Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Pymetrozine Degrad (CGA-180777)

MRID #: 444113-39

Matrix: Soil

Analysis: HPLC/UV

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444113-39

VOLUME 48 OF 58 OF SUBMISSION

ANALYTICAL METHOD

CGA-215944

METHOD TITLE

Analytical Method for the Determination of CGA-180777, A Metabolite of CGA-215944, In Soil by High Performance Liquid Chromatography with UV and MS Detection Including Validation Data

DATA REQUIREMENT

40 CFR 158, Subdivision N, 164-1

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METHOD COMPLETION DATE

September 9, 1996

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PROJECT ID

Ciba Method No. AG-660
Ciba Study No. 285-96

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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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SPONSOR CERTIFICATION OF GOOD LABORATORY PRACTICE

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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9-16-97
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CERTIFICATION OF AUTHENTICITY

This report contains an unaltered copy of Ciba Analytical Method No. AG-660 (except for changes required to comply with PR Notice 86-5).

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9-16-97

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Ciba Analytical Method No. AG-660

Analytical Method for the Determination of CGA-180777, a Metabolite of CGA-215944, in Soil by High Performance Liquid Chromatography with UV and MS Detection Including Validation Data

ANALYTICAL METHOD FOR THE DETERMINATION OF CGA-180777, A METABOLITE OF CGA-215944, IN SOIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH UV AND MS DETECTION INCLUDING VALIDATION DATA

METHOD NO. AG-660

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Protocol Number: 285-96
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I. INTRODUCTION/SUMMARY

A. Scope

This method is used for the determination of CGA-180777, a metabolite of the insecticide CGA-215944, in soil. The analyte is separated by high performance liquid chromatography (HPLC), utilizing a cation exchange HPLC column, and detected by UV absorption detection (LC/UV). A confirmatory, or alternative, analysis procedure which utilizes HPLC with mass spectrometric detection (LC/MS) is also presented. The structures, chemical names, and Chemical Abstracts Registry numbers of the analyte and active ingredient are presented in Figure 1.

The limit of detection (smallest standard amount injected during the chromatographic run) is 2.5 ng. The limit of determination (the lowest fortification specified by the method which gives adequate recovery according to EPA guidelines) is 10 ppb in soil.

B. Principle

Soil samples (20 g) are extracted two times with 1/9/90% ammonium hydroxide/water/methanol using mechanical shaking at room temperature. The samples are centrifuged and filtered. Methanol is removed via rotary evaporation until only aqueous remains. The aqueous is acidified and then passed through a C18 solid phase extraction (SPE) cartridge which is attached piggy-back style to a cation exchange (SCX) extraction cartridge. The analyte is retained on the SCX SPE column and subsequently eluted with 1/99% ammonium hydroxide/methanol. The eluate is reduced to dryness via rotary evaporation. The residue is dissolved in the HPLC mobile phase with sonication and vortex mixing. The sample is injected onto the HPLC system with the analyte detected by UV absorbance. Alternatively, the confirmatory LC/MS analysis procedure may be used. 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, 150-mL (Fisher cat. #02-540J) 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. Must be centrifugable.

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 Concentration tube, 50-mL (Fisher cat. #05-538-40B) or equivalent.

7.0 Cylinder, graduated, 50-mL, 100-mL, and 1000-mL (Fisher cat. #08-556C, #08-556D, #08-556G), or equivalent.

8.0 Filter, paper, for filtering soil extracts prior to rotary evaporation, 24-cm prepleated circles, Whatman Reeve Angel 802 (Fisher cat. #09-901D) or equivalent.

9.0 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).

10.0 Flasks, round bottom, 250-mL (Fisher cat. #10-067E) and 100-mL (Fisher cat. #10-067D), or equivalent.

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

12.0 Mixer, vortex (Fisher cat. #12-810-10) or equivalent.

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

14.0 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).

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

16.0 Rotary evaporator, Buchi (Fisher cat. #09-548-105F) or equivalent, with rotary evaporator traps (Fisher cat. #K570210-0124) or equivalent.

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

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

B. Reagents and Analytical Standards

All reagents and polypropylene glycols (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 Acetic acid, glacial, HPLC grade (Fisher cat. #A35-500) or equivalent.

2.0 Acetic acid, 0.1%, pH adjusted to 3.25. Add 1.0 mL acetic acid (glacial) to 999 mL of

water. Adjust pH to 3.25 ± 0.05 using ammonium hydroxide while accurately monitoring with a pH meter (pH indicating paper is not sufficiently accurate for use in this step).

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

4.0 Ammonium formate, (Fisher cat. #A666-500) or equivalent.

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

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

7.0 Extraction solvent: 1/9/90% (v/v) ammonium hydroxide/water/methanol. Add 10 mL of ammonium hydroxide to 900 mL of methanol and 90 mL of purified water.

8.0 C18 SPE extraction column, 1 gram size (Varian cat. #1225-6001).

9.0 LC/MS tuning solution: Mix 0.5 mL of the 100 ng/µL stock solution of CGA-180777 with 9.5 mL of HPLC mobile phase. The final solution concentration will be 5 ng/µL.

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

11.0 Mobile phase for SCX column (analysis mobile phase): 25/75% methanol/water (0.1% acetic acid, pH adjusted to 3.25 ± 0.05). Mix 250 mL of methanol with 750 mL of the 0.1% acetic acid solution, pH adjusted to 3.25 ± 0.05.

12.0 Mobile phase for SCX column (cleanup mobile phase): 25/75% methanol/0.02 M ammonium acetate. Mix 250 mL of methanol with 750 mL of water and 1.16 grams of ammonium acetate.

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

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

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

16.0 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.

17.0 Phosphoric acid, 85%, HPLC grade (Fisher cat. #A260-500) or equivalent.

18.0 Phosphoric acid solution, 1%. Add 1 mL of phosphoric acid to 99 mL of purified water. This solution is used to condition/rinse the SPE columns.

19.0 Sample diluent: 25/75% methanol/water (0.1% acetic acid, pH adjusted to 3.25 ± 0.05).

20.0 SCX SPE extraction column, 0.5 gram size (Varian cat. #1211-3039).

21.0 SCX eluting solvent: 1/99% ammonium hydroxide/methanol. Add 1.0 mL of ammonium hydroxide to 99 mL of methanol.

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

23.0 CGA-180777, 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 acetic acid and ammonium hydroxide that are used in this method are irritants and should be used in a well-ventilated area (i.e., a fume hood).

D. Analytical Procedure

1.0 Soil Moisture Determination

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

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

1.2 Add 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:

$$m = \frac{W_{1.2} - W_{1.3}}{W_{1.2} - W_{1.1}}$$

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 $W_{1.3}$ is the weight of the dry soil plus container (from Step 1.3).

2.0 Soil Extraction/Cleanup

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

2.1 Weigh and record 20 ± 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 100 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. (Use 500-mL round bottom flasks if excessive bumping or foaming of the samples are observed during rotary evaporation of the methanol.)

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. Combine this extract with the extract from Step 2.5 in the round bottom flask.

2.7 Add approximately 25 mL of water to each sample to help prevent it from going dry during the rotary evaporation step.

2.8 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. (Note: Periodic venting of the sample may be required to prevent losses due to bumping.) Remove the methanol until only water remains.

2.9 If the volume of water remaining is less than approximately 25 mL, add water to reach this volume. Add 0.5 mL of phosphoric acid. (The pH of the aqueous should be < 2.) Note: A precipitate will form in some soils after addition of the acid. This precipitate may clog the filters on the C18 SPE column. The sample may be centrifuged after addition of the acid and the extract then decanted into the C18 SPE reservoir, if it is deemed necessary.

2.10 Load the sample into a reservoir that is attached to a preconditioned C18 SPE extraction column which is attached piggy-back style to a SCX SPE. (Note: The SPE columns are preconditioned by passing approximately 5 mL each of methanol and 1% phosphoric acid through the columns. Add approximately 3 mL of 1% phosphoric acid to the SCX SPE before attaching the C18 SPE and beginning the sample loading step.) The sample loading speed should not exceed a fast drip rate. Drain the extract through the SPE columns until the liquid level reaches the top of the filter frit on the C18 SPE. (Note: If the SCX SPE drains faster than the C18 SPE,

disconnect the C18 SPE and add 1% phosporic acid to the SCX SPE as needed to prevent it from going dry. Reconnect the C18 SPE and proceed.)

2.11 Add approximately 5 mL of 1% phosphoric acid to the 250-mL round bottom flask in which the rotary evaporation step was done. Vortex the solvent along the sides of the flask to dissolve any residues. Load this rinse onto the C18/SCX SPE columns and drain.

2.12 Disconnect the C18 SPE column from the SCX SPE. Discard the C18 SPE. Rinse the SCX SPE with approximately 3 mL of methanol.

2.13 Place a 50-mL concentration tube underneath the SCX SPE. Elute CGA-180777 from the SCX SPE with 10 mL of 1% ammonium hydroxide in methanol. Collect the eluate in the concentration tube.

2.14 Place the sample on a rotary evaporator with a water bath temperature of approximately 40 to 45°C and remove all solvents until the sample is dry. Use methanol to azeotrope the water, if needed. Use a solvent trap to minimize losses due to bumping. (Note: Periodic venting of the sample may be required to prevent losses due to bumping.)

2.15 Dissolve the residue with 2.0 mL of sample diluent. (Additional dilution may be needed for samples containing high levels of residue.) Sonicate and vortex mix the sample.

2.16 Filter the sample with an Anotop sample filter, if necessary.

2.17 Analyze the sample by HPLC with UV detection (or alternatively, use the LC/MS confirmation procedure). Store the sample in a freezer (<-10°C) if it will not be analyzed the same day the sample was processed.

E. Instrumentation

1.0 Description and Operating Conditions: HPLC

See Table II for a description of the HPLC system and operating conditions and Table III for a description and operating conditions of the mass spectrometry system used for LC/MS detection.

2.0 Calibration and Standardization: LC/UV and LC/MS

2.1 Determine the retention time of CGA-180777 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.

2.2 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 2.5 to 50 ng (50 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. Calibration data are presented for each sample set analyzed (Tables IV-IX).

3.0 Mass Calibration and Standardization: LC/MS (Not required if only LC/UV analyses will be performed.)

3.1 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 presented in Figure 3. Mass calibration must be performed on a daily basis, or after each instrument recycle period.

3.2 CGA-180777 is detected as a protonated molecular ion at mass 124.0. State file conditions for the analyte are optimized while infusing a 5 ng/µL solution in sample diluent. The mass spectrum obtained for the analyte in the MS mode is presented in Figure 4.

3.3 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. Typical state file values optimized for PPG and for the CGA-180777 are presented in Table III.

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 Confirmation of residues can be obtained by LC/MS analysis. See Table III for a description. No additional confirmation of residues is required if LC/MS is used as the analysis technique.

H. Time Required

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

2.0 Each HPLC analysis requires approximately 12 minutes.

I. Modifications and Potential Problems

1.0 Analytical Method AG-660 was validated only for the soil types listed in the final method. Other soil types, or soil samples from different locations, may exhibit binding or interference problems which were not observed with these samples.

2.0 "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. A 500-mL round bottom flask may be necessary for the initial evaporation of solvent from the raw extract (Step 2.8) if severe bumping or foaming occurs in a 250-mL round bottom flask.

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

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

5.0 The procedure for analyte isolation by SCX SPE with subsequent elution with basic methanol was optimized with Varian SCX SPE cartridges. It is not known whether other brands of SCX SPE cartridges will provide equivalent results.

6.0 Minor changes in the mobile phase pH will have a significant effect on the retention time of the analyte when using the SCX analysis column. Other brands of SCX analysis columns may require the pH to be adjusted to provide the same retention as observed with the Zorbax brand column. Analyte retention will increase as the pH of the mobile phase decreases.

7.0 Matrix residues that are retained on the SCX HPLC column may be cleaned off at the end of a sample run by passing 25/75% methanol/0.02 M ammonium acetate through the column at a flow rate of 1.0-1.5 mL/min for 30 minutes. The column will need to be re-equilibrated with the SCX mobile phase for at least 30 minutes at 1.5 mL/min prior to running another set of samples.

8.0 The C18 and SCX SPE cleanup cartridges must be properly conditioned prior to use, as detailed in Section II.D.2.10, to ensure proper and reproducible behavior.

9.0 CGA-180777 forms a very stable protonated molecular ion. Daughter ions (i.e., a MS/MS experiment) are difficult to form and have very poor sensitivity. While LC/MS exhibits good sensitivity, LC/MS/MS with a characteristic daughter ion fragment has poor sensitivity in comparison and thus is not very useful.

10.0 CGA-180777 is commonly known as niacin. This compound naturally occurs in plants at levels as high as several hundred parts per million. Thus, there is a distinct possibility that CGA-180777 may be observed in soils that have not been treated with the Ciba insecticide CGA-2159444.

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.

1.0 Prepare a 100 ng/µL stock solution in methanol. Weigh approximately 10.0 mg of analyte. Determine the appropriate volume of methanol 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 methanol 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/ul; and 10^3 is a conversion factor.

For example:

The volume of methanol required to dilute 9.9 mg of an analyte, of 98.0% purity, to a final concentration of 100 ng/uL is:

$$V\,(mL) = \frac{9.9\ mg \times 0.98}{100\ ng/uL} \times 10^3 = 97.02\ mL$$

2.0 Fortification standards are prepared by dilution of the 100 ng/uL standard with methanol. 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 20 g soil sample, the addition of 1.0 mL of a 0.2 ng/µL fortification solution will result in a fortification level of 10 ppb.)

3.0 A 1.0 ng/µL analytical standard for HPLC calibration use is prepared by pipetting

0.5 mL of the 100 ng/µL stock solution into a 50-mL volumetric flask and diluting to the mark using sample diluent. Subsequent serial dilutions are made with the sample diluent to prepare additional calibration standards.

K. Methods of Calculation

1.0 Determination of Residues in Samples

1.1 Inject the sample solution from Step II.D.2.17 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.2.2) 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 6-8, 10-12.

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.2.0) to the sample prior to any of the cleanup steps. The total volume of the added fortification solution should not exceed 1.0 mL.

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

3.0 Calculations

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

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

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

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).

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

where, g is the grams of soil (wet weight) used, V_a is the aliquot volume of extracted sample used for analysis, V_e is the volume 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.2.15) (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 for the cleanup process, the term "V_e + (m x g)" will equal the aliquot volume, V_a.)

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 metabolite 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 (217.2) and MW(m) is the average molecular weight of the metabolite, 123.1 for CGA-180777.

