SAM No. 1810

BIOCHEMISTRY DEPARTMENT
CIBA-GEIGY CORPORATION
GREENSBORO, N.C.

PAGE 1 of 32	METHOD NO. AG-454A	SUBJECT DETERMINATION OF TOTAL RESIDUES OF PROPICONAZOLE IN CROPS AS 2,4-DICHLOROBENZOIC ACID BY CAPILLARY GAS CHROMATOGRAPHY
EDITION 12/8/86		
SUBMITTED BY: J. Toth, P. J. Manuli		
John Toth P. J. Manuli		APPROVED BY: M. W. Chang

I. SUMMARY/INTRODUCTION

Ia. SCOPE

This method is used for the determination of total residues of propiconazole, 1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole in crops as 2,4-dichlorobenzoic acid (DCBA) (see Figure 1 for structures). Analytical Method Ag-454A is an updated version of AG-454. In this new version, the following modifications and additions were included:

Ia.1. Several analytical steps and statements were clarified;

Ia.2. The validation results were expanded to include the latest recovery data; and

Ia.3. This version was written to conform to the new requirements according to EPA publication, "Pesticide Assessment Guidelines, Subdivision O, Addendum 2, Residue Chemistry, Series 171-4, Analytical Method(s), Magnitude of the Residue: Crop Field Trials and Storage Stability Study."

Ib. PRINCIPLE

Samples are extracted by refluxing with 20% concentrated ammonium hydroxide/methanol for one hour. The mixture is cooled and filtered. An aliquot of the extract is evaporated to dryness, and the residue dissolved in NaOH. The sample is then heated for one hour and fifteen minutes with potassium permanganate, where propiconazole and its metabolites are converted to 2,4-dichlorobenzoic acid. After addition of water, the sample is partitioned with 10% diethyl ether/hexane. The organic phase containing 2,4-dichlorobenzoic acid is evaporated to dryness and derivatized with diazomethane in the presence of dodecane which acts as a keeper to reduce losses, due to the volatility of the derivative, in subsequent steps. The derivative is cleaned up using an acidic alumina Sep-Pak®. The cleaned extract is analyzed by capillary gas chromatography.

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The limit of detection for the method is 0.05 ppm expressed as propiconazole equivalents.

The flow diagram for the method is shown in Figure 2.

II. MATERIALS/METHODS

IIa. Equipment

IIa.1 Concentration tubes, 50 ml (Fisher Catalog No. 05-538-40B, Kimax Brand or equivalent).

IIa.2 Cotton, absorbent (Fisher Catalog No. 07-900 or equivalent).

IIa.3 Distillation column, Snyder, 3 ball (Kontes Catalog No. K-503000-012 or equivalent).

IIa.5 Food chopper, Hobart or equivalent.

IIa.6 Funnel, 12.5 cm. size.

IIa.7 Glascol heating mantle, 500 ml.

IIa.8 Multi-Blok Heater, (Cole-Parmer, Catalog No. J-3128-00 or Thomas, Catalog No. 5891-C10 or equivalent).

IIa.9 N-evap or equivalent.

IIa.10 Rotary evaporator, Buchi or equivalent.

IIa.11 Sample vials, GC autosampler.

IIa.12 Sample concentrator (Thomas, Catalog No. 4367-B20 or equivalent)

IIa.13 Separatory funnel, 125 ml with Teflon stopcock.

IIa.14 Sep-Pak, acidic alumina (Waters Associates, Catalog No. 51800).

IIa.15 Syringe, 25 ml, LuerLok®.

IIa.16 Test tubes, 24/40 joint, 18.5 cm x 22 mm. (Ace Glass Co., Catalog No. 8645-38) or equivalent.

IIa.17 Thermometer, -10 to 360°C.

IIa.18 Thermometer, -20 to 110°C.

IIa.19 Variable transformer, Powerstat.

IIa.20 Vortex mixer or equivalent.

IIa.21 Stirring rods, 10 x 300 mm.

IIb. Reagents and Standards

IIb.1 Acetone Pesticide Grade (Fisher Catalog No. A-40-4 or equivalent).

IIb.2 Ammonium hydroxide, concentrated (Fisher Catalog No. A-669 or equivalent).

IIb.3 20% (v/v) Ammonium Hydroxide, concentrated/ methanol.

IIb.4 Propiconazole analytical standard.

IIb.5 Diazomethane, diethyl ether solution, prepared according to AG-345[1].

IIb.6 2,4-Dichlorobenzoic acid (DCBA), Aldrich Chemical Co., Catalog No. 13957-2.

IIb.7 Diethyl ether, distilled in glass (American Scientific, Catalog No. 106-40 or equivalent).

IIb.8 10% (v/v) Diethyl ether in hexane.

IIb.9 Distilled water.

IIb.10 Dodecane, 99%, Aldrich Chemical Co., Catalog No. D22, 110-4.

IIb.11 1% (w/v) Dodecane in acetone.

IIb.12 Ethyl acetate, Certified Grade (Fisher Scientific, Catalog No. E145-4 or equivalent).

IIb.13 1.0% (v/v) distilled water in ethyl acetate.

IIb.14 Hexane, HPLC Grade (Fisher, Catalog No. H302-4 or equivalent).

IIb.15 Methanol, Certified Grade (Fisher, Catalog No. A412-4 or equivalent).

IIb.16 Potassium permanganate, Reagent Grade (Fisher, Catalog No. P287).

IIb.17 Sodium meta-bisulfite, reagent grade, Baker.

IIb.18 Sodium hydroxide, reagent grade, Baker.

IIb.19 Sodium hydroxide, one normal solution.

IIb.20 Hydrochloric acid, reagent grade.

IIb.21 Hydrochloric acid, six normal solution.

IIc. Analytical Procedure

IIc.1 Preparation of Sample

IIc.1.1 Grind 300-400 g of crop sample using a Hobart food cutter and dry ice. Dry samples such as grains or nut meats are milled using a Wiley Mill.

IIc.2 Extraction and Fortification

IIc.2.1 Weigh a 15-g representative sample (Section IIc.1.1) into a 500-ml round bottom flask.

IIc.2.2 Fortification of one or more control samples will be performed at this step.

IIc.2.2.1 Prepare the fortification standard by dissolving 100 ± 0.1 mg propiconazole (using an analytical balance) in 100 ml of hexane in a volumetric flask. Make serial dilution of the standard such that the fortification volume will not exceed 1.0 ml.

IIc.2.2.2 Add parent propiconazole standard in 1.0 ml or less of hexane to the control samples before extraction.

IIc.2.2.3 Let the spiked samples stand for at least 30 minutes before adding extraction solvent.

IIc.2.3 Add 200 ml of 20% conc. ammonium hydroxide/methanol. Fit the flask to a reflux condenser and heat under reflux for one hour using a Glascol heating mantle with a variable transformer setting of 65. Allow to cool to room temperature.

IIc.2.4 Filter the extract through a Reeve Angel Grade 802 filter paper inside a Whatman 2V filter paper into an 8 oz. bottle.

IIc.2.5 Transfer a 0.225 g crop equivalent aliquot (3.0 ml) to a 24/40 test tube (18.5 cm x 22 mm) and add 0.1 ml of conc. acetic acid. Concentrate the solution to dryness at a temperature $\leq$ 40°C.

The acetic acid prevents possible losses of parent propiconazole during the concentration step.

IIc.3 Potassium Permanganate Reflux

IIc.3.1 Add 0.4 g of potassium permanganate to the test tube.

IIc.3.2 Add 6 ml of 1N sodium hydroxide. Stopper and mix well on a vortex mixer.

NOTE: After addition of NaOH and $KMnO_4$ and mixing well, the sample color must be dark purple. If sample appears to be brownish to dark green, additional $KMnO_4$ must be added in increments of 0.1 g. Mix sample well each time.

IIc.3.3 Rinse sides of test tube with 2 ml of 1N sodium hydroxide. Add boiling chips.

IIc.3.4 Place test tubes fitted with Snyder columns on a heating block which has been pre-heated to 125°C, and heat for one hour and fifteen minutes.

IIc.3.5 Add 5 ml of water through the top of the Snyder column, and allow to cool for 15 minutes.

IIc.3.6 Add 6 g of sodium meta-bisulfite, stopper and mix well on a vortex mixer. Sample will gradually turn white.

IIc.3.7 Add 14 ml of 6N hydrochloric acid slowly. Sample will effervesce. Mix sample carefully using glass stirring rod until completely clear.

NOTE: Stained glassware which has been used in the $KMnO_4$ reaction may be rinsed with a dilute solution of sodium meta-bisulfite to remove stains. Continue with usual wash and rinse.

IIc.4 Diethyl Ether/Hexane Partition

IIc.4.1 Transfer the sample solution in the test tube to a 125-ml separatory funnel. Add 15 ml of 10% (v/v) diethyl ether/hexane and stopper. Partition for one minute and allow the layers to separate. Drain the lower aqueous layer back into the test tube. Transfer the organic phase through a filter tube or powder funnel containing absorbent cotton into a 100-ml round bottom flask.

IIc.4.2 Repeat the partition with 15 ml of 10% diethyl ether/hexane.

IIc.4.3 Rinse the separatory funnel with 10 ml of 10% diethyl ether/hexane. Use this and an additional 20 ml of 10% diethyl ether/hexane to rinse the absorbent cotton.

IIc.4.4 Add 2 ml of 1% (w/v) dodecane solution in acetone to the flask and evaporate to dryness using a rotary evaporator (bath temperature $\leq 40^{\circ}C$).

NOTE: The added dodecane coats the surface of the round bottom flask uniformly after the evaporation step and acts as a keeper to reduce volatility losses of the methyl derivative of 2,4-dichloro-benzoic acid during the subsequent evaporation steps.

IIc.5 Derivatization with Diazomethane

IIc.5.1 To the residues remaining after evaporation of the diethyl ether/ hexane extract of Step IIc.4.4, add 2 ml of diazomethane/diethyl ether reagent solution (AG-345[1]).

IIc.5.2 Allow the solution to stand for at least 30 minutes with occasional gentle swirling. Add more diazomethane as required to maintain a yellow color.

CAUTION: Add diazomethane inside a well ventilated hood. Extreme care should be exercised in handling diazomethane because of the potential toxic effects and explosion hazards on contact with sharp or rough surfaces.

IIc.5.3 Evaporate the diethyl ether off after the derivatization using a rotary evaporator at room temperature (do not use a bath). Continue the evaporation at room temperature for an additional one and no longer than two minutes to ensure the complete removal of diethyl ether.

NOTE: Evaporation at higher temperature to total dryness may cause losses of the derivative.

IIc.6 Alumina Sep-Pak Cleanup

IIc.6.1 Fit an acidic alumina Sep-Pak cartridge to the LuerLok end of a 25 ml syringe with the plunger removed (see Figure 3).

IIc.6.2 Add 10 ml of 1.0% (v/v) water/ethyl acetate to the barrel of the syringe and allow it to flow by gravity through the Sep-Pak. (A rate of 2-3 ml per minute is normal).

NOTE: The 1.0% (v/v) water/ethyl acetate wash is used to deactivate the acidic alumina.

IIc.6.3 Prewash the Sep-Pak cartridge with 10 ml of hexane by gravity flow.

IIc.6.4 Dissolve the residue from IIC.5.3 in 5 ml of hexane and transfer into the barrel of the syringe. Elute the hexane through the Sep-Pak by gravity flow.

IIc.6.5 Rinse the round bottom flask with 7 ml of 10% (v/v) diethyl ether/hexane and transfer to the barrel of the syringe. Elute the acidic alumina Sep-Pak by gravity flow, collecting the eluant in a 50-ml concentration tube.

NOTE: Distilled in glass diethyl ether is used for the elution steps.

IIc.6.6 Add hexane to bring to a volume of 10.0 ml. Dilute with hexane if necessary.

IId. Instrumentation

IId.1 Description

The sample in Step IIc.6.6 is analyzed by capillary gas chromatography using an electron capture detector. The gas chromatographic conditions are given in Table I.

