TR-34-91-64

Lab Memo No. 34-89-79

I. Summary

The RH-7592 residues are extracted from soil by shaking with methanol. The methanol extract is partitioned with 10% sodium chloride solution and methylene chloride. The methylene chloride is evaporated and the samples are cleaned up by silica gel column chromatography.

RH-7592 and its metabolites are determined by gas chromatography using a megabore 0.53 mm ID capillary column (SPB-608) and a capillary thermionic specific detector optimized for nitrogen selectivity. A flow diagram of the method is shown on page 3.

II. Introduction

RH-7592 is a triazole fungicide being developed for use on stone fruit, wheat, nuts and other crops. In order to obtain commercial registration, terrestrial field dissipation studies are required to determine patterns of pesticide residue dissipation under actual field conditions. To obtain data on the persistence, degradation and mobility of RH-7592 residues in soil a residue analytical method is needed. This report gives a detailed description of an analytical procedure that measures the residues of RH-7592 found in soil.

Soil metabolism studies conducted in the laboratory with radiolabelled RH-7592 have characterized the residues that contain the backbone of the original molecule as principally parent RH-7592 and lesser amounts of a mixture of the lactone diastereomers of RH-7592 and the benzylic-ketone of RH-7592. Diastereomers are materials with multiple asymmetric centers which differ only in the relative configuration of these centers. Since there are two unsymmetrical substituted quaternary carbons in the lactone structure, this material can exist as two diastereomeric isomers which can have different physical and chemical properties. The structure of these compounds are shown below.

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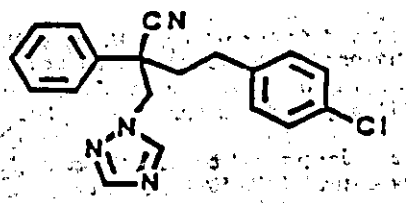
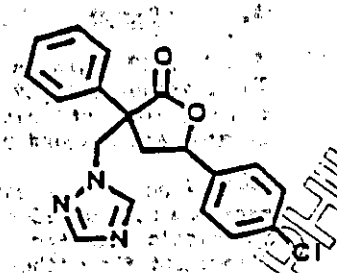
2.



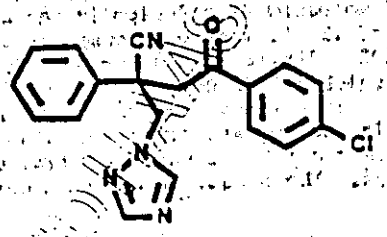
RH-7592

2-(2-[4-chlorophenyl]ethyl)-2-phenyl-3-(1H-1,2,4-triazole)-1-propanenitrile



5-(4-chlorophenyl)dihydro-3-phenyl-3-(1H-1,2,4-triazole-1-yl)methyl)-2(3H)-furanone

RH-99129 - Lactone A - 3R5R/3S5S
RH-99130 - Lactone B - 3R5S/3S5R



Ketone (RH6467)

4-(4-chlorophenyl)-2-(methyl-1H-1,2,4-triazole)-4-oxo-2-phenyl butanenitrile

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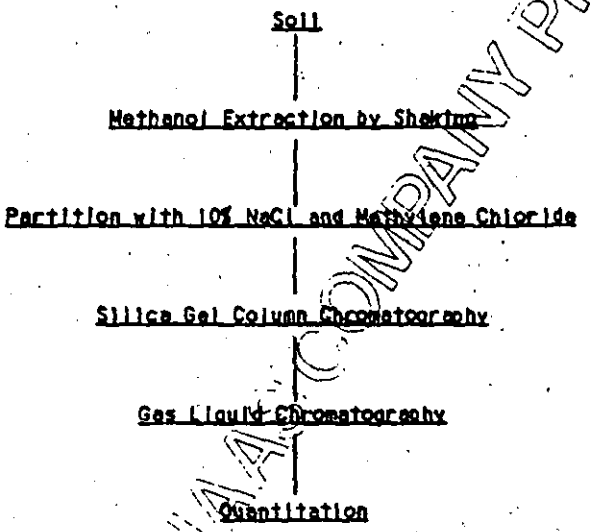
FLOW DIAGRAM



