Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Pyriproxyfen 4'OH

MRID #: 440369-17

Matrix: Soil

Analysis: HPLC/UV

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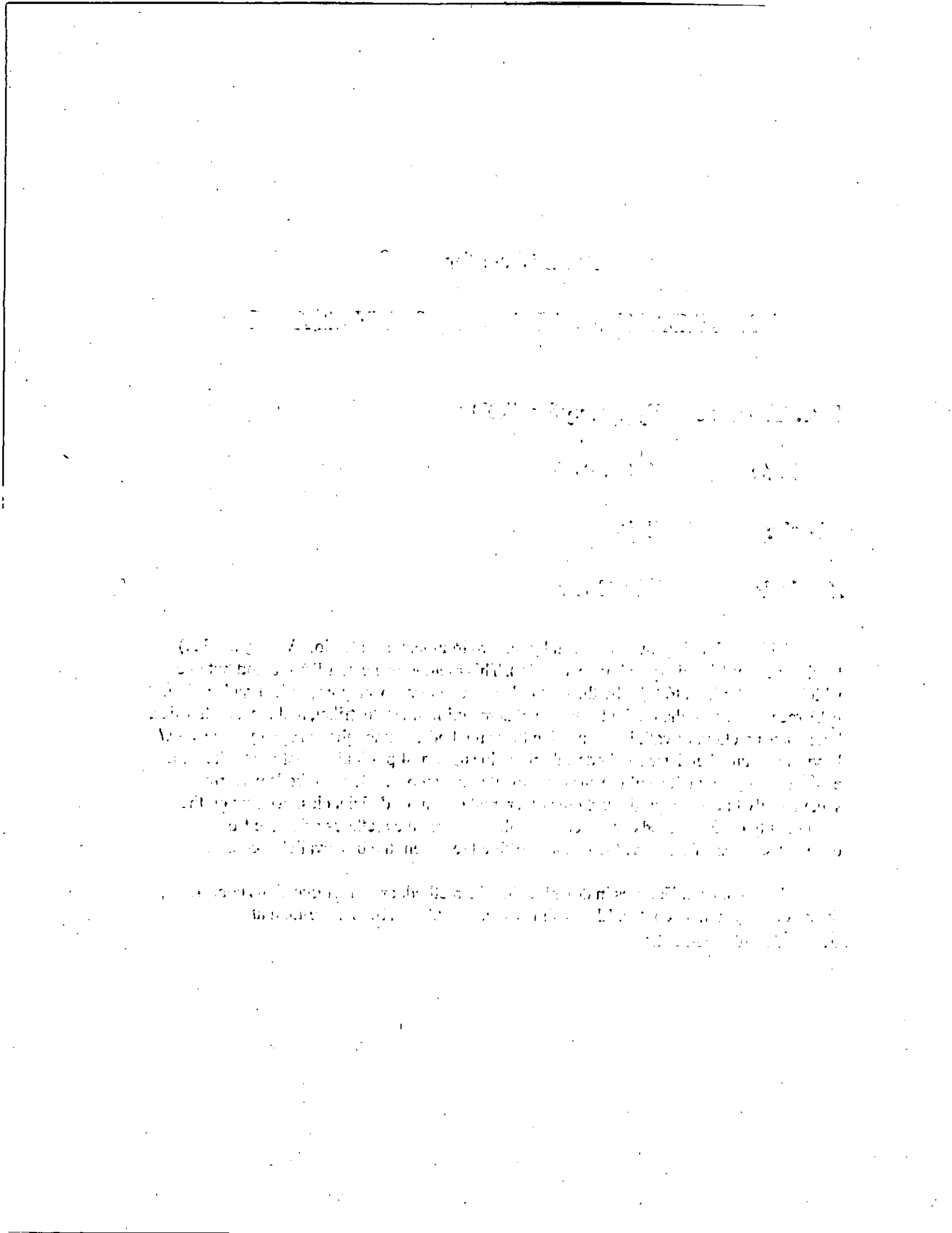

440369-17

APPENDIX 3

ANALYTICAL METHOD

VALENT U.S.A. CORPORATION
VALENT TECHNICAL CENTER
DUBLIN, CALIFORNIA

DETERMINATION OF 4'-OH-PYRIPROXYFEN
RESIDUES IN SOIL AND SOIL SEDIMENT
METHOD RM-33S-3-3

DATE: JUNE 20, 1994
REVISED: AUGUST 18, 1995

INTRODUCTION

This method determines residues of 4'-OH-pyriproxyfen, one of the metabolites of pyriproxyfen, in soil and soil sediment. 4'-OH-pyriproxyfen is extracted from soil using a mixture of methanol and Hastings-Sendroy buffer and partitioned between ethyl acetate and water. The ethyl acetate extract is cleaned-up with a silica gel solid phase extraction (SPE) column and analyzed by high performance liquid chromatography (HPLC) using either a fluorescence or UV detector.

