

US EPA ARCHIVE DOCUMENT

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

DETERMINATION OF PYRIPROXYFEN
PYPA, AND PYPA CONJUGATES IN
TOMATOES AND PEPPERS
METHOD RM-33P-9

DATE: JULY 22, 1996
REVISED: SEPTEMBER 8, 1997

INTRODUCTION

This method determines residues of pyriproxyfen [V-71639; 4-phenoxyphenyl(*RS*)-2-(2-pyridyloxy) propyl ether], its degradate, PYPA [(*RS*)-2-(2-pyridyloxy) propanol], and conjugates of PYPA in tomatoes and peppers. Pyriproxyfen, PYPA, and PYPA conjugates are extracted from the crop matrix using acetonitrile or acetonitrile:water (4:1, v/v). After evaporation of the acetonitrile, the aqueous extract is hydrolyzed by refluxing for two hours in 1 *N* HCl. Pyriproxyfen is extracted from the acidic aqueous extract using dichloromethane, cleaned-up with silica gel column chromatography, and analyzed by gas chromatography. PYPA is extracted from the neutralized aqueous extract with dichloromethane, cleaned-up with solid phase extraction (SPE) and analyzed by gas chromatography. GC analysis of both analytes is performed using nitrogen-phosphorus specific flame-ionization detectors.

This method was revised on September 8, 1997 to make minor changes to the SPE cleanup step to allow the method to be used for the analysis of peppers.

REAGENTS

Acetone - pesticide quality or equivalent.

Acetonitrile - pesticide quality or equivalent.

Dichloromethane - pesticide quality or equivalent.

Diethyl ether - pesticide quality or equivalent.

Ethyl acetate - pesticide quality or equivalent.

Hexane - pesticide quality or equivalent.

Hydrochloric acid - 36.5-38.0%, Baker-Analyzed, J.T. Baker Cat # 9530-00, or equivalent. Prepare a 5 *N* solution by carefully adding 416 mL of concentrated acid to a 1L volumetric flask, partially filled with deionized water, and diluting to volume with deionized water.

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Page 2**REAGENTS (CONTINUED)**

1-Octanol - 99+%, Aldrich Cat # 29,324-5 or equivalent.

Silica Gel 60 - Mesh size 70-230, 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. Prepare a 5% (w/v) solution by adding 50 grams to a 1L volumetric flask and diluting to volume with deionized water.

Sodium hydroxide - 50% aqueous solution, EM Science Cat # SX0597-3 or equivalent. Prepare a 10% (v/v) aqueous solution by adding 20 mL of the 50% solution to a 100 mL graduated cylinder and diluting to volume with deionized water. Mix well and store at room temperature.

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

Toluene - pesticide quality or equivalent.

Water - deionized.

REFERENCE STANDARDS

Pyriproxyfen - analytical standard of known purity. Prepare a stock solution containing 1.0 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 µg/mL (see Note 2). Prepare a calibrating solution containing 1.0 µg/mL by diluting the stock solution with toluene. (The calibrating solution may be used as one the four required linearity standards). Prepare a fortifying solution by diluting the stock solution to 1.0 µg/mL with acetone. All solutions should be kept refrigerated when not in use.

PYPA - analytical standard of known purity. Prepare a stock solution containing 1.0 mg/mL in acetone. Prepare a minimum of four linearity standards by diluting this stock solution with hexane:ethyl acetate (5:2, v/v) to concentrations ranging from 0.10 to 2.0 µg/mL (see Note 2). Prepare a calibrating solution containing 1.0 µg/mL by diluting the stock solution with hexane:ethyl acetate (5:2, v/v). (This calibrating solution may be used as one the four required linearity standards). Prepare a fortifying solution by diluting the stock solution to 1.0 µg/mL with acetone. All solutions should be kept refrigerated when not in use.

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EQUIPMENT

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

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

Beakers - 400 mL

Büchner funnels - 10 cm diameter.

Filter flasks - 500 mL.

Filter funnels - approximately 10 cm diameter.

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

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 a Hewlett-Packard ChemStation or equivalent system.

Glass chromatography column - 10.5 mm ID x 250 mm with 200 mL reservoir and Teflon stopcock, Kontes Cat. # K-420280-0213 or equivalent.

Glass wool.

Graduated cylinders - 50 mL or 100 mL.

Heating mantles - sized for 100 mL round-bottom flasks.

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

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

pH Meter - Corning Model 240 or equivalent.

Reflux condensers, with ground glass joint.

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

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Page 4**EQUIPMENT (CONTINUED)**

Round-bottom flasks - 50, 100, 250, 500, and 1000 mL.

Separatory funnels - 500 mL.

Syringes - Yale hypodermic, 10 mL, glass Luer tip.

Ultrasonic cleaner - Branson 3200 or equivalent.

Volumetric flasks - 5 mL.

ANALYTICAL PROCEDURES**1. Extraction of Samples**

Weigh 50 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 recoveries may be fortified with pyriproxyfen and PYPA (see Note 4). Add 100 mL of acetonitrile [use acetonitrile:water (4:1, v/v) for dry matrices, e.g., tomato pomace] to the sample and blend on the Omni-Mixer for 5 minutes.

