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STUDY TITLE

DETERMINATION OF RESIDUES OF CGA-219417 IN CROPS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH COLUMN SWITCHING

DATA REQUIREMENT

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AUTHOR

ROBERT E. M. WURZ, Ph.D.

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PERFORMING LABORATORY

Biochemistry Department
Ciba Crop Protection
Ciba-Geigy Corporation
Greensboro, NC 27419

LABORATORY PROJECT IDENTIFICATION

ANALYTICAL METHOD AG-631

Ciba Crop Protection
Ciba-Geigy Corporation
Post Office Box 18300
Greensboro, NC 27419-8300

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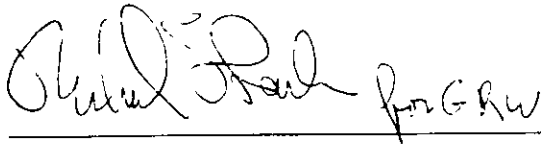
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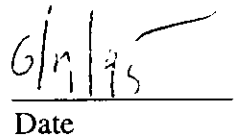
Company: Ciba Plant Protection, Ciba-Geigy Corporation

Company Representative: Gregory R. Watson, Ph.D.

Title: Senior Regulatory Manager

Handwritten signature of Gregory R. Watson, with the initials "GRW" written below it.

Signature

Handwritten date "6/11/95" with a horizontal line underneath.

Date

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STATEMENT CONCERNING GOOD LABORATORY PRACTICES

This document is an analytical method and contains data from other referenced studies. Certification of compliance with Good Laboratory Practice Standards in 40 CFR Part 160 (October 16, 1989) is not appropriate.

Refer to GLP Statements in separate studies being submitted with this volume.

W. T. Beidler

W. T. Beidler, Ph.D.
Agent of Submitter/Sponsor
And Manager, Residue Chemistry
Biochemistry Department

6-1-95

Date

SUBMITTER/SPONSOR: Ciba Crop Protection, Ciba-Geigy Corporation, P. O. Box 18300, Greensboro, NC 27419-18300

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Ciba-Geigy Corporation
Ciba Crop Protection
Biochemistry Department
P. O. Box 18300
Greensboro, NC 27419

ANALYTICAL METHOD AG-631

DETERMINATION OF RESIDUES OF CGA-219417 IN CROPS
BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH COLUMN
SWITCHING

Submitted By: R.E.M. Wurz Title: Scientist III

K. L. Van Geluwe Title: Scientist I

Approved By: W. T. Beidler Title: Manager
Residue Chemistry

Signature: *W.T. Beidler*

Approval Date: *5-23-95*

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I. SUMMARY/INTRODUCTION

A. Scope

Analytical Method AG-631 is used for the determination of residues of CGA-219417, in crops (see Figure 1 for the chemical name and structure). This method is a revised version of Analytical Method REM 141.01¹. This method contains radiolabeled CGA-219417 validation data² and confirmatory GC procedures. The limit of detection, as determined by the smallest standard amount injected, is 1.0 ng. The limits of quantitation (LOQ), as demonstrated by the lowest fortifications successfully recovered, are as follows:

Fruit, grain, juice	0.02 ppm
Forage, fodder, straw	0.05 ppm ¹
Grapes	0.01 ppm ¹

B. Principle

Homogenized samples are extracted with 80% methanol/water by shaking for 1 hour. After filtration, an aliquot of the extract is acidified with dilute hydrochloric acid and loaded onto a Bond-Elut SCX cartridge. After washing the Bond-Elut with aqueous methanol, CGA-219417 is eluted with 75% methanol/aqueous ammonia. The eluent is evaporated and reconstituted for analysis. Quantitation is by HPLC with column switching (Figure 2) and UV detection or by confirmatory GC/NPD.

The method flow diagrams for plant materials and wine are shown in Figures 3 and 4, respectively.

II. MATERIALS/METHODS

A. Equipment

- 1.0 Filter Paper, Reeve Angel or equivalent
- 2.0 Jars, amber, 8-oz.
- 3.0 Syringe, disposable Luer-lock
- 4.0 Syringe filter, ACRODISC LC13 PVDF, 0.2 μ m(Gelman #4450)

- 5.0 Mechanical Shaker
- 6.0 N-evap or equivalent.
- 7.0 Rotary evaporator, Büchii or equivalent.
- 8.0 Sample vials, Wheaton, 2-ml or equivalent.
- 9.0 Round bottom flasks, 25-ml.
- 10.0 Bond Elut SCX cartridge, 3ml/500mg,
Varian Co., Harbor City, CA 90710, USA;
Cat. No. 917303.
- 11.0 Test tubes, 24/40 joint, 18.5 cm x 22 mm.
(Ace Glass Co., Catalog No. 8645-38) or
equivalent.
- 12.0 Vortex mixer or equivalent.
- 13.0 Graduated cylinders, 25-ml.
- 14.0 Volumetric pipettes, 2-, 5-, 20-ml.
- 15.0 Top loading balance, Mettler PE 3600 or
equivalent.

B. Reagents and Standards

- 1.0 Methanol (MeOH), HPLC grade.
- 2.0 Water, HPLC grade.
- 3.0 Acetone, HPLC grade.
- 4.0 Hydrochloric Acid 32% (HCL), analytical
grade.
- 5.0 Ammonia solution 25%, analytical grade.
- 6.0 Diethylene glycol diethyl ether.
- 7.0 CGA-219417 analytical standard, Ciba Crop
Protection PTAS.

C. Analytical Procedures

1.0 Sample Preparation

Samples are received and stored frozen at -20°C. Samples are prepared under the general guidelines of the U.S. Food and Drug Administration Pesticide Analytical Manual Volume I, Section. Analyze samples immediately after preparation or store at -20°C until analysis.

2.0 Extraction

2.1 PLANT MATERIAL: Weigh a 10-g aliquot of crop substrate into an 8-oz round wide mouth bottle. Add 100 ml of MeOH:H₂O (8:2 v/v) to the subsample and shake the tightly sealed containers mechanically for 1 hour. Then filter through fluted filter paper into a bottle and cap tightly.

2.2 WINE: No extraction procedure needed.

3.0 SPE Cleanup

Preparing Samples for SPE Cleanup

3.1 EDIBLE PLANT MATERIAL (EXCEPT GRAPES): Transfer 20-ml extract (including grape extract) into a 25-ml test tube, add 2-ml 1M HCl and mix.

3.2 NON-EDIBLE PLANT MATERIAL AND GRAPE: Transfer 2-ml extract into a 5-ml test tube, add 0.2-ml 1M HCl and mix.

NOTE: If more than 2-ml extract is transferred onto Bond Elut SCX cartridge, overloading of the cartridge may occur.

3.3 WINE: Add 1-ml MeOH and 0.5-ml 1M HCl to the subsample of 5 ml and mix.

Loading Solid Phase Cartridge

- 3.4 Precondition a Bond-Elut SCX cartridge by consecutive passage of 5-ml MeOH and 5-ml 0.1M HCl. Attach a plastic reservoir to the top of the Bond Elut SCX cartridge. Transfer the acidified extract to the cartridge using a Pasteur pipette and drain the solvent to the top of the packing. Rinse the tube with 12 ml of MeOH:H₂O (1:1 v/v), transfer the rinsing to the cartridge and drain the solvent to the top of the cartridge bed. Discard the eluate. Elute CGA-219417 with 8 ml of MeOH:25% ammonia solution (95:5 v/v) into a 25-ml round bottom flask. Wash down the sides of flask with additional elution solvent (use a Pasteur pipette).
- 3.5 Add three drops of diethylene glycol dithyl ether and evaporate the eluate to dryness using a rotary evaporator (bath temp. ~40°C) and dissolve the residue in an appropriate amount of mobile phase as listed below in order to obtain the requested lower practical level. Evaporate controls back-to-back (if more than one set is being analyzed together) on clean rotary evaporator.

<u>Matrix</u>	<u>Final Volume (ml)</u>
Edible plant material (except grapes)	2
Non-edible plant material and grapes	?
Wine	2.5

D. Instrumentation

1.0 Description

The sample from Section II.C.3.5 is analyzed by High Performance Liquid Chromatography (HPLC).

Note: A two-column HPLC switching system is used for the final determination of CGA-219417 in stone and pome fruits, non-edible plant material, juice and wine samples. For other matrices a one-column system may be sufficient, but can be replaced at any time by the two-column system (e.g. if the quantitation of CGA-219417 is not possible due to interfering peaks). In such a case, the detector sensitivity must be increased.

- 1.1 Install the HPLC system according to Table I and Figure 2. Control of the switching valve is accomplished via time-programmed contact closures of the detector, autoinjector or other timing sources.
- 1.2 Determine the retention time of CGA-219417 on Column #1 by connecting Column #1 directly to the detector and injecting 10 ng of the analyte. (Inject 100 μ l of the 0.10 ng/ μ l standard solution prepared in Section II.I.1.3.)
- 1.3 Program the column switching valve to switch to the INJECT POSITION at the beginning of the CGA-219417 analyte peak and to return to the LOAD POSITION at the end of the CGA-219417 peak.
- 1.4 Inject 10 ng of CGA-219417 to determine its retention time through the two columns and to confirm that the valve time programming is correct.

2.0 Standardization

- 2.1 The HPLC system should be calibrated with each analytical run, by checking the retention time and detector response relative to previous runs. Retention time must not vary more than 2% within a run and detector response should not vary more than 10% between runs.
- 2.2 Standardize the HPLC system by injecting 100- μ l aliquots of standard solutions of CGA-219417 in a working range of 1-20 ng/injection (Figure 5). Generate a linear regression plot from the data by comparing detector response and ng injected.

E. Interferences

Some SCX cartridges used gave interferences in chromatograms which hindered the quantitation of CGA-219417. The use of 5-ml MeOH and 5-ml 0.1M HCl for conditioning of the cartridges prevents this problem.

F. Confirmatory Techniques

Residue analyses of CGA-219417 may be confirmed by capillary GC or by online GC-MS. All samples must be reconstituted in acetone for GC analysis. Standards in acetone must also be prepared (Section II.I.1.4). The GC conditions can be found in Table II.

G. Time Required for Analysis

One working day is required to process a series of 14 samples to the point of injection. Automated HPLC-analysis can be performed overnight.

H. Modifications and Potential Problems

1.0 Analytical Procedures For Juice Samples

- 1.1 EXTRACTION: No extraction procedures are needed.

- 1.2 SOLID PHASE EXTRACTION: Thaw a subsample of juice and using a 5-ml volumetric pipette transfer to a screw-top tube. Add 20 ml of MeOH using a volumetric pipette. Add 2 to 4 ml of 1M HCl to the sample (pH ~ 3.0). Tightly cap the tube and mix. Place a large loose portion of glass wool in the bottom of the reservoir attached to the SCX cartridge to prevent the SCX fruit from clogging. Refer to Section II.C.3.4 to II.C.3.5 for the remainder of the procedures.

The following modifications can be made to the parameters in Analytical Method AG-631. The HPLC columns used can be 150 mm long instead of 120 mm as specified if the latter size is not readily available.

The vapor pressure of CGA-219417 is relatively high. Although no remarkable loss occurred during evaporation, specimens with high residues contaminated rotary evaporators used after the clean-up step with SCX cartridges. The next specimen solutions to be evaporated were then contaminated with CGA-219417. In order to avoid this contamination, 3 drops of diethylene glycol diethyl ether are added to solutions to be evaporated.

I. Preparation of Standard Solutions

1.0 Preparation of Standard CGA-219417 Solutions

HPLC STANDARDS

- 1.1 Weigh 10 mg of CGA-219417 analytical standard into a 100-ml volumetric flask and dilute to the mark with MeOH. This produces a 100 µg/ml stock solution.
- 1.2 Make serial dilutions of the 100 µg/ml stock solution with mobile phase (MeOH:WATER 7:3 v/v) to give fortification standards of 1.0 and 0.10 µg/ml CGA-219417 concentrations.

Store these standard solutions in glass bottles at -18°C to -30°C .

- 1.3 Make serial dilutions of the 100 $\mu\text{g}/\text{ml}$ stock solution with mobile phase (MeOH:WATER 7:3 v/v) to give a series of injection standards in a range of 0.01 - 0.20 $\text{ng}/\mu\text{l}$ of CGA-219417. Store these standard solutions in glass bottles at approximately 3°C . Typical chromatograms of standards are shown in Figure 6.

GC STANDARDS

- 1.4 Pipette 1.0 ml of the 100 $\mu\text{g}/\text{ml}$ stock solution of CGA-219417 in MeOH (Section II.I.1.1) into a 100-ml volumetric flask and dilute with acetone. This produces a 1.0 $\mu\text{g}/\text{ml}$ solution.
- 1.5 Make serial dilutions of the 1.0 $\mu\text{g}/\text{ml}$ solution (Section II.I.1.4) using acetone as the diluent, to produce fortification standards in the range of the LOQ of 0.020 ppm and any other level consistent with expected residues.
- 1.6 Make serial dilutions of the 1.0 $\mu\text{g}/\text{ml}$ solution (Section II.I.1.4) using acetone as the diluent, to produce injection standards in a range of 0.01 $\text{ng}/\mu\text{l}$ to 0.20 $\text{ng}/\mu\text{l}$ of CGA-219417. Store these standard solutions in glass bottles at approximately 3°C . Typical GC chromatograms of standards are also shown in Figure 6.

J. Methods of Calculation

1.0 Determination of Sample Residues

- 1.1 Inject a 100- μl aliquot of the sample from Section II.C.3.5 into the HPLC. Make appropriate dilutions of the sample to have the

sample peak height within the range of the standard curve. Compare peak heights of unknown samples with the standard curve, manually or by either using an electronic calculator or a computer system to determine the amounts of CGA-217419 in the aliquots injected. Typical chromatograms of control and recovery samples are shown in Figure 7. Inject 3- μ l aliquots of sample reconstituted with acetone. The control and recovery samples analyzed by confirmatory procedures are shown in Figure 8.

- 1.2 To calculate the residue results in terms of ppm of CGA-219417, the mg sample injected must be first calculated as follows (Equation 1):

$$(1) \text{ mg inj.} = \frac{(G)(V_i)(V_a)}{(V_f)[V + W(m/100)]}$$

G = milligrams sample extracted
V_i = injection volume (μ l)
V_f = total volume of final
V_a = aliquot volume
V_e = extraction volume
W = Weight of sample in grams
M = percent moisture of sample

To determine the ppm analyte found in the sample, use Equation 2.

$$(2) \text{ ppm} = \frac{(\text{ng CGA} - 219417 \text{ Found})(100)}{(\text{mg sample injected})(R\%)}$$

R% = recovery ratio given by Equation 4, expressed as a decimal (not used for tolerance enforcement analyses)

2.0 Fortification Experiments

- 2.1 To regularly check the performance of the method, analyze two fortified control samples with each series of analyses. To prepare these samples add known amounts of CGA-219417 to

control samples prior to extraction. Make sure that the control samples neither are contaminated nor show signals interfering with the signal of CGA-219417.

2.2 Add 1.0 mL of the appropriate standard solution of CGA-219417 (Section II.I.1.0) to 10 g of control crop prior to the addition of extraction solvent. Use correspondingly larger amounts of standards (volume should not exceed 2 mL) for higher fortifications. Analyze control and freshly fortified samples along with the treated samples according to the procedures of the method.

2.3 Calculate the final ppm value of the control and fortified samples according to the following equation:

$$(3) \text{ ppm CGA - 219417} = \frac{(\text{ng CGA - 219417})}{(\text{mg sample injected})}$$

Determine the recovery factor by first subtracting the background detector response, if any, in the control sample from the CGA-219417 response in the recovery sample. Calculate the recovery factor as a percentage (R) by the equation:

$$(4) R\% = \frac{\text{ppm CGA - 219417 found} - \text{ppm control}}{\text{ppm CGA - 219417 added}} \times 100$$

III. RESULTS AND DISCUSSION

A. Ruggedness Testing

Ruggedness was demonstrated by analysis of cherry samples using REM 141.01 at and above the limit of quantitation (LOQ) (0.02 ppm for cherries).

Control cherry samples were fortified with CGA-219417 to verify that acceptable recoveries of the analytes are obtained when the samples are analyzed according to the procedures of Analytical Method REM 141.01 and GC confirmatory procedures (see TABLE III and FIGURES 7-8). The

average recovery of CGA-219417 for the successfully analyzed set of cherry samples was 94% (s.d. = 3%, n = 6) for HPLC. No interferences were found in reagent blank samples (Figure 9). Additional recovery data from pome and stone fruit residue studies are presented in Tables IV-VI. Recovery data from REM 141.01 is presented in Table VII.

B. ACCURACY

The accuracy of method AG-631 is determined by the mean recovery observed in the analysis of all of the fortified control samples of the stone and pome fruits in Tables IV-VI (see FIGURES 10-22 for representative chromatograms). Results from Figures 19 and 21 are NOT tabulated in Tables. The mean recovery for all control samples fortified with CGA-219417 is 98% (n = 41).