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III. Method

A. Chemicals/Supplies

Acetone, Pesticide Grade	Fisher
BioSil A, 100-200 mesh (Activate at 200°C for 24 hours. Bottle and cap immediately. Store in a desiccator cabinet. Standardize before use.)	Bio-Rad
Celite-545	Johns Manville
Cotton, Sterile, Absorbent	Johnson & Johnson
Filter paper, 7 cm, No. 2	Whatman
Methanol, Pesticide Grade	Baker
Methylene Chloride, Pesticide Grade	Baker
Sodium Chloride, Certified A.C.S.	Fisher
Sodium Sulfate, Anhydrous, Granular AR	Mallinckrodt
Toluene, Pesticide Grade	Baker
RH-7592 Analytical Standard	Rohm and Haas Co.
RH-99,129 Analytical Standard	Rohm and Haas Co.
RH-99,130 Analytical Standard	Rohm and Haas Co.
RH-6467 Analytical Standard	Rohm and Haas Co.
Water, Milli-Q	Millipore

B. Preparation of Solutions

1. Prepare a 10% sodium chloride solution by dissolving 400 g of sodium chloride in four liters of Milli-Q water.

2. Prepare 100/50 and 100/10 (v/v) toluene/acetone solutions by adding 500, and 100 ml of acetone to 1000 ml of toluene.

3. Prepare 100/3 (v/v) toluene/methanol solution by adding 30 ml of methanol to 1000 ml of toluene.

4. Prepare GLC standard and fortification solutions by carefully weighing on an analytical balance 0.100 g of each of the analytical standards (RH-7592, RH-99,129, RH-99,130 and RH-6467) into individual 100 ml volumetric flasks. Add approximately 80 ml of the toluene/methanol (100/3) solution and sonicate until dissolution occurs. Bring to volume with toluene/methanol (100/3). These are the primary standards of 1000 ug/ml. Into a 100 ml volumetric flask carefully pipet 10 ml of each of the primary standards and bring to volume with toluene/methanol (100/3). This is the primary multi-component standard of 100 ug/ml. Make serial dilutions of the multi-component standard to 10, 5, 1, 0.5, 0.1, 0.05 and 0.02 ug/ml for working standards.

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C. Equipment

Buchner Funnel, Porcelain, 83 mm ID	A. H. Thomas
Chromatographic Columns, 14.5 mm ID x 30 cm length with 250 ml integral reservoir at the top	Ace Glass, Inc.
Filter flasks, 400 ml	Kimax
Horizontal Shaker	A. H. Thomas
Rotary Evaporator, Buchi Rotovap R with dry ice trap	Brinkman
Round bottom flasks, 500 and 300 ml	Kimax
Separatory funnels, 500 ml	Pyrex
Standard laboratory equipment, balances, beakers, etc.	
Standard Testing Sieve	Fisher
Ultrasonic Cleaner	Branson
Volumetric flasks, 100 ml	Kimble
Wide mouth jars, 4 oz. with foil lined caps	Kerr Glass Mfg. Corp.

D. Instrumentation

Varian 3500 Capillary Gas Chromatograph equipped with a Varian Model 8035 Autosampler, a 1040 Megabore Injector and a Capillary Thermionic Specific Detector. Data are obtained with an HP 300 Data Acquisition and Processing Station with Hewlett-Packard Extrachrom Software. Data are processed by Nelson Analytical Software.

Column: Fused silica capillary, SPB-608, 0.53 mm ID, 15 meters 0.5 um df - Supelco

Temperatures: Column - 245°C
Injector - 255°C
Detector - 300°C

Flows: Air (zero grade) - 175 ml/min
Hydrogen (UPC) - 4.5 ml/min
Helium (UPC) - 20 ml/min, 10 ml/min purge

Bead Current: 3.5 amps (varies)

Under these conditions, the retention times are as follows:

RH-7592	-	3.69 minutes
RH-99,130	-	4.66 minutes
RH-99,129	-	5.18 minutes
RH-6467	-	6.17 minutes

E. Analytical Procedure

1. Sample Processing

All samples are received frozen (dry ice) at the Rohm and Haas Research Laboratories. The soil is sieved through a 2 mm mesh screen (ASTM No. 10) to remove stones and plant debris, then well mixed. Representative subsamples are taken for moisture analysis.

