Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Pymetrozine (CGA215944 & CGA249257)

MRID #: 444113-33

Matrix: Soil

Analysis: HPLC/MSD

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444113-33

VOLUME 40 OF 58 OF SUBMISSION

ANALYTICAL METHOD

CGA-215944

METHOD TITLE

Analytical Method for the Determination of CGA-215944 and its Metabolites CGA-249257 and 2U in Soil by High Performance Liquid Chromatography with Mass Spectrometric Detection Including Validation Data

DATA REQUIREMENT

40 CFR 158, Subdivision N, 164-1

AUTHOR

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METHOD COMPLETION DATE

November 10, 1995

PERFORMING LABORATORY

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PROJECT ID

Ciba Method No. AG-641
Ciba Study No. 334-95

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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.

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DATE: 9/16/97

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The Good Laboratory Practice Compliance Statement as defined by 40 CFR Part 160, found on page 32 of this volume is truthful and accurate.

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CERTIFICATION OF AUTHENTICITY

This report contains an unaltered copy of Ciba Analytical Method No. AG-641 (except for changes required to comply with PR Notice 86-5).

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Ciba Analytical Method No. AG-641

Analytical Method for the Determination of CGA-215944 and its Metabolites CGA-249257 and 2U in Soil by High Performance Liquid Chromatography with Mass Spectrometric Detection Including Validation Data

ANALYTICAL METHOD FOR THE DETERMINATION OF CGA-215944 AND ITS METABOLITES CGA-249257 AND 2U IN SOIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH MASS SPECTROMETRIC DETECTION INCLUDING VALIDATION DATA

METHOD NO. AG-641

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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.

III. RESULTS AND DISCUSSION

This method was validated under Protocol 334-95[1] and Amendment 1 for the analysis of control and fortified control soil. The objective of Protocol 334-95 was to validate "Draft" Analytical Method AG-641 for the determination of CGA-215944 and its metabolites CGA-249257 and 2U with a limit of determination of 10 ppb.

Recovery data for fortified soil samples are presented in Tables V-VII. These Tables contain raw data for both the 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.) The recovery data for all soil types are tabulated and summarized in Table VIII. Typical chromatograms for control and fortified soils along with their respective calibration standards are presented in Figures 7-9.

In these validation studies, a limit of detection (defined as the lowest standard injected during an analysis set) of 0.5 ng injected was achieved for all three sets of data. The sensitivity of analyte CGA-249257 limits the lower detection limit. The absolute mass detection limit is influenced by the sensitivity of the mass spectrometer (cleanliness and how well the system is optimized), the sharpness of the eluted analyte peaks from the analytical column, and the signal-to-noise ratio that the analyst is willing to accept. The upper limit of the calibration curve was effected by the linearity of the detector response to CGA-215944. The first set of samples analyzed (California) showed excellent linearity for all analytes from 0.5 to 10 ng injected. After this set was analyzed, the performance of the column degraded so that a new column was used, and the mass spectrometer and interface were better optimized for the analytes. After these changes, more signal was obtained for analytical standards with the result being that the system was not linearly responding to an upper standard of 10 ng injected for CGA-215944. Therefore, the highest amount of standard injected was decreased to 5 ng or 7.5 ng for subsequent sample sets.

The accuracy of the method is measured by the recovery values obtained for fortified samples. The precision of the method is estimated by the percent relative standard deviation of fortified samples. Good recoveries were obtained at all fortification levels of analytes added to the soils from the three study locations. A limit of quantitation of 10 ppb (defined as the lowest fortification amount used in the study which gave acceptable recoveries) was

achieved for all soil locations. At the 10 ppb level, the average recovery (n=12) and percent relative standard deviation for data combined for the three soil locations were 81% ± 12.0%, 78% ± 12.8%, and 79% ± 13.3% for CGA-249257, 2U, and CGA-215944, respectively. This demonstrates good accuracy and precision for the method at the lowest fortification level, where the poorest method performance typically occurs. The method accuracy and precision is significantly better at higher fortification levels for all three analytes as demonstrated in Table VIII. The average recovery and precision for each analyte within a set of fortified soil samples for a specific location was very good: 82% ± 9.0%, 80% ± 12.0%, and 87% ± 3.3% for CGA-249257 in California, Georgia, and New York soils, respectively; 81% + 9.2%, 83% ± 13.5%, and 81% ± 3.5% for 2U in California, Georgia, and New York soils, respectively; and 80% ± 8.5%, 82% ± 13.1%, and 85% ± 7.2% for CGA-215944 in California, Georgia, and New York soils, respectively.

No residues, or interferences, were observed for any analyte in the method blank and soil control samples with the exception of a trace amount of CGA-215944 observed in the control sample for the New York soil (VAL26). The peak area (area = 206) in this sample is less than 0.5% of the peak area of the lowest standard of 0.5 ng (area = 67643). It is most likely that this trace level of analyte is due to unknown contamination of laboratory glassware or other equipment that was in contact with the samples during processing of the samples.

An independent laboratory validation of this method will be conducted at a future date. This data will be included with the data package that will be submitted when the active ingredient is submitted for registration.

Reference substance ID, test system ID, protocol amendments, protocol deviations, and circumstances affecting the quality and integrity of data are reported in the Residue Test Report[2], RI-MV-009-95. All raw data associated with this study and the original final report and protocol will be archived in the Agricultural Group Archive Facility at Ciba-Geigy Corporation, 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 Biochemistry Group Sample Storage Facility, Greensboro, NC, until the registration studies have been accepted by the EPA. Disposal of soil samples will be verified by the Ciba Quality Assurance Unit.

IV. CONCLUSION

Analytical method AG-641 was successfully validated for all three analytes in all three soil locations. The accuracy and precision of the method was very good for all three analytes in all three soil locations and at all fortification levels, easily exceeding the EPA requirements for accuracy and precision: average recoveries of 70-120% and a relative standard deviation of less than 20% at all fortification levels.

V. CERTIFICATION

This report and experimental results included in this study, laboratory Project I.D. AG-641, are certified to be authentic accounts of the experiments.

11-10-95
Date

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VI. CERTIFICATION OF GOOD LABORATORY PRACTICES

The analytical work reported in AG-641 was performed in accordance with Good Laboratory Practice Standards, 40 CFR Part 160.

11-10-95
Date

John D. Vargo
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Study Director

11/10/95
Date

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CIBA CROP PROTECTION
CIBA-GEIGY CORPORATION
QUALITY ASSURANCE UNIT

QUALITY ASSURANCE STATEMENT

Report Title: ANALYTICAL METHOD FOR THE DETERMINATION OF CGA-215944 AND ITS METABOLITES CGA-249257 AND 2U IN SOIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH MASS SPECTROMETRIC DETECTION INCLUDING VALIDATION DATA

METHOD AG-641

Study Director: J. Vargo **Project Number:** 344001

Report Number: AG-641

Ciba Study Number: 334-95 with Amendment

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 DATE(S)	REPORTING DATE
Protocol Audit	08/25/95	08/25/95
In-Progress Inspection	09/27/95	09/27/95
Final Report Audit	10/17-19/95	10/19/95

Prepared By: Kent Ediger Date: 10/19/95

Kent Ediger

VIII. TABLES AND FIGURES

TABLE I. SOIL CHARACTERIZATION

	California	Georgia	New York
County	Madera	Mitchell	Columbia
Soil Depth	0-6"	0-6"	0-6"
pH	5.6	6.8	5.8
Cation Exchange Capacity (meq/100 g)	7.2	6.8	7.77
% Organic Matter	0.2	0.9	2.35
% Water Holding Capacity @ 1/3 Bar	7.5	8.5	21.39
% Sand	71	80	39.2
% Silt	21	10	42.4
% Clay	8	10	18.4
Soil Classification	Sandy Loam	Loamy Sand	Loam
Bulk Density (g/cc)	1.43	1.38	1.26

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

TABLE V. CALIBRATION AND RECOVERY DATA FOR FORTIFIED CALIFORNIA SOIL

CGA-249257

Sample Code	Amount Added (ppb)	Sample Weight Extr. (g)	(V_f) Final Volume (mL)	Sample Wt. Inj. (g)	Retention Time (min)	Peak Area	Analyte Found (ng)	Residue Found (ppb)	% Recovery
VAL1	0 (blank)	10.00	10	0.100000	-	0	< 0.5	< 10	-
VAL2	0 (control)	10.08	10	0.100800	-	0	< 0.5	< 10	-
VAL3	10	10.00	10	0.100000	3.94	10991	0.692	6.917	69.17
VAL4	10	10.04	10	0.100400	3.96	13697	0.871	8.674	86.74
VAL5	10	10.02	10	0.100200	3.97	11734	0.741	7.394	73.94
VAL6	10	10.02	10	0.100200	3.94	11878	0.750	7.489	74.89
VAL7	25	10.12	10	0.101200	3.96	30252	1.967	19.437	77.75
VAL8	25	10.08	10	0.100800	3.97	33615	2.190	21.724	86.89
VAL9	100	10.03	20	0.050150	3.94	65170	4.279	85.327	85.33
VAL10	100	10.02	20	0.050100	3.97	67969	4.464	89.112	89.11
VAL11	1000	10.03	200	0.005015	3.97	67374	4.425	882.372	88.24
VAL12	1000	10.05	200	0.005025	3.92	67814	4.454	886.414	88.64

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

m = % moisture = 6.44%
Ve = volume of extraction solvent used = 150 mL
Va = aliquot volume of extract used for cleanup = 150 mL + (0.0644 x grams extr.)
Vi = HPLC injection volume = 0.1 mL

Calibration Standards

Concentration (ng/µL)	Injection Volume (µL)	Amount Inj.(ng)	Retention Time (min)	Peak Area	
0.005	100	0.5	3.97	7738	slope = 15102.31553
0.01	100	1	4.01	15416	Y-intercept = 544.8325
0.02	100	2	3.96	30823	correlation coeff. = 0.999952
0.05	100	5	3.96	77035	
0.1	100	10	3.99	151105	

*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 V. CALIBRATION AND RECOVERY DATA FOR FORTIFIED CALIFORNIA SOIL (Continued)

2U

Sample Code	Sample Amount Added (ppb)	(V_f) Weight Extr. (g)	Sample Final Volume (mL)	Wt. Inj. (g)	Retention Time (min)	Analyte Peak Area	Residue Found (ng)	Residue Found (ppb)	% Recovery
VAL1	0 (blank)	10.00	10	0.100000	-	0	< 0.5	< 10	-
VAL2	0 (control)	10.08	10	0.100800	-	0	< 0.5	< 10	-
VAL3	10	10.00	10	0.100000	8.66	31268	0.694	6.943	69.43
VAL4	10	10.04	10	0.100400	8.67	36685	0.827	8.240	82.40
VAL5	10	10.02	10	0.100200	8.71	31673	0.704	7.028	70.28
VAL6	10	10.02	10	0.100200	8.68	34136	0.765	7.632	76.32
VAL7	25	10.12	10	0.101200	8.68	83515	1.977	19.539	78.16
VAL8	25	10.08	10	0.100800	8.68	90217	2.142	21.250	85.00
VAL9	100	10.03	20	0.050150	8.67	174555	4.213	84.011	84.01
VAL10	100	10.02	20	0.050100	8.71	185245	4.476	89.335	89.34
VAL11	1000	10.03	200	0.005015	8.72	186889	4.516	900.511	90.05
VAL12	1000	10.05	200	0.005025	8.62	182558	4.410	877.552	87.76

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

m = % moisture = 6.44%

V_e = volume of extraction solvent used = 150 mL

V_a = aliquot volume of extract used for cleanup = 150 mL + (0.0644 x grams extr.)

V_i = HPLC injection volume = 0.1 mL

Calibration Standards

Concentration (ng/µL)	Injection Volume (µL)	Amount Inj.(ng)	Retention Time (min)	Peak Area	
0.005	100	0.5	8.69	19541	slope = 40719.74595
0.01	100	1	8.68	42916	Y-intercept = 2996.1400
0.02	100	2	8.69	85212	correlation coeff. = 0.999589
0.05	100	5	8.69	214041	
0.1	100	10	8.68	406586	

*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 V. CALIBRATION AND RECOVERY DATA FOR FORTIFIED CALIFORNIA SOIL (Continued)

CGA-215944

Sample Code	Sample Amount Added (ppb)	(V_t) Weight Extr. (g)	Sample Final Volume (mL)	Wt. Inj. (g)	Retention Time (min)	Analyte Peak Area	Residue Found (ng)	Residue Found (ppb)	% Recovery
VAL1	0 (blank)	10.00	10	0.100000	-	0	< 0.5	< 10	-
VAL2	0 (control)	10.08	10	0.100800	-	0	< 0.5	< 10	-
VAL3	10	10.00	10	0.100000	9.03	85690	0.704	7.041	70.41
VAL4	10	10.04	10	0.100400	9.03	98324	0.813	8.098	80.98
VAL5	10	10.02	10	0.100200	9.08	87318	0.718	7.167	71.67
VAL6	10	10.02	10	0.100200	9.04	89659	0.738	7.368	73.68
VAL7	25	10.12	10	0.101200	9.03	224706	1.903	18.806	75.22
VAL8	25	10.08	10	0.100800	9.04	243579	2.066	20.496	81.98
VAL9	100	10.03	20	0.050150	9.04	498512	4.265	85.044	85.04
VAL10	100	10.02	20	0.050100	9.06	517681	4.430	88.429	88.43
VAL11	1000	10.03	200	0.005015	9.08	511713	4.379	873.147	87.31
VAL12	1000	10.05	200	0.005025	8.99	507912	4.346	864.885	86.49

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

m = % moisture = 6.44%

V_e = volume of extraction solvent used = 150 mL

V_a = aliquot volume of extract used for cleanup = 150 mL + (0.0644 x grams extr.)

V_i = HPLC injection volume = 0.1 mL

Calibration Standards

Concentration (ng/µL)	Injection Volume (µL)	Amount Inj.(ng)	Retention Time (min)	Peak Area	
0.005	100	0.5	9.06	56761	slope = 115931.75890
0.01	100	1	9.04	119078	Y-intercept = 4067.2921
0.02	100	2	9.06	233749	correlation coeff. = 0.999814
0.05	100	5	9.06	598892	
0.1	100	10	9.03	1156594	

*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 GEORGIA SOIL

CGA-249257

Sample Code	Sample Amount Added (ppb)	(V_f) Weight Extr. (g)	Sample Final Volume (mL)	Wt. Inj. (g)	Retention Time (min)	Analyte Peak Area	Residue Found (ng)	Residue Found (ppb)	% Recovery
VAL13	0 (blank)	10.00	10	0.100000	-	0	< 0.5	< 10	-
VAL14	0 (control)	10.00	10	0.100000	-	0	< 0.5	< 10	-
VAL15	10	10.05	10	0.100500	4.01	19556	0.751	7.473	74.73
VAL16	10	10.00	10	0.100000	3.97	15685	0.619	6.186	61.86
VAL17	10	10.00	10	0.100000	3.99	24662	0.926	9.257	92.57
VAL18	10	10.00	10	0.100000	3.96	23884	0.899	8.991	89.91
VAL19	25	10.03	10	0.100300	3.99	52556	1.880	18.745	74.98
VAL20	25	10.04	10	0.100400	4.01	50822	1.821	18.136	72.54
VAL21	100	10.02	20	0.050100	3.99	106071	3.711	74.076	74.08
VAL22	100	10.09	20	0.050450	4.01	121626	4.243	84.111	84.11
VAL23	1000	10.38	200	0.005190	4.01	126376	4.406	848.930	84.89
VAL24	1000	10.05	200	0.005025	3.99	125409	4.373	870.221	87.02

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

m = % moisture = 10.52%

V_e = volume of extraction solvent used = 150 mL

V_a = aliquot volume of extract used for cleanup = 150 mL + (0.1052 x grams extr.)

V_i = HPLC injection volume = 0.1 mL

Calibration Standards

Concentration (ng/µL)	Injection Volume (µL)	Amount Inj. (ng)	Retention Time (min)	Peak Area
0.005	100	0.5	3.97	12354
0.01	100	1	3.98	26072
0.02	100	2	3.97	52598
0.05	100	5	3.99	131453
0.1	100	10	3.97	268198
0.1	100	10	3.99	308980
0.05	100	5	4.02	162672

slope = 29226.47931
Y-intercept = -2394.2938
correlation coeff. = 0.991737

TABLE VI. CALIBRATION AND RECOVERY DATA FOR FORTIFIED GEORGIA SOIL (Continued)

2U

Sample Code	Amount Added (ppb)	Sample Weight Extr. (g)	(V_f) Final Volume (mL)	Sample Wt. Inj. (g)	Retention Time (min)	Analyte Peak Area	Residue Found (ng)	Residue Found (ppb)	% Recovery
VAL13	0 (blank)	10.00	10	0.100000	-	0	< 0.5	<10	-
VAL14	0 (control)	10.00	10	0.100000	-	0	< 0.5	< 10	-
VAL15	10	10.05	10	0.100500	8.62	33046	0.742	7.387	73.87
VAL16	10	10.00	10	0.100000	8.64	27105	0.611	6.112	61.12
VAL17	10	10.00	10	0.100000	8.62	43684	0.977	9.772	97.72
VAL18	10	10.00	10	0.100000	8.64	41833	0.936	9.364	93.64
VAL19	25	10.03	10	0.100300	8.62	87890	1.953	19.472	77.89
VAL20	25	10.04	10	0.100400	8.62	84489	1.878	18.705	74.82
VAL21	100	10.02	20	0.050100	8.62	177223	3.925	78.345	78.35
VAL22	100	10.09	20	0.050450	8.64	203283	4.500	89.204	89.20
VAL23	1000	10.38	200	0.005190	8.64	205435	4.548	876.274	87.63
VAL24	1000	10.05	200	0.005025	8.64	205951	4.559	907.314	90.73

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

m = % moisture = 10.52%

V_e = volume of extraction solvent used = 150 mL

V_a = aliquot volume of extract used for cleanup = 150 mL + (0.1052 x grams extr.)

V_i = HPLC injection volume = 0.1 mL

Calibration Standards

Concentration (ng/µL)	Injection Volume (µL)	Amount Inj.(ng)	Retention Time (min)	Peak Area
0.005	100	0.5	8.62	21906
0.01	100	1	8.62	44825
0.02	100	2	8.66	89818
0.05	100	5	8.62	214193
0.1	100	10	8.62	436860
0.1	100	10	8.66	467801
0.05	100	5	8.67	238065

slope = 45300.25583
Y-intercept = -584.3672
correlation coeff. = 0.998045

TABLE VI. CALIBRATION AND RECOVERY DATA FOR FORTIFIED GEORGIA SOIL (Continued)

CGA-215944

Sample Code	Sample Amount Added (ppb)	(V_f) Weight Extr. (g)	Sample Final Volume (mL)	Wt. Inj. (g)	Retention Time (min)	Analyte Peak Area	Residue Found (ng)	Residue Found (ppb)	% Recovery
VAL13	0 (blank)	10.00	10	0.100000	-	0	< 0.5	< 10	-
VAL14	0 (control)	10.00	10	0.100000	-	0	< 0.5	< 10	-
VAL15	10	10.05	10	0.100500	8.96	102392	0.737	7.338	73.38
VAL16	10	10.00	10	0.100000	8.96	84631	0.616	6.157	61.57
VAL17	10	10.00	10	0.100000	8.97	133170	0.948	9.484	94.84
VAL18	10	10.00	10	0.100000	8.97	133180	0.948	9.484	94.84
VAL19	25	10.03	10	0.100300	8.94	283245	1.977	19.710	78.84
VAL20	25	10.04	10	0.100400	8.94	270478	1.889	18.819	75.28
VAL21	100	10.02	20	0.050100	8.96	556842	3.852	76.887	76.89
VAL22	100	10.09	20	0.050450	8.96	608840	4.208	83.417	83.42
VAL23	1000	10.38	200	0.005190	8.96	658462	4.548	876.396	87.64
VAL24	1000	10.05	200	0.005025	8.95	668443	4.617	918.786	91.88

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

m = % moisture = 10.52%

V_o = volume of extraction solvent used = 150 mL

V_a = aliquot volume of extract used for cleanup = 150 mL + (0.1052 x grams extr.)

V_i = HPLC injection volume = 0.1 mL

Calibration Standards

Concentration (ng/µL)	Injection Volume (µL)	Amount Inj.(ng)	Retention Time (min)	Peak Area
0.005	100	0.5	8.94	70482
0.01	100	1	8.94	138796
0.02	100	2	8.98	285059
0.05	100	5	8.96	694389
0.1*	100	10	8.96	859717*
0.1*	100	10	8.96	945851*
0.05	100	5	8.99	755023

slope = 145909.18617
Y-intercept = -5205.0027
correlation coeff. = 0.997694

* Values not used in calibration. Linear range of electron multiplier tube exceeded.

TABLE VII. CALIBRATION AND RECOVERY DATA FOR FORTIFIED NEW YORK SOIL

CGA-249257

Sample Code	Amount Added (ppb)	Sample Weight Extr. (g)	(V_f) Final Volume (mL)	Sample Wt. Inj. (g)	Retention Time (min)	Analyte Peak Area	Residue Found (ng)	Residue Found (ppb)	% Recovery
VAL25	0 (blank)	10.00	10	0.100000	-	0	< 0.5	< 10	-
VAL26	0 (control)	10.00	10	0.100000	-	0	< 0.5	< 10	-
VAL27	10	9.99	10	0.099900	4.04	18372	0.858	8.586	85.86
VAL28	10	10.00	10	0.100000	4.04	17980	0.838	8.385	83.85
VAL29	10	10.04	10	0.100400	4.02	18372	0.858	8.543	85.43
VAL30	10	10.01	10	0.100100	4.06	19463	0.911	9.104	91.04
VAL31	25	10.09	10	0.100900	4.03	46952	2.261	22.407	89.63
VAL32	25	10.08	10	0.100800	4.03	45885	2.208	21.909	87.64
VAL33	100	9.99	30	0.033300	4.03	59270	2.866	86.053	86.05
VAL34	100	10.10	30	0.033667	4.04	58031	2.805	83.309	83.31
VAL35	1000	10.05	300	0.003350	4.02	63515	3.074	917.599	91.76
VAL36	1000	10.10	300	0.003367	4.01	61792	2.989	887.932	88.79

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

m = % moisture = 12.27%

V_e = volume of extraction solvent used = 150 mL

V_a = aliquot volume of extract used for cleanup = 150 mL + (0.1227 x grams extr.)

V_i = HPLC injection volume = 0.1 mL

Calibration Standards

Concentration (ng/µL)	Injection Volume (µL)	Amount Inj. (ng)	Retention Time (min)	Peak Area
0.005	100	0.5	4.04	10948
0.01	100	1	4.03	20969
0.02	100	2	4.01	40454
0.05	100	5	4.04	106521
0.075	100	7.5	3.99	151520

slope = 20369.33590
Y-intercept = 900.5232
correlation coeff. = 0.999305

TABLE VII. CALIBRATION AND RECOVERY DATA FOR FORTIFIED NEW YORK SOIL (Continued)

2U

Sample Code	Amount Added (ppb)	Sample Weight Extr. (g)	(V_f) Final Volume (mL)	Sample Wt. Inj. (g)	Retention Time (min)	Analyte Peak Area	Residue Found (ng)	Residue Found (ppb)	% Recovery
VAL25	0 (blank)	10.00	10	0.100000	-	0	< 0.5	< 10	-
VAL26	0 (control)	10.00	10	0.100000	-	0	< 0.5	< 10	-
VAL27	10	9.99	10	0.099900	8.72	28302	0.799	7.998	79.98
VAL28	10	10.00	10	0.100000	8.74	28209	0.796	7.963	79.63
VAL29	10	10.04	10	0.100400	8.71	27122	0.764	7.614	76.14
VAL30	10	10.01	10	0.100100	8.72	28793	0.813	8.126	81.26
VAL31	25	10.09	10	0.100900	8.72	73651	2.130	21.113	84.45
VAL32	25	10.08	10	0.100800	8.72	72070	2.084	20.674	82.70
VAL33	100	9.99	30	0.033300	8.72	96754	2.809	84.341	84.34
VAL34	100	10.10	30	0.033667	8.72	90495	2.625	77.695	77.96
VAL35	1000	10.05	300	0.003350	8.74	96848	2.811	839.200	83.92
VAL36	1000	10.10	300	0.003367	8.72	93248	2.706	803.654	80.37

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

m = % moisture = 12.27%

V_e = volume of extraction solvent used = 150 mL

V_a = aliquot volume of extract used for cleanup = 150 mL + (0.1227 x grams extr.)

V_i = HPLC injection volume = 0.1 mL

Calibration Standards

Concentration (ng/µL)	Injection Volume (µL)	Amount Inj. (ng)	Retention Time (min)	Peak Area	
0.005	100	0.5	8.71	18870	slope = 34063.86490
0.01	100	1	8.71	36432	Y-intercept = 1083.6291
0.02	100	2	8.71	67839	correlation coeff. = 0.999840
0.05	100	5	8.72	168970	
0.075	100	7.5	8.71	258329	

TABLE VII. CALIBRATION AND RECOVERY DATA FOR FORTIFIED NEW YORK SOIL (Continued)

CGA-215944

Sample Code	Sample Amount Added (ppb)	(V_f) Weight Extr. (g)	Sample Final Volume (mL)	Wt. Inj. (g)	Retention Time (min)	Analyte Peak Area	Residue Found (ng)	Residue Found (ppb)	% Recovery
VAL25	0 (blank)	10.00	10	0.100000	-	0	< 0.5	< 10	-
VAL26	0 (control)	10.00	10	0.100000	9.04	209	< 0.5	< 10	-
VAL27	10	9.99	10	0.099900	9.06	107686	0.775	7.754	77.54
VAL28	10	10.00	10	0.100000	9.08	105261	0.753	7.531	75.31
VAL29	10	10.04	10	0.100400	9.04	110107	0.796	7.930	79.30
VAL30	10	10.01	10	0.100100	9.06	125668	0.935	9.338	93.38
VAL31	25	10.09	10	0.100900	9.04	278525	2.295	22.748	90.99
VAL32	25	10.08	10	0.100800	9.06	274002	2.255	22.371	89.48
VAL33	100	9.99	30	0.033300	9.06	354973	2.976	89.361	89.36
VAL34	100	10.10	30	0.033667	9.06	336115	2.808	83.402	83.40
VAL35	1000	10.05	300	0.003350	9.08	347309	2.907	867.909	86.79
VAL36	1000	10.10	300	0.003367	9.06	355714	2.982	885.834	88.58

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

m = % moisture = 12.27%

V_o = volume of extraction solvent used = 150 mL

V_a = aliquot volume of extract used for cleanup = 150 mL + (0.1227 x grams extr.)

V_i = HPLC injection volume = 0.1 mL

Calibration Standards

Concentration (ng/µL)	Injection Volume (µL)	Amount Inj.(ng)	Retention Time (min)	Peak Area	
0.005	100	0.5	9.04	67643	slope = 112348.59391
0.01	100	1	9.04	136098	Y-intercept = 20655.8888
0.02	100	2	9.04	247133	correlation coeff. = 0.999578
0.05	100	5	9.06	596164	
0.075	100	7.5	9.04	853819	

TABLE VIII. SUMMARY DATA FOR FORTIFIED SOIL

Fortification Level (ppb)	% Recovery for CGA-249257 California	Georgia	New York
10	69.17	74.73	85.86
10	86.74	61.86	83.85
10	73.94	92.57	85.43
10	74.89	89.91	91.04
25	77.75	74.98	89.63
25	86.89	72.54	87.64
100	85.33	74.08	86.05
100	89.11	84.11	83.31
1000	88.24	84.89	91.76
1000	88.64	87.02	88.79
Average	82.07	79.67	87.34
Standard Deviation	7.37	9.53	2.92
% Relative Std. Dev.	8.98	11.97	3.34

Pooled Recovery Data for all Soils by Fortification Level

	10 ppb	25 ppb	100 ppb	1000 ppb
Average	80.83	81.57	83.67	88.22
Standard Deviation	9.68	7.34	5.10	2.26
% Relative Std. Dev.	11.98	9.00	6.10	2.56
Range	61.86-92.57	72.54-89.63	74.08-89.11	84.89-91.76
Number of Samples	12	6	6	6

TABLE VIII. SUMMARY DATA FOR FORTIFIED SOIL (Continued)

Fortification Level (ppb)	% Recovery for 2U		
	California	Georgia	New York
10	69.43	73.87	79.98
10	82.40	61.12	79.63
10	70.28	97.72	76.14
10	76.32	93.64	81.26
25	78.16	77.89	84.45
25	85.00	74.82	82.70
100	84.01	78.35	84.34
100	89.34	89.20	77.96
1000	90.05	87.63	83.92
1000	87.76	90.73	80.37
Average	81.28	82.50	81.08
Standard Deviation	7.47	11.17	2.80
% Relative Std. Dev.	9.19	13.54	3.46

Pooled Recovery Data for all Soils by Fortification Level

	10 ppb	25 ppb	100 ppb	1000 ppb
Average	78.48	80.50	83.87	86.74
Standard Deviation	10.04	4.13	4.98	3.93
% Relative Std. Dev.	12.79	5.13	5.94	4.53
Range	61.12-97.72	74.82-85.00	77.96-89.34	80.37-90.73
Number of Samples	12	6	6	6

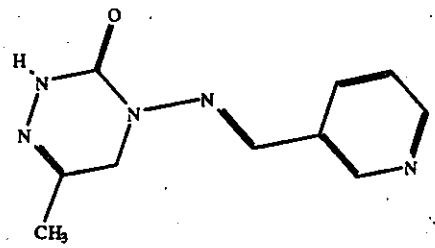
TABLE VIII. SUMMARY DATA FOR FORTIFIED SOIL (Continued)

Fortification Level (ppb)	% Recovery for CGA-215944		
	California	Georgia	New York
10	70.41	73.38	77.54
10	80.98	61.57	75.31
10	71.67	94.84	79.30
10	73.68	94.84	93.38
25	75.22	78.84	90.99
25	81.98	75.28	89.48
100	85.04	76.89	89.36
100	88.43	83.42	83.40
1000	87.31	87.64	86.79
1000	86.49	91.88	88.58
Average	80.12	81.86	85.41
Standard Deviation	6.84	10.72	6.18
% Relative Std. Dev.	8.54	13.09	7.24

Pooled Recovery Data for all Soils by Fortification Level

	10 ppb	25 ppb	100 ppb	1000 ppb
Average	78.91	81.97	84.42	88.12
Standard Deviation	10.53	6.70	4.46	1.98
% Relative Std. Dev.	13.34	8.42	5.29	2.25
Range	61.57-94.84	75.22-90.99	76.89-89.36	86.49-91.88
Number of Samples	12	6	6	6

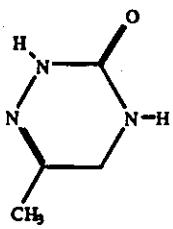
FIGURE 1. CHEMICAL NAMES AND STRUCTURES



CGA-215944

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

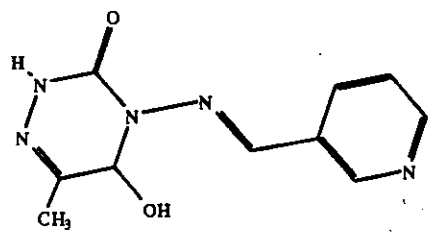
Chemical purity: 98.3%



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.

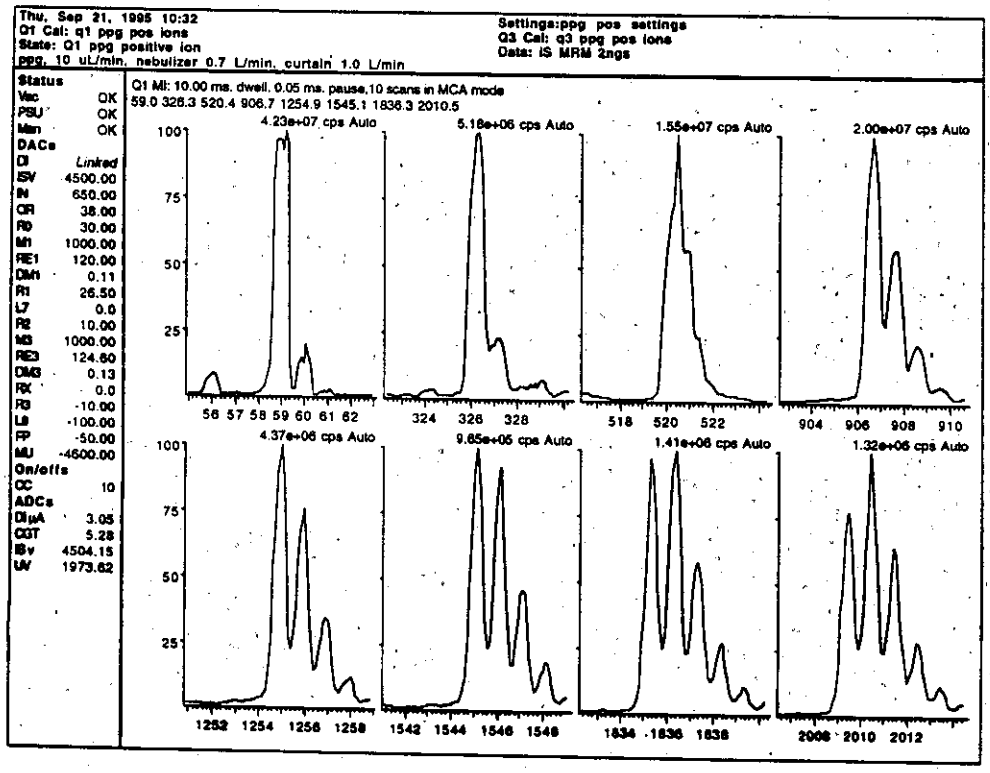
↓

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.

FIGURE 3. TYPICAL PPG MASS CALIBRATION TUNE



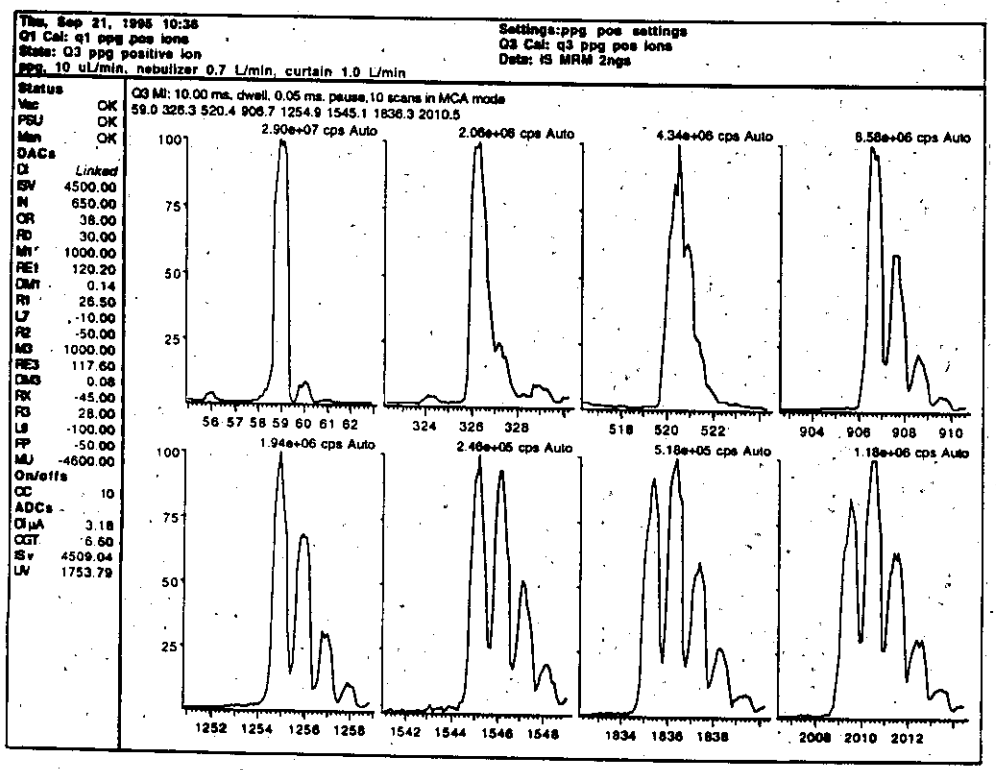


FIGURE 4. TYPICAL MASS CALIBRATION TUNE WITH ANALYTES

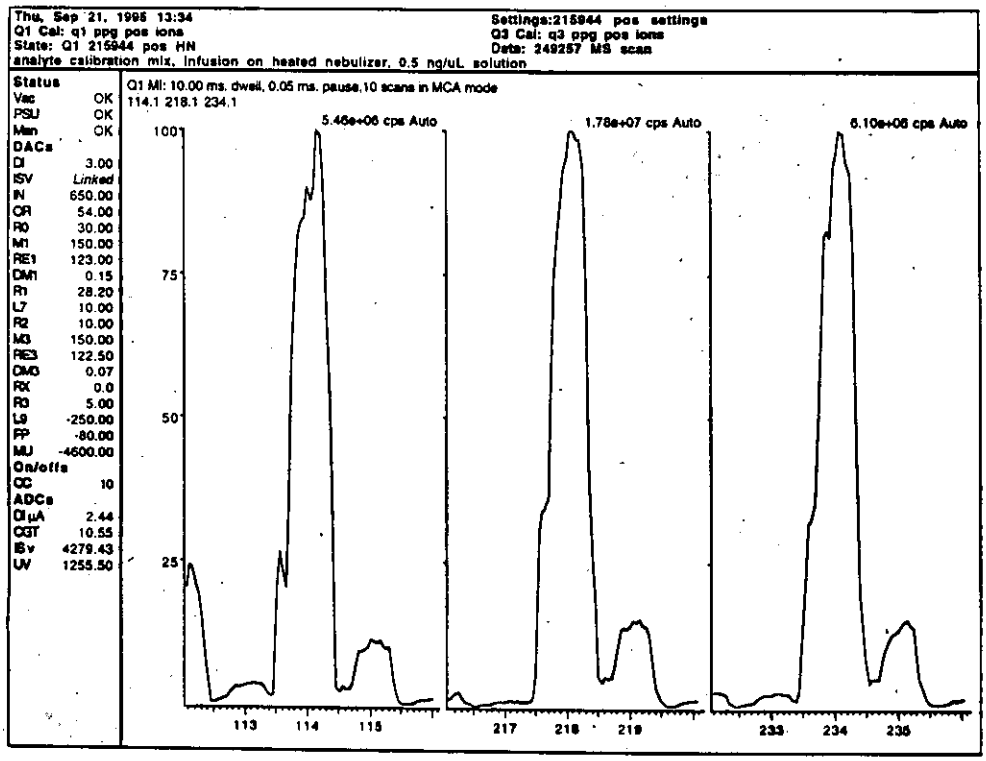
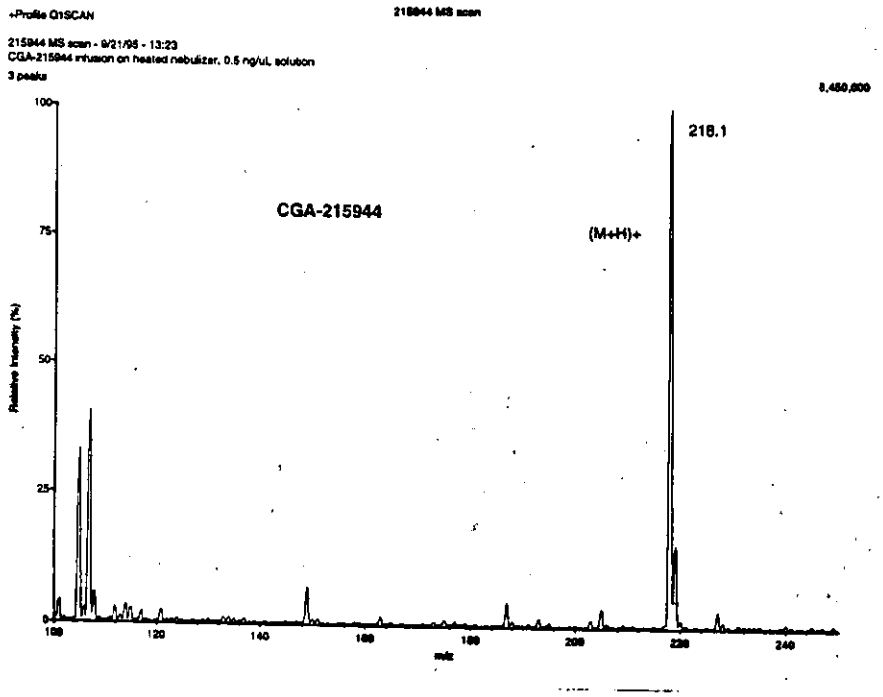


FIGURE 5. TYPICAL Q1 PARENT ION MASS FRAGMENTATION SPECTRA



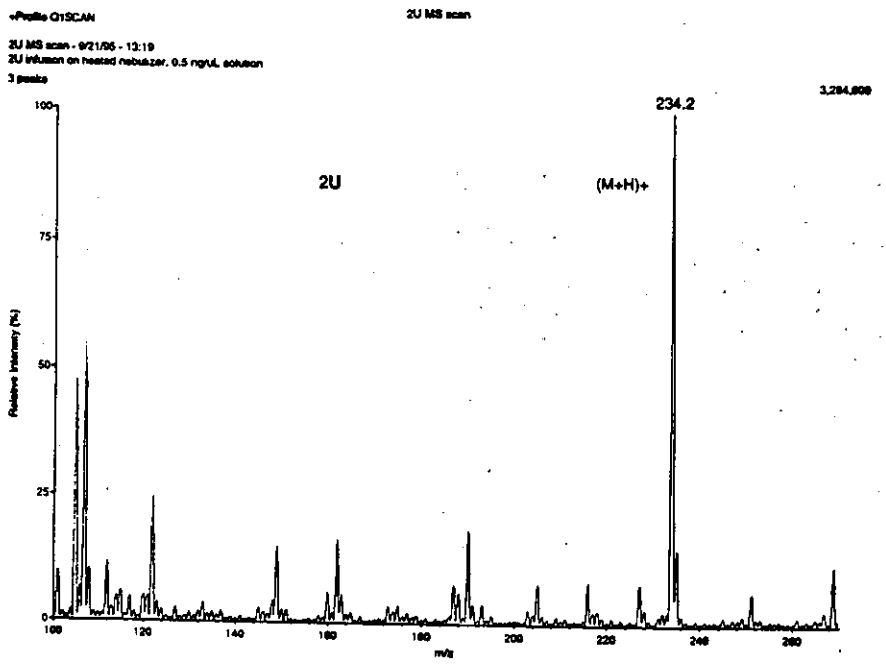


FIGURE 5. TYPICAL Q1 PARENT ION MASS FRAGMENTATION SPECTRA (Continued)

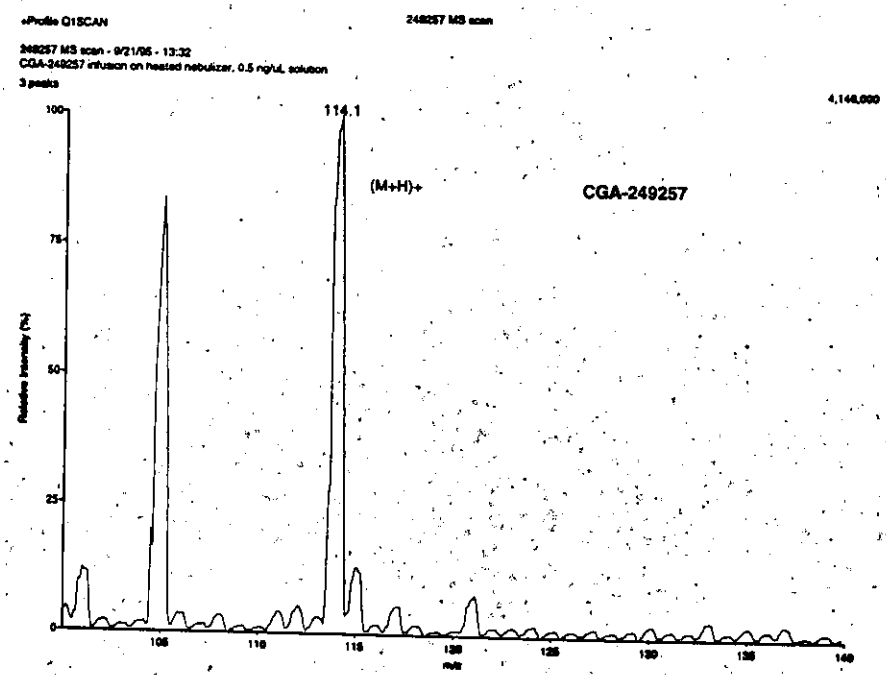
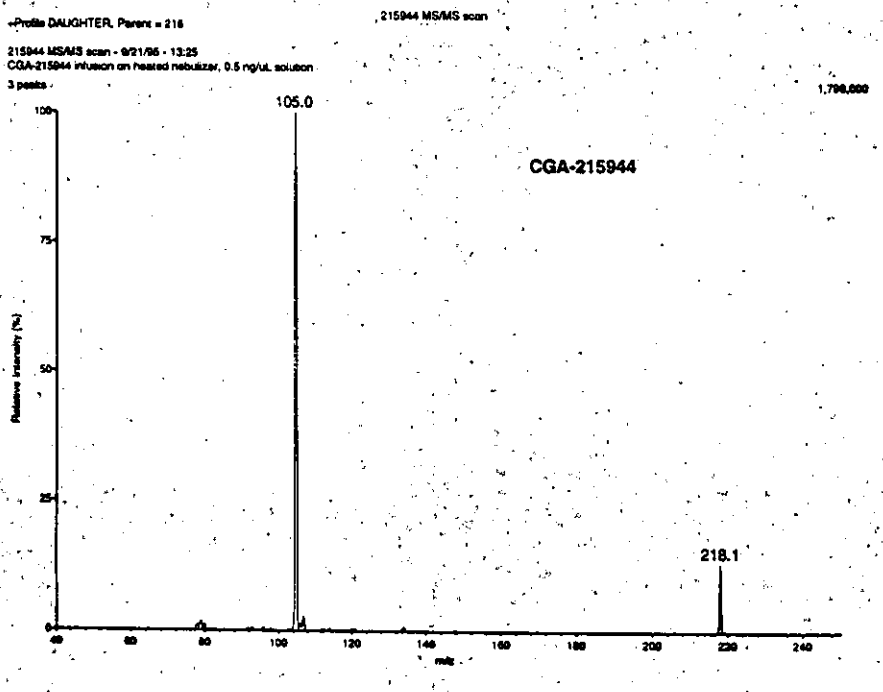


FIGURE 6. TYPICAL MS/MS DAUGHTER ION MASS FRAGMENTATION SPECTRA



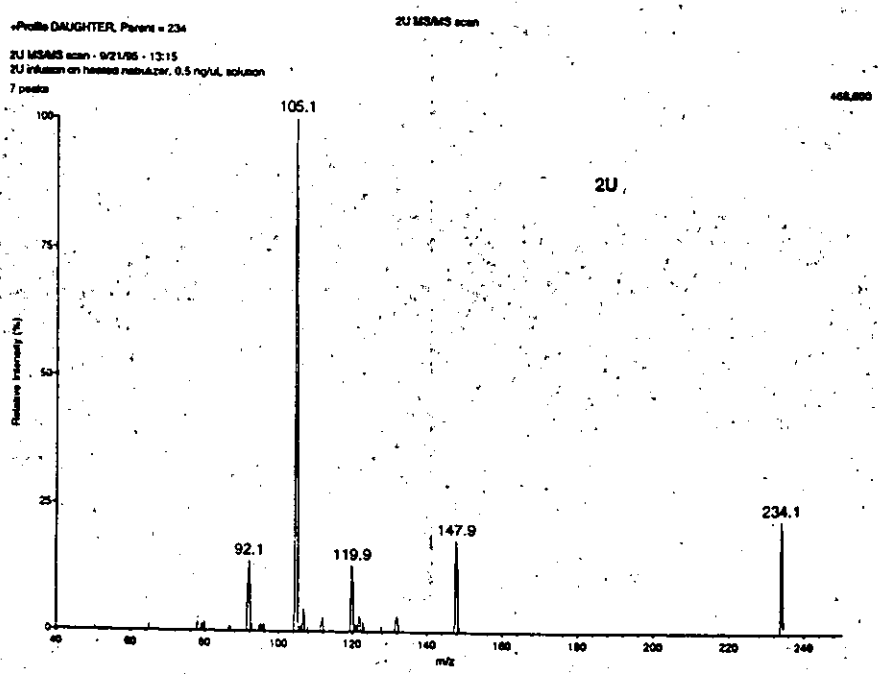


FIGURE 6. TYPICAL MS/MS DAUGHTER ION MASS FRAGMENTATION SPECTRA (Continued)

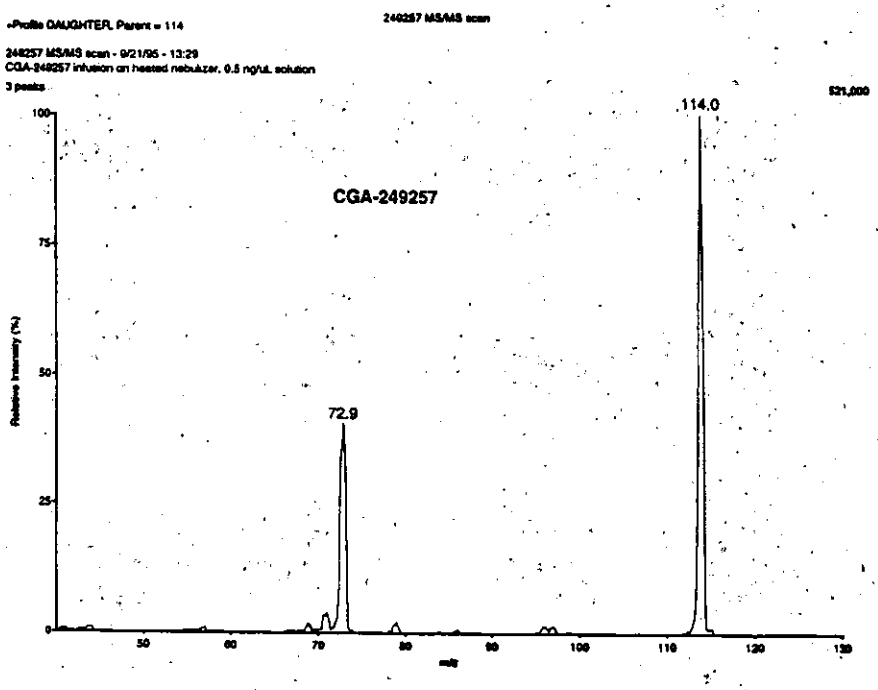
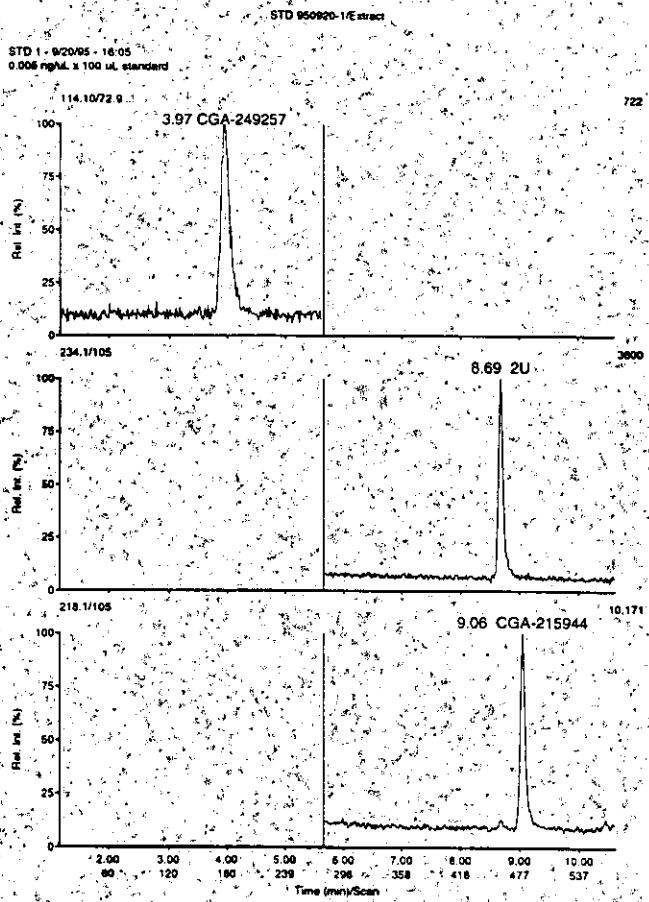
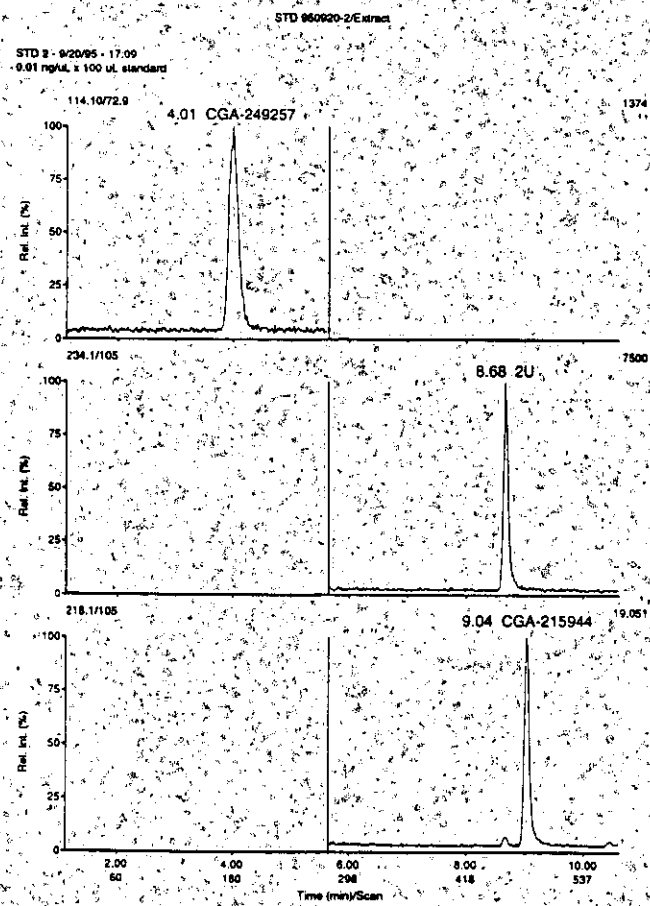


FIGURE 7. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS AND CALIFORNIA SOIL SAMPLES



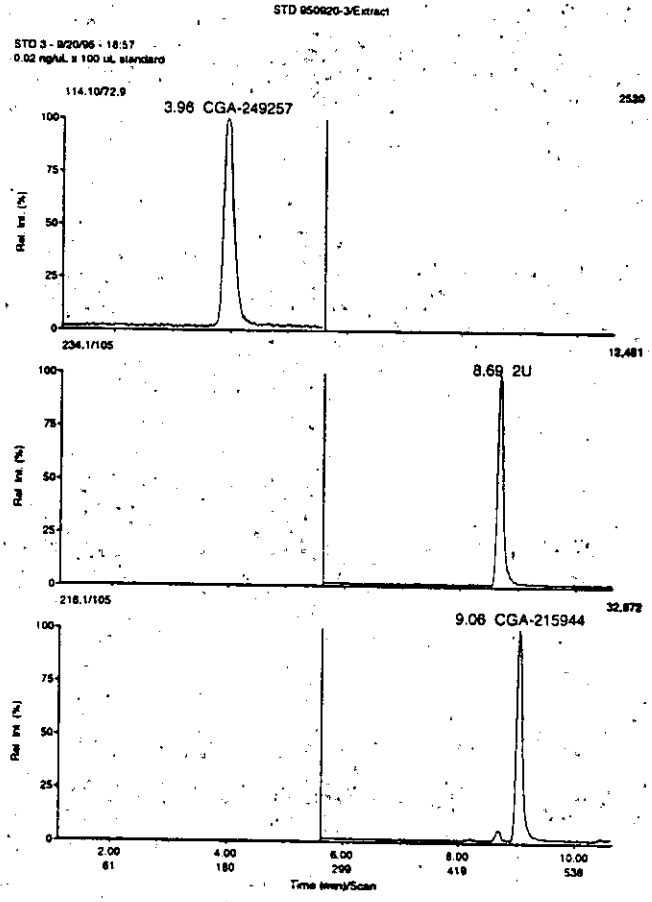
Sample Code: Standard 1
Description: 0.5 ng inj.



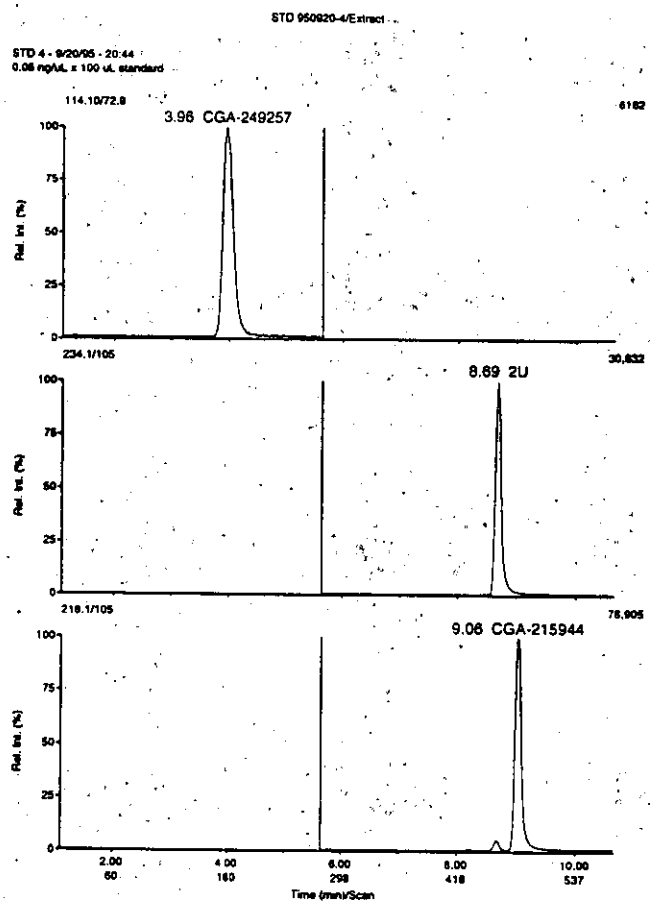
Sample Code: Standard 2
Description: 1 ng inj.

* Specific data regarding amounts injected, peak area, amount found, recovery, etc. are detailed in Table V.

FIGURE 7. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS AND CALIFORNIA SOIL SAMPLES (Continued)



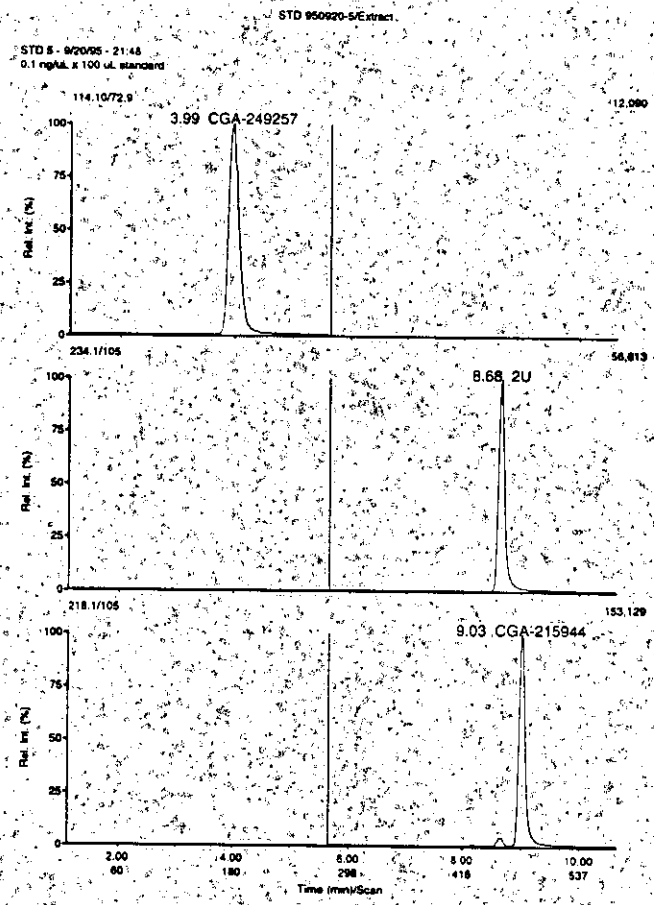
Sample Code: Standard 3
Description: 2 ng inj.



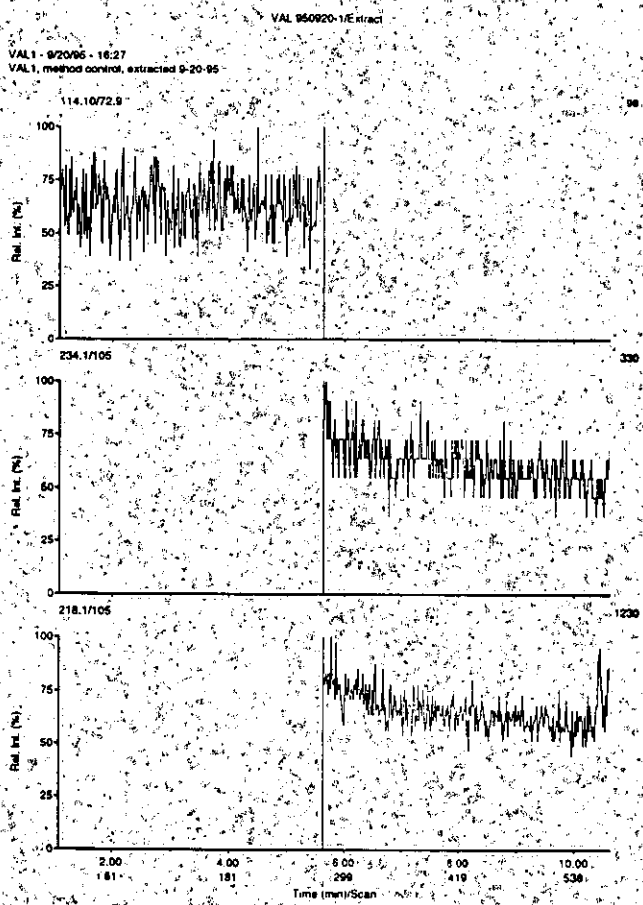
Sample Code: Standard 4
Description: 5 ng inj.

* Specific data regarding amounts injected, peak area, amount found, recovery, etc. are detailed in Table V.

FIGURE 7. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS AND CALIFORNIA SOIL SAMPLES (Continued)



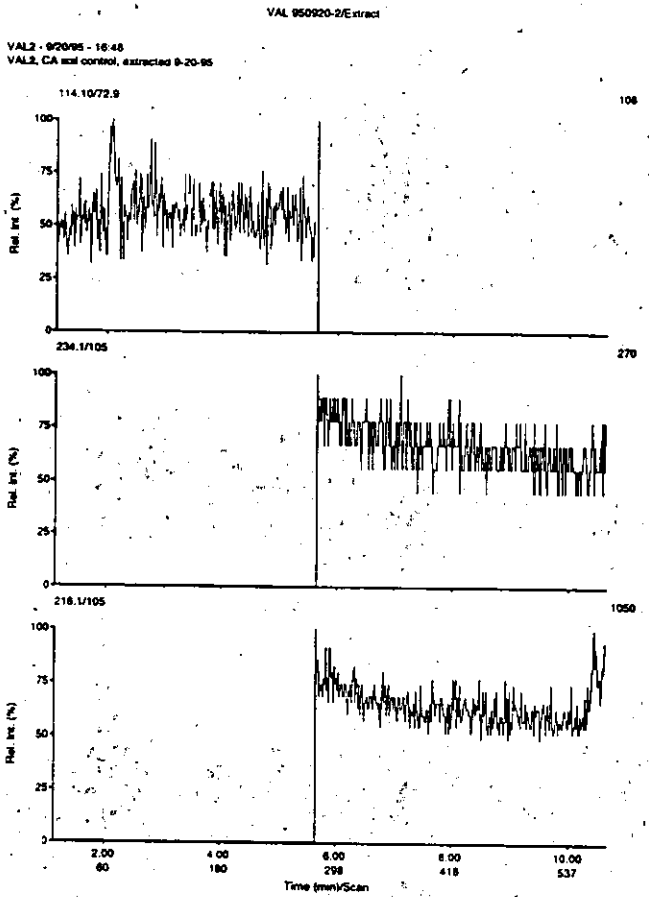
Sample Code: Standard 5
Description: 10 ng inj.



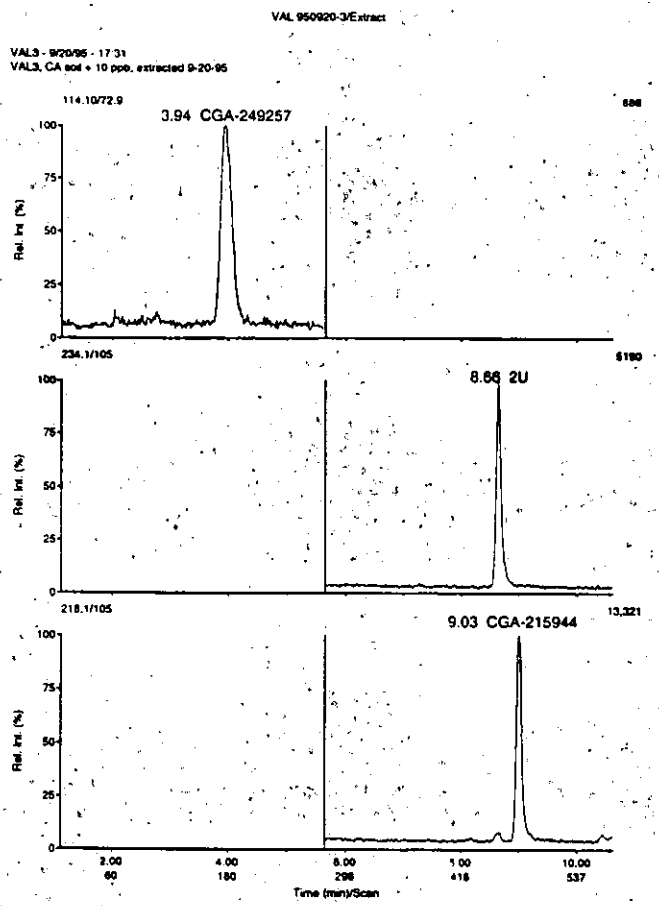
Sample Code: VAL1
Description: Method Blank

* Specific data regarding amounts injected, peak area, amount found, recovery, etc. are detailed in Table V.

FIGURE 7. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS AND CALIFORNIA SOIL SAMPLES (Continued)



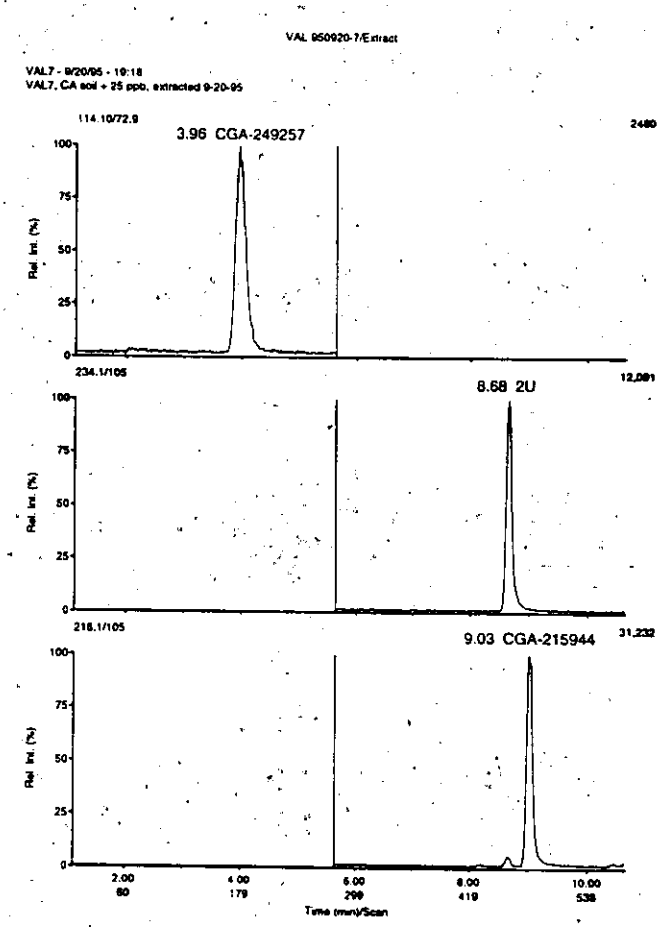
Sample Code: VAL2
Description: Soil Control



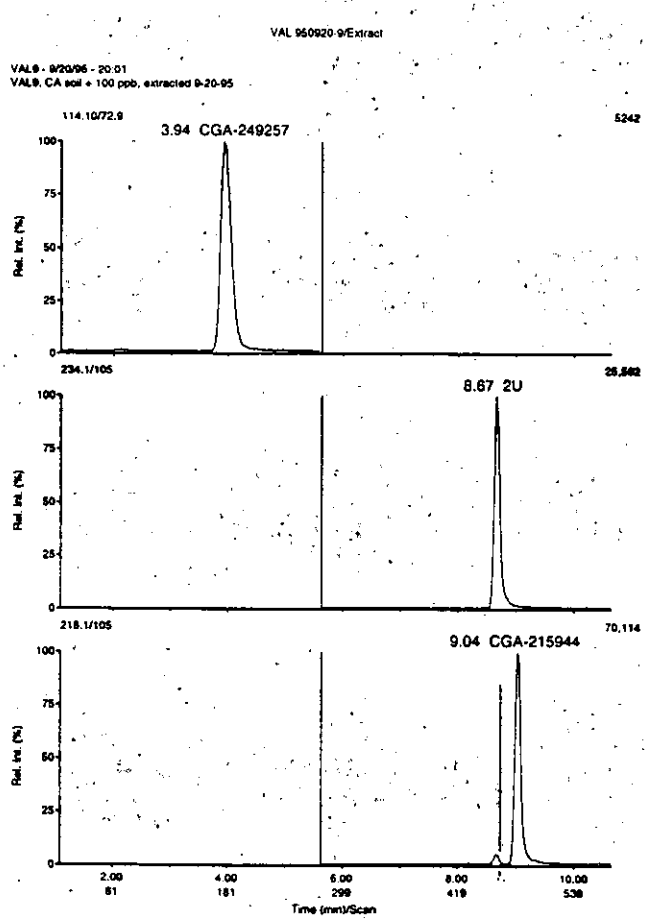
Sample Code: VAL3
Description: Control + 10 ppb

* Specific data regarding amounts injected, peak area, amount found, recovery, etc. are detailed in Table V.

FIGURE 7. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS AND CALIFORNIA SOIL SAMPLES (Continued)



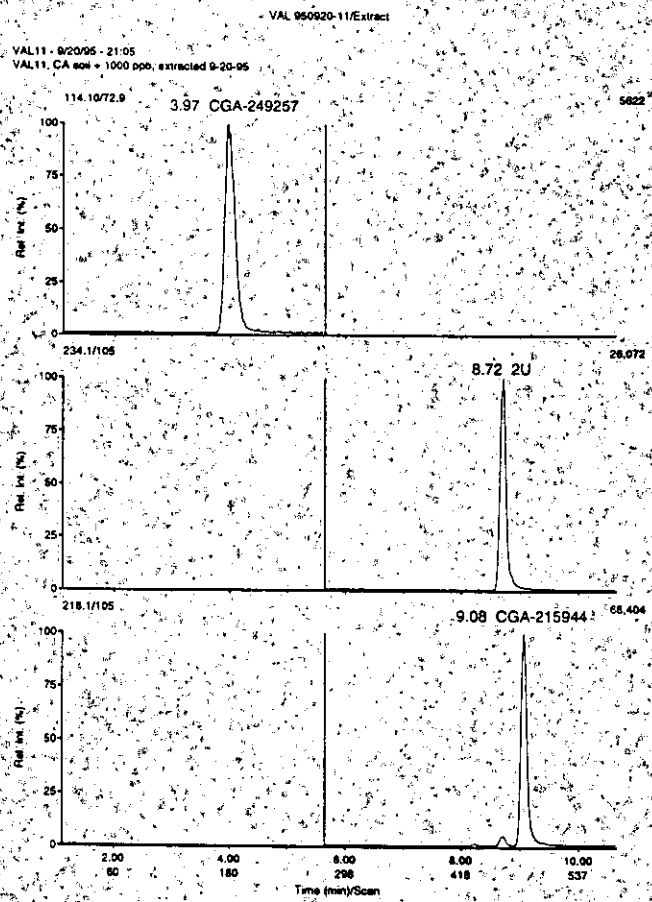
Sample Code: VAL7
Description: Control + 25 ppb



Sample Code: VAL9
Description: Control + 100 ppb

* Specific data regarding amounts injected, peak area, amount found, recovery, etc. are detailed in Table V.

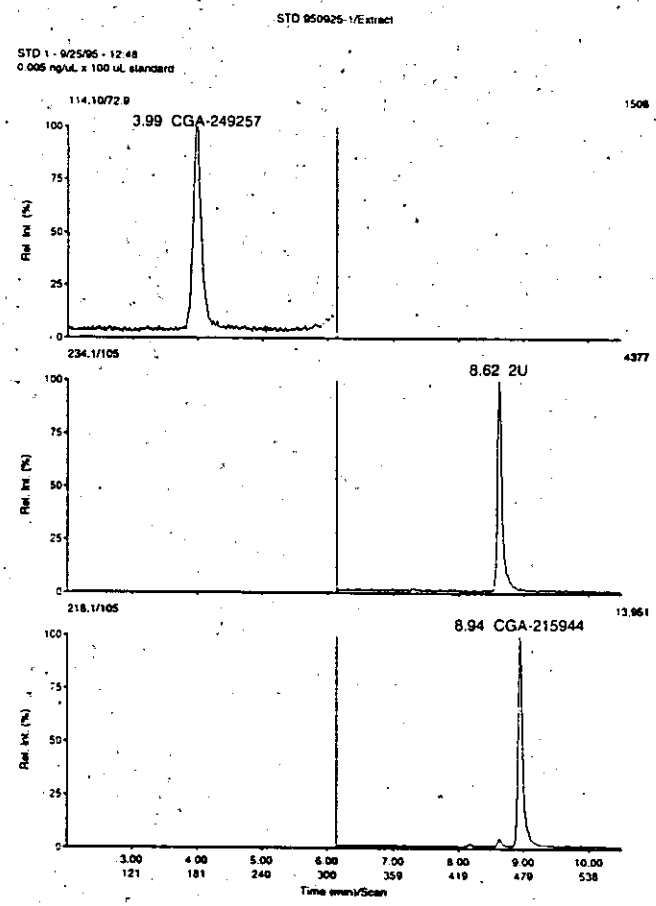
FIGURE 7. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS AND CALIFORNIA SOIL SAMPLES (Continued)



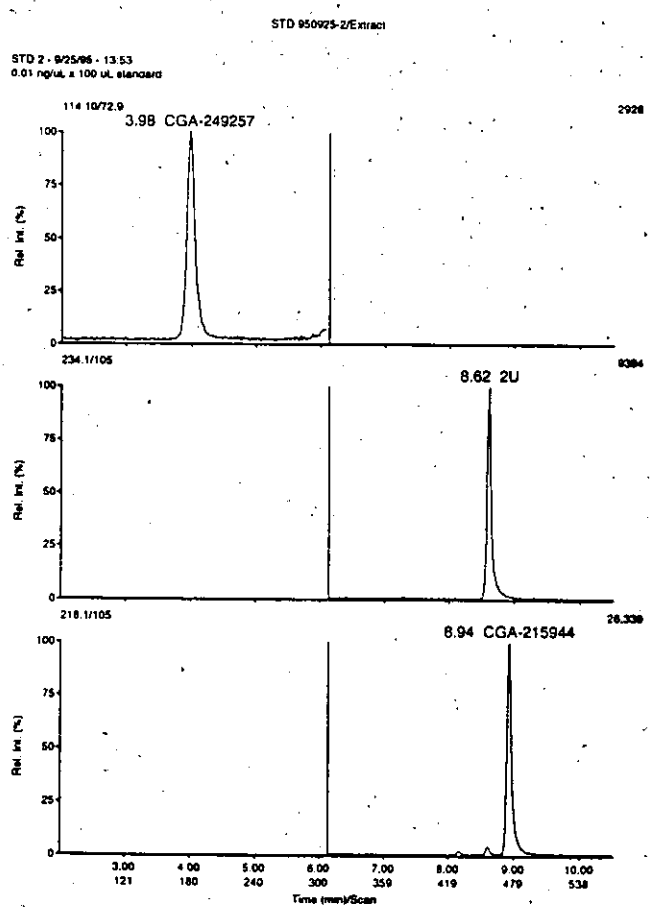
Sample Code: VAL11
Description: Control + 1000 ppb

* Specific data regarding amounts injected, peak area, amount found, recovery, etc. are detailed in Table V.

FIGURE 8. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS AND GEORGIA SOIL SAMPLES



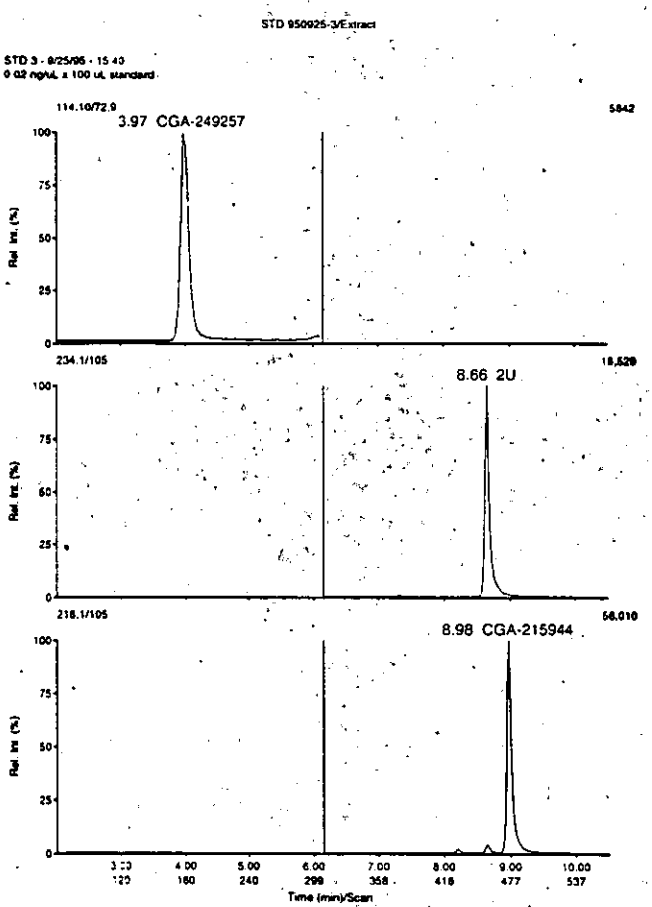
Sample Code: Standard 1
Description: 0.5 ng inj.



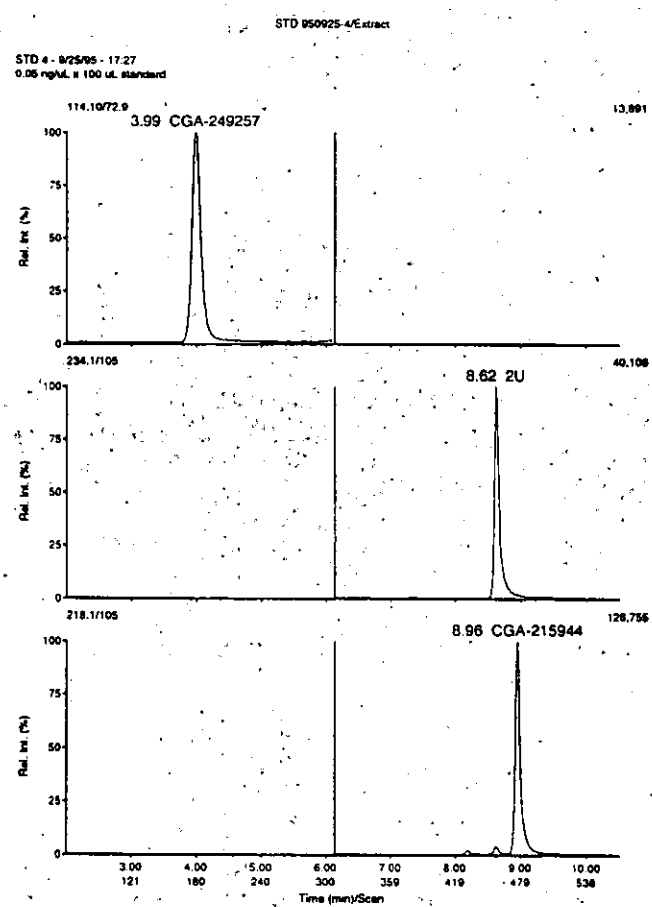
Sample Code: Standard 2
Description: 1 ng inj.

* Specific data regarding amounts injected, peak area, amount found, recovery, etc. are detailed in Table VI.

FIGURE 8. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS AND GEORGIA SOIL SAMPLES (Continued)



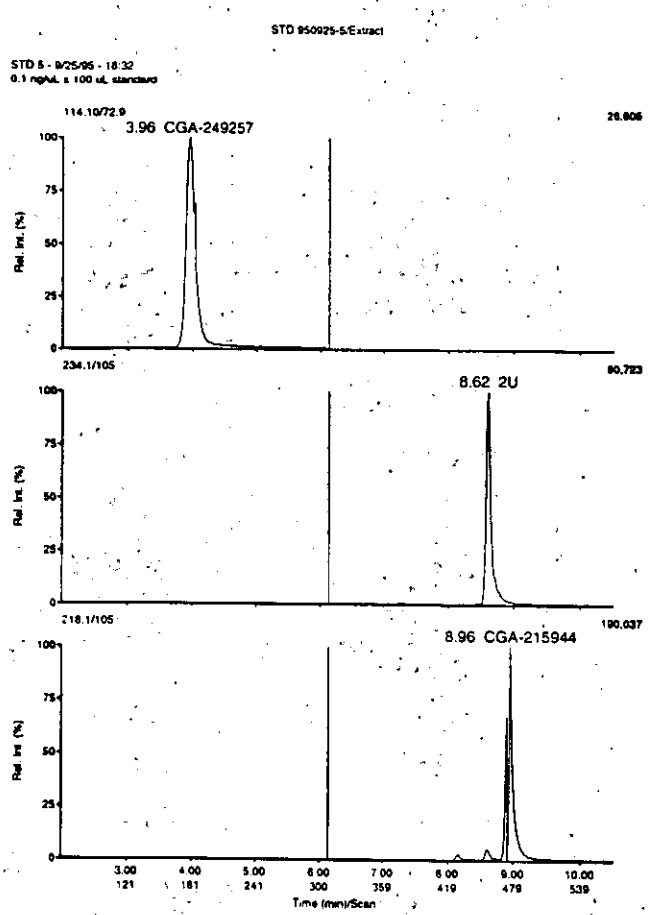
Sample Code: Standard 3
Description: 2 ng inj.



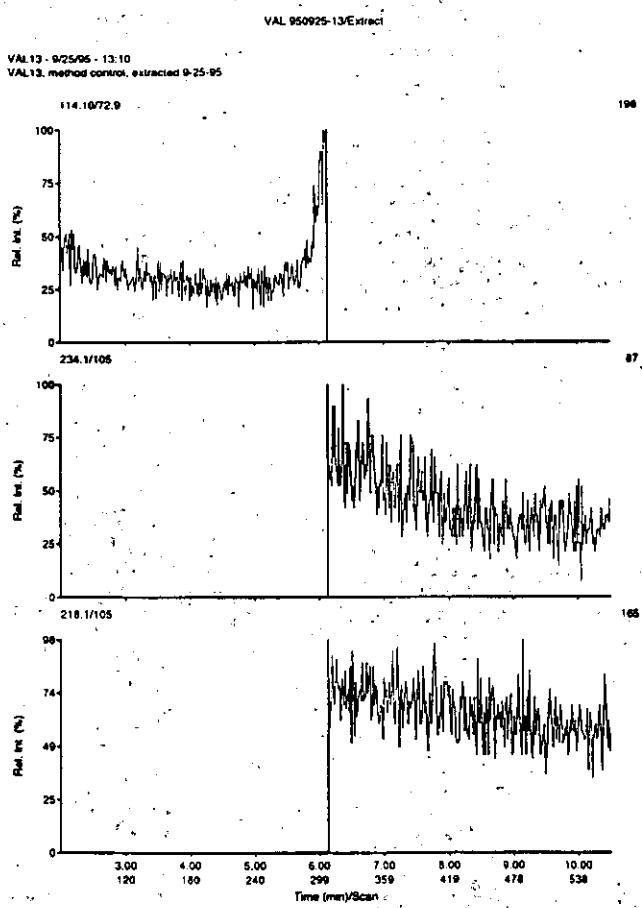
Sample Code: Standard 4
Description: 5 ng inj.

* Specific data regarding amounts injected, peak area, amount found, recovery, etc. are detailed in Table VI.

FIGURE 8. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS AND GEORGIA SOIL SAMPLES (Continued)



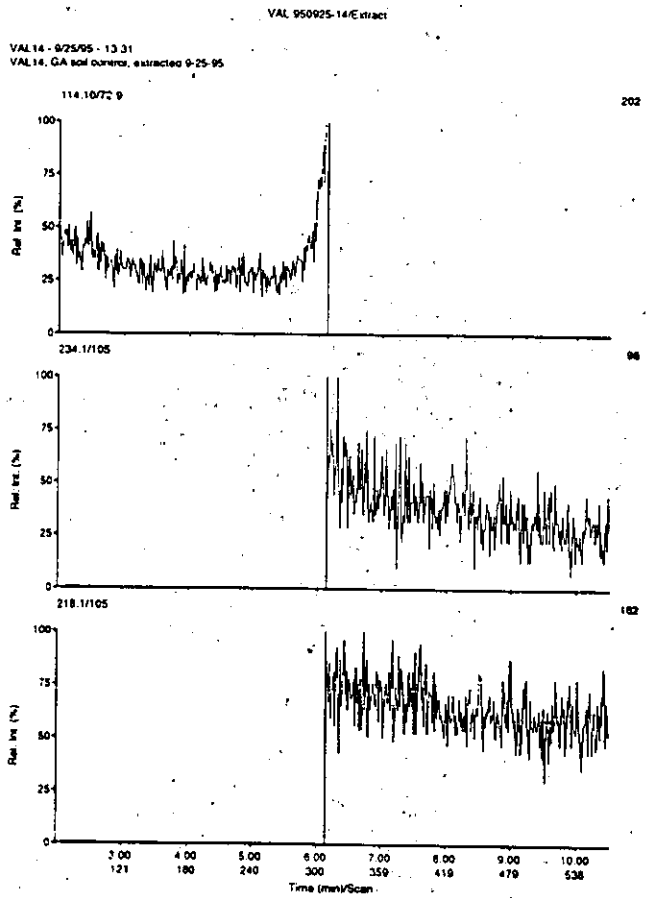
Sample Code: Standard 5
Description: 10 ng inj.



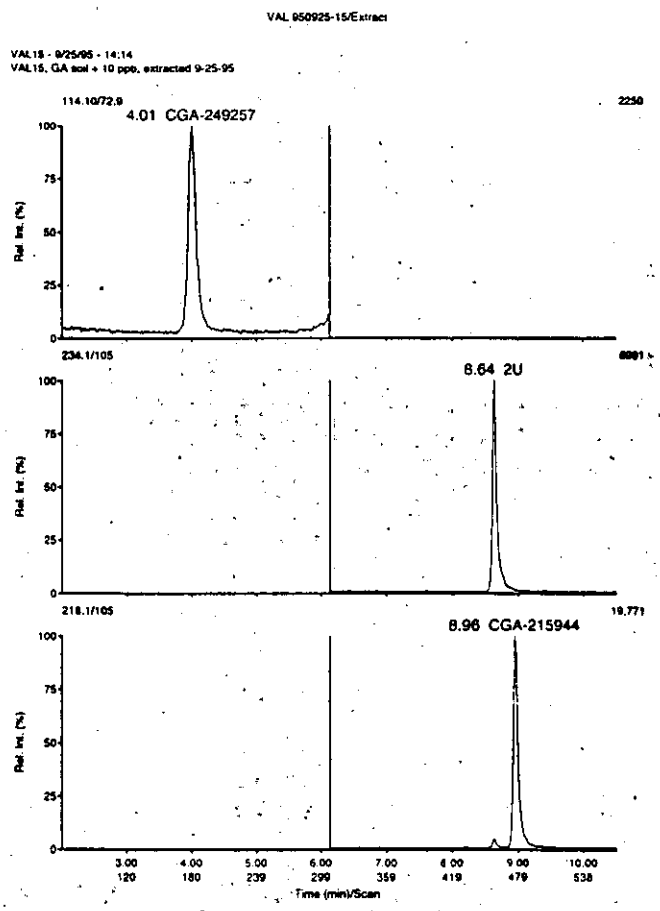
Sample Code: VAL13
Description: Method Blank

* Specific data regarding amounts injected, peak area, amount found, recovery, etc. are detailed in Table VI.

FIGURE 8. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS AND GEORGIA SOIL SAMPLES (Continued)



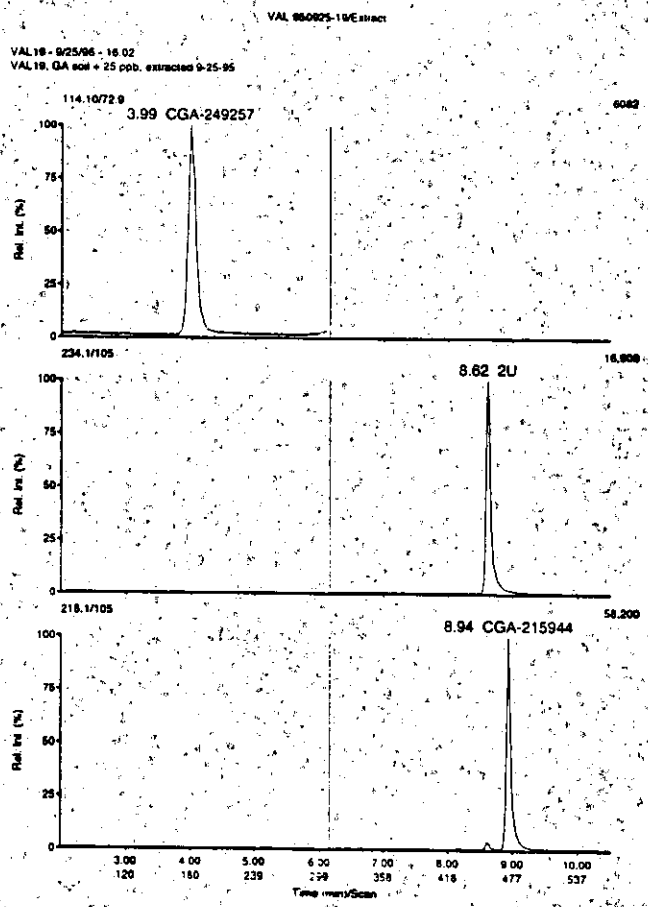
Sample Code: VAL14
Description: Soil Control

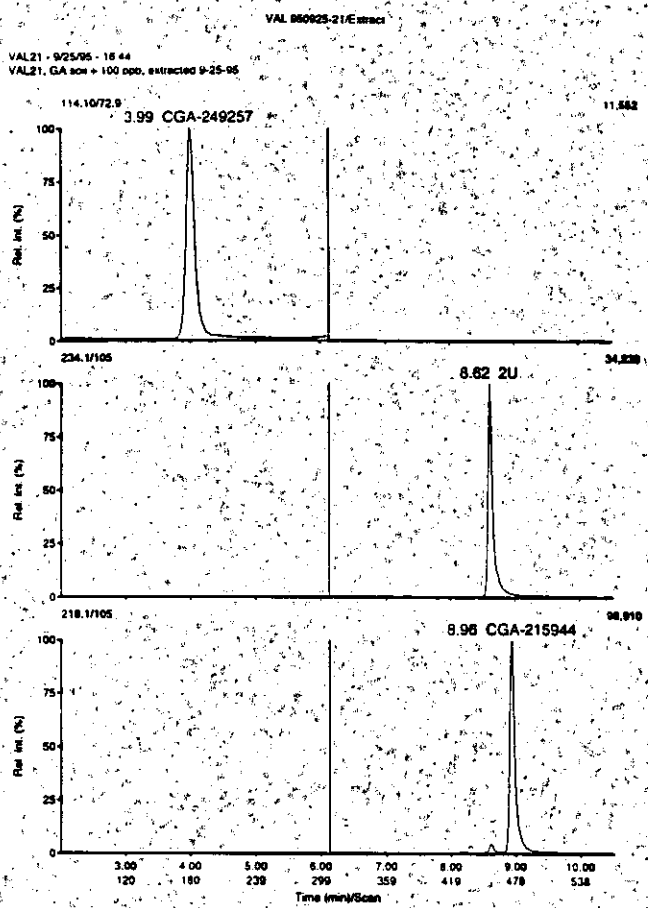


Sample Code: VAL15
Description: Control + 10 ppb

* Specific data regarding amounts injected, peak area, amount found, recovery, etc. are detailed in Table VI.

FIGURE 8. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS AND GEORGIA SOIL SAMPLES (Continued)



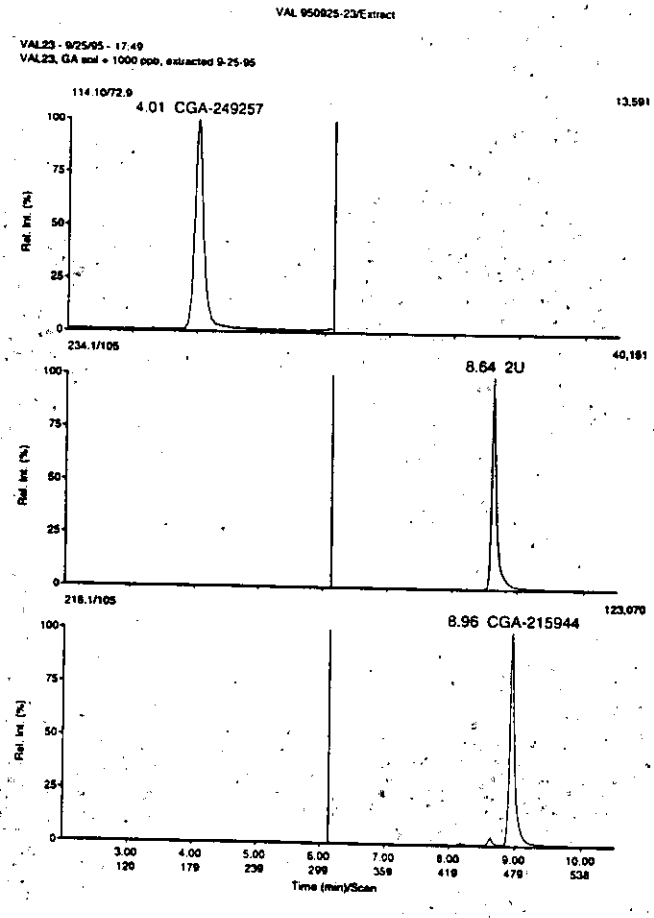


Sample Code: VAL19
Description: Control + 25 ppb

Sample Code: VAL21
Description: Control + 100 ppb

* Specific data regarding amounts injected, peak area, amount found, recovery, etc. are detailed in Table VI.

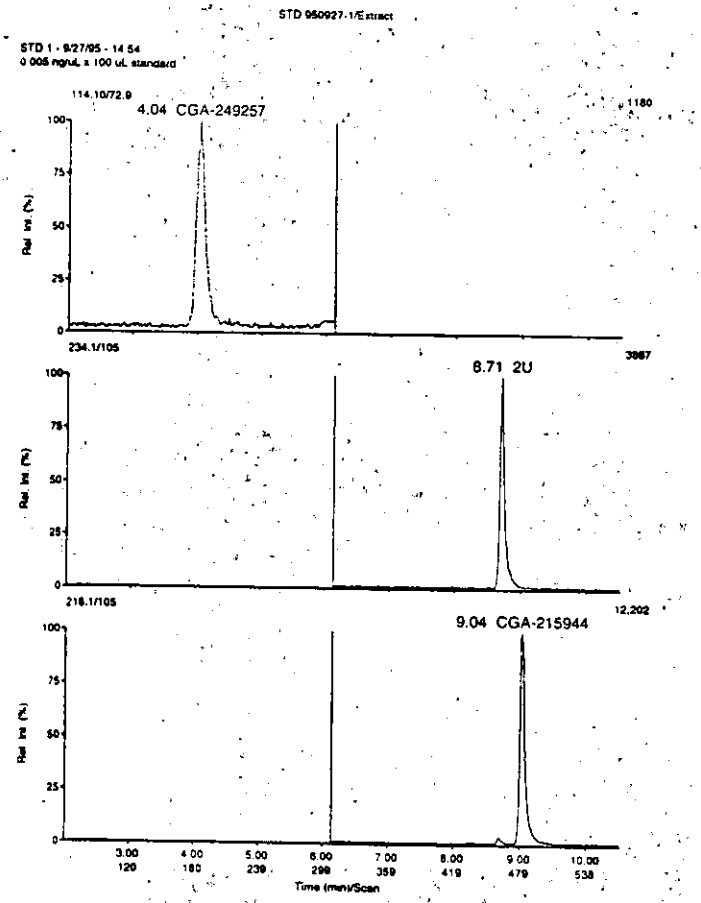
FIGURE 8. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS AND GEORGIA SOIL SAMPLES (Continued)



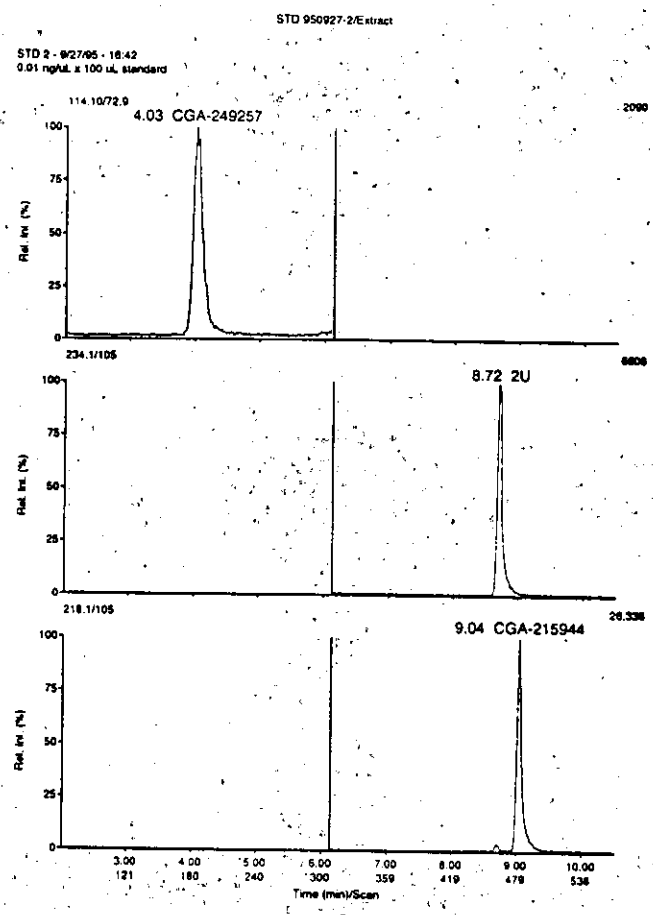
Sample Code: VAL23
Description: Control + 1000 ppb

* Specific data regarding amounts injected, peak area, amount found, recovery, etc. are detailed in Table VI.

FIGURE 9. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS AND NEW YORK SOIL SAMPLES



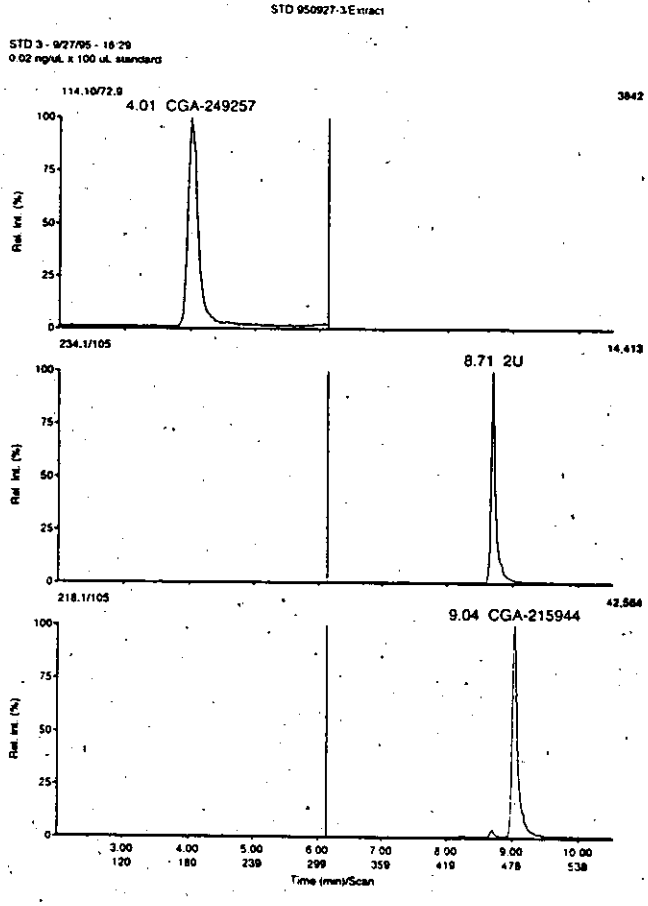
Sample Code: Standard 1
Description: 0.5 ng inj.



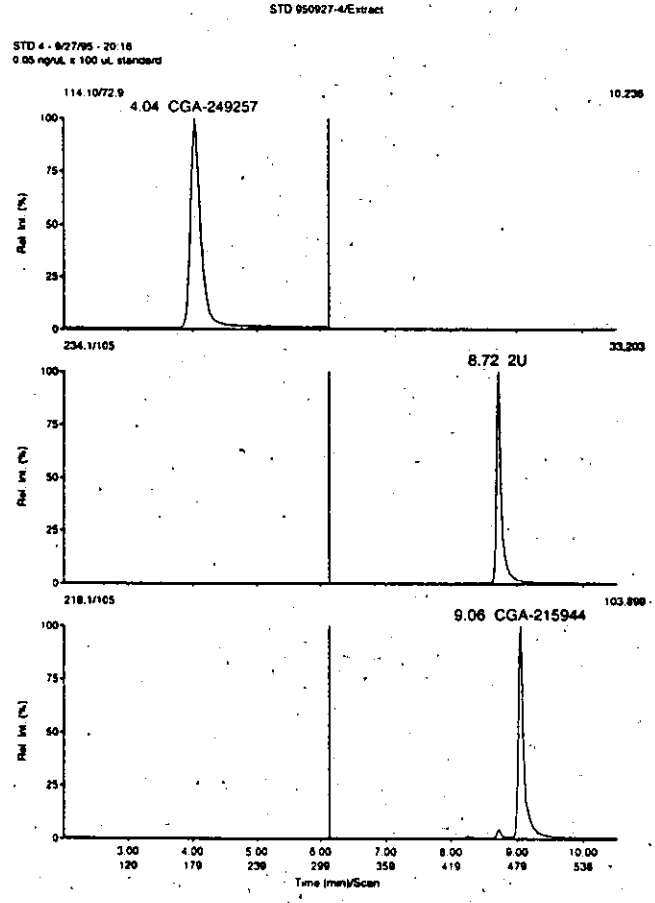
Sample Code: Standard 2
Description: 1 ng inj.

* Specific data regarding amounts injected, peak area, amount found, recovery, etc. are detailed in Table VII.

FIGURE 9. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS AND NEW YORK SOIL SAMPLES (Continued)



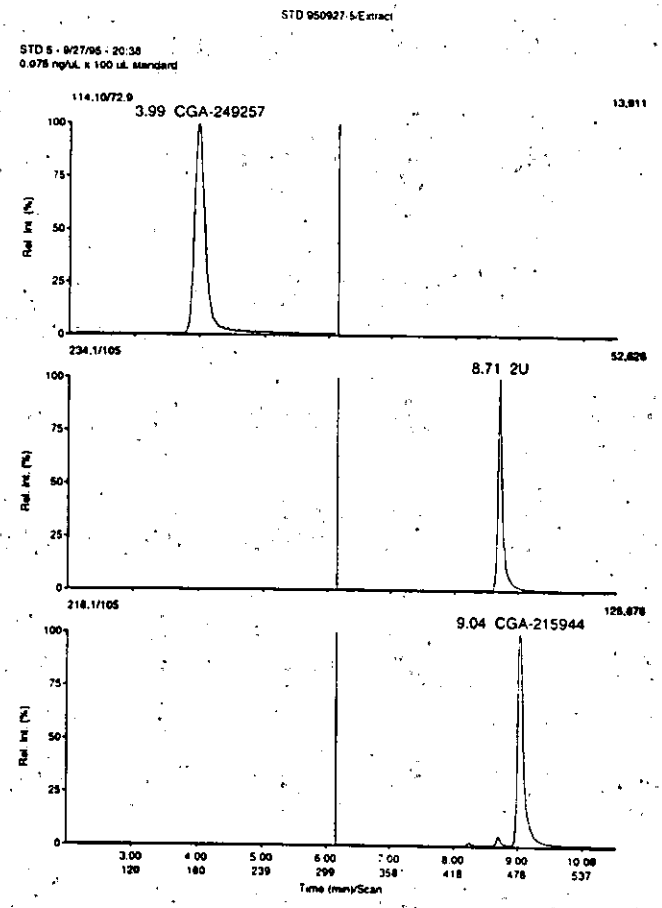
Sample Code: Standard 3
Description: 2 ng inj.



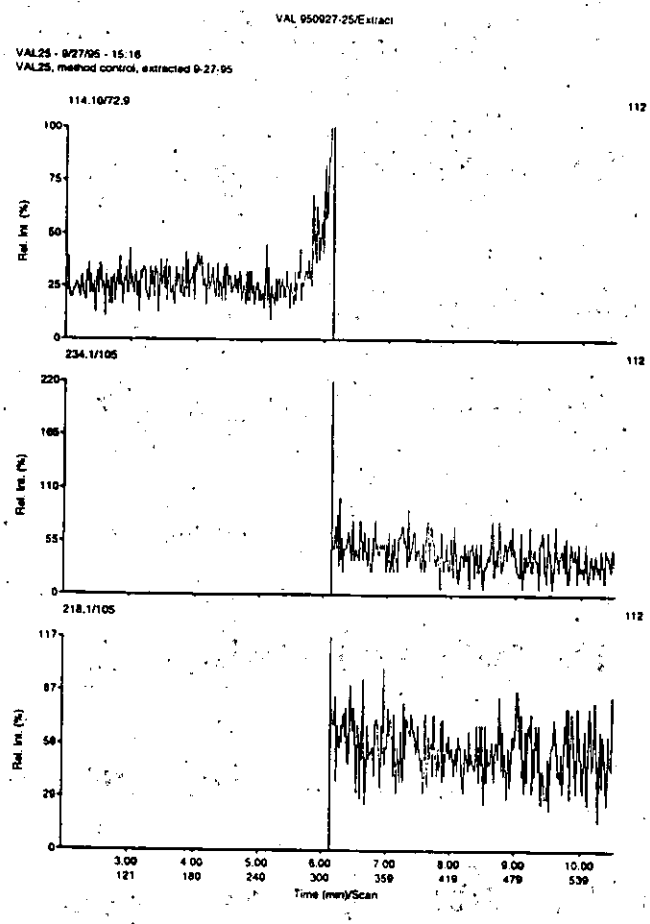
Sample Code: Standard 4
Description: 5 ng inj.

* Specific data regarding amounts injected, peak area, amount found, recovery, etc. are detailed in Table VII.

FIGURE 9. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS AND NEW YORK SOIL SAMPLES (Continued)



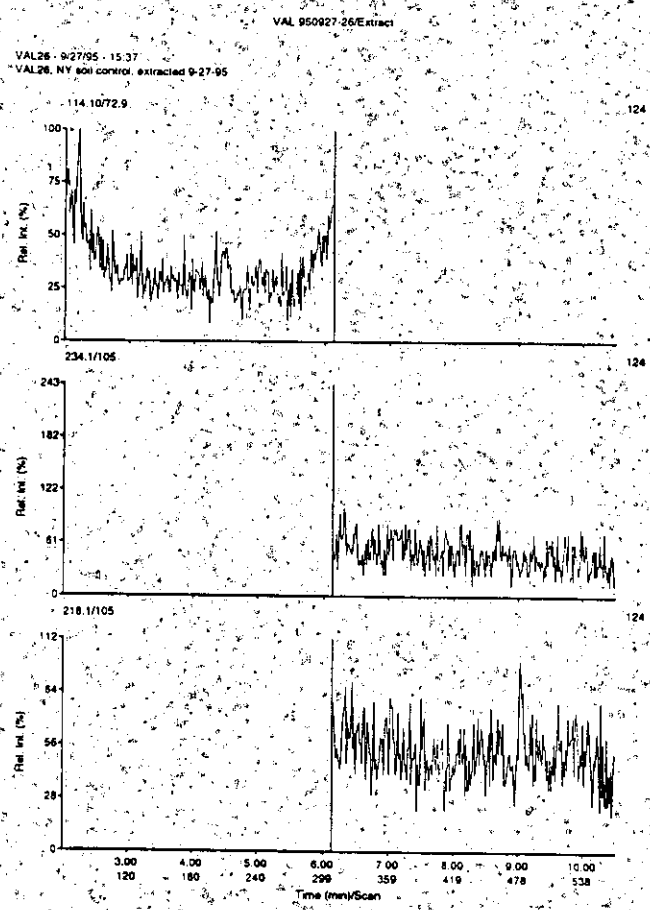
Sample Code: Standard 5
Description: 7.5 ng inj.



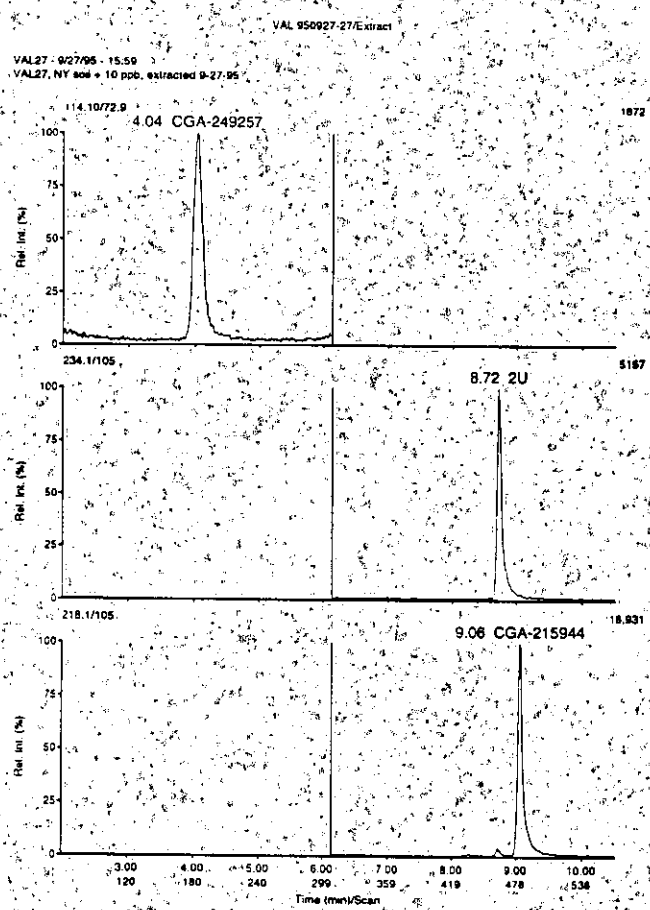
Sample Code: VAL25
Description: Method Blank

* Specific data regarding amounts injected, peak area, amount found, recovery, etc. are detailed in Table VII.

FIGURE 9. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS AND NEW YORK SOIL SAMPLES (Continued)



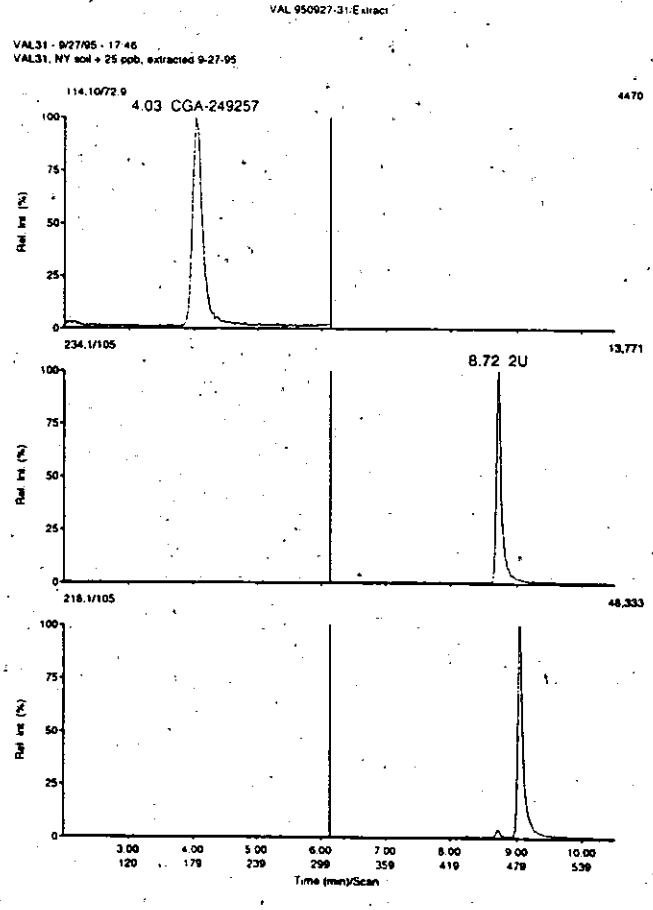
Sample Code: VAL26
Description: Soil Control



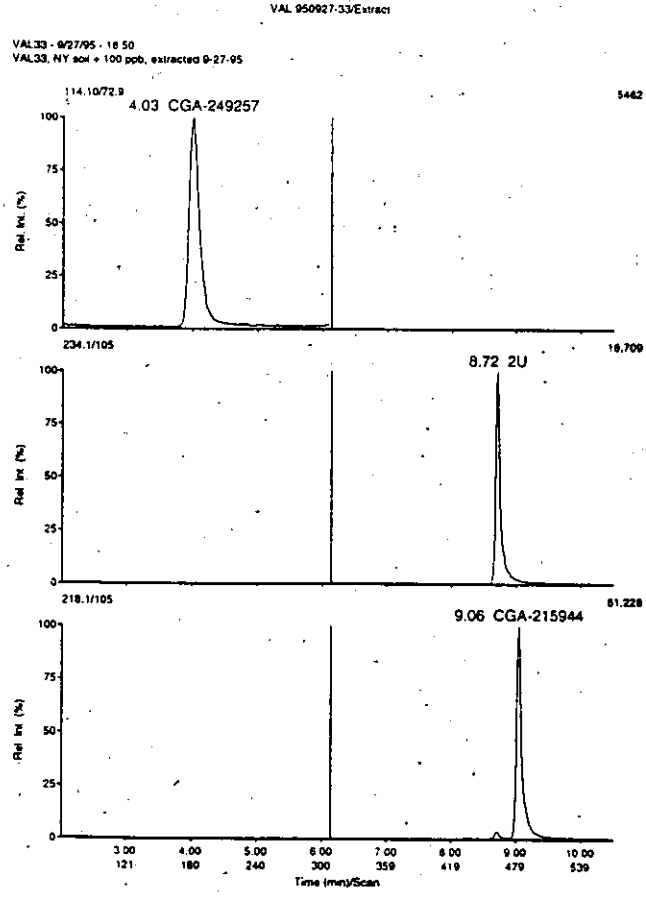
Sample Code: VAL27
Description: Control + 10 ppb

* Specific data regarding amounts injected, peak area, amount found, recovery, etc. are detailed in Table VII.

FIGURE 9. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS AND NEW YORK SOIL SAMPLES (Continued)



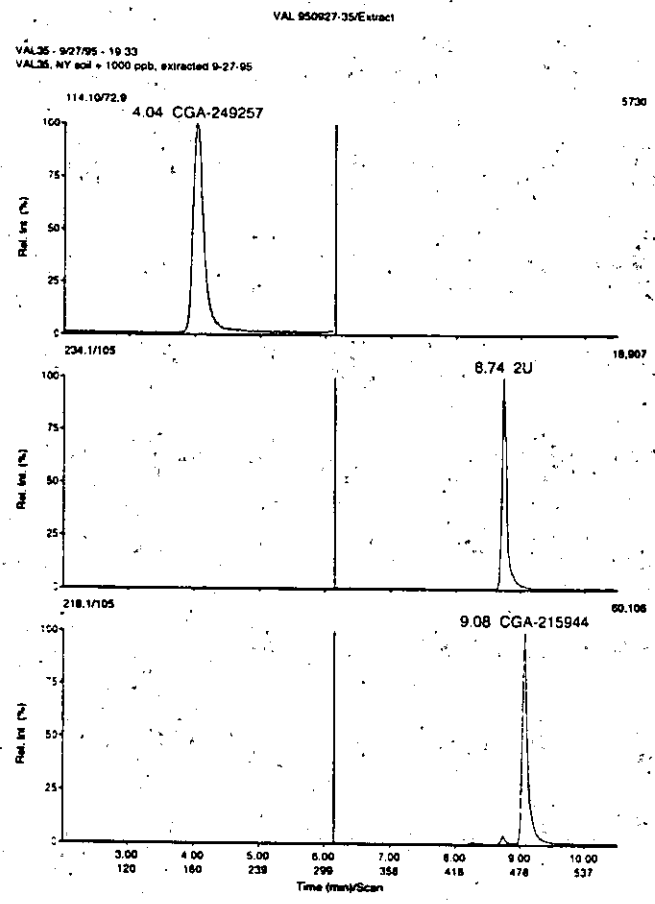
Sample Code: VAL31
Description: Control + 25 ppb



Sample Code: VAL33
Description: Control + 100 ppb

* Specific data regarding amounts injected, peak area, amount found, recovery, etc. are detailed in Table VII.

FIGURE 9. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS AND NEW YORK SOIL SAMPLES (Continued)



Sample Code: VAL35
Description: Control + 1000 ppb

* Specific data regarding amounts injected, peak area, amount found, recovery, etc. are detailed in Table VII.

IX. REFERENCES

1. Vargo, J. D., Ciba Protocol 334-95, "Validation of "Draft" Analytical Method AG-641 for the Determination of CGA-215944 and its Metabolites CGA-249257 and 2U in Soil by High Performance Liquid Chromatography with Mass Spectrometric Detection," including Protocol Amendment 1.

2. Vargo, J. D., and Chamkasem, N., Ciba Residue Test Report RI-MV-009-95, Report Number 1.