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Analytical Method for the Determination of CGA-215944 in Crops by HPLC

Analytical Method No. AG-643

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I. INTRODUCTION/SUMMARY

A. Scope

This method is used in the determination of residues of CGA-215944 (4,5-dihydro-6-methyl-4-[(3-pyridinylmethylene)-amino]-1,2,4-triazin-3(2H)-one), also known as pymetrozine, in crops. The structure and chemical name of the analyte is given in Figure 1.

The limit of detection (smallest standard amount injected during the chromatographic run) for CGA-215944 is 1.0 ng. The limit of quantitation (the lowest fortification specified by the method which gives adequate recovery according to EPA guidelines) is 0.02 ppm for CGA-215944.

B. Principle

A 5 gram sample of substrate is extracted by shaking with a solution of borax buffer in methanol for one hour. The extract is diluted to 50 mL (100 mL for dry samples) and a 10 mL aliquot is carried through the method.

The CGA-215944 aliquot is evaporated to a reduced volume and the extract is applied to an Extrelut® column and removed using ethyl acetate (EtOAc). The samples are then cleaned up using a silica solid-phase extraction (SPE) cartridge. Cured tobacco samples are further cleaned up using a C-18 SPE cartridge. The samples are reconstituted in mobile phase and analyzed by HPLC using a C-18 column and a mobile phase of 91/9 (v/v) 0.005M PIC B7/acetonitrile. A column switching system is required for tobacco and fruiting vegetables in which a cyano column with a mobile phase of 95/5 (v/v) 0.005M PIC B7/acetonitrile is used for initial separation and the C-18 column with mobile phase of 91/9 (v/v) 0.005M PIC B7/acetonitrile is used for final separation.

II. MATERIALS AND METHODS

A. Apparatus

1.0 Bottles, square amber wide mouth, 8-oz, with lids.

2.0 Column, HPLC, Inertsil ODS II 5 µm, 250 mm x 4.6 mm (Phenomenex No. 00G-3125-EO).

3.0 Column, HPLC, Spherisorb 5 CN 5 µm, 150 mm x 4.6 mm (Phenomenex No. 00F-0107-RO).

4.0 Cylinder, graduated, 50-mL, 100-mL (Fisher No. 08-554D, 08-554E) or equivalent.

5.0 Extrelut QE cartridge, 10 mL capacity (EM Separations No. 901010-1).

6.0 Filter paper, Whatman #5, 7 cm (Fisher No. 09830K) or equivalent.

7.0 Flask, round-bottom, 50-mL, 100-mL (Fisher No. 10-067B, 10-067C) or equivalent.

8.0 Funnel, Buchner, Coors Porcelain w/fixed perforated plate, plate diameter 83 mm, height 143 mm, (Fisher No. 10-356C) or equivalent.

9.0 Pipette, Pasteur, Flint Glass, 9 inch, disposable, (Fisher No. 13-678-7C) or equivalent.

10.0 Pipette, volumetric, 0.5, 1.0, and 2.0-mL capacities (Fisher Nos. 13-649A, 13-649B, 13-649C) or equivalent.

11.0 Solid-Phase Extraction cartridge, silica, 1000-mg, 6-cc Bakerbond (Baker No. 7086-07)

12.0 Solid-Phase Extraction cartridge, C-18, 1000-mg, 6-cc Bakerbond (Baker No. 7020-07), for cured tobacco only.

13.0 Traps, pear-shaped distilling (Kontes No. 517000-2400).

14.0 Vials, 1.5-mL E-Z Auto Vial (Wheaton Part No. 225175SP) or equivalent, with 11 mm E-Z Auto Seal (Wheaton Part no. 224212SP) or equivalent.

B. Reagents and Analytical Standards

All reagents are stored at room temperature. Analytical standards and stock solutions are stored frozen and standard solutions are refrigerated.

1.0 Acetone, Optima (Fisher No. A929-4) or equivalent.

2.0 Acetonitrile, HPLC grade (Fisher No. A998-4) or equivalent.

3.0 Borax, Sigma, approx. 99% (Sigma No. B-9876) or equivalent.

4.0 0.05 M Borax - Dilute 19.1 g of Borax to 1000 mL with water.

5.0 Ethyl Acetate, Optima (Fisher No. E196-4) or equivalent.

6.0 O-Phosphoric Acid, 85% (Fisher No. A242-4) or equivalent.

7.0 PIC B7 ion pairing reagent (Waters No. WAT085103).

8.0 0.005M PIC B7 (aq) - add the contents of one vial of PIC B7 to 1000 mL of water.

9.0 Methanol, HPLC grade (Fisher No. A452-4) or equivalent.

10.0 Mobile Phase - 91/9 (v/v) 0.005M PIC B7 (aq)/acetonitrile.

11.0 Mobile Phase - 95/5 (v/v) 0.005M PIC B7 (aq)/acetonitrile (tobacco and fruiting vegetables).

12.0 Water, deionized and purified in-house using a HYDRO™ purification system, or HPLC grade equivalent.

13.0 CGA-215944 analytical standard, Ciba-Geigy Corporation, 410 Swing Road, Greensboro, NC 27407.

C. Analytical Procedure

A flow diagram for the procedure is presented in Figure 2.

1.0 Preparation of sample

Prepare samples according to Ciba SOP 7.21. Store samples frozen at -20°C until just before analysis.

Weigh 5.0 g of plant material into an 8-oz. wide mouth jar. Weigh the samples in a semi-frozen state to avoid losing sample moisture. Allow the samples to thaw fully before extraction.

Prepare procedural fortifications at this point according to section II.J.2.0.

2.0 Extraction

General Procedure

2.1 Add 4 mL of 0.05 M sodium borate to the sample and mix carefully by shaking.

2.2 Add 36 mL of methanol to the sample.

Dry samples (e.g. cured tobacco, tomato dry pomace)

2.1 Add 8 mL of 0.05 M sodium borate to the sample and mix carefully by shaking.

2.2 Add 80 mL of methanol to the sample.

For all samples

2.3 Seal the wide mouth jar tightly and shake at approximately 250

oscillations per minute for 1 hour using a laboratory shaker.

2.4 Vacuum filter the extract using Whatman #5 filter paper. Wash the filter cake with 5 mL of methanol, collecting the washing.

2.5 Adjust the volume of the extract solution to 50.0 mL (100.0 mL for dry samples) with methanol using a volumetric flask.

3.0 CGA-215944 Cleanup

3.1 Transfer into buffer solution

Weigh a 100-mL round-bottom flask. Transfer a 10-mL aliquot of the extract solution into the tared 100-mL round-bottom flask and add 5 mL of 0.05 M sodium borate. Evaporate the solution to ≤ 6 g using a rotary evaporator (water bath ≤ 30°C).

3.2 Extrelut® partition

Vibrate or knock the Extrelut column for 10-20 seconds to settle the contents. Transfer the aqueous extract solution onto the column. Rinse the 100-mL round-bottom flask with ~3 mL of sodium borate buffer and transfer the washing to the Extrelut column. Wait for 15 minutes to allow the aqueous solution to penetrate the matrix. Rinse the 100-mL round-bottom flask with four 25 mL portions of ethyl acetate and use the rinses to extract the analyte from the Extrelut. Collect the eluate in a 250-mL round-bottom flask and evaporate to dryness by rotary evaporation (water bath ≤30°C). Reconstitute the residue from the partition in 5 mL of acetone.

3.3 Silica SPE

Attach a silica cartridge to a vacuum manifold. Precondition the cartridge with 5 mL of acetone. Pass the solution from step 3.2 through the silica cartridge. Discard the eluate. Wash the round-bottom flask with 5 mL of acetone and use this rinse to wash the cartridge, discarding the eluate. Rinse the round-bottom flask with 10 mL of methanol and use the rinse to elute the analyte from the cartridge, collecting the eluate in a 15-mL test tube. Transfer to a 50-mL round-bottom flask rinsing the test tube with 15-mL of methanol. Evaporate to dryness by rotary evaporation (water bath $\leq$30°C).

3.4 C-18 SPE (cured tobacco only)

Reconstitute the residue in 1 mL of methanol. Add 9 mL of water to the flask and mix the solution. Attach a C-18 cartridge to a vacuum manifold. Precondition the cartridge with 5 mL of methanol followed by 5 mL of water. Pass the solution from silica SPE through the cartridge. Discard the eluate. Wash the round-bottom flask with 10 mL of 10/90 (v/v) methanol/water and use this rinse to wash the cartridge, discarding the eluate. Elute the analyte from the cartridge using 10 mL of 30/70 methanol/water, collecting the eluate in a 15-mL test tube. Transfer the solution from the test tube to a 50-mL round bottom flask with a rinse of 15 mL of methanol. Evaporate to dryness by rotary evaporation (water bath $\leq$40°C). If water remains, continue to add methanol and evaporate repetitively until all of the solvent has evaporated.

3.5 Final solution

Reconstitute the residue from solid-phase extraction in mobile phase. Sonicate the solution for ~30 seconds while swirling the flask, being sure to contact all inside surfaces. Transfer to a vial for HPLC analysis.

D. Instrumentation

1.0 Description and Operating Conditions

See Table I for a description of the system components and chromatographic conditions.

2.0 Calibration and Standardization

The instrument is calibrated by construction of a calibration curve from detector response (chromatographic peak height or area and the amount of analyte standard injected), encompassing the range of 1.0 ng to 20 ng. The response curve can be constructed manually, with a calculator, or using a computer. Typical standardization data of analytical standards are presented in Tables II and III and typical standard chromatograms and calibration plots are presented in Figures 3 through 6.

E. Interferences

There are no known interferences resulting from the chemical reagents used in the procedure.

F. Confirmatory Techniques

The presence of CGA-215944 can be confirmed using analytical method REM 154.01, "Determination of Residues of Parent Compound by Liquid Chromatography."[1]

G. Time Required for Analysis

1.0 Twelve samples can be taken through the extraction and cleanup procedure in eight hours.

2.0 Samples can be analyzed overnight if an autosampler is available.

H. Modifications and Potential Problems

1.0 CGA-215944 is sensitive to light and to low pH. It is recommended that extraction and analysis of the CGA-215944 fraction be performed in one day. Initial extracts may be stored refrigerated in the dark in organic solvent.

2.0 New analytical standards of CGA-215944 must be prepared from stock at least every five days. For this study new standards were prepared daily.

3.0 The same extract can be used for analysis of both CGA-215944 by this method and for its metabolite, GS-23199, by method AG-647[2].

I. Preparation of Standards

1.0 CGA-215944 Stock Solution - CGA-215944 is not highly soluble in organic solvents. It is necessary to sonicate the stock solution for some time in order to achieve complete dissolution.

1.1 Weigh 20 mg of CGA-215944 into a 100-mL volumetric flask. Add a few milliliters of methanol to the flask and shake to dissolve the CGA-215944. Dilute to the mark with methanol. The concentration of this stock is 200 µg/mL. Store frozen and in the dark. Stock solutions expire 6 months after preparation.

2.0 Fortification Standards -

Prepare fortification standards of CGA-215944 in methanol such that no more than 1 mL of the standard is required to fortify recovery samples. Fortification standards expire 6 months after preparation.

3.0 Analytical Standards -

Prepare at least four standards of CGA-215944 in 0.005 M PIC B7 in the range of 0.01 to 0.2 µg/mL. New CGA-215944 standards must be prepared from stock at least every five days.

J. Methods of Calculation

1.0 Determination of Sample Residues

Inject 100-µL aliquots of sample final fractions from Sections II.C.3.6 into the HPLC under the same conditions as for the standards. Make appropriate dilutions of the stock to bring the sample response within the range of the standard curve. Compare the response of the unknown samples to the standard curve or enter the value of the response into a least squares program to determine the nanograms of CGA-215944 in the injected aliquot. Typical chromatograms for control and fortified control samples are presented in Figures 7 through 14.

To calculate the residue results the milligrams injected must first be calculated as follows;

$$(1)\quad \text{mg sample injected} = \frac{mg \times V_i}{V_f}$$

mg = milligrams of sample used
V_i = injection volume (mL)
V_f = total volume of final injection solution (mL)

To determine ppm CGA-215944 found in the samples use equation 2:

$$(2)\quad \text{ppm analyte} = \frac{(\text{ng analyte found})}{(\text{mg sample injected})\ (R)}$$

R = recovery factor expressed as a fraction (e.g. 100% = 1.0) and is calculated from equation 4.

Note: R is not used for tolerance enforcement.

2.0 Fortification Experiments

This method is validated for each set of samples analyzed by including an untreated control sample and one or more control samples fortified immediately prior to extraction with CGA-215944.

2.1 Add an appropriate amount of each fortification standard (from section II.I.2.1) to a sample of control crop sample prior to extraction. The total volume of the added fortification solutions should not exceed 1 mL.

2.2 Proceed to step II.C.2.0. Typical recovery data for control and fortified control samples are given in Table IV.

2.3 Calculate the analyte concentration using equation 3:

$$(3)\quad \text{ppm analyte found} = \frac{(\text{ng analyte})}{(\text{mg sample injected})}$$

Determine the recovery factor by first subtracting the background detector response, if any, in the control sample from the analyte response in the recovery sample. Calculate the recovery factor as a percentage (R) by the equation:

$$(4)\ R = \frac{\text{ppm analyte found}}{\text{ppm analyte added}}.$$

Note: R is not used for tolerance enforcement.

III. RESULTS AND DISCUSSION

This method has been validated under Ciba-Geigy Residue Chemistry Protocol 627-95 and Amendment 1[3]. The objective of Protocol 627-95 was to validate Analytical Method AG-643 for the determination of CGA-215944 in crops. The limit of quantitation of Analytical Method AG-643 is 0.02 ppm. The limit of detection of Analytical Method AG-643 is 1.0 ng.

Analytical Method AG-643 was used to generate residue data for tobacco, fruiting vegetables, and cucurbits, and will be proposed as the tolerance enforcement method for fruiting vegetables and cucurbits. This method is not a regulatory method for tobacco as no tolerances are required for tobacco. The method will be used to generate data for risk assessment for tobacco.

The average recovery of CGA-215944 achieved in validation using the analytical method was 81% (sd=16%, CV=20%, n=58). The average recovery of CGA-215944 achieved for substrates fortified at the limit of quantitation (0.02 ppm) was 96% (sd=21%, CV=22%, n=16).

The average recovery was between 70-120% for all substrates except peppers and cured tobacco. The average recovery for peppers was 69% with a standard deviation of 5% (CV=8%). The average recovery for cured tobacco was 68% with a standard deviation of 6% (CV=8%). The results are considered acceptable due to the good precision. Recovery results can be found in Table IV.

No detectable residues were found in the controls for any of the substrates except for green tobacco. This will not pose a problem as the method will not be used for tolerance enforcement purposes on tobacco as was noted above and incurred residues in tobacco will be well above the control amounts. No detectable residues were found in the reagent blanks. Chromatograms from the analysis of control and fortified control samples are presented in Figures 7 through 14.

Sample identification and test and reference substance information can be found in Residue Test Report RI-MV-015-95[4]. The raw data Residue Test Report and Final Report are stored in the Ciba Agricultural Group Archives in Greensboro, NC. No samples were archived.

There were no deviations from the protocol, nor any circumstances affecting the quality or integrity of the data.

IV. CONCLUSION

Analytical Method AG-643 is a valid and accurate method for the determination CGA-215944 in crops.

V. CERTIFICATION

The report and experimental results included in this study, laboratory Project I. D. AG-643 are certified to be authentic accounts of the experiments.

3/18/96
Date

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CERTIFICATION OF GOOD LABORATORY PRACTICES

The analytical work reported in AG-643 was performed in accordance with Good Laboratory Practice Standards, 40 CFR Part 160.

3/18/96
Date

Timothy Joseph
Study Director

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VI. QUALITY ASSURANCE STATEMENT

CIBA CROP PROTECTION
CIBA-GEIGY CORPORATION
QUALITY ASSURANCE UNIT

QUALITY ASSURANCE STATEMENT

Report Title: ANALYTICAL METHOD FOR THE DETERMINATION OF CGA-215944 IN CUCURBITS, TOBACCO, AND FRUITING VEGETABLES, INCLUDING TOMATO PROCESSED FRACTIONS, BY HPLC

Analytical Method No. AG-643

Study Director: T. Joseph

Report Number: AG-643

Ciba Study Number: 627-95 with Amendments

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	11/03/95	11/03/95
In-Progress Inspection	12/06/95	12/06/95
Final Report Audit	01/12, 15-16/96	01/17/95

Prepared By: Kent Ediger Date: 1/17/95

Kent Ediger

VII. TABLES AND FIGURES

TABLE I. HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC CONDITIONS

CGA-215944

Instrumentation:

Pump -	Perkin-Elmer 250
Autoinjector -	Perkin-Elmer ISS-100
Detector -	Applied Biosystems 783
Column -	Inertsil ODS II 5 µm, 250 mm x 4.6 mm

Conditions:

Mobile Phase -	91/9 (v/v) 0.005M PIC B7 (aq)/acetonitrile
Flow Rate -	1 mL/min.
Injection Volume -	100 µL
Detection -	300 nm

Approximate retention time - 9 min.

TABLE I. HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC CONDITIONS (Continued)

CGA-215944 column switching for tobacco and fruiting vegetables

Instrumentation:

Pump 1 -	Perkin-Elmer 250
Pump 2 -	Perkin-Elmer 250
Autoinjector -	Perkin-Elmer ISS 100
Detector -	Applied Biosystems 783
Column 1 -	Spherisorb 5 CN 5 µm, 150 mm x 4.6 mm
Column 2 -	Inertsil ODS II 5 µm, 250 mm x 4.6 mm

Conditions:

Mobile Phase 1-	95/5 (v/v) 0.005M PIC B7 (aq)/acetonitrile
Mobile Phase 2-	91/9 (v/v) 0.005M PIC B7 (aq)/acetonitrile
Flow Rate -	1 mL/min.
Injection Volume -	100 µL
Detection -	300 nm

Approximate cut time - 6.5 - 7.5 min.

Approximate retention time - 13 min.

TABLE II. TYPICAL STANDARDIZATION DATA FOR CGA-215944

ng of CGA-215944 injected	Peak Height
1.0	123
5.0	669
5.0	595
10.0	1290
10.0	1183
20.0	2715

Correlation Coefficient	0.99727
Slope	136
Intercept	-60.0

Data is from the analysis of cantaloupes.

TABLE III. TYPICAL STANDARDIZATION DATA FOR CGA-215944 WITH COLUMN SWITCHING

ng of CGA-215944 injected	Peak Height
1.0	95
5.0	487
5.0	466
10.0	1083
10.0	991
20.0	2286

Correlation Coefficient	0.99755
Slope	117
Intercept	-89.5

Data is from the analysis of tomatoes, mature fruit.

TABLE IV. RECOVERY RESULTS FOR CGA-215944 AND CGA-215944 FORTIFIED IN CONTROL SUBSTRATES

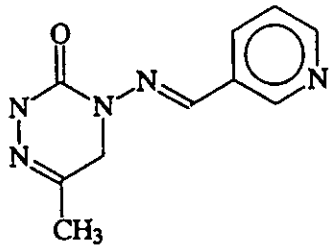
Sample Code	Substrate	Fortification Level (ppm)	% Recovery CGA-215944
CC.CA	Cucumbers	Control	<0.02 ppm
CC.CB	Cucumbers	Control	<0.02 ppm
CC.02A	Cucumbers	0.02	118
CC.02B	Cucumbers	0.02	122
CC.05A	Cucumbers	0.05	83
CC.05B	Cucumbers	0.05	93
CC.10A	Cucumbers	0.10	a
CC.10B	Cucumbers	0.10	67
CC.20A	Cucumbers	0.20	64
CC.20B	Cucumbers	0.20	71
Average Recovery			88
Standard Deviation			24
CN.C	Cantaloupes	Control	<0.02 ppm
CN.02A	Cantaloupes	0.02	100
CN.02B	Cantaloupes	0.02	122
CN.05A	Cantaloupes	0.05	86
CN.05B	Cantaloupes	0.05	74
CN.10A	Cantaloupes	0.10	73
CN.10B	Cantaloupes	0.10	80
CN.20A	Cantaloupes	0.20	73
CN.RB	Cantaloupes	reagent blank	<0.02 ppm
Average Recovery			87
Standard Deviation			19
TM.CA	Tomatoes	Control	<0.02 ppm
TM.CB	Tomatoes	Control	<0.02 ppm
TM.02A	Tomatoes	0.02	116
TM.02B	Tomatoes	0.02	114
TM.05A	Tomatoes	0.05	88
TM.05B	Tomatoes	0.05	86
TM.10A	Tomatoes	0.10	80
TM.10B	Tomatoes	0.10	80
TM.20A	Tomatoes	0.20	72
TM.20B	Tomatoes	0.20	74
Average Recovery			89
Standard Deviation			17
TW.CA	Tomato wet pomace	Control	<0.02 ppm
TW.02A	Tomato wet pomace	0.02	113
TW.02B	Tomato wet pomace	0.02	103
TW.05A	Tomato wet pomace	0.05	87
TW.05B	Tomato wet pomace	0.05	85
TW.10A	Tomato wet pomace	0.10	79
TW.10B	Tomato wet pomace	0.10	77
TW.20A	Tomato wet pomace	0.20	68
Average Recovery			87
Standard Deviation			16

[a] Flask was broken while transferring solution onto silica SPE cartridge.