C. PRECISION

The precision of method AG-631 is determined by the standard deviation (sd) and coefficient of variation (% CV) for all of the fortified control sample recovery values as presented in Tables IV-VI (see FIGURES 10-22 for representative chromatograms). The coefficient of variation is determined by dividing the standard deviation by the mean, multiplied by 100. The standard deviation (sd) is 11% for all of the recovery values presented in Tables IV-VI and the coefficient of variation (% CV) is 12%, (n = 41).

D. EXTRACTABILITY

Extractability was determined by liquid scintillation counting (LSC) of an aliquot of the extracts of ¹⁴C-CGA-219417 treated tomato fruits. The percent extractability was determined by comparing the ppm ¹⁴C in the extract to the total radioactive residue (TRR) of the sample, as determined by combustion analysis of replicate subsamples. Extractability results are shown in Table VIII. The mean %-extractability is 116%.

E. ACCOUNTABILITY

Accountability results are calculated by comparing the total ppm obtained from HPLC analysis using method REM 141.01 to the TRR. Accountability results are shown in Table VIII. The mean accountability is 47%.

IV. CONCLUSION:

Analytical Method AG-631 is a valid and accurate method for the determination of parent residues of CGA-219417. The overall accuracy and precision of Analytical Method AG-631 are acceptable.

TABLE I. HPLC OPERATING PARAMETERS

Autoinjector:	Perkin-Elmer ISS-100
Pumps:	Perkin-Elmer 250 Isocratic LC pump
Detector:	ABI 783A Variable wavelength UV detector
Switching Valve:	Valco 6-port valve with electric actuator
Columns (1 and 2):	Nucleosil 100 C18, 5 μ m, 150 mm x 4.6 mm i.d. (Alltech Assoc.)
Mobile Phase 1:	65:35 methanol:water (HPLC)
Mobile Phase 2:	70:30 methanol:water (HPLC)
Flow Rate:	1 mL/min both columns
Injection Volume:	100 μ L
Wavelength:	270 nm

TABLE II. GC OPERATING PARAMETERS

Gas Chromatograph:	HP 5890A with nitrogen/phosphorus detector (NPD) and packed column injection port (Hewlett-Packard, USA)
Sampler:	HP 7673A (Hewlett-Packard)
Column:	Fused silica, ca. 30 m x 530 μ m i.d., DB-5 (J&W Scientific, USA)
Carrier gas:	Helium, approx. 10 mL/min.
Detector gases:	Helium, approx. 16 mL/min. (makeup gas) Hydrogen, approx. 3.0 mL/min. Air, approx. 98 mL/min.
Oven temp.:	Initial - 110°C for 0.30 min Temp. program rate - 30°C/min Final temp. - 250°C for 7 min
Injector temp.:	250°C
Detector temp.:	300°C
Injection volume:	3 μ L

TABLE III. SUMMARY OF HPLC AND GC ANALYSES FOR FORTIFIED CONTROL CHERRIES USING METHOD REM 141.01 (DATA FROM RUGGEDNESS ABR-94088)

<u>Code #</u>	<u>Type*</u>	<u>CGA-219417 Added</u>	<u>% Rec. by HPLC</u>	<u>% Rec. by GC</u>
CH.0CA	Control	--	(<0.02 ppm)	(<0.02 ppm)
CH.0CB	"	--	(<0.02 ppm)	(<0.02 ppm)
CH.02A	Recovery	0.02 ppm	93	86
CH.02B	"	"	94	90
CH.05A	"	0.05 ppm	93	99
CH.05B	"	"	90	83
CH.10A	"	0.10 ppm	100	101
CH.10B	"	"	93	112
<u>CH.RB</u>	<u>Control</u>	<u>Blank</u>	<u>(<0.02 ppm)</u>	<u>(<0.02 ppm)</u>
			Mean = 94%	Mean = 95%
			s.d. = 3.3%	s.d. = 11%

TABLE IV. PROCEDURAL RECOVERIES OF CGA-219417 FROM FORTIFIED CONTROLS OF APPLES AND PEARS USING ANALYTICAL METHOD REM 141.01 (DATA FROM IN-PROGRESS STUDY 67-94)

<u>Field Test Number</u>	<u>Location</u>	<u>Mature Fruit Commodity</u>	<u>ppm in Control</u>	<u>Fortification Level (ppm)</u>	<u>CGA-219417 Percent Recovered</u>
OW-FR-641-94	Washington	Apples	<0.02	0.20	105
OW-FR-642-94	Washington	Apples	<0.02	0.02	91
05-FR-003-94	New York	Apples	<0.02	0.02	94
			<0.02	0.02	96
NE-FR-712-94	Michigan	Apples	<0.02	0.02	112
02-FR-031-94	California	Apples	<0.02	0.05	97
			<0.02	0.02	92
NR-FR-807-94	Pennsylvania	Apples			
OS-FR-602-94	North Carolina	Apples	<0.02	0.10	112
			<0.02	0.02	117
NE-FR-304-94	West Virginia	Apples	<0.02	0.10	103
02-FR-032-94	California	Pears	<0.02	0.05	97
OW-FR-404-94	California	Pears	<0.02	0.02	90
05-FR-004-94	New York	Pears	<0.02	0.02	93
OW-FR-643-94	Washington	Pears	<0.02	0.05	97
OW-FR-644-94	Washington	Pears	<0.02	0.02	86
OW-FR-653-94	Oregon	Pears	<0.02	0.05	99
				Average:	99
				SD:	9
				n=	16

TABLE V. PROCEDURAL RECOVERIES OF CGA-219417 FROM FORTIFIED CONTROLS OF PEACHES, PLUMS, PRUNES AND CHERRIES USING ANALYTICAL METHOD REM 141.01 (DATA FROM ABR-94093)

<u>Field Test Number</u>	<u>Location</u>	<u>Mature Fruit Commodity</u>	<u>ppm in Control</u>	<u>Fortification Level (ppm)</u>	<u>CGA-219417 Percent Recovered</u>
02-FR-033-94	California	Peaches	<0.02	0.30	109
OS-FR-830-94	Georgia	Peaches	<0.02	0.02	118
NE-FR-808-94	Pennsylvania	Peaches	<0.02	1.0	97
		Peaches	<0.02	1.0	99
OW-FR-645-94	Washington	Peaches	<0.02	2.00	99
		Peaches	<0.02	0.20	99
NE-FR-714-94	Michigan	Peaches	<0.02	0.50	93
02-FR-034-94	California	Plums	<0.02	1.0	78
OW-FR-646-94	Oregon	Plums	<0.02	0.20	107
NE-FR-715-94	Michigan	Plums	<0.02	0.50	105
		Plums	<0.02	0.50	98
OW-FR-647-94	Idaho	Plums	<0.02	1.0	102
02-FR-035-94	California	Prunes	<0.02	0.02	83
OW-FR-648-94	Oregon	Prunes	<0.02	0.50	101
		Prunes	<0.02	0.50	115
OW-FR-649-94	Washington	Sweet cherries	<0.02	0.02	118
OW-FR-650-94	Oregon	Sweet cherries	<0.02	1.0	118
02-FR-036-94	California	Sweet cherries	<0.02	3.0	75
NE-FR-716-94	Michigan	Sweet cherries	<0.02	0.50	109
NE-FR-717-94	Michigan	Tart cherries	<0.02	0.02	110
NE-FR-403-94	New York	Tart cherries	<0.02	0.50	86
Average:					101
Standard deviation:					12.6
Number:					21
Range:					75-118

TABLE VI. PROCEDURAL RECOVERIES OF CGA-219417 FROM FORTIFIED CONTROLS OF PROCESSED PRUNES USING ANALYTICAL METHOD REM 141.01 (DATA FROM ABR-94093)

<u>Field Test Number</u>	<u>Location</u>	<u>Mature Fruit Commodity</u>	<u>ppm in Control</u>	<u>Fortification Level (ppm)</u>	<u>CGA-219417 Percent Recovered</u>
02-FR-035-94	California	Whole fruit-fresh	<0.02	1.0	101
		Whole fruit-dried	<0.02	0.02	73
OW-FR-648-94	Oregon	Whole fruit-fresh	<0.02	0.50	84
		Whole fruit-dried	<0.02	1.0	79
Average:					84
Standard deviation:					12.0
Number:					4
Range:					73-101

TABLE VII. RECOVERIES OF CGA-219417 IN GRAPES, APPLES, GRAIN AND STRAW OF BARLEY AND WHEAT, SOIL AND WINE (FROM ANALYTICAL METHOD REM 141.01)¹

<u>Matrix</u>	<u>Substrate</u>	<u>Fortification Level [mg/kg]</u>	<u>Recoveries Found [%]</u>	<u>Average [%]</u>
Grapes		0.02	90,98,93,89	93
		0.1	89,94,85,77	86
Apple		0.04	100,96	98
		0.2	96,91	94
Barley	Grain	0.04	93,90	92
		0.2	96,97	97
	Straw	0.1	84,100,96,83,107	94
		0.5	88,82,81,81	83
Wheat	Grain	0.04	101,93,97,109	100
		0.2	97,99,95,97	97
	Straw	0.1	97,97,97,94	96
		0.5	91,85,87,87	88
Soil		0.02	93,93,89	92
		0.1	93,93,94	93
Wine		0.01	90,96,96,91	93
		0.05	101,81,84,85	88

Average Recoveries: 92% (Sabs. = 7%), n = 55 values

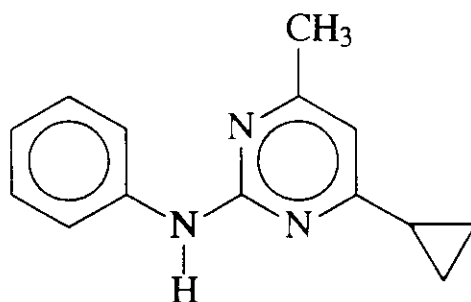
Acceptable Recovery Range: 72 - 112%

TABLE VIII. SUMMARY TABLE FOR THE EXTRACTABILITY AND ACCOUNTABILITY OF ¹⁴C-CGA-219417 TREATED TOMATO FRUIT (SPECIAL STUDY REPORT 100/92)

Mean TRR	ppm ¹⁴ C in extract	% Extractability	ppm ¹⁴ C in final HPLC fraction	ppm by HPLC/UV	% Accountability*
4.19	5.21	124	3.82	1.87	45
	4.69	112	3.69	1.81	43
	5.19	124	3.95	2.22	53
	4.4	105	3.41	1.88	45
MEAN	4.87	116	3.72	1.94	47

*Accountability determined from HPLC/UV detection.

