TR 34-97-110

I. Summary

This report details the analytical method for dithiopyr and three acidic metabolites for soil samples. The parent compound, dithiopyr, is selectively extracted from soil by first shaking the sample with a 95% solution of acetonitrile/0.2 M HCl and secondly with petroleum ether (PE). The PE phase containing dithiopyr is retained for further cleanup. The aqueous phase containing all the acidic metabolites are acidified, and then partitioned into diethyl ether (DE), followed by methylation with diazomethane. Water is added to the methylated solution, and methylated metabolites are extracted from the aqueous solution with PE. The extracts of the parent compound dithiopyr and the methylated metabolites are then combined and purified using a Florisil column. This combined extract is brought to final volume in isooctane. Quantitation is performed by gas liquid chromatography using electron capture detection (GLC/ECD).

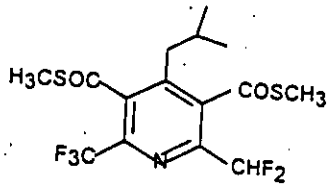
II. Introduction

Dithiopyr, RH-1664 (also known as RH-131664) is the active ingredient of turf herbicide Dimension®. A soil method is developed to analyze dithiopyr and three acid metabolites with a limit of quantitation (LOQ) for each compound at 0.01 ppm. The limit of detection (LOD) is 0.003 ppm for each compound. The three metabolites are normal monoacid, RH-1765 (also known as RH-131765), reverse monoacid, RH-1766 (known as RH-131766) and diacid, RH-3972 (known as RH-133972). The soil samples used to validate this method were collected in East Hampton, Long Island, New York.

III. Standard Compounds

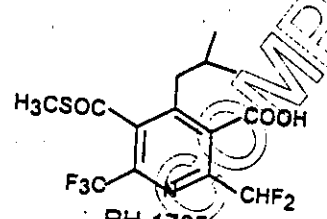
Standard	RH-No
Dithiopyr	RH-1664
Normal Monoacid	RH-1765
Reverse Monoacid	RH-1766
Diacid	RH-3972
*N-methyl RH-1765	RH-4218
*N-methyl RH-1766	RH-4286

*At time of method development, these standards were prepared by Centre Analytical Laboratories, Inc. The structures were confirmed by mass spectrometry. See section V.B.4. for preparation of these standards and see TR 34-97-106 for GLC/MS confirmation.



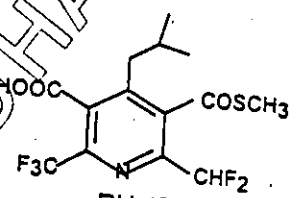
RH-1664

3,5-Pyridinedicarbothioic acid, 2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-S,S-dimethylester



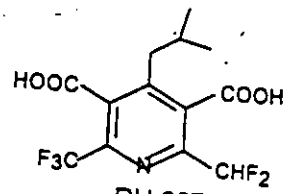
RH-1765

3-Pyridinecarboxylic acid, 2-(difluoromethyl)-4-(2-methylpropyl)-5-[(methylthio) carbonyl]-6-(trifluoromethyl)



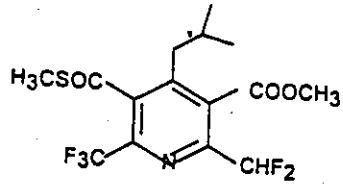
RH-1766

5-Pyridinecarboxylic acid, (difluoromethyl)-4-(2-methylpropyl)-3-[(methylthio) carbonyl]-2-(trifluoromethyl)



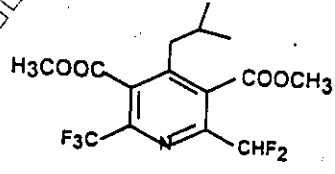
RH-3972

[3,5-pyridinedicarboxylic acid, 2-(difluoromethyl)-4-(2-methylpropyl)-6 (trifluoromethyl)]



