

US EPA ARCHIVE DOCUMENT

VOLUME 139 OF 151 OF SUBMISSION

B99-(64-72)

CGA-279202

444968-36

TITLE

ANALYTICAL METHOD FOR THE DETERMINATION OF RESIDUES CGA-279202
AND THE ACID METABOLITE, CGA-321113, IN CROP AND ANIMAL
SUBSTRATES BY GAS CHROMATOGRAPHY

DATA REQUIREMENT

EPA Guideline No. 171-4 (j)
(EPA Guideline No. 860-1480)

AUTHOR

D. D. CAMPBELL, SCIENTIST I

COMPLETION DATE

January 14, 1998

PERFORMING LABORATORY

Human Safety Department
Novartis Crop Protection, Inc. (Formerly Ciba Crop Protection)
Greensboro, NC 27419

LABORATORY PROJECT IDENTIFICATION

AG- 659A
(Supersedes Method No. 659)
Novartis Number 276-96

SUBMITTER/SPONSOR

Novartis Crop Protection, Inc.
(Formerly Ciba Crop Protection)
Post Office Box 18300
Greensboro, NC 27419-8300

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: Robert E. M. Wurz, Ph.D.

Title: Senior Regulatory Manager

	<u>1/15/98</u>
Signature	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 Good Laboratory Practice Compliance Statement regarding the U. S. Environmental Protection Agency's Good Laboratory Practice Standards (40 CFR Part 160, October 16 1989) provided on page 8 this submittal volume for AG-659A and signed by the Study Director, is truthful and accurate.



Robert K. Williams

Manager of Residue Chemistry and
Representative of Submitter/Sponsor

1/15/98
Date

SUBMITTER/SPONSOR: Novartis Crop Protection, Inc. P. O. Box 18300, Greensboro, NC 27419-8300

TABLE OF CONTENTS OF SUBMITTAL VOLUME

<u>TITLE</u>	<u>PAGE NO.</u>
AG-659A: Analytical Method for The Determination of Residues CGA-279202 and the Acid Metabolite, CGA-321113, In Crop and Animal Substrates By Gas Chromatography	5
Appendix III: Separate Documents Accompanying this Submission	215

SUBMITTER/SPONSOR: Novartis Crop Protection, Inc. P. O. Box 18300, Greensboro, NC 27419-8300

Human Safety Department
Novartis Crop Protection, Inc.
(formerly Ciba Crop Protection)
Greensboro, North Carolina

ANALYTICAL METHOD FOR THE DETERMINATION OF RESIDUES
CGA-279202 AND THE ACID METABOLITE, CGA-321113, IN
CROPS AND ANIMAL SUBSTRATES BY GAS CHROMATOGRAPHY

ANALYTICAL METHOD NO. AG-659A
(SUPERSEDES METHOD AG-659)

Protocol No.: 276-96

Project No.: 433000

Study Director: D. D. Campbell

Approved by: Robert K. Williams

Title: Scientist I

Title: Manager, Residue
Chemistry

Signature:



Signature:



Date:

1/14/98

Date:

1/14/98

Sponsor: Novartis Crop Protection
Human Safety Department
Residue Chemistry Laboratory
Post Office Box 18300
Greensboro, NC 27419

Study Initiation Date: June 27, 1996

TABLE OF CONTENTS

	<u>PAGE NO.</u>
STATEMENT CONCERNING GOOD LABORATORY PRACTICES	8
QUALITY ASSURANCE STATEMENT	9
I. SUMMARY AND INTRODUCTION	10
A. Scope	10
B. Principle	10
II. MATERIALS AND METHODS	11
A. Apparatus/Equipment	11
B. Reagents	13
C. Analytical Procedure	15
D. Instrumentation	22
E. Interferences	23
F. Confirmatory Techniques	23
G. Time Required	24
H. Modifications and Potential Problems	24
I. Preparation of Standard Solutions and Sample Fortification Procedures	27
J. Methods of Calculation	28
III. RESULTS AND DISCUSSION	29
A. Accuracy	31
B. Precision	32
C. Extractability	34

TABLE OF CONTENTS
(Continued)

	<u>PAGE NO.</u>
D. Accountability	36
IV. CONCLUSION.....	37
V. CERTIFICATION.....	39
VI. TABLES AND FIGURES.....	40
TABLE I: GAS CHROMATOGRAPHY OPERATING PARAMETERS FOR THE DETERMINATION OF CGA-321113 AND CGA-279202 RESIDUES	40
TABLE II: GC/MS OPERATING PARAMETERS FOR THE CONFIRMATION OF CGA-321113 AND CGA-279202 RESIDUES	41
TABLE IIA. GC/MS OPERATING PARAMETERS FOR THE CONFIRMATION OF CGA-321113 AND CGA-279202 RESIDUES; TYPICAL MASS SPECTRA	42
TABLE III: TYPICAL STANDARDIZATION DATA AND CALIBRATION PLOTS (FROM THE ANALYSIS OF APPLES)	43
TABLE IV: RESULTS FROM THE ANALYSIS OF CONTROL AND FORTIFIED CONTROL SUBSTRATES	44
TABLE IVA: STATISTICAL RESULTS FROM THE ANALYSIS OF FORTIFIED CONTROLS BASED UPON PPM LEVEL	49
TABLE V: RESULTS FROM THE ANALYSIS OF ¹⁴ C-CGA-279202 TREATED SAMPLES	50
TABLE VI: PRECISION OF ANALYTICAL METHOD FROM THE ANALYSIS OF ¹⁴ C-CGA-279202 TREATED SAMPLES	52

TABLE OF CONTENTS
(Continued)

	<u>PAGE</u> <u>NO.</u>
TABLE VII: RESULTS FROM THE ANALYSIS OF PEANUT HAY BY THE GC/MS CONFIRMATORY METHOD	54
TABLE VIII: COMPARISON OF RESULTS OBTAINED FROM ANALYSIS OF ¹⁴ C-CGA-279202 INCURRED SAMPLES BY AG-659 TO RESULTS FROM METABOLISM STUDIES	55
FIGURE 1: STRUCTURES AND CHEMICAL NAMES	56
FIGURE 2: ANALYTICAL PROCEDURAL FLOWCHART FOR AG-659	57
FIGURE 3: REPRESENTATIVE STANDARD CHROMATOGRAMS (FROM THE ANALYSIS OF APPLES)	59
FIGURE 4: CHROMATOGRAMS FROM THE ANALYSIS OF REAGENT BLANKS	63
FIGURE 5: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CROP RACs	65
FIGURE 6: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CROP PROCESSED COMMODITIES	71
FIGURE 7: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF ANIMAL SUBSTRATES	78
FIGURE 8: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF PEANUT HAY BY THE CONFIRMATORY GC/MS METHOD	83
VII. REFERENCES	88

TABLE OF CONTENTS
(Continued)

	<u>PAGE NO.</u>
APPENDIX I: VALIDATION REPORT	1
A-I. GENERAL INFORMATION	2
A-II. ANALYTICAL SECTION	3
1.0 METHOD SUMMARY	3
2.0 TEST AND REFERENCE SUBSTANCE	4
3.0 ANALYSES	5
A-III. BIOLOGICAL SECTION	6
A-IV. CIRCUMSTANCES EFFECTING THE INTEGRITY OF THE STUDY	7
1.0 REJECTED ANALYSES	7
2.0 SAMPLES NOT ANALYZED	9
A-V. CHROMATOGRAMS AND CALCULATION DATA FROM ALL ANALYSES	10
FIGURE A-1. CHROMATOGRAMS FROM THE ANALYSIS OF GRAPE RAC (FRUIT)	11
FIGURE A-2. CHROMATOGRAMS FROM THE ANALYSIS OF GRAPE PROCESSED COMMODITIES (RAISINS, JUICE)	14
FIGURE A-3. CHROMATOGRAMS FROM THE ANALYSIS OF APPLE RAC (FRUIT)	19
FIGURE A-4. CHROMATOGRAMS FROM THE ANALYSIS OF APPLE PROCESSED COMMODITIES (WET POMACE, JUICE)	24

TABLE OF CONTENTS
(Continued)

	<u>PAGE NO.</u>
FIGURE A-5. CHROMATOGRAMS FROM THE ANALYSIS OF PEANUT NUTMEAT	30
FIGURE A-6. CHROMATOGRAMS FROM THE ANALYSIS OF PEANUT HAY	34
FIGURE A-7. CHROMATOGRAMS FROM THE ANALYSIS OF PEANUT PROCESSED COMMODITY (OIL)	40
FIGURE A-8. CHROMATOGRAMS FROM THE ANALYSIS OF POTATO RAC (TUBERS)	45
FIGURE A-9. CHROMATOGRAMS FROM THE ANALYSIS OF POTATO PROCESSED COMMODITIES (GRANULES, WET PEEL)	50
FIGURE A-10. CHROMATOGRAMS FROM THE ANALYSIS OF CANTALOUPE	55
FIGURE A-11. CHROMATOGRAMS FROM THE ANALYSIS OF CUCUMBERS	60
FIGURE A-12. CHROMATOGRAMS FROM THE ANALYSIS MEAT	67
FIGURE A-13. CHROMATOGRAMS FROM THE ANALYSIS OF LIVER	70
FIGURE A-14. CHROMATOGRAMS FROM THE ANALYSIS OF ANIMAL FAT	76
FIGURE A-15. CHROMATOGRAMS FROM THE ANALYSIS OF COW KIDNEY	81
FIGURE A-16. CHROMATOGRAMS FROM THE ANALYSIS OF MILK	84

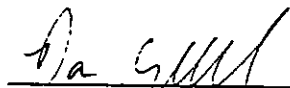
TABLE OF CONTENTS
(Continued)

	<u>PAGE NO.</u>
FIGURE A-17. CHROMATOGRAMS FROM THE ANALYSIS OF CHICKEN EGGS	87
FIGURE A-18. CHROMATOGRAMS FROM THE ANALYSIS OF PEANUT HAY BY THE CONFIRMATORY GC/MS METHOD	92
APPENDIX II: VALIDATION REPORT FOR ¹⁴ C-CGA-279202 TREATED ANIMAL SUBSTRATES.	1
A2-I. GENERAL INFORMATION	2
A2-II. ANALYTICAL SECTION	3
A2-III. BIOLOGICAL SECTION	5
A2-IV. CHROMATOGRAMS AND CALCULATION DATA FROM ALL ANALYSES	6
FIGURE A2-1. CHROMATOGRAMS FROM THE ANALYSIS POULTRY LEAN MEAT	7
FIGURE A2-2. CHROMATOGRAMS FROM THE ANALYSIS GOAT MEAT	10
FIGURE A2-3. CHROMATOGRAMS FROM THE ANALYSIS GOAT MILK	13

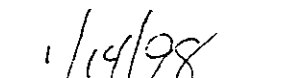
STATEMENT CONCERNING GOOD LABORATORY PRACTICES

This study was conducted in accordance with the applicable EPA Good Laboratory Practice Standards (40 CFR Part 160) with the following exception(s):

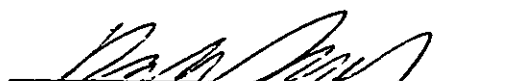
1. The SOP 10.2, Revision 1 detailing the report format for analytical methods is being revised. The current SOP was not followed in every detail.



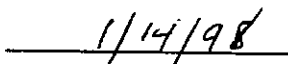
D. D. Campbell
Study Director



Date



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



Date

Submitter/Sponsor:
Novartis Crop Protection
Post Office Box 18300
Greensboro, NC 27419

QUALITY ASSURANCE STATEMENT

Method Title: Analytical Method for the Determination of Residues CGA-279202 and the Acid Metabolite, CGA-321113, in Crops and Animal Substrates By Gas Chromatography

Study Director: D Campbell

Novartis Study Number: 276-96 with Amendments

Project Number: 433000

Method Number: AG-659A

Pursuant to Good Laboratory Practice Standards, this statement verifies that the aforementioned study was inspected and/or audited and the findings reported to Management and to the Study Director by the Novartis Crop Protection Quality Assurance Unit on the dates listed below.

<u>INSPECTION/AUDIT TYPE</u>	<u>INSPECTION/AUDIT DATE(S)</u>	<u>REPORTING DATE</u>
Protocol Audit	06/27/96	06/27/96
In-Progress Inspection	08/29/96	09/04/96
Final Report Audit (AG-659)	05/6-9,15/97	05/15/97
Revised Method AG-659A	12/05,08/97	12/08/97

Prepared by: Roger A. Edwards Date: 12/8/97

Roger A. Edwards
Senior Quality Assurance Auditor
Novartis Crop Protection
Quality Assurance Unit

I. SUMMARY AND INTRODUCTION

A. SCOPE

This method is for the determination of residues of CGA-279202 and the metabolite CGA-321113 in animal tissues, milk, poultry eggs, and the following crop substrates: grapes, pome fruit, cucurbits, peanuts, and tuberous vegetables (including processed fractions). The limit of detection (LOD) for each analyte of this method, defined as the lowest standard concentration injected, is 0.04 ng/uL, or 0.08 ng injected. The limit of quantitation (LOQ) is defined as the lowest fortification level used in this study. For all substrates, except milk and peanut hay, the LOQ is 0.02 ppm. For milk, the LOQ is lowered to 0.01 ppm, and for the analysis of peanut hay, the LOQ is increased to 0.05 ppm (Peanut hay LOD = 0.2 ng, see alternative in Section II.H.8.0). The chemical structures of CGA-279202 and CGA-321113 are shown in Figure 1.

This method was validated under Protocol 276-96¹. A summary of the validation results and representative chromatograms are provided in the body of the method. Appendix I includes all chromatograms from all commodities analyzed, all validation data needed to reproduce calculations and other information related to the validation study.

Method AG-659 was issued on June 6, 1997. Protocol 276-96 was re-opened on October 10, 1997 to include the analysis of tissue samples from animals dosed with ¹⁴C-CGA-279202 during the goat metabolism⁷ and poultry metabolism studies⁸. Method AG-659A includes the extractability and accountability results from the ¹⁴C-CGA-279202 animal sample validations. Method AG-659A also includes minor changes that have been made to the method over time to improved ruggedness during the magnitude of the residue studies, as well as suggestions from the Independent Laboratory Validation Study⁹.

B. PRINCIPLE

Samples are extracted twice by homogenization in acetonitrile (ACN):water (80:20, volume to volume or

v:v). Liquid samples such as milk or juices are extracted by shaking for 15 minutes in ACN:water (80:20, v:v). After filtration, the extract volume is measured (and adjusted to 200 mL if necessary) and an 80 mL aliquot is taken (160 mL for milk samples). A 3-layer liquid-liquid partition is performed by adding water saturated with sodium chloride, toluene and hexane (peanut oil samples are extracted by adding four grams directly to a separatory funnel and forming the same three layer partition). The middle layer is collected, partitioned a second time with hexane, and evaporated. The sample is reconstituted in 0.085% aqueous phosphoric acid:acetone (95:5, v:v) and subjected to a C18 solid-phase extraction cleanup. After elution with 0.085% aqueous phosphoric acid:acetone (30:70, v:v), the sample is evaporated to aqueous and partitioned into methyl-tert butyl ether:hexane (1:1, v:v). The methyl-tert butyl ether:hexane is evaporated to dryness and the sample is reconstituted in 0.1% polyethylene glycol in acetone (v:v) for analysis by gas chromatography (GC). The GC analytical column is a capillary DBWAX, and detection is by Nitrogen-Phosphorus Detection (NPD). Confirmation of residues is by GC/MS on a capillary DB-1701 column.

II. MATERIALS AND METHODS

A. APPARATUS/EQUIPMENT

- 1.0 Bottles, Owens-Brockway square amber, 8-oz. with wide mouth, or equivalent (Penn Bottle Co.)
- 2.0 Concentration Tubes, conical-bottom 50-mL capacity (Fisher #05-507-5C or equivalent)
- 3.0 Filter, Acrodisc CR PTFE, 0.2 μ m, 13.0 mm (Gelman Product #4423, or equivalent)
- 4.0 Filter Flask Adapter, neoprene 46 x 29 mm (OD top x bottom) (Fisher #10-18-4, or equivalent)

- 5.0 Filter Paper, Whatman Qualitative #5, 7 cm (Whatman #1005070, or equivalent)
- 6.0 Flasks, KIMAX with side-arm, 500-mL (Fisher #10-181E, or equivalent)
- 7.0 Flasks, Boiling with flat bottom, 500-mL (Fisher #09-552C, or equivalent)
- 8.0 Flasks, Boiling with round bottom, 50-mL (Fisher #K601000-0124, or equivalent)
- 9.0 Flasks, Erlenmeyer, 250-mL (Fisher #10-047C, or equivalent)
- 10.0 Funnel, Buchner porcelain (Fisher #10-356-C or equivalent)
- 11.0 Funnel, Separatory, 60-mL and 125-mL (Fisher #10-437-A, and 10-437-10B or equivalent)
- 12.0 Graduated cylinder, 10-mL, 50-mL, 100-mL, 250-mL (Fisher #08-551A, 08-551C, 08-551D, and 08-551E or equivalent)
- 13.0 Homogenizer, Polytron (Brinkman Instruments or equivalent)
- 14.0 Pasteur pipettes (Kimble 13-678-30B, 13-678-30C or equivalent)
- 15.0 Pipettes, volumetric class A, 1-mL (Fisher #13-650-2B, or equivalent)
- 16.0 Reservoir, 15-mL for solid phase extraction (J. T. Baker #7119-01 or equivalent), with adapter (J. T. Baker #7122-00 or equivalent).
- 17.0 Rotary Evaporator, Buchii or equivalent, with warm water bath (Buchler Instruments or equivalent), and aspirator pump (Cole-Parmer or equivalent). The use of a bump trap is suggested (Fisher #K570200-2524, or equivalent)

- 18.0 Shaker, Orbital, IKA Labortechnik #KS501, or equivalent.
- 19.0 Solid Phase Extraction, Varian Bond Elut C18, 6cc/1 gram (Varian Part Number 1225-6001)
- 20.0 Ultrasonicator (Heatsystems Inc. or equivalent)
- 21.0 Vacuum Manifold for solid phase extraction (Supelco Part Number 5-7250, or equivalent)
- 22.0 Vials, crimp top borosilicate (Sun Broker, Inc. or equivalent)

B. REAGENTS

- 1.0 Acetone, HPLC grade (Fisher #A949-4 or equivalent)
- 2.0 Acetonitrile, HPLC grade (Fisher #A998-4, or equivalent)
- 3.0 Celite 545 Filter Aid (Fisher #C212-500, or equivalent)
- 4.0 Hexane, HPLC grade (Fisher #H302-4 or equivalent)
- 5.0 Isopropyl Alcohol (2-propanol), HPLC grade (Fisher #A451-4, or equivalent)
- 6.0 Methyl-tert Butyl Ether, HPLC grade (Fisher #E127-4, or equivalent)
- 7.0 Phosphoric acid, 85%, Certified ACS grade (Fisher #A242 or equivalent)
- 8.0 Polyethylene Glycol, Average Molecular Weight 200 (SIGMA # P3015 or equivalent)
- 9.0 Sodium Chloride, Certified ACS grade (Fisher #S271-3 or equivalent)
- 10.0 Toluene, High Purity Solvent grade (Burdick and Jackson #347-4 or equivalent)

- 11.0 Water, HPLC grade (picopure or equivalent)
- 12.0 Acetonitrile:water (80:20, v:v) - prepare 1 liter by adding 800 mL of acetonitrile + 200 mL of water.
- 13.0 0.085% Phosphoric acid (v:v) - prepare 1 liter by adding 1 mL of 85% phosphoric acid + 999 mL of HPLC grade water
- 14.0 0.085% Phosphoric acid:acetone (95:5, v:v) - prepare 300 mL by adding 285 mL of 0.085% phosphoric acid + 15 mL acetone.
- 15.0 0.085% Phosphoric acid:acetone (60:40, v:v) - prepare 300 mL by adding 180 mL of 0.085% phosphoric acid + 120 mL acetone.
- 16.0 0.085% Phosphoric acid:acetone (30:70, v:v) - prepare 300 mL by adding 90 mL of 0.085% phosphoric acid + 210 mL acetone.
- 17.0 Water saturated with sodium chloride - prepare 1 liter by adding 357 grams per liter of water (at room temperature) and mix well.
- 18.0 0.1% Polyethylene Glycol in Acetone (v:v) - prepare 1 L by adding 1 mL polyethylene glycol to a 1-Liter volumetric flask and diluting to the line with acetone.
- 19.0 Methyl-tert Butyl Ether:Hexane (1:1, v:v) - prepare 1 L by mixing 500 mL of methyl-tert butyl ether + 500 mL of hexane.
- 20.0 CGA-279202, analytical standard supplied by Chemical Synthesis Group, Novartis Crop Protection, 410 Swing Rd., Greensboro, NC, 27419
- 21.0 CGA-321113, analytical standard supplied by Chemical Synthesis Group, Novartis Crop Protection, 410 Swing Rd., Greensboro, NC, 27419

C. ANALYTICAL PROCEDURE

NOTE - All liquid mixtures are volume to volume, as is shown in Section II.B. (REAGENTS)

1.0 Extraction

1.1 Sample Preparation

Animal samples are prepared using the procedures of Ciba Biochemistry Standard Operating Procedure (SOP) 7.27, current revision². Crop substrates are prepared using the procedures of Ciba Biochemistry SOP 7.21, current revision³. These SOPs follow the guidelines of the US Food and Drug Administration Pesticide Analytical Manual, Volume I, Sections 102 and 203. Samples are cut into 1-2 inch pieces and ground in a Hobart food cutter, using dry ice as necessary to keep the sample frozen. Liquid samples such as juices, milk, or eggs are fully thawed and will be mixed or blended. Nut samples (e.g., peanuts) are separated into shells and nutmeat prior to preparation.

1.2 Extraction of All Substrates Except Milk, Juices and Peanut Oil

Weigh a 10-gram subsample of homogenized crop sample, animal tissue, or egg sample into a tared extraction bottle. For recovery samples, fortify as specified in section II.I.2.0. Add 150 mL of ACN:water (80:20) to the substrate and homogenize for 1 minute with the Polytron at high speed (~8000-10000 rpm). Allow the solid material in the extract to settle out (approximately 2-3 minutes). Place a Whatman #5 filter paper in a Buchner funnel on a 500-mL side-arm flask equipped with a filter adapter (attached to a vacuum system). Pour approximately 5 grams of Celite

onto the filter. Decant the solvent and any suspended material from the settled solid material and filter through the Celite. Add 50 mL of ACN:water (80:20) to the solid material left in the extraction bottle. Homogenize a second time for 30 seconds with the Polytron at high speed. Pour the extract through the same filter and into the flask containing the first extract. Measure the final extract volume, and adjust the volume to 200 mL using the extraction solvent (if necessary).

Measure an 80-mL aliquot of the extract using a 100-mL graduated cylinder. Pour the aliquot into a 125-mL separatory funnel, and proceed to step II.C.2.1. The remaining extract should be stored refrigerated in an amber bottle.

1.3 Extraction of Milk and Juices

Weigh a 10 gram sample of milk or juice into a tared extraction bottle. For recovery samples, fortify as specified in section II.I.2.0. Add 200 mL of ACN:water (80:20) and shake on an orbital shaker for 15 minutes. Place a Whatman #5 filter paper in a Buchner funnel on a 500-mL side-arm flask equipped with a filter adapter (attached to a vacuum system). Pour approximately 5 grams of Celite onto the filter. Decant and filter the entire sample. Measure the final extract volume.

For juices, measure an 80-mL aliquot of the extract using a 100-mL graduated cylinder. For milk, measure an 160-mL aliquot of the extract using a graduated cylinder. Pour the aliquot into a 125-mL separatory funnel (250-mL separatory funnel for milk), and proceed to step II.C.2.1. The remaining extract

should be stored refrigerated in an amber bottle.

1.4 Extraction of Peanut Oil

Weigh 4 grams of peanut oil directly into a tared 125-mL separatory funnel (the separatory funnel will need to be held upright on the balance - e.g. place it in a large beaker before the balance is tared). For recovery samples, fortify as specified in section II.I.2.0, based upon a total sample weight of 4 grams. Add 80 mL of ACN:water (80:20) to the separatory funnel. Proceed to step II.C.2.1.

2.0 Partition of extract

- 2.1 For all substrates except milk, add 5 mL of water saturated with sodium chloride, 2 mL of toluene, and 10 mL of hexane to the sample in the 125-mL separatory funnel from section II.C.1.2, II.C.1.3 or II.C.1.4. For milk samples, add 10 mL of water saturated with sodium chloride, 4 mL of toluene, and 20 mL of hexane to the sample in the 250-mL separatory funnel from section II.C.1.3. Partition all samples by shaking for 1 minute. Allow the layers to separate after the partition. This partition forms three layers; a bottom aqueous layer, a middle layer comprised primarily of ACN and toluene, and a top hexane layer. CGA-279202 and CGA-321113 will be in the ACN and toluene middle layer after the partition. Drain the lower aqueous layer and discard. Drain the middle ACN and toluene layer into an appropriate flask (250 mL Erlenmeyer or the 500-mL boiling flask used in next step) and save. Drain the top hexane layer and discard.

- 2.2 For all samples, pour the middle ACN and toluene layer back into the 125-mL separatory funnel. Add 10 mL of hexane (20 mL for milk samples) and partition by shaking for 1 minute. Allow the layers to separate after the partition. This partition forms two layers; a bottom ACN and toluene layer and a top hexane layer. Drain the bottom layer into a 500-mL boiling flask and discard the top hexane layer. For peanut oil and animal fat samples only: repeat the above step (II.C.2.2) to remove residual lipophilic compounds.
- 2.3 Concentrate the sample in the 500-mL boiling flask (section II.C.2.2) using a vacuum rotary evaporator fitted with a bump trap and a water bath at 30-35°C. Evaporate to near dryness, so only the aqueous portion of the sample remains. Water condensation will be observed on the walls of the bump trap when the aqueous contents of the boiling flask begins to evaporate, indicating that all of the ACN and toluene has been removed. NOTE: foaming may occur as the samples are nearing dryness, and in some cases the foam may boil over the top of the flask. The use of a bump trap is suggested. The samples should be monitored closely as they are nearing dryness. If the samples start to foam over, the vacuum should be released momentarily until the foam dissipates.
- 2.4 When evaporation is complete, add 10 mL of 0.085% phosphoric acid:acetone (95:5). Swirl the flask, and sonicate to release material adhering to the walls of the boiling flask. Note that for some substrates, a small amount of solid material may remain on the flask at this step.

3.0 C18 Silica Solid Phase Extraction

3.1 Column Conditioning

Attach a 15-mL reservoir to a Varian Bond Elut C18, 6cc/1 gram Solid Phase Extraction (SPE) column with an adapter, and place the column on a vacuum manifold system. Condition the column by flushing with 5 mL acetone followed by 10 mL 0.085% Phosphoric acid:acetone (95:5). Let each solvent used in the following procedure pass through the column until it reaches the top of the C18 packing, and do not let the packing become dry. The flow rate through the SPE column should be maintained at about 1 to 2 drops per second with the vacuum manifold system.

3.2 Sample Addition

Pour the sample from section II.C.2.4 from the 500-mL boiling flask into the reservoir above the column. Allow the sample to pass through to the top of the column without drying the C18 packing. Discard the load eluate.

3.3 Column Wash

Add 10 mL of 0.085% phosphoric acid:acetone (60:40) to the 500-mL boiling flask, swirl, and sonicate if any material remains on the walls of the boiling flask. Remove as much material as possible (note that for some substrates, material may remain on the glassware at this step). Pour the rinse into the column reservoir and allow it to pass through to the top of the column without drying the C18 packing. Discard the rinse eluate.

3.4 Sample Elution

Add 25 mL of 0.085% phosphoric acid:acetone (30:70) to the 500-mL boiling flask, swirl, and sonicate if any material remains on the walls of the boiling flask. Remove as much material as possible. Pour the elution solvent into the column reservoir and allow it to pass through the column. Collect the eluate in a 50-mL round bottom boiling flask.