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IId.2 Operating Conditions

See Table No. 1

IId.3 Calibration

IId.3.1 The GC system should be calibrated with each analytical run, by checking the retention time of DCBA methyl ester. The retention time should not vary by more than ± 2% on a daily basis, otherwise the system should be inspected and proper maintenance should be performed in order to achieve the narrow range of variation.

IId.3.2 Method of Calculation

The gas chromatograph is standardized by injecting 2 µl aliquots of the diluted DCBA-methyl ester solutions during residue analysis. This represents a working range of 0.5 to 10.0 picograms of the derivative (expressed as dichlorobenzoic acid).

IIe. Interference(s)

IIe.1 Sample matrices. Analytical Method AG-454A has been used to analyze a large variety of crop matrices (Table II). No interferences (<0.05 ppm) were found in corn grain, soybean beans, wheat grain, peanut nutmeat, beans or peas and the majority of corn fodder, forage or soybean hay samples. A few corn forage, corn fodder and soybean hay samples showed a maximum of 0.15 ppm and an average of 0.083 ± 0.034 ppm (N = 8) of propiconazole residues detected as DCBA-methyl ester. These residues could have been real propiconazole

residues resulting from contamination due to drift from adjacent treated plots. Rotational celery stem control samples showed a maximum of 0.13 ppm total residue with an average of 0.10 ± 0.029 ppm (N = 6). This again might have resulted from contamination.

IIe.2 No interferences from chemicals having permanent or Section 18 tolerances in or on pecans, peanuts, grapes or apples were detected when determined as 2,4-dichlorobenzoic acid. Two studies were conducted (ABR-83075[2] and ABR-85040[3]). In one of the studies, ABR-85040, maximum tolerance level amounts of the pesticide chemicals having permanent tolerances or Section 18 tolerances in or on grapes and apples were subjected to the procedures of Analytical Method AG-445[4]", Determination of CGA-71818 Residues in Grapes and Apples by Conversion to 2,4-Dichlorobenzoic Acid and Analysis by Capillary Gas Chromatography." Analytical Methods AG-454A and AG-445 are nearly identical in every step with slight differences in the length of basic permanganate hydrolysis time (1.25 vs. 2.0 hours), in the partition solvents (diethyl ether/hexane vs. methyl tert-butyl ether) and in the Sep-Pak columns (acidic alumina vs. silica gel). Propiconazole and CGA-71818 are also very similar in chemical structures and properties. In the second study (ABR-83075), pesticidal chemicals having permanent or Section 18 tolerances in or on pecans or peanuts were subjected to the analytical procedures of AG-356[5] which was an earlier version of AG-454A. No interferences were detected.

IIe.3 No interference from the solvents used in this method has been detected.

IIe.4. The roto evaporator should be rinsed with fresh acetone solvent between each sample evaporation to eliminate possible cross contamination.

IIf. Confirmatory Techniques

IIf.1 GC/MS according to Analytical Method AG-356[5].

IIg. Time Required

IIg.1 A total of twelve hours is needed. This includes the cooling of hot extract and the actual injection time. When several sets of samples are being worked up, many steps can be overlapped and performed concurrently.

IIh. Modifications

IIh.1 None

IIi. Preparation of Standard 2,4-Dichlorobenzoic Acid Methyl Derivative

IIi.1 Calibration Factors

IIi.1.1 Weigh 20.0 mg of 2,4-dichlorobenzoic acid into a 200-ml volumetric flask.

IIi.1.2 Add 3 ml of diazomethane as in Steps IIc.5.1 to IIc.5.2.

IIi.1.3 Bring to volume with hexane. The standard solution of the derivative is 100 ng/µl expressed as 2,4-dichlorobenzoic acid equivalents. Serial dilutions of the standard solution are made with hexane until working solutions containing 0.25, 0.50, 1.0, 2.5, and 5.0 picograms per microliter are achieved.

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IIi.1.4 Inject 2 µl aliquots of the diluted solutions during residue analysis. This represents a working range of 0.5 to 10.0 picograms of the derivative (expressed as dichlorobenzoic acid).

IIi.1.5 Determine the peak height for the injected standards. Typical chromatograms of standards are shown in Figure 4.

IIi.1.6 Construct a standard curve by plotting detector response versus picograms injected or enter the standardization data into an appropriate electronic calculator (e.g., Hewlett-Packard Model HP-11C) or a computer system (e.g., HP-1000 Lab Automation System [LAS]) which utilizes integration software to calculate a least square standard curve. A typical standard curve is shown in Figure 5.

IIi.2 Detection of Sample Residues

IIi.2.1 Inject a 2-µl aliquot of the sample in Step IIc.6.6 into a gas chromatograph equipped with an electron capture detector. Make appropriate dilutions of the sample to have the sample peak height within the range of the standard curve. Compare peak heights of unknown samples with the standard curve, manually or by either using an electronic calculator or a computer system as mentioned in IIi.1.6, to determine the amounts of

the derivative in the aliquot injected. Typical chromatograms of checks and recovery samples are shown in Figures 6 through 9.

IIi.2.2 Calculate residue results as ppm equivalents of propiconazole using the following equation:

$$\text{PPM Found} = \frac{\text{Amount 2,4-Dichlorobenzoic Acid Found (pg)}}{\text{(mg injected)}\ \ (1000\ \text{pg/ng})} \times 1.79$$

Correct the ppm found in recoveries by subtracting the ppm found, real or apparent, in the controls. Calculate the recovery factor by the following equation:

$$R = \frac{\text{Corrected PPM Found in Fortified Sample}}{\text{PPM Added}}$$

where R is the recovery factor determined using a fortified control sample carried through the procedure and is expressed as a decimal (100% = 1.00, etc.). If the recovery is >100%, use the factor 1.00.

Correct the propiconazole ppm found in samples by the following equation:

$$\text{Corrected ppm} = \frac{\text{(PPM Found in Sample)}}{R}$$

The factor 1.79 is used to convert residues of 2,4-dichlorobenzoic acid found into propiconazole equivalents.

IIj. DISCUSSION

IIj.1 Preparation of diazomethane should be carried out as specified in AG-345.

IIj.2 The average recovery of propiconazole from crop samples fortified at 0.05 to 2.0 ppm was 88.2 ± 15.0%; N = 118.

IIj.3 The extraction procedure used in this method was shown in ABR-83078[6] to remove a significant portion of the total ^{14}C-residue (88%, 89%) present in winter wheat straw and corn stalks. Accountability of the total method was tested, using five propiconazole related metabolites present in crops. Average recovery of all metabolites was 93 ± 11% (see Table III). In addition, the method was validated using ^{14}C-treated crop. Validation details are presented in ABR-85021[7].

IIj.4 Corn fraction samples, feedstock, meal, flour, soap-stock and oils can be analyzed by this method. Soapstock samples require cooling of sample in refrigerator after extraction, and filtering of sample while cool to remove precipitate. It also requires 1.0 g of potassium permanganate.

IIj.5 For pineapple samples, refer to Analytical Method AG-448[8].

III. RESULTS AND DISCUSSION

IIIa. Accuracy

IIIa.1 The average recovery was 88.2 ± 15.0%, N = 118, at a fortification range of 0.05 ppm to 2.0 ppm. No dependency of percent recovery on fortification level was found.

IIIb. Precision

IIIb.1 Not performed.

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IIIc. Limits of Detection and Quantitation

IIIc.1 The limit of detection in crops is 0.05 ppm detected as 2,4-DCBA and reported as propiconazole equivalents.

IIId. Ruggedness

Testing not performed.

IIIe. Limitation

None

IV. REFERENCES

1. Ross, J. A., AG-345, "Preparation of Diazomethane."

2. R. K. Williams, P. J. Manuli, J. A. Ross, ABR-83075, "Specificity of Analytical Method AG-356 for the Determination of Total Residues of CGA-64250 as 2,4-Dichlorobenzoic Acid (Methyl Ester)."

3. W. T. Beidler, J. A. Ross, ABR-85040, "Specificity of Analytical Method AG-445 for the Determination of the Total Residues of CGA-71818 in Grapes and Apples."

4. P. Manuli, J. W. Smith, S. G. Burge, Ag-445, "Determination of CGA-71818 Residues in Grapes and Apples by Conversion to 2,4-Dichlorobenzoic Acid and Analysis by Capillary Gas Chromatography."

5. K. Balasubramanian, B. Gold, M. W. Cheung, AG-356, "Determination of Total CGA-64250 Residues in Crops by Conversion to 2,4-Dichlorobenzoic Acid and Analysis by Gas Chromatography - Mass Spectrometry."

6. Nixon, W. B., Rhoads, W. D., ABR-83078, "Validation of Analytical Methods AG-356, AG-407, and AG-415 for the Determination of Residues of CGA-64250 in Crops by Conversion to 2,4-Dichlorobenzoic Acid."

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IV. REFERENCES (continued)

7. Perez, R., Toth, J., ABR-85021, "Validation of Analytical Methods AG-448 and AG-454 for the Determination of Residues of Propiconazole in Crops by Conversion to 2,4-Dichlorobenzoic Acid."

8. Perez, R., Toth, J., AG-448, "Determination of Total Residues of CGA-64250 in Pineapples as 2,4-Dichlorobenzoic Acid by Capillary Gas Chromatography."

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TABLE I: CAPILLARY GAS CHROMATOGRAPHIC CONDITIONS

Instrument: Hewlett-Packard Model 5880 Capillary Gas Chromatograph with Model 7672A Automatic Sampler.

Carrier Gas: Helium, flow adjusted to give 17 psi (1-2 ml per minute).

Makeup Gas: 5% argon/methane, 30 ml per minute.

Column: J & W capillary, DB-5, 30 meter, 0.25 µm film thickness, 0.32 mm i.d.

Injection: Splitless.

Detector: Electron capture.

Temperatures:

Injector: 250°C
Detector: 300°C

Oven Program and Run Table

PROGRAM·	(ANNOTATION OFF)
10	VALVE 6 ON
15	SIGNAL C DEVICE# 5
20	OVEN TEMP 60
30	OVEN TEMP EQUIB TIME 1
40	OVEN TEMP INITIAL VALUE 60
50	OVEN TEMP INITIAL TIME 1
60	OVEN TEMP PRGM RATE 30
70	OVEN TEMP FINAL VALUE 125
80	OVEN TEMP FINAL TIME 5
90	OVEN TEMP POST VALUE 240
100	OVEN TEMP POST TIME 8
110	ATTN 2,10

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TABLE I: CAPILLARY GAS CHROMATOGRAPHIC CONDITIONS
(continued)

```
120          ATTN 2↑10 DEVICE# 15
130          CHART SPEED 0.2
140          % OFFSET 10
150          RUN TIME ANNOTATION OFF
160          RUN TBL ANNOTATION OFF
170          REPORT ANNOTATION ON
180          REPORT ON
190          DELETE RUN TBL
200          DELETE REPORT - TBL
210          PEAK WIDTH 0.04
220          THRESHOLD 1
230          RUN TIME 0 VALUE 6 ON
240          RUN TIME 0.1 INTG OFF
250          RUN TIME 0.5 VALVE 6 OFF
260          RUN TIME 6.71 ATTN 2↑4
270          RUN TIME 6.72 CHART SPEED 2
280          RUN TIME 6.73 ZERO
290          RUN TIME 6.74 % OFFSET 10
300          RUN TIME 6.9 INTG ON
310          RUN TIME 6.91 RUN TIME ANNOTATION ON
320          RUN TIME 6.7 ATTN 2↑2 DEVICE# 5
330          RUN TIME 7.4 INTG OFF
340          RUN TIME 7.41 RUN TIME ANNOTATION OFF
350          RUN TIME 7.51 VALVE 6 ON
360          OVEN TEMP ANNOTATION OFF
370          EDIT AUTO SEQ 1,2
380          SIGNAL C DEVICE# 1
390          AREA %
```

