Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pesticide Name: **Difenoconazole**

MRID #: **469501-28**

Matrix: **Soil**

Analysis: **LC/MS/MS**

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DIFENOCONAZOLE: METHOD

TITLE

Determination of Difenoconazole and Its Metabolites CGA-205375, CGA-142856 and CGA-71019 in Soil, Using Liquid Chromatography–Electrospray Ionization Tandem Mass Spectrometry

DATA REQUIREMENT

EPA Guideline No. 164-1

COMPLETION DATE

December 15, 2005

AUTHOR

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PERFORMING LABORATORY

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LABORATORY STUDY IDENTIFICATION

Enviro-Test Method M 314
Syngenta Number T013656-05

SUBMITTER/SPONSOR

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VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 70

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Company: Syngenta Crop Protection, Inc.

Company Representative: Patrick McCain

Title: Regulatory Product Manager

Signature: Patrick McCain Date: 1/3/06

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GOOD LABORATORY PRACTICE STATEMENT

The analytical work described in this report was performed in accordance with U.S. EPA Good Laboratory Practice Standards, 40 CFR Part 160.

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REPORT APPROVAL

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QUALITY ASSURANCE STATEMENT

Method validation experiments whose results are reported in this report were conducted per protocol as part of the analytical phase of the following Syngenta studies, T002983-03, T002984-03 and T002985-03. The Quality Assurance Unit of Enviro-Test Laboratories has inspected and/or audited the analytical phase of these studies, and this report, and has reported its findings to the Sponsor management, to the Study Director and to ETL Management. The raw data is complete, consistent, well documented and accurately reflects the method in which the analytical phases have been conducted to date.

Dates of Inspection	Dates Reported to P.I./ Management	Reported to Study Director/ Management
T002983-03 In-phase: July 13 & 14/05	July 18/05	July 18/05
In-phase: Aug. 17 & 18/05	Aug. 22/05	Aug. 22/05
T002984-03 In-phase: May 17 & 18/05	July 18/05	July 18/05
In-phase: Aug. 24 & 25/05	Aug. 29/05	Aug. 29/05
T002985-03 In-phase: June 9-10, & 14/05	July 18/05	July 18/05
In-phase: Sept. 8 & 9/05	Sept. 12/05	Sept. 12/05
Method Report: Dec. 1 & 9/05	Dec. 15/05	Dec. 15/05

Anne Beaubien

Date Dec. 15, 2005

Signature of Anne Beaubien,
Quality Assurance Officer

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GENERAL INFORMATION

Study Title:	Determination of Difenoconazole and Its Metabolites CGA-205375, CGA-142856 and CGA-71019 in Soil, using Liquid Chromatography–Electrospray Ionization Tandem Mass Spectrometry
Data Requirement	Subdivision N: Guideline 164-1
Study Identification Number:	Enviro-Test Analytical Method M 314 Syngenta Number T013656-05
Performing Laboratory:	Enviro-Test Laboratories 9936 - 67 Avenue Edmonton, Alberta T6E 0P5 Canada
Analytical Timetable:	Experimental Initiation Date: Nov. 18, 2004 Experimental Completion Date: Feb. 11, 2005
Archive Location:	The raw data and final method report will be archived at the Syngenta Crop Protection Archives, Syngenta Crop Protection, Greensboro, North Carolina at the end of the study. All non-study specific data (log books, etc.) will be archived according to applicable ETL SOPs.

Reference Substance Information:

Common Name	CAS Number	IUPAC Name	Lot Number	Purity	Reassay Date
Difenoconazole	119446-68-3	cis,trans-3-chloro-4-[4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether	593-1669	96.0%	4/2006
CGA-205375	117018-19-6	1-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-2-[1,2,4]triazaol-1-yl-ethanol	NV-XXVIII-45	97.2%	1/31/2006
CGA-71019	288-88-0	1,2,4-triazole	WFH-IV-5	99.2%	3/31/2007
CGA-142856	28711-29-7	[1,2,4]Triazol-1-yl-acetic acid	GAN-VI-84-111	99.0%	3/31/2006

ABBREVIATIONS AND SYMBOLS

Abbreviation	Definition
amu	atomic mass unit
C	Celsius or Centigrade
CAS	Chemical Abstract Services
CFR	Code of Federal Regulations
cm	centimeter
EPA	Environmental Protection Agency (U.S.)
EU	European Union
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act (U.S.)
g	gram
GC	gas chromatography
GLPs	Good Laboratory Practices
i.d.	inside diameter
ID	identification
IUPAC	International Union of Pure and Applied Chemistry
kg	kilogram
L	liter
LC	liquid chromatography
LC/MS/MS	tandem liquid chromatography/mass spectrometry/mass spectrometry
LOD	limit of detection
LOQ	limit of quantitation
m	meter
m/z	mass to charge ratio
µg	microgram
µL	microliter
µm	micrometer
MDL	method detection limit
mg	milligram
mL	milliliter
mm	millimeter
mmol	millimole
min	minute
mol	mole
MS	mass spectrometry

ABBREVIATIONS AND SYMBOLS (continued)

Abbreviation	Definition
MS/MS	tandem mass spectrometry/mass spectrometry
mV	millivolt
MW	molecular weight
N/A	not applicable
ND or nd	Non detect (below limit of detection)
ng	nanogram
No.	number
PMRA	Pest Management Regulatory Agency, Canada
ppb	parts per billion or micrograms per kilogram
ppm	parts per million or microgram per gram or milligrams per kilogram
pg	picogram
psi	pounds per square inch
QAU	quality assurance unit
R^2 (or r^2)	square of correlation coefficient
RSD	relative standard deviation
Rt	retention time
s	second
SD	standard deviation
USDA	United States Department of Agriculture
V	volume
Wt	weight

1.0 Introduction and Summary

1.1 Scope and Chemical Structures

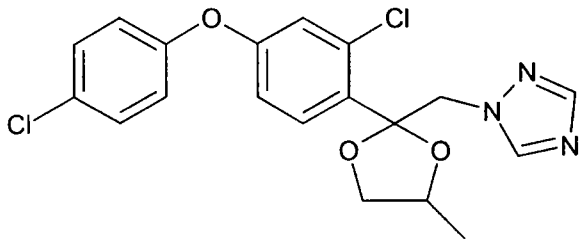
The method described herein is suitable for the determination of residues of difenoconazole and its metabolites, CGA-205375, CGA-142856 and CGA-71019 in soil. This method combines and modifies the existing Syngenta Methods RAM 435/01 and RAM 448/01 (References 1 and 2). The method LOQ has been established at 1.0 ppb (ng/g). Chemical structures and CAS information for the method analytes are shown below.

Name/Synonym: Difenoconazole

IUPAC Name: cis,trans-3-chloro-4-[4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether

CAS Number: 119446-68-3

Structure:

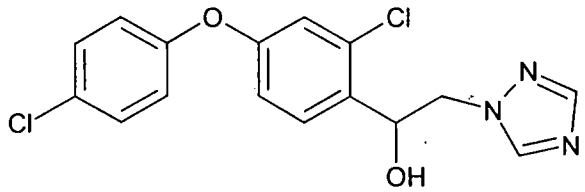


Name/Synonym: CGA-205375

IUPAC Name: 1-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-2-[1,2,4]triazaol-1-yl-ethanol

CAS Number: 117018-19-6

Structure:

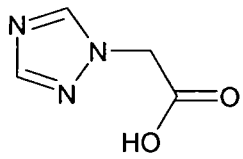


Name/Synonym: CGA-142856

IUPAC Name: Benzoic acid, 4-(methylsulfonyl)-2-nitro-

CAS Number: 110964-79-9

Structure:

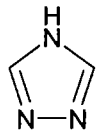


Name/Synonym: CGA-71019

IUPAC Name: 1,2,4 triazole

CAS Number: 288-88-0

Structure:



1.2 Method Summary

A 10.0 g sub sample of soil is extracted by reflux with acetonitrile/0.3% formic solution (70:30, v/v) for one hour. For difenoconazole and CGA-205375 analysis, an aliquot of the centrifuged soil extract is taken and diluted with water. It is analyzed by high performance liquid chromatography with triple quadrupole mass spectrometry detection (LC/MS/MS) using reverse phase.

For CGA-71019 analysis, a second aliquot of the centrifuged extract is taken and reacted with dansyl chloride to form the dansyl triazole derivative. The dansyl triazole (DT) is partitioned into dichloromethane, the dichloromethane evaporated to dryness and the sample re-dissolved in acetonitrile/water solution. It is analyzed by reverse phase LC/MS/MS.

For CGA-142856, a third aliquot of the extract is subjected to a cation exchange resin concentration procedure. The column eluate containing CGA-142856 is rotary evaporated to dryness and re-dissolved in acetonitrile/0.3% formic acid solution. It is analyzed using normal phase LC/MS/MS.

The limit of quantification (LOQ) of the method is 1.00 ng/g (ppb).

2.0 Materials and Apparatus

The recommended equipment and apparatus are listed in Appendix 1. Equipment with equivalent performance specifications may be substituted.

2.1 Reagents and Analytical Standards

All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used. Reagents of comparable purity may be substituted as long as acceptable performance is demonstrated. See Appendix 2 for a list of reagents, solutions and analytical standards for the method.

2.2 Preparation of Analytical Stock Standard Solutions

It is recommended that the following precautions should be taken when weighing the analytical materials.

1. Ensure good ventilation.
2. Wear approved eye protection, gloves and a laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area(s) immediately.

These are suggested concentrations and preparation procedures. Different schemes may be used to prepare different and/or additional standard solutions.

Accurately weigh with a five-figure balance approximately 10.0 mg each of difenoconazole, CGA-205375, CGA-71019 and CGA-142856 analytical standard into 10.0 mL volumetric flasks. Fill the volumetric to the calibration mark with acetonitrile/water solution (1:1, v/v). Mix. This gives four stock standards each of a nominal 1000 ppm. Calculate the exact concentration of the stocks using the following formula.

$$C\,(ppm) = \frac{Wt.\,(mg) \times P \times 1000}{V\,(mL)}$$

Where "V" is the volume of diluting solvent; "Wt." is the weight, in mg, of the solid analytical standard; "P" is the purity, in decimal form, of the analytical standard; and 1000 is conversion factor from ppm to ppb.

Prepare an exact 10.0 ppm mixed fortification solution containing the 4 analytes. Use the following formula to determine the required delivery volume of each stock standard.

$$V\,(mL) = \frac{10.0\,ppm \times 10.0\,mL}{C_{stock}\,(ppm)}$$

Use an adjustable 250 uL or 100 uL Microman pipette and deliver the appropriate volumes to a 10.0 mL volumetric flask. Fill to the calibration mark with acetonitrile. Mix.

Prepare the following solutions from the mixed fortification solution with acetonitrile/water solution (1:1, v/v).

1.00 µg/mL(ppm) - 1.0 mL of the 10.0 ppm fortification solution is diluted with 9.0 mL acetonitrile/water solution (1:1, v/v) to give a 1.00 ppm fortification solution.

100 ng/mL(ppb) - 1.0 mL of the 1.00 ppm fortification solution is diluted with 9.0 mL acetonitrile/water solution (1:1, v/v) to give a 100 ppb fortification solution.

10 ng/mL(ppb) - 1.0 mL of the 100 ppb fortification solution is diluted with 9.0 mL acetonitrile/water solution (1:1, v/v) to give a 10 ppb fortification solution.

When not in use, always store the standard solutions in a refrigerator at about 5°C to prevent decomposition and/or concentration of the standard. An expiration date of one year is recommended for the stock and fortification standards unless additional data is generated that shows a longer storage period.

2.3 Preparation of Cation Resin and Cation Exchange Columns for CGA-142856 Analysis

Cation Resin Preparation Procedure:

Add 500 mL high purity water to approximately 200 g of Bio-Rad AG 50W-X4 (200-400 mesh size) resin in a 1 L conical flask and swirl gently to mix. Allow the resin to settle and decant the water. Add a further 500 mL ultra pure water and 1.0 mL of concentrated formic acid and swirl gently to mix. Cover or stopper the flask and leave to equilibrate overnight.

Cation Exchange Column Preparation Procedure:

Place a 10 mL (16mm ID x 80 mm) polypropylene column onto a suitable vacuum manifold (e.g. Supelco Visiprep®). Tightly pack the bottom of the column with a double layer formed by two 21 mm glass fiber filter papers (Whatman 934AH). It is helpful to have a 14 mm piston that fits within the column to help fit the papers tightly and securely. Keep the manifold tap closed and use a large bore 5 mL pipette to transfer small volumes of the resin slurry to the column. Attain a resin bed height of 2.5 cm (5 mL). Allow the resin to settle and adjust the height as required. Open the manifold tap and allow the water from the slurry to drain **under gravity** until the level is approximately 1-2 mm above the resin bed surface. Keep the flow rate at approximately 2 mL min^{-1}. Close the manifold tap. Do not allow the resin bed to go dry. Add 10 mL water to the column. Drain through the resin bed **under gravity** discarding the wash. Keep the flow rate at approximately 2 mL min^{-1}. Stop when the level is approximately 1-2 mm above the resin bed surface. Add 10 mL acetonitrile/2%

formic acid in water 70:30 (v/v) solution and drain through the resin bed **under gravity**. Keep the flow rate at approximately 2 mL min^{-1}. Discard the wash. Stop when the level is approximately 1-2 mm above the resin bed. Close the manifold taps. The cation exchange column is now ready to use.

2.4 Safety Precautions and Hazards

Whereas most of the chemicals in this method have not been completely characterized, general laboratory safety precautions are advised (e.g., safety glasses, gloves, etc.). The user(s) should consult the relevant MSDS for commonly used reagents and materials.

Solvent Hazards

	Acetonitrile	Acetic Acid	Dichloro-methane	Acetone	Formic acid
Harmful Vapour	✓	✓	✓	✓	✓
Highly Flammable	✓	×	×	✓	×
Harmful by Skin Absorption	✓	✓	✓	×	✓
OES Short Term (mg m^{-3})	**105**	**37**	**870 (MEL)**	**3560**	-

In all cases avoid breathing vapors. Avoid contact with eyes and skin.

Difenoconazole and CGA-71019 have been assigned a Syngenta toxicity classification of 4. At present there is insufficient data available to assign a Syngenta toxicity classification for CGA-205375. It should be treated as a class 3 compound until further information indicates otherwise. The toxicity classification scale rates highly toxic chemicals as class 1 and non toxic chemicals as class 5.

Dansyl chloride has been assigned a Syngenta toxicity classification of 3. It causes burns therefore suitable eye and skin protection should be used.

3.0 Analytical Procedure

Note: Due to the low detection limit of the method it is important that precautions be taken to avoid cross contamination in the laboratory.
Specifically:

- Where possible disposable glassware/plastic-ware has been specified, clean glassware/plastic-ware should be used for each batch of samples.
- High purity distilled-in-glass pesticide grade solvents should be used.

- Existing glassware should be washed and solvent (acetone or methanol) rinsed, before use in the method and between batches of samples

3.1 Sample Preparation

It is important that a homogeneous soil sample be available for analysis. All samples should be prepared using an approved method of preparation for residue analysis prior to analysis. (References 3, 4 and 5).

3.2 Extraction

a) Weigh a representative amount of soil (10.0 g) into a round-bottomed flask (250 mL size). At least one untreated control and two control samples fortified with known amounts of difenoconazole, CGA-205375, CGA-71019 and CGA-142856 in acetonitrile/water solution 1:1 (v/v) should be analyzed alongside each batch of samples to demonstrate acceptable performance of the method.

b) Add 100 mL acetonitrile/0.3% formic acid in water 70:30 (v/v) to the sample and place the round-bottomed flask on a heating mantle and heat the sample under reflux for 1 hour. Allow the sample to cool to room temperature. Pour 45 mL of the sample into a 50 mL polypropylene centrifuge tube. Discard the remainder. This solution will be cloudy and so it is necessary to centrifuge it. Centrifuge the sample at a speed that separates the particulate matter from the liquid e.g. 3500 rpm for five minutes. Store cool.

3.3 Difenoconazole and CGA-205375 Analysis

a) Transfer an aliquot of the soil extract from Section 3.2 (b) equivalent to 0.050 g soil (0.50 mL) into an autosampler vial. Add water (0.50 mL) to the sample, cap the vial and vortex for a few seconds to mix the sample. The sample is ready for LC/MS/MS analysis.