4.0 Partition of C18 Eluate

- 4.1 Evaporate the sample from Section II.C.3.4 using a vacuum rotary evaporator fitted with a bump trap (water bath at 30-35°C). Evaporate until all the acetone has been removed. Aqueous condensation will be observed on the walls of the bump trap when the aqueous contents of the boiling flask begin to evaporate, indicating that all of the acetone has been removed (an alternative way to ensure acetone removal is by measuring the volume after evaporation; the volume should be less than 6-7 mL after evaporation). The removal of all acetone is crucial to prevent emulsion formation in the partition steps below. Some samples will foam during this evaporation step and should be monitored carefully. If the samples start to foam over, the vacuum should be released momentarily until the foaming stops [See note in Modifications and Potential Problems Section (II.H.1.0 and II.H.2.0)].
- 4.2 Pour the sample (from section II.C.4.1) into a 60-mL separatory funnel. Add 5 mL of 0.085% phosphoric acid to the 50-mL round bottom flask, swirl, and transfer to a 60-mL separatory funnel. Add 10 mL of methyl-tert butyl ether (MTBE):hexane

(1:1) to the 50-mL round bottom, swirl, and transfer to the 60-mL separatory funnel. Add 1 mL of water saturated with sodium chloride to the 60-mL separatory funnel. For peanut hay, liver and kidney samples, use 5 mL of water saturated with sodium chloride (to help prevent emulsions with these substrates). Stopper the separatory funnel with a Teflon or plastic stopper and partition by shaking for 1 minute. Allow the layers to separate (this may take up to approximately one-half hour or more for peanut hay). [See note on emulsions in Modifications and Potential Problems (Section II.H.2.0)]

- 4.3 Drain the bottom aqueous layer back into the 50-mL boiling flask. Use a Pasteur pipette to remove the MTBE:hexane (1:1) from the top of the 60-mL separatory funnel [instead of draining the MTBE:hexane (1:1) layer through the stopcock] and transfer to a 50-mL concentration tube. NOTE: It is crucial that no water droplets are transferred with the MTBE:hexane (1:1) layer. The use of a drying agent to remove water from the MTBE:hexane (1:1) (e.g. sodium sulfate) must be avoided at this step, since the metabolite CGA-321113 will bind to such agents when dissolved in MTBE:hexane (1:1). See Modifications and Potential Problems (Section II.H.3.0) if water droplets are accidentally transferred with the hexane).
- 4.4 Pour the aqueous layer back into the 60-mL separatory funnel. Add 10 mL of MTBE:hexane (1:1) and partition a second time according to section II.C.4.3. Use the same procedures to prevent transfer of droplets of the aqueous phase into the MTBE:hexane (1:1) layer. Add the second MTBE:hexane (1:1) layer to the

same 50-mL concentration tube from
section II.C.4.3.

5.0 Preparation of Final Sample

Evaporate the sample from section II.C.4.4 to dryness using a vacuum rotary evaporator fitted with a bump trap with a water bath of 30-35°C. Reconstitute in the appropriate volume of 0.1% polyethylene glycol in acetone. A 1 mL volume of 0.1% polyethylene glycol in acetone is used for recovery samples fortified at the LOQ of 0.02 ppm (0.01 ppm for milk). The volume may be increased as needed to bring the sample concentration within the range of the standards. See note for peanut hay in section II.H.8.0. Transfer the sample to a GC vial for automated analysis.

D. INSTRUMENTATION

1.0 Description and Operating Conditions

Residues of CGA-321113 and CGA-279202 are determined by Gas Chromatography (GC) using a Nitrogen-phosphorus detector (NPD) and a DBWAX analytical column. The instrumental conditions are shown in Table I.

2.0 Standardization

Standardize the GC system by injecting 2 µL aliquots of standard solutions containing CGA-279202 and CGA-321113 in a range of 0.08 to 1.0 ng/injection. Generate a linear regression analysis from the data by comparing detector response to the nanograms injected. See Section II.I.1.0 for preparation of analytical standards. It is necessary to run a set of standards with each analytical set to achieve accurate quantification of residues.

E. INTERFERENCES

None observed. A highly specific GC/Mass Spectrometry system (GC/MS) confirmatory procedure that uses an alternative GC column (DB-1701) is presented in Section II.F. The confirmatory procedure will distinguish CGA-279202 and CGA-321113 from any other pesticide or co-extractant if present at similar retention times on the original DBWAX column.

F. CONFIRMATORY TECHNIQUES

Confirmation of residues can be achieved by analysis of the samples by GC/MS. Use selected ion monitoring (SIM) to detect the following ions: CGA-279202 ($m/z = 116, 131, 222$), CGA-321113 ($m/z = 116, 145, 317$). Confirmation of residue concentration can be achieved by quantitating on the target ion for each analyte ($m/z = 116$). Confirmation of compound identity can be determined by taking a ratio of the ions specified for each compound. Structural confirmation can be made if the comparison of the ratios determined in a suspected residue match within $\pm 20\%$ of the ratio determined in the standard. Typical examples of ratios, obtained from the injection of the 0.5 ng/ μ l standard (1.0 ng injected), are shown in Table II.

The conditions listed in Table II were used for validation of the confirmatory method by the analysis of peanut hay. A DB-1701 GC column was used, providing a mode of separation that is alternative to the DBWAX used in the analytical method. Typical mass spectra for CGA-279202 and CGA-321113 are included in Table IIA. Results for the confirmatory analysis of peanut hay are shown in Table VII and typical chromatograms are shown in Figure 8. Chromatogram 1 of Figure 8 is the solvent blank, showing the peaks expected in all injections that occur due to the presence of Polyethylene Glycol in the injection solvent. In chromatogram 3 (control) and 4 (0.05 ppm recovery), the ratio of the ions did not match those expected for CGA-321113 or CGA-279202 due to the presence of interferences.

The ratios did match at the higher fortification (10 ppm) and with the ^{14}C -CGA-279202 treated peanut hay samples due the large residues found. The confirmatory technique is valid for peanut hay at the expected tolerance level (4 ppm).

NOTE: ions used for analysis of peanut hay were the following: CGA-279202 (m/z = 116, 131, 145), CGA-321113 (m/z = 116, 145).

G. TIME REQUIRED

The extraction and purification of a set of 6 samples may be completed within a time period of approximately 8 hours. GC analyses should take about 3 - 4 hours using automatic injection.

H. MODIFICATIONS AND POTENTIAL PROBLEMS

1.0 Evaporation Flask Used In Section II.C.4.1

Evaporate the samples in the 50-mL round bottom flask specified and not in a 50-mL conical concentration tube. Some samples were observed to boil over rapidly and shoot into the rotary evaporator when the conical tube was used. A larger round bottom flask may be used if bumping problems persist. Use of a bump trap is essential for the difficult samples.

2.0 Emulsion Formation in Section II.C.4.2

If all the acetone has been removed in the previous step (II.C.4.1), no emulsions should occur in the hexane layer. If emulsions do occur in the hexane layer, drain the entire sample (including the emulsions and hexane) back into the 50-mL boiling flask. Evaporate off all organic solvent using the vacuum rotary evaporator. Ensure that the acetone has been removed entirely (volume must be <6 - 7 mL). Pour the sample back into the 60-mL separatory funnel, add 10 mL hexane, and repeat the partition.

Peanut hay, liver and kidney samples tend to produce emulsions at the interface of the organic and aqueous layers even when all of the acetone has been removed. The amount of saturated aqueous sodium chloride was increased to help prevent these emulsions. The emulsions should break after about one-half hour, with occasional agitation by a stir bar. If emulsions persist, collect the emulsions with the aqueous and remove all recoverable MTBE:Hexane (1:1) as described in Section II.C.4.0. If the emulsions persist after the second partition, perform a third partition to ensure that all of the organic soluble material is recovered.

3.0 Presence of Water Droplets in the Hexane Sample in Section II.C.4.3

If water droplets are accidentally transferred with the hexane, first minimize them by removal with a Pasteur pipette. Make sure any remaining aqueous droplets are evaporated to dryness during the subsequent evaporation step (II.C.5.0). Add 1-2 mL of isopropyl alcohol to azeotrope off the water if necessary. It is possible that salt crystals will form upon reconstitution of the sample in the final solvent (acetone). If salt crystals are visible, filter the sample using a Gelman CR PTFE 13 mm filter attached to a glass syringe (pre-rinse the filter with 2-3 mL of acetone and dry by forcing air through the filter prior to sample filtration).

4.0 Stability of CGA-321113 in Acetone

CGA-321113 has been shown to be stable in acetone under refrigerator conditions (0-4°C) for at least 28 days, but was shown to degrade in acetone at room temperature (0-4°C) after 4 days. It is essential that the standard be stored under refrigerated conditions. The final fractions that are analyzed by GC should be stored in the refrigerator at all times.

except during analyses. DO NOT STORE AT ROOM TEMPERATURE. Standards have been stable in the refrigerator for as long as 4 months in 0:1% PEG/Acetone.

5.0 Method Stopping Points

Stop at the following steps if the samples need to be stored overnight during the work-up procedure. Stop after sections II.C.2.2 or II.C.4.4 so that the samples will be stored in organic solvent mixtures (ACN/toluene or MTBE:hexane). The extract may also be stored in the refrigerator (0-4°C).

6.0 Potential Carryover of CGA-321113

CGA-321113 has the potential to adhere to glassware when high concentrations (e.g. >1 ppm) are analyzed. If high concentration samples are undergoing analysis, isolate the glassware used for these analyses and include an acid wash in the glassware cleaning procedures.

7.0 GC Inlet Seal

The use of a Restek Silcosteel inlet seal (Part No. 21319) was shown to cause breakdown of CGA-279202 during injection. The problem was fixed by using the Hewlett-Packard gold plated inlet seal (Part No. 18740-20885).

8.0 Final Volume For Peanut Hay Samples

The increase of LOQ to 0.05 ppm for peanut hay was achieved in this study by using a final volume of 1 mL and increasing the LOD to 0.2 ng. A valid alternative approach is to increase the final volume to 2.5 mL and having the LOD remain at 0.08 ng. The larger volume was shown to give better chromatography during the magnitude of the residue study.

I. PREPARATION OF STANDARD SOLUTIONS AND SAMPLE
FORTIFICATION PROCEDURES

1.0 Preparation of Fortification and Analytical
Standards

Weigh 10 mg of CGA-279202 analytical standard into a 100-mL volumetric flask and dilute to the mark with acetone. Repeat for CGA-321113 to obtain two separate stock solutions of 100 ng/ μ L. Add 1 mL of each to a 100-mL volumetric flask and dilute to the mark with 0.1% polyethylene glycol in acetone to give a stock solution containing 1 ng/ μ L of both analytes. Alternatively, weigh the CGA-279202 and CGA-321113 into the same 100-mL volumetric flask and dilute to the line with acetone to obtain a stock solution containing 100 ng/ μ L of both analytes. Make serial dilutions of the stock solutions with 0.1% polyethylene glycol in acetone to give a series of fortification/analytical standards in a range of 0.04 to 0.5 ng/ μ L. Store the standards in a refrigerator in capped amber bottles when not in use.

2.0 Sample Fortification Procedures

Add 1 mL of 0.2 ng/ μ L standard to 10 grams of substrate to obtain a sample fortified at 0.02 ppm (for milk samples, add 1 mL of a 0.1 ng/ μ L standard to 10 grams of milk to get an 0.01 ppm sample, and for peanut oil, add 1 mL of an 0.08 ng/ μ L standard to 4 grams oil for an 0.02 ppm sample). Other volumes and standard concentrations may be used for fortification, although the total volume of fortification standard should not exceed 2 mL. See Section II.J.2.0 for recovery calculation procedures.

J. METHODS OF CALCULATION

1.0 Determination of Sample Residues

Inject 2- μ L aliquots of the final fractions prepared in Section II.C.5.0 into the GC system under the same conditions as for the standards. Compare the peak heights of the unknown samples to the standard curve or enter the peak heights into a least squares program to determine the nanograms of CGA-279202 and CGA-321113 in the injected aliquot.

To calculate the residue results, the mg injected must first be calculated as follows:

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

G = milligrams sample extracted

V_a = aliquot volume

V_e = extraction volume

V_i = injection volume

Vf = total volume of final injection
solution (μ L)

R% = recovery ratio given by equation 5
below

M = % moisture of sample

W = weight of sample in grams

To determine ppm of each analyte found in samples, use equation 2.

$$(2) \text{ ppm} = \frac{(\text{ng analyte found}) (100)}{(\text{mg sample injected}) (R\%)}$$

To convert the ppm found for CGA-321113 to parent CGA-279202 equivalents, multiply the results from equation 2 by the ratio of the molecular weights, defined as F:

$$(3) F = \text{ratio of molecular weights} = \frac{\text{MW parent}}{\text{MW of the metabolite}}$$

See Figure 1 for the molecular weights of parent and metabolite.

2.0 Determination of Procedural Recoveries

2.1 Determine the ng of CGA-321113 and CGA-279202 injected in the sample, and calculate the mg injected using the procedures described in Section II.J.1.0. Calculate the final ppm values of the control and fortified samples according to the following equation:

$$(4) \text{ ppm} = \frac{\text{ng analyte found}}{\text{mg sample injected}}$$

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

$$(5) R\% = \frac{(\text{ppm analyte found} - \text{ppm control})}{\text{ppm analyte added}} \times 100$$

Note: Analyses of controls, correction of recovery results for small control values, and use of the recovery factors are not required for tolerance enforcement purposes. They are used for method generation and validation of residue data, where applicable.

III. RESULTS AND DISCUSSION

The objective of Protocol 276-96 was to validate Analytical Method AG-659 for the quantitation of residues of CGA-279202 and CGA-321113 in crops and animal substrates. The precision and accuracy of the method was verified by analyzing controls and control samples fortified with CGA-321113 and CGA-279202 in the following commodities:

Dairy: Tenderloin, Fat, Liver, Kidney, and Milk.
Poultry: Fat, Liver, Eggs
Grape: Fruit, Raisins, Juice
Apple: Fruit, Wet Pomace, Juice
Peanut: Nutmeat, Hay, Refined Oil
Potato: Tubers, Granules, Wet Peel
Cucurbits: Cantaloupes, Cucumbers

The method precision, the extractability of weathered samples with incurred residues, and the accountability expected for this method, was affirmed by analyses of the following ^{14}C -CGA-279202 metabolism samples:

Apple: Fruit (Metabolism Study 95GN30⁴)
Peanut: Nutmeat and Hay (Metabolism Study 75-95⁵)
Cucurbits: Cucumbers (Metabolism Study 96JS23⁶)

^{14}C -CGA-279202 treated goat and poultry metabolism samples (goat meat, liver, milk, and poultry meat) were not available for analysis at the time of the issuance of the original method AG-659. These samples were validated at a later time and are reported in method AG-659A and Appendix II.

The Limit of Quantitation (LOQ), as determined by the smallest acceptable recovery level, is 0.02 ppm of CGA-321113 and 0.02 ppm of CGA-279202 for all substrates except milk and peanut hay. The LOQ for milk is 0.01 ppm, and for peanut hay is 0.05 ppm. The Limit of Determination (LOD), as determined by the smallest standard injected (which is defined as one-half the ng injected at LOQ) is 0.08 ng CGA-321113 and 0.08 ng CGA-279202. For the analysis of peanut hay, the LOD is 0.2 ng. Typical standard chromatograms, which were injected for the analysis of apples, are shown in Figure 3. The calibration plots for each analyte are shown in Table III. The results from these standard injections is typical of the results observed when using this method. Chromatograms from reagent blanks are shown in Figure 4. The reagent blanks were run during the analysis of cow omental fat, milk, apple juice, and peanut oil.

Representative chromatograms from the analysis of crop raw agricultural commodities (RACs), crop processed commodities, and animal substrates are shown in

Figures 5, 6 and 7, respectively. Appendix I and II show all chromatograms from the validation study. Figure 8 shows representative chromatograms from the GC/MS confirmatory procedure.

A. ACCURACY

Table IV shows the individual results from analyses of controls and control samples fortified with CGA-321113 and CGA-279202 over the range of 0.02 (0.01 for milk) to 10.0 ppm. Only 15 of the 216 recovery values determined during the validation of method AG-659 fell outside of the desired range of 70% to 120% [see Table IV: cow liver (sample 17), cow omental fat (sample 12R), poultry liver (samples 80 and 81), egg (sample 84), raisin (sample 100), grape juice (sample 103), apple (sample 109), apple wet pomace (sample 117), and peanut oil (sample 146), poultry lean meat (sample A2-2), and goat milk (sample A2-15)]. The overall mean for all recoveries for CGA-321113 was 89% (standard deviation = 14, coefficient of variation (%CV) = 16, n = 108). The overall mean for all CGA-279202 recoveries was 95% (standard deviation = 12, %CV = 12, n = 108).

Table IVA shows the accuracy obtained at each fortification level. The average recovery for CGA-321113 ranged from 80% (at the 1.0 - 10.0 ppm fortification level) to 92% (method LOQ fortifications and at 0.1 ppm level). The average recovery for CGA-279202 ranged from 89% (0.2 - 0.3 ppm fortification level) to 98% (at the method LOQ). At the LOQ of 0.01 ppm for milk and 0.02 ppm for all other substrates (except peanut hay), the average recovery was 92% and 98% for CGA-321113 and CGA-279202, respectively. The peanut hay LOQ of 0.05 ppm level had an average recovery of 87% and 96% for CGA-321113 and CGA-279202, respectively.

Mean recoveries of CGA-321113 for the crop substrates ranged from 71% for grape juice to 102% for peanut nutmeat. The overall mean recovery for all crop substrates for CGA-321113 was 88% (standard deviation = 12%, %CV = 14, n = 67). Mean recoveries of CGA-279202 for the crop substrates ranged from 86% for peanut oil to 107 for peanut nutmeat. The

overall mean recovery for crop substrates for CGA-279202 was 95% (standard deviation = 9%, %CV = 10, n = 67).

Mean recoveries of CGA-321113 for the animal substrates ranged from 76% for cow kidney to 120% for poultry liver (note that the average for the poultry lean meat analysis was 70%, but that only 2 recovery samples were run for this set). The overall mean of CGA-321113 for animal substrates was 89% (standard deviation = 17%, %CV = 19, n = 41). Mean recoveries of CGA-279202 for the animal substrates ranged from 74% for cow liver to 109% for milk (note that the goat milk analysis had an average of 111%, but that only 2 recoveries were run in this set). The overall mean of CGA-279202 for animal substrates was 96% (standard deviation = 15%, %CV = 16, n = 41).

Table VII shows the results obtained when the recovery samples of peanut nutmeat are injected on the confirmatory GC/MS system. Quantitation is shown when monitoring on a single ion (m/z = 116). The mean percent recoveries fall within the acceptable range (>70%-<120%).

B. PRECISION

The precision was obtained by the analyses of all fortified control samples and was measured by the overall Standard Deviation (SD), and Coefficient of Variation (%CV) as follows:

COMPOUND	CROP SUBSTRATES	ANIMAL SUBSTRATES	ALL ANALYSES
CGA-321113	SD=12%, %CV=14 (n=67)	SD=17%, %CV=19 (n=41)	SD=14%, %CV=16 (n=108)
CGA-279202	SD=9%, %CV=10 (n=67)	SD=15%, %CV=16 (n=41)	SD=12%, %CV=12 (n=108)

Table IVA shows the precision obtained at each fortification level. The %CV obtained from the analyses of CGA-321113 ranged from 11% (1.0 - 10 ppm and 0.2 - 0.3 ppm fortification levels) to 23% (0.05 ppm level). The %CV obtained from the analyses of CGA-279202 ranged from 9% (0.1 ppm levels) to 15%

(0.2 - 0.3 ppm level). The %CV obtained at the LOQ (0.01 ppm for milk, and 0.02 ppm for all other substrates) was 14% for CGA-321113 and 12% for CGA-279202.

Table VI demonstrates the precision obtained from the analyses of crop and animal samples treated with ¹⁴C-CGA-279202 from apple, cucumber, peanut, poultry and goat metabolism studies. For crops, the %CV for CGA-321113 ranged from 6.5% for cucumbers to 14% for peanut hay. The %CV for CGA-279202 ranged from 4.8% for cucumbers to 18% for apples. When the results are expressed as CGA-279202 equivalents, the %CV ranged from 4.7% for cucumbers to 17 % for apples. No statistical calculations were possible for peanut nut meat since all results were <LOD. The results for CGA-321113 obtained from the analyses of cucumbers are all less than the LOQ of 0.02 ppm, but are greater than the LOD (<0.08 ng), and are reported here only as a measure of method precision.

For the animal substrates, the %cv determined were 13% for poultry meat, 53% for goat muscle, and 35% for goat milk. One of the 3 repetition of the goat meat and milk were low for unknown reasons, skewing the results and unrealistically increasing the %cv from these analyses. Liquid scintillation counting results indicate that the losses in these samples occurred during the sample cleanup steps (extractability count shows no loss, but count of final fraction shows loss - see Table V), and the low results are considered anomalies.

It should be noted that the ¹⁴C-CGA-279202 treated apple samples thawed during shipping from Switzerland (testing facility). These samples had been homogenized prior to shipment. The results for CGA-321113 after shipping are higher than the preliminary results reported from apples Metabolism study 95GN30⁴ (See Table VIII). These results do not reflect the values expected for an apple sample that has not been compromised. They are included to provide chromatograms for method evaluation, and to provide data on method precision only.

C. EXTRACTABILITY

Table V shows the extractability obtained from the analyses of ^{14}C -CGA-279202 treated samples from apple, cucumber, peanut, goat and poultry metabolism studies. These results are a demonstration of the method's ability to extract residues from weathered plant matrices. The extractability is determined by Liquid Scintillation Counting (LSC) of an aliquot of the extracted sample to obtain a disintegration per minute (dpm)/per mL value. Total radioactivity in the extract is calculated by multiplying the dpm/mL by the total volume of the extract. Percent extracted is determined by converting the total radioactivity to parts per million, and dividing this value by the total radioactive residue (TRR) of the sample, multiplied by 100. The calculation of the mean extractability of Peanut hay is provided as an example.

Three 10 gram sub-samples of peanut hay (treated by ^{14}C -CGA-279202 of a specific activity of 23.8 $\mu\text{Ci}/\text{mg}$) are extracted with a total volume of 200 mL extraction solvent, and an 0.5 mL aliquot is removed to a scintillation vial. Approximately 15 mL of scintillation cocktail is added, and the aliquot is assayed by LSC for radioactivity. For the first sub-sample, sample number 138, the aliquot gave a dpm value of 31,356. Parts per million in the extract for this sample is calculated as:

$$\frac{\text{DPM}(\text{alig})}{\text{mL}(\text{alig})} * \frac{\text{mL of Extract}}{\text{Sample Wt. (g)}} * \frac{(\text{mg } ^{14}\text{C - parent})}{(\mu\text{Ci})} * \frac{1 \mu\text{Ci}}{2.22 \times 10^6 \text{ dpm}} * \frac{1000 \mu\text{g}}{1 \text{ mg}}$$

$$= \frac{\mu\text{g}}{\text{g}} = \text{ppm in extract}$$

$$\frac{(31356)}{0.5 \text{ mL}} * \frac{200 \text{ mL}}{10 \text{ (g)}} * \frac{(1 \text{ mg } ^{14}\text{C - parent})}{23.8 \mu\text{Ci}} * \frac{1 \mu\text{Ci}}{2.22 \times 10^6 \text{ dpm}} * \frac{1000 \mu\text{g}}{1 \text{ mg}}$$

$$= 23.7 \frac{\mu\text{g}}{\text{g}} = 23.7 \text{ ppm in extract}$$

The TRR for peanut hay, as determined by oxidation (metabolism study 75-97), is 26.3 ppm. The extractability for sample 138 is 90% (23.7 ppm divided by 26.3 ppm, multiplied by 100). The other 2 sub-samples (139 and 140, giving aliquot dpm of 27882 and 26278, respectively) are calculated in the same manner. Extractibility for sub-samples 139 and 140 are 80% and 75.6%, respectively. The mean extractability is obtained by adding together the extractability results from the three sub-samples, and dividing by three, to obtain a mean extractability in peanut hay of 82%.

Results from the analysis of ^{14}C -CGA-279202 incurred samples to determine method extractability and accountability are summarized below. These results are compared to the preliminary data available from the metabolism studies.

SUBSTRATE	AVERAGE % EXTRACTED DURING VALIDATION OF AG-659A	% EXTRACTED DURING METABOLISM STUDIES (preliminary data)
Apples	99	100
Peanut Nutmeat	52	45
Peanut Hay	82	70
Cucumbers	120	100
Goat Meat	66	88
Goat Milk	90	92
Poultry Meat	80	75

The results from the validation of method AG-659 are in good agreement with the preliminary extractability reported from the metabolism studies. The largest deviation is the goat meat, which is 22% low in AG-659 compared to the metabolism study. The variability is likely due to the analysis of a different sub-samples of the goat meat than was analyzed in the metabolism study.

Extractability from a dry sample, which would be expected to be the most difficult extraction, is demonstrated from the analyses of peanut hay. Extractability of peanut hay from the three hay sub-samples analyzed ranged from 76% to 90%, with a mean extractability of 82%. Extractability from a primarily aqueous matrix is demonstrated by the

analysis of cucumbers. Extractability from the analyses of the three cucumber sub-samples ranged from 116% to 124%, with a mean extractability of 120%. Extractability from an oily matrix is demonstrated by the analysis of peanut nutmeat. Extractability from the analysis of the three nutmeat sub-samples analyzed ranged from 50% to 54%, with a mean extractability of 52%. Extractability in the apple samples ranged from 85% to 109%, with a mean extractability of 99%.

Meat samples of goat and poultry, and goat milk from animals dosed with ^{14}C -CGA-279202 were analyzed to determine the extractability from animal commodities. Results are included in Table V and Appendix II. Percent extractability ranged from 72% to 89% from poultry meat (mean = 80%), 60% to 71% in goat meat (mean = 66%), and 84% - 91% in goat milk (mean = 90%).

D. ACCOUNTABILITY

Table V shows the method accountability obtained from the analyses of ^{14}C -CGA-279202 treated samples from apple, cucumber, peanut, goat and poultry metabolism studies. Method accountability is calculated by comparing the total ppm obtained from GC analysis by Method AG-659 to the TRR of the initial sample. The results obtained by GC are corrected for recovery and converted to total parent equivalents (see Section II.J.1.0). The mean of the triplicate analyses is calculated and expressed as a percent of the TRR. In addition, aliquot portions of the final fractions obtained in Section II.C.5.0 were transferred for LSC to determine the ppm of the radioactive residue found in the final fraction. The results obtained from the LSC of the final fraction are converted to ppm using calculations similar to the extractability previously described.

The accountability results obtained when using method AG-659A are consistent with the results obtained in the metabolism studies. For the ^{14}C -CGA-279202 treated crop samples, mean % accountabilities of 0% in peanut nutmeat, 33% in peanut hay, 116% in cucumbers, and 111% in apples

were observed using method AG-659A. Parent CGA-279202 was the major compound detected, which is consistent with the metabolism results obtained for all crops except apples. As noted previously in section III.B. Precision, the apple samples thawed during shipping. High residues of CGA-321113 found in the apples during validation are attributed to breakdown occurring as the homogenized samples were thawed. For the ¹⁴C-CGA-279202 dosed animal samples, mean % accountabilities were 30% in poultry meat, 33% in goat meat, and 43% in goat milk. In the goat meat, CGA-321113 was the only residue detected, and CGA-279202 was less than the method LOQ. In the goat milk and the poultry meat, the parent CGA-279202 was the residue observed, and no metabolite above the method LOQ was detected. These results are also consistent with the metabolism studies. See Table VIII for a comparison of the results obtained in the metabolism and method validation studies.

Table VII shows the accountability obtained when the peanut hay samples are re-injected on the confirmatory method. The results are in good agreement with those obtained from the primary GC/NPD method.

IV. CONCLUSION

Method AG-659 is a valid and accurate method for the determination of CGA-321113 and CGA-279202 in crop and animal substrates. This conclusion is based on the accuracy, precision, extractability and accountability results presented in the validation study.

Precision and accuracy results from the analysis of control samples fortified with CGA-321113 and CGA-279202 are summarized below:

	CGA-321113			CGA-279202		
	Average % Recovery	Standard Deviation	%CV	Average % Recovery	Standard Deviation	%CV
All fortification (n=108)	89	14	16	95	12	12
Fortifications at method LOQ (n=49)	92	13	14	98	12	12


Method precision obtained from the analysis of the ^{14}C -CGA-279202 incurred samples from metabolism studies ranged from %CV of 4.7% in cucumbers to 53% in goat muscle. One of the 3 repetitions in the goat milk and goat muscle samples was unrealistically low due to unusual losses during the clean-up procedures. When the goat substrates are not considered, the %CV ranged from 4.7% to 17% (in apples).

Extractability and accountability results from the analysis of ^{14}C -CGA-279202 incurred samples during validation of this method were in agreement with the extractability achieved in the metabolism studies. Extractability from the analysis of the ^{14}C -CGA-279202 incurred samples using method AG-659A ranged from 52% in peanut nutmeat to >100% in cucumbers. Accountability results ranged from 0% in peanut nutmeat to >100% in apples and cucumbers.

A GC/MS method to confirm residues is included in this method. Confirmation is possible through the re-analysis of the final fractions using a different GC column (DB-1701 vs. The DBWAX) and analysis by a second detection method (MS vs. NPD). Structural confirmation is also possible by comparing the ratio of fragment ions obtained in the mass spectra from a suspected residue to those obtained from a standard. The confirmatory method was used successfully to re-analyze peanut hay samples as part of the method validation.