TABLE IV. RECOVERY RESULTS FOR CGA-215944 AND CGA-215944 FORTIFIED IN CONTROL SUBSTRATES (Continued)

Sample Code	Substrate	Fortification Level (ppm)	% Recovery CGA-215944
TD.CA	Tomato dry pomace	Control	<0.02 ppm
TD.02A	Tomato dry pomace	0.02	97
TD.02B	Tomato dry pomace	0.02	98
TD.05A	Tomato dry pomace	0.05	70
TD.05B	Tomato dry pomace	0.05	84
TD.10A	Tomato dry pomace	0.10	81
TD.10B	Tomato dry pomace	0.10	79
TD.20A	Tomato dry pomace	0.20	67
Average Recovery			82
Standard Deviation			12
PP.CA	Peppers	Control	<0.02 ppm
PP.02A	Peppers	0.02	65
PP.02B	Peppers	0.02	65
PP.05A	Peppers	0.05	78
PP.05B	Peppers	0.05	71
PP.10A	Peppers	0.10	63
PP.10B	Peppers	0.10	67
PP.20A	Peppers	0.20	74
Average Recovery			69
Standard Deviation			5.4
TG.CA	Tobacco, green	Control	<0.02 ppm
TG.02A	Tobacco, green	0.02	81
TG.02B	Tobacco, green	0.02	84
TG.05A	Tobacco, green	0.05	85
TG.05B	Tobacco, green	0.05	65
TG.10A	Tobacco, green	0.10	73
TG.10B	Tobacco, green	0.10	77
TG.20A	Tobacco, green	0.20	73
TG.RB	Tobacco, green	Reagent Blank	<0.02 ppm
Average Recovery			77
Standard Deviation			7.2
TC.CA	Tobacco, cured	Control	<0.02 ppm
TC.CB	Tobacco, cured	Control	<0.02 ppm
TC.02A	Tobacco, cured	0.02	77
TC.02B	Tobacco, cured	0.02	65
TC.05A	Tobacco, cured	0.05	69
TC.05B	Tobacco, cured	0.05	74
TC.10A	Tobacco, cured	0.10	62
TC.10B	Tobacco, cured	0.10	73
TC.20A	Tobacco, cured	0.20	64
TC.20B	Tobacco, cured	0.20	62
TC.RB	Tobacco, cured	Reagent Blank	<0.02 ppm
Average Recovery			68
Standard Deviation			5.7

FIGURE 1. CHEMICAL NAME AND STRUCTURE



CGA-215944

4,5-Dihydro-6-methyl-4-[(3-pyridinylmethylene)-amino]-1,2,4-triazin-3(2H)-one

CAS # 123312-89-0

FIGURE 2. FLOW DIAGRAM FOR THE DETERMINATION OF CGA-215944 IN CROPS

Extraction

5-gram sample

↓

General Procedure	**Dry Crops**
↓	↓
Add 4 mL of Borax + 36 mL of methanol	Add 8 mL of Borax + 80 mL of methanol
↓	↓
Shake for 1 hour	Shake for 1 hour
↓	↓
Filter	Filter
↓	↓
Wash filter cake with 5 mL of methanol	Wash filter cake with 5 mL of methanol
↓	↓
Adjust volume to 50 mL with methanol	Adjust volume to 100 mL with methanol
↓	↓

Take a 10-mL aliquot and add 5 mL of borax buffer

↓

Evaporate to ≤6 g

↓

Transfer onto Extrelut column with 3 mL borax rinse

↓

Elute with 4x25 mL of ethyl acetate

↓

Evaporate to dryness, reconstitute in 5 mL of acetone

↓

Condition silica SPE cartridge with 5 mL of acetone

↓

Apply extract to cartridge

↓

Wash flask with 5 mL of acetone, apply to cartridge

↓

Wash flask with 10 mL of methanol, use to elute analyte

↓

FIGURE 2. FLOW DIAGRAM FOR THE DETERMINATION OF CGA-215944 IN CROPS (Continued)

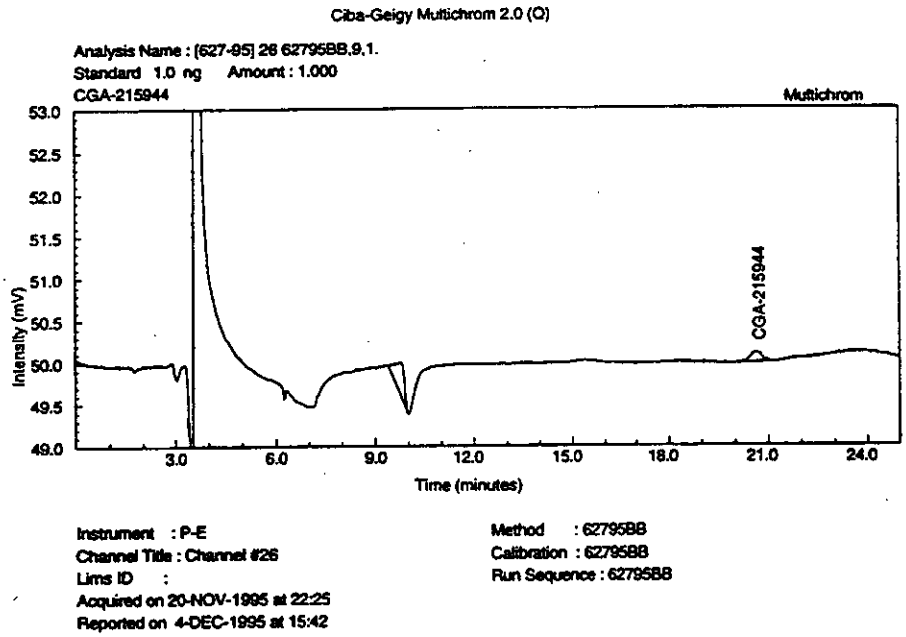
Cured Tobacco	**All other substrates**
↓	↓
Evaporate to dryness, reconstitute in 10/90 methanol/water	Evaporate to dryness
↓	↓
Condition C-18 cartridge with 5 mL of methanol followed by 5 mL of water	↓
↓	↓
Apply solution to cartridge	↓
↓	↓
Wash flask with 10 mL of 10/90 methanol/water, apply to cartridge	↓
↓	↓
Wash flask with 10 mL of 30/70 methanol/water, use to elute analyte	↓
↓	↓
Evaporate to dryness	↓
↓	↓

Reconstitute in mobile phase

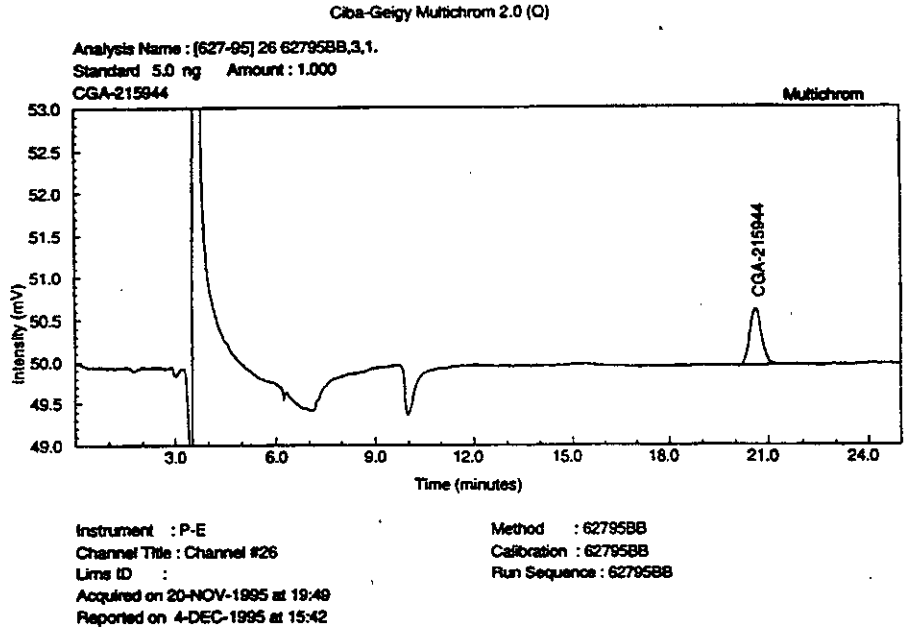
↓

Analyze by HPLC

FIGURE 3. TYPICAL CHROMATOGRAMS OF CGA-215944 STANDARD SOLUTIONS

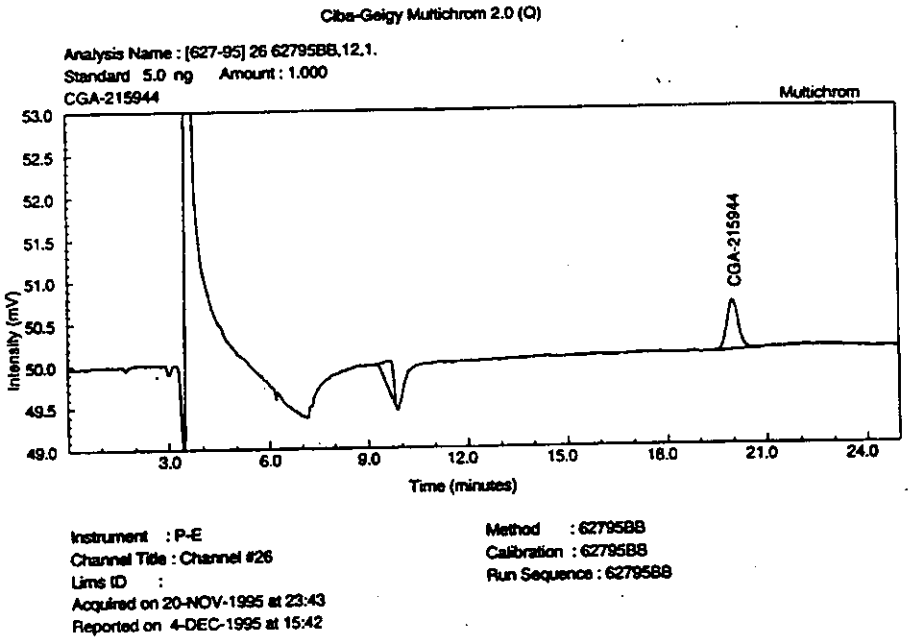


1. 0.01 ng/µL, 100 µL injected

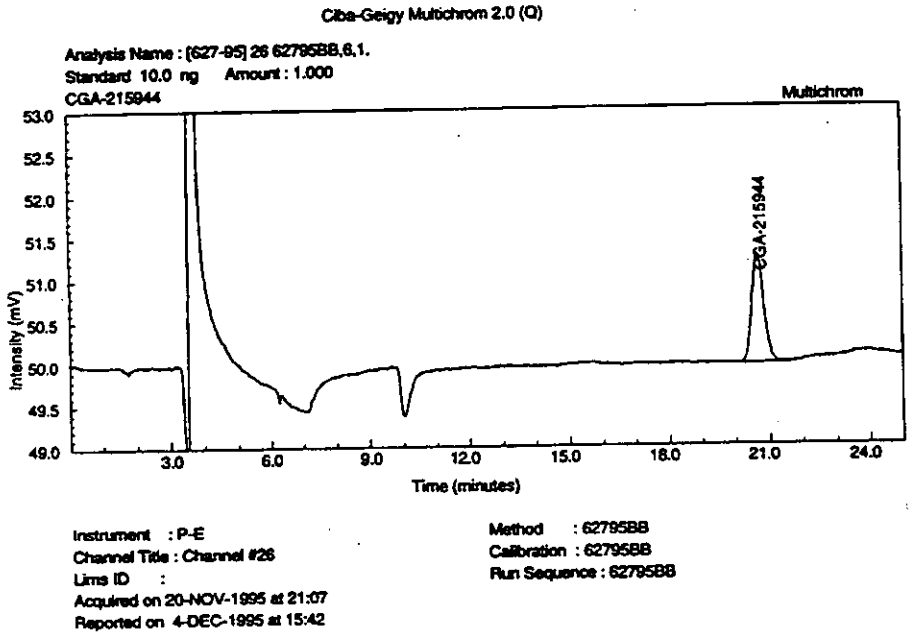


2. 0.05 ng/µL, 100 µL injected

FIGURE 3. TYPICAL CHROMATOGRAMS OF CGA-215944 STANDARD SOLUTIONS (Continued)

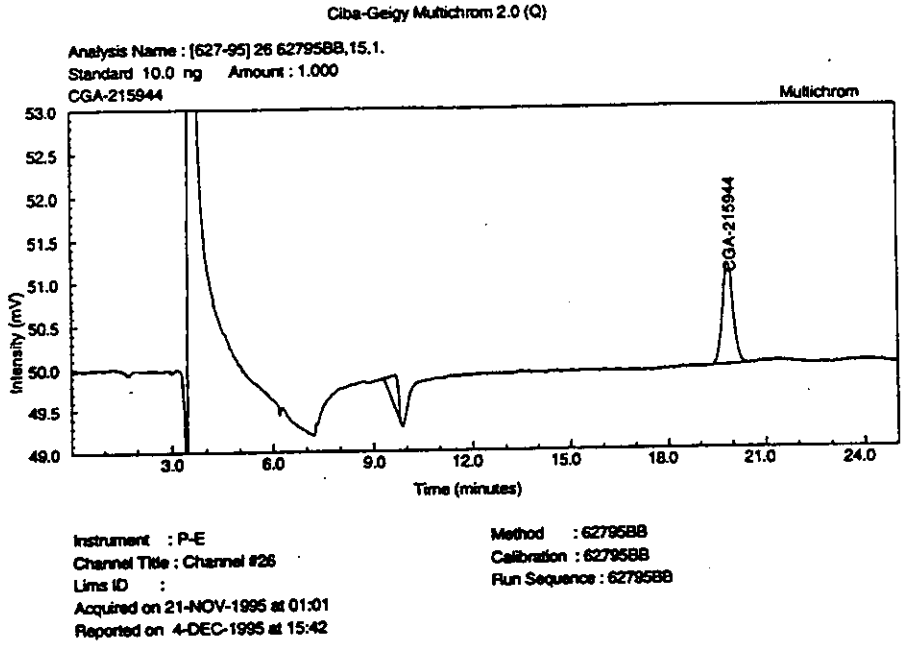


3. 0.05 ng/µL, 100 µL injected

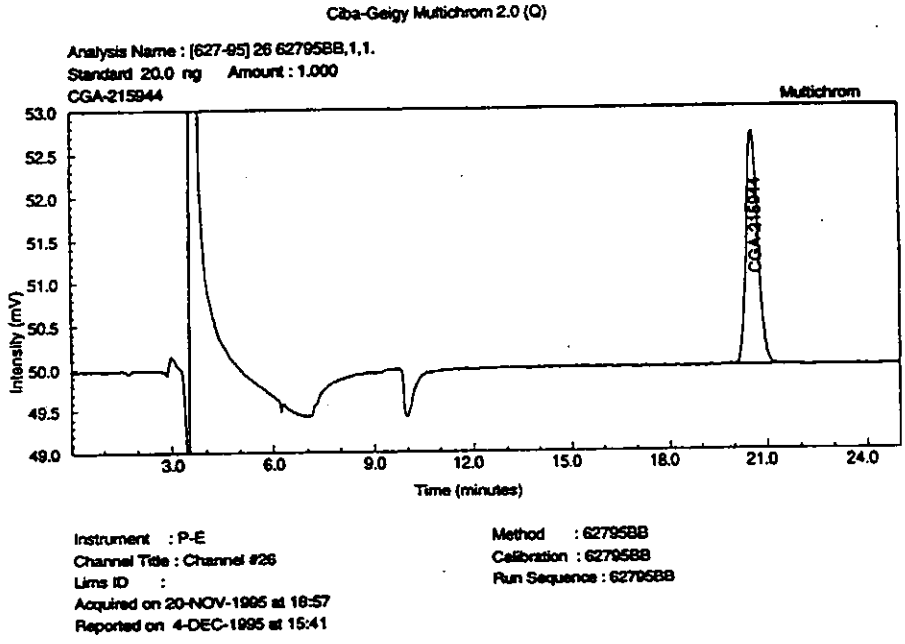


4. 0.1 ng/µL, 100 µL injected

FIGURE 3. TYPICAL CHROMATOGRAMS OF CGA-215944 STANDARD SOLUTIONS (Continued)

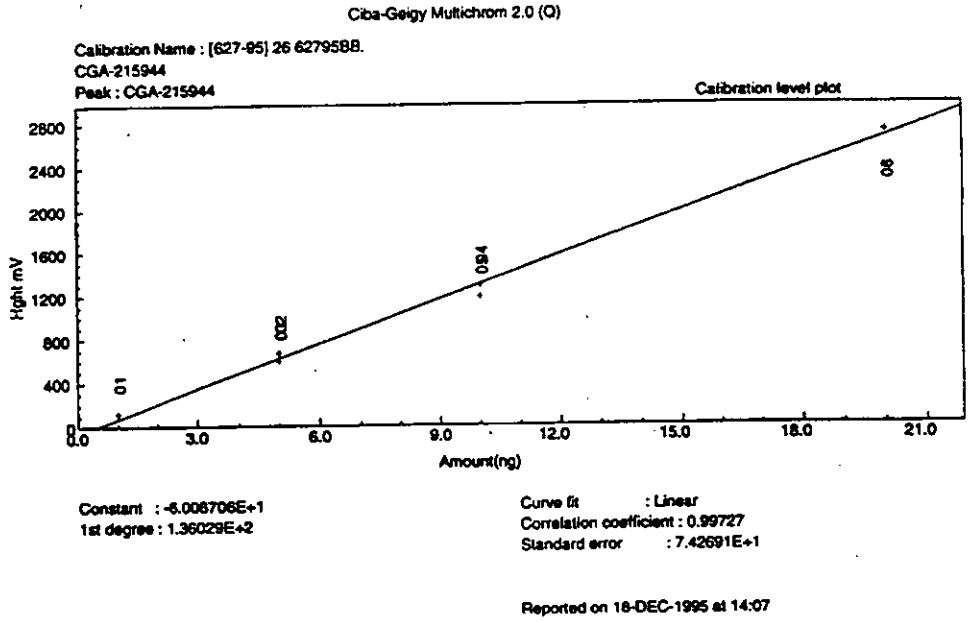


5. 0.1 ng/µL, 100 µL injected



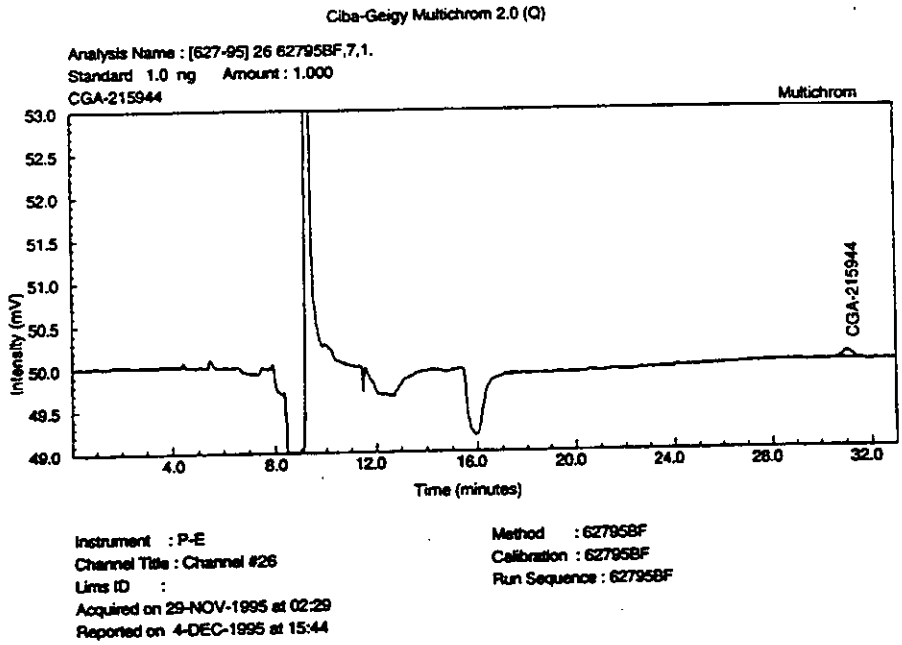
6. 0.2 ng/µL, 100 µL injected
Chromatograms from the analysis of cantaloupes

FIGURE 4. TYPICAL CALIBRATION PLOT OF CGA-215944 STANDARD SOLUTIONS

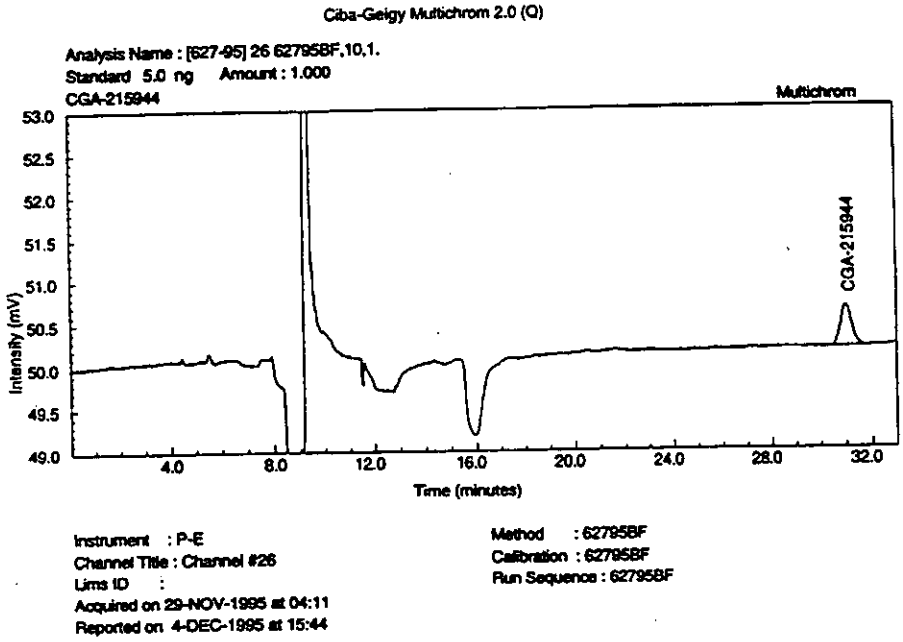


Curve from the analysis of cantaloupes

FIGURE 5. TYPICAL CHROMATOGRAMS OF CGA-215944 STANDARD SOLUTIONS WITH COLUMN SWITCHING

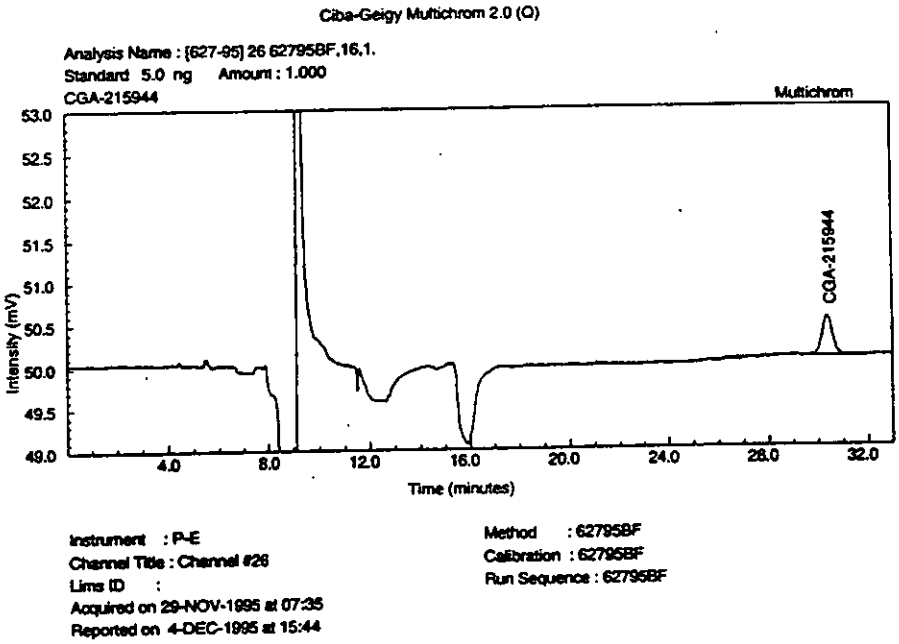


1. 0.01 ng/µL, 100 µL injected

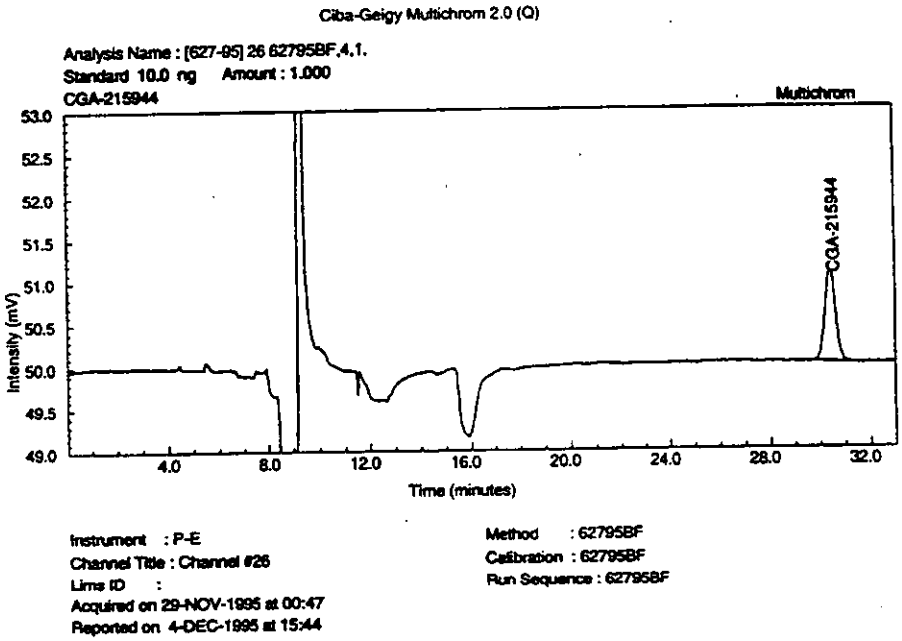


2. 0.05 ng/µL, 100 µL injected

FIGURE 5. TYPICAL CHROMATOGRAMS OF CGA-215944 STANDARD SOLUTIONS WITH COLUMN SWITCHING (Continued)

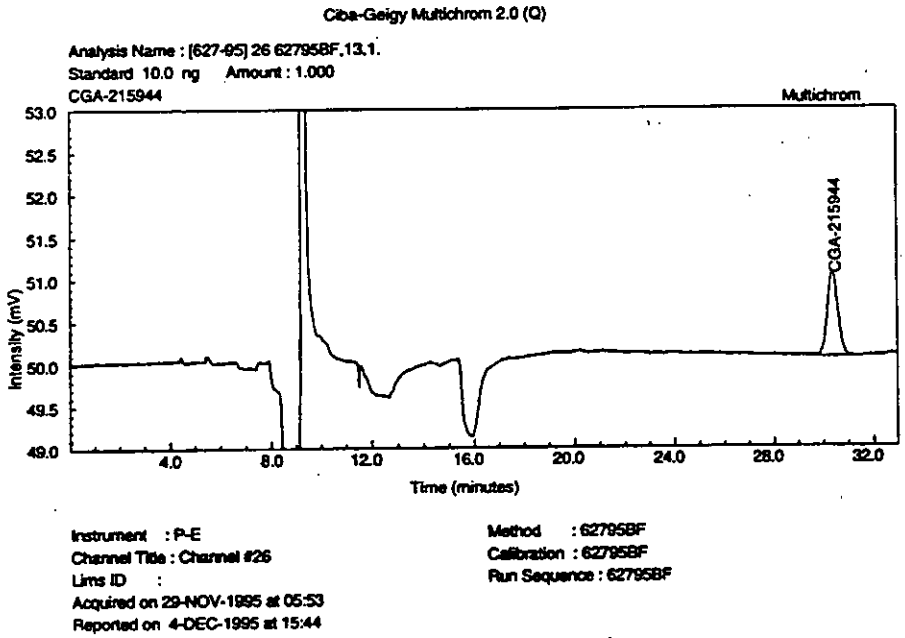


3. 0.05 ng/µL, 100 µL injected

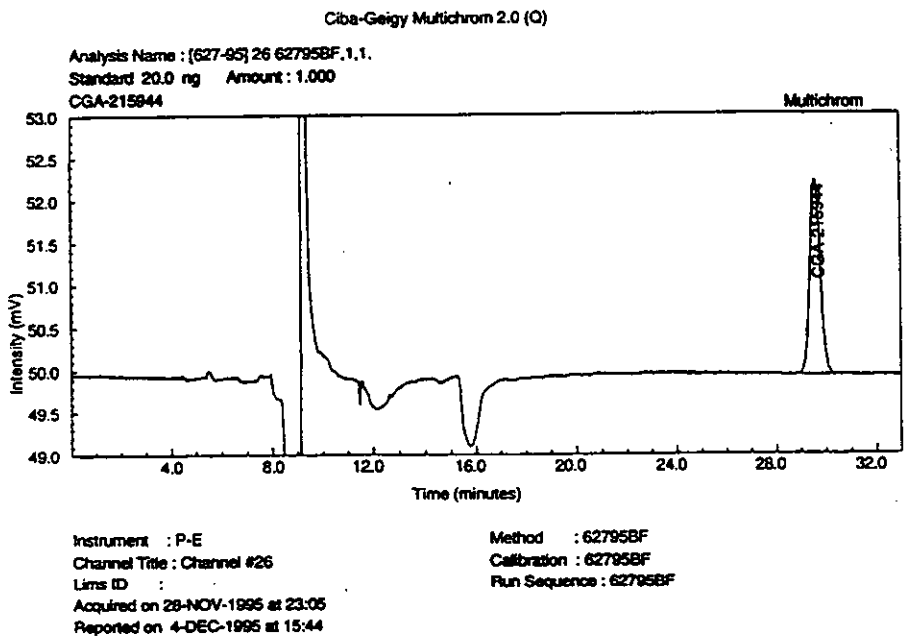


4. 0.1 ng/µL, 100 µL injected

FIGURE 5. TYPICAL CHROMATOGRAMS OF CGA-215944 STANDARD SOLUTIONS WITH COLUMN SWITCHING (Continued)