Filter the sample into a 500 mL filter flask using a 10 cm 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 a second 100 mL portion of acetonitrile [or acetonitrile:water (4:1, v/v)] as described above. Filter the sample into the 500 mL filter flask, combining this extract with the first. Rinse the Mason jar with two 20 mL portions of acetonitrile 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 acetonitrile and one 5 mL portion of deionized water. Add these rinsates to the round-bottom flask. Evaporate the acetonitrile using a rotary-evaporator and water bath set to ambient temperature ($<30^{\circ}\text{C}$). Approximately 35 mL of water will remain.

2. Hydrolysis of Samples

Transfer the aqueous residue to a 100 mL round-bottom flask. Rinse the 1000 mL flask first with two 5 mL portions of acetonitrile then with one 5 mL portion of deionized water, sonicating each rinse for at least 15 seconds. Transfer these rinsates to the 100 mL round-bottom flask. Evaporate the acetonitrile again using a rotary-evaporator and water bath set to ambient temperature ($<30^{\circ}\text{C}$).

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Transfer the aqueous extract to a graduated cylinder and determine its volume. Return the extract to the 100 mL round-bottom flask and add a volume of 5 N HCl to the flask so that the concentration of HCl is 1.0 N. (For example, if the volume of the extract is 40 mL, add 10 mL of 5 N HCl). Use this acid to rinse the 1000 mL round-bottom flask and graduated cylinder.

Place the 100 round-bottom flask in a heating mantle and attach a water-cooled reflux condenser to the top of the flask. Heat the sample to 70-80°C for approximately 2 hours. When the hydrolysis is complete, allow the sample to cool. At this point, the sample may be stored overnight at $\leq 0^{\circ}\text{C}$.

3. Extraction of Pyriproxyfen

Transfer the hydrolyzate to a 500 mL separatory funnel and add 150 mL of the 5% NaCl solution, using portions of this solution to rinse the 100 mL round-bottom flask. Add 80 mL of dichloromethane to the separatory funnel and vigorously shake for approximately one minute. Drain the lower dichloromethane layer into a 500 mL round-bottom flask through a 10 cm filter funnel containing approximately 50 grams of sodium sulfate, suspended on a plug of glass wool.

Repeat this extraction with two additional 80 mL portions of dichloromethane. Drain the lower dichloromethane layers through the funnel containing the sodium sulfate into the 500 mL round-bottom flask containing the first extract. Rinse the sodium sulfate with two 10 mL portions of dichloromethane. At this point, the sample may be stored overnight at $\leq 0^{\circ}\text{C}$. **Save the aqueous phase for Step 6, Extraction of PYPA.** (The aqueous phase may also be stored overnight at $\leq 0^{\circ}\text{C}$). Evaporate the pyriproxyfen extract to dryness using a rotary-evaporator and water bath set to $< 40^{\circ}\text{C}$. (The extract must be dry at this point. If any water is present when the dichloromethane is removed, add approximately 10 mL of dichloromethane and re-evaporate).

4. Silica Gel Column Cleanup For Pyriproxyfen

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, dilute with 2 mL of hexane, and sonicate for 15 seconds if necessary to dissolve any residue adhering to the walls of the flask. Transfer the extract to the column. Rinse the flask with three 3 mL portions of hexane and transfer each rinse to 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). Discard these eluates.

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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). Evaporate the pyriproxyfen eluate **just to dryness** using a rotary-evaporator and water bath set to $<40^{\circ}\text{C}$. Add 5.0 mL of toluene to the round-bottom flask, swirl and sonicate for 15 seconds to dissolve the sample. Transfer to a screw cap vial and store at $\leq 0^{\circ}\text{C}$ until GC analysis.

5. GC Analysis For Pyriproxyfen

Transfer a portion of the pyriproxyfen sample extract to an auto-sampler vial and analyze, along with calibrating standard solutions, using the following operating conditions:

Column: DB-17 (15 M x 530 μm) wide bore capillary (1.0 μm film thickness), J & W Scientific Cat # 125-1732 or equivalent.

Column Oven Temperature Program:

Initial Temp: 225°C

Hold Time: 2.5 minutes

Prog Rate: $10^{\circ}\text{C}/\text{minute}$

Final Temp: 260°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: 110 mL/min

Hydrogen: 3.6 mL/min

Injection Size: 1.0 μl

Retention Time: 4.8 minutes (See Figure 1)

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 labeled with the GC parameters used. See Note 5 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 $\mu\text{g}/\text{mL}$ of pyriproxyfen). This sequence may, however, be modified if the reproducibility requirement is met. (See Note 6). Each sequence must begin and end with a calibrating standard.

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6. Extraction of PYPA

Transfer the aqueous phase from Step 3, Extraction of Pyriproxfen, to a 400 mL beaker. Carefully add approximately 2 mL of 50% sodium hydroxide solution and mix well. Adjust the pH to between 7.0 and 7.5 by adding, dropwise, a 10%(v/v) solution of sodium hydroxide while monitoring the pH with a pH meter.

