Field Corn

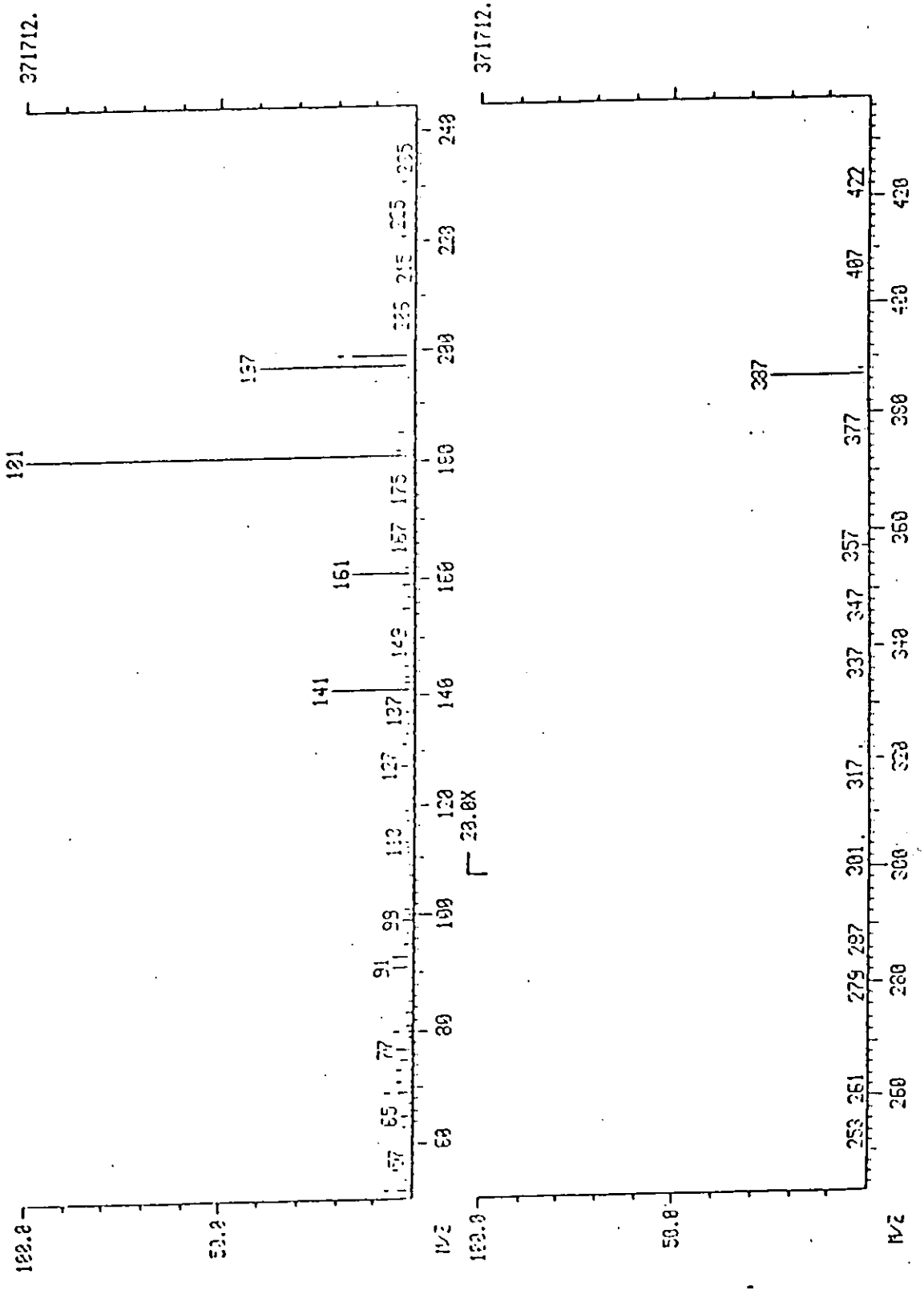
Crop Description		Fortification Level, ppm	% Recovery
A.	Field Corn		
	(fodder/forage)	0.02	108, 90.5, 87.5
		0.05	96.1, 77.3, 82.9
		0.10	113, 89.9, 98.3
	(grain)	0.02	92.0, 83.1
		0.05	83.5
		Mean	91.8
		RSD	11.5