3.4 CGA-71019 Analysis

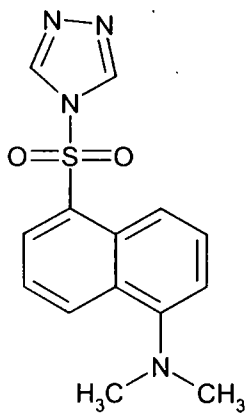
a) Transfer an aliquot of the soil extract from Section 3.2 (b) equivalent to 0.1 g soil (1.0 mL) into a screw capped glass test tube (15 mL size). Add 1 mL of 0.1 M sodium bicarbonate solution, 20 µL of 10% ammonium hydroxide, 100 µL of 10% EDTA and 100 µL of 50 mM dansyl chloride in acetone solution to the sample. Cap the tube and vortex for a few seconds to mix.
Note: The dansyl chloride solution should be prepared weekly and stored in an amber bottle in a refrigerator when not in use. Dansyl chloride neat material is prone to degradation and is moisture sensitive. It is to be kept cold and in a dessicator.

b) Place the vial in a heating block at 40°C for 30 minutes. Do not expose samples to direct light during this procedure. Cover the samples with aluminum foil during the derivatization process.

c) After 30 minutes, remove the samples from the heating block and cool for 10 minutes. Add 2 mL of dichloromethane to the sample, cap the vial and vortex for 30 seconds. Add 5 mL of water. Centrifuge at 1000 ppm for 1 minute to cleanly separate the phases. Carefully pipette the lower dichloromethane layer containing the dansyl triazole into a test tube (4 mL size). Evaporate the dichloromethane to dryness under a stream of clean, dry nitrogen.

d) Re-dissolve the sample in 1.0 mL pH 11 water/acetonitrile 60:40 (v/v) and ultrasonicate. Transfer the sample to an autosampler vial ready for LC/MS/MS analysis.

Dansyl Triazole Derivative



Note: Due to the limited stability of the dansyl triazole derivative, samples need to be kept stored deep-frozen if not immediately analyzed. Stored samples should be removed from the freezer just prior to analysis, thawed and well mixed. The LC/MS/MS should have an autosampler equipped with a chiller. Maintain the sample tray between 3 to 7°C.

3.5 CGA-142856 Analysis

a) Transfer an aliquot of the soil extract from Section 3.2 (b) equivalent to 2.0 g soil (20 mL) into a disposable 40 mL screw capped test tube and acidify with 400 µL concentrated formic acid. Cap the tube and invert it several times to mix it thoroughly.

b) Transfer the sample to a cation exchange column (Section 2.3) attached to a vacuum manifold and allow it to drain through the bed under gravity. Adjust the flow to approximately 2 mL min^{-1}. Discard the column eluate.

Stop when the level is approximately 1-2 mm above the resin bed surface. Close the manifold taps.

c) Rinse the tube with 5 mL water and add to the cation exchange column. Allow the water to drain through the bed under gravity. Adjust the flow to approximately 2 mL min^{-1}. Discard the wash. Stop when the level is approximately 1-2 mm above the resin bed surface.

d) Remove the cartridge from the vacuum manifold and insert it into the neck of a 125 mL round bottom flask with a 24/40 ground glass joint. Add 20 mL methanol/conc. ammonia 75:25 (v/v) to the column. Allow the solvent to drain through the bed under gravity eluting the CGA-142856. Remove the cation exchange column from the neck of the 125 mL flask.

e) Rotary evaporate the sample to dryness under reduced pressure with a water bath temperature of 35-40°C.

f) Re-dissolve the sample in 4.0 mL acetonitrile/0.3% formic acid in water 50:50 (v/v). Ultrasonicate.

g) Transfer 1.0 mL of sample to an autosampler vial ready for final determination by LC/MS/MS. Store cool.

3.6 Time Required for Analysis

The methodology is normally performed with a batch of 15 samples. A method flow diagram is shown in Figure 1. One person can complete the reflux of 15 samples in 1 hour. Preparation of the difenoconazole and CGA-205375 extracts takes about 1 hour. Preparation of the dansyl derivative extracts of CGA-71019 takes about 4 hours. Preparation of the CGA-142856 extracts takes about 5 hours. The total time required for preparation of 15 samples is 11 working hours. Instrumental analysis requires three separate runs. A single set of extracts can be run in about 4 hours. Total run time is about 12 hours.

3.7 Method Stopping Points

The analytical procedure can be stopped after the soil extraction step (section 3.2). The preparation of the cleaned up extracts of each analyte should be completed entirely without stopping. (Section 3.3, 3.4 or 3.5). The raw soil extract of Section 3.2 should be stored in sealed container and refrigerated. Final extracts suitable for analysis can be stored indefinitely as long as they kept deep-frozen (< -10° C). Acceptable recoveries (70-120%) of procedural fortification(s) prepared together with an analytical set validate the extract storage.

3.8 Preparation of Calibration Standards for LC/MS/MS

A minimum of five standard levels is recommended for generation of the external calibration curves. Solutions of analytical standards are interspersed with the samples to form a sequence of analyses. The first and the last injections used in an analytical set must be standards. The smallest standard within a set will determine the limit of detection (LOD) for the set. The smallest standard generally corresponds to about 50% of the limit of quantitation (LOQ) of the analytical method.

The MS/MS response of the samples should fall within the limits of the standard curve. One exception would be for control samples containing residues less than the method LOQ. Any samples with residues less than the method LOQ are typically reported as <1.0 ppb. See section 5.0 of this report for details regarding calculation of results.

The calibration standards should be stored in glass and refrigerated. An expiration date of six months is recommended unless additional data are generated that show a longer expiration date. The expiration date may be extended to a maximum of one year.

3.8.1 Difenoconazole and CGA-205375

LC/MS/MS calibration standards should be prepared at suitable concentrations in acetonitrile/water solution (1:1, v/v). For example, to prepare a 10.0 ppb calibration standard, transfer 1.0 mL of a 100 ppb difenoconazole and CGA-205375 mixed standard to a volumetric flask (10.0 mL) and dilute to 10.0 mL volume with acetonitrile/water solution 1:1 (v/v).

3.8.2 CGA-71019

A 100 ppb stock dansyl triazole LC/MS/MS calibration standard should be derivatized alongside each analysis batch. Prepare the 100 ppb dansyl triazole calibration standard by transferring 100 µL of a 1.00 ppm CGA-71019 standard to a 15 mL screw capped tube. Add 1.0 mL of acetonitrile/0.3% formic (70:30, v/v) and derivatize according to the procedure of Section 3.4 (b to d). Prepare suitable calibration standards from the derivatized stock by serial dilutions in pH 11 water/acetonitrile 60:40 (v/v).

3.8.3 CGA-142856

Standards suitable for external calibration should be prepared in acetonitrile/0.3% formic acid in water 50:50 (v/v). For example, to prepare a 10.0 ppb calibration standard, transfer 1.0 mL of a 100 ppb of mixed standard to a volumetric flask (10.0 mL) and dilute to 10.0 mL volume with acetonitrile/0.3% formic acid in water 50:50 (v/v).

4.0 Final Determination by LC/MS/MS

Three different LC systems are recommended for the analysis of difenoconazole, CGA-205375, CGA-71019 and CGA-142856. Difenoconazole and CGA-205375 are analyzed together. The metabolites CGA-71019 (triazole) and CGA-205375 (triazole acetic acid) are analyzed using separate systems.

The difenoconazole and CGA-205375 utilize a reverse phase silica based LC column. A formic acid mobile phase is required to achieve good sensitivity.

The dansyl derivative of CGA-71019 also utilizes a reverse phase silica based LC column. An acetic acid mobile phase is required. Substitution of the acetic mobile phase with a formic mobile phase significantly reduces the dansyl derivative sensitivity.

CGA-142856 is analyzed by normal phase chromatography on a pentafluorophenyl phase with a propyl spacer (pPFP). The column is highly retentive for basic analytes. CGA-42856 elution can be readily increased or decreased by small changes (~ 1%) of the aqueous percentage. The mobile phase linear gradient needs to be adjusted to give suitable retention and peak shape on the pPFP column. An aqueous composition somewhere in the range of 10% is generally suitable for elution of the analyte.

Difenoconazole, CGA-205375 and the dansyl derivative of CGA-71019 analytes are determined by positive ion electrospray MS/MS. CGA-142856 (triazole acetic acid) is determined by negative ion electrospray MS/MS.

The following instrumentation and conditions have been found suitable for this analysis. Other instrumentation can be used, however optimization may be required to achieve the desired separation and sensitivity. The operating manuals for the instruments should be consulted to ensure safe and optimum use.

4.1 LC/MS/MS System Description and Operating Conditions: System 1

4.1.1 System 1: Difenoconazole and CGA-205375

LC: Instrumentation: CTC HTS PAL Autosampler
Perkin Elmer Series 200 Micropumps

Column	:	Aquasil C18 3.0 x 150 mm,3 um
Column Oven Temperature	:	40°C
Flow rate	:	0.500 mL min^{-1}
Injection volume	:	25 µL
Stop Time	:	10 minutes
Mobile phase	:	Solvent A = 0.2% formic acid in water (v/v) Solvent B = Acetonitrile

Mobile Phase Program (linear)

Time (min.)	% A	% B
0	50	50
1.0	50	50
4.0	10	90
7.0	10	90
7.1	50	50
10	50	50

Typical Analyte LC Retention Times:

Analyte	Approx. Retention time, min.
Difenoconazole	6.0 (doublet)
CGA-205375	4.9

Note: To help minimize ion source contamination, it is recommended that a timed event controlled switching valve be used to divert the LC stream to waste during periods of no data collection (e.g., from injection to 4.0 minutes and 7.0 minutes to run completion).

Typical chromatograms are shown in Figures 3, 4, 5 and 6.

4.1.2 Mass Spectrometer System Description and Operating Conditions

Applied Biosystems Sciex API 4000 LC/MS/MS triple quadrupole mass spectrometer.
Sciex Turbo Ion Spray (TIS) sample introduction unit for the API 4000 mass spectrometer.
Computer: Dell Computer Precision Workstation 360 x86- based PC
Software: OS Microsoft Windows 2000 Professional Version 5.0.2195
Applied Biosystems Analyst 4.1

General Operating Conditions

Interface	:	Turbo Spray	
Polarity	:	Positive	
GS1 (NEB)	:	Air set at 60 (arbitrary units)	
GS2 (AUX)	:	Air set at 60 (arbitrary units)	
Curtain gas (CUR)	:	Nitrogen set at 10 (arbitrary units)	
Temperature (TEM)	:	600°C	
Ionspray voltage	:	5500 V	
Collision gas setting (CAD)	:	Nitrogen set at 6 (arbitrary units)	
Scan type	:	MRM	
		Difenoconazole	CGA-205375
Q1 mass	:	406.0	350.1
Q3 mass	:	251.1	69.9
Dwell time	:	100 ms	100ms
Resolution Q1	:	Unit	Unit
Resolution Q3	:	Unit	Unit
Declustering potential (DP)	:	116 V	81 V
Entrance potential (EP)	:	10 V	10 V
Collision energy (CE)	:	37 V	45 V
Collision cell exit potential (CXP)	:	8 V	6 V
Electron multiplier setting (CEM)	:	2300 V	2300 V

4.2 LC/MS/MS System Description and Operating Conditions: System 2

4.2.1 System 2: CGA-71019 (triazole) as Dansyl Derivative

LC: Instrumentation: CTC HTS PAL Autosampler
Perkin Elmer Series 200 Micropumps

Column	:	Aquasil C18 3.0 x 150 mm,3 um
Column Oven Temperature	:	40°C
Flow rate	:	0.500 mL min^{-1}
Injection volume	:	50 µL
Stop Time	:	10 minutes
Mobile phase	:	Solvent A = 0.2% acetic acid in water (v/v) Solvent B = Acetonitrile

Mobile Phase Program (linear)

Time (min.)	% A	% B
0	60	40
1.0	60	40
4.0	10	90
8.0	10	90
8.1	60	40
10	60	40

Typical Analyte LC Retention Times:

Analyte	Approx. Retention time, min.
Dansyl triazole	5.3

Note: To help minimize ion source contamination, it is recommended that a timed event controlled switching valve be used to divert the LC stream to waste during periods of no data collection (e.g., from injection to 4.0 minutes and 8.0 minutes to run completion).

Typical chromatograms are shown in Figures 7 and 8.

4.2.2 Mass Spectrometer System Description and Operating Conditions

Applied Biosystems Sciex API 4000 LC/MS/MS triple quadrupole mass spectrometer.
Sciex Turbo Ion Spray (TIS) sample introduction unit for the API 4000 mass spectrometer.
Computer: Dell Computer Precision Workstation 360 x86- based PC
Software: OS Microsoft Windows 2000 Professional Version 5.0.2195
Applied Biosystems Analyst 4.1

General Operating Conditions

Interface	:	Turbo Spray
Polarity	:	Positive
GS1 (NEB)	:	Air set at 60 (arbitrary units)
GS2 (AUX)	:	Air set at 60 (arbitrary units)
Curtain gas (CUR)	:	Nitrogen set at 6 (arbitrary units)
Temperature (TEM)	:	600°C
Ionspray voltage	:	5500 V
Collision gas setting (CAD)	:	Nitrogen set at 6 (arbitrary units)
Scan type	:	MRM
		Dansyl triazole
Q1 mass	:	303.0
Q3 mass	:	181.00
Dwell time	:	250 ms
Resolution Q1	:	Unit
Resolution Q3	:	Unit
Declustering potential (DP)	:	76 V
Entrance potential (EP)	:	10 V
Collision energy (CE)	:	39 V
Collision cell exit potential (CXP)	:	12 V
Electron multiplier setting (CEM)	:	2300 V

4.3 LC/MS/MS System Description and Operating Conditions: System 3

4.3.1 System 3: CGA-142856 (triazole acetic acid)

LC: Instrumentation: CTC HTS PAL Autosampler,
Perkin Elmer Series 200 Micropumps

Column	:	Allure PFP Propyl 3.2 x 250, 5um
Column Oven Temperature	:	40°C
Injection volume	:	50 µL
Stop Time	:	10 minutes
Mobile phase	:	Solvent A = 20% 5 mM AmAc at pH 4.5 in ACN Solvent B = ACN

Mobile Phase Program (linear)

Time (min.)	% A	% B	Flow (mL min^{-1})
0	0	100	1.0
2.0	0	100	1.0
6.0	100	0	0.8
8.0	100	0	0.8
10.0	0	100	1.0

Typical Analyte LC Retention Times:

Analyte	Approx. Retention time, min.
CGA-142856	4.2

It is recommended to equilibrate the Allure pPFP column prior to use. Condition with 100% acetonitrile at 1.0 mL min^{-1} for 1 hour, followed by 20% 5 mM AmAc at pH 4.5 in acetonitrile at 0.5 mL min^{-1} for 1 hour, followed by 5% AmAc at pH 4.5 in acetonitrile at 1.0 mL min^{-1} for 1 hour. Monitor the column pressure.

The column backpressure may increase significantly after 80-100 runs. Reverse the column and rinse with 50:50 Acetonitrile water for 2 to 4 hours to remove salts that can precipitate on the head of the column. Flushing will restore the column function.

The retention time of CGA-142856 will vary between 3.5 to 6 minutes on the column depending on the aqueous percentage. Optimize the chromatography to obtain the best possible peak shape.

Note: To help minimize ion source contamination, it is recommended that a timed event controlled switching valve be used to divert the LC stream to waste during periods of no data collection (e.g., from injection to 1.0 minutes and 6.0 minutes to run completion).

Typical chromatograms are shown in Figure 9 and 10.

4.3.2 Mass Spectrometer System Description and Operating Conditions

Applied Biosystems Sciex API 4000 LC/MS/MS triple quadrupole mass spectrometer.
Sciex Turbo Ion Spray (TIS) sample introduction unit for the API 4000 mass spectrometer.
Computer: Dell Computer Precision Workstation 360 x86- based PC
Software: OS Microsoft Windows 2000 Professional Version 5.0.2195
Applied Biosystems Analyst 4.1

General Operating Conditions

Interface	:	Turbo Spray
Polarity	:	Negative
GS1 (NEB)	:	Air set at 60 (arbitrary units)
GS2 (AUX)	:	Air set at 60 (arbitrary units)
Curtain gas (CUR)	:	Nitrogen set at 10 (arbitrary units)
Temperature (TEM)	:	600°C
Ionspray voltage	:	-3800 V
Collision gas setting (CAD)	:	Nitrogen set at 9 (arbitrary units)
Scan type	:	MRM
		CGA-142856
Q1 mass	:	125.80
Q3 mass	:	81.9
Dwell time	:	1000 ms
Resolution Q1	:	Unit
Resolution Q3	:	Unit
Declustering potential (DP)	:	-46 V
Entrance potential (EP)	:	-10 V
Collision energy (CE)	:	-14 V
Collision cell exit potential (CXP)	:	-3 V
Electron multiplier setting (CEM)	:	2200 V

4.3.3 MS/MS Ion transitions

Representative MS/MS product ion scans for difenoconazole and its metabolites CGA-205375, CGA-71019 and CGA-142856 are shown in Figure 2. For difenoconazole, the most intense MS/MS transition is from the positive parent ion at m/z 406 → 251. For CGA-205375 the monitored transition is 350 → 70 and for CGA-71019 as the dansyl derivative the monitored transition is 303 → 181. For CGA-142856 the most intense MS/MS transition is from the negative parent ion at m/z 126 → 82.