Transfer the sample to a 500 mL separatory funnel, add 80 mL of dichloromethane, and vigorously shake for approximately one minute. Drain the lower dichloromethane layer into a 500 mL round-bottom flask through a 10 cm filter funnel containing approximately 50 grams of sodium sulfate, suspended on a plug of glass wool.

Repeat this extraction with two additional 80 mL portions of dichloromethane. Drain the lower dichloromethane layers through the funnel containing the sodium sulfate into the 500 mL round-bottom flask containing the first extract. Rinse the sodium sulfate with two 10 mL portions of dichloromethane. At this point, the sample may be stored overnight at $\leq 0^{\circ}\text{C}$.

Add 100 μL of 1-octanol to the combined dichloromethane layers, evaporate to <10 mL using a rotary-evaporator and water bath set to $<30^{\circ}\text{C}$, and transfer to a 50 mL round-bottom flask. Rinse the 500 mL round-bottom flask with three 3 mL portions of dichloromethane and transfer these rinses to the 50 mL round-bottom flask. Carefully evaporate the dichloromethane using a rotary-evaporator and water bath set to ambient temperature ($<30^{\circ}\text{C}$). (Only the octanol should remain at this point. If any water is present when the dichloromethane is removed, add approximately 10 mL of dichloromethane and carefully re-evaporate. Remove from the evaporator immediately).

7. SPE Cleanup For PYPA

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 hexane. Do not exceed a flow rate of 5 mL/minute. Do not allow the column to dry before the sample is applied. Vacuum may be used for this pre-conditioning, but gravity flow only should be used for the following application/elution procedure.

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Remove the glass syringe from the column. Re-dissolve the sample extract in 1 mL of hexane:ethyl acetate (9:1, v/v), sonicate briefly, and transfer to the column using a Pasteur pipet. Allow the solvent to drain to the top of the packing using gravity flow only. Elute the column with an additional 6 mL of hexane:ethyl acetate (9:1, v/v) as follows: Rinse and sonicate the 50 mL round-bottom flask with two 1 mL portions of hexane:ethyl acetate (9:1, v/v) and add each portion to the column. Allow each portion of solvent to reach the top of the packing using gravity flow only before adding the next. Add 4 mL of hexane:ethyl acetate (9:1, v/v) to the column and allow this solvent to reach the top of the packing. Discard this eluate.

Place a 5 mL volumetric flask under the column and re-attach the 10 mL glass syringe (plunger removed) to the column. Add 5 mL of hexane:ethyl acetate (5:2, v/v) to the 50 mL round-bottom flask, swirl, and transfer to the column reservoir. Allow the solvent to drain to the top of the packing using gravity flow only, collecting the eluate in the 5 mL volumetric flask. Adjust the volume of the extract to exactly 5.0 mL with hexane:ethyl acetate (5:2, v/v), if necessary, then stopper the flask and shake. The sample may be stored at $\leq 0^{\circ}\text{C}$ until GC analysis.

8. GC Analysis For PYPA (See Note 8)

Transfer a portion of the PYPA sample extract to an auto-sampler vial and analyze, along with calibrating standard solutions, using the following operating conditions:

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

Column Oven Temperature Program:

Initial Temp: 130°C
Hold Time: 2.0 minutes
Prog Rate: 10°C/minute
Final Temp: 150°C
Hold Time: 5 minutes
Prog Rate A: 25°C/minute
Final Temp: 200°C
Hold Time: 5 minutes

Detector Temperature: 300°C

Injector Temperature: 250°C

Carrier Gas: Helium at 10 mL/min

Make-Up Gas: Helium at 20 mL/min

Air: 102 mL/min

Hydrogen: 3.6 mL/min

Injection Size: 1.0 μl

Retention Time: 5.1 minutes (See Figure 4).

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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 labeled with the GC parameters used. See Note 7 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 $\mu\text{g/mL}$ of PYPA). This sequence may, however, be modified if the reproducibility requirement is met. (See Note 6). Each sequence must begin and end with a calibrating standard.

9. Calculations For Pyriproxyfen and PYPA

The amount of pyriproxyfen and PYPA in each sample is calculated using the following formula:

$$\text{ppm Pyriproxyfen / PYPA} = \frac{B \times C \times V \times DF}{A \times W}$$

Where:

- B = integration counts for pyriproxyfen or PYPA in the sample.
- C = concentration of pyriproxyfen or PYPA in the calibrating standard (1.0 $\mu\text{g/mL}$)
- V = final volume of the sample extract (5.0 mL).
- DF = dilution factor, used if the sample extract is diluted prior to analysis.
- A = mean integration counts for pyriproxyfen or PYPA in the calibrating standards.
- W = sample weight (50 grams)

LIMITS OF DETECTION AND QUANTITATION

The limit of detection (LOD) of pyriproxyfen and PYPA in fruit 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, PYPA, and PYPA conjugates in approximately 16 hours. The results are available within 48 hours of initiating the analysis.

