Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Iprodione

MRID #: 437183-01

Matrix: Soil

Analysis: LC/MS/MS

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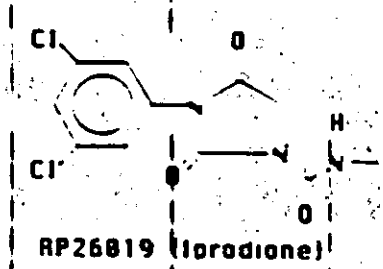
METHOD OF ANALYSIS FOR THE DETERMINATION OF IPRODIONE (RP26019) AND ITS METABOLITES (RP32490, RP37176, RP32596, RP36221, AND RP30228) IN SOIL

INTRODUCTION

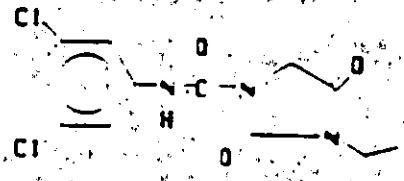
A method of analysis was developed to determine possible residues of iprodione and its metabolites (RP32490, RP37176, RP32596, RP36221, and RP30228) in soil. The validity of the method was demonstrated by fortifying Texas and Mississippi soil samples over the range of 10 to 50 ppb with iprodione plus five metabolites. All six analytes have estimated limits of quantitation below 10 ppb with this method.

Residues are extracted from soil with methanol. The extract is filtered into a small volume of pH 3 water, evaporated to a small volume, then diluted with acetonitrile/pH 3 water. Residues are quantified by HPLC/MS/MS.

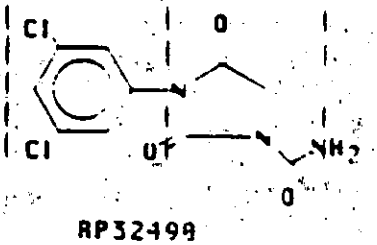
STRUCTURES



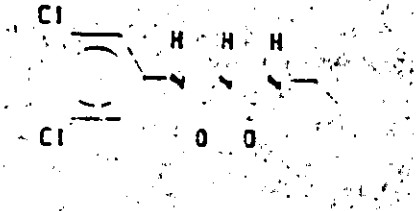
RP26819 (Iprodione)



RP30228



RP32490



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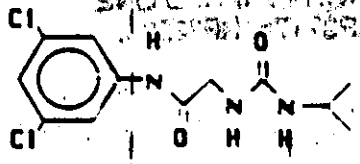
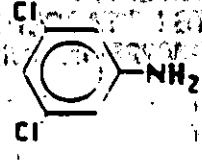


RP37176



RP32596

REAGENTS

1. Methanol, B&J ChromPure, HPLC grade (Burdick & Jackson)
2. Water, HPLC grade (Burdick & Jackson
3. Acetonitrile UV, B&J brand (Burdick & Jackson)
4. Hydrochloric Acid, concentrated (Fisher Brand)
5. RP32490 (Rhone-Poulenc)
6. RP37176 (Rhone-Poulenc)
7. RP26019 (Rhone-Poulenc)
8. RP36221 (Rhone-Poulenc)
9. RP30228 (Rhone-Poulenc)
10. RP32596 (Rhone-Poulenc)

EQUIPMENT

1. Rotary Evaporator (Buchi, Model R-124, or equivalent)
2. Junior Orbital Lab Shaker (Lab Line), or equivalent
3. Nalgene 250 ml screw-cap bottles (NalgeCo.#2105-0008)
4. Centrifuge (Fisher Marathon 10K, or equivalent)
5. Filter paper (Whatman #1, 7.0 cm diameter, or equivalent)
6. Filter (Anotop 25, 0.2 µm, 25 mm diameter filter units, or equivalent)
7. Sonicator (Branson model 2200, or equivalent)
8. Perkin Elmer Sciex API III+ LC/MS/MS system with Hitachi L6200 pump, Sciex heated nebulizer LC/MS interface, and Hitachi AS2000 autosampler
9. pH meter (Cole-Parmer model 05669-20, or equivalent)
10. Boiling flasks, 500 mL with 24/40 standard taper neck, or equivalent

SOLVENTS

1. Prepare pH 3 water (pH 2.6 - 3.0) by adding 5 M HCl to HPLC grade water. Check with a pH meter. (About 20 drops 5 M HCl in 2 liters water).

