Cover Sheet for

Environmental Chemistry Method

Pesticide Name: Thiamethoxam

MRID#: 449754-02

Matrix: Soil

Analysis: LC/MS

449754-02

VOLUME 4 OF 5 OF SUBMISSION

CGA-293343: METHOD

TITLE

Analytical Method For The Determination of CGA-293343 And Its Degradates CGA-322704, CGA-355190, CGA-353042, NOA-404617, And NOA-407475 In Soil By High Performance Liquid Chromatography With Mass Spectrometric Detection Including Validation Data

DATA REQUIREMENT

EPA Guideline Number 164-1

AUTHOR

Narong Chamksem, Ph.D.

COMPLETION DATE

January 22, 1999

PERFORMING LABORATORY

Environmental Safety Department
Novartis Crop Protection, Inc.
Greensboro, NC 27419

LABORATORY STUDY IDENTIFICATION

Novartis Number 9-96
Analytical Method No. AG-679B

SUBMITTER/SPONSOR

Novartis Crop Protection, Inc.
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419

VOLUME 1 OF 1 OF STUDY

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d)(1)(A), (B) or (C).

Company: Novartis Crop Protection, Inc.

Company Representative: Robert E.M. Wurz, Ph.D.

Title: Senior Regulatory Manager

Signature: Robert E.M. Wurz Date: 10/27/99

These data are the property of Novartis Crop Protection, Inc., and, as such, are considered to be confidential for all purposes other than compliance with the regulations implementing FIFRA Section 10.

Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other provision of common law or statute or in any other country.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA FIFRA GLP Standards, 40 CFR Part 160. The Good Laboratory Practice Compliance Statement found on page 38 in this final report is truthful and accurate.

10-13-99

Narong Chamkasem, Ph.D.
Study Director, Environmental Residue Studies
Environmental Safety Department

Date

10/26/99

Sunmao Chen, Ph.D.
Manager, Environmental Residue Studies
Environmental Safety Department
Representative of Submitter/Sponsor

Date

Submitter/Sponsor: Novartis Crop Protection, Inc.
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419

CERTIFICATION OF AUTHENTICITY

This final report contains a complete and unaltered copies (except for changes required to comply with PR Notice 86-5) of the Novartis Method No. AG-679B, *Analytical Method for the Determination of CGA-293343 and its Degradates CGA-322704, CGA-355190, CGA-353042, NOA-404617, and NOA-407475 in Soil by High Performance Liquid Chromatography with Mass Spectrometric Detection Including Validation Data.*

10-13-99

Narong Chamkasem, Ph.D.
Study Director, Environmental Residue Studies
Environmental Safety Department

Date

ANALYTICAL METHOD FOR THE DETERMINATION OF CGA-293343 AND ITS DEGRADATES CGA-322704, CGA-355190, CGA-353042, NOA-404617, AND NOA-407475 IN SOIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH MASS SPECTROMETRIC DETECTION INCLUDING VALIDATION DATA

METHOD NO. AG-679B

SPONSOR AND TESTING FACILITY:

Novartis Crop Protection, Inc.
Environmental Safety Department
Environmental Residue Studies
410 Swing Road
P. O. Box 18300
Greensboro, NC 27419-8300

Project Number: 346001

Protocol Number: 9-96 and Amendment 1, 2, and 3

Study Initiation Date: November 10, 1997

Study Director:
Narong Chamkasem, Ph.D.

Title: Scientist III,
Environmental Residue Studies

Signature:

Completion Date:
1-22-99

Approved By:
Sunmao Chen, Ph.D.

Title: Manager,
Environmental Residue Studies

Signature:

Date:
1/22/99

TABLE OF CONTENTS

TABLE OF CONTENTS
(Continued)

PAGE NO.

TABLE OF CONTENTS
(Continued)

PAGE NO.

I. INTRODUCTION/SUMMARY

A. Scope

This method is used for the determination of CGA-293343 (Chemical Abstracts Registry (CAS) Number: 153719-23-4, 4*H*-1,3,5-Oxadiazin-4-imine 3-[(2-chloro-5-thiazolyl)methyl]tetrahydro-5-methyl-*N*-nitro-) and its degradates CGA-322704, CGA-355190, CGA-353042, NOA-404617, and NOA-407475 in soil. The compounds are separated by high performance liquid chromatography (HPLC) and detected by mass spectrometry (MS). An Ion-Spray or Turbo Ion-Spray atmospheric pressure ionization (API) interface is used to introduce the HPLC effluent into the mass spectrometer. The analytes are detected in the triple quadrupole mode (MS/MS) by passing the positive molecular ion through Q1, inducing fragmentation in Q2, and then monitoring a characteristic product ion in Q3. The chemical structures, chemical names, and Chemical Abstracts Registry numbers of the analytes are presented in Figure 1.

The limit of detection (LOD) by LC/MS (smallest standard amount injected during the chromatographic run) is 0.063 ng for all analytes. The limit of quantification (the lowest fortification specified by the method which gives adequate recovery according to EPA guidelines) for LC/MS analyses is 1 ppb for all analytes in soil. **Analytical Method AG-679B supersedes Method AG679A** and includes additional validation data of California II soil samples analyzed with Turbo Ion-Spray interface.

B. Principle

A 20 gram of soil is extracted twice with 100 mL of 20% 10 mM ammonium acetate/acetonitrile for thirty minutes at room temperature using mechanical shaking. The sample is centrifuged and filtered. Solvent is evaporated via rotary evaporation until the final volume is approximately 20-25 mL. The sample is made basic and passed through an ENVI-Carb SPE cartridge attached on top of a C-18 SPE cartridge. CGA-293343, CGA-322704, CGA-355190, NOA-404617, NOA-407475 and a large portion of CGA-353042 are retained on the ENVI-Carb SPE.

The remaining portion of CGA-353042 is retained on the lower C-18 SPE cartridge. Analytes in the ENVI-Carb SPE cartridge are eluted with 20% MeOH/$MeCl_2$ + 0.2% formic acid. Water is added to the sample to prevent the sample from going dry while the organic content is removed via rotary evaporation. The sample is then passed through the C-18 SPE cartridge that is used previously. The eluate is collected along with the analytes that are later eluted from the cartridge with 50% methanol/0.1 N acetic acid. After rotary evaporation to remove the organic solvent to near dryness, the sample is reconstituted with 10% acetonitrile/water with 0.1% acetic acid. An aliquot of the sample is transferred into an HPLC autosampler vial for analysis by LC/MS. A flow diagram for the soil method is presented in Figure 2.

II. MATERIALS AND METHODS

A. Apparatus

1.0 Balance, analytical (Sartorius R160P) or equivalent.

2.0 Bottle, amber Boston round, with Polyseal-lined cap (Fisher cat. #05-563-2E) or equivalent.

3.0 Bottle, 250-mL polypropylene with screw cap, (Fisher cat. #05-562-23) or equivalent.

4.0 Centrifuge, Sorvall Superspeed RC5-B (DuPont Instruments cat. #55228-9) or equivalent, with 6-place GSA rotor head (DuPont, Sorvall GSA cat. #08136) or equivalent.

5.0 Cylinder, graduated, 100-mL and 50-mL (Fisher cat. #08-551D and 08-551C) or equivalent.

6.0 Filter paper, 15-cm prepleated circles, Whatman 114V (Whatman cat. #1214 150) or equivalent.

7.0 Flasks, round bottom, 500-mL and 100-mL(Fisher cat. #10-067T and 10-067C) or equivalent.

8.0 Funnel, filter, 147-mm (Fisher cat. #10-373B) or equivalent.

9.0 Mechanical shaker, orbital (Fisher cat. #15-456-6) or equivalent.

10.0 Pasteur pipette (Fisher cat. #13-678-7C) or equivalent.

11.0 Pipettes, glass, class A certified, assorted volumes. These pipets are used when an exact addition of liquid is required (i.e., final addition of solvent to samples).

12.0 Pipetters, Oxford BenchMate adjustable, 40-200 μL volume range (Fisher cat. #21-231), 200-1000 μL volume range (Fisher cat. #21-229) or equivalent. (Note: These adjustable pipetters may only be used for addition of liquid where an exact volume added is not critical, i.e., addition of acid or base.)

