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DETERMINATION OF TOTAL RESIDUES OF PROPICONAZOLE IN CROPS  
AS 2,4-DICHLOROBENZOIC ACID METHYL ESTER BY CAPILLARY GAS  
CHROMATOGRAPHY

ANALYTICAL METHOD NO. AG-626

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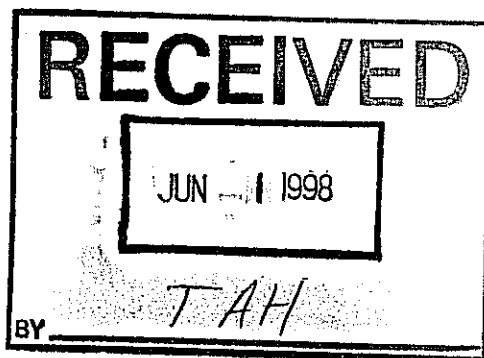


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## I. SUMMARY/INTRODUCTION

### A. SCOPE

Novartis Analytical Method AG-626 is a revised version of AG-454B<sup>1</sup> for determination of the total residues of propiconazole as 2,4-dichlorobenzoic acid methyl ester (2,4-DCBAME) in crops. In response to the Phase 3 Submission received from USEPA (memo dated on 9/30/1993), Novartis Crop Protection, Inc. (formerly Ciba Crop Protection) was requested to replace diazomethane<sup>2</sup> with a safe and commercially available derivatization reagent for 2,4-dichlorobenzoic acid (2,4-DCBA). Methyl iodide is presented herein as a methylation reagent for the conversion of 2,4-DCBA to 2,4-DCBAME. This reagent is also utilized in standard FDA multiresidue methods<sup>3</sup>. In a previous Ciba study<sup>4</sup>, methyl iodide was found to react effectively with 2,4-DCBA to form 2,4-DCBAME (equivalent to diazomethane). Therefore, AG-626 was issued to use methyl iodide as the primary derivatization reagent with diazomethane as an alternative for quantitation of propiconazole in crops. This method includes an optional Alumina A Sep-Pak cartridge purification procedure. According to USEPA Residue Chemistry Guidelines (OPPTS 860.1340<sup>5</sup>), AG-626 was validated and radiovalidated with control, fortified and <sup>14</sup>C-propiconazole-treated crop samples<sup>6,7</sup>. The limit of detection (LOD), as determined by the smallest standard amount injected, is 0.50 pg of 2,4-DCBAME (expressed as 2,4-DCBA). The limit of quantitation (LOQ), as demonstrated by the lowest fortification successfully recovered, is 0.05 ppm of propiconazole. The chemical names and structures of propiconazole, 2,4-DCBA and 2,4-DCBAME are shown in Figure 1.

### B. PRINCIPLE

A 15-g crop sample is reflux-extracted with 80/20 methanol (MeOH)/ammonium hydroxide (NH<sub>4</sub>OH) for 60 minutes. An aliquot of the sample extract is taken and evaporated to dryness. The residues are dissolved in aqueous sodium hydroxide (NaOH) and heated for 75 minutes ( $\leq 125^{\circ}\text{C}$ ) in the presence of potassium permanganate (KMnO<sub>4</sub>) for oxidation of propiconazole and metabolites to 2,4-DCBA. The sample is acidified with hydrochloric acid (HCl) and partitioned with 90/10 hexane/ethyl ether. The organic phase is evaporated to dryness and derivatized with methyl iodide (CH<sub>3</sub>I) in the presence of tetrabutyl ammonium hydroxide (TBAH) to form the methyl ester of 2,4-DCBA. Hexane is added to the derivatized solution and is partitioned with water to remove salts. The organic phase is loaded onto a pre-conditioned Alumina A Sep-Pak cartridge and the cartridge is eluted with 90/10 hexane/ethyl ether. The sample is evaporated briefly to remove ethyl ether and the final volume is adjusted for analysis.

The sample final fraction is analyzed with a gas chromatography (GC) system equipped with an electron capture detector (ECD) for quantitation of the total residues of propiconazole as 2,4-DCBAME. A flow diagram for the analytical procedure is shown in Figure 2.

## II. MATERIALS AND METHODS

### A. EQUIPMENT

- 1.0 Sample bottles, amber narrow mouth, 8 oz., or equivalent
- 2.0 Round bottom flask with 24/40 ground glass joint, 100-ml, 250-ml and 500-ml
- 3.0 Funnel with long stem, 12.5 cm size
- 4.0 Filter paper, prepleated circle, 24 cm, Whatman 2V, or equivalent
- 5.0 Filter paper, prepleated circle, 24 cm, Reeve Angel Grade 802, or equivalent
- 6.0 Heating mantle, 500 ml
- 7.0 Nitrogen evaporator and dri-block heater, Techne SC-3, or equivalent
- 8.0 Rotary evaporator, Buchi, Fisher Scientific Cat. #09-548-105F, or equivalent
- 9.0 Water bath, Büchi, or equivalent
- 10.0 Crimp-top glass GC injection vial, 2-ml, Wheaton, or equivalent
- 11.0 Separatory funnel with Teflon stopcock, 125-ml
- 12.0 Cotton, absorbent, Fisher Cat. #07-900, or equivalent
- 13.0 Alumina A Sep-Pak cartridge, Waters Associates, Cat. #51800
- 14.0 Glass syringe, LuerLok®, 25-ml
- 15.0 Test tubes, 24/40 joint, 18.5 cm x 22 mm, Ace Glass Co., Cat. #8645-38, or equivalent
- 16.0 Snyder distillation column, 3 ball, Kontes Cat. #K-503000-012, or equivalent
- 17.0 Multi-Blok Heater, Cole-Palmer, Cat. #J-3128-00, or equivalent
- 18.0 Volumetric pipette, 0.5 ml, 1-ml, 2-ml, 3-ml, 5-ml, 6-ml, 9-ml, 10-ml and 15-ml
- 19.0 Graduated cylinder, 10-ml, 25-ml, 250-ml and 1000-ml
- 20.0 Kimax heavy duty graduated concentration tube, 50-ml, Fisher Cat. #05-538-40B, or equivalent
- 21.0 Thermometers, -10 to 360°C and 0 to 100°C
- 22.0 Vortex mixer, or equivalent
- 23.0 Disposable Pasteur pipettes with 2-ml bulb
- 24.0 Filtering crucible holder (also identified as carbon filter tube), Fisher Scientific Cat. #08-261A, or equivalent
- 25.0 Balance, top loading, Mettler PE-600, or equivalent
- 26.0 Condenser, Allihn, 30-cm jacket, Fisher Scientific Cat. #07-734A, or equivalent

- 27.0 Boiling stones, Teflon, Fisher Scientific Cat. #09-191-20, or equivalent
- 28.0 Ultrasonic bath, Fisher Scientific Cat. #FS-14, or equivalent
- 29.0 Syringe, 100- $\mu$ l, Hamilton, or equivalent

B. REAGENTS AND STANDARDS

- 1.0 Acetone, Optima Grade, Fisher Cat. #A929-4, or equivalent
- 2.0 Methanol (MeOH), HPLC Grade, Fisher Cat. #A452-4, or equivalent
- 3.0 Ammonium hydroxide (NH<sub>4</sub>OH), concentrated, ACS Certified, Fisher Cat. #A669-212, or equivalent
- 4.0 80/20 MeOH/NH<sub>4</sub>OH (v/v)
- 5.0 Acetic acid, glacial, ACS Certified, Fisher Cat. #A38-500, or equivalent
- 6.0 Potassium permanganate (KMnO<sub>4</sub>), ACS Certified, Fisher Cat. #P279-500, or equivalent
- 7.0 Sodium hydroxide (NaOH), 50% w/w, ACS Certified, Fisher, Cat. #SS254-4, or equivalent
- 8.0 Sodium hydroxide solution (1M)
- 9.0 Sodium meta-bisulfite (Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>), ACS Certified, Fisher Cat. #S244-500, or equivalent
- 10.0 Hydrochloric acid (HCl), Reagent Grade, Fisher Cat. #A144-212, or equivalent
- 11.0 Hydrochloric acid solution (6M)
- 12.0 Ethyl ether, HPLC Grade, Fisher Cat. #E138-1, or equivalent
- 13.0 Hexane, HPLC Grade, Fisher Cat. #302-4, or equivalent
- 14.0 90/10 Hexane/ethyl ether (v/v)
- 15.0 Dodecane, 99% purity, Aldrich Chemical Co., Cat. #D22-110-4
- 13.0 99/1 Acetone/dodecane (v/w)
- 15.0 Diazomethane, prepared as described in AG-345<sup>2</sup>
- 16.0 Methyl iodide (CH<sub>3</sub>I), Fisher Cat. #M212-100, or equivalent
- 17.0 Tetrabutyl ammonium hydroxide (TBAH), 1M in MeOH, Fisher Cat. #04576-100
- 18.0 Water (H<sub>2</sub>O), HPLC Grade, or equivalent
- 19.0 Ethyl acetate, HPLC Grade, Fisher Cat. #E195-4, or equivalent
- 20.0 99/1 Ethyl acetate/H<sub>2</sub>O, (v/v)
- 21.0 FL-70® Detergent, Fisher Scientific (Cat. #SF105-4)
- 22.0 Propiconazole and 2,4-DCBA, analytical standards supplied by Analytical and Production Chemistry Department, Novartis Crop Protection, Inc., P. O. Box 18300, Greensboro, NC 27419-8300

C. ANALYTICAL PROCEDURES

1.0 Sample Preparation

The sample is received and stored frozen at -20°C. The sample is prepared under the general guidelines of the U.S. Food and Drug Administration Pesticide Analytical Manual<sup>3</sup> Volume I, Sections 102 and 203.

2.0 Extraction

**All glassware must be hand washed to avoid any cross-contamination. See Section II.H.1.0 for the glassware washing procedures.**

2.1 Weigh a 15-g representative sample into a 500-ml round bottom flask. Add a few boiling stones.

2.2 Fortify samples with propiconazole at this point if applicable (see Section 2.0 for fortification details).

2.3 Add 200 ml of 80/20 MeOH/NH<sub>4</sub>OH and fit the flask to an Allihn condenser. Set the round bottom flask in a heating mantle and reflux for 60 minutes. After refluxing, allow the extract to reach room temperature.

2.4 Filter the extract through a Reeve Angel Grade 802 filter paper placed inside a Whatman 2V filter paper into an 8 oz. amber narrow mouth bottle. Store the extract under refrigerated conditions if the next procedure (Section II.C.3.1) cannot be performed immediately.

3.0 Potassium Permanganate Oxidation

3.1 Transfer a 3 - 9 ml aliquot of the extract to a 24/40 ground glass joint test tube using a volumetric pipette. Note: Allow refrigerated extract to reach room temperature before transferring.

3.2 Add 0.1 ml of acetic acid to the sample and vortex well. Evaporate the sample to incipient dryness using a nitrogen evaporator equipped with a dri-block heater. Maintain the heater temperature at 35-40°C. Note: The evaporation may be performed overnight.

- 3.3 Weigh 1.0 g of  $\text{KMnO}_4$  into the test tube from Section II.C.3.2.
  - 3.4 Add 6 ml of 1M NaOH solution to the test tube (above). Stopper and dissolve the residues with a vortex mixer or sonicator. Note: A well-mixed solution is dark purple in color. If sample appears to be brownish to dark green, additional  $\text{KMnO}_4$  must be added in increments of 0.10 g. Vortex to mix sample after each addition.
  - 3.5 Rinse sides of test tube with 2 ml of 1M aqueous NaOH. Add several boiling chips.
  - 3.6 Fit the test tube to a Snyder column and place the tube/column assembly on a heating block pre-heated to  $\leq 125^\circ\text{C}$ . Reflux the sample for 75 minutes. Vortex the test tube twice briefly during the reflux period. Note: It is normal that the heating block temperature decreases after the test tube/column assembly is in the heating block.
  - 3.7 Remove the test tube/column assembly (Section II.C.3.6) from the heating block and allow to cool for a minimum of 15 minutes. Add 5 ml of  $\text{H}_2\text{O}$  to the tube through the top of the Snyder column.
  - 3.8 Remove the Snyder column and add 6 g of  $\text{Na}_2\text{S}_2\text{O}_5$  to the sample. Fit the test tube to the Snyder column again and mix well with a vortex mixer or sonicator. When the sample turns white, add ~5-ml of 90/10 hexane/ethyl ether to the tube through the top of the Snyder column.
  - 3.9 Slowly add 14 ml of 6M HCl to the test tube through the top of the Snyder column. The sample will effervesce. Mix the sample carefully using a glass stirring rod until a completely clear solution is achieved. Proceed to Section II.C.4.1.
- 4.0 Partition
- 4.1 Plug a filtering crucible holder (also identified as carbon filter tube) with a minimal, but functional amount of absorbent cotton. Rinse the cotton with 2 x 30 ml of 90/10 hexane/ethyl ether.

