

VALENT U.S.A. CORPORATION
VALENT TECHNICAL CENTER
Dublin, California

**DETERMINATION OF *cis*- AND *trans*-METCONAZOLE
IN CROPS**

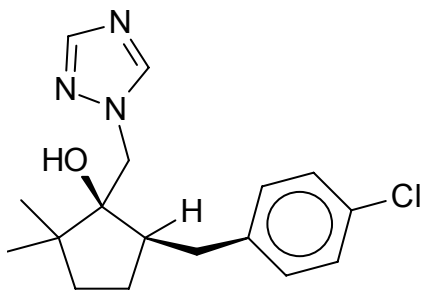
Method: RM-41C-1

Date: November 13, 2003

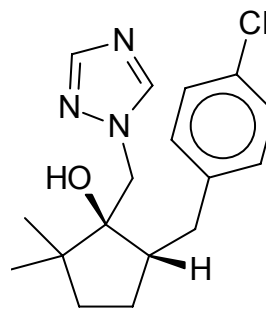
I. INTRODUCTION

This method describes the determination of *cis*- and *trans*-Metconazole, 1*RS*,5*RS*-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol and 1*RS*,5*SR*-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol, in crops. Briefly, the method involves extraction with an aqueous acetonitrile mixture (70:30 acetonitrile/water), filtration to remove solids, rotary evaporation of the acetonitrile in the filtrate to obtain an aqueous residue, and partition of the residues into hexane. The residues are cleaned up by an acetonitrile/hexane partition, the acetonitrile is removed by rotary evaporation, and the residues are then cleaned up using a C18 SPE cartridge. The column eluant is rotary evaporated and the residues are dissolved in toluene. Analysis of the extracts is performed using a gas chromatograph with a nitrogen-phosphorous detector (GC/NPD).

II. ANALYTICAL STANDARDS



cis-Metconazole reference standard
- Valent U.S.A. Corporation



trans-Metconazole reference standard
- Valent U.S.A. Corporation

cis-Metconazole Standard, 1.0 mg/mL Stock solution.

Weigh 0.100 grams (to ensure a 1.0 mg/mL concentration, correct the amount of standard weighed for the purity of the standard) into 100 mL volumetric flask. Dilute to volume with acetone, and store refrigerated.

trans-Metconazole Standard, 1.0 mg/mL Stock solution.

Weigh 0.100 grams (to ensure a 1.0 mg/mL concentration, correct the amount of standard weighed for the purity of the standard) into 100 mL volumetric flask. Dilute to volume with acetone, and store refrigerated.

cis- and *trans*-Metconazole Standard, 10 µg/mL solution (of each, in acetone).

Pipet 1.0 mL of each of the 1.0 mg/mL Stock solutions (*cis*- and *trans*-Metconazole) into a 100 mL volumetric flask and dilute to volume with acetone. Store refrigerated.

cis- and *trans*- Metconazole Standard, 1.0 µg/mL solution (of each, in acetone).

Pipet 10.0 mL of the 10 µg/mL *cis*- and *trans*-Metconazole solution (in acetone) into a 100 mL volumetric flask and dilute to volume with acetone. Store refrigerated.

cis- and *trans*-Metconazole Standard, 10 µg/mL solution (in toluene).

Pipet 1.0 mL of each of the 1.0 mg/mL Stock solutions (*cis*- and *trans*-Metconazole) into a 100 mL volumetric flask and dilute to volume with toluene. Store refrigerated.

cis- and *trans*-Metconazole Standard, 2.0 µg/mL solution (in toluene).

Pipet 10.0 mL of the 10 µg/mL solution (in toluene) into a 50 mL volumetric flask and dilute to volume with toluene. Store refrigerated.

cis- and *trans*-Metconazole Standard, 1.0 µg/mL solution (in toluene).

Pipet 10.0 mL of the 10 µg/mL solution (in toluene) into a 100 mL volumetric flask and dilute to volume with toluene. Store refrigerated.

cis- and *trans*-Metconazole Standard, 0.5 µg/mL solution (in toluene).

Pipet 5.0 mL of the 10 µg/mL solution (in toluene) into a 100 mL volumetric flask and dilute to volume with toluene. Store refrigerated.

cis- and *trans*-Metconazole Standard, 0.1 µg/mL solution (in toluene).

Pipet 10.0 mL of the 1.0 µg/mL solution (in toluene) into a 100 mL volumetric flask and dilute to volume with toluene. Store refrigerated.