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1. Each batch of silica gel must be checked for recovery of pyriproxyfen as follows: Transfer 5.0 mL of the 1.0 $\mu\text{g/mL}$ pyriproxyfen fortifying solution to a 50 mL round-bottom flask and evaporate 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 as described under Step 4, Silica Gel Column Cleanup For Pyriproxyfen. Evaporate the pyriproxyfen eluate to dryness using a rotary-evaporator and water bath set to $<40^{\circ}\text{C}$. Add 5 mL of toluene to the flask, swirl and sonicate for at least 15 seconds. Analyze this eluate and the 1.0 $\mu\text{g/mL}$ calibrating standard as described under Step 5, GC Analysis For Pyriproxyfen. If the pyriproxyfen peak for the eluate is less than 90% of the calibrating standard, then the elution profile of pyriproxyfen must be determined.
2. At Valent, linearity of the gas 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 $\mu\text{g/mL}$ of either pyriproxyfen or PYPA. The response for each standard is normalized to response per 1.0 $\mu\text{g/mL}$ 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. Each batch of SPE columns must be checked for recovery of PYPA as follows: Transfer 5.0 mL of the 1.0 $\mu\text{g/mL}$ PYPA fortifying solution to a 50 mL round-bottom flask, add 100 μL of octanol, and evaporate just to dryness using a rotary-evaporator and water bath set to ambient temperature ($<30^{\circ}\text{C}$). Transfer the residue to an SPE column and elute the PYPA as described under Step 7, SPE Cleanup For PYPA. Analyze this eluate and the 1.0 $\mu\text{g/mL}$ calibrating standard as described under Step 8, GC Analysis For PYPA. If the PYPA peak for the eluate is less than 90% of the calibrating standard, then the elution profile of PYPA must be determined.
4. 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 1.0 mL and 5.0 mL, respectively, of the 1.0 $\mu\text{g/mL}$ fortifying solutions to a 50 gram sample. Method recoveries must be 70% to 120% to be acceptable unless approved by the chemist responsible for the analysis.

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5. If matrix interferences are encountered during the analysis of pyriproxyfen, the following 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 10 mL/min

Make-Up Gas: Helium at 20 mL/min

Air: 110 mL/min

Hydrogen: 3.6 mL/min

Injection Size: 1.0 μ l

Retention Time: 5.3 minutes (See Figure 7).

6. 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).
7. If matrix interferences are encountered during the analysis of PYP A, the following GC parameters may be used:

Column: DB-17 (15 M x 530 μ m) wide bore capillary (1.0 μ m film thickness), J & W Scientific Cat # 125-1732 or equivalent.

Column Oven Temperature Program:

Initial Temp: 100°C
Hold Time: 10.0 minutes
Prog Rate: 25°C/minute
Final Temp: 200°C
Hold Time: 2 minutes

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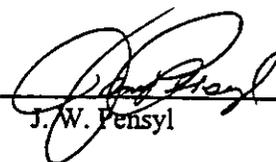
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Detector Temperature: 300°C
Injector Temperature: 250°C
Carrier Gas: Helium at 10 mL/min
Detector Makeup Gas: Helium at 20 mL/min
Air: 110 mL/min
Hydrogen: 3.6 mL/min
Injection Size: 1.0 µl
Retention Time: 5.0 minutes (See Figure 10)

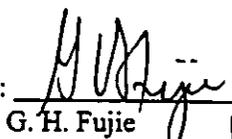
8. PYPA is susceptible to decomposition during GC analysis. If decomposition occurs, a secondary PYPA peak may be observed eluting immediately before (DB-17) or immediately after (DB-5) the primary PYPA peak or the PYPA peak may appear as a doublet. To minimize the secondary PYPA peak, replace the injection port liner/glass wool, and, if necessary, remove a short section from the front of the column. Also, the injection port temperature must not exceed 250°C.

METHOD APPROVAL

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Reviewed by:


