

US EPA ARCHIVE DOCUMENT

B99-59

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

DETERMINATION OF PYRIPROXYFEN
AND 4'-OH-PYRIPROXYFEN IN NUTMEATS
METHOD RM-33N-2

DATE: AUGUST 26, 1996
REVISED: JANUARY 14, 1997

INTRODUCTION

This method determines residues of pyriproxyfen [V-71639; 4-phenoxyphenyl(RS)-2-(2-pyridyloxy) propyl ether] and its metabolite/degradate, 4'-OH-pyriproxyfen [4-(4-hydroxyphenoxy) phenyl(RS)-2-(2-pyridyloxy) propyl ether], in nutmeats. This method is based on procedures developed by Sumitomo Chemical Company, Ltd, Environmental Health Science Laboratory, Takarazuka, Japan (Report No. ER-MT-8925).

Briefly, pyriproxyfen and 4'-OH-pyriproxyfen are extracted from nutmeats using acetone and partitioned first with dichloromethane/water, then with hexane/acetonitrile to remove oils. The pyriproxyfen and 4'-OH-pyriproxyfen residues are separated by silica gel column chromatography and analyzed separately. The pyriproxyfen residues are quantified by gas chromatography using a nitrogen-phosphorus specific flame-ionization detector (NPD). The 4'-OH-pyriproxyfen residues are quantified by high performance liquid chromatography (HPLC) using a fluorescence detector.

This method was revised on January 14, 1997 to add an additional hexane/acetonitrile partitioning step (Step 5).

REAGENTS

Acetone - pesticide quality or equivalent.

Acetonitrile - pesticide quality or equivalent.

Dichloromethane - pesticide quality or equivalent.

Diethyl ether - anhydrous, Analytical Reagent, Mallinckrodt or equivalent.

Hexane - pesticide quality or equivalent.

Methanol - pesticide quality or equivalent.

Phosphoric acid, 85% - reagent grade or equivalent.

Valent U.S.A. Corporation

RM-33N-2

Page 2

REAGENTS (CONTINUED)

Silica Gel 60 - 70-230 mesh, EM Science Cat# 7734-7 or equivalent. Prepare for use as follows: Activate by heating for at least 16 hours at 130°C. Cool to room temperature and add 10% (w/w) of deionized water and gently rotate until all lumps are removed. Allow to equilibrate for at least 16 hours before use. Store at room temperature in a tightly capped bottle. See Note 1.

Sodium chloride - reagent grade or equivalent.

Sodium sulfate - anhydrous, granular, AR grade or equivalent.

Tetrahydrofuran - HPLC grade or equivalent.

Toluene - pesticide quality or equivalent.

Water - deionized.

Water- HPLC grade.

REAGENT SOLUTIONS

Methanol/Water Solution, 4:1 (v/v) - Combine 4 parts methanol with 1 part HPLC grade water. For example, add 800 mL methanol and 200 mL of HPLC grade water sequentially to a reagent bottle. Store at room temperature.

— Methanol/Tetrahydrofuran Solution, 2:3 (v/v) - Combine 2 parts methanol with 3 parts tetrahydrofuran. For example, add 400 mL methanol and 600 mL of tetrahydrofuran sequentially to a reagent bottle. Store at room temperature.

✓ Hexane/acetone, 7:3 (v/v) - Combine 7 parts of hexane with 3 parts of acetone. For example, add 700 mL of hexane and 300 mL of acetone sequentially to a reagent bottle. Store at room temperature.

✓ Hexane/diethyl ether, 15:1 (v/v) - Combine 15 parts of hexane with 1 part of diethyl ether. For example, add 600 mL of hexane and 40 mL of diethyl ether sequentially to a reagent bottle. Store at room temperature.

✓ Sodium chloride, 5% (w/v) - Add 50 grams of sodium chloride to a 1L flask and dilute to volume with deionized water. Stopper and shake.

Water, acidified (0.04% phosphoric acid, v/v) - Add 0.5 mL of 85% phosphoric acid to 1 liter of HPLC grade water. Store at room temperature.

