I. INTRODUCTION/SUMMARY

A. Scope

This method is used for the determination of CGA-215944 and its metabolites CGA-249257 and 2U in soil. The compounds are separated by high performance liquid chromatography (HPLC) and detected by mass spectrometry (LC/MS). For LC/MS detection, a heated nebulizer interface is used to introduce the HPLC effluent into the mass spectrometer. The analytes are detected in the triple quadrupole mode (MS/MS) by monitoring characteristic daughter ions resulting from passage of their parent molecular ions through the first quadrupole (Q1) into the collision cell (Q2), where fragmentation of the parent ions occurs, with the resulting fragments separated in the second mass analyzing quadrupole (Q3). The structures, chemical names, and Chemical Abstracts Registry numbers of the analytes are presented in Figure 1.

The limit of detection by LC/MS/MS (smallest standard amount injected during the chromatographic run) is 0.5 ng for all analytes. The limit of determination (the lowest fortification specified by the method which gives adequate recovery according to EPA guidelines) for LC/MS/MS analyses is 10 ppb in soil.

B. Principle

Soil samples (10 g) are extracted three times with 10% water/methanol, 1% in ammonium hydroxide, using mechanical shaking at room temperature. The samples are centrifuged and filtered. Several drops of ethylene glycol are added to serve as an analyte trap. The extracting solvent is removed via rotary evaporation. 10% methanol/water is added to dilute the residue to its final volume. The samples are analyzed by LC/MS/MS. A flow diagram for the method is presented in Figure 2.

II. MATERIALS AND METHODS

A. Apparatus

1.0 Balance, analytical (Sartorius R160P) or equivalent.

2.0 Beaker, glass, 250-mL (Fisher cat. #02-540K) or equivalent.

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

4.0 Bottle, polypropylene, (Fisher cat. #05-562-23) or equivalent with cap, appropriate size for soil extractions.

5.0 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.

6.0 Cylinder, graduated, 50-mL (Fisher cat. #08-552-10C) or equivalent.

7.0 Filter, paper, for filtering soil extracts prior to rotary evaporation, 24-cm prepleated circles, Whatman 114V (Fisher cat. #09-834D) or equivalent.

8.0 Filter, sample, for filtering final sample prior to analysis, Whatman Anotop 25 Inorganic Membrane Filter, 0.2 µm pore, 25 mm diameter (Whatman cat. #6809-2022).

9.0 Flasks, round bottom, 250-mL (Fisher cat. #10-067E) or equivalent.

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

11.0 Pasteur pipet, disposable (Fisher cat. #13-678-7C) or equivalent.

12.0 pH meter, Corning Checkmate M90 (Fisher cat. #13-641-140) or equivalent.

13.0 Pipets, 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).

14.0 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 base.)

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

16.0 Shaker, Eberbach 6010 two-speed (Baxter cat. #S1105) with utility carrier (Baxter cat. #S110) or equivalent.

17.0 Ultrasonic bath, (Fisher cat. #15-336-6) or equivalent.

18.0 Vials, 1.8-mL (Perkin-Elmer, cat. #N930-1385) or equivalent, with polyethylene caps (Perkin-Elmer, cat. #0494-8532) or equivalent.

B. Reagents and Analytical Standards

All reagents and polypropylene glycols (PPG) are stored at room temperature. The PPG mass calibration solution is stored refrigerated. Solid analytical standards are stored in a freezer (temperature < -10°C).

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

2.0 Acetic acid, glacial, double distilled "Optima" grade (Fisher cat. #A465-250).

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

4.0 Ammonium acetate buffer, pH = 5.8 ± 0.05, 0.1 M solution. Dissolve 7.7 grams of ammonium acetate with purified water. Dilute to 1 L. Adjust pH with acetic acid while monitoring pH with a calibrated pH meter.

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

6.0 Extraction solvent: 10% (v/v) water in methanol, with 1% ammonium hydroxide added. Add 10 mL of ammonium hydroxide to 900 mL of methanol. Dilute to 1000 mL with purified water.