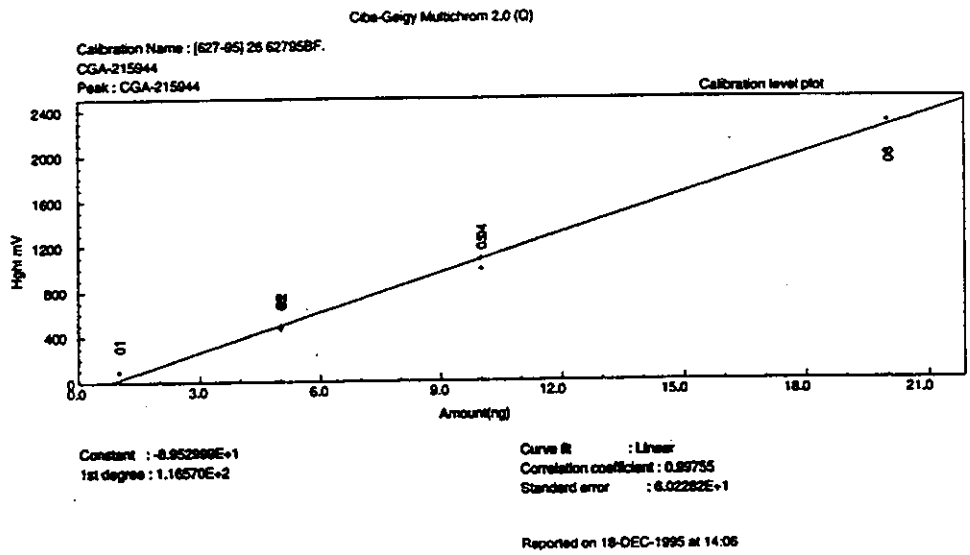


5. 0.1 ng/µL, 100 µL injected



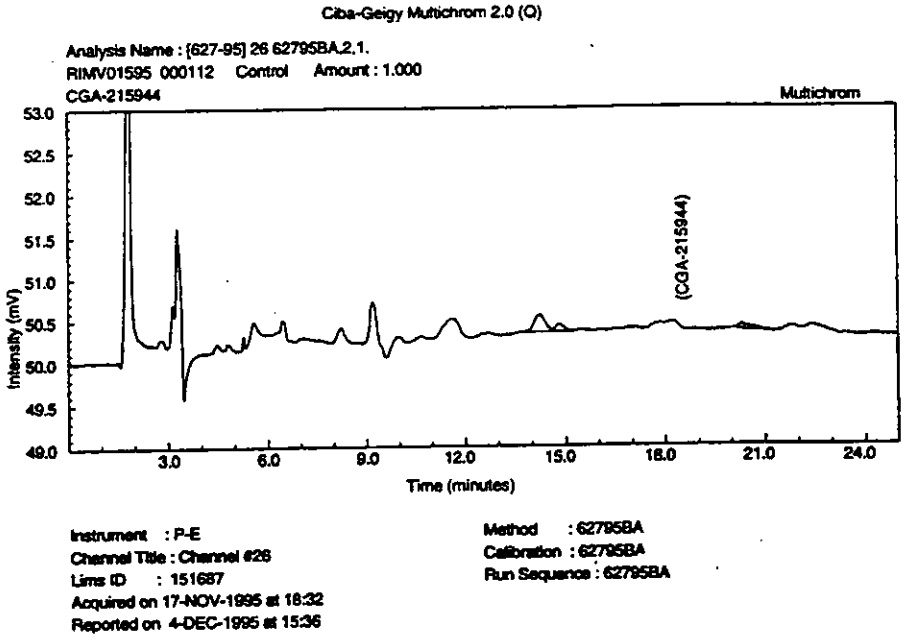
6. 0.2 ng/µL, 100 µL injected
Chromatograms from the analysis of tomatoes

FIGURE 6. TYPICAL CALIBRATION PLOT OF CGA-215944 STANDARD SOLUTIONS

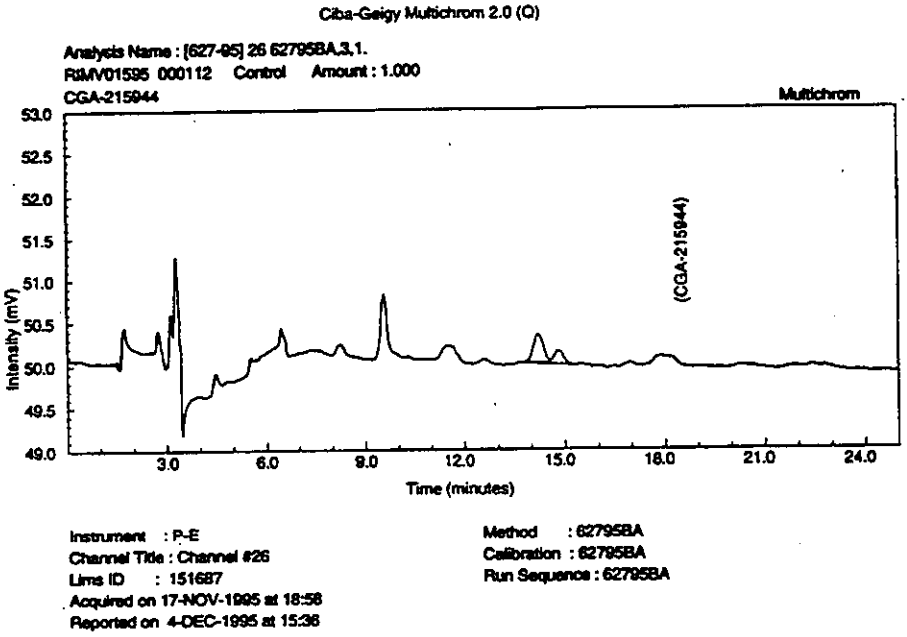


Curve from the analysis of tomatoes

FIGURE 7. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CUCUMBERS FOR CGA-215944

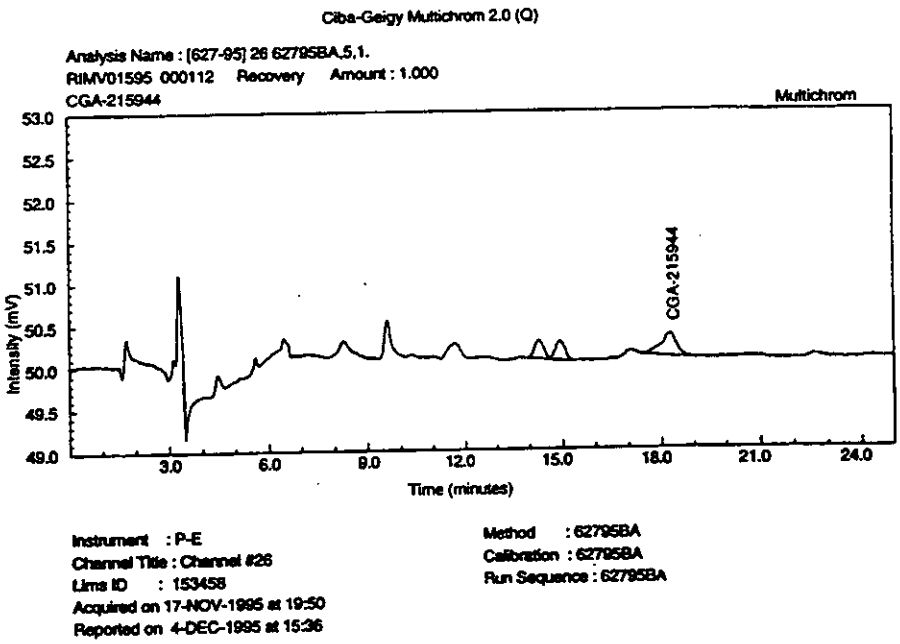


1. CC.CA, control, 100 mg injected, <2.0 ng found, <0.02 ppm CGA-215944 found

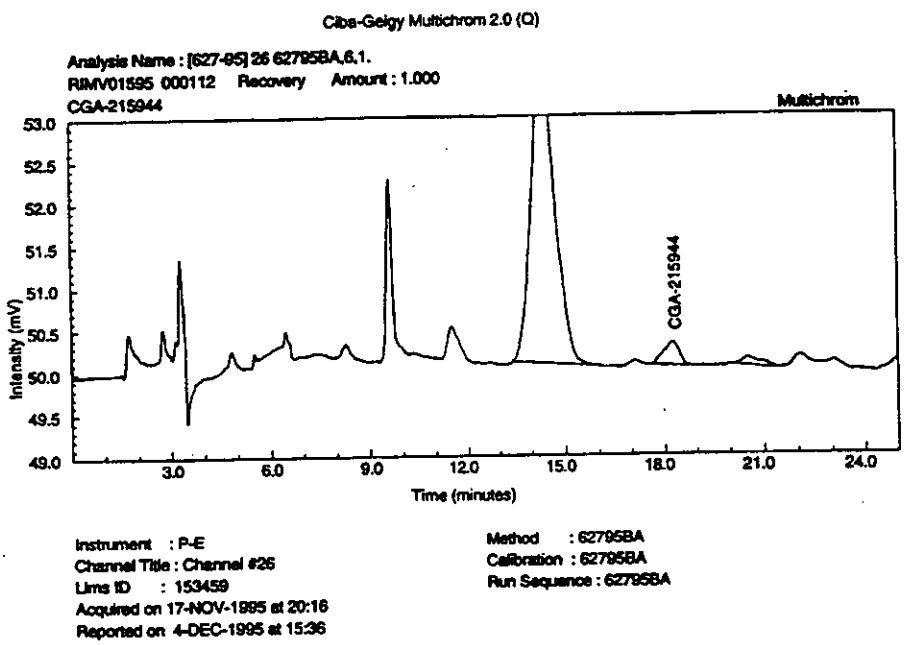


2. CC.CB, control, 100 mg injected, <2.0 ng found, <0.02 ppm CGA-215944 found

FIGURE 7. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CUCUMBERS FOR CGA-215944 (Continued)

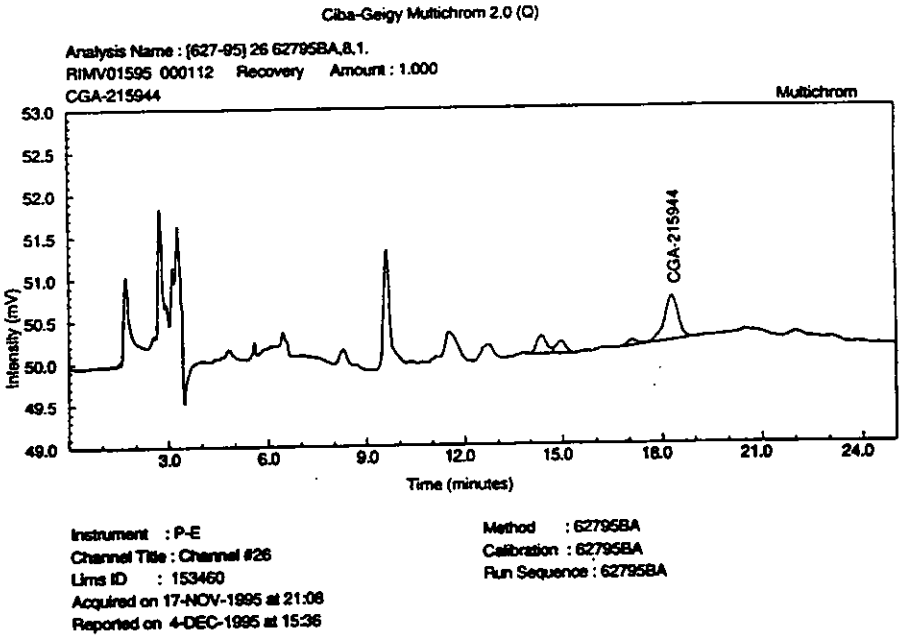


3. CC.02A, 0.02 ppm fortification, 100 mg injected, 2.36 ng CGA-215944 found, 0.024 ppm CGA-215944 found, 118% recovery

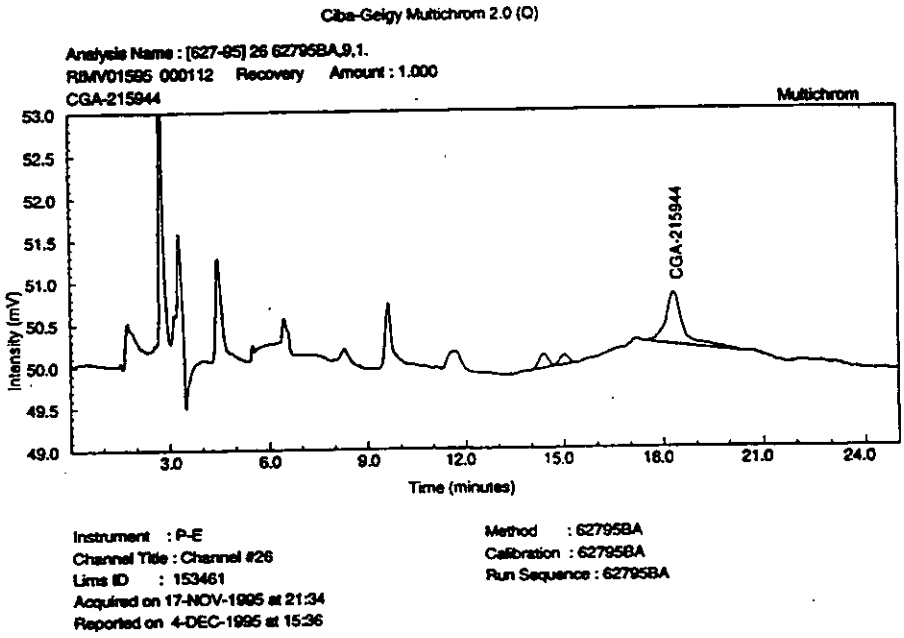


4. CC.02B, 0.02 ppm fortification, 100 mg injected, 2.43 ng CGA-215944 found, 0.024 ppm CGA-215944 found, 122% recovery

FIGURE 7. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CUCUMBERS FOR CGA-215944 (Continued)

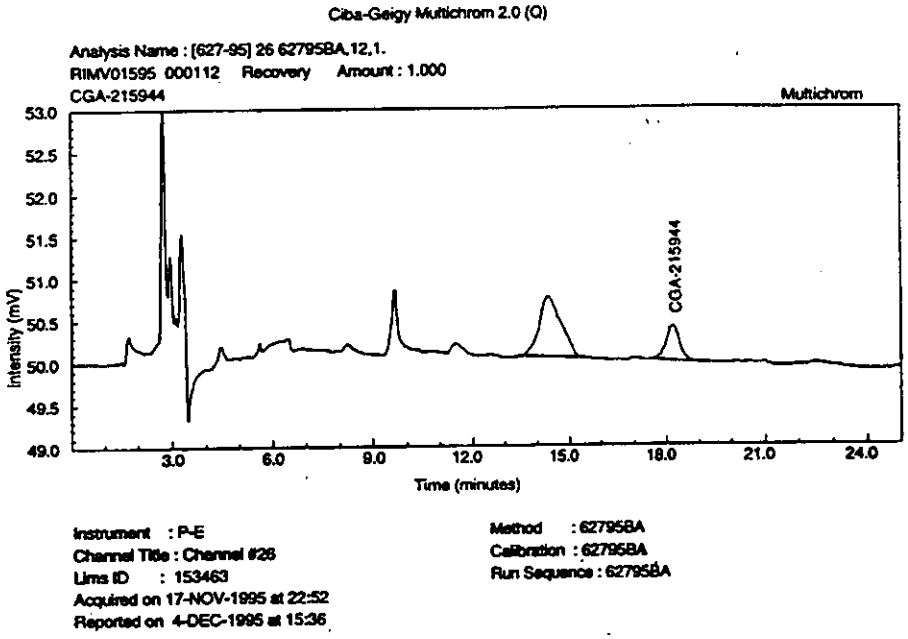


5. CC.05A, 0.05 ppm fortification, 99 mg injected, 4.11 ng CGA-215944 found, 0.041 ppm CGA-215944 found, 83% recovery

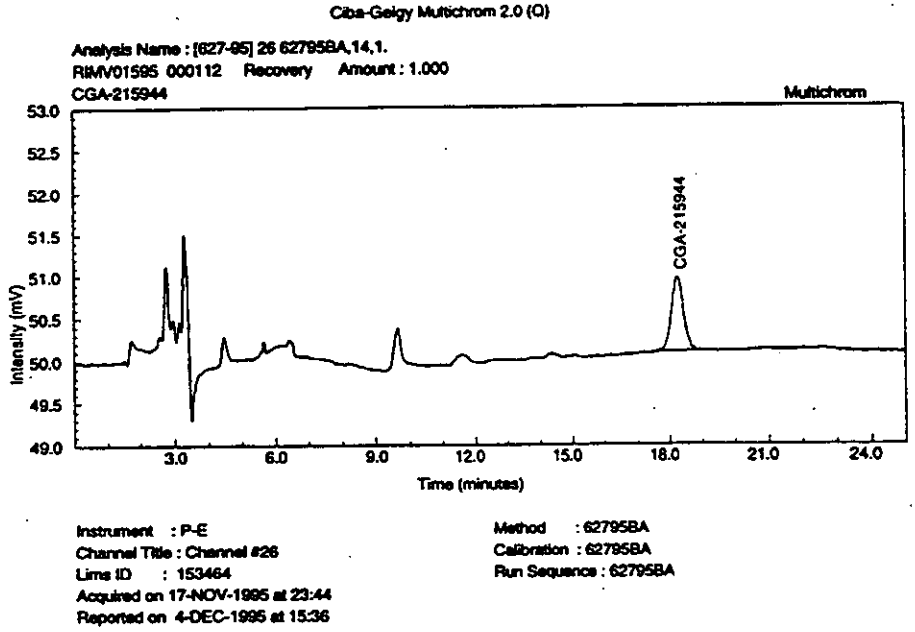


6. CC.05B, 0.05 ppm fortification, 101 mg injected, 4.66 ng CGA-215944 found, 0.046 ppm CGA-215944 found, 93% recovery

FIGURE 7. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CUCUMBERS FOR CGA-215944 (Continued)

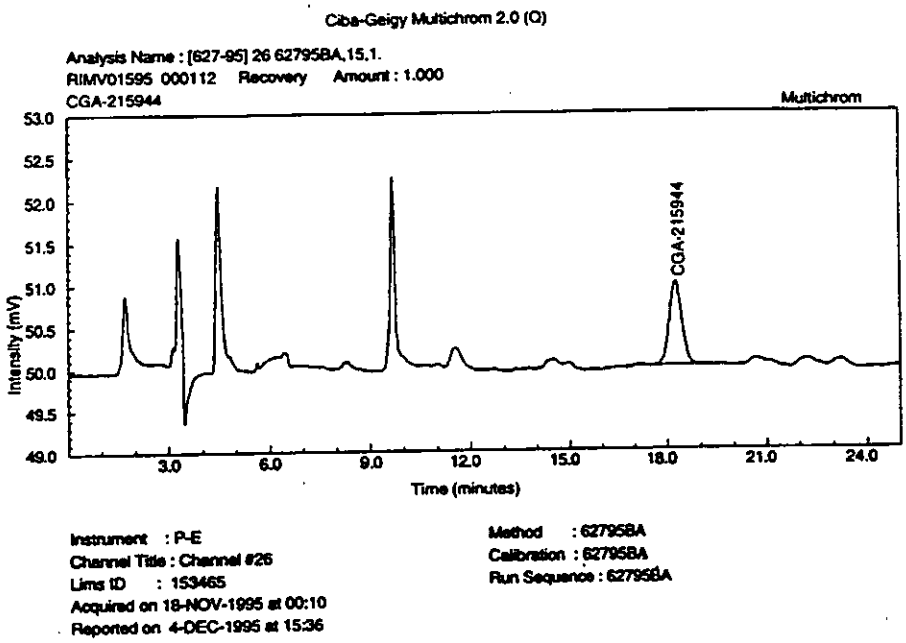


7. CC.10B, 0.10 ppm fortification, 50 mg injected, 3.35 ng CGA-215944 found, 0.067 ppm CGA-215944 found, 67% recovery



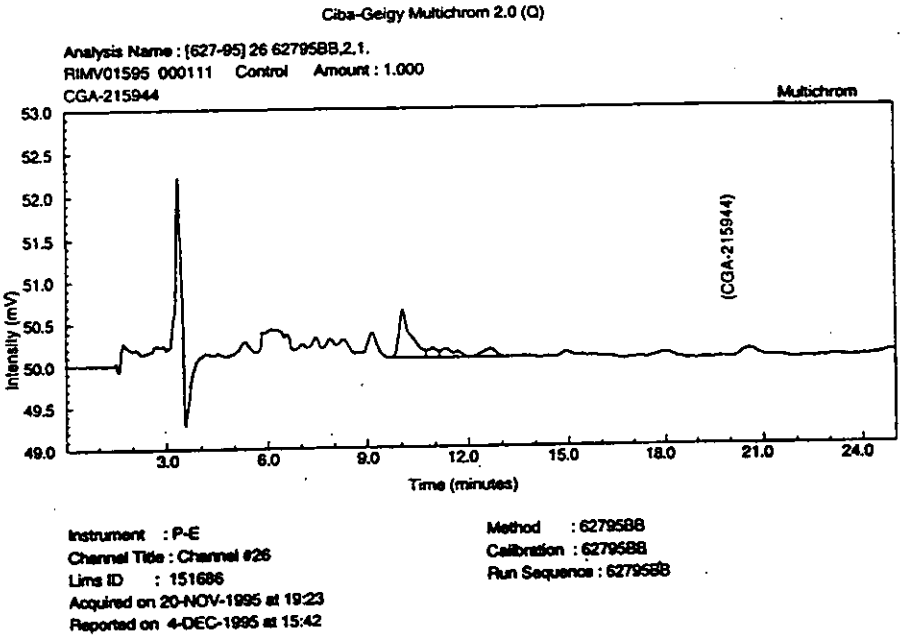
8. CC.20A, 0.20 ppm fortification, 50 mg injected, 6.40 ng CGA-215944 found, 0.13 ppm CGA-215944 found, 64% recovery

FIGURE 7. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CUCUMBERS FOR CGA-215944 (Continued)

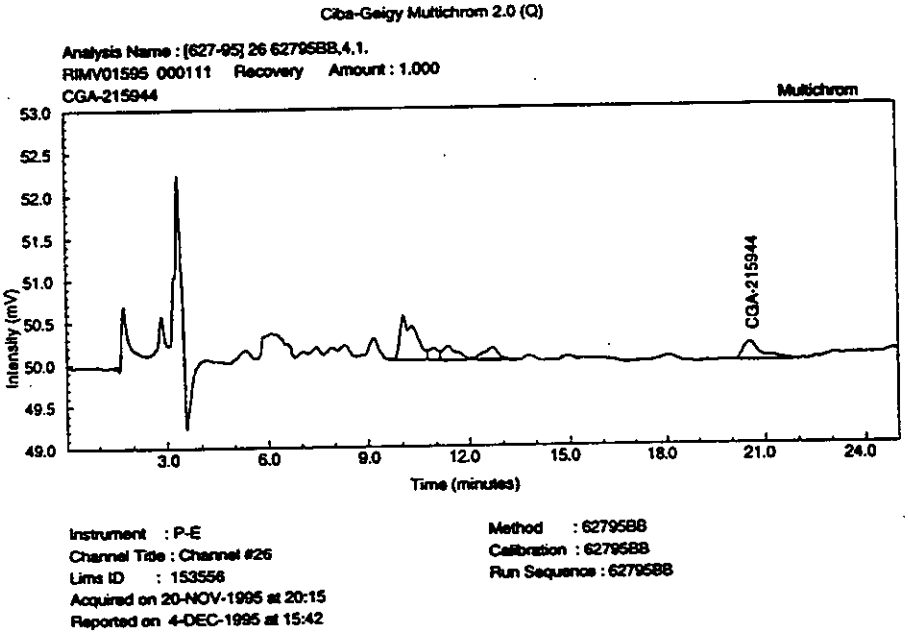


9. CC.20B, 0.20 ppm fortification, 50 mg injected, 7.19 ng CGA-215944 found, 0.14 ppm CGA-215944 found, 71% recovery

FIGURE 8. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CANTALOUPES FOR CGA-215944