QAUDate: 9/24/97

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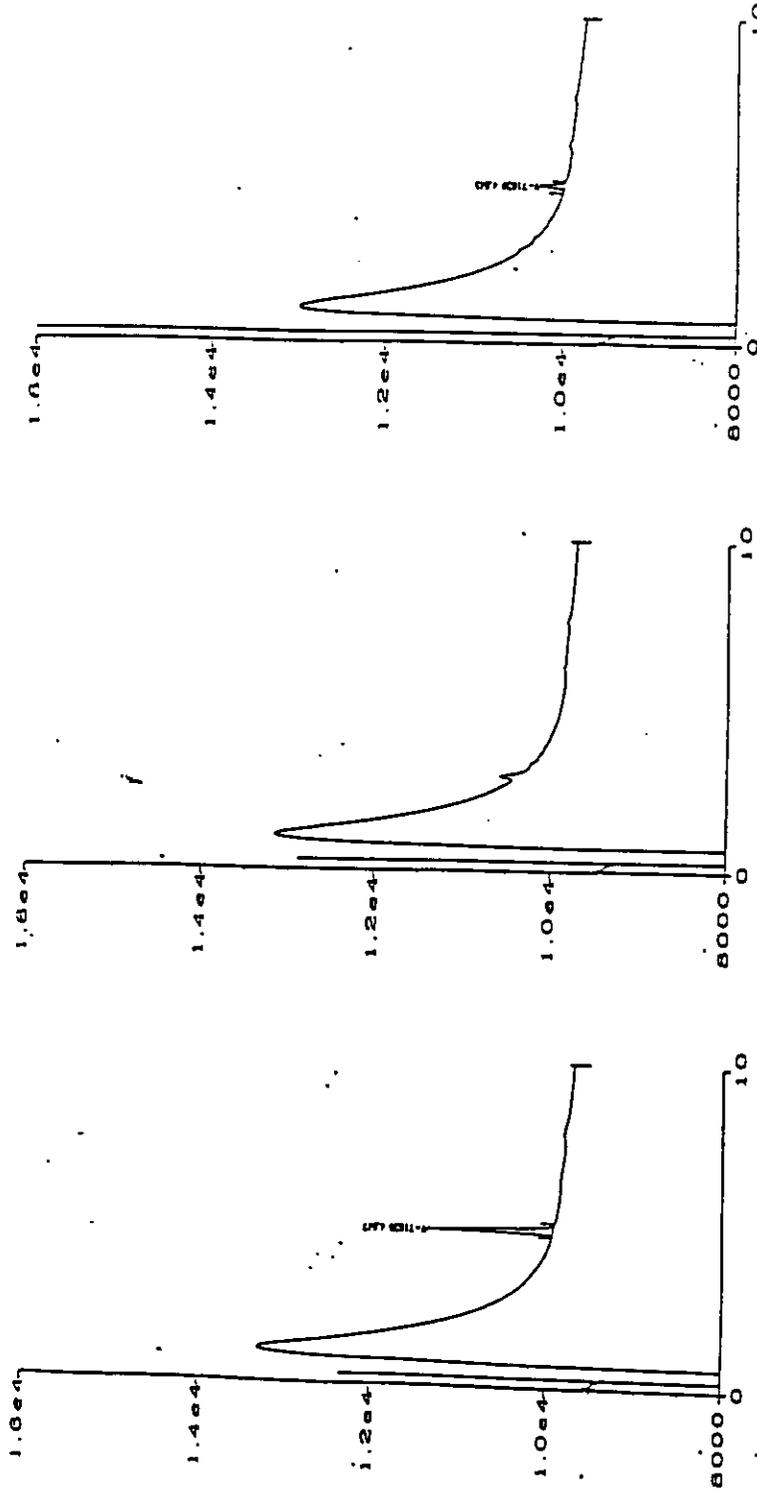


Figure 1

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

Figure 2

UTC Tomato for pyriproxyfen
10 mg crop equivalents
Injected on DB-17 column

Figure 3

Tomato fortified with 0.02 ppm pyriproxyfen
10 mg crop equivalents
Injected on DB-17 column

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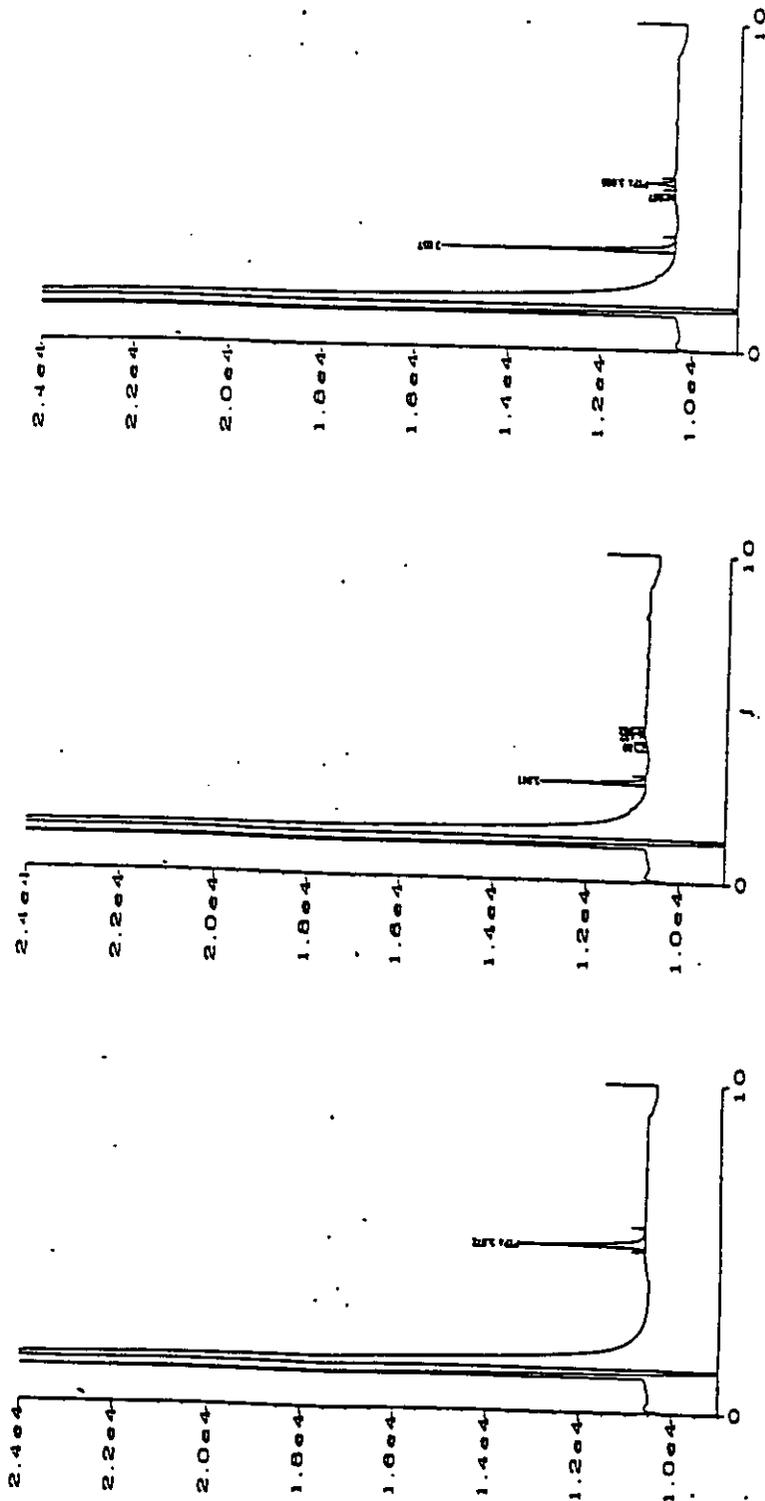