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

III. RESULTS AND DISCUSSION

This analytical method was validated under Protocol 285-96[1] for the analysis of control and fortified control soil. The objective of Protocol 285-96 was to

validate "Draft" Analytical Method AG-660 for the determination of CGA-180777 in soil with a limit of determination of 10 ppb.

Recovery data for fortified soil samples are presented in Tables IV-IX. 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 for each analysis procedure are tabulated and summarized in Tables X-XI. Typical chromatograms for analytical standards and for fortified soil samples are presented in Figures 5-12.

In these validation studies, a limit of detection (defined as the lowest analytical standard injected during an analysis set) of 2.5 ng was obtained for both LC/UV and LC/MS analyses. The absolute mass detection limit is influenced by the sensitivity and cleanliness of the mass spectrometer (for LC/MS analyses), the sharpness of the eluted peak, the volume of sample injected, and the signal-to-noise ratio that the analyst is willing to accept. In these experiments, the sensitivities displayed by the LC/MS analysis of the New York soil samples with the API-III+ instrument are not as good as those displayed on the API-I system for California and Georgia soils due to the API-III+ system not being as well optimized. The displayed sensitivities on the API-III+ system are adequate, however.

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 for all samples, with the exception of one unexplained low recovery for Georgia soil fortified at 10 ppb, by both LC/UV and LC/MS analyses in all three soils. A limit of quantitation (defined as the lowest fortification amount used in the study which gave acceptable recoveries) of 10 ppb was achieved for all three soil locations.

At the 10 ppb fortification level, the average recoveries (n=11) and percent relative standard deviations for data combined for all three soil

locations were 84.5 ± 15.2% and 79.7 ± 19.0% for LC/UV and LC/MS analyses, respectively. This demonstrates acceptable accuracy and precision at the lowest fortification level where poorest method performance is typically encountered. The accuracy and precision of the method are acceptable at the higher fortification levels of 100 ppb and 1000 ppb as demonstrated in Tables X-XI. The accuracy and precision are good for each set of soil samples; 83.4 ± 5.6% and 77.3 ± 8.1% for LC/UV and LC/MS analyses of California soil, 72.7 ± 4.7% and 76.3 ± 6.7% for LC/UV and LC/MS analyses of Georgia soil, and 85.5 ± 14.9% and 89.8 ± 14.7% for LC/UV and LC/MS analyses of New York soil.

Trace levels of CGA-180777 were observed in soil control samples. The largest amount observed, in which the calibration curve was reasonably accurate at levels far below the lowest calibration standard, was 0.19 ppb which was found in the LC/UV analysis of New York soil. CGA-180777, commonly known as niacin, is known to naturally occur in various plants at levels as high as several hundred parts per million. Therefore, it would not be unexpected to find the analyte present in control soil. No residues of CGA-180777 were observed in any of the method blank 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]. 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 and QA verification has been performed.

IV. CONCLUSION

The results of these validation experiments indicate that Analytical Method AG-660 is an accurate method for the determination of CGA-180777 in soil.

V. CERTIFICATION

This report and experimental results included in this study, laboratory Project I.D. AG-660, are certified to be authentic accounts of the experiments.

9-9-96
Date

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VI. CERTIFICATION OF GOOD LABORATORY PRACTICES

The analytical work reported in AG-660 was performed in accordance with Good Laboratory Practice Standards, 40 CFR Part 160.

9-9-96
Date

John D. Vargo
John D. Vargo, Ph.D.
Study Director

9/4/96
Date

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VII. QUALITY ASSURANCE STATEMENT

Report Title: ANALYTICAL METHOD FOR THE DETERMINATION OF CGA-180777, A METABOLITE OF CGA-215944, IN SOIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH UV AND MS DETECTION INCLUDING VALIDATION DATA

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

Method Number: AG-660

Ciba Study Number: 285-96 and 1 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	7/22/96	7/22/96
In-Progress Inspection	8/6/96	8/6/96
Final Report Audit	9/3/96	9/3/96

Prepared by: Teresa S Cox Date: 9/3/96

Teresa S. Cox
Senior Quality Assurance Auditor
Quality Assurance Unit
Ciba Crop Protection
Ciba-Geigy Corporation

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
Soil Moisture Percent	6.0	9.9	11.3

The soil samples were collected and the soil characterized under protocol numbers: 189-95 for New York, 190-95 for California, and 191-95 for Georgia. Soil moisture percentages were obtained under protocol 285-96.

TABLE II. HPLC SYSTEM AND OPERATING CONDITIONS

Instrumentation:

Perkin-Elmer Model Series 4 Gradient Pump
Perkin-Elmer Model ISS 200 Autosampler
Eppendorf Model CH-30 Column Heater
Perkin-Elmer Model LC-95 UV Detector

Operating Conditions:

Column Heater: 30°C
Detection:
Absorbance: 265 nm, rise time = 100 msec
Mass Spectrometry: see Table II
Injection Volume: 50 µL
Mobile Phase Flow Rate: 1.5 ml/min
Column: Zorbax SCX (Mac-Mod Analytical, Inc.), 15 cm x 4.6 mm, dp = 5 µm, equipped with an Upchurch (#A-318) pre-column filter (0.5 µm), or equivalent
Mobile Phase: 25/75% methanol/water (0.1% acetic acid, pH adjusted to 3.25 ± 0.05)

Total Run Time: 12 min.

Analyte Retention Time: 4.5 min

Data Collection and Processing: Lab Systems Multichrom, version 2.0 and Ciba Worksheet, version 1.6.1.

TABLE III. MASS SPECTROMETRY SYSTEM AND OPERATING CONDITIONS

Instrumentation:

PE Sciex API-I Mass Spectrometer
PE Sciex API-III+ Mass Spectrometer (operated in MS mode)
Ionspray 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
Curtain Gas Flow: 1.0 L/min
Nebulizer Gas Flow: 0.7 L/min
Ionspray Split Ratio: Approximately 50 µL/min delivered through the interface to the mass spectrometer

HPLC equipment and conditions: See Table I

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

Typical State File Values

	API-I PPG	API-I Analyte	API-III+ Q1 PPG	API-III+ Q1 Analyte
ISV	5000	4800	4000	4500
IN	650	650	650	650
OR	35	55	35	55
R0	30	30	30	30
M1	200	200	1000	150
RE1	120.0	119.5	120.7	125
DM1	0.07	0.10	0.08	0.16
R1	28.0	28.0	26.0	26.5
L7	*	*	-40	10
R2	*	*	-45	15
M3	*	*	1000	150
RE3	*	*	124.6	122.5
DM3	*	*	0.13	0.07
RX	*	*	-10	5
R3	*	*	-70	5
L9	-250	-250	-250	-250
FP	-250	-250	-250	-250
MU	-3400	-3400	-4100	-4100
CC	10	10	10	10

* Value is applicable to API-III+ instrument only.

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

RAD Acquisition Parameters

Mode	Duration	ADC's	Threshold
Profile	10.0	None	0 Counts

Period Name	Scan Type	State File
Period 1	Q1MI	180777

Delay	Acquire	Scan Rate	Dwell Time	Pause Time
0.00	10.0	1.00	499.98	0.02

Mass	Width	Defect
124.0	0.0	0.0

TABLE IV. CALIBRATION AND RECOVERY DATA FOR FORTIFIED CALIFORNIA SOIL: LC/UV ANALYSIS

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)	20.00	2.0	0.500	-	0	< 2.5	< 10	-
VAL2	0 (control)	20.02	2.0	0.501	-	0	< 2.5	< 10	-
VAL3	10	20.02	2.0	0.501	4.56	5442	4.29	8.57	86
VAL4	10	19.97	2.0	0.499	4.50	4966	3.90	7.81	78
VAL5	10	19.98	2.0	0.500	4.57	5689	4.50	9.00	90
VAL6	10	20.01	2.0	0.500	4.54	5720	4.52	9.04	90
VAL7	100	20.02	5.0	0.200	4.55	19691	16.0	80.0	80
VAL8	100	20.02	5.0	0.200	4.56	19895	16.2	80.9	81
VAL9	1000	20.00	50	0.020	4.56	19900	16.2	810	81
VAL10	1000	19.99	50	0.020	4.55	19945	16.2	812	81

These values are common to all samples and are used in the calculations detailed in Section II.K.3.0
m = % moisture = 6.0%
Ve = volume of extraction solvent used = 150 mL
Va = aliquot volume of extract used for cleanup = 150 mL + (0.060 x grams extr.)
Vi = HPLC injection volume = 0.05 mL

Calibration Standards

Concentration (ng/µL)	Injection Volume (µL)	Amount Inj.(ng)	Retention Time (min)	Peak Area	Amount Found (ng)	
5ppb 0.05	50	2.5	4.52	2920	2.22	slope = 1214.35
10ppb 0.1	50	5	4.55	5966	4.72	Y-intercept = 230.703
0.25	50	12.5	4.55	16225	13.2	corr. coeff. = 0.99979
0.5	50	25	4.53	30630	25.0	
100ppb 1.0	50	50	4.53	60775	49.9	

*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: LC/MS ANALYSIS

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)	20.00	2.0	0.500	-	0	< 2.5	< 10	-
VAL2	0 (control)	20.02	2.0	0.501	4.58	7877	< 2.5	< 10	-
VAL3	10	20.02	2.0	0.501	4.59	671749	3.84	7.67	77
VAL4	10	19.97	2.0	0.499	4.49	634343	3.53	7.07	71
VAL5	10	19.98	2.0	0.500	4.56	616552	3.38	6.77	68
VAL6	10	20.01	2.0	0.500	4.54	646010	3.62	7.24	72
VAL7	100	20.02	5.0	0.200	4.56	2266860	17.0	85.1	85
VAL8	100	20.02	5.0	0.200	4.56	2214498	16.6	83.0	83
VAL9	1000	20.00	50	0.020	4.56	2188134	16.4	819	82
VAL10	1000	19.99	50	0.020	4.56	2133792	15.9	797	80

These values are common to all samples and are used in the calculations detailed in Section II.K.3.0
m = % moisture = 6.0%
Ve = volume of extraction solvent used = 150 mL
Va = aliquot volume of extract used for cleanup = 150 mL + (0.060 x grams extr.)
Vi = HPLC injection volume = 0.05 mL

Calibration Standards

Concentration (ng/µL)	Injection Volume (µL)	Amount Inj.(ng)	Retention Time (min)	Peak Area	Amount Found (ng)	
0.05	50	2.5	4.54	420663	1.76	slope = 120804.64643
0.1	50	5	4.59	809674	4.98	Y-intercept = 208245.1177
0.25	50	12.5	4.58	1786216	13.1	corr. coeff. = 0.999538
0.5	50	25	4.56	3301390	25.6	
1.0	50	50	4.56	6199724	49.6	

*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: LC/UV ANALYSIS

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
VAL11	0 (blank)	20.00	2.0	0.500	-	0	< 2.5	< 10	-
VAL12	0 (control)	20.04	2.0	0.501	-	0	< 2.5	< 10	-
VAL13	10	19.97	2.0	0.499	4.52	4195	3.34	6.69	67
VAL14	10	20.02	2.0	0.501	4.54	4408	3.51	7.02	70
VAL15	10	19.99	2.0	0.500	4.51	4813	3.85	7.70	77
VAL16	10	20.05	2.0	0.501	4.50	3069	2.42	4.82	48
VAL17	100	20.02	5	0.200	4.45	18022	14.7	73.2	73
VAL18	100	19.99	5	0.200	4.48	17824	14.5	72.5	72
VAL19	1000	20.01	50	0.020	4.49	18650	15.2	758	76
VAL20	1000	20.02	50	0.020	4.49	18167	14.8	738	74

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

m = % moisture = 9.9%
Ve = volume of extraction solvent used = 150 mL
Va = aliquot volume of extract used for cleanup = 150 mL + (0.099 x grams extr.)
Vi = HPLC injection volume = 0.05 mL

Calibration Standards

Concentration (ng/µL)	Injection Volume (µL)	Amount Inj.(ng)	Retention Time (min)	Peak Area	Amount Found (ng)	
0.05	50	2.5	4.45	3138	2.47	slope = 114.422
0.1	50	5	4.45	6766	5.44	Y-intercept = 1222.15
0.25	50	12.5	4.45	14729	12.0	corr. coeff. = 0.99983
0.5	50	25	4.46	30750	25.1	
1.0	50	50	4.49	61294	50.1	

*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 VII. CALIBRATION AND RECOVERY DATA FOR FORTIFIED GEORGIA SOIL: LC/MS ANALYSIS

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
VAL11	0 (blank)	20.00	2.0	0.500	-	0	< 2.5	< 10	-
VAL12	0 (control)	20.04	2.0	0.501	4.47	17424	< 2.5	< 10	-
VAL13	10	19.97	2.0	0.499	4.41	412905	3.41	6.82	68
VAL14	10	20.02	2.0	0.501	4.42	428122	3.59	7.17	72
VAL15	10	19.99	2.0	0.500	4.37	445447	3.80	7.60	76
VAL16	10	20.05	2.0	0.501	4.44	206502	0.92	1.83	18
VAL17	100	20.02	5	0.200	4.46	1474925	16.2	80.9	81
VAL18	100	19.99	5	0.200	4.44	1412136	15.4	77.3	77
VAL19	1000	20.01	50	0.020	4.47	1506037	16.6	828	83
VAL20	1000	20.02	50	0.020	4.42	1412404	15.4	772	77

These values are common to all samples and are used in the calculations detailed in Section II.K.3.0
m = % moisture = 9.9%
Ve = volume of extraction solvent used = 150 mL
Va = aliquot volume of extract used for cleanup = 150 mL + (0.099 x grams extr.)
Vi = HPLC injection volume = 0.05 mL

Calibration Standards

Concentration (ng/µL)	Injection Volume (µL)	Amount Inj.(ng)	Retention Time (min)	Peak Area	Amount Found (ng)	
0.05	50	2.5	4.51	299591	2.04	slope = 82989.3466
0.1	50	5	4.44	544077	4.99	Y-intercept = 130270.4146
0.25	50	12.5	4.49	1213895	13.1	corr. coeff. = 0.999818
0.5	50	25	4.44	2210160	25.1	
1.0	50	50	4.44	4267617	49.9	

*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 VIII. CALIBRATION AND RECOVERY DATA FOR FORTIFIED NEW YORK SOIL: LC/UV ANALYSIS

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
VAL21	0 (blank)	20.00	2.0	0.500	-	0	< 2.5	< 10	-
VAL22	0 (control)	20.00	2.0	0.500	5.17	648	< 2.5 (0.094)	< 10 (0.19)	-
VAL23	10	20.00	2.0	0.500	5.11	5199	3.95	7.89	77
VAL24	10	20.00	2.0	0.500	5.17	5661	4.34	8.67	85
VAL25	10	20.00	2.0	0.500	5.16	7285	5.71	11.4	112
VAL26	10	20.00	2.0	0.500	5.14	6409	4.97	9.94	97
VAL27	100	20.00	5	0.200	5.16	18888	15.5	77.6	77
VAL28	100	19.99	5	0.200	5.16	19716	16.2	81.2	81
VAL29	1000	20.00	50	0.020	5.19	19235	15.8	791	79
VAL30	1000	20.00	50	0.020	5.16	18610	15.3	765	76