V. CERTIFICATION


The reports and experimental results included in this study, Laboratory Project ID AG-659, are certified to be authentic accounts of the experiments.



Daniel D. Campbell
Scientist I
Residue Chemistry
Human Safety Department

1-21-98

Date



Robert K. Williams,
Manager, Residue Chemistry
Agent of Sponsor

1/21/98

Date

Sponsor/submitter: Novartis Crop Protection
 Human Safety Department
 Residue Chemistry Laboratory
 Post Office Box 18300
 Greensboro, NC 27419

VI. TABLES AND FIGURES

TABLE I: GAS CHROMATOGRAPHY OPERATING PARAMETERS FOR THE
DETERMINATION OF CGA-321113 AND CGA-279202
RESIDUES

Instrument: Hewlett-Packard 5890 Series II with a 7673
Autoinjector and an NPD detector (or equivalent).

Column: DBWAX - 30 meters length, 0.32 mm Internal
Diameter, 0.25 mm film thickness, J&W Scientific
Part Number 123-7032.

Gas Flows: Measured at an oven temperature of 120°C:
Carrier (helium) - ~2 mL/minute
Air - ~100 mL/minute
Hydrogen - ~4mL/minute
Makeup - ~30 mL/minute
Purge Vent - ~3.6 mL/minute
Total Flow - 135-140 mL/minute

Injection: 2 µL injection volume

Temperatures: Oven Program: Initial Temperature = 120°C
Initial hold = 1 minute
Program Ramp Rate = 30°C/min.
Final temperature = 240°C
Final Hold Time = 15 minutes
(increase if late eluting peaks are
observed).

Injector: 170°C (splitless); a straight liner
was used.

Detector: 300°C

Purge Valve: On at 1 minute (off at start of run).

Run Time: 20 minutes (increase if late eluting peaks are
observed)

Retention Time: CGA-321113 = ~11 minutes
CGA-279202 = ~17 minutes
(these times will vary with changes in column
length, conditions, and carrier gas flow)

Instrument: Data Acquisition: Microvax II (Q) Operating System,
VMS Version 5.3-1 Application Software VG Multichrom Version 2.0
Worksheet Version: Ws.pas 1.3.1.

TABLE II: GC/MS OPERATING PARAMETERS FOR THE CONFIRMATION OF
CGA-321113 AND CGA-279202 RESIDUES

Instrument: Hewlett-Packard 5890 Series II with a HP 5971A MSD
and a HP 7673 injector.

Column: DB-1701 - 30 meters length, 0.25 mm Internal
Diameter, 0.25 mm film thickness, J&W Scientific
Part Number 122-0732.

Gas Flows: Similar to the GC/NPD method; changes made for
requirements of MSD detector.

Injection: 2 µL injection volume

Temperatures: Oven Program: Initial Temperature = 120°C
Initial hold = 1 minute
Program Ramp Rate = 30°C/min.
Final temperature = 240°C
Hold Time = 15 minutes
Final ramp at 30°C/min to 280°C
(hold 3 minutes).

Injector: 170°C

Run Time: 25 minutes

Retention Time: CGA-321113 = 8.4 minutes
(on DB-1701) CGA-279202 = 11.5 minutes

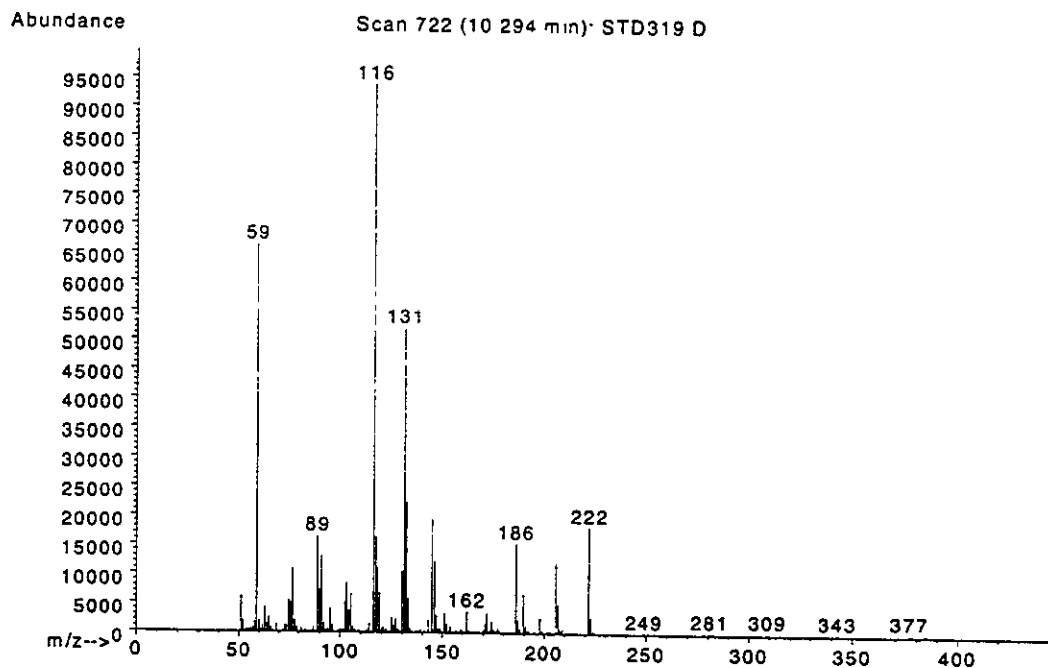
Monitoring Ions: CGA-321113 m:z = 116, 145
CGA-279202 m:z = 116, 131, 145

Ion Ratio example*:

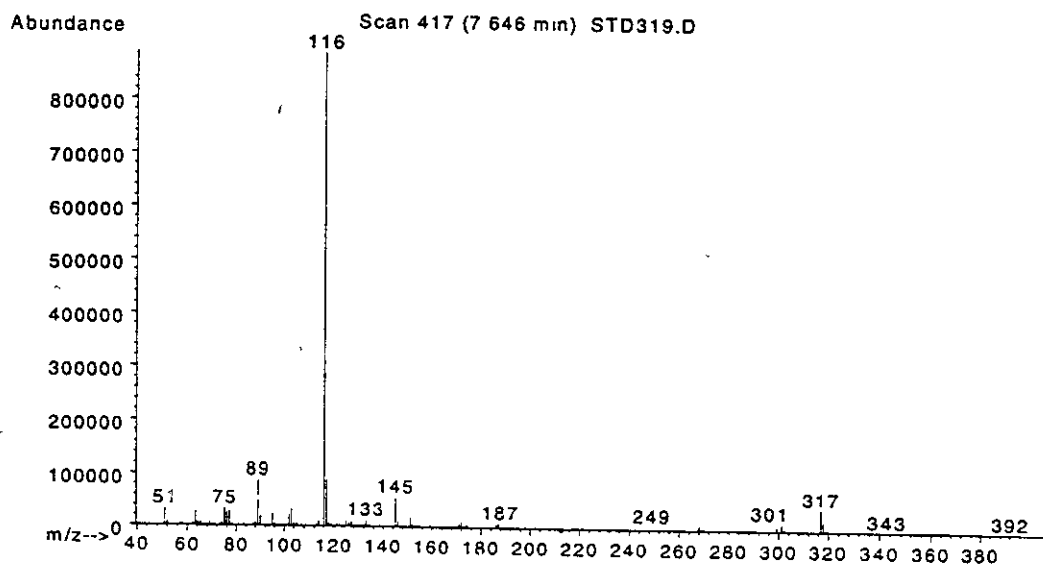
Compound	Ions Compared	Ratio (%)
CGA-321113	$[(m:z\ 145) \div (m:z\ 116)] \times 100$	6.6
CGA-279202	$[(m:z\ 145) \div (m:z\ 116)] \times 100$	68.7
	$[(m:z\ 131) \div (m:z\ 116)] \times 100$	25.9

* Obtained from injection of the 0.5 ng/µl standard during the
confirmatory analysis of peanut hay.

TABLE IIA: GC/MS OPERATING PARAMETERS FOR THE CONFIRMATION OF
CGA-321113 AND CGA-279202 RESIDUES; TYPICAL MASS
SPECTRA

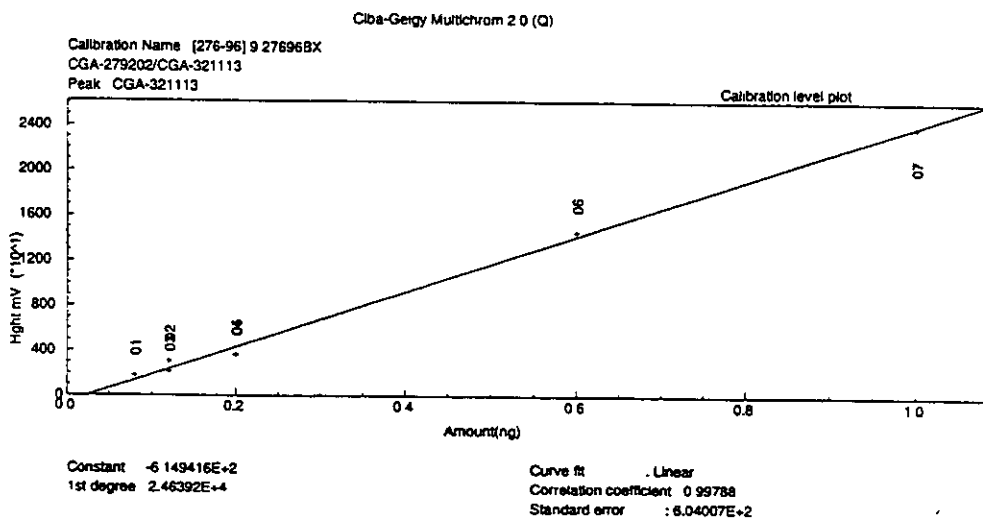


- 1) Mass Spectrum of CGA-279202 from a 20 ng standard injection (abundance threshold = 100)



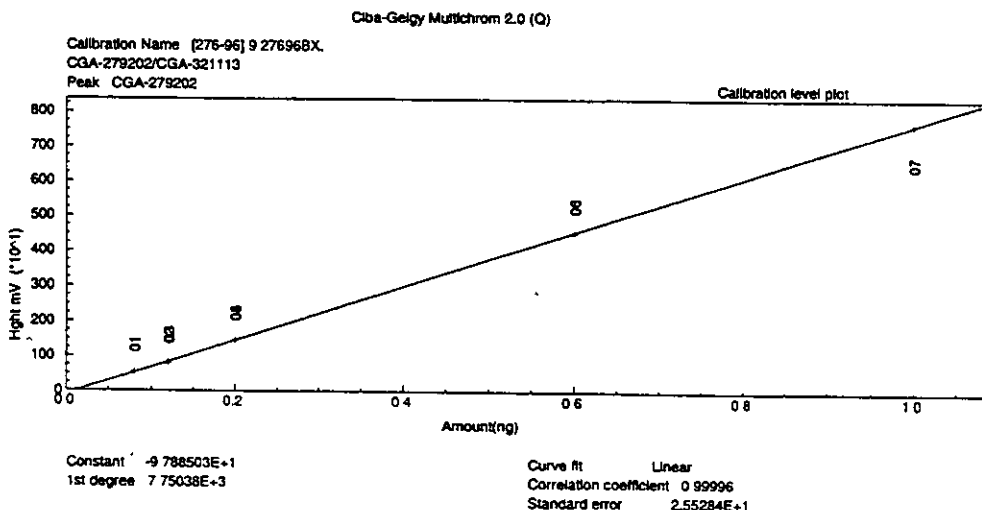
- 2) Mass Spectrum of CGA-321113 from a 20 ng standard injection (abundance threshold = 100)

TABLE III: TYPICAL STANDARDIZATION DATA AND CALIBRATION PLOTS
(FROM THE ANALYSIS OF APPLES)



Reported on 26-FEB-1997 at 13:52

- 1) Calibration plot from the analysis of CGA-321113 in apples. Standard peak heights (provided in order injected): 0.04 ng/uL = 1806 mV, 0.06 ng/uL = 3081 mV, 0.1 ng/uL = 3607 mV, 0.3 ng/uL = 14565 mV, 0.5 ng/uL = 23948 mV, 0.06 ng/uL = 2151 mV, 0.1 ng/uL = 3700 mV.



Reported on 26-FEB-1997 at 13:52

- 2) Calibration plot from the analysis of CGA-279202 in apples. Standard peak heights (provided in order injected): 0.04 ng/uL = 549 mV, 0.06 ng/uL = 790 mV, 0.1 ng/uL = 1469 mV, 0.3 ng/uL = 4535 mV, 0.5 ng/uL = 7661 mV, 0.06 ng/uL = 833 mV, 0.1 ng/uL = 1459 mV.

TABLE IV: RESULTS FROM THE ANALYSIS OF CONTROL AND FORTIFIED CONTROL SUBSTRATES

<u>SUBSTRATE</u>	<u>PROTOCOL SAMPLE #</u>	<u>RECOVERY LEVEL (ppm)</u>	<u>CGA-321113 RESULTS</u>		<u>CGA-279202 RESULTS</u>	
			<u>(ppm)</u>	<u>% REC.</u>	<u>(ppm)</u>	<u>% REC.</u>
<u>COW</u>						
Tenderloin	1	Control	<0.02		<0.02	
	2	0.02	0.02	113	0.02	119
	3	0.02	0.02	106	0.02	105
	4	0.10	0.08	84	0.09	85
	5	0.10	0.10	<u>98</u>	0.09	<u>91</u>
			% MEAN:	100	% MEAN:	100
Omental Fat	6R	Reagent Blank	<0.02		<0.02	
	7R	Control	<0.02		<0.02	
	9R	0.02	0.02	93	0.02	89
	10R	0.02	0.02	101	0.02	93
	11R	0.20	0.18	91	0.17	86
	12R	0.20	<u>0.14</u>	<u>69</u>	<u>0.12</u>	<u>58</u>
		% MEAN:	88	% MEAN:	81	
Liver	13R	Control	<0.02		<0.02	
	14R	0.02	0.02	86	0.02	75
	15R	0.02	0.02	84	0.02	74
	16R	0.50	0.46	91	0.43	85
	17R	0.50	0.34	<u>67</u>	0.31	<u>63</u>
		% MEAN:	82	% MEAN:	74	
Kidney	18	Control	<0.02		<0.02	
	19	0.02	0.02	82	0.02	103
	20	0.02	0.02	81	0.02	90
	21	1.0	0.70	70	0.89	89
	22	1.0	<u>0.70</u>	<u>70</u>	<u>0.90</u>	<u>90</u>
		% MEAN:	76	% MEAN:	93	
Milk	23	Reagent Blank	<0.01		<0.01	
	24	Control	<0.01		<0.01	
	25	0.01	0.01	95	0.01	102
	26	0.01	0.01	109	0.01	117
	27	0.10	0.08	79	0.11	109
	28	0.10	<u>0.08</u>	<u>76</u>	<u>0.11</u>	<u>107</u>
		% MEAN:	90	% MEAN:	109	

TABLE IV: RESULTS FROM THE ANALYSIS OF CONTROL AND FORTIFIED CONTROL SUBSTRATES (Continued)

SUBSTRATE	PROTOCOL SAMPLE #	RECOVERY LEVEL (ppm)	CGA-321113 RESULTS		CGA-279202 RESULTS	
			(ppm)	% REC.	(ppm)	% REC.
CHICKEN						
Fat	71	Control	<0.02		<0.02	
	72	0.02	0.02	107	0.02	109
	73	0.02	0.02	88	0.02	84
	74	1.0	0.83	83	0.99	99
	75	1.0	0.75	75	0.89	89
			% MEAN:	88	% MEAN:	95
Liver	76	Control	<0.02		<0.02	
	77	Control	<0.02		<0.02	
	78	0.02	0.02	108	0.02	93
	79	0.02	0.02	117	0.02	97
	80	0.10	0.13	129	0.11	106
	81	0.10	0.13	127	0.11	108
			% MEAN:	120	% MEAN:	101
Eggs	82	Control	<0.02		<0.02	
	83	Control	<0.02		<0.02	
	84	0.02	0.02	110	0.03	125
	85	0.02	0.02	98	0.02	110
	86	0.02	0.02	96	0.02	98
	87	0.10	0.09	87	0.11	110
	88	0.10	0.09	86	0.10	99
	89	1.0	0.83	83	1.1	106
	90	1.0	0.84	84	1.0	101
	91	1.0	0.76	76	0.97	97
			% MEAN:	90	% MEAN:	106
Lean Meat	A2-1	Control	<0.02		<0.02	
	A2-2	0.02	0.014	68	0.02	92
	A2-3	0.05	0.04	72	0.04	82
			% MEAN:	70	% MEAN:	87
GOAT						
Meat	A2-7	Control	<0.02		<0.02	
	A2-8	0.02	0.02	82	0.02	100
Milk	A2-13	Control	<0.02		<0.02	
	A2-14	0.01	0.009	93	0.01	131
	A2-15	0.05	0.03	58	0.05	91
			% MEAN:	75	% MEAN:	111

TABLE IV: RESULTS FROM THE ANALYSIS OF CONTROL AND FORTIFIED CONTROL SUBSTRATES (Continued)

SUBSTRATE	PROTOCOL SAMPLE #	RECOVERY LEVEL (ppm)	CGA-321113 RESULTS		CGA-279202 RESULTS	
			(ppm)	% REC.	(ppm)	% REC.
<u>GRAPES</u>						
Fruit	92	Control	<0.02		<0.02	
	93	0.02	0.02	92	0.02	94
	94	0.02	0.02	116	0.02	98
	95	0.10	0.09	86	0.09	88
	96	0.10	0.09	<u>86</u>	0.09	<u>88</u>
		% MEAN:	95	% MEAN:	92	
Raisins	97	Control	<0.02		<0.02	
	98	0.02	0.02	85	0.02	95
	99	0.02	0.02	85	0.02	84
	100	0.20	0.14	69	0.15	76
	101	0.20	0.18	<u>89</u>	0.20	<u>98</u>
		% MEAN:	82	% MEAN:	88	
Juice	102	Control	<0.02		<0.02	
	103	0.02	0.01	68	0.02	95
	104	0.02	0.02	75	0.02	102
	105	0.50	0.35	71	0.47	94
	106	0.50	0.35	<u>70</u>	0.44	<u>88</u>
		% MEAN:	71	% MEAN:	95	
<u>APPLES</u>						
Fruit	107	Control	<0.02		<0.02	
	108	0.02	0.02	84	0.02	105
	109	0.02	0.01	62	0.01	67
	110	1.0	<u>0.92</u>	<u>92</u>	<u>1.1</u>	<u>108</u>
		% MEAN:	79	% MEAN:	93	
Wet Pomace	114	Control	<0.02		<0.02	
	115	0.02	0.01	72	0.02	101
	116	0.02	0.01	71	0.02	108
	117	0.05	0.03	67	0.05	104
	118	0.20	0.16	79	0.19	94
	119	0.20	0.16	81	0.19	94
	120	0.20	0.16	<u>81</u>	0.19	<u>94</u>
		% MEAN:	75	% MEAN:	99	
Juice	121	Reagent Blank	<0.02		<0.02	
	122	Control	<0.02		<0.02	
	123	0.02	0.02	87	0.02	97
	124	0.02	0.02	98	0.02	110
	125	0.10	0.10	99	0.10	101
	126	0.10	0.09	<u>88</u>	0.10	<u>97</u>
			% MEAN:	93	% MEAN:	101

TABLE IV: RESULTS FROM THE ANALYSIS OF CONTROL AND FORTIFIED CONTROL SUBSTRATES (Continued)

	PROTOCOL	RECOVERY	CGA-321113 RESULTS		CGA-279202 RESULTS		
SUBSTRATE	SAMPLE #	LEVEL (ppm)	(ppm)	% REC.	(ppm)	% REC.	
PEANUTS							
Nutmeat	127	Control	<0.02		<0.02		
	128	0.02	0.02	98	0.02	107	
	129	0.02	0.02	110	0.02	106	
	130	0.30	<u>0.29</u>	<u>98</u>	<u>0.33</u>	<u>109</u>	
			% MEAN:	102	% MEAN:	107	
Hay	134	Control	<0.05		<0.05		
	134a	Control	<0.05		<0.05		
	136a	0.05	0.05	106	0.05	99	
	136b	0.05	0.05	90	0.05	81	
	137	10.0	<u>9.7</u>	<u>97</u>	<u>8.4</u>	<u>84</u>	
			% MEAN:	98	% MEAN:	88	
Refined Oil	141	Reagent Blank	<0.02		<0.02		
	142	Control	<0.02		<0.02		
	143	0.02	0.02	90	0.02	83	
	144	**	**	**	**	**	
	145	1.0	0.71	71	0.78	78	
	146	1.0	0.67	67	0.78	78	
	141r	Reagent Blank	<0.02		<0.02		
	142r	Control	<0.02		<0.02		
	143r	0.02	0.02	91	0.02	98	
	144r	0.02	0.02	85	0.02	92	
	145r	1.0	0.87	87	0.89	89	
	146r	1.0	0.86	<u>86</u>	0.87	<u>87</u>	
			% MEAN:	82	% MEAN:	86	
	POTATOES						
	Tubers	147	Control	<0.02		<0.02	
148		Control	<0.02		<0.02		
149		0.02	0.02	83	0.02	96	
150		0.02	0.02	87	0.02	94	
151		0.02	0.02	91	0.02	105	
152		0.10	0.08	81	0.09	88	
153		0.10	0.09	86	0.09	90	
154		0.50	0.44	87	0.49	99	
155		0.50	0.42	83	0.46	92	
156		0.50	0.45	<u>89</u>	0.52	<u>105</u>	
			% MEAN:	86	% MEAN:	96	
Granules		157	Control	<0.02		<0.02	
		158	0.02	**	**	**	**
	159	0.02	0.020	90	0.02	99	
	160	0.50	0.42	84	0.47	94	
	161	0.50	<u>0.46</u>	<u>92</u>	<u>0.51</u>	<u>102</u>	
			% MEAN:	89	% MEAN:	98	

TABLE IV: RESULTS FROM THE ANALYSIS OF CONTROL AND FORTIFIED CONTROL SUBSTRATES (Continued)

<u>SUBSTRATE</u>	<u>PROTOCOL SAMPLE #</u>	<u>RECOVERY LEVEL (ppm)</u>	<u>CGA-321113 RESULTS</u>		<u>CGA-279202 RESULTS</u>	
			<u>(ppm)</u>	<u>% REC.</u>	<u>(ppm)</u>	<u>% REC.</u>
Wet Peel	162	Control	<0.02		<0.02	
	163	0.02	0.02	97	0.02	101
	164	0.02	0.02	83	0.02	93
	165	0.20	0.17	87	0.19	96
	166	0.20	<u>0.16</u>	<u>79</u>	<u>0.18</u>	<u>89</u>
			% MEAN:	86	% MEAN:	95
<u>CUCURBITS</u>						
Cantaloupe	167	Control	<0.02		<0.02	
	168	Control	<0.02		<0.02	
	169	0.02	0.02	99	0.02	100
	170	0.02	0.02	97	0.02	88
	171	0.02	0.02	90	0.02	84
	172	0.05	0.06	114	0.06	111
	173	0.05	0.05	105	0.05	104
	174	0.50	0.48	95	0.47	94
	175	0.50	0.50	100	0.49	99
	176	0.50	<u>0.53</u>	<u>107</u>	<u>0.51</u>	<u>103</u>
			% MEAN:	101	% MEAN:	98
Cucumbers	177	Control	<0.02		<0.02	
	178	0.02	0.02	84	0.02	88
	179	0.02	**	**	**	**
	180	0.10	0.10	96	0.09	86
	181	0.10	0.09	90	0.09	86
Cucumbers	182R	Control	<0.02		<0.02	
	183R	0.02	0.02	110	0.02	96
	184R	0.02	0.02	109	0.02	100
	185R	0.50	<u>0.48</u>	<u>97</u>	<u>0.41</u>	<u>83</u>
			% MEAN:	98	% MEAN:	90

** Samples 144, 158, and 179 did not have valid results due to errors during the samples cleanup or injection steps.

TABLE IVA: STATISTICAL RESULTS FROM THE ANALYSIS OF FORTIFIED CONTROLS BASED UPON PPM LEVEL

Fortification Level (ppm)	Number of Analyses	<u>CGA-321113</u>			<u>CGA-279202</u>		
		Average % Recovery	SD	%CV	Average % Recovery	SD	%CV
0.01 - 0.02	49	92	13	14	98	12	12
0.05	7	87	20	23	96	11	11
0.1	16	92	15	16	96	9	9
0.2 - 0.03	10	82	9	11	89	13	15
0.5	13	87	12	13	92	11	12
1.0 - 10.0	13	80	9	11	92	9	10

TABLE V: RESULTS FROM THE ANALYSIS OF ^{14}C -CGA-279202
TREATED SAMPLES

SAMPLE INFORMATION			EXTRACTABILITY		ACCOUNTABILITY					
Substrate and Sample Code	Sample No	(1) TRR	(2)	(3)	LSC Analysis Final Fraction ¹⁴ C PPM (4)	GC Analysis				(8) Mean % Account- ability
			Extractable PPM	% Extractability		(5) Final Fraction Analyte PPM	(6) Final Fraction Corrected	(7) Total PPM (CGA-279202 Equivalents)		
Apple Fruit ^a	111	0.95	1.04	109	0.86	CGA-321113	0.16	0.20	111	
	112	0.95	0.97	102	0.82	CGA-279202	0.88	0.94		
						CGA-321113	0.16	0.20		
						CGA-279202	0.89	0.96		
	113	0.95	0.81	85	0.70	CGA-321113	0.13	0.16		
CGA-279202						0.63	0.68			
		MEAN % = 99		MEAN= 0.79						
Peanut Nutmeat	131	0.27	0.15	53	0.017	CGA-321113	<0.02	--	0	
	132	0.27	0.15	54	0.016	CGA-279202	<0.02	--		
						CGA-321113	<0.02	--		
						CGA-279202	<0.02	--		
	133	0.27	0.14	50	0.011	CGA-321113	<0.02	--		
CGA-279202						<0.02	--			
		MEAN % = 52		MEAN= 0.015						
Peanut Hay	138	26	24	90	9.8	CGA-321113	1.31	1.34	33	
	139	26	21	80	9.3	CGA-279202	7.00	7.98		
						CGA-321113	1.03	1.06		
						CGA-279202	7.00	7.98		
	140	26	20	76	9.1	CGA-321113	1.05	1.08		
CGA-279202						5.57	6.36			
		MEAN % = 82		MEAN= 9.4						
Cucumbers ^b	186R	0.44	0.52	119	0.36	CGA-321113	0.017	0.017	116	
	187R	0.44	0.51	116	0.36	CGA-279202	0.47	0.50		
						CGA-321113	0.019	0.019		
						CGA-279202	0.47	0.51		
	188R	0.44	0.54	124	0.35	CGA-321113	0.017	0.017		
CGA-279202						0.43	0.46			
		MEAN % = 120		MEAN= 0.36						

- (1) Total radioactive residue, as determined by combustion analysis of samples
(2) Determined by liquid scintillation counting of aliquots of the extract.
(3) Mean % is average of the three extractions shown (% Extractability) divided by the Total Radioactive Residue, multiplied by 100.
(4) Determined by liquid scintillation counting of aliquots of the final fractions
(5) As determined by GC analysis using method AG-659
(6) Corrected by mean % recovery from fortified controls, as shown in Table IV of AG-659.
(7) Sum of the ppm value of the two analytes after converting the CGA-321113 value into CGA-279202 equivalents (as per section II J.1.0 of AG-659)
(8) Average of the total ppm (as CGA-279202 equivalents) of the three analyses, divided by the Total Radioactive Residue, multiplied by 100

- ^a Apple samples thawed during shipment, and the ppm of CGA-321113 does not mimic that found in metabolism study 95GN30. Results are reported here as a measure of method performance only, and do not reflect the ppm obtained from an uncompromised sample.
^b Results for CGA-321113 are slightly below the LOQ of 0.02 ppm, and would be reported as <0.02 ppm. Values are included here as a measure of method accuracy only.