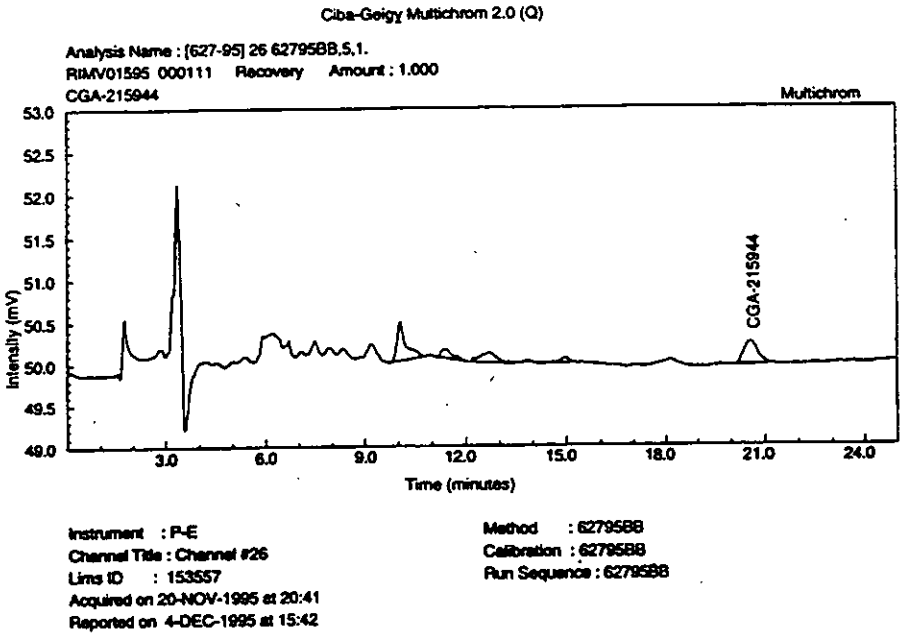


1. CN.C, control, 100 mg injected, <2.0 ng found, <0.02 ppm CGA-215944 found

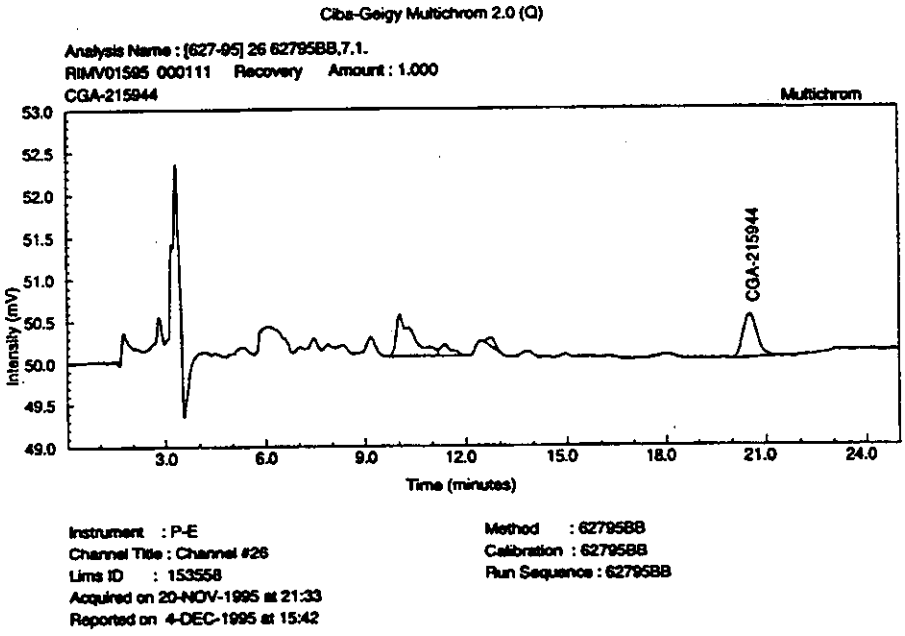


2. CN.02A, 0.02 ppm fortification, 100 mg injected, 2.02 ng CGA-215944 found, 0.020 ppm CGA-215944 found, 100% recovery

FIGURE 8. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CANTALOUPES FOR CGA-215944 (Continued)

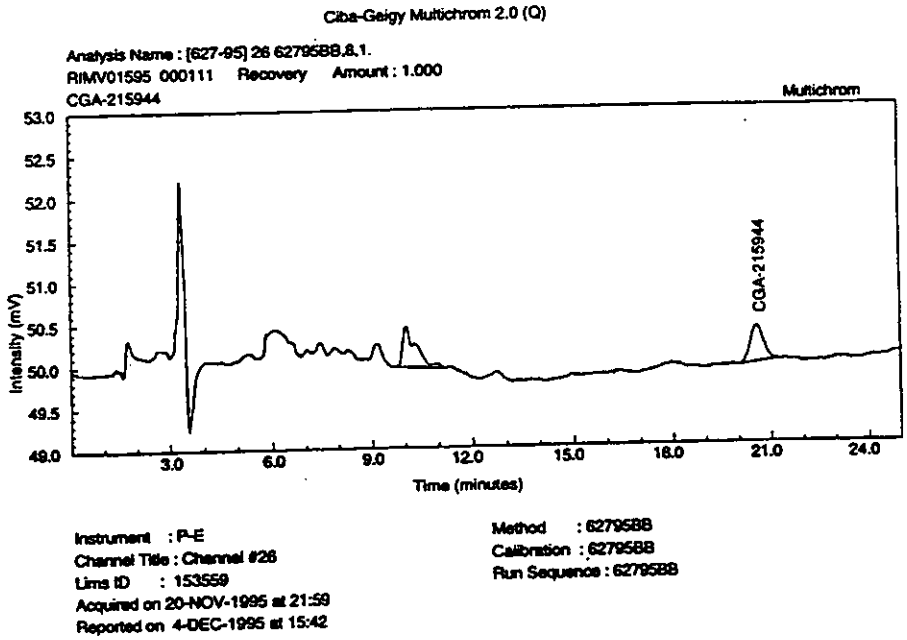


3. CN.02B, 0.02 ppm fortification, 101 mg injected, 2.46 ng CGA-215944 found, 0.024 ppm CGA-215944 found, 122% recovery

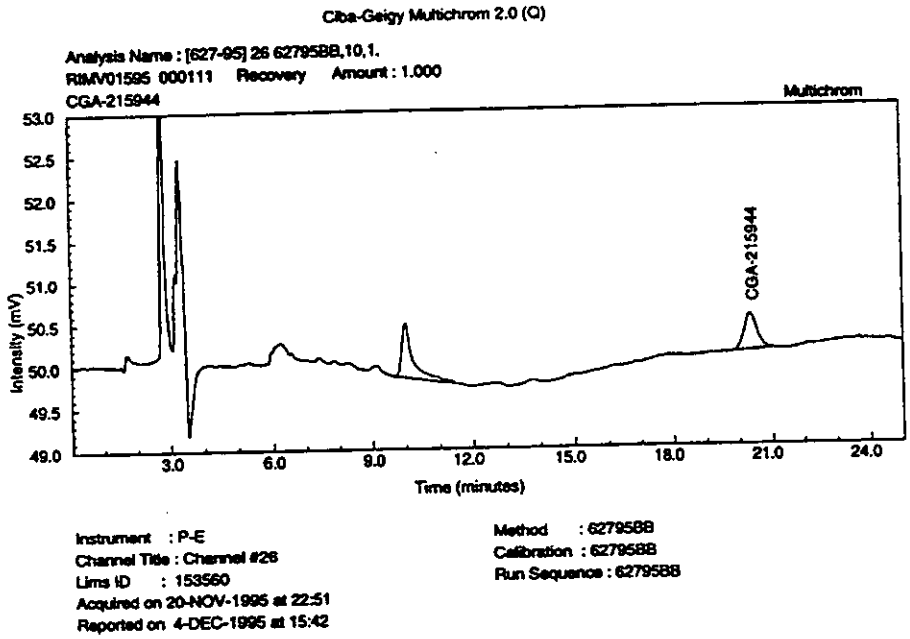


4. CN.05A, 0.05 ppm fortification, 100 mg injected, 4.29 ng CGA-215944 found, 0.043 ppm CGA-215944 found, 86% recovery

FIGURE 8. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CANTALOUPES FOR CGA-215944 (Continued)

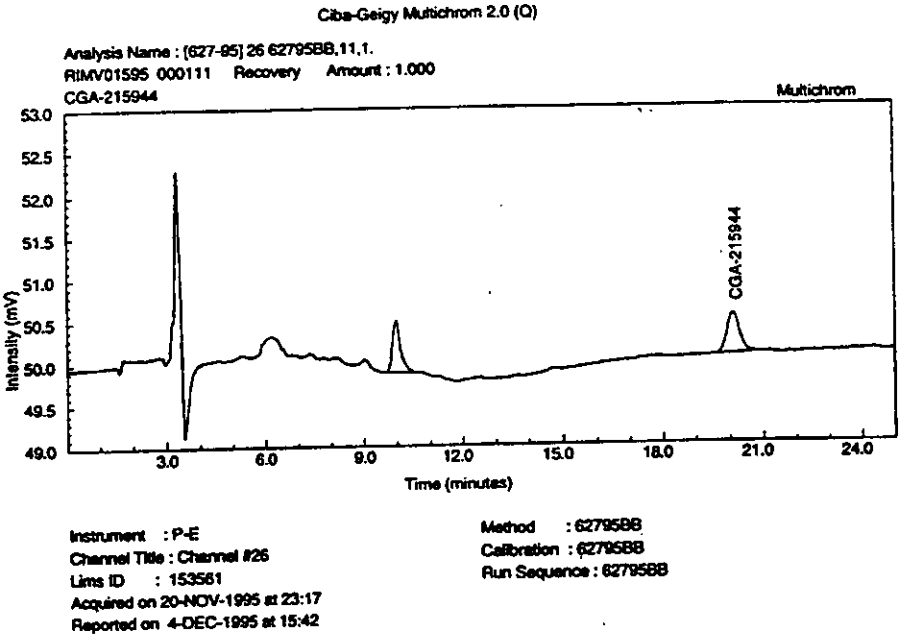


5. CN.05B, 0.05 ppm fortification, 100 mg injected, 3.68 ng CGA-215944 found, 0.037 ppm CGA-215944 found, 74% recovery

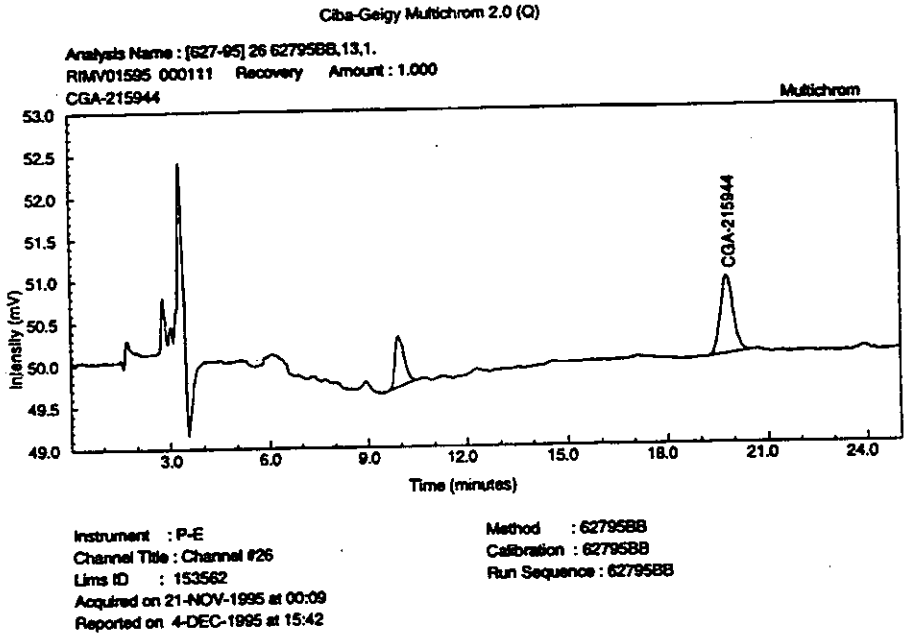


6. CN.10A, 0.10 ppm fortification, 50 mg injected, 3.65 ng CGA-215944 found, 0.073 ppm CGA-215944 found, 73% recovery

FIGURE 8. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CANTALOUPES FOR CGA-215944 (Continued)

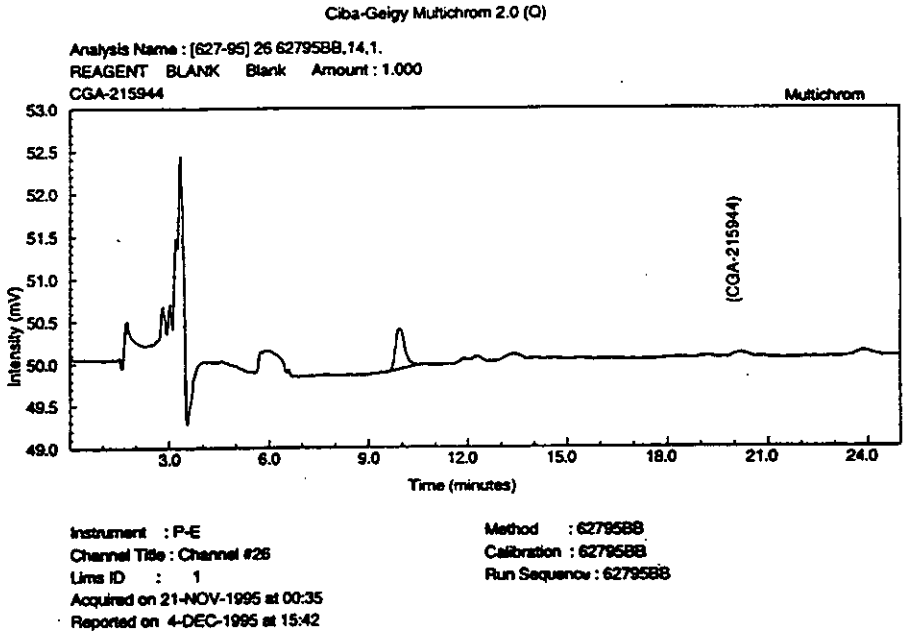


7. CN.10B, 0.10 ppm fortification, 50 mg injected, 3.97 ng CGA-215944 found, 0.080 ppm CGA-215944 found, 80% recovery



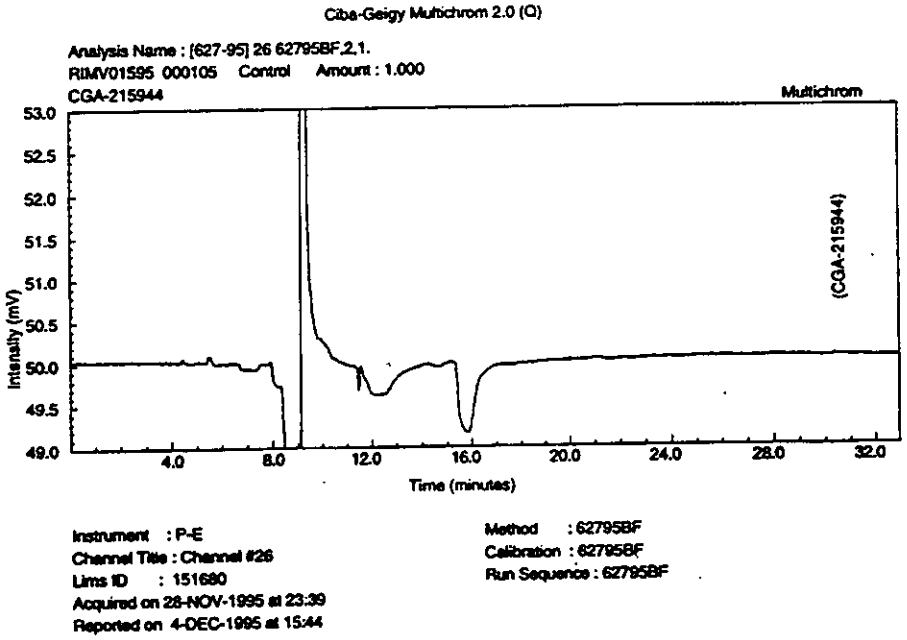
8. CN.20A, 0.20 ppm fortification, 50 mg injected, 7.26 ng CGA-215944 found, 0.15 ppm CGA-215944 found, 73% recovery

FIGURE 8. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CANTALOUPES FOR CGA-215944 (Continued)

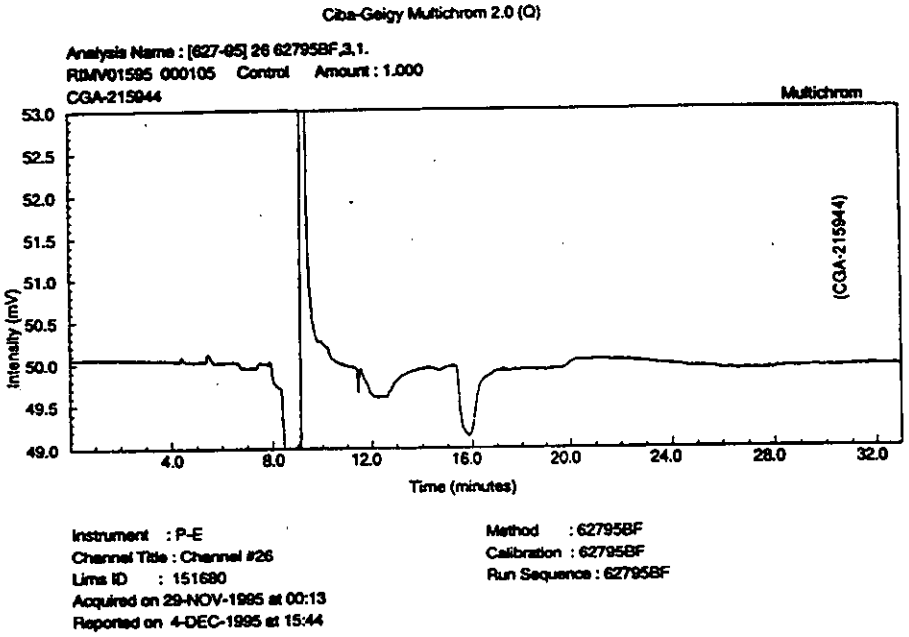


9. CN.RB, reagent blank, 100 mg injected, <2.0 ng found, <0.02 ppm CGA-215944 found

FIGURE 9. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF TOMATOES FOR CGA-215944

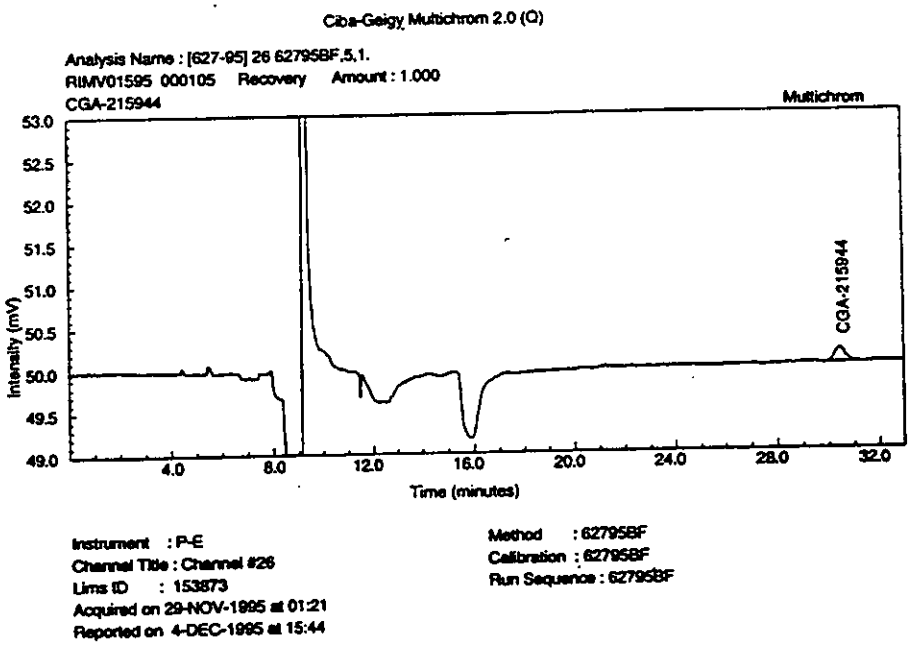


1. TM.CA, control, 101 mg injected, <2.0 ng found, <0.02 ppm CGA-215944 found

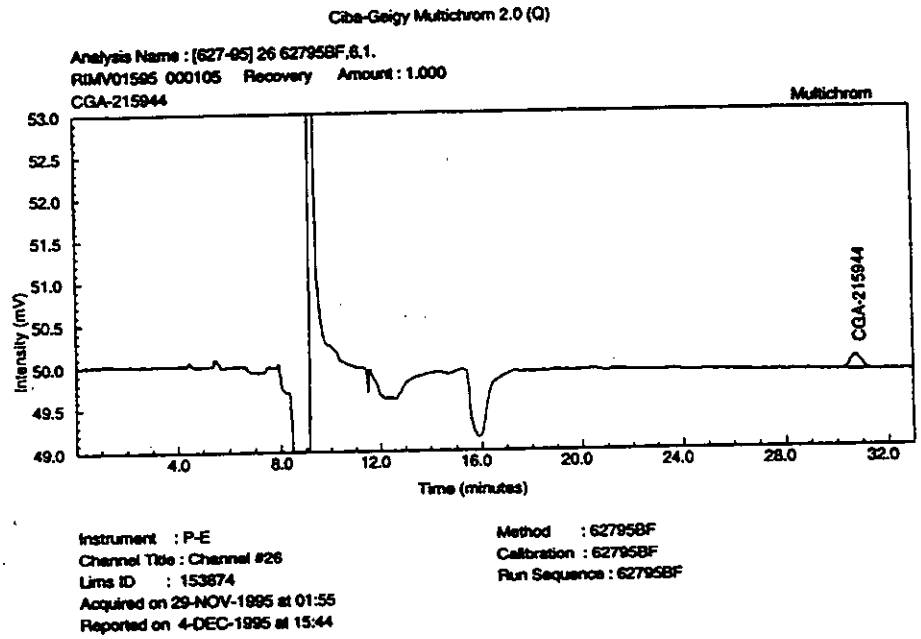


2. TM.CB, control, 99 mg injected, <2.0 ng found, <0.02 ppm CGA-215944 found

FIGURE 9. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF TOMATOES FOR CGA-215944 (Continued)