RDF-1-1/RSI

FIGURE 1

THE MASS SPECTRUM OF THE PENTAFLUOROBENZYL DERIVITIVE OF

(1RS)-CIS-3-(ZE-2-CHLORO-3,3,3-TRIFLUOROPROP-1-ENYL)-2,2-DIMETHYL-CYCLOPROPANECARBOXYLIC ACID (PP890)



APPENDIX 1

Typical Mass Spectra for PP890/PFB Determined in Field Corn and Cottonseed.

Figure 1: 0.125 ug/ml PP890/PFB Standard.

Figure 2: 85-8078 Untreated Field Corn Fodder Sample at 0.893 g ml^{-1}. Residue = <0.02 ppm.

Figure 3: 85-7219 Untreated Field Corn Grain Sample at 0.893 g ml^{-1}. Residue = <0.02 ppm.

Figure 4: 86-8078 Fortified Field Corn Fodder Sample (0.02 ppm) at 0.893 g ml^{-1}. Recovery = 90.5% PP890.

Figure 5: 86-8078 Fortified Field Corn Fodder Sample (0.10 ppm) at 0.893 g ml^{-1}. Recovery = 96.1% PP890.

Figure 6: 86-7219 Fortified Field Corn Grain Sample (0.02 ppm) at 0.893 g ml^{-1}. Recovery = 92.0% PP890.

Figure 7: 86-7219 Fortified Field Corn Grain Sample (0.05 ppm) at 0.893 g ml^{-1}. Recovery = 83.5% PP890.