TABLE V: RESULTS FROM THE ANALYSIS OF ¹⁴C-CGA-279202
TREATED SAMPLES (Continued)

SAMPLE INFORMATION			EXTRACTABILITY		ACCOUNTABILITY				
Substrate and Sample Code	(1) Total Sample Radioactive No Residue		(2) Extractable PPM	(3) % Extractability	LSC Analysis Final Fraction ¹⁴ C PPM (4)	GC Analysis			
						(5) Final Fraction Analyte PPM	(6) Final Fraction Corrected	(7) Total PPM (CGA-279202 Equivalents)	(8) Mean % Account- ability
Poultry Meat	A2-4	0.27	0.24	90	0.05	CGA-321113 <0.02	--	0.09	30
						CGA-279202 0.08	0.09		
	A2-5	0.27	0.19	72	0.05	CGA-321113 <0.02	--		
						CGA-279202 0.06	0.07		
	A2-6	0.27	0.21	78	0.05	CGA-321113 <0.02	--		
						CGA-279202 0.07	0.08	0.08	
			MEAN % = 80		MEAN= 0.05				
Goat Muscle	A2-10	0.07	0.05	71	0.017	CGA-321113 0.03	0.03	0.03	33
						CGA-279202 <0.02	--		
	A2-11	0.07	0.04	65	0.017	CGA-321113 0.02	0.03		
						CGA-279202 <0.02	--		
	A2-12	0.07	0.04	60	0.005	CGA-321113 (9) 0.007	0.009		
						CGA-279202 <0.02	--	0.009	
			MEAN % = 66		MEAN= 0.013				
Goat Milk	A2-16	0.08	0.08	94	0.04	CGA-321113 <0.01	--	0.04	43
						CGA-279202 0.04	0.04		
	A2-17	0.08	0.07	84	0.04	CGA-321113 <0.01	--		
						CGA-279202 0.04	0.04		
	A2-18	0.08	0.07	91	0.02	CGA-321113 <0.01	--		
						CGA-279202 0.02	0.02	0.02	
			MEAN % = 90		MEAN= 0.03				

- (1) As determined by combustion analysis as part of the metabolism studies.
(2) Determined by liquid scintillation counting of aliquots of the extract.
(3) Average of the three extractions shown (Extractable PPM) divided by the Total Radioactive Residue, multiplied by 100.
(4) Determined by liquid scintillation counting of aliquots of the final fractions.
(5) As determined by GC analysis using method AG-659.
(6) Corrected by mean % recovery from fortified controls as shown in Table A2-1 of AG-659, Appendix 2.
(7) Sum of the ppm value of the two analytes after converting the CGA-321113 value into CGA-279202 equivalents, if found (as per section II.J.1.0 of AG-659).
(8) Average of the total ppm (as CGA-279202 equivalents) of the three analyses, divided by the Total Radioactive Residue, multiplied by 100.
(9) Results for CGA-321113 are slightly below the LOQ of 0.02 ppm, and would be reported as <0.02 ppm. Values are included here as a measure of method accuracy only

TABLE VI: PRECISION OF ANALYTICAL METHOD FROM THE ANALYSIS OF ^{14}C -CGA-279202 TREATED SAMPLES

Substrate and Sample Numeric ID	Metabolism Reference (Study #/Numeric ID)	276-96 Sample Number	GC Results		Total ppm (as CGA-279202 equivalents)
			CGA-321113	CGA-279202	
Apple Fruit 174242	Study #95GN30	111	0.20	0.94	1.1
		112	0.20	0.96	1.2
		113	<u>0.16</u>	<u>0.68</u>	<u>0.84</u>
		Mean:	0.19	0.86	1.05
		Range:	0.16 - 0.20	0.68 - 0.96	0.84 - 1.2
		Standard Deviation:	0.02	0.16	0.18
		CV (%):	12	18	17

NOTE - Values for CGA-321113 apples are suspect due to thawing of samples during international shipment. These values included here to demonstrate method precision only.

Peanut Nutmeat 174240	Study 75-95 Sample # 129651	131	<0.02	<0.02	<0.02
		132	<0.02	<0.02	<0.02
		133	<u><0.02</u>	<u><0.02</u>	<u><0.02</u>
		Mean:	<0.02	<0.02	<0.02
		Range:	na	na	na
		Standard Deviation:	na	na	na
		CV (%):	na	na	na
Peanut Hay 174241	Study 75-95 Sample # 129649	138	1.3	8.0	9.4
		139	1.1	8.0	9.1
		140	<u>1.1</u>	<u>6.4</u>	<u>7.5</u>
		Mean:	1.2	7.44	8.6
		Range:	1.1 - 1.3	6.4 - 8.0	7.5 - 9.4
		Standard Deviation:	0.16	0.94	1.0
		CV (%):	14	13	12
Cucumbers 177053	Study 96JS23	186R	0.017	0.50	0.52
		187R	0.019	0.51	0.52
		188R	<u>0.017</u>	<u>0.46</u>	<u>0.48</u>
		Mean:	0.018	0.49	0.51
		Range:	0.017 - 0.019	0.46 - 0.51	0.48 - 0.52
		Standard Deviation:	0.0012	0.02	0.02
		CV (%):	6.5	4.8	4.7

NOTE - Values for CGA-321113 cucumbers would be reported as <LOQ of 0.02 ppm. These values included here to demonstrate method precision only.
na = not applicable

TABLE VI: PRECISION OF ANALYTICAL METHOD FROM THE ANALYSIS OF ¹⁴C-CGA-279202 TREATED SAMPLES (Continued)

Substrate and Sample Numeric ID	Metabolism Reference (Study #/Numeric ID)	276-96 Sample Number	GC Results		Total ppm (as CGA-279202 equivalents)
			CGA-321113	CGA-279202	
Poultry Lean Meat 210164	Study 021AM04	A2-4	<0.02	0.09	0.09
		A2-5	<0.02	0.07	0.07
		A2-6	<u><0.02</u>	<u>0.08</u>	<u>0.08</u>
		Mean:	<0.02	0.08	0.08
		Range:	na	0.07 - 0.09	0.07 - 0.09
		Standard Deviation:	na	0.01	0.01
		CV (%):	na	13	13
Goat Muscle 210162	Study 021AM03	A2-10	0.03	<0.02	0.03
		A2-11	0.03	<0.02	0.03
		A2-12	<u>0.01</u>	<u><0.02</u>	<u>0.01</u>
		Mean:	0.02	<0.02	0.02
		Range:	0.01 - 0.03	na	0.01 - 0.03
		Standard Deviation:	0.01	na	0.01
		CV (%):	53	na	53
		Note - value for sample A2-12 would be reported as <LOQ of 0.02 ppm. These values included here to demonstrate method precision only			
Goat Milk 210163	Study 021AM03	A2-16	<0.01	0.04	0.04
		A2-17	<0.01	0.04	0.04
		A2-18	<u><0.01</u>	<u>0.02</u>	<u>0.02</u>
		Mean:	<0.01	0.04	0.04
		Range:	na	0.02 - 0.04	0.02 - 0.04
		Standard Deviation:	na	0.01	0.01
			CV (%):	35	35

na = not applicable

TABLE VII: RESULTS FROM THE ANALYSIS OF PEANUT HAY BY THE GC/MS CONFIRMATORY METHOD

Results From GC/MS Analysis of the Control and Fortified Control Peanut Hay Samples Using the Target Ion (m:z = 116):

PROTOCOL 276-96 SAMPLE #	FORTIFICATION (ppm)	DETERMINED CGA-321113 (ppm)	% RECOVERY	DETERMINED CGA-279202 (ppm)	% RECOVERY
134	control	<0.05	--	<0.05	--
134A	control	<0.05	--	<0.05	--
136A	0.05	0.035	69	0.037	73
136B	0.05	0.032	64	0.033	67
137	10.0	8.5	85	8.0	80
		% mean:	73	% mean:	73

Results From GC/MS Analysis of ¹⁴C-CGA-279202 Incurred Crop Metabolism Samples:

Ion(s) Used For Quantitation	PROTOCOL 276-96 SAMPLE #	Analyte	(1) PPM by GC/MS	(2) Corrected PPM	(3) Total ppm as CGA-279202 equivalents	(4) Mean % accountability
m:z = 116	138	CGA-321113	1.0	1.4	10.9	40%
		CGA-279202	7.0	9.5		
	139	CGA-321113	0.88	1.2	10.2	
		CGA-279202	6.6	8.9		
	140	CGA-321113	0.85	1.2	10.6	
		CGA-279202	6.9	9.4		

- (1) Analyzed by GC/MS method described in Table II.
- (2) Corrected by mean % recovery from fortified controls, as shown above.
- (3) Sum of the PPM value of the two analyses after converting the CGA-321113 result into CGA-279202 equivalents (as per Section II.J.1.0 of AG-659).
- (4) Average of the total ppm (as CGA-279202 equivalents) of the three analyses, divided by the Total Radioactive Residue (26.3 ppm), multiplied by 100.

TABLE VIII: COMPARISON OF RESULTS OBTAINED FROM ANALYSIS OF ^{14}C -CGA-279202 INCURRED SAMPLES BY AG-659 TO RESULTS FROM METABOLISM STUDIES

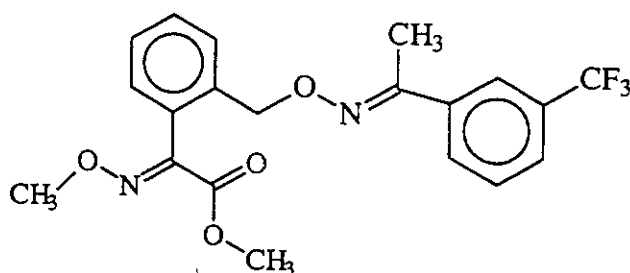
Description of Metabolism Samples	Metabolism Results ¹				AG-659 Results ²			
	TRR ³	ppm Parent	ppm Metabolite	% Account-ability	TRR ³	ppm Parent	ppm Metabolite	% Account-ability
<u>Apples:</u> Study 95GN30, Glyoxyl-phenyl-(U) label	1.3	1.07	0.006	83%	0.95	0.85	0.19	111%
<u>Cucumbers:</u> Study 96JS23, Glyoxyl-phenyl-(U) label	0.3	0.24	0.01	80%	0.44	0.49	0.018	116%
<u>Peanut Hay:</u> Study 75-95, Glyoxyl-phenyl-(U) label	26	8.6	1.4	38%	26	7.4	1.2	33%
<u>Peanut Nutmeat:</u> Study 75-95, Glyoxyl-phenyl-(U) label	0.27	<0.02	<0.02	0%	0.27	<0.02	<0.02	0%
<u>Goat Meat</u> Study 021AM03; Trifluomethyl-phenyl-(U) label	0.06	<0.02	0.03	57% (CGA-321113 only)	0.07	<0.02	0.02	33%
<u>Goat Milk</u> Study 021AM03; Trifluomethyl-phenyl-(U) label	0.09	0.04	<0.01	52% (CGA-279202 only)	0.08	0.04	<0.01	43%
<u>Poultry Meat</u> Study 021AM04; Trifluomethyl-phenyl-(U) label	0.21	0.06	<0.02	29% (CGA-279202 only)	0.27	0.08	<0.02	30%

¹ Metabolism studies were in progress at the time of issue of this method, and the results reported in this table are preliminary and are not meant to represent final values.

² Results from AG-659 were determined by GC analysis, and are corrected for recovery. The numbers reported in this table are the average of the triplicate analyses from each substrate. Individual results are reported in Table V in the column titled "Final Fraction Corrected".

³ TRR = Total Radioactive Residue as determined by combustion analysis. All values were determined as part of metabolism studies, except the TRR reported under AG-659 results for apples and cucumbers, which are new combustion analyses of the sub-samples analyzed during the method validation.

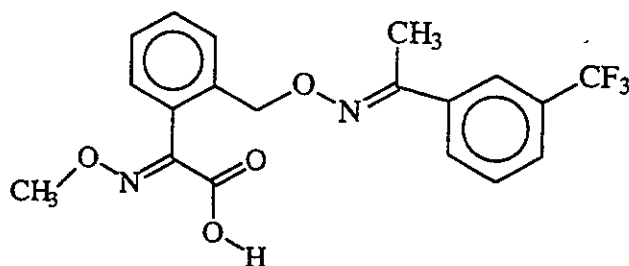
FIGURE 1. STRUCTURES AND CHEMICALS NAMES



CGA-279202

Benzeneacetic acid, .alpha.-(methoxyimino)-2-[[[1-[3-trifluoromethyl)phenyl]ethylidene]amino]oxy]methyl]-, methyl ester, (E,E)-

Molecular Weight = 408



CGA-321113

Benzeneacetic acid, .alpha.-(methoxyimino)-2-[[[1-[3-trifluoromethyl)phenyl]ethylidene]amino]oxy]methyl]-, (E,E)-

Molecular Weight = 394

FIGURE 2. ANALYTICAL PROCEDURE FLOWCHART FOR AG-659

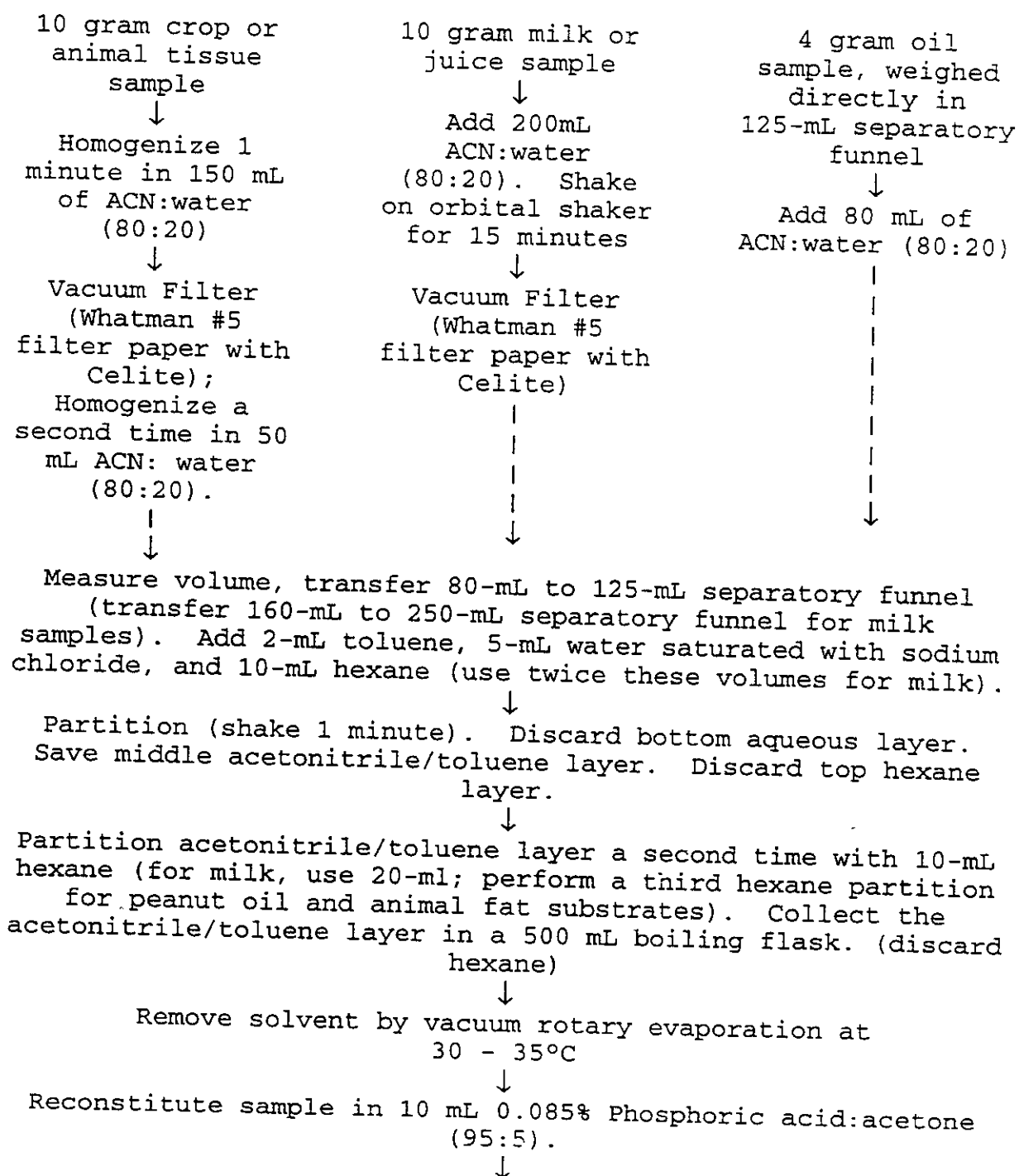


FIGURE 2. ANALYTICAL PROCEDURE FLOWCHART FOR AG-659
(Continued)

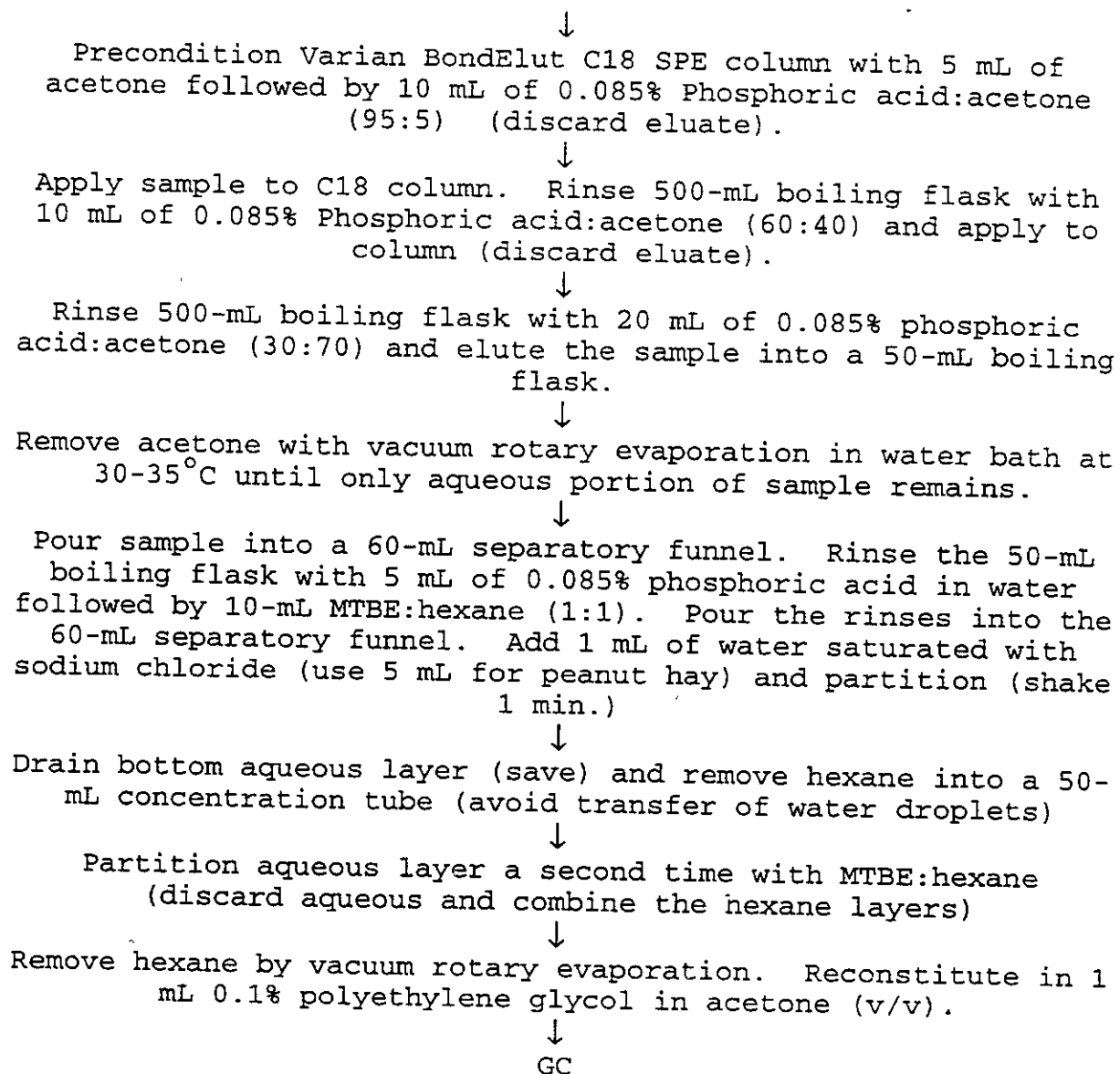
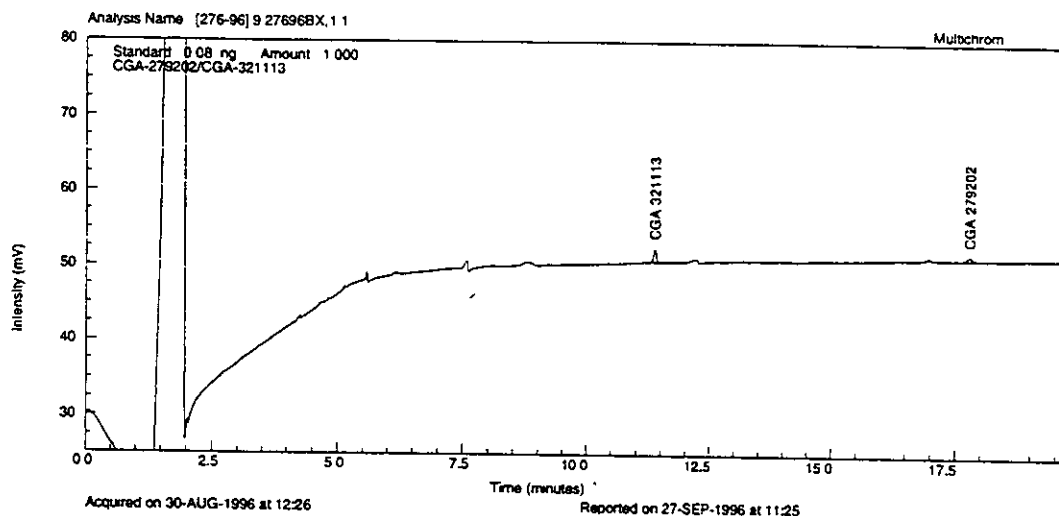
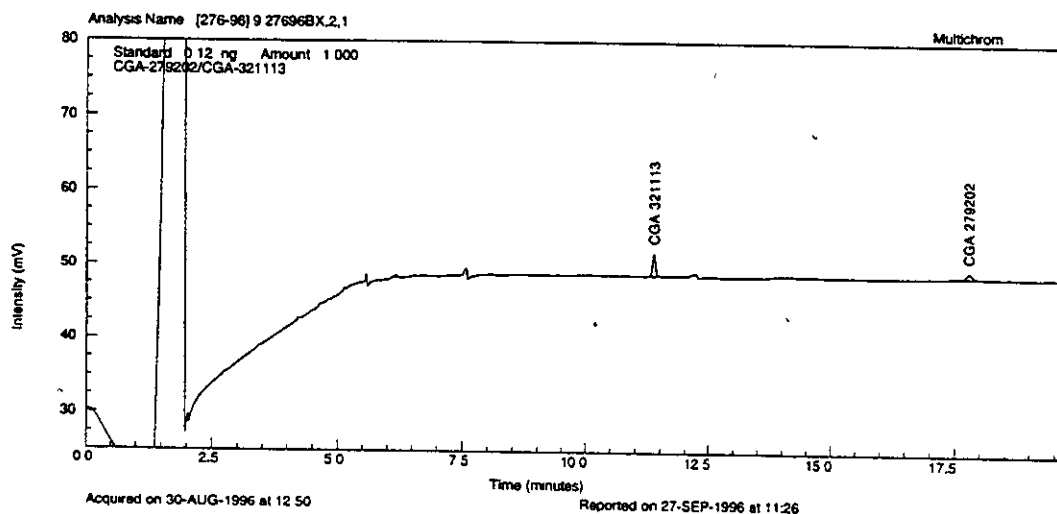


FIGURE 3: REPRESENTATIVE STANDARD CHROMATOGRAMS
(FROM THE ANALYSIS OF APPLES)

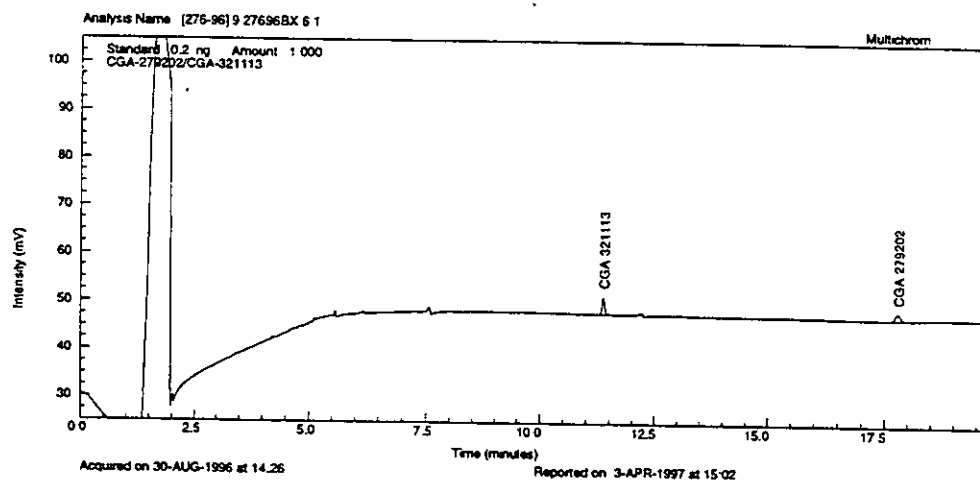


1) 0.04 ng/ μ L standard; 0.08 ng injected

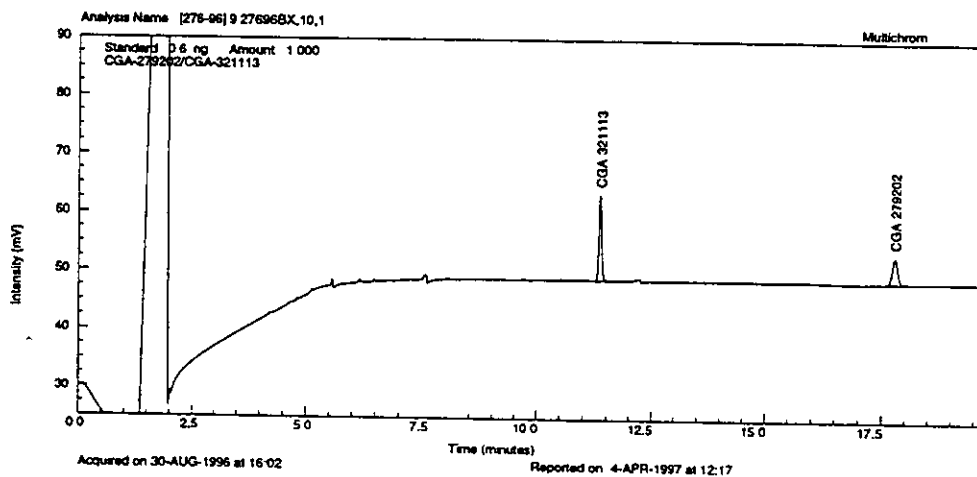


2) 0.06 ng/ μ L standard; 0.12 ng injected

FIGURE 3: REPRESENTATIVE STANDARD CHROMATOGRAMS
(FROM THE ANALYSIS OF APPLES) (Continued)

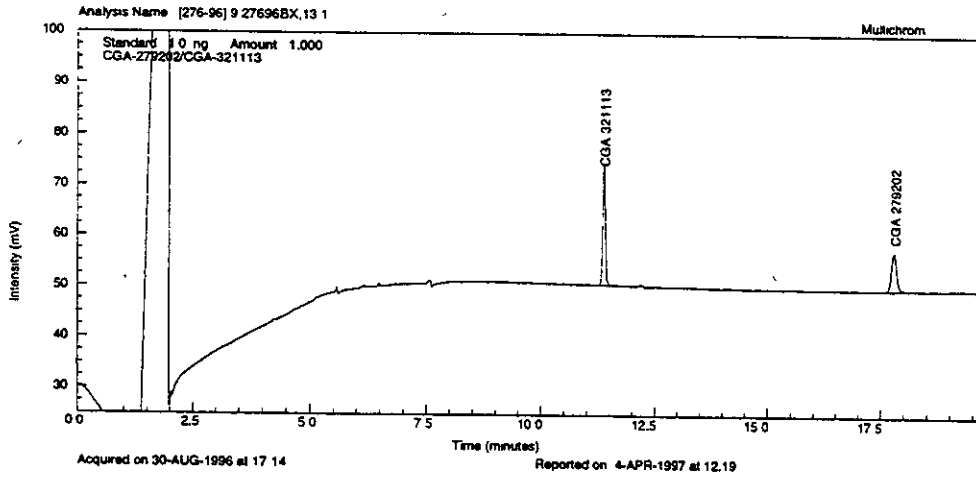


3) 0.1 ng/uL standard, 0.2 ng injected.