13.0 Rotary evaporator, Buchi (Fisher cat. #09-548-105F) or equivalent.

14.0 Solid phase extraction (SPE) columns: (1) ENVI-Carb, 1 gram/12 mL capacity/volume (Supelco cat. #5-7127) and (2) Mega Bond Elut C-18, 1 gram/6 mL capacity/volume (Varian cat. #1225-6001).
Note: Substitutions for these items have not been investigated.

15.0 Vacuum manifold, (J.T. Baker #7018-00) or equivalent.

16.0 SPE Adapter, (J.T. Baker #7122-00) or equivalent.

17.0 Vials, clear or amber, 1.5-mL (Sun Brokers, Inc. cat. #200-002) or equivalent, with Teflon-lined, crimp-top seals (Sun Brokers, Inc. cat. #200-152) or equivalent.

B. Reagents and Analytical Standards

All reagents and polypropylene glycols are stored at room temperature. Solid analytical standards are stored in a freezer (temperature <-10°C).

1.0 Acetic acid, concentrated, HPLC grade (Fisher cat. #A35-500) or equivalent.

2.0 Acetic acid, 2%: Mix 20.0 mL of conc. acetic acid with 980 mL of purified water.

3.0 Acetic acid, 0.1 N: Pipette 5.75 mL of acetic acid into a 1-L volumetric flask and dilute to volume with purified water.

4.0 Acetic acid, 0.1% solution: Mix 1 mL of acetic acid with 999 mL of purified water.

5.0 Formic acid, concentrated (90%), ACS grade (Fisher cat. #A119P-500) or equivalent.

6.0 Acetonitrile, HPLC grade (Fisher cat. #A998-4) or equivalent.

7.0 Ammonium acetate, HPLC grade (Fisher cat. #A639-500) or equivalent.

8.0 Ammonium acetate, 10 mM: Mix 0.77 g of ammonium acetate with 1 L of purified water.

9.0 Ammonium acetate, 25 mM: Mix 1.927 g of ammonium acetate with 1 L of purified water and adjust the pH to 5.8-6.0 with acetic acid (approx. 50 µL)

10.0 Ammonium hydroxide, ACS grade, (Fisher cat. #A669-500) or equivalent.

11.0 Extraction solvent: 20% 10 mM ammonium acetate/acetonitrile. Mix 200 mL of 10 mM ammonium acetate with 800 mL of acetonitrile.

12.0 Methanol, HPLC grade (Fisher cat. #A452-4) or equivalent.

Purified H_2O

13.0 Methylene Chloride, HPLC grade (Burdick & Jackson cat. #300-4) or equivalent. (Note: it may contain Cyclohexene as preservative.

14.0 Polypropylene glycol, M.W. 425 (Aldrich cat. #20,230-4).

15.0 Polypropylene glycol, M.W. 1000 (Aldrich cat. #20,232-0).

16.0 Polypropylene glycol, M.W. 2000 (Aldrich cat. #20,233-9).

17.0 PPG tuning solution (for mass calibration of the LC/MS system). Dissolve 0.0014 g PPG 425, 0.0100 g PPG 1000, 0.0400 g PPG 2000, and 0.0126 g of ammonium formate in 50 mL of methanol, 50 mL water, and 0.1 mL of acetonitrile. Mix well. Store refrigerated in an amber bottle.

18.0 Sample diluent: 10% acetonitrile/water + 0.1% acetic acid. Mix 100 mL of acetonitrile with 900 mL water and 1 mL of acetic acid.

19.0 ENVI-Carb SPE eluting solvent: 20% MeOH/$MeCl_2$ + 0.2% formic acid. Mix 200 mL of methanol with 800 mL of methylene chloride and add 2 mL of concentrated formic acid (90%).

20.0 C-18 SPE eluting solvent: 50% methanol/0.1 N acetic acid. Mix 500 mL methanol with 500 mL of 0.1 N acetic acid.

21.0 Test analytes tuning solution, 2.5 ng/µL. Mix one volume of a 10 ng/µL mixed solution of analytes in acetonitrile with three volumes of 0.1% acetic acid. Store at refrigerated or frozen temperature.

22.0 Water, HPLC grade, purified in-house with a HYDRO™ purification system or equivalent.

23.0 Mobile phase A: 0.1% acetic acid in water. Mix 1 mL of acetic acid with 999 mL of water

24.0 Mobile phase B: 0.1% acetic acid in acetonitrile. Mix 1 mL of acetic acid with 999 mL of acetonitrile

25.0 Mobile phase C: 50% acetonitrile/25 mM ammonium acetate. Mix 500 mL of acetonitrile with 500 mL of 25 mM ammonium acetate.

26.0 CGA-293343, CGA-322704, CGA-355190, CGA-353042, NOA-404617, and NOA-407475, Novartis Crop Protection, Inc., P. O. Box 18300, Greensboro, NC 27419-8300.

C. Safety and Health

Whereas most of the chemicals used and analyzed for in this method have not been completely characterized, general laboratory safety is advised (e.g., safety glasses, gloves, etc. should be used). Formic acid, acetic acid, and ammonium hydroxide used in this method are caustic and irritants and should be used in a well-ventilated area (i.e., a fume hood).

D. Analytical Procedure

Note: All glassware, including the polypropylene bottles for extraction, should be thoroughly cleaned and followed with a rinse of acetonitrile or methanol prior to use. The analysis system is very sensitive and may detect contamination from previous samples if all glassware and extraction bottles are not properly cleaned prior to each use.

1.0 Soil Moisture Determination

Soil characterization data for the soils used in this validation study is presented in Table I.

1.1 Label and record the actual weight of an appropriate-sized glass beaker or aluminum weighing pan that will be used to determine the soil moisture content.

1.2 Add 10.0 g of soil sample to the beaker or pan. Record the weight of the container plus wet soil.

1.3 Place the sample in an oven set at 100-120°C and let it dry overnight, or 12-16 hours.

1.4 Remove the sample and allow it to cool to room temperature.

1.5 Record the weight of the container plus dry soil.

1.6 Calculate the moisture content using the equation:

$$\%\ \text{Moisture} = \frac{W_{1.2} - W_{1.3}}{W_{1.2} - W_{1.1}} \times 100$$

where m is the moisture content expressed in percent, $W_{1.1}$ is the weight of the container (from Step 1.1), $W_{1.2}$ is the weight of wet soil plus container (from Step 1.2), and $W_{1.3}$ is the weight of the dry soil plus container (from Step 1.5).

2.0 Soil

(Note: Samples must be homogenized prior to analysis using suitable sample preparation techniques.)

2.1 Weigh and record 20 ± 0.1 g of soil sample into a 250-mL polypropylene bottle.

2.2 Sample fortification, if required for this particular sample, is to be done at this time (refer to Section II.K.2.0).

2.3 Add 100 mL of extraction solvent, i.e. 20% 10 mM ammonium acetate/acetonitrile. Replace screw cap and swirl briefly to mix the content.

2.4 Place the bottle on a mechanical shaker and agitate at room temperature for approximately 30 minutes.

2.5 Centrifuge the sample at approximately 9,000 RPM for 10 minutes, or at an alternate speed and time if the results are considered satisfactory.

2.6 Decant the sample extract through a filter paper into a 500-mL round bottom flask.

2.7 Repeat step 2.3 to 2.6 one more time.

2.8 Place the sample on a rotary evaporator with a water bath temperature of approximately 40°C. Use a solvent trap to minimize losses due to bumping. Evaporate organic solvent until approximately 20-25 mL of extract remains in the flask.

2.9 Condition a 1-g ENVI-Carb cartridge with approximately 10 mL of 20% MeOH/$MeCl_2$ + 0.2% formic acid using an SPE vacuum manifold at a steady drip rate. When the liquid level almost reaches the top of the cartridge, add approximately 3 mL of methanol and allow it to pass through the SPE at a steady drip rate. (Do not allow the cartridge to dry out between rinses). Add approximately 10 mL of 2% acetic acid/water after the methanol level almost reaches the top of the cartridge and allow it to pass through the cartridge. Add approximately 24 mL purified water after the acidified water level almost reaches the top of the cartridge and allow water to pass through the cartridge. Add approximately 1 mL of water into the cartridge and stop the flow by turning off the stopcock of the vacuum manifold underneath the cartridge. Do not allow the cartridge to go dry.