- 4.2 Transfer the sample (Section II.C.3.9) to a 125-ml separatory funnel. Add 15 ml of 90/10 hexane/ethyl ether to the funnel and stopper. Gently shake the funnel for one minute and allow the two layers to separate. Drain the lower aqueous layer back into the test tube. Transfer the organic phase (top) through the filtering crucible holder containing cotton (Section II.C.4.1) and into a 250-ml round bottom flask. Note: It is extremely important that no water residue is transferred to the round bottom flask.
- 4.3 Decant the aqueous layer collected in the test tube back into the separatory funnel and repeat the Section II.C.4.2 partitioning procedure two more times each using 15 ml of 90/10 hexane/ethyl ether (each partition). Collect the organic phases into the same 250-ml round bottom flask filtering through absorbent cotton.
- 4.4 Rinse the separatory funnel with ~10 ml of 90/10 hexane/ethyl ether. Pass the rinse through the cotton and combine the rinse with the sample. Pass an additional ~30 ml of 90/10 hexane/ethyl ether through the cotton and combine the rinse with the sample. Add 2 ml of 99/1 acetone/dodecane to the 250-ml round bottom flask. Derivatization is performed with methyl iodide (Section II.C.5.0) or with diazomethane (Section II.C.6.0).

#### 5.0 Methylation with Methyl Iodide

- 5.1 Evaporate the sample (Section II.C.4.4) to ~5 ml with a rotary evaporator. Maintain the water bath temperature at 35-40°C. Transfer the sample to a 50-ml graduated concentration tube. Rinse the 250-ml round bottom flask with 2 x 10 ml of acetone. Sonicate approximately 30-60 seconds each to rinse all the sides of the flask. Combine the rinses with the sample. Add 1-2 ml 99/1 acetone/dodecane to the concentration tube.
- 5.2 Evaporate the sample (Section II.C.5.1) to near dryness with a rotary evaporator. Maintain the water bath temperature at 35-40°C. Add acetone to the 3-ml mark and vortex for 10 seconds.
- 5.3 Add 80 µl of TBAH to the concentration tube using a syringe. Add 40 µl of CH<sub>3</sub>I using a second syringe. Stopper immediately and vortex for 30 seconds. Note: The addition of

methyl iodide should be performed inside a well ventilated hood.

- 5.4 Immediately place the sample into a water bath for 90 minutes maintaining the water bath temperature at 40-45°C. Allow the methylated solution to reach room temperature. Proceed to Section II.C.5.5. For difficult substrates, the Alumina A Sep-Pak purification is required and analyst should proceed to Section II.C.5.6 for an extra partition step to be performed prior to the Alumina A Sep-Pak purification (see Section II.C.7.1).
- 5.5 Evaporate the sample (Section II.C 5.4) to 0.2-0.3 ml at room temperature with a rotary evaporator. Do not use a water bath at this point. Evaporation is rapid and analyst should monitor closely. Add 15 ml of hexane to the concentration tube and sonicate for 30 seconds. Add 5 ml of water to the tube, stopper and shake for one minute. Allow the two phases to separate. Adjust the volume (top layer) in the concentration tube with hexane. Inject the sample into the GC/ECD system for analysis.
- 5.6 **Optional** - Add 15 ml of hexane to the sample (Section II.C.5.4) followed by ~5 ml of water. Stopper and shake for 30-60 seconds to remove salts from the hexane phase (top layer). Transfer the hexane phase to a 100-ml round bottom flask using a disposable Pasteur pipette. Add an additional 2 x 10-15 ml of hexane to the 50-ml concentration tube and shake for 30-60 seconds each time. Transfer the hexane phases (top layer) to the 100-ml round bottom flask. Note: Do not transfer water from the concentration tube to the 100-ml round bottom flask.
- 5.7 Evaporate the sample (Section II.C.5.6) to 5-15 ml with a rotary evaporator (water bath temperature  $\leq 20^{\circ}\text{C}$ ). Proceed to Section II.C.7.1 for the Alumina A Sep-Pak purification procedure.
- 6.0 Methylation with Diazomethane (Alternative)

Note: The diazomethane derivatization should be performed inside a well ventilated hood. Extreme care should be exercised because of potential toxic effects (lung) and explosion (with scratched, etched or chipped glass). It is imperative that glassware is free of cracks, scratches or nicks. Use new glassware if possible.

- 6.1 Evaporate the sample (Section II.C.4.4) to dryness with a rotary evaporator. Maintain the water bath temperature at 35-40°C.
  - 6.2 Add 2.0 ml of diazomethane reagent solution<sup>2</sup>. Allow the sample to stand for  $\geq 30$  minutes with occasional gentle swirling. Add more diazomethane to maintain a yellow color, if necessary.
  - 6.3 Evaporate the sample (Section II.C.6.2) to dryness with a rotary evaporator at room temperature. Do not use a water bath at this point. Evaporation is rapid and analyst should monitor closely. Continue to evaporate the sample at room temperature for an additional one and no longer than two minutes to ensure the ethyl ether is completely removed. Note: Evaporation beyond 2 minutes may lead to loss of sample.
  - 6.4 Add 5 ml of hexane to the round bottom flask (Section II.C.6.3) and swirl to dissolve the residues. Proceed to Section II.C. 7.4 for the Alumina A Sep-Pak cleanup procedure.
- 7.0 Alumina A Sep-Pak Cleanup
- 7.1 Fit an Alumina A Sep-Pak cartridge to the LuerLok end of a 25-ml syringe with the plunger removed. Note: Do not allow the cartridge bed to go dry between any of the following steps. It is essential to monitor elutions closely to ensure the Sep-Pak does not go dry. A stopcock can be fitted between the cartridge and the receiving flask for control of the elutions.
  - 7.2 Use a volumetric pipette to load 10 ml of 1/99 water/ethyl acetate into the syringe barrel and allow to pass through the Alumina A Sep-Pak cartridge under gravity. If there are air bubbles between the syringe barrel and cartridge, swirl with a disposable Pasteur pipette to remove the air bubbles. Discard the eluate.
  - 7.3 Pass 10 ml of hexane through the Sep-Pak cartridge under gravity and discard the eluate.

- 7.4 Load the sample (Section II.C. 5.7 or II.C. 6.4) into the syringe barrel. Pass through the Sep-Pak cartridge under gravity and collect the eluate into a 50-ml concentration tube.
- 7.5 Pass 10-15 ml of 90/10 hexane/ethyl ether through the Sep-Pak cartridge under gravity and collect the eluate into the same 50-ml concentration tube.
- 7.6 Evaporate the eluate to 15-20 ml with a rotary evaporator (water bath at  $\leq 20^{\circ}\text{C}$ ). Adjust the sample final volume with hexane and sonicate for 30-60 seconds. Transfer a ~1-ml aliquot of the final fraction into a GC injection vial and inject into the GC/ECD system (Section II.D.1.0) for residue analysis.

D. INSTRUMENTATION

1.0 Description and Operating Conditions

Inject a 2- $\mu\text{l}$  aliquot of the sample final fraction from Section II.C.7.6 (or Section II.C.5.5) into a GC/ECD system for residue analysis. The GC/ECD system and operating conditions are given in Table I.

2.0 Standardization

Prepare calibration standards of 2,4-DCBAME as described in Section II.I.2.0 (below). Calibrate the GC/ECD system by injecting 2- $\mu\text{l}$  aliquots of the calibration standards to represent a working range from 0.5 to 20 pg of 2,4-DCBAME (expressed as 2,4-DCBA). Figure 3 shows typical GC chromatograms of 2,4-DCBAME standards. Compare the detector responses (GC peak height) to the injected standard amount (pg) manually or with a computer data acquisition system. Construct the calibration curve by plotting GC peak height versus pg of standard injected or enter the data into an appropriate computer system for a least square linear regression analysis. Express a calibration curve as follows:

Peak Height = Slope x 2,4-DCBAME (pg) + Intercept

$$(y = mx + b)$$

Figure 4 shows a typical calibration curve constructed by the Novartis Residue Chemistry Data Acquisition System (VG Multichrom).

E. INTERFERENCES

Analytical Method AG-626 was used to analyze several crop samples in two previous validation studies<sup>6,7</sup>. No significant interferences (<0.05 ppm) were observed in control citrus, lentils, and wheat (immature whole plant and wheat grain). Control background residues were detected as 2,4-DCBAME in wheat forage (0.11-0.15 ppm), celery (0.054-0.060 ppm), wheat chaff (0.040 -0.012 ppm) and dry bulb onion (0.030-0.16 ppm). The same interferences were encountered when Analytical Method AG-454B was validated<sup>1</sup>.

F. CONFIRMATORY TECHNIQUES

The quantitation of 2,4-DCBAME was confirmed by a GC/MSD system<sup>4</sup>. Therefore, the GC/MSD operating procedures are confirmatory for AG-626. The GC/MSD operating conditions are presented as follows:

Column (30m x 0.25 mm, 1.0 µm):	DB-17
Initial Oven Temp:	70° C
Initial Time:	1 min.
Temp Rate (level 1):	30° C/min.
Final Oven Temp:	130° C
Hold Time:	15 min.
Temp Rate (level 2):	50° C
Final Oven Temp:	260° C
Final Time:	5 min.
Injector Temp:	250° C
MS Detector Temp:	300° C
Equilibration Time:	1 min.
Injector Purge Time:	0.8 min.
Carrier Gas (He) Flow rate:	2 ml/min.
Solvent Delay:	3 min.
Acquisition Mode:	Scan
Scan Range:	50-500 amu

G. TIME REQUIRED FOR ANALYSIS

A total of 16 hours is required to analyze a set of 5-6 samples. This includes the cooling of hot extract and the actual injection time. Sections II.C. 2.4, 3.2, 4.4 and 5.7 can be stopping points for this method. When several sets of samples are being worked up, many steps can be overlapped and performed concurrently.

H. MODIFICATION AND POTENTIAL PROBLEMS

- 1.0 Hand-wash the glassware used for analysis of propiconazole according to the following procedures:
  - (a) Remove brown stains from glassware (potassium permanganate) by dipping test tubes in a solution of water and  $\text{Na}_2\text{S}_2\text{O}_5$ . This solution may be kept for an indefinite period. Discard any waste appropriately (heavy metal).
  - (b) Rinse all glassware with tap water prior to soaking in detergent.
  - (c) Soak water-rinsed glassware in a 10% FL-70 (Fisher Scientific) aqueous solution.
  - (d) Using a brush, thoroughly scrub all glassware. Remove any residual remaining. Extreme care should be taken to scrub the 50-ml concentration tube tip.
  - (e) Rinse each piece with hot water no less than three times and until no more bubbles (caused by detergent) are apparent. At this point carefully inspect glassware, especially 250/100-ml round bottom flasks and 50-ml concentration tubes. Discard any glassware that does not exhibit sheeting action or glassware that has an apparent ring of water beads.
  - (f) Sonicate to rinse glassware with acetone for 1-2 minutes. Add hexane to concentration tubes and 250/100-ml round bottom flasks and sonicate to rinse for 1-2 minutes.
  - (g) Prior to use, rinse glassware with an appropriate solvent and place upside down to dry.
- 2.0 Rinse the rotary evaporator with fresh acetone between evaporations to eliminate possible cross contamination.
- 3.0 The GC system should be well maintained and serviced routinely. The injection port parts (septum, liner and golden seal) should be inspected and changed routinely.

I. PREPARATION OF STANDARD SOLUTIONS

1.0 Fortification Standards

- 1.1 Weigh 100 mg of propiconazole into a 100-ml volumetric flask and bring to volume with hexane.
- 1.2 Make serial dilutions with hexane to achieve concentrations of 30 µg/ml, 15 µg/ml, 3.75 µg/ml, 1.5 µg/ml and 0.75 µg/ml. Refrigerate the fortification standards in amber bottles when not in use.

2.0 Calibration Standards

- 2.1 Dissolve 10 mg of 2,4-DCBA (50-ml volumetric flask) with acetone. Transfer 0.5 ml of the 2,4-DCBA acetone solution into a 50-ml graduate concentration tube. Add acetone to the 3-ml mark. Add 100 µl of TBAH and 50 µl of CH<sub>3</sub>I. Stopper and vortex well. Place the solution into a water bath at 40-45°C for 90 minutes.
- 2.2 Allow the methylated solution to reach room temperature. Add 15 ml of hexane and 5 ml of water respectively to the methylated solution. Shake for 30-60 seconds and transfer the hexane phase into a 100-ml volumetric flask. Add an additional 3 x 15 ml of hexane and shake for 30-60 seconds after each transfer. Transfer the hexane phases to the 100-ml volumetric flask. Bring to volume with hexane to produce a 1000 pg/µl calibration standard (expressed as 2,4-DCBA). Transfer 10 ml of the standard into a 100-ml volumetric flask and bring to volume with hexane to produce a 100 pg/µl standard. Make serial dilutions with hexane to achieve concentrations of 0.2, 0.5, 1, 2, 3, 4, 5, 10 and 20 pg/µl (expressed as 2,4-DCBA). Refrigerate the calibration standards in amber bottles when not in use.