These values are common to all samples and are used in the calculations detailed in Section II.K.3.0
m = % moisture = 11.3%
Ve = volume of extraction solvent used = 150 mL
Va = aliquot volume of extract used for cleanup = 150 mL + (0.113 x grams extr.)
Vi = HPLC injection volume = 0.05 mL

Calibration Standards

Concentration (ng/µL)	Injection Volume (µL)	Amount Inj.(ng)	Retention Time (min)	Peak Area	Amount Found (ng)	
0.05	50	2.5	5.12	3231	2.28	slope = 536.592
0.1	50	5	5.17	6881	5.37	Y-intercept = 1181.94
0.25	50	12.5	5.17	15237	12.4	corr. coeff. = 0.99993
0.5	50	25	5.16	29910	24.9	
1.0	50	50	5.18	59710	50.1	

*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 IX. CALIBRATION AND RECOVERY DATA FOR FORTIFIED NEW YORK SOIL: LC/MS ANALYSIS

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
VAL21	0 (blank)	20.00	2.0	0.500	-	0	< 2.5	< 10	-
VAL22	0 (control)	20.00	2.0	0.500	5.38	35527	< 2.5	< 10	-
VAL23	10	20.00	2.0	0.500	5.38	175607	4.40	8.80	88
VAL24	10	20.00	2.0	0.500	5.43	162205	3.96	7.93	79
VAL25	10	20.00	2.0	0.500	5.41	226735	6.07	12.1	121
VAL26	10	20.00	2.0	0.500	5.45	170231	4.23	8.45	85
VAL27	100	20.00	5	0.200	5.48	567723	17.2	85.9	86
VAL28	100	19.99	5	0.200	5.38	602432	18.3	91.6	92
VAL29	1000	20.00	50	0.020	5.53	550077	16.6	831	83
VAL30	1000	20.00	50	0.020	5.48	552991	16.7	835	84

These values are common to all samples and are used in the calculations detailed in Section II.K.3.0
m = % moisture = 11.3%
Ve = volume of extraction solvent used = 150 mL
Va = aliquot volume of extract used for cleanup = 150 mL + (0.113 x grams extr.)
Vi = HPLC injection volume = 0.05 mL

Calibration Standards

Concentration (ng/µL)	Injection Volume (µL)	Amount Inj.(ng)	Retention Time (min)	Peak Area	Amount Found (ng)	
0.05	50	2.5	5.31	106677	2.15	slope = 30667.17678
0.1	50	5	5.45	201676	5.25	Y-intercept = 40661.4411
0.25	50	12.5	5.43	423251	12.5	corr. coeff. = 0.999914
0.5	50	25	5.43	814884	25.2	
1.0	50	50	5.41	1570201	49.9	

*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 X. SUMMARY DATA FOR FORTIFIED SOIL: LC/UV ANALYSES

Fortification Level (ppb)	California	Georgia	New York
10	86	67	77
10	78	70	85
10	90	77	112
10	90	48*	97
100	80	73	77
100	81	72	81
1000	81	76	79
1000	81	74	76
Average	83.4	72.7	85.5
Standard Deviation	4.7	3.5	12.7
% Relative Std. Dev.	5.6	4.7	14.9

* Recovery not used in statistical calculations.
Uncharacteristic low recovery for unknown reason.

Pooled Recovery Data for all Soils by Fortification Level

	10 ppb	100 ppb	1000 ppb
Average	84.5	77.3	77.8
Standard Deviation	12.8	4.0	2.9
% Relative Std. Dev.	15.2	5.2	3.8
Range	67 - 112	72 - 81	74 - 81
Number of Samples	11	6	6

TABLE XI. SUMMARY DATA FOR FORTIFIED SOIL: LC/MS ANALYSES

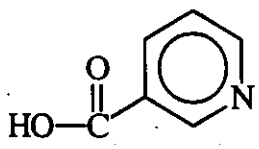
Fortification Level (ppb)	California	Georgia	New York
10	77	68	88
10	71	72	79
10	68	76	121
10	72	18*	85
100	85	81	86
100	83	77	92
1000	82	83	83
1000	80	77	84
Average	77.3	76.3	89.8
Standard Deviation	6.3	5.1	13.2
% Relative Std. Dev.	8.1	6.7	14.7

* Recovery not used in statistical calculations. Uncharacteristic low recovery for unknown reason.

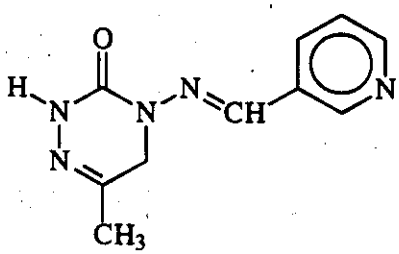
Pooled Recovery Data for all Soils by Fortification Level

	10 ppb	100 ppb	1000 ppb
Average	79.7	84.0	81.5
Standard Deviation	15.1	5.1	2.6
% Relative Std. Dev.	19.0	6.0	3.2
Range	68 - 121	77 - 92	77 - 84
Number of Samples	11	6	6

FIGURE 1. CHEMICAL NAMES AND STRUCTURES



CGA-180777
CAS Name: 3-Pyridinecarboxylic acid
CAS No.: 59-67-6
Average Molecular Weight: 123.1



CGA-215944
CAS Name: (E)-4,5-Dihydro-6-methyl-4-[(3-pyridinyl methylene)amino]-1,2,4-triazin-3(2H)-one
CAS No.: 123312-89-0
Average Molecular Weight: 217.2

FIGURE 2. AG-660 FLOW DIAGRAM FOR SOIL

Weigh 20 gram soil sample. Fortify, if necessary.
Add 100 mL of 1/9/90% ammonium hydroxide/water/methanol.
Extract by mechanical shaking for 30 min at room temperature.
Centrifuge and filter sample.
Repeat extraction with 50 mL of solvent.

⇓

Remove methanol from sample via rotary evaporation.
Acidify sample.
Pass through C18 SPE piggy-backed to SCX SPE.
Elute analyte from SCX SPE using 1/99% ammonium hydroxide/methanol.

⇓

Remove all solvent via rotary evaporation.
Dissolve residue with sample diluent.

⇓

Analyze

FIGURE 3. TYPICAL PPG MASS CALIBRATION TUNE

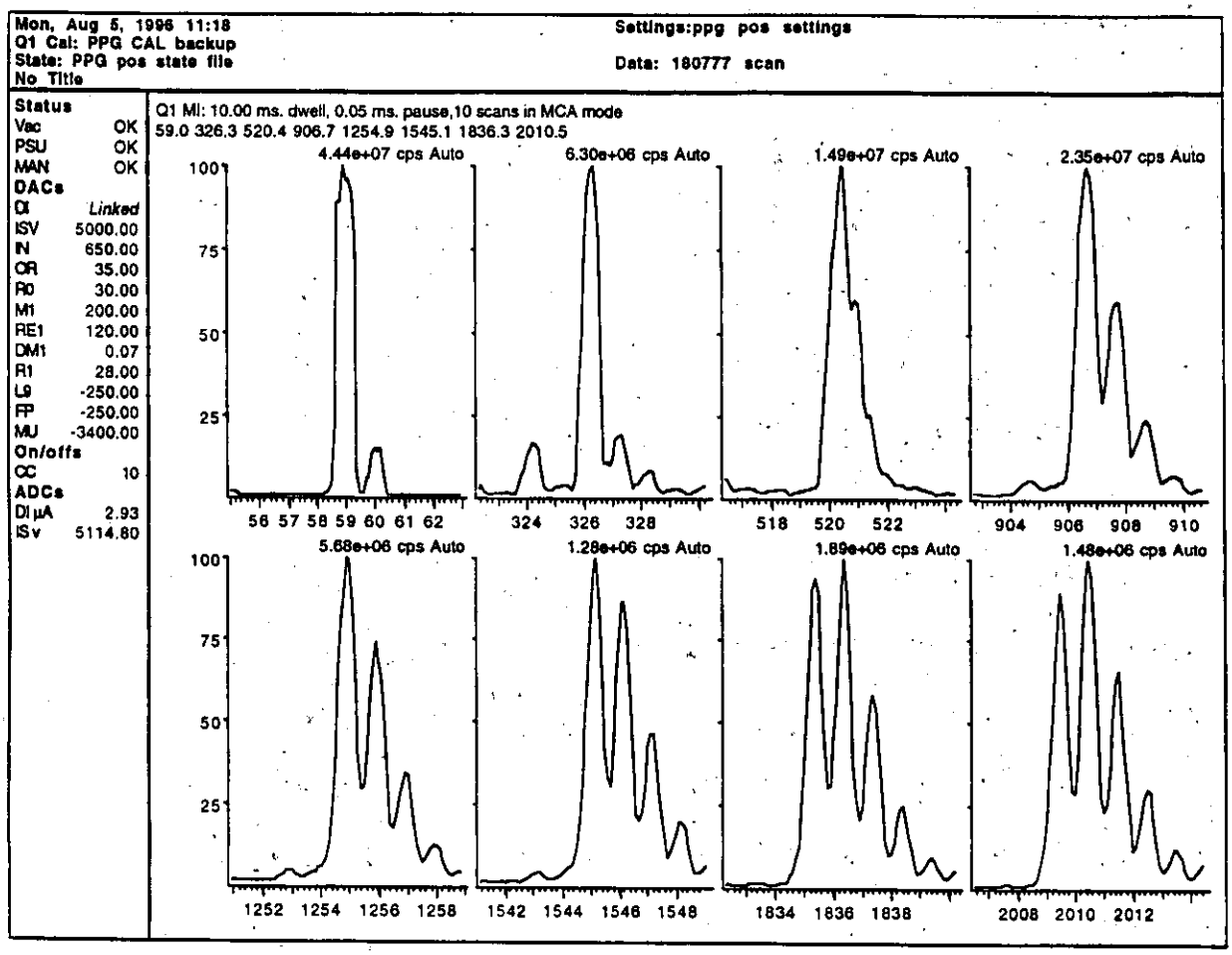


FIGURE 4. TYPICAL MASS SPECTRUM FOR CGA-180777

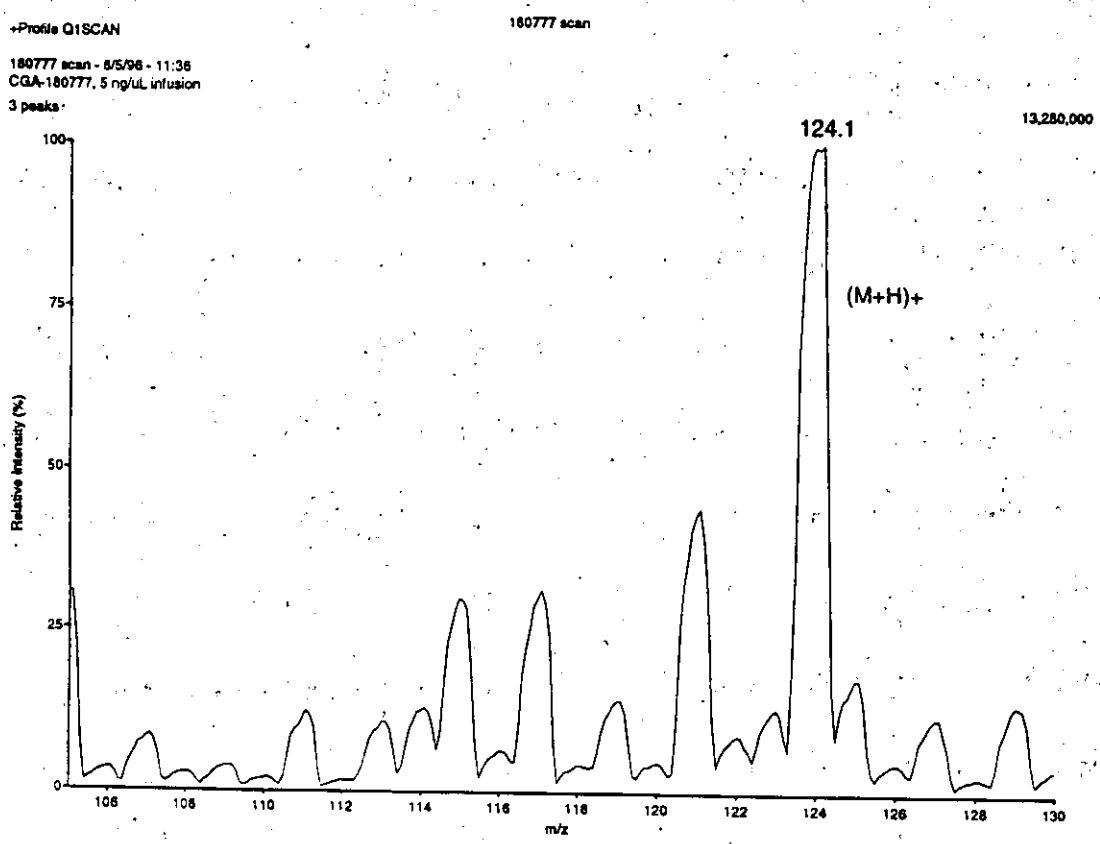
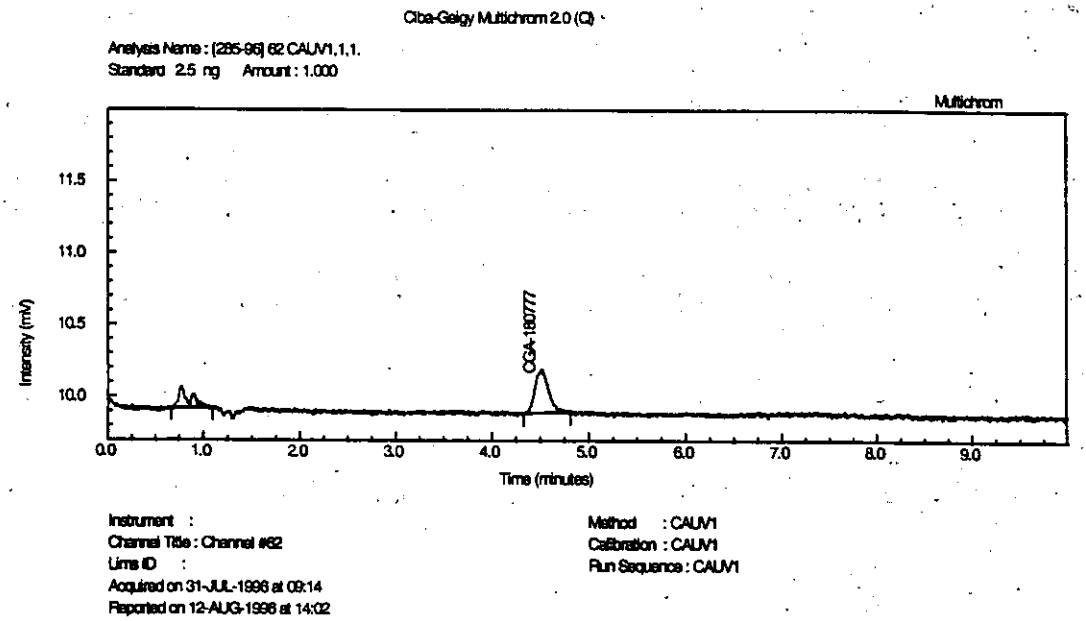
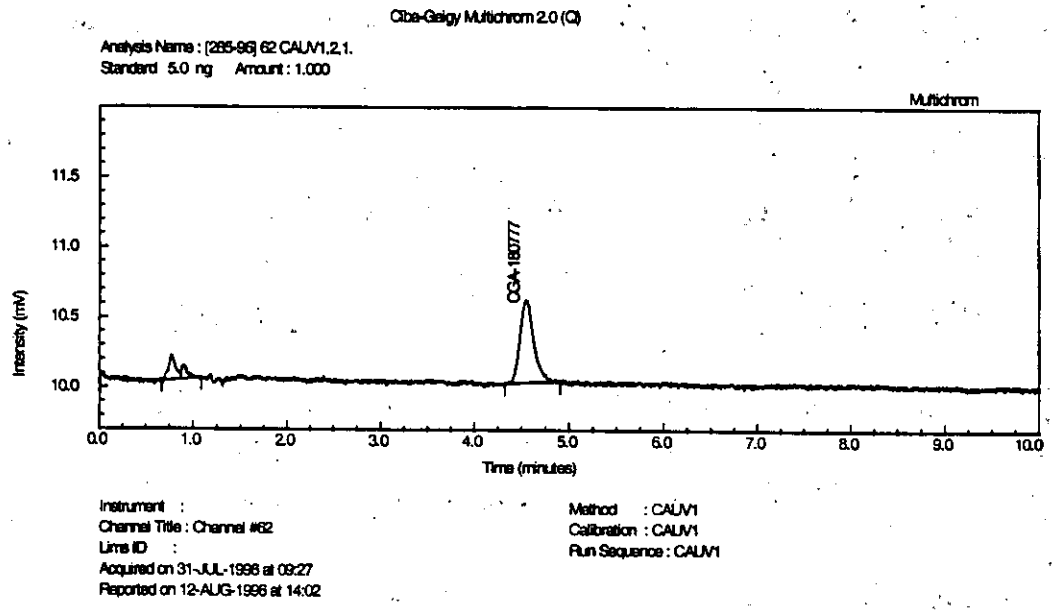