This method is a modification of method number 4OH0993, developed by Analytical Bio-Chemistry Laboratories, Inc[1]. The method was revised on April 12, 1995 to change the HPLC system (column and mobile phase) from normal phase to reverse phase and on June 21, 1995 to change the sample weight. The method was revised on August 18, 1995 to include analysis with a UV detector.

REAGENTS

Acetic acid - 99.8%, Aldrich Cat# 10,908-8, or equivalent.

Ethyl acetate - Pesticide quality or equivalent.

Hexane - Pesticide quality or equivalent.

Methanol - Pesticide quality or equivalent.

2-Propanol - Pesticide quality or equivalent.

Phosphoric acid - concentrated (85%), reagent grade.

Potassium phosphate, monobasic reagent grade or equivalent.

Sodium chloride - reagent grade or equivalent.

Sodium phosphate, dibasic, heptahydrate ($Na_2HPO_4 \cdot 7H_2O$), reagent grade or equivalent.

Sodium sulfate - reagent grade or equivalent.

REAGENTS (CONTINUED)

Tetrahydrofuran - Pesticide quality or equivalent.

Water - HPLC grade or equivalent.

REAGENT SOLUTIONS

1/15 M Disodium hydrogen phosphate - dissolve 17.9 grams of $Na_2HPO_4 \cdot 7H_2O$ in 1 liter of deionized water. Mix well.

1/15 M Potassium phosphate - dissolve 9.07 grams of KH_2PO_4 in 1 liter of deionized water. Mix well.

Hastings-Sendroy buffer - combine 611 mL of 1/15 M disodium hydrogen phosphate with 389 mL of 1/15 M potassium phosphate. Mix well.

Methanol:Hastings-Sendroy buffer (4:1, v/v) - mix 3200 mL of methanol with 800 mL of Hastings-Sendroy buffer. Mix well. (Note: Precipitation may occur after mixing. Shake well before each use).

Eluting solvent (3:1 Hexane:ethyl acetate with 1% acetic acid, v/v/v)- mix 300 mL of hexane with 100 mL of ethyl acetate, then add 4 mL of acetic acid. Mix well.

Methanol-water (4:1, v/v)- mix 4 parts of methanol with 1 part of HPLC water. Mix well.

Mobile phase (Reservoir A) - mix 2 parts of methanol and 3 parts of THF. Mix well and degas for 15 minutes before pumping to column.

Mobile phase (Reservoir B) - add 0.05% (v/v) of 85% phosphoric acid to HPLC water. Mix well and degas for 15 minutes before pumping to column.

Water saturated with sodium chloride - add 1000 grams of sodium chloride to 3 L of deionized water and stir or shake intermittently for at least one hour. Decant the supernatant solution into another vessel for use.

REFERENCE STANDARDS

4'-OH-pyriproxyfen - analytical standard of known purity available from Valent U.S.A. Corporation. Prepare a stock solution containing 1 mg/mL in acetone. Prepare calibrating/ linearity standards by diluting this stock solution to 0.20, 0.50, 1.0, and 2.0 µg/mL with methanol:water (4:1, v/v). The 1.0 µg/mL solution serves as the calibrating and fortifying solution. All solutions should be kept at <0° C when not in use.

EQUIPMENT

Baker SPE-12G Column Processor (12-port vacuum manifold) - J.T. Baker Product Number 7018-00, or equivalent system.

Bakerbond SPE® silica gel disposable columns - 3 mL, J.T. Baker Product Number 7086-03 or equivalent.

Büchner funnels - 9 cm diameter.

Centrifuge tubes - 15 mL, glass, graduated.

Filter flasks - 500 mL.