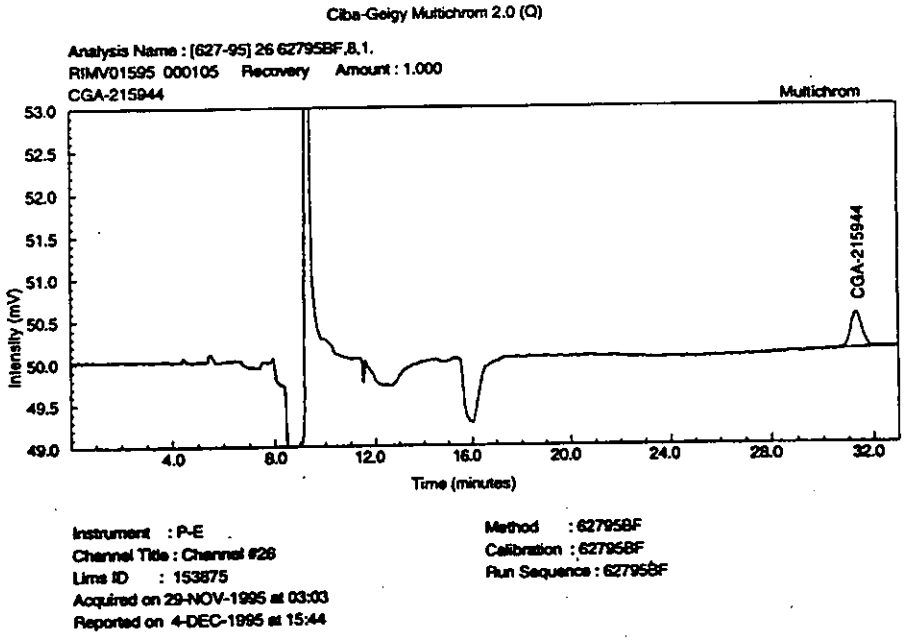


3. TM.02A, 0.02 ppm fortification, 99 mg injected, 2.30 ng CGA-215944 found, 0.023 ppm CGA-215944 found, 116% recovery

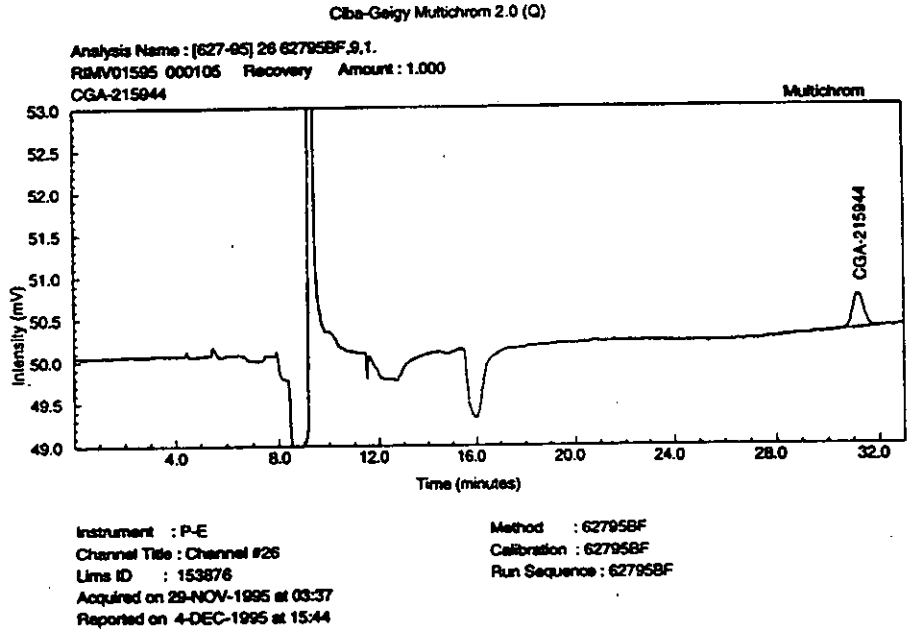


4. TM.02B, 0.02 ppm fortification, 100 mg injected, 2.29 ng CGA-215944 found, 0.023 ppm CGA-215944 found, 114% recovery

FIGURE 9. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF TOMATOES FOR CGA-215944 (Continued)

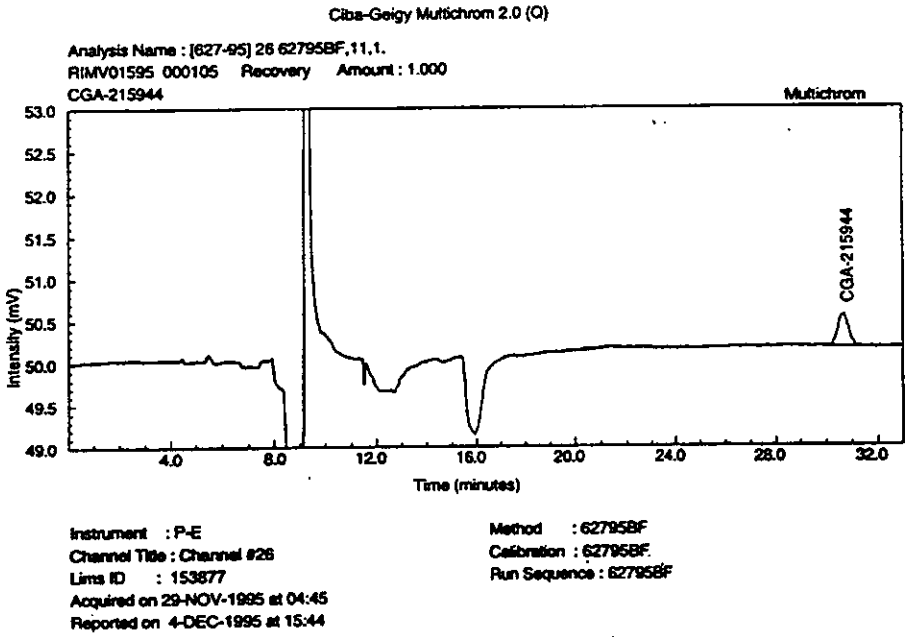


5. TM.05A, 0.05 ppm fortification, 100 mg injected, 4.41 ng CGA-215944 found, 0.044 ppm CGA-215944 found, 88% recovery

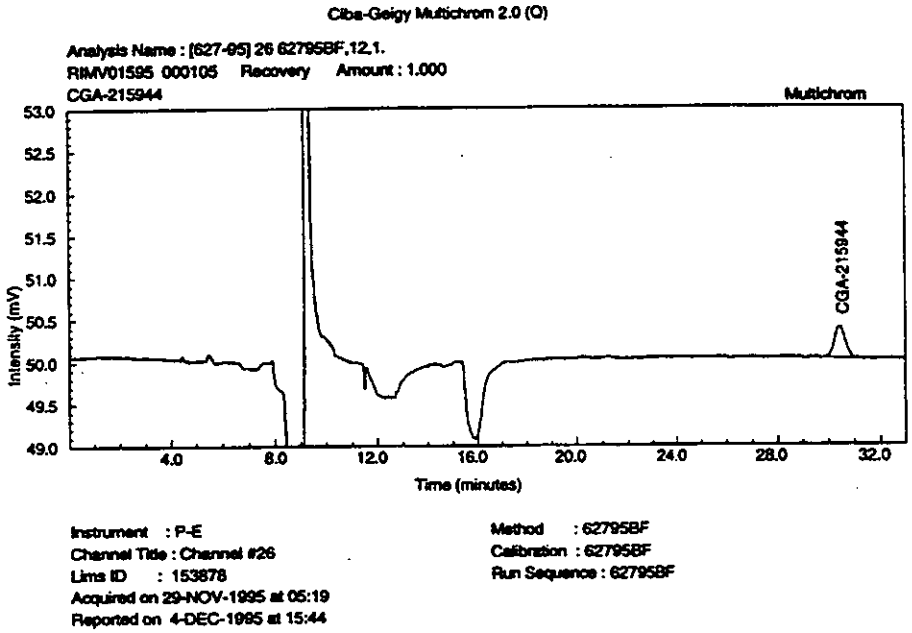


6. TM.05B, 0.05 ppm fortification, 100 mg injected, 4.32 ng CGA-215944 found, 0.043 ppm CGA-215944 found, 86% recovery

FIGURE 9. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF TOMATOES FOR CGA-215944 (Continued)

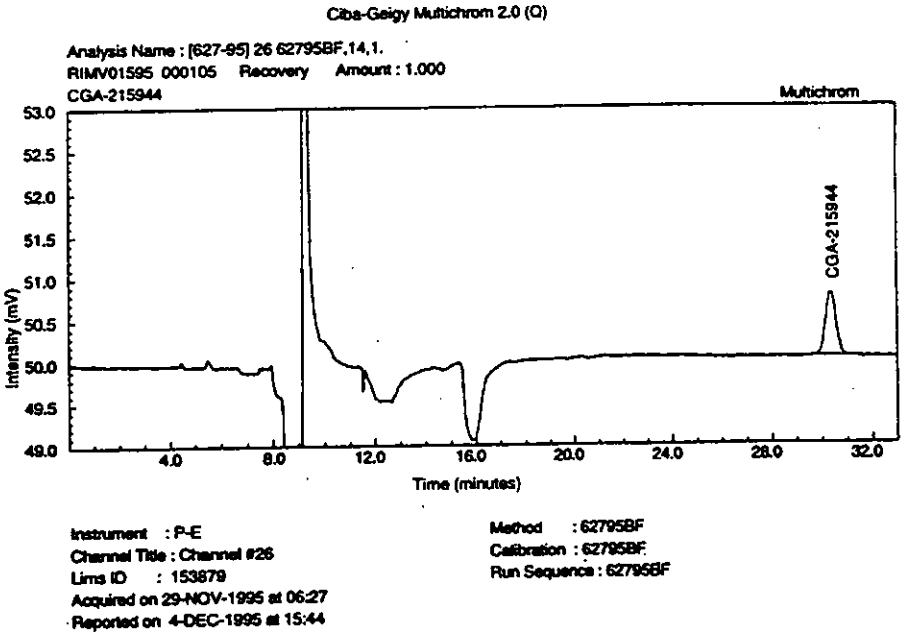


7. TM.10A, 0.10 ppm fortification, 50 mg injected, 3.99 ng CGA-215944 found, 0.080 ppm CGA-215944 found, 80% recovery

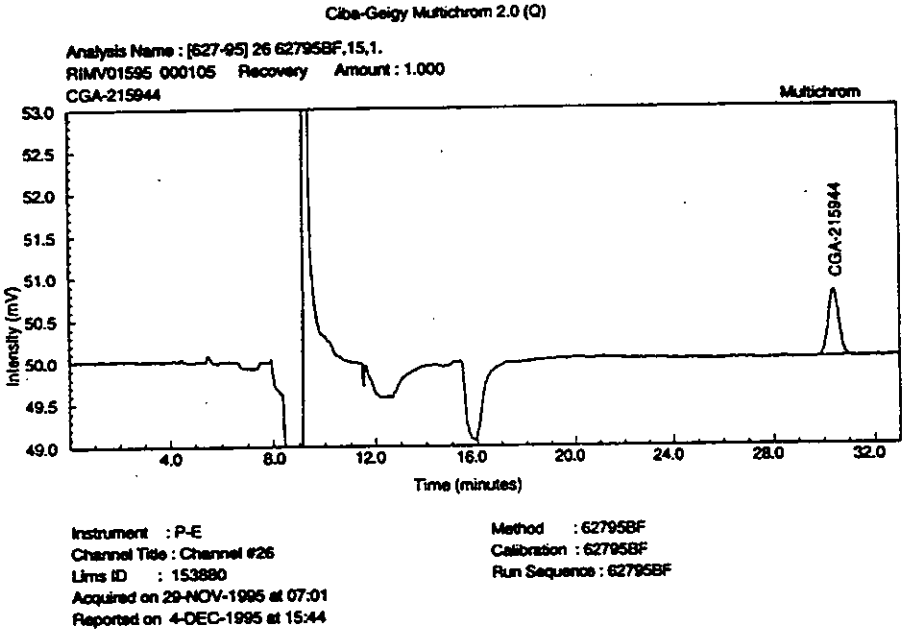


8. TM.10B, 0.10 ppm fortification, 50 mg injected, 4.02 ng CGA-215944 found, 0.080 ppm CGA-215944 found, 80% recovery

FIGURE 9. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF TOMATOES FOR CGA-215944 (Continued)

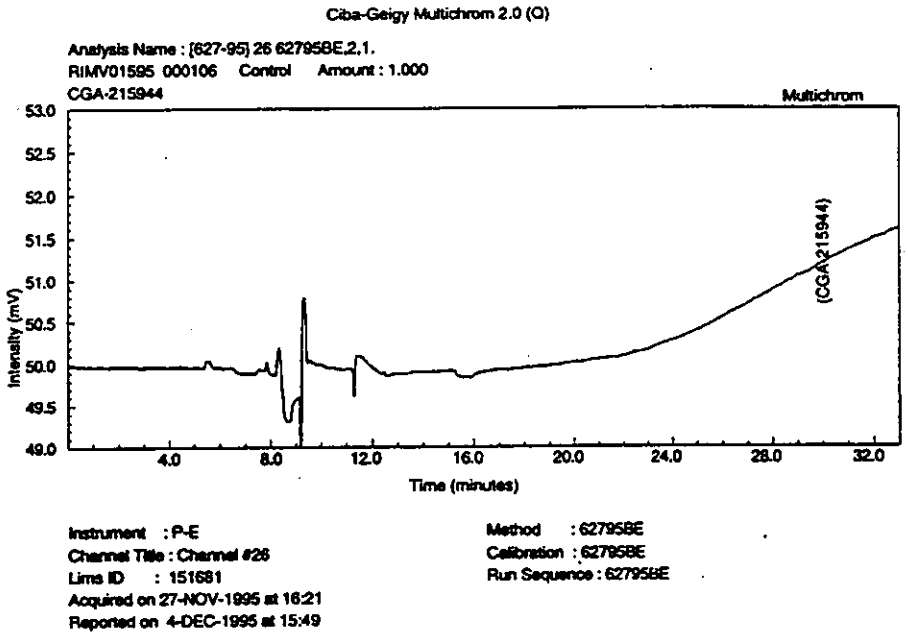


9. TM.20A, 0.20 ppm fortification, 50 mg injected, 7.21 ng CGA-215944 found, 0.14 ppm CGA-215944 found, 72% recovery

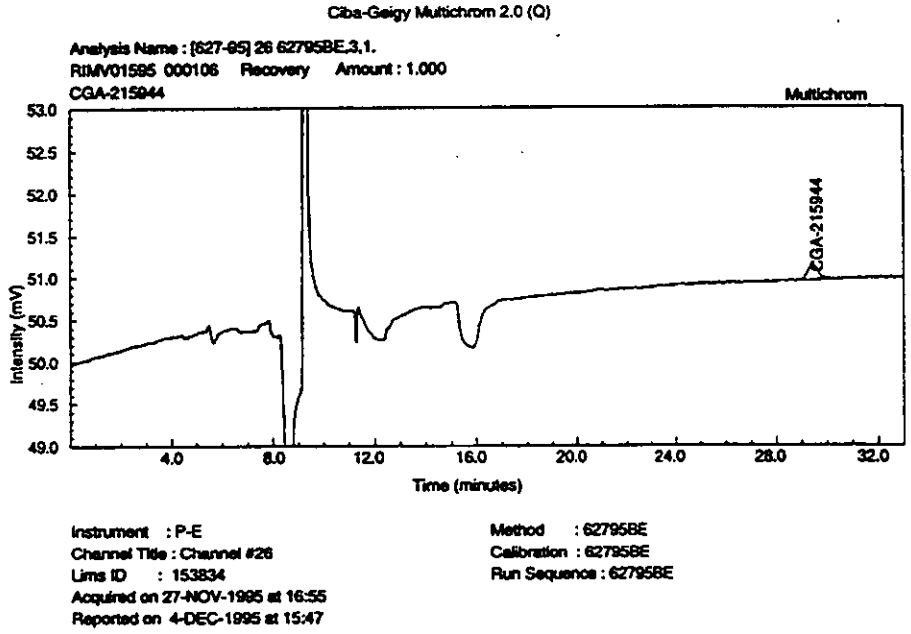


10. TM.20B, 0.20 ppm fortification, 50 mg injected, 7.48 ng CGA-215944 found, 0.15 ppm CGA-215944 found, 74% recovery

FIGURE 10. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF TOMATO WET POMACE FOR CGA-215944

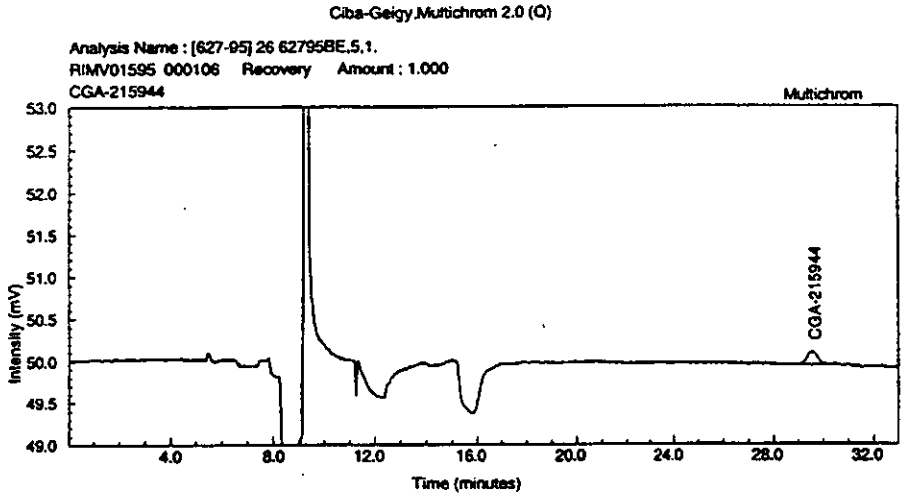


1. TW.CA, control, 99 mg injected, <2.0 ng found, <0.02 ppm CGA-215944 found

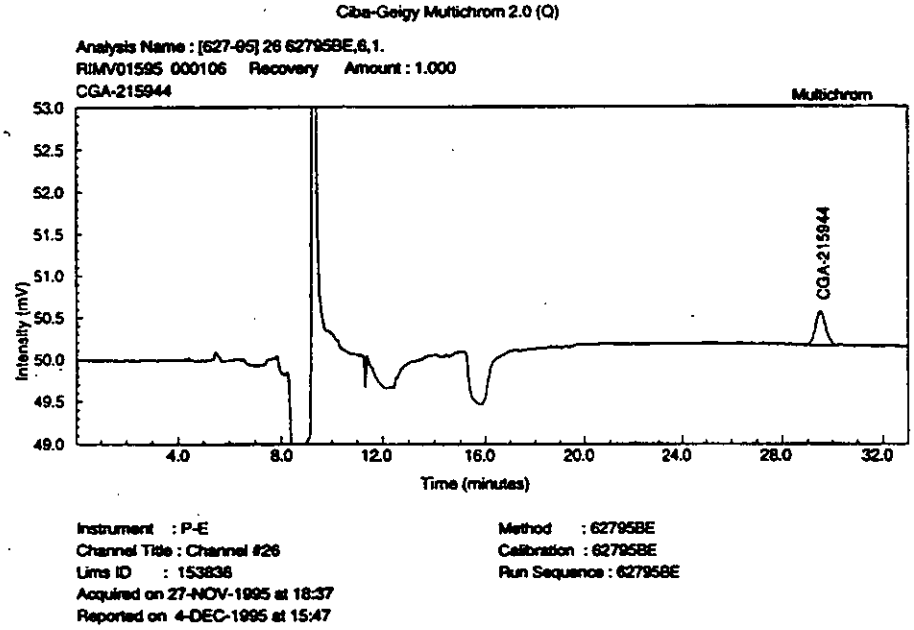


2. TW.02A, 0.02 ppm fortification, 99 mg injected, 2.24 ng CGA-215944 found, 0.023 ppm CGA-215944 found, 113% recovery

FIGURE 10. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF TOMATO WET POMACE FOR CGA-215944 (Continued)

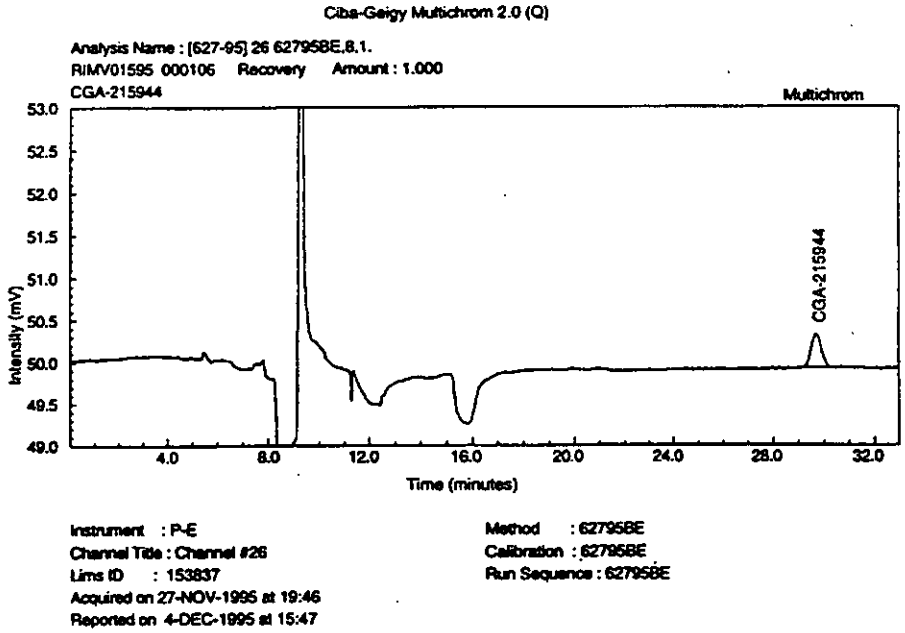


3. TW.02B, 0.02 ppm fortification, 99 mg injected, 2.06 ng CGA-215944 found, 0.021 ppm CGA-215944 found, 103% recovery

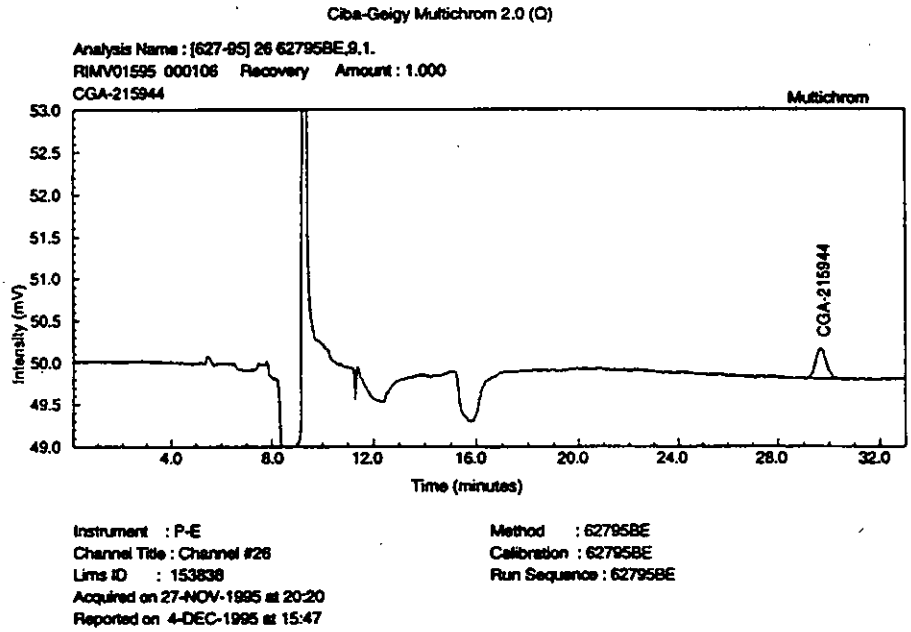


4. TW.05A, 0.05 ppm fortification, 100 mg injected, 4.32 ng CGA-215944 found, 0.043 ppm CGA-215944 found, 87% recovery

FIGURE 10. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF TOMATO WET POMACE FOR CGA-215944 (Continued)

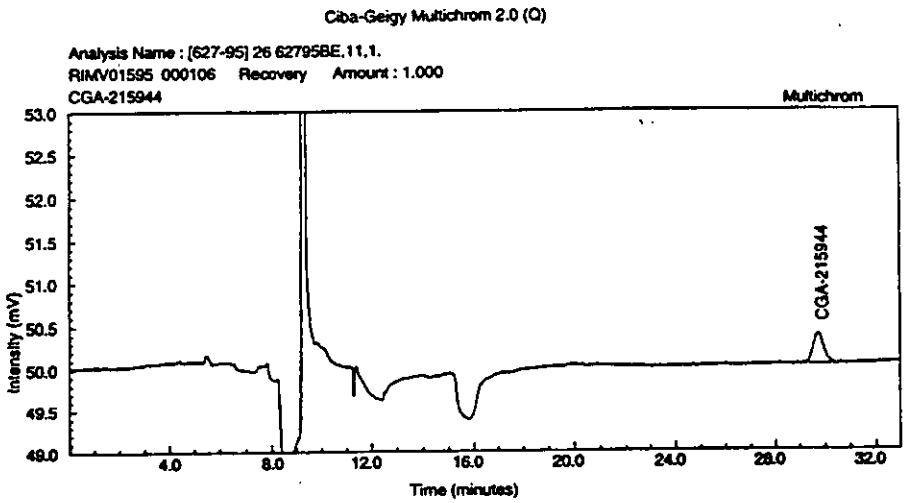


5. TW.05B, 0.05 ppm fortification, 99 mg injected, 4.23 ng CGA-215944 found, 0.043 ppm CGA-215944 found, 85% recovery

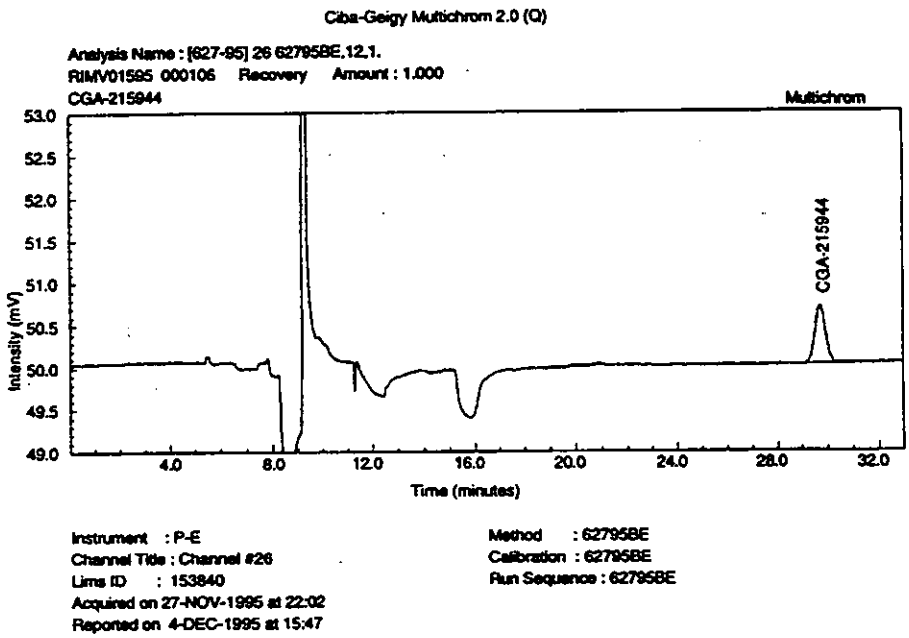


6. TW.10A, 0.10 ppm fortification, 50 mg injected, 3.93 ng CGA-215944 found, 0.079 ppm CGA-215944 found, 79% recovery

FIGURE 10. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF TOMATO WET POMACE FOR CGA-215944 (Continued)