Figure 4

PYPA Calibration Standard
1.0 µg/mL in hexane:ethyl acetate
1.0 ng injected on DB-5 column

Figure 5

UTC Tomato for PYPA
10 mg crop equivalents
Injected on DB-5 column

Figure 6

Tomato fortified with 0.02 ppm PYPA
10 mg crop equivalents
Injected on DB-5 column

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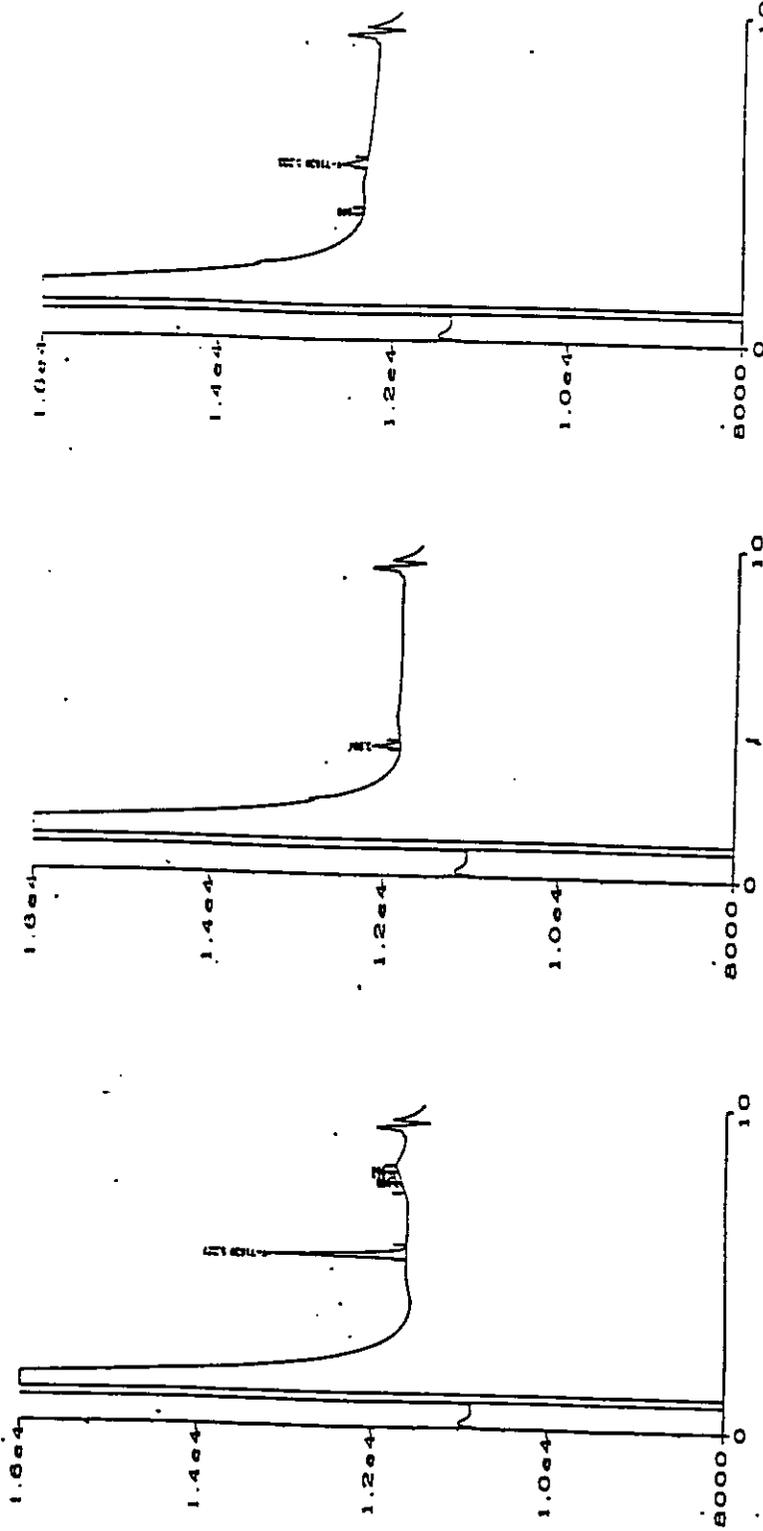