Minimum Detection Limit: 0.5 picogram

Volume Injected: 2 µl

Retention Time: 8.06 ± 0.1 minutes

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TABLE II: TYPICAL PROPICONAZOLE RECOVERIES

Substrate	Fortification Range (ppm)	Average Recovery (%)
Wheat (including wheat straw and grain)	0.10 - 1.0	79.8 ± 3.8 (n = 4)
Soybean (including soybean hay, dry beans, and fractions [hulls, meal, crude oil, refine oil, refine B.H. oil, R.B.H.D. oil and soapstock]	0.05 - 2.0	91.7 ± 16.0 (n = 15)
Corn (including silage, fodder, grain, ears, and fractins [feed stock, meals, and flour]	0.05 - 2.0	87.4 ± 15.5 (n = 58)
Celery (stems)	0.05 - 2.0	86.0 ± 14.6 (n = 17)
Peanut (including hay and nuts)	0.05 - 1.0	99.5 ± 12.2 (n = 6)
Beans and Peas (including hay, kidney beans, pinto beans, and lima beans)	0.05 - 2.0	87.8 ± 14.5 (n = 18)

Overall recovery = 88.2 ± 15.0 (n = 118)

Reference: AG-As 8863; 8796, 2-3; 8669; 8642; 8583, 1-2; 8621, 1-2; 8596; 8589, 1-2, 8578; 8570; 8566, 1-2; 8560, 1-2; 8544, 1-2; 8471; 8459, 1-2, 8386, 1-2; 8304, 1-3; 8054, 1-4; 8027, 2; 8008, 1-2; 8745; 8693; 8599; 8542; 8160; 7976, 1-2; 8739; 8698; 8629; 8614; 8597; 8567; 8457; 8456, 1-2; 8441, 1-2; 8437

TABLE III: RECOVERIES OF RELATED METABOLITES

Metabolite	Recovery
CGA-91305, alkanol	101%
CGA-91304, ketone	76%
CGA-104284, olefin	105%
CGA-118244, β-hydroxy	89%
CGA-121676, γ-acid	93%

Average recovery 93 ± 11%

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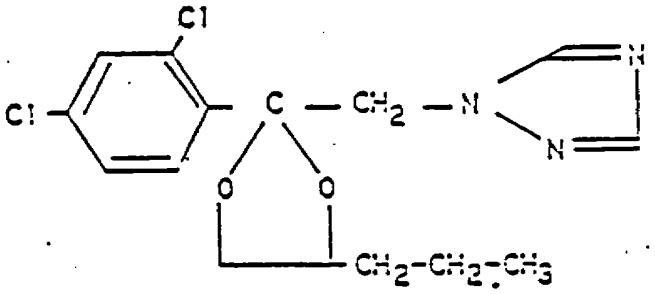
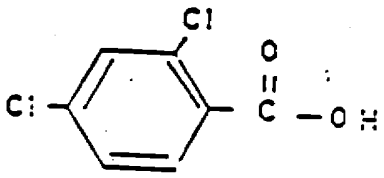
FIGURE 1: STRUCTURES

CGA-64250



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



2,4-Dichlorobenzoic Acid

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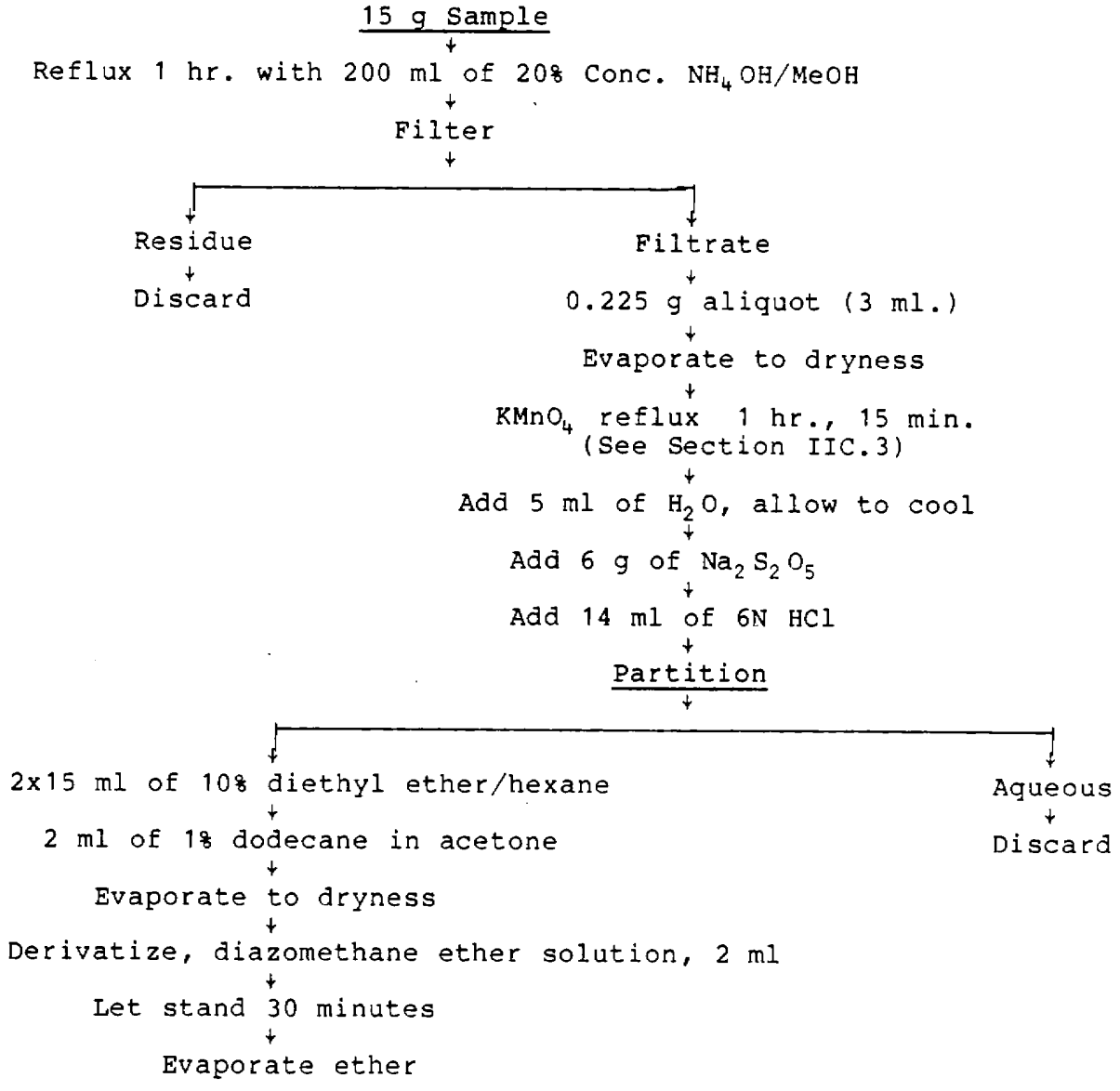
EDITION		
SUBMITTED BY: J. Toth, P. J. Manuli		
		APPROVED BY:

FIGURE 2: FLOW DIAGRAM OF METHOD



(Continued on following page)

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FIGURE 2: FLOW DIAGRAM FOR THE DETERMINATION OF TOTAL CGA-64250 (Continued)

Sep-Pak Cleanup

↓

Prewash acidic alumina with 10 ml of 1.0% (v/v) water/ethyl acetate, and 10 ml of hexane.

↓

Load sample in 5 ml of hexane, and elute.

↓

Rinse sample flask with 7 ml of 10% diethyl ether/hexane, transfer to column and elute.

↓

Dilute to 10.0 ml.

↓

Analyze by Capillary GC.

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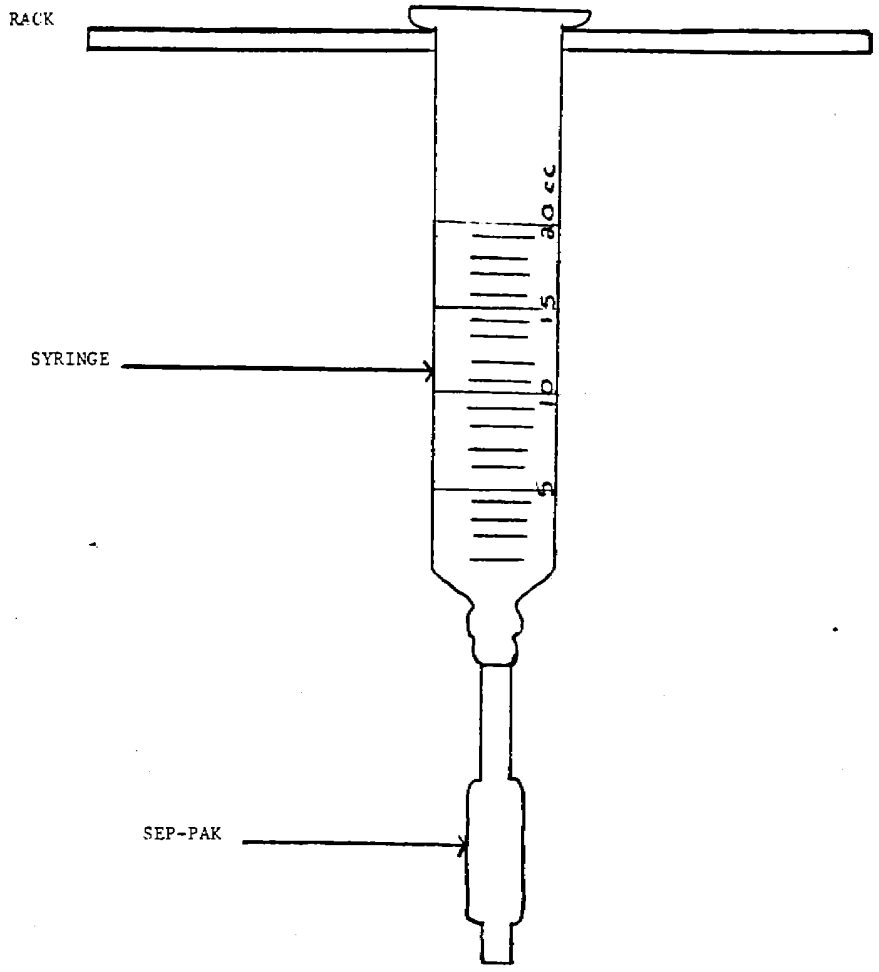
		APPROVED BY:

FIGURE 3: DIAGRAM OF SEP-PAK CLEANUP



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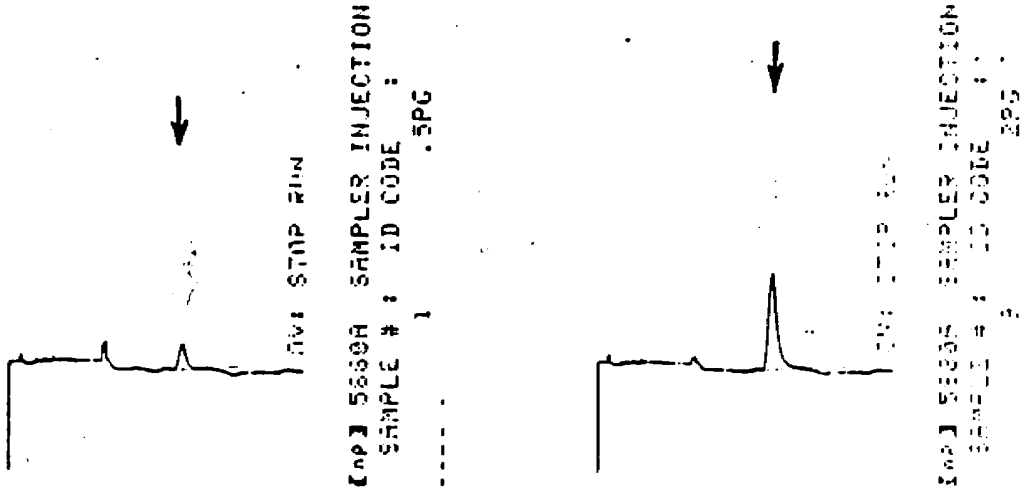
		APPROVED BY:

FIGURE 4: TYPICAL STANDARD CHROMATOGRAMS OF 2,4-DICHLOROBENZOIC ACID METHYL DERIVATIVE



0.5 pg. Standard

2.0 pg. Standard

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FIGURE 5: TYPICAL STANDARD CURVE

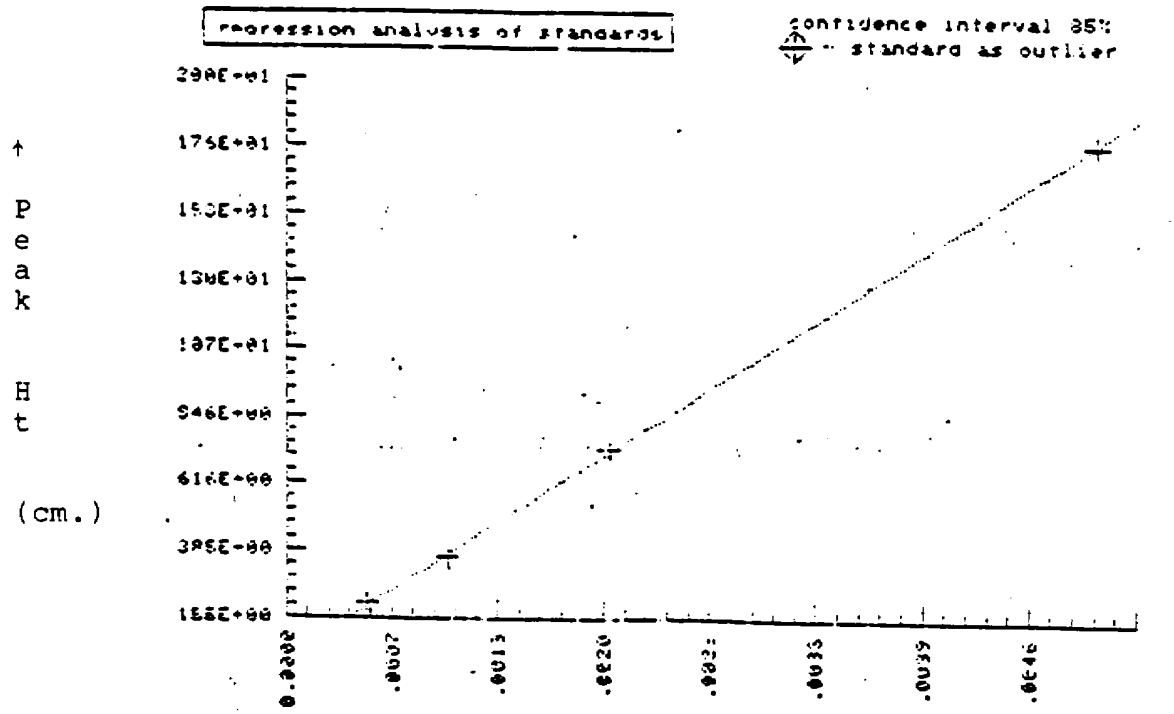

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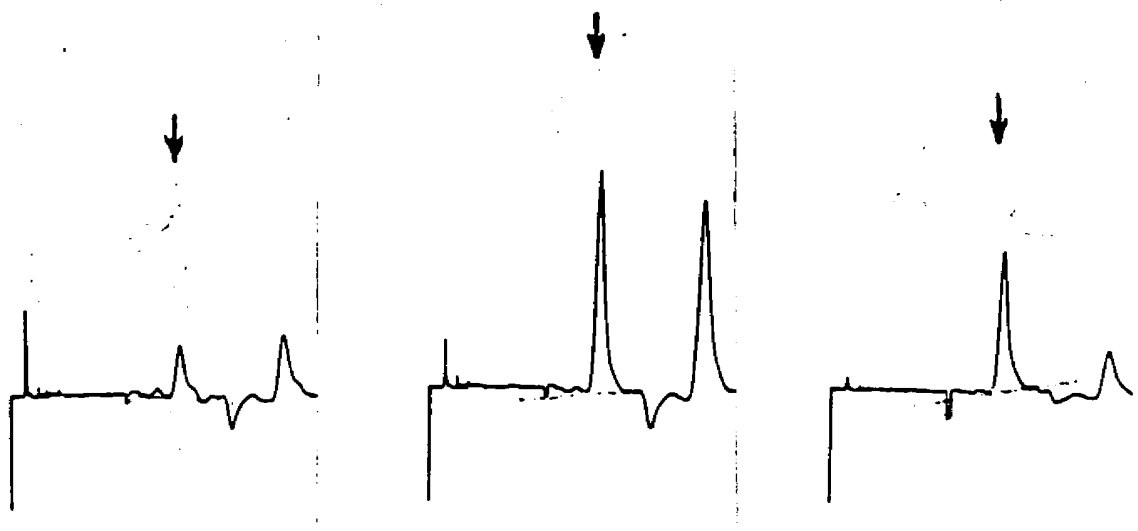
FIGURE 6: TYPICAL CHROMATOGRAMS FOR THE DETERMINATION OF RESIDUES OF PROPICONAZOLE IN CELERY



Check sample
0.045 mg injected
Found: 0.07 ppm
of propiconazole

Check + 0.2 ppm
0.045 mg injected
Found: 0.26 ppm
of propiconazole
95% recovery

Sample 3-1B
0.022 mg injected
Found: 0.34 ppm
of propiconazole

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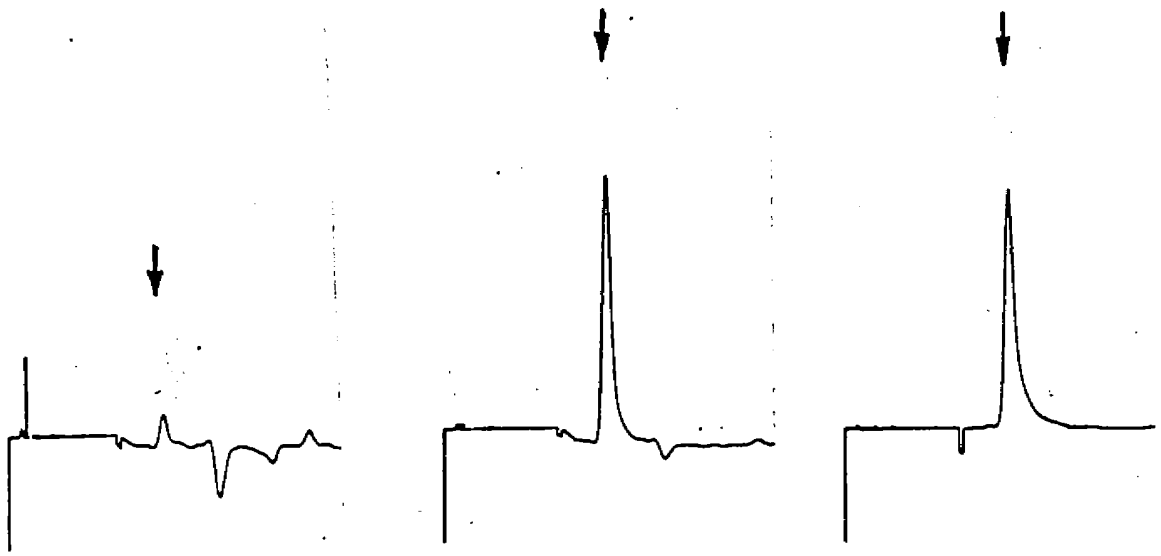
FIGURE 7: TYPICAL CHROMATOGRAMS FOR THE DETERMINATION OF RESIDUES OF PROPICONAZOLE IN CORN SILAGE



Check sample
0.045 mg injected
Found: <0.05 ppm
of propiconazole

Check + 1.0 ppm
0.018 mg injected
Found: 0.85 ppm
of propiconazole
81% recovery

Sample 6-1A
0.001 mg injected
Found: 9.3 ppm
of propiconazole

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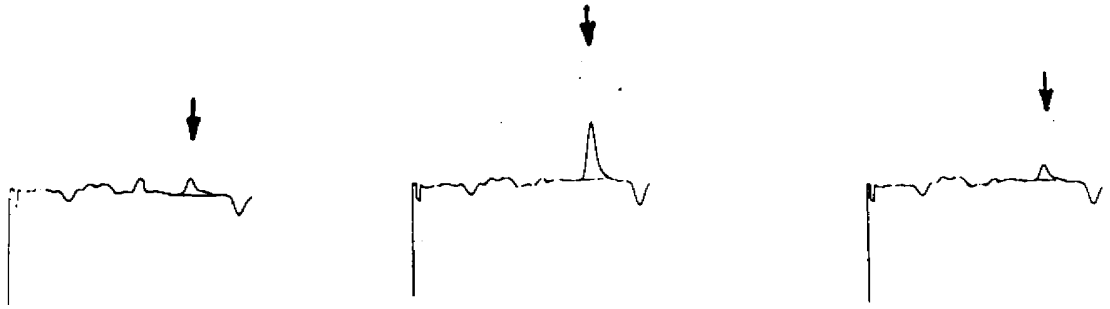
		APPROVED BY:

FIGURE 8: TYPICAL CHROMATOGRAMS FOR THE DETERMINATION OF RESIDUES OF PROPICONAZOLE IN WHEAT GRAIN



Check sample
0.045 mg injected
Found: <0.05 ppm
of propiconazole
Ref. AG-A 8661, 02

Check + 0.05 ppm
0.045 mg injected
Found: 0.05 ppm
of propiconazole
107% recovery

Treated Sample
(200 g ai/A)
0.045 mg injected
Found: <0.05 ppm
of propiconazole

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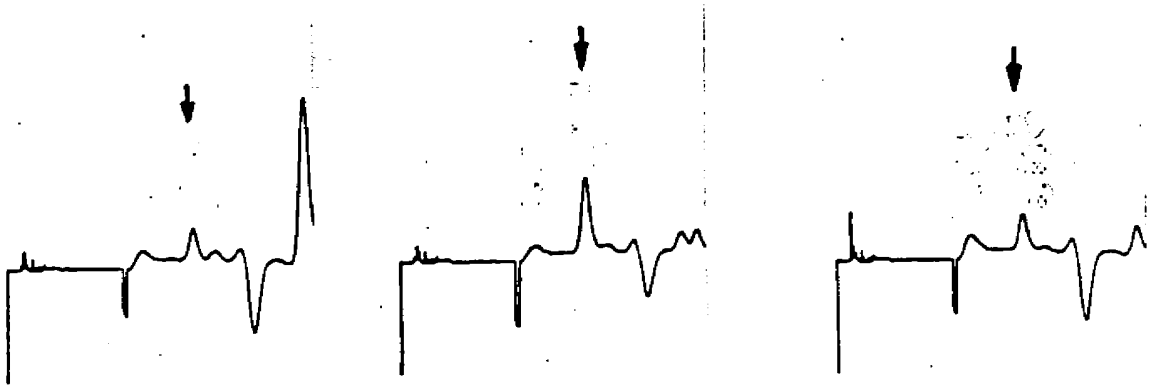
FIGURE 9: TYPICAL CHROMATOGRAMS FOR THE DETERMINATION OF RESIDUES OF PROPICONAZOLE IN CORN GRAIN



Check sample
0.045 mg injected
Found: <0.05 ppm
of propiconazole

Check + 0.05 ppm
0.045 mg injected
Found: 0.08 ppm
of propiconazole
95% recovery
(corrected)

Sample 6-3A
0.045 mg injected
Found: <0.05 ppm
of propiconazole

CERTIFICATION

The reports and the experimental results included in this study, Laboratory Project I.D. AG-454A, are certified to be authentic accounts of the experiments.

12/8/86
Date

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ANALYTICAL METHOD AG-454B

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

Project No. 411000

Submitted By: John Toth Title: Assistant Chemist
Method Development

Signature: John Toth

P. J. Manuli Title: Senior Chemist
Contract Studies

Signature: P. J. Manuli

Approved By: M. W. Cheung Title: Senior Group Leader
Residue Analysis

Signature: M. W. Cheung

Approval Date: December 20, 1989

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I. SUMMARY/INTRODUCTION

Ia. SCOPE

This method is used for the determination of total residues of propiconazole, 1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole in crops as 2,4-dichlorobenzoic acid (DCBA) (see Figure 1 for structures). Analytical Method AG-454B is an updated version of AG-454A. In this new version, the following modifications and additions were included:

Ia.1. Clarifications on sample size (Section IIc.2.5) and on number of partitions (Section IIc.4.2) were incorporated.