FIGURE 1. CHEMICAL NAME AND STRUCTURE



CGA-219417: 4-cyclopropyl-6-methyl-N-phenyl-2-pyrimidinamine

FIGURE 2. SCHEMATIC DIAGRAM OF THE HPLC COLUMN SWITCHING SYSTEM

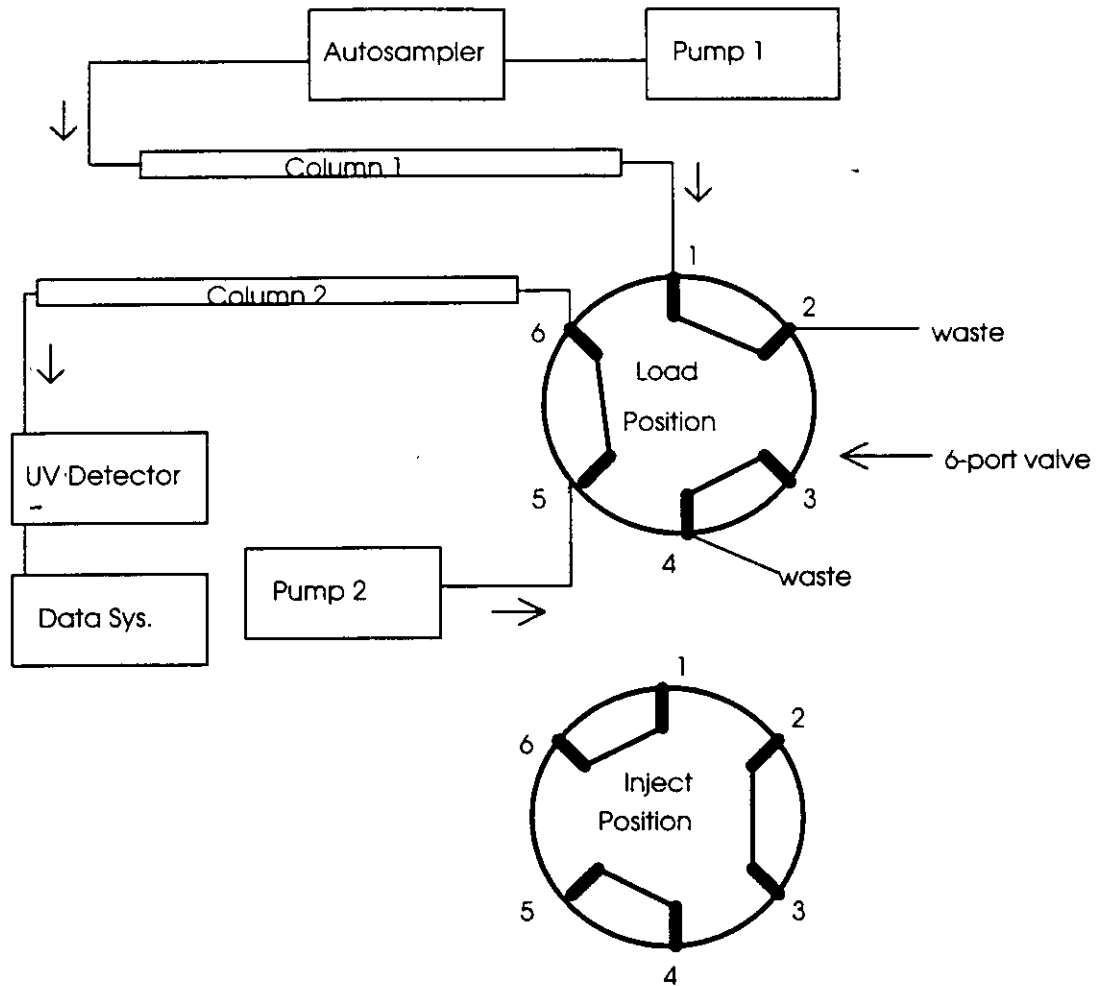


FIGURE 3. FLOW DIAGRAM OF METHOD FOR PLANT MATERIAL

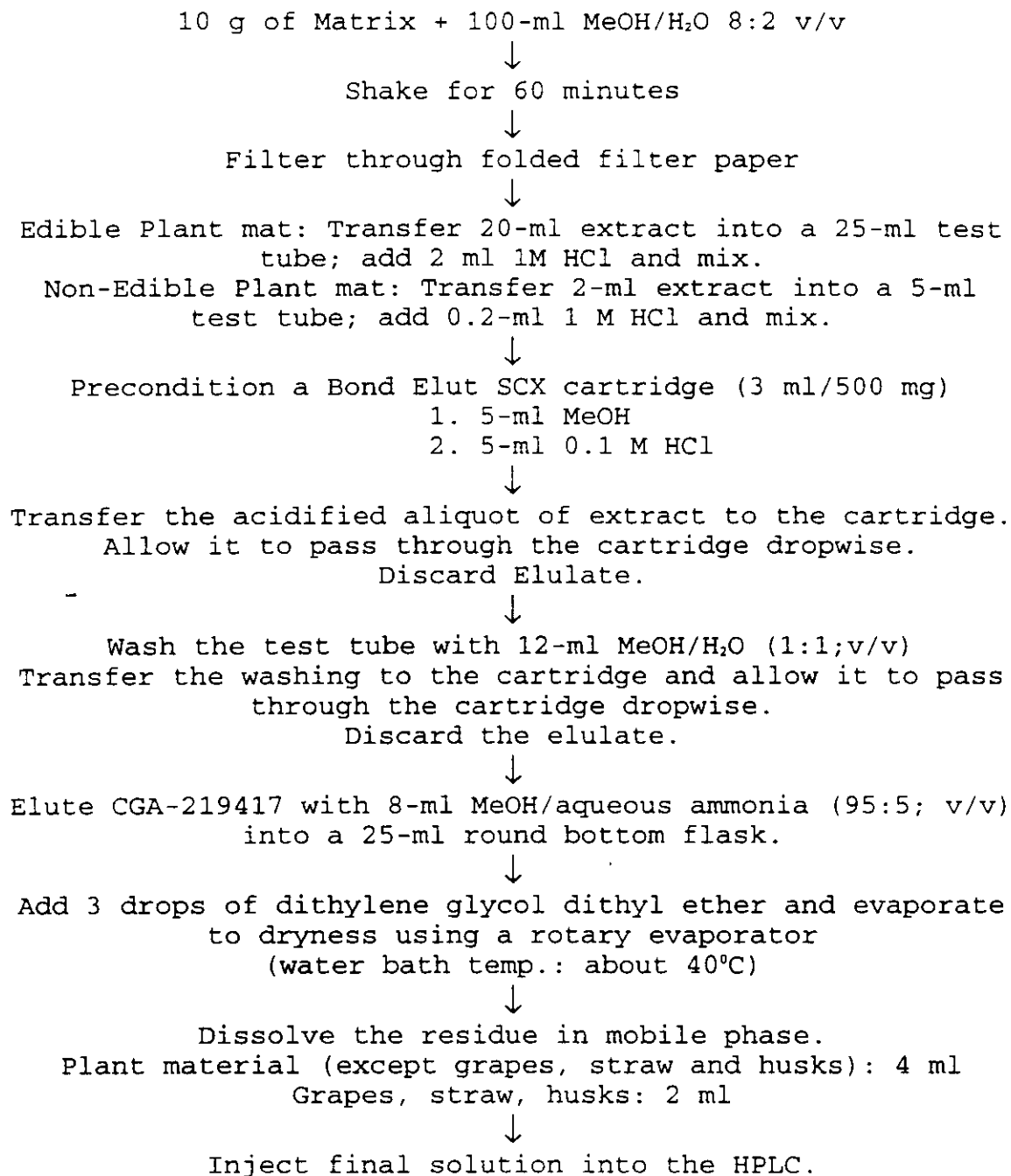


FIGURE 4. FLOW DIAGRAM OF METHOD FOR WINE

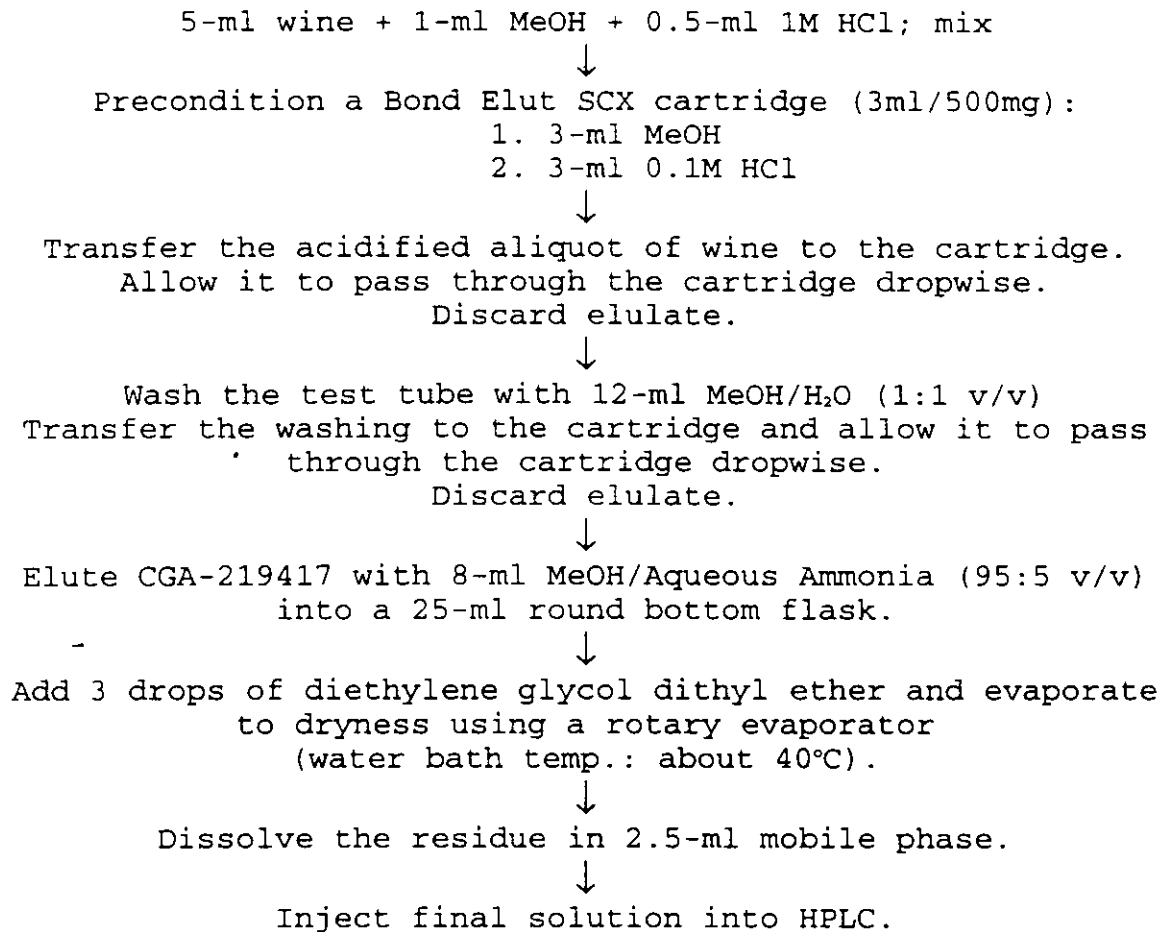
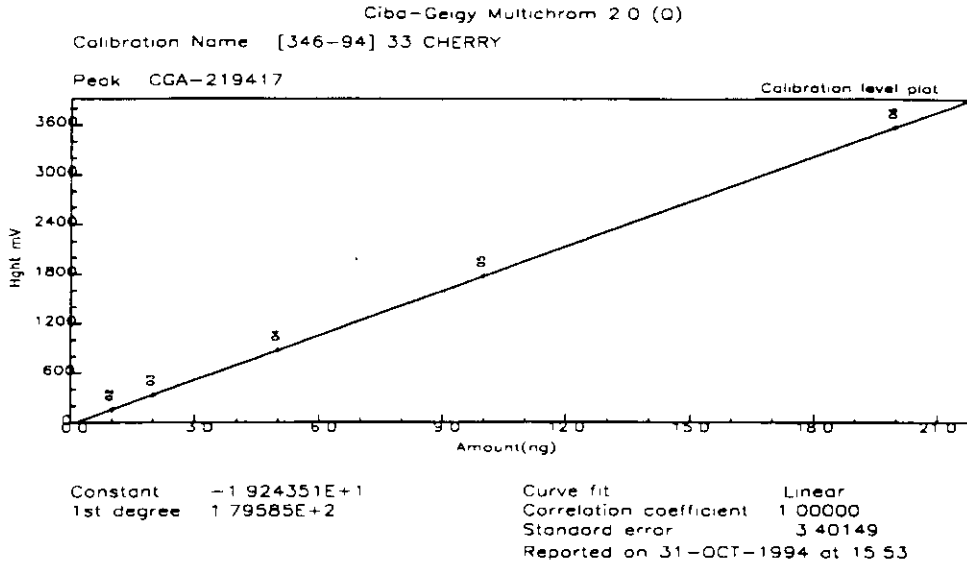
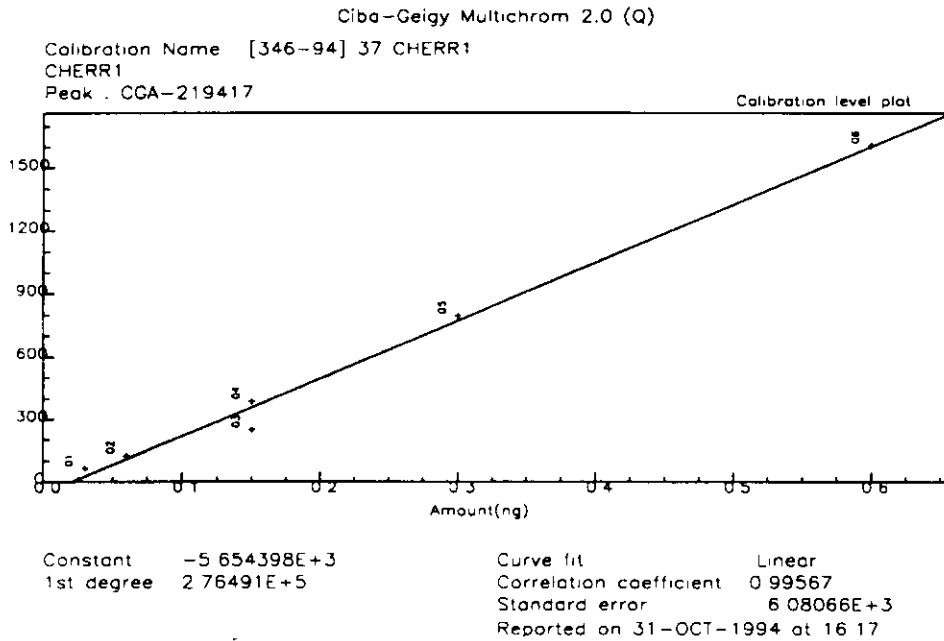


FIGURE 5. CGA-219417 CALIBRATION CURVES

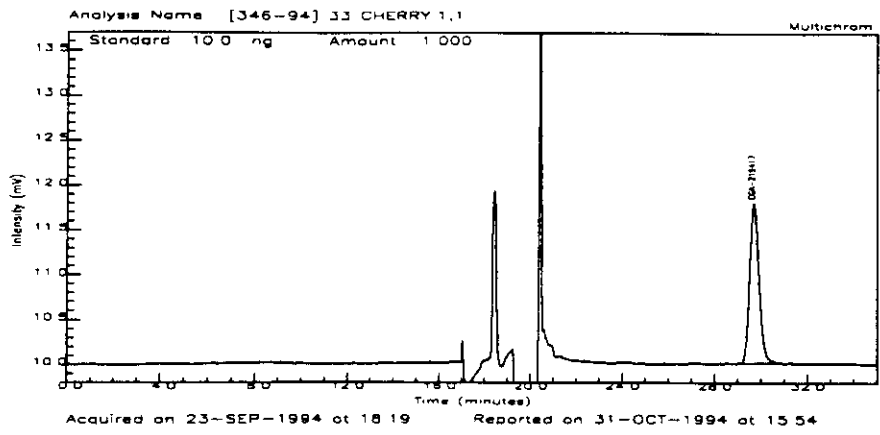


Curve for HPLC Standards

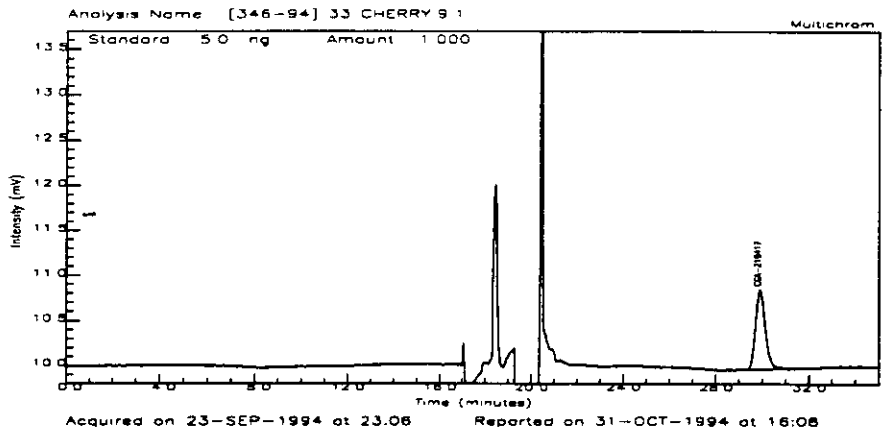


Curve for GC Standards

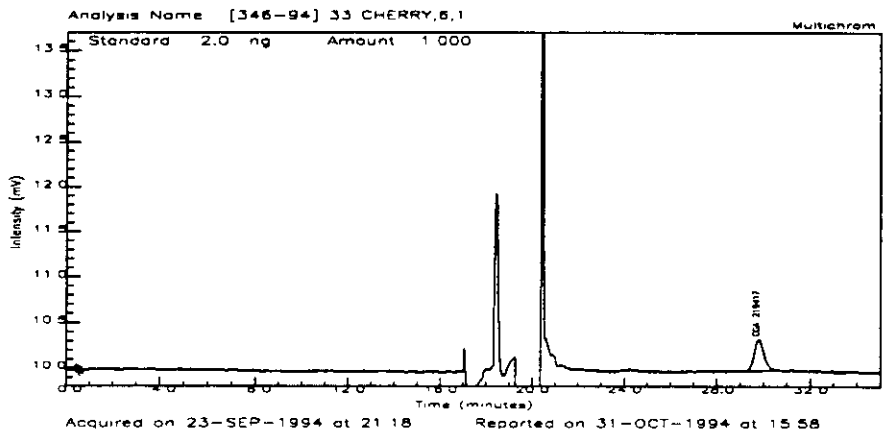
FIGURE 6. CGA-219417 STANDARD CHROMATOGRAMS



HPLC
Standard
CGA-219417
10.0 ng injected

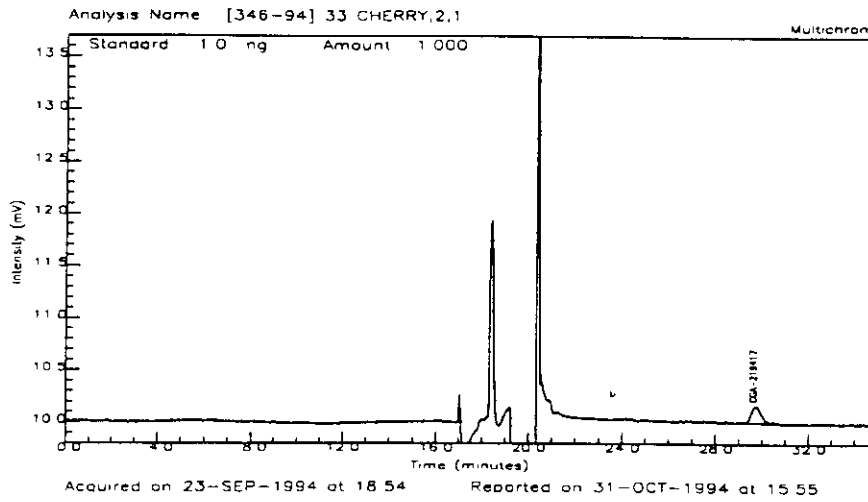


HPLC
Standard
CGA-219417
5.0 ng injected

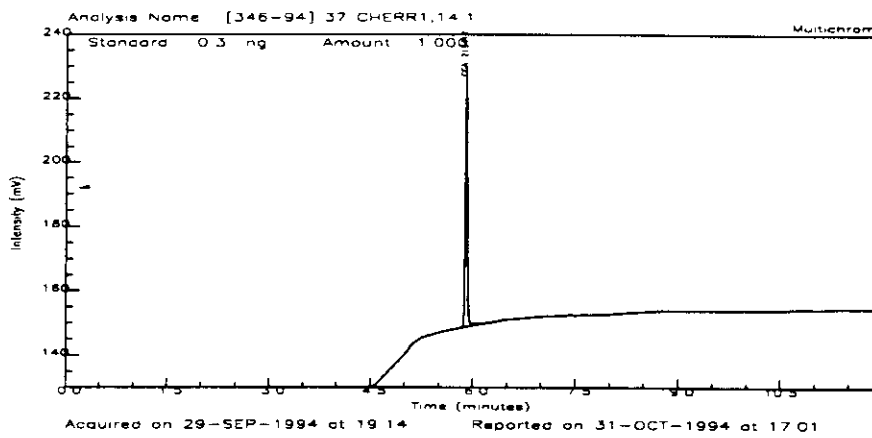


HPLC
Standard
CGA-219417
2.0 ng injected

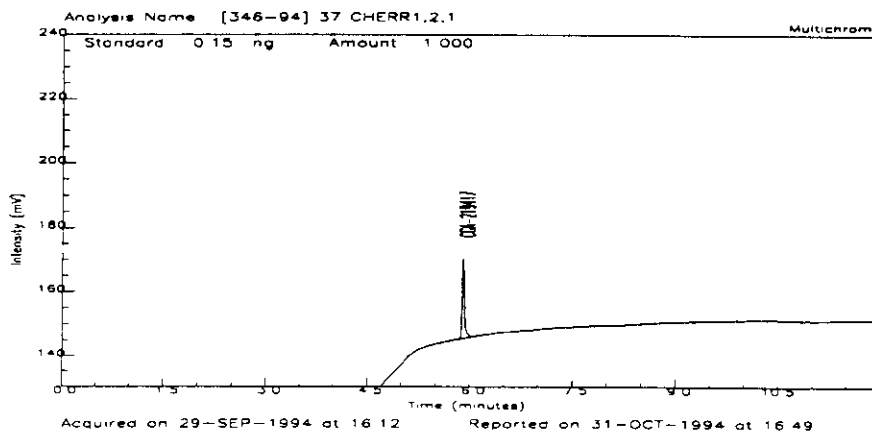
FIGURE 6. CGA-219417 STANDARD CHROMATOGRAMS (Continued)