RDF-1-1/RSI

FIGURE 2

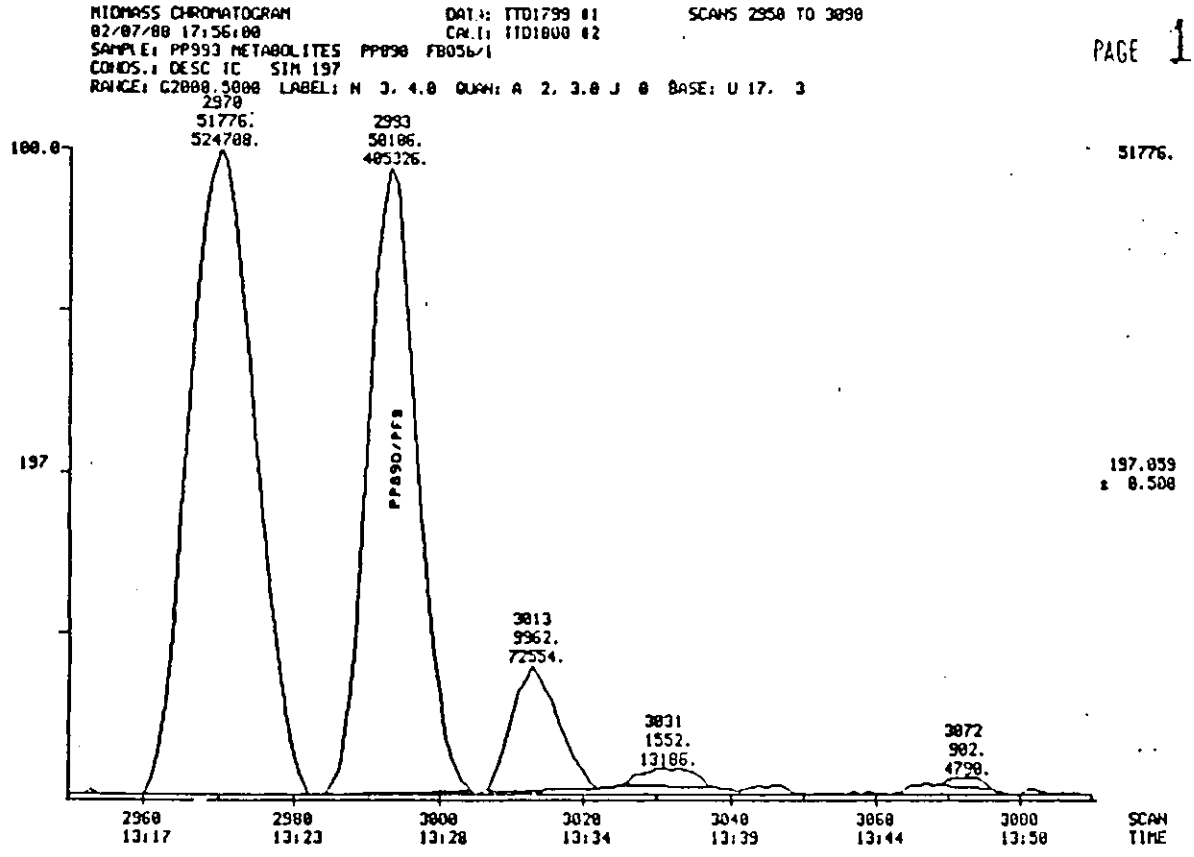


FIGURE 3