5.0 Calculation of Results

5.1 Determination of Residues in Samples:

Inject the sample extract from 3.3(a), 3.4 (d) and 3.5 (g) into its analysis system. The sample solution must be diluted if the analyte response exceeds the linear range of the calibration curve. Quantitation is achieved using a linear least squares curve fit to the external standards. Acceptable calibration curve fits include linear, linear forced through zero, or linear weighted 1/x, as appropriate.

5.2 Determination of Residues in Fortified Samples:

Verify the method performance for each set of samples analyzed by including a control sample and two or more control samples fortified with known amounts of difenoconazole, CGA-205375, CGA-71019 and CGA-142856 prior to the extraction procedure. One fortification should be performed at the LOQ and the second fortification level should approximate the expected residue levels in the study samples.

Recovery data are generally considered acceptable when the mean values are between 70% and 120% with a relative standard deviation of ≤20%.

Calculations:

Calculations may be performed with a computer program (preferred) or manually as shown below.

Calculate the analyte concentration (in ppb) for field-incurred residues using the equation:

$$RES(ppb) = \frac{\text{Analyte found (ng)}}{\text{SWI (g)}}$$

where RES is the residue value in ppb (ng/g), analyte found (ng) is calculated from a standard calibration curve, and SWI is the sample weight injected (g).

The amount, in milligrams, of sample weight injected (SWI) can be calculated using the equation:

$$SWI(g) = \frac{FW(g) \times IV(\mu L)}{FV(mL)} \times 1000$$

where FW = final sample weight (g), IV = LC injection volume (µL) and FV = final volume in which sample is dissolved (mL).

The final sample weight (FW) is calculated by the equation:

$$FW(g) = \left[\frac{SWE(g) \times A1(mL)}{EV(mL) + \{SWE(g) \times M(\%)/100\}}\right] \times \left[\frac{A2(mL)}{INV(mL)}\right]$$

where FW = final weight (g), SWE = sample weight extracted (g), A1 = aliquot 1 volume (mL), EV = total extraction solvent volume (mL), M = sample moisture in percent, A2 = aliquot 2 volume (mL), if needed, INV = interim volume (mL) is the total volume from which the 2nd aliquot is taken.

NOTES: Either actual or nominal sample weights may be used in the calculations. All of the calculations performed in this report used the 10.0 g nominal sample weight. For method performance (recovery) samples, the M% (moisture) value is set to zero since

the fortifications are based upon their wet weights. If no sample dilutions are performed, the second term in the equation (i.e., A2/INV) is equal to one.

The recovery factor, expressed as a percentage (R%), is calculated using the following equation.

$$R\% = \frac{\text{RES fortified (ppb)} - \text{RES control (ppb)}}{\text{ppb analyte added}} \times 100$$

To correct a residue value to its dry weight value, the following equation may be used:

$$\text{SDW(ppb)} = \frac{\text{CR (ppb)}}{\frac{(100 - \text{M(\%)})}{100}}$$

where SDW = soil dry weight residue (ppb), CR = corrected soil residue (ppb), and M = soil moisture (%). For study samples, soil moistures should be determined following the appropriate SOP.

6.0 Interferences and Confirmation

Final determination by LC/MS/MS is considered to be highly specific; therefore no confirmatory conditions are included. It is recommended that batches of solvent and/or reagents be checked for potential contamination if residue levels are detected in the method blanks. This method uses disposable lab ware, where possible. All reusable glassware should be detergent washed then rinsed with HPLC grade methanol, acetone or acetonitrile prior to use.

7.0 Modifications and Potential Problems

7.1 Modifications

This method is a combination of two existing methods of analysis, Syngenta Method RAM 435/01 for the determination of difenoconazole and its metabolites CGA-205375 and CGA-71019 in soil [1] and Syngenta Method RAM 448/01 for the determination of residues of CGA-142856 in soil [2]. The major modifications to the Syngenta methods are:

1. The use of a single extraction of all residues with an acetonitrile/0.3% formic solution (70:30, v/v) reflux in place of two separate extractions, by reflux in RAM 435/01 and by accelerated solvent extraction (ASE) in RAM 448/01.
2. The derivatization conditions for the dansyl triazole in RAM 435/01 were altered to improve the ruggedness of the procedure. Modification to the derivatization procedure include:
   1) Increase of the concentration of the dansyl chloride derivatizing reagent from 5 mM to 50 mM.

2) Addition of a small amount of ammonium hydroxide to the derivatization extract prior to heating to increase its pH.
3) Addition of EDTA to the derivatization extract prior to heating to chelate any divalent cations present. Cations will reduce the reaction efficiency.

3. Simplification of the cleanup methodology for CGA-142856 in RAM 448/01. The Oasis SPE cartridge cleanup step following the cation exchange column was removed.
4. Improvement in the ruggedness of cation exchange procedure. The bed volume was increased from 1 mL to 5 mL (2.5 cm bed in a 16 x 80 mm column). The volume and strength of the elution solvent was increased to 20 mL of 75:25 methanol/conc. ammonia (v/v).
5. The alteration of the chromatography conditions for CGA-142856 in RAM 448/01. The cleaned extracts from the cation exchange column were chromatographed by normal phase chromatography on an Allure pPFP column.

7.2 Potential Problems

1. The preparation of the dansyl triazole derivative can be problematic. Alternate lot numbers of the neat dansyl chloride material may need to be obtained from the supplier if the reaction fails. The neat dansyl chloride is a hygroscopic, light sensitive material and is prone to degradation. It should be stored frozen in the dark in a dessicator. Fresh derivatization solution in acetone should be prepared only as required and discarded within 7 days.
2. CGA-71019 (triazole) is a ubiquitous compound and may be present at significant concentrations in soil, even soils of non-agricultural origin. Soil chosen for controls may need to be analyzed and evaluated for residues prior to use.
3. The capacity of the cation exchange cartridge may need to be increased if the recoveries of CGA-142856 are low or erratic. Increase the length of the resin bed from 2.5 cm (5 mL) to 3 cm (6 mL). Additional elution solvent may be necessary to completely remove the CGA-142856 from the resin.
4. Ensure that the cation exchange column is prepared so that no resin can escape from the bed into the extract. Contaminating resin will appear as an oily film coating the round bottom used for evaporation.
5. Poor sensitivity of the LC/MS/MS system for CGA-142856 may require that the final volume of the CGA-142856 extract be reduced from 4.0 mL (section 3.5 (f)). For instance, if the LC/MS/MS system cannot be optimized to detect a 0.250 ppb calibration standard (Section 8.4) the extract residue from Section 3.5 (f) may be reconstituted in 1.0 mL of acetonitrile/0.3% formic acid in water 50:50 (v/v). For this scenario optimization of a 1.0 ppb LOD standard would be sufficient.
6. The chromatography on the Allure pPFP column may need to be optimized by altering the aqueous percentage of the mobile phase. Reducing the aqueous by 1-2% will retain the CGA-142856 longer. Increasing the aqueous will decrease the retention time.

8.0 Method Validation

8.1 Recovery and Reproducibility

Method validation experiments demonstrating acceptable recovery data and repeatability were carried out on the procedures described in Section 3. The method validation experiments were performed per protocol as a part of the analytical phases for Syngenta soil dissipation studies, T002983-03 (North Dakota), T002984-03 (California) and T002985-03 (Georgia). (References 6, 7 and 8).

The soils used as the test system for the validations were collected from the difenoconazole soil dissipation test sites in Grand Forks Co., North Dakota, Tulare Co., California and Macon Co., Georgia (USA). The soils used were bulk control soil samples collected from the test plots prior to application of difenoconazole. A summary of the soil characterization data for the sites is shown in Table 1. Complete characterization data are provided in Appendix 3.

Fortification levels of 1.00 ppb and 10.0 ppb and 100 ppb were analyzed to demonstrate the validity of the method. Validation sample sets consisted of an unfortified control sample, three control samples fortified at 1.00 ppb, three control samples fortified at 10.0 ppb, three control samples fortified at 100 ppb and a reagent (or method) blank that contained no soil. The reagent blank served to demonstrate that no contamination occurred from either the reagents or apparatus used in the procedure.

The accuracy of the method was measured by the average recovery for each analyte at each fortification level. The precision of the method was indicated by the percent relative standard deviation observed at each fortification level for each analyte.

8.2 Results

The method validation analytical results are summarized in Tables 2 through 5. Trace residues of difenoconazole (0.228 ppb), CGA-205375 (peak area 689) and CGA-71019 (0.0744 ppb) were detected in the ND sandy clay loam control soil. These residues were at levels lower than 1/3 the LOQ. See Tables 6, 7 and 8 and Figures 4, 6 and 7. No traces of CGA-142856 were noted. The source of the difenoconazole in the control soil was not determined. Cross contamination during work up or glass residues from the reflux apparatus used during extraction may be a possible source. No residues were detected in the reagent blank prepared with the set.

Due to the unknown source for the contamination, additional cleaning steps of the reflux apparatus were put in place. The reflux apparatus was now thoroughly rinsed with caustic methanol (5% ammonium hydroxide in methanol) and methanol between samples. This was done to reduce the possibility that a high treated sample or high fortification contaminated

the condenser tubes. Greater care was taken during the validations with the CA loam and GA loamy sand soil and no residues or interferences were noted in these soil controls.

The recovery results indicate excellent accuracy and precision for this method in the soil types analyzed. All mean recoveries lie within the range of 70-120 % at the 1.00, 10.0 and 100 ppb fortification levels. Calculated RSDs are less than 20%. Detailed examples of recovery data of the sandy clay loam soil are shown in Tables 6 to 9. A summary of the calibration data for the ND sandy clay loam analysis set is presented in Table 10.

Representative chromatograms of external standards are shown in Figures 3, 5, 7 and 9 for difenoconazole, CGA-205375, CGA-71019 and CGA-142856, respectively. Chromatograms for the lowest and highest standard concentrations are shown. Example control soil, reagent blank and LOQ fortified and 100 X LOQ fortified control soil chromatograms for the ND sandy clay loam validation set are presented in Figures 4, 6, 8 and 10 for difenoconazole, CGA-205375, CGA-71019 and CGA-142856, respectively. All reagent blank chromatograms are free of residues. As mentioned above a trace of difenoconazole and CGA-205375 were detected in the ND sandy clay loam control soil. The chromatograms of the fortified control samples demonstrate very good signal to noise response, excellent peak shape, and minimal matrix interferences.

8.3 Limit of Quantitation (LOQ)

The LOQ of the method is defined as the lowest analyte concentration in a sample at which the methodology has been validated. Validation indicates that a mean recovery of 70-120% with an RSD of ≤20% has been obtained at the LOQ fortification level. The LOQ has been set and validated at 1.0 ppb (ng/g) in soil, using LC/MS/MS determination.

8.4 Limit of Detection (LOD)

The method LOD is defined as the smallest standard amount injected during the chromatographic run. This LOD typically corresponds to an amount of analyte equivalent to one half of the theoretical amount for a recovery sample at the method LOQ. For difenoconazole and CGA-205375 the lowest external standard concentration run during validation was 0.0250 ppb (pg/uL). Based on an injection volume of 25 µL this yields an LOD of 0.625 pg injected. For the triazole the lowest external standard concentration run during validation was 0.050 ppb. Based on an injection volume of 50 µL this yields an LOD of 2.5 pg injected. For CGA-142856 the lowest external standard concentration run during validation was 0.250 ppb. Based on an injection volume of 50 µL this yields an LOD of 12.5 pg injected.

8.5 Detector Linearity

For accurate quantification of residue concentrations, analyses should be carried out within the linear range of the detector response. Detector linearity graphs are given in Figures 11, 12, 13, and 14. In this laboratory the linearity of the LC-MS/MS detector response for difenoconazole and CGA-205375 was tested in the range from 0.5 pg to 100 pg injected on column (equivalent to 0.025 ppb to 5.00 ppb standards when using a 25 µL injection volume) and was found to be linear.

The linearity of the LC-MS/MS detector response for CGA-71019 (as the dansyl triazole derivative) was tested in the range from 2.5 pg to 1000 pg injected on column (equivalent to 0.050 to 20.0 ppb standards when using a 50 µL injection volume) and was found to be linear.

The linearity of the LC-MS/MS detector response for CGA-142856 was tested in the range from 6.25 pg to 2500 pg injected on column (equivalent to 0.250 to 100 ppb standards when using a 50 µL injection volume) and was found to be linear.

If a residue beyond the tested concentration range is expected, the extract should be diluted appropriately to bring it within the tested linear range prior to quantification.

8.6 Limitations

This method has been validated only for the soil types listed in this method. Samples from other locations may exhibit binding or interference problems, which were not observed during method validation. For the ND sandy clay loam soil this method was evaluated using an Applied Biosystems API 4000 LC/MS. Validations were also performed on an Applied Biosystems API 3000 LC/MS with the CA loam and the GA loamy sand soil. The following Table shows the soil type/analytes and the LC/MS/MS systems used for analysis. For brevity the instrument conditions for the API 3000 are not shown in the method. Analysis of CGA-71019 was performed only on an API 4000. Both instruments are suitable for analysis.

Soil Type/Site Location	Difenoconazole and CGA205375	CGA-71019	CGA-142856
Sandy Clay Loam Grand forks Co., ND	API 4000	API 4000	API 4000
Loam Tulare Co., CA	API 3000	API 4000	API 4000
Loamy Sand Macon Co., GA	API 3000	API 4000	API 3000

9.0 Conclusions

Validation experiments have demonstrated that ETL Method M314 is a reliable and accurate procedure for the determination of difenoconazole,°CGA-205375, CGA-71019 and CGA-142856 in soil. The method was validated with three distinctly different soil types, as far as texture and physiochemical properties. There were no significant interferences observed in any of the control soils or method blank analyses. This method was validated at fortification levels of 1.00, 10 ppb and 100 ppb. The demonstrated LOQ for the method is 1.00 ppb. The estimated LOD is 0.50 ppb. All analytes demonstrated a linear response for external standard calibration over the ranges injected.

10.0 References

1. Robinson NJ. Residue Analytical Method for the determination of residues of difenoconazole and its metabolites CGA-205375 and CGA-71019 in soil. RAM 435/01 Syngenta Crop Protection, Inc. Jealott's Hill International Research Center, Bracknell Berkshire, UK.

2. Robinson NJ. Residue Analytical Method for the determination of residues of CGA-142856 in soil. RAM 448/01 Syngenta Crop Protection, Inc. Jealott's Hill International Research Center, Bracknell Berkshire, UK.

3. GLP11_4.** Enviro-Test Standard Operating Procedure, Preparation of Soil and Crop Samples for Residue Analysis

4. GLP 8_5.** Enviro-Test Standard Operating Procedure, Sample Preparation Equipment

5. GLP11_7.** Enviro-Test Standard Operating Procedure, Handling Preparation and Disposal of Potential "High Risk" Soils, Crop and Related Materials.

6. Protocol No. T002983-03. Dissipation of Difenoconazole in Soil Under Potato Production Conditions and in a Bare Soil Plot in North Dakota. Syngenta Crop Protection, Inc., Environmental Residue Group, Greensboro, NC. (Including Amendment 1.)

7. Protocol No. T002984-03. Dissipation of Difenoconazole in a Bare Soil Plot Under Simulated Tomato Production Conditions in the Central Valley of California. Syngenta Crop Protection, Inc., Environmental Residue Group, Greensboro, NC. (Including Amendment 1.)

8. Protocol No. T002985-03. Dissipation of Difenoconazole in a Bare Soil Plot Under Simulated Fall Squash Production Conditions in Georgia. Syngenta Crop Protection, Inc., Environmental Residue Group, Greensboro, NC. (Including Amendment 1.)

9. Marlow, D. A., McDaniel, D. D., Dupuy, Jr., A. E. and Leovey, E. M., 1995. Data Reporting Guideline for Environmental Chemistry Methods – Pesticide Assessment Guidelines, Subdivisions E, K, and N. U.S. Environmental Protection Agency, Office of Pesticide Programs, EPA 733-B-95-001.