Note: Similar dilutions may also be performed to generate appropriate standards.

III. REAGENTS

Acetone - Pesticide quality

Acetonitrile - Pesticide quality

Ethyl Acetate - Pesticide quality

Hexane - Pesticide quality

BAKERBOND spe Octadecyl (C18) Disposable Extraction Columns (6 mL/1000 mg size)
[J.T.Baker, Box of 30, Part # 7020-07]

Methanol – Pesticide quality

Toluene – Pesticide quality

Water - Deionized

IV. REAGENT SOLUTIONS

Acetonitrile/Water 70:30 (v/v).

Combine 7 parts acetone with 3 parts deionized water. For example, add 700 mL of acetone and 300 mL of deionized water sequentially to a reagent bottle. Store at room temperature.

Methanol/Water, 1/1 (v/v).

Combine 1 part methanol with 1 part water. For example, add 200 mL of methanol and 200 mL of water sequentially to a reagent bottle. Store at room temperature.

Methanol/Water, 5/1 (v/v).

Combine 5 parts methanol with 1 part water. For example, add 250 mL of methanol and 50 mL of water sequentially to a reagent bottle. Store at room temperature.

V. EQUIPMENT

Balances, Analytical and Top Loading

Büchner Funnels – 7 or 9 cm

Filter Flasks, Vacuum – 250 or 500 mL

Filter Funnels (approximately 100 mm diameter)

Filters, Paper - Whatman No. 1 (7 or 9 cm diameter)

Filters, Glass Fibre – Whatman GF/A (7 or 9 cm diameter)

Graduated Cylinders (1000, 250, 100, 50, 10 mL)

Heated Water Bath (temperature <35°C)

Hobart® Food Chopper (or equivalent)

Mason® Jars - pint (or equivalent)

Omni-Mixers® (or similar top-drive blender)

Pipettor, Automatic - capable of accurately dispensing volumes of 0.2 to 1.0 mL

Pipettes, Volumetric – 10.0, 5.0, and 1.0 mL

Round-bottom Flasks – 1000, 500, 250, and 100 mL

Refrigerator

Rotary Vacuum Evaporators

Separatory Funnels - 500 mL

Wiley® Mill

VI. INSTRUMENTATION

GAS CHROMATOGRAPH (GC)

Hewlett-Packard Model 5890 GC equipped with a nitrogen-phosphorus detector (GC/NPD), autosampler, and integrator (or equivalent). The conditions shown are suggested for this analysis, and these may be changed as appropriate (*see Note 1 for additional parameters*).

Column: DB - 5 (J & W Scientific, Inc.), 30 m x 0.53 mm I.D. , 1.5 µm film thickness (or equivalent).

Gases: Carrier gas : Helium, 10 mL/min.
Make-up gas: Helium, 20 mL/min.
Detector: Hydrogen, 3.4 mL/min.
Air, 110 mL/min.

Injector: 2.0 µL, glass liner with quartz wool in packed column adapter

Temperatures: Injector - 280°C
Detector - 285°C
Column Oven - Initial - 220°C Initial (Hold 2 min.)
Rate - 10°C/min.
Final - 290°C (Hold 3 min.)

Retention Times: 7.8 min. *cis*-Metconazole
8.1 min. *trans*-Metconazole (*Figure 1*)

VII. ANALYTICAL PROCEDURES

1. Sample Setup

Macerate or grind the sample using the Hobart® food chopper or the Wiley® Mill. Weigh 10 g (± 0.1 g) of the sample into a pint Mason® jar. At this point, if required by the testing facility, a control sample to be used for method recoveries may be fortified with *cis*- and *trans*-Metconazole (*see Note 2*).

2. Extraction with Acetone/Water

Add 120 mL of acetonitrile/water (70:30, v/v) into the pint jar. Blend using an Omni-Mixer® for 15 minutes. Assemble the filtration apparatus using a two layer filter, Whatman No. 1 over GF/A, in the Büchner funnel (7 or 9 cm) and a vacuum flask (250 or 500 mL). Apply vacuum and filter the mixture through the Büchner funnel, transferring both the liquid and the solids from the jar into the funnel. Rinse the jar with 20 mL of acetonitrile/water (70:30). Once the filtrate has been collected, release the vacuum to the filter flask and flood the filter cake with the rinse. Reapply vacuum and pass this rinse through the filter cake in the funnel, combining the original filtrate and the rinse.