HPLC
Standard
CGA-219417
1.0 ng injected

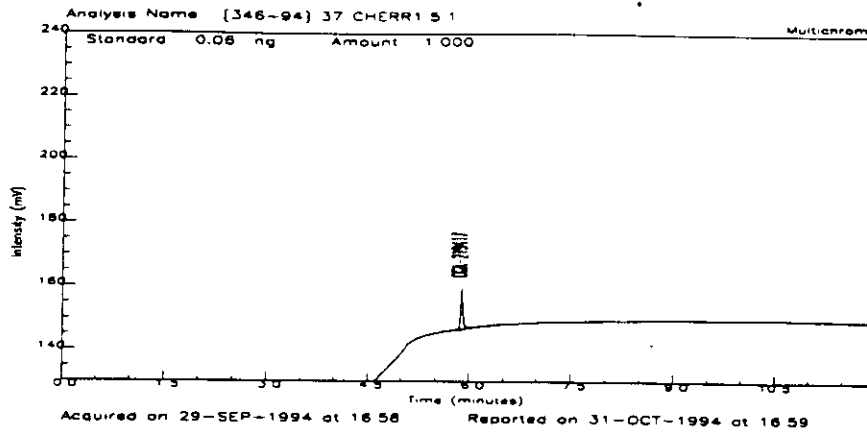


GC
Standard
CGA-219417
0.3 ng injected

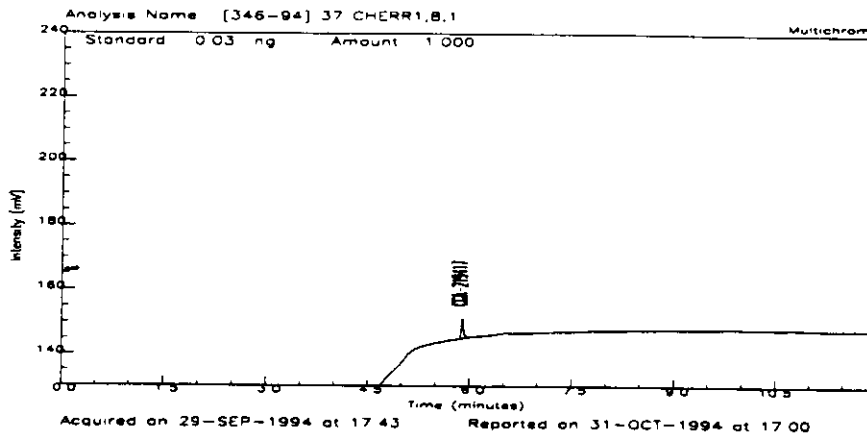


GC
Standard
CGA-219417
0.15 ng injected

FIGURE 6. CGA-219417 STANDARD CHROMATOGRAMS (Continued)

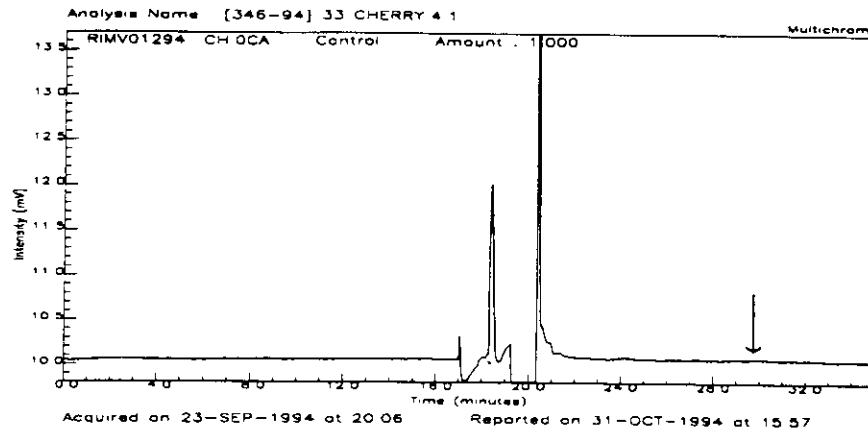


GC
Standard
CGA-219417
0.06 ng injected

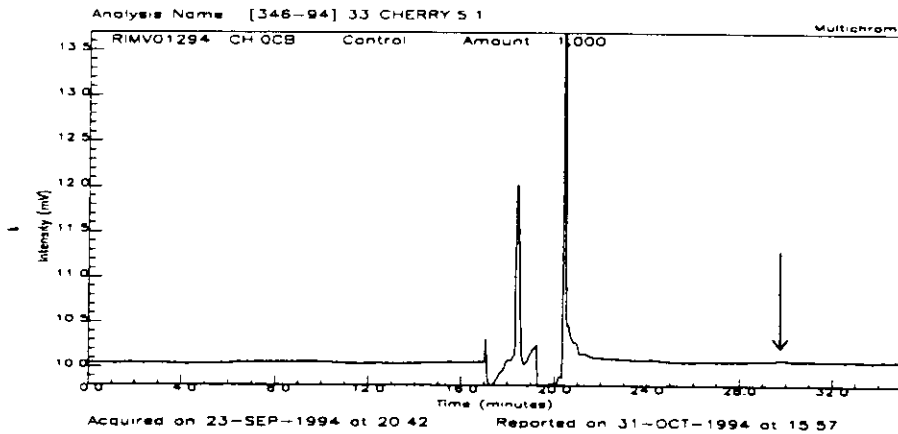


GC
Standard
CGA-219417
0.03 ng injected

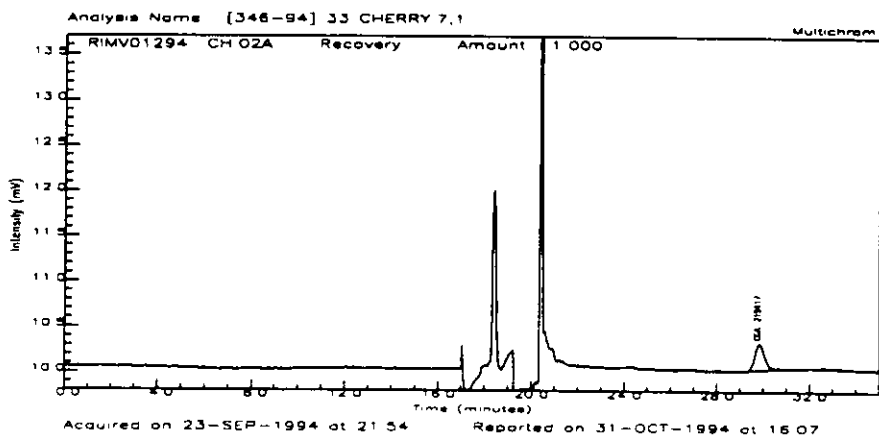
FIGURE 7. CONTROL AND CGA-219417 FORTIFIED CHERRY SAMPLE
HPLC CHROMATOGRAMS



CH.0CA, Control
92.1 mg inj.
<1.0 ng found
<0.02 ppm

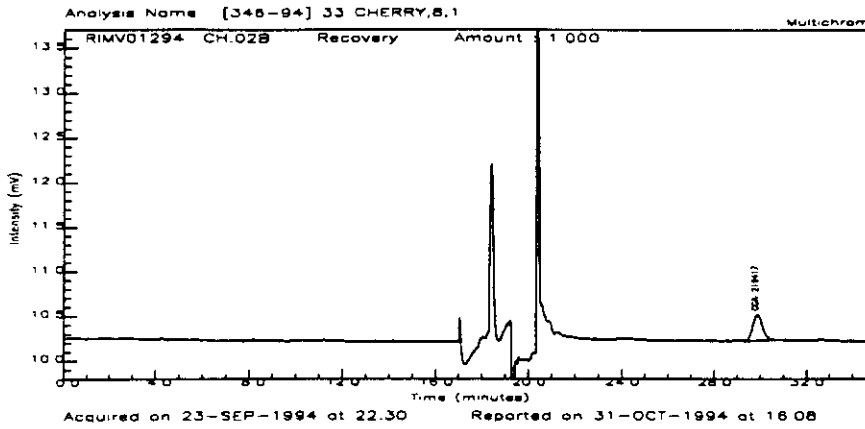


CH.0CB, Control
92.1 mg inj.
<1.0 ng found
<0.02 ppm

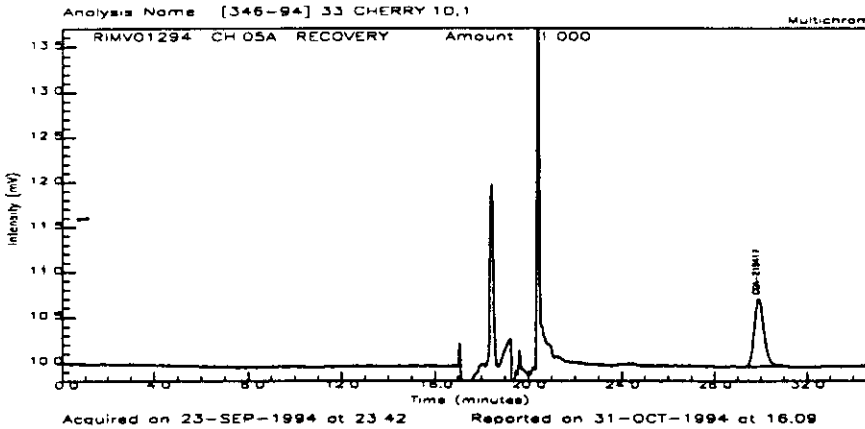


CH.02A,
Control + 0.02 ppm
CGA-219417
92.1 mg inj.
1.71 ng found
0.019 ppm
93% Recovery

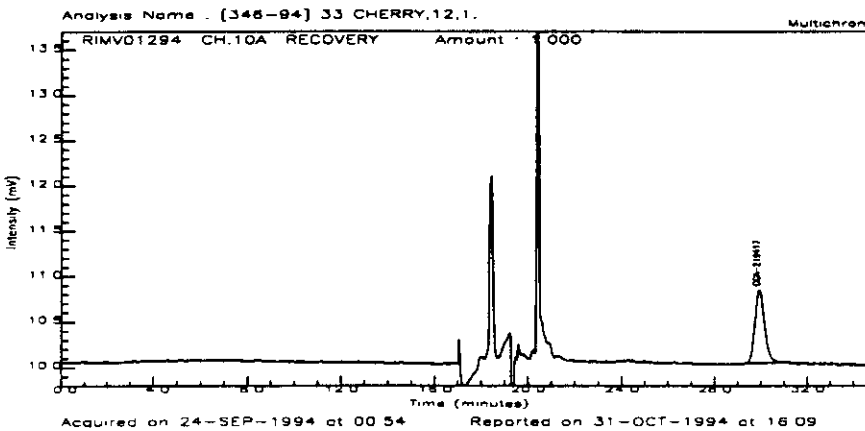
FIGURE 7. CONTROL AND CGA-219417 FORTIFIED CHERRY SAMPLE
HPLC CHROMATOGRAMS (Continued)



CH.02B,
Control + 0.02 ppm
CGA-219417
92.1 mg inj.
1.73 ng found
0.019 ppm
94% Recovery

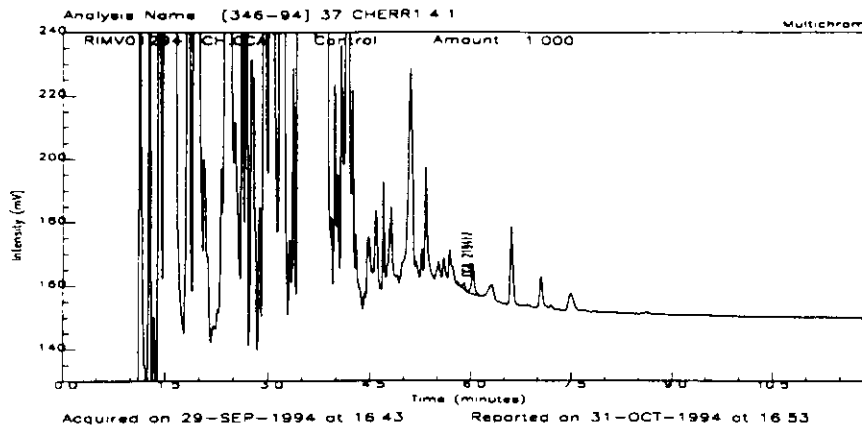


CH.05A,
Control + 0.05 ppm
CGA-219417
92.1 mg inj.
4.26 ng found
0.046 ppm
93% Recovery

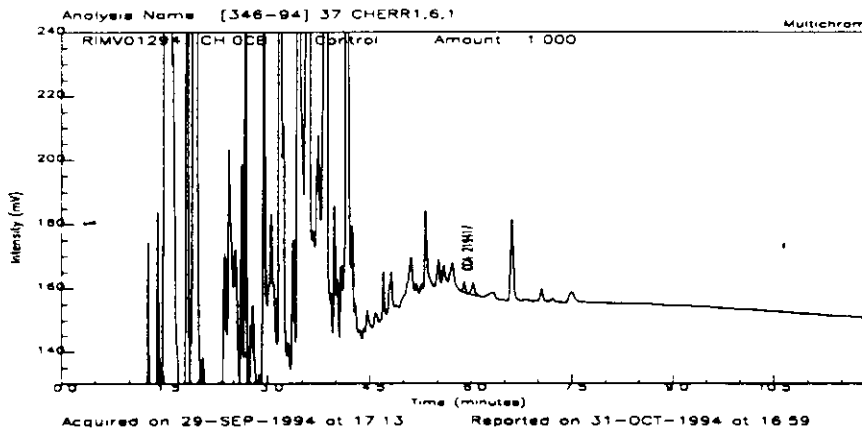


CH.10A,
Control + 0.10 ppm
CGA-219417
46.0 mg inj.
4.6 ng found
0.10 ppm
100% Recovery

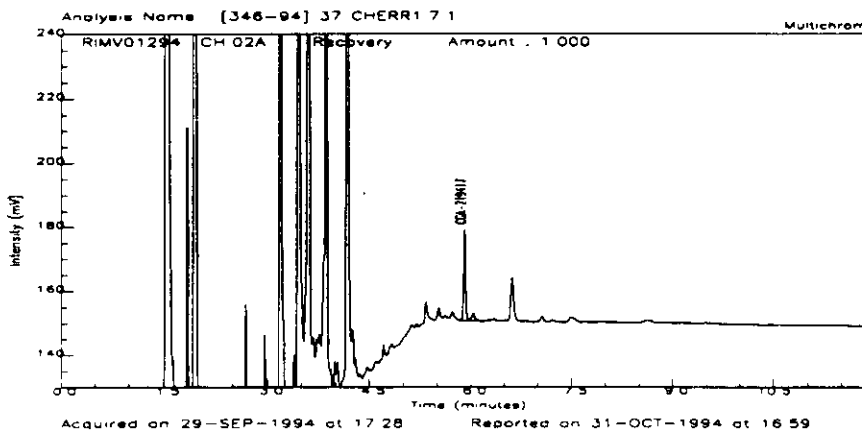
FIGURE 8. CONTROL AND CGA-219417 FORTIFIED CHERRY SAMPLE
GC CHROMATOGRAMS



CH.0CA,
Control
5.53 mg inj.
<0.03 ng (0.028 ng)
found
<0.02 ppm
(0.005 ppm)



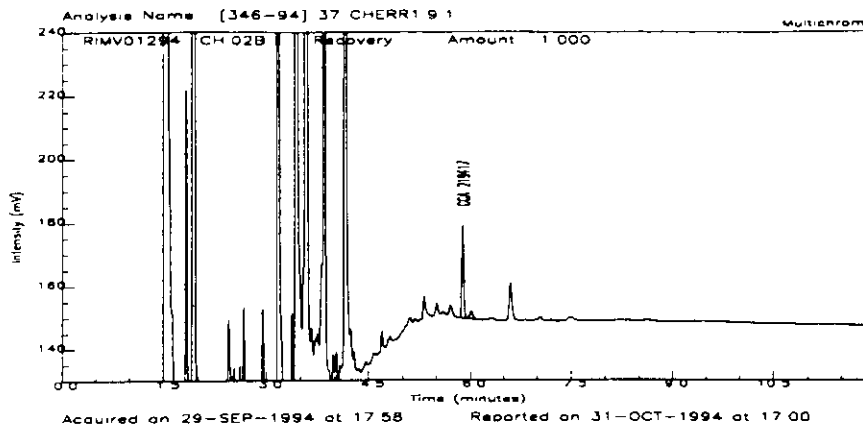
CH.0CB,
Control
5.53 mg inj.
<0.03 ng (0.033 ng)
found
<0.02 ppm
(0.006 ppm)



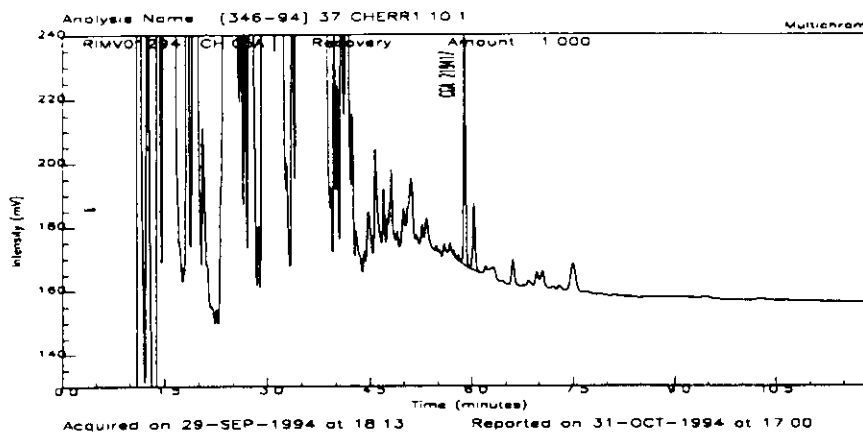
CH.02A,
Control + 0.02 ppm
CGA-219417
5.53 mg inj.
0.123 ng found
0.022 ppm
0.017 ppm corrected
86% Recovery