2.10 Condition a 1-g C-18 SPE cartridge by passing approximately 5 mL of methanol through the cartridge at a steady drip rate using the SPE vacuum manifold. Do not let the cartridge to go dry before adding 5-6 mL of purified water. Pass water through at a steady drip rate until just a small amount of water remains on top of the cartridge. Turn off the stopcock underneath the cartridge.

2.11 Add an additional 5 mL of purified water into the C-18 cartridge, allow about half of the water to drip through, then attach the conditioned ENVI-Carb SPE cartridge (from step 2.9) on top of the C-18 SPE cartridge using an adapter.

2.12 Add approximately 200 µL of ammonium hydroxide into the sample (step 2.8) and quickly transfer it into the ENVI-Carb SPE cartridge. Allow the sample to pass through both cartridges at a steady drip rate (1-2 drop/second) and do not allow the cartridge to go dry. Discard the eluate.

2.13 Rinse the round bottom flask with approximately 10-12 mL of purified water, swirl briefly, and transfer the rinsate into the cartridge just before the sample extract reaches the top of the ENVI-Carb cartridge.

2.14 Add approximately 2 mL of purified water into the ENVI-Carb SPE cartridge as soon as the rinsate (from step 2.13) reaches the top of the cartridge. Let it pass through the cartridge at a steady drip rate. After the liquid level reaches the top of the ENVI-Carb SPE cartridge, disconnect the adapter from the C-18 SPE cartridge and use a pipette bulb to push the remaining liquid in the ENVI-Carb SPE cartridge into the C-18 SPE cartridge (keep the C-18 SPE cartridge wet at all time). As the liquid level almost reaches the top of the C-18 SPE cartridge, stop the flow by turning off the stopcock underneath the cartridge.

2.15 Elute the analytes from the ENVI-Carb SPE with approximately 3 mL of methanol, collect the solvent in a 100-mL round bottom flask. Use a pipette bulb to assist the flow. Methanol is used to get rid of water residue in the cartridge. After the methanol has passed through the cartridge, add approximately 24-mL (two cartridgeful) of 20% MeOH/ $MeCl_2$ + 0.2% formic acid into the cartridge and let it drip slowly by gravity. Use a pipette bulb to push the remaining of the solvent in the cartridge into the flask. Collect this eluate in the 100-mL round bottom flask containing the methanol rinse.

2.16 Add approximately 3 mL of water (as a keeper) into the sample and evaporate organic solvent using the rotary evaporator at a water bath temperature of 40°C until approximately 2-3 mL of water is left in the flask.

2.17 Transfer the sample from step 2.16 into the C-18 SPE from step 2.14 and collect the non-retained eluate in a new 100-mL round bottom flask. Use a pipette bulb to assist the flow at a steady drip rate.

2.18 Rinse the sample flask from step 2.16 with approximately ~~12~~ 24 mL (two cartridgeful) of 50% methanol/0.1 N acetic acid, sonicate approx. 5 minutes and transfer the rinsate into the C-18 SPE just after the sample extract from step 2.17 reaches the top of the cartridge. Use the pipette bulb to assist the flow at a steady drip rate. Collect the eluate in the 100-mL round bottom flask as described in step 2.17.

2.19 Evaporate sample to near dryness (approx. 50 µL) using a rotary evaporator at a water bath temperature of 40°C. Methanol may be added to assist the evaporation of water. (**Note**: CGA-353042 may be lost due to the evaporation, don't let the sample dry to dryness).

2.20 Pipette 4 mL of sample diluent to the flask and sonicate for approximately 5 minutes to dissolve the residue. The sample may be diluted with the sample diluent if the analyte response exceeds the range of the calibration curve. Store the samples in a refrigerator (< 5°C) until the time of analysis.

2.21 Transfer the sample into an autosampler vial and analyze by LC/MS system I with a reversed-phase HPLC as detailed in Table II for the presence of CGA-293343, CGA-322704, CGA-355190, and NOA-404617.

2.22 Use LC/MS system II with a cation exchange HPLC as detailed in Table III to determine CGA-353042 and NOA-407475.

E. Instrumentation

1.0 Description and Operating Conditions: HPLC

See Tables II and III for a description of the reversed phase HPLC system and chromatographic conditions. See Table IV for a description of typical MS state file values and for conditions used with the Ion-Spray interface in Analytical Method AG-679B. The optimized values for the analytes state files may vary with time and need periodic re-optimization by infusion of the analytes into the mass spectrometer. Ion-Spray MS/MS infusion spectra are presented in Figure 4.

2.0 Description and Operating Conditions: LC/MS

CGA-293343, CGA-322704, CGA-355190, CGA-353042, NOA-404617 and NOA-407475 are monitored as positive ions. Triple stage quadrupole analysis (MS/MS) of the unique precursor/product ion pair is suggested, although single stage quadrupole analysis (MS) utilizing the molecular ion may be performed provided that no interferences are present in the sample matrix. The optimized values for the Ion-Spray interface may vary with time and may need to be periodically re-optimized. See Table IV for a description of typical Ion-Spray and Turbo Ion-Spray operating conditions used with the analytes in Analytical Method AG-679B.

3.0 Calibration and Standardization: LC/MS

3.1 Calibrate and tune the mass spectrometer prior to analyzing samples. Check the calibration and tune by infusing a standard solution of polypropylene glycol (PPG) into the mass spectrometer using the Ion-Spray interface while monitoring positive ions. Typical mass calibration tune files with PPG are presented in Figure 3. Weekly calibrations and tunes with the PPG solution are considered sufficient provided that instrument mass calibration stability is demonstrated for that time interval.

3.2 Determine the specific ion to monitor for each analyte by infusion of an analyte test solution (approx. 2.5 ng/µL in 50% acetonitrile/water, 0.1% acetic acid) while scanning the Q1 quadrupole mass analyzer to find the optimum ion. Determine the specific product ion fragment to monitor for each analyte in the MS/MS mode by passing the characteristic precursor ion through Q1, fragmenting the ion in Q2, and scanning the resulting ion fragments in Q3. The selected product ion chosen to monitor will depend on the intensity of the ion fragment along with the possibility that an interference also has the same fragment ion. Table V lists the precursor ion and monitored product ion for each analyte.

3.3 Determine the retention time of the analytes by injecting a standard solution into the HPLC. During a series of analyses, the analyte retention time should vary no more than 2% from its mean value, on a daily basis.

3.4 Calibrate the instrument by constructing a calibration curve from detector response (chromatographic peak height or area) and the amount of analyte injected, encompassing a range from 1 to 10 ng for Ion-Spray interface and 0.063 to 1.25 ng for Turbo Ion-Spray interface. The response curve can be constructed manually or, preferably, by generation of a linear regression equation by use of a computer or appropriate calculator. Typical chromatograms of calibration standards are presented in Figures 5-6 and 13-14.

F. Interferences

1.0 There are no known interferences originating from the sample cleanup procedure. However, interferences can originate from impure chemicals, solvents, contaminated glassware, and the HPLC water supply.

G. Confirmatory Techniques

1.0 No confirmatory analysis procedure is included in this method. This method employs highly specific LC/MS/MS for the detection mode, coupled with the characteristic retention time observed for the analyte on the appropriate HPLC column.

H. Time Required

1.0 The sample extraction and cleanup procedure can be completed for a set of seven samples in an eight-hour working day.

2.0 Each HPLC analysis requires approximately 15-17 minutes.

I. Modifications and Potential Problems

1.0 Contaminants from chemicals, solvents, glassware, and the HPLC water supply can interfere with the analysis. It is recommended that a reagent blank be analyzed with an analysis set to verify that no interferences are originating from the chemicals and reagents used in this procedure. MS techniques are so very sensitive that all glassware should be solvent rinsed before use to prevent inadvertent contamination of control or low level samples.

2.0 Analytical Method AG-679B was validated only for the soil type listed in Table I. Other soil samples from different locations may exhibit binding or interference problems which were not observed with these samples.