3.0 Calibration Standards (by Diazomethane Methylation)

Note: These standards (prepared below) are for the analysis of diazomethane derivatized samples only (Section II.C.6.0). The diazomethane derivatization should be performed inside a well ventilated hood. Extreme care should be exercised because of potential toxic effects (lung) and explosion (with scratched,

etched or chipped glass). It is imperative that glassware is free of cracks, scratches or nicks. Use new glassware if possible.

- 3.1 Weigh 20 mg of 2,4-DCBA into a 200-mL volumetric flask.
- 3.2 Add 3 ml of diazomethane<sup>2</sup> to derivatize 2,4-DCBA as described in Section II.C.6.2.
- 3.3 Add hexane to the mark of the 200-ml volumetric flask to produce a calibration standard of 100 pg/μl (expressed as 2,4-DCBA). Make serial dilutions with hexane to achieve concentrations of 0.2, 0.5, 1, 2, 3, 4, 5, 10 and 20 pg/μl (expressed as 2,4-DCBA). Refrigerate standards in amber bottles when not in use.

## J. METHODS OF CALCULATION

### 1.0 Determination of Sample Residues

Inject a 2-μl aliquot of the sample final solution (Section II.C.7.6 or II.C.5.5) into the GC/ECD system (Section II.D.10) for residue analysis. Inject at least four calibration standards into the GC/ECD system for each analysis of samples and construct a calibration curve as specified in Section II.D.2.0. Determine 2,4-DCBAME (pg) in the injected aliquot by comparing the GC peak height with the calibration curve (or enter the peak height into the calibration equation). Representative GC chromatograms from analyses of citrus, dry bulb onion, lentils, celery, wheat whole plant, wheat forage, wheat chaff and wheat grain are shown in Figure 5-12.

Calculate the total residues of propiconazole from 2,4-DCBAME (pg) (GC analysis) as follows:

$$(1) \text{ Propiconazole (ppm)} = \frac{2,4\text{-DCBAME (pg) quantitated by GC analysis} \times 1.79}{\text{sample weight } (\mu\text{g}) \text{ injected}}$$

Note : The factor of 1.79 is the molecular weight ratio of propiconazole (MW = 342) to 2,4-DCBA (MW = 191)

Calculate sample weight (μg) injected into the GC system according to the following equation:

$$(2) \text{ Sample weight injected } (\mu\text{g}) = \frac{(G) \times (V_i) \times (V_a) \times 1000}{(V_f) \times [V_e + G \times M]}$$

G = sample weight (g) extracted  
V<sub>e</sub> = extraction volume (ml)  
V<sub>i</sub> = GC injection volume (μl)  
V<sub>f</sub> = volume of sample final fraction (ml)  
V<sub>a</sub> = volume of aliquot taken from extract (ml)  
M = sample moisture (%)

## 2.0 Determination of Procedural Recoveries

Prepare fortification standards as described in Section II.I.1.0. Fortify a sample by adding an appropriate amount (0.5-2 ml) to a control sample prior to extraction (Section II.C.2.2). (For example, add 1 ml of the 0.75 μg/ml propiconazole fortification standard to a 15-g control sample to yield a 0.05 ppm fortified sample).

Analyze the fortified sample by following the analytical procedures as described in Section II.C 2.0-7.0. Determine total residues of propiconazole in the fortified sample as described in Section II. J.1.0. Calculate procedural recovery (R%) as a percentage according to the following equation:

$$(3) R (\%) = \frac{\text{propiconazole (pp m) in fortified sample} - \text{control background (ppm)}}{\text{propiconazole (pp m) added}} \times 100$$

Note: The residue concentration detected in a fortified sample should be corrected by subtracting the control residue background if there is any

Use the procedural recovery(s) to validate a method or to correct residues found in a sample when the method is used for residue data collection. Correct residue concentrations according to the following equation:

$$(4) \text{ Corrected Propiconazole (pp m)} = \frac{2,4 - \text{DCBAME (pg) found by GC} \times 1.79}{\text{sample weight (}\mu\text{g) injected}} \times \frac{1}{R}$$

Note: Do not use procedural recovery data for tolerance enforcement calculations. Use Equation 1 to determine residues when the method is used for tolerance enforcement purposes.

## III. RESULTS AND DISCUSSION

AG-626 was validated by analyzing control and fortified crop samples<sup>6,7</sup>. The average procedural recoveries were 83% for citrus, 99% for lentils, 84% for dry bulb

onions, 92% for celery, 94% for wheat immature whole plant, 88% for wheat forage, 96% for wheat chaff and 84% for wheat grain (Table II). The coefficients of variation ranged from 0.0-12% for triplicate residue analyses with AG-626 (Table III). The extractability ranged from 82% for wheat forage to 121% for celery. The GC accountability ranged from 68% for wheat chaff to 87% for wheat immature plant (Table IV).

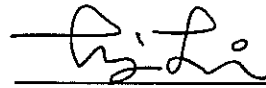
#### IV. CONCLUSION

Novartis Analytical Method AG-626 is a valid method for the determination of total residues of propiconazole in crops. The validity is demonstrated by the accuracy, precision, extractability and accountability as reported in the validation studies<sup>6,7</sup>.

V. CERTIFICATE

The report and experimental results included in this study are certified to be authentic accounts of the experiments.

10/10/97  
Date

  
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VI. TABLES AND FIGURES

TABLE I. GC OPERATING CONDITIONS

Instrument:	Hewlett-Packard 5880A Gas Chromatograph with an electron capture detector and HP 7673A automatic sampler, or equivalent.	
Column:	J & W capillary column, DB-5 (30 m x 0.32 mm i. d., 0.25 $\mu$ m film thickness), or DB-5.625 (30 m x 0.25 mm i. d., 0.25 $\mu$ m film thickness)	
Temperatures:	Injector:	250°C
	Detector:	300°C
	Oven Initial:	60°C/70°C for 1 minute
	Oven Final:	120°C/130°C for 18 minutes
	Program Rate:	30°C/minute
Oven Post:	320°C for 5 minutes	
	Note: GC oven temperature program rate can be alternated to 20°C/minute when interferences are encountered.	
Flows:	Carrier gas: Helium, 1-2 ml/minute Makeup: 95% argon/methane, 30 ml/minute	
Injection:	Mode:	Splitless.
	Volume:	2 $\mu$ l

TABLE II. PROCEDURAL RECOVERY DATA\*

Substrate	Sample ID	Fortification Level (ppm)	Propiconazole Residues Found by GC analysis <sup>1</sup>	Procedural Recovery <sup>2</sup>	Average Recovery by Substrate
Citrus (I)	CIT-01A	Control	0.038 ppm**	---	
Citrus (I)	CIT-01B	Control	0.037 ppm	---	
Citrus (I)	CIT-02A	0.05 ppm	0.083 ppm	89%	
Citrus (I)	CIT-02B	0.05 ppm	0.078 ppm	80%	
Citrus (I)	CIT-03A	0.10 ppm	0.14 ppm	98%	
Citrus (I)	CIT-04A	0.50 ppm	0.50 ppm	93%	
Citrus (I)	CIT-04B	0.50 ppm	0.47 ppm	87%	
Citrus (I)	CIT-04C	0.50 ppm	0.45 ppm	83%	
Citrus (II)	CIT-01C	Control	0.017 ppm**	----	
Citrus (II)	CIT-03B	0.10 ppm	0.11 ppm	90%	
Citrus (II)	CIT-05	1.0 ppm	0.88 ppm	86%	83 ± 5.7%
Lentils (I)	LEN-01A	Control	0.015 ppm**	----	
Lentils (I)	LEN-04A	1.0 ppm	1.0 ppm	102%	
Lentils (I)	LEN-04B	1.0 ppm	0.94 ppm	92%	
Lentils (I)	LEN-04C	1.0 ppm	1.0 ppm	101%	
Lentils (II)	LEN-01B	Control	0.027 ppm**	----	
Lentils (II)	LEN-02A	0.05 ppm	0.073 ppm	92%	
Lentils (II)	LEN-02B	0.05 ppm	0.070 ppm	86%	
Lentils (II)	LEN-03A	0.25 ppm	0.28 ppm	103%	
Lentils (II)	LEN-03B	0.25 ppm	0.28 ppm	103%	
Lentils (II)	LEN-03C	0.25 ppm	0.31 ppm	112%	99 ± 8.3%
Bulb Onion (I)	DBO-01A	Control	0.16 ppm	----	
Bulb Onion (I)	DBO-01B	Control	0.074 ppm**	----	
Bulb Onion (I)	DBO-02A	0.05 ppm	0.11 ppm	80%	
Bulb Onion (I)	DBO-02B	0.05 ppm	0.11 ppm	79%	
Bulb Onion (I)	DBO-03A	0.10 ppm	0.15 ppm	79%	
Bulb Onion (I)	DBO-03B	0.10 ppm	0.15 ppm	77%	
Bulb Onion (I)	DBO-03C	0.10 ppm	0.15 ppm	72%	
Bulb Onion (I)	DBO-04A	0.50 ppm	0.55 ppm	95%	
Bulb Onion (II)	DBO-01C	Control	0.030 ppm**	----	
Bulb Onion (II)	DBO-04B	0.50 ppm	0.52 ppm	98%	
Bulb Onion (II)	DBO-05	1.0 ppm	0.97 ppm	94%	84 ± 9.8%
Celery (I)	CEL-1-01	Control	0.054 ppm**	----	
Celery (I)	CEL-1-02	0.05 ppm	0.12 ppm	122%	
Celery (I)	CEL-1-03	1.0 ppm	0.87 ppm	81%	
Celery (II)	CEL-5-01	Control	0.060 ppm**	----	
Celery (II)	CEL-5-02	0.05 ppm	0.10 ppm	81%	
Celery (II)	CEL-5-03	3.0 ppm	2.5 ppm	82%	92 ± 20%

TABLE II. PROCEDURAL RECOVERY DATA (CONTINUED)

Substrate	Sample ID	Fortification Level (ppm)	Propiconazole Residues Found by GC analysis <sup>1</sup>	Procedural Recovery <sup>2</sup>	Average Recovery by Substrate
Immature Whole Plant (I)	IWP-01A	Control	0.013 ppm**	----	
Immature Whole Plant (I)	IWP-02A	0.05 ppm	0.052 ppm	77%	
Immature Whole Plant (I)	IWP-03A	1.0 ppm	1.1 ppm	106%	
Immature Whole Plant (I)	IWP-03B	1.0 ppm	1.0 ppm	102%	
Immature Whole Plant (I)	IWP-03C	1.0 ppm	1.2 ppm	121%	
Immature Whole Plant (II)	IWP-01B	Control	0.018 ppm**	----	
Immature Whole Plant (II)	IWP-02B	0.05 ppm	0.055 ppm	73%	
Immature Whole Plant (II)	IWP-04	3.0 ppm	2.6 ppm	85%	94%
Wheat Forage (I)	WF-01A	Control	0.11 ppm**	----	
Wheat Forage (I)	WF-02A	0.05 ppm	0.15 ppm	77%	
Wheat Forage (I)	WF-03A	0.50 ppm	0.60 ppm	98%	
Wheat Forage (I)	WF-03B	0.50 ppm	0.51 ppm	80%	
Wheat Forage (I)	WF-03C	0.50 ppm	0.53 ppm	85%	
Wheat Forage (I)	WF-04A	3.0 ppm	2.8 ppm	88%	
Wheat Forage (II)	WF-01B	Control	0.15 ppm**	----	
Wheat Forage (II)	WF-02B	0.05 ppm	0.20 ppm	111%	
Wheat Forage (II)	WF-04B	3.0 ppm	2.4 ppm	74%	88%
Wheat Chaff (I)	WC-01A	Control	0.040 ppm**	----	
Wheat Chaff (I)	WC-05A	0.05 ppm	0.089 ppm	98%	
Wheat Chaff (I)	WC-02A	0.10 ppm	0.13 ppm	92%	
Wheat Chaff (I)	WC-03A	0.20 ppm	0.20 ppm	79%	
Wheat Chaff (I)	WC-03B	0.20 ppm	0.20 ppm	81%	
Wheat Chaff (I)	WC-03C	0.20 ppm	0.22 ppm	91%	
Wheat Chaff (I)	WC-04A	0.40 ppm	0.40 ppm	89%	
Wheat Chaff (II)	WF-01B	Control	0.12 ppm**	----	
Wheat Chaff (II)	WF-05B	0.05 ppm	0.18 ppm	119%	
Wheat Chaff (II)	WF-02B	0.10 ppm	0.23 ppm	115%	
Wheat Chaff (II)	WF-04B	0.40 ppm	0.51 ppm	97%	96%
Wheat Grain (I)	WG-01A	Control	0.020 ppm**	----	
Wheat Grain (I)	WG-02A	0.05 ppm	0.060 ppm	80%	
Wheat Grain (I)	WG-02B	0.05 ppm	0.060 ppm	80%	
Wheat Grain (I)	WG-02C	0.05 ppm	0.059 ppm	78%	
Wheat Grain (I)	WG-04A	0.50 ppm	0.47 ppm	91%	
Wheat Grain (I)	WG-04B	0.50 ppm	0.51 ppm	99%	
Wheat Grain (II)	WG-01B	Control	0.019 ppm**	----	
Wheat Grain (II)	WG-03A	0.10 ppm	0.091 ppm	72%	
Wheat Grain (II)	WG-03B	0.10 ppm	0.11 ppm	86%	84%