Valent U.S.A. Corporation

RM-33N-2

Page 3

REFERENCE STANDARDS

Pyriproxyfen - analytical standard of known purity. Prepare a stock solution containing 1 mg/mL in acetone. Prepare a minimum of four linearity standards by diluting this stock solution with toluene to concentrations ranging from 0.10 to 2.0 $\mu\text{g/mL}$. (See Note 2). Prepare a calibrating solution containing 1.0 $\mu\text{g/mL}$ by diluting the stock solution with toluene. (The calibrating solution may be used as one of the four required linearity standards). Prepare a fortifying solution by diluting the stock solution to 1.0 $\mu\text{g/mL}$ with acetone. All solutions should be kept refrigerated when not in use.

4'-OH-pyriproxyfen - analytical standard of known purity. Prepare a stock solution containing 1 mg/mL in acetone. Prepare a fortifying solution by diluting the stock solution to 1.0 $\mu\text{g/mL}$ with acetone. Prepare a minimum of four linearity standards by diluting the stock solution with methanol/water (4/1, v/v) to concentrations ranging from 0.20 to 2.0 $\mu\text{g/mL}$. (See Note 2). Prepare a calibrating solution containing 1.0 $\mu\text{g/mL}$ by diluting the stock solution with methanol/water (4/1, v/v). (The calibrating solution may be used as one of the four required linearity standards). All solutions should be kept refrigerated when not in use.

EQUIPMENT

Acrodisc® LC 13 PVDF Syringe Filters - 0.45 μm pore size, Luerlock inlet and 13 mm minispike outlet or equivalent.

Büchner funnels - 9 cm diameter.

Centrifuge - Clay Adams Safety Head or equivalent.

Centrifuge tubes - 15 mL, glass, with stoppers

Filter flasks - 500 mL.

Filter funnels - approximately 10 cm diameter.

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

Gas Chromatograph - Hewlett-Packard Model 5890, equipped with a packed column glass insert for splitless injection (HP Part No. 5080-8732, packed with approximately 5 mm of silanized glass wool), an NP detector, automatic sampler, and 3392A recording integrator or equivalent system.

Valent U.S.A. Corporation

RM-33N-2

Page 4

EQUIPMENT (CONTINUED)

Glass chromatography column - 19 mm ID x 300 mm with 250 mL reservoir and Teflon stopcock, Kontes Cat. # K-420280-0232 or equivalent.

Glass wool - Pyrex® or equivalent.

HPLC - Hewlett-Packard Model 1050 equipped with a tertiary pump, an autosampler, a recording integrator, and an HP Model 1046 fluorescence detector (or HP Model 79853 Variable Wavelength Detector) or equivalent system.

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

Omni-Mixer with adaptor for use with 1-pint Mason jars.

Pasteur pipets - 5¼" and 9".

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

Round-bottom flasks - 50 mL, 250 mL, 500 mL, and 1000 mL.

Separatory funnels - 500 mL

Syringe - Tuberculin, with glass Luer-Tip, 1 mL capacity, VWR Cat # BD 2004 or equivalent.

Syringe needles - 22 gauge, blunt tip, 2 inches long, Rainin Cat # DF 722-3 or equivalent.

Ultrasonic cleaner - Branson 3200 or equivalent.

Vortex mixer - Vortex Genie 2, VWR or equivalent.

Valent U.S.A. Corporation

RM-33N-2

Page 5

ANALYTICAL PROCEDURES**1. Extraction**

Weigh 20 grams (± 0.1 grams) of sample into a one pint Mason jar. At this point, if required by the testing facility, control samples for method recovery should be fortified with pyriproxyfen and 4'-OH-pyriproxyfen (See Note 3). Add 150 mL of acetone to the sample and blend on the Omni-Mixer for 5 minutes.

Filter the sample into a 500 mL filter flask using a Büchner funnel and Whatman GF/A glass fiber filter paper. Transfer the filter cake back into the Mason jar and re-extract the sample with two additional 150 mL portions of acetone as described above. Filter each extract into the 500 mL filter flask, combining these extracts with the first. Rinse the Mason jar with two 20 mL portions of acetone and add to the Büchner funnel.

Transfer the combined filtrates to a 1000 mL round-bottom flask. Rinse the filter flask with two 20 mL portions of acetone and add to the round-bottom flask. Evaporate the acetone using a rotary-evaporator and water bath set to $<40^{\circ}\text{C}$.