3.0 "Bumping" is sometimes observed for soil samples during the solvent removal steps via rotary evaporation. Periodic venting of the vacuum and the use of solvent traps helps minimize inadvertent losses during these steps.

4.0 No analyte stability or solubility problems have been observed when solutions have been prepared and stored as detailed in Section II.J.

5.0 Long-term optimization of the LC/MS signal by infusion of a test mixture of analytes into the system will result in lingering high backgrounds for the molecular ions. While the background signals will decrease with time or cleaning of the orifice plate, it may be severe enough to affect the ability to achieve desired signal to noise ratios for lowest standards. For this reason it is highly recommended that optimizing/calibrating with analytical standards be done with dilute solutions and the optimizing/calibrating time be minimized. It is also recommended after calibrating/optimizing with test analytes, to turn the power off to the electronics, remove the Ion-Spray interface, and thoroughly wipe clean the orifice plate using a lint-free tissue wetted with methanol. Repeat several times.

6.0 This method has been tested on a PE Sciex API-III+ LC/MS system using the Ion-Spray or Turbo Ion-Spray interface and on a PE Sciex API 365 system equipped with the Turbo Ion-Spray interface. The ability of LC/MS systems of different manufacturer to demonstrate acceptable sensitivity, stability, and reproducibility is unknown.

7.0 The cation exchange HPLC column may be cleaned of bound components by passing 25 mM ammonium acetate through the column for approximately 30 minutes. The column should then be equilibrated for approximately 30 minutes with the mobile phase used for analysis prior to starting a new sample run sequence. It is recommended to perform this cleaning procedure on a frequent basis. The mobile phase may be modified to compensate for the column-to-column variability.

8.0 Reversed-phase columns from other manufacturers may be substituted for the column used in this study provided that the analyst demonstrates acceptable peak shape and sensitivity.

9.0 To maintain the analytical column integrity, it is recommended to flush the reversed-phase column with several column volumes of 20% water/acetonitrile to get rid of acid and non-polar compounds that may strongly retain in the column. Store the column in this solvent.

10.0 The cleanup procedure was optimized for use with the SPE cartridges specified in the method. The ability of other SPE cartridges of different manufacturers to satisfactorily perform the cleanup steps as written is unknown.

11.0 The HPLC column and LC/MS interface should be equilibrated for a period of time before starting the analysis. This will ensure the reproducibility of retention time and ionization efficiency of the analytes. Several injections of standard should be performed to obtain reproducible retention time and peak area. This is especially important when the analyst switches from HPLC system II to HPLC system I. Ammonium acetate in the mobile phase of HPLC system II may affect the ionization of analytes determined by HPLC system I. It is recommended to clean the orifice plate and plenum chamber with methanol and water to get rid of ammonium acetate residue and equilibrate HPLC system I for at least one hour before injecting the samples.

J. Preparation of Standard Solutions

All stock solutions are stored in amber bottles in a refrigerator(< 5°C) when not in use. Mixed standards should also be stored in a refrigerated (< 5°C). No analyte stability or solubility problems have been observed in the standard solutions used in this study. The mixed standards are used for fortifications and as HPLC standards. (**Note**: CGA-353042 quantitative GLP standard was not available at the time of this validation and the qualitative GLP standard was used in this study. However, this did not affect integrity of the data)

1.0 Prepare individual 200 ng/µL stock solutions for CGA-293343, CGA-322704, CGA-355190, CGA-353042, NOA-407475, and NOA-404617. Weigh approximately 10.0 mg of analyte. Determine the appropriate volume of solvent to add using the equation presented below. The concentration of the analytical standard is corrected for its chemical purity. Acetonitrile is used as the solvent for CGA-293343, CGA-322704, and CGA-355190. NOA-404617, CGA-353042 and NOA-407475 are dissolved in 30% water/acetonitrile because they do not dissolve well in 100% acetonitrile.

$$V\ (\mathrm{mL}) = \frac{W(\mathrm{mg}) \times P}{C\ (\mathrm{ng/uL})} \times 10^3$$

Where V is the volume of solvent needed; W is the weight, in mg, of the solid analytical standard; P is the purity, in decimal form, of the analytical standard; C is the desired concentration of the final solution, in ng/µL; and 10^3 is a conversion factor.

For example:

The volume of solvent required to dilute 9.9 mg of an analyte, of 98.0% purity, to a final concentration of 200 ng/µL is:

$$V\ (\text{mL}) = \frac{9.9\ \text{mg} \times 0.98}{200\ \text{ng/uL}} \times 10^3 = 48.5\ \text{mL}$$

2.0 Fortification standards are prepared by mixing the individual stock solutions and diluting with 30% water/acetonitrile. Prepare a 10 ng/µL mixed solution by pipetting 5.0 mL of each analyte 200 ng/µL stock solution into a 100-mL volumetric flask and then diluting to the calibration mark with 30% water/acetonitrile. Subsequent dilutions of this solution with 30% water/acetonitrile will depend upon the desired fortification level(s). Fortification standards should be prepared such that no more than 1.0 mL of the fortification solution is added to a sample. (Example: For a 20 g of soil sample, the addition of 1.0 mL of a 0.1 ng/µL fortification solution will result in a fortification level of 5.0 ppb.)

3.0 Prepare a 1 ng/µL mixed standard for generating external calibration curves for CGA-293343, CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475 on the LC/MS system. Pipette 5.0 mL from the 10 ng/µL stock solutions for CGA-293343, CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475 into a 50-mL volumetric flask and dilute to the calibration mark using 10% acetonitrile/0.1% acetic. Subsequent dilutions using 10% acetonitrile/0.1% acetic are made to prepare a series of calibration standards.

K. Methods of Calculation

1.0 Determination of Residues in Samples

1.1 Inject the sample solution from Step II.D.2.21 into the analysis system. The sample solution may be diluted if the analyte response exceeds the range of the calibration curve. The amount of analyte injected (ng) is determined by entering the value of the chromatographic peak height, or area, in the calibration response curve (Step II.E.3.4) and calculating (by computer, calculator, or manual means) the corresponding value of nanograms injected. Typical chromatograms for fortified soil are presented in Figures 7-12 and 15-16.

2.0 Determination of Residues in Fortified Samples

Validate the method for each set of samples analyzed by including a control sample and one or more control samples fortified prior to the extraction procedure with 5 ppb or more of each analyte in soil.

2.1 Add an appropriate volume of a fortification solution (from Step II.J.2.0) to the sample prior to any of the cleanup steps. The total volume of the added fortification solution should not exceed 1.0 mL.

2.2 Proceed with the sample cleanup procedure (Step II.D.2.3).

3.0 Calculations

Calculations may be performed by computer program or manually as follows (soil concentrations are based on their wet weight):

3.1 Calculate the analyte concentration (in ppb) for field samples from equation (1):

$$(1)\quad \text{ppb analyte} = \frac{\text{ng analyte found}}{\text{g sample injected}} \times \frac{1}{R}$$

where R is the recovery factor expressed in decimal form (i.e., 0.8 = 80%) and is calculated from equation (3), and the chemical purity of the analytical standard has been accounted for in the preparation of the standard solutions. The use of the recovery correction factor "1/R" is left to the discretion of the study director.

The grams of sample injected for soil is calculated from equation (2).

$$(2)\quad \text{g soil injected} = \text{g} \times \frac{V_i}{V_f}$$

where, g is the grams of soil (wet weight) used, V_i is the volume (mL) injected onto the HPLC column, and V_f is the final volume (mL) of the cleaned-up sample (from Step II.D.2.20).

The recovery factor, expressed as a percentage (R%), is calculated from fortification experiments and is presented in equation (3).

$$(3)\quad R\% = \frac{\text{ppb analyte found} - \text{ppb analyte (control)}}{\text{ppb analyte added}} \times 100\%$$

The amount (ppb) of analyte found is calculated from equation (4).

$$(4)\quad \text{ppb analyte found} = \frac{\text{ng analyte found}}{\text{g sample injected}}$$

Residues of degradates found in test samples may also be expressed as parent equivalents by multiplying the amount found by the ratio of the molecular weight of CGA-293343 to that of the degradates (equation (5)).

$$(5)\quad \text{ppb CGA-293343 equiv.} = \text{ppb metabolite} \times \frac{\text{MW (p)}}{\text{MW (m)}}$$

where MW(p) is the average molecular weight of CGA-293343 (291.71) and MW(m) is the average molecular weight of the degradates, 249.67 for CGA-322704, 247.70 for CGA-355190, 115.13 for CGA-353042, 246.71 for NOA-407475, and 236.63 for NOA-404617.