1. Propiconazole residues (ppm) = [2,4-DCBA (pg) detected by GC analysis x 1.79] + sample weight injected (µg)
  2. Recovery (%) = [(propiconazole (ppm) in sample - control background (ppm)) x 100] + propiconazole spiked (ppm)
- \* Data obtained from Studies 402-94<sup>6</sup> and 412-97<sup>7</sup>
- \* Control background values used to calculate recoveries

TABLE III. PRECISION DATA\*

Substrate	Sample No.	Fortification Level (ppm)	Propiconazole Found (ppm)	Mean (ppm)	Std. Dev.	CV
Citrus	CIT-04A	0.5 ppm	0.50 ppm			
Citrus	CIT-04B	0.5 ppm	0.47 ppm			
Citrus	CIT-04C	0.5 ppm	0.45 ppm	0.47	0.025	5.3%
Dry Bulb Onion	DBO-03A	0.1 ppm	0.15 ppm			
Dry Bulb Onion	DBO-03B	0.1 ppm	0.15 ppm			
Dry Bulb Onion	DBO-03C	0.1 ppm	0.15 ppm	0.15	0.0	0.0%
Lentils	LEN-04A	1.0 ppm	1.0 ppm			
Lentils	LEN-04B	1.0 ppm	0.94 ppm			
Lentils	LEN-04C	1.0 ppm	1.0 ppm	0.98	0.035	3.6%
lentils	LEN-03A	0.25 ppm	0.28 ppm			
Lentils	LEN-03B	0.25 ppm	0.28 ppm			
Lentils	LEN-03C	0.25 ppm	0.31 ppm	0.29	0.017	5.9%
Celery	CEL-1-04A	Treated	0.77 ppm			
Celery	CEL-1-04B	Treated	0.71 ppm			
Celery	CEL-1-04C	Treated	0.67 ppm	0.72	0.050	7.0%
Celery	CEL-5-04A	Treated	1.7 ppm			
Celery	CEL-5-04B	Treated	1.9 ppm			
Celery	CEL-5-04C	Treated	1.9 ppm	1.8	0.12	6.7%
Wheat Whole Plant	IWP-03A	1.0 ppm	1.1 ppm			
Wheat Whole Plant	IWP-03B	1.0 ppm	1.0 ppm			
Wheat Whole Plant	IWP-03C	1.0 ppm	1.2 ppm	1.1	0.10	9.1%
Wheat Whole Plant	IWP-5xA	Treated	3.7 ppm			
Wheat Whole Plant	IWP-5xB	Treated	3.1 ppm			
Wheat Whole Plant	IWP-5xC	Treated	3.0 ppm	3.3	0.38	12%
Wheat Forage	WF-03A	0.5 ppm	0.60 ppm			
Wheat Forage	WF-03B	0.5 ppm	0.51 ppm			
Wheat Forage	WF-03C	0.5 ppm	0.53 ppm	0.55	0.047	8.5%
Wheat Forage	WF-1xA	Treated	0.60 ppm			
Wheat Forage	WF-1xB	Treated	0.51 ppm			
Wheat Forage	WF-1xC	Treated	0.53 ppm	0.55	0.047	8.5%
Wheat Chaff	WC-03A	0.2 ppm	0.20 ppm			
Wheat Chaff	WC-03B	0.2 ppm	0.20 ppm			
Wheat Chaff	WC-03C	0.2 ppm	0.22 ppm	0.21	0.012	5.7%
Wheat Chaff	WC-5xA	Treated	0.19 ppm			
Wheat Chaff	WC-5xB	Treated	0.21 ppm			
Wheat Chaff	WC-5xC	Treated	0.18 ppm	0.19	0.015	7.9%
Wheat Grain	WG-02A	0.05 ppm	0.060 ppm			
Wheat Grain	WG-02B	0.05 ppm	0.060 ppm			
Wheat Grain	WG-02C	0.05 ppm	0.059 ppm	0.060	0.00058	1.0%
Wheat Grain	WG-5xA	Treated	0.068 ppm			
Wheat Grain	WG-5xB	Treated	0.062 ppm			
Wheat Grain	WG-5xC	Treated	0.062 ppm	0.064	0.0035	5.5%

\* Data obtained from Studies 402-94<sup>6</sup> and 412-97<sup>7</sup>

TABLE IV. EXTRACTABILITY AND ACCOUNTABILITY\*

Substrates	Sample ID	<sup>14</sup> C-Propiconazole Treatment Rate	TRR <sup>1</sup> (ppm)	<sup>14</sup> C-Residues (LSC) in Extracts <sup>2</sup> (ppm)	Extraction Efficiency <sup>3</sup>	Total Residues <sup>4</sup> (GC) in Final Solution (ppm)	GC Accountability <sup>5</sup>
Immature Whole Plant	IWP-5xA	0.5 lb. a.i./acre	3.78	3.25		3.7	
Immature Whole Plant	IWP-5xB	0.5 lb. a.i./acre	3.78	3.49		3.1	
Immature Whole Plant	IWP-5xC	0.5 lb. a.i./acre	3.78	3.34	89%	3.0	87%
Wheat Forage	WF-1xA	0.1 lb. a.i./acre	3.45	2.76		2.4	
Wheat Forage	WF-1xB	0.1 lb. a.i./acre	3.45	2.87		2.3	
Wheat Forage	WF-1xC	0.1 lb. a.i./acre	3.45	2.88	82%	2.4	70%
Wheat Chaff	WC-5xA	0.5 lb. a.i./acre	0.28	0.23		0.19	
Wheat Chaff	WC-5xB	0.5 lb. a.i./acre	0.28	0.22		0.21	
Wheat Chaff	WC-5xC	0.5 lb. a.i./acre	0.28	0.25	82%	0.18	68%
Celery	CEL-1-04A	0.5 lb. a.i./acre	0.85	1.10		0.77	
Celery	CEL-1-04B	0.5 lb. a.i./acre	0.85	1.04		0.71	
Celery	CEL-1-04C	0.5 lb. a.i./acre	0.85	0.95	121%	0.67	84%
Celery	CEL-5-04A	2.5 lb. a.i./acre	3.1	3.0		2.1	
Celery	CEL-5-04B	2.5 lb. a.i./acre	3.1	2.9		2.3	
Celery	CEL-5-04C	2.5 lb. a.i./acre	3.1	2.9	95%	2.4	73%

\* Data obtained from Studies 402-94<sup>1</sup> and 412-97<sup>2</sup>

1. measured by combustion/LSC analysis

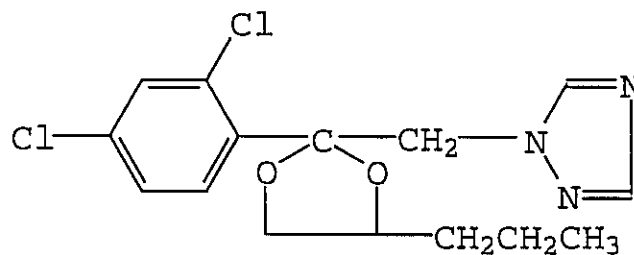
2. measured by LSC analysis

3. Extraction Efficiency = (<sup>14</sup>C-residues in sample extracts + TRR) x 100%

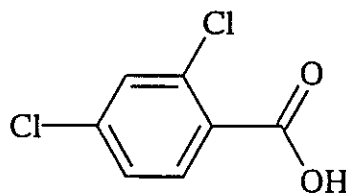
4. measured by GC analysis

5. GC Accountability = (propiconazole determined by GC analysis + TRR ) x 100%

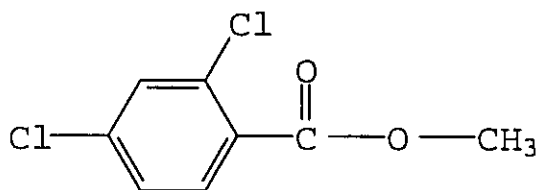
FIGURE 1. CHEMICAL NAMES AND STRUCTURES OF PROPICONAZOLE, 2,4-DICHLOROBENZOIC ACID AND 2,4-DICHLOROBENZOIC ACID METHYL ESTER



Propiconazole (Tilt)  
1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1-H-1,2,4-triazole



2,4-Dichlorobenzoic Acid (2,4-DCBA)



2,4-Dichlorobenzoic Acid Methyl Ester (2,4-DCBAME)

FIGURE 2. FLOW DIAGRAM FOR ANALYTICAL PROCEDURES

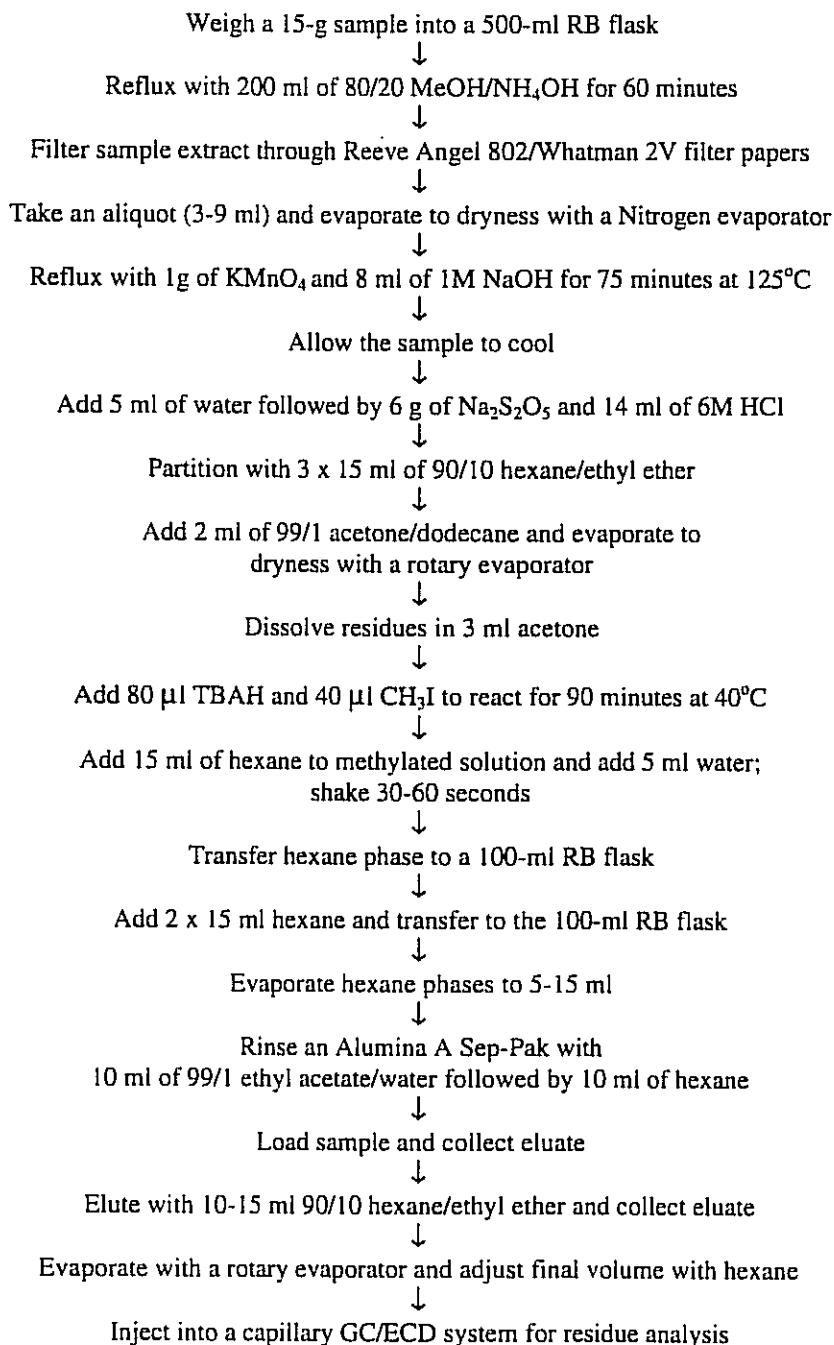
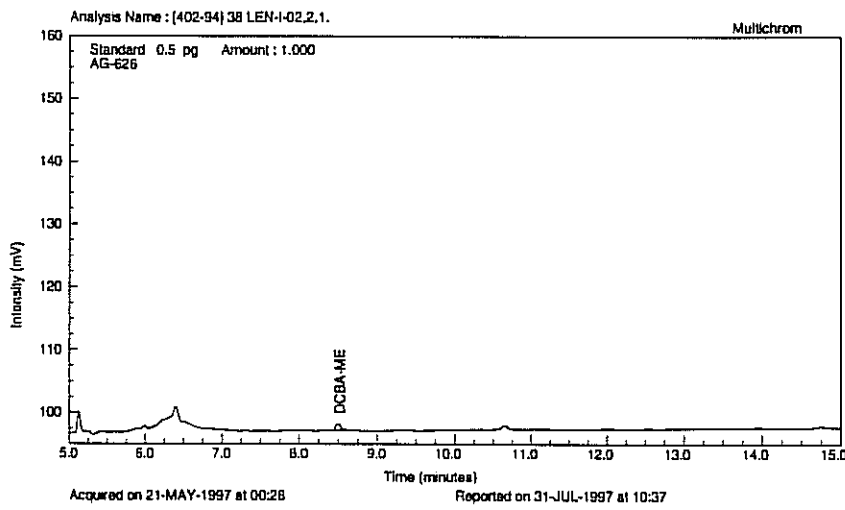
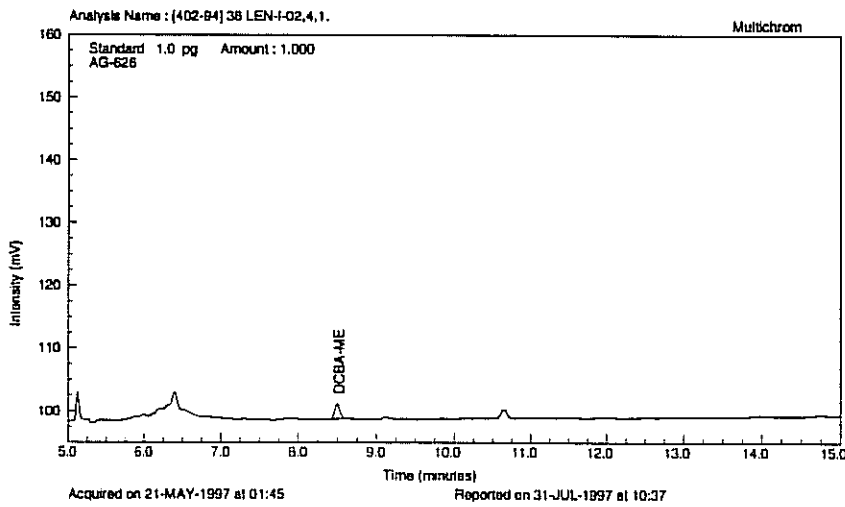