** = current version

11.0 Tables

Table 1. Select Characterization Data for Soil Types Used In Method Validation Experiments

Soil Type/Site Location	pH (water)	Sand Content (% w/w)	Silt Content (% w/w)	Clay Content (% w/w)	% Organic Matter
Sandy Clay Loam[1] Grand Forks Co., ND	6.0	56	22	22	3.5
Loam[2] Tulare Co., CA	8.2	48	40	12	0.8
Loamy Sand[3] Macon Co., GA	7.1	85	10	5	0.9

[1]Data from Syngenta Study No. T002983-03.
[2]Data from Syngenta Study No. T002984-03.
[3]Data from Syngenta Study No. T002985-03.

Table 2. Difenoconazole Recovery Data Obtained During Method Validation in All Soil Types

Matrix	Fortification Level (ppb)	Recovery (%)**	Mean (%)	RSD (%)	Range (%)
Sandy Clay Loam	1.00 *	108,108,104	107	2.2	104-108
	10.0	108,104,103	105	2.5	103-108
	100	98,107,82	96	13	82-107
		Overall	**102**	**8.1**	**82-108**
Loam	1.00 *	81,113,90	95	17	81-113
	10.0	81,81,77	80	2.9	77-81
	100	90,89,88	89	1.1	88-90
		Overall	**88**	**12**	**77-113**
Loamy Sand	1.00 *	111,109,120	113	5.2	109-120
	10.0	116,96,106	106	9.4	96-116
	100	118,112,105	112	5.8	105-118
		Overall	**110**	**6.7**	**96-120**

* Limit of quantification, defined by the lowest validated fortification level
**Residues in control samples were less than 1/3 of the LOQ

Table 3. CGA-205375 Recovery Data Obtained During Method Validation in All Soil Types

Matrix	Fortification Level (ppb)	Recovery (%)**	Mean (%)	RSD (%)	Range (%)
Sandy Clay Loam	1.00 *	88,91,83	87	4.6	83-91
	10.0	97,100,99	99	1.5	97-100
	100	98,116,81	98	18	81-116
		Overall	**95**	**11**	**81-116**
Loam	1.00 *	78,89,87	85	6.9	78-89
	10.0	86,90,81	86	5.3	81-90
	100	89,85,90	88	3.0	85-90
		Overall	**86**	**4.9**	**78-90**
Loamy Sand	1.00 *	114,116,119	116	2.2	114-119
	10.0	114,93,99	102	11	93-114
	100	108,103,104	105	2.5	103-108
		Overall	**108**	**8.1**	**93-119**

* Limit of quantification, defined by the lowest validated fortification level.
**Residues in control samples were less than 1/3 of the LOQ.

Table 4. CGA-71019 Recovery Data Obtained During Method Validation in All Soil Types

Matrix	Fortification Level (ppb)	Recovery (%)**	Mean (%)	RSD (%)	Range (%)
Sandy Clay Loam	1.00 *	98,102,91	97	5.7	91-102
	10.0	94,100,106	100	6.0	94-106
	100	114,93,114	107	11	93-114
		Overall	**101**	**8.5**	**91-114**
Loam	1.00 *	111,98,95	101	8.4	95-111
	10.0	73,97,98	89	16	73-98
	100	103,109,75	96	19	75-109
		Overall	**95**	**14**	**73-111**
Loamy Sand	1.00 *	98,110,119	109	9.7	98-119
	10.0	102,105,103	103	1.5	102-105
	100	109,105,105	106	2.2	105-109
		Overall	**106**	**5.7**	**98-119**

* Limit of quantification, defined by the lowest validated fortification level.
**Residues in control samples were less than 1/3 of the LOQ.

Table 5. CGA-142856 Recovery Data Obtained During Method Validation in All Soil Types

Matrix	Fortification Level (ppb)	Recovery (%)**	Mean (%)	RSD (%)	Range (%)
Sandy Clay Loam	1.00 *	93,76,102	90	15	76-102
	10.0	75,74,86	78	8.5	74-86
	100	76,83,79	79	4.4	76-83
		Overall	**83**	**11**	**74-102**
Loam	1.00 *	87,95,111	98	13	87-111
	10.0	87,97,104	96	8.9	87-104
	100	91,94,96	94	2.7	91-96
		Overall	**96**	**8.1**	**87-111**
Loamy Sand	1.00 *	102,103,98	101	2.6	98-103
	10.0	98,102,104	101	3.0	98-104
	100	84,109,93	95	13	84-109
		Overall	**99**	**7.4**	**84-109**

* Limit of quantification, defined by the lowest validated fortification level.
**Residues in control samples were less than 1/3 of the LOQ.

Table 6. Difenoconazole Example Recovery Data – ND Sandy Clay Loam Soil

Analyte: Difenoconazole

Sample Code	Fort. Level ppb	Retention Time (minutes)	Peak Area	Amount Found ppb	Recovery (percent)
RB	method blank	-	0	-	-
V-1	control	5.99	3125	* 0.228	-
V+1A	1.00	5.99	11641	1.08	108
V+1B	1.00	5.99	11707	1.08	108
V+1C	1.00	5.99	11253	1.04	104
V+2A	10.0	5.99	109113	10.8	108
V+2B	10.0	5.99	105448	10.4	104
V+2C	10.0	5.99	103892	10.3	103
V+3A	100	5.99	980804	97.7	98
V+3B	100	6.00	1077889	107	107
V+3C	100	6.00	825564	82.2	82

Statistics for Difenoconazole:

	1.0 ppb (LOQ)	10 ppb (10 x LOQ)	100 ppb (100 x LOQ)
Mean, %:	107	105	96
Std. Dev.:	2.3	2.6	13
RSD, %:	2.2	2.5	13
Range, %:	104-108	103-108	82-107

Data for Calculations (see section 5.0): SWE = 10 g, A1 = 0.5 mL, EV = 100 mL, M = 0%, IV = 25 µL, FV = 1.0 mL.

* LOD is 0.50 ppb. Control residue is less than 1/3 of LOQ and is not subtracted from the fortifications.

Table 7. CGA-205375 Example Recovery Data – ND Sandy Clay Loam Soil

Analyte: CGA-205375

Sample Code	Fort. Level ppb	Retention Time (minutes)	Peak Area	Amount Found ppb	Recovery (percent)
RB	method blank	-	0	-	
V-1	control	4.94	689	-	
V+1A	1.00	4.94	7552	0.884	88
V+1B	1.00	4.94	7715	0.909	91
V+1C	1.00	4.94	7171	0.827	83
V+2A	10.0	4.94	65980	9.65	97
V+2B	10.0	4.94	68582	10.0	100
V+2C	10.0	4.94	67299	9.85	99
V+3A	100	4.94	653544	97.8	98
V+3B	100	4.94	775571	116	116
V+3C	100	4.95	543154	81.3	81

Statistics for CGA-205375:

	1.0 ppb (LOQ)	10 ppb (10 x LOQ)	100 ppb (100 x LOQ)
Mean, %:	87	99	98
Std. Dev.:	4.0	1.5	18
RSD, %:	4.6	1.5	18
Range, %:	83-91	97-100	81-116

Data for Calculations (see section 5.0): SWE = 10 g, A1 = 0.5 mL, EV = 100 mL, M = 0%, IV = 25 µL, FV = 1.0 mL.

Table 8. CGA-71019 Example Recovery Data – ND Sandy Clay Loam Soil

Analyte: CGA-71019

Sample Code	Fort. Level ppb	Retention Time (minutes)	Peak Area	Amount Found ppb	Recovery (percent)
RB	method blank	-	0	-	-
V-1	control	5.27	258	* 0.0744	-
V+1A	1.00	5.28	1570	0.983	98
V+1B	1.00	5.28	1627	1.02	102
V+1C	1.00	5.28	1459	0.907	91
V+2A	10.0	5.28	13710	9.39	94
V+2B	10.0	5.28	14640	10.0	100
V+2C	10.0	5.28	15431	10.6	106
V+3A	100	5.28	164559	114	114
V+3B	100	5.28	134545	93.1	93
V+3C	100	5.28	164179	114	114

Statistics for CGA-71019:

	1.0 ppb (LOQ)	10 ppb (10 x LOQ)	100 ppb (100 x LOQ)
Mean, %:	97	100	107
Std. Dev.:	5.6	6.0	12
RSD, %:	5.7	6.0	11
Range, %:	91-102	94-106	93-114

Data for Calculations (see section 5.0): SWE = 10 g, A1 = 1.0 mL, EV = 100 mL, M = 0%, IV = 50 µL, FV = 1.0 mL.

* - LOD is 0.50 ppb. Control residue is less than 1/3 of LOQ and is not subtracted from the fortifications.

Table 9. CGA-142856 Example Recovery Data – ND Sandy Clay Loam Soil

Analyte: CGA-142856

Sample Code	Fort. Level ppb	Retention Time (minutes)	Peak Area	Amount Found ppb	Recovery (percent)
RB	method blank	-	0	-	-
T-1	control	-	0	-	-
T+1A	1.00	4.31	2959	0.934	93
T+1B	1.00	4.33	2287	0.756	76
T+1C	1.00	4.32	3287	1.02	102
T+2A	10.0	4.21	10689	7.45	75
T+2B	10.0	4.22	10661	7.43	74
T+2C	10.0	4.21	12454	8.62	86
T+3A	100	4.21	57039	76.3	76
T+3B	100	4.19	61988	82.8	83
T+3C	100	4.19	59322	79.3	79

Statistics for CGA-142856:

	1.0 ppb (LOQ)	10 ppb (10 x LOQ)	100 ppb (100 x LOQ)
Mean, %:	90	78	79
Std. Dev.:	13	6.7	3.5
RSD, %:	15	8.5	4.4
Range, %:	93-102	74-86	76-83

Data for Calculations (see section 5.0): SWE = 10 g, A1 = 20 mL, EV = 100 mL, M = 0%, IV = 50 µL, FV = 4.0 mL.

Table 10. LC/MS/MS Example Instrument Calibration Data – ND Sandy Clay Loam Analysis

Sample Lists: RG112604Acal_DF_V (for Difenoconazole and CGA-205375)
RG112904Bcal_DT_V (for CGA-71019)
RG120304Acal_TAA_V (for CGA-142856)

	Std. Conc. Inj. (ppb)	Retention Time (min.)	Peak Area	Calculated Amount (ppb)	Accuracy, %
Difenoconazole	0.0250	5.99	5767	0.0246	98
(25 µL injection)	0.0500	5.99	9439	0.0429	86
	0.100	5.98	20341	0.0972	97
	0.250	5.99	51907	0.255	102
	1.00	5.99	188725	0.937	94
	2.50	5.99	524901	2.61	105
	5.00	5.99	994868	4.96	99
CGA-205375	0.0250	4.93	3588	0.0145	58
(25 µL injection)	0.0500	4.93	7234	0.0418	84
	0.100	4.93	12670	0.0826	83
	0.250	4.93	34464	0.246	99
	1.00	4.93	135056	1.00	100
	2.50	4.93	347088	2.59	104
	5.00	4.93	662053	4.95	99
CGA-71019	0.0500	5.27	883	0.0507	101
(50 µL injection)	0.100	5.27	1477	0.0919	92
	0.500	5.28	6096	0.412	82
	1.00	5.27	13476	0.923	92
	5.00	5.27	75730	5.23	105
	10.0	5.27	144271	9.98	100
	20.0	5.28	288306	20.0	100
CGA-142856	0.250	4.15	1680	0.298	119
(50 µL injection)	0.500	4.15	3371	0.521	104
	1.00	4.15	6614	0.951	95
	2.50	4.17	16064	2.20	88
	5.00	4.17	32947	4.44	89
	10.0	4.14	79611	10.6	106
	25.0	4.14	182131	24.2	97
	50.0	4.14	383412	50.8	102
	100	4.17	756285	100	100

Calibration Data Summary:

	Curve Fit	Correlation Coeff.	Slope	y-Intercept
Difenoconazole	Linear (no weighting)	0.9996	2.01e+005	842
CGA-205375	Linear (no weighting)	0.9998	1.33e+005	1660
CGA-71019	Linear (no weighting)	0.9999	1.44e+004	150
CGA-142856	Linear [Weighted (1/x)]	0.9995	7.55e+003	-567

FIGURES

Figure 1. Method Flow Diagram

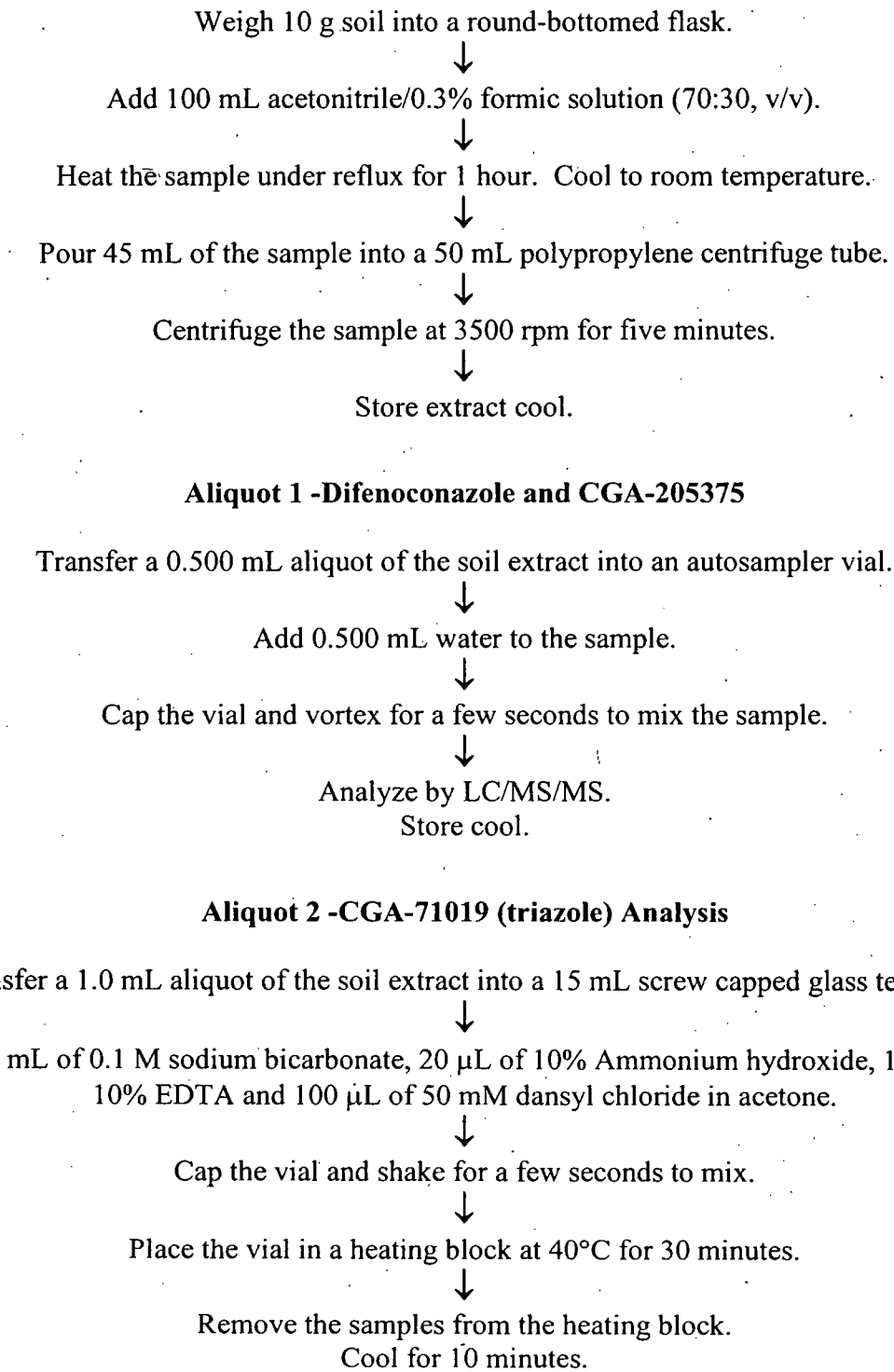


Figure 1. Method Flow Diagram (Continued)

↓

Add 2 mL dichloromethane to the sample, cap and vortex for 30 seconds.

↓

Add 5 mL of water and centrifuge at 1000 rpm for 1 minute or until the phases cleanly separate.

↓

Carefully pipette the lower layer into a 4 mL test tube.

↓

Evaporate the dichloromethane to dryness under a stream of clean, dry nitrogen.

↓

Re-dissolve the sample with 1.0 mL acetonitrile/water (60:40) at pH 11 and ultrasonicate.

↓

Transfer to an autosampler vial ready for LC/MS/MS analysis.
Store deep-frozen.

Aliquot 3 -CGA-142856 (triazole acetic acid) Analysis

Transfer a 20 mL aliquot of the soil extract into a disposable 40 mL screw capped test tube and acidify with 400 µL concentrated formic acid.