2. Prepare 50/50:acetonitrile/pH 3 water by mixing equal parts acetonitrile and pH 3 water.

STANDARD SOLUTIONS

1. Weigh 0.0100 g of RP32490, RP37176, RP26019, RP36221, RP30228, and RP32596 individually into a 100 mL volumetric flask. Add 50 mL UV grade acetonitrile and sonicate to dissolve. Add pH 3 water to the mark to make a stock solution containing 100 µg/mL of each component.
2. Dilute 25 mL of the stock solution prepared in step 1 above to 100 mL with 50/50 acetonitrile/ pH 3 water to make a 25 µg/mL mixed standard.
3. By further dilution of the 25 µg/mL mixed standard, with 50/50:acetonitrile/pH 3 water, prepare a series of mixed standards to serve as both spiking and calibration standards.
4. After preparation, standards should be transferred from volumetric flasks into screw-capped brown glass bottles with teflon lined caps.
5. Store standards at room temperature.

PROCEDURE

1. Weigh 25 g sample into a 250 mL Nalgene screw-capped bottle. Fortification of untreated control samples for the purpose of recovery determination should be done at this point.
2. Add 50 mL methanol to each sample and shake for 15-20 minutes on a Junior Orbit shaker.
3. Centrifuge for 9 minutes at 2500 to 3500 rpm.
4. Place 1 mL pH 3 water into a 250 mL filter flask. Place a 7 cm Buchner funnel containing a 7 cm Whatman #1 filter paper circle on top of the filter flask.
5. Filter the supernatant from step 3 through the filter apparatus prepared in step 4.
6. Repeat extraction procedure two more times, centrifuging and filtering the supernatant each time into the same filter flask in step 5.
7. Transfer the filtrate into a 500 mL boiling flask. Evaporate the extract to approximately 1 mL on a rotary evaporator. The water bath temperature should be kept below 35°C, and the vacuum kept at approximately 28-30 inches Hg.
8. Add 5 mL UV grade acetronitrile to the boiling flask, swirl, and sonicate.
9. Transfer to a 10 mL volumetric flask.

10. Add approximately 4 mL pH 3 water to the boiling flask, sonicate, and add to the same 10 mL volumetric flask as step 9. Bring to 10 mL volume with pH 3 water.
11. Filter thru Anotop 25 filter into appropriate vial for injection on LC/MS/MS.

LC/MS/MS OPERATING CONDITIONS

1. Heated nebulizer temperature: 450°C
2. Collision gas: Argon at approximately 220 X 10^{12} atoms/cm^2
3. Injection size: 40 µL, cut injection mode, in 100 µL loop
4. MS Mode: MS/MS with multiple reaction monitoring (MRM)
5. Scan Rate: 1.33 scans/sec
6. Mass Transitions:

RP32490:	243/42
RP37176:	302/217
RP32596:	162/127
RP26019:	243/42
RP36221:	288/160
RP30228:	328/141

7. The mass spectrometer run is divided into four periods:
   The first period is for warming up the positive ion electronics.
   RP32490 and RP37176 are determined in the second period.
   RP32596 in the third period, and RP26019, RP36221, and RP30228 in the final period.

Period	1	2	3	4
Approximate Duration (minutes)	5.35	4.0	0.9	3.15
Mode	positive ion	negative ion	positive ion	negative ion

8. Column: Burdick and Jackson ODS, 250 mm X 4.6 mm, 5 µm particles, or equivalent
9. Mobile phase flow rate: 1.0 mL/min
10. Mobile phase composition: A=acetonitrile
    B=0.1% acetic acid in HPLC grade water

11. Gradient and autosampler programs:

Time (min)	%A	%B	
0.0	40.0	60.0	
7.0	90.0	10.0	
9.0	90.0	10.0	
9.1	40.0	60.0	
12.0	40.0	60.0	
14.1			end ms run time
15.0			end autosampler run time
15.6			next injection

Adjust the gradient as needed to keep the peaks within their corresponding mass spectral acquisition period.