2. Dichloromethane/Water Partitioning

Add 75 mL of dichloromethane to the round-bottom flask and briefly rotate the flask in an ultrasonic water bath to assist removal of residue adhering to the walls of the flask. Transfer to a 500 mL separatory funnel. Rinse the round-bottom flask with another 75 mL portion of dichloromethane, as described above and transfer to the separatory funnel. Add 100 mL of a 5% aqueous solution of sodium chloride to the separatory funnel and shake vigorously for approximately one minute. Allow the phases to separate, then drain the lower dichloromethane layer through a 10 cm filter funnel containing approximately 50 grams of sodium sulfate (suspended on a plug of glass wool) into a 1000 mL round-bottom flask.

Re-extract the aqueous phase with two additional 100 mL portions of dichloromethane as described above. Drain each dichloromethane layer through the funnel containing the sodium sulfate into the 1000 mL round-bottom flask containing the first extract. Rinse the sodium sulfate with two 10 mL portions of dichloromethane. At this point, the sample extract may be stored overnight at $\leq 0^{\circ}\text{C}$. Evaporate the combined dichloromethane layers just to dryness using a rotary-evaporator and water bath set to $<40^{\circ}\text{C}$.

Valent U.S.A. Corporation

RM-33N-2

Page 6

3. Hexane/Acetonitrile Partitioning

Transfer the residue to a 500 mL separatory funnel by rinsing the round-bottom flask first with two 50 mL portions of hexane (saturated with acetonitrile) followed by 100 mL of acetonitrile (saturated with hexane). Shake vigorously for one minute and drain the acetonitrile (bottom phase) into a 1000 mL round-bottom flask. Re-extract the hexane phase with two additional 100 mL portions of acetonitrile (saturated with hexane) and combine these extracts with the first. Evaporate the combined acetonitrile phases to <10 mL and transfer to a 100 mL round-bottom flask. Rinse the 1000 mL round-bottom flask with three 3 mL portions of acetonitrile and add to the extract in the 100 mL round-bottom flask. At this point, the sample extract may be stored overnight at $\leq 0^{\circ}\text{C}$. Evaporate the combined acetonitrile phases just to dryness using a rotary-evaporator and a water bath set to $<40^{\circ}\text{C}$ before proceeding to Step 4, Silica Gel Column Cleanup.

4. Silica Gel Column Cleanup

Place a glass wool plug at the bottom of the glass chromatography column. Weigh 15 grams of silica gel (deactivated with 10% water, see Note 1) into a beaker and suspend in approximately 60 mL of hexane. Pour the silica gel slurry into the column while tapping the side of the column. Wash the sides of the column with hexane as needed and cap the silica gel with 1-3 grams of sodium sulfate. Drain the hexane to the top of the sodium sulfate layer.

Re-dissolve the sample extract in 1 mL of toluene followed by 2 mL of hexane. Sonicate the round-bottom flask for 15 seconds if necessary to dissolve any residue adhering to the walls of the flask and transfer the sample extract to the column. Rinse the round-bottom flask with three 3 mL portions of hexane and transfer each rinse to the top of the column. Drain the solvent to the top of the sodium sulfate layer after each rinse. Elute the column with an additional 88 mL of hexane (total of 100 mL), then 50 mL of hexane:diethyl ether (15:1, v/v). (Rinse the round-bottom flask with the hexane:diethyl ether and sonicate for 15 seconds before transferring to the column). Discard these eluants.

Place a 250 mL round-bottom flask under the column and elute the pyriproxyfen with 50 mL of hexane:diethyl ether (15:1, v/v) followed by 20 mL of hexane:acetone (7:3, v/v). (Rinse the 500 mL round-bottom sample flask with each eluting solvent and sonicate for 15 seconds before transferring to the column). Evaporate the eluate just to dryness using a rotary-evaporator and water bath set to $<40^{\circ}\text{C}$.

Place another 250 mL round-bottom flask under the column and elute the 4'-OH-pyriproxyfen with 60 mL of hexane:acetone (7:3, v/v). Evaporate the eluate just to dryness using a rotary-evaporator and water bath set to $<40^{\circ}\text{C}$.

