VOLUME _____ OF _____ OF SUBMISSION

CGA-219417

TITLE

ANALYTICAL METHOD AG-631B
(Supersedes Analytical Method AG-631 and AG-631A)

ANALYTICAL METHOD FOR THE DETERMINATION OF RESIDUES OF CGA-219417 IN CROPS BY HIGH LIQUID CHROMATOGRAPHY WITH COLUMN SWITCHING

DATA REQUIREMENT

EPA New Guideline No. 860-1340

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Residue Chemistry

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PERFORMING LABORATORY

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LABORATORY PROJECT IDENTIFICATION

Analytical Method No. AG-631B
Novartis Number 601-98
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SUBMITTER/SPONSOR

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VOLUME 1 OF 1 OF STUDY

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10 (d)(1)(A), (B) or (C).

Company: Novartis Crop Protection, Inc.

Company Representative: H. Lee Hubbard, Ph.D.

Title: Senior Regulatory Manager

Lee Hubbard /ccs — Signature

7/30/98 — Date

These data are the property of Novartis Crop Protection, Inc. and, as such, are considered confidential for all purposes other than compliance with FIFRA Section 10.

Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality that may exist under any other statute or in any other country.

STATEMENT CONCERNING GOOD LABORATORY PRACTICES

The document in this submission, is an analytical method and is not considered a final report because validation of the method is not included. Therefore, certification of compliance with Environmental Protection Agency's Good Laboratory Practice Standards (40 CFR Part 160, October 16, 1989) is not required.

Robert K. Williams
Agent of Submitter/Sponsor
And Manager, Residue Chemistry
Human Safety Department

Date 7/30/98

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Novartis Crop Protection, Inc.
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ANALYTICAL METHOD AG-631B

(Supersedes Analytical Method AG-631 and AG-631A)

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

Novartis No: 601-98

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I. SUMMARY/INTRODUCTION

A. Scope

Analytical Method AG-631B is a revision of AG-631. AG-631B. It clarifies issues resulting from the Petition Method Validation (PMV) trials discussed in Memoranda related to PP#5G04553 and PP#6F04656 from the United States Environmental Protection Agency.

Analytical Method AG-631B 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 141.01[1]. This method contains radiolabeled CGA-219417 validation data[2] 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	0.02 ppm
Forage, fodder, straw	0.05 ppm
Juice	0.01 ppm

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 95% 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 µm (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. 1210-2040.

11.0 Test tubes, 24/40 joint, 18.5 cm x 22 mm. (Ace Glass Co., Catalog No. 8645-38) or equivalent.

12.0 Test tubes, screw top, 13 x 100 mm. (Fisher Catalog No. 14-957-76A) or equivalent.

13.0 Vortex mixer or equivalent.

14.0 Graduated cylinders, 25-ml.

15.0 Volumetric pipettes, 2-, 5-, 20-ml.

16.0 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/GRAPE JUICE: Weigh a 10-g aliquot of crop/juice substrate into an 8-oz round wide mouth bottle. Add 100 ml of MeOH:H_2O (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: Transfer 20-ml extract into a 25-ml test tube, add 2-ml 1M HCl and mix.

3.2 NON-EDIBLE PLANT MATERIAL: 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:H_2O (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 diethyl ether and evaporate the eluate to dryness using a rotary evaporator or an N-evaporator with a gentle stream of nitrogen (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.

Matrix	Final Volume (ml)
Edible plant material	2
Non-edible plant material	2
Wine	2.5
Apple juice	1.5
Grape juice	1.0

Filter the final volume through an Acrodisc LC 13 PVDF 0.2 µm filter into an injection vial.

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 µl of the 0.10 ng/µl 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-µl 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. For MS confirmation ions with m/z = 210, 224 and 225 should be monitored in SIM mode.

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 Apple Juice Samples

1.1 EXTRACTION: No extraction procedures are needed.

1.2 SOLID PHASE EXTRACTION: Thaw a subsample of apple 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 µg/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 ng/µl 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 µg/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 µg/ml solution.

1.5 Make serial dilutions of the 1.0 µg/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 µg/ml solution (Section II.I.1.4) using acetone as the diluent, to produce injection standards in a range of 0.01 ng/µl to 0.20 ng/µl 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. To analyze samples using the confirmatory GC technique, inject 3-µl aliquots of the sample reconstituted in acetone. Reconstitution should be done using final volumes of acetone sufficient to give equivalent substrate LOQ's as shown in Section I.A. 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):

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

G = milligrams sample extracted
V_i = injection volume (µl)
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

(Determined from OPPTS 860.1000 Table I %DM for feedstuffs and FDA Pesticide Analytical Manual, Volume I, Section 201 for food)

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

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

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

2.0 Fortification Experiments

2.1 This method is validated, for data collection purposes only, with each set of samples analyzed. This is accomplished by including an untreated control sample and one or more control samples fortified immediately prior to extraction. The use of fortified control samples is not necessary for tolerance enforcement method applications. To prepare fortified control 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:

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

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-631B 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-631A 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 ^{14}C-CGA-219417 treated tomato fruits. The percent extractability was determined by comparing the ppm ^{14}C 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-631B is a valid and accurate method for the determination of parent residues of CGA-219417. The overall accuracy and precision of Analytical Method AG-631B 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 µm, 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 µL
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 µm 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 µL

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

Code #	Type*	CGA-219417 Added	% Rec. by HPLC	% Rec. by GC
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
CH.RB	Control	Blank	(<0.02 ppm)	(<0.02 ppm)
			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)

Field Test Number	Location	Mature Fruit Commodity	ppm in Control	Fortification Level (ppm)	CGA-219417 Percent Recovered
0W-FR-641-94	Washington	Apples	<0.02	0.20	105
0W-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
0W-FR-404-94	California	Pears	<0.02	0.02	90
05-FR-004-94	New York	Pears	<0.02	0.02	93
0W-FR-643-94	Washington	Pears	<0.02	0.05	97
0W-FR-644-94	Washington	Pears	<0.02	0.02	86
0W-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)

Field Test Number	Location	Mature Fruit Commodity	ppm in Control	Fortification Level (ppm)	CGA-219417 Percent Recovered
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
0W-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
0W-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
0W-FR-647-94	Idaho	Plums	<0.02	1.0	102
02-FR-035-94	California	Prunes	<0.02	0.02	83
0W-FR-648-94	Oregon	Prunes	<0.02	0.50	101
		Prunes	<0.02	0.50	115
0W-FR-649-94	Washington	Sweet cherries	<0.02	0.02	118
0W-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)

Field Test Number	Location	Mature Fruit Commodity	ppm in Control	Fortification Level (ppm)	CGA-219417 Percent Recovered
02-FR-035-94	California	Whole fruit-fresh	<0.02	1.0	101
		Whole fruit-dried	<0.02	0.02	73
0W-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]

Matrix	Substrate	Fortification Level [mg/kg]	Recoveries Found [%]	Average [%]
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

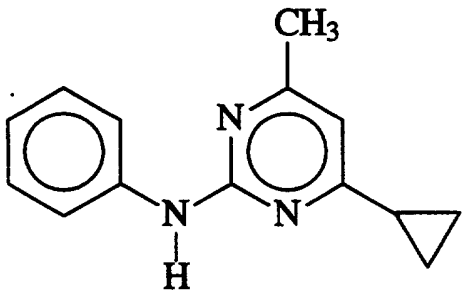
Acceptable Recovery Range: 72 - 112%

TABLE VIII. SUMMARY TABLE FOR THE EXTRACTABILITY AND ACCOUNTABILITY OF ^{14}C-CGA-219417 TREATED TOMATO FRUIT (SPECIAL STUDY REPORT 100/92[2])

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

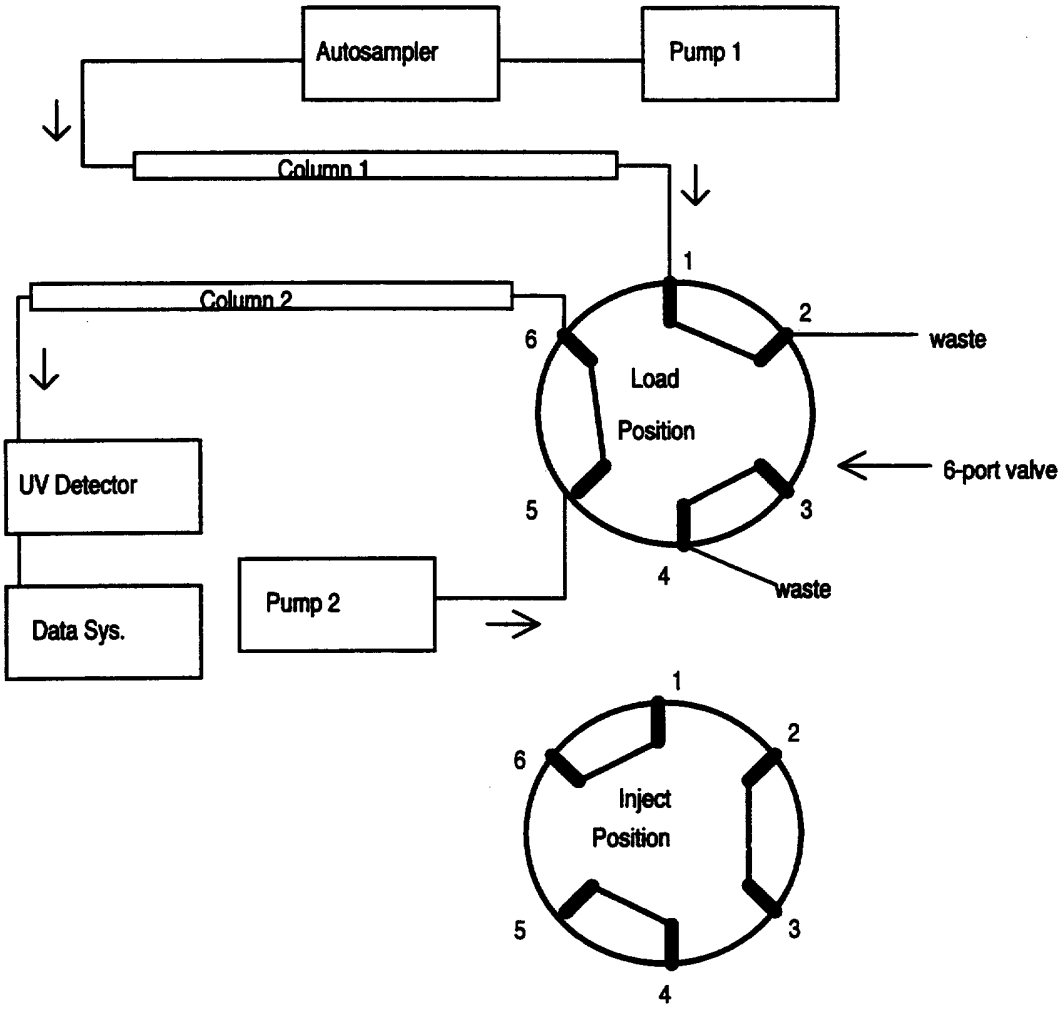


FIGURE 3. FLOW DIAGRAM OF METHOD FOR PLANT MATERIAL

10 g of Matrix + 100-ml MeOH/H_2O 8:2 v/v

↓

Shake for 60 minutes

↓

Filter through folded filter paper

↓

Edible Plant mat: Transfer 20-ml extract into a 25-ml test tube; add 2 ml 1M HCl and mix.
Non-Edible Plant mat: Transfer 2-ml extract into a 5-ml test tube; add 0.2-ml 1 M HCl and mix.

↓

Precondition a Bond Elut SCX cartridge (3 ml/500 mg)
1. 5-ml MeOH
2. 5-ml 0.1 M HCl

↓

Transfer the acidified aliquot of extract to the cartridge. Allow it to pass through the cartridge dropwise. Discard Elulate.