N-Methyl RH-1765

N-Methyl RH-1766



N-Methyl, Methyl RH-3972

IV. Materials

A. Reagents and Supplies

Reagents	Grade	Source*
Diazomethane in diethyl ether		Aldrich
Diethyl ether	residue grade	Baker
Hydrochloric acid, (36.5- 38.0%)		Baker
Methanol	pesticide grade	Baker
Sodium chloride	certified A.C.S.	Baker
Sodium hydroxide	certified A.C.S.	Baker
Sodium sulfate, Anhydrous, granular	AR	Baker
Acetonitrile	residue grade	Baker
Petroleum Ether	residue grade	Baker
Isooctane	residue grade	OmniSolv

B. Equipment

Description	Model*
Separatory Funnels	500 mL
Graduated cylinder	500 mL, 50 mL, 25 mL, 10 mL
Pipettes	(2 mL)
Kimax Chromatography Columns	2 x 30 cm
Pear shaped flasks	300 mL, 100 mL
Rotary Evaporator	Buchi
GLC System	HP 5890II
Auto Sampler	HP 7673A
Injector	HP 7673
Detector	ECD
Column	J & W DB-210, 30 m, 0.53 mm ID, 1 µm df

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

C. Solutions

	Composition (V/V)
Acetonitrile/0.2 M HCl	95%
Diazomethane in Diethyl ether	1.25%
Diethyl ether in Pet Ether	(5:95)
Diethyl ether in Pet Ether	(50:50)
NaOH	0.02 M
NaCl	10%
NaCl	saturated

V. Method

A. Flow Diagram and Detailed Method

The flow diagram of the method is listed below, followed by steps of the detailed method.

Method (Flow Chart)

Soil Sample
↓
Acetonitrile/HCl/Liquid Extraction
↓
Pet Ether/Liquid partition
↓
Diethyl Ether/liquid partition
↓
Methylation
↓
Florisil Column Cleanup
↓
GLC Quantitation

Step I. Extraction

Weigh 10 grams of soil into a 200-mL Teflon® sample bottle. Fortifications should be made at this point. Add 50 mL of 95% acetonitrile/0.2 M HCl and shake for 15 minutes on a linear reciprocating shaker. Filter sample into a 250-mL filtering flask, then wash sample bottle and soil cake with 2 x 25 mL of 95% acetonitrile/0.2 M HCl and add to filtrate. Transfer the sample into a 300-mL pear shaped flask and concentrate the sample to 2 to 3 mL using a rotary evaporator (approximately 35°C) under pressure.

Step II: Partition

Transfer the concentrated sample to a 500-mL separatory funnel. Rinse the pear shaped flask with 2 x 25 mL of 0.02M NaOH. Add 1 to 2 mL of 1 M NaOH by pipette, *10 mL of saturated NaCl solution and 50 mL of petroleum ether (PE) to the separatory funnel. Shake vigorously for 2 min. Allow phases to separate (approximately 10 min.). The PE (upper) phase contains the parent RH-1664, and the aqueous (lower) phase contains the acidic metabolites. Retain the PE phase in the separatory funnel, and reserve for subsequent purification. Drain the aqueous phase to a second separatory funnel.

*It was found later that 20 mL of 10% NaCl may improve recoveries for certain soils.

Add 1 to 2 mL of 6 M HCl by pipette and 50 mL of diethyl ether (DE) to the separatory funnel containing the metabolites (aqueous phase). Shake vigorously for 2 minutes and allow the phases to separate (approximately 10 min.). Discard the aqueous (lower) layer. Pass the DE fraction through a 2 x 30 cm column packed with 2 spoonfuls (about 20 g) of Na_2SO_4. Collect the sample in a 100 mL pear-shaped flask. Rinse the Na_2SO_4 column with two 10 mL aliquots of DE, collecting and combining the rinses in the 100 mL pear-shaped flask.