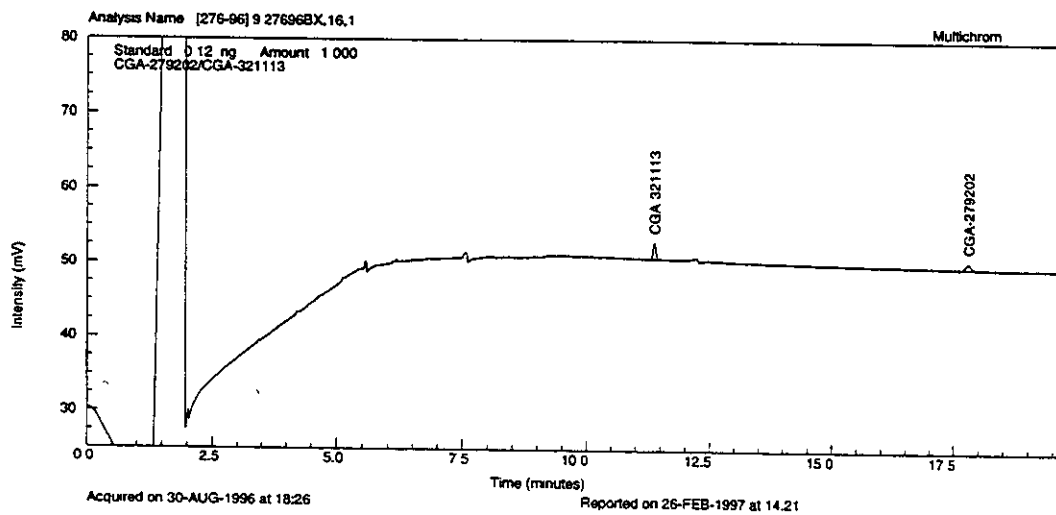


4) 0.3 ng/uL standard: 0.6 ng injected

FIGURE 3: REPRESENTATIVE STANDARD CHROMATOGRAMS
(FROM THE ANALYSIS OF APPLES) (Continued)

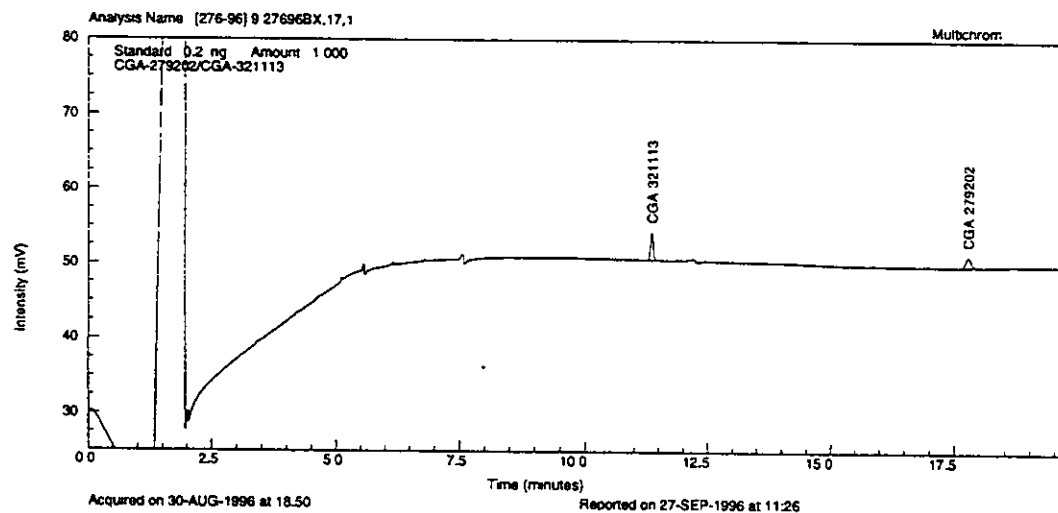


5) 0.5 ng/ μ L standard; 1.0 ng injected



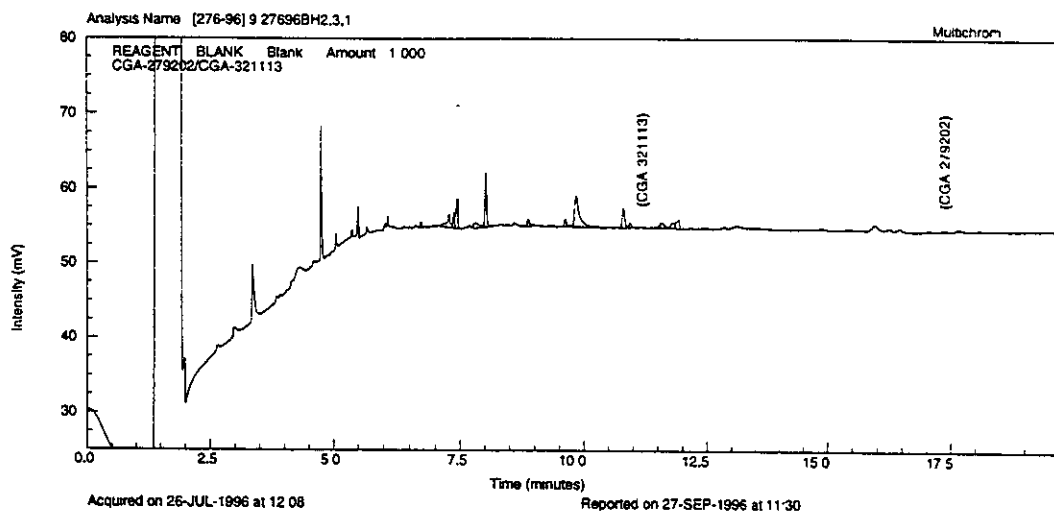
6) 0.06 ng/ μ L standard; 0.12 ng injected

FIGURE 3: REPRESENTATIVE STANDARD CHROMATOGRAMS
(FROM THE ANALYSIS OF APPLES) (Continued)

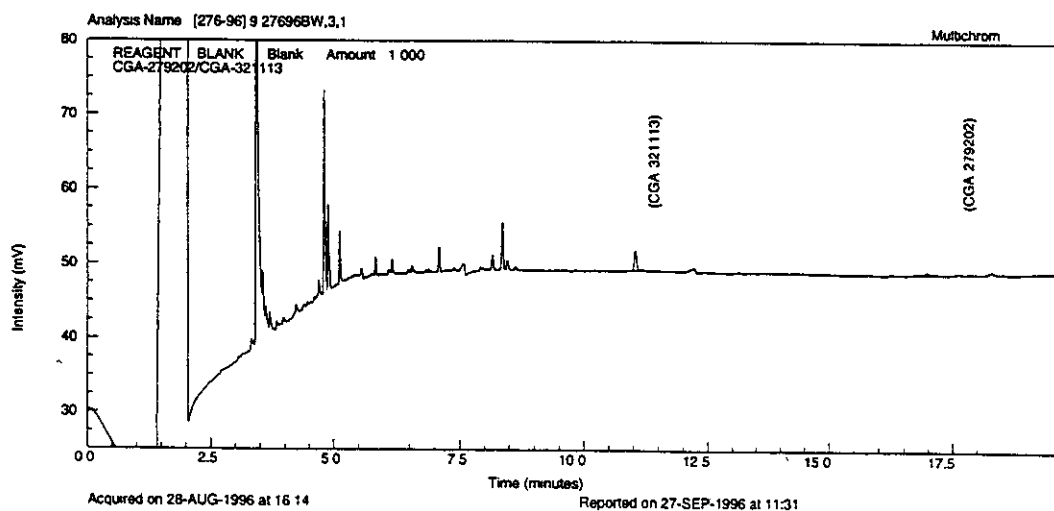


7) 0.1 ng/ μ L standard; 0.2 ng injected

FIGURE 4: CHROMATOGRAMS FROM THE ANALYSIS OF REAGENT BLANKS

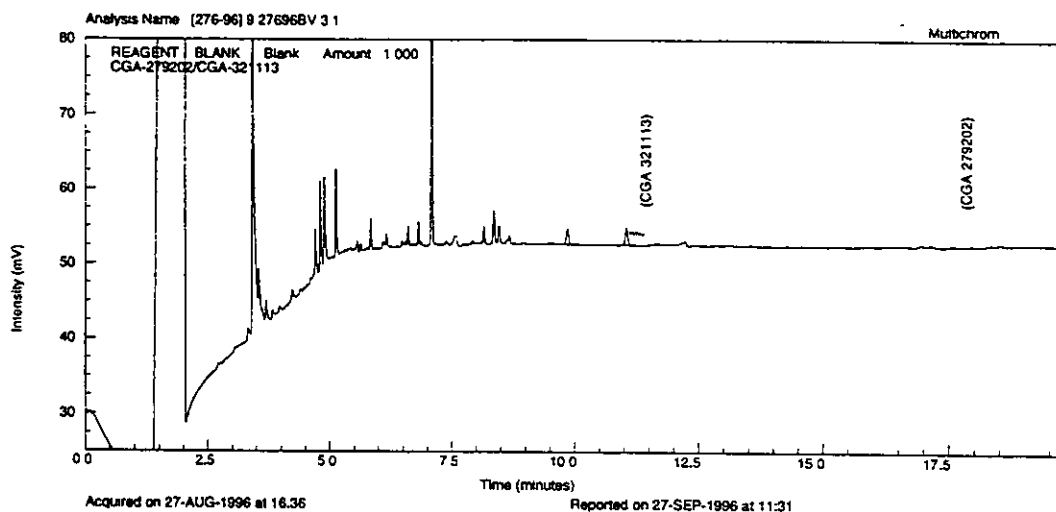


- 1) Sample # 6R; Reagent Blank analyzed with cow omental fat; 8 mg injected, <0.08 ng CGA-321113 found (<0.02 ppm), <0.08 ng CGA-279202 found (<0.02 ppm)

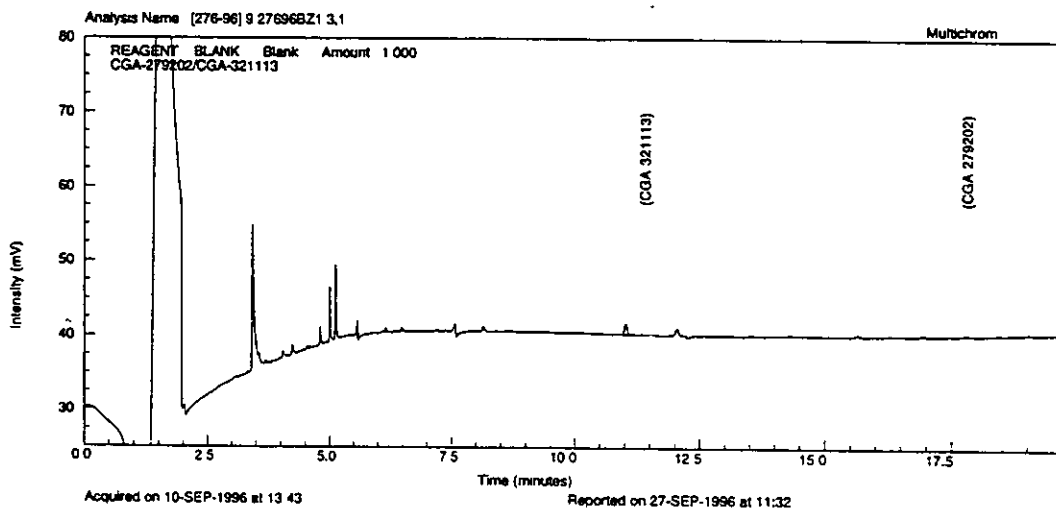


- 2) Sample # 23; Reagent Blank analyzed with milk; 15.3 mg injected, <0.08 ng CGA-321113 found (<0.01 ppm), <0.08 ng CGA-279202 found (<0.01 ppm)

FIGURE 4: CHROMATOGRAMS FROM THE ANALYSIS OF REAGENT BLANKS
(Continued)

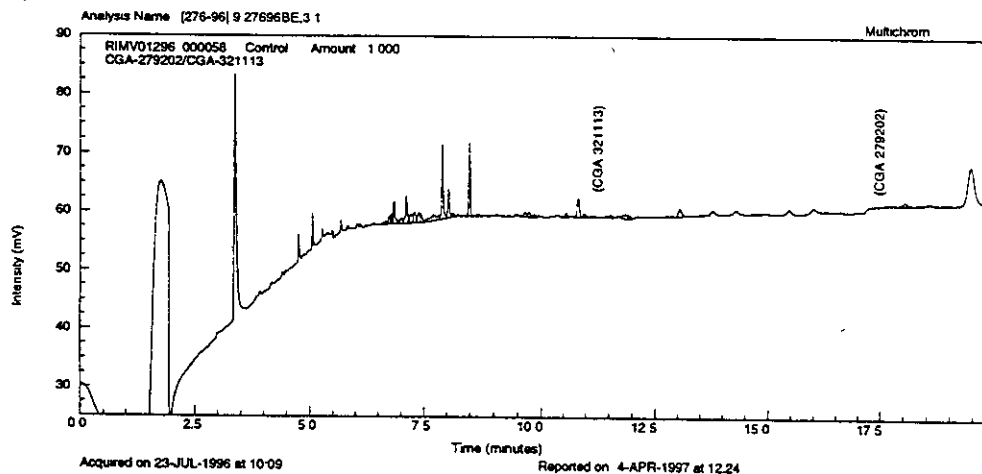


- 3) Sample # 121; Reagent Blank analyzed with apple juice;
7.66 mg injected, <0.08 ng CGA-321113 found (<0.02 ppm),
<0.08 ng CGA-279202 found (<0.02 ppm)

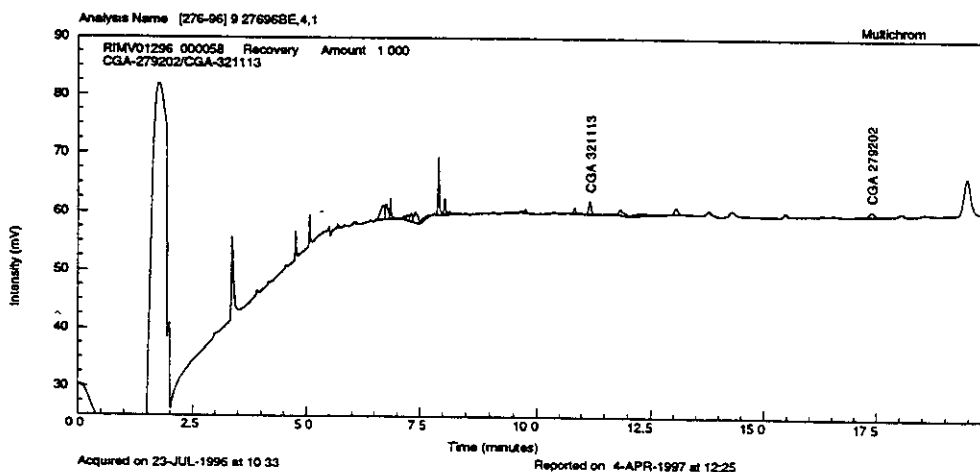


- 4) Sample # 141; Reagent Blank analyzed with peanut oil; 8
mg injected, <0.08 ng CGA-321113 found (<0.02 ppm),
<0.08 ng CGA-279202 found (<0.02 ppm)

FIGURE 5: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CROP RACS

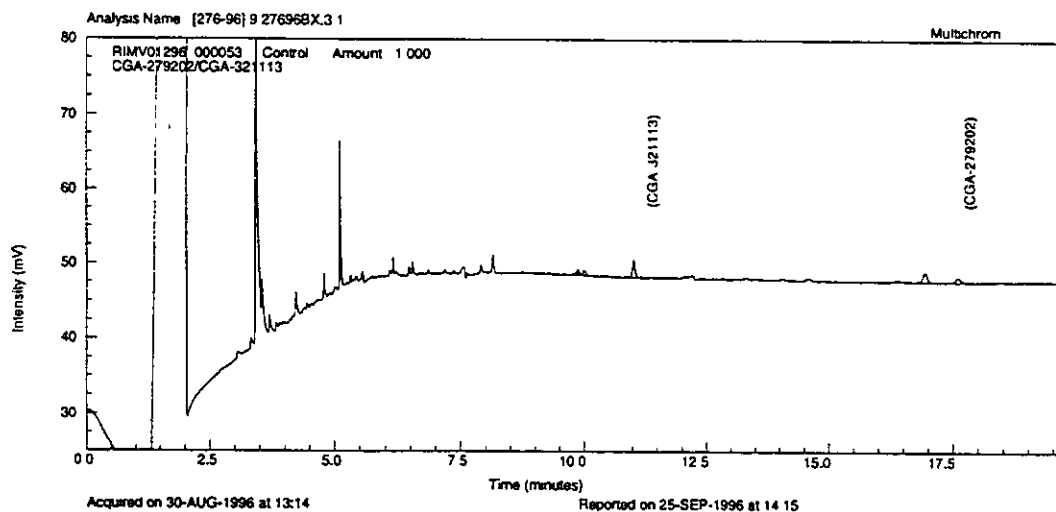


- 1) Sample # 92; Grapes (fruit) control; 7.69 mg injected, <0.08 ng CGA-321113 found (<0.02 ppm), <0.08 ng CGA-279202 found (<0.02 ppm)

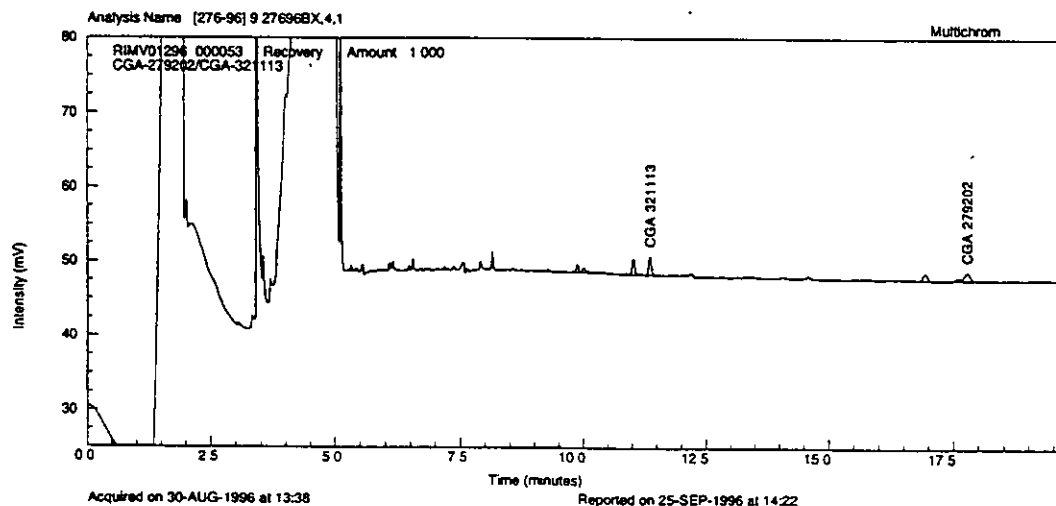


- 2) Sample # 93; Grapes (fruit) control + 0.02 ppm CGA-321113 and 0.02 ppm CGA-279202; 7.69 mg injected, 0.14 ng CGA-321113 found, 0.018 ppm, 92% recovered; 0.14 ng CGA-279202 found, 0.019 ppm, 94% recovered.

FIGURE 5: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CROP RACs (Continued)

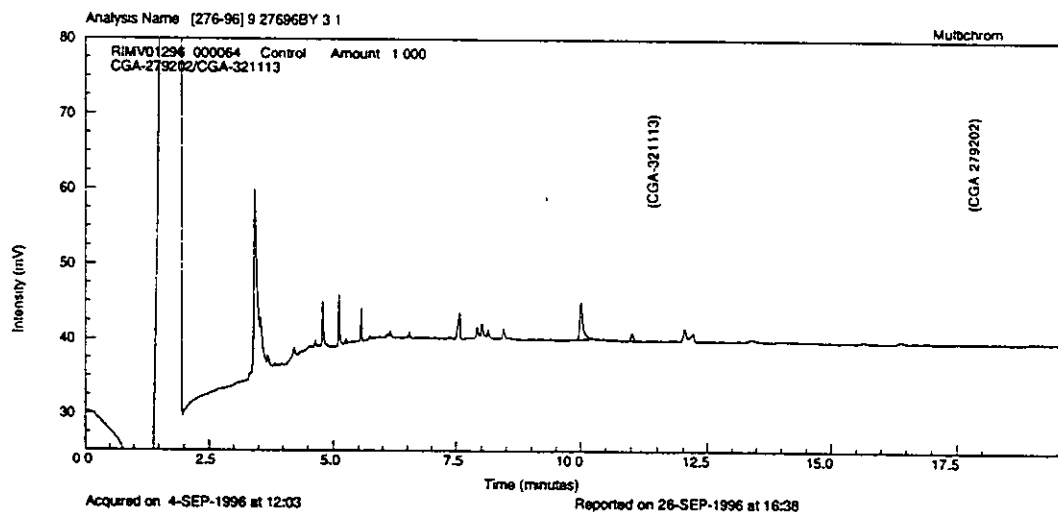


- 3) Sample # 107; Apples (fruit) control; 7.68 mg injected, <0.08 ng CGA-321113 found (<0.02 ppm), <0.08 ng CGA-279202 found (<0.02 ppm)

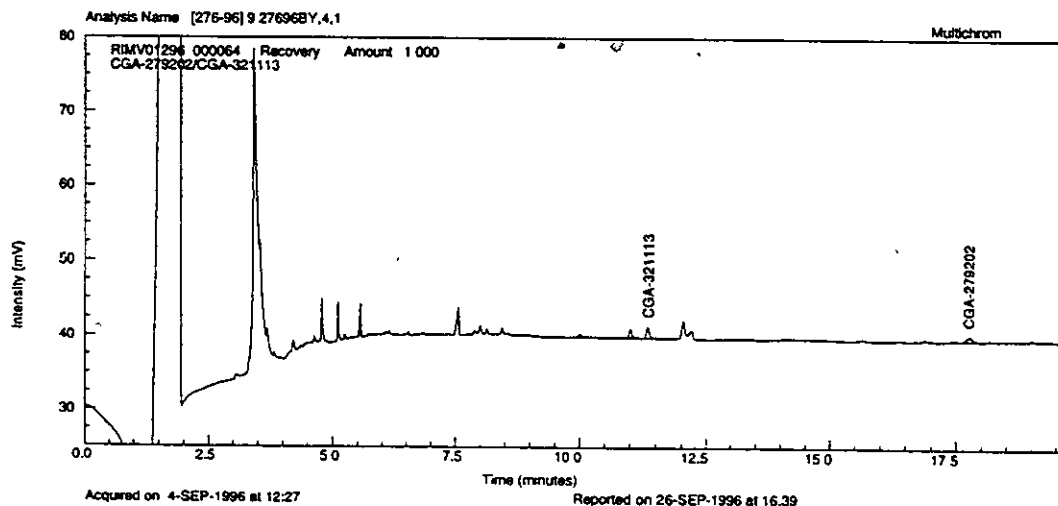


- 4) Sample # 108; Apples (fruit) control + 0.02 ppm CGA-321113 and 0.02 ppm CGA-279202; 7.68 mg injected, 0.13 ng CGA-321113 found, 0.017 ppm, 84% recovered; 0.16 ng CGA-279202 found, 0.021 ppm, 105% recovered.

FIGURE 5: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CROP RACs (Continued)

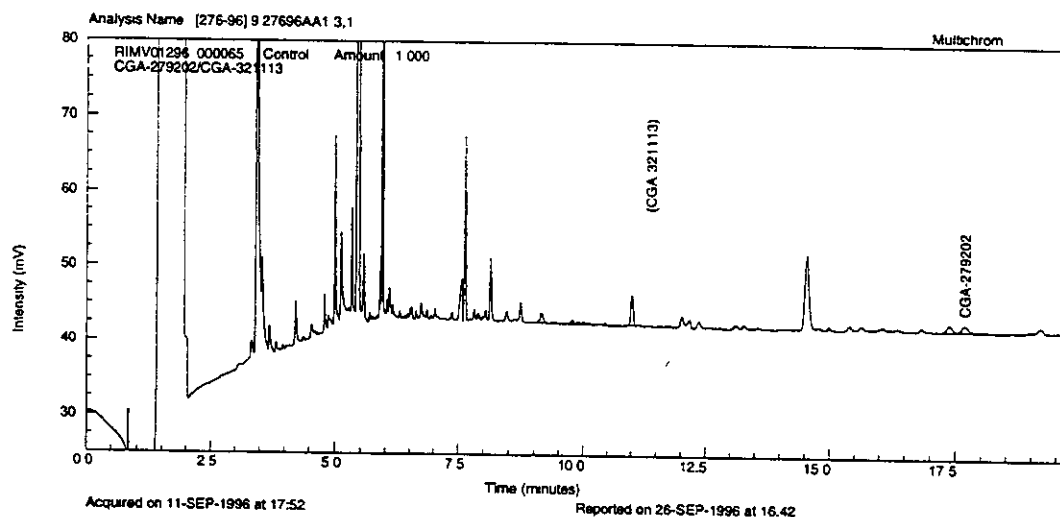


- 5) Sample # 127; Peanut nutmeat control; 7.97 mg injected, <0.08 ng CGA-321113 found (<0.02 ppm), <0.08 ng CGA-279202 found (<0.02 ppm)

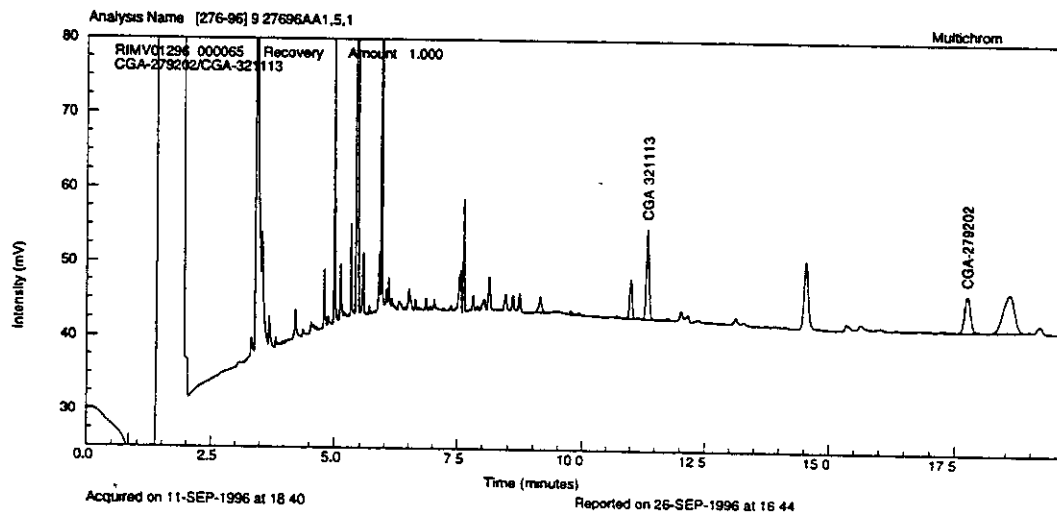


- 6) Sample # 128; Peanut nutmeat control + 0.02 ppm CGA-321113 and 0.02 ppm CGA-279202; 7.97 mg injected, 0.16 ng CGA-321113 found, 0.020 ppm, 98% recovered; 0.17 ng CGA-279202 found, 0.021 ppm, 107% recovered.

FIGURE 5: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CROP RACs (Continued)

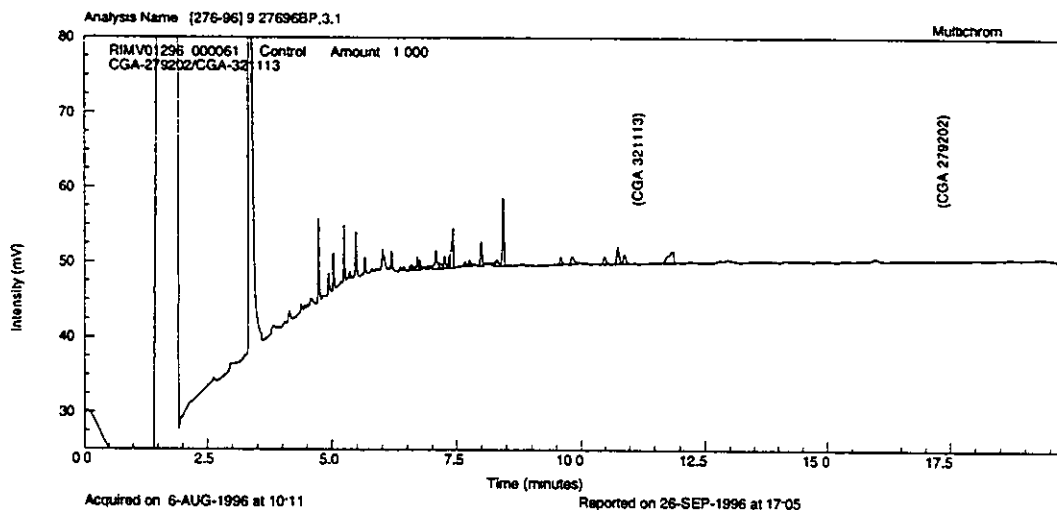


- 7) Sample # 134A; Peanut hay control; 7.97 mg injected, <0.2 ng CGA-321113 found (<0.05 ppm), <0.2 ng (0.034) CGA-279202 found, <0.05 ppm (0.004 ppm).