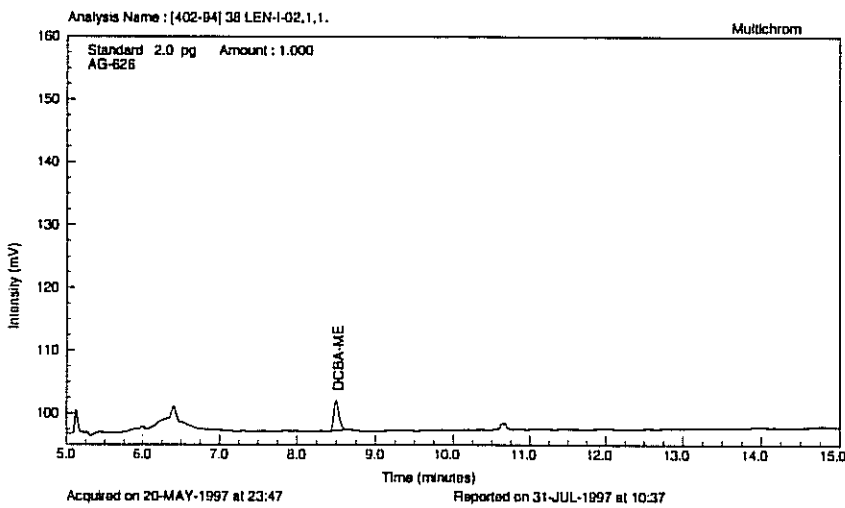
FIGURE 3. REPRESENTATIVE GC CHROMATOGRAMS FOR CALIBRATION STANDARDS



1. 0.5 pg 2,4-DCBAME injected (expressed as 2,4-DCBA equivalent)

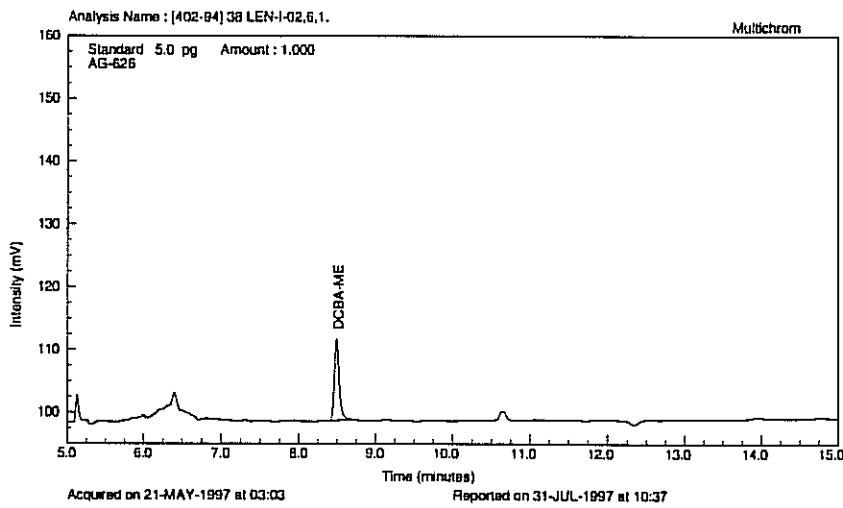


2. 1.0 pg 2,4-DCBAME injected (expressed as 2,4-DCBA equivalent)

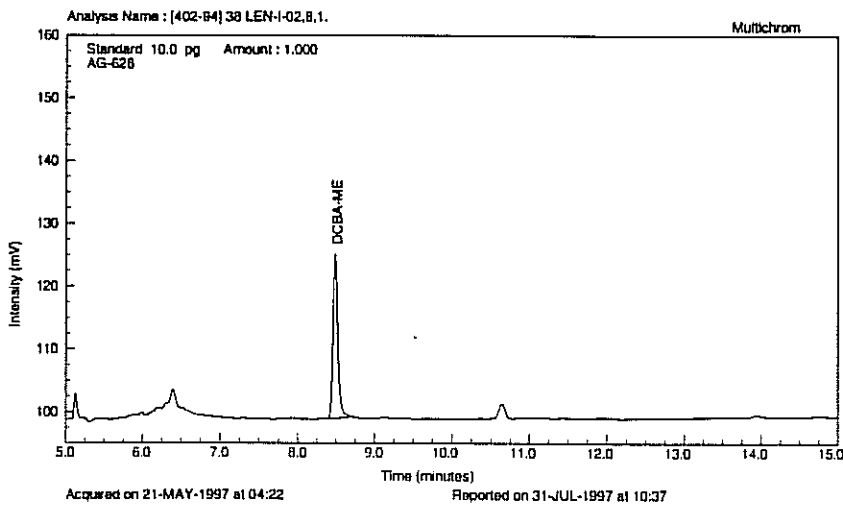


3. 2.0 pg 2,4-DCBAME injected (expressed as 2,4-DCBA equivalent)

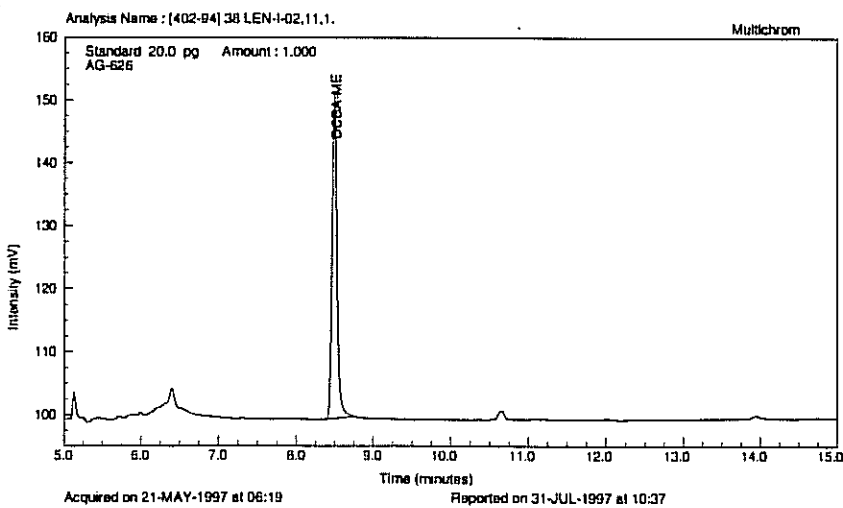
FIGURE 3. REPRESENTATIVE GC CHROMATOGRAMS FOR CALIBRATION  
STANDARDS (CONTINUED)



4. 5.0 pg 2,4-DCBAME  
injected (expressed as  
2,4-DCBA equivalent)

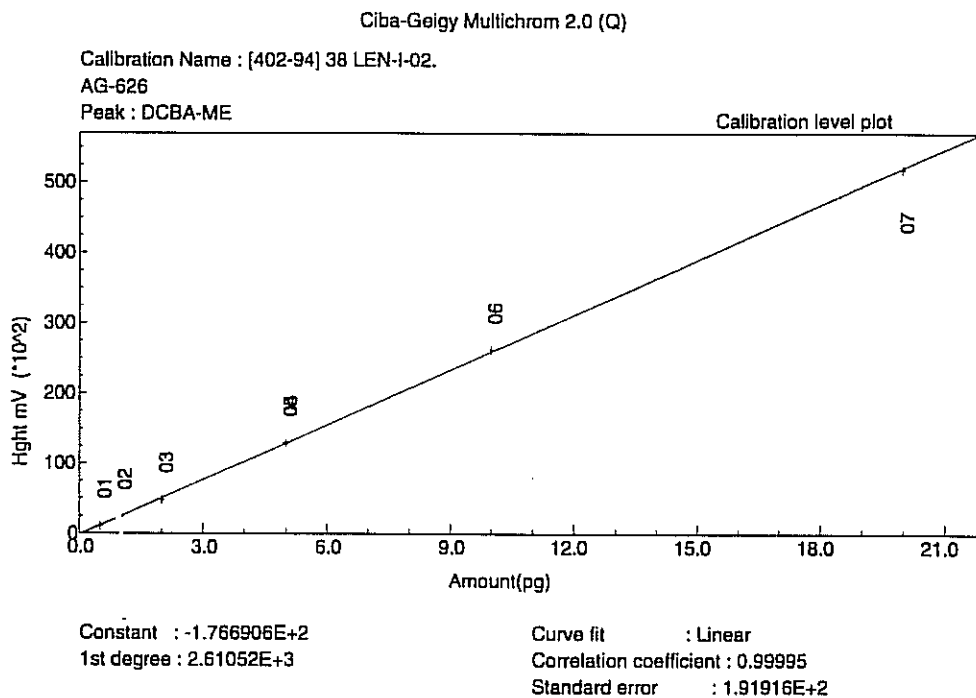


5. 10 pg 2,4-DCBAME  
injected (expressed as  
2,4-DCBA equivalent)



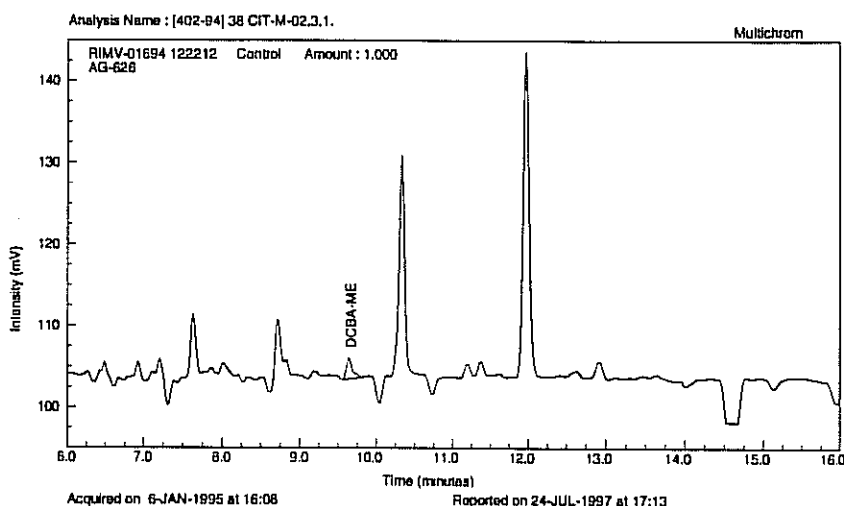
6. 20 pg 2,4-DCBAME  
injected (expressed as  
2,4-DCBA equivalent)