FIGURE 5. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS: LC/UV

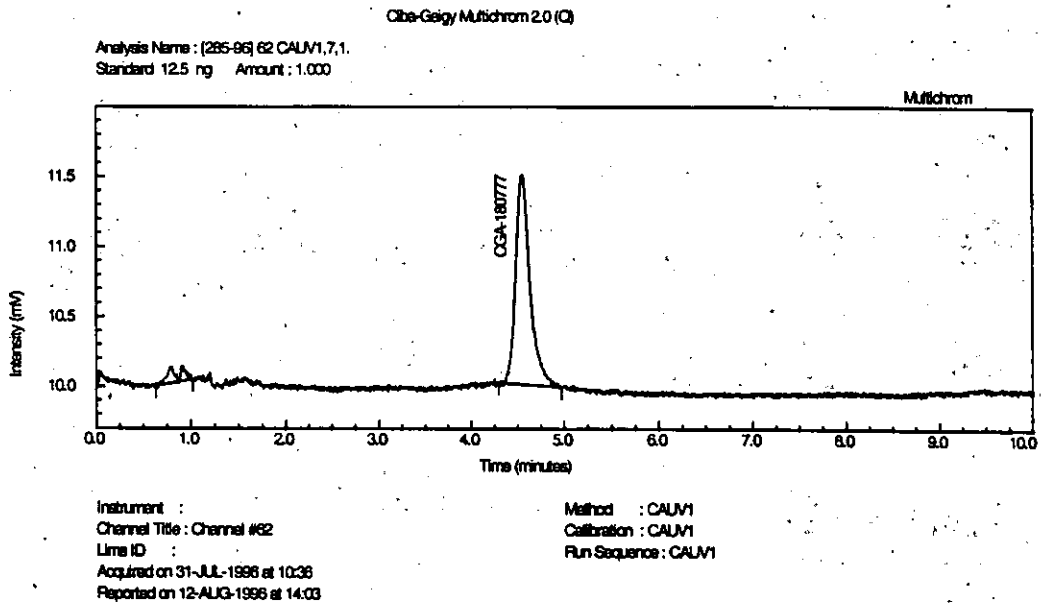


Standard: 2.5 ng injected

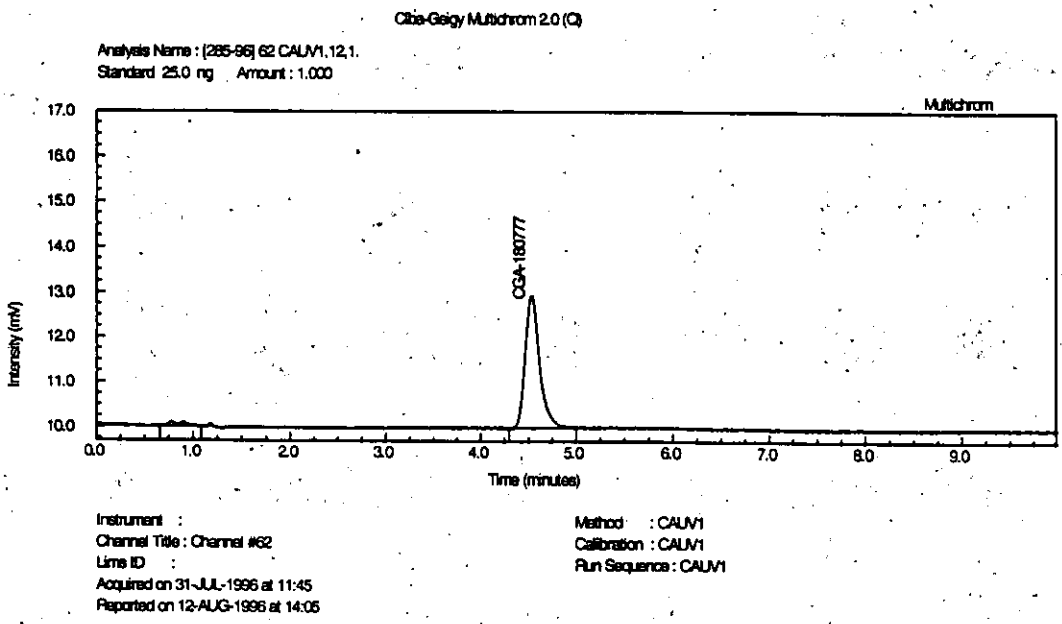


Standard: 5 ng injected

FIGURE 5. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS: LC/UV (Continued)

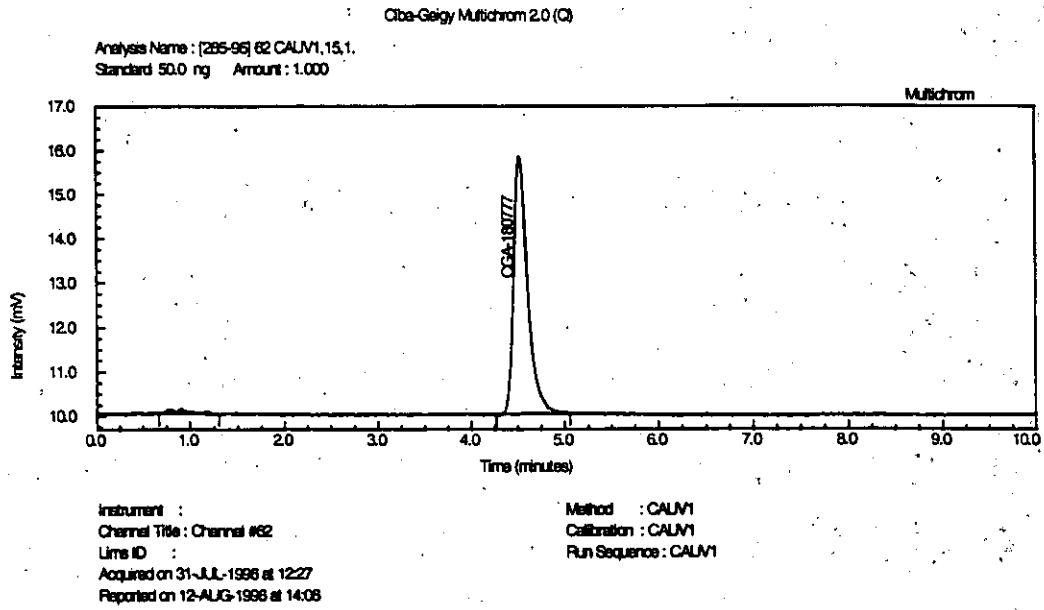


Standard: 12.5 ng injected



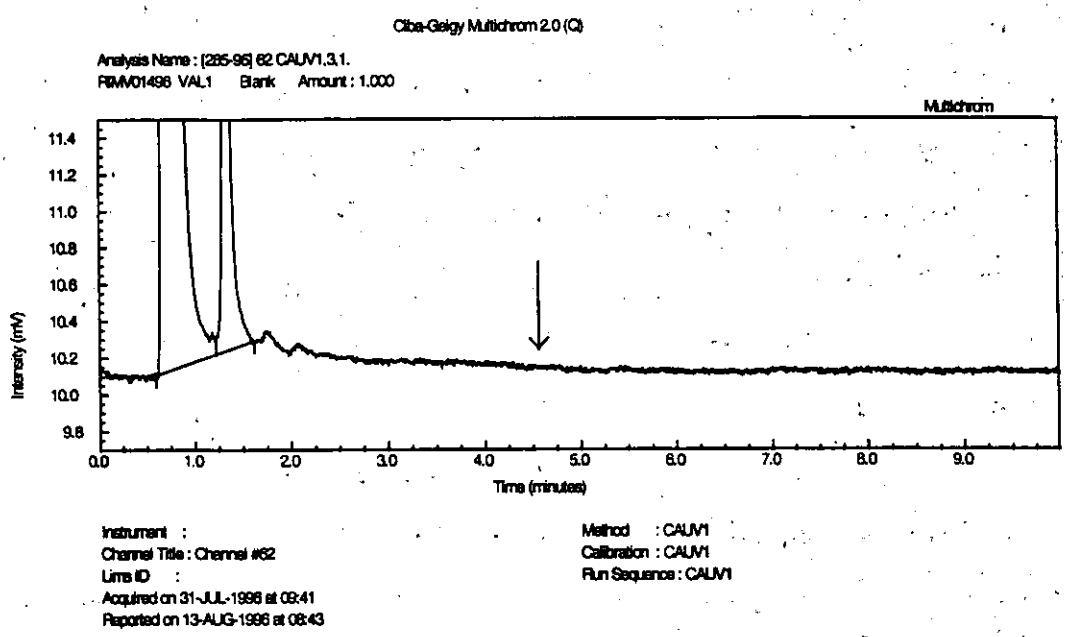
Standard: 25 ng injected

FIGURE 5. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS: LC/UV (Continued)

