CK-244-042

DATE SUBMITTED: NO.: VOLUME:

BASF Agro Research
P. O. Box 400
Princeton, NJ 08543-0400

REPORT NUMBER

RES 99-151

STUDY TITLE

CL 303630 (chlorfenapyr): Independent Laboratory Validation of GC Determinative and GC/MS Confirmatory Method M 2686 for the Determination of CL 303630 Residues in Various Fruits (such as Stone Fruits, Pome Fruits, Strawberries, and Grapes)

DATA REQUIREMENTS
40 CFR 158.240
EPA Residue Chemistry Test Guidelines
OPPTS 860.1340 (171-4)

AUTHOR
Huns Nejad

STUDY COMPLETION DATE

June 28, 2001

PERFORMING LABORATORY
ABC Laboratories, Inc.
7200 East ABC Lane
Columbia, MO 65202-8015

LABORATORY PROJECT IDENTIFICATION
BASF Protocol XD96PT03
ABC Laboratories, Inc. Project ID #43899

Total Number of Pages
89

(This page left intentionally blank
for Statement of Confidentiality Claim)

BASF Agro Research
Residue Sciences
Princeton, New Jersey

STATEMENT OF GLP COMPLIANCE

Report No.: RES 99-151
Protocol No.: XD96PT03

This study was conducted in accordance with Good Laboratory Practice (GLP) Regulation 40 CFR part 160 established by the Federal Insecticide, Fungicide, and Rodenticide Act.

Huns Nejad — 6/28/01

Huns Nejad
Study Director — Date

Richard Braddock — 6/28/01

Richard Braddock
Senior Group Leader — Date

Submitter — Date

BASF AGRO RESEARCH

Study Number: XD96PT03 **QAU File Number:** 970064

Study Title: Independent Laboratory Validation of GC Determinative and GC/MS Confirmatory Method M 2686 for the Determination of CL 303,630 Residues in Various Fruits (such as Stone Fruits, Pome Fruits, Strawberries, and Grapes)

Testing Facility: BASF Agro Research
P.O. Box 400
Princeton, NJ 08543-0400

Study Director: H. Nejad

Quality Assurance Unit Statement

Pursuant to Good Laboratory Practice regulations, this statement verifies that the aforementioned study was inspected/audited and the audit findings were reported to management, study director, and principal investigator (if appplicaple) by the Quality Assurance Unit on the dates shown below. These inspections/audits are in addition to those performed by the test site(s) QAU:

#	Phase Inspected	Trial Number	Audit Start Date	Audit End Date	Total Days	Date Reported
1	PR	N/A	07-Apr-1997	07-Apr-1997	1	07-Apr-1997
2	RP	N/A	08-Feb-2000	09-Feb-2000	2	10-Feb-2000
3	RV	N/A	01-Jun-2001	01-Jun-2001	1	01-Jun-2001

DA	Data Audit	RP	Report Audit	SP	Sample/Specimen Activities
FA	Facility Audit	RV	Report Verification	SY	Test System Activities
PR	Protocol Audit	SI	Special Audit	TS	Test Substance Activities
AD	Test Substance Administration Activities				

Jennifer P. Leonard
Group Leader, Quality Assurance
BASF Agro Research
Princeton, New Jersey

June 13 2001
Date

Report No.:	RES 99-151	Study Initiation Date:	97MAR19
Type of Report:	Final	Exptl. Start Date:	97APR01
Type of Study:	Independent Laboratory Validation	Exptl. Termination Date:	97APR16
Protocol No.:	XD96PT03	Study Completion Date:	01JUN28
Reported By:	Huns Nejad	Report Issue Date:	01JUN28

TITLE

CL 303630 (chlorfenapyr): Independent Laboratory Validation of GC Determinative and GC/MS Confirmatory Method M 2686 for the Determination of CL 303630 Residues in Various Fruits (such as Stone Fruits, Pome Fruits, Strawberries, and Grapes)

PURPOSE

To have ABC Laboratories, Inc. conduct an independent laboratory validation of method M 2686 for the determination of CL 303630 residues in various fruits (such as stone fruits, pome fruits, strawberries, and grapes).

SUMMARY

This study was conducted according to BASF Agro Research Protocol XD96PT03 (Appendix A) in accordance with EPA PR Notice 96-1. Method M 2686 (Appendix B) was successfully validated by ABC Laboratories, Inc. and was found to be satisfactory for the determination of CL 303630 residues in various fruits. The validated sensitivity (LOQ, limit of quantitation) of the method is 0.05 ppm.

Recoveries were run by fortifying various untreated fruits with solutions of analytical grade CL 303630. Fortification levels were 0.00 (control). 0.05, 0.10 and 0.50 ppm. The first trial produced acceptable recoveries for peaches, plums, prunes, cherries. pears, apples, strawberries and grapes. Detailed analytical data for CL 303630 recoveries are presented in Tables I - VIII of ABC Laboratories, Inc. Report #43899 (Appendix C).