Figure 7

Pyriproxyfen Calibration Standard
1.0 µg/mL in toluene
1.0 ng injected on DB-5 column

Figure 8

UTC Tomato for pyriproxyfen
10 mg crop equivalents
Injected on DB-5 column

Figure 9

Tomato fortified with 0.02 ppm pyriproxyfen
10 mg crop equivalents
Injected on DB-5 column

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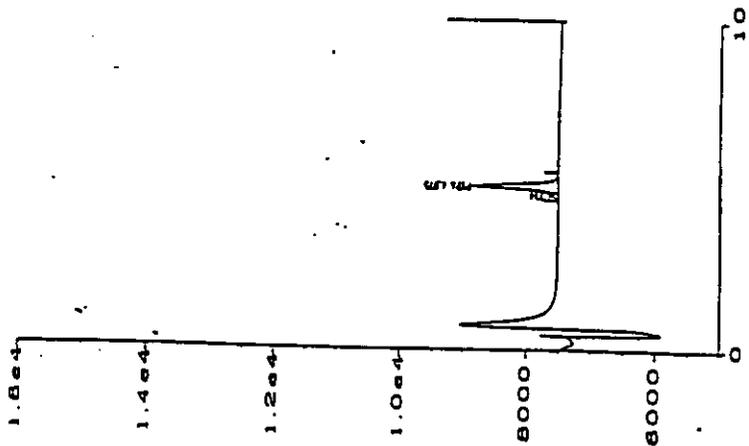


Figure 10

PYPA Calibration Standard
1.0 µg/mL in hexane:ethyl acetate
1.0 ng injected on DB-17 column

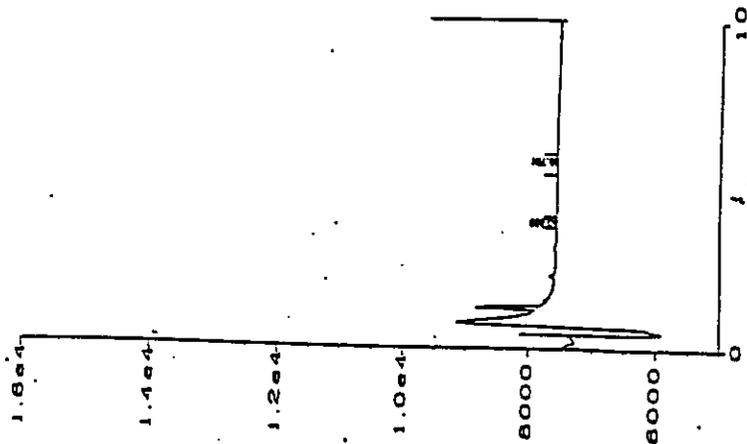


Figure 11

UTC Tomato for PYPA
10 mg crop equivalents
Injected on DB-17 column

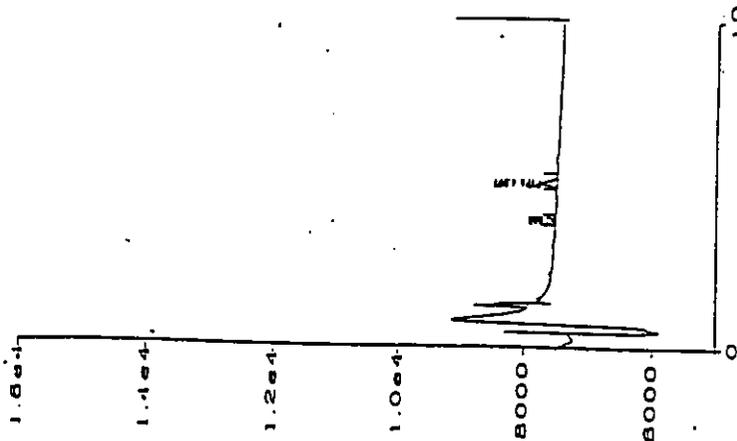


Figure 12

Tomato fortified with 0.02 ppm PYPA
10 mg crop equivalents
Injected on DB-17 column