Ia.2. Other minor clarifications were also added (Sections IIb.7, IIc.3.5, Table I, Figures 2, 6, 7, 8, and 9).

Ib. PRINCIPLE

Samples are extracted by refluxing with 20% concentrated ammonium hydroxide/methanol for one hour. The mixture is cooled and filtered. An aliquot of the extract is evaporated to dryness, and the residue dissolved in NaOH. The sample is then heated for one hour and fifteen minutes with potassium permanganate, where propiconazole and its metabolites are converted to 2,4-dichlorobenzoic acid. After addition of water, the sample is partitioned with 10% diethyl ether/hexane. The organic phase containing 2,4-dichlorobenzoic acid is evaporated to dryness and derivatized with diazomethane in the presence of dodecane which acts as a keeper to reduce losses, due to the volatility of the derivative, in subsequent steps. The derivative is cleaned up using an acidic alumina Sep-Pak®. The cleaned extract is analyzed by capillary gas chromatography.

The limit of detection for the method is 0.05 ppm expressed as propiconazole equivalents.

The flow diagram for the method is shown in Figure 2.

II. MATERIALS/METHODS

IIa. Equipment

IIa.1 Concentration tubes, 50 ml (Fisher Catalog No. 05-538-40B, Kimax Brand or equivalent).

IIa.2 Cotton, absorbent (Fisher Catalog No. 07-900 or equivalent).

IIa.3 Distillation column, Snyder, 3 ball (Kontes Catalog No. K-503000-012 or equivalent).

IIa.5 Food chopper, Hobart or equivalent.

IIa.6 Funnel, 12.5 cm. size.

IIa.7 Glascol heating mantle, 500 ml.

IIa.8 Multi-Blok Heater, (Cole-Parmer, Catalog No. J-3128-00 or Thomas, Catalog No. 5891-C10 or equivalent).

IIa.9 N-evap or equivalent.

IIa.10 Rotary evaporator, Büchi or equivalent.

IIa.11 Sample vials, GC autosampler.

IIa.12 Sample concentrator (Thomas, Catalog No. 4367-B20 or equivalent)

IIa.13 Separatory funnel, 125 ml with Teflon stopcock.

IIa.14 Sep-Pak, acidic alumina (Waters Associates, Catalog No. 51800).

IIa.15 Syringe, 25 ml, LuerLok®.

IIa.16 Test tubes, 24/40 joint, 18.5 cm x 22 mm. (Ace Glass Co., Catalog No. 8645-38) or equivalent.

IIa.17 Thermometer, -10 to 360°C.

IIa.18 Thermometer, -20 to 110°C.

IIa.19 Variable transformer, Powerstat.

IIa.20 Vortex mixer or equivalent.

IIa.21 Stirring rods, 10 x 300 mm.

IIb. Reagents and Standards

IIb.1 Acetone Pesticide Grade (Fisher Catalog No. A-40-4 or equivalent).

IIb.2 Ammonium hydroxide, concentrated (Fisher Catalog No. A-669 or equivalent).

IIb.3 20% (v/v) Ammonium Hydroxide, concentrated/ methanol.

IIb.4 Propiconazole analytical standard.

IIb.5 Diazomethane, diethyl ether solution, prepared according to AG-345[1].

IIb.6 2,4-Dichlorobenzoic acid (DCBA), Aldrich Chemical Co., Catalog No. 13957-2.

IIb.7 Diethyl ether, distilled in glass, product should contain 2% ethanol added by manufacturer as preservative, (American Scientific, Catalog No. 106-40 or equivalent).

IIb.8 10% (v/v) Diethyl ether in hexane.

IIb.9 Distilled water.

IIb.10 Dodecane, 99%, Aldrich Chemical Co., Catalog No. D22, 110-4.

IIb.11 1% (w/v) Dodecane in acetone.

IIb.12 Ethyl acetate, Certified Grade (Fisher Scientific, Catalog No. E145-4 or equivalent).

IIb.13 1.0% (v/v) distilled water in ethyl acetate.

IIb.14 Hexane, HPLC Grade (Fisher, Catalog No. H302-4 or equivalent).

IIb.15 Methanol, Certified Grade (Fisher, Catalog No. A412-4 or equivalent).

IIb.16 Potassium permanganate, Reagent Grade (Fisher, Catalog No. P287).

IIb.17 Sodium meta-bisulfite, reagent grade, Baker.

IIb.18 Sodium hydroxide, reagent grade, Baker.

IIb.19 Sodium hydroxide, one normal solution.

IIb.20 Hydrochloric acid, reagent grade.

IIb.21 Hydrochloric acid, six normal solution.

IIc. Analytical Procedure

IIc.1 Preparation of Sample

IIc.1.1 Grind 300-400 g of crop sample using a Hobart food cutter and dry ice. Dry samples such as grains or nut meats are milled using a Wiley Mill.

IIc.2 Extraction and Fortification

IIc.2.1 Weigh a 15-g representative sample (Section IIc.1.1) into a 500-ml round bottom flask.

IIc.2.2 Fortification of one or more control samples will be performed at this step.

IIc.2.2.1 Prepare the fortification standard by dissolving 100 ± 0.1 mg propiconazole (using an analytical balance) in 100 ml of hexane in a volumetric flask. Make serial dilution of the standard such that the fortification volume will not exceed 1.0 ml.

IIc.2.2.2 Add parent propiconazole standard in 1.0 ml or less of hexane to the control samples before extraction.

IIc.2.2.3 Let the spiked samples stand for at least 30 minutes before adding extraction solvent.

IIc.2.3 Add 200 ml of 20% conc. ammonium hydroxide/methanol. Fit the flask to a reflux condenser and heat under reflux for one hour using a Glascol heating mantle with a variable transformer setting of 65. Allow to cool to room temperature.

IIc.2.4 Filter the extract through a Reeve Angel Grade 802 filter paper inside a Whatman 2V filter paper into an 8 oz. bottle.

IIc.2.5 Transfer a 0.225 g to 0.675 g crop equivalent aliquot (3.0 ml to 9.0 ml) to a 24/40 test tube (18.5 cm x 22 mm) and add 0.1 ml of conc. acetic acid. Concentrate the solution to incipient dryness at a temperature $\leq$ 40°C.

NOTE: A larger crop size helps to reduce chromatographic interferences, by dilution, resulting from reagents. The acetic acid prevents possible losses of parent propiconazole during the concentration step.

IIc.3 Potassium Permanganate Reflux

IIc.3.1 Add 0.4 g of potassium permanganate to the test tube.

IIc.3.2 Add 6 ml of 1N sodium hydroxide. Stopper and mix well on a vortex mixer.

NOTE: After addition of NaOH and $KMnO_4$ and mixing well, the sample color must be dark purple. If sample appears to be brownish to dark green, additional $KMnO_4$ must be added in increments of 0.1 g. Mix sample well each time.

IIc.3.3 Rinse sides of test tube with 2 ml of 1N sodium hydroxide. Add boiling chips.

IIc.3.4 Place test tubes fitted with Snyder columns on a heating block which has been pre-heated to 125°C, and heat for one hour and fifteen minutes.

IIc.3.5 Add 5 ml of water through the top of the Snyder column, and allow to cool for a minimum of 15 minutes.

IIc.3.6 Add 6 g of sodium meta-bisulfite, stopper and mix well on a vortex mixer. Sample will gradually turn white.

IIc.3.7 Add 14 ml of 6N hydrochloric acid slowly. Sample will effervesce. Mix sample carefully using glass stirring rod until completely clear.

NOTE: Stained glassware which has been used in the $KMnO_4$ reaction may be rinsed with a dilute solution of sodium meta-bisulfite to remove stains. Continue with usual wash and rinse.

IIc.4 Diethyl Ether/Hexane Partition

IIc.4.1 Transfer the sample solution in the test tube to a 125-ml separatory funnel. Add 15 ml of 10% (v/v) diethyl ether/hexane and stopper. Partition for one minute and allow the layers to separate. Drain the lower aqueous layer back into the test tube. Transfer the organic phase through a filter tube or powder funnel containing absorbent cotton into a 100-ml round bottom flask.

IIc.4.2 Repeat the partition twice using 15 ml of 10% diethyl ether/hexane.

IIc.4.3 Rinse the separatory funnel with 10 ml of 10% diethyl ether/hexane. Use this and an additional 20 ml of 10% diethyl ether/hexane to rinse the absorbent cotton.

IIc.4.4 Add 2 ml of 1% (w/v) dodecane solution in acetone to the flask and evaporate to dryness using a rotary evaporator (bath temperature $\leq$40°C).

NOTE: The added dodecane coats the surface of the round bottom flask uniformly after the evaporation step and acts as a keeper to reduce volatility losses of the methyl derivative of 2,4-dichloro-benzoic acid during the subsequent evaporation steps.

IIc.5 Derivatization with Diazomethane

IIc.5.1 To the residues remaining after evaporation of the diethyl ether/ hexane extract of Step IIc.4.4, add 2 ml of diazomethane/diethyl ether reagent solution (AG-345[1]).

IIc.5.2 Allow the solution to stand for at least 30 minutes with occasional gentle swirling. Add more diazomethane as required to maintain a yellow color.

CAUTION: Add diazomethane inside a well ventilated hood. Extreme care should be exercised in handling diazomethane because of the potential toxic effects and explosion hazards on contact with sharp or rough surfaces.

IIc.5.3 Evaporate the diethyl ether off after the derivatization using a rotary evaporator at room temperature (do not use a bath). Continue the evaporation at room temperature for an additional one and no longer than two minutes to ensure the complete removal of diethyl ether.

NOTE: Evaporation at higher temperature to total dryness may cause losses of the derivative.

IIc.6 Alumina Sep-Pak Cleanup

IIc.6.1 Fit an acidic alumina Sep-Pak cartridge to the LuerLok end of a 25 ml syringe with the plunger removed (see Figure 3).

IIc.6.2 Add 10 ml of 1.0% (v/v) water/ethyl acetate to the barrel of the syringe and allow it to flow by gravity through the Sep-Pak. (A rate of 2-3 ml per minute is normal).

NOTE: The 1.0% (v/v) water/ethyl acetate wash is used to deactivate the acidic alumina.

IIc.6.3 Prewash the Sep-Pak cartridge with 10 ml of hexane by gravity flow.

IIc.6.4 Dissolve the residue from IIC.5.3 in 5 ml of hexane and transfer into the barrel of the syringe. Elute the hexane through the Sep-Pak by gravity flow.

IIc.6.5 Rinse the round bottom flask with 7 ml of 10% (v/v) diethyl ether/hexane and transfer to the barrel of the syringe. Elute the acidic alumina Sep-Pak by gravity flow, collecting the eluant in a 50-ml concentration tube.

NOTE: Distilled in glass diethyl ether is used for the elution steps.

IIc.6.6 Add hexane to bring to a volume of 10.0 ml. Dilute with hexane if necessary.

IId. Instrumentation

IId.1 Description

The sample in Step IIc.6.6 is analyzed by capillary gas chromatography using an electron capture detector. The gas chromatographic conditions are given in Table I.

IId.2 Operating Conditions

See Table No. 1

IId.3 Calibration

IId.3.1 The GC system should be calibrated with each analytical run, by checking the retention time of DCBA methyl ester. The retention time should not vary by more than ± 2% on a daily basis, otherwise the system should be inspected and proper maintenance should be performed in order to achieve the narrow range of variation.

IId.3.2 Method of Calculation

The gas chromatograph is standardized by injecting 2 µl aliquots of the diluted DCBA-methyl ester solutions during residue analysis. This represents a working range of 0.5 to 10.0 picograms of the derivative (expressed as dichlorobenzoic acid). Detailed method of calculation is presented in Section II.i.2.