Recovery results corrected for control values

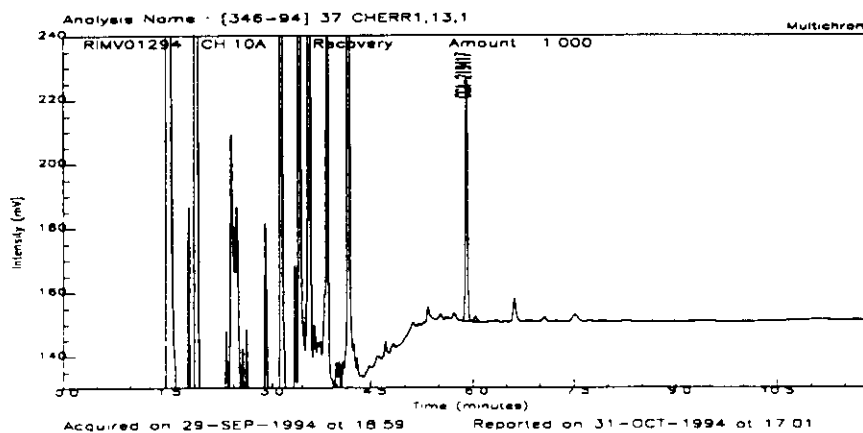
FIGURE 8. CONTROL AND CGA-219417 FORTIFIED CHERRY SAMPLE
GC CHROMATOGRAMS (Continued)



CH.02A,
Control + 0.02 ppm
CGA-219417
5.53 mg inj.
0.127 ng found
0.023 ppm
0.018 ppm corrected
86% Recovery

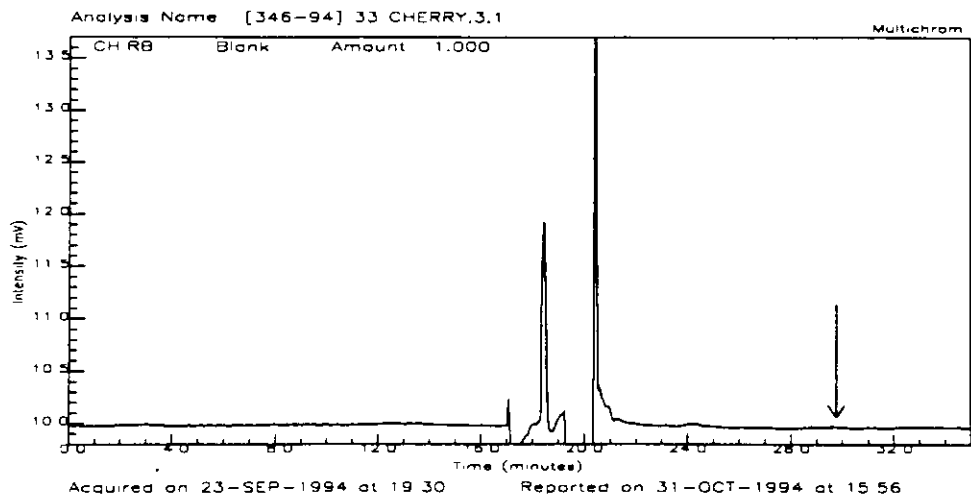


CH.05A,
Control + 0.05 ppm
CGA-219417
5.53 mg inj.
0.301 ng found
0.055 ppm
0.050 ppm corrected
99% Recovery

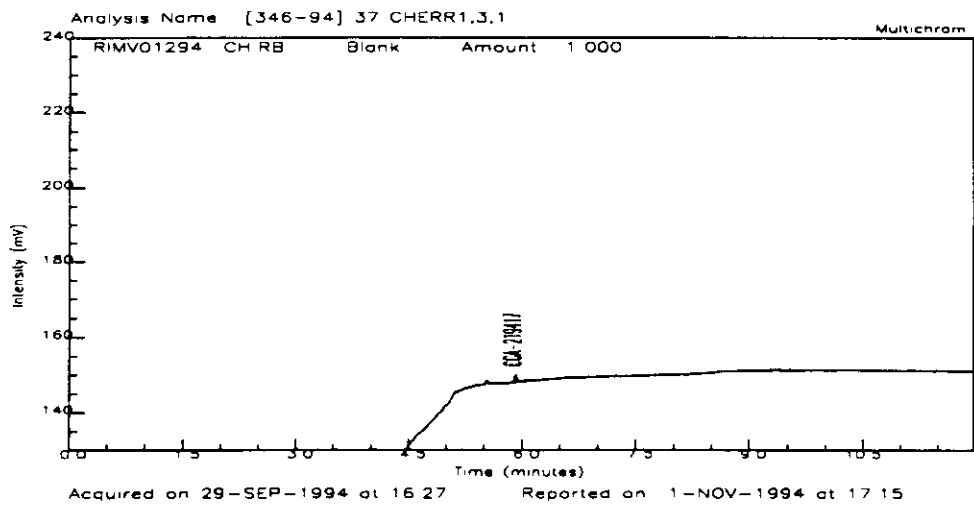


CH.10A,
Control + 0.10 ppm
CGA-219417
2.76 mg inj.
0.294 ng found
0.107 ppm
0.102 ppm corrected
101% Recovery

FIGURE 9. REAGENT BLANK CHROMATOGRAMS



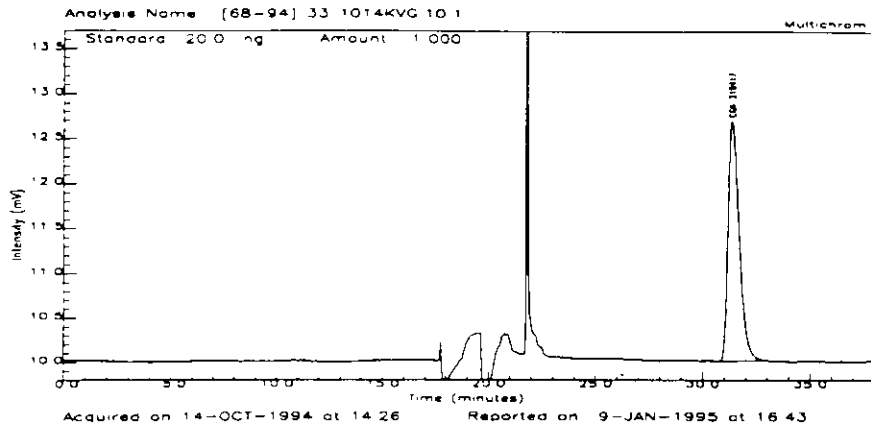
HPLC: CH.RB, 100 mg inj. <1.0 ng, <0.02 ppm



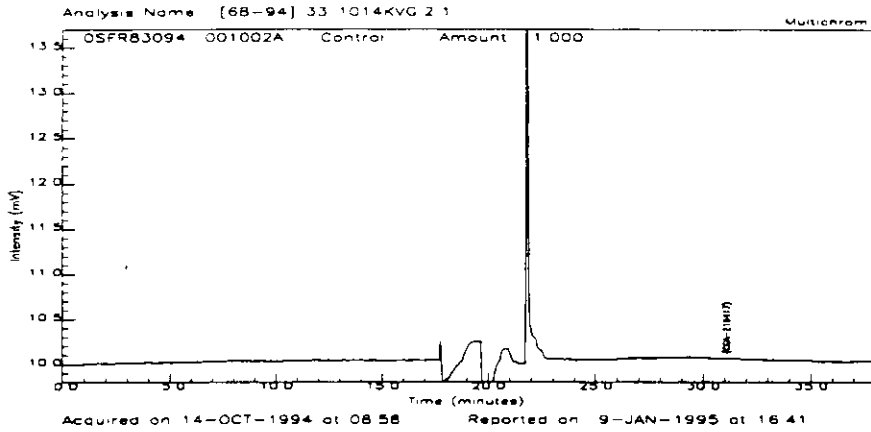
GC: CH.RB, 6.0 mg inj. <1.0 ng, <0.02 ppm

FIGURE 10. REPRESENTATIVE CHROMATOGRAMS - PEACHES

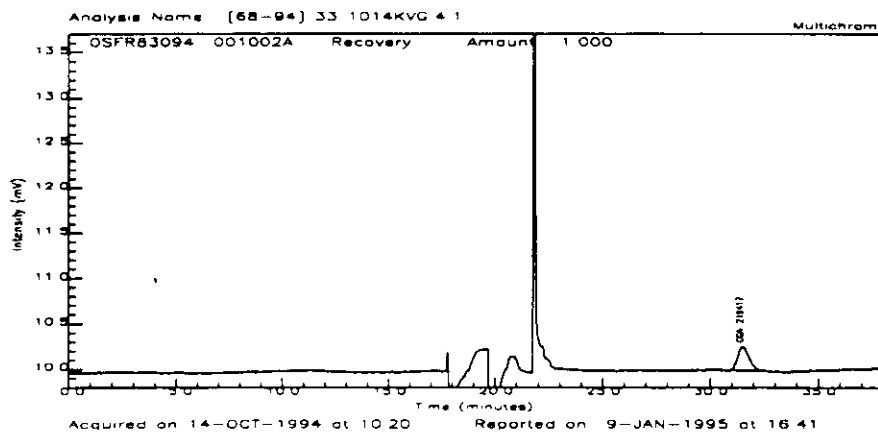
1)



2)



3)

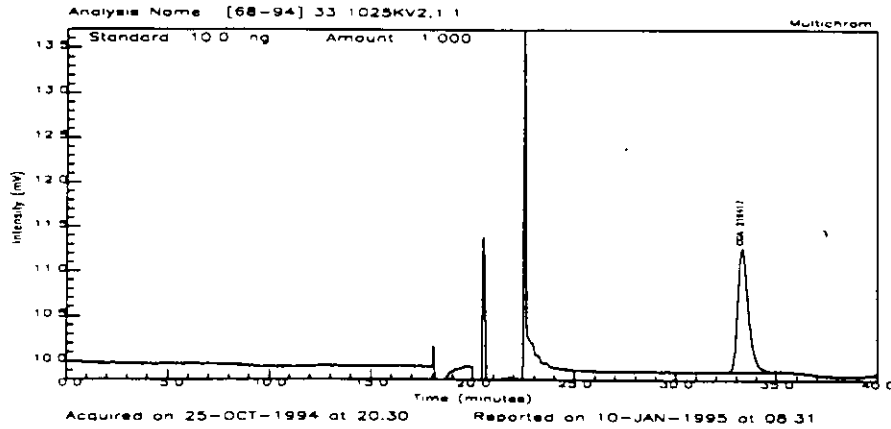


- 1) Standard, CGA-219417, 20. ng
- 2) 1-2-A, control, 91.8 mg injected, <1.0 ng found, <0.02 ppm found (ND)
- 3) 1-2-A, control + 0.02 ppm CGA-219417, 91.8 mg injected, 2.2 ng found, 0.024 ppm found, 0.024 ppm found (corrected for control), 118% recovery

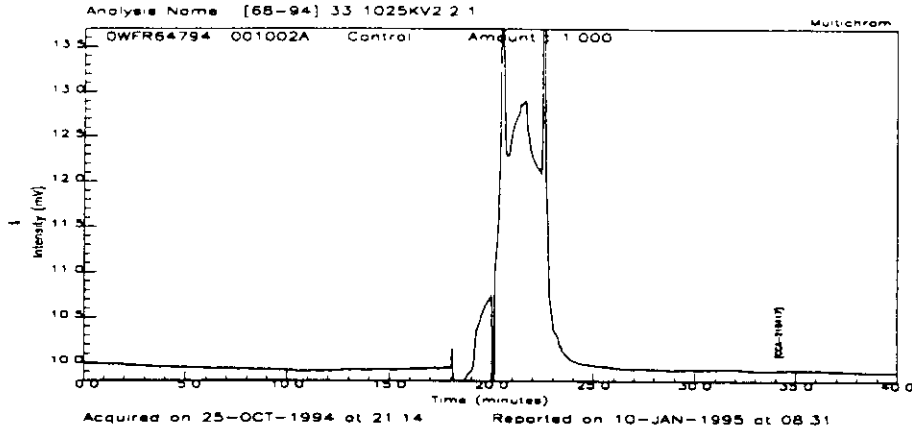
Control results are corrected for procedural recoveries <100%.
ND - No residue detected

FIGURE 11. REPRESENTATIVE CHROMATOGRAMS - PLUMS

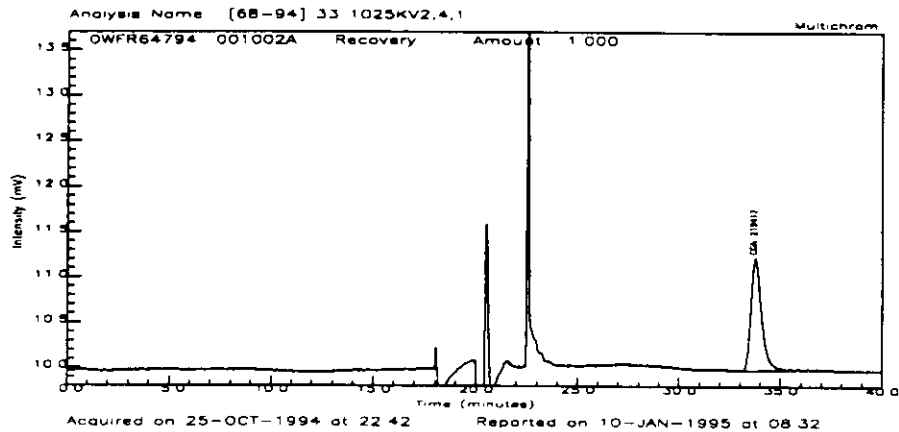
1)



2)



3)

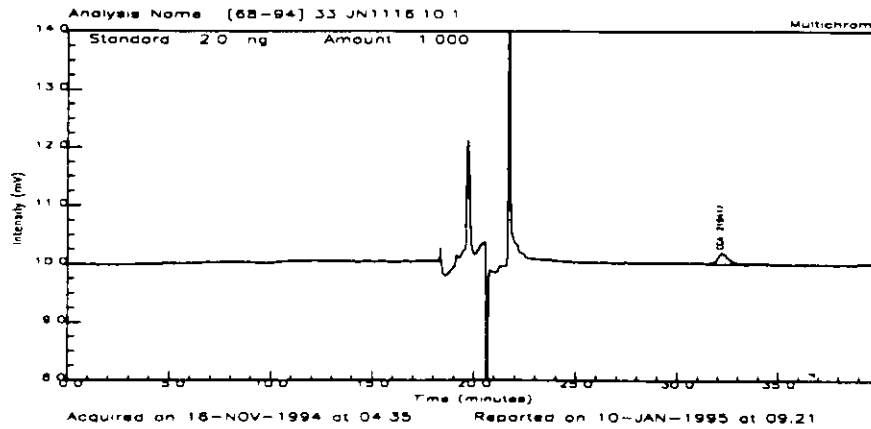


- 1) Standard, CGA-219417, 10 ng
- 2) 1-2-A, control, 92.3 mg injected, <1.0 ng found, <0.02 ppm found (ND)
- 3) 1-2-A, control + 1.0 ppm CGA-219417, 9.2 mg injected, 9.4 ng found, 1.02 ppm found, 1.02 ppm found (corrected for control), 102% recovery

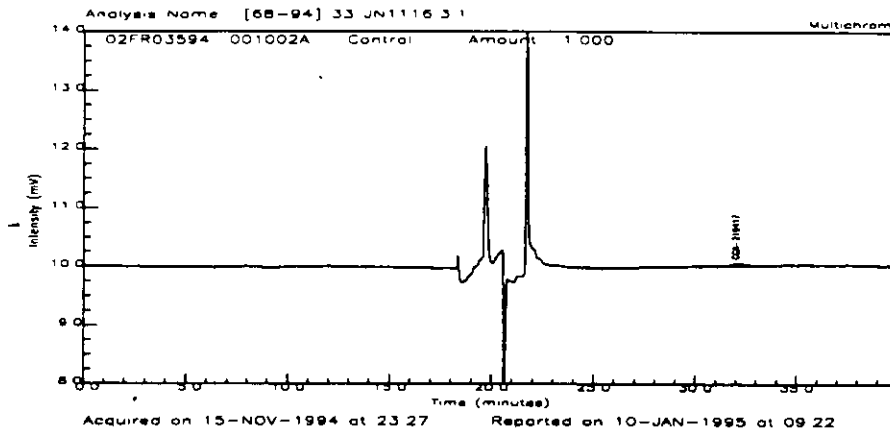
Control results are corrected for procedural recoveries <100%