↓

Cap the tubes and invert several times to mix.
Transfer the sample to a cation resin exchange column

↓

Draw through under gravity at a rate of approximately 2 mL min^{-1}.

↓

Stop when the level is approximately 1-2 mm above the resin bed surface.
Discard the column eluate.

↓

Rinse the tube with 5 mL water and it to the column.

↓

Draw through the resin bed at a flow rate of approximately 2 mL min^{-1}.

↓

Stop when the level is approximately 1-2 mm above the resin bed surface.
Discard the wash.

↓

Remove the SPE column from the vacuum manifold and place directly into a 125 mL round bottom flask.

↓

Add 20 mL methanol/ concentrated ammonia solution (75:25, v/v) to the column.

Figure 1. Method Flow Diagram (Continued)

↓

Allow to drip by gravity to elute the CGA-142856.

↓

Rotary evaporate the sample to dryness under reduced pressure with water bath temperature at 35-40 °C.

↓

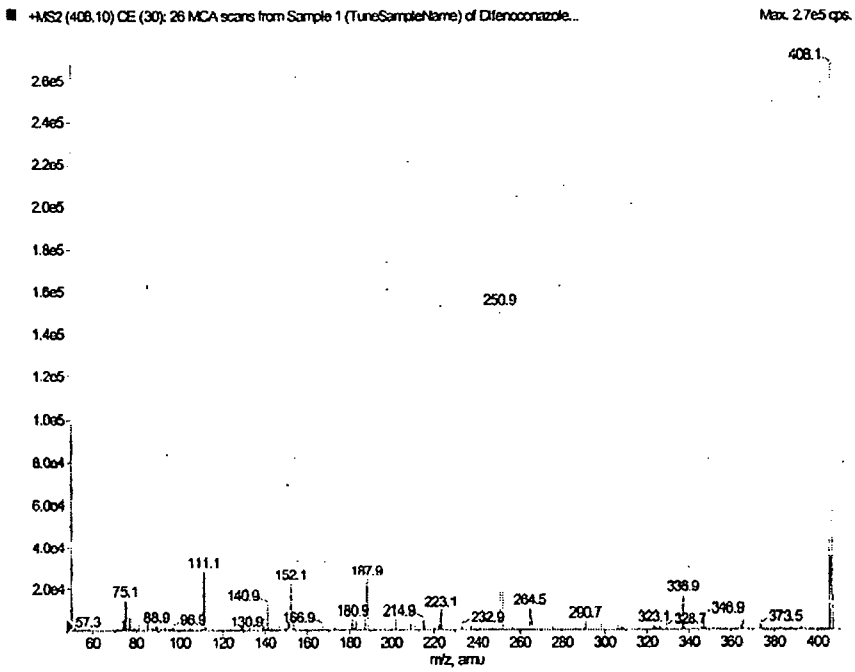
Re-dissolve the sample in 4.00 mL acetonitrile/0.3% formic (50:50). Ultrasonicate.

↓

Transfer 1.0 mL of sample to an autosampler vial for analysis by LC/MS/MS. Store cool.

Figure 2. MS/MS Product Ion Spectra

Product Ion Scan for Difenoconazole



Product Ion Scan for CGA-205375 (Positive Ion)

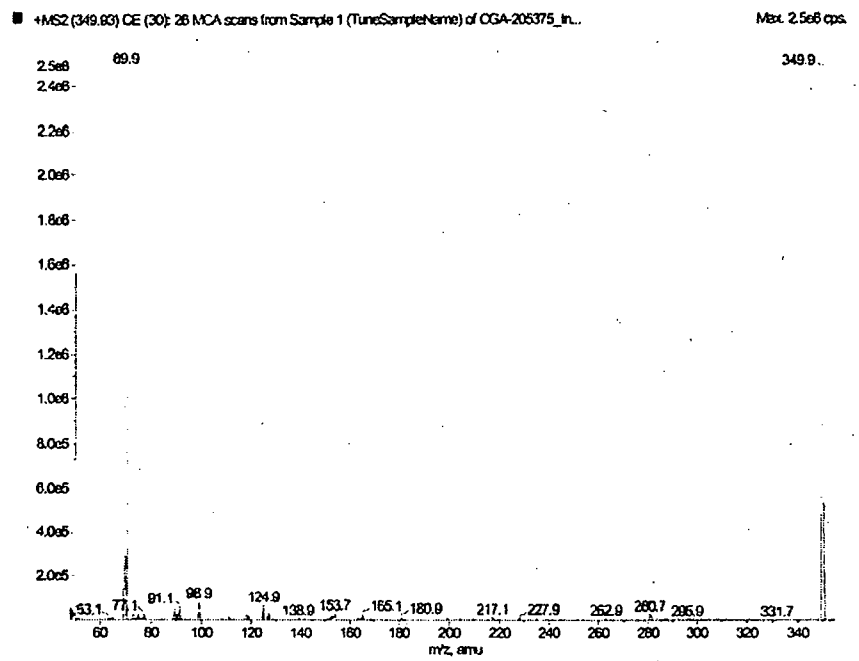
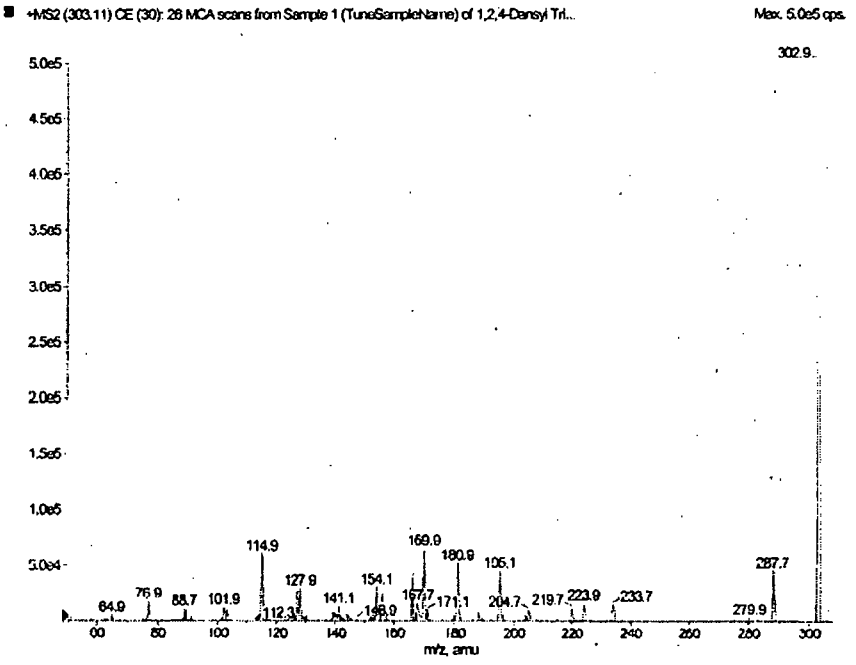


Figure 2. MS/MS Product Ion Spectra (Continued)

Product Ion Scan for CGA-71019 Dansyl Triazole Derivative (Positive Ion)



Product Ion Scan for CGA-142856 (Negative Ion)

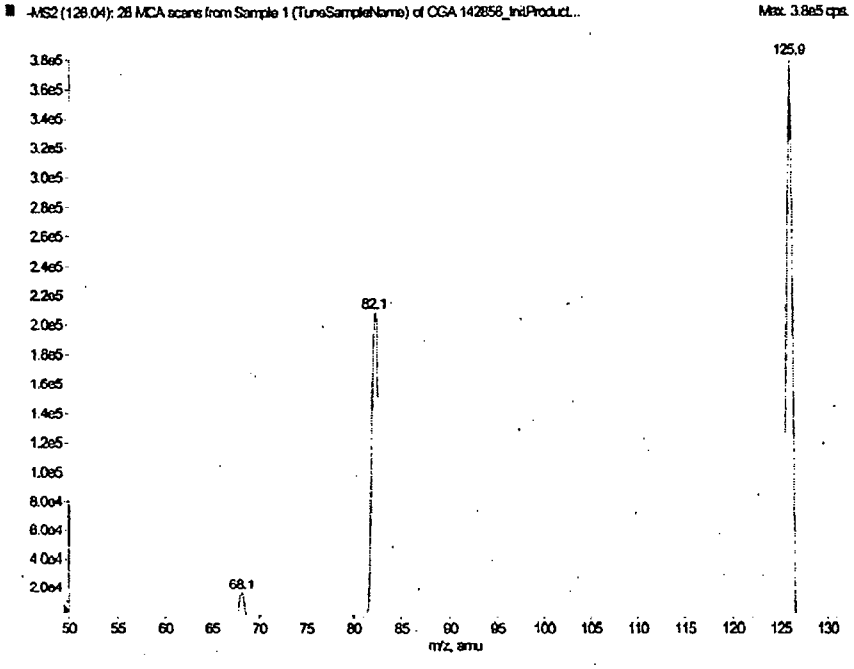
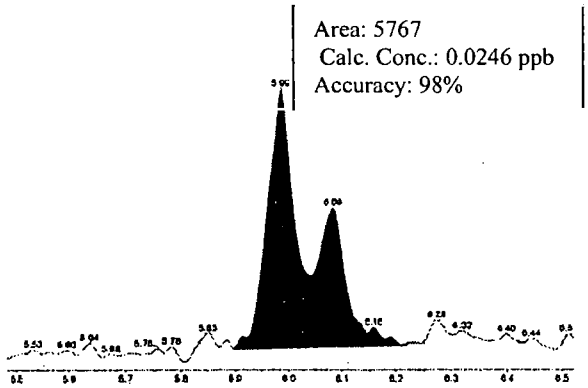
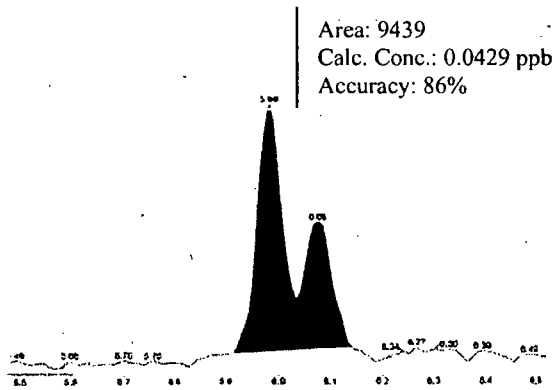


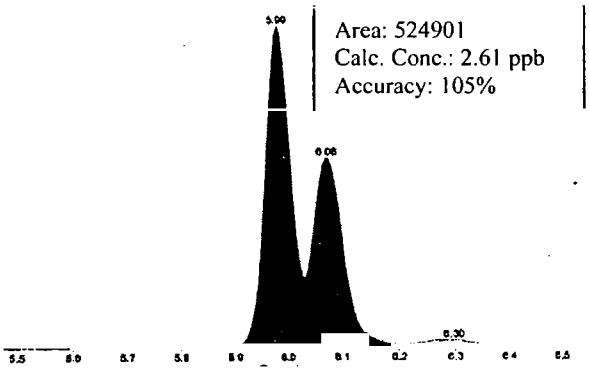
Figure 3. MS/MS Chromatograms of External Standards (Difenoconazole)



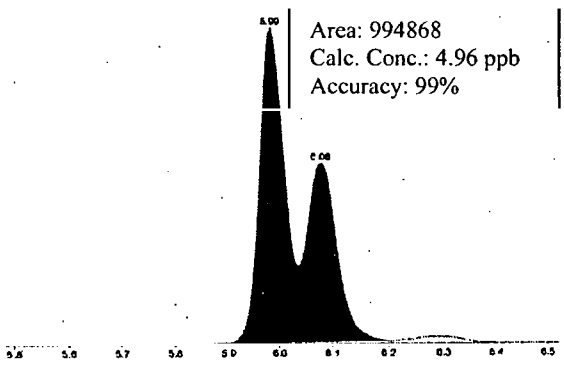
Difenoconazole, 0.0250 ppb standard (LOD)



Difenoconazole, 0.0500 ppb standard (LOQ)

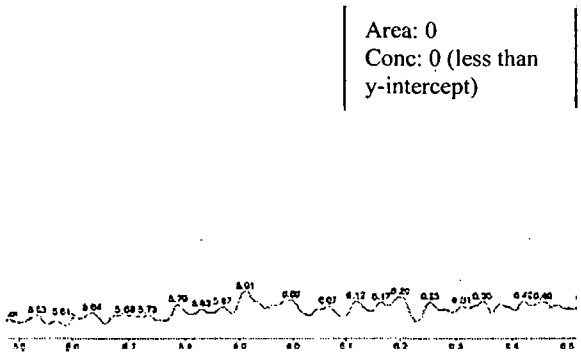


Difenoconazole, 2.50 ppb standard

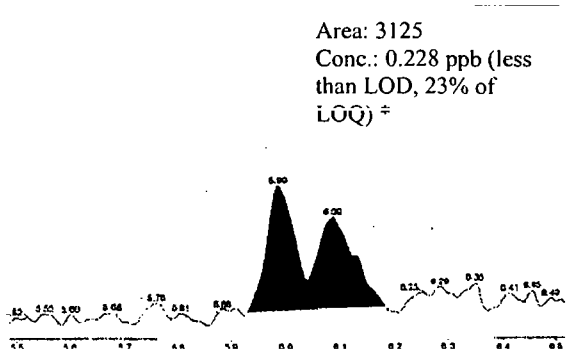


Difenoconazole, 5.00 ppb standard

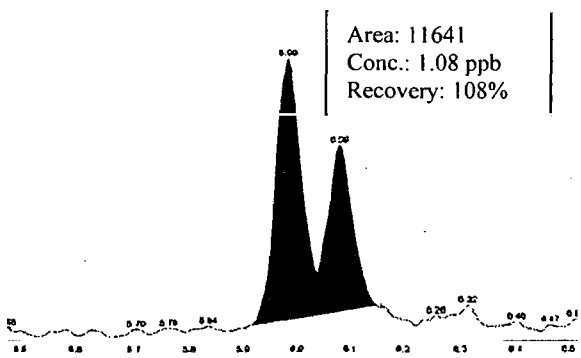
Figure 4. MS/MS Chromatograms of Sandy Clay Loam, North Dakota Soil (Difenoconazole)



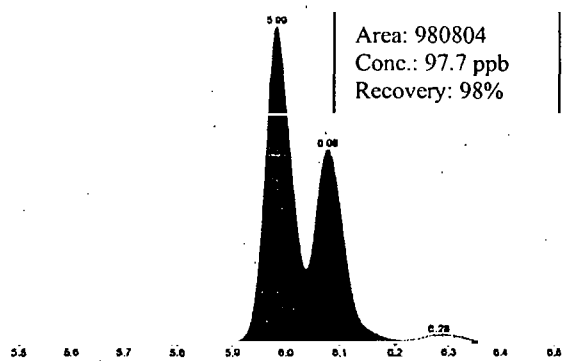
Reagent Blank (Difenoconazole)



V-1, Control Soil (Difenoconazole)



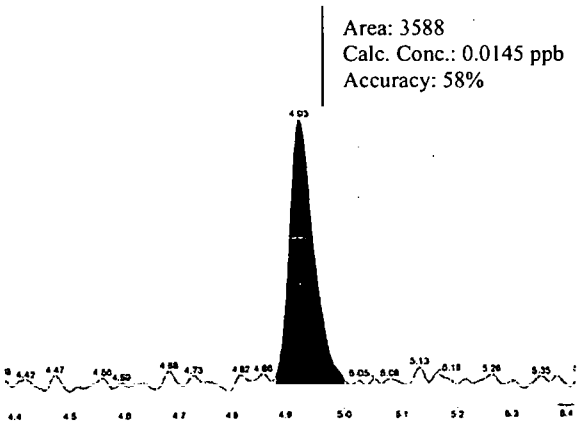
V+1A, Control + 1.00 ppb, (Difenoconazole)



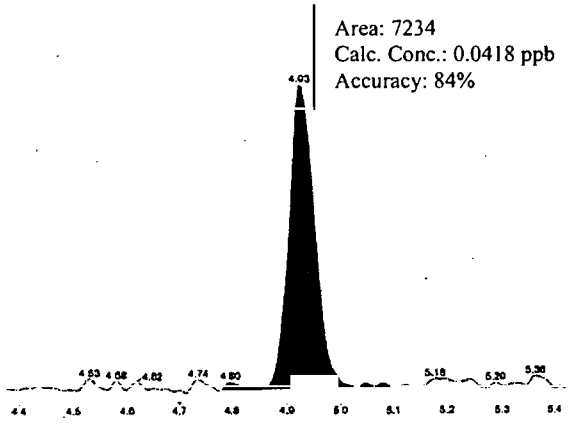
V+3A, Control + 100 ppb, (Difenoconazole)

* = Trace background, source unknown.