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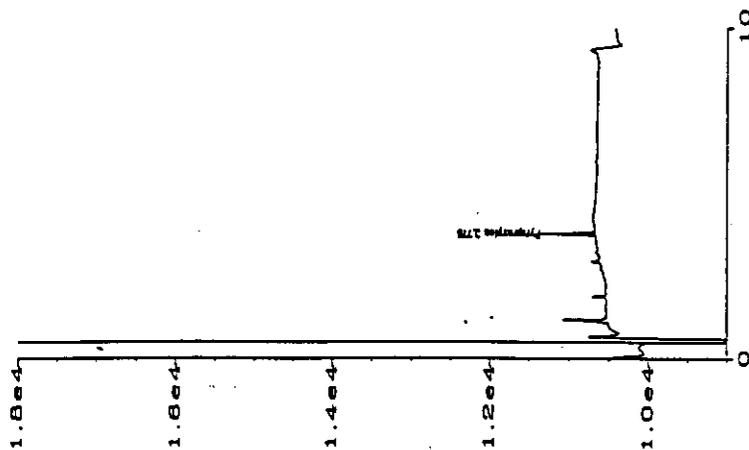


Figure 14

Pepper fortified with 0.02 ppm pyriproxyfen
10 mg crop equivalents
Injected on DB-5 column

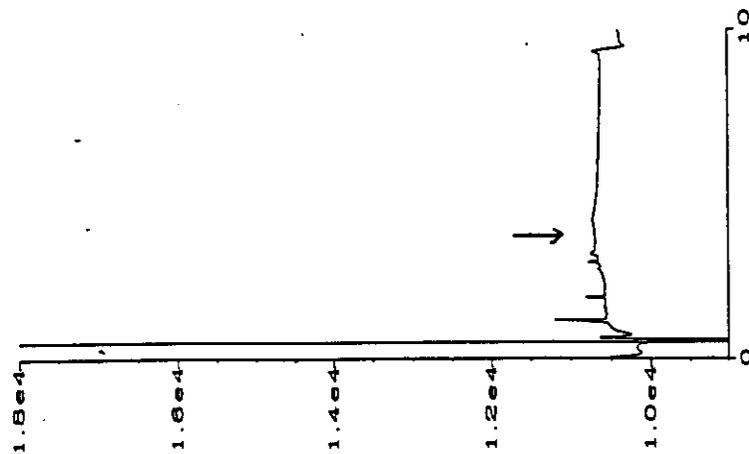


Figure 13

UTC Pepper for Pyriproxyfen
10 mg crop equivalents
Injected on DB-5 column

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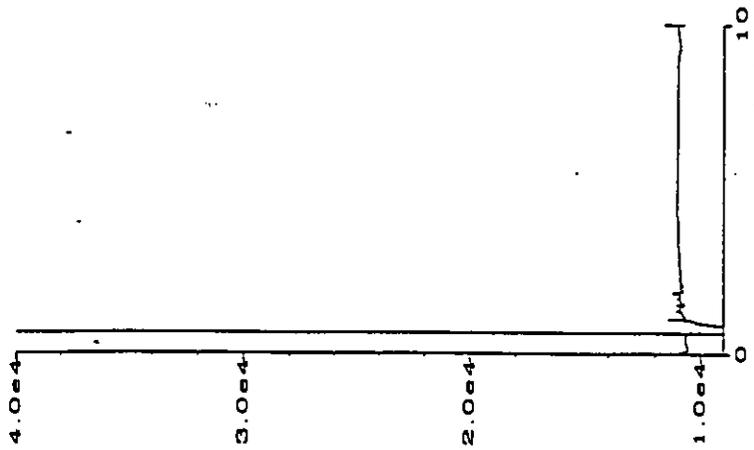


Figure 15

UTC Pepper for PYPA
10 mg crop equivalents
Injected on DB-5 column

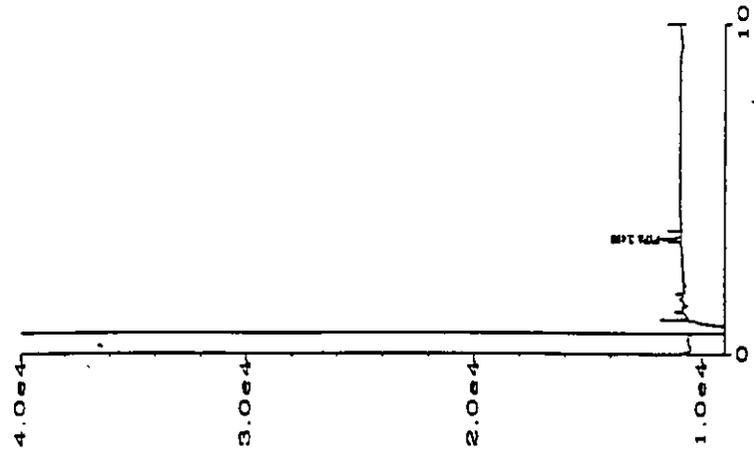


Figure 16

Pepper fortified with 0.02 ppm PYPA
10 mg crop equivalents
Injected on DB-5 column

**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

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(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).