↓

Wash the test tube with 12-ml MeOH/H_2O (1:1;v/v) Transfer the washing to the cartridge and allow it to pass through the cartridge dropwise. Discard the elulate.

↓

Elute CGA-219417 with 8-ml MeOH/aqueous ammonia (95:5; v/v) into a 25-ml round bottom flask.

↓

Add 3 drops of dithylene glycol dithyl ether and evaporate to dryness using a rotary evaporator (water bath temp.: about 40°C)

↓

Dissolve the residue in mobile phase.
Plant material (except grapes, straw and husks): 4 ml
Grapes, straw, husks: 2 ml

↓

Inject final solution into the HPLC.

FIGURE 4. FLOW DIAGRAM OF METHOD FOR WINE

5-ml wine + 1-ml MeOH + 0.5-ml 1M HCl; mix

↓

Precondition a Bond Elut SCX cartridge (3ml/500mg):
1. 3-ml MeOH
2. 3-ml 0.1M HCl

↓

Transfer the acidified aliquot of wine to the cartridge.
Allow it to pass through the cartridge dropwise.
Discard elulate.

↓

Wash the test tube with 12-ml MeOH/H_2O (1:1 v/v)
Transfer the washing to the cartridge and allow it to pass through the cartridge dropwise.
Discard elulate.

↓

Elute CGA-219417 with 8-ml MeOH/Aqueous Ammonia (95:5 v/v) into a 25-ml round bottom flask.

↓

Add 3 drops of diethylene glycol dithyl ether and evaporate to dryness using a rotary evaporator
(water bath temp.: about 40°C).

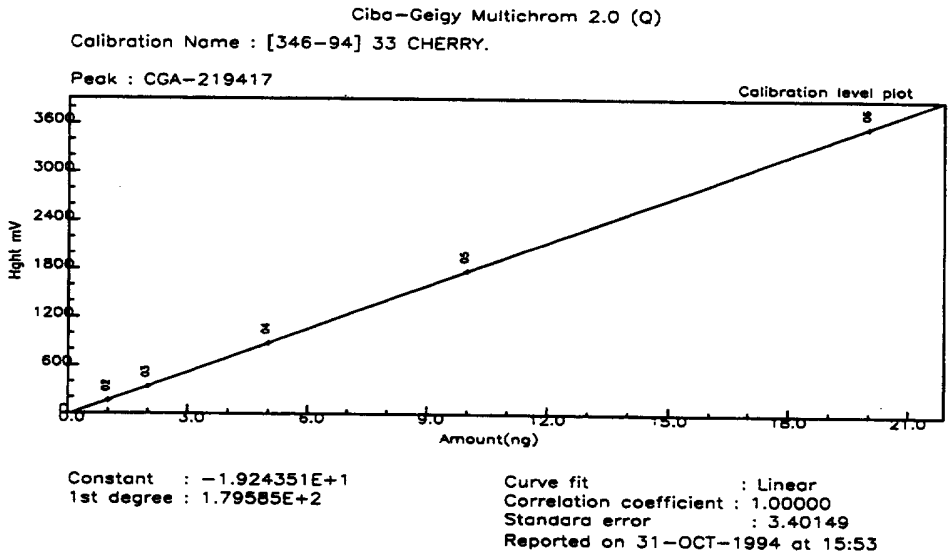
↓

Dissolve the residue in 2.5-ml mobile phase.

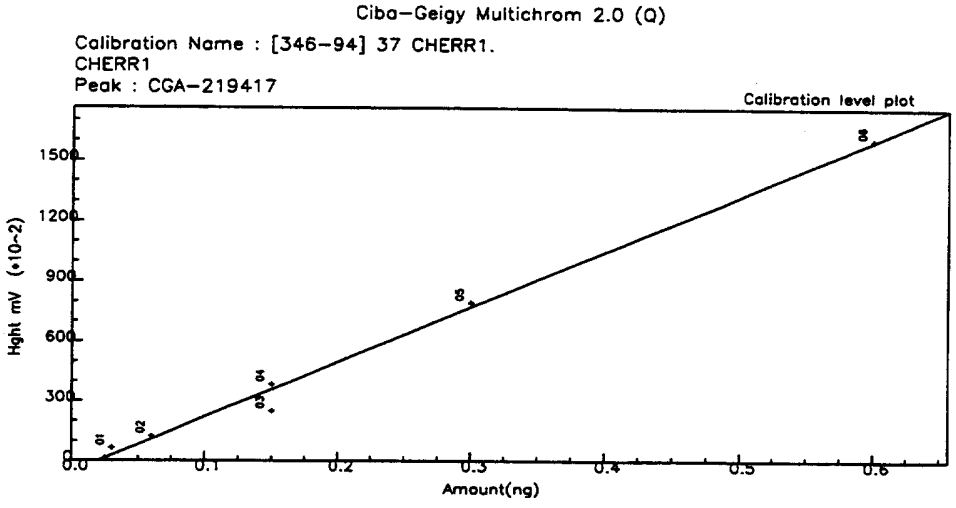
↓

Inject final solution into HPLC.

FIGURE 5. CGA-219417 CALIBRATION CURVES

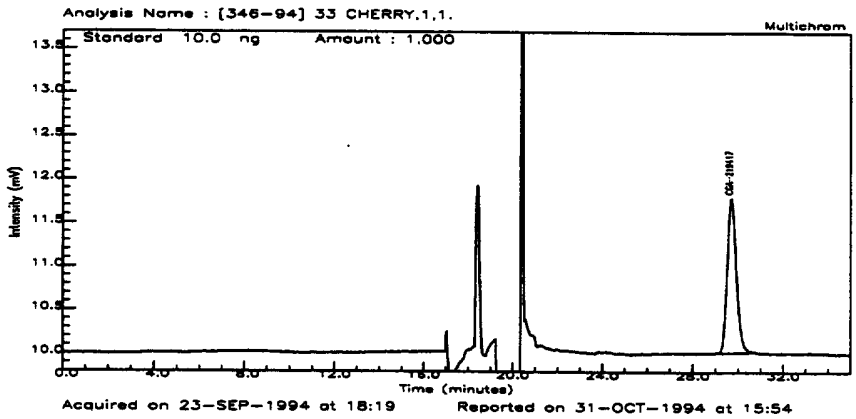


Curve for HPLC Standards

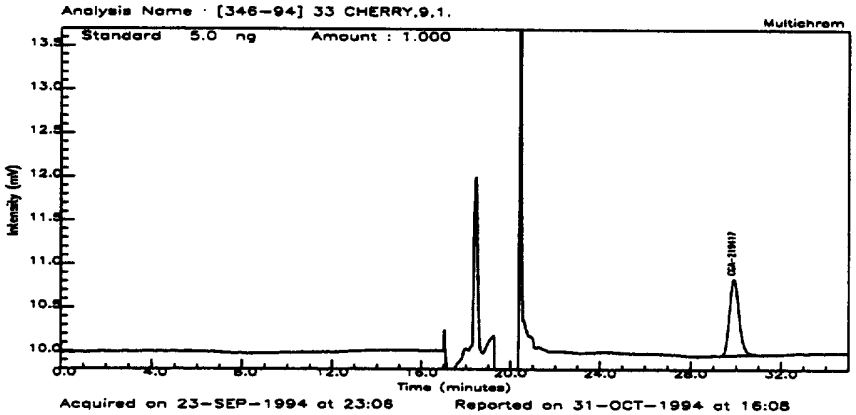


Curve for GC Standards

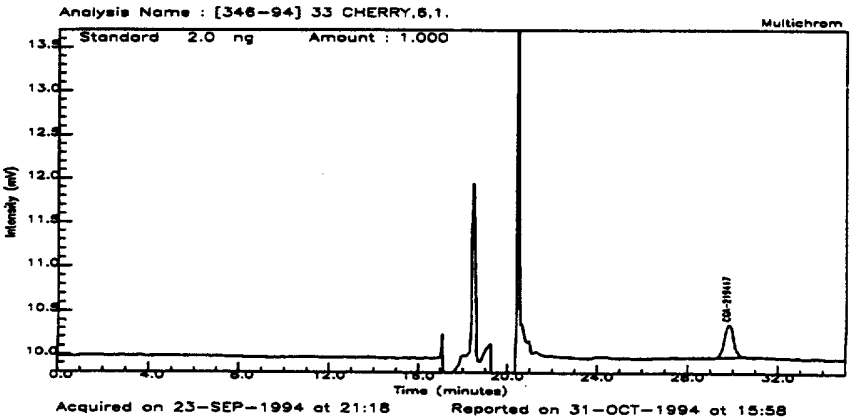
FIGURE 6. CGA-219417 STANDARD CHROMATOGRAMS



HPLC
Standard
CGA-219417
10.0 ng injected

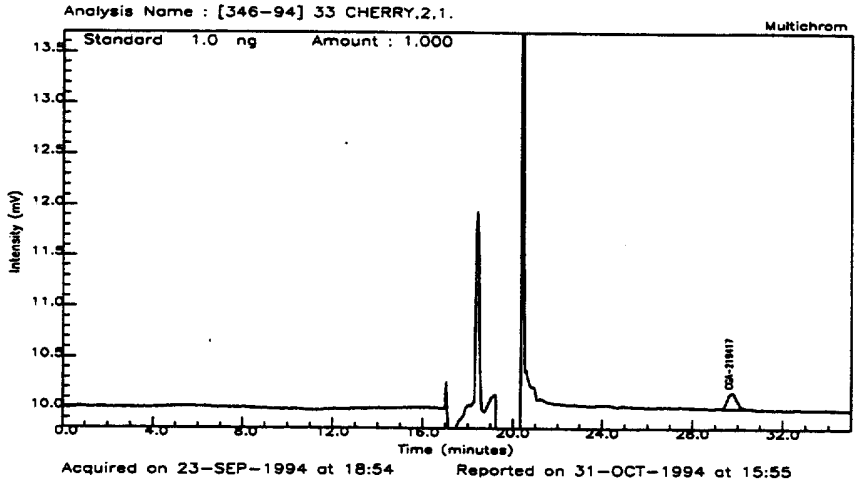


HPLC
Standard
CGA-219417
5.0 ng injected

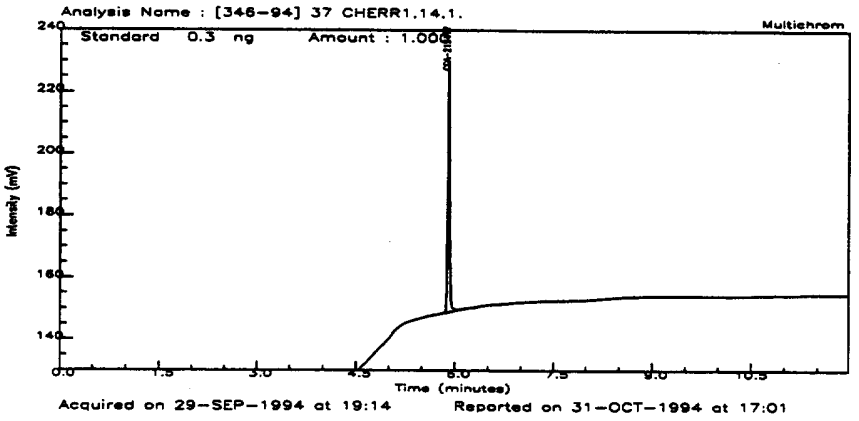


HPLC
Standard
CGA-219417
2.0 ng injected

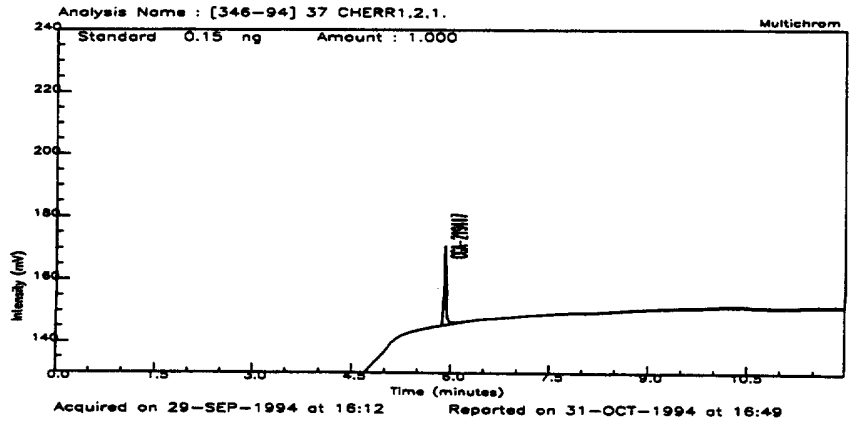
FIGURE 6. CGA-219417 STANDARD CHROMATOGRAMS (Continued)