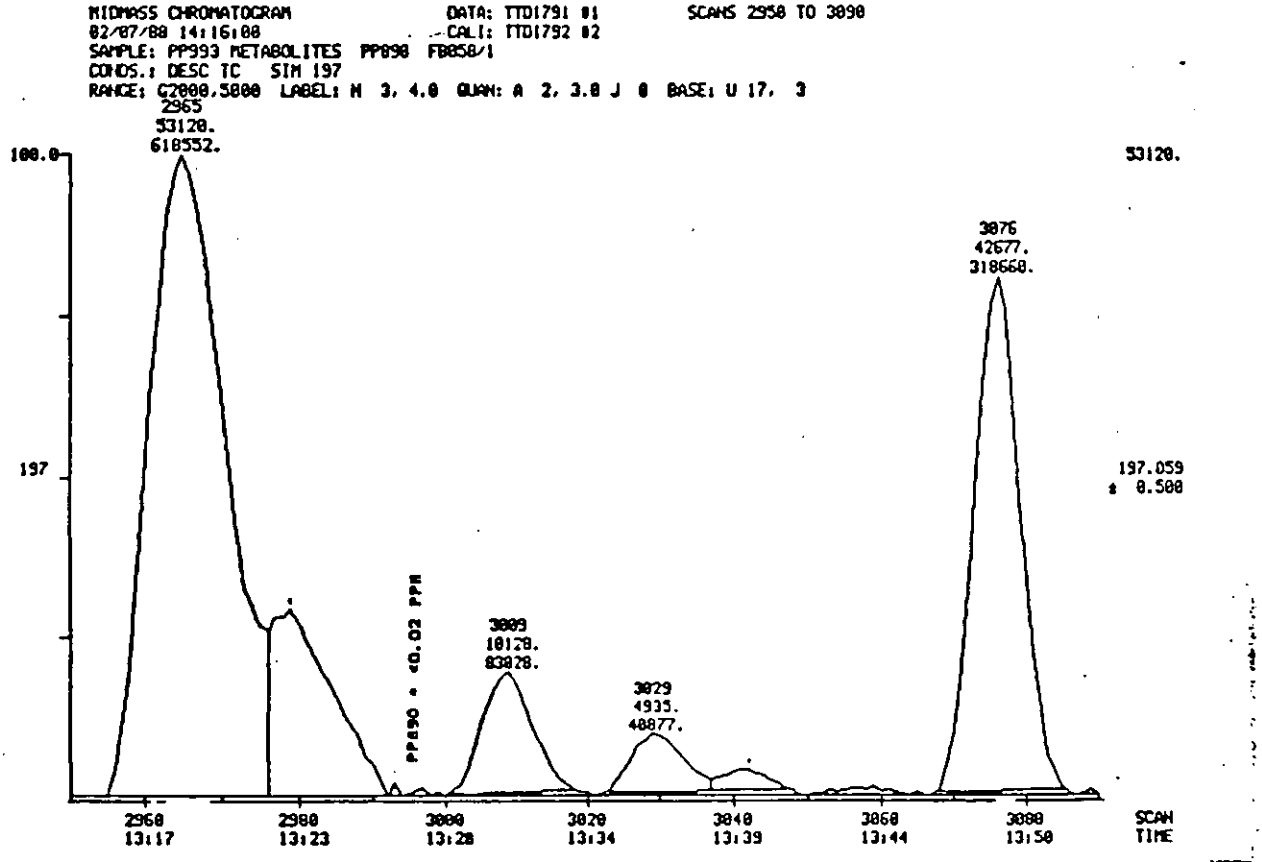


FIGURE 4