IIe. Interferences

IIe.1 Sample matrices. Analytical Method AG-454A has been used to analyze a large variety of crop matrices (Table II). No interferences (<0.05 ppm) were found in corn grain, soybean beans, wheat grain, peanut nutmeat, beans or peas and the majority of corn fodder, forage or soybean hay samples. A few corn forage, corn fodder and soybean hay samples showed a maximum of 0.15 ppm and an average of 0.083 ± 0.034 ppm (N = 8) of propiconazole residues detected as DCBA-methyl ester. These residues could have been real propiconazole

residues resulting from contamination due to drift from adjacent treated plots. Rotational celery stem control samples showed a maximum of 0.13 ppm total residue with an average of 0.10 ± 0.029 ppm (N = 6). This again might have resulted from contamination.

IIe.2 No interferences from chemicals having permanent or Section 18 tolerances in or on pecans, peanuts, grapes or apples were detected when determined as 2,4-dichlorobenzoic acid. Two studies were conducted (ABR-83075[2] and ABR-85040[3]). In one of the studies, ABR-85040, maximum tolerance level amounts of the pesticide chemicals having permanent tolerances or Section 18 tolerances in or on grapes and apples were subjected to the procedures of Analytical Method AG-445[4]", Determination of CGA-71818 Residues in Grapes and Apples by Conversion to 2,4-Dichlorobenzoic Acid and Analysis by Capillary Gas Chromatography." Analytical Methods AG-454B and AG-445 are nearly identical in every step with slight differences in the length of basic permanganate hydrolysis time (1.25 vs. 2.0 hours), in the partition solvents (diethyl ether/hexane vs. methyl tert-butyl ether) and in the Sep-Pak columns (acidic alumina vs. silica gel). Propiconazole and CGA-71818 are also very similar in chemical structures and properties. In the second study (ABR-83075), pesticidal chemicals having permanent or Section 18 tolerances in or on pecans or peanuts were subjected to the analytical procedures of AG-356[5] which was an earlier version of AG-454B. No interferences were detected.

IIe.3 No interference from the solvents used in this method has been detected.

IIe.4. The roto evaporator should be rinsed with fresh acetone solvent between each sample evaporation to eliminate possible cross contamination.

IIf. Confirmatory Techniques

IIf.1 GC/MS according to Analytical Method AG-356[5].

IIg. Time Required for Analysis

IIg.1 A total of twelve hours is needed. This includes the cooling of hot extract and the actual injection time. When several sets of samples are being worked up, many steps can be overlapped and performed concurrently.

IIh. Modifications

IIh.1 None

IIi. Preparation of Standard 2,4-Dichlorobenzoic Acid Methyl Derivative

IIi.1 Calibration Factors

IIi.1.1 Weigh 20.0 mg of 2,4-dichlorobenzoic acid into a 200-ml volumetric flask.

IIi.1.2 Add 3 ml of diazomethane as in Steps IIc.5.1 to IIc.5.2.

IIi.1.3 Bring to volume with hexane. The standard solution of the derivative is 100 ng/µl expressed as 2,4-dichlorobenzoic acid equivalents. Serial dilutions of the standard solution are made with hexane until working solutions containing 0.25, 0.50, 1.0, 2.5, and 5.0 picograms per microliter are achieved.

IIi.1.4 Inject 2 µl aliquots of the diluted solutions during residue analysis. This represents a working range of 0.5 to 10.0 picograms of the derivative (expressed as dichlorobenzoic acid).

IIi.1.5 Determine the peak height for the injected standards. Typical chromatograms of standards are shown in Figure 4.

IIi.1.6 Construct a standard curve by plotting detector response versus picograms injected or enter the standardization data into an appropriate electronic calculator (e.g., Hewlett-Packard Model HP-11C or HP-32S) or a computer system (e.g., HP-1000 Lab Automation System [LAS]) which utilizes integration software to calculate a least square standard curve. A typical standard curve is shown in Figure 5.

IIi.2 Detection of Sample Residues

IIi.2.1 Inject a 2-µl aliquot of the sample in Step IIc.6.6 into a gas chromatograph equipped with an electron capture detector. Make appropriate dilutions of the sample to have the sample peak height within the range of the standard curve. Compare peak heights of unknown samples with the standard curve, manually or by either using an electronic calculator or a computer system as mentioned in IIi.1.6, to determine the amounts of

the derivative in the aliquot injected. Typical chromatograms of checks and recovery samples are shown in Figures 6 through 9.

IIi.2.2 Calculate residue results as ppm equivalents of propiconazole using the following equation:

$$\text{PPM Found} = \frac{\text{Amount 2,4-Dichlorobenzoic Acid Found (pg)}}{\text{(mg injected)}\quad\text{(1000 pg/ng)}} \times 1.79$$

Correct the ppm found in recoveries by subtracting the ppm found, real or apparent, in the controls. Calculate the recovery factor by the following equation:

$$R = \frac{\text{Corrected PPM Found in Fortified Sample}}{\text{PPM Added}}$$

where R is the recovery factor determined using a fortified control sample carried through the procedure and is expressed as a decimal (100% = 1.00, etc.). If the recovery is >100%, use the factor 1.00.

Correct the propiconazole ppm found in samples by the following equation:

$$\text{Corrected ppm} = \frac{\text{(PPM Found in Sample)}}{R}$$

The factor 1.79 is used to convert residues of 2,4-dichlorobenzoic acid found into propiconazole equivalents.

IIj. Discussion

IIj.1 Preparation of diazomethane should be carried out as specified in AG-345.

IIj.2 The average recovery of propiconazole from crop samples fortified at 0.05 to 2.0 ppm was 88.2 ± 15.0%; N = 118.

IIj.3 The extraction procedure used in this method was shown in ABR-83078[6] to remove a significant portion of the total ^{14}C-residue (88%, 89%) present in winter wheat straw and corn stalks. Accountability of the total method was tested, using five propiconazole related metabolites present in crops. Average recovery of all metabolites was 93 ± 11% (see Table III). In addition, the method was validated using ^{14}C-treated crop. Validation details are presented in ABR-85021[7].

IIj.4 Corn fraction samples, feedstock, meal, flour, soap-stock and oils can be analyzed by this method. Soapstock samples require cooling of sample in refrigerator after extraction, and filtering of sample while cool to remove precipitate. It also requires 1.0 g of potassium permanganate.

IIj.5 For pineapple samples, refer to Analytical Method AG-448[8].

III. RESULTS AND DISCUSSION

IIIa. Accuracy

IIIa.1 The average recovery was 88.2 ± 15.0%, N = 118, at a fortification range of 0.05 ppm to 2.0 ppm. No dependency of percent recovery on fortification level was found.

IIIb. Precision

IIIb.1 Not performed.

IIIc. Limits of Detection and Quantitation

IIIc.1 The limit of detection in crops is 0.05 ppm detected as 2,4-DCBA and reported as propiconazole equivalents.

IIId. Ruggedness

Testing not performed.

IIIe. Limitation

None

IV. REFERENCES

1. Ross, J. A., AG-345, "Preparation of Diazomethane."

2. R. K. Williams, P. J. Manuli, J. A. Ross, ABR-83075, "Specificity of Analytical Method AG-356 for the Determination of Total Residues of CGA-64250 as 2,4-Dichlorobenzoic Acid (Methyl Ester)."

3. W. T. Beidler, J. A. Ross, ABR-85040, "Specificity of Analytical Method AG-445 for the Determination of the Total Residues of CGA-71818 in Grapes and Apples."

4. P. Manuli, J. W. Smith, S. G. Burge, Ag-445, "Determination of CGA-71818 Residues in Grapes and Apples by Conversion to 2,4-Dichlorobenzoic Acid and Analysis by Capillary Gas Chromatography."

5. K. Balasubramanian, B. Gold, M. W. Cheung, AG-356, "Determination of Total CGA-64250 Residues in Crops by Conversion to 2,4-Dichlorobenzoic Acid and Analysis by Gas Chromatography - Mass Spectrometry."

6. Nixon, W. B., Rhoads, W. D., ABR-83078, "Validation of Analytical Methods AG-356, AG-407, and AG-415 for the Determination of Residues of CGA-64250 in Crops by Conversion to 2,4-Dichlorobenzoic Acid."

IV. REFERENCES (continued)

7. Perez, R., Toth, J., ABR-85021, "Validation of Analytical Methods AG-448 and AG-454 for the Determination of Residues of Propiconazole in Crops by Conversion to 2,4-Dichlorobenzoic Acid."

8. Perez, R., Toth, J., AG-448, "Determination of Total Residues of CGA-64250 in Pineapples as 2,4-Dichlorobenzoic Acid by Capillary Gas Chromatography."

V. TABLES AND FIGURES

Va. TABLES

TABLE I: CAPILLARY GAS CHROMATOGRAPHIC CONDITIONS

Instrument:	Hewlett-Packard Model 5880 Capillary Gas Chromatograph with Model 7672A Automatic Sampler, or equivalents.
Carrier Gas:	Helium, flow adjusted to give 17 psi (1-2 ml per minute).
Makeup Gas:	5% argon/methane, 30 ml per minute.
Column:	J & W capillary, DB-5, 30 meter, 0.25 μm film thickness, 0.32 mm i.d.
Injection:	Splitless.
Detector:	Electron capture.
Temperatures:	
Injector:	250°C
Detector:	300°C

Oven Program and Run Table

Program	(Annotation Off)
10	Valve 6 On
15	Signal C Device #5
20	Oven Temp 60
30	Oven Temp Equib Time 1
40	Oven Temp Initial Value 60
50	Oven Temp Initial Time 1
60	Oven Temp Prgm Rate 30
70	Oven Temp Final Value 125
80	Oven Temp Final Time 5
90	Oven Temp Post Value 240
100	Oven Temp Post Time 8
110	Attn 2↑10

TABLE I: CAPILLARY GAS CHROMATOGRAPHIC CONDITIONS (continued)

Oven Program and Run Table (Continued)

Program

120	Attn 2↑10 Device #15
130	Chart Speed 0.2
140	% Offset 10
150	Run Time Annotation Off
160	Run Tbl Annotation Off
170	Report Annotation On
180	Report On
190	Delete Run Tbl
200	Delete Report - Tbl
210	Peak Width 0.04
220	Threshold 1
230	Run Time 0 Value 6 On
240	Run Time 0.1 Intg Off
250	Run Time 0.5 Valve 6 Off
260	Run Time 6.71 Attn 2↑4
270	Run Time 6.72 Chart Speed 2
280	Run Time 6.73 Zero
290	Run Time 6.74 % Offset 10
300	Run Time 6.9 Intg On
310	Run Time 6.91 Run Time Annotation On
320	Run Time 6.7 Attn 2↑2 Device #5
330	Run Time 7.4 Intg Off
340	Run Time 7.41 Run Time Annotation Off
350	Run Time 7.51 Valve 6 On
360	Oven Temp Annotation Off
370	Edit Auto Seq 1,2
380	Signal C Device #1
390	Area %

Minimum Detection Limit: 0.5 picogram

Volume Injected: 2 µl

Retention Time: 8.06 ± 0.1 minutes (Retention time may vary depending on column conditions.)