Transfer the combined extract into a 500 mL round-bottom flask. Remove the acetonitrile by rotary evaporation using a heated water bath (temperature < 35 °C) to obtain an aqueous residue (approximately 45 mL).

3. Hexane Partition

Transfer the aqueous residue into a 500 mL separatory funnel. Rinse the round-bottom flask with 150 mL of hexane (in two or three portions), adding the hexane rinses to the separatory funnel. Partition the residues into the hexane by shaking vigorously for 1 minute. Allow the phases to separate, drain the aqueous layer back into the 500 mL round bottom flask, and drain the hexane layer into a clean 250 mL round-bottom flask (or similar vessel). Transfer the aqueous layer back into the separatory funnel, rinse the round-bottom flask with 100 mL of hexane, and transfer the rinsate into the separatory funnel. Repeat the partition, allow the phases to separate, and then drain the aqueous layer into the 500 mL round-bottom flask. Drain the hexane layer into the 250 mL round-bottom flask, combining the hexane extracts.

The 500 mL separatory funnel may be reused for the acetonitrile/hexane partition after rinsing briefly with acetone to remove water. A second clean 500 mL separatory funnel could also be used instead.

4. Acetonitrile/Hexane Partition

Decant the hexane extract back into the 500 mL separatory funnel, taking care to retain any water that accumulated in the flask. Add 150 mL of acetonitrile to the separatory funnel, shake (with appropriate venting) for 1 minute, and then allow the phases to separate. Drain the

acetonitrile layer into a clean 1000 mL round-bottom flask, add a 100 mL portion of acetonitrile to the hexane remaining in the separatory funnel, and repeat the partition. Combine the acetonitrile with the original extract in the 1000 mL round-bottom flask.

Rotary evaporate the acetonitrile (temperature <35°C), to approximately 20-30 mL. Transfer the residues to a 250 mL round-bottom flask with two or three 10 to 15 mL ethyl acetate rinses. Continue rotary evaporation, just to dryness. Add 5 mL of methanol/water (1:1, v/v) to the residues, and sonicate briefly to dissolve the residues.

5. C18 SPE Cleanup

Precondition the BAKERBOND C18 cartridge by passing 5 mL of methanol and 10 mL of methanol/water (1:1) sequentially through the cartridge (with suction). *Do not allow air to be pulled into the cartridge.* Transfer the residues in methanol/water to the preconditioned cartridge, sequentially rinsing the round-bottom flask with two additional 5-mL portions of methanol/water (1:1) to ensure quantitative transfer. Discard the accumulated eluant.

Elute the *cis*- and *trans*-Metconazole residues into a beaker (or vial) by passing 10 mL (2 x 5 mL) of methanol/water (5:1) through the 250 mL round-bottom flask, and then through the cartridge.

Transfer the eluant into a 100 mL round-bottom flask with 2 or 3 10-mL portions of ethyl acetate. Rotary evaporate to obtain an aqueous residue (temperature <35°C), and then add 50 mL ethyl acetate to azeotrope the water. Continue rotary evaporation, just to dryness. [Note that additional ethyl acetate may be required to remove the water.] Add 1.0 mL of toluene, stopper and sonicate briefly to dissolve the residues, and then transfer the final extract into an autosampler vial (or vials) for storage under refrigeration. Samples in autosampler vials may also be analyzed directly.

6. GC/NPD Measurement

Condition the instrument with at least three injections of a sample extract prior to initiating an analytical sequence. Analyze a range of at least four standard concentrations to establish the linear response of the GC/NPD, including a 0.1 µg/mL (or less) standard. [A typical set of standards would be 0.1, 0.5, 1.0, and 2.0 µg/mL; with an injection volume of 2.0 µL.] To verify the linear response, calculate the response factor for each of the four standards [by dividing the peak height (or area) of the standard by its concentration], determine the standard deviation of the four response factors, and then divide this value by the average response factor. The result (the coefficient of variation) must be 10% or less for the instrument response to be considered linear for that range of standards. Exceptions will be allowed only with supervisory approval. The calibration may also be performed using a linear fit with a non-zero intercept [this is probably required for GC/NPD analyses performed with a more polar column (such as a DB-17 column), *see Note 3*]. The linearity of the instrument response should be verified on a daily basis.