Valent U.S.A. Corporation

RM-33N-2

Page 7

5. Hexane/Acetonitrile Partitioning For Pyriproxyfen

(Note: this step removes the last traces of oil from the extract and significantly improves the chromatography of the pyriproxyfen). Transfer the pyriproxyfen eluate to a 15 mL centrifuge tube using 2 mL of hexane (saturated with acetonitrile) followed by 2 mL of acetonitrile (saturated with hexane) to rinse the round-bottom flask. Sonicate each rinse for approximately 15 seconds. Stopper the centrifuge tube and vortex for approximately 30 seconds and allow the phases to separate (centrifuge for approximately 2 minutes if necessary). Carefully withdraw the acetonitrile phase (bottom) from the centrifuge tube using a 1 mL glass syringe and needle and transfer to a 50 mL round-bottom flask.

Extract the hexane phase with two additional 2 mL portions of acetonitrile (saturated with hexane) as described above, rinsing the 250 mL round-bottom flask with each portion of acetonitrile. Transfer each acetonitrile phase to the 50 mL round-bottom flask containing the first extract. Evaporate the combined acetonitrile phases in the 50 mL round-bottom flask just to dryness using a rotary-evaporator and water bath set to 40°C. Add 2.0 mL of toluene to the flask, stopper, swirl, and sonicate for approximately 15 seconds to completely dissolve the residue. Transfer the extract to an autosampler vial and store at $\leq 0^{\circ}\text{C}$ until GC analysis.

6. Hexane/Acetonitrile Partitioning For 4'-OH-Pyriproxyfen

(Note: this step removes the last traces of oil from the sample extract and enabling the sample extract to dissolve in the LC mobile phase). Transfer the 4'-OH-pyriproxyfen eluate to a 15 mL centrifuge tube using 2 mL of hexane (saturated with acetonitrile) followed by 2 mL of acetonitrile (saturated with hexane) to rinse the round-bottom flask. Sonicate each rinse for approximately 15 seconds. Stopper the centrifuge tube and vortex for approximately 30 seconds and allow the phases to separate (centrifuge for approximately 2 minutes if necessary). Carefully withdraw the acetonitrile phase (bottom) from the centrifuge tube using a 1 mL glass syringe and needle and transfer to a 50 mL round-bottom flask. Discard the hexane phase and transfer the acetonitrile phase back to the centrifuge tube.

Extract this acetonitrile phase with two additional 2 mL portions of hexane (saturated with acetonitrile) as described above. Rinse the 250 mL round-bottom flask with each portion of hexane. Transfer the resulting acetonitrile phase to the 50 mL round-bottom flask and evaporate just to dryness using a rotary-evaporator and water bath set to 40°C.

Add 1.0 mL of methanol/water (4:1, v/v) to the 50 mL round-bottom flask. Stopper, swirl and sonicate for approximately 15 seconds to completely dissolve the residue. Attach an Acrodisc filter to the glass syringe (plunger removed) and transfer the extract to the syringe barrel using a Pastuer pipet. Insert the plunger into the syringe barrel and gently push the sample extract through the filter into an autosampler vial. Seal and store at $\leq 0^{\circ}\text{C}$ until HPLC analysis.

Valent U.S.A. Corporation

RM-33N-2

Page 8

7. Gas Chromatography Measurement For Pyriproxyfen

Analyze the sample extracts for pyriproxyfen, along with calibrating standard solutions, using the following (or similar) operating conditions:

Column: DB-17 (15 M x 530 μ m) wide bore capillary (1.0 μ m film thickness)
J & W Scientific Cat # 125-1712 or equivalent.

Column Oven Temperature Program:

Initial Temp: 205°C
Hold Time: 2.5 minutes
Prog Rate: 10°C/minute
Final Temp: 240°C
Hold Time: 6 minutes

Detector Temperature: 300°C
Injector Temperature: 250°C
Carrier Gas: Helium at 10 mL/min
Detector Makeup Gas: Helium at 20 mL/min
Air: 100 mL/min
Hydrogen: 3.6 mL/min
Injection Size: 1.0 μ l
Retention Time: 5.4 minutes

The GC 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 GC parameters used. See Note 4 for alternative GC parameters.

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 μ g/mL of pyriproxyfen in toluene). This sequence may, however, be modified if the reproducibility requirement is met. (See Note 5). Each sequence must begin and end with a calibration standard.