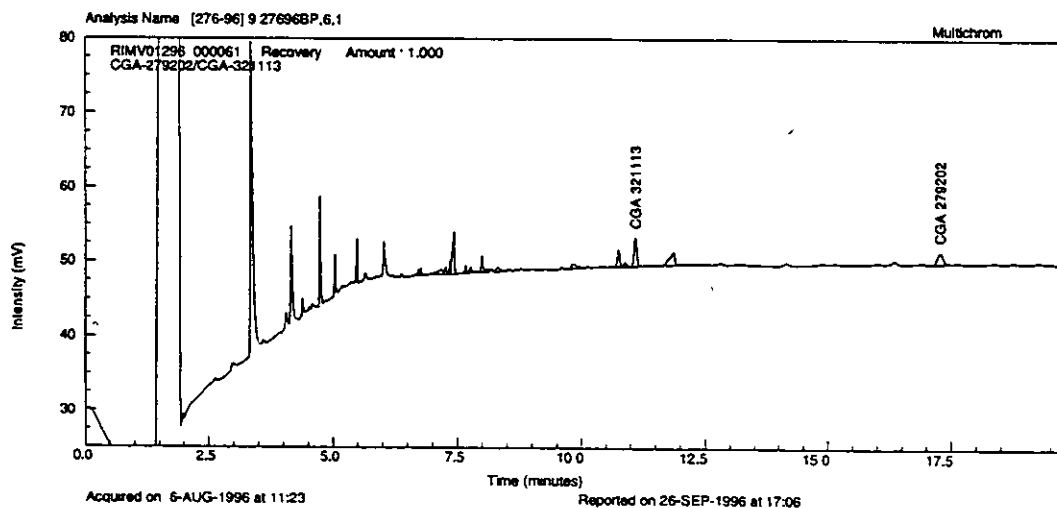


- 8) Sample # 136A; Peanut hay control + 0.05 ppm CGA-321113 and 0.05 ppm CGA-279202; 7.97 mg injected, 0.42 ng CGA-321113 found, 0.05 ppm, 105% recovered; 0.43 ng CGA-279202 found, 0.054 ppm (0.05 ppm corrected for control value), 99% recovered.

FIGURE 5: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CROP RACs (Continued)

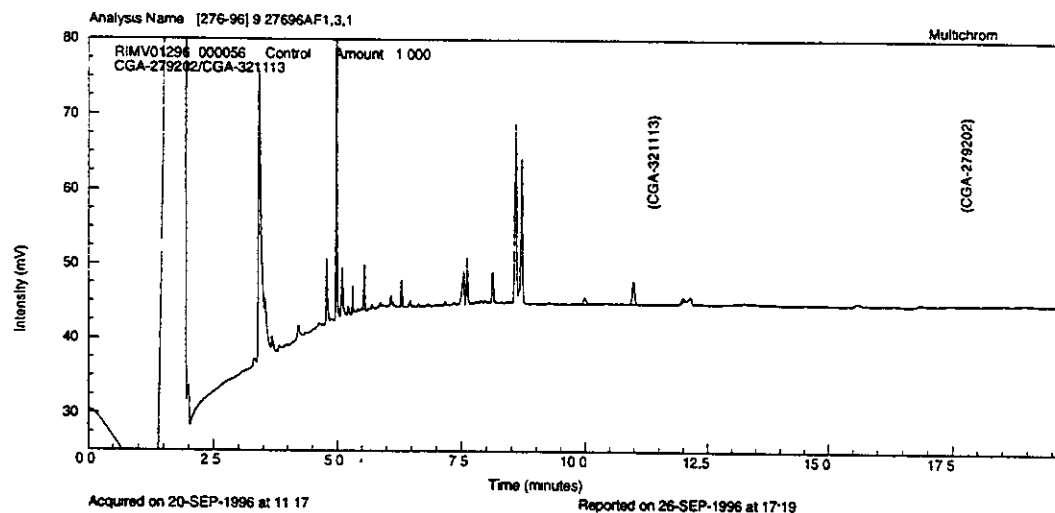


- 9) Sample # 147; Potato tuber control; 7.68 mg injected, <0.08 ng CGA-321113 found (<0.02 ppm), <0.08 ng CGA-279202 found, (<0.02 ppm).

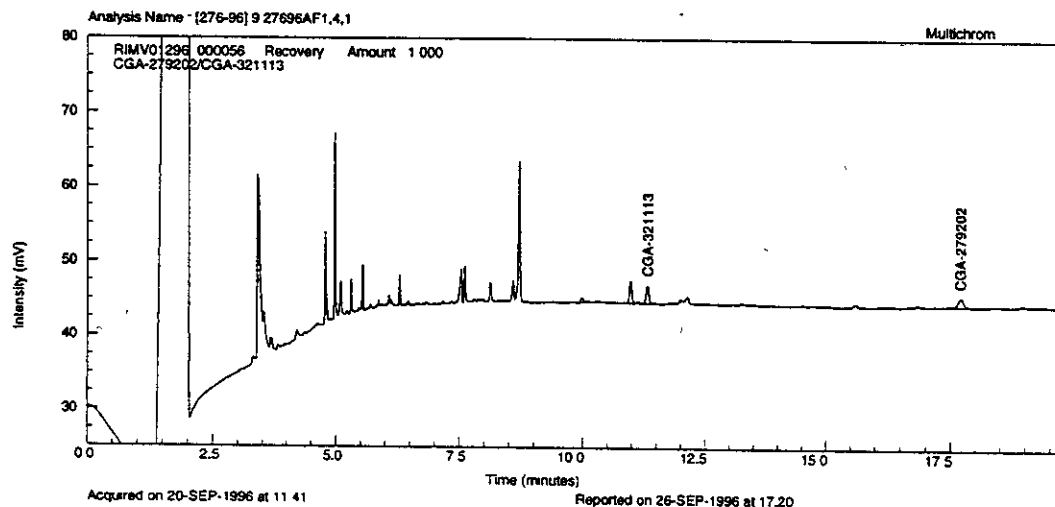


- 10) Sample # 149; Potato tuber control + 0.02 ppm CGA-321113 and 0.02 ppm CGA-279202; 7.68 mg injected, 0.13 ng CGA-321113 found, 0.017 ppm, 83% recovered; 0.15 ng CGA-279202 found, 0.019 ppm, 96% recovered.

FIGURE 5: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CROP RACs (Continued)

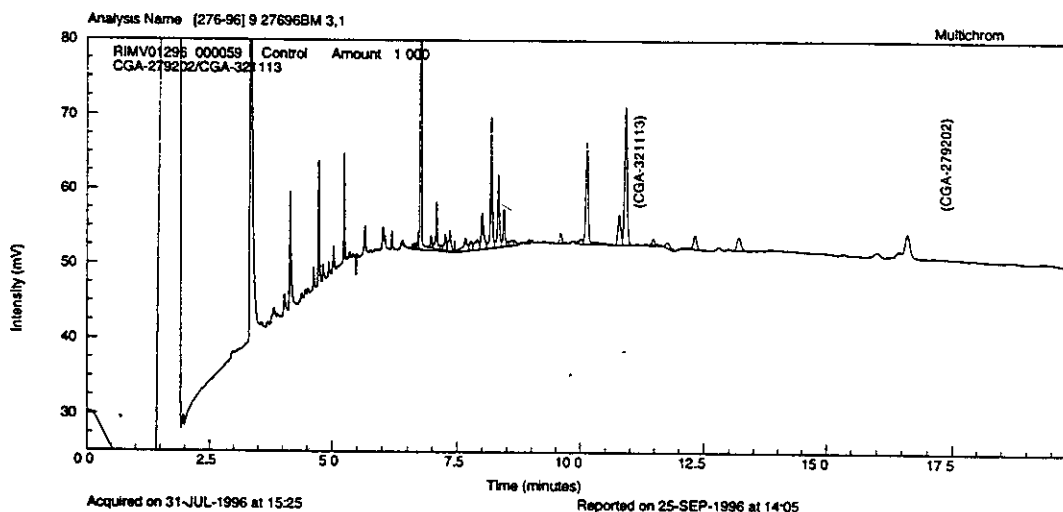


- 11) Sample # 182; Cucumber control; 7.63 mg injected, <0.08 ng CGA-321113 found (<0.02 ppm), <0.08 ng CGA-279202 found, (<0.02 ppm).

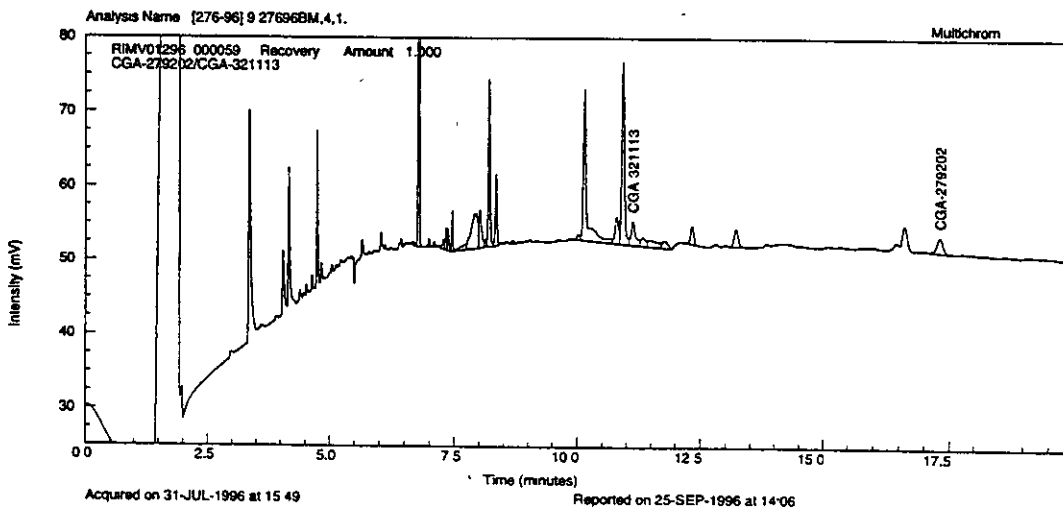


- 12) Sample # 183; Cucumber control + 0.02 ppm CGA-321113 and 0.02 ppm CGA-279202; 7.63 mg injected, 0.17 ng CGA-321113 found, 0.022 ppm, 110% recovered; 0.15 ng CGA-279202 found, 0.019 ppm, 96% recovered.

FIGURE 6: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CROP PROCESSED COMMODITIES

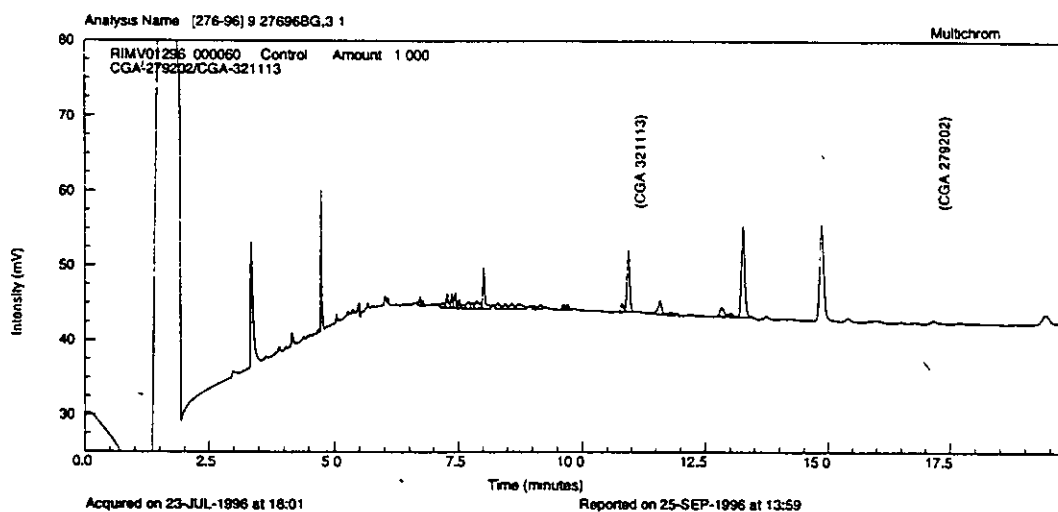


- 1) Sample # 102; Grape juice control; 7.68 mg injected, <0.08 ng CGA-321113 found (<0.02 ppm), <0.08 ng CGA-279202 found, (<0.02 ppm).

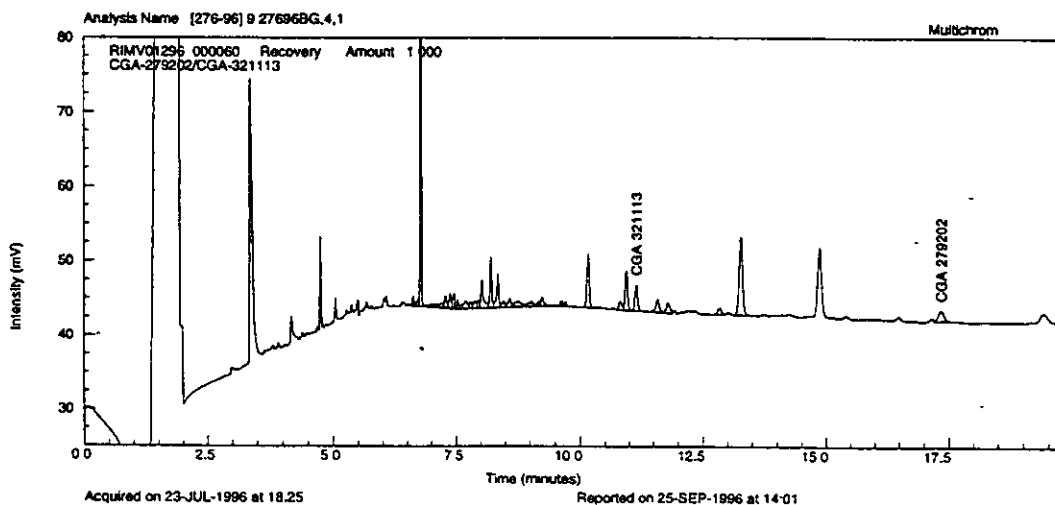


- 2) Sample # 103; Grape juice control + 0.02 ppm CGA-321113 and 0.02 ppm CGA-279202; 7.68 mg injected, 0.10 ng CGA-321113 found, 0.014 ppm, 68% recovered; 0.15 ng CGA-279202 found, 0.019 ppm, 95% recovered.

FIGURE 6: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CROP PROCESSED COMMODITIES (Continued)

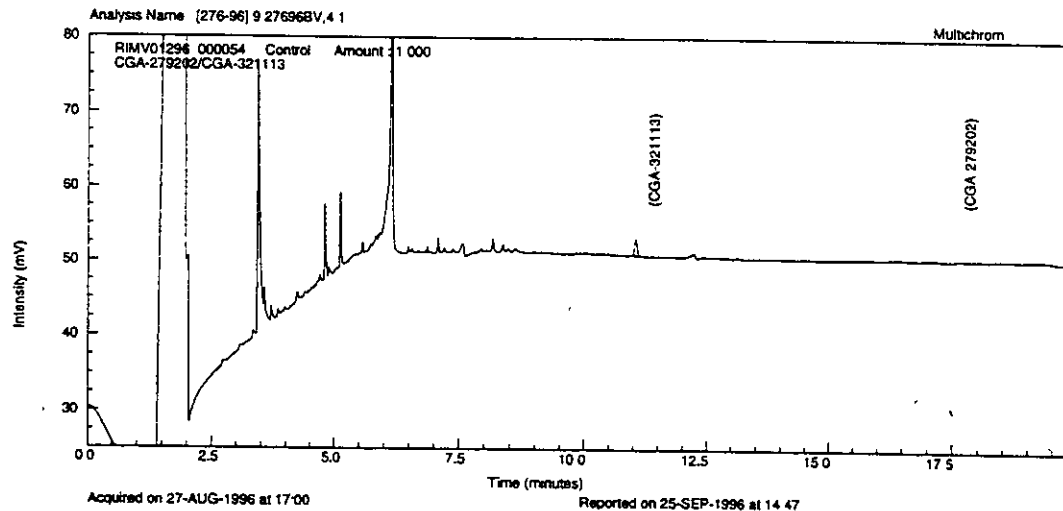


- 3) Sample # 97; Grape raisin control; 7.94 mg injected, <0.08 ng CGA-321113 found (<0.02 ppm), <0.08 ng CGA-279202 found, (<0.02 ppm).

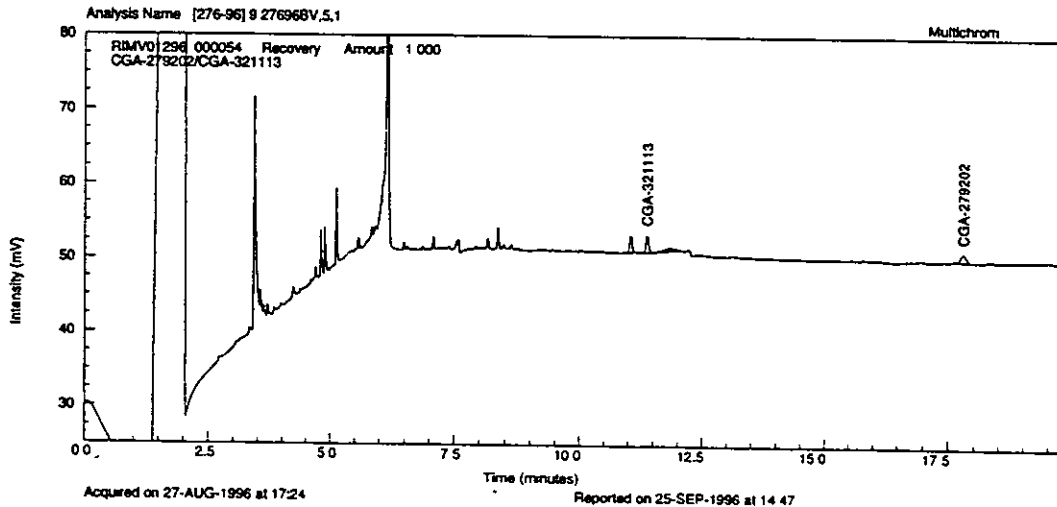


- 4) Sample # 98; Grape raisin control + 0.02 ppm CGA-321113 and 0.02 ppm CGA-279202; 7.94 mg injected, 0.13 ng CGA-321113 found, 0.017 ppm, 85% recovered; 0.15 ng CGA-279202 found, 0.019 ppm, 95% recovered.

FIGURE 6: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CROP PROCESSED COMMODITIES (Continued)

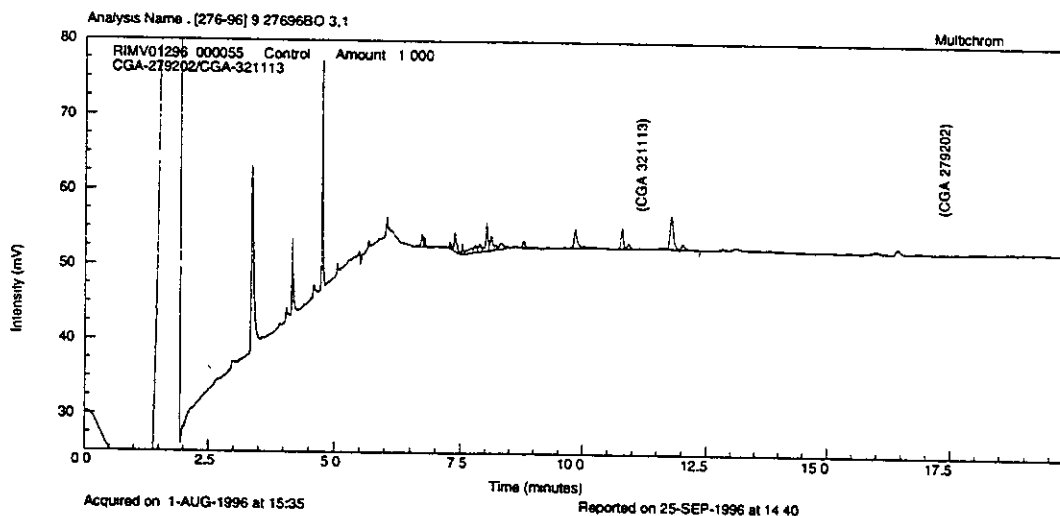


- 5) Sample # 122; Apple juice control; 7.66 mg injected, <0.08 ng CGA-321113 found (<0.02 ppm), <0.08 ng CGA-279202 found, (<0.02 ppm).

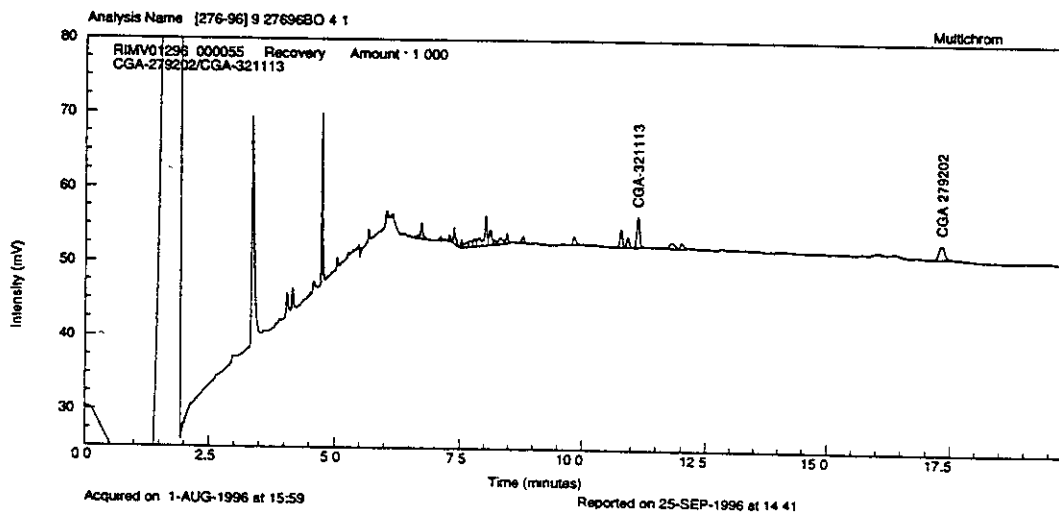


- 6) Sample # 123; Apple juice control + 0.02 ppm CGA-321113 and 0.02 ppm CGA-279202; 7.66 mg injected, 0.13 ng CGA-321113 found, 0.017 ppm, 87% recovered; 0.15 ng CGA-279202 found, 0.019 ppm, 97% recovered.

FIGURE 6: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CROP PROCESSED COMMODITIES (Continued)

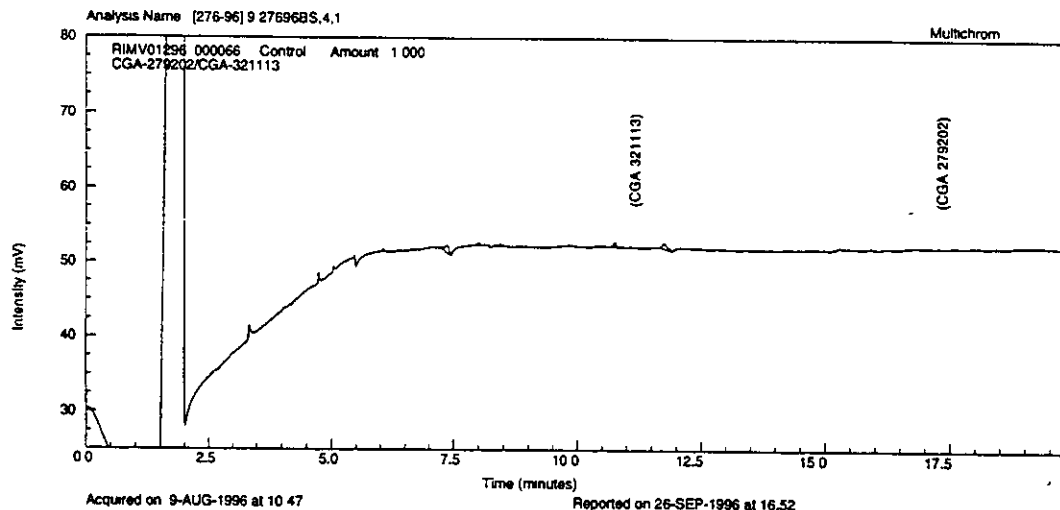


- 7) Sample # 114; Apple wet pomace control; 7.7 mg injected, <0.08 ng CGA-321113 found (<0.02 ppm), <0.08 ng CGA-279202 found, (<0.02 ppm).

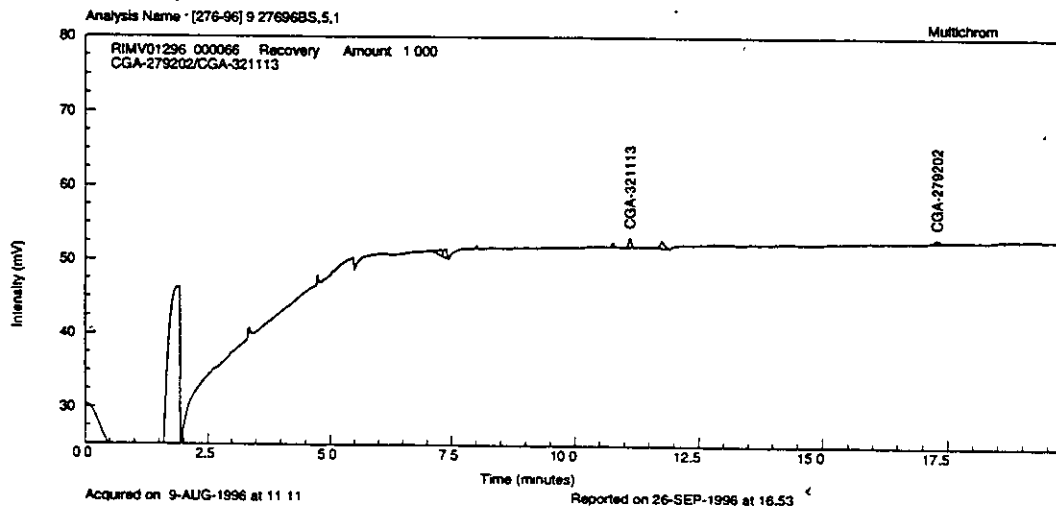


- 8) Sample # 115; Apple wet pomace control + 0.02 ppm CGA-321113 and 0.02 ppm CGA-279202; 7.7 mg injected, 0.11-ng CGA-321113 found, 0.014 ppm, 72% recovered; 0.16 ng CGA-279202 found, 0.02 ppm, 101% recovered.

FIGURE 6: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CROP PROCESSED COMMODITIES (Continued)

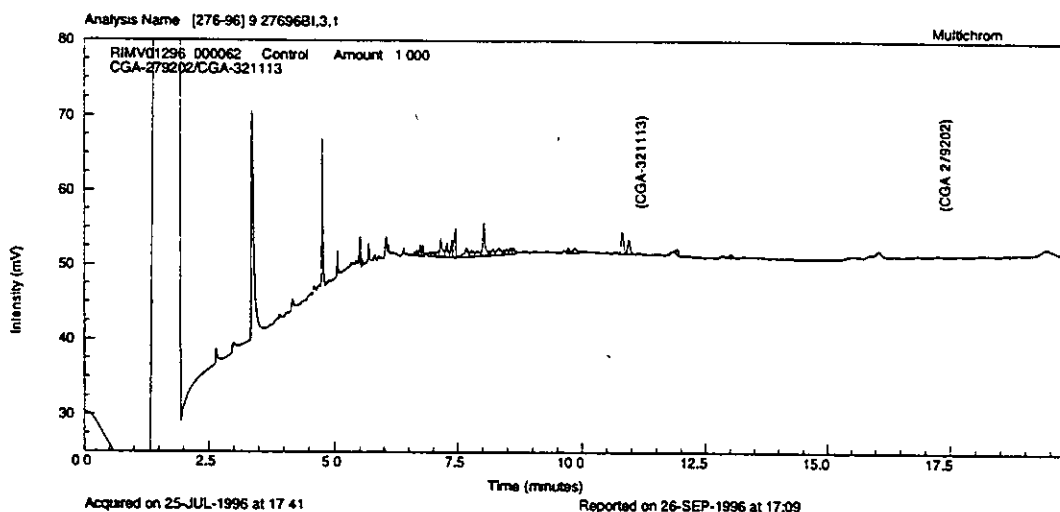


- 9) Sample # 142; Peanut oil control; 8 mg injected, <0.08 ng CGA-321113 found (<0.02 ppm), <0.08 ng CGA-279202 found, (<0.02 ppm).