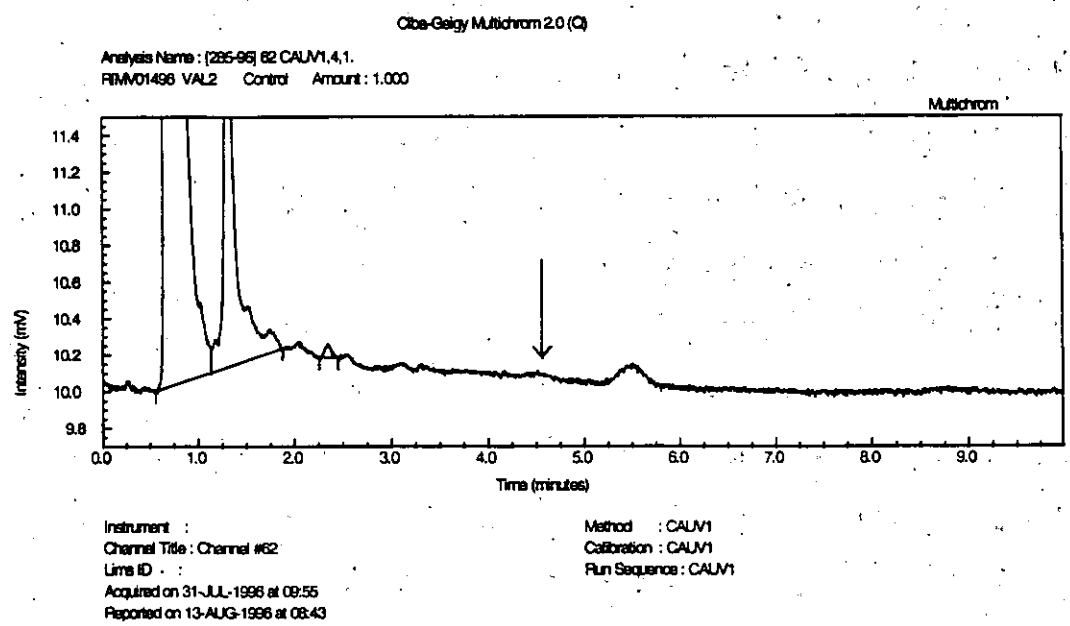


Standard: 50 ng injected

FIGURE 6. TYPICAL CHROMATOGRAMS FOR CONTROL AND FORTIFIED CALIFORNIA SOIL: LC/UV

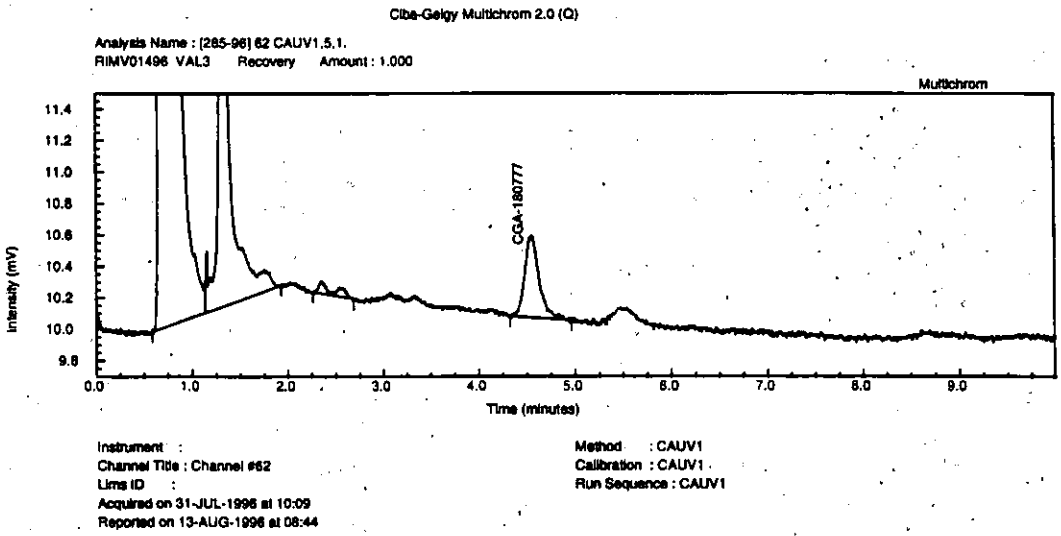


Sample Code: VAL1, Method Blank

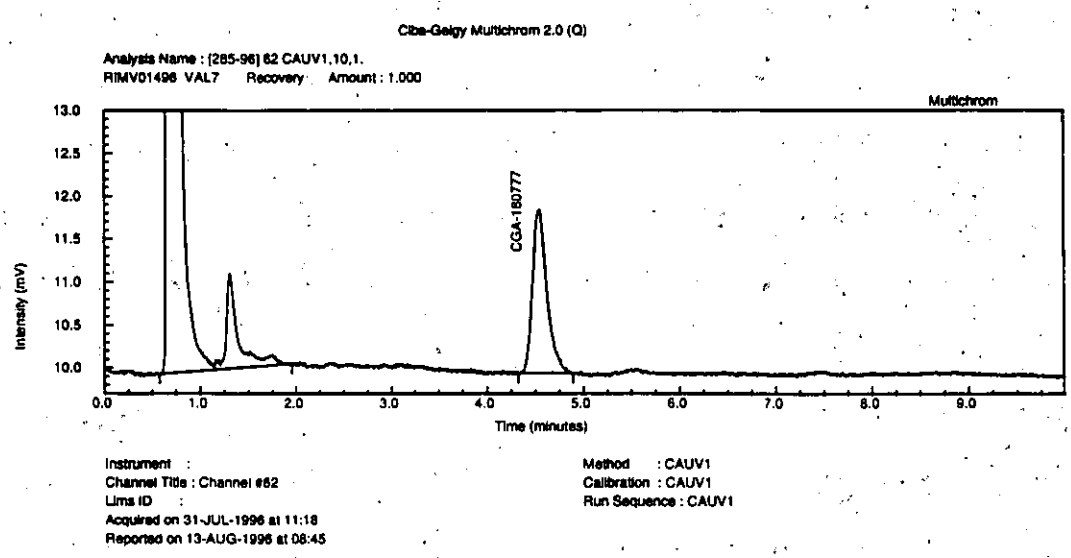


Sample Code: VAL2, Soil Control

FIGURE 6. TYPICAL CHROMATOGRAMS FOR CONTROL AND FORTIFIED CALIFORNIA SOIL: LC/UV (Continued)

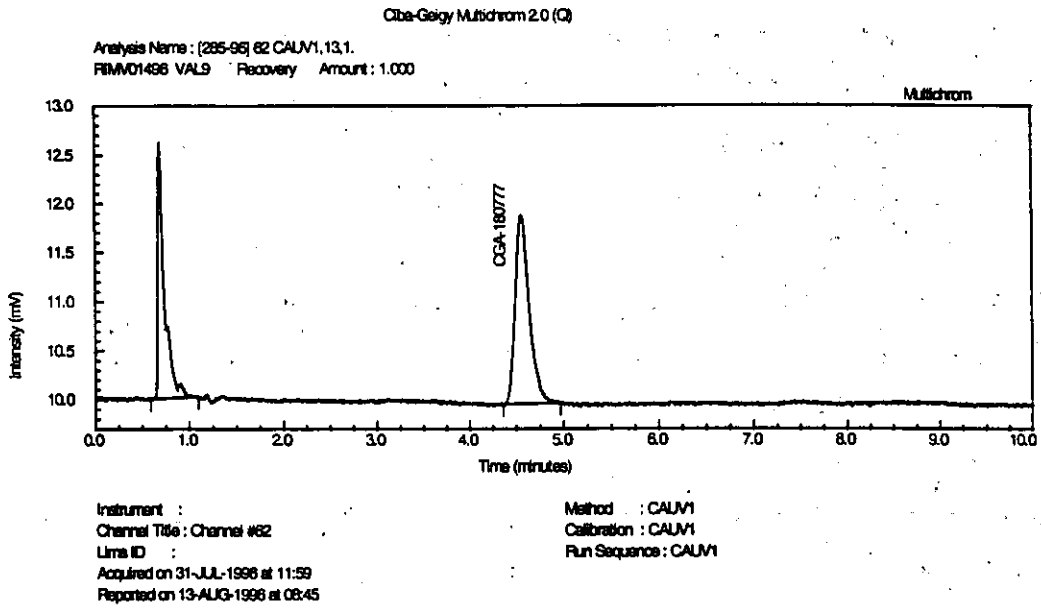


Sample Code: VAL3, Soil + 10 ppb



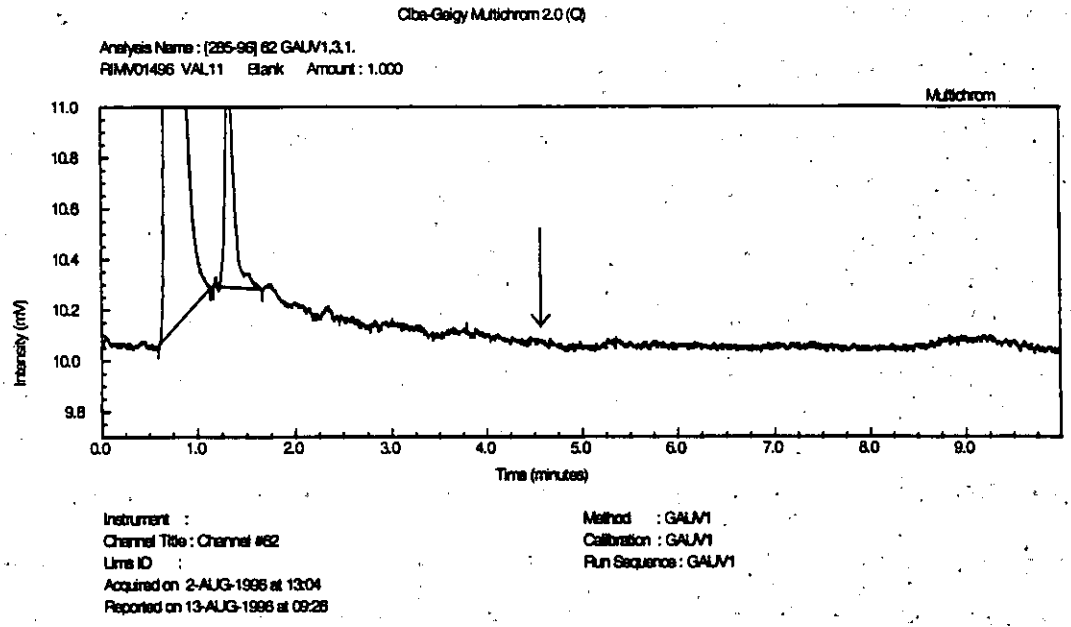
Sample Code: VAL7, Soil + 100 ppb

FIGURE 6. TYPICAL CHROMATOGRAMS FOR CONTROL AND FORTIFIED CALIFORNIA SOIL: LC/UV (Continued)

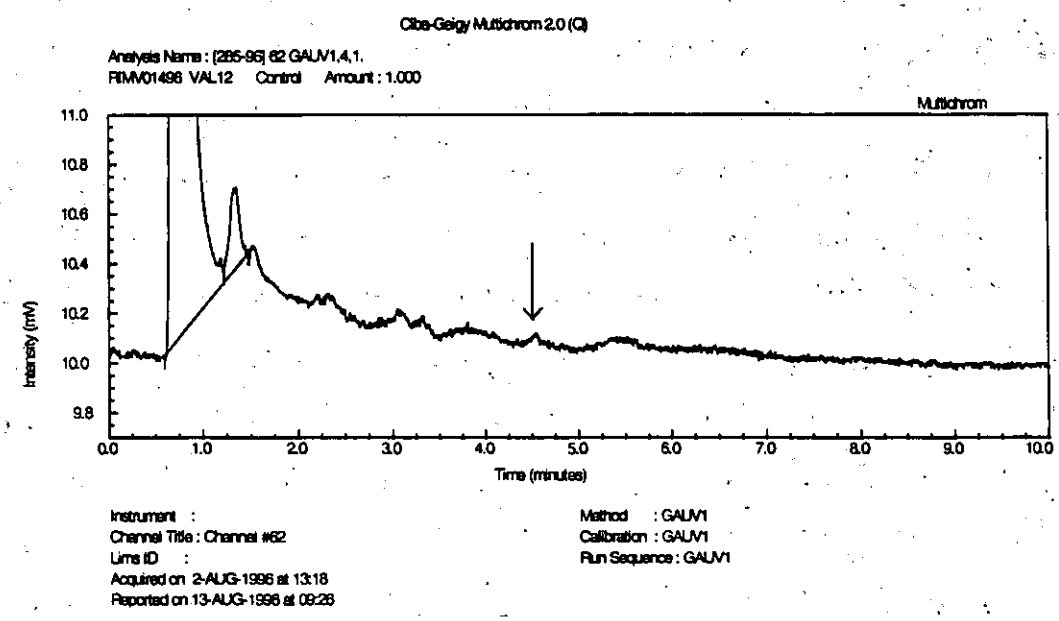


Sample Code: VAL9, Soil + 1000 ppb

FIGURE 7. TYPICAL CHROMATOGRAMS FOR CONTROL AND FORTIFIED GEORGIA SOIL: LC/UV

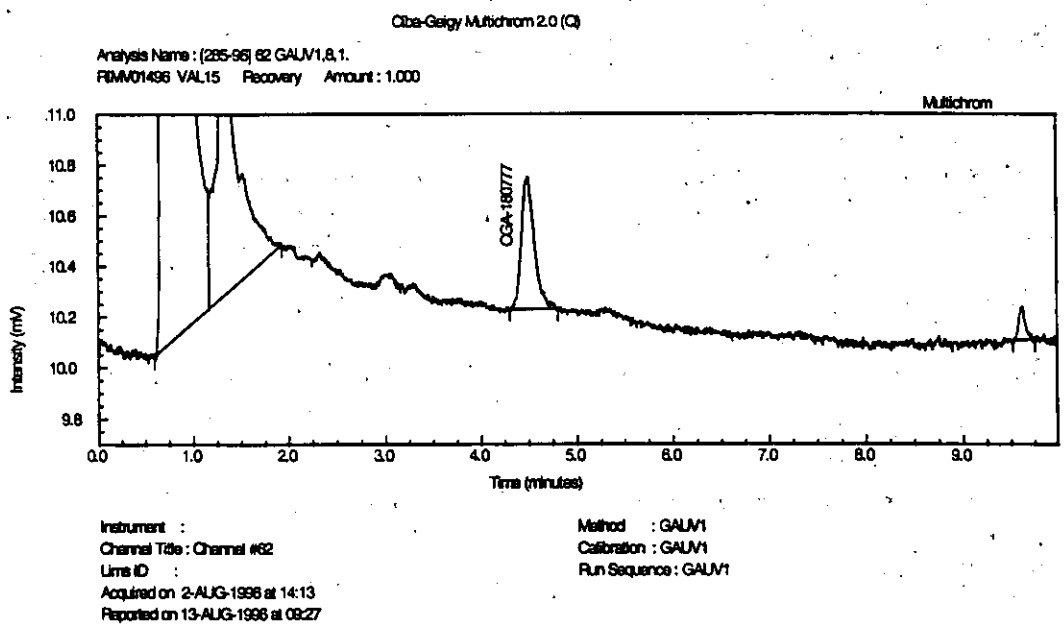


Sample Code: VAL11, Method Blank

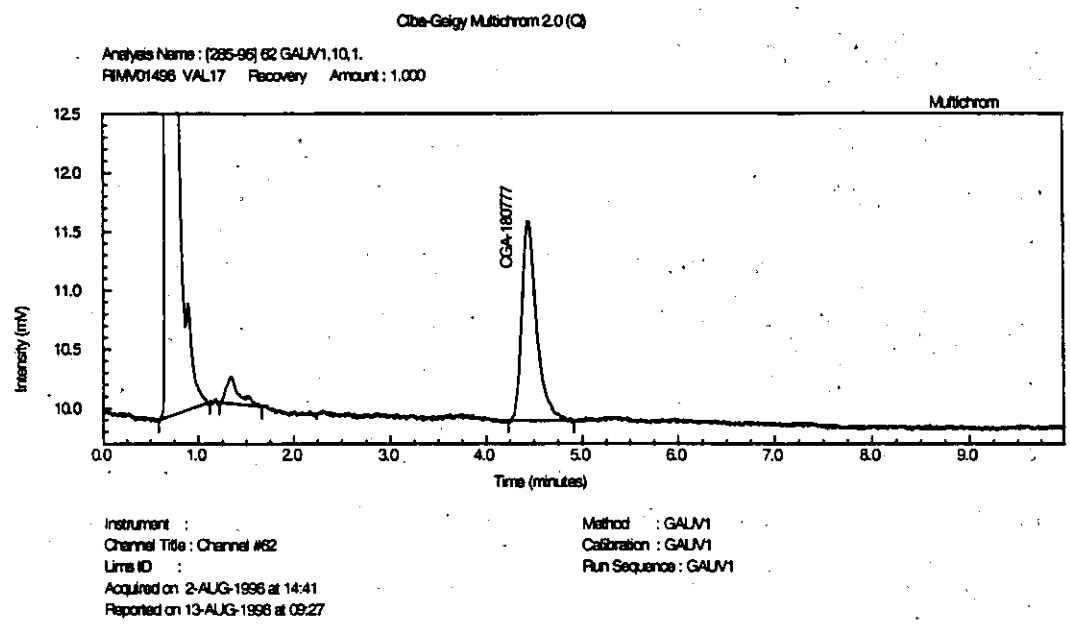


Sample Code: VAL12, Soil Control

FIGURE 7. TYPICAL CHROMATOGRAMS FOR CONTROL AND FORTIFIED GEORGIA SOIL: LC/UV (Continued)

