1. Summary

This report details the RH-7281 residue analytical method for soil. The recovery data in this report is cited from the preliminary soil method (TR34-96-91), the method validation studies (34P-97-53 and 34P-97-59), and the field dissipation study (TR34-98-42).

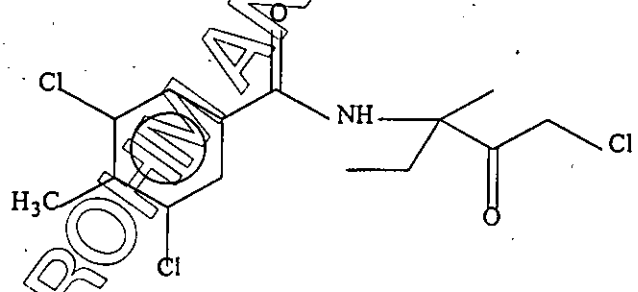
RH-7281 residues are extracted from the soil matrices by shaking with acetonitrile. The extract is partially purified by liquid-liquid partition, Florisil column chromatography, and Alumina-B solid phase extraction (SPE). Quantitation is performed by gas-liquid chromatography using electron capture detection (GC/ECD). Confirmation is done by gas-liquid chromatography/mass selective detection (GC/MSD).

2. Introduction

RH-7281 is a fungicide recently developed at Rohm and Haas Company and targeted to be used on variety of crops. In order to file for the commercial registration, an analytical residue method for soil is required to obtain residue data from soil dissipation studies.

3. Experimental Compound

The structure of RH-117,281 (CAS number: 156052-68-5) is shown below:



CA Name: 3,5-dichloro-N-(3-chloro-1-ethyl-1-methyl-2-oxopropyl)-4-methylbenzamide

Proposed Generic Name: Zoxamide

R&H I.D.*	Lot #	Purity	Appearance	Expiration Date
RH-117,281	ELM1157	97.9%	White Solid	Nov. 11, 2001

*The full R&H number is RH-117,821. The designation typically used in studies is the four digit abbreviation: RH-7281.

4, Chemicals and Supplies

Chemicals	Grade	Supplier*
Acetonitrile	HPLC	Baker
Alumina B Cartridge	LC-Alumina B(0.5 g)	Supelco
Dichloromethane	HPLC	Baker
Ethyl Acetate	HPLC	Baker
Florisil	60-100 mesh (PR Grade)	U.S. Silica Co.
Hexane	HPLC	Baker
Petroleum Ether	Optima	Fisher Chemical
Sodium Sulfate (Na_2SO_4)	Analytical	Fisher Chemical
Water	Mill-Q	Millipore System

* Other manufacturer brands may be substituted if shown to be suitable.

Prepared Solutions and Packing Materials

3/97 (v/v) and 30/70 (v/v) ethyl acetate/hexane solutions:

3/97 (v/v) and 30/70 (v/v) ethyl acetate/hexane solutions were prepared by mixing 30 mL and 300 mL of ethyl acetate in 970 mL and 700 mL of hexane in a 1000 mL flat bottom flask, respectively.

5% deactivated Florisil:

Florisil was baked in an oven at 150°C for at least 4 hours. After cooling to room temperature in a dissector, 5%(w/w) water was added to it dropwisely with a pipette. The deactivated Florisil (tightly capped) then was homogenized on a shaker for 2 hours.

5. Equipment

Equipment	Description	Source*
Centrifuge	J2-21M/E	Beckman
Cotton		J & J
Flat Bottom Flask, 24/40 ST	1000 mL	Fisher Scientific
Glass Chromatography Column	11 mm I.D. x 25 cm	KMAX

Laboratory Shaker	G10 Gyrotory	New Brunswick
Polypropylene Centrifuge Bottle	250 mL	Fisher Scientific
Rotary Evaporator	Model R114	Brinkman
Round Bottom Flasks, 24/40 ST	100 mL and 250 mL	Fisher Scientific
Separatory Funnels	500 mL	Fisher Scientific
Sieve	No.8, 2.36 mm	W.S. Tyler Co.
Sonicator	Model 2210	Branson
SPE Manifold	Visiprep	Supelco
Test Tubes	50 mL	Pyrex

Standard laboratory equipment, balance, beakers, etc.

* Other manufacturer brands may be substituted if shown to be suitable.

6. Method

6.1 The method flow diagram

Soil Sample (10 g)

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Acetonitrile Extraction

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Dichloromethane/Water Liquid-Liquid Partition

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Florisil Column Chromatography

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Basic Alumina Solid Phase Extraction

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GC/ECD or GC/MSD Quantitation

6.2 Sample Preparation

Two soil samples were used in this study:

Soil Name	Soil Type	Location
Soil 1	Loamy Sand, 0-24 inches	Madera, CA
Soil 2	Sand, 0-24 inches	Lyon, NY

Break soil clods into smaller pieces with a cleaver and rubber mallet or by any other appropriate means (To prepare frozen soil samples, for example, chop the soil with a food chopper with dry ice and allow dry ice to sublime overnight in a freezer). Remove stones and debris as found. Sieve and homogenize the soil through a No. 8 sieve (2.36 mm).