Valent U.S.A. Corporation

RM-33N-2

Page 9

8. HPLC Measurement For 4'-OH-Pyriproxyfen

Analyze the sample extracts, along with calibrating standard solutions, using the following (or similar) operating conditions:

Column: Phenomenex Prodigy® ODS (3) (250 mm x 4.6 mm, 5 μ m particle size), Phenomenex Cat # 00G-4097-E0 or equivalent.

Column Temperature: 35°C

Mobile phase: A = methanol:THF (2:3, v/v); B = water + 0.05% 85% H₃PO₄ (v/v).

Gradient: T = 0, 45% A + 55% B
T = 8, 45% A + 55% B
T = 28, 75% A + 25% B
T = 36, 75% A + 25% B

Flow rate: 1.0 mL/min.

Injection volume: 50 μ l

Detector 1: HP Model 1046A Fluorescence Detector (FLD)
Excitation wavelength: 235 nm
Emission wavelength: 327 nm

Detector 2: HP Model 79853A Variable Wavelength Detector (VWD)
Wavelength: 272 nm
Retention Time: 22.6 min.

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. See Note 6 for alternate HPLC parameters.

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 μ g/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 5. Each sequence must begin and end with a calibration standard.

Valent U.S.A. Corporation

RM-33N-2

Page 10

9. Calculations

The amount of each analyte in each sample is calculated using the following formula:

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

where:

- B = integration counts for the analyte in the sample.
- C = concentration of analyte in the calibrating standard (1.0 $\mu\text{g/mL}$).
- V = final volume of the sample extract (2.0 mL for pyriproxyfen and 1.0 mL for 4'-pyriproxyfen).
- DF = dilution factor, used if the sample extract is diluted prior to analysis.
- A = mean integration counts for analyte in the calibrating standards.
- W = sample weight (20 grams)

LIMITS OF DETECTION AND QUANTITATION

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

ANALYSIS TIME

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

Valent U.S.A. Corporation

RM-33N-2

Page 11

NOTES

1. Each batch of silica gel must be checked for recovery of pyriproxyfen and 4'-OH-pyriproxyfen as follows: Transfer 1.0 mL of the 1.0 $\mu\text{g}/\text{mL}$ pyriproxyfen fortifying solution and 1.0 mL of the 1.0 $\mu\text{g}/\text{mL}$ 4'-OH-pyriproxyfen fortifying solution to a 50 mL round-bottom flask and evaporate **just to dryness** using a rotary-evaporator and water bath set to $<40^{\circ}\text{C}$. Transfer the residue to a silica gel column and elute the pyriproxyfen and 4'-OH-pyriproxyfen as described under Step 4, Silica Gel Column Cleanup. Evaporate the eluates **just to dryness** using a rotary-evaporator and water bath set to $<40^{\circ}\text{C}$. Add 1.0 mL of toluene to the flask containing the pyriproxyfen eluate and 1.0 mL of methanol/water (4:1, v/v) to the flask containing the 4'-OH-pyriproxyfen eluate. Swirl each flask to completely dissolve the residues.

Analyze the pyriproxyfen eluant and the 1.0 $\mu\text{g}/\text{mL}$ calibrating standard as described under Step 6, Gas Chromatography Measurement For Pyriproxyfen. Analyze the 4'-OH-pyriproxyfen eluant and the 1.0 $\mu\text{g}/\text{mL}$ calibrating standard as described under Step 7, HPLC Measurement For 4'-OH-Pyriproxyfen. If either eluate peak is less than 90% of its corresponding calibrating standard, then the elution profile of the analyte must be determined.

2. At Valent, linearity of the gas chromatograph and liquid chromatograph must be determined each day that samples are analyzed (Valent SOP #VR-007). Linearity is determined by analyzing a series of linearity standards containing 0.10 to 2.0 $\mu\text{g}/\text{mL}$ of pyriproxyfen and 0.2 to 2.0 $\mu\text{g}/\text{mL}$ of 4'-OH-pyriproxyfen. The response for each standard is normalized to response per 1.0 $\mu\text{g}/\text{mL}$ 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 each analyte within the range of linearity established.
3. 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 0.4 mL and 2.0 mL, respectively, of the 1.0 $\mu\text{g}/\text{mL}$ fortifying solution to a 20 gram sample. Method recoveries must be 70% to 120% to be acceptable unless approved by the chemist responsible for the analysis.