Note: It was found later in sample analysis study that samples without passing through the Na_2SO_4 column had better recoveries. After sample volumes were reduced to about 5 mL in the beginning of Step II, a small amount of Na_2SO_4 was added to the pear shaped flask to absorb the residue moisture. In the methylation step increase the rinse volume of the pear shaped flask from 2 mL to 10 mL.

Step III. Methylation

Reduce the volume of metabolites in DE extract to about 5 mL by using a rotary evaporator (30°C, reduced pressure). Transfer this concentrated DE extract to a 50-mL conical graduated centrifuge tube with a ground glass fitting. Rinse the pear-shaped flask twice with 2 mL DE, and combine in the centrifuge tube. Reduce the DE extract volume to 1 mL under a gentle stream of nitrogen. Add 1 mL of diazomethane (Prepared in house using the Aldrich's DIAZALD Kit, concentration approximately 1.25% in DE) to the extract. Stopper the centrifuge tube and mix gently using a vortex mixer. Let the methylation reaction proceed for 20 minutes at room temperature.

10 mL

After the methylation is complete (20 min.), add 1 mL of methanol to the reaction mixture and evaporate the mixture to about 0.5 mL (so that DE is evaporated completely) under a gentle stream of nitrogen. Add 4 mL of water and 10 mL of PE to the centrifuge tube. Using a pasteur pipette, thoroughly mix the liquid phases by gently drawing the liquid up and down. Allow the phases to separate (about 2 min.). Transfer the PE (upper) phase to a column packed with 2 spoonfuls (about 20 g) of Na_2SO_4, and collect the elute to a 100 mL pear-shaped flask. Repeat the PE extraction by adding another 10 mL of PE to the aqueous phase remaining in the centrifuge tube. Stopper the centrifuge tube and shake for 1 min. Transfer the second PE extract to the Na_2SO_4 column, and collect in the elute to the same 100 mL pear-shaped flask. Pass the PE fraction containing the parent compound RH-1664 (retained from the first partition in the initial separatory funnel) through the same Na_2SO_4 column and combine with the metabolites in the same 100 mL pear-shaped flask. Reduce the volume of this combined extract to 10 mL by rotary evaporation (30 °C, reduced pressure). The sample is now ready for purification by Florisil cleanup.

Step IV. : Florisil Column Cleanup

Fill a Kimax 2 x 30 cm chromatography column with PE. Add 20 mL (about 15 g) of Florisil and top with 1 spoonful of Na_2SO_4. Drain the PE just to the top of the bed. Load the sample. Rinse the column with 30 mL of 5% DE in PE. Elute the sample into a 100 mL pear-shaped flask using 50 mL of 50% DE in PE. Add 5 mL of isooctane to the flask. Reduce the volume to less than 5 mL using rotary evaporation (30°C, reduced pressure). Transfer the sample to a 15 mL graduated test tube. Rinse the flask twice with 2 mL of isooctane, add the rinses to the test tube, and adjust the final volume to 10 mL with isooctane.

B. GLC Chromatography

1) Instrumentation and Conditions

A Hewlett Packard 5890 Series II Gas Chromatograph equipped with a Hewlett Packard Model 7673 autosampler and a ^{63}Ni electron capture detector is used for separation and quantitation of the compounds. Data are obtained with an HP-300 Data Acquisition and Processing Station with Hewlett-Packard Extrachrom Software. Data are processed by Nelson Analytical Software. The Hewlett Packard system is detailed below:

Column:	J & W DB-210, 30 m, 0.53 mm ID, 1 µm df		
Temperature Program:	Initial hold: 100°C for 3 min. ramp: 100 to 210°C at 10°C/min. final hold: 210°C for 10 min.		
Injector Temperature:	225°C		
Detector Temperature:	300°C		
Carrier Gas:	Helium 10 mL/min.		
Makeup gas:	30 mL/min.		
Injection Volume:	1 µL		
Typical Retention Times	RH-1664	=	17.39 minutes
	Methyl-RH-1765	=	15.35 minutes
	Methyl-RH-1766	=	15.57 minutes
	Methyl-RH-3972	=	13.97 minutes

2) Preparation of Stock Standards

Stock standard solution for RH-1664 is prepared by weighing an accurate amount (approximately 10 mg) of analytical standard and placing into a 100-mL volumetric flask. Dissolve the compound in methanol and bring to volume with methanol. Calculate and record the exact concentration of RH-1664, correcting for the standard purity. Repeat this process for RH-1765, RH-1766, and RH-3972.