Filter funnels - 10 cm diameter.

Filter paper - Whatman GF/A glass fiber or equivalent, 9 cm circles.

Linear shaker - Eberbach or equivalent.

Liquid Chromatograph - Hewlett-Packard Model 1090, equipped with an HP Model 1046A Programmable Fluorescence Detector (or HP Model 79853 Variable Wavelength Detector), automatic sampler, and HP 3396A recording integrator or an equivalent system.

Mason jars - 1 pint with plastic screw cap lids or equivalent.

Pasteur pipets - 5¾" and 9".

Rotary evaporator - Büchi (Brinkman) or equivalent, equipped with a temperature controlled water bath.

Round-bottom flasks - 50 mL, 250 mL and 500 mL.

Separatory funnels - 500 mL.

Syringes - Tuberculin, with glass Luer-Tip, 1 mL and 10 mL.

Syringe filters - Gelman Acrodisc LC13 (PVDF, 0.45 µm).

Ultrasonic cleaner - Branson Model 3200 or equivalent.

ANALYTICAL PROCEDURES

Extraction

Weigh 20 grams (± 0.1 grams) of soil into a one pint Mason jar. At this point, if required by the testing facility, control samples to be used for method recoveries may be fortified with 4'-OH-pyriproxyfen. (See Note 1). Add 40 mL of methanol/Hastings-Sendroy buffer (4:1, v/v) to the sample, cap and place on the shaker. Shake on HIGH for 15 minutes.

Filter the sample into a 500 mL filter flask using a 9 cm Büchner funnel and Whatman GF/A filter paper. Rinse the Mason jar with 20 mL of extraction solvent and add to the Büchner funnel.

Transfer the filter cake back into the Mason jar and re-extract with a second 40 mL portion of methanol/Hastings-Sendroy buffer (4:1, v/v). Filter as above, combining this extract with the first extract. Rinse the Mason jar with 20 mL of extraction solvent and add to the Büchner funnel.

Transfer the combined filtrates to a 500 mL round-bottom flask. Rinse the filter flask with two 20 mL portions of methanol/Hastings-Sendroy buffer (4:1, v/v) and add to the 500 mL round-bottom flask. Evaporate to approximately 20 mL using a rotary-evaporator and water bath set to <40°C.

Ethyl Acetate/Water Partitioning

Transfer the concentrated extract to a 500 mL separatory funnel. Rinse the round-bottom flask with 80 mL of ethyl acetate and add this rinse to the separatory funnel. Add 80 mL of water saturated with sodium chloride to the separatory funnel and shake vigorously for approximately 1 minute. Drain the lower aqueous layer into the original 500 mL round-bottom flask. Drain the upper ethyl acetate layer into a clean 250 mL round-bottom flask through a 10 cm filter funnel containing approximately 50 grams of sodium sulfate supported on a plug of glass wool.

Return the aqueous layer to the separatory funnel, add 40 mL of ethyl acetate, and partition as above. Discard the lower aqueous layer. Drain the upper ethyl acetate layer through the filter funnel containing the sodium sulfate into the 250 mL round-bottom flask containing the first extract. Rinse the sodium sulfate with 2 x 10 mL of ethyl acetate. Evaporate the combined ethyl acetate layers to dryness using a rotary-evaporator and water bath set to <40°C. Add 3 mL of eluting solvent (3:1 hexane:ethyl acetate with 1% acetic acid, v/v/v) to the evaporated extract, swirl and sonicate for 30 seconds.

Silica Gel SPE Cleanup

Attach a Bakerbond SPE® silica gel disposable column to the vacuum manifold. Attach a 10 mL glass syringe (plunger removed) to the column and pre-condition the column with 10 mL of eluting solvent. Do not exceed a flow rate of 5 mL/minute. Do not allow the column to dry before the sample is applied.

Remove the glass syringe from the column and place a 15 mL centrifuge tube under the column. Transfer the sample extract to the column using a Pasteur pipet and apply vacuum until the solvent reaches the top of the packing. Rinse the round-bottom flask with two 3 mL portions of eluting solvent and transfer to the column using a Pasteur pipet, allowing each portion to reach the top of the packing before adding the next.