HPLC
Standard
CGA-219417
1.0 ng injected

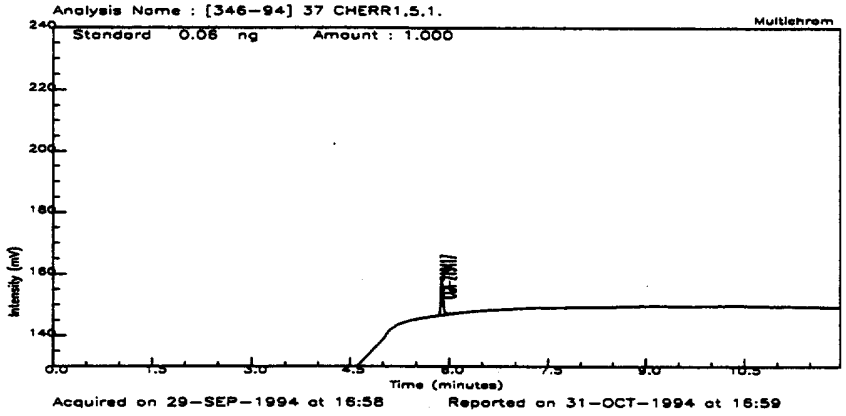


GC
Standard
CGA-219417
0.3 ng injected

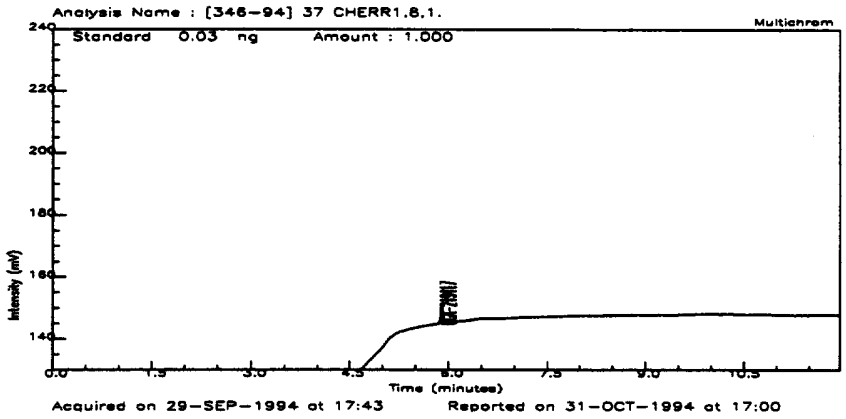


GC
Standard
CGA-219417
0.15 ng injected

FIGURE 6. CGA-219417 STANDARD CHROMATOGRAMS (Continued)

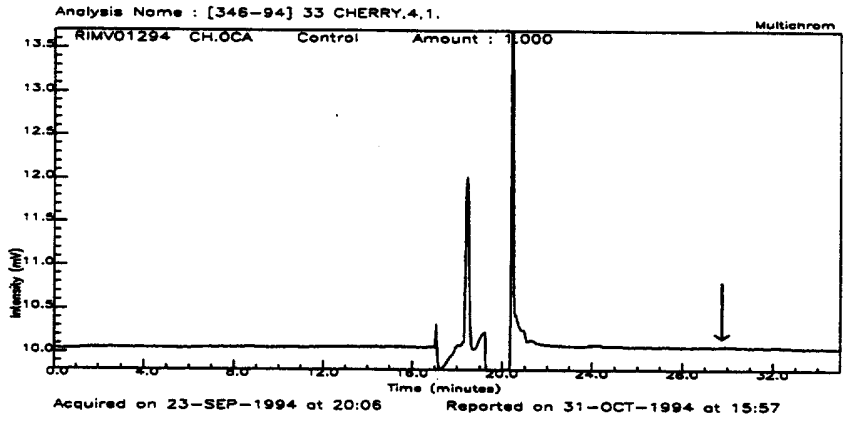


GC
Standard
CGA-219417
0.06 ng injected

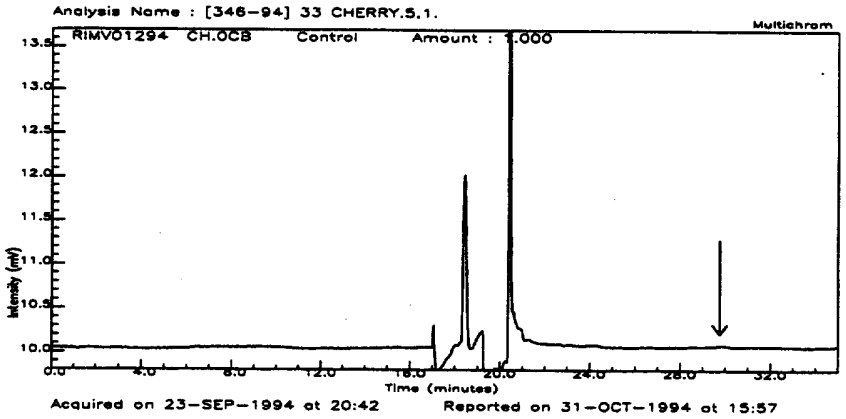


GC
Standard
CGA-219417
0.03 ng injected

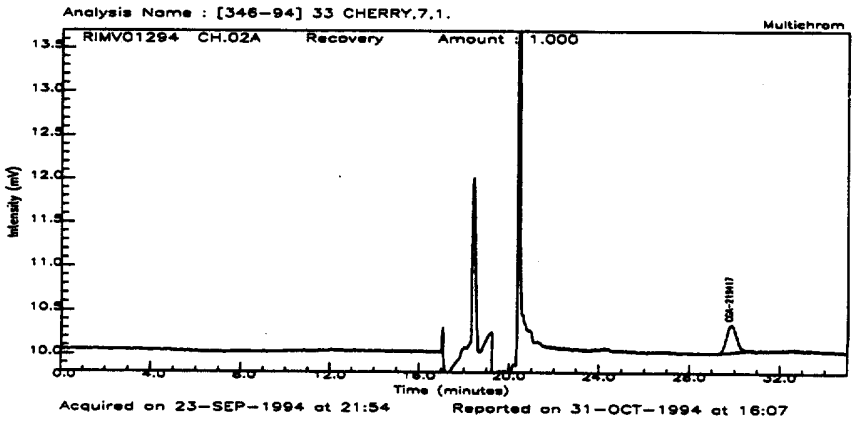
FIGURE 7. CONTROL AND CGA-219417 FORTIFIED CHERRY SAMPLE HPLC CHROMATOGRAMS



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

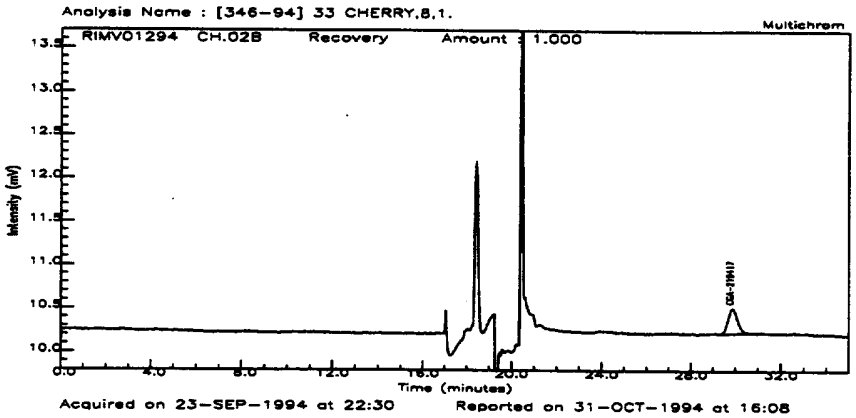


CH.0CB, Control
92.1 mg inj.
<1.0 ng found
<0.02 ppm

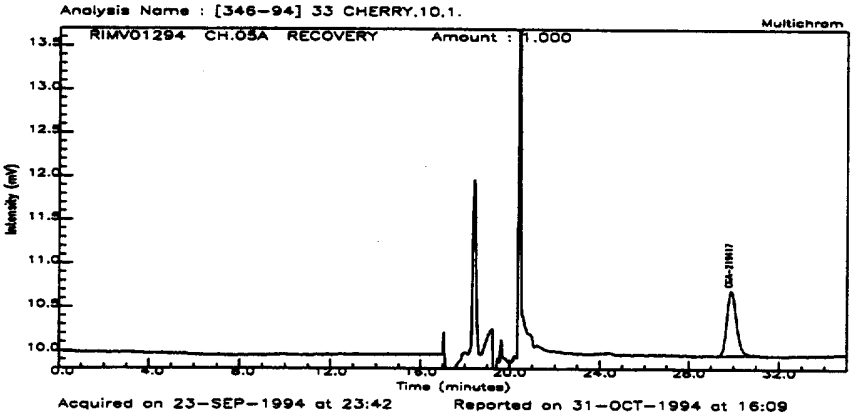


CH.02A,
Control + 0.02 ppm
CGA-219417
92.1 mg inj.
1.71 ng found
0.019 ppm
93% Recovery

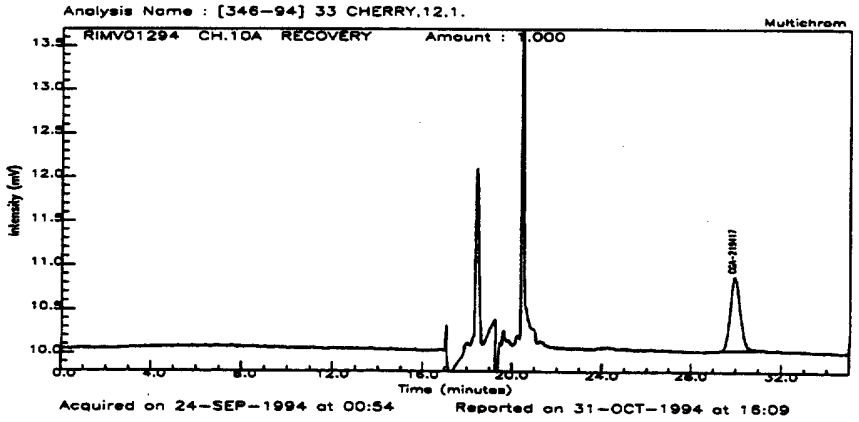
FIGURE 7. CONTROL AND CGA-219417 FORTIFIED CHERRY SAMPLE HPLC CHROMATOGRAMS (Continued)