Continuing calibration standards (1.0 µg/mL) are analyzed as part of the analytical sequence. For calculation of analytical results based on the response of the continuing calibration standards, only continuing calibration standards are required to be interspersed within the sequence. If the analytical results will be calculated from a linear fit with a non-zero intercept, both continuing calibration standards and linearity standards are interspersed with the samples. Typically, the sequence is constructed with the following order: a reference standard (1.0 µg/mL), 1 or 2 sample extracts, a reference standard or linearity standard, 1 or 2 sample extracts, ..., and a reference standard. *The sequence must begin and end with reference standards. [For analytical sequences to be calculated using a linear fit with a non-zero intercept, analysis of a minimum of three continuing calibration standards is recommended.]* The coefficient of variation of the reference standard responses must be 10% or less for the analysis set to be acceptable.

If the peak area observed for a sample is greater than the peak area of the highest linearity standard, the sample extract must be diluted and the diluted extract analyzed. The sample extract must be diluted (with toluene) such that the peaks obtained are within the documented linear response range of the gas chromatograph.

7. Calculations

For analytical results based on average response factors (from the continuing calibration standards), the amount of each analyte in each sample is calculated as follows:

$$\text{Analyte Concentration, ppm (mg/kg)} = \frac{A \times B \times C \times D}{E \times F}$$

where:

- A = Sample response (Peak Area or Height)
- B = Continuing calibration standard concentration (typically, 1.0 µg/mL)
- C = Final volume (typically, 1.0 mL)
- D = Dilution factor, used if the sample extract is diluted prior to analysis
- E = Average continuing calibration standard response
- F = Sample weight (typically, 10 g)

For analytical results based on a linear fit with a non-zero intercept, see *Note 3*.

VIII. LIMIT OF DETECTION

The limit of detection (LOD) of this method is 0.01 ppm (µg/g), based on a 10 g sample with a 1.0 mL final volume and a 0.1 µg/mL linearity standard in the linearity verification.

$$\text{Limit of Detection} = \frac{1.0 \text{ mL Final Volume} \times 0.1 \text{ } \mu\text{g/mL}}{10 \text{ g}} = 0.01 \text{ } \mu\text{g/g} = 0.01 \text{ ppm}$$

IX. DISCUSSION

The method was verified by fortifying peach samples with *cis*- and *trans*-Metconazole at 0.02 and 0.1 ppm, and then analyzing the sample extracts by GC/NPD. The mean recoveries were 90 and 96% for *cis*-Metconazole, and the mean recoveries were 92 and 97% for *trans*-Metconazole. Example chromatograms are shown in *Figures 2* through *4*. The recovery data are presented in Attachments 1 and 2.

X. NOTES

1. The parameters shown below are suggested for GC/NPD analysis with a DB-17 column:

Column: DB - 17 (J & W Scientific, Inc.), 30 m x 0.53 mm I.D., 1.0 µm film thickness (or equivalent).

Gases: Carrier gas : Helium, 6 mL/min.
Make-up gas: Helium, 21 mL/min.
Detector: Hydrogen, 3.5 mL/min.
Air, 110 mL/min.

Injector: 2.0 µL, glass liner with quartz wool in packed column adapter

Temperatures: Injector - 250°C
Detector - 280°C
Column Oven - Initial - 250°C Initial (Hold 2 min.)
Rate - 10°C/min.
Final - 280°C (Hold 7 min.)

Retention Times: 6.9 min. *cis*-Metconazole
7.5 min. *trans*-Metconazole (*Figure 5*)

2. Valent Standard Operating Procedure VR-002 specifies that fortified control samples are to be analyzed with each set of samples. If the testing facility does not require concurrent analysis of fortified control samples, or if an untreated control (UTC) sample is not available, this method requirement may be waived.

Typically, samples are fortified at 0.02 ppm and/or 0.1 ppm. For example, add 0.20 mL of the 1.0 µg/mL *cis*- and *trans*-Metconazole Standard solution (in acetone) and/or 1.0 mL of the 1.0 µg/mL *cis*- and *trans*-Metconazole Standard solution (in acetone) to a 10 g sample. Method recoveries must be 70 to 120% to be acceptable, unless approved by the supervising chemist responsible for the analysis.