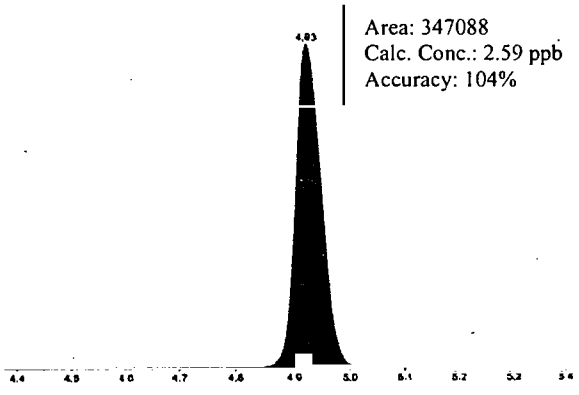
Figure 5. MS/MS Chromatograms of External Standards (CGA-205375)



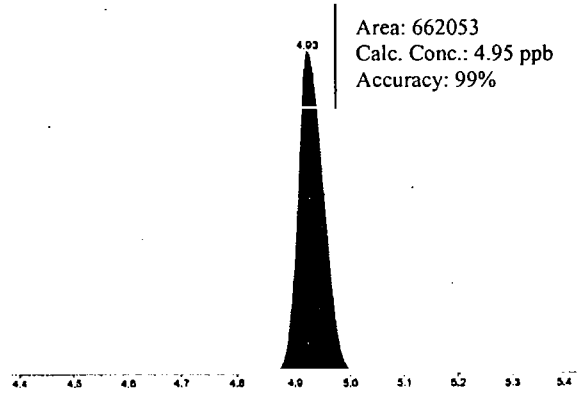
CGA-205375, 0.0250 ppb standard (LOD)



CGA-205375, 0.0500 ppb standard (LOQ)

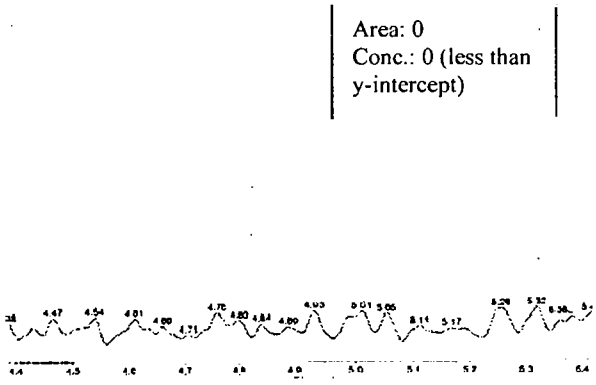


CGA-205375, 2.50 ppb standard

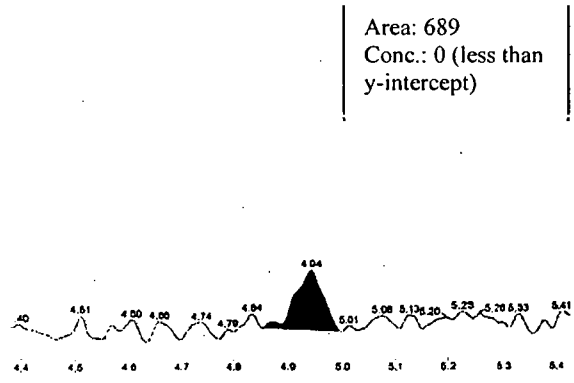


CGA-205375, 5.00 ppb standard

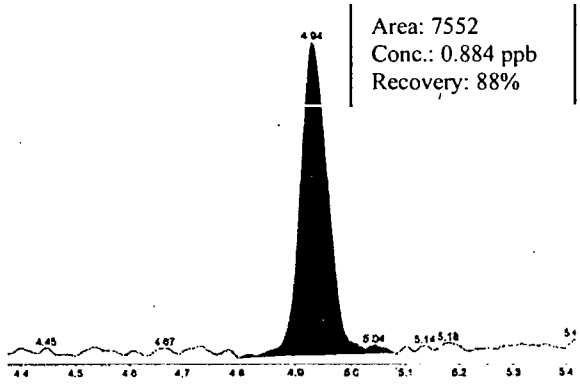
Figure 6. MS/MS Chromatograms of Sandy Clay Loam, North Dakota Soil (CGA-205375)



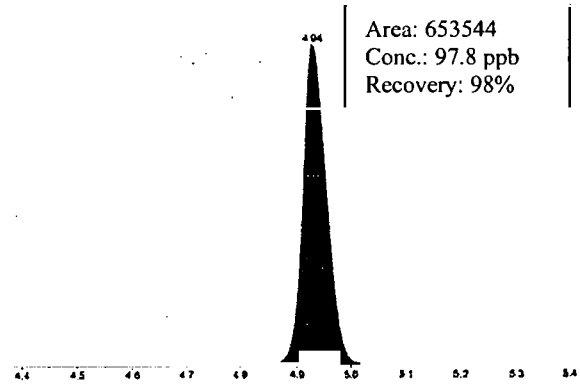
Method Blank (CGA-205375)



V-1, Control Soil (CGA-205375)

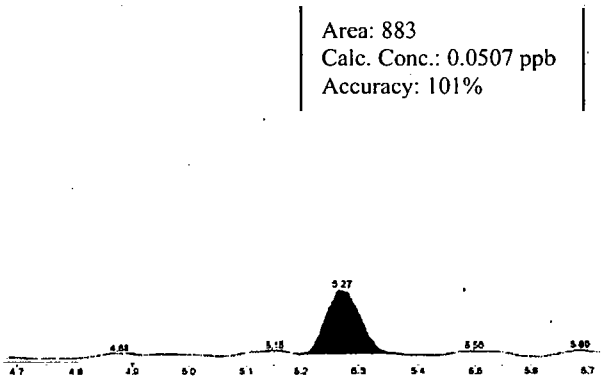


V+1A, Control + 1.00 ppb, (CGA-205375)

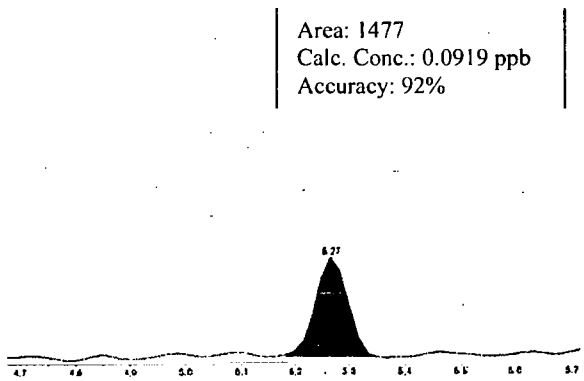


V+3A, Control + 100 ppb, (CGA-205375)

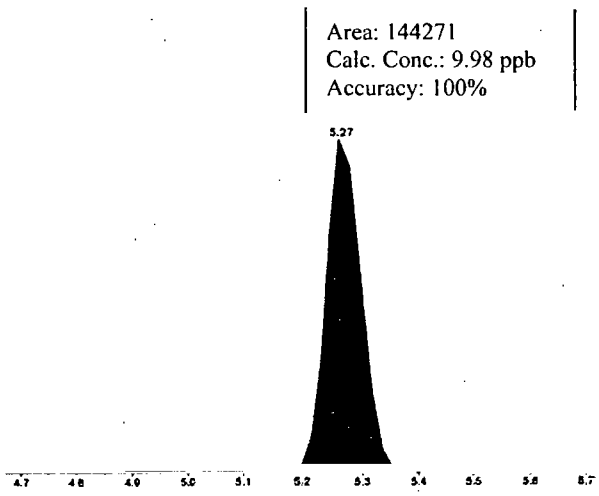
Figure 7. MS/MS Chromatograms of External Standards (CGA-71019)



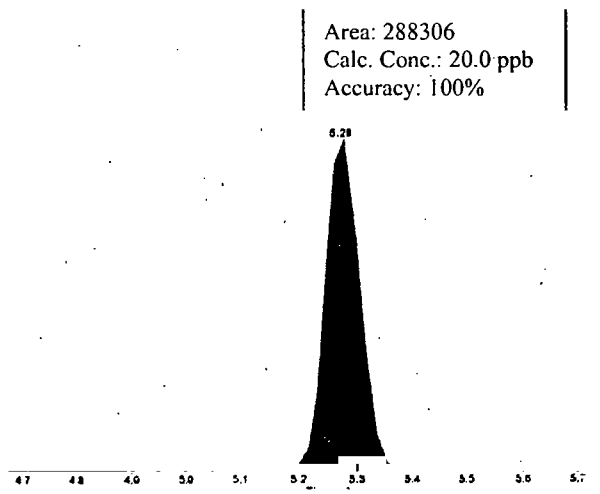
CGA-71019, 0.050 ppb standard (LOD)



CGA-71019, 0.100 ppb standard (LOQ)

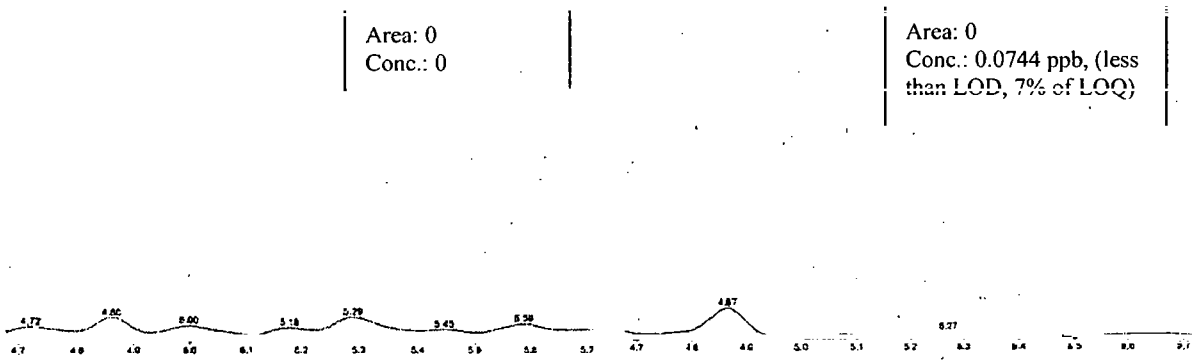


CGA-71019, 10.0 ppb standard



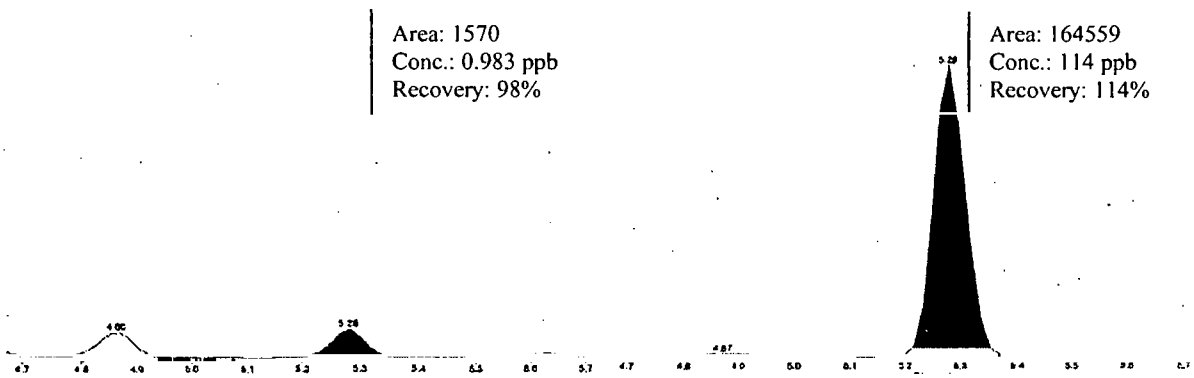
CGA-71019, 20.0 ppb standard

Figure 8. MS/MS Chromatograms of Sandy Clay Loam, North Dakota Soil (CGA-71019)



Method Blank (CGA-71019)

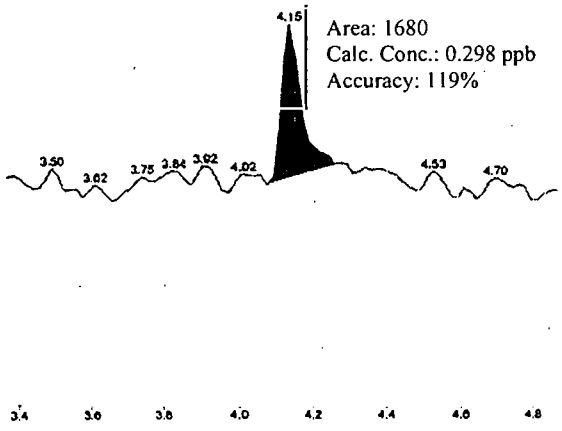
V-1, Control Soil (CGA-71019)



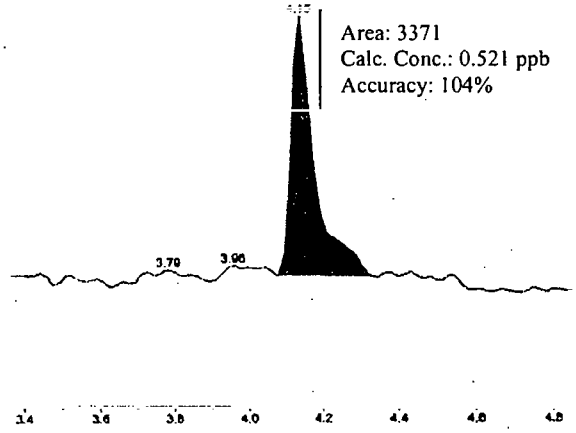
V+1A, Control + 1.00 ppb, (CGA-71019)

V+3A, Control + 100 ppb, (CGA-71019)

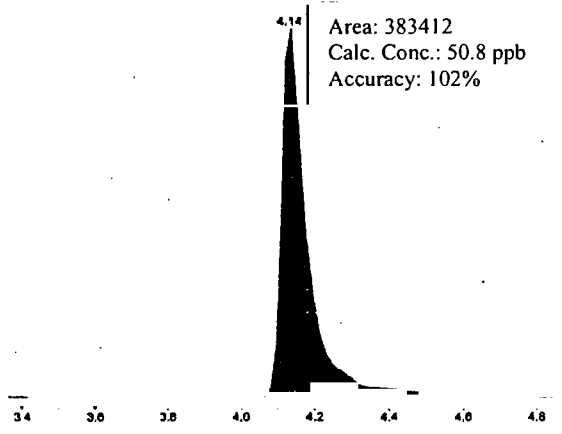
Figure 9. MS/MS Chromatograms of External Standards (CGA-142856)



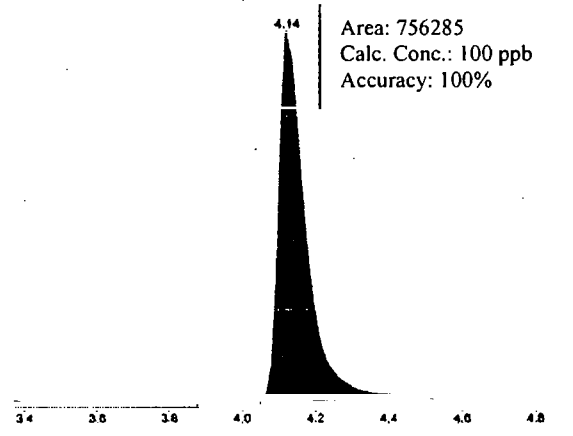
CGA-142856, 0.250 ppb standard



CGA-142856, 0.500 ppb standard

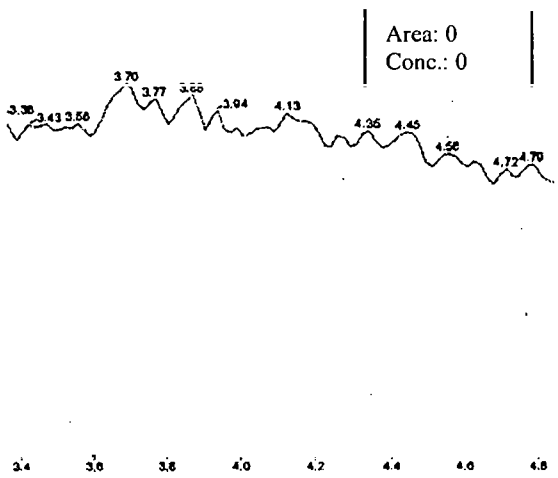


CGA-142856, 50.0 ppb standard

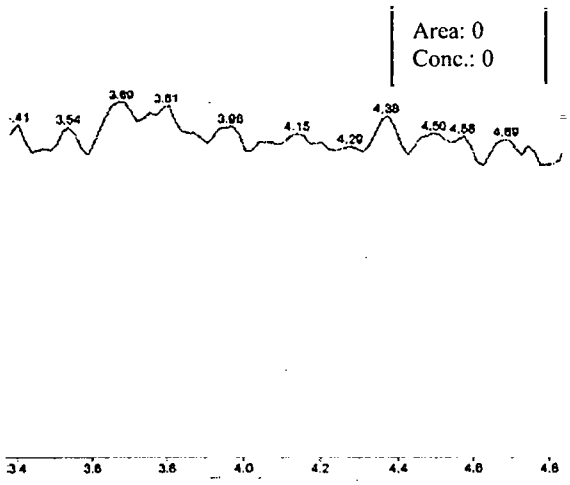


CGA-142856, 100 ppb standard

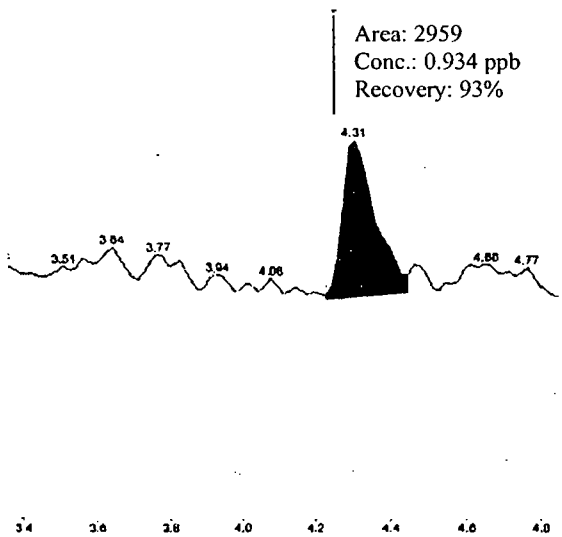
Figure 10. MS/MS Chromatograms of Sandy Clay Loam, North Dakota Soil (CGA-142856)