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

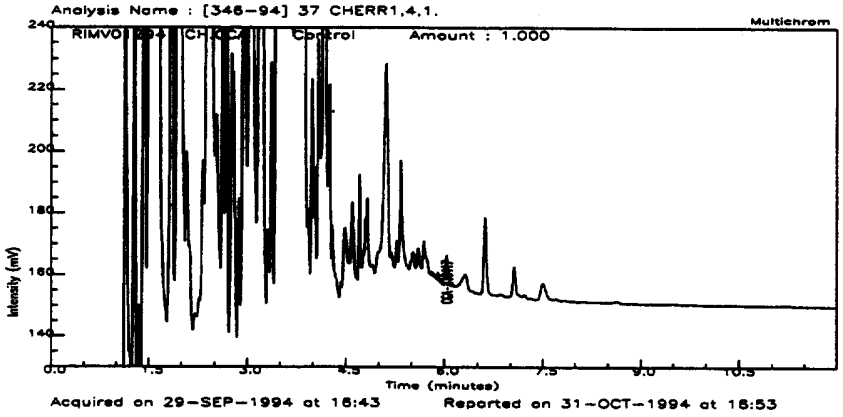


CH.05A,
Control + 0.05 ppm
CGA-219417
92.1 mg inj.
4.26 ng found
0.046 ppm
93% Recovery

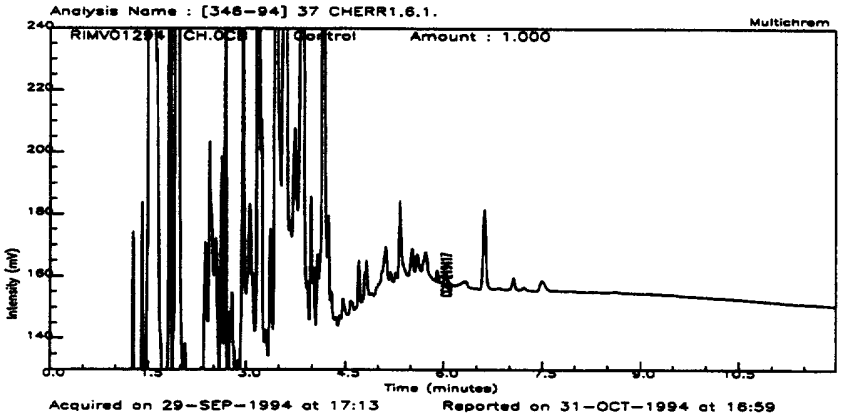


CH.10A,
Control + 0.10 ppm
CGA-219417
46.0 mg inj.
4.6 ng found
0.10 ppm
100% Recovery

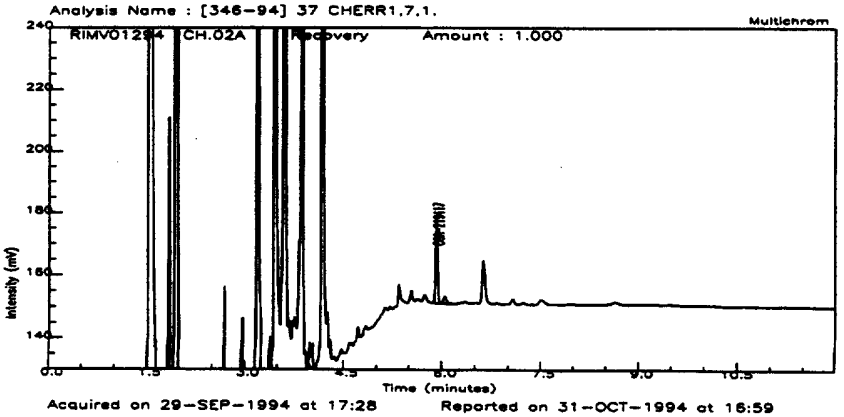
FIGURE 8. CONTROL AND CGA-219417 FORTIFIED CHERRY SAMPLE GC CHROMATOGRAMS



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



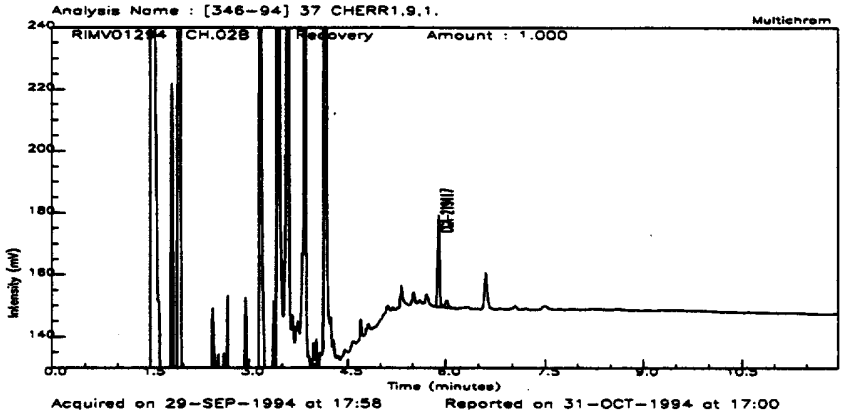
CH.0CB,
Control
5.53 mg inj.
<0.03 ng (0.033 ng)
found
<0.02 ppm
(0.006 ppm)



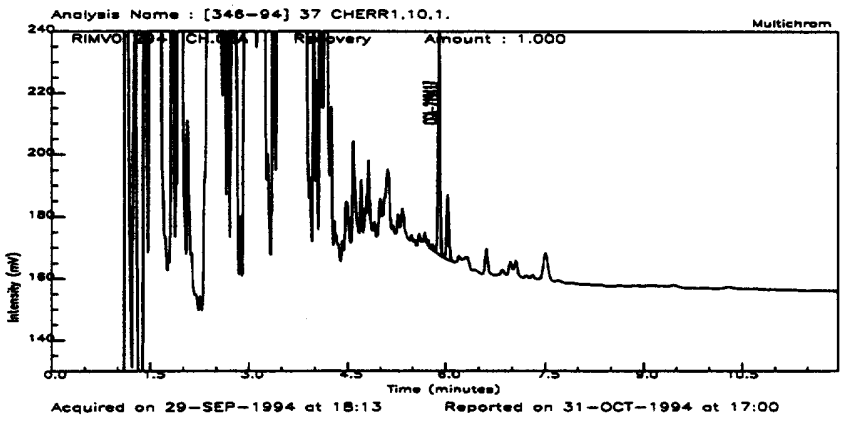
CH.02A,
Control + 0.02 ppm
CGA-219417
5.53 mg inj.
0.123 ng found
0.022 ppm
0.017 ppm corrected
86% Recovery

Recovery results corrected for control values

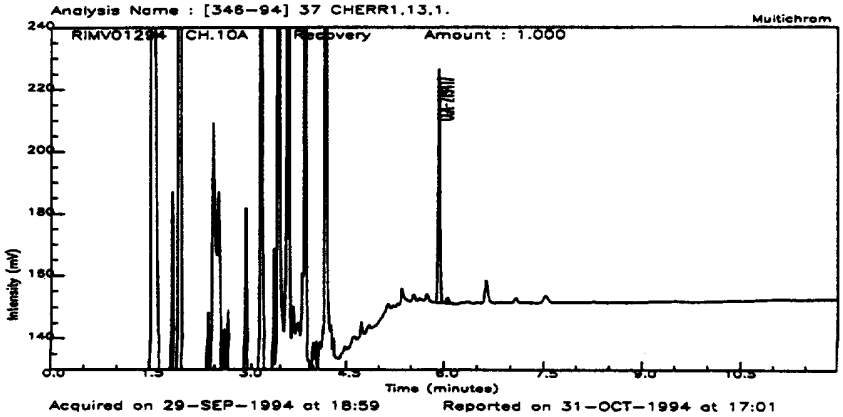
FIGURE 8. CONTROL AND CGA-219417 FORTIFIED CHERRY SAMPLE GC CHROMATOGRAMS (Continued)



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

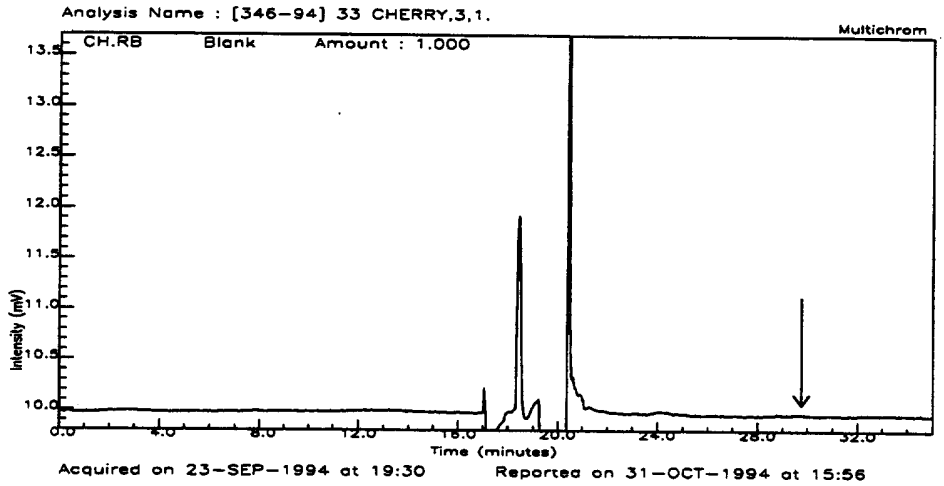


CH.05A,
Control + 0.05 ppm
CGA-219417
5.53 mg inj.
0.301 ng found
0.055 ppm
0.050 ppm corrected
99% Recovery

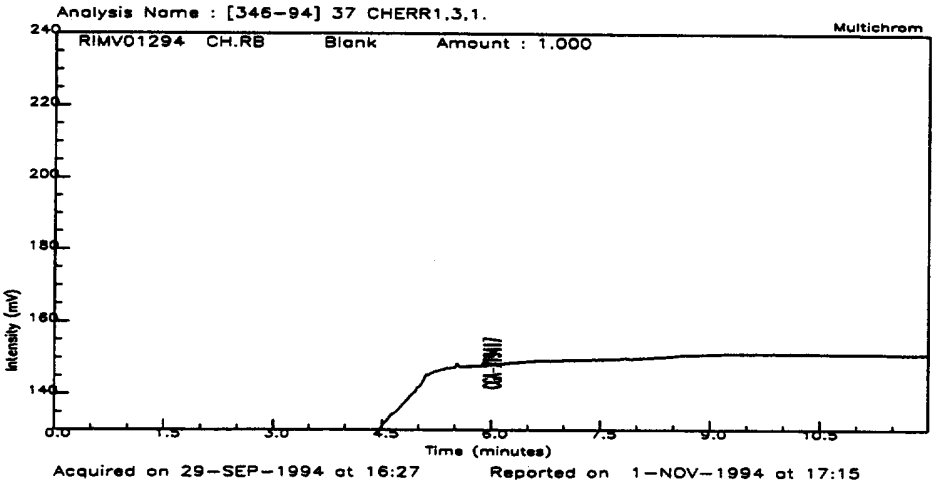


CH.10A,
Control + 0.10 ppm
CGA-219417
2.76 mg inj.
0.294 ng found
0.107 ppm
0.102 ppm corrected
101% Recovery

FIGURE 9. REAGENT BLANK CHROMATOGRAMS



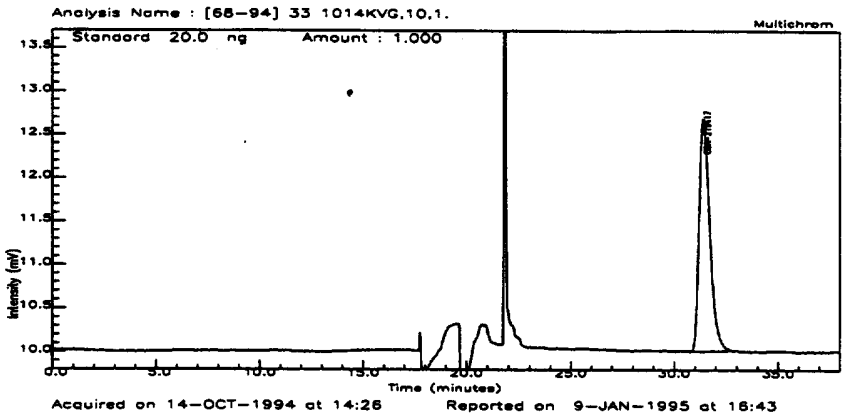
HPLC: CH.RB, 100 mg inj. <1.0 ng, <0.02 ppm