7.0 Mobile phase A. Dilute 200 mL of the pH = 5.8 ammonium acetate buffer with 700 mL of purified water. Add 100 mL of methanol. Filter and degass prior to use.

8.0 Mobile phase B. Dilute 200 mL of the pH = 5.8 buffer with 300 mL of purified water and 500 mL of acetonitrile. Filter and degass prior to use.

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

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

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

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

13.0 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.

14.0 Test analytes tuning solution, 0.5 ng/µl. Mix 0.5 mL of a 10 ng/uL mixed solution with 2.0 mL of the pH = 5.8 ammonium acetate buffer solution, 5.5 mL of purified water, and 2.0 mL of acetonitrile. Store at frozen temperature (< -10°C).

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

16.0 CGA-215944, CGA-249257, and 2U, Ciba-Geigy Corp., 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). The ammonium hydroxide and acetic acid that are 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 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 the glassware is not properly cleaned prior to each use.

1.0 Soil Moisture Determination

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 approximately 10-20 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:

$$m = \frac{W_{1.2} - W_{1.5}}{W_{1.2} - W_{1.1}}$$

where m is the moisture content expressed in decimal form (i.e., 0.1 = 10%), $W_{1.1}$ 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 $W_{1.5}$ is the weight of the dry soil plus container (from Step 1.5).

2.0 Soil Extraction/Cleanup

Soil samples must be homogenized prior to analysis using suitable sample preparation techniques.

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

2.1 Weigh and record 10 ± 0.1 g of soil sample and place in an appropriate-sized, centrifugable 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 50 mL of the soil extraction solvent. Swirl the contents briefly. Place the bottle in a mechanical shaker and agitate the sample at room temperature for approximately 30 minutes.

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

2.5 Decant the sample extract through filter paper into a 250-mL round bottom flask.

2.6 Pour a second aliquot of 50 mL of the soil extracting solvent into the plastic bottle containing the sample and extract, centrifuge, and filter the sample as detailed in Steps 2.3-2.5.

2.7 Pour a third aliquot of 50 mL of the soil extracting solvent into the plastic bottle containing the sample and extract, centrifuge, and filter the sample as detailed in Steps 2.3-2.5.

2.8 Place 5 drops of ethylene glycol (acts as an analyte trap) into the round bottom flask.

2.9 Place the sample on a rotary evaporator with a water bath temperature of approximately 40 to 45°C. Use a solvent trap to minimize losses due to bumping. Remove the methanol and water from the sample until only the ethylene glycol drops remain. (Note:

Periodic venting of the sample may be required to prevent losses due to bumping.) Add methanol to azeotrope the water, as needed.

2.10 Remove the sample from the rotary evaporator. Add an appropriate volume of 10% methanol/water, via calibrated pipette, to dissolve the residue. Sonicate the sample for several minutes to aid dissolution.

2.11 Attach a Whatman Anotop 25 sample filter to a clean syringe equipped with a Leur-lock end fitting. Transfer approximately 1.5 mL of the sample from Step 2.10 to the syringe, via a disposable pipette. Place a sample vial underneath the filter to receive the filtered sample. Push the sample through the filter into the vial. Analyze the sample by LC/MS/MS. Samples which are not to be analyzed the same working day as the extraction/ cleanup procedure should be stored under frozen conditions (< -10°C) until the time of analysis.

E. Instrumentation

1.0 Description and Operating Conditions: HPLC

See Table II for a description of the HPLC systems and chromatographic conditions.

2.0 Description and Operating Conditions: LC/MS/MS

All analytes are monitored as characteristic positive ions (daughter ions) which result from the fragmentation of the analyte molecular ion. See Table III for a description of the mass spectrometer instrumentation and operating conditions.