3. For calibration with a linear fit and a non-zero intercept, condition the instrument with at least three injections of a sample extract. Analyze a range of at least four standard concentrations *within the analytical sequence* to establish the linear response of the GC/NPD, including a 0.1 µg/mL (or less) standard. As the linear response has a non-zero intercept, the results are calculated based on the set of linearity standards. A typical set of standards would include concentrations of 0.1, 0.5, 1.0, and 2.0 µg/mL (with an injection

volume of 2.0 μL). To calculate the linear fit, the peak area and the concentration of each of the standards is input into an Excel spreadsheet.

$$\text{Excel calculates the slope for the regression line as } b = \frac{n \sum xy - (\sum x)(\sum y)}{n \sum x^2 - (\sum x)^2}$$

$$\text{and calculates the intercept for the regression line as } a = \bar{Y} - b\bar{X}$$

The slope and the intercept are calculated using a linear regression weighed by 1/concentration. Typically, the linear regression is based on standard concentration and Peak Units (Area/1000), and replicate entries are included in the data set prior to performing the linear regression in Excel (to provide weighting by 1/concentration):

Standard	Number of Entries in Data Set
2.0 $\mu\text{g/mL}$	1
1.0 $\mu\text{g/mL}$	2
0.5 $\mu\text{g/mL}$	4
0.1 $\mu\text{g/mL}$	20

The r-squared value is also calculated from this data set, and this value must be greater than 0.99 for the instrument response to be considered linear for the range of standard concentrations. In addition, the concentration calculated from the peak area of each of the standards, using the slope and intercept from the linear fit, must be within 15% of the corresponding standard concentrations.

$$\text{Analyte Concentration, ppm (mg/kg)} = \frac{\text{Extract Conc} \times \text{Final Vol} \times \text{Dilution}}{\text{Sample Weight}} = \frac{(A \times B + C) \times D \times E}{F}$$

where:

- A = Sample peak area
- B = Slope from linear regression (weighted 1/concentration)
- C = Intercept from linear regression (weighted 1/concentration)
- D = Final extract volume (typically, 1.0 mL)
- E = Dilution factor, used if the sample extract is diluted prior to analysis
- F = Sample weight (typically, 10 grams)

XI. SIGNATURES

Written by Charles Green
Charles Green

Date 11/24/03

Approved by Glenn Fujie
Glenn Fujie, Laboratory Manager

Date 11/24/03

Reviewed by Michelle Chen
Michelle Chen, QAU

Date 11/24/03

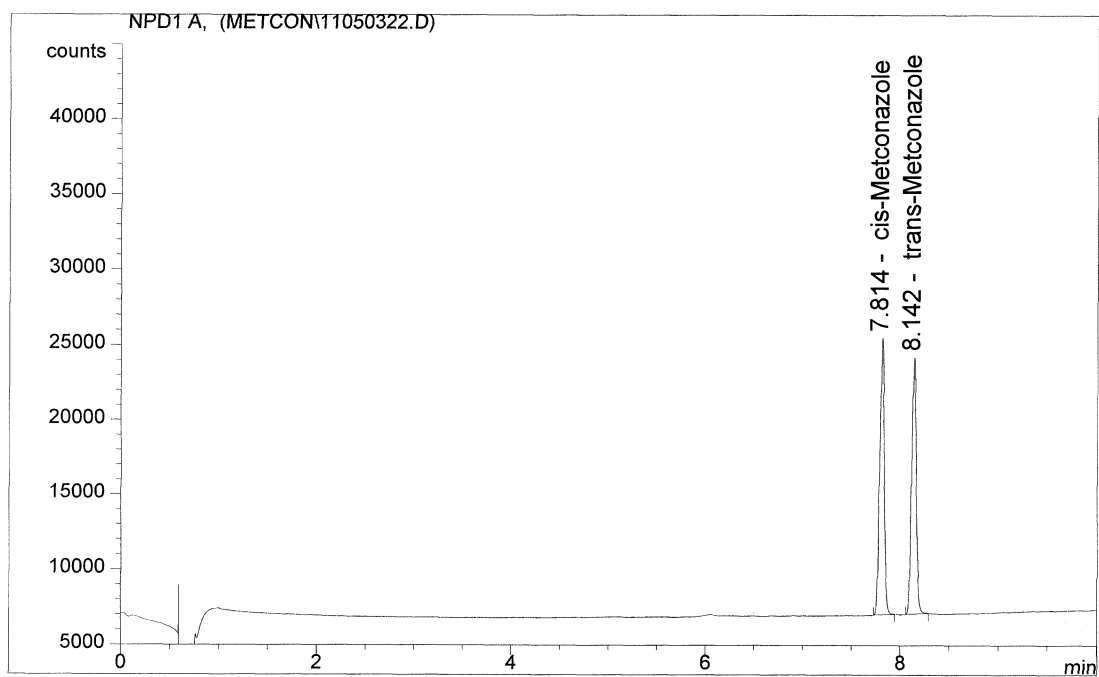


Figure 1. *cis*- and *trans*-Metconazole Standard, 1.0 $\mu\text{g}/\text{mL}$ (DB-5 Column).