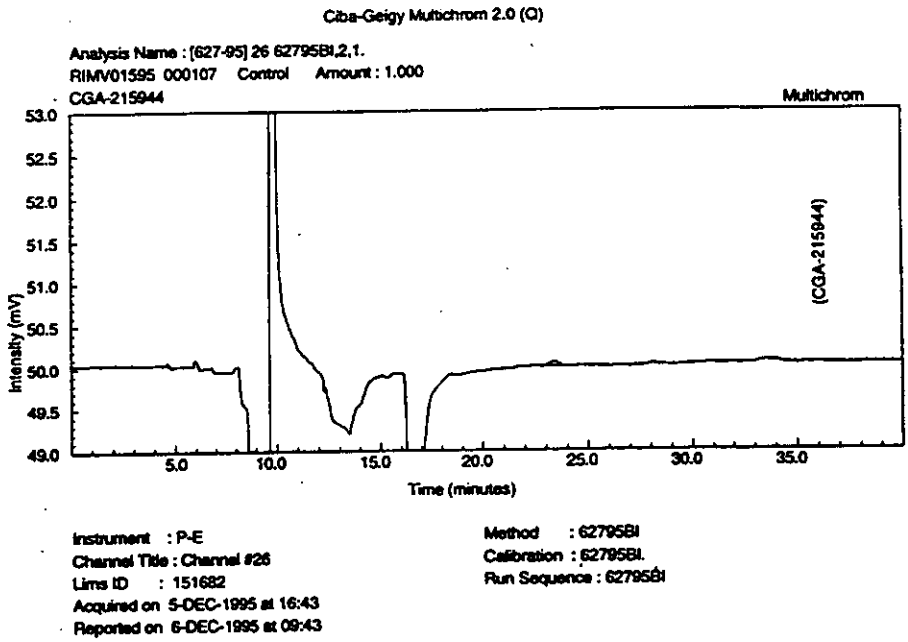


7. TW.10B, 0.10 ppm fortification, 50 mg injected, 3.88 ng CGA-215944 found, 0.077 ppm CGA-215944 found, 77% recovery

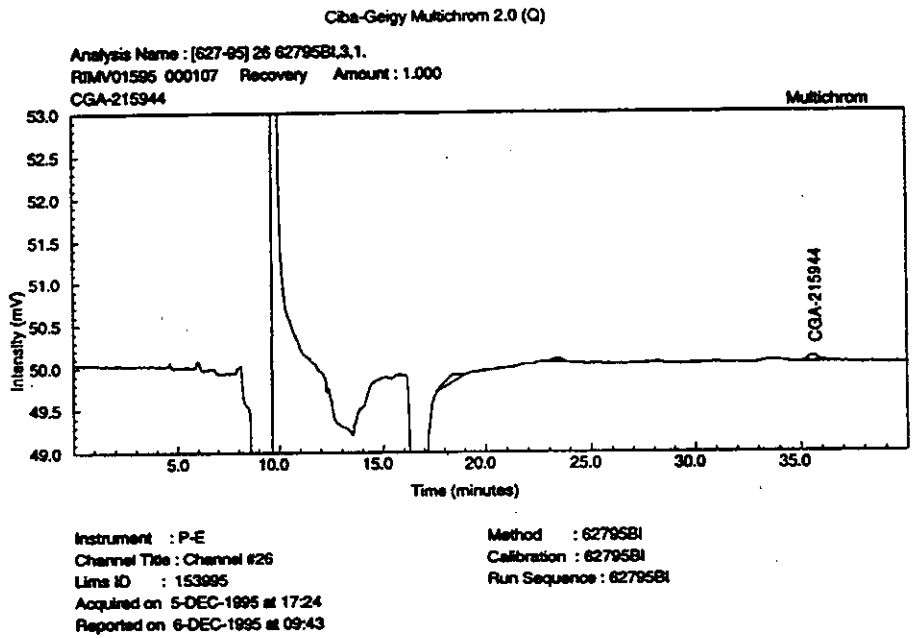


7. TW.20A, 0.20 ppm fortification, 50 mg injected, 6.79 ng CGA-215944 found, 0.14 ppm CGA-215944 found, 68% recovery

FIGURE 11. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF TOMATO DRY POMACE FOR CGA-215944

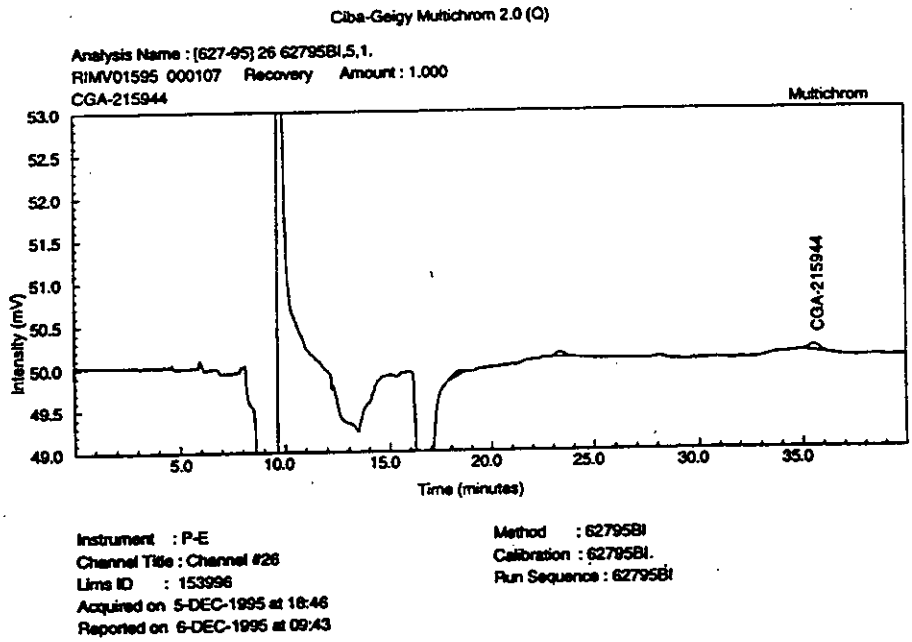


1. TD.CA, control, 50 mg injected, <1.0 ng found, <0.02 ppm CGA-215944 found

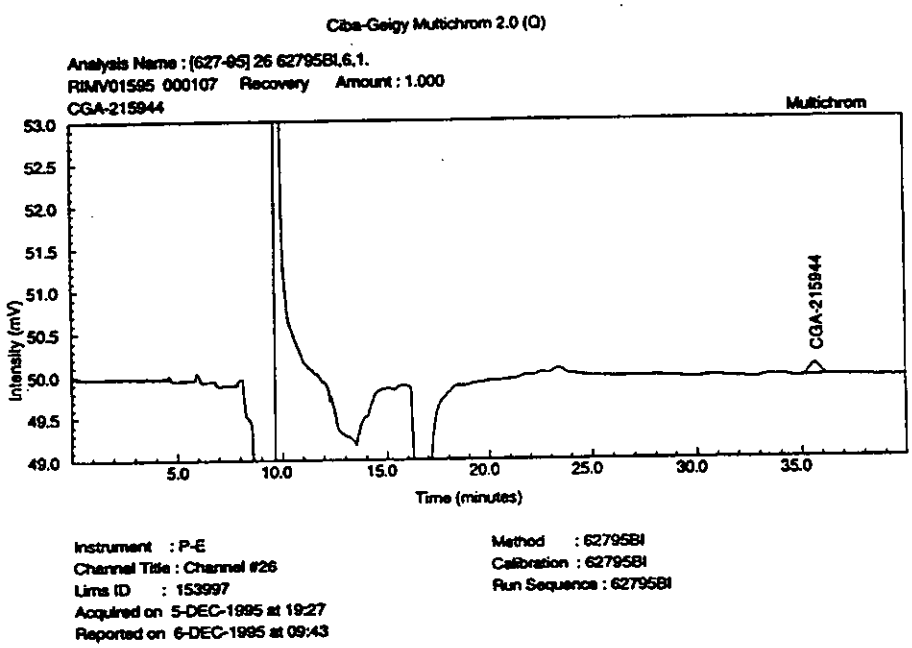


2. TD.02A, 0.02 ppm fortification, 50 mg injected, 0.98 ng CGA-215944 found, 0.019 ppm CGA-215944 found, 97% recovery

FIGURE 11. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF TOMATO DRY POMACE FOR CGA-215944 (Continued)

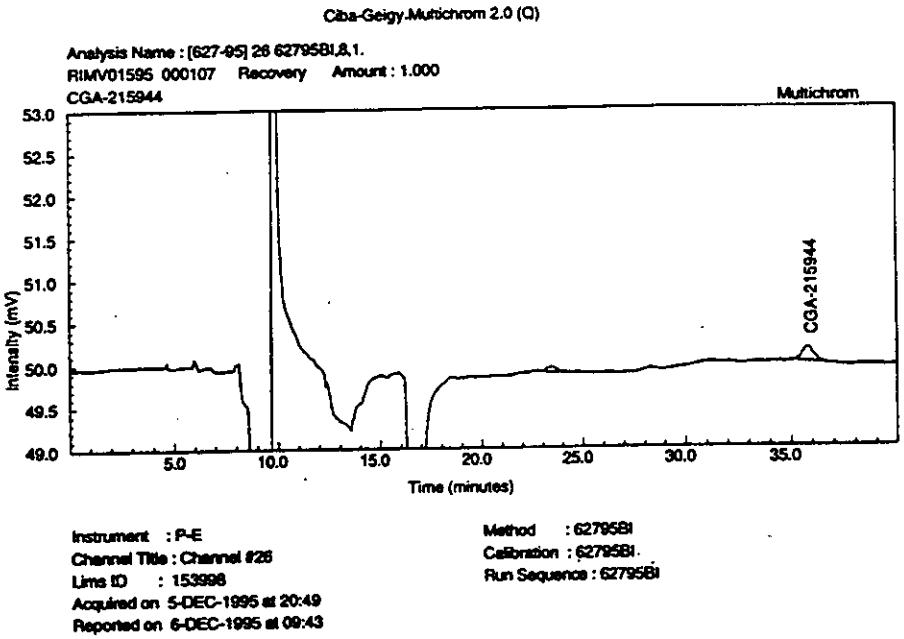


3. TD.02B, 0.02 ppm fortification, 51 mg injected, 0.99 ng CGA-215944 found, 0.020 ppm CGA-215944 found, 98% recovery

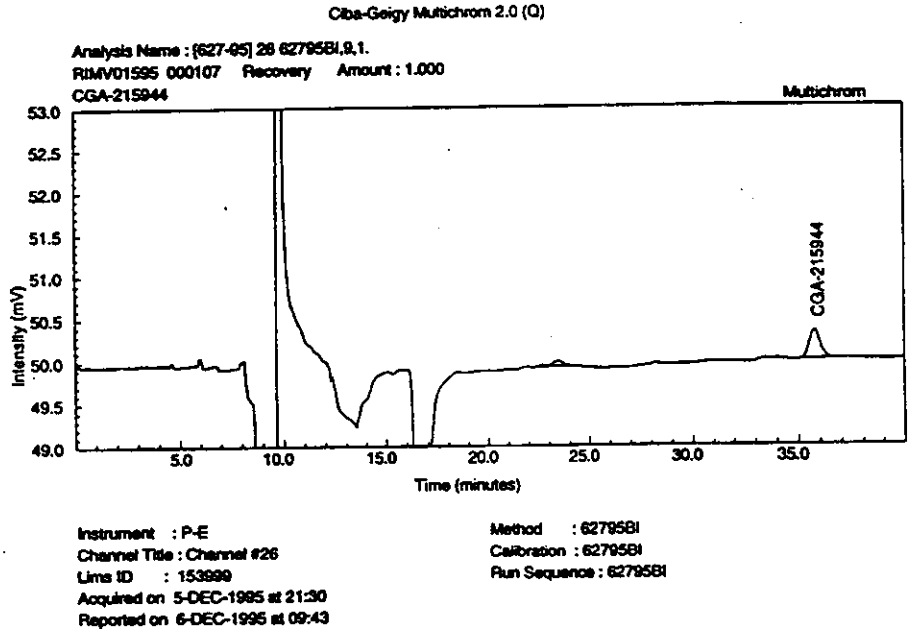


4. TD.05A, 0.05 ppm fortification, 50 mg injected, 1.73 ng CGA-215944 found, 0.035 ppm CGA-215944 found, 70% recovery

FIGURE 11. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF TOMATO DRY POMACE FOR CGA-215944 (Continued)

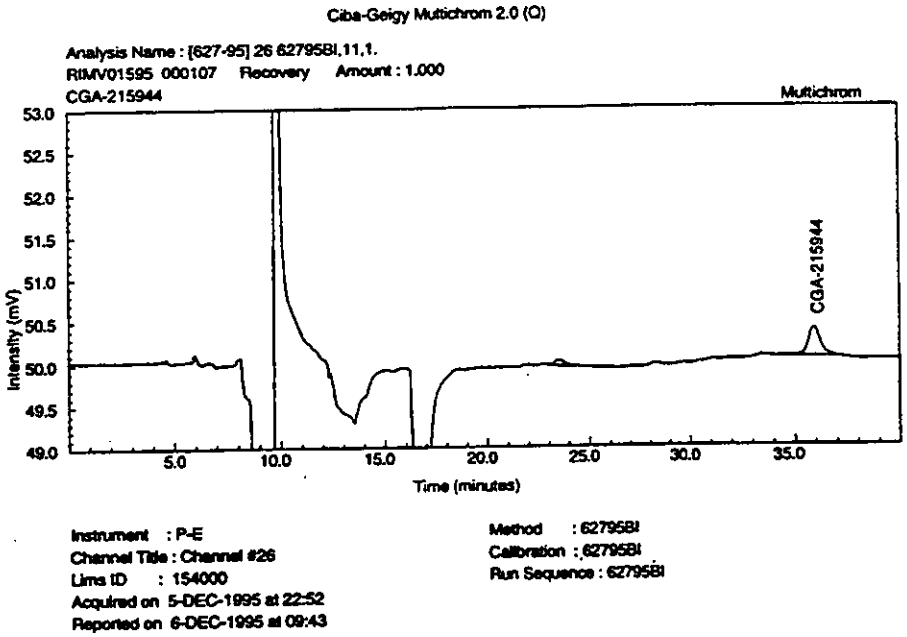


5. TD.05B, 0.05 ppm fortification, 50 mg injected, 2.09 ng CGA-215944 found, 0.042 ppm CGA-215944 found, 84% recovery

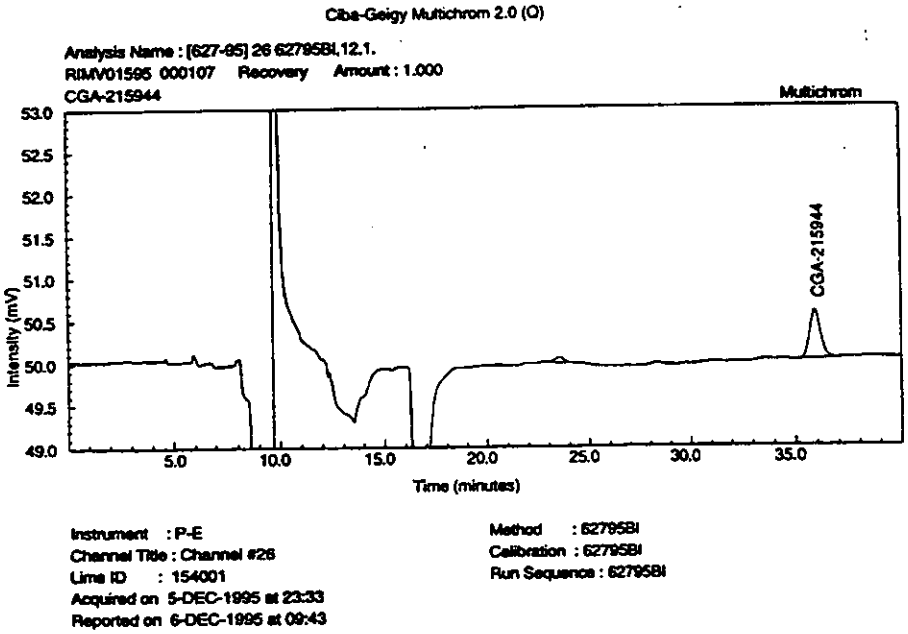


6. TD.10A, 0.10 ppm fortification, 50 mg injected, 3.99 ng CGA-215944 found, 0.081 ppm CGA-215944 found, 81% recovery

FIGURE 11. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF TOMATO DRY POMACE FOR CGA-215944 (Continued)

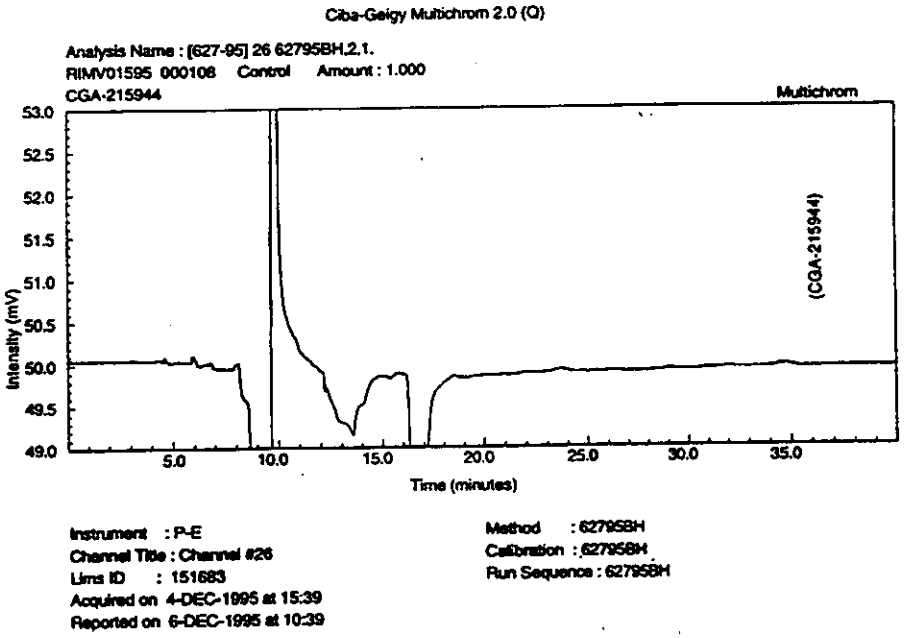


7. TD.10B, 0.10 ppm fortification, 50 mg injected, 3.98 ng CGA-215944 found, 0.079 ppm CGA-215944 found, 79% recovery

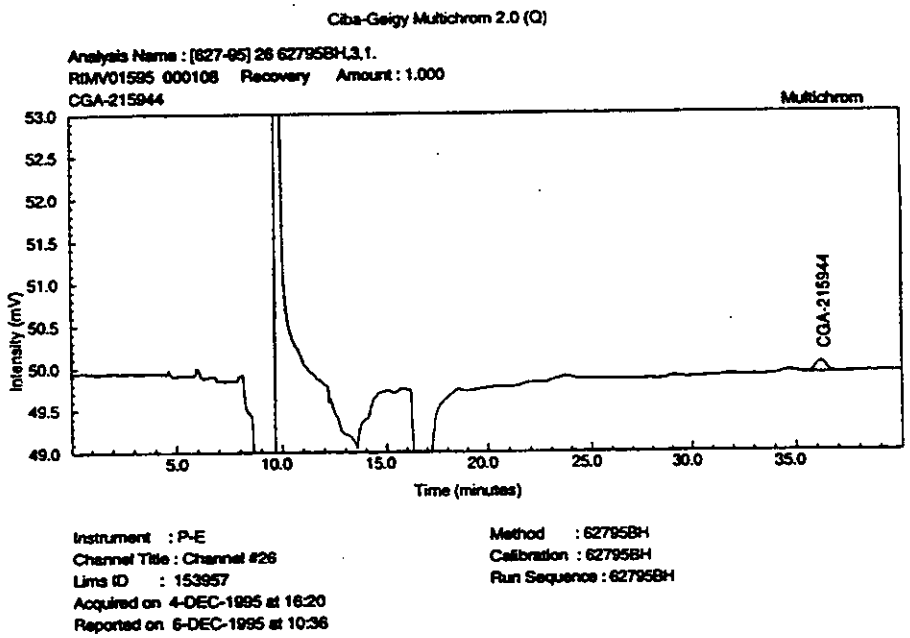


8. TD.20A, 0.20 ppm fortification, 50 mg injected, 6.72 ng CGA-215944 found, 0.13 ppm CGA-215944 found, 67% recovery

FIGURE 12. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF PEPPERS FOR CGA-215944

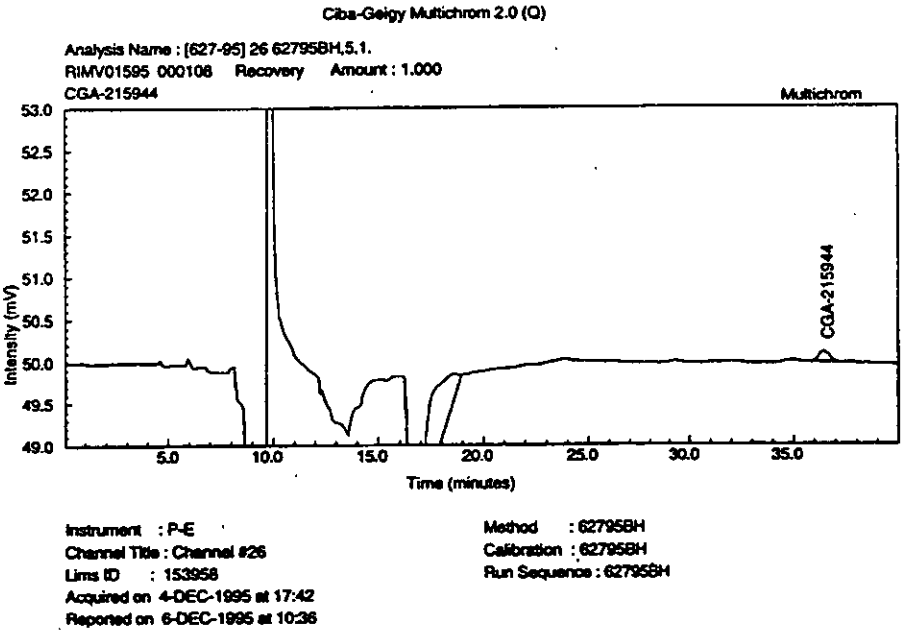


1. PP.CA, control, 100 mg injected, <2.0 ng found, <0.02 ppm CGA-215944 found

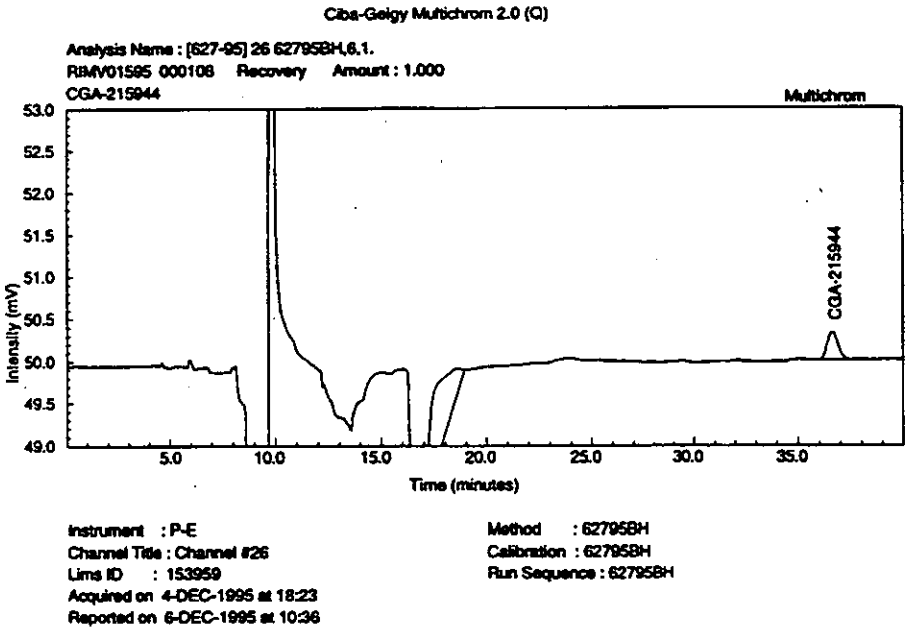


2. PP.02A, 0.02 ppm fortification, 99 mg injected, 1.29 ng CGA-215944 found, 0.013 ppm CGA-215944 found, 65% recovery

FIGURE 12. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF PEPPERS FOR CGA-215944 (Continued)