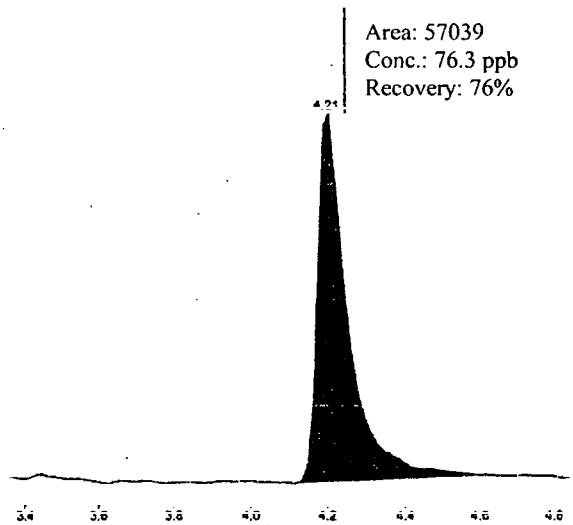
Method Blank (CGA-142856)



Control Soil (CGA-142856)



Control + 1.00 ppb, (CGA-142856)



Control + 100 ppb, (CGA-142856)

Figure 11. Calibration Curve for Difenoconazole

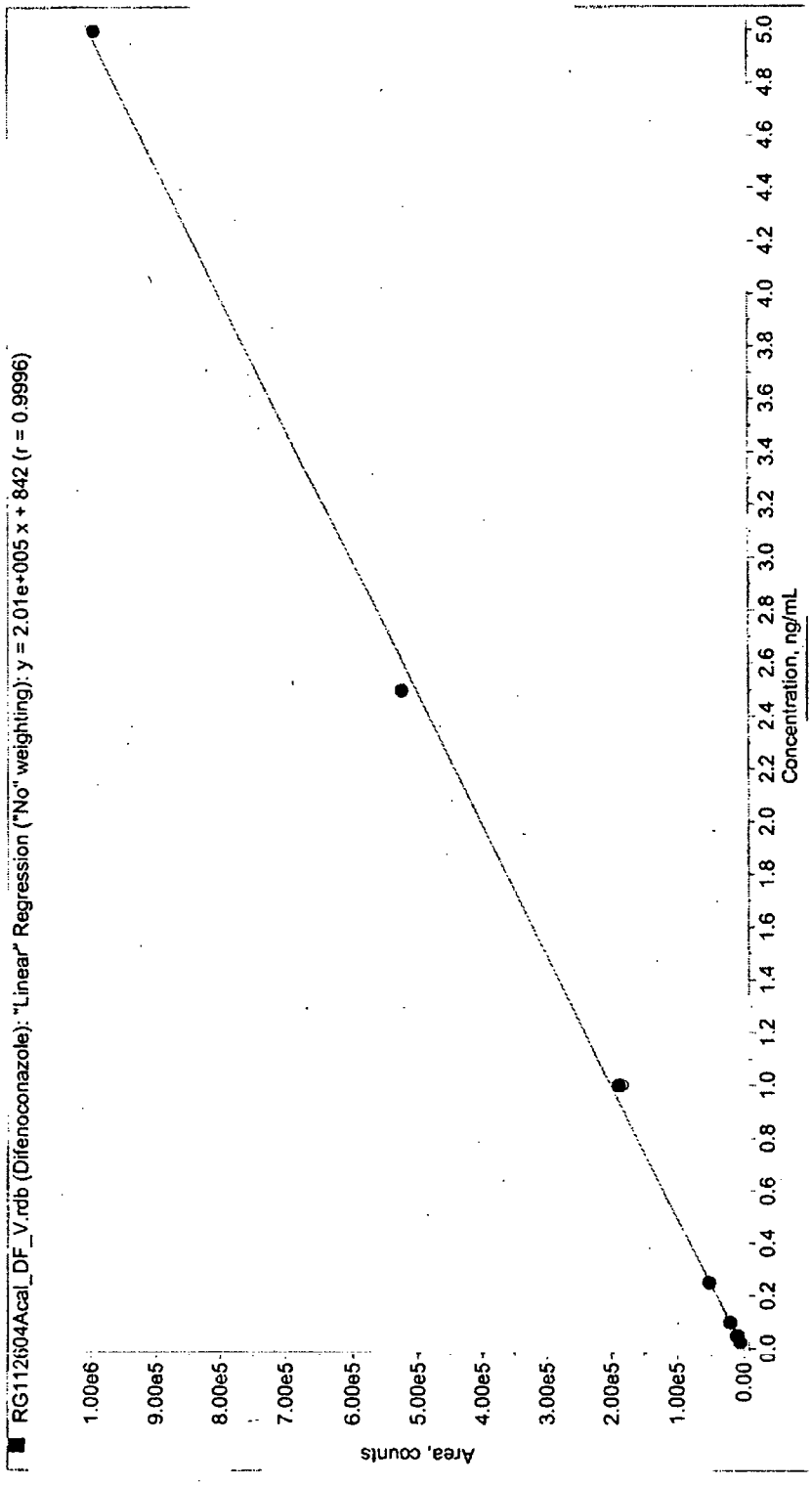


Figure 12. Calibration Curve for CGA-205375

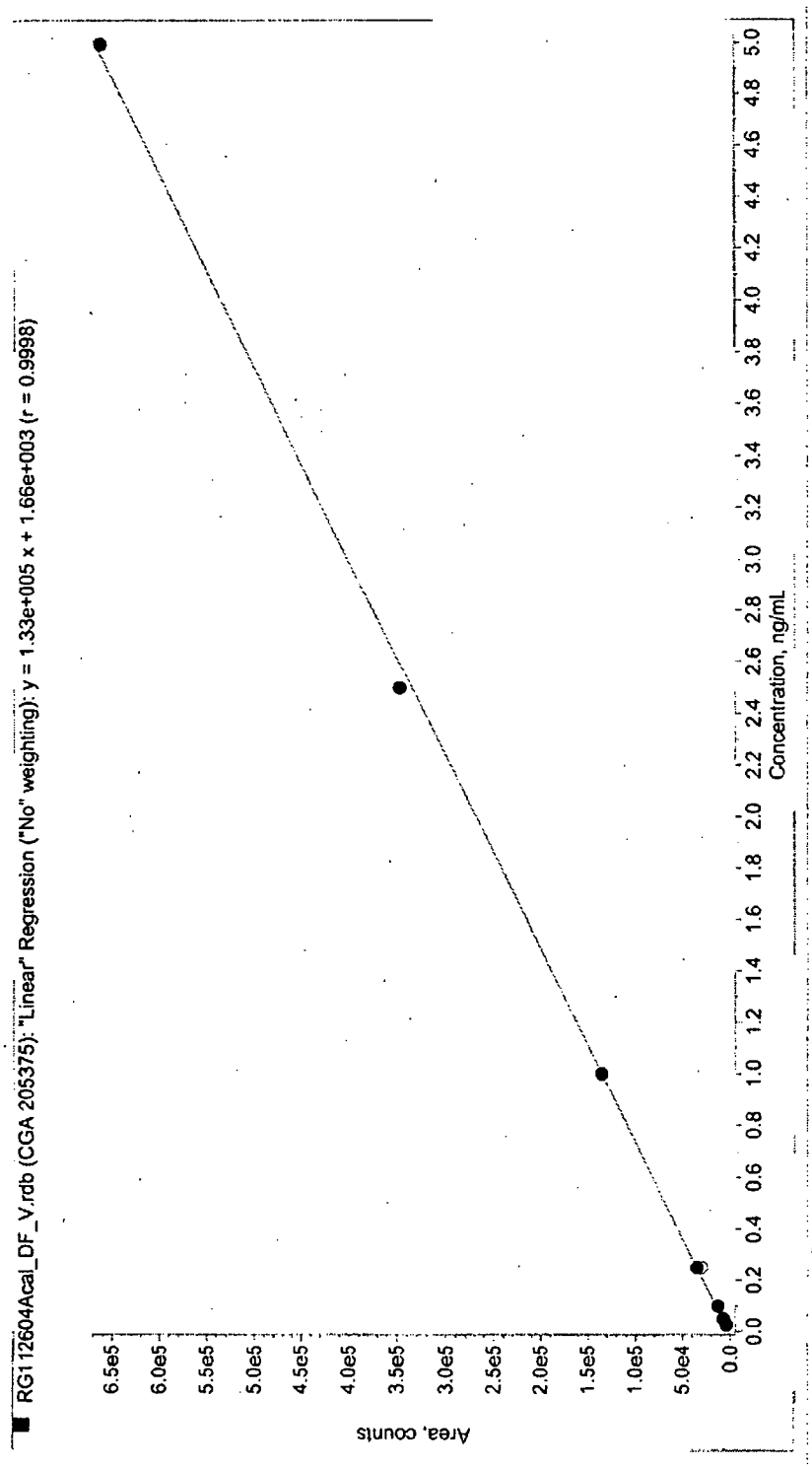


Figure 13. Calibration Curve for CGA-71019

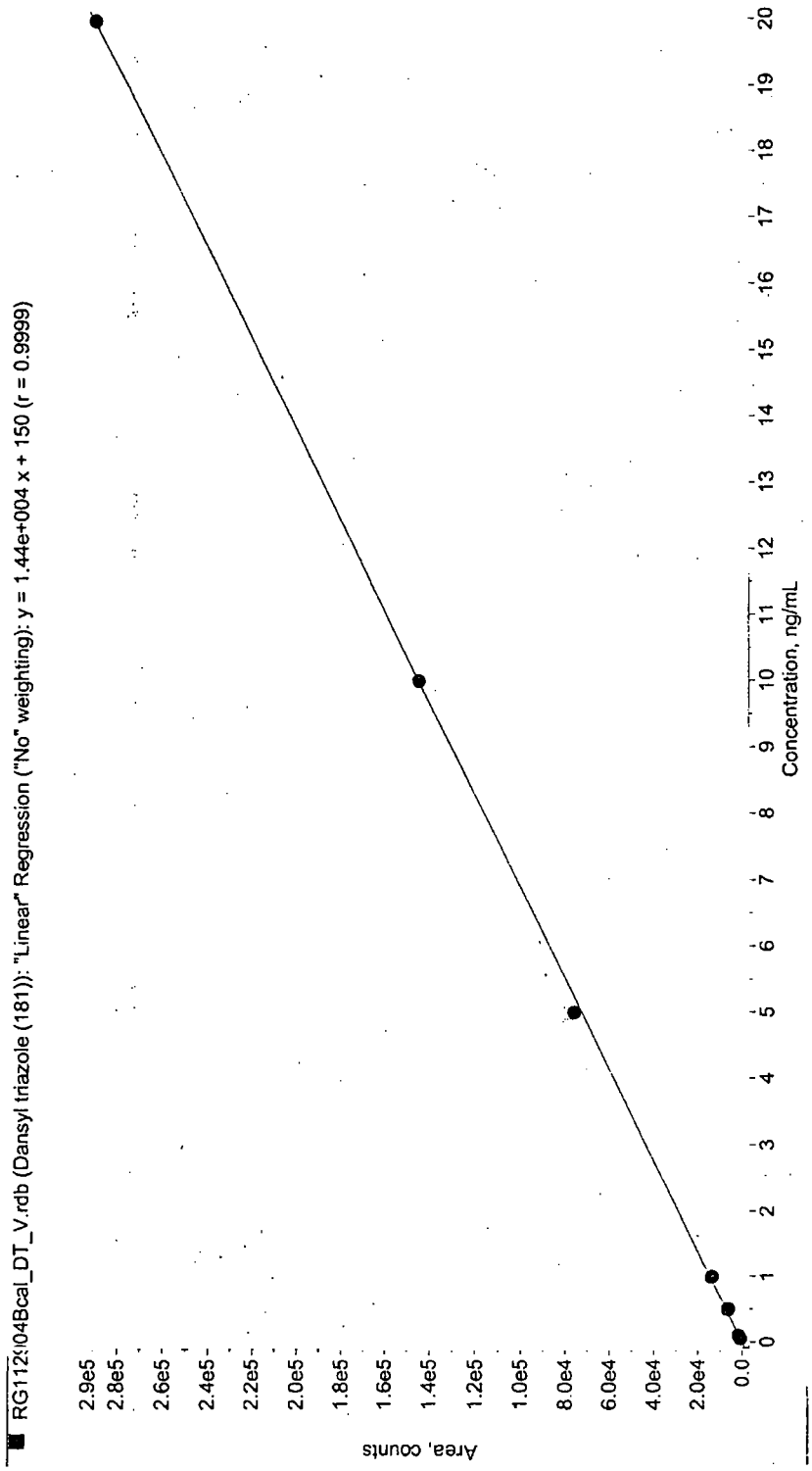
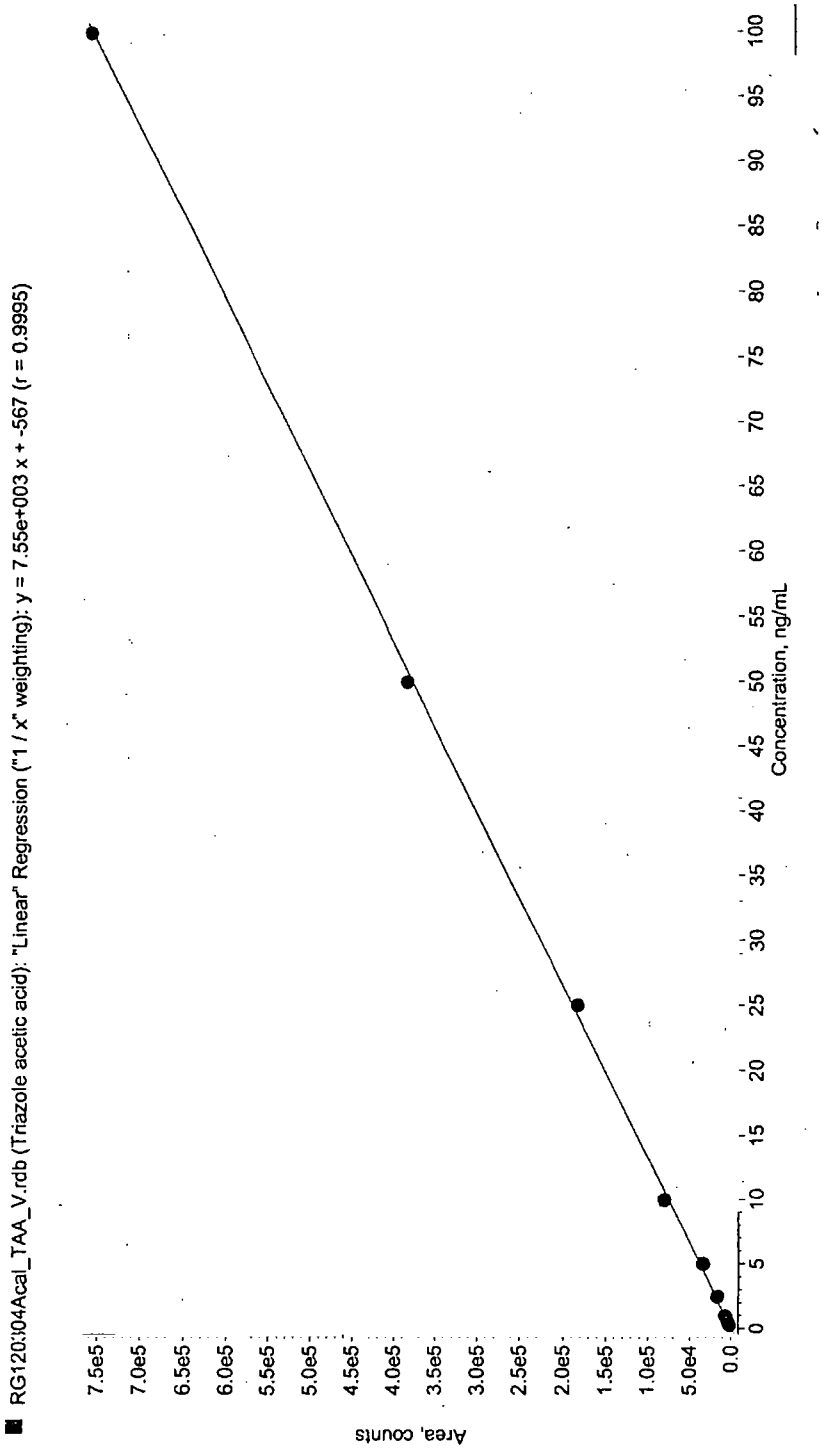


Figure 14. Calibration Curve for CGA-142856



APPENDICES

Appendix 1. Apparatus

General laboratory glassware (beakers, graduated cylinders, disposable pipettes, pipette bulbs, etc.) available from a general laboratory supply company.