3.2 The accuracy of the method is determined by the average recovery of the analytes fortified into the test substrate. The precision is estimated by the relative standard deviation of the determined concentration.

If background interference is found in the matrix blank (soil control), it will be reported in the data table. In addition, an indication must be made as to whether or not these amounts were taken into account in the recovery calculations. The decision of whether to subtract any amount found in the matrix blank from the recovery sample(s) is left to the discretion of the study director.

III. RESULTS AND DISCUSSION

This method was validated under Protocol 9-96 for the analysis of control and fortified control soil. The objective of Protocol 9-96 is to validate "Draft" Analytical Method AG-679 for the determination of CGA-293343 and its degradates CGA-322704, CGA-355190, CGA-353042, NOA-404617 and NOA-407475 in soil.[1]

With Ion-Spray interface, the method has a limit of detection (the lowest amount of standard injected) of 1 ng and a limit of quantification (the lowest concentration fortified in the control sample) of 5 ppb for all analytes. Very good linearity (1 - 10 ng range) was observed for calibration curves for all analytes. The accuracy of the method was measured by the mean recovery values obtained for fortified samples. The precision of the method was estimated by the percent relative standard deviation of the recovery values for the fortified samples.

Please note that CGA-353042 GLP qualitative standard was used in this study (it contains 27.8% NH_4Cl). The quantitative GLP standard was not available at the time; however, this did not affect the integrity of the recovery data.

The results for fortification experiment are presented in Tables VI - XI for soil samples from Florida, California I, and Michigan. These tables contain raw data for samples and calibration standards which permits the manual calculation of recovery values. Attempts to duplicate calculations from data in these tables will be subject to round-off errors. Summary tables are also presented in Tables XIV and XV. Typical chromatograms for method blanks, soil controls, and fortified control samples are presented in Figures 7-12.

Soil samples from Florida, California I, and Michigan were used to evaluate the method. Two HPLC systems with Inertsil ODS-2 and Zorbax SCX columns were used to obtain reliable response and acceptable separation for all analytes.

Recovery data for CGA-293343 demonstrated excellent accuracy and precision of the method within each soil sample set at 5, 25, and 100 ppb fortification levels. The mean recovery values and percent relative standard deviation for all levels within a set combined were 91 ± 9.0% (n=16), 94 ± 4.9% (n=8), and 87 ± 8.1% (n=8) for analyses conducted on soil samples from Florida, California I, and Michigan, respectively. No quantifiable interferences were observed in the method blank and soil control. When the recovery data were pooled and examined for each fortification level, mean recovery values and percent relative standard deviations were 87 ± 9.4 (n=16), 94 ± 6.0 (n=10), and 95 ± 2.5 (n=6) for fortification levels of 5 ppb, 25 ppb, and 100 ppb, respectively (see Table XIV).

Recovery data for CGA-322704 were good at all fortification levels for all three soil types. The mean recovery values and percent relative standard deviation for all levels within a set combined were 83 ± 12.4% (n=16), 93 ± 3.4% (n=8), and 82 ± 13.6% (n=8) for analyses conducted on soil samples from Florida, California I, and Michigan, respectively. No quantifiable interferences were observed in the method blank and soil control. When the recovery data were pooled and examined for each fortification level, mean recovery values and percent relative standard deviations were 80 ± 11.9 (n=16), 91 ± 9.4 (n=10), and 92 ± 5.6 (n=6) for fortification levels of 5 ppb, 25 ppb, and 100 ppb, respectively (see Table XIV).

Recovery data for CGA-355190 were excellent at all fortification levels for all three soil types. The mean recovery values and percent relative standard deviation for all levels within a set combined were 91 ± 8.4% (n=16), 98 ± 2.9% (n=8), and 92 ± 8.9% (n=8) for analyses conducted on soil samples from Florida, California I, and Michigan, respectively. No quantifiable interferences were observed in the method blank. There was trace amount of CGA-355190 (0.13 ppb) found in Florida soil set 1 (FL2B). These residues were attributed to glassware and apparatus contamination and were not significant in comparison to the lowest analytical standard used in the calibration. When the recovery data were pooled and examined for each fortification level, mean recovery values and percent relative standard deviations were 91 ± 8.5 (n=16), 96 ± 7.2 (n=10), and 95 ± 5.9 (n=6) for fortification levels of 5 ppb, 25 ppb, and 100 ppb, respectively (see Table XIV).

Recovery data for NOA-404617 were acceptable at all fortification levels for all three soil types. The mean recovery values and percent relative standard deviation for all levels within a set combined were 77 ± 13.1% (n=16), 86 ± 5.5% (n=8), and 78 ± 14.0% (n=8) for analyses conducted on soil samples from Florida, California I, and Michigan, respectively. No quantifiable interferences were observed in the method blank and soil control. When the recovery data were pooled and examined for each fortification level, mean recovery values and percent relative standard deviations were 73 ± 11.6 (n=16), 83 ± 8.4 (n=10), and 89 ± 4.3 (n=6) for fortification levels of 5 ppb, 25 ppb, and 100 ppb, respectively (see Table XIV).

Recovery data for CGA-353042 were acceptable at all fortification levels for all three soil types. The mean recovery values and percent relative standard deviation for all levels within a set combined were 70 ± 17.2% (n=16), 79 ± 12.0% (n=8), and 76 ± 16.4% (n=8) for analyses conducted on soil samples from Florida, California I, and Michigan, respectively. No quantifiable interferences were observed in the method blank and soil control. When the recovery data were pooled and examined for each fortification level, mean recovery values and percent relative standard deviations were 76 ± 16.0 (n=16), 64 ± 10.7 (n=10), and 83 ± 5.3 (n=6) for fortification levels of 5 ppb, 25 ppb, and 100 ppb, respectively (see Table XV).

Recovery data for NOA-407475 were good at all fortification levels for all three soil types. The mean recovery values and percent relative standard deviation (for all levels within a set combined were 85 ± 8.5% (n=16), 93 ± 9.4% (n=8), and 86 ± 9.5% (n=8) for analyses conducted on soil samples from Florida, California I, and Michigan, respectively. No quantifiable interferences were observed in the method blank and soil control. When the recovery data were pooled and examined for each fortification level, mean recovery values and percent relative standard deviations were 88 ± 10.8 (n=16), 84 ± 8.4 (n=10), and 90 ± 7.5 (n=6) for fortification levels of 5 ppb, 25 ppb, and 100 ppb, respectively (see Table XV).

The method was also validated with Turbo Ion-Spray interface to improve method sensitivity. With Turbo Ion-Spray interface, the method has a limit of detection (the lowest amount of standard injected) of 0.063 ng and a limit of quantification (the lowest concentration fortified in the control sample) of 1 ppb for all analytes. Very good linearity (0.063 - 1.25 ng range) was observed for calibration curves for all analytes.

The results for fortification experiment are presented in Tables XII - XIII for soil samples from California II. These tables contain raw data for samples and calibration standards which permits the manual calculation of recovery values. Attempts to duplicate calculations from data in these tables will be subject to round-off errors. Summary tables are also presented in Tables XVI and XVII. Typical chromatograms for soil controls, and fortified control samples are presented in Figures 13 - 16.

Recovery data for CGA-293343 analyzed with Turbo Ion-Spray interface, demonstrated excellent accuracy and precision of the method for California II soil sample at 1, 2, 5, 50, 100, 500 and 1000 ppb fortification levels. The mean recovery value and percent relative standard deviation for all levels within a set were 95 and 9.3 (n=19) respectively. No quantifiable interferences were observed in the soil control. When the recovery data were pooled and examined for each fortification level, mean recovery values and percent relative standard deviations were 109 ± 7.4% (n=3), 92 ± 4.7% (n=3), 89 ± 10.6% (n=3), 95 ± 1.6% (n=3), 90 ± 3.4% (n=3), 98 ± 8.7% (n=2), and 89 ± 8.8% (n=2) for fortification levels of 1, 2, 5, 50, 100, 500 and 1000 ppb, respectively (see Table XVI).