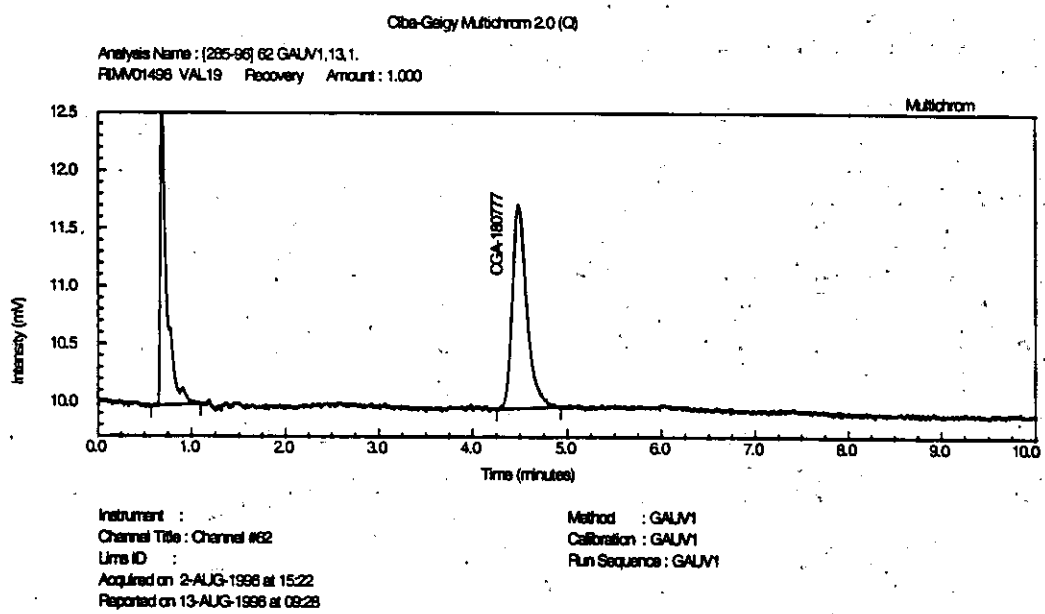


Sample Code: VAL15, Soil + 10 ppb



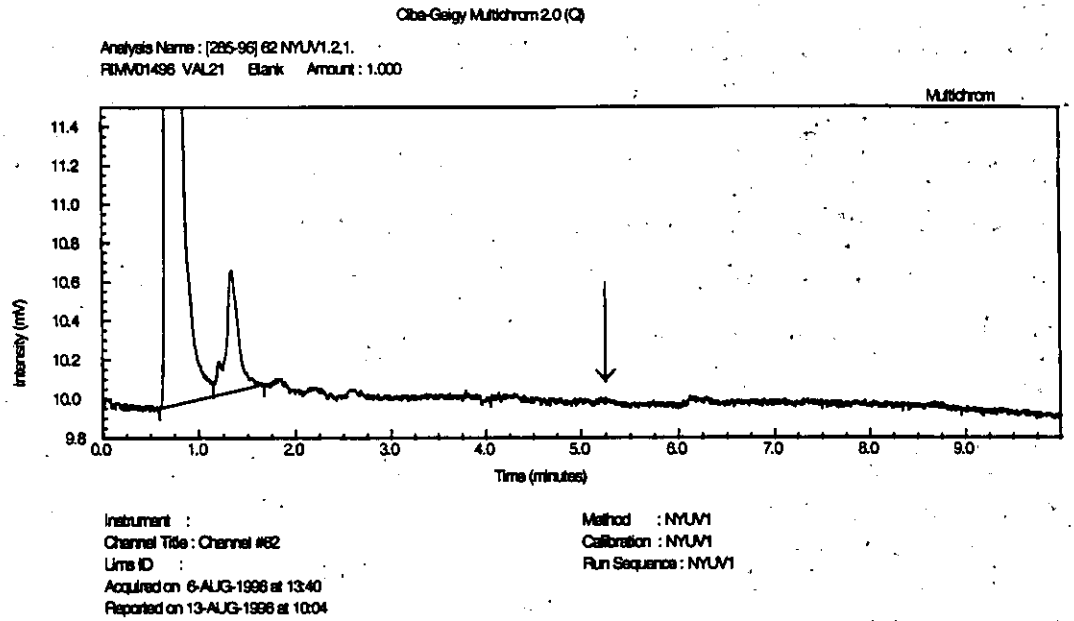
Sample Code: VAL17, Soil + 100 ppb

FIGURE 7. TYPICAL CHROMATOGRAMS FOR CONTROL AND FORTIFIED GEORGIA SOIL: LC/UV (Continued)

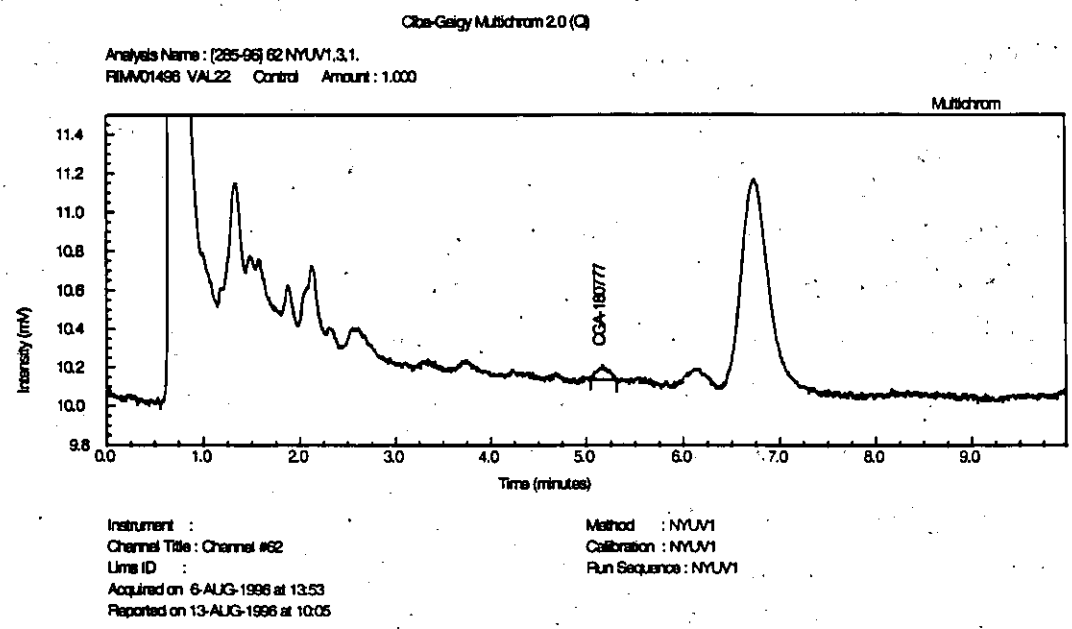


Sample Code: VAL19, Soil + 1000 ppb

FIGURE 8. TYPICAL CHROMATOGRAMS FOR CONTROL AND FORTIFIED NEW YORK SOIL: LC/UV

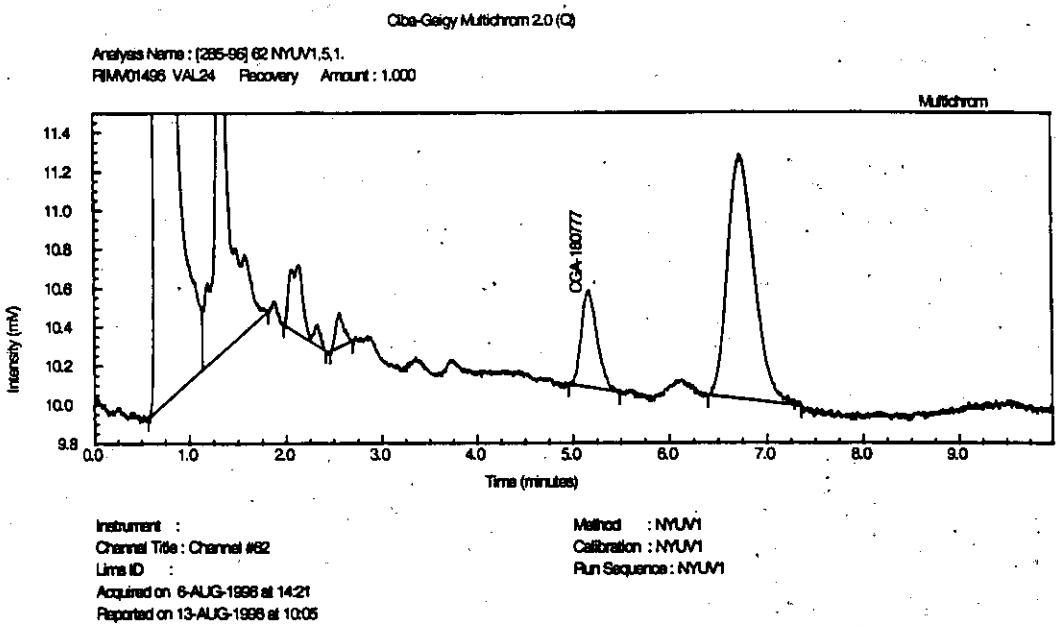


Sample Code: VAL21, Method Blank

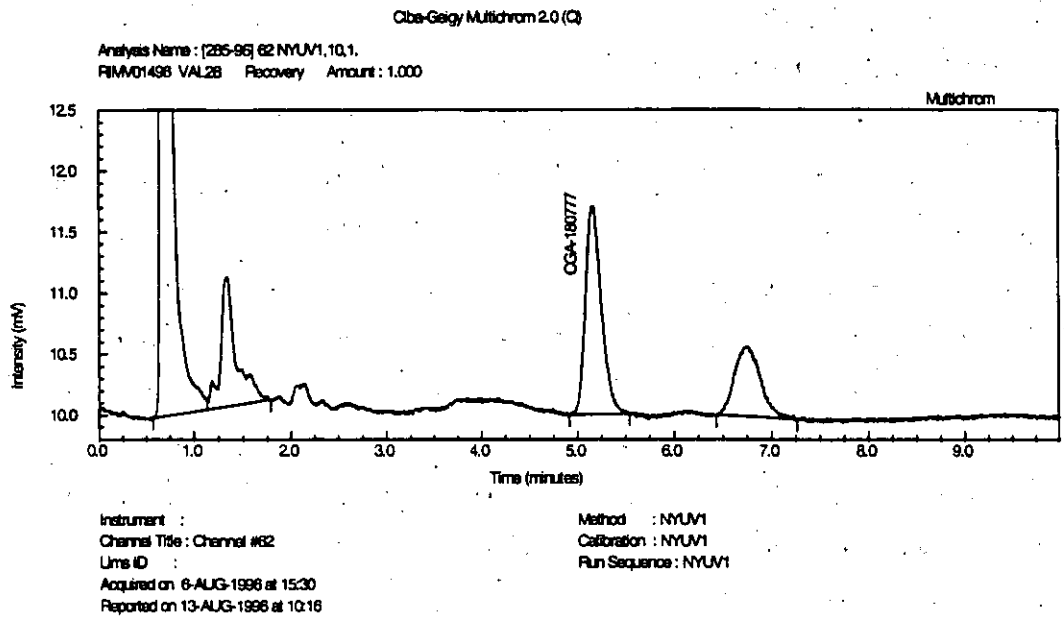


Sample Code: VAL22, Soil Control

FIGURE 8. TYPICAL CHROMATOGRAMS FOR CONTROL AND FORTIFIED NEW YORK SOIL: LC/UV (Continued)

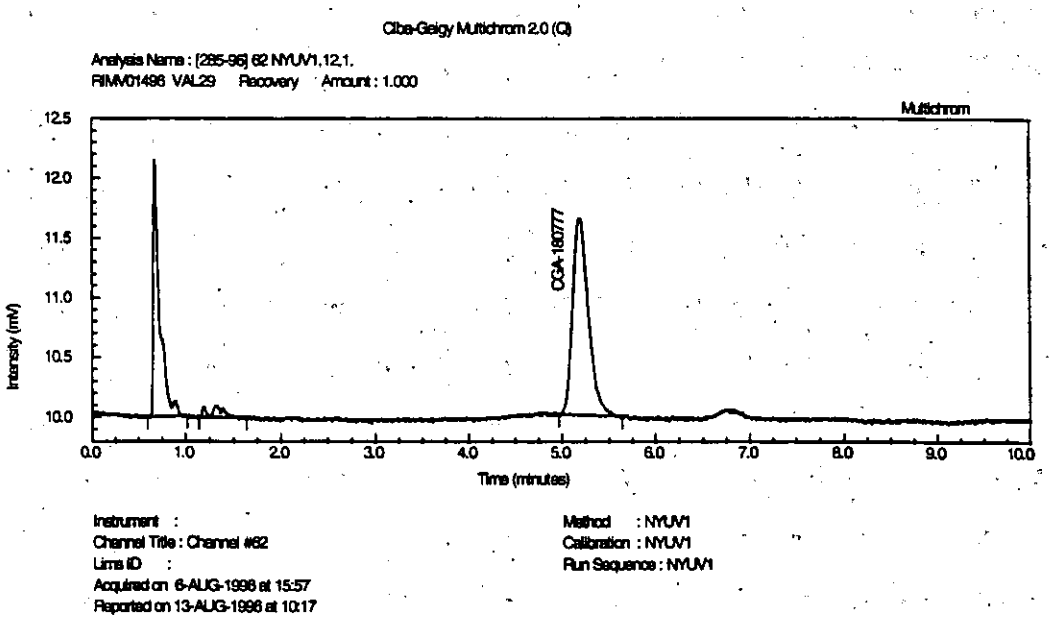


Sample Code: VAL24, Soil + 10 ppb



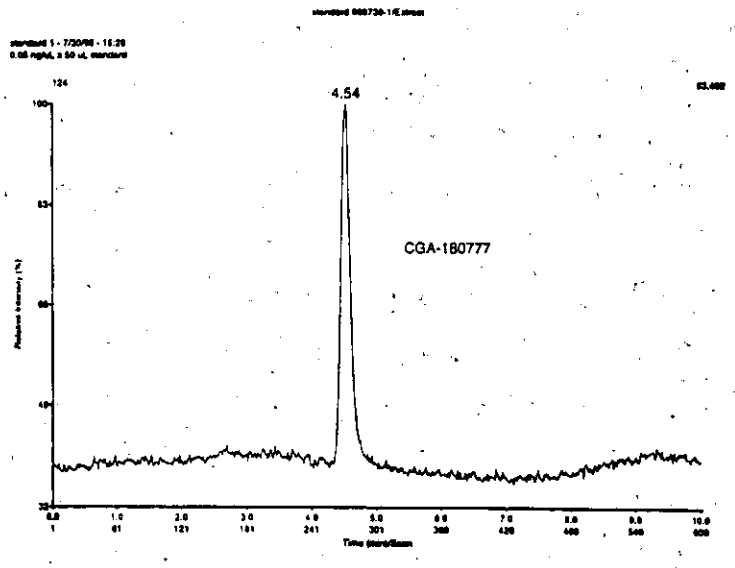
Sample Code: VAL28, Soil + 100 ppb

FIGURE 8. TYPICAL CHROMATOGRAMS FOR CONTROL AND FORTIFIED NEW YORK SOIL: LC/UV (Continued)