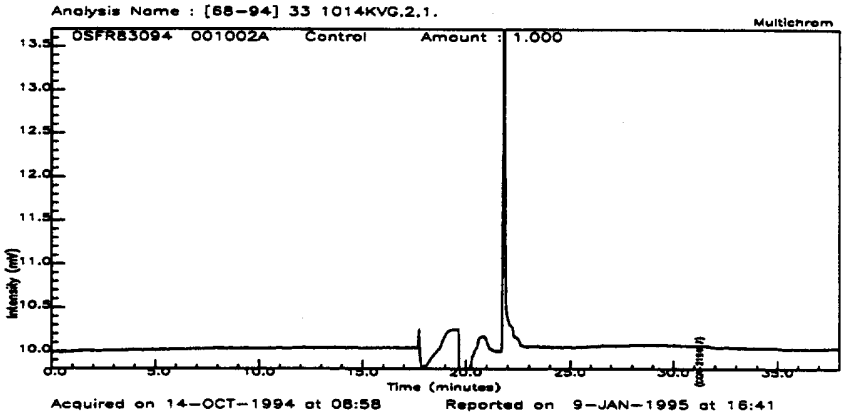
GC: CH.RB, 6.0 mg inj. <1.0 ng, <0.02 ppm

FIGURE 10. REPRESENTATIVE CHROMATOGRAMS - PEACHES

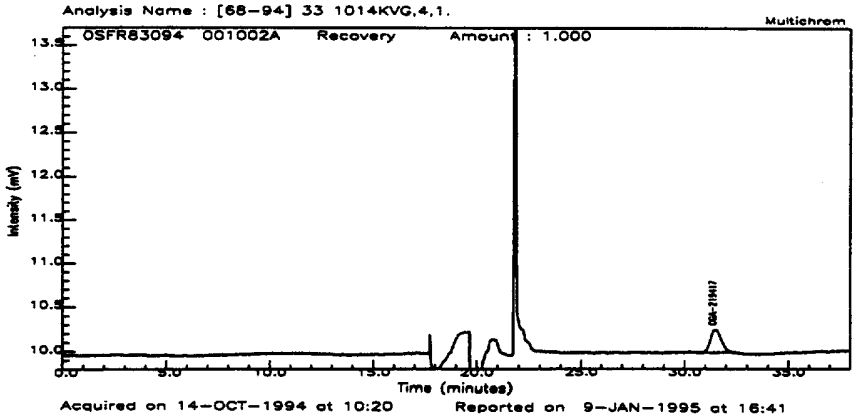
1)



2)



3)

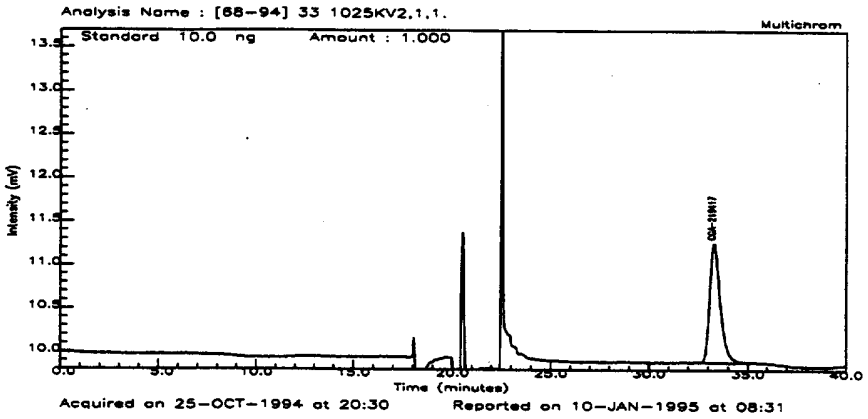


1) Standard, CGA-219417, 20. ng
2) 1-2-A, control, 91.8 mg injected, <1.0 ng found, <0.02 ppm found (ND)
3) 1-2-A, control + 0.02 ppm CGA-219417, 91.8 mg injected, 2.2 ng found, 0.024 ppm found, 0.024 ppm found (corrected for control), 118% recovery

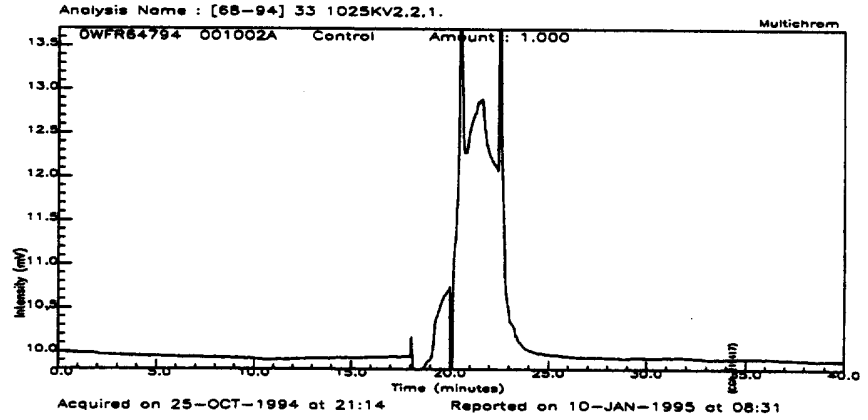
Control results are corrected for procedural recoveries <100%.
ND - No residue detected

FIGURE 11. REPRESENTATIVE CHROMATOGRAMS - PLUMS

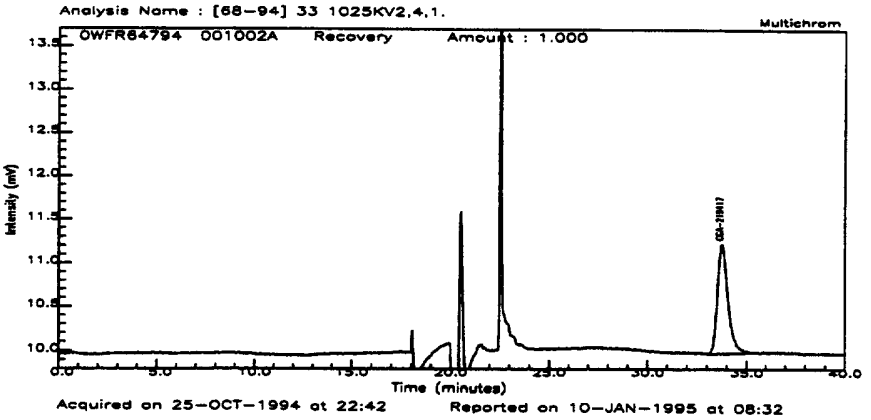
1)



2)



3)

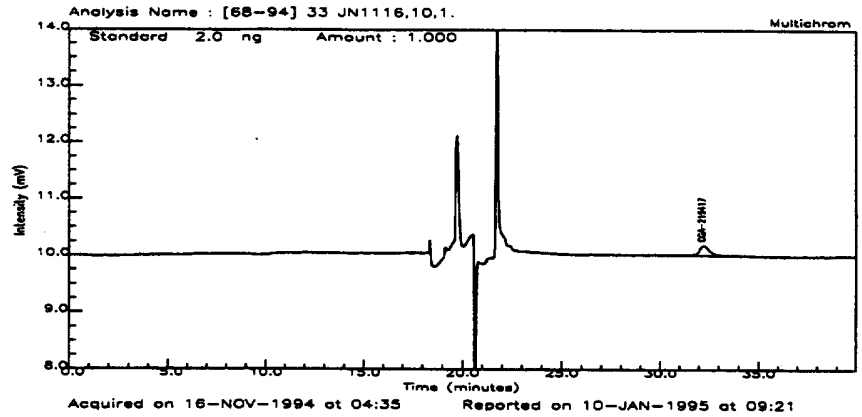


1) Standard, CGA-219417, 10 ng
2) 1-2-A, control, 92.3 mg injected, <1.0 ng found, <0.02 ppm found (ND)
3) 1-2-A, control + 1.0 ppm CGA-219417, 9.2 mg injected, 9.4 ng found, 1.02 ppm found, 1.02 ppm found (corrected for control), 102% recovery

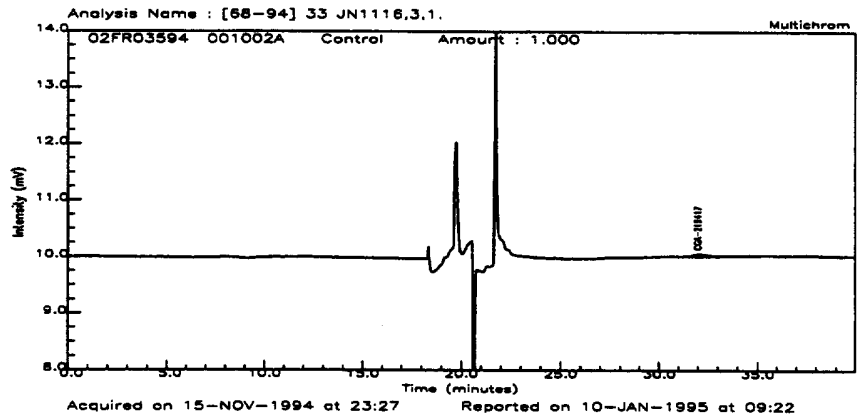
Control results are corrected for procedural recoveries <100%.

FIGURE 12. REPRESENTATIVE CHROMATOGRAMS - PRUNES

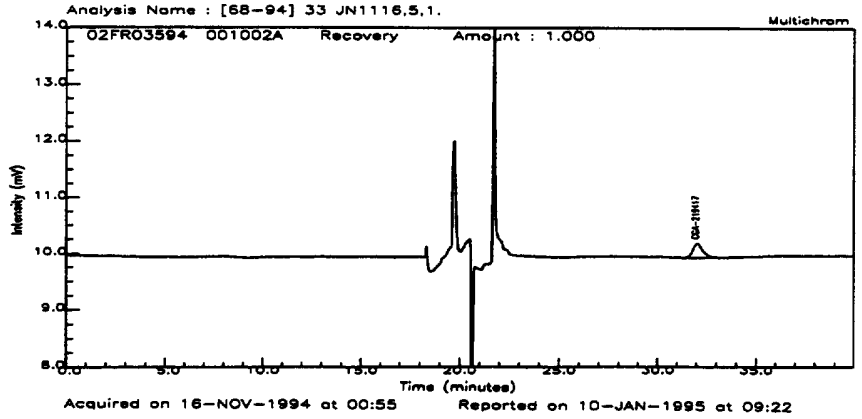
1)



2)



3)

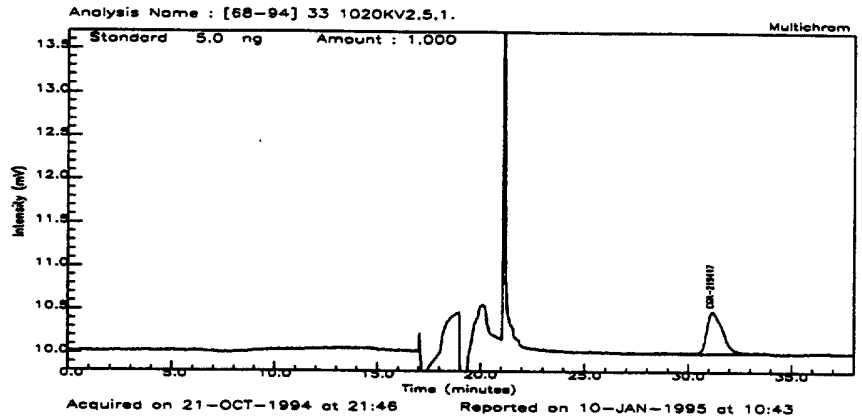


1) Standard, CGA-219417, 2.0 ng
2) 1-2-A, control, 92.7 mg injected, <1.0 ng found, <0.02 ppm found (0.009 ppm)
3) 1-2-A, control + 0.02 ppm CGA-219417, 92.7 mg injected, 2.4 ng found, 0.026 ppm found, 0.017 ppm found (corrected for control), 83% recovery.