FIGURE 12. REPRESENTATIVE CHROMATOGRAMS - PRUNES

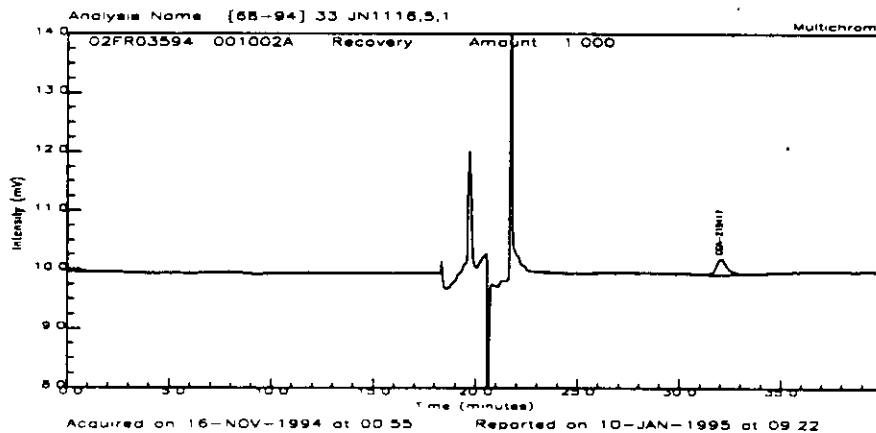
1)



2)



3)

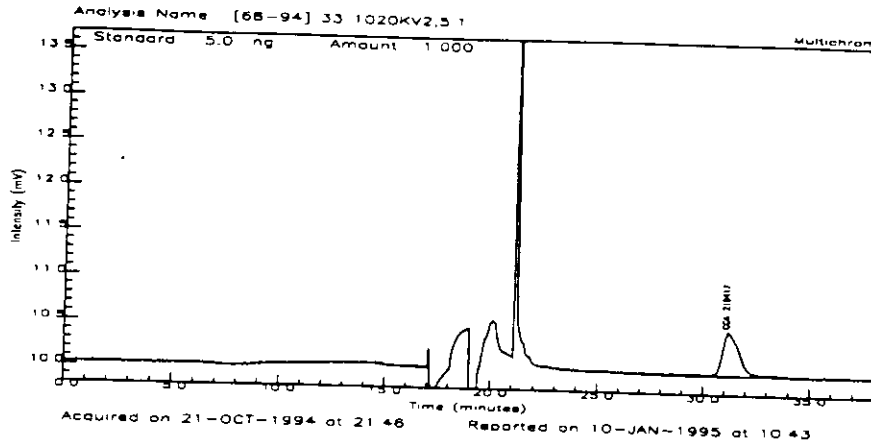


- 1) Standard, CGA-219417, 2.0 ng
- 2) 1-2-A, control, 92.7 mg injected, <1.0 ng found, <0.02 ppm found (0.009 ppm)
- 3) 1-2-A, control + 0.02 ppm CGA-219417, 92.7 mg injected, 2.4 ng found, 0.026 ppm found, 0.017 ppm found (corrected for control), 83% recovery.

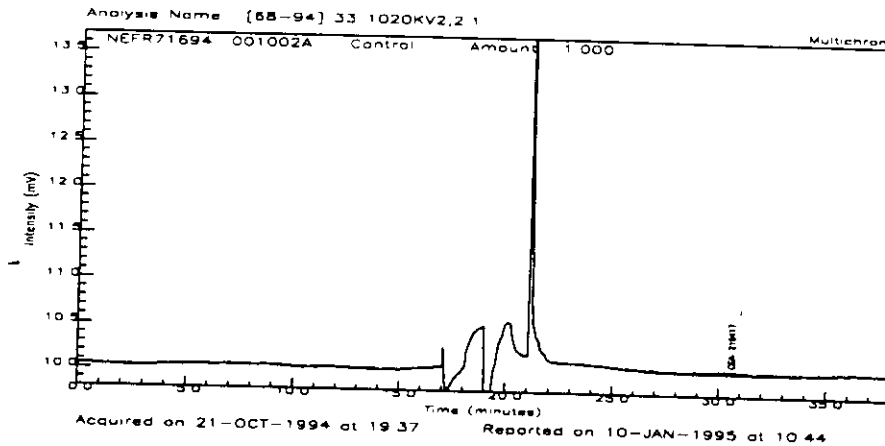
Control results are corrected for procedural recoveries <100%.

FIGURE 13. REPRESENTATIVE CHROMATOGRAMS - SWEET CHERRIES

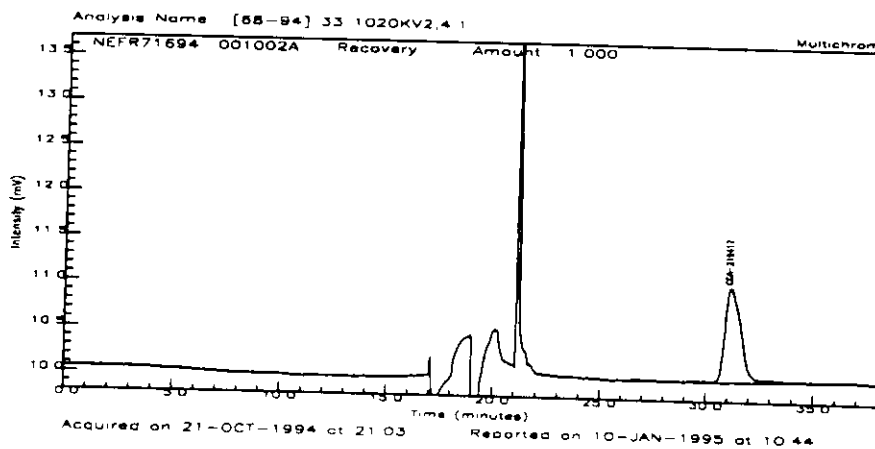
1)



2)



3)

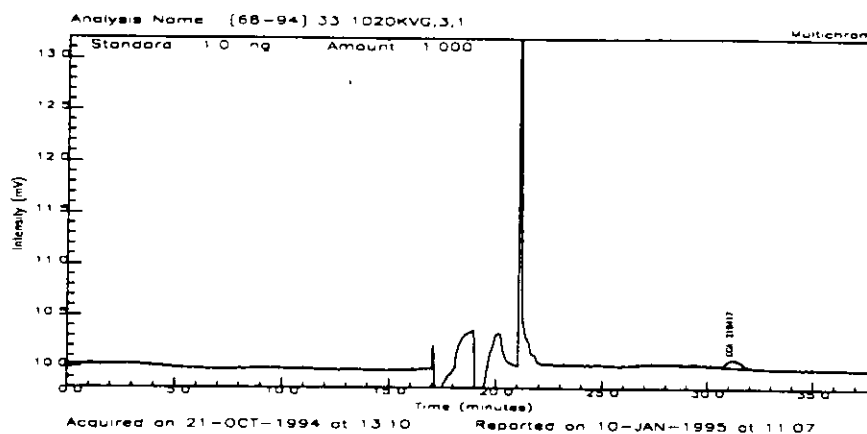


- 1) Standard, CGA-219417, 5.0 ng
- 2) 1-2-A, control, 92.6 mg injected, <1.0 ng found, <0.02 ppm found (0.005 ppm)
- 3) 1-2-A, control + 0.50 ppm CGA-219417, 18.5 mg injected, 10.2 ng found, 0.55 ppm found, 0.55 ppm found (corrected for control), 109% recovery

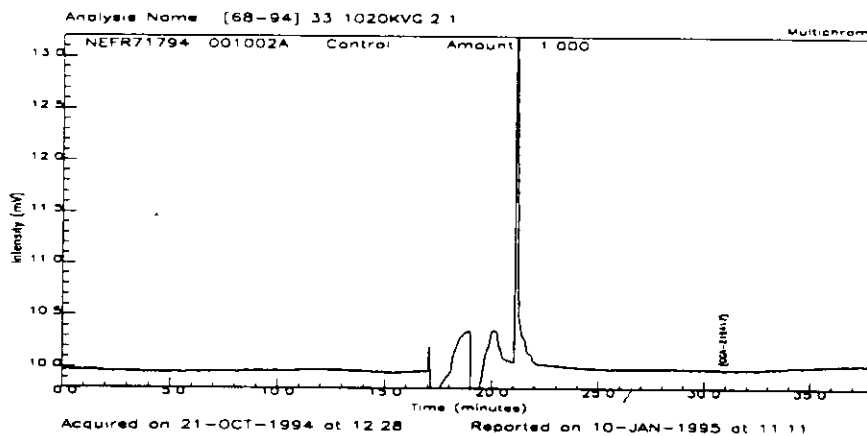
Control results are corrected for procedural recoveries <100%

FIGURE 14. REPRESENTATIVE CHROMATOGRAMS - TART CHERRIES

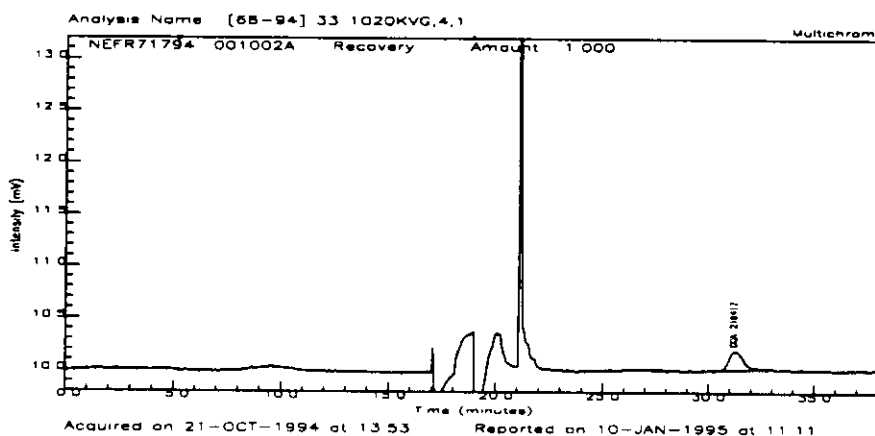
1)



2)



3)

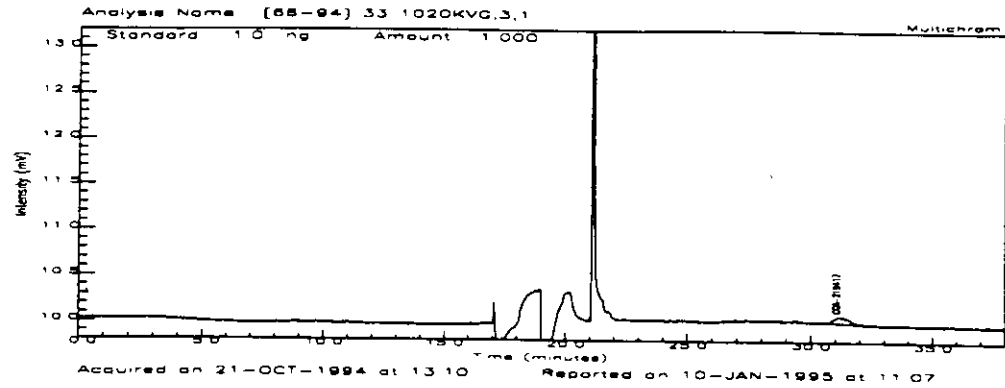


- 1) Standard, CGA-219417, 1.0 ng
- 2) 1-2-A, control, 92.3 mg injected, <1.0 ng found, <0.02 ppm found (ND)
- 3) 1-2-A, control + 0.02 ppm CGA-219417, 92.3 mg injected, 2.0 ng found, 0.022 ppm found, 0.022 ppm found (corrected for control), 110% recovery

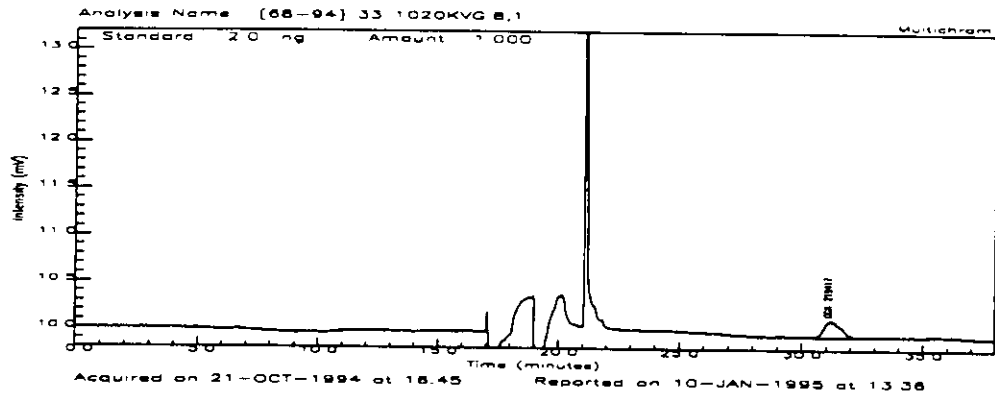
Control results are corrected for procedural recoveries <100%.
ND - No residue detected

FIGURE 15. REPRESENTATIVE CHROMATOGRAMS OF CGA-219417
STANDARDS FOR TART CHERRIES

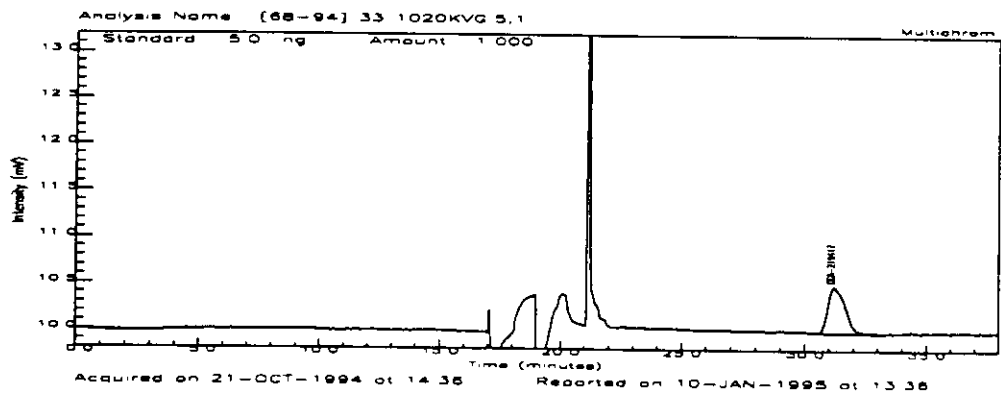
1)



2)



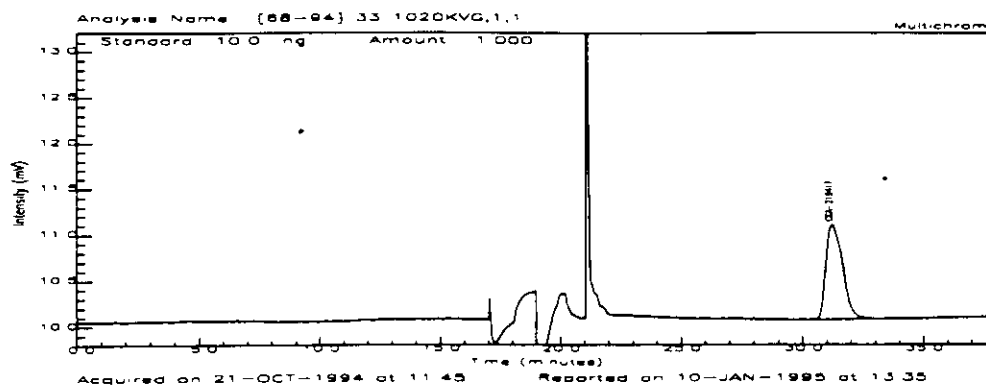
3)



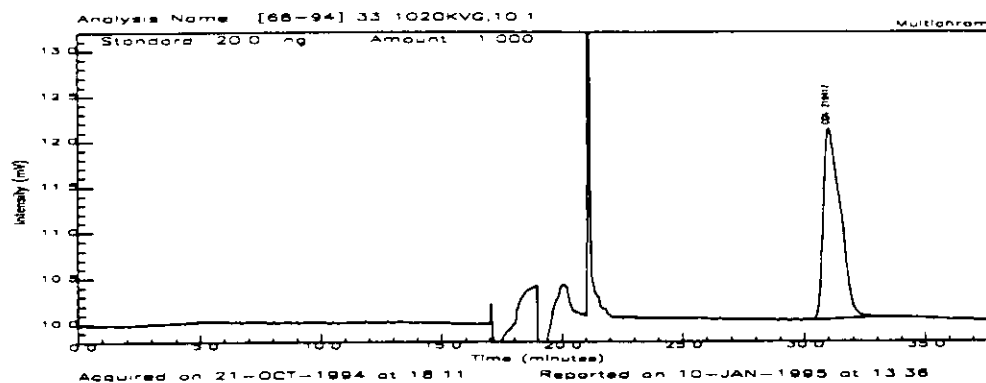
- 1) Standard, 1.0 ng (Response Factor = 77)
- 2) Standard, 2.0 ng (Response Factor = 179)
- 3) Standard, 5.0 ng (Response Factor = 500)