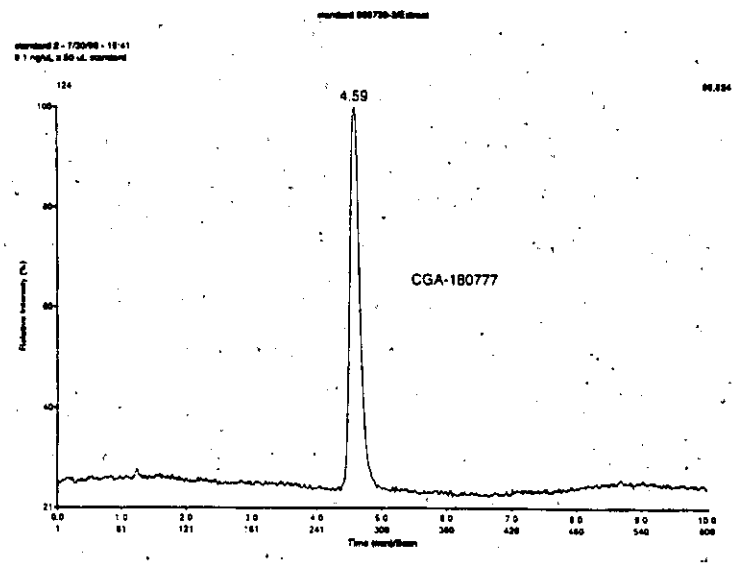


Sample Code: VAL29, Soil + 1000 ppb

FIGURE 9. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS: LC/MS

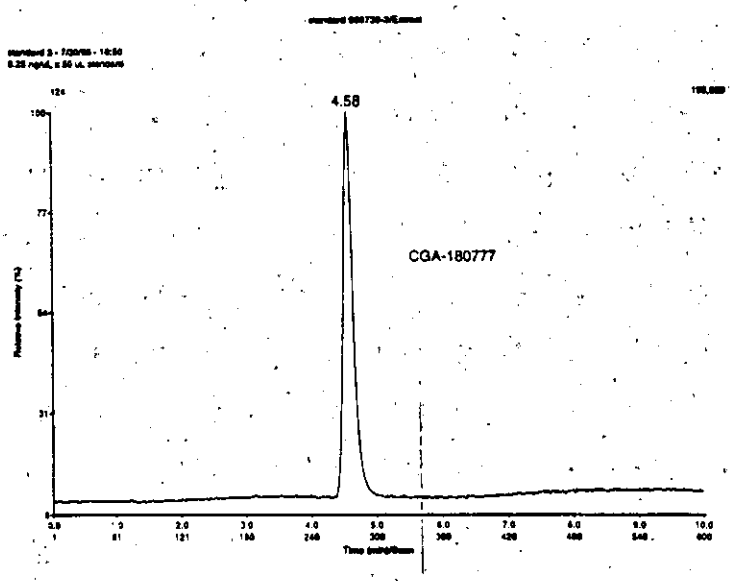


Standard: 2.5 ng injected

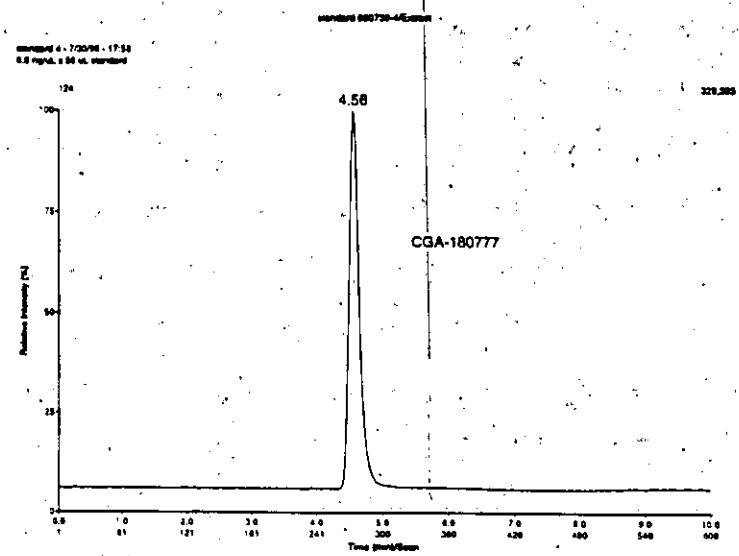


Standard: 5 ng injected

FIGURE 9. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS: LC/MS (Continued)

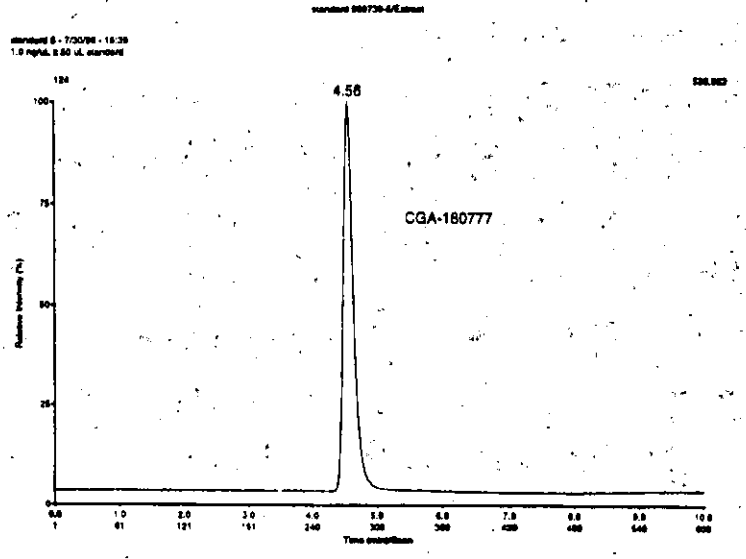


Standard: 12.5 ng injected



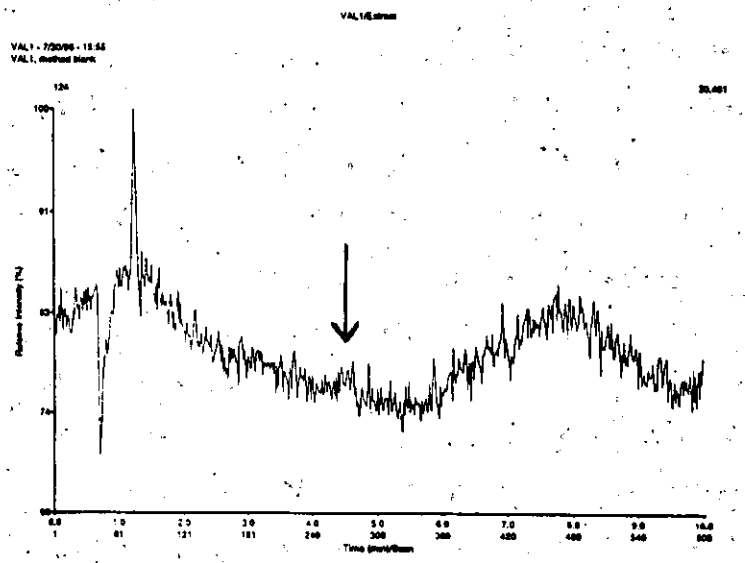
Standard: 25 ng injected

FIGURE 9. TYPICAL CHROMATOGRAMS FOR ANALYTICAL STANDARDS: LC/MS (Continued)

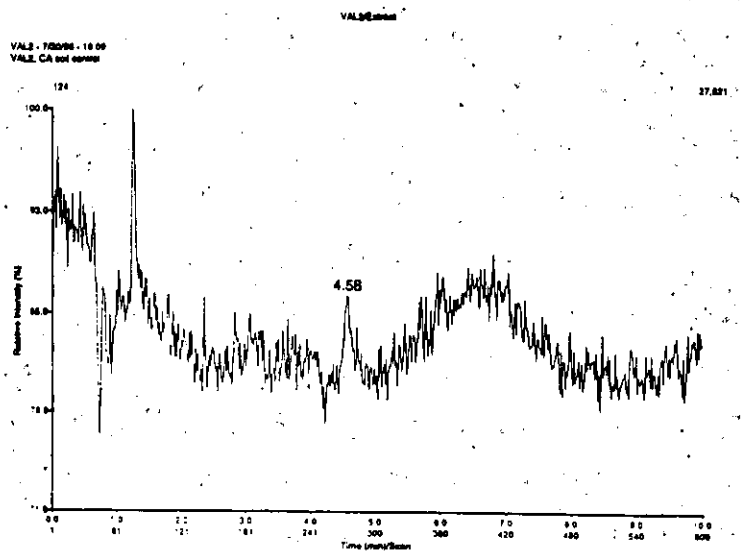


Standard: 50 ng injected

FIGURE 10. TYPICAL CHROMATOGRAMS FOR CONTROL AND FORTIFIED CALIFORNIA SOIL: LC/MS

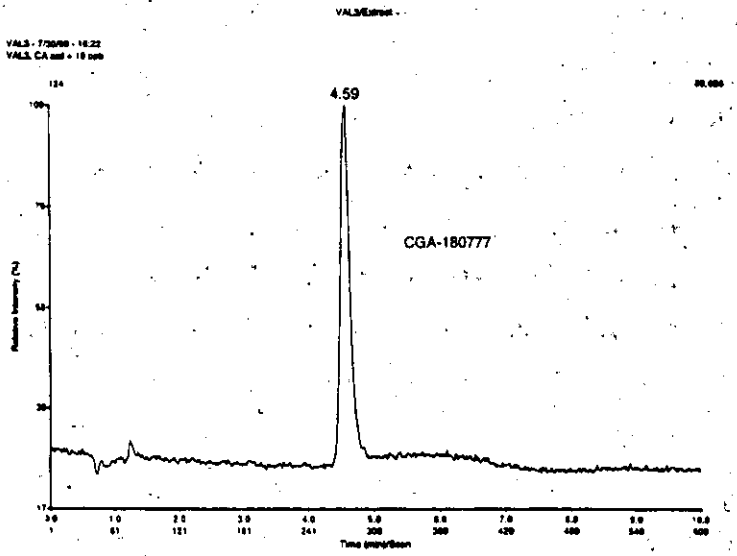


Sample Code: VAL1, Method Blank

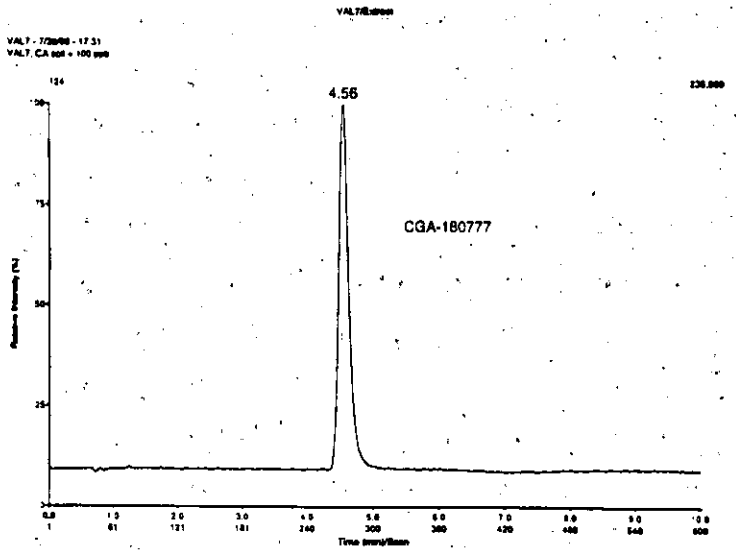


Sample Code: VAL2, Soil Control

FIGURE 10. TYPICAL CHROMATOGRAMS FOR CONTROL AND FORTIFIED CALIFORNIA SOIL: LC/MS (Continued)

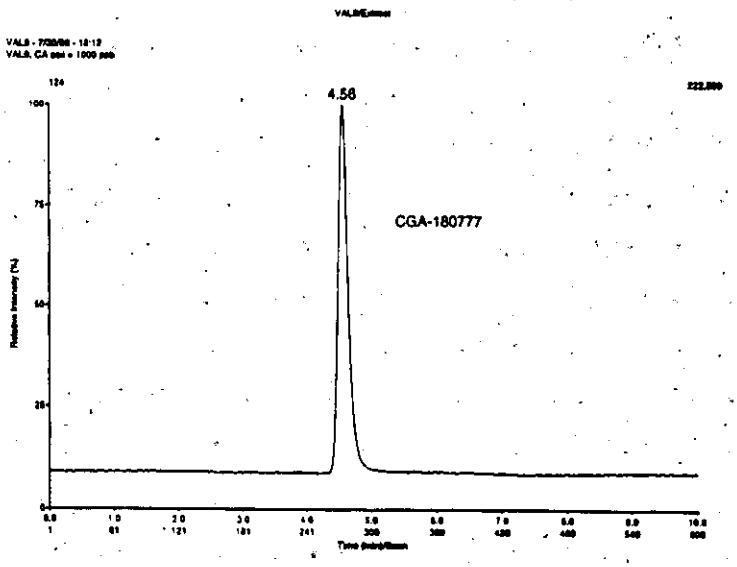


Sample Code: VAL3, Soil + 10 ppb



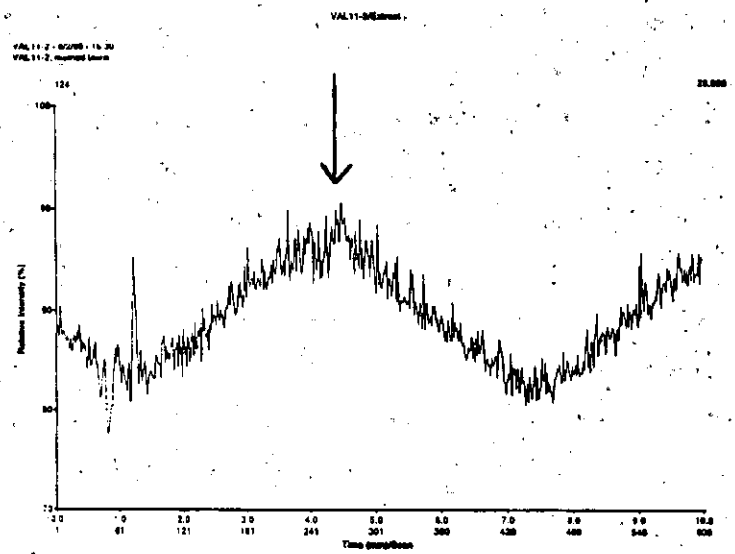
Sample Code: VAL7, Soil + 100 ppb

FIGURE 10. TYPICAL CHROMATOGRAMS FOR CONTROL AND FORTIFIED CALIFORNIA SOIL: LC/MS (Continued)