TABLE II: TYPICAL PROPICONAZOLE RECOVERIES

Substrate	Fortification Range (ppm)	Average Recovery (%)
Wheat (including wheat straw and grain)	0.10 - 1.0	79.8 ± 3.8 (n = 4)
Soybean (including soybean hay, dry beans, and fractions [hulls, meal, crude oil, refine oil, refine B.H. oil, R.B.H.D. oil and soapstock]	0.05 - 2.0	91.7 ± 16.0 (n = 15)
Corn (including silage, fodder, grain, ears, and fractins [feed stock, meals, and flour]	0.05 - 2.0	87.4 ± 15.5 (n = 58)
Celery (stems)	0.05 - 2.0	86.0 ± 14.6 (n = 17)
Peanut (including hay and nuts)	0.05 - 1.0	99.5 ± 12.2 (n = 6)
Beans and Peas (including hay, kidney beans, pinto beans, and lima beans)	0.05 - 2.0	87.8 ± 14.5 (n = 18)

Overall recovery = 88.2 ± 15.0 (n = 118)

Reference: AG-As 8863; 8796, 2-3; 8669; 8642; 8583, 1-2; 8621, 1-2; 8596; 8589, 1-2, 8578; 8570; 8566, 1-2; 8560, 1-2; 8544, 1-2; 8471; 8459, 1-2, 8386, 1-2; 8304, 1-3; 8054, 1-4; 8027, 2; 8008, 1-2; 8745; 8693; 8599; 8542; 8160; 7976, 1-2; 8739; 8698; 8629; 8614; 8597; 8567; 8457; 8456, 1-2; 8441, 1-2; 8437

TABLE III: RECOVERIES OF RELATED METABOLITES

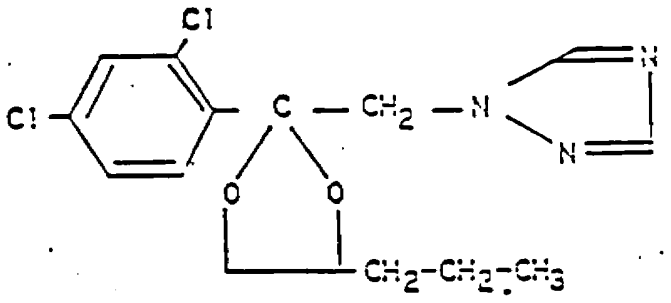
Metabolite	Recovery
CGA-91305, alkanol	101%
CGA-91304, ketone	76%
CGA-104284, olefin	105%
CGA-118244, ß-hydroxy	89%
CGA-121676, γ-acid	93%

Average recovery 93 ± 11%

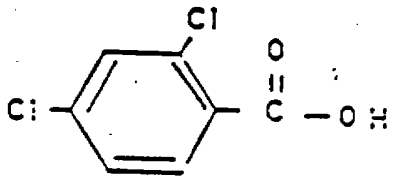
Vb. FIGURES

FIGURE 1: STRUCTURES

CGA-64250

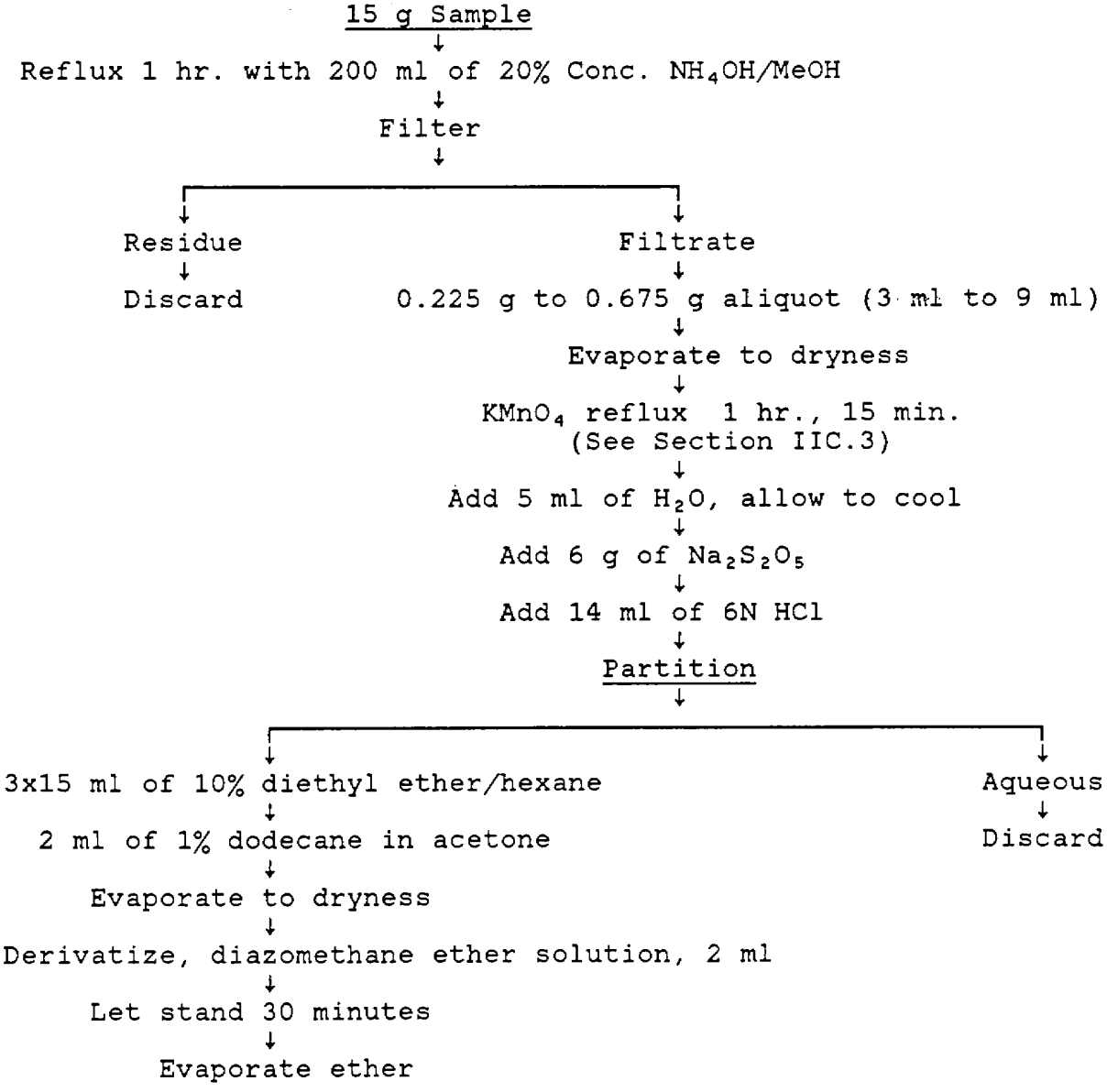


1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1-H-1,2,4-triazole



2,4-Dichlorobenzoic Acid

FIGURE 2: FLOW DIAGRAM OF METHOD



(Continued on following page)

FIGURE 2: FLOW DIAGRAM OF METHOD
(Continued)

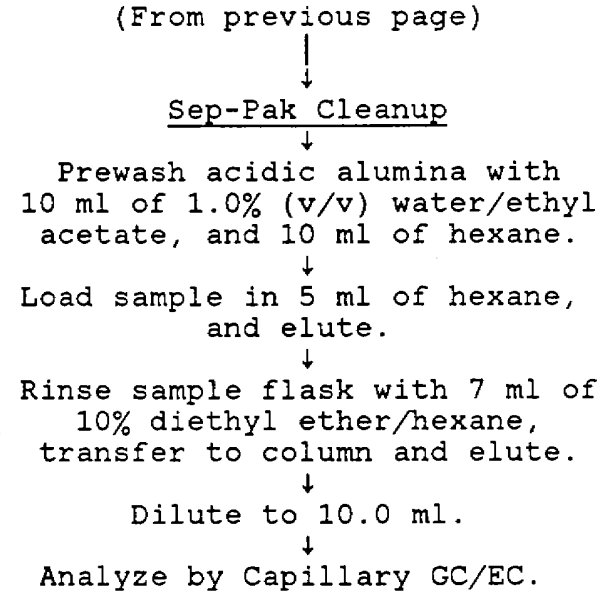


FIGURE 3: DIAGRAM OF SEP-PAK CLEANUP

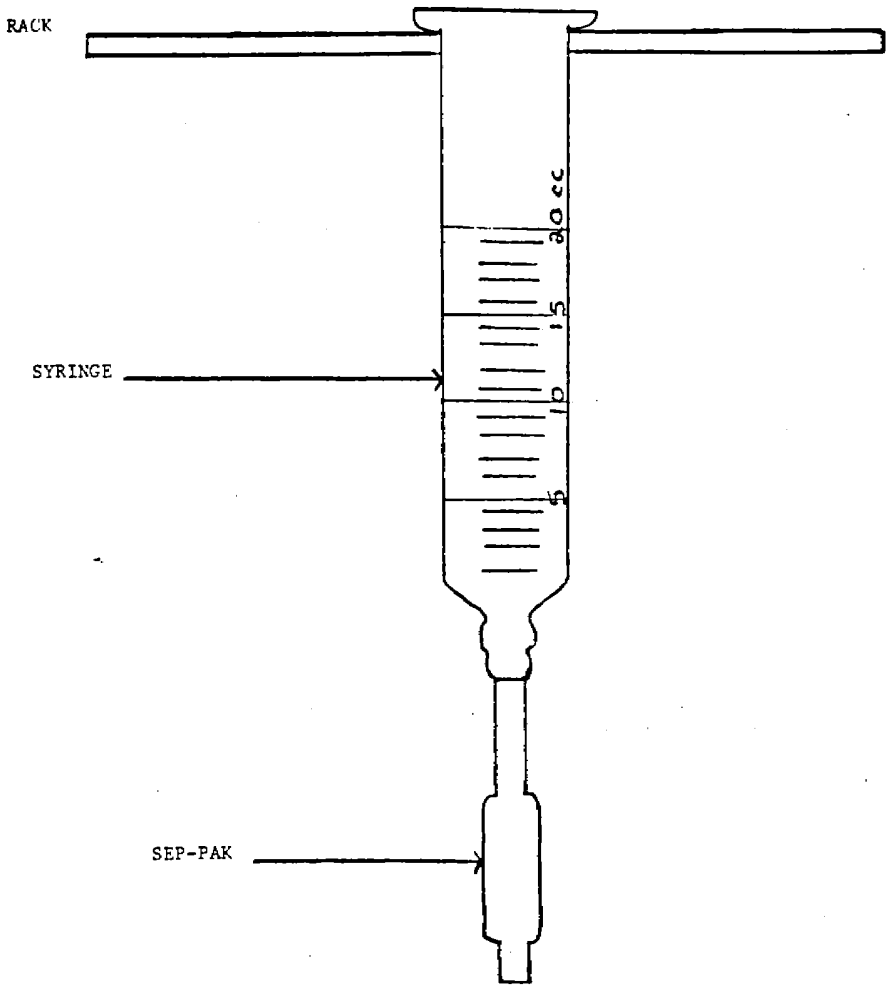
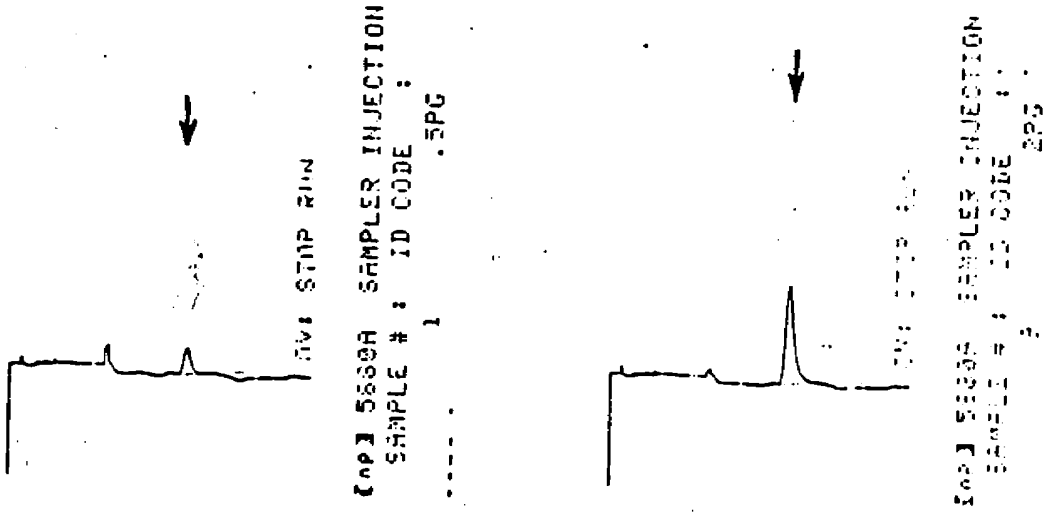