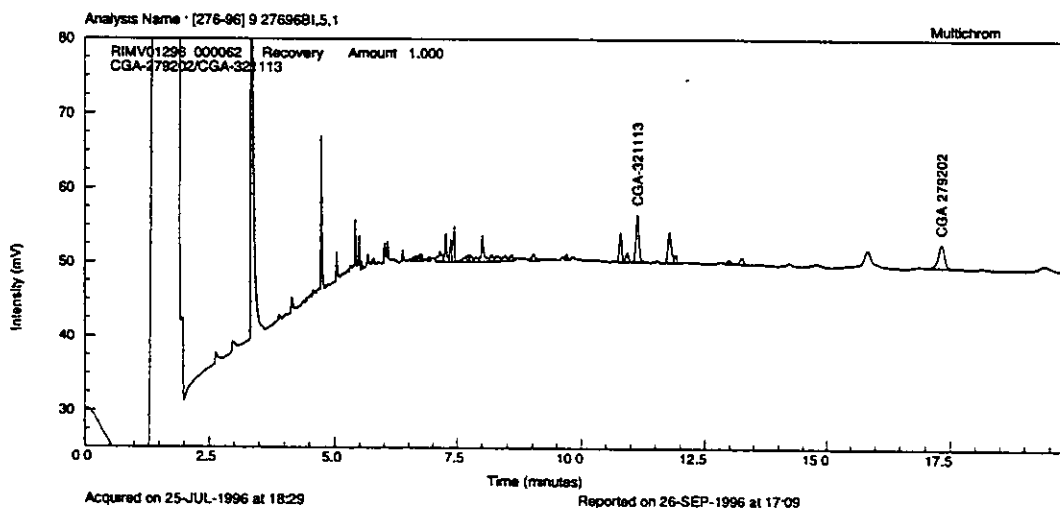


- 10) Sample # 143; Peanut oil control + 0.02 ppm CGA-321113 and 0.02 ppm CGA-279202; 8 mg injected, 0.15 ng CGA-321113 found, 0.018 ppm, 90% recovered; 0.13 ng CGA-279202 found, 0.017 ppm, 83% recovered.

FIGURE 6: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CROP PROCESSED COMMODITIES (Continued)

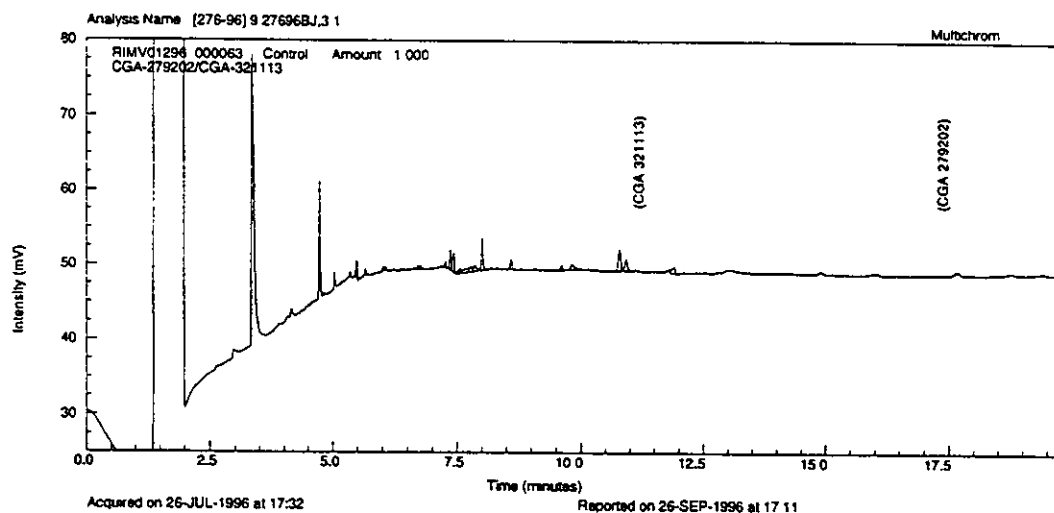


- 11) Sample # 157; Potato granule control; 8 mg injected,
<0.08 ng CGA-321113 found (<0.02 ppm), <0.08 ng
CGA-279202 found, (<0.02 ppm).

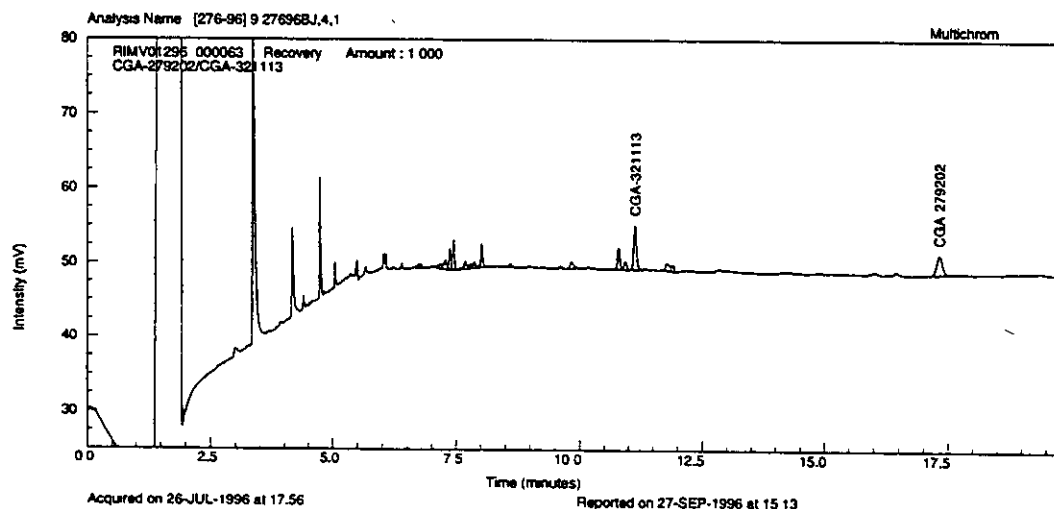


- 12) Sample # 159; Potato granule control + 0.02 ppm
CGA-321113 and 0.02 ppm CGA-279202; 8 mg injected, 0.15
ng CGA-321113 found, 0.018 ppm, 90% recovered; 0.16 ng
CGA-279202 found, 0.02 ppm, 99% recovered.

FIGURE 6: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CROP PROCESSED COMMODITIES (Continued)

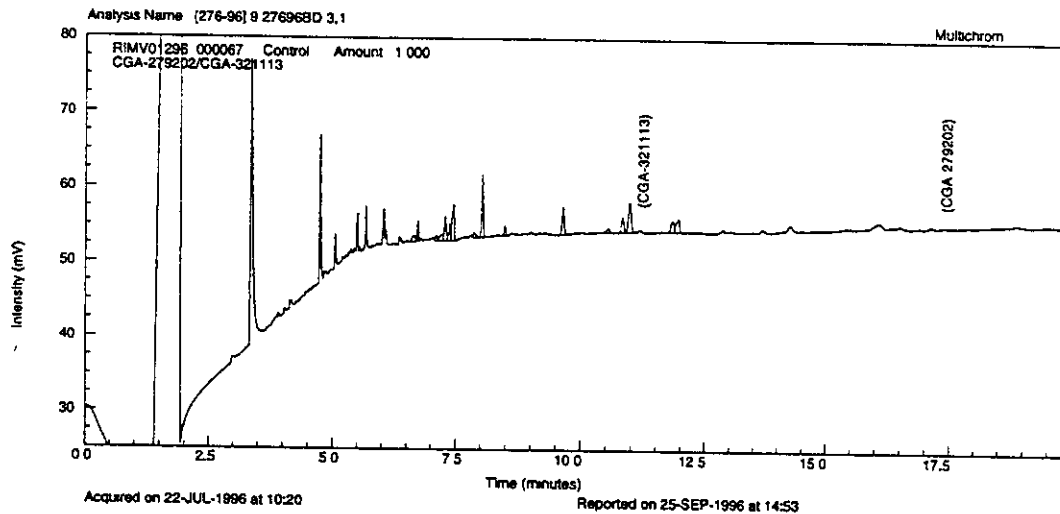


- 13) Sample # 162; Potato wet peel control; 7.67 mg injected, <0.08 ng CGA-321113 found (<0.02 ppm), <0.08 ng CGA-279202 found, (<0.02 ppm).

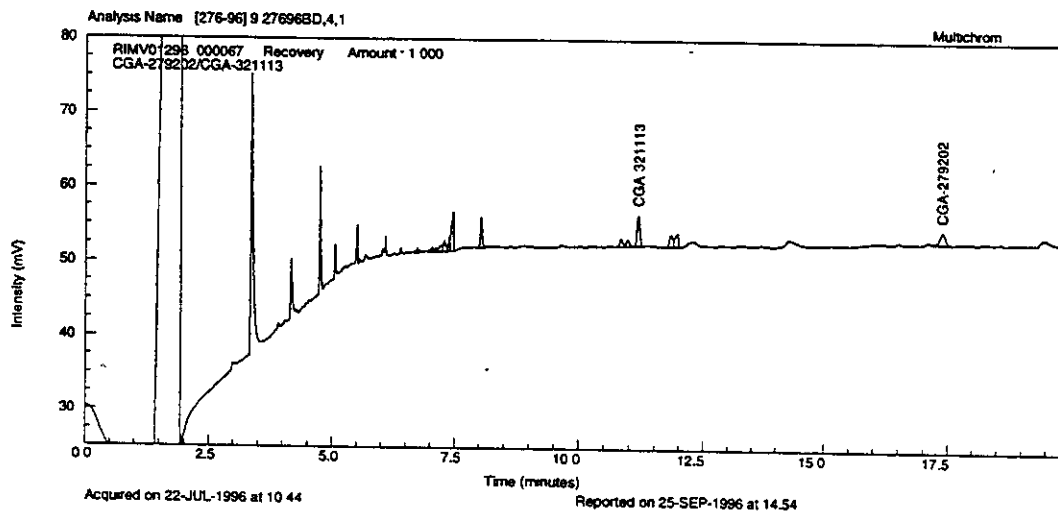


- 14) Sample # 163; Potato wet peel control + 0.02 ppm CGA-321113 and 0.02 ppm CGA-279202; 7.67 mg injected, 0.15 ng CGA-321113 found, 0.019 ppm, 97% recovered; 0.15 ng CGA-279202 found, 0.02 ppm, 101% recovered.

FIGURE 7: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF ANIMAL SUBSTRATES

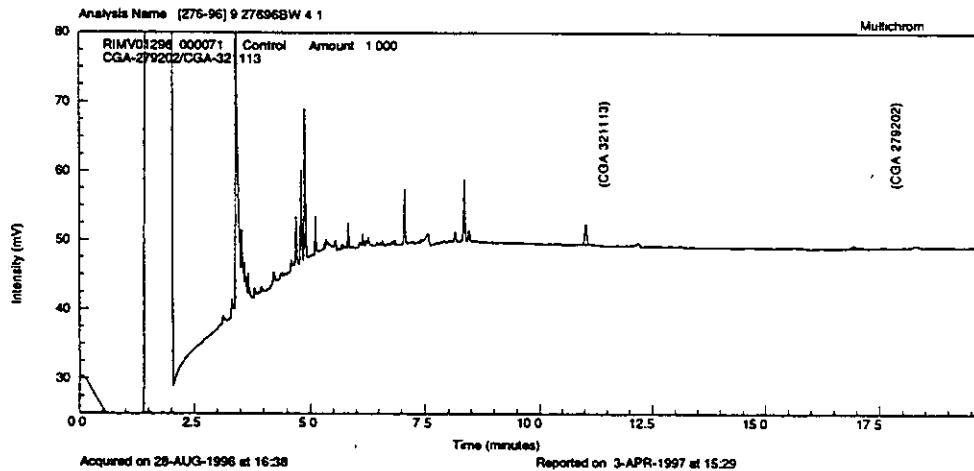


- 1) Sample #1, Cow Tenderloin control; 7.69 mg injected, <0.08 ng CGA-321113 found (<0.02 ppm), <0.08 ng CGA-279202 found, (<0.02 ppm).

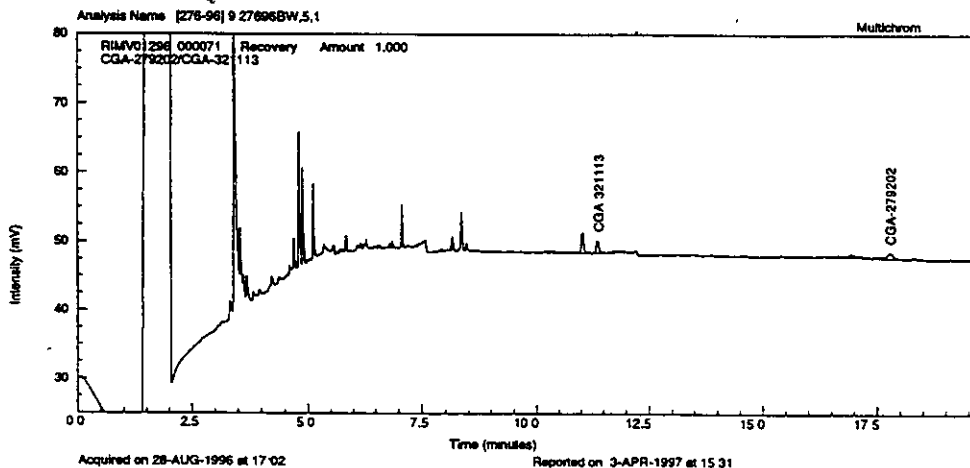


- 2) Sample #2, Cow tenderloin control + 0.02 ppm CGA-321113 and 0.02 ppm CGA-279202; 7.69 mg injected, 0.17 ng CGA-321113 found, 0.023 ppm, 113% recovered; 0.18 ng CGA-279202 found, 0.024 ppm, 119% recovered.

FIGURE 7: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF ANIMAL SUBSTRATES (Continued)

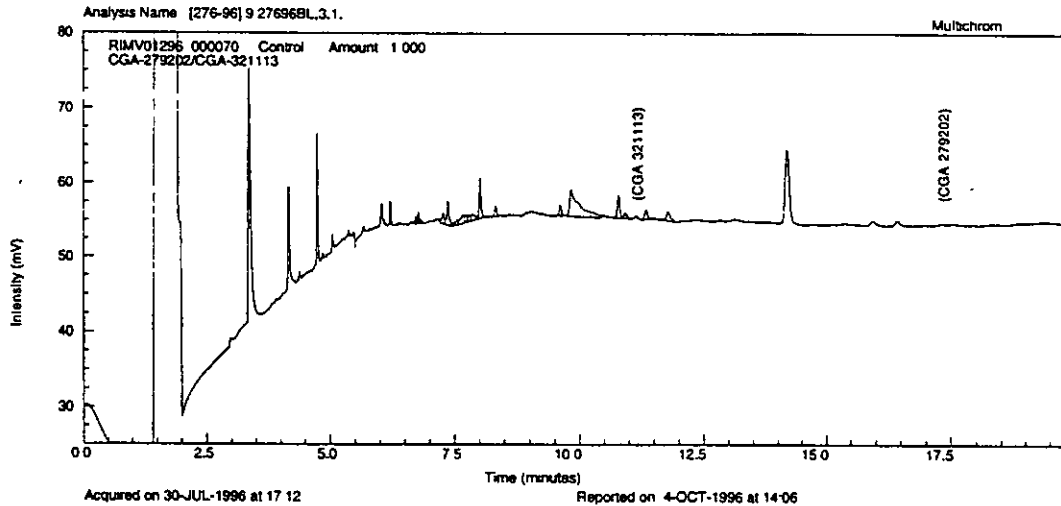


- 3) Sample #24, Milk control; 15.3 mg injected, <0.08 ng CGA-321113 found (<0.01 ppm), <0.08 ng CGA-279202 found, (<0.01 ppm).

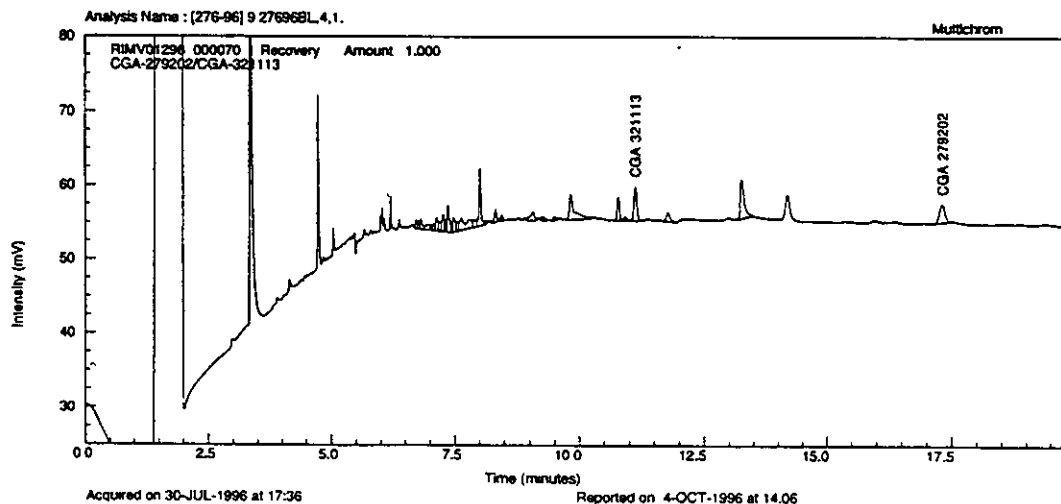


- 4) Sample #25, Milk control + 0.01 ppm CGA-321113 and 0.01 ppm CGA-279202; 15.3 mg injected, 0.15 ng CGA-321113 found, 0.010 ppm, 95% recovered; 0.16 ng CGA-279202 found, 0.010 ppm, 102% recovered.

FIGURE 7: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF ANIMAL SUBSTRATES (Continued)

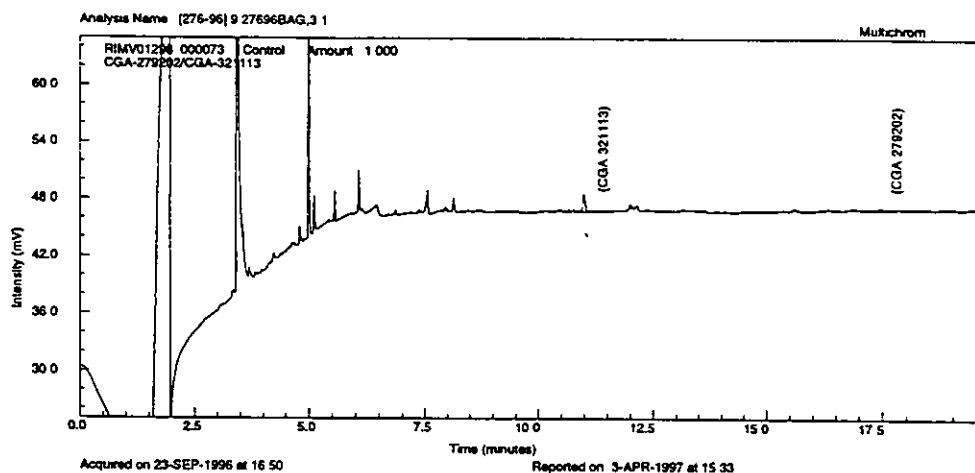


- 5) Sample # 18, Cow kidney control; 7.69 mg injected, <0.08 ng CGA-321113 found (<0.02 ppm), <0.08 ng CGA-279202 found, (<0.08 ppm).

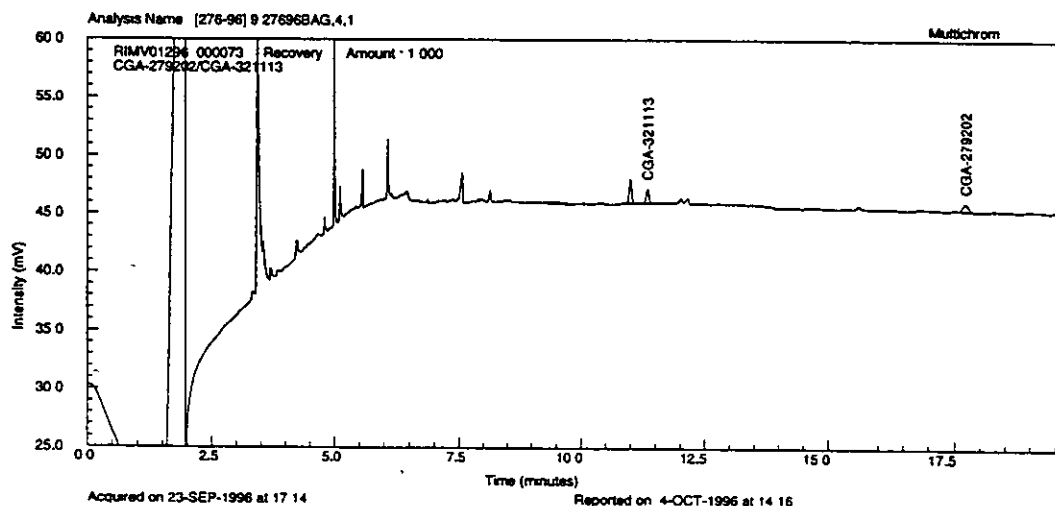


- 6) Sample # 19, Cow Kidney control + 0.02 ppm CGA-321113, and 0.02 ppm CGA-279202; 7.69 mg injected, 0.13 ng CGA-321113 found, 0.016 ppm, 82% recovered; 0.16 ng CGA-279202 found, 0.021 ppm, 103% recovered.

FIGURE 7: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF ANIMAL SUBSTRATES (Continued)

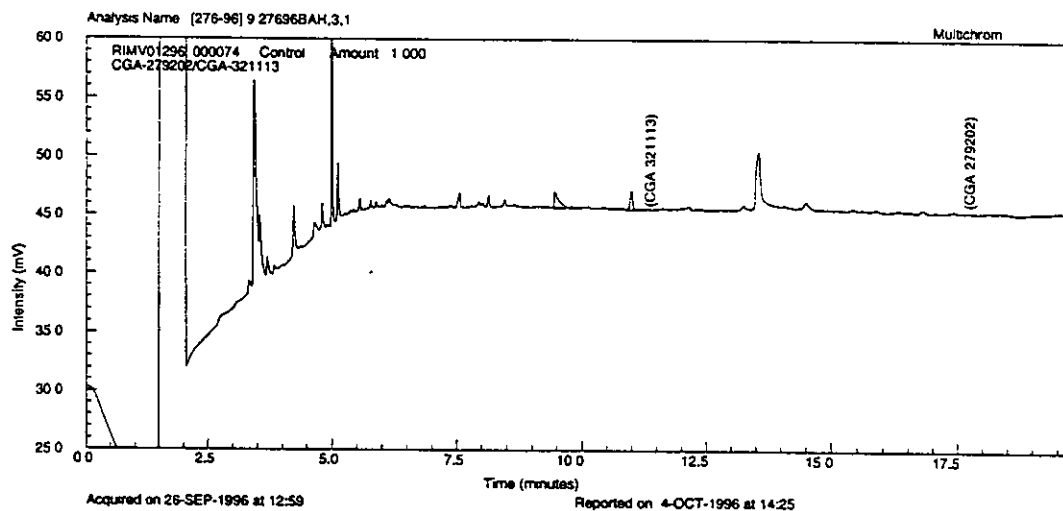


- 7) Sample #71, Chicken fat control; 8 mg injected, <0.08 ng CGA-321113 found (<0.02 ppm), <0.08 ng CGA-279202 found, (<0.08 ppm).

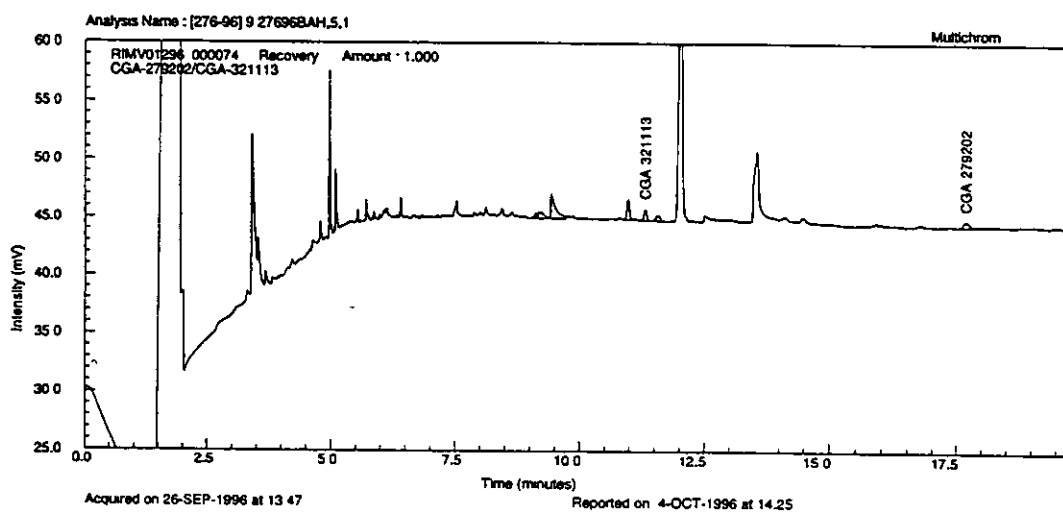


- 8) Sample # 72, Chicken fat control + 0.02 ppm CGA-321113 and 0.02 ppm CGA-279202; 8 mg injected, 0.17 ng CGA-321113 found, 0.021 ppm, 107% recovered; 0.18 ng CGA-279202 found, 0.022 ppm, 109% recovered.

FIGURE 7: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF ANIMAL SUBSTRATES (Continued)

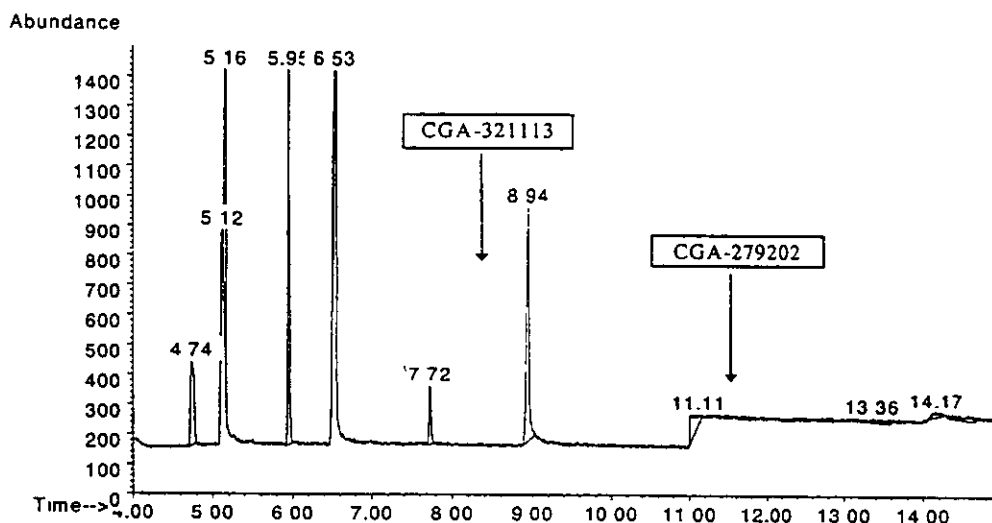


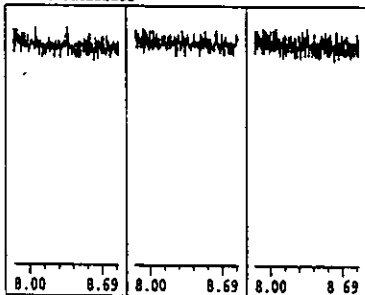
- 9) Sample # 76, Chicken liver control; 7.69 mg injected, <0.08 ng CGA-321113 found (<0.02 ppm), <0.08 ng CGA-279202 found, (<0.08 ppm).