Transfer the eluate to a 50 mL round bottom flask using two 3 mL portions of eluting solvent to rinse the centrifuge tube. Evaporate to dryness using the rotary evaporator and water bath set at <40°C. Dissolve the residue in exactly 1.0 mL of methanol:water (4:1, v/v) and sonicate to dissolve the residue (See Note 4). Attach an Acrodisc filter to a 1 mL glass syringe (plunger removed) and transfer the extract to the syringe with a Pasteur pipet. Insert the syringe plunger into the syringe and slowly push the extract through the filter into an autosampler vial for storage until analysis. Store at <0°C until analysis.

HPLC Measurement (See Notes 2, 3, and 4)

Analyze the sample extracts, along with calibrating standard solutions, using the following operating conditions:

Column: ODS-Hypersil (15 cm x 4.6 mm, 3 μm particle size), Phenomenex Cat # 00F-0145-E0 or equivalent.

Mobile phase: A = methanol:THF (2:3, v/v)
B = water + 0.05% 85% H_3PO_4 (v/v).

Gradient: T = 0, 45% A + 55% B
T = 8, 45% A + 55% B
T = 18, 75% A + 25% B
T = 30, 75% A + 25% B

Flow rate: 1.0 mL/min.
Injection volume: 50 μl

Detector 1: HP Model 1046A Fluorescence
Excitation wavelength: 275 nm
Emission wavelength: 327 nm
Retention Time: 15.8 min. (See Figure 1).

Detector 2: HP Model 79853A Variable Wavelength
Wavelength: 275 nm
Retention Time: 14.9 min. (See Figure 4)

The HPLC parameters shown above are given only as a guide. They may be modified as needed to optimize the chromatography or to resolve matrix interferences. Each set of chromatograms must be clearly labelled with the parameters used.

The recommended sequence of samples and standards for analysis is: calibrating standard, sample, sample, sample, calibrating standard, etc. The calibrating standard vials contain 1.0 µg/mL of 4'-OH-pyriproxyfen in methanol:water (4:1, v/v). This sequence may, however, be modified if the reproducibility requirement is met (See Note 3).

Calculations

The amount of 4'-OH-pyriproxyfen in each sample is calculated using the following formula:

$$ppm\ 4'\text{-}OH\text{-}Pyriproxyfen = \frac{B \times C \times V \times DF}{A \times W}$$

where:

B = integration counts for 4'-OH-pyriproxyfen in the sample.
C = concentration of the 4'-OH-pyriproxyfen calibrating standard (1.0 µg/mL).
V = final volume of the sample extract (1.0 mL).
DF = dilution factor, used if the sample extract is diluted prior to analysis.
A = mean integration counts for the 4'-OH-pyriproxyfen standards.
W = sample weight (20 grams)

LIMITS OF DETECTION AND QUANTITATION

The limit of detection (LOD) of 4'-OH-pyriproxyfen in soil and soil sediment analyzed by this method is 0.01 ppm. The validated limit of quantitation (LOQ) is 0.02 ppm.

ANALYSIS TIME

A trained analyst can complete the analysis of a set of eight samples for 4'-OH-pyriproxyfen in approximately 8 hours. The results are available within 24 hours of initiating the analysis.

NOTES

1. At Valent, a standard operating procedure (SOP# VR-002) requires that fortified control samples be analyzed with each set of samples. If the testing facility does not require concurrent analysis of fortified control samples, or if a UTC sample is not available, this method requirement may be waived.

   The level of fortification is generally 0.02 ppm (the LOQ of the method) and/or 0.1 ppm. These fortifications are made by adding 0.40 mL and 2.0 mL, respectively, of the 1.0 µg/mL fortifying solutions to a 20 gram sample. Method recoveries must be 70% to 120% to be acceptable unless approved by the chemist responsible for the analysis.

2. At Valent, the linearity of the detector must be determined each day that samples are analyzed (Valent SOP # VR-007). Linearity is determined by analyzing a minimum of four linearity standards including the 0.10 and 1.0 µg/mL solutions. The response for each standard is normalized to response per 1.0 µg/mL by dividing the response of each standard by its concentration. The coefficient of variation (CV) of these responses must be 10% or less. Sample extracts must be diluted to bring the concentration of the analyte within the range of linearity established.

3. At Valent, the reproducibility of an analytical run is determined by calculating the CV from the peak units obtained for the calibrating standards analyzed during the run. For a run to be acceptable, these CV's must be 10% or less unless approved by the chemist responsible for the analysis (Valent SOP# VR-013).