Recovery data for CGA-322704 analyzed with Turbo Ion-Spray interface, demonstrated excellent accuracy and precision of the method for California II soil sample at 1, 2, 5, 50, 100, 500 and 1000 ppb fortification levels. The mean recovery value and percent relative standard deviation for all levels within a set were 96 and 10.4 (n=19) respectively. No quantifiable interferences were observed in the soil control. When the recovery data were pooled and examined for each fortification level, mean recovery values and percent relative standard deviations were 92 ± 5.5% (n=3), 94 ± 12.4% (n=3), 86 ± 9.3% (n=3), 109 ± 8.2% (n=3), 101 ± 4.1% (n=3), 100 ± 7.8% (n=2), and 88 ± 0.8% (n=2) for fortification levels of 1, 2, 5, 50, 100, 500 and 1000 ppb, respectively (see Table XVI).

Recovery data for CGA-355190 analyzed with Turbo Ion-Spray interface, demonstrated excellent accuracy and precision of the method for California II soil sample at 1, 2, 5, 50, 100, 500 and 1000 ppb fortification levels. The mean recovery value and percent relative standard deviation for all levels within a set were 95 and 6.9 (n=19) respectively. No quantifiable interferences were observed in the soil control. When the recovery data were pooled and examined for each fortification level, mean recovery values and percent relative standard deviations were 93 ± 1.6% (n=3), 96 ± 8.0% (n=3), 89 ± 2.2% (n=3), 104 ± 8.4% (n=3), 93 ± 3.3% (n=3), 99 ± 6.5% (n=2), and 92 ± 1.5% (n=2) for fortification levels of 1, 2, 5, 50, 100, 500 and 1000 ppb, respectively (see Table XVI).

Recovery data for NOA-404617 analyzed with Turbo Ion-Spray interface, demonstrated excellent accuracy and precision of the method for California II soil sample at 1, 2, 5, 50, 100, 500 and 1000 ppb fortification levels. The mean recovery value and percent relative standard deviation for all levels within a set were 86 and 11.8 (n=19) respectively. No quantifiable interferences were observed in the soil control. When the recovery data were pooled and examined for each fortification level, mean recovery values and percent relative standard deviations were 98 ± 0.0% (n=3), 91 ± 5.5% (n=3), 80 ± 12.3% (n=3), 88 ± 6.9% (n=3), 83 ± 15.8% (n=3), 87 ± 2.5% (n=2), and 72 ± 15.7% (n=2) for fortification levels of 1, 2, 5, 50, 100, 500 and 1000 ppb, respectively (see Table XVI).

Recovery data for CGA-353042 analyzed with Turbo Ion-Spray interface, demonstrated excellent accuracy and precision of the method for California II soil sample at 1, 2, 5, 50, 100, 500 and 1000 ppb fortification levels. The mean recovery value and percent relative standard deviation for all levels within a set were 91 and 8.4 (n=19) respectively. No quantifiable interferences were observed in the soil control. When the recovery data were pooled and examined for each fortification level, mean recovery values and percent relative standard deviations were 83 ± 9.1% (n=3), 94 ± 7.1% (n=3), 86 ± 5.3% (n=3), 99 ± 2.1% (n=3), 99 ± 2.0% (n=3), 87 ± 4.1% (n=2), and 86 ± 5.8% (n=2) for fortification levels of 1, 2, 5, 50, 100, 500 and 1000 ppb, respectively (see Table XVII).

Recovery data for NOA-407475 analyzed with Turbo Ion-Spray interface, demonstrated excellent accuracy and precision of the method for California II soil sample at 1, 2, 5, 50, 100, 500 and 1000 ppb fortification levels. The mean recovery value and percent relative standard deviation for all levels within a set were 92 and 9.8 (n=19) respectively. No quantifiable interferences were observed in the soil control. When the recovery data were pooled and examined for each fortification level, mean recovery values and percent relative standard deviations were 82 ± 4.6% (n=3), 96 ± 8.7% (n=3), 84 ± 4.1% (n=3), 100 ± 2.1% (n=3), 103 ± 5.3% (n=3), 89 ± 7.2% (n=2), and 91 ± 3.9% (n=2) for fortification levels of 1, 2, 5, 50, 100, 500 and 1000 ppb, respectively (see Table XVII).

Reference substance ID, test system ID, some of the significant method changes listed in the protocol amendment, protocol deviations, and circumstances affecting the quality and integrity of data are reported in the Residue Test Report RI-MV-018-97.[2] All raw data associated with this study and the original final report and protocol will be archived in the Crop Protection Archives at Novartis Crop Protection, Inc., Greensboro, NC. All non-study specific data (i.e., instrument logbooks, etc.) will be stored in the previously mentioned archives when all entry pages are filled or when the logbook is replaced. Soil samples will be archived in the Sample Storage Facility, Greensboro, NC, until the registration studies have been accepted by the EPA.

IV. CONCLUSION

Validation experiments have demonstrated that analytical method AG-679B is rugged, reliable, and accurate for the determination of CGA-293343, CGA-322704, CGA-355190, CGA-353042, NOA-404617, and NOA-407475 in soil. LC/MS is suitable for the determination of these residue in samples. No interferences were observed in soil control samples.

V. CERTIFICATION

This report and experimental results included in this study, laboratory Project I.D. AG-679B, are certified to be authentic accounts of the experiments.

1-22-99
Date

Narong Chamkasem, Ph.D.
Scientist III
Environmental Residue Studies
Environmental Safety Department
336-632-2312

Novartis Crop Protection, Inc.
Post Office Box 18300
Greensboro, NC 27419-8300

VI. CERTIFICATION OF GOOD LABORATORY PRACTICES

The analytical work reported in AG-679B was performed in accordance with Good Laboratory Practice Standards, 40 CFR Part 160.

Please note that CGA-353042 GLP qualitative standard was used in this study (it contains 27.8% NH_4Cl). The quantitative GLP standard was not available at the time; however, this did not significantly affect integrity of the data.

1-22-99
Date

Narong Chamkasem, Ph.D.
Study Director

1/22/99
Date

Sunmao Chen, Ph.D.
Manager
Environmental Residue Studies
Environmental Safety Department
336-632-6333

Novartis Crop Protection, Inc.
Post Office Box 18300
Greensboro, NC 27419-8300

VII. QUALITY ASSURANCE STATEMENT

QUALITY ASSURANCE STATEMENT

Study Title: Analytical Method for the Determination of CGA-293343 and its Degradates CGA-322704, CGA-355190, CGA-353042, NOA-404617, and NOA-407475 in Soil by High Performance Liquid Chromatography with Mass Spectrometric Detection Including Validation Data

Study Director: Narong Chamkasem

Study Number: 9-96

Pursuant to Good Laboratory Practice Regulations, this statement verifies that the aforementioned study was inspected and/or audited and the findings reported to Management and to the Study Director by the Quality Assurance Unit on the dates listed below.

Inspection/Audit Type	Inspection/Audit Dates	Reporting Date
Audit Protocol	10/24/97 - 10/24/97	10/24/97
Inspect Analytical	11/19/97 - 11/19/97	11/19/97
Inspect Sample Verification	11/19/97 - 11/19/97	11/19/97
Audit Final Report	01/26/98 - 01/28/98	01/28/98
Audit Final Report, AG-679B	01/12/99 - 01/14/99	01/15/99

Prepared by: Karen S. Price Date: 1/19/99

Karen S. Price
Sr. Quality Assurance Auditor
Quality Assurance Unit
Novartis Crop Protection, Inc.

VIII. TABLES AND FIGURES

TABLE I. SOIL CHARACTERIZATION DATA

	Florida	California I	Michigan	California II**
County	Putnam	Madera	Ingham	Madera
Soil Depth	0-6″	0-6″	0-6″	0-6″
pH	7.7	7.4	6.9	7.5
Cation Exchange Capacity (meq/100 g)	9.5	5.4	9.5	5.9
% Organic Matter	1.0	0.4	1.4	0.5
% Water Holding Capacity @ 1/3 Bar	3.2	7.2	8.8	7.9
% Sand	90	73	84	77
% Silt	3	21	11	18
% Clay	7	6	5	5
Soil Classification	Sand	Sandy Loam	Loamy Sand	Sandy Loam
Bulk Density (g/cc)	1.5	1.46	1.45	1.47
Soil Moisture Percent*	10.4	2.1	7.4	6.0

The soil samples were collected under protocol number 176-97, 177-97, and 178-97 for soil samples from California I, Florida, and Michigan, respectively.