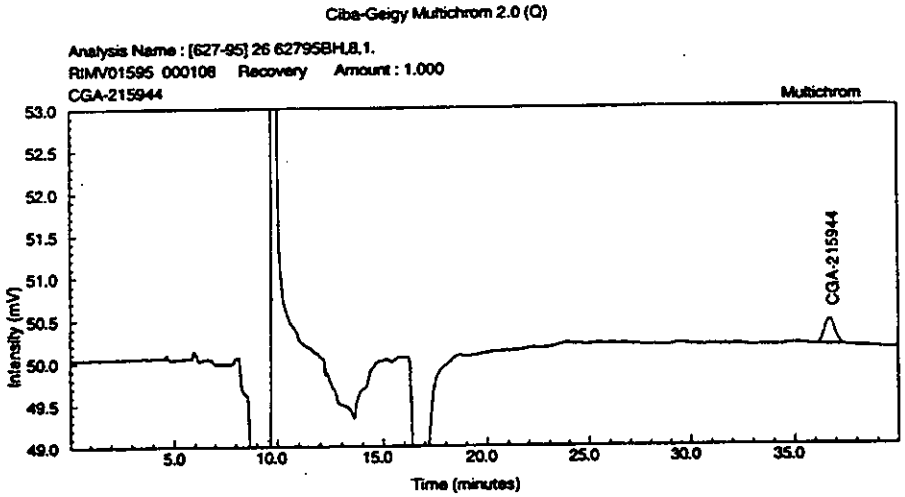


3. PP.02B, 0.02 ppm fortification, 99 mg injected, 1.28 ng CGA-215944 found, 0.013 ppm CGA-215944 found, 65% recovery

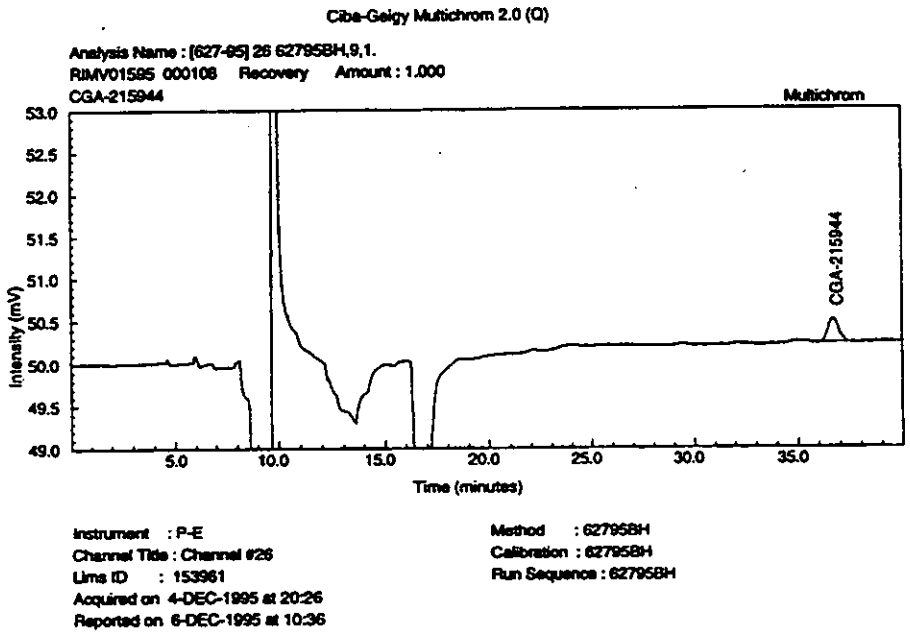


4. PP.05A, 0.05 ppm fortification, 99 mg injected, 3.86 ng CGA-215944 found, 0.039 ppm CGA-215944 found, 78% recovery

FIGURE 12. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF PEPPERS FOR CGA-215944 (Continued)

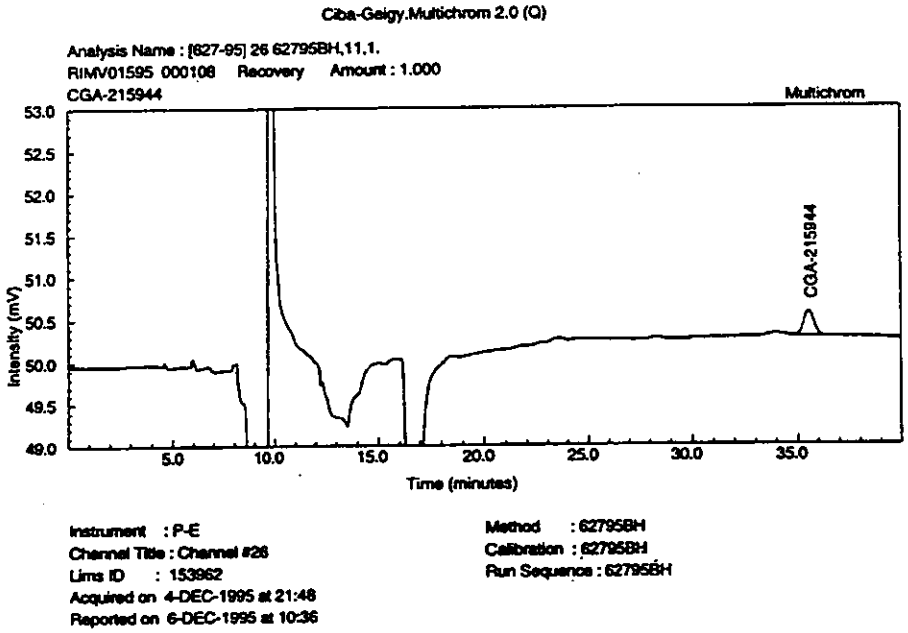


5. PP.05B, 0.05 ppm fortification, 100 mg injected, 3.52 ng CGA-215944 found, 0.035 ppm CGA-215944 found, 71% recovery

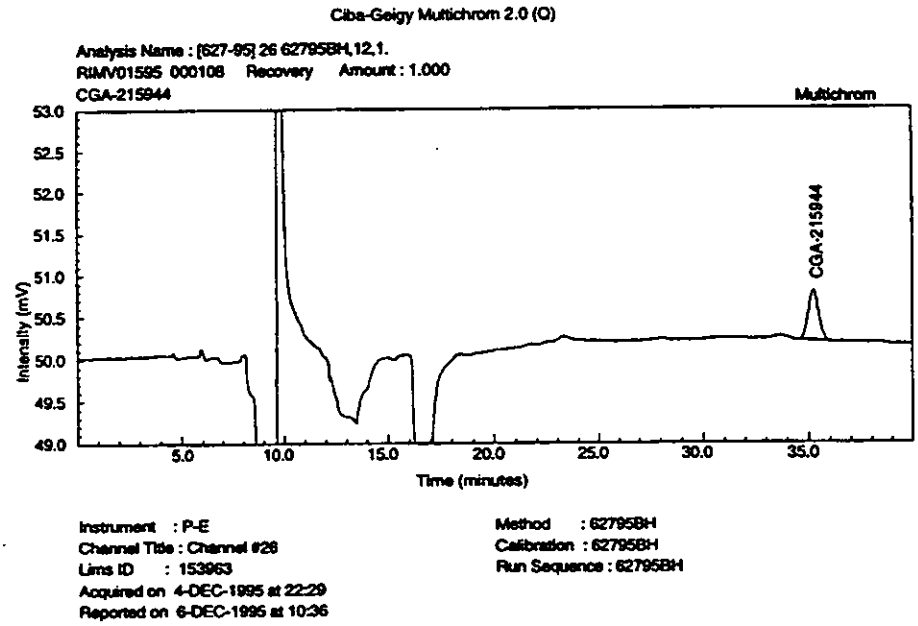


6. PP.10A, 0.10 ppm fortification, 50 mg injected, 3.17 ng CGA-215944 found, 0.063 ppm CGA-215944 found, 63% recovery

FIGURE 12. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF PEPPERS FOR CGA-215944 (Continued)

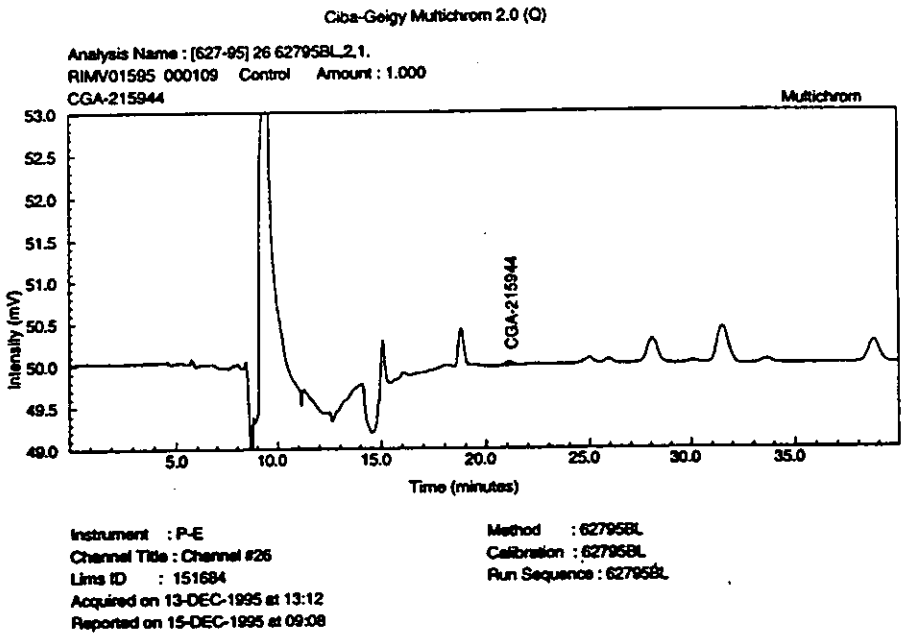


7. PP.10B, 0.10 ppm fortification, 50 mg injected, 3.36 ng CGA-215944 found, 0.067 ppm CGA-215944 found, 67% recovery

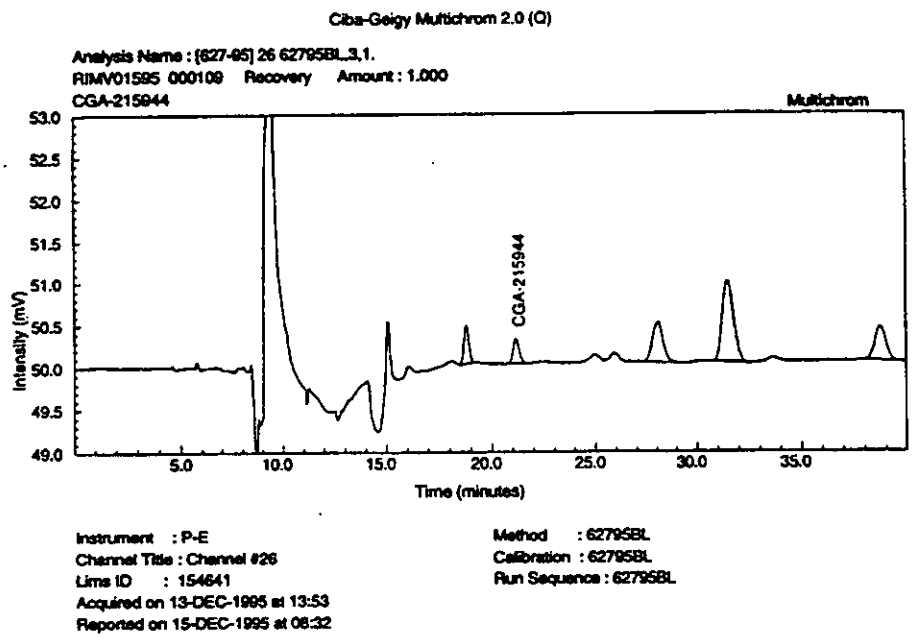


8. PP.20A, 0.20 ppm fortification, 50 mg injected, 7.33 ng CGA-215944 found, 0.15 ppm CGA-215944 found, 74% recovery

FIGURE 13. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GREEN TOBACCO FOR CGA-215944

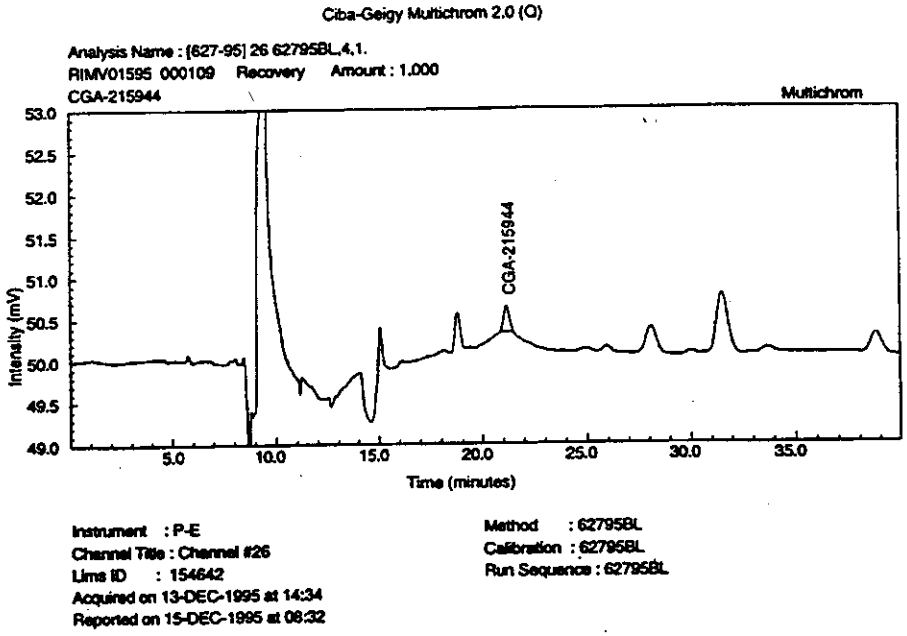


1. TG.CA, control, 100 mg injected, <2.0 ng found (0.95 ng), <0.02 ppm CGA-215944 found (0.010 ppm)

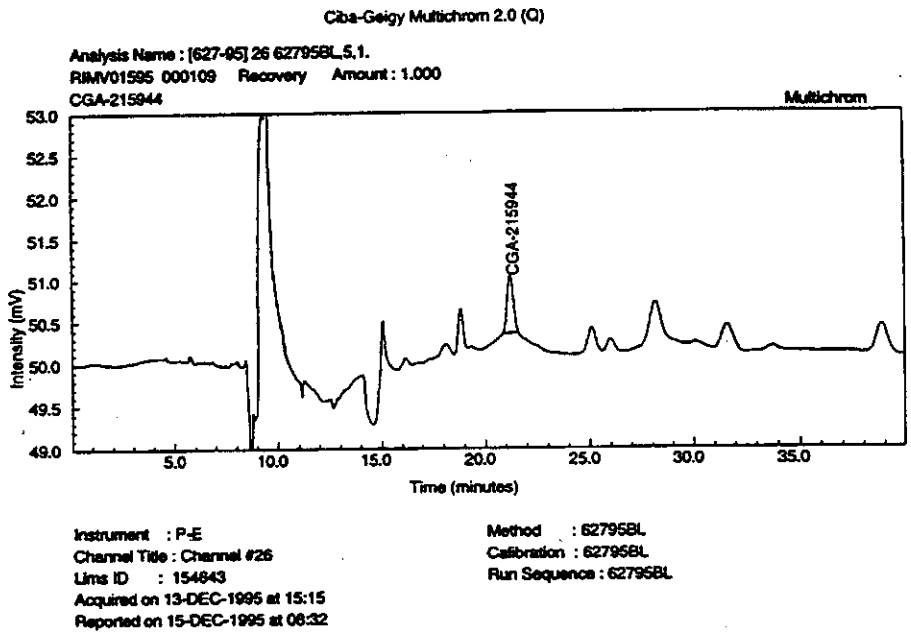


2. TG.02A, 0.02 ppm fortification, 100 mg injected, 2.57 ng CGA-215944 found, 0.026 ppm CGA-215944 found, 81% recovery

FIGURE 13. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GREEN TOBACCO FOR CGA-215944 (Continued)

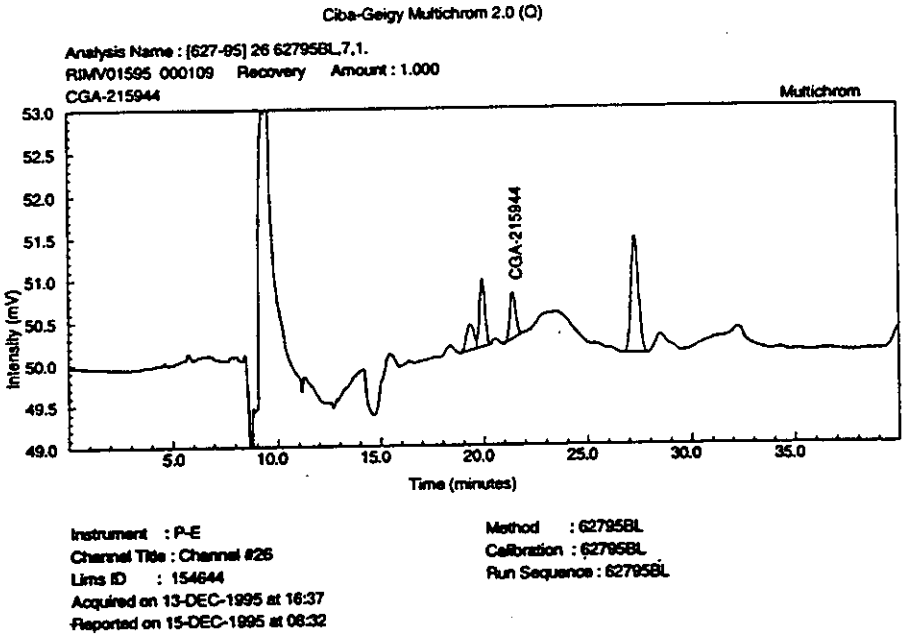


3. TG.02B, 0.02 ppm fortification, 100 mg injected, 2.63 ng CGA-215944 found, 0.026 ppm CGA-215944 found, 84% recovery

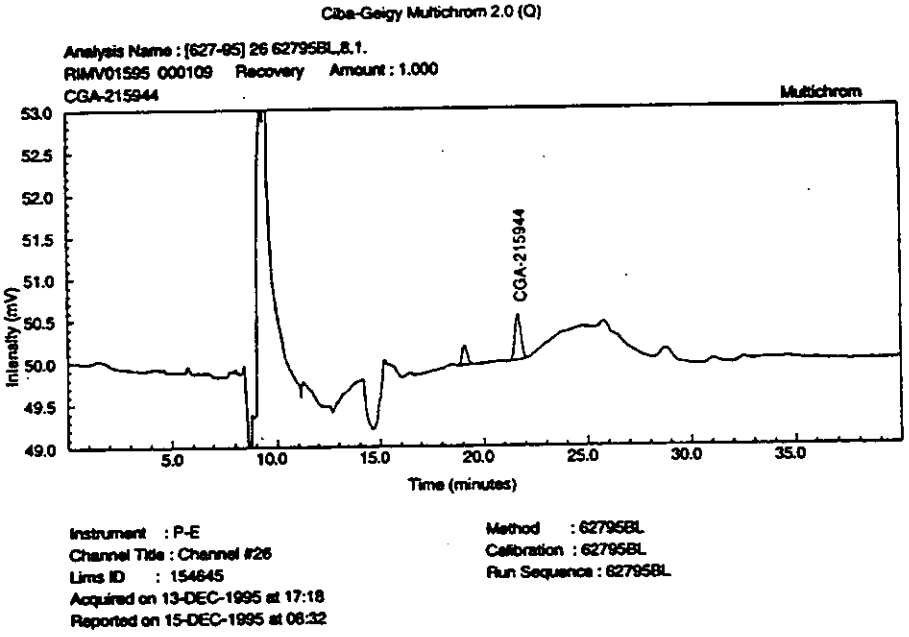


4. TG.05A, 0.05 ppm fortification, 100 mg injected, 5.23 ng CGA-215944 found, 0.052 ppm CGA-215944 found, 85% recovery

FIGURE 13. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GREEN TOBACCO FOR CGA-215944 (Continued)

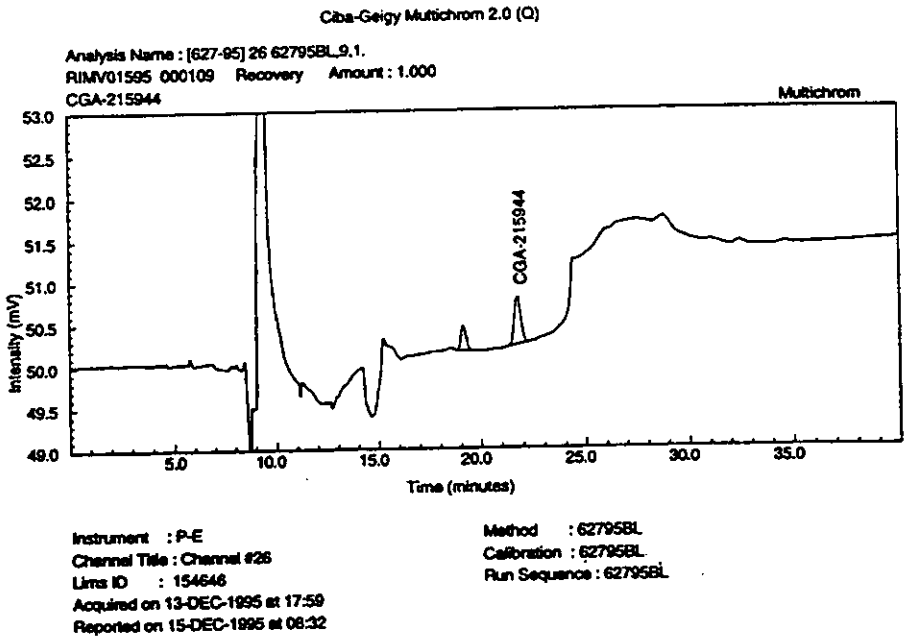


5. TG.05B, 0.05 ppm fortification, 100 mg injected, 4.19 ng CGA-215944 found, 0.042 ppm CGA-215944 found, 65% recovery

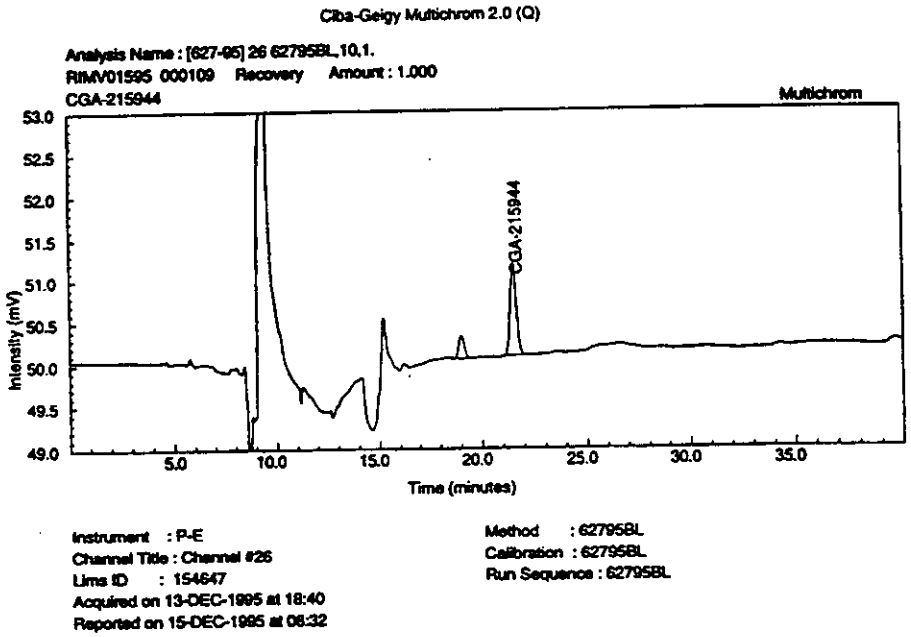


6. TG.10A, 0.10 ppm fortification, 50 mg injected, 4.14 ng CGA-215944 found, 0.083 ppm CGA-215944 found, 73% recovery

FIGURE 13. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GREEN TOBACCO FOR CGA-215944 (Continued)