FIGURE 4. REPRESENTATIVE CALIBRATION CURVE

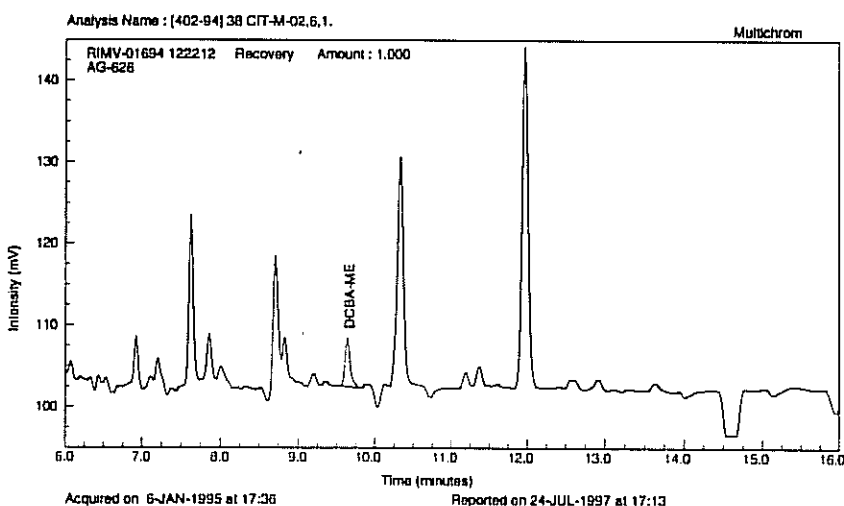


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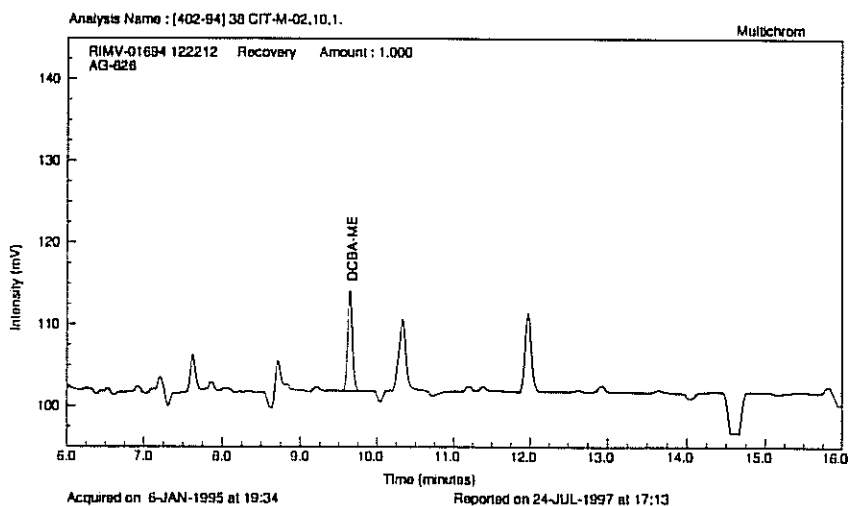
FIGURE 5. REPRESENTATIVE GC CHROMATOGRAMS FROM ANALYSIS OF CITRUS



1. CIT-01A (Set I), control, 127  $\mu$ g sample injected, 2.70 pg 2,4-DCBAME found, <0.05 ppm (0.038 ppm) propiconazole determined

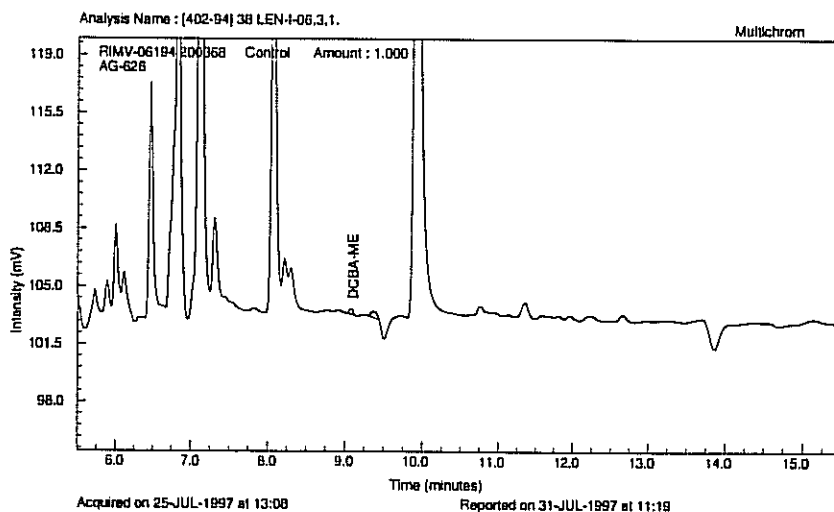


2. CIT-02A (Set I), control + 0.05 ppm propiconazole, 127  $\mu$ g sample injected, 5.84 pg 2,4-DCBAME found, 0.083 ppm propiconazole determined, 89% recovered

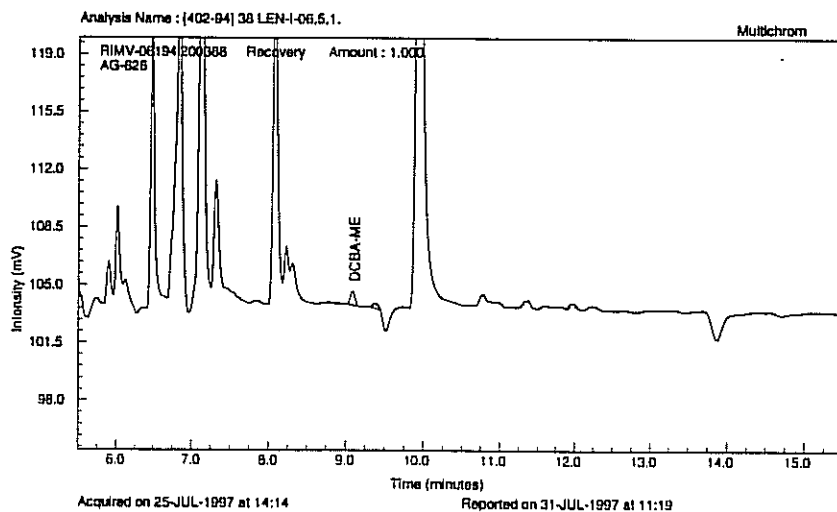


3. CIT-04A (Set I), control + 0.5 ppm propiconazole, 42.2  $\mu$ g sample injected, 11.8 pg 2,4-DCBAME found, 0.50 ppm propiconazole determined, 93% recovered

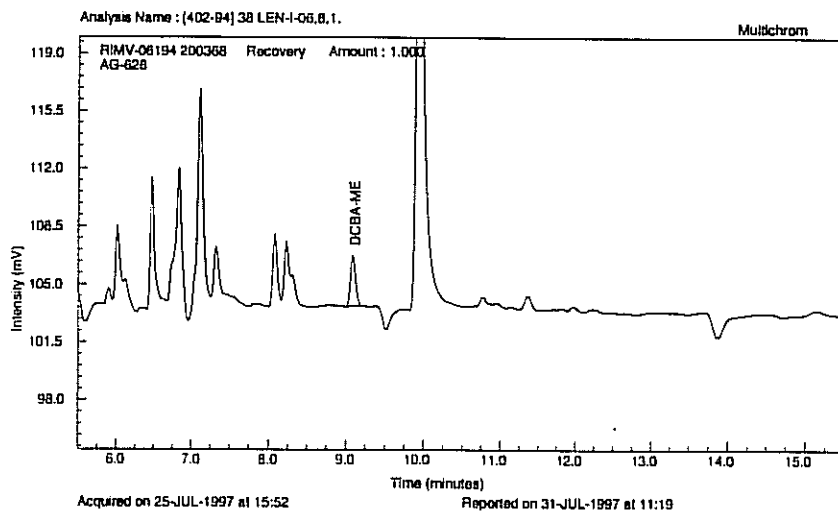
FIGURE 6. REPRESENTATIVE GC CHROMATOGRAMS FROM ANALYSIS OF LENTILS



1. LEN-01B (Set II),  
control, 29.8  $\mu$ g sample  
injected, 0.442  $\mu$ g 2,4-  
DCBAME found, <0.05  
ppm (0.027 ppm)  
propiconazole  
determined

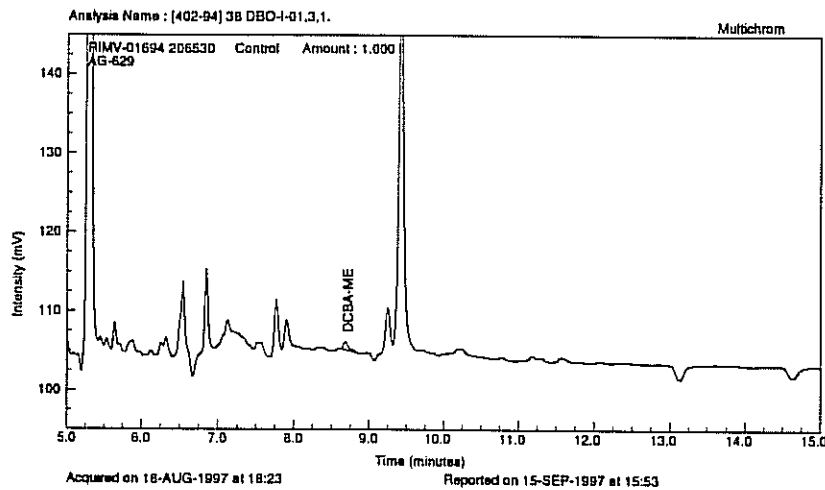


2. LEN-02A (Set II),  
control + 0.05 ppm  
propiconazole, 29.8  $\mu$ g  
sample injected, 1.21  $\mu$ g  
2,4-DCBAME found,  
0.073 ppm  
propiconazole  
determined, 92%  
recovered

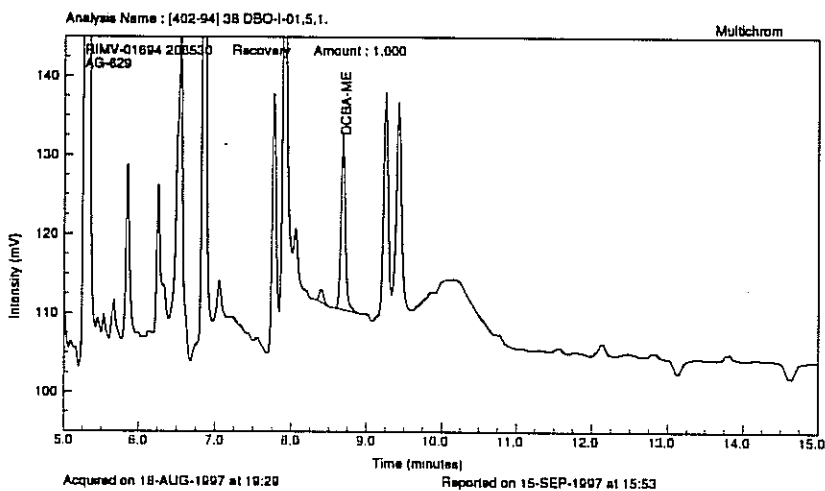


3. LEN-03A (Set II),  
control + 0.25 ppm  
propiconazole, 29.8  $\mu$ g  
sample injected, 4.71  
 $\mu$ g 2,4-DCBAME  
found, 0.28 ppm  
propiconazole  
determined, 103%  
recovered

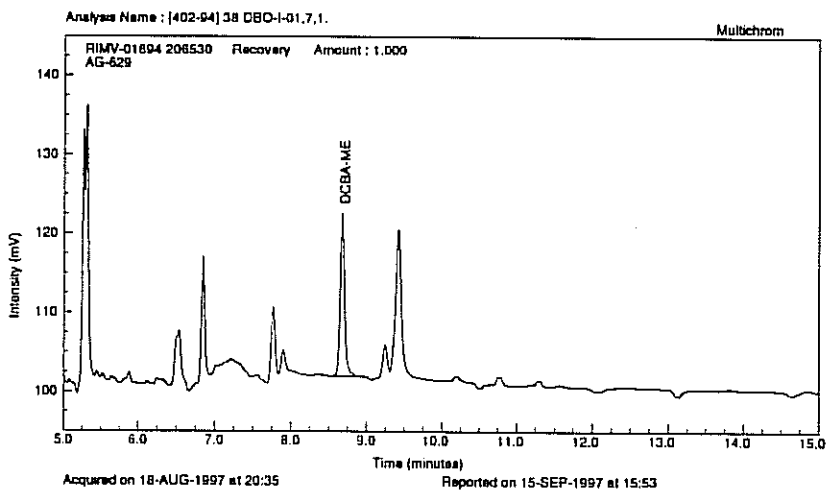
FIGURE 7. REPRESENTATIVE GC CHROMATOGRAMS FROM ANALYSIS OF DRY BULB ONIONS



1. DBO-01C (Set I), control  
42.2 µg sample injected,  
0.718 pg 2,4-DCBAME  
found, 0.030 ppm  
propiconazole determined