Overall, sixty-four analyses were conducted. For each fruit type, eight analyses were conducted consisting of two unfortified samples and six fortified samples. The overall average recovery and standard deviation of the forty-eight analyses of fortified fruit was

94 ± 9% (Table I). Mass spectrometric confirmation was performed on the controls and the control samples fortified at 0.05 ppm.

Method M 2686 was followed as written by ABC Laboratories, Inc. with minor modifications made to the gas chromatographic conditions for some commodities. There was no communication regarding the validation of the method between personnel at ABC Laboratories, Inc. and BASF prior to or during the first trial. The highlights of the communication between the Study Director and ABC Laboratories, Inc. have been summarized and included in Appendix D.

Key Personnel Involved in Study XD96PT03

Study Director	Huns Nejad	BASF
Sr. Group Leader	Phillip Miller (3/97-12/98)	BASF
Sr. Group Leader	Robert Herrick (1/99-2/1/00)	BASF
Group Leader	Timothy Mahl (2/1/00 – 1/5/01)	BASF
Sr. Group Leader	Richard Braddock (1/5/01 – present)	BASF
Principal Analyst	Richard F. Kennedy	ABC Laboratories, Inc.
Scientist	Leanne Forbis	ABC Laboratories, Inc.
Technical Scientist	Carol Doerge	ABC Laboratories, Inc.
Associate Technician	J. Craig Harris	ABC Laboratories, Inc.
Senior Technician	Martha A. Pezold	ABC Laboratories, Inc.
Associate Technician	Thomas P. Sanders	ABC Laboratories, Inc.
Assistant Technician	Deborah Kerr	ABC Laboratories, Inc.

ARCHIVING STATEMENT

All original raw data, protocol, amendments, appropriate samples, documentation and records, related to this study and the final report are stored in the Archives of BASF Agro Research, Princeton, New Jersey. Original site-specific raw data are archived at the appropriate facilities.

GLP COMPLIANCE

A statement of compliance which is signed by the Study Director and Sponsor Representative is included on page 3.

STATEMENT OF STUDY INTEGRITY

There were no known circumstances that may have adversely affected the quality or integrity of the data in this study.

QUALITY ASSURANCE

A Quality Assurance statement from the Quality Assurance Unit of ABC Laboratories, Inc. can be found on page 28 of Appendix C. A Quality Assurance statement from the BASF Quality Assurance Unit is included on page 4 of this report.

Huns Nejad — 6/28/01

Huns Nejad
Study Director — Date

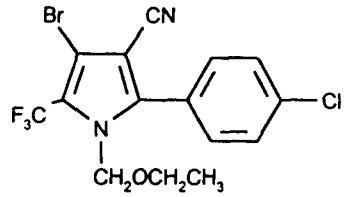
Richard Braddock — 6/28/01

Richard Braddock
Senior Group Leader — Date

/ct

TABLE OF CONTENTS

Table of Compound



CL 303630

4-bromo-2-(4-chlorophenyl)-1-(ethoxy-methyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile

Table A: Overall Average Percent Recovery and Standard Deviation for Residues of CL 303630 in Various Fruits

$$Average = \bar{x} = \frac{\sum_i x_i}{n} = 94\%$$

$$Standard\ Deviation = s = \sqrt{\frac{\sum_i (x_i - \bar{x})^2}{n-1}} = 9.0\%$$

% Recoveries = x =	Peaches:	102, 143, 80, 81, 98, 98
	Plums:	99, 99, 91, 92, 93, 92
	Prunes:	90, 88, 84, 87, 91, 91
	Cherries:	101, 96, 88, 82, 87, 87
	Pears:	95, 97, 94, 94, 93, 93
	Apples:	95, 96, 90, 92, 94, 94
	Strawberries	95, 101, 90, 92, 93, 96
	Grapes:	104, 102, 90, 91, 91, 93

number of recoveries = n = 48

APPENDIX A

Protocol XD96PT03 and Amendments

CYANAMID

RESIDUE SUPPORT STUDY PROTOCOL

Distribution: ES File
Quality Assurance Unit
Those Signing Protocol
Laboratory Personnel
Residue Chemistry Manager

PROTOCOL NUMBER: XD96PT03

PROJECT NUMBER: 593

TITLE: Independent Laboratory Validation of GC Determinative and GC/MS Confirmatory Method M 2686 for the Determination of CL 303,630 Residues in Various Fruits (such as Stone Fruits, Pome Fruits, Strawberries, and Grapes)

PURPOSE: To have ABC Laboratories, Inc. conduct an independent laboratory validation in accordance with PR Notice 96-1 (Attachment I) of method M 2686 for the determination of CL 303,630 residues in Various Fruits (such as Stone Fruits, Pome Fruits, Strawberries, and Grapes).