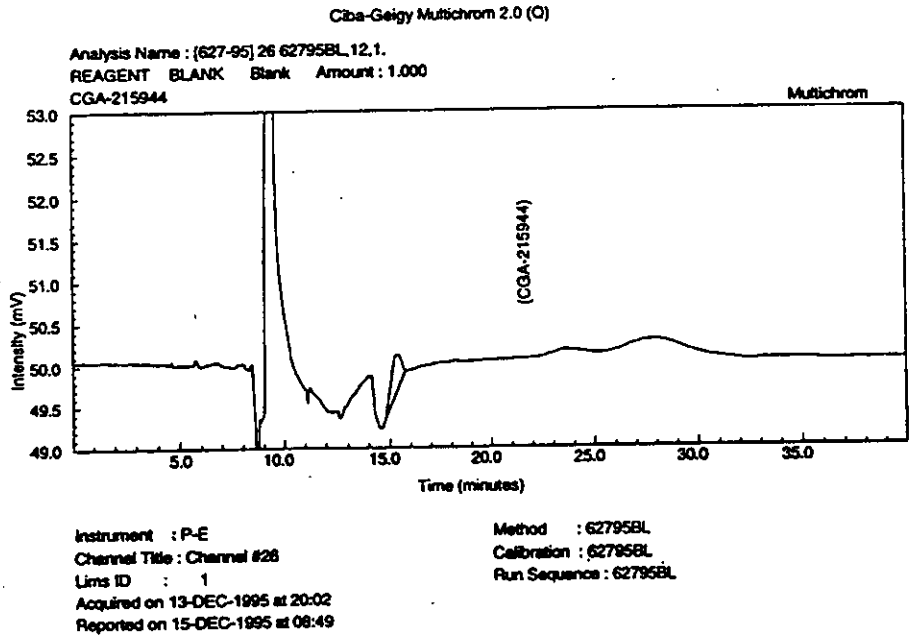


7. TG.10B, 0.10 ppm fortification, 50 mg injected, 4.33 ng CGA-215944 found, 0.087 ppm CGA-215944 found, 77% recovery



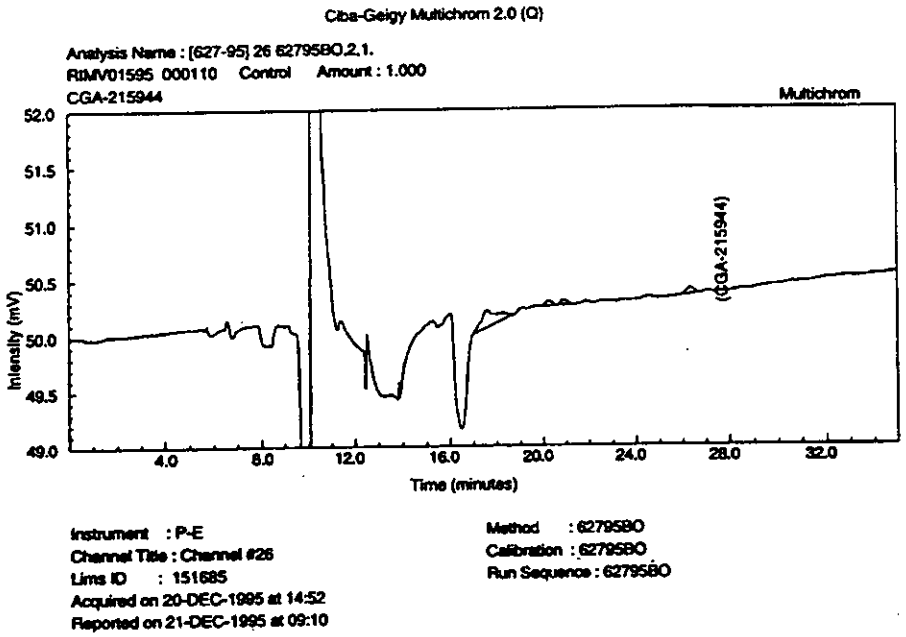
8. TG.20A, 0.20 ppm fortification, 50 mg injected, 7.78 ng CGA-215944 found, 0.16 ppm CGA-215944 found, 73% recovery

FIGURE 13. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF GREEN TOBACCO FOR CGA-215944 (Continued)

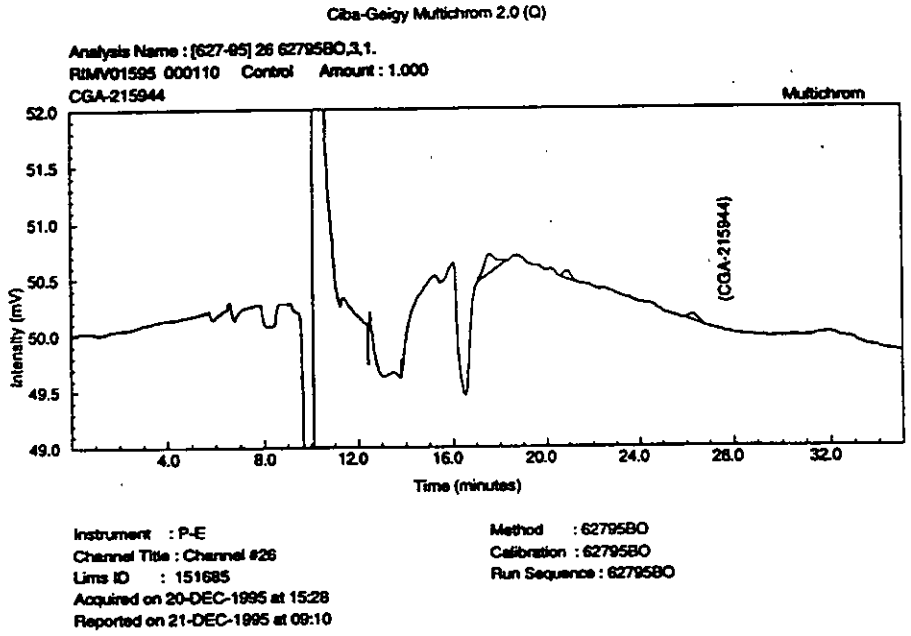


9. TG.RB, reagent blank, 100 mg injected, <2.0 ng CGA-215944 found, <0.02 ppm CGA-215944 found

FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CURED TOBACCO FOR CGA-215944

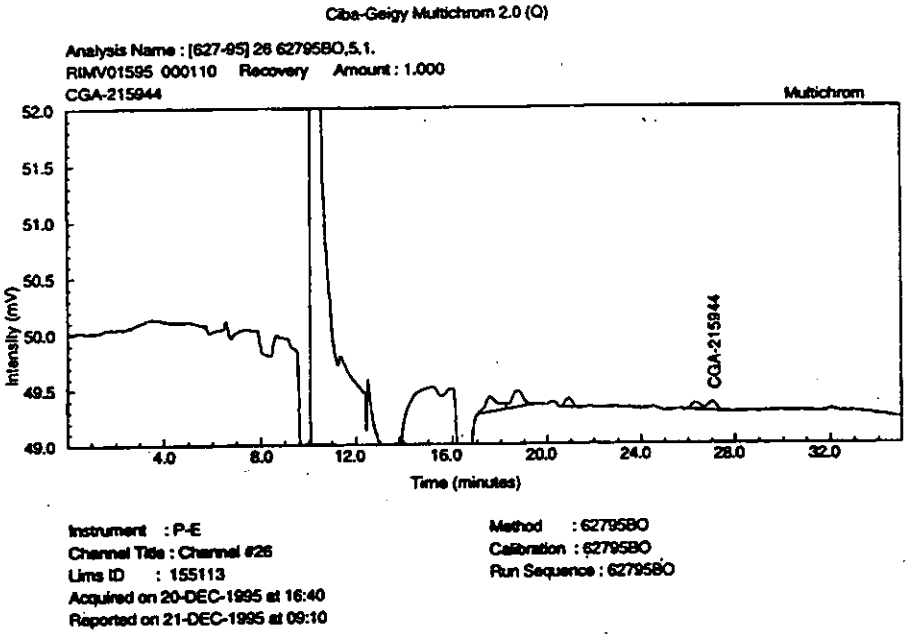


1. TC.CA, control, 50 mg injected, <1.0 ng found, <0.02 ppm CGA-215944 found

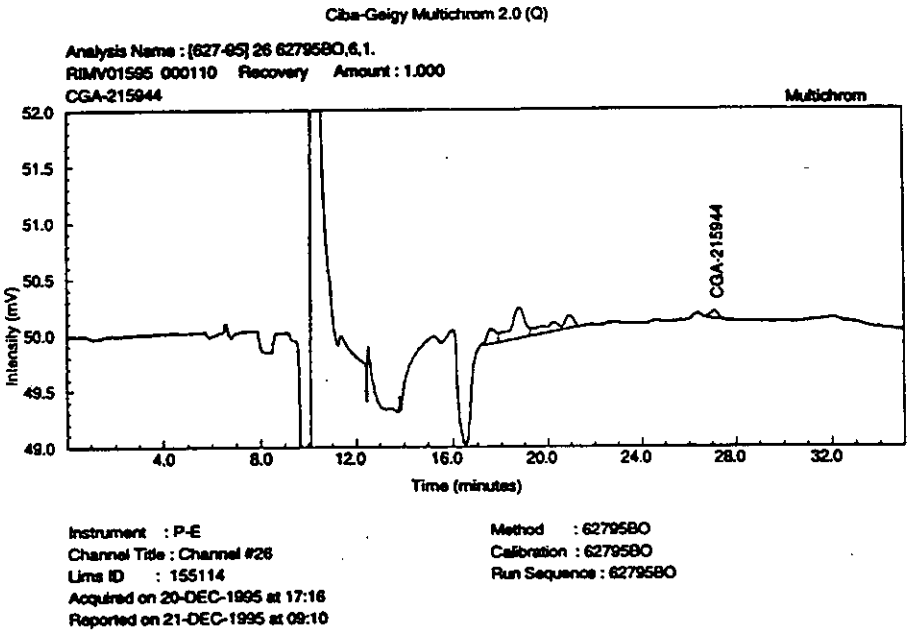


2. TC.CB, control, 50 mg injected, <1.0 ng found, <0.02 ppm CGA-215944 found

FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CURED TOBACCO FOR CGA-215944 (Continued)

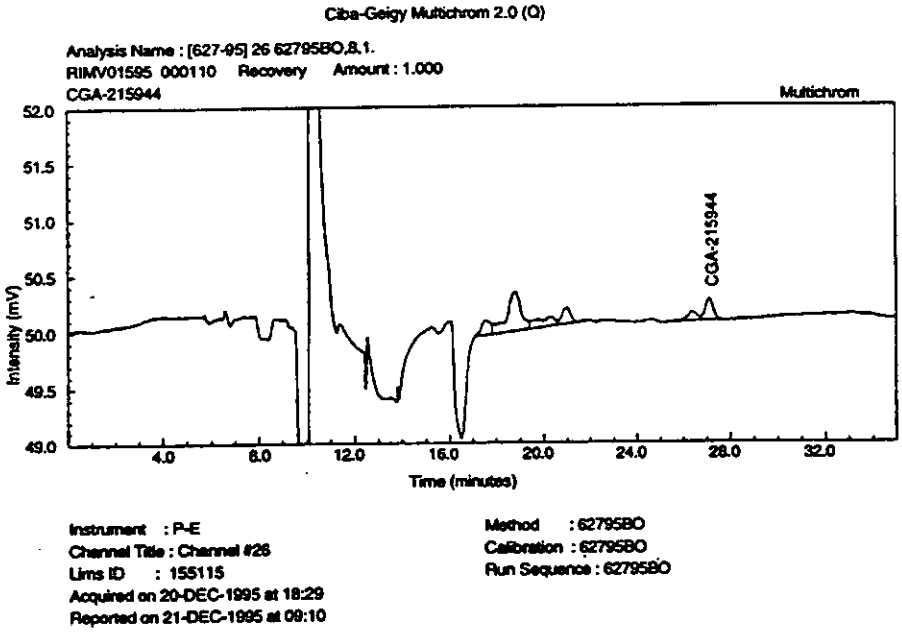


3. TC.02A, 0.02 ppm fortification, 50 mg injected, 0.77 ng CGA-215944 found, 0.015 ppm CGA-215944 found, 77% recovery

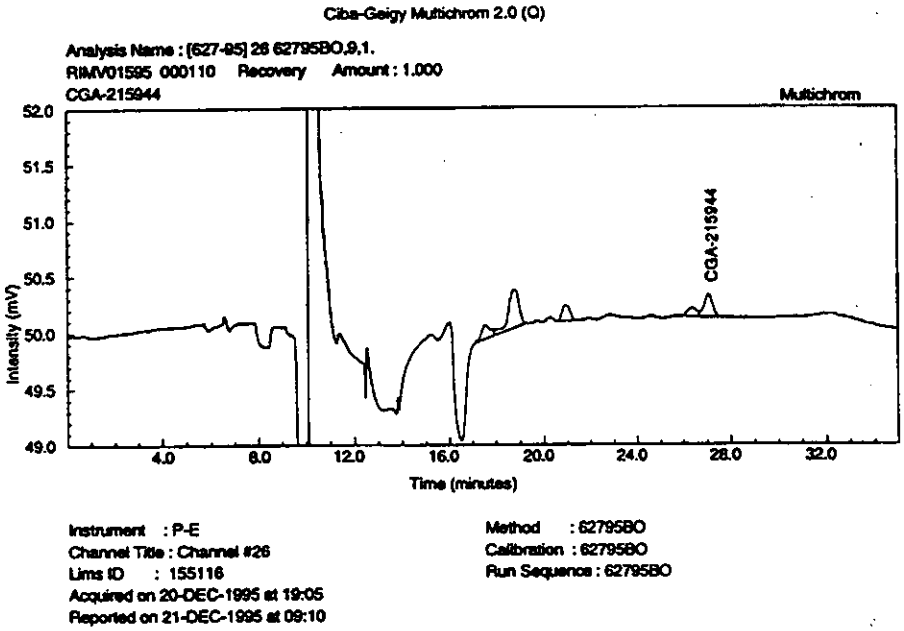


4. TC.02B, 0.02 ppm fortification, 50 mg injected, 0.65 ng CGA-215944 found, 0.013 ppm CGA-215944 found, 65% recovery

FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CURED TOBACCO FOR CGA-215944 (Continued)

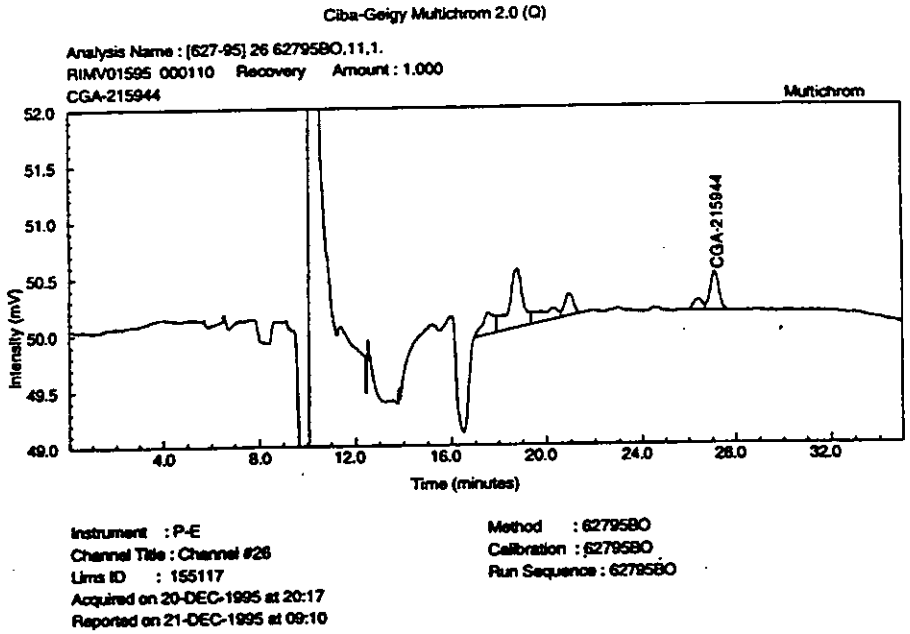


5. TC.05A, 0.05 ppm fortification, 50 mg injected, 1.73 ng CGA-215944 found, 0.034 ppm CGA-215944 found, 69% recovery

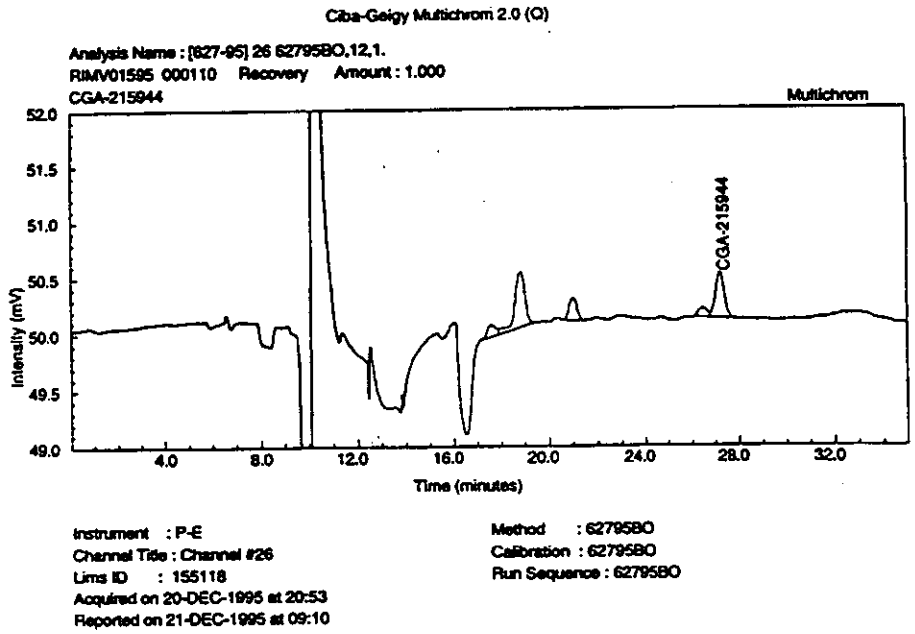


6. TC.05B, 0.05 ppm fortification, 50 mg injected, 1.85 ng CGA-215944 found, 0.037 ppm CGA-215944 found, 74% recovery

FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CURED TOBACCO FOR CGA-215944 (Continued)

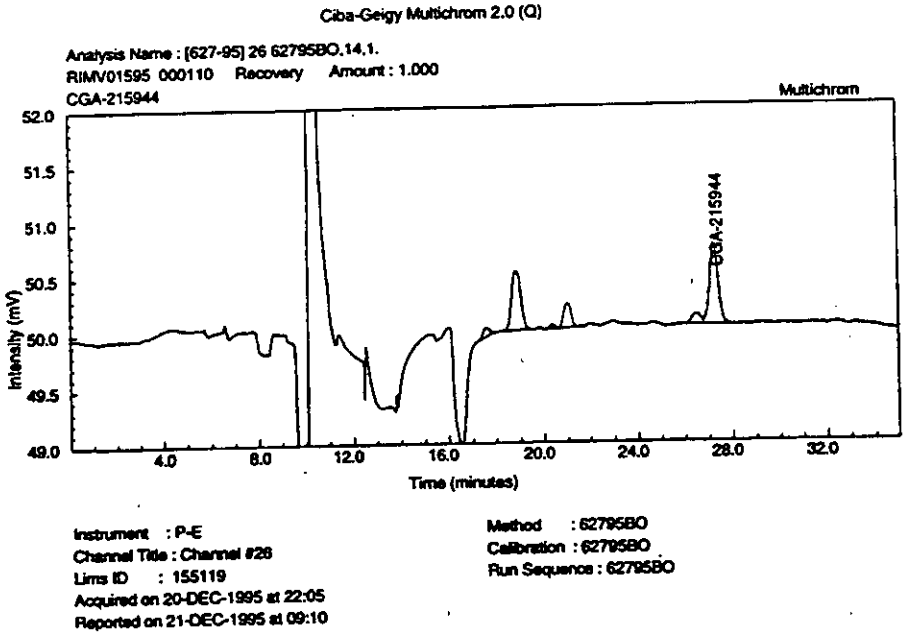


7. TC.10A, 0.10 ppm fortification, 50 mg injected, 3.11 ng CGA-215944 found, 0.062 ppm CGA-215944 found, 62% recovery

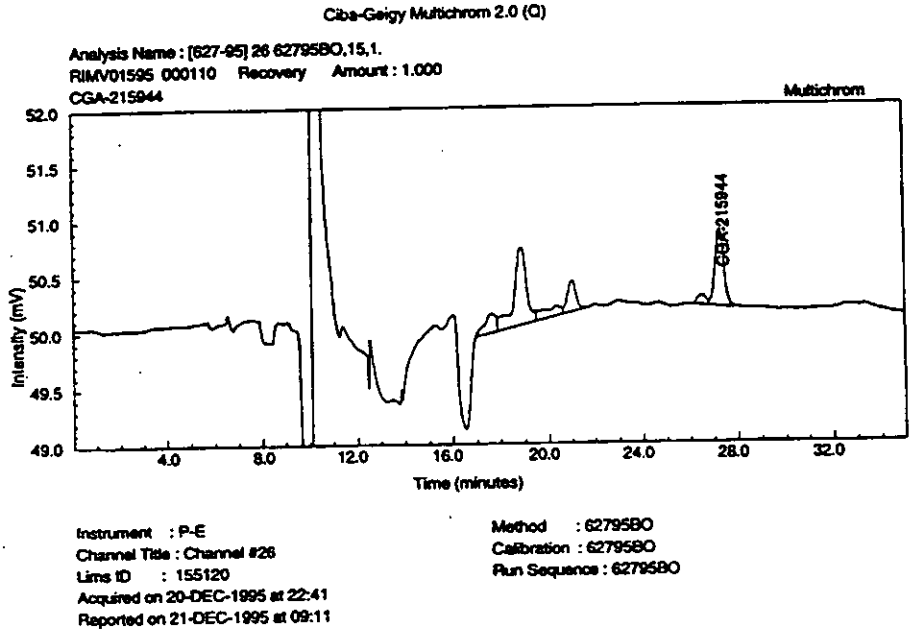


8. TC.10B, 0.10 ppm fortification, 50 mg injected, 3.66 ng CGA-215944 found, 0.073 ppm CGA-215944 found, 73% recovery

FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CURED TOBACCO FOR CGA-215944 (Continued)

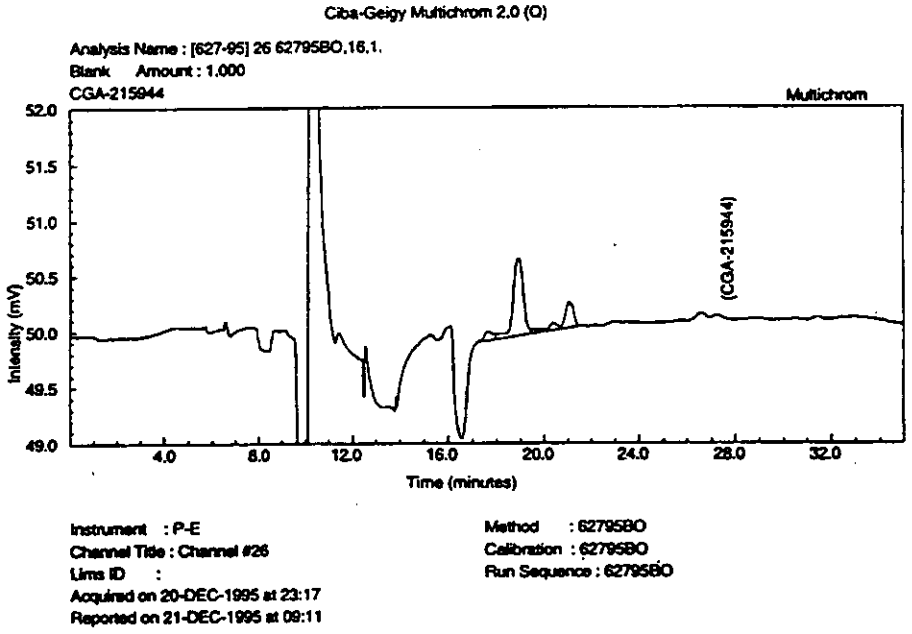


9. TC.20A, 0.20 ppm fortification, 50 mg injected, 6.40 ng CGA-215944 found, 0.13 ppm CGA-215944 found, 64% recovery



10. TC.20B, 0.20 ppm fortification, 50 mg injected, 6.16 ng CGA-215944 found, 0.12 ppm CGA-215944 found, 62% recovery

FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CURED TOBACCO FOR CGA-215944 (Continued)



11. TC.RB, reagent blank, 50 mg injected, <1.0 ng found, <0.02 ppm CGA-215944 found

VIII. REFERENCES

1. Tribolet, R., REM 154.01, "Determination of Residues of Parent Compound by Liquid Chromatography."

2. Joseph, T. A., Thorn, T., Analytical Method AG-647, "Analytical Method for the Determination of GS-23199 in Crops by HPLC."

3. Joseph, T. A., Thorn, T., Ciba Protocol 627-95, "Analytical Method for the Determination of CGA-215944 in Crops by HPLC."

4. Joseph, T. A., Thorn, T., Residue Test Report RI-MV-015-95.