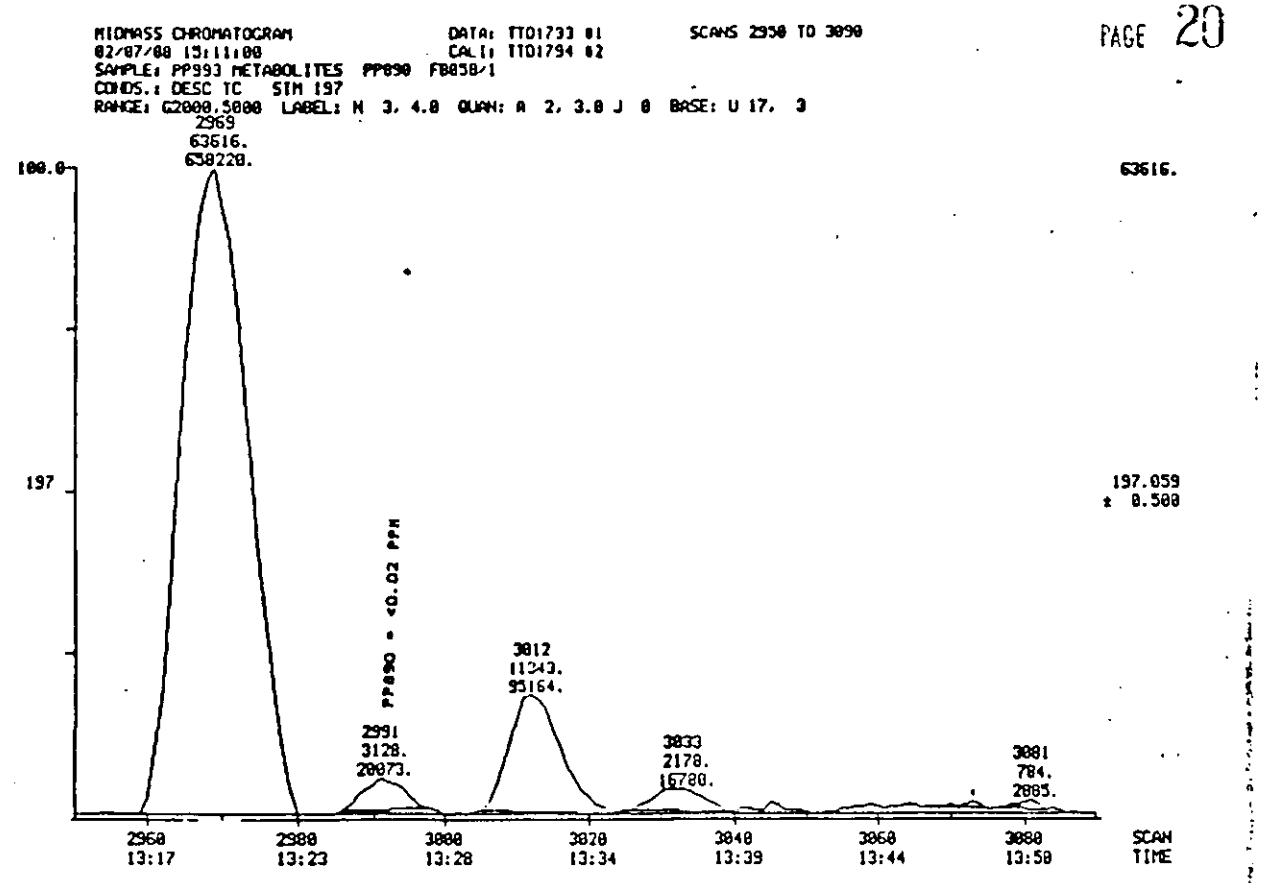


FIGURE 5

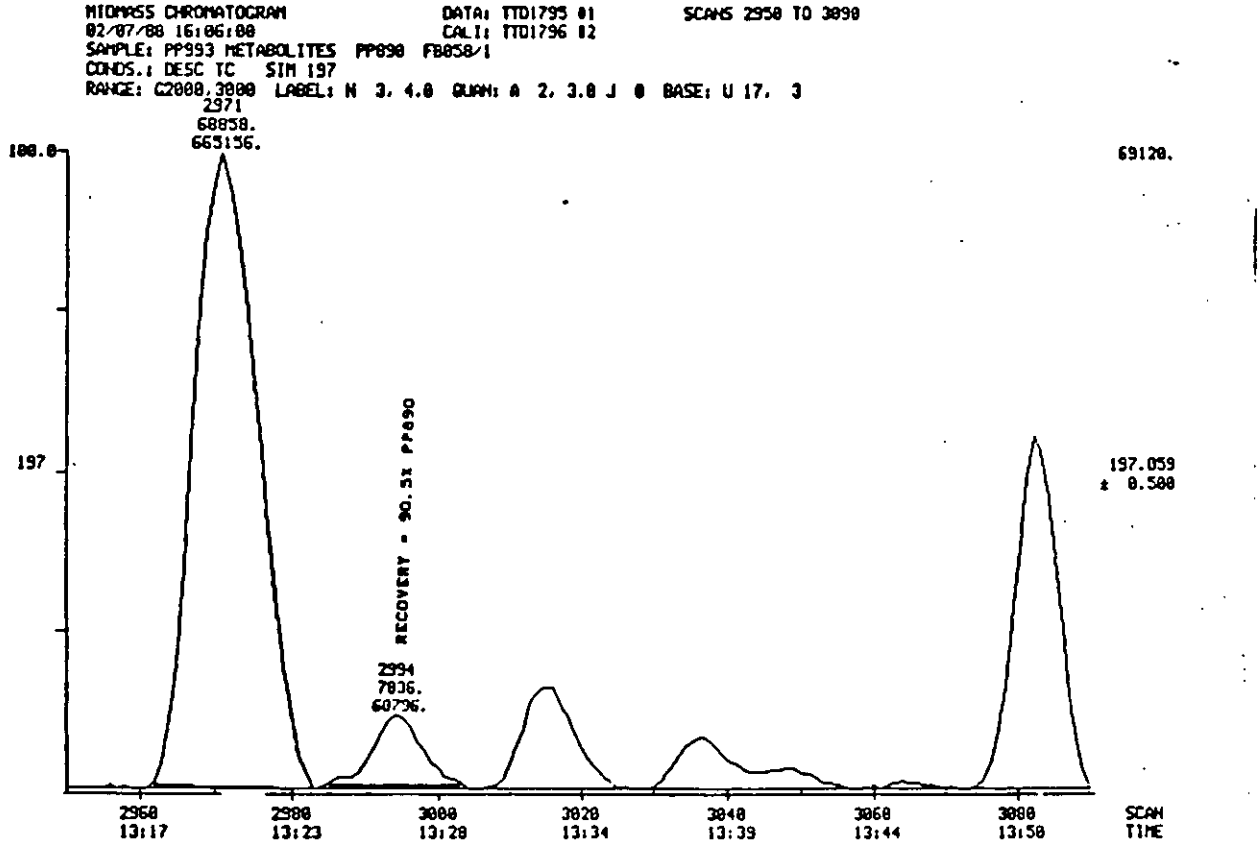