FIGURE 15. REPRESENTATIVE CHROMATOGRAMS OF CGA-219417
STANDARDS FOR TART CHERRIES (Continued)

4)



5)



- 4) Standard, 10. ng (Response Factor = 1022)
- 5) Standard, 20. ng (Response Factor = 2090)

FIGURE 16. REPRESENTATIVE STANDARD CALIBRATION CURVE
USING STANDARDS FROM FIGURE 15

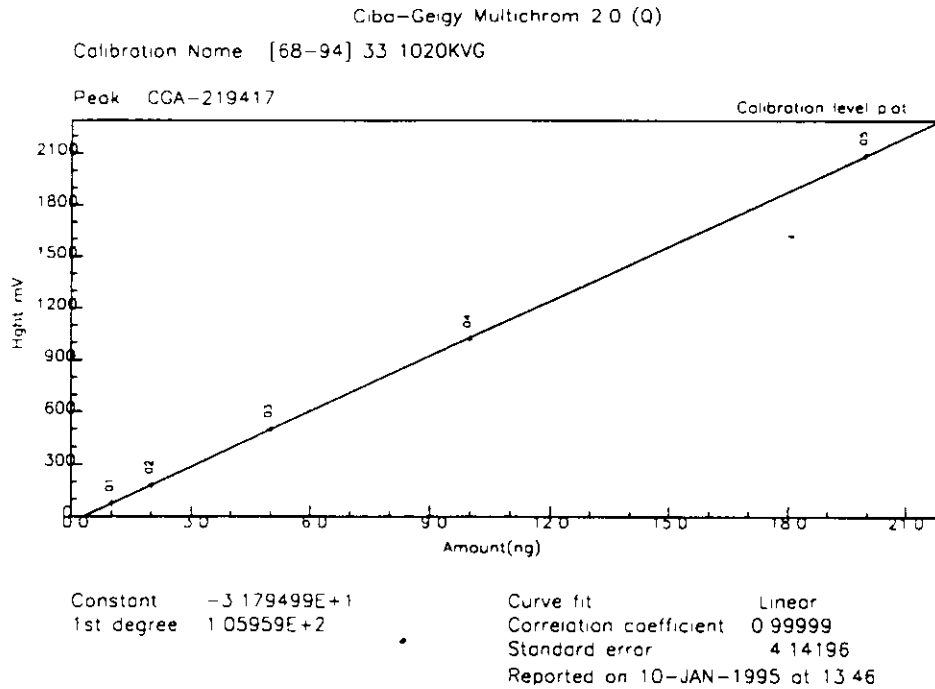
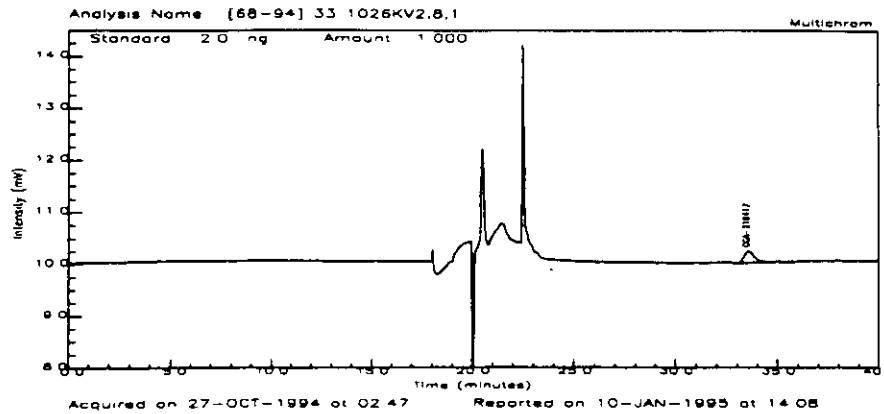
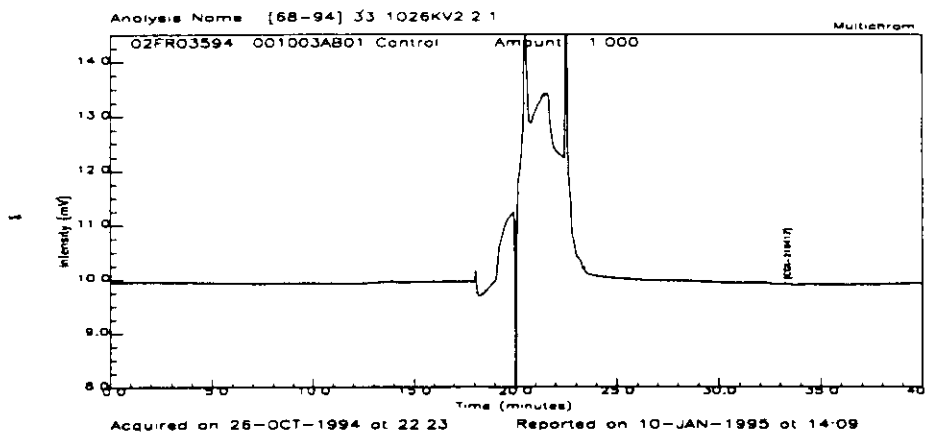


FIGURE 17. REPRESENTATIVE CHROMATOGRAMS - PROCESSED
PRUNES - FRESH

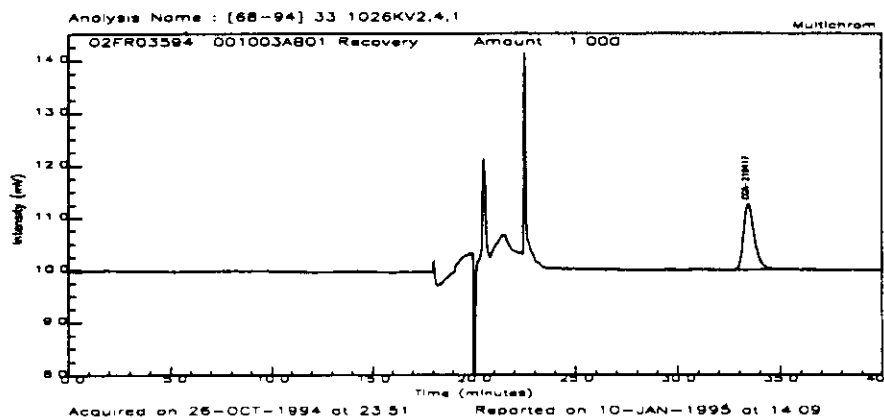
1)



2)



3)

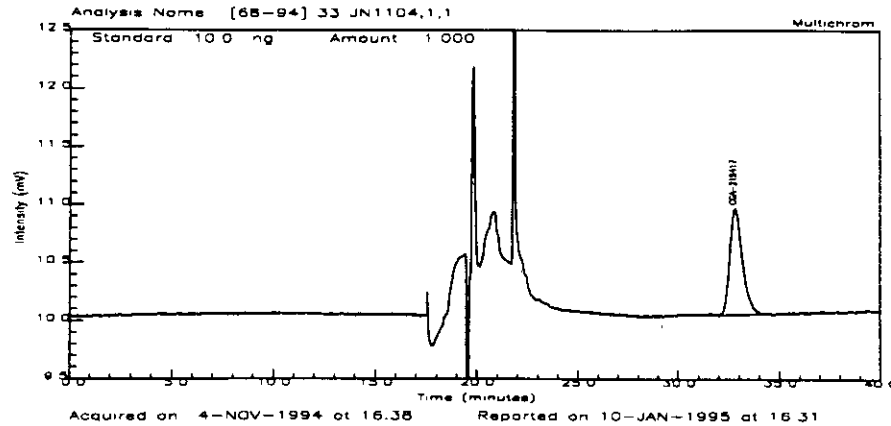


- 1) Standard, CGA-219417, 2.0 ng
- 2) 1-3-AB-01, control, 92.7 mg injected, <1.0 ng found, <0.02 ppm found (ND)
- 3) 1-3-AB-1, control + 1.0 ppm CGA-219417, 9.3 mg injected, 9.4 ng found, 1.01 ppm found, 1.01 ppm found (corrected for control), 101% recovery

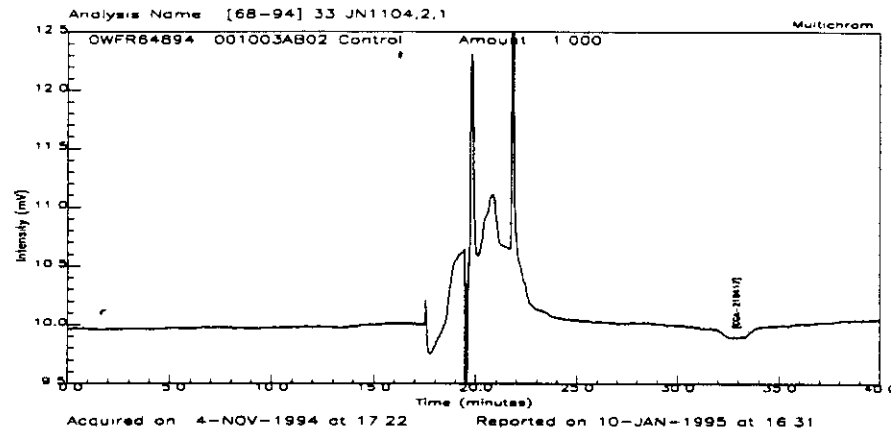
Control results are corrected for procedural recoveries <100%.
ND - No residue detected

FIGURE 18. REPRESENTATIVE CHROMATOGRAMS - PROCESSED PRUNES - DRIED

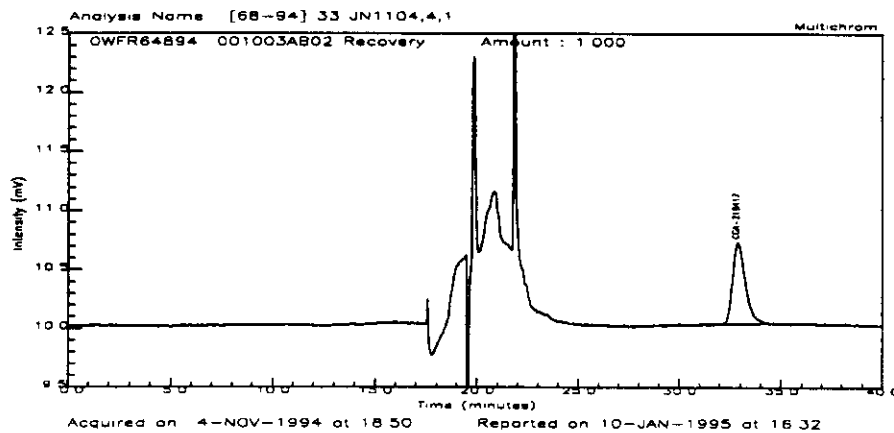
1)



2)



3)

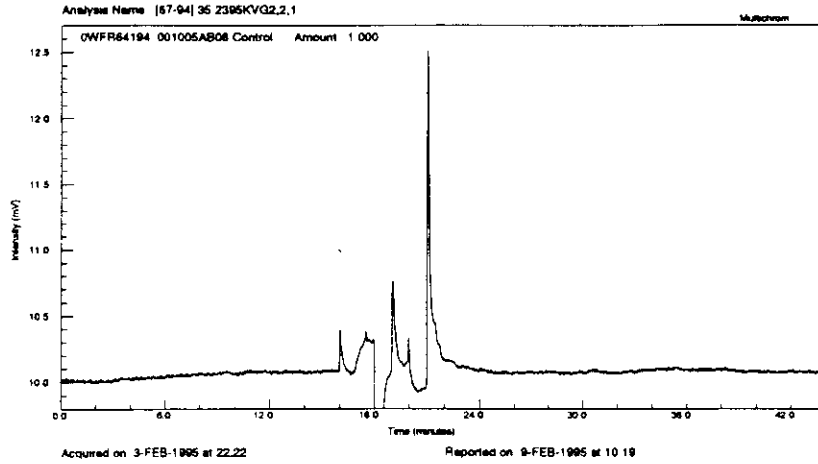


- 1) Standard, CGA-219417, 10.0 ng
- 2) 1-3-AB-02, control, 97.3 mg injected, <1.0 ng found, <0.02 ppm found (ND)
- 3) 1-3-AB-2, control + 1.0 ppm CGA-219417, 9.7 mg injected, 7.7 ng found, 0.79 ppm found, 0.79 ppm found (corrected for control), 79% recovery

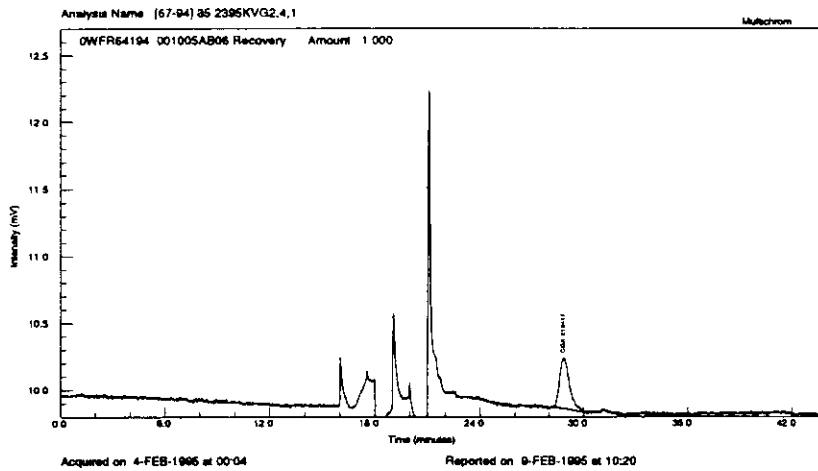
Control results are corrected for procedural recoveries <100%.
ND - No residue detected

FIGURE 19. REPRESENTATIVE CHROMATOGRAMS - APPLE JUICE

1)



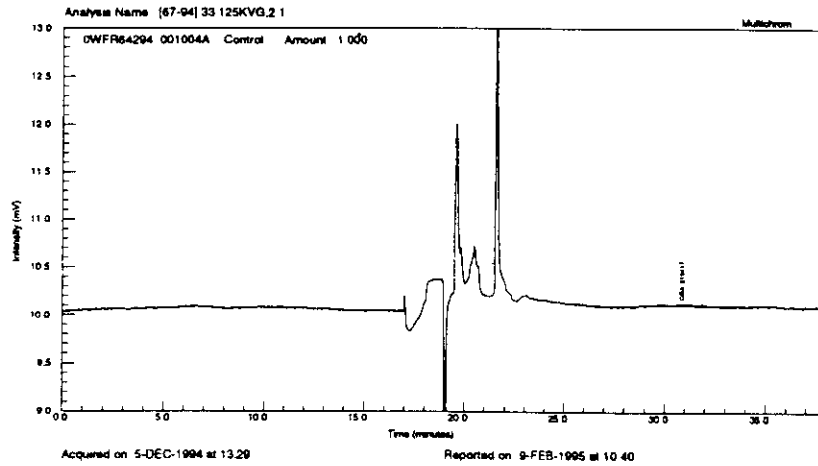
2)



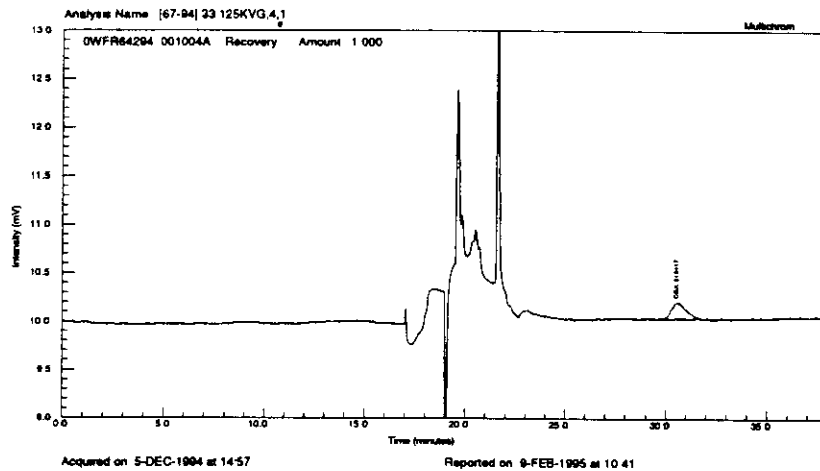
- 1) Apple juice control:1-5AB-6, control, 333 mg inj., <0.731 ng found, <0.010 ppm found (ND)
- 2) Apple juice recovery:1-5AB-6, control + 0.01 ppm, 333 mg inj., 3.2'ng found, 0.01 ppm found, 96% recovery.