Valent U.S.A. Corporation

RM-33N-2

Page 12

NOTES (CONTINUED)

4. If matrix interferences are encountered during the analysis of pyriproxyfen, the following (or similar) GC parameters may be used:

Column: DB-5 (30 M x 530 μ m) wide bore capillary (1.5 μ m film thickness).
J & W Scientific Cat # 125-5032 or equivalent.

Column Oven Temperature Program:

Initial Temp: 265°C
Hold Time: 2.0 minutes
Prog Rate: 10°C/minute
Final Temp: 285°C
Hold Time: 5 minutes

Detector Temperature: 300°C
Injector Temperature: 250°C
Carrier Gas: Helium at 20 mL/min
Make-Up Gas: Helium at 10 mL/min
Air: 102 mL/min
Hydrogen: 3.8 mL/min
Injection Size: 1.0 μ l
Retention Time: 3.9 minutes

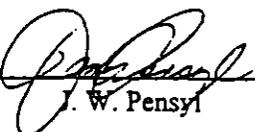
5. At Valent, reproducibility of an analytical run is determined by calculating the CV from the peak units obtained for the calibrating standards analyzed during the run. For a run to be acceptable, these CV's must be 10% or less unless approved by the chemist responsible for the analysis (Valent SOP #VR-013).
6. If matrix interferences are encountered during the analysis of 4'-OH-pyriproxyfen, the following HPLC column may be used: ODS-Hypersil (150 mm x 4.6 mm, 3 μ m particle size), Phenomenex Cat # 00F-0145-EO or equivalent. Use of the alternate detector may also be helpful.

Valent U.S.A. Corporation

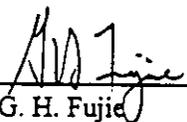
RM-33N-2

Page 13

METHOD APPROVAL

Written by: 
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Date: 1/15/97

Reviewed by: 
G. H. Fujie

Date: 1/15/97

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Date: 1/20/97

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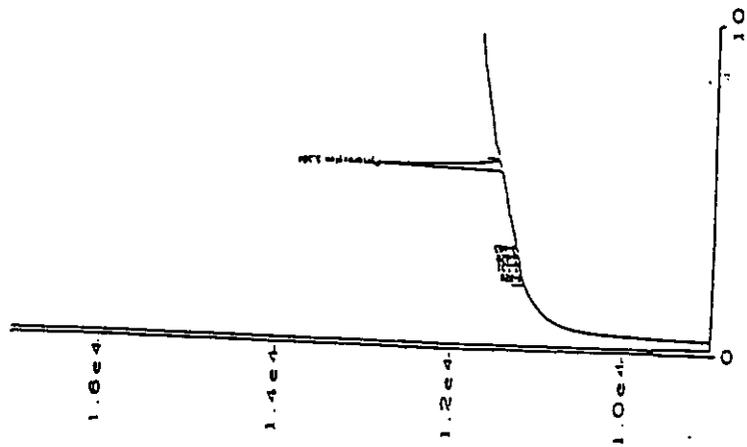


Figure 1

Pyriproxyfen Calibration Standard
1.0 µg/mL in toluene
1.0 ng injected on DB-17 column