SPONSOR/TEST FACILITY:

American Cyanamid Company
Agricultural Products Research Division
P.O. Box 400
Princeton, NJ 08543-0400
Telephone Number: (609) 716-2000

TEST SITE:

ABC Laboratories, Inc.
7200 East ABC Lane
Columbia, MO 65202-8015
Telephone Number: (573) 474-8579

PERSONNEL:

Study Director:	Huns Nejad American Cyanamid Company
Principal Analyst:	Richard F. Kennedy ABC Laboratories, Inc.
Other Laboratory Personnel:	Steven J. Stout American Cyanamid Company
	Adrian daCunha American Cyanamid Company
Quality Assurance:	American Cyanamid Quality Assurance Unit
	ABC Laboratories, Inc. Quality Assurance Unit

TEST MATERIAL:

Analytical standard of the following:

CL 303,630, Lot No. AC 9389-90, purity of 99.7%, expiration date 4/13/01

1. Characterization data for the test materials is on file with the Analytical, Physical and Biochemical Research (APBR) section of the American Cyanamid Company, Agricultural Products Research Division, Princeton, New Jersey.

2. The test material has been shown to be soluble under the conditions of the study as described in the analytical method and the raw data stored in the Archives of Cyanamid Agricultural Research Center, Princeton, New Jersey (Reference Report RES 93-039).

3. Solutions of the test material have been shown to be stable under the conditions of the study and the raw data stored in the Archives of Cyanamid Agricultural Research Center, Princeton, New Jersey (Reference Report RES 93-039).

TEST SYSTEM:

1. Sample Type: Various Fruits (such as Stone Fruits, Pome Fruits, Strawberries, and Grapes)

2. Source: Control Peaches (AC 10555.72)
   Control Plums (AC 10555.48)
   Control Prunes (AC 10555.49)
   Control Cherries (AC 10555.46)
   Control Pears (AC 9088.63)
   Control Apples (AC 10555.44)
   Control Strawberries (AC 10555.62)
   Control Grapes (AC 10555.61)

   Samples will be obtained from American Cyanamid Company Sample Preparation Laboratory.

3. Justification: Various fruits (such as stone fruits, pome fruits, strawberries, and grape) commodities resulting from fruit field samples treated with CL 303,630 may contain CL 303,630 residues. A residue method needs to be validated for measuring CL 303,630 residues in various fruits (such as stone fruits, pome fruits, strawberries, and grape) commodities.

4. Procedure for Identification: Samples will be labeled with indelible ink to unequivocally identify them by sample type and fortification level.

METHOD OF ANALYSIS:

American Cyanamid Company Method M 2686, Draft Dated 3/18/97, entitled: CL 303,630 (chlorphenapyr): GC Determinative and GC/MS Confirmatory Method for the Determination of CL 303,630 Residues in Various Fruits (such as Stone Fruits, Pome Fruits, Strawberries, and Grapes)

TIME FRAME:

Proposed Experimental Start Date: March 24, 1997

Proposed Experimental Completion Date: September 24, 1997

EXPERIMENTAL DESIGN:

1. Since the residue levels from treated samples are not known, add, by pipet, known amounts of the appropriate standard solution of CL 303,630 to control samples to give the following fortification levels. Run each fortification level in duplicate to cover a range of possible residue levels:

CL 303,630 Fortification Levels (ppm)

0.00 ppm (control)
0.05 ppm
0.10 ppm
0.50 ppm

2. Follow method M 2686, draft dated 3/17/97, as written and determine the percent recovery by dividing the amount found by the amount of standard added. No modifications to the method are to be made.

3. If the majority of the recoveries do not fall in the range of 70-120%, the Study Director should be notified to determine the cause of the unacceptable recovery values using the following guidelines:

   a. The laboratory may contact the Study Director, developers or previous users of the method prior to the analysis of the first set of samples; however, all communications must be documented in the final report. The laboratory conducting the validation trial will not contact the sponsor during the analysis of the first set of samples (see Attachment I for a copy of PR Notice 96-1).

   b. If this set, or subsequent sample sets, are unsuccessful, the laboratory may contact the developer of the method and/or Study Director of the method validation. This communication is to be documented in the final report. Any modifications or additions to the method will be incorporated into the method write-up that is sent to the EPA for validation.

   c. If after three attempts, the validation trial has failed the established criteria, a new method must be submitted for another independent laboratory validation trial.

4. At least one control and one control sample fortified at the validated sensitivity (LOQ, limit of quantitation) of the method for each fruit commodity, along with a 0.01 mcg/mL standard, will be packed in ice and sent to American Cyanamid Company where they will be analyzed for mass spectrometric confirmation.

HANDLING OF DATA:

All data related to the conduct of this study will be maintained by ABC Laboratories, Inc. during the course of the experimental work and writing of the analytical report. Prior to the completion of the study, all the original raw data will be sent to American Cyanamid Company where it will be stored in the Archives along with the final report. ABC Laboratories, Inc. shall retain a copy of the raw data. Original site-specific raw data (such as CVs or temperature logs) will be archived at ABC Laboratories, Inc. Certified true copies of these data will be stored at American Cyanamid.

Records to be maintained for this study include the following (as appropriate):

1. Study protocol, amendments and deviations.
2. Documentation for preparation of standard