FIGURE 4: TYPICAL STANDARD CHROMATOGRAMS OF 2,4-DICHLOROBENZOIC ACID METHYL DERIVATIVE



0.5 pg. Standard

2.0 pg. Standard

FIGURE 5: TYPICAL STANDARD CURVE

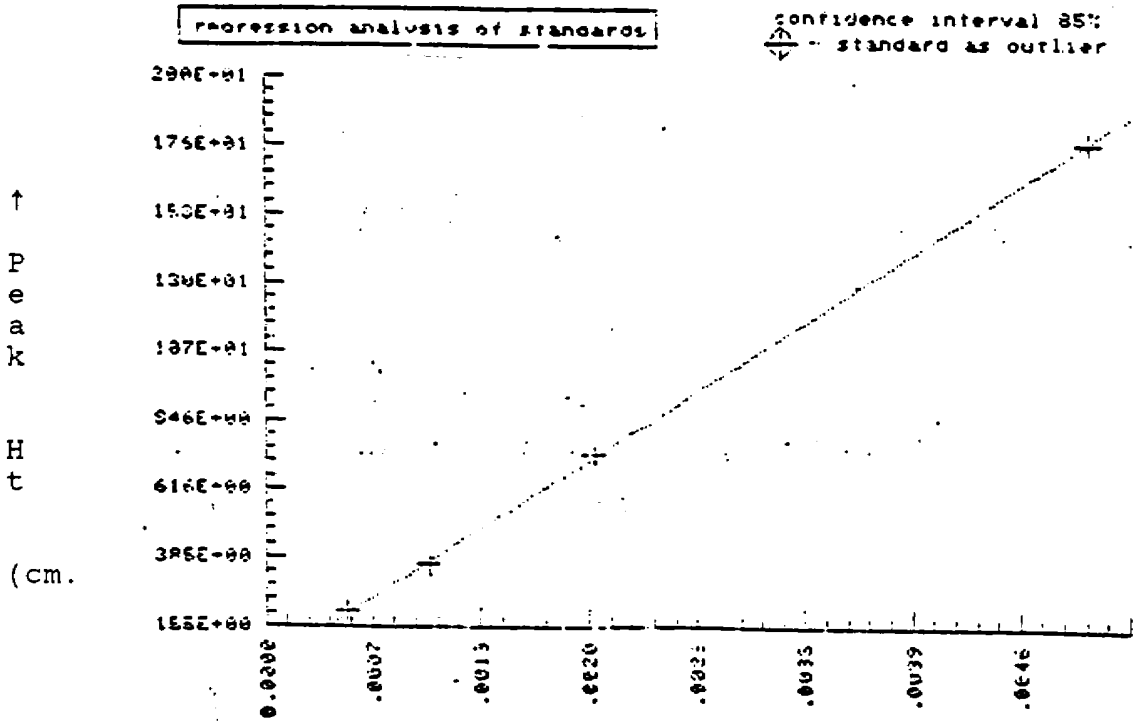
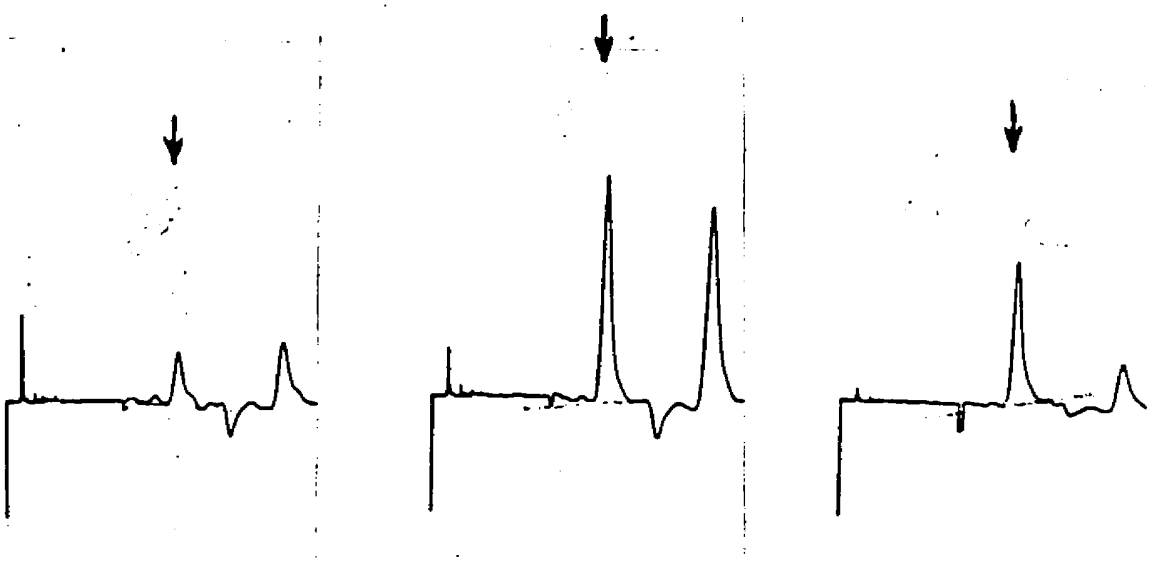


FIGURE 6: TYPICAL CHROMATOGRAMS FOR THE DETERMINATION OF RESIDUES OF PROPICONAZOLE IN CELERY

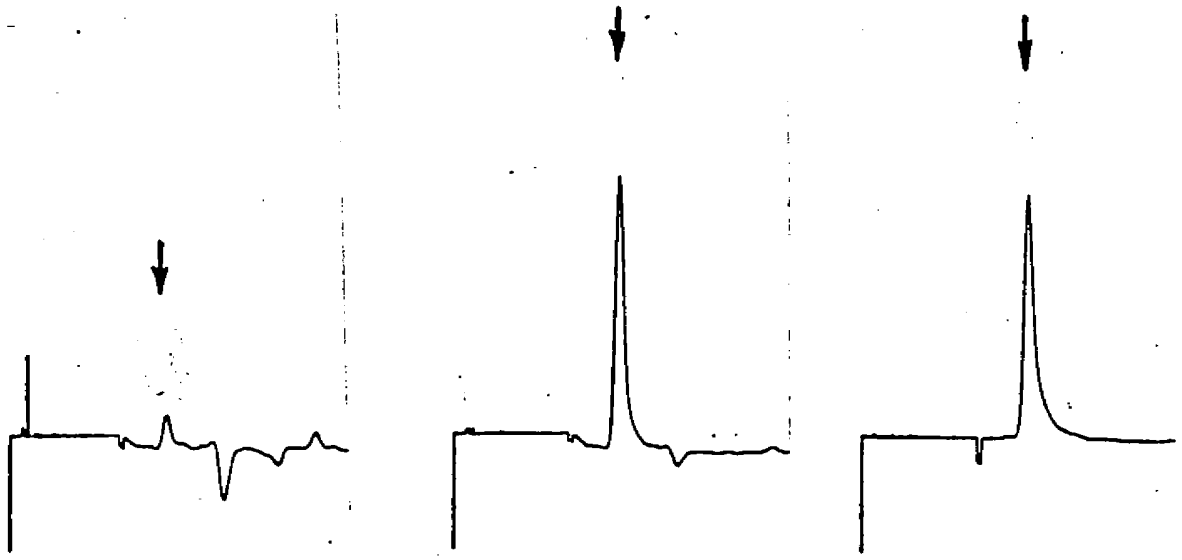


Check sample
0.045 mg injected
Found: 0.07 ppm
of propiconazole

Check + 0.2 ppm Propiconazole
0.045 mg injected
Found: 0.26 ppm
of propiconazole
95% recovery

Sample 3-1B
0.022 mg injected
Found: 0.34 ppm
of propiconazole

FIGURE 7: TYPICAL CHROMATOGRAMS FOR THE DETERMINATION OF RESIDUES OF PROPICONAZOLE IN CORN SILAGE

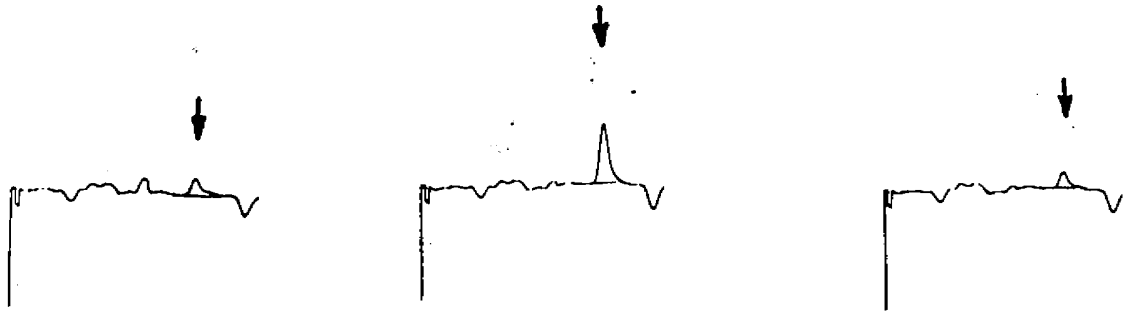


Check sample
0.045 mg injected
Found: <0.05 ppm
of propiconazole

Check + 1.0 ppm Propiconazole
0.018 mg injected
Found: 0.85 ppm
of propiconazole
81% recovery

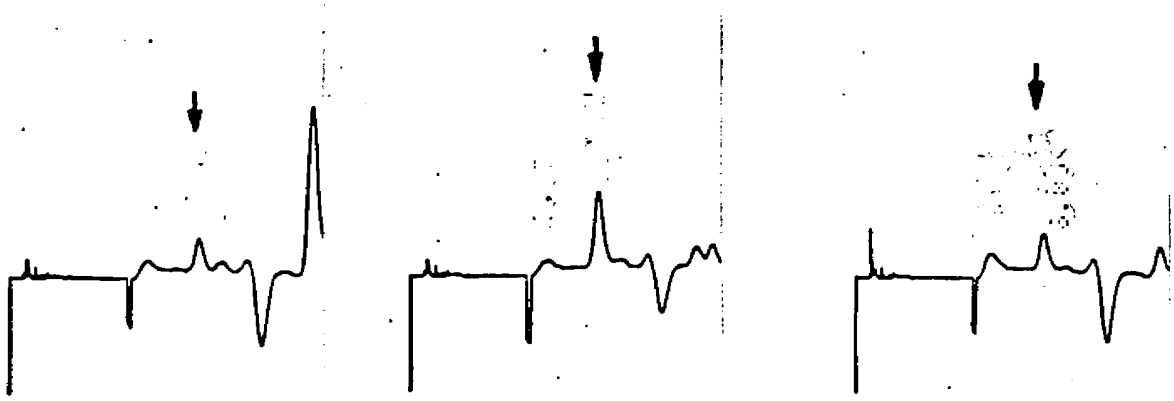
Sample 6-1A
0.001 mg injected
Found: 9.3 ppm
of propiconazole

FIGURE 8: TYPICAL CHROMATOGRAMS FOR THE DETERMINATION OF RESIDUES OF PROPICONAZOLE IN WHEAT GRAIN



Check sample	Check + 0.05 ppm Propiconazole	Treated Sample
0.045 mg injected	0.045 mg injected	(200 g ai/A)
Found: <0.05 ppm	Found: 0.05 ppm	0.045 mg injected
of propiconazole	of propiconazole	Found: <0.05 ppm
Ref. AG-A 8661, 02	107% recovery	of propiconazole

FIGURE 9: TYPICAL CHROMATOGRAMS FOR THE DETERMINATION OF RESIDUES OF PROPICONAZOLE IN CORN GRAIN



Check sample
0.045 mg injected
Found: <0.05 ppm
of propiconazole

Check + 0.05 ppm Propiconazole
0.045 mg injected
Found: 0.08 ppm
of propiconazole
95% recovery
(corrected)

Sample 6-3A
0.045 mg injected
Found: <0.05 ppm
of propiconazole

VI. CERTIFICATION

The reports and the experimental results included in this study, Laboratory Project I.D. AG-454B, are certified to be authentic accounts of the experiments.

December 20, 1989
Date

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