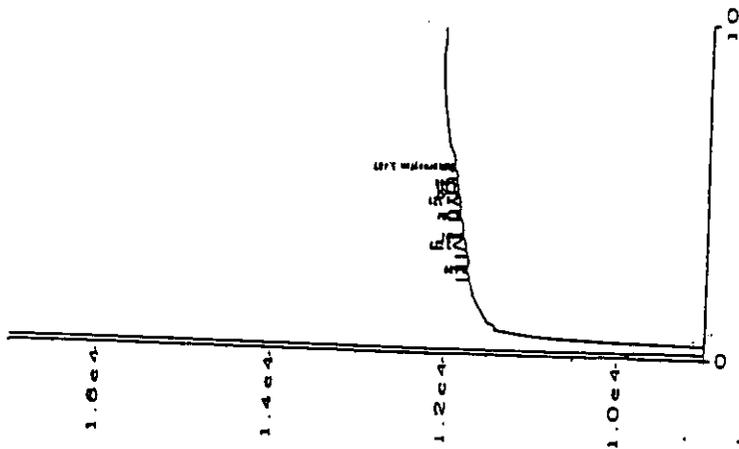


Figure 2

Untreated Control Walnut
10 mg crop equivalents
Injected on DB-17 column

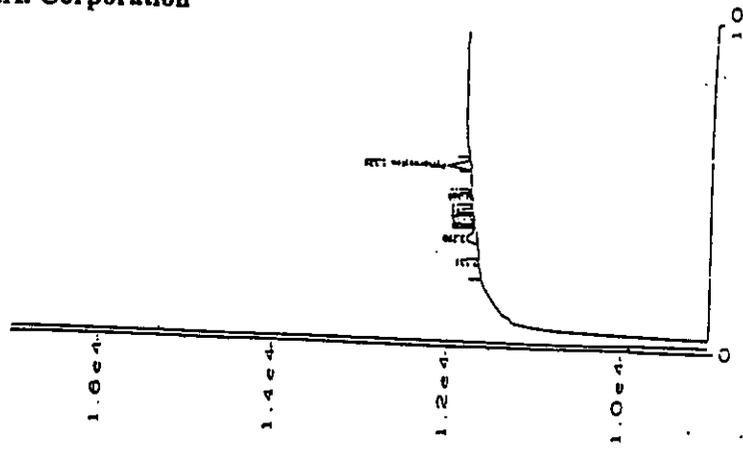


Figure 3

RM-33N-2
Page 14
UTC Walnut Fortified with 0.02 ppm Pyr
10 mg crop equivalents
Injected on DB-17 column

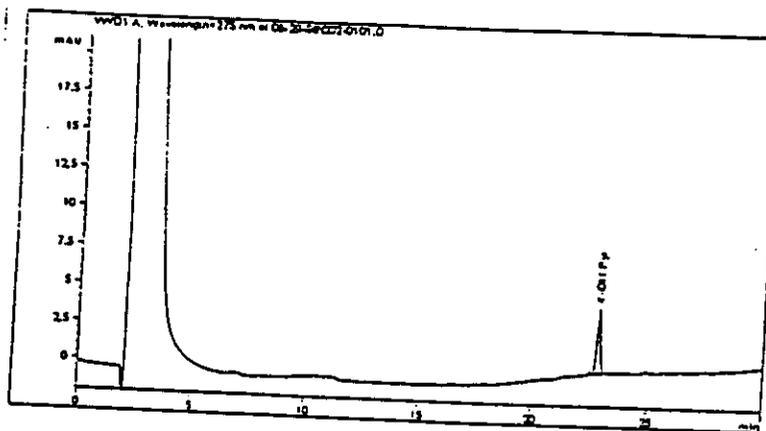


Figure 4

4'-OH-Pyr Calibration Standard
1.0 $\mu\text{g/mL}$ in methanol:water
50 ng injected