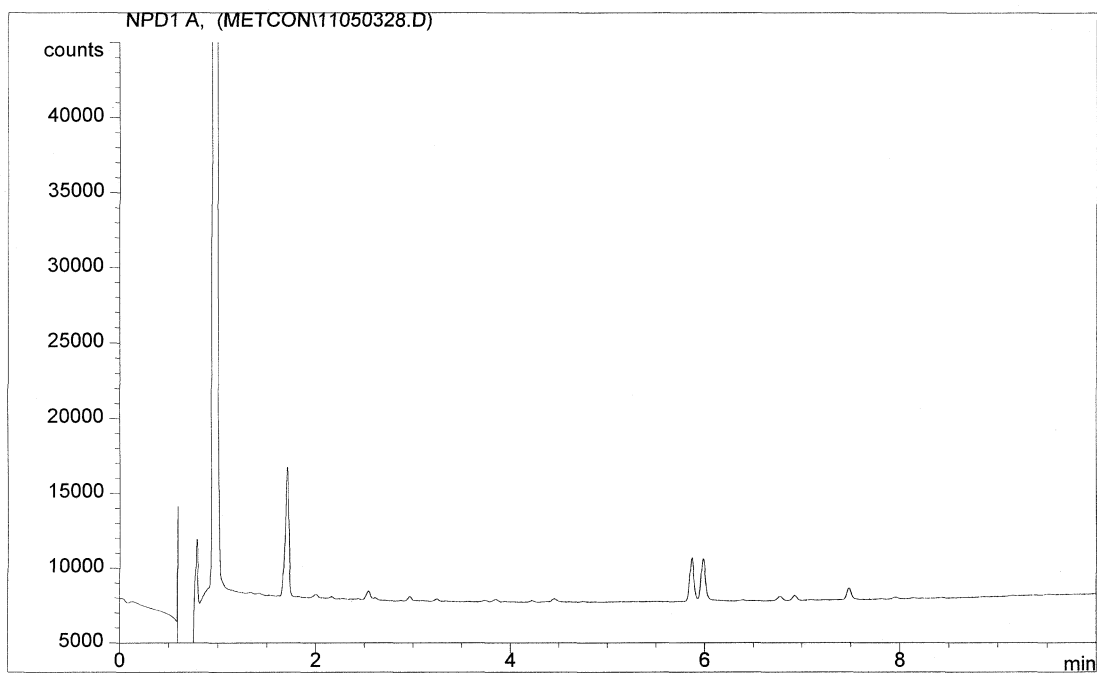


Figure 2. Control Peach Sample (DB-5 Column)