FIGURE 6

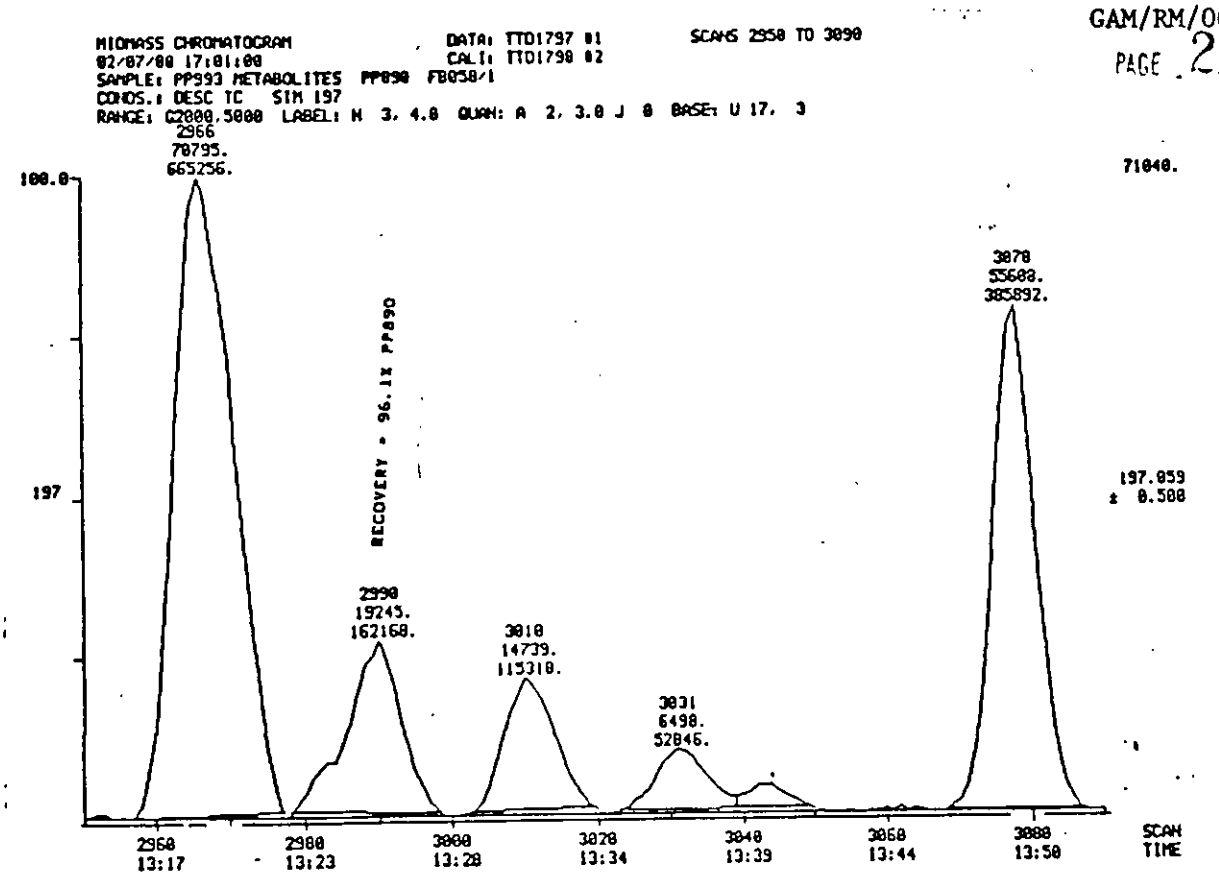


FIGURE 7