3.0 Description and Operating Conditions: LC/MS/MS Heated Nebulizer Interface

The optimized values for the analyte state file may vary with time and may need to be periodically re-optimized by infusion of the analytes into the mass spectrometer. See Table III for a description of typical MS state file values and for conditions used with the heated nebulizer interface in Analytical Method AG-641.

4.0 Calibration and Standardization

4.1 Calibrate and tune the mass spectrometer on a daily basis prior to analyzing samples. Check the calibration and tune by infusing a standard solution of polypropylene glycol (PPG) into the mass spectrometer using the ionspray interface while monitoring positive ions. A typical mass calibration tune with PPG is shown in Figure 3. The calibrations are additionally checked by infusion of a 2.5 ng/µL solution of the test analytes dissolved in 25% acetonitrile/water, 0.02 M in ammonium acetate, pH = 5.8. A typical analyte mass calibration tune is presented in Figure 4. Both mass analyzing quadrupoles (Q1 and Q3) must be calibrated when operating in the MS/MS mode. It is recommended that the analyte calibration be added to the PPG calibration table to ensure that the maximum ion intensity for each analyte will always be at its exact calculated mass. Mass calibration must be performed on a daily basis, or after each instrument recycle period.

(Note: The ionspray interface is used for mass calibration purposes while the heated nebulizer is used

for the actual analyses. The ionspray interface may also be used to determine optimum state file values for the analytes. The optimum state file values are generally independent of the interface used. A more concentrated solution of 2-5 ng/µL is used for analyte infusion work with the ionspray interface. The heated nebulizer interface, by its design, is somewhat difficult to use for infusion work. In addition, infusion work may contaminate the heated nebulizer interface which will then require extensive cleaning prior to use.)

4.2 Detect the analytes at their specific monitoring ions. Determine the parent ion to monitor by infusing the analyte into the mass spectrometer while scanning on the Q1 mass analyzer. Determine the specific daughter ion fragment to monitor for each analyte in the MS/MS mode by passing the characteristic parent ion through Q1, fragmenting the ion in Q2, and scanning the resulting ion fragments in Q3. The selected daughter 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 IV lists the parent ion and monitored daughter ion for each analyte. Typical ionspray mass fragmentation spectra for the MS and MS/MS modes are presented in Figures 5 and 6.

4.3 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.

4.4 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 0.5 to 7.5 ng (100 µL injections). The response curve can be constructed manually or, preferably, by generation of a linear regression equation by use of a computer or appropriate calculator. Standard calibrations are presented with the recovery data in Tables V, VI, and VII for the three soil types. Chromatograms of analytical standards are presented in with chromatograms from California, Georgia, and New York soil samples in Figures 7-9.

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 method is presented. LC/MS/MS is considered to be a highly specific method which combines unique MS/MS data coupled with chromatographic retention time.

H. Time Required

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

2.0 Each HPLC analysis requires approximately 20 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 run with an analysis set to verify that no interferences are originating from the chemicals and reagents used in this procedure. MS techniques are very sensitive. All glassware should be solvent rinsed before use to prevent inadvertent contamination of control or low level samples.

2.0 Analytical Method AG-641 was validated only for the soil types listed in this method. Other soil types, or 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 optimizing/ calibrating with test analytes, to turn the power off to the electronics, remove the LC/MS interface, and thoroughly wipe clean the orifice plate using a lint-free tissue paper wetted with methanol. Repeat several times.

6.0 No analytes have been observed binding to the Whatman Anotop 25 sample filters during the final sample filtration step. It is unknown whether the analytes will bind to other brands/types of sample filters.

7.0 Most analytes will exhibit binding in soil containing high organic content if the analytes are permitted to sit at room temperature for an extended period of time after the fortification step. For this reason, storage stability samples should immediately be placed in a freezer (< -10°C) after fortification. Extraction solvent should be added to fortified samples within 10 minutes of fortification.

8.0 During method development experiments it was noted that 2U degrades significantly under reflux conditions while CGA-215944 and CGA-249257 are stable. For this reason the samples are extracted at room temperature via mechanical shaking.

9.0 LC/MS/MS sensitivity for CGA-215944 is very good while sensitivity for CGA-249257 limits the lower level standard that can be observed. Saturation of the electron multiplier has been observed on occasions when 10 ng of CGA-215944 was injected into the system, while neither CGA-249257 or 2U exhibited this symptom due to less sensitivity on the instrument. This saturation effect

may limit the upper level amount that may be injected.

10.0 The YMC ODS-AQ column was selected for this method as it provided much greater retention of the polar metabolite CGA-249257, compared to other C8 or C18 columns. However, this column has been observed to degrade rapidly when using buffered solution of ammonium acetate alone (pH = 6.9). The manufacturer's literature indicates the column has an upper pH stability limit of 6.3. Therefore, we selected pH = 5.8 for the mobile phases. (Poor peak shapes result if the mobile phase pH is made acidic.) If the YMC column is deemed unstable by the analyst, we recommend that an Intersil 5 ODS-2 column be used (15-cm x 4.6-mm, d_p = 5 µm, MetaChem Technologies Inc., cat. #0296-150X046) with the same mobile phase gradient as used with the YMC column. The retention for CGA-249257 is less than observed on the YMC column, but is acceptable.

11.0 This analysis procedure was developed and validated for use with a PE Sciex MS/MS system using a heated nebulizer interface. No claims are made that this method will work acceptably as written if a different MS system or interface are used.

J. Preparation of Standard Solutions

All standards are stored in amber bottles in a freezer (< -10°C) when not in use. 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.

1.0 Prepare individual 100 ng/µL stock solutions for each analyte. Weigh approximately 10.0 mg of analyte. Determine the appropriate volume of acetonitrile to add using the equation

presented below. The concentration of the analytical standard is corrected for its chemical purity.

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

Where V is the volume of acetonitrile 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 required to dilute 9.9 mg of an analyte, of 98.0% purity, to a final concentration of 200 ng/µL is:

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

2.0 Prepare a 10 ng/µL mixed standard solution in acetonitrile/water by pipetting 5.0 mL of each analyte (from its 100 ng/µL stock solution in Step 1.0) into a 50-mL volumetric flask and diluting to the mark with 25% acetonitrile/water. Store the solution in an appropriate size amber bottle. This solution is used to prepare all subsequent dilutions.

3.0 Fortification standards are prepared by dilution of the 10 ng/µL mixed standard with 10% methanol/water. The concentration of the solutions to be prepared 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 10 g soil sample, the addition of 1.0 mL of a 0.1 ng/µL fortification

solution will result in a fortification level of 10 ppb.)

4.0 Analytical standards for generating calibration curves are prepared by serial dilution of the 10 ng/µL mixed standard (Step 1.0 above) with 10% methanol/water.

K. Methods of Calculation

1.0 Determination of Residues in Samples

1.1 Inject the sample solution from Step II.D.2.11 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.4.4) and calculating (by computer, calculator, or manual means) the corresponding value of nanograms injected. Typical chromatograms for control and fortified soil samples are presented in Figures 7-9.

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 10 ppb or more of each analyte in soil.

2.1 Add an appropriate volume of a fortification solution (from Step II.J.3.0) 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):

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

where R is the recovery factor expressed in decimal form (i.e., 0.8 = 80%) and is calculated from equation (4), and the chemical purity of the analytical standard has been accounted for in the preparation of the standard solutions.

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

$$(2)\ \text{g sample injected} = \frac{g}{V_e + (m \times g)} \times \frac{V_a}{V_f} V_i$$

where, g is the grams of soil (wet weight) used, V_a is the aliquot volume (mL) of extracted sample used for analysis, V_e is the volume (mL) of extract solvent used, V_i is the volume (mL) injected onto the HPLC column, m is the percent moisture in the sample, expressed in decimal form (ex. 0.1 = 10%), and V_f is the final volume (mL) of the cleaned-up sample (from Step II.D.2.11) (Note: the term "(m x g)" is a dilution correction factor due to the moisture in the soil, where 1.0 g = 1.0 mL. When the entire extract volume is used, $V_a = V_e + (mxg)$.)

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

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

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

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

Residues of metabolites 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-215944 to that of the metabolite (equation (5)).

$$(5)\ \text{ppb CGA-215944 equiv.} = \text{ppb metabolite} \times \frac{MW(p)}{MW(m)}$$

where MW(p) is the average molecular weight of CGA-215944 (217.23) and MW(m) is the average molecular weight of the metabolite, 113.12 for CGA-249257, and 233.23 for 2U.

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 percent relative standard deviation of the determined concentration.

TABLE II. HPLC SYSTEM AND OPERATING CONDITIONS

LC/MS System

Instrumentation:

Perkin-Elmer Model 410 Gradient Pump
Perkin-Elmer Model ISS 200 Autosampler
Eppendorf Model CH-30 Column Heater

Operating Conditions:

Column Heater: 30°C
Injection Volume: 100 µL
Mobile Phase Flow Rate: 1.5 mL/min
Column: YMC ODS-AQ, 25 cm x 4.6 mm, dp = 5 µm equipped with an Upchurch (#A-318) pre-column filter (0.5 µm), or equivalent column
Mobile Phase A: 10% (v/v) methanol in water, 0.02 M in ammonium acetate, pH = 5.8
Mobile Phase B: 50% (v/v) acetonitrile in water, 0.02 M in ammonium acetate, pH = 5.8
Mobile Phase C: methanol

Mobile Phase Gradient Program:

Time (min.)	% A	% B	% C	Curve
0.0	100	0	0	-
10.0	0	100	0	1
0.5	0	0	100	1
2.0	0	0	100	-
0.5	100	0	0	1
7.0	100	0	0	-

Total Run Time: 20 min.

Analyte Retention Times:

CGA-249257	4.0 min
2U	8.6 min
CGA-215944	9.0 min

TABLE III. MASS SPECTROMETRY SYSTEM AND OPERATING CONDITIONS

Instrumentation:

PE Sciex API III+ Mass Spectrometer
Heated Nebulizer Liquid Introduction Interface
Instrument Control and Data Collection: Apple MacIntosh Quadra 950 Computer

Software:

Apple System 7.5
Calibration and Mass Tuning: Tune 2.5
Acquisition: RAD 2.6
Quantitation: MacQuan 1.3
Display: MacSpec 3.3

All software programs 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:

Interface Heater: 70 °C
Heated Nebulizer Temperature: 500 °C
Curtain Gas Flow: 1.0 L/min
Nebulizer Gas Flow: 0.6 L/min
Auxiliary Gas Flow: 1.8 L/min

TABLE III. MASS SPECTROMETRY SYSTEM AND OPERATING CONDITIONS (Continued)

Typical State File Values:

	Q1 PPG	Q3 PPG	Q1 Analyte	MS/MS Analyte
Interface	**ISV**	**ISV**	**HN**	**HN**
DI	*	*	5	5
ISV	4500	4500	*	*
IN	650	650	650	650
OR	38	38	54	48
R0	30	30	30	30
M1	1000	1000	150	150
RE1	120.9	120.2	123	122
DM1	0.10	0.14	0.15	0.17
R1	26.5	26.5	28.2	21
L7	0	-10	10	24
R2	10	-50	10	20.5
M3	1000	1000	150	150
RE3	124.6	117.5	122.5	127
DM3	0.13	0.08	0.07	0.15
RX	0	-45	0	0
R3	-10	28	5	14.5
L9	-100	-100	-250	-150
FP	-50	-50	-80	-150
MU	-4600	-4600	-4600	-4600
CC	10	10	10	1
CGT	0	0	0	250

ISV = Ionspray interface
HN = Heated nebulizer interface

* Value not applicable for this interface type.

Note: State file values will vary slightly from instrument to instrument. The values often will be changed slightly on a daily basis during instrument optimization procedures.

TABLE IV. TYPICAL ANALYTE MONITORING IONS

Analyte	Exact Molecular Weight	Q1 Parent Ion (M+H)+	Q3 Daughter Ion
CGA-249257	113.06	114.1	72.9
2U	233.09	234.1	105.0
CGA-215944	217.10	218.1	105.0

RAD Acquisition Parameters

Mode	Duration	ADC's	Threshold
Profile	10.70	None	0 Counts

Period Name	Scan Type	State File
Period 1	Q1MI	Q1 Analyte

Delay	Acquire	Scan Rate	Dwell Time	Pause Time
0.00	0.10	1.00	499.98	0.02

Mass	Width	Defect
400.0	0.0	0.0

Period Name	Scan Type	State File
Period 2	MRM	MS/MS Analyte

Delay	Acquire	Scan Rate	Dwell Time	Pause Time
1.00	5.00	1.00	499.98	0.02

Q1 Mass	Q3 Mass	Width	Defect
114.1	72.9	0.0	0.0

Period Name	Scan Type	State File
Period 3	MRM	MS/MS Analyte

Delay	Acquire	Scan Rate	Dwell Time	Pause Time
0.00	4.50	1.00	333.31	0.02

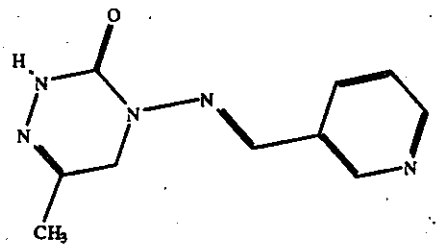
Q1 Mass	Q3 Mass	Width	Defect
234.1	105.0	0.0	0.0
218.1	105.0	0.0	0.0

Period Name	Scan Type	State File
Period 4	Q1MI	Q1 Analyte

Delay	Acquire	Scan Rate	Dwell Time	Pause Time
0.00	0.10	1.00	499.98	0.02

Mass	Width	Defect
400.0	0.0	0.0

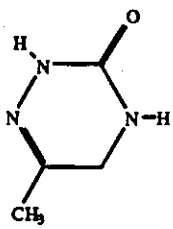
FIGURE 1. CHEMICAL NAMES AND STRUCTURES



CGA-215944

CAS Reg. No. 123312-89-0
4,5-Dihydro-6-methyl-4-[(3-pyridinylmethylene) amino]-1,2,3-triazin-3(2H)-one

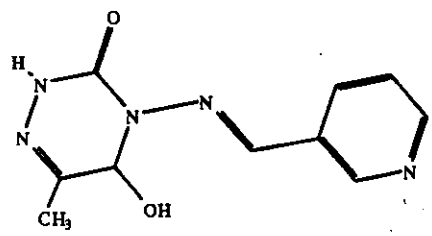
Chemical purity: 98.3%



CGA-249257

CAS Reg. No. 78830-97-4
4,5-Dihydro-6-methyl-1,2,4-triazin-3(2H)-one

Chemical purity: 98.9%



2U (CGA-359009)

CAS Reg. No. Not Assigned

Chemical purity: 99.9%

FIGURE 2. AG-641 FLOW DIAGRAM FOR SOIL

Weigh 10-g aliquot of soil.

↓

Extract with 50 mL of 90% methanol/water, 1% in ammonium hydroxide, using mechanical shaking at room temperature.

↓

Centrifuge and filter extract.

↓

Repeat extraction for total of 3X.
Combine extracts in round bottom flask.

↓

Add ethylene glycol to act as solute trap.

↓

Remove extraction solvent via rotary evaporation.

↓

Dilute sample to volume with 10% methanol/water.

↓

Analyze sample by LC/MS/MS using a PE Sciex MS/MS system equipped with a heated nebulizer interface.