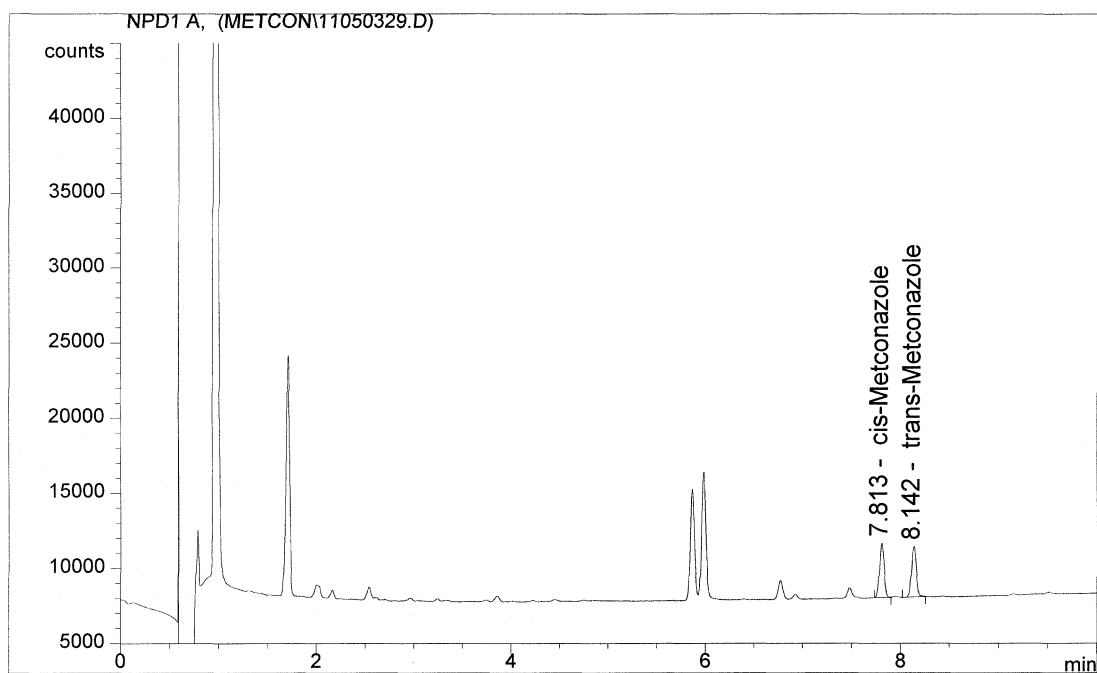


Figure 3. Fortified Control Peach Sample (0.02 ppm, DB-5 Column)

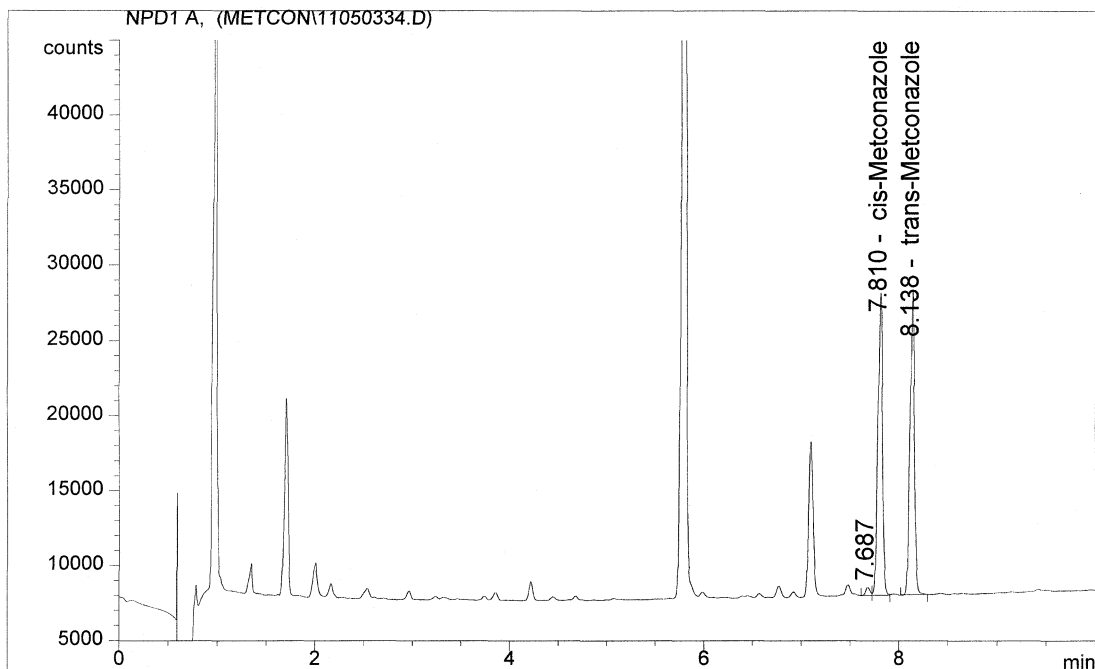


Figure 4. Fortified Control Peach Sample (0.10 ppm, DB-5 Column)