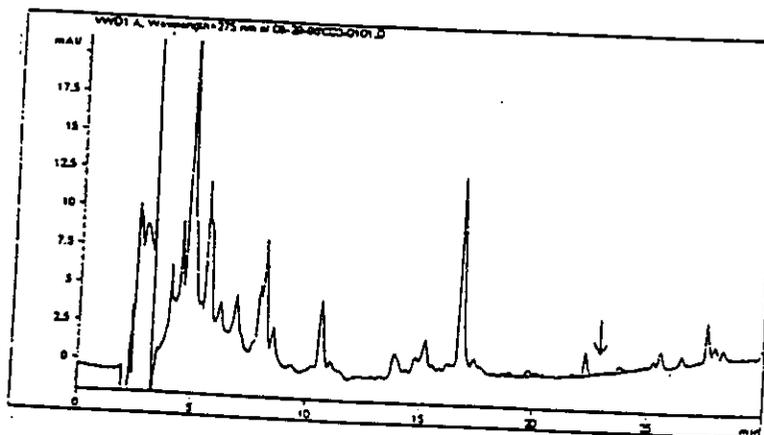


Figure 5

Untreated Control Walnut
1.0 g crop equivalents injected

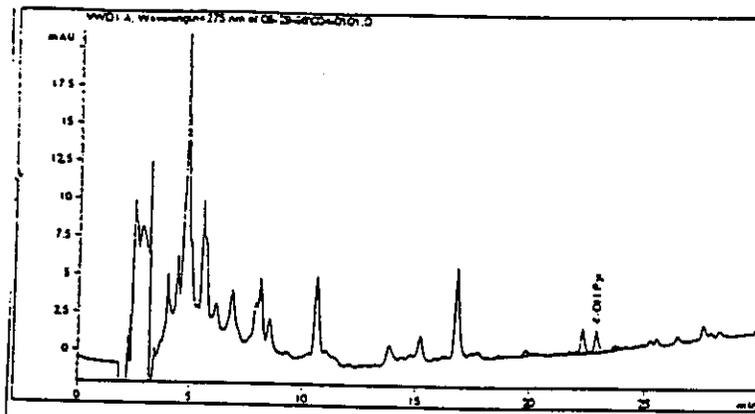


Figure 6

UTC Walnut Fortified with 0.02 ppm 4'-OH-Pyr
1.0 g crop equivalents injected

**EPA ADDENDUM For Residue Analytical Methods
PP#s 7F04882 and 8F05022**

1) ACLB made slight modifications to the GC/NPD instrument parameters. The parameters were the same for all six commodities.

The parameters for the GC/NPD were:

Gas Chromatograph: HP 6890 GC with NPD.
Column: DB-17, 15m x 0.53mm i.d., 1 um film thickness
Carrier gas: Helium, 10.0/min.

Oven Temperature: Initial 80°C, Hold for 1.0 min.
40°C/min to 205°C, Hold for 2.5 min.
10°C/min. to 240°C, Hold for 6.0 min.

Injector temperature: 250°C
Injection volume: 4 uL

Detector temperature: 320°C
Hydrogen Flow: 3.0 mL/min
Air Flow: 60 mL/min
Makeup: 20 mL/min Helium

2) The flow rate for the silica gel column chromatography procedure was not specified. ACB used a flow rate of 10 mL/min for all six commodities.

3a) Valent has not submitted an enforcement method for Orange Oil. Per conversation with Charles Green (Valent) on 4/20/99, it was suggested to perform a Hexane/Acetonitrile partitioning on the orange oil aliquot (5 gram sample). The partitioning step involved dissolving the orange oil in 70 mL acetonitrile (hexane saturated) and partitioning it with 100 mL hexane (acetonitrile saturated). The acetonitrile layer was kept and the hexane layer was partitioned with two additional 70 mL portions of acetonitrile (hexane saturated). The acetonitrile layers were combined and evaporated to dryness. The sample was taken through the methylene chloride partitioning step, as stated in Method RM-33P-1-3 for citrus. The remaining procedure was to be carried out as stated in the citrus method.

ACB tried the approach with control and spiking at the 0.02 ppm level and was unsuccessful. The analyte was not recovered and appeared oily. ACB modified the procedure as follows:

3b) ACB's Modification For Orange Oil: A 5 gram portion of orange oil was partitioned with acetonitrile/hexane as stated in 4a. After evaporating the acetonitrile layer, ACB skipped the dichloromethane partitioning step and cleaned up the sample using the silica gel column chromatography procedure, as stated in Method RM-33H

EPA ADDENDUM For Residue Analytical Methods
PP#s 7F04882 and 8F05022
(Continued)

(Almond Hulls) and Method RM-33N-2 (Nutmeat). Prior to GC analysis, the extract was taken to dryness and dissolved in 2 mL hexane (acetonitrile saturated) and partitioned three times with 2 mL acetonitrile (hexane saturated). The acetonitrile layers were combined, evaporated to dryness, and dissolved to the appropriate volume with toluene for GC analysis. The acetonitrile/hexane partitioning step, prior to GC analysis, was the same as described in the nutmeat procedure (RM-33N-2). This additional cleanup greatly improved the quality of the chromatography for oily crops.

4) Prior to GC analysis, for the orange commodities, ACB added the same acetonitrile/hexane partitioning step, as described in 4b. This final cleanup step greatly reduced the deep yellow color and remaining oils, contained in the final extract. This was ACB's only modification to Method RM-33P-1-3 (citrus).