6.3 Fortification

Weigh 10 g of soil in a 250 mL polypropylene centrifuge bottle. Fortify the soil with a known amount of RH-7281 in hexane. Allow the solvent to evaporate in a fume hood until the soil is free of solvent (~ 30 min.).

6.4 Extraction

Extract the soil sample with 100 mL of acetonitrile by shaking for one hour on a shaker. Sonicate the sample for 10 minutes and centrifuge at 5,000 rpm and 4°C for 10 minutes. Decant the supernatant into a 250 mL (24/40 ST) round bottom flask. Add another 50 mL of acetonitrile to the centrifuge bottle and extract the soil by hand shaking for 30 seconds, sonicating and centrifuging as before. Combine the second extract into the 250 mL round bottom flask with the first extract and concentrate the final extract to about 30 mL (**Solution A**) on a rotovap at 30°C and diminished pressure (50 mm Hg).

6.5 Liquid/Liquid Partition

Transfer **Solution A** to a 500 mL separatory funnel containing 200 mL of Milli-Q water and 75 mL of dichloromethane. Rinse the flask with a small amount of dichloromethane. Shake the funnel for 30 seconds with frequent venting. Allow the two phase to separate. Transfer the organic phase (lower layer) to a 250 mL (24/40 ST) round bottom flask and partition the aqueous phase with another 75 mL of dichloromethane in the same manner. Discard the aqueous

phase to waste. Combine both organic layers and concentrate it to dryness on a rotovap at 30°C under diminished pressure (50 mm Hg). Dissolve the residue with 10 mL of hexane with sonication for 10 seconds (**Solution B**).

6.6 Florisil Column Chromatography*

Insert a small cotton plug into a 11 mm ID x 25 cm length glass chromatographic column. Dry pack the column with 15 mL of 5% deactivated Florisil topped with about 1 g of Na_2SO_4. Condition the column with 20 mL of ethyl acetate followed by 20 mL of hexane. Apply **Solution B** to the column and elute to the top of the bed. Rinse the 250 mL round bottom flask with 20 mL of hexane and apply to the column. Rinse the 250 mL round bottom flask again with 25 mL of 3/97 ethyl acetate/hexane and apply to the column. Discard all effluents obtained.

Elute RH-7281 from the column with 75 mL 30/70 ethyl acetate/hexane into a 250 mL (24/40 ST) round bottom flask and concentrate the eluate to dryness on a rotovap at 30°C under diminished pressure (50 mm Hg). Dissolve the residues in 20 mL of hexane with sonication for 10 seconds (**Solution C**) and proceed to section 6.7.

6.7 Alumina-B Solid Phase Extraction*

Insert an Alumina-B solid phase extraction cartridge into a solid phase extraction manifold. Condition the cartridge with 5 mL of ethyl acetate followed by 5 mL of hexane. Apply **Solution C** to the cartridge and elute to the top of the bed. Rinse the 250 mL round bottom flask with 10 mL of hexane and apply the wash to the cartridge. Wash the loaded column with 25 mL each of petroleum ether and 3/97(v/v) ethyl acetate/hexane. Discard all effluents at this stage.

Elute RH-7281 from the cartridge with 25 mL of 30/70 (v/v) ethyl acetate/hexane solution into a test tube. Transfer the eluate from the test tube to a 100 mL (24/40 ST) round bottom flask. Wash the tube with about 1 mL of 30/70 ethyl acetate/hexane and add to the 100 mL round bottom flask. Concentrate the eluate to dryness on a rotovap at about 30 °C under diminished pressure (about 50 mm Hg). Dissolve the residues in 10 mL of hexane with sonication for 10 seconds and proceed to quantitation of RH-7281 (section 7).

*The analyte elution profile should be checked for each new lot of Florisil and Alumina-B cartridge. Minimum volume of solvent should be used to elute most of target analyte from the column or cartridge. An example procedure for testing the elution profile for a new lot of Florisil or Alumina-B cartridge is as follows:

1). Using 10 mL of 0.10 µg/mL RH-7281 standard, follow the elution schemes as outlined under section 6.6, or section 6.7, which ever is desirable.

2). Collect the pre-elution cuts as well as the target elution cut. Wash the cartridge with 30 mL of ethyl acetate and collect this post-elution cut.

3). Concentrate the pre-elution cuts, the target cut, and the post-elution cut to dryness.

4). Dissolve the cuts in 10 mL of hexane and inject them as outlined in the GC/ECD quantitation section.

5). If the target cut contains a minimum of 85% of RH-7281 standard, it may be considered acceptable.