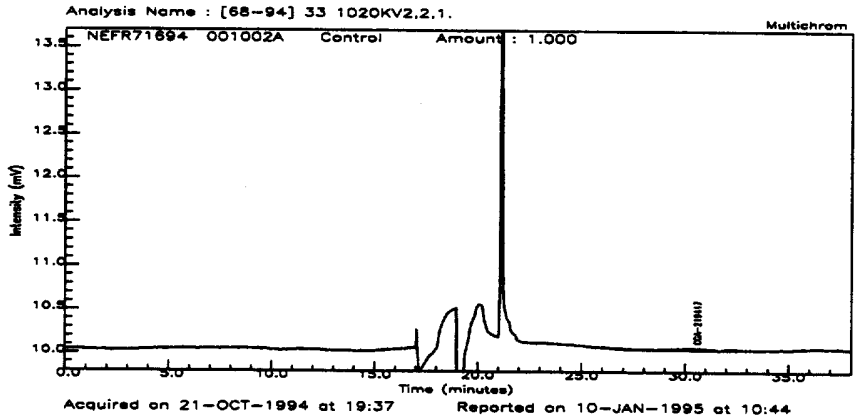
Control results are corrected for procedural recoveries <100%.

FIGURE 13. REPRESENTATIVE CHROMATOGRAMS - SWEET CHERRIES

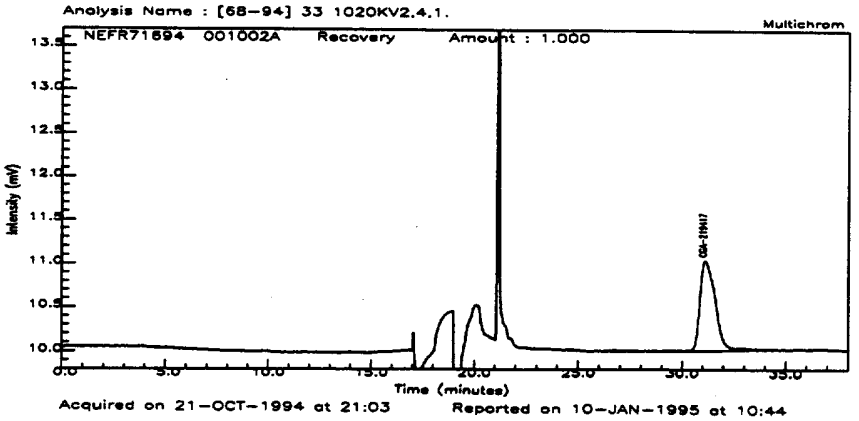
1)



2)



3)

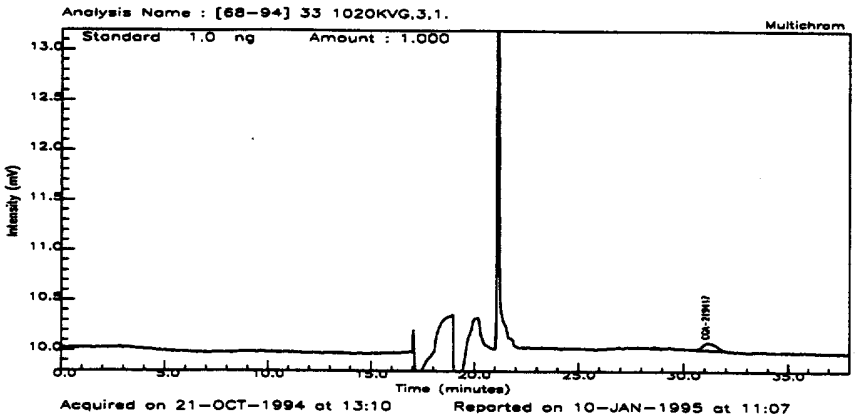


1) Standard, CGA-219417, 5.0 ng
2) 1-2-A, control, 92.6 mg injected, <1.0 ng found, <0.02 ppm found (0.005 ppm)
3) 1-2-A, control + 0.50 ppm CGA-219417, 18.5 mg injected, 10.2 ng found, 0.55 ppm found, 0.55 ppm found (corrected for control), 109% recovery.

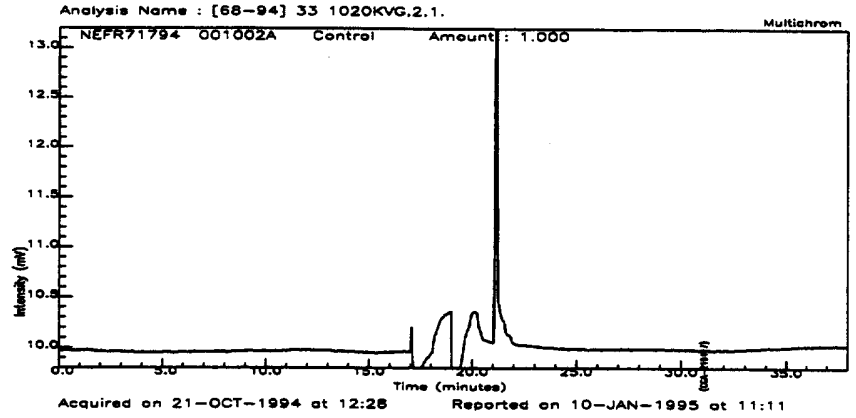
Control results are corrected for procedural recoveries <100%.

FIGURE 14. REPRESENTATIVE CHROMATOGRAMS - TART CHERRIES

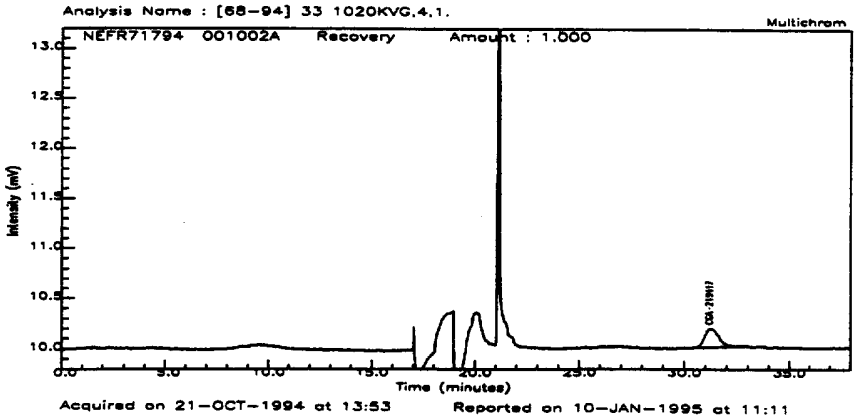
1)



2)



3)

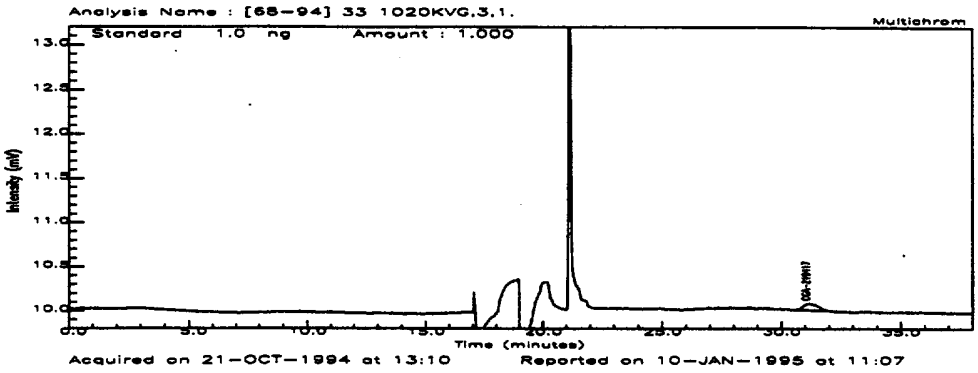


1) Standard, CGA-219417, 1.0 ng
2) 1-2-A, control, 92.3 mg injected, <1.0 ng found, <0.02 ppm found (ND)
3) 1-2-A, control + 0.02 ppm CGA-219417, 92.3 mg injected, 2.0 ng found, 0.022 ppm found, 0.022 ppm found (corrected for control), 110% recovery

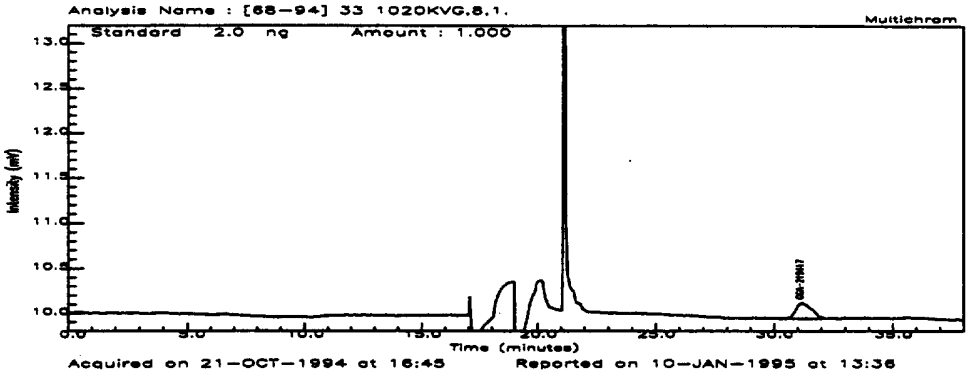
Control results are corrected for procedural recoveries <100%.
ND - No residue detected

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

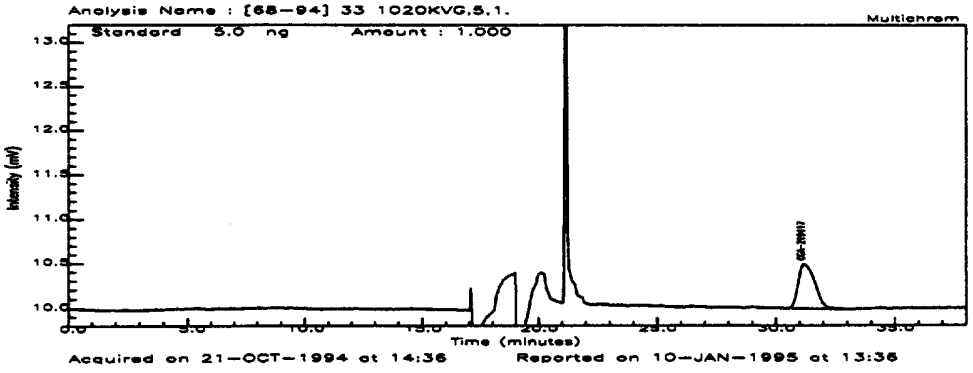
1)



2)



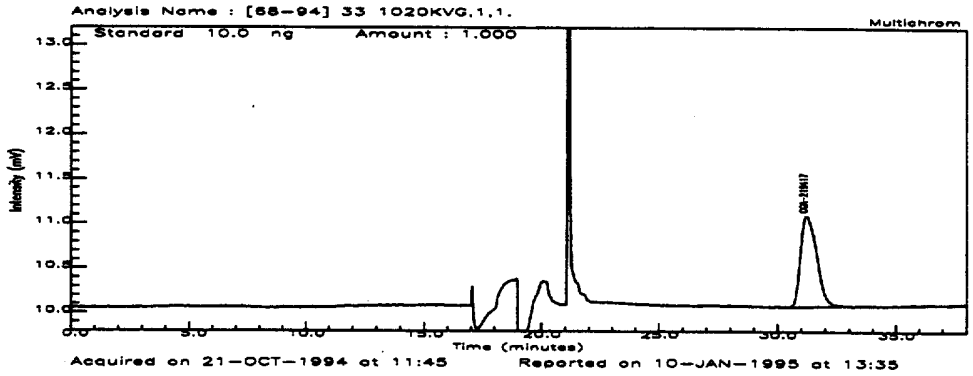
3)