2. Extraction

Weigh a representative 25 g sample of soil into a 4 oz. wide mouth jar. Add 5 - 6 g of Celite-545 filter aid. Add 100 ml of methanol and cap the jar with a foil lined lid. Extract for 30 minutes by shaking on the horizontal shaker. Vacuum suction filter the extracted soil sample through the 7 cm No. 2 filter paper using a porcelain filter funnel and a 500 ml filter flask. Wash the 4 oz. jar and remaining soil sample with 25 ml of methanol and pour over the filter cake into the 500 ml flask. Transfer the filtrate to a 500 ml separatory funnel. Wash the 500 ml filter flask with 10 ml methanol and add it to the separatory funnel. Proceed to the partition step.

3. Partition

To the 500 ml separatory funnel containing the methanol extract, add 150 ml of methylene chloride and 250 ml of 10% sodium chloride solution. Let the solution stand for one or two minutes to allow the release of pressure. Cap and shake for approximately 10 seconds. Invert the separatory funnel and release the pressure by opening the stopcock. Close the stopcock and shake vigorously for one minute. When the phases separate, draw down the lower methylene chloride phase into a 500 ml round bottom flask. Evaporate the methylene chloride on the rotary evaporator at 45-50°C at atmospheric pressure. Remove the final traces of methylene chloride under vacuum. Add 25 ml of toluene/acetone (100/10) and swirl to dissolve the residue. Proceed to the BioSil cleanup step.

4. BioSil A Column Cleanup

Insert a small cotton plug into a 14.5 mm ID chromatography column and dry pack the column with 5 cc of the activated BioSil A (measure in a 10 cc graduated cylinder). Top the column with 1 inch of anhydrous sodium sulfate. Add the 25 ml of toluene/acetone (100/10) solution in the 500 ml round bottom flask from the partition step to the column and collect the eluent in a 250 ml Erlenmeyer flask. Rinse the 500 ml round bottom flask with 10 ml of the toluene/acetone (100/10) and add to the column when the previous addition just enters the top of the column and collect the eluent in the 250 ml Erlenmeyer flask. Discard the combined eluent. Collect 150 ml of toluene/acetone (100/50) in a 300 ml round bottom flask. Concentrate the eluent to dryness on the rotary evaporator at 70-75°C under reduced pressure. Add the appropriate

volume (5 ml) of toluene/methanol (100/3) and proceed to the gas chromatography step. The elution pattern for each batch of BioSil should be checked before use, and adjustments made to the elution solvents, if necessary.

5. Gas Chromatography

a. Preparation of Standard Curves

A 3 ul aliquot of each multi-component standard in the range of 1.0 to 0.02 ug/ml is injected into the gas chromatograph. The resulting peak heights are measured and plotted vs. concentration (ug/ml) of the appropriate standard to obtain 4 standard calibration curves. Standard curves are prepared for each analysis day.

b. Sample Analysis

A 3 ul aliquot of the final GLC sample is injected into the gas chromatograph. If necessary, the sample is diluted with toluene/methanol (100/3) to give a response within the standard curve range. The peak heights are measured and the concentration of each component is determined from the standard curves. The limit of detection is set by the data system at one-half the value of the peak height of the lowest standard injected (0.5 x lowest peak height of the 0.02 ug/ml standard).

6. Method of Calculation

The RH-7592, RH-99,129, RH-99,130 and RH-6467 residue concentration are determined as follows:

a. Fortification Recovery

For samples fortified with known amounts of RH-7592, RH-99,129, RH-99,130 and RH-6467 prior to extraction, measure the peak heights, determine the concentration (ug/ml) from the standard curves and calculate the percent recovery from the equation 1.

Eq. 1

$$\frac{(\text{ug/ml Found}) \times \text{Final Sample Volume (ml)} - \text{Ctl correction}}{\text{Fortification (ug)}} \times 100 = \% \text{ Recovery}$$

b. Component Residue Concentration

The component residue concentration is determined as follows:

Eq. 2 $$\frac{\text{Final Sample Vol. (ml)} \times \text{Component Conc. (ug/ml)}}{\text{Average Recovery (\%)} \times \text{Sample Weight (g)}^{*}} \times 100 = \text{ppm}$$

*A correction for moisture content can be made if desired.