3) Preparation of Fortification Solutions

Prepare 10 µg/mL combined fortification solutions by pipetting an appropriate amount of each stock solution to deliver 1000 µg each of RH-1664, RH-1765, RH-1766, and RH-3972 into a 100-mL flask and bring to volume with methanol. Dilute 10 mL of this 10 µg/mL solution to volume with methanol in a 100-mL volumetric flask to make a 1 µg/mL fortification solution.

4) Preparation of Calibration Solutions

A 1 µg/mL calibration solution for RH-1664 is prepared by measuring an appropriate aliquot of the stock solution to deliver 100 µg of RH-1664 into a 100-mL volumetric flask. Bring to volume with isooctane to make a 1 µg/mL calibration solution.

Calibration solutions of the metabolites are prepared by measuring an appropriate aliquot of stock solution into separate 100-mL pear-shaped flasks to deliver 100 µg of each metabolite. Add 1 mL of diazomethane and let rest at room temperature for 30 minutes. Dry completely under a nitrogen stream, resuspend in 25 mL of isooctane and transfer to a 100-mL volumetric flask. Rinse the flask two more times with 25 mL of isooctane and add to the flask. Bring to volume with isooctane making a 1 µg/mL methlyated solution of each compound. Measure an aliquot of each methylated solution into a 100-mL volumetric flask and bring to volume with isooctane making a 0.1 µg/mL combined solution. Use the 0.1 µg/mL standard to make serial dilutions of 0.025 µg/mL, 0.01 µg/mL and 0.005 µg/mL. One microliter of standard is injected into the GLC and the resulting standard curve is constructed by plotting peak heights or peak areas measured versus concentration (µg/mL). The standard curves are constructed by linear regression. However when a linear regression does not fit, a quadratic regression will be used. Standard curves are prepared for each analysis day. The mass spectrum of each standard compound was determined by GLC/MS to confirm the structures of the methylated standards.

*See TR 34-97-106 for GLC/MS confirmation.

5) Fortifications

Control samples are fortified with known amounts of combined fortification solution. For example, add 0.1 mL of 1.0 µg/mL fortification solution or 1 mL of 1.0 µg/mL fortification solution to 10 g of soil to make the fortification levels of 0.01 ppm and 0.1 ppm respectively in the soil sample. Recovery data is generated from Equation 2 on the next page.

6) Quantitation and Fortification Recovery

One microliter of the sample is injected into the GLC. If necessary, the sample is diluted to an appropriate volume to give a response within the standard curve range. The peak height or peak area is measured and the concentration is determined from the standard curve. The residue concentration is determined by using the equations on the next page.

Equation 1:

$$\frac{\text{Analyte Concentration } (\mu g/mL) \times \text{Final Volume (mL)}}{\text{Sample Volume (mL)}} = \text{ppm Found } (\mu g/mL)$$

Fortification Recovery

For samples fortified with known amounts of spiking solution prior to extraction, measure the peak height or peak area, determine the µg/mL from the standard curve, correct for any background in the control sample and calculate percent recovery for the following equation:

Equation 2:

$$\frac{\mu g/mL \text{ Found} \times \text{Final Sample Volume}}{\mu g \text{ Added}} \times 100 = \% \text{ Recovery}$$

C. Time Required for Analysis

A set of 8 samples can be prepared for analysis in 8 hours.