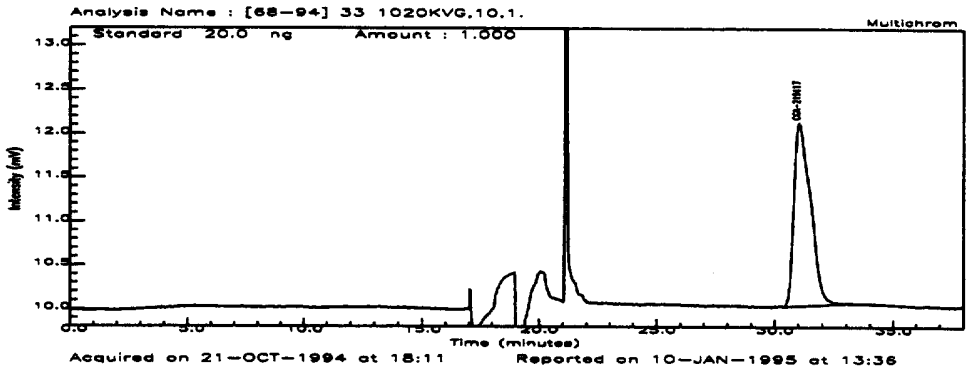
1) Standard, 1.0 ng (Response Factor = 77)
2) Standard, 2.0 ng (Response Factor = 179)
3) Standard, 5.0 ng (Response Factor = 500)

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

4)

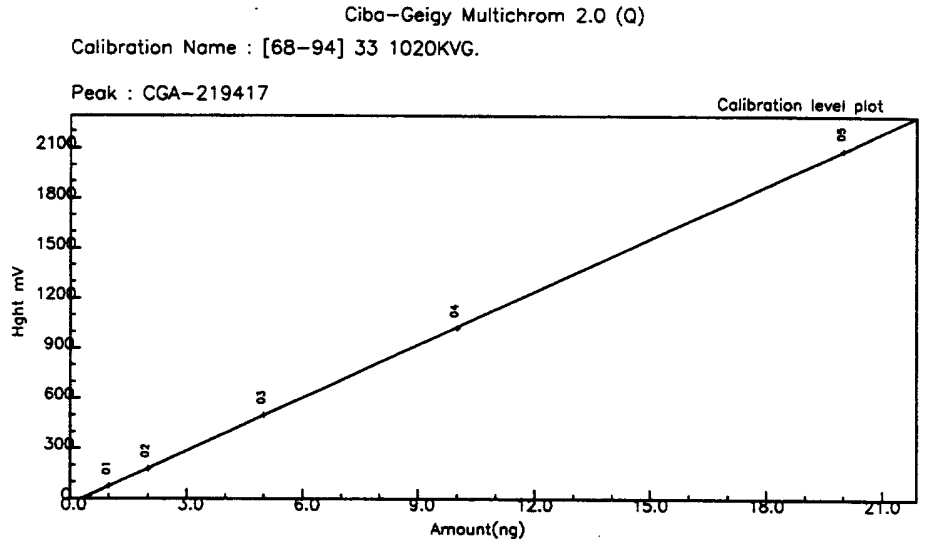


5)



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

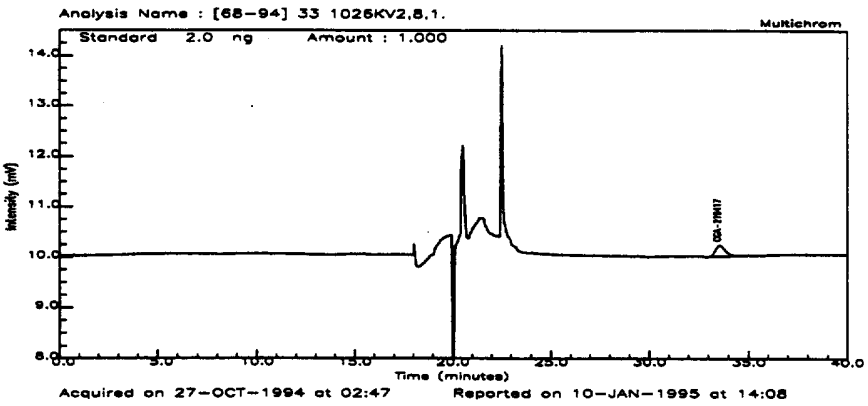


Constant : -3.179499E+1
1st degree : 1.05959E+2

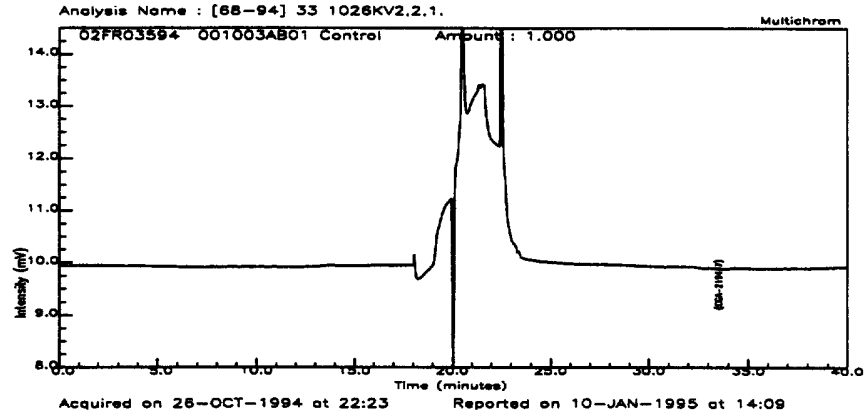
Curve fit : Linear
Correlation coefficient : 0.99999
Standard error : 4.14196
Reported on 10-JAN-1995 at 13:46

FIGURE 17. REPRESENTATIVE CHROMATOGRAMS - PROCESSED PRUNES - FRESH

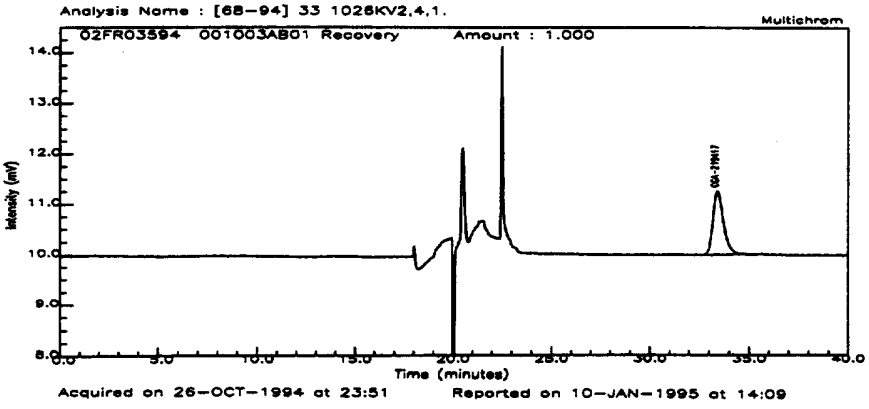
1)



2)



3)

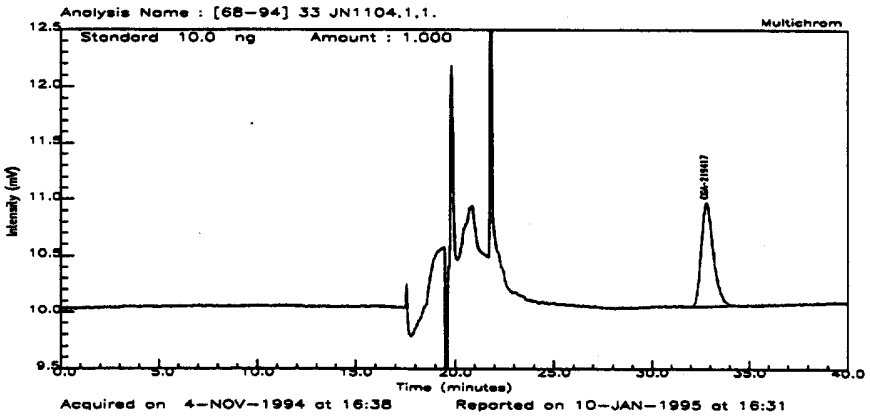


1) Standard, CGA-219417, 2.0 ng
2) 1-3-AB-01, control, 92.7 mg injected, <1.0 ng found, <0.02 ppm found (ND)
3) 1-3-AB-1, control + 1.0 ppm CGA-219417, 9.3 mg injected, 9.4 ng found, 1.01 ppm found, 1.01 ppm found (corrected for control), 101% recovery

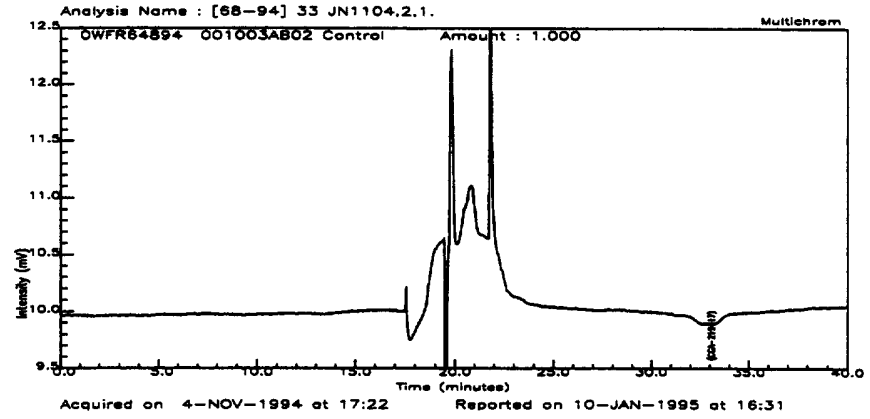
Control results are corrected for procedural recoveries <100%.
ND - No residue detected

FIGURE 18. REPRESENTATIVE CHROMATOGRAMS - PROCESSED PRUNES - DRIED

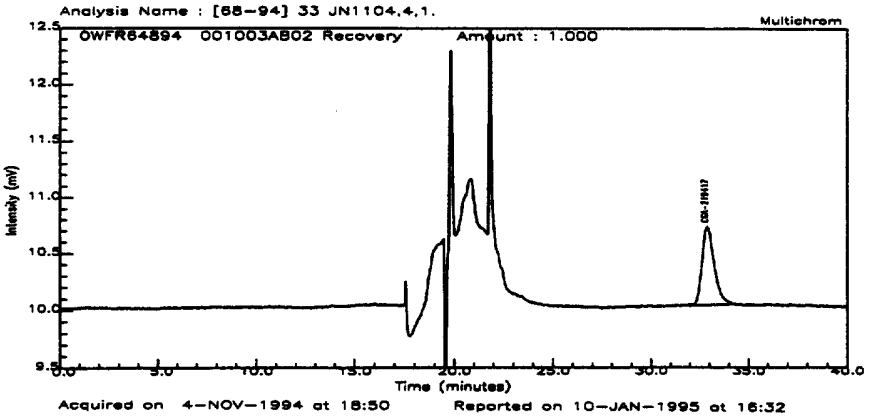
1)



2)



3)