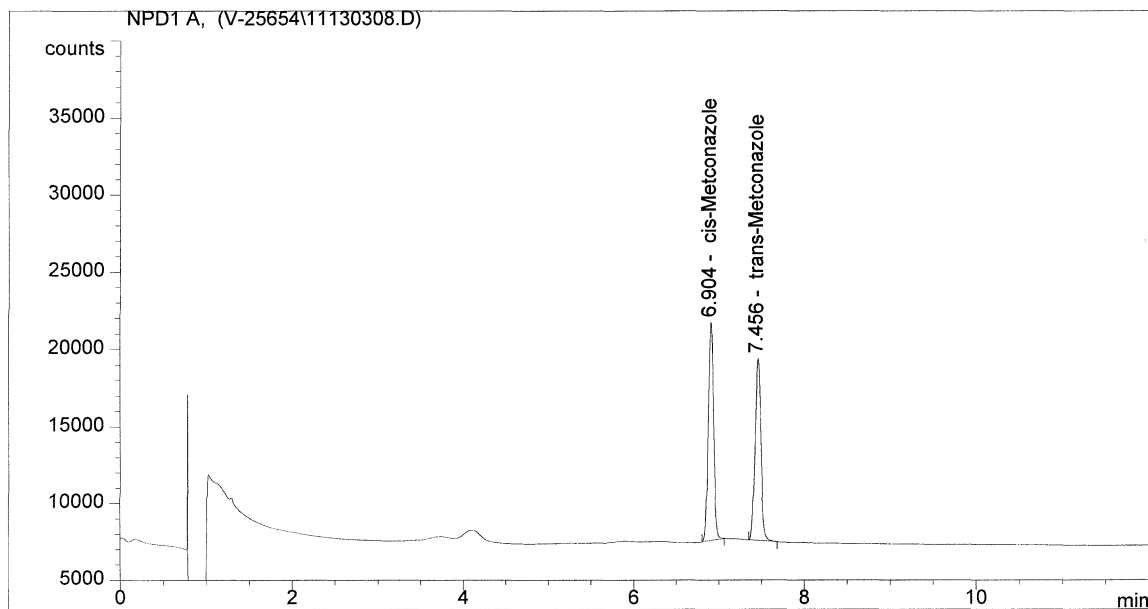


Figure 5. *cis*- and *trans*-Metconazole Standard, 1.0 µg/mL (DB-17 Column).

ATTACHMENT 1

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

VALIDATION REPORT FOR RESIDUE METHOD **RM-41C-1**
 REPRODUCIBILITY OF ANALYSIS (REF. SOP VR-002)

DETERMINATION OF RESIDUES OF *cis*-Metconazole IN CROPS

CHEMICAL: *cis*-Metconazole

Sample Matrix	Sample ID	Extraction Date	Fortification Level (ppm)	Result (ppm)	Percent Recovery	
Peaches	F1	11/3/03	0.020	0.0183	91.7	Mean = 90.3% CV = 3.4% n = 3
Peaches	F2	11/3/03	0.020	0.0174	86.8	
Peaches	F3	11/3/03	0.020	0.0185	92.4	
Peaches	F4	11/3/03	0.100	0.0984	98.4	Mean = 95.6% CV = 2.2% n = 6
Peaches	F5	11/4/03	0.100	0.0939	93.9	
Peaches	F6	11/4/03	0.100	0.0948	94.8	
Peaches	F7	11/4/03	0.100	0.0935	93.5	
Peaches	F8	11/4/03	0.100	0.0948	94.8	
Peaches	F9	11/4/03	0.100	0.0979	97.9	

ppm = mg/kg

Bill Kumboly
Analyst

11-13-03
Date

Charles Green
Approved by

11/13/03
Date

Michelle Chen
Reviewed by

11-17-03
Date

ATTACHMENT 2

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

VALIDATION REPORT FOR RESIDUE METHOD RM-41C-1
 REPRODUCIBILITY OF ANALYSIS (REF. SOP VR-002)

DETERMINATION OF RESIDUES OF *trans*-Metconazole IN CROPS

CHEMICAL: *trans*-Metconazole

Sample Matrix	Sample ID	Extraction Date	Fortification Level (ppm)	Result (ppm)	Percent Recovery	
Peaches	F1	11/3/03	0.020	0.0181	90.7	Mean = 91.7% CV = 4.4% n = 3
Peaches	F2	11/3/03	0.020	0.0177	88.3	
Peaches	F3	11/3/03	0.020	0.0192	96.1	
Peaches	F4	11/3/03	0.100	0.1028	102.8	Mean = 97.1% CV = 4.9% n = 6
Peaches	F5	11/4/03	0.100	0.0906	90.6	
Peaches	F6	11/4/03	0.100	0.0985	98.5	
Peaches	F7	11/4/03	0.100	0.0939	93.9	
Peaches	F8	11/4/03	0.100	0.0952	95.2	
Peaches	F9	11/4/03	0.100	0.1018	101.8	

ppm = mg/kg

Bill Kowalsky
Analyst

11-13-03
Date

Charles J. Green
Approved by

11/13/03
Date

Michen Chan
Reviewed by

11-17-03
Date