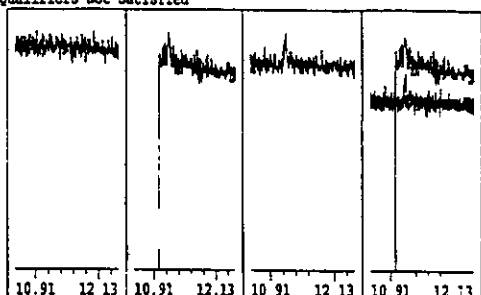


- 10) Sample # 78, Control chicken liver + 0.02 ppm CGA-321113 and CGA-279202; 7.69 mg injected, 0.17 ng CGA-321113 found, 0.022 ppm, 108% recovered; 0.14 ng CGA-279202 found, 0.019 ppm, 93% recovered

FIGURE 8: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF PEANUT HAY BY THE CONFIRMATORY GC/MS METHOD

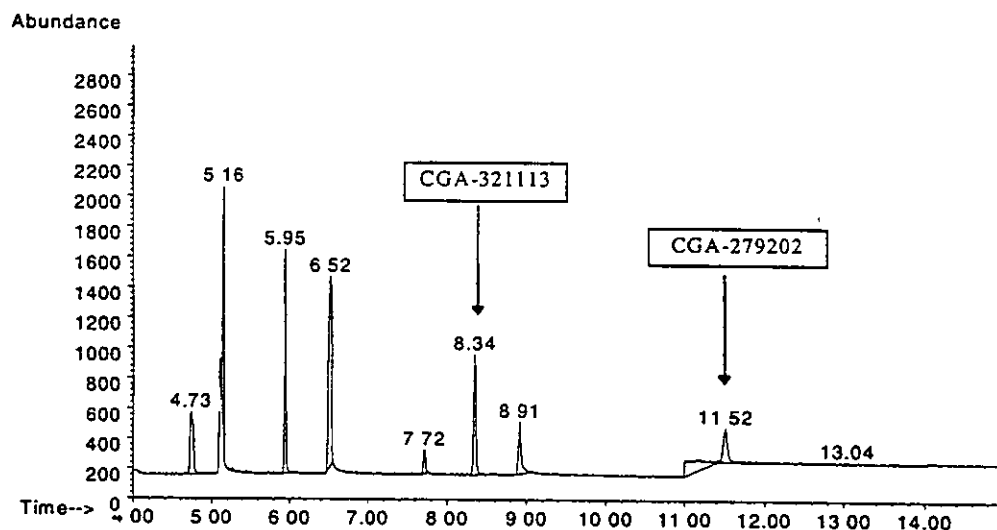


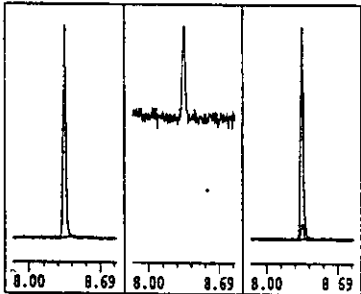
CGA-321113																																																	
Compound: CGA-321113																																																	
Ret Time: 8.47																																																	
Concentration: 0.03 ng																																																	
Pk # and Type: 1 RTEINT used																																																	
# Qualifiers Not Satisfied																																																	
																																																	
<table><thead><tr><th>Signal</th><th>Ratios</th><th>Limits</th><th>RT</th><th>Limits</th><th>Resp</th><th>Integ</th><th>Type</th></tr></thead><tbody><tr><td>Tgt</td><td>116.00</td><td>100.0%</td><td></td><td></td><td></td><td></td><td></td></tr><tr><td>Q1</td><td>145.00</td><td>120.0%</td><td>5.3-</td><td>8.0</td><td>8.47</td><td>8.14</td><td>5 sys def</td></tr><tr><td>Q2</td><td>0.00</td><td>0.0</td><td>0.0-</td><td>0.0</td><td>0.00</td><td>8.56</td><td>0 sys def</td></tr><tr><td>Q3</td><td>0.00</td><td>0.0</td><td>0.0-</td><td>0.0</td><td>0.00</td><td></td><td>0 sys def</td></tr></tbody></table>										Signal	Ratios	Limits	RT	Limits	Resp	Integ	Type	Tgt	116.00	100.0%						Q1	145.00	120.0%	5.3-	8.0	8.47	8.14	5 sys def	Q2	0.00	0.0	0.0-	0.0	0.00	8.56	0 sys def	Q3	0.00	0.0	0.0-	0.0	0.00		0 sys def
Signal	Ratios	Limits	RT	Limits	Resp	Integ	Type																																										
Tgt	116.00	100.0%																																															
Q1	145.00	120.0%	5.3-	8.0	8.47	8.14	5 sys def																																										
Q2	0.00	0.0	0.0-	0.0	0.00	8.56	0 sys def																																										
Q3	0.00	0.0	0.0-	0.0	0.00		0 sys def																																										

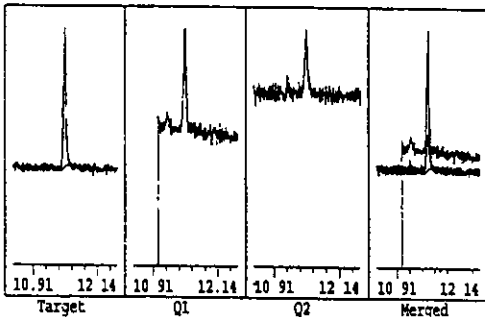
CGA-279202																																																	
Compound: CGA-279202																																																	
Ret Time: 11.60																																																	
Concentration: 0.06 ng																																																	
Pk # and Type: 2 RTEINT used																																																	
# Qualifiers Not Satisfied																																																	
																																																	
<table><thead><tr><th>Signal</th><th>Ratios</th><th>Limits</th><th>RT</th><th>Limits</th><th>Resp</th><th>Integ</th><th>Type</th></tr></thead><tbody><tr><td>Tgt</td><td>116.00</td><td>100.0%</td><td></td><td></td><td></td><td></td><td></td></tr><tr><td>Q1</td><td>131.00</td><td>0.0</td><td>55.0-</td><td>82.5</td><td>0.00</td><td>to</td><td>8 sys def</td></tr><tr><td>Q2</td><td>145.00</td><td>0.0</td><td>20.8-</td><td>31.1</td><td>0.00</td><td>11.81</td><td>0 sys def</td></tr><tr><td>Q3</td><td>0.00</td><td>0.0</td><td>0.0-</td><td>0.0</td><td>0.00</td><td></td><td>0 sys def</td></tr></tbody></table>										Signal	Ratios	Limits	RT	Limits	Resp	Integ	Type	Tgt	116.00	100.0%						Q1	131.00	0.0	55.0-	82.5	0.00	to	8 sys def	Q2	145.00	0.0	20.8-	31.1	0.00	11.81	0 sys def	Q3	0.00	0.0	0.0-	0.0	0.00		0 sys def
Signal	Ratios	Limits	RT	Limits	Resp	Integ	Type																																										
Tgt	116.00	100.0%																																															
Q1	131.00	0.0	55.0-	82.5	0.00	to	8 sys def																																										
Q2	145.00	0.0	20.8-	31.1	0.00	11.81	0 sys def																																										
Q3	0.00	0.0	0.0-	0.0	0.00		0 sys def																																										

- 1) Solvent Blank: Selected Ion Monitoring, (0-11 minutes, m:z = 116, 145; 11-15 minutes, m:z = 116, 131, 145), 7.94 mg injected, <0.2 ng CGA-321113 found, <0.05 ppm, <0.2 ng CGA-279202 found, <0.05 ppm.

FIGURE 8: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF PEANUT HAY BY THE CONFIRMATORY GC/MS METHOD (Continued)

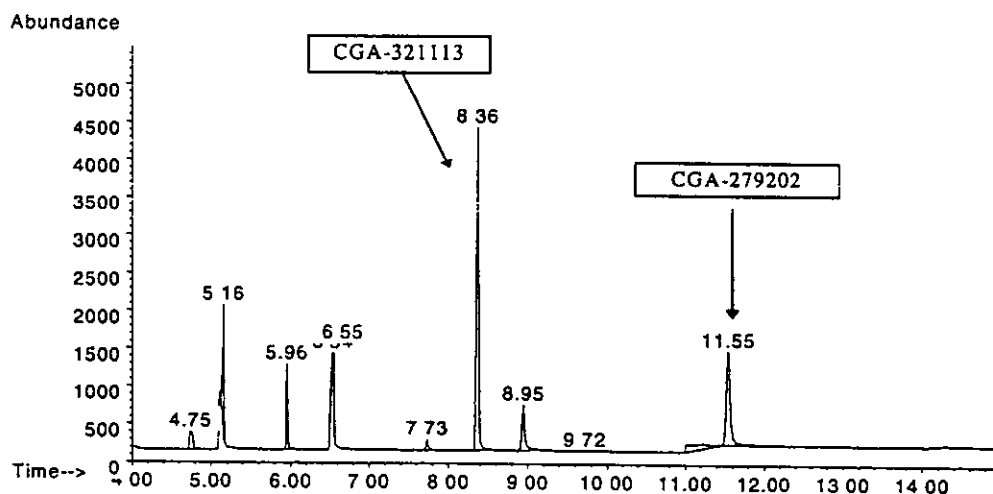


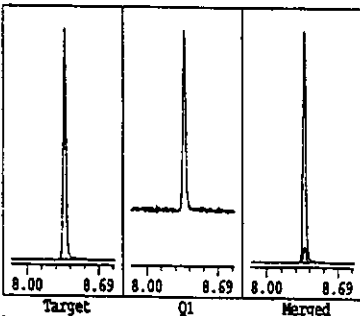
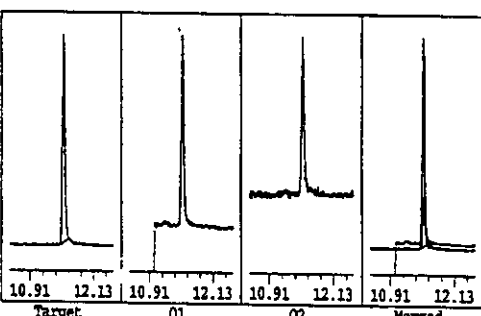
CGA-321113																																																	
Compound:		CGA-321113																																															
Ret Time:		8.34																																															
Concentration:		0.20 ng																																															
Pk # and Type:		1 RTEINT used																																															
																																																	
<table border="1"><thead><tr><th>Signal</th><th>Ratios</th><th>Limits</th><th>RT</th><th>Limits</th><th>Resp</th><th>Integ</th><th>Type</th></tr></thead><tbody><tr><td>Tgt</td><td>116.00</td><td>100.0%</td><td></td><td>8.34</td><td>8.14</td><td>1410</td><td>sys def</td></tr><tr><td>Q1</td><td>145.00</td><td>7.7</td><td>5.3- 8.0</td><td>8.34</td><td>to</td><td>108</td><td>sys def</td></tr><tr><td>Q2</td><td>0.00</td><td>0.0</td><td>0.0- 0.0</td><td>0.00</td><td>8.56</td><td>0</td><td>sys def</td></tr><tr><td>Q3</td><td>0.00</td><td>0.0</td><td>0.0- 0.0</td><td>0.00</td><td></td><td>0</td><td>sys def</td></tr></tbody></table>										Signal	Ratios	Limits	RT	Limits	Resp	Integ	Type	Tgt	116.00	100.0%		8.34	8.14	1410	sys def	Q1	145.00	7.7	5.3- 8.0	8.34	to	108	sys def	Q2	0.00	0.0	0.0- 0.0	0.00	8.56	0	sys def	Q3	0.00	0.0	0.0- 0.0	0.00		0	sys def
Signal	Ratios	Limits	RT	Limits	Resp	Integ	Type																																										
Tgt	116.00	100.0%		8.34	8.14	1410	sys def																																										
Q1	145.00	7.7	5.3- 8.0	8.34	to	108	sys def																																										
Q2	0.00	0.0	0.0- 0.0	0.00	8.56	0	sys def																																										
Q3	0.00	0.0	0.0- 0.0	0.00		0	sys def																																										

CGA-279202																																																	
Compound:		CGA-279202																																															
Ret Time:		11.52																																															
Concentration:		0.22 ng																																															
Pk # and Type:		2 RTEINT used																																															
																																																	
<table border="1"><thead><tr><th>Signal</th><th>Ratios</th><th>Limits</th><th>RT</th><th>Limits</th><th>Resp</th><th>Integ</th><th>Type</th></tr></thead><tbody><tr><td>Tgt</td><td>116.00</td><td>100.0%</td><td></td><td>11.52</td><td>11.23</td><td>391</td><td>sys def</td></tr><tr><td>Q1</td><td>131.00</td><td>60.9</td><td>55.0- 82.5</td><td>11.52</td><td>to</td><td>238</td><td>sys def</td></tr><tr><td>Q2</td><td>145.00</td><td>24.8</td><td>20.8- 31.1</td><td>11.52</td><td>11.81</td><td>97</td><td>sys def</td></tr><tr><td>Q3</td><td>0.00</td><td>0.0</td><td>0.0- 0.0</td><td>0.00</td><td></td><td>0</td><td>sys def</td></tr></tbody></table>										Signal	Ratios	Limits	RT	Limits	Resp	Integ	Type	Tgt	116.00	100.0%		11.52	11.23	391	sys def	Q1	131.00	60.9	55.0- 82.5	11.52	to	238	sys def	Q2	145.00	24.8	20.8- 31.1	11.52	11.81	97	sys def	Q3	0.00	0.0	0.0- 0.0	0.00		0	sys def
Signal	Ratios	Limits	RT	Limits	Resp	Integ	Type																																										
Tgt	116.00	100.0%		11.52	11.23	391	sys def																																										
Q1	131.00	60.9	55.0- 82.5	11.52	to	238	sys def																																										
Q2	145.00	24.8	20.8- 31.1	11.52	11.81	97	sys def																																										
Q3	0.00	0.0	0.0- 0.0	0.00		0	sys def																																										

- 2) 0.1 ng/uL Standard - Selected Ion Monitoring, (0-11 minutes, m:z = 116, 145; 11-15 minutes, m:z = 116, 131, 145)

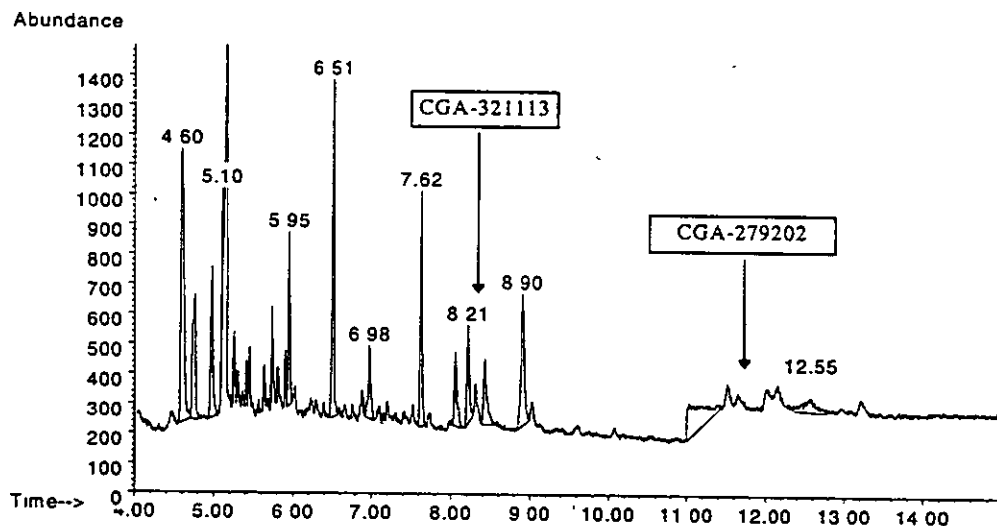
FIGURE 8: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF PEANUT HAY BY THE CONFIRMATORY GC/MS METHOD (Continued)



CGA-321113								CGA-279202							
Compound: CGA-321113								Compound: CGA-279202							
Ret Time: 8.36								Ret Time: 11.54							
Concentration: 0.99 ng								Concentration: 1.01 ng							
Pk # and Type: 1 RTINT used								Pk # and Type: 2 RTINT used							
															
Signal	Ratio	Limits		RT	Limits	Resp	Integ Type	Signal	Ratio	Limits		RT	Limits	Resp	Integ Type
Tgt	116.00	100.0%		8.36	8.14	7906	sys def	Tgt	116.00	100.0%		11.54	11.23	2232	sys def
Q1	145.00	6.6	5.3- 8.0	8.36	to	521	sys def	Q1	131.00	68.7	55.0- 82.5	11.55	to	1534	sys def
Q2	0.00	0.0	0.0- 0.0	0.00	8.56	0	sys def	Q2	145.00	25.9	20.8- 31.1	11.55	11.81	578	sys def
Q3	0.00	0.0	0.0- 0.0	0.00		0	sys def	Q3	0.00	0.0	0.0- 0.0	0.00		0	sys def

- 3) 0.5 ng/uL Standard, Selected Ion Monitoring, (0-11 minutes, m/z = 116, 145; 11-15 minutes, m/z = 116, 131, 145).

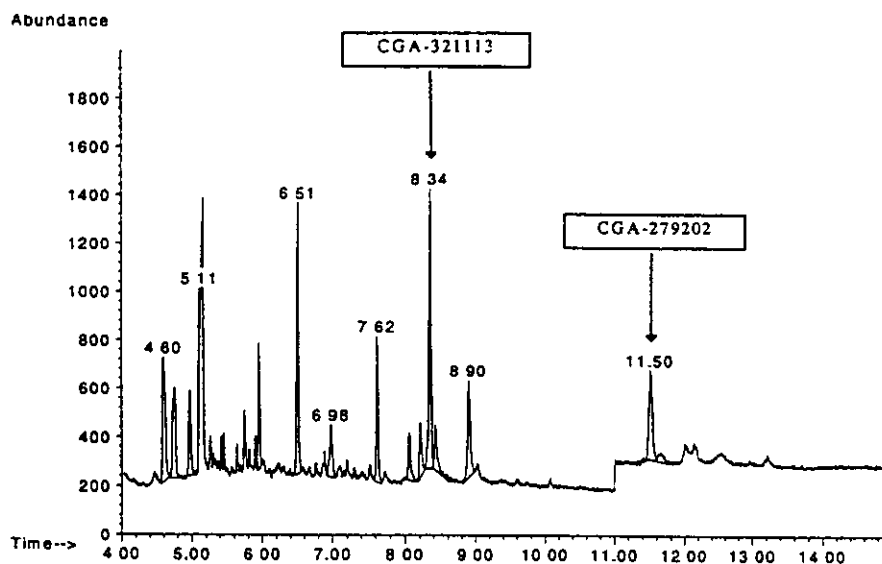
FIGURE 8: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF PEANUT HAY BY THE CONFIRMATORY GC/MS METHOD (Continued)

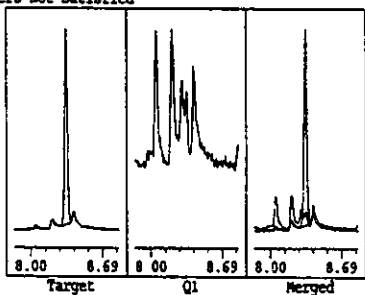


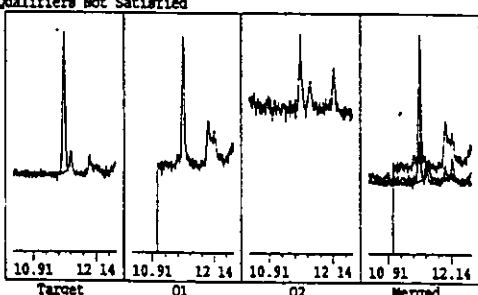
CGA-321113								CGA-279202							
Compound: CGA-321113 Ret Time: 8.34 Concentration: 0.04 ng Pk # and Type: 1 RTEINT used # Qualifiers Not Satisfied								Compound: CGA-279202 Ret Time: 11.48 Concentration: 0.06 ng Pk # and Type: 2 RTEINT used # Qualifiers Not Satisfied							
Signal	Ratio	Limits	RT	Limits	Resp	Integ	Type	Signal	Ratio	Limits	RT	Limits	Resp	Integ	Type
Tgt	116.00	100.0%		8.34	8.14	41	sys def	Tgt	116.00	100.0%		11.48	11.23	14	sys def
Q1	145.00	0.0	5.3-	8.0	0.00	to	0 sys def	Q1	131.00	0.0	55.0-	82.5	0.00	to	0 sys def
Q2	0.00	0.0	0.0-	0.0	0.00	8.56	0 sys def	Q2	145.00	0.0	20.8-	31.1	0.00	11.81	0 sys def
Q3	0.00	0.0	0.0-	0.0	0.00		0 sys def	Q3	0.00	0.0	0.0-	0.0	0.00		0 sys def

- 4) Sample 134A, Control Peanut Hay, Selected Ion Monitoring, (0-11 minutes, m/z = 116, 145; 11-15 minutes, m/z = 116, 131, 145), 7.97 ng injected, <0.2 ng CGA-321113 found, <0.05 ppm, <0.2 ng CGA-279202 found, <0.05 ppm.

FIGURE 8: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF
PEANUT HAY BY THE CONFIRMATORY GC/MS METHOD
(Continued)



CGA-321113																																																						
Compound		CGA-321113																																																				
Ret Time		8.34																																																				
Concentration		0.27 ng																																																				
Pk # and Type		1 RTKINT used																																																				
# Qualifiers Not Satisfied																																																						
																																																						
<table><thead><tr><th></th><th>Signal</th><th>Ratios</th><th>Limits</th><th>RT</th><th>Limits</th><th>Resp</th><th>Integ</th><th>Type</th></tr></thead><tbody><tr><td>Tgt</td><td>116.00</td><td>100.0%</td><td></td><td>8.34</td><td>8.14</td><td>1993</td><td></td><td>sys def</td></tr><tr><td>Q1</td><td>145.00</td><td>3.2</td><td>5.3- 8.0</td><td>8.35</td><td>to</td><td>63</td><td></td><td>sys def</td></tr><tr><td>Q2</td><td>0.00</td><td>0.0</td><td>0.0- 0.0</td><td>0.00</td><td>8.56</td><td>0</td><td></td><td>sys def</td></tr><tr><td>Q3</td><td>0.00</td><td>0.0</td><td>0.0- 0.0</td><td>0.00</td><td></td><td>0</td><td></td><td>sys def</td></tr></tbody></table>											Signal	Ratios	Limits	RT	Limits	Resp	Integ	Type	Tgt	116.00	100.0%		8.34	8.14	1993		sys def	Q1	145.00	3.2	5.3- 8.0	8.35	to	63		sys def	Q2	0.00	0.0	0.0- 0.0	0.00	8.56	0		sys def	Q3	0.00	0.0	0.0- 0.0	0.00		0		sys def
	Signal	Ratios	Limits	RT	Limits	Resp	Integ	Type																																														
Tgt	116.00	100.0%		8.34	8.14	1993		sys def																																														
Q1	145.00	3.2	5.3- 8.0	8.35	to	63		sys def																																														
Q2	0.00	0.0	0.0- 0.0	0.00	8.56	0		sys def																																														
Q3	0.00	0.0	0.0- 0.0	0.00		0		sys def																																														

CGA-279202																																																						
Compound		CGA-279202																																																				
Ret Time		11.50																																																				
Concentration		0.29 ng																																																				
Pk # and Type		2 RTKINT used																																																				
# Qualifiers Not Satisfied																																																						
																																																						
<table><thead><tr><th></th><th>Signal</th><th>Ratios</th><th>Limits</th><th>RT</th><th>Limits</th><th>Resp</th><th>Integ</th><th>Type</th></tr></thead><tbody><tr><td>Tgt</td><td>116.00</td><td>100.0%</td><td></td><td>11.50</td><td>11.23</td><td>551</td><td></td><td>sys def</td></tr><tr><td>Q1</td><td>131.00</td><td>104.4</td><td>55.0- 82.5</td><td>11.52</td><td>to</td><td>575</td><td></td><td>sys def</td></tr><tr><td>Q2</td><td>145.00</td><td>33.2</td><td>20.8- 31.1</td><td>11.50</td><td>11.81</td><td>183</td><td></td><td>sys def</td></tr><tr><td>Q3</td><td>0.00</td><td>0.0</td><td>0.0- 0.0</td><td>0.00</td><td></td><td>0</td><td></td><td>sys def</td></tr></tbody></table>											Signal	Ratios	Limits	RT	Limits	Resp	Integ	Type	Tgt	116.00	100.0%		11.50	11.23	551		sys def	Q1	131.00	104.4	55.0- 82.5	11.52	to	575		sys def	Q2	145.00	33.2	20.8- 31.1	11.50	11.81	183		sys def	Q3	0.00	0.0	0.0- 0.0	0.00		0		sys def
	Signal	Ratios	Limits	RT	Limits	Resp	Integ	Type																																														
Tgt	116.00	100.0%		11.50	11.23	551		sys def																																														
Q1	131.00	104.4	55.0- 82.5	11.52	to	575		sys def																																														
Q2	145.00	33.2	20.8- 31.1	11.50	11.81	183		sys def																																														
Q3	0.00	0.0	0.0- 0.0	0.00		0		sys def																																														

- 5) Sample 136A, Control Peanut Hay + 0.05 ppm CGA-321113 and 0.05 ppm CGA-279202, Selected Ion Monitoring, (0-11 minutes, m/z = 116, 145; 11-15 minutes, m/z = 116, 131, 145), 7.97 mg injected, 0.28 ng CGA-321113 found, 0.035 ppm, 69% recovered, 0.29 ng CGA-279202 found, 0.037 ppm, 73% recovered. Note: Ratios skewed by the contribution of control peaks; data is accepted.

VII. REFERENCES

1. Campbell, D. D., "Validation of Draft Analytical Method AG-659: Analytical Method for the Determination of Residues of CGA-279202 and the Acid Metabolite CGA-321113, in Crops and Animal Substrates by Gas Chromatography, Including Validation Data", Novartis Crop Protection Protocol 276-96, Project Number 433000, June 27, 1996.
2. Alemanni, A., "Preparation of Animal Tissues/ Products for Residue Analysis", Novartis Crop Protection SOP Number 7.27, Revision 2, November 11, 1994, MRID# 42928403.
3. Sprink, J., "Preparation of Crop Samples For Residue Analysis", Novartis Crop Protection SOP Number 7.21, Revision 1, July 16, 1992, MRID# 42885707. NOTE: Since the time of this study, this SOP has been revised to: Moore, M. E., "Preparation of Crop Samples for Residue Analysis", Novartis Crop Protection SOP Number 7.21, Revision 2, February 25, 1997
4. Nicollier, G., "Metabolism of CGA-279202 in Greenhouse Grown Apple Trees After Application of [Glyoxyl-Phenyl-(u)-¹⁴C] Labelled Material." Novartis Crop Protection (Basle, Switzerland) Study Number 95GN30, September 21, 1997.
5. Rezaaiyan, R., "Uptake and Metabolism of CGA-279202 in Field grown Peanuts After Spray Treatment with ¹⁴C-Phenyl(A)-CGA-279202 and ¹⁴C-Phenyl(B)-CGA-279202," Novartis Crop Protection Protocol Number 75-95, In Progress.
6. Stingelin, J., "Behavior and Metabolism of CGA-279202 in Greenhouse Grown Cucumbers After Treatment with [Glyoxyl-Phenyl-(u)-¹⁴C] Labelled Material." Novartis Crop Protection (Basle, Switzerland) Study Number 96JS23, October 14, 1997.
7. Rumbeli, R., "The Metabolism of [Trifluormethyl-Phenyl-(U)-¹⁴C] CGA-279202 after Multiple Oral Administration to Lactating Goats", Novartis Crop Protection (Basle, Switzerland) Study Number 021AM03, August 27, 1997.

VII. REFERENCES (Continued)

8. Rumbeli, R., "The Metabolism of [Trifluormethyl-Phenyl-(U)-¹⁴C] CGA-279202 after Multiple Oral Administration to Laying Hens", Novartis Crop Protection (Basle, Switzerland) Study Number 021AM04, In-Progress.

EPA ADDENDUM

PP#8F04955

Novartis Method AG-659A

Conditions and Substitutions used by ACB

1 ACB used a Hewlett-Packard 5890 Series II Gas Chromatograph (GC) equipped with a nitrogen-phosphorus detector (NPD) and operated under the same conditions as directed in the analytical method

2a TurboVap®II and TurboVap®LV (Zymark Corporation, Hopkinton, MA) concentrators operated at 35 °C under a nitrogen stream were used in place of the vacuum rotary evaporators to concentrate samples during work-up. This substitution completely eliminated the need to monitor the samples during concentration steps against any bumping and foaming potential that was forewarned in the method had the rotary evaporator been used.

2b Most of the extracts were reconstituted to a 1 mL final volume (for some commodities, a larger final volume was necessary) before sample analysis which left little room for dilution errors. ACB also used a vortex mixer to homogenize the final sample extracts just prior to aliquoting into autosampler vials for instrument analysis. Several initial data sets yielded unexpectedly high recoveries. On closer inspection, it was discovered that the samples from these high recovery sets may have actually been concentrating during the vortexing of the final extract. Subsequent data sets (and re-extraction of high recovery sets) were then reconstituted by dilution to the mark in **calibrated** graduated concentration tubes before an aliquot was removed for sample analysis. This technique proved to yield more confidence in the final extract volumes and in turn the recovery data determined as well.

3 The method suggests the use of calibration curves to determine sample concentrations. ACB determined sample concentrations from a ratio of sample responses to the average of standard responses that bracketed the samples. All other parameters were followed as written in the method.

ACB's Recommended Changes to the Method

4 Due to the interferences levels found in the peanut hay control samples by the registrant, the ILV and ACB, we recommend that the LOQ be raised to 0.2 ppm for this substrate only. The proposed tolerance (4 ppm) does not need to be altered.

5 Care needs to be taken to ensure the accuracy of the final extract volumes due to the volatility of acetone used as the diluent.

6 TurboVap® concentrators or other equivalent evaporators should be referenced for permitted use to eliminate the bumping and/or foaming concerns inherent with the vacuum rotary evaporator.