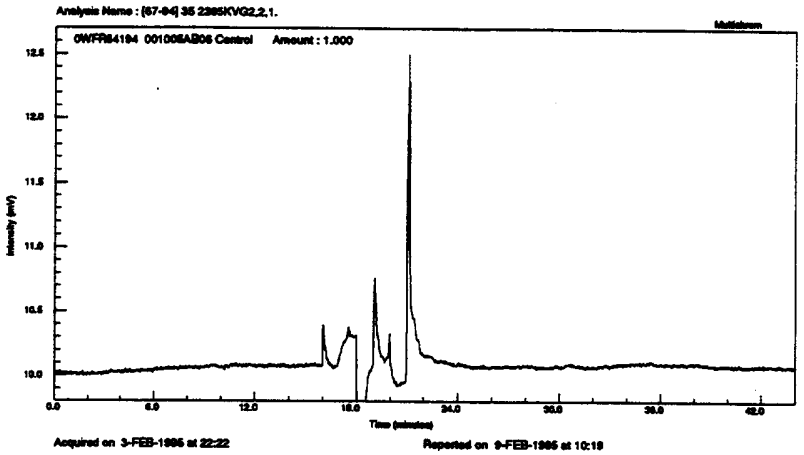


1) Standard, CGA-219417, 10. ng
2) 1-3-AB-02, control, 97.3 mg injected, <1.0 ng found, <0.02 ppm found (ND)
3) 1-3-AB-2, control + 1.0 ppm CGA-219417, 9.7 mg injected, 7.7 ng found, 0.79 ppm found, 0.79 ppm found (corrected for control), 79% recovery

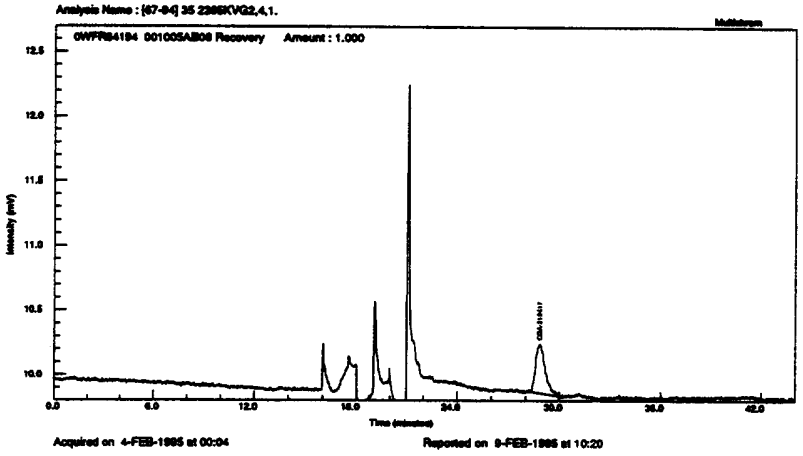
Control results are corrected for procedural recoveries <100%.
ND - No residue detected

FIGURE 19. REPRESENTATIVE CHROMATOGRAMS - APPLE JUICE

1)



2)

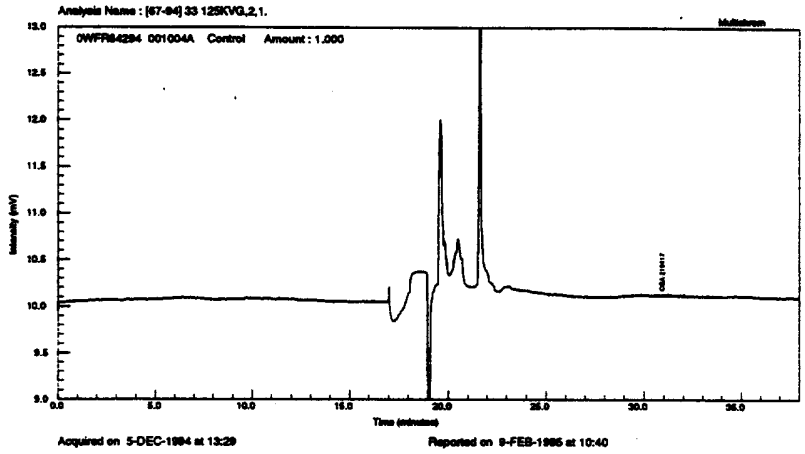


1) Apple juice control:1-5AB-6, control, 333 mg inj., <0.731 ng found, <0.010 ppm found (ND).

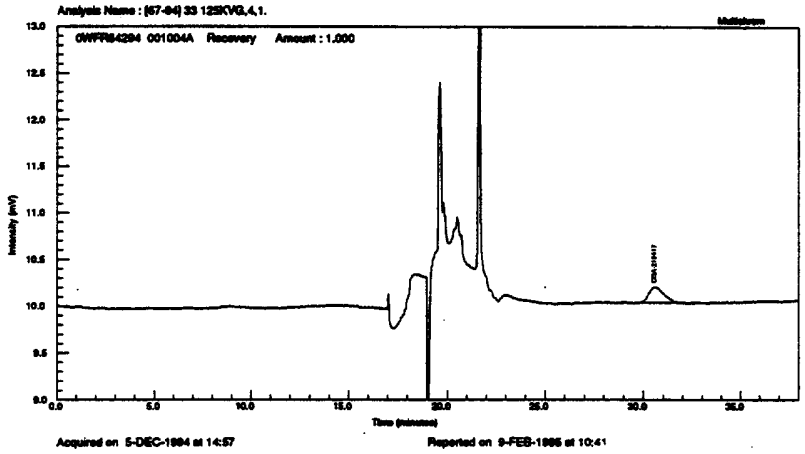
2) Apple juice recovery:1-5AB-6, control + 0.01 ppm, 333 mg inj., 3.2 ng found, 0.01 ppm found, 96% recovery.

FIGURE 20. REPRESENTATIVE CHROMATOGRAMS - APPLE FRUIT

1)



2)

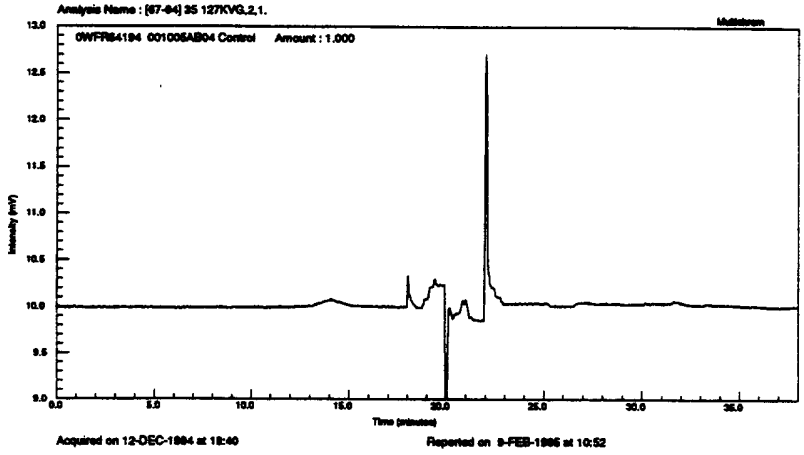


1) Apple fruit control:1-4-A, control, 92 mg inj., <0.95 ng found, <0.020 ppm found (ND).

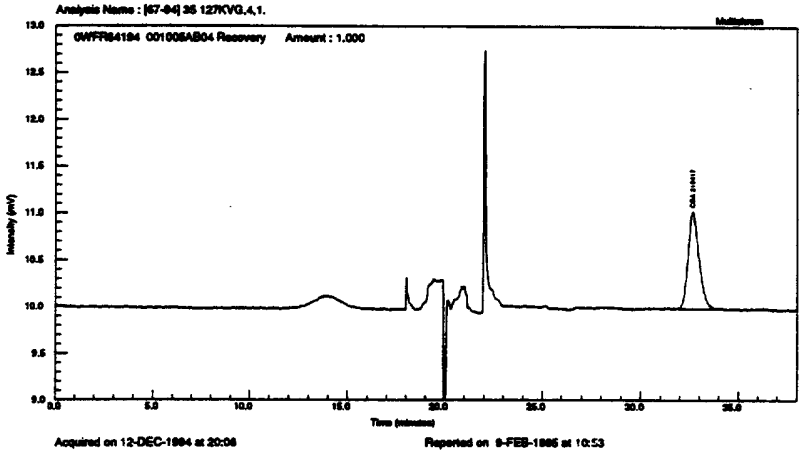
2) Apple fruit recovery:1-4-A + 0.02 ppm, 92 mg inj., 1.7 ng found, 0.018 ppm found, 91% recovery.

FIGURE 21. REPRESENTATIVE CHROMATOGRAMS - APPLE WET POMACE

1)



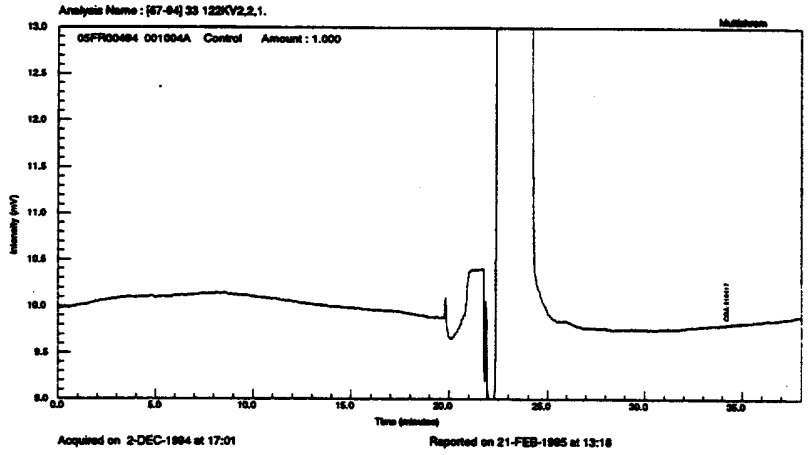
2)



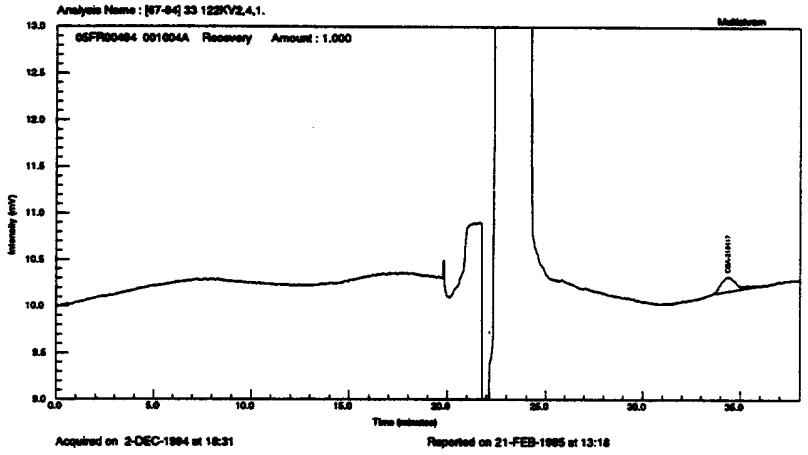
1) Apple wet pomace control:1-5AB-4, control, 94 mg inj., <0.98 ng found, <0.020 ppm found (ND).
2) Apple wet pomace recovery:1-5AB-4 + 0.5 ppm, 19 mg inj., 7.9 ng found, 0.42 ppm, 84% recovery.

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. MRID No. 43709054

2. Dieterle, R., Report of Special Study, 100/92, "CGA-219417 Validation of Method REM 141.01 on Tomatoes," Ciba-Geigy Limited, July, 1992. MRID No. 43709052

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. MRID No. 43709051

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. MRID No. 43737604

APPENDIX I

PREVIOUSLY SUBMITTED DOCUMENTS WITH EPA MRID NUMBERS

Dieterle, R., "Determination of Residues of Parent Compound by High Performance Liquid Chromatography (HPLC)," Ciba-Geigy Limited REM 141.01, December, 1989. MRID No. 43709054

Dieterle, R., Report of Special Study, 100/92, "CGA-219417 Validation of Method REM 141.01 on Tomatoes," Ciba-Geigy Limited, July, 1992. MRID No. 43709052

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. MRID No. 43709051

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. MRID No. 43737604