* Soil Moisture was determined during the method validation as described in section II.D.1.

** The first three soil samples (Florida, California I, and Michigan) were analyzed with a Ion-Spray Interface.
The last soil samples (California II) was analyzed with a Turbo Ion-Spray Interface.

TABLE II. HPLC SYSTEM I AND OPERATING CONDITIONS

This system is used for the determination of CGA-293343, CGA-322704, CGA-355190, and NOA-404617

Instrumentation:
Perkin-Elmer Series 4 Gradient Pump
Perkin-Elmer Series 200 Autosampler
Eppendorf Model CH-30 Column Heater

Operating Conditions
Column Heater: 30°C
Injector Volume: 100 µL (with Ion-Spray) 25 uL (with Turbo Ion-Spray)
Mobile Phase Flow Rate: 1.5 mL/min
Column: Inertsil ODS-2 guard column (cat# 0296-CS, Metachem) equipped with a guard column holder (cat# 5001-CS, Metachem)
Inertsil ODS-2, 5µm 100x4.6 mm (cat# 0296-100x046, Metachem)
An Upchurch (A-318) pre-column filter (0.5 µm) is also installed between the autosampler and columns to prevent fine particles from the sample blocking the columns.

Mobile phase A = 0.1% acetic acid in water
B = 0.1% acetic acid in acetonitrile

Mobile Phase Gradient Program:

Time (min.)	% A	% B	Curve
0	90	10	
3	30	70	1
3	30	70	
0.1	90	10	0
8	90	10	

Total Run Time: 15 min

Analyte Retention Times:		
	CGA-293343	4.5 min
	CGA-322704	4.8 min
	CGA-355190	4.9 min
	NOA-404617	5.8 min

TABLE III. HPLC SYSTEM II AND OPERATING CONDITIONS

This system is used for the determination of CGA-353042 and NOA-407475.

Instrumentation:
Perkin-Elmer Series 4 Gradient Pump
Perkin-Elmer Series 200 Autosampler
Eppendorf Model CH-30 Column Heater

Operating Conditions
Column Heater: 30°C
Injector Volume: 100 µL (with Ion-Spray) 25 uL (with Turbo Ion-Spray)
Mobile Phase Flow Rate: 1.5 mL/min
Column: An Upchurch (A-318) pre-column filter (0.5 µm) is installed between the autosampler and columns to prevent fine particles from the sample blocking the columns.
Zorbax SCX guard column (cat# 820950-904, MAC-MOD ✓ Analytical) equipped with a guard column holder ✓ ?(cat# 820582-001, MAC-MOD Analytical) ✓
Zorbax SCX, 5 um 150x4.6 mm (cat# 883952-704✓, MAC-MOD Analytical)

Mobile phase C = 50% acetonitrile/25 mM ammonium acetate.

Total Run Time: 15 min

Analyte Retention Times:		
	CGA-353042	9.5 min
	NOA-407475	10.5 min

TABLE IV. MASS SPECTROMETRY SYSTEM AND OPERATING CONDITIONS: LC/MS/MS

Instrumentation:

PE Sciex API-III+ Triple Quadrupole Mass Spectrometer
Ion-Spray Liquid Introduction Interface
Instrument Control and Data Collection: Apple MacIntosh Computer, Model Quadra 950

Software:

Apple System 7.5
Calibration and Mass Tuning: Tune 2.5
Acquisition: RAD 2.6
Quantitation: MacQuan 1.3

All software programs are written and provided by PE Sciex., except the system software by Apple.
Different versions of the system and applications software may be used provided they are able to collect and process the data properly.

Operating Conditions:

Ion-Spray Interface (used when LOQ is 5 ppb or more)

Interface Heater: 70°C
Curtain Gas Flow: 1.0-1.2 L/min
Nebulizer Gas Pressure (Nitrogen): generally 40-45 psi (optimize for maximum analyte response)

Turbo Ion-Spray Interface (used when LOQ is 1 ppb or more)

Interface Heater: 70°C
Curtain Gas Flow: 1.0-1.2 L/min
Nebulizer Gas Pressure (Nitrogen): generally 40-45 psi (optimize for maximum analyte response)
Auxiliary Gas Pressure (Nitrogen): 7 L /min
Nebulizer Temperature 500°C
Delta P: 1.5 inches of water

LC Flow into the Mass Spectrometer: split the LC flow so that approximately 40 uL/min is directed towards the interface plate (a small amount of liquid will just be visible on the plate surface).

TABLE IV. MASS SPECTROMETRY SYSTEM AND OPERATING CONDITIONS: LC/MS/MS (Continued)

Typical State File Values

	Q1 293343 positive ion*	File for first four analytes **	File for last two analytes***
ISV	4500.00	4500.00	4500.00
IN	650.00	650.00	650.00
OR	50.00	60.00	49.00
R0	30.00	30.00	30.00
M1	1000.00	150.00	150.00
RE1	118.00	122.50	122.50
DM1	0.20	0.15	0.15
R1	27.50	26.00	26.00
L7	-35.00	22.00	22.00
R2	-20.00	19.00	19.00
M3	1000.00	150.00	150.00
RE3	124.60	120.00	120.00
DM3	0.13	0.15	0.15
RX	-10.00	0.00	0.00
R3	-70.0	13.00	13.00
L9	-250.00	-250.00	-250.00
FP	-250.00	-250.00	-250.00
MU	-4600.00	-4600.00	-4600.00
CC	10	1	1
CGT	off	ca. 225-235	ca. 225-235

Notes:

State file values will vary slightly from instrument to instrument. These values may need slight adjustment during regular instrument optimization procedures

* This state file is used to turn on and off argon collision gas needed to generate the Q3 product ion.
** This group includes CGA-293343, CGA-322704, CGA-355190, and NOA-404617
*** This group includes CGA-353042 and NOA-407475

TABLE V. MONITORING IONS AND ACQUISITION PARAMETERS (LC/MS/MS)

For CGA-293343, CGA-322704, CGA-355190, and NOA-404617

Ions Monitored:

Analyte	Exact Mass	Molecular Ion (Mode)	Product Ion Monitored
CGA-293343	291.02	292.2 (pos. ion)	210.8
CGA-322704	249.01	249.8 (pos. ion)	168.8
CGA-355190	247.02	247.8 (pos. ion)	174.8
NOA-404617	235.97	236.8 (pos. ion)	174.8

RAD Acquisition Parameters:

Mode	Duration	ADC's		Threshold
Profile	11.20	None		0 Counts
Period Name	Scan Type	Duration	State File	
Period 1	Q1MI	0.1	Q1 pos ion 293343	
Delay	Acquire	Scan Rate	Dwell Time	Pause Time
0.0	0.1	1.00	499.98	0.02
Step (a.m.u.)	Mass	Width	Defect	
1.00	400.00	0.00	0.00	
Period Name	Scan Type	Duration	State File	
Period 2	MRM	8.48	293343 first group	
Delay	Acquire	Scan Rate	Dwell Time	Pause Time
0.5	15.0	0.80	249.98	0.02
Step (a.m.u.)	Q1 Mass	Q3 Mass	Defect	
1.00	292.20	210.80	0.00	
	249.80	168.80	0.00	
	247.80	174.80	0.00	
	236.80	174.80	0.00	
Period Name	Scan Type	Duration	State File	
Period 3	Q1MI	0.1	Q1 pos ion 293343	
Delay	Acquire	Scan Rate	Dwell Time	Pause Time
0.0	0.1	1.00	499.98	0.02
Step (a.m.u.)	Mass	Width	Defect	
1.00	400.00	0.00	0.00	

Note: Period 1 and 3 are used solely to turn on and off, respectively, the argon collision gas needed to generate the Q3 product ions.