4. If interferences are encountered with this analysis, the following HPLC conditions may be used. Dissolve sample extracts, after silica gel SPE cleanup, in 1.0 mL of hexane:2-propanol (50:1, v/v) and omit the Acrodisc filtration. Calibrating and linearity standards must also be prepared in hexane:2-propanol (50:1, v/v):

   Columns: Spherisorb Si (25 cm x 4.6 mm, 5 µm particle size), 2 in series. Phenomenex Cat # 00G-0100-E0 or equivalent.

   Mobile phase: 95% A + 5% B, where
   A = hexane
   B = 2-propanol + 2.0% (v/v) acetic acid + 2.0% (v/v) water.

   Flow rate: 2.0 mL/min.
   Detector: HP Model 1046A Fluorescence or equivalent.
   Excitation wavelength: 275 nm
   Emission wavelength: 327 nm
   Injection volume: 50 µl
   Retention Time: 8.3 min. (See Figure 7)

REFERENCE

1. Serak, K., Gresham, M. E., "Analysis of Soil for 4'-OH Pyriproxyfen", ABC Method Number 4OH0993, Revision 03, 01-17-94.

Written by: _____ Date: 10/10/95
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Reviewed by: _____ Date: 10/10/95
G. H. Fujie

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October 10, 1995

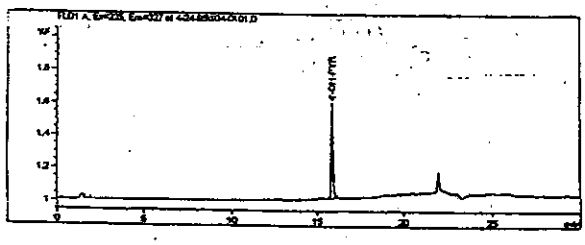

Figure 1. 4'-OH-Pyriproxyfen Calibration Standard
1.0 µg/mL in methanol:water
50 µl injected (50 ng on column)
Reverse phase system (Fluorescence Detector)

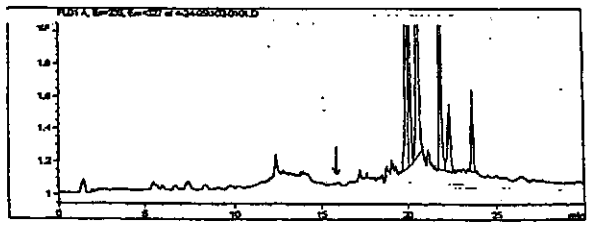

Figure 2. Control Soil from Grant County, WA
50 µl injected (500 mg soil equivalent)
Reverse phase system (Fluorescence Detector)

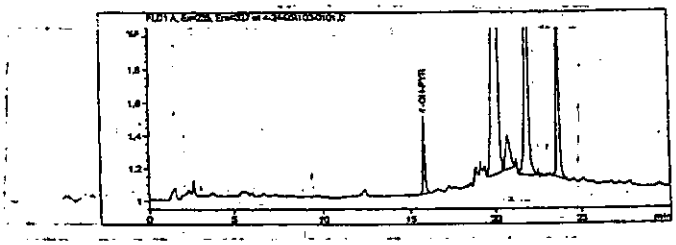

Figure 3. Fortified Soil from Grant County, WA
Fortified with 0.10 ppm of 4'-OH-PYR
50 µl injected (500 mg soil equivalent)
Reverse phase system (Fluorescence Detector)

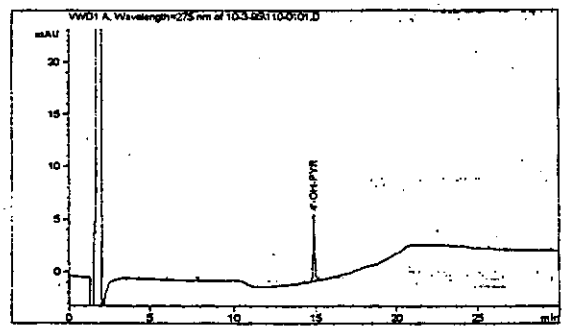

Figure 4. 4'-OH-Pyriproxyfen Calibration Standard
1.0 μg/mL in methanol:water
50 μl injected (50 ng on column)
Reverse phase system (UV Detector)