FIGURE 20. REPRESENTATIVE CHROMATOGRAMS - APPLE FRUIT

1)



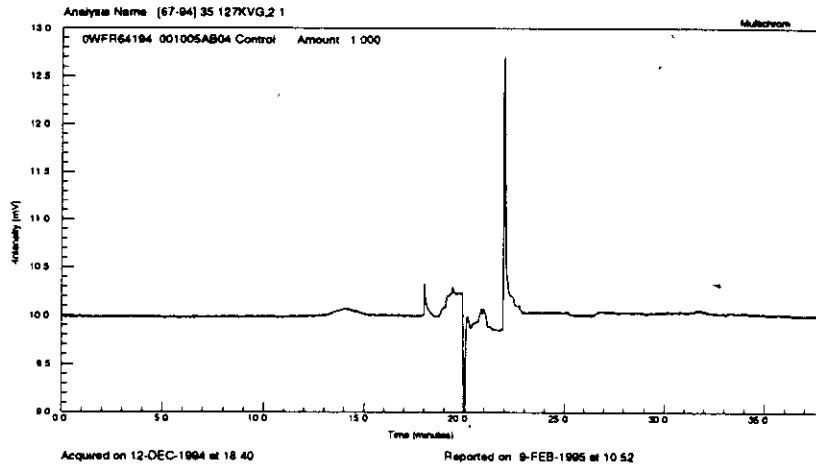
2)



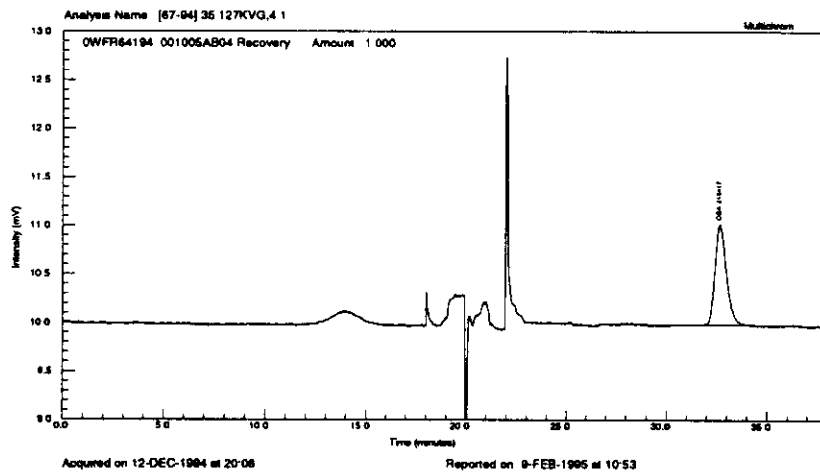
- 1) Apple fruit control:1-4-A, control, 92 mg inj., <0.95 ng found, <0.020 ppm found (ND).
- 2) Apple fruit recovery:1-4-A + 0.02 ppm, 92 mg inj., 1.7 ng found, 0.018 ppm found, 91% recovery.

FIGURE 21. REPRESENTATIVE CHROMATOGRAMS - APPLE WET POMACE

1)



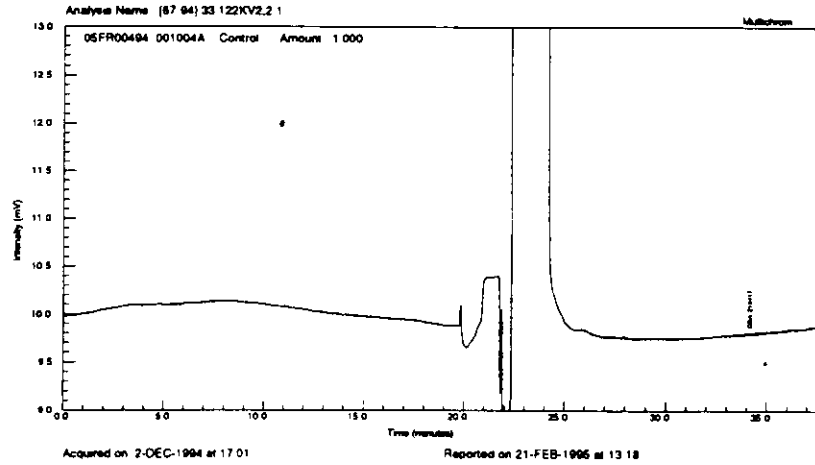
2)



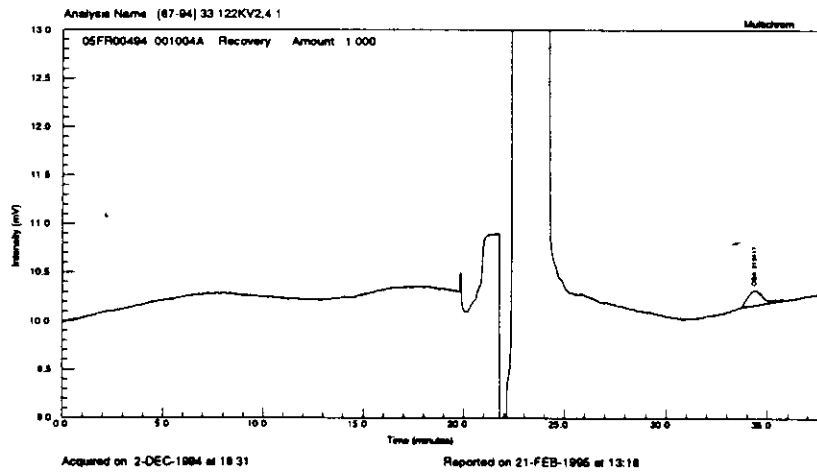
- 1) Apple wet pomace control:1-5AB-4, control, 94 mg inj., <0.98 ng found, <0.020 ppm found (ND).
- 2) Apple wet pomace recovery:1-5AB-4 + 0.5 ppm, 19 mg inj., 7.9 ng found. 0.42 ppm, 84% recovery.

FIGURE 22. REPRESENTATIVE CHROMATOGRAMS - PEAR FRUIT

1)



2)



- 1) Pear fruit control:1-4-A, control 92 mg inj., <1.2 ng found, <0.02 ppm found (ND)
- 2) Pear fruit recovery:1-4-A + 0.02 ppm, 92 mg inj., 2.2 ng found, 0.024 ppm, 93% recovery.

IV. REFERENCES

1. Dieterle, R., "Determination of Residues of Parent Compound by High Performance Liquid Chromatography (HPLC)," Ciba-Geigy Limited REM 141.01, December, 1989.
2. Dieterle, R., Report of Special Study, 100/92, "CGA-219417 Validation of Method REM 141.01 on Tomatoes," Ciba-Geigy Limited, July, 1992.
3. Wurz, R., "Method Validation Ruggedness Trial for the Determination of CGA-219417 in Cherries Using Analytical Method REM 141.01," "Determination of Residues of Parent Compound by High Performance Liquid Chromatography (HPLC)," Ciba Crop Protection ABR-94088, April, 1995.
4. Van Geluwe, K., "CGA-219417 and Propiconazole - Magnitude of the Residues in or on Representative Commodities of the Pome Fruits Group, Including Processed Apple Fractions, Following Foliar Applications of CGA-219417 75WP and Tilt 45WP," Protocol 67-94, Ciba Crop Protection (in progress).
5. Van Geluwe, K., "CGA-219417 - Magnitude of the Residues in or on Representative Commodities of the Stone Fruits Group, Including Processed Prunes, Following Post Foliar Spray Applications," Ciba Crop Protection ABR-94093, March, 1995.

APPENDIX I

SEPARATE DOCUMENTS ACCOMPANYING THIS REPORT

1. Dieterle, R., Ciba-Geigy Limited REM 141.01, "Determination of Residues of Parent Compound by High Performance Liquid Chromatography (HPLC)."
2. Wurz, R., Ciba Crop Protection 's ABR-94088, "Method Validation Ruggedness Trial for the Determination of CGA-219417 in Cherries Using Analytical Method REM 141.01, 'Determination of Residues of Parent Compound by High Performance Liquid Chromatography (HPLC).'"
3. Van Geluwe, K., ABR-94093. "CGA-219417 - Magnitude of the Residues In or On Representative Commodities of the Stone Fruits Group, Including Processed Prunes, Following Post Foliar Spray Applications,"

SUBMITTER/SPONSOR: Ciba Plant Protection, Ciba-Geigy Corporation, Post Office Box 18300, Greensboro, NC 27419

APPENDIX II

REPORT OF SPECIAL STUDY 100/92

"CGA-219417 - VALIDATION OF METHOD REM 141.01 ON TOMATOES"

AUTHOR

DR. ROLAND MARIO DIETERLE

LABORATORY

CIBA-GEIGY LIMITED
BASEL, SWITZERLAND

Ciba Crop Protection, Ciba-Geigy Corporation, P. O. Box 18300, Greensboro, NC 27419-8300

CGA 219417

Validation of Method REM 141.01 on Tomatoes

1 OBJECTIVE

Determination of the extractability and accountability of analysis method REM 141.01 for tomatoes.

2 SUBSTRATE

Washed fruits of tomatoes treated with ¹⁴C-CGA 219417 (= active ingredient a.i., spec. activity: 15 µCi/mg).
 Two spray applications with 750 g a.i./ha each (WP 50); sampling: 15 days after last application.
 Origin: Ciba-Geigy AG, Metabolism PP 2.52, Basel, CH, project No. 91CN02.

3 ANALYTICAL METHOD

Method REM 141.01 issued 21 Dec 1989 (CIBA-GEIGY AG, Residue Analysis, Basel, Switzerland). Modifications are included in REM 141.01 Appendix 1 from 3 April 1992.

Extraction of specimen with methanol/water (8 vol.+ 2 vol.); clean-up on Bond Elut® SCX; determination by HPLC, UV-detection.

4 RESULTS

4.1 Total Residues

Table 1: total residues determined by measurement of total radioactivity (r.a.)

matrix	CGA 219417-equivalents [mg/kg]	
	individual analyses	mean value
tomatoes fruits	3.56 / 4.24 / 4.16 4.50 / 4.61 / 4.09	4.19 ± 0.37

4.2 Efficiency of Extraction (Extractability)

Table 2: residues in extracts and extractability

total residue [mg a.i.-eq./kg]	residues in extracts individual values [mg a.i.-eq./kg]	extractability [%]
4.19	5.21 / 4.69 / 5.19 / 4.40	124 / 112 / 124 / 105

Mean value of extractability: 116 %

4.3 Accountability

Table 3: residues in final solutions for HPLC and in HPLC-fractions containing CGA 219417 determined by r.a. measurements and residues determined by HPLC/UV-detection

final solution [mg a.i.-eq./kg]	HPLC-fraction		HPLC/UV-detection	
	[mg a.i.-eq./kg]	[% accountab.]	[mg/kg]	[% accountab.]
3.82	2.80	67	1.87	45
3.69	2.80	67	1.81	43
3.95	3.10	74	2.22	53
3.41	2.61	62	1.88	45

Mean value of accountability obtained by HPLC and r.a.-measurements: 68 %
Mean value of accountability obtained by HPLC/UV-detection: 47 %

Residue results are not corrected for recoveries.

5 Remark

In metabolism project 91CN02, 47 % of total ¹⁴C-residues in tomatoe fruits were found as unchanged CGA 219417.

1. July 1992 *Mich*

date

Dr. R. Dieterle
(study director)

GENERAL

sponsor CIBA-GEIGY AG, Plant Protection, Safety Evaluation,
4002 Basel, Switzerland (Dr. U. Leuthold)

testing facility CIBA-GEIGY AG, Plant Protection, Residue Analysis,
4002 Basel, Switzerland (Dr. R. Dieterle, study director)

quality assurance The project was under surveillance of the Quality Assurance
Unit of Ciba-Geigy AG, 4002 Basel, Switzerland

archives Archives of Residue Analysis PP 2.53; no archives for specimen

guidelines Internal Standard Operating Procedures according to Swiss
GLP-Procedures and Principles (Department of Interior, 1986).

study dates Protocol issued 27 Mar 1992
project, start 2 Apr 1992
end 28 Apr 1992

analyst Mrs. E. Schönfeld

DETAILS ON TEST SYSTEM

Greenhouse tomatoes from project No. 91CN02 (CIBA-GEIGY AG, Plant Protection, Metabolism, PP 2.52, Basel, Switzerland, Dr. C. Neumann).

Ten weeks old plants (fruits: ca. 2 cm Ø) were treated by spray application with WP 50 formulated [¹⁴C-phenyl]-CGA 219417 at a rate of 750 g a.i./ha. The application was repeated after 28 days. The tomatoes were harvested 15 days after last application.

The tomatoes were washed, homogenized and frozen by PP 2.52. The specimen was delivered on 28 Jan 1992 in frozen condition. It was stored at ca. -20 °C until analysis.

The determination of total ¹⁴C-residues in specimen delivered was performed by PP 2.52 on 27 Jan 1992. Result: 139'623 dpm/g. With a specific radioactivity of 15 µCi/mg a.i., the total residue is 4.19 mg CGA 219417-equivalents per kg tomatoes.

DETAILS ON ANALYTICS

Analysis of CGA 219417

storage and preparation cf. details on test system (above)

analyt. method REM 141.01 including REM 141.01 Appendix 1 (CIBA-GEIGY AG, Basel, Switzerland)
sample size for analyses: 3 g
final determination: HPLC, 1-column system, according to REM 141.01 Appendix 1 (HPLC-column: 250 mm length, 2 mm i.d.)

quantitation	HPLC, UV detection, calibration by external standards (alternate injections of samples and standards; linear regression, method of weighted least squares of peak heights)
ref. substance	CGA 219417
recoveries	0.04 mg/kg: 93 %, 95 %; 2 mg/kg: 88 %, 93 %
control	origin: Exp. No. 1012/89; <0.02 mg/kg, <0.02 mg/kg
corrections of results	corrections were neither made for check values nor for recoveries
remarks	2 series of 2 analyses each were performed

Measurement of Radioactivity

Equipment and Chemicals:

- Biological Oxidizer, Model OX 300 (R.J. Harvey Instr. Corp., Hillsdale, NJ, USA)
- Packard Tricarb Scintillation Counter Model 2200 (Canberra-Packard Instr. Inc., Downers Grove, IL, USA)
- Oxysolve C-400 (Zinsser Analytic, Frankfurt, FRG)
- Irgascint A 300 (Ciba-Geigy AG, Basel, CH)

Determination of total residues (performed by PP 2.52):

The total radioactivity was determined by combustion of a subspecimen and collection of the carbondioxid in Oxysolve C-400. The radioactivity was measured on the scintillation counter. 6 combustions were performed; the total r.a. was transformed in CGA 219417 equivalents.

Determination of residues in extracts, final solutions for HPLC and HPLC fractions:

0.25 mL extract or final solution were added to 15 mL scintillation mixture Irgascint A 300. The HPLC fraction containing CGA 219417 was either evaporated to dryness, redissolved in 1 mL methanol from which 0.5 mL were added to 15 mL Irgascint A 300 or directly transferred into 15 mL Irgascint A 300. The radioactivities were measured on the scintillation counter.

Calculation of Extractability and Accountabilities

$$\text{Extr.} = \frac{\text{radioactivity of whole extract}}{\text{total radioactivity of subspecimen}} \times 100 \text{ [\%]}$$

$$\text{A-UV} = \frac{\text{mg CGA 219417/kg found by UV-detection} \times 100 \text{ [\%]}}{\text{total radioactivity of specimen in mg CGA 219417-equivalents/kg}}$$

$$\text{A-r.a.} = \frac{\text{mg CGA 219417/kg found in eluent fraction} \times 100 \text{ [\%]}}{\text{total radioactivity of specimen in mg CGA 219417-equivalents/kg}}$$

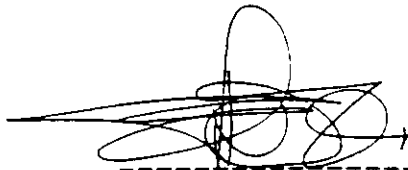
QUALITY ASSURANCE STATEMENT

Test Number : 100/92
Test Substance: CGA 219417
Study Title : Special Study
Study Director: Dr. R. Dieterle

I hereby certify that the following Quality Assurance activities were performed :

QA-Activity	Date performed	Date reported
1) Facility Inspection	26.11.91	16.01.92
2) Protocol Audit	22.04.92	22.04.92
3) Final Report Audit	30.06.92	01.07.92

Quality Assurance
Inspector :



P. Scherrer

2.7.92

(Date)

AMENDMENT NO. 1 TO REPORT OF SPECIAL STUDY 100/92

(Validation of Method REM 141.01 on Tomatoes)

This amendment corrects section 5 of report 100/92. It has no influence on the results obtained.

Old version.

5 Remark

In metabolism project 91CN02, 47 % of total ¹⁴C-residues in tomatoe fruits were found as unchanged CGA 219417

New Version:

5 Remark

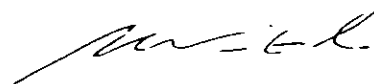
In metabolism project 91CN03, 47 % of the extractable ¹⁴C-residues in tomatoe fruits were found as unchanged CGA 219417.

Explanations:

In project 91CN03, the pyrimidine label of ¹⁴C-CGA 219417 was used instead of the phenyl label. The metabolite patterns of the two labels were qualitatively and quantitatively almost similar.

As nearly all ¹⁴C-residues could be extracted, the total ¹⁴C-residues approximately correspond to the extractable ¹⁴C-residues.

17. Juli 1992



date

Dr. R. Dieterle
(study director)