TABLE V. MONITORING IONS AND ACQUISITION PARAMETERS (LC/MS/MS) (Continued)

For CGA-353042 and NOA-407475

Ions Monitored:

Analyte	**Exact Mass**	**Molecular Ion (Mode)**	**Product Ion Monitored**
CGA-353042	115.07	115.8 (pos. ion)	86.0
NOA-407475	246.03	247.0 (pos. ion)	160.8

RAD Acquisition Parameters:

Mode	Duration	ADC's		Threshold
Profile	11.20	None		0 Counts
Period Name	Scan Type	Duration	State File	
Period 1	Q1MI	0.1	Q1 pos ion 293343	
Delay	Acquire	Scan Rate	Dwell Time	Pause Time
0.0	0.1	1.00	499.98	0.02
Step (a.m.u.)	Mass	Width	Defect	
1.00	400.00	0.00	0.00	
Period Name	Scan Type	Duration	State File	
Period 2	MRM	10.0	293343 second group	
Delay	Acquire	Scan Rate	Dwell Time	Pause Time
0.0	15	1.00	249.98	0.02
Step (a.m.u.)	Q1 Mass	Q3 Mass	Defect	
1.00	247.00	160.80	0.00	
	116.00	86.00	0.00	
Period Name	Scan Type	Duration	State File	
Period 3	Q1MI	0.1	Q1 pos ion 293343	
Delay	Acquire	Scan Rate	Dwell Time	Pause Time
0.0	0.1	1.00	499.98	0.02
Step (a.m.u.)	Mass	Width	Defect	
1.00	400.00	0.00	0.00	

Note: Period 1 and 3 are used solely to turn on and off, respectively, the argon collision gas needed to generate the Q3 product ions.

TABLE VI. CALIBRATION AND RECOVERY DATA FOR FORTIFIED FLORIDA SOIL: INERTSIL ODS-2 COLUMN (**ANALYZED WITH ION-SPRAY INTERFACE**) PER PROTOCOL 9-96

CGA-293343

Sample Code	Amount Added (ppb)	Sample Weight (g)	(V_f) Final Volume (mL)	Sample Wt. Inj. (mg)	Retention Time (min)	Peak Area	Analyte Found (ng)	Residue Found (ppb)	% Recovery
FL1B	0 (blank)	-	4	-	-	0	< 1	< 5	-
FL2B	0 (control)	20.0	4	500	-	0	< 1	< 5	-
FL3B	5	20.0	4	500	4.09	32446	1.930	3.859	77
FL4B	5	20.0	4	500	4.10	32356	1.924	3.848	77
FL5B	5	20.0	4	500	4.08	35604	2.120	4.240	85
FL6B	5	20.0	4	500	4.10	32248	1.978	3.956	79
FL7B	25	20.0	10	200	4.08	71249	4.267	21.334	85
FL8B	25	20.0	10	200	4.08	71368	4.274	21.370	85
FL9B	100	20.0	25	80	4.09	122554	7.357	91.966	92
FL10B	100	20.0	25	80	4.08	122647	7.363	92.036	92

These values are common to all samples and are used in the calculations detailed in Section II.K.3.0
V_i = HPLC injection volume = 0.1 mL

Calibration Standards

Concentration (ng/µL)	Injection Volume (µL)	Amount Inj. (ng)	Retention Time (min)	Peak Area	Amount Found (ng)
0.01	100	1.0	4.10	17207	1.012
0.01	100	1.0	4.10	16477	0.968
0.025	100	2.5	4.09	41764	2.491
0.05	100	5.0	4.08	84267	5.051
0.1	100	10.0	4.09	167782	10.082
0.1	100	10.0	4.09	164722	9.897

slope = 16601.5182
Y-intercept = 412.3689
corr. coeff. = 0.999881

Note: The displayed calculated values were taken from the Ciba Worksheet program. Attempts to duplicate these calculated values are subject to computer round-off error.

TABLE VI. CALIBRATION AND RECOVERY DATA FOR FORTIFIED FLORIDA SOIL: INERTSIL ODS-2 COLUMN (**ANALYZED WITH ION-SPRAY INTERFACE**) PER PROTOCOL 9-96 (Continued)

CGA-293343

Sample Code	Amount Added (ppb)	Sample Weight (g)	(V_f) Final Volume (mL)	Sample Wt. Inj. (mg)	Retention Time (min)	Peak Area	Analyte Found (ng)	Residue Found (ppb)	% Recovery
FL1C	0 (blank)	-	4	-	-	0	< 1	< 5	-
FL2C	0 (control)	20.0	4	500	4.08	40	< 1	< 5	-
FL3C	5	20.0	4	500	4.08	58985	2.474	4.948	99
FL4C	5	20.0	4	500	4.07	57920	2.427	4.855	97
FL5C	5	20.0	4	500	4.09	58136	2.437	4.874	97
FL6C	5	20.0	4	500	4.08	54262	2.266	4.533	91
FL7C	25	20.0	10	200	4.09	118851	5.107	25.537	102
FL8C	25	20.0	10	200	4.08	112385	4.823	24.115	96
FL9C	25	20.0	10	200	4.08	113608	4.877	24.384	98
FL10C	25	20.0	10	200	4.08	112105	4.811	24.054	96

These values are common to all samples and are used in the calculations detailed in Section II.K.3.0
V_i = HPLC injection volume = 0.1 mL

Calibration Standards

Concentration (ng/μL)	Injection Volume (μL)	Amount Inj. (ng)	Retention Time (min)	Peak Area	Amount Found (ng)
0.01	100	1.0	4.18	23525	0.914
0.01	100	1.0	4.04	18774	0.705
0.01	100	1.0	4.06	28843	1.148
0.025	100	2.5	4.07	65375	2.755
0.05	100	5.0	4.07	117190	5.034
0.1	100	10.0	4.07	228765	9.942

slope = 22734.3310
Y-intercept = 2736.3692
corr. coeff. = 0.998556

Note: The displayed calculated values were taken from the Ciba Worksheet program. Attempts to duplicate these calculated values are subject to computer round-off error.

TABLE VI. CALIBRATION AND RECOVERY DATA FOR FORTIFIED FLORIDA SOIL: INERTSIL ODS-2 COLUMN (**ANALYZED WITH ION-SPRAY INTERFACE**) PER PROTOCOL 9-96 (Continued)

CGA-322704

Sample Code	Amount Added (ppb)	Sample Weight (g)	(V_f) Final Volume (mL)	Sample Wt. Inj. (mg)	Retention Time (min)	Peak Area	Analyte Found (ng)	Residue Found (ppb)	% Recovery
FL1B	0 (blank)	-	4	-	-	0	< 1	< 5	-
FL2B	0 (control)	20.0	4	500	4.24	33	< 1	< 5	-
FL3B	5	20.0	4	500	4.26	38714	1.770	3.539	71
FL4B	5	20.0	4	500	4.27	37654	1.721	3.442	69
FL5B	5	20.0	4	500	4.25	38986	1.782	3.564	71
FL6B	5	20.0	4	500	4.27	38149	1.744	3.487	70
FL7B	25	20.0	10	200	4.26	84325	3.854	19.271	77
FL8B	25	20.0	10	200	4.26	84894	3.880	19.401	78
FL9B	100	20.0	25	80	4.26	151242	6.912	86.406	86
FL10B	100	20.0	25	80	4.26	148580	6.791	84.885	85

These values are common to all samples and are used in the calculations detailed in Section II.K.3.0

V_i = HPLC injection volume = 0.1 mL

Calibration Standards

Concentration (ng/µL)	Injection Volume (µL)	Amount Inj. (ng)	Retention Time (min)	Peak Area	Amount Found (ng)
0.01	100	1.0	4.27	21878	1.000
0.01	100	1.0	4.27	21545	0.968
0.025	100	2.5	4.27	55632	2.491
0.05	100	5.0	4.26	108589	5.051
0.1	100	10.0	4.27	221846	10.082
0.1	100	10.0	4.27	215952	9.897

slope = 21880.0737
Y-intercept = -3.3623
corr. coeff. = 0.999775

Note: The displayed calculated values were taken from the Ciba Worksheet program. Attempts to duplicate these calculated values are subject to computer round-off error.