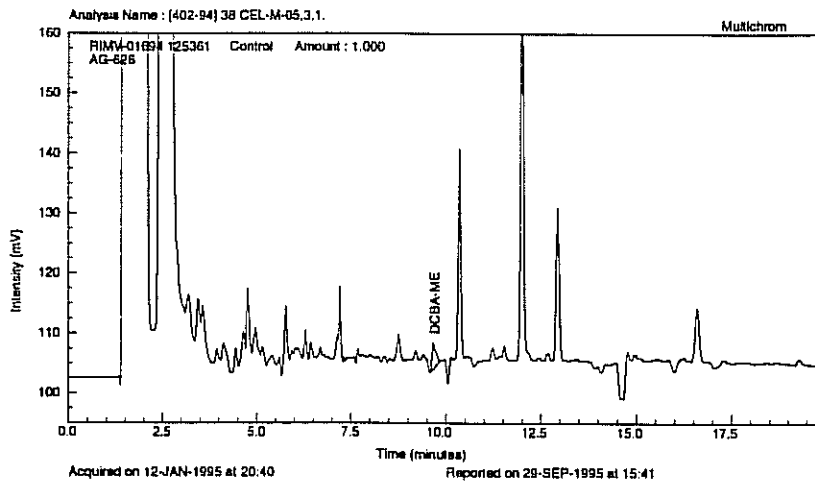


2. DBO-04B (Set I), control  
+ 0.5 ppm propiconazole,  
42.2 µg sample injected,  
12.2 pg 2,4-DCBAME  
found, 0.52 ppm  
propiconazole determined,  
98% recovered

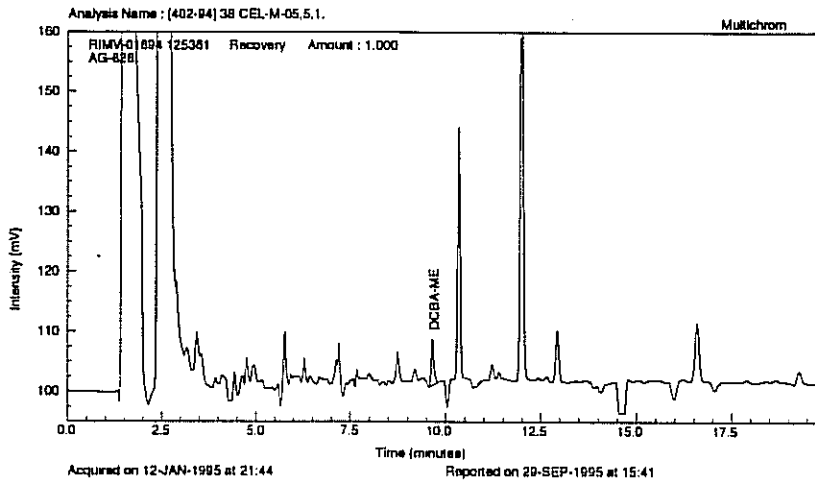


3. DBO-05 (Set I), control +  
1.0 ppm propiconazole,  
21.1 µg sample injected,  
11.5 pg 2,4-DCBAME  
found, 0.97 ppm  
propiconazole determined,  
94% recovered

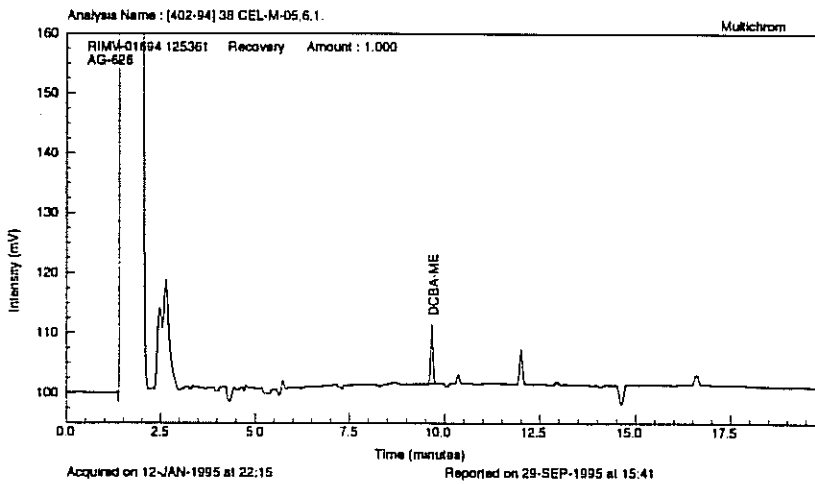
FIGURE 8. REPRESENTATIVE GC CHROMATOGRAMS FROM ANALYSIS OF CELERY



1. CEL-5-01 (Set II), control, 126  $\mu\text{g}$  sample injected, 4.26  $\mu\text{g}$  2,4-DCBAME found, 0.060 ppm propiconazole determined

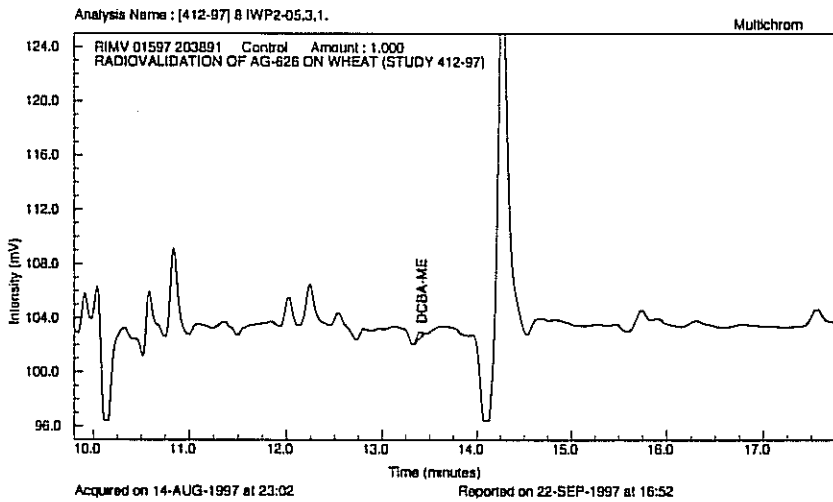


2. CEL-5-02 (Set II), control + 0.05 ppm propiconazole, 126  $\mu\text{g}$  sample injected, 7.13  $\mu\text{g}$  2,4-DCBAME found, 0.10 ppm propiconazole determined, 81% recovered

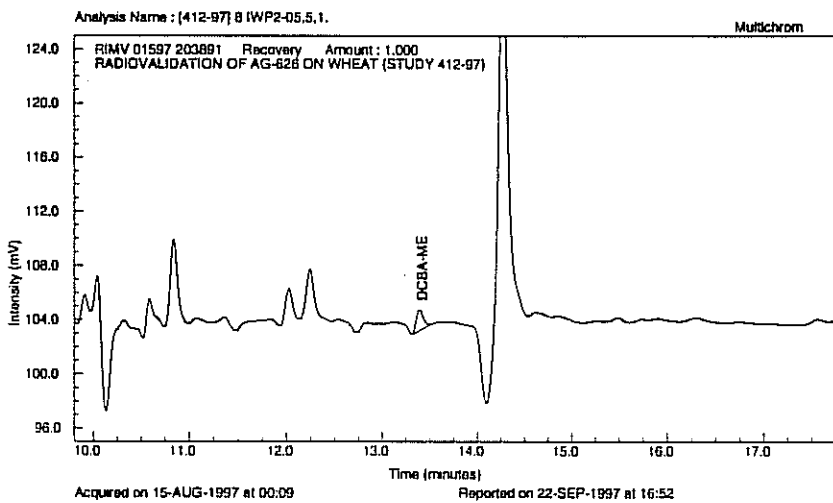


3. CEL-5-03 (Set II), control + 3.0 ppm propiconazole, 6.31  $\mu\text{g}$  sample injected, 8.92  $\mu\text{g}$  2,4-DCBAME found, 2.5 ppm propiconazole determined, 82% recovered

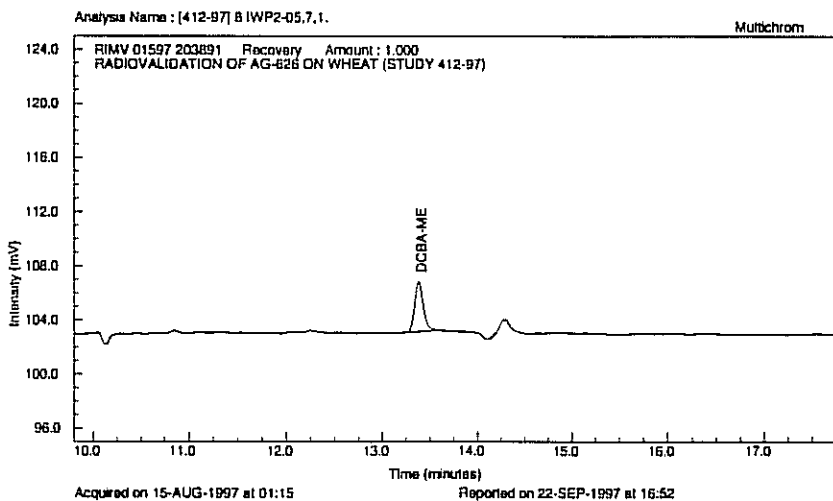
FIGURE 9. REPRESENTATIVE GC CHROMATOGRAMS FROM ANALYSIS OF IMMATURE WHEAT WHOLE PLANT



1. IWP-01B (Set II), control, 127  $\mu$ g sample injected, 3.88 pg 2,4-DCBAME found, <0.05 ppm (0.018 ppm) propiconazole determined

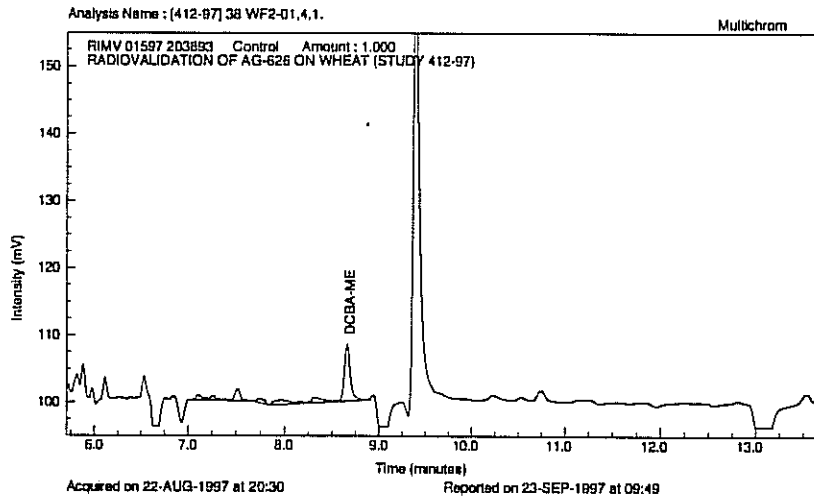


2. IWP-02B (Set II), control + 0.05 ppm propiconazole, 127  $\mu$ g sample injected, 3.88 pg 2,4-DCBAME found, 0.055 ppm propiconazole determined, 73% recovered

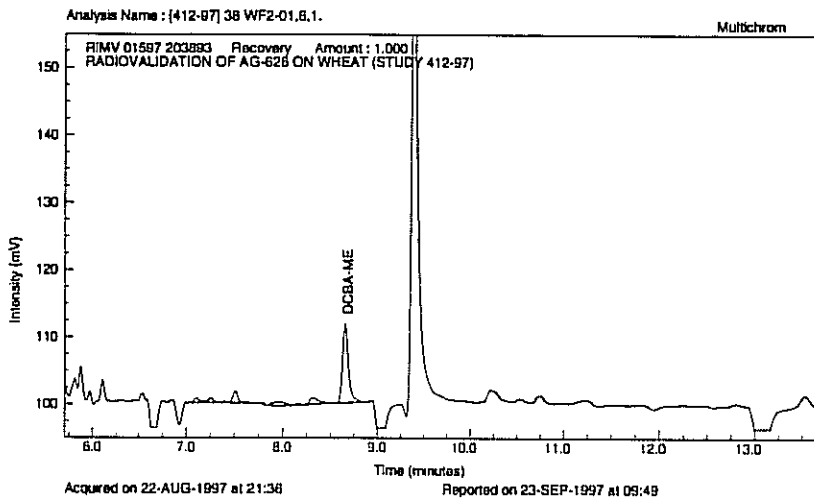


3. IWP-04 (Set II), control + 3.0 ppm propiconazole, 6.36  $\mu$ g sample injected, 9.10 pg 2,4-DCBAME found, 2.6 ppm propiconazole determined, 85% recovered