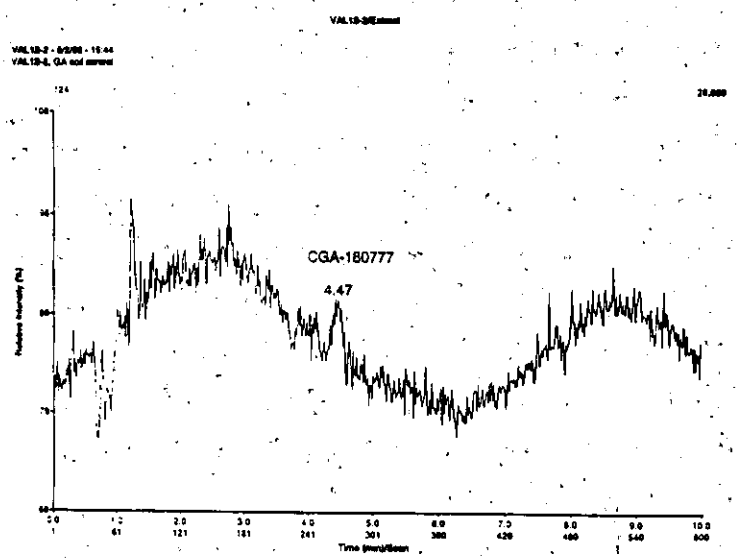


Sample Code: VAL9, Soil + 1000 ppb

FIGURE 11. TYPICAL CHROMATOGRAMS FOR CONTROL AND FORTIFIED GEORGIA SOIL: LC/MS

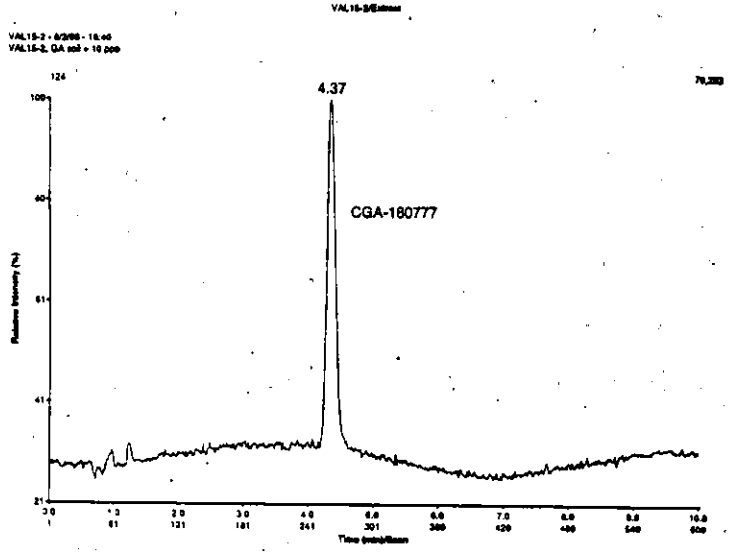


Sample Code: VAL11, Method Blank

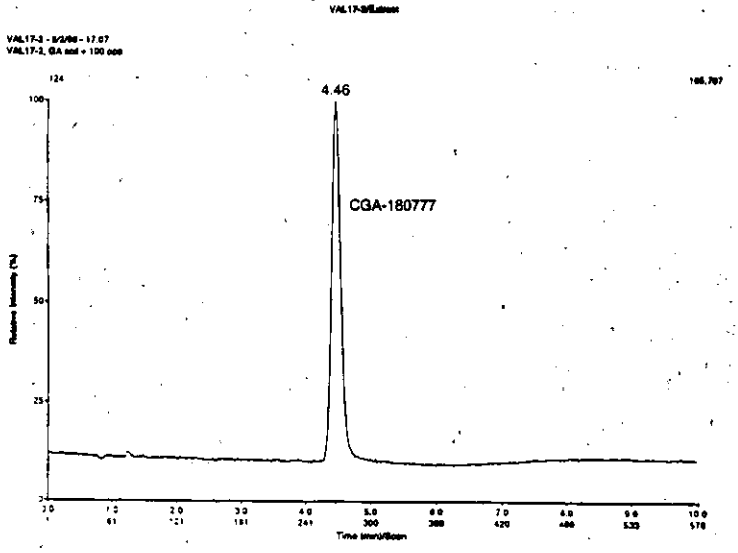


Sample Code: VAL12, Soil Control

FIGURE 11. TYPICAL CHROMATOGRAMS FOR CONTROL AND FORTIFIED GEORGIA SOIL: LC/MS (Continued)

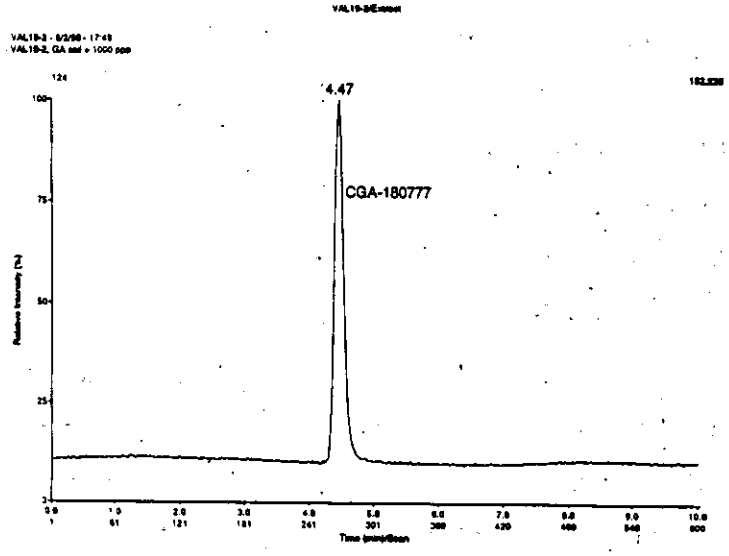


Sample Code: VAL15, Soil + 10 ppb



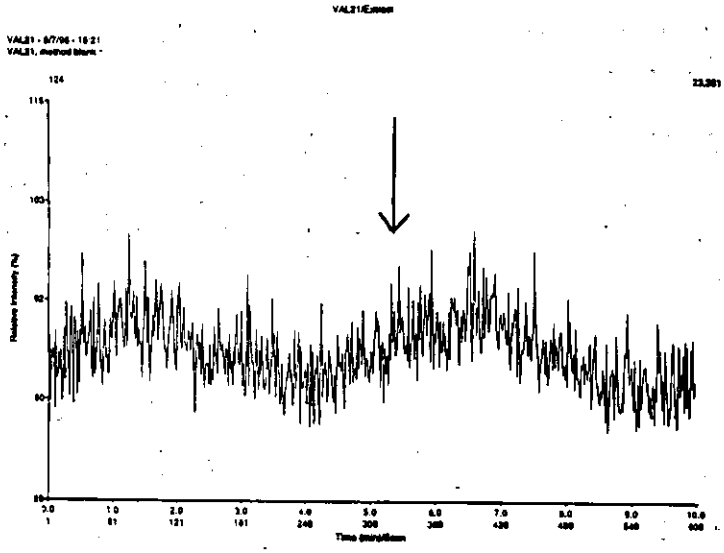
Sample Code: VAL17, Soil + 100 ppb

FIGURE 11. TYPICAL CHROMATOGRAMS FOR CONTROL AND FORTIFIED GEORGIA SOIL: LC/MS (Continued)

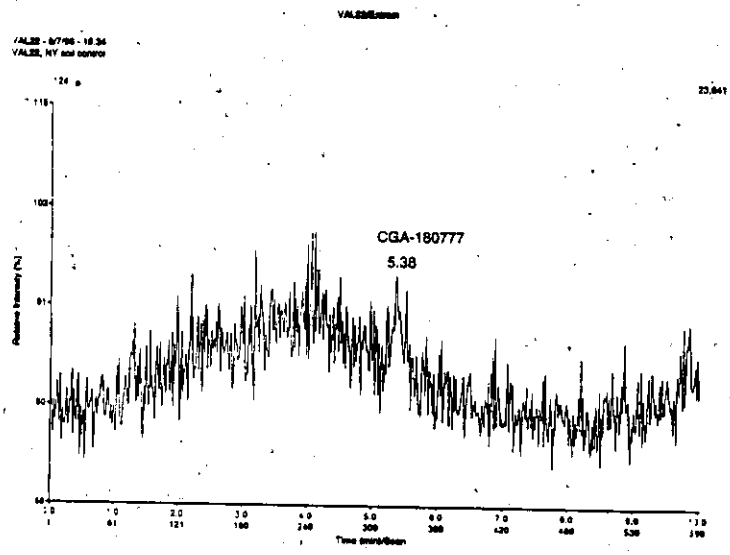


Sample Code: VAL19, Soil + 1000 ppb

FIGURE 12. TYPICAL CHROMATOGRAMS FOR CONTROL AND FORTIFIED NEW YORK SOIL: LC/MS

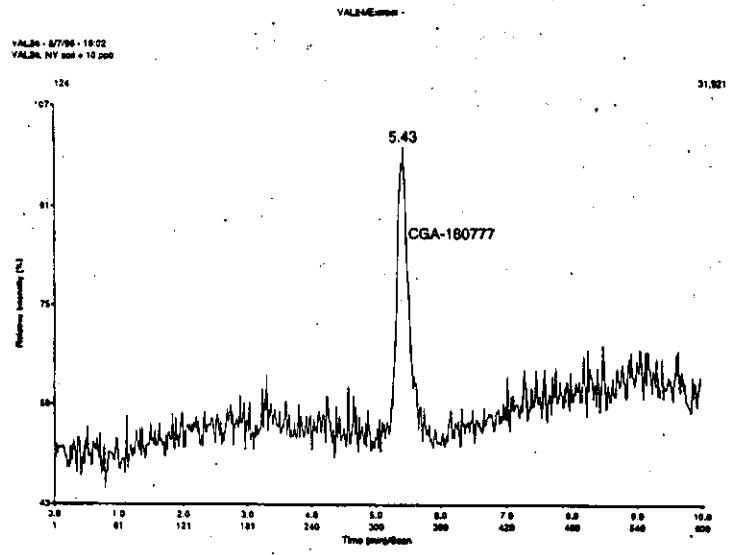


Sample Code: VAL21, Method Blank

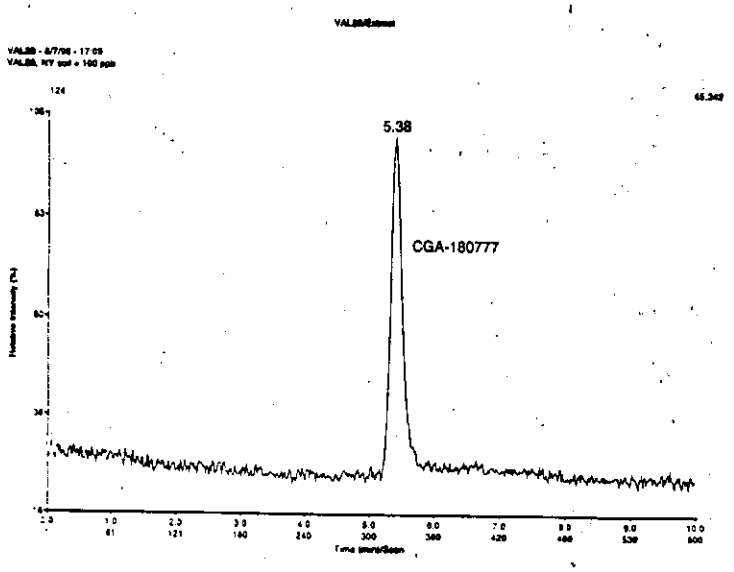


Sample Code: VAL22, Soil Control

FIGURE 12. TYPICAL CHROMATOGRAMS FOR CONTROL AND FORTIFIED NEW YORK SOIL: LC/MS (Continued)

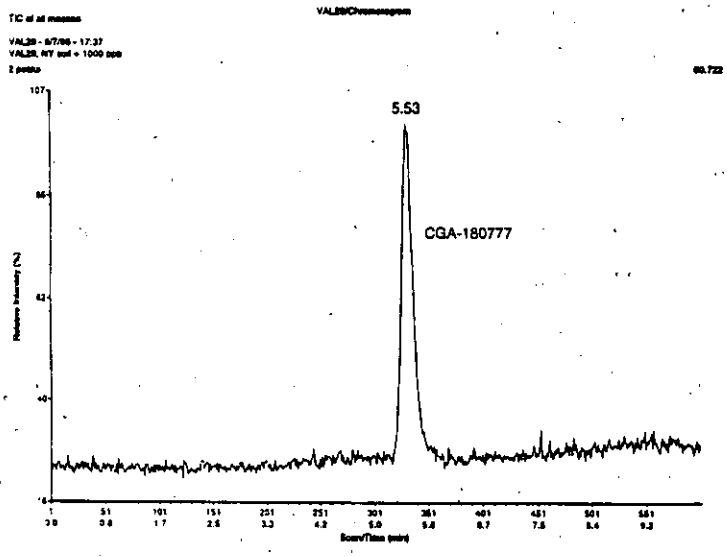


Sample Code: VAL24, Soil + 10 ppb



Sample Code: VAL28, Soil + 100 ppb

FIGURE 12. TYPICAL CHROMATOGRAMS FOR CONTROL AND FORTIFIED NEW YORK SOIL: LC/MS (Continued)



Sample Code: VAL29, Soil + 1000 ppb

IX. REFERENCES

1. Vargo, J. D., Ciba Protocol 285-96, "Validation of "Draft" Analytical Method AG-660 for the Determination of CGA-180777, a Metabolite of CGA-215944, by High Performance Liquid Chromatography with UV Detection," including Protocol Amendment 1.

2. Vargo, J. D., Ciba Residue Test Report RI-MV-014-96, Report Number 1.