12. Approximate retention times (minutes):

RP32490:	8.11
RP37176:	8.68
RP32596:	10.44
RP26019:	11.32
RP36221:	12.22
RP30228:	12.86

Note: LC/MS/MS conditions may be varied to achieve separation and quantitation of the analytes. For example, the final extract (from step 11 in the procedure) may be analyzed in two or more separate injections. In such a case, the MS may be operated in the negative ion mode for the entire run for the analysis of RP32490, RP37176, RP26019, RP36221 and RP30228. RP32596 may then be quantified in a separate injection of the same extract with the MS operated in the positive ion mode.

QUANTITATION OF RESIDUES

Linear regression is used to generate calibration curves for each analyte. At least 4 different non-zero standards and one zero standard (a solvent blank consisting of 50% acetonitrile/50% pH 3 water) should be run with each set of samples. Standards should be interspersed with samples to compensate for any minor change in instrument response. Sample extracts should be diluted such that the peak areas (or heights) obtained are less than the area (or height) of the highest standard injected.

2. Linear regression coefficients are calculated on peak area (or height) versus µg/mL injected. The data from the analytical standards, including the zero level standard, are fit to a linear model:

$$y = a + bx$$

where: y = peak height or area
a = calibration line intercept
b = calibration line slope
x = conc of analyte in injected solution

The coefficient of determination, R^2, is also calculated.

3. Calculate the concentration of analyte in the injected solution by:

$$\text{Conc in injected solution} = \frac{\text{(peak height or area) - (calibration line intercept)}}{\text{calibration line slope}}$$

4. Calculate the concentration of analyte in the original sample by:

$$\text{Conc in original sample} = \frac{\text{(conc in injected soln) X (dilution volume, mL)}}{\text{weight of original sample}}$$

5. Calculate the percent recovery by:

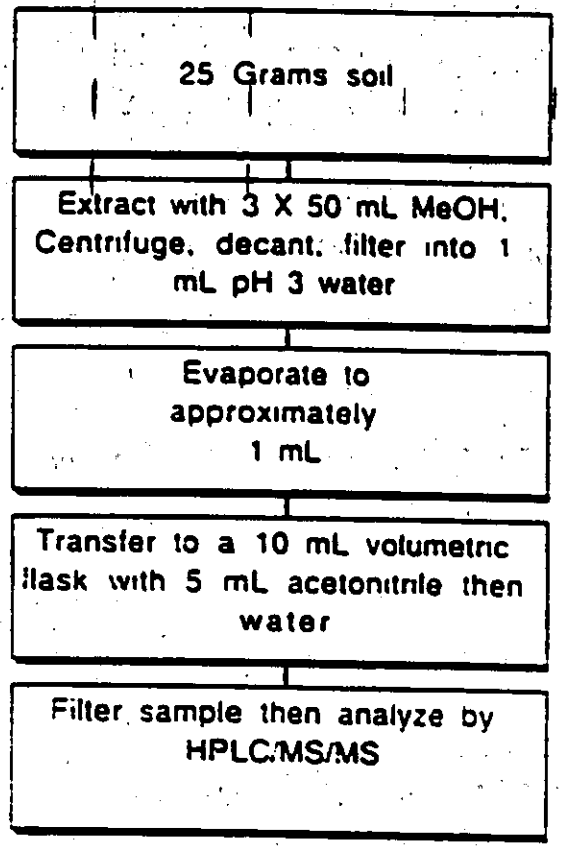
$$\text{Percent Recovery} = \frac{\text{(ppm found in spiked UTC) - (ppm found in UTC)}}{\text{ppm added to UTC}}$$

NOTE: Other regression models may be used for calibration and quantitation at the discretion of the analyst.

COMMENTS

Acidic conditions (obtained through the use of pH 3 water) must be maintained in the standard solutions, filtered sample extract, and the final sample solution in order to prevent decompostion of RP26019 and RP32490 in these solutions.

METHOD FLOW CHART



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APPROVAL:

Alan Y. Chene, Group Leader — Date

APPENDIX G

LIST OF REFERENCED STANDARD OPERATING PROCEDURES