6). If the 85% of recovery criteria for standard is not met, the following adjustment in elution scheme should be made:

a). For case where the pre-wash cut contains significant standard; either the pre-wash amount or eluting solvent percentage may be decreased.

b). For cases where the post-wash cut contains significant standard; the percentage of the more polar component in the eluting solvent may be increased.

7. Quantitation

Primary quantitation is GC/ECD and confirmatory quantitation is GC/MSD.

7.1 Instrumentation and Conditions

Gas Chromatography/Electron Capture Detector

GC/ECD: Varian 3500*

*Any other suitable GC/ECD system may be used for analysis after verifying system suitability.

Injector:	Varian 8100 Auto-Sampler	
	On-column	
	Guard column:	Rtx-1 (0.53 mm I.D. x 1 m, 0.25 µm film)
	Injection temp.:	150°C
	Injection volume:	1.0 µL
Column:	Rtx-1 (0.25mm I.D. x 15 m; 0.25µm film)	
	Carrier gas:	Nitrogen
	Head Pressure:	6 psi
	Flow rate:	1.0 mL/min.

Detector temp:300°C

Oven temperature program:
Initial temp. = 100°C, hold for 1 min.
Ramp at 20°C/min.,
Temp. = 250°C, hold for 5 min.

Typical Retention Time:

RH-7281: about 13 min.

Gas Chromatography/Mass Selective Detector

GC/MSD: HP5890/5972*

* Any other suitable GC/MSD system may be used for analysis after verifying system suitability.

Injector: HP18593 Auto-Sampler
On column
Injection temp.: 65°C
Injection volume: 2.0 µL

Column: Rtx-5 (0.25mm I.D. x 30 m; 0.25 µm film)
Carrier gas: Helium
Head Pressure: 12 psi
Flow rate: 1.0 mL/min.

Detector temperature: 280°C

Oven temperature program:
Initial temp. = 60°C, hold for 1 min.
Ramp at 30°C/min.,
Temp. = 250°C, hold for 6 min.

SIM: m/z 187, 189, and 258 (quantitation ion)

Typical Retention Time:

RH-7281: about 11 min.

7.2 Preparation of Standard Curves

Prepare a 100 µg/mL standard stock solution by weighing 10 mg of RH-7281 into a 100 mL volumetric flask. Bring to the volume with ethyl acetate.

Prepare a 1.0 µg/mL stock solution by pipetting 1.0 mL of 100 µg/mL stock solution into a 100 mL volumetric flask and bringing to volume with hexane.

Prepare a series of working standard solutions by diluting the 1.0 µg/mL stock solution with hexane:

Concentration(µg/g)	Volume Taken (mL)	Final Volume (mL)
0.010	1.0	100
0.020	2.0	100
0.050	5.0	100
0.10	10.0	100
0.15	15.0	100

Different concentration series of standards can be made with the same procedure.

Store standards in the refrigerator at 4-8 °C. The shelf-life of RH-7281 stock standard is one year. However, remaking of the stock standard at interval no more than 6 months is recommended.

A minimum of four standard solutions are prepared in a desired concentration range. Standards are preferably quantitated by peak area, although height may be used. A linear regression is used to fit the instrument response into the equation: $Y = mX + b$ although a quadratic equation $Y = aX^2 + bX + c$ can also be used. The concentration of RH-7281 in soil samples is then determined from the standard curve.

7.3 Fortification Recovery

Percent recovery is calculated by measuring the peak area or peak height, calculating the µg/mL found from the standard curve, and correcting any background from the control sample as shown in Equation 1:

$$\frac{[\text{Found (µg/mL)} \times \text{Final Vol. (mL)}] - \text{Control(µg)}}{\text{Fortification Amount (µg)}} \times 100\% = \% \text{ Recovery} \qquad \text{Eq.1}$$

7.4 Sample Analysis

If necessary, the samples are diluted (or concentrated) to an appropriate volume to give a final concentration within the standard curve range. The residue concentration is determined as follows:

$$\frac{[\text{Found } (\mu g/mL) \times \text{Final Vol. } (mL)]}{\text{Sample Weight } (g)} = \text{ppm} \qquad \text{Eq.2}$$

7.5 Sample Calculations

A typical calculation for the recovery of a 0.10 ppm (1.0µg/10g wet soil) fortification of soil (Table 2 and Figure 8) is demonstrated as follows:

$$\frac{[0.0771\ (\mu g/mL) \times 10\ mL - 0.0\ (\mu g)]}{1.0\ \mu g} \times 100\% = 77.1\% \qquad \text{Eq.3}$$

A typical calculation for the concentration in treated sample #254 (Figure 15):

$$\frac{[0.0348\ (\mu g/mL) \times 10\ (mL)}{10\ g\ soil} = 0.0348\ ppm \qquad \text{Eq.4}$$