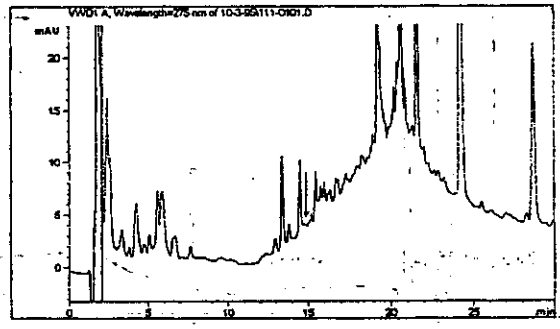

Figure 5. Control Soil from Fresno County, CA
50 µl injected (500 mg soil equivalent)
Reverse phase system (UV Detector)

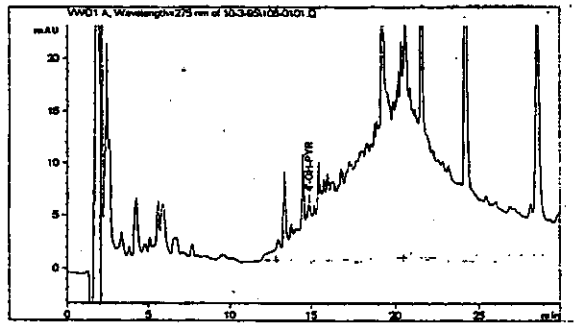

Figure 6. Fortified Soil from Fresno County, CA
Fortified with 0.02 ppm of 4'-OH-PYR
50 μl injected (500 mg soil equivalent)
Reverse phase system (UV Detector)

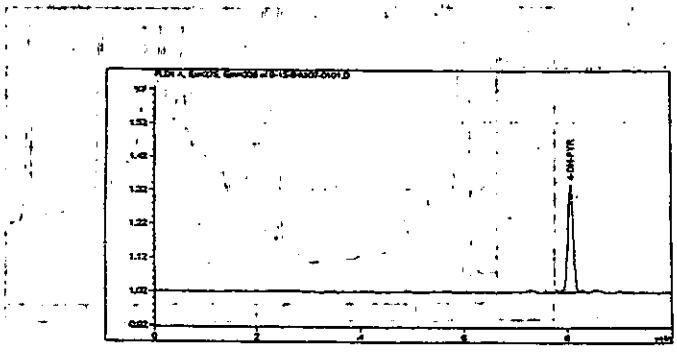

Figure 7. 4'-OH-Pyriproxyfen Calibration Standard
1.0 µg/mL in hexane:2-propanol
50 µl injected (50 ng on column)
Normal phase system (Fluorescence Detector)

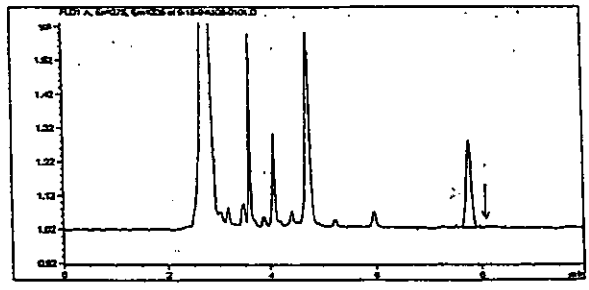

Figure 8. Control Soil from Fresno County, CA
50 µl injected (500 mg soil equivalent)
Normal phase system (Fluorescence Detector)

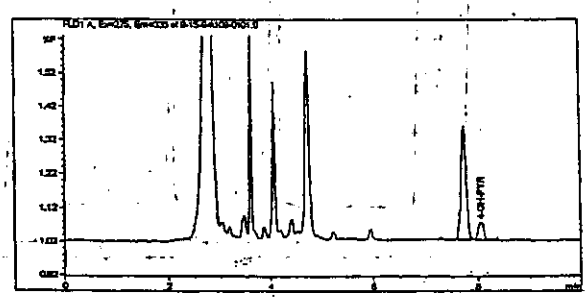

Figure 9. Fortified Soil from Fresno County, CA
Fortified with 0.02 ppm of 4'-OH-PYR
50 µl injected (500 mg soil equivalent)
Normal phase system (Fluorescence Detector)