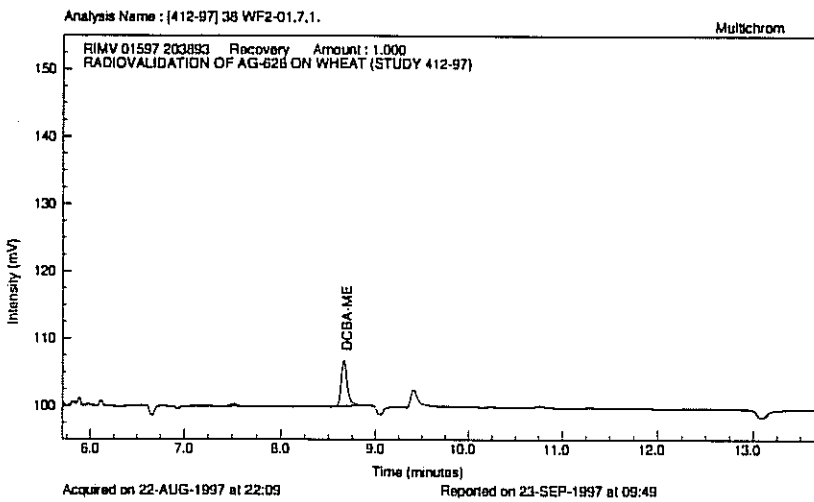
FIGURE 10. REPRESENTATIVE GC CHROMATOGRAMS FROM ANALYSIS OF WHEAT FORAGE



1. WF-01B (Set II), control, 67.2  $\mu$ g sample injected, 5.51 pg 2,4-DCBAME found, 0.15 ppm propiconazole determined

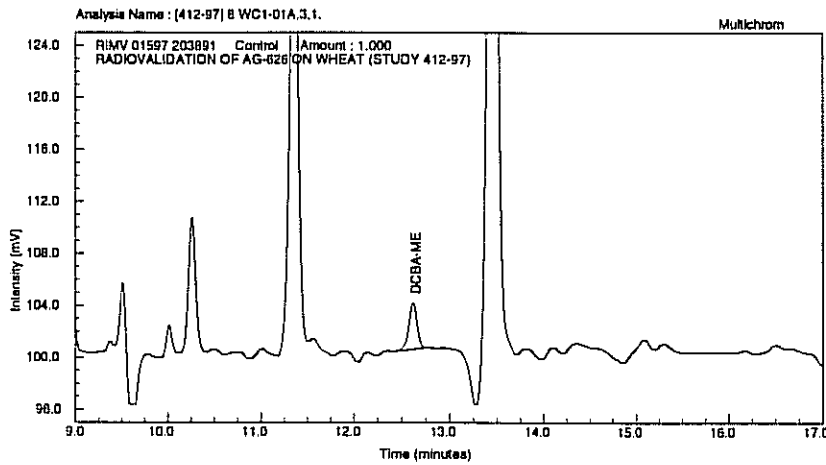


2. WF-02B (Set II), control + 0.05 ppm propiconazole, 67.2  $\mu$ g sample injected, 7.59 pg 2,4-DCBAME found, 0.20 ppm propiconazole determined, 111% recovered

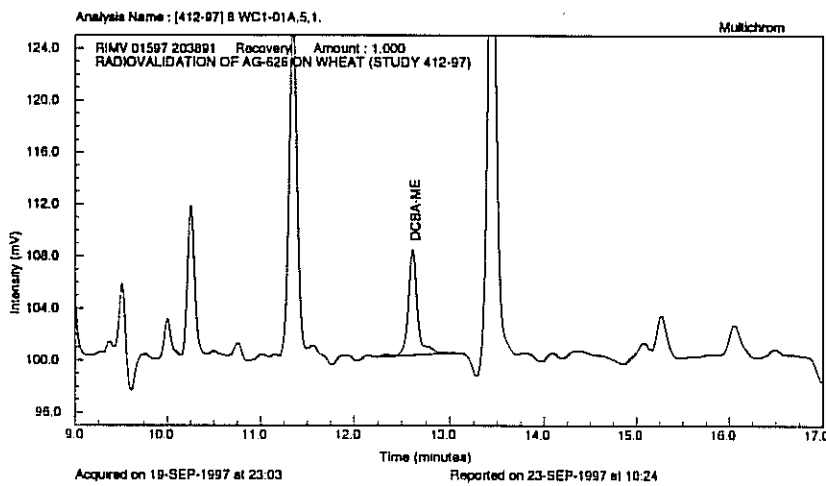


3. WF-04B (Set II), control + 3.0 ppm propiconazole, 3.36  $\mu$ g sample injected, 4.44 pg 2,4-DCBAME found, 2.4 ppm propiconazole determined, 74% recovered

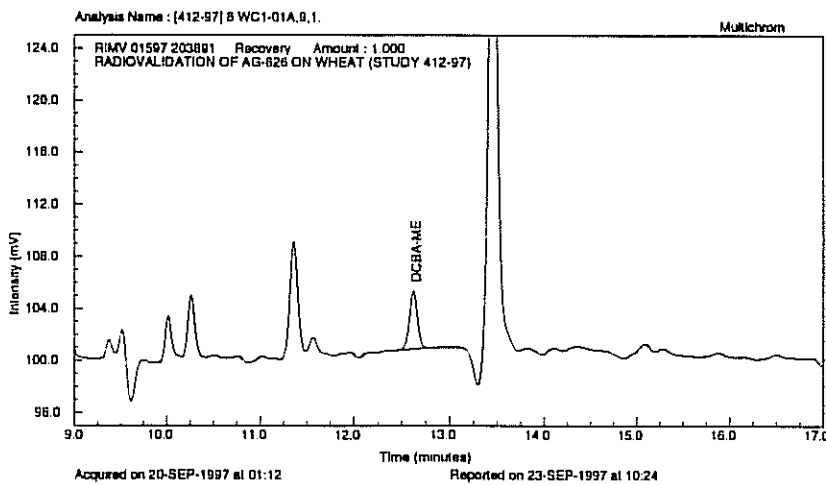
FIGURE 11. REPRESENTATIVE GC CHROMATOGRAMS FROM ANALYSIS OF WHEAT CHAFF



1. WC-01A (Set I), control, 134  $\mu$ g sample injected, 2.98 pg 2,4-DCBAME found, <0.05 ppm (0.04 ppm) propiconazole determined

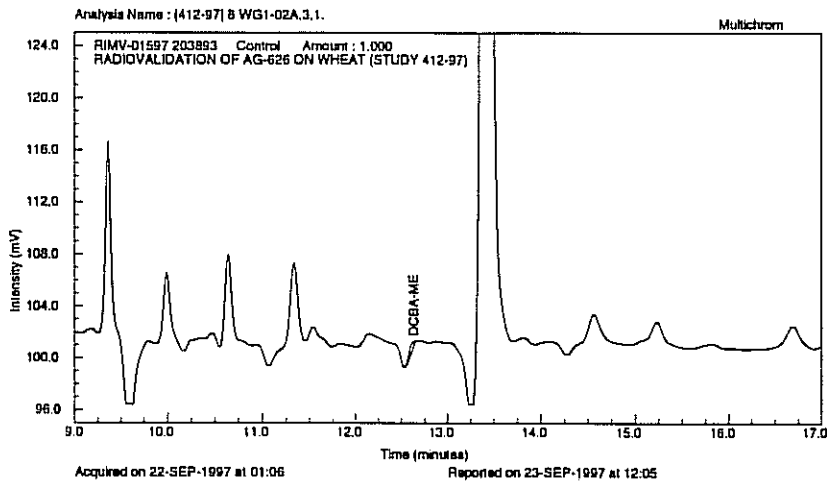


2. WC-05A (Set I), control + 0.05 ppm propiconazole, 134  $\mu$ g sample injected, 6.67 pg 2,4-DCBAME found, 0.089 ppm propiconazole determined, 98% recovered

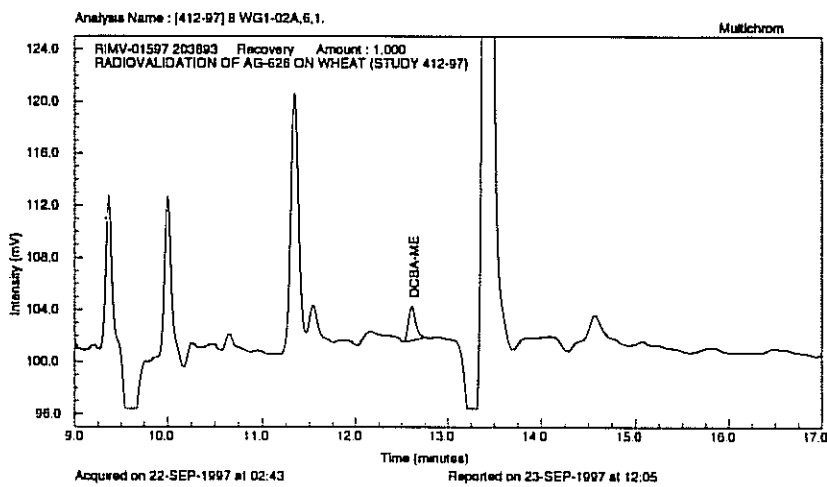


3. WC-03A (Set I), control + 0.20 ppm propiconazole, 33.5  $\mu$ g sample injected, 3.70 pg 2,4-DCBAME found, 0.20 ppm propiconazole determined, 79% recovered

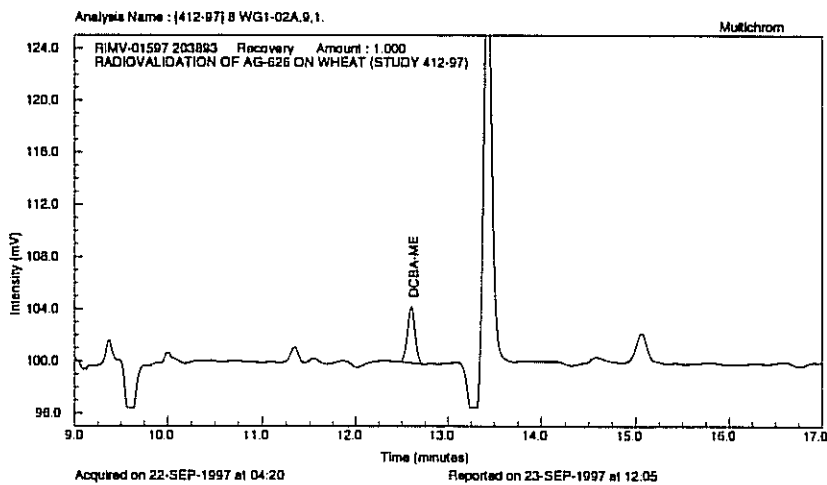
FIGURE 12. REPRESENTATIVE GC CHROMATOGRAMS FROM ANALYSIS OF WHEAT GRAIN



1. WG-01A (Set I), control, 67.0  $\mu$ g sample injected, 0.739 pg 2,4-DCBAME found, <0.05 ppm (0.020 ppm) propiconazole determined



2. WG-02B (Set I), control + 0.05 ppm propiconazole, 67.0  $\mu$ g sample injected, 2.23 pg 2,4-DCBAME found, 0.060 ppm propiconazole determined, 80% recovered



3. WG-04A (Set I), control + 0.50 ppm propiconazole, 13.4  $\mu$ g sample injected, 3.54 pg 2,4-DCBAME found, 0.47 ppm propiconazole determined, 91% recovered

VII. REFERENCES

1. J. Toth and P. Manuli, Analytical Method AG-454B, "Determination of Total Residues of Propiconazole in Crops as 2,4-Dichlorobenzoic Acid Methyl Ester by Capillary Gas Chromatography" MRID No. 41823303
2. J. A. Ross, AG-345, "Preparation of Diazomethane" MRID No. 41664505
3. Pesticide Analytical Manual (PAM) , Vol. I, 3rd Edition, Multiresidue Methods, Section 402b, Transmittal No. 94-1, 1/94.
4. K. Lin, ABR-94065, "Investigations into the methylation of 2,4-Dichlorobenzoic Acid as a part of Analytical Methods AG-517 and AG-454B" MRID No. 43825401
5. Residue Chemistry Guidelines, OPPTS 860.1340, Residue Analytical Method, USEPA, Office of Prevention, Pesticides and Toxic Substances (7101), EPA 712-C-96-174, 8/1996
6. K. Lin, ABR-95061, "Validation of Analytical Methods AG-626 and AG-629 for the Determination of Total Residues of Propiconazole in Crops and in Meat, Milk and Eggs as 2,4-Dichlorobenzoic Acid Methyl Ester by Capillary Gas Chromatography"
7. K. Lin, ABR-97092, "Validation of Analytical Method AG-626 for the Determination of Total Residues of Propiconazole in Wheat"