Balance, analytical (Mettler model AG245) or equivalent. Electronic display of 0.01 mg, for weighing neat standard materials.

Balance, laboratory (Mettler model PB4002-S or PJ6000), or equivalent. Electronic display of 0.01 g, for weighing soil samples.

Micropipettes, Microman® 25, 50, 100, 250 and 1000 µL, Gilson, (models M25, M50, M100 and M1000). (These pipettes should be used for sample fortification and standard solution preparations).

Suitable Microman® plastic tips to match, Gilson (Cat. No. CP25, CP50, CP100 and CP1000).

Pipette, Socorex, 0.5 - 5 mL, (model 831).

Suitable Socorex 5.0 mL plastic tips to match.

Class A Volumetric Flasks 10 mL, Kimble Glass Inc., (Cat. No. 28014).

Round-bottom flasks, 250 mL and 125 mL with 24/40 ground glass necks, Pyrex (Cat. No. 4100).

1.0 L Erlenmeyer flask, Pyrex (Cat. No. 5100).

Reflux condensors 24/40, Pyrex, (Cat. No. 2480-300).

Heating Mantle 6 position, Barnstead Electrothermal.

pH meter Fisher Scientific. Accumet Research. AR-15.

Glass fiber filter papers, Whatman 934AH, (Cat. No. 1827-150).

Mixer, Vortex-Genie 2, (Fisher Scientific Cat. No. 12-812) or equivalent.

Centrifuge, Centrific Model 225, (Fisher Scientific Cat. No. 04-978-50), or equivalent.

N-Evap Model 111 nitrogen evaporator (Organomation Associates, Berlin, MA), or equivalent.

Tubes, disposable centrifuge, polypropylene, 50-mL, VWR, Cat. No. 21008-240) or equivalent.

Ultrasonic bath, VWR-Scientific Products, 550-T Aquasonic, or equivalent.

Autosampler vials, amber, 1.5 mL, National Scientific, Inc. (Cat. No. C4013), with caps (Cat No. C4013-A).

Autosampler vials, clear, National Scientific Inc. (Cat no. C4013-1) with caps (Cat No. C4013-A).

10 mL disposable glass test tubes, Fisher Scientific (Cat No. 14-961-29).

15 mL screw capped borosilicate glass tubes with Teflon lined solid phenolic closures, available from Kimble Glass Inc. (Cat. No. 45066A-16125).

40 mL screw capped borosilicate glass tubes with Teflon lined solid phenolic closures, available from Kimble Glass Inc. (Kimax. Cat. No. 45066A-25150).

Poly-prep chromatography columns, 10 mL size (16 mm ID x 80 mm), available from Reservoir Analytical, International. (Cat. No. D6).

Vacuum manifold Supelco Visiprep® 12 position.

Vacuum rotary evaporator (Heidolph, Laborota-4000 coupled with Buchi Vac. (Cat. No. V-500) equipped with thermostatically controlled water bath, from IKA

API 3000 LC/MS/MS system equipped with a Turbo Ion Spray source, available from Applied Biosystems, 850 Lincoln Center, Foster City, CA 94404-1128, USA.

API 4000 LC/MS/MS system equipped with a Turbo Ion Spray source, available from Applied Biosystems, 850 Lincoln Center, Foster City, CA 94404-1128, USA.

CTC HTS PAL autosampler, available from LEAP Technologies Inc., P.O. Box 969, Carrboro, NC 27510 USA.

Perkin-Elmer Series 200 Autosampler, available from Perkin-Elmer Corp., 761 Main Ave. Norwalk, CT 06859-0012, USA.

Perkin-Elmer Series 200 HPLC Micro Pump system, available from Perkin-Elmer Corp. 761 Main Ave. Norwalk, CT 06859-0012, USA.

HPLC column Allure PFP Propyl 5 um 250x3.2 mm available from Restek 110 Benner Circle Bellefonte PA 16823, USA

HPLC column, Aquasil C18 3 um 150 x 3.0 mm available from Thermo Electron Corp. Penn Eagle Industrial Park 320 Rolling Ridge Drive Bellefonte PA 16823, USA.

Appendix 2. Reagents, Solution Preparation and Analytical Standards

Reagents:
All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents. All reagents except dansyl chloride are stored at room temperature.

Acetic acid, glacial, EMD Chemical Inc. AnalaR®. (Cat. No. B1001-78), or equivalent.

Acetone, HPLC grade, EMD Chemical Inc. OmniSolv®. (Cat. No. AX0116-1), or equivalent.

Acetonitrile, HPLC grade, EMD Chemical Inc. OmniSolv®. (Cat. No. AX0142-1), or equivalent.

Ammonium acetate, BDH Inc., (Cat. No. B10013), or equivalent.

Ammonium hydroxide, (0.88 specific gravity) Fisher Scientific. (Cat. No. A669-212), or equivalent.

Dansyl chloride, 98% Aldrich (Cat. No. D14,335-9), or equivalent.

Dichloromethane, HPLC grade, EMD Chemical Inc. OmniSolv®. (Cat. No. DX0831-1), or equivalent.

EDTA (ethylenediamine-tetra acetic acid tetrasodium salt dihydrate) certified ACS (EM Science. Cat. No. B10093-34.), or equivalent.

Formic acid, 90%, certified ACS, Fisher Chemicals (Cat. No. A118P-100), or equivalent.

Mass Spectrometer Standard Kit (PPGs) PE Applied Biosystems, Part No. 410936

Methanol, HPLC grade, EMD Chemical Inc. OmniSolv®. (Cat. No. MX0488-1.), or equivalent.

Sodium Bicarbonate, certified ACS (Fisher Chemicals. Cat. No. BP328-500.), or equivalent.

Strong cation exchange resin AG 50W-X4, 200-400 mesh size, Bio-Rad Laboratories, (Cat. No. 142-1351).

Water, ultra pure or HPLC grade (EMD Chemical Inc. OmniSolv®. Cat. No. WX0004-1.) or purified in-house with a Millipore® purification system or equivalent.

Preparation of Solutions:

A. Acetonitrile/0.3% formic acid in water (70:30 v/v) - Add 3.6 mL of formic acid and 1200 mL of de-ionized water to 2800 mL of acetonitrile. Mix well.

B. 0.1M sodium bicarbonate – Stir and dissolve 8.4 g of sodium bicarbonate in 1000 mL of de-ionized water.

C. 50 mM dansyl chloride in acetone - Stir and dissolve 133 mg of dansyl chloride in 10.0 mL acetone. PREPARE WEEKLY/EXPIRES WEEKLY.

D. 10% NH_4OH - SLOWLY stir 10 mL of conc. Ammonium hydroxide into 90 mL of de-ionized water.

E. 10% EDTA – Stir and dissolve 90 g of ethylenediamine-tetra acetic acid tetrasodium salt dihydrate (EDTA) in 900 mL of de-ionized water.

F. pH 11 water/acetonitrile 60:40 (v/v) - Mix 400 mL of acetonitrile with 600 mL of de-ionized water. Add conc. ammonium hydroxide (approx. 5 mL), adjusting the pH to 11. Verify with pH meter.

G. Acetonitrile/2% formic acid in water 70:30 (v/v) - Add 24 mL of formic acid and 1176 mL of bottled water to 2800 mL of acetonitrile. Mix well.

H. Methanol/conc. ammonia 75:25 (v/v) - SLOWLY stir 750 mL of conc. Ammonium hydroxide into 2250 mL of methanol

I. Acetonitrile/0.3% formic acid in water 50:50 (v/v) - Add 3.0 mL of formic acid and 1000 mL of de-ionized water to 1000 mL of acetonitrile. Mix well.

J. Cation exchange resin - Add 500 mL of de-ionized water to approx. 200 g of Bio-Rad AG 500W-X4 (200-400 mesh size) resin in a 1 L conical flask and swirl gently. Allow the resin to settle and decant the water. Add another 500 mL of bottled water followed by 1 mL of conc. formic acid. Swirl gently to mix. Cover and equilibrate overnight. Expires in 1 week.

K. 1000 mM Ammonium acetate (AmAc) - Stir and dissolve 77.1 g ammonium acetate into 1000 mL of HPLC grade water.

L. 5 mM AmAc at pH 4.5 - Add 5 mL of 1000 mM AmAc to 1000 mL HPLC grade water. Mix. Stir and adjust to pH 4.5 with ammonium hydroxide.

M. 5% ammonium hydroxide in methanol - Stir 50 mL of conc. ammonium hydroxide into 1000 mL of methanol. Store in a squirt bottle.

Preparation of Mobile Phases:

A. 0.2 % formic acid in water (v/v) - Add 2.0 mL formic acid (90%) to 1000 mL HPLC grade water. Mix. Degas.

B. 0.2% acetic acid in water (v/v) - Add 2.0 mL glacial acetic acid to 1000 mL HPLC grade water. Degas.

C. 10% 5mM AmAc at pH 4.5 in acetonitrile (v/v) - Add 100 mL of 5 mM AmAc at pH 4.5 to 900 mL acetonitrile. Mix. Degas.

D. 20% 5mM AmAc at pH 4.5 in acetonitrile (v/v) - Add 200 mL of 5 mM AmAc at pH 4.5 to 800 mL acetonitrile. Mix. Degas.

Analytical Standards:

Solid analytical standards are stored in a freezer (temperature < -10°C) unless specified otherwise on the sample shipment paperwork.

Difenoconazole, obtained from Syngenta Crop Protection, Inc., P. O. Box 18300, Greensboro, NC 27419-8300.

CGA-205375 obtained from Syngenta Crop Protection, Inc., P. O. Box 18300, Greensboro, NC 27419-8300.

CGA-71019, obtained from Syngenta Crop Protection, Inc., P. O. Box 18300, Greensboro, NC 27419-8300.

CGA-142856 obtained from Syngenta Crop Protection, Inc., P. O. Box 18300, Greensboro, NC 27419-8300.

AGVISE LABORATORIES

Highway 15
P.O. Box 510
Northwood, ND 58267

(701) 587-6010
FAX (701) 587-6013
email: agvise@polarcomm.com
Homepage: agviselabs.com

AGVISE GLP Soil Characterization Report

Submitting firm = AGVISE RES/SYNGENTA
Protocol or Study No = T002983-03
Trial ID. = 242.75
Date Received = 8-6-04
Date Reported = 08-12-2004

Lab #	Sample ID	% SA	% SI	% Cl	USDA Texture	Bulk Dens.	CEC	1/3 bar	15 bar	% OM	pH	ppm Ca	ppm Mg	ppm K	ppm Na	ppm H
04-0695	0-6	56	22	22	Sandy Clay Loam	1.14	19.8	23.6	13.4	3.5	6.0	2400	477	206	15	33
04-0696	6-12	58	23	19	Sandy Loam	1.10	21.1	24.1	14.1	2.0	7.7	2780	628	110	27	15
04-0697	12-18	58	21	21	Sandy Clay Loam	1.14	20.6	24.4	13.6	1.2	8.3	2490	811	96	31	10
04-0698	18-24	56	21	23	Sandy Clay Loam	1.10	19.4	23.4	13.2	0.9	8.5	1980	1010	97	34	7
04-0699	24-30	57	20	23	Sandy Clay Loam	1.18	18.7	22.3	12.4	0.7	8.6	1600	1170	82	39	6
04-0700	30-36	52	26	22	Sandy Clay Loam	1.16	18.7	22.6	12.2	0.5	8.6	1450	1280	71	49	4

These tests were completed in compliance of 40 CFR Part 160.

Robert Deutsch
Soil Scientist/Analytical Investigator

8-12-04
Date

VERIFIED COPY
Initial 3-2-05
Date

Agricultural Testing

AGVISE LABORATORIES
604 Highway 15
P.O. Box 510
[illegible]
[illegible]
FAX (701) 567-6013
email: agvise@polarcomm.com
Homepage: www.agvise.com

AGVISE Soil Characterization Report

Submitting firm = RESEARCH FOR HIRE
Protocol or Study No = T002984-03
Sample ID. = PA.CA.TBS.CHAR.0-6"
Trial ID. = NA
Date Received = 9-21-04
Date Reported = 09-28-2004

AGVISE Lab No	04- 863
Percent Sand	48
Percent Silt	40
Percent Clay	12
USDA Textural Class (hydrometer method)	Loam
Bulk Density (disturbed) gm/cc	1.14
Cation Exchange Capacity (meq/100 g)	11.6
% Moisture at 1/3 Bar	16.5
% Moisture at 15 Bar	5.7
% Organic Matter--Walkley Black	0.8
pH in 1:1 soil:water ratio	8.2

Base Saturation Data

Cation	Percent	ppm
Calcium	64.2	1490
Magnesium	6.0	84
Sodium	16.1	429
Potassium	8.2	369
Hydrogen	5.5	6

These tests were completed in compliance of 40 CFR Part 160.

Robert Deutsch — Date
Soil Scientist/Analytical Investigator

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Initial 3-2-05
Date

AGVISE LABORATORIES

Highway 15
P.O. Box 510
Northwood, ND 58267

(701) 587-6010
FAX (701) 587-6013

email: agvise@polarcomm.com
Homepage: agviselabs.com

AGVISE GLP Soil Characterization Report

Submitting firm = RESEARCH OPTIONS/SYNGENTA
Protocol or Study No = T002985-03
Trial ID. = NA
Date Received = 10-28-04
Date Reported = 11-05-2004

Lab #	Sample ID	% SA	% SI	% Cl	USDA Texture	Bulk Dens.	CEC	1/3 bar	15 bar	% OM	pH	ppm Ca	ppm Mg	ppm K	ppm Na	ppm H
04-1007	SID.PA.GA.TBS 0-6"	85	10	5	Loamy Sand	1.44	4.1	6.1	1.9	0.9	7.1	527	62	57	28	7
04-1008	SID.PA.GA.TBS 6-12"	83	11	6	Loamy Sand	1.39	3.0	5.9	2.1	0.5	6.2	231	43	47	30	13
04-1009	SID.PA.GA.TBS 12-18"	83	9	8	Loamy Sand	1.40	2.5	6.3	2.3	0.2	5.4	131	35	38	27	14
04-1010	SID.PA.GA.TBS 18-24"	81	11	8	Loamy Sand	1.37	2.8	6.5	2.8	0.1	4.8	105	32	34	28	18
04-1011	SID.PA.GA.TBS 24-30"	79	10	11	Sandy Loam	1.37	3.3	7.3	3.5	0.1	4.7	115	35	30	28	22
04-1012	SID.PA.GA.TBS 30-36"	77	9	14	Sandy Loam	1.32	3.9	9.3	4.7	0.1	4.8	154	39	31	30	26

These tests were completed in compliance of 40 CFR Part 160.

Robert Deutsch
Soil Scientist/Analytical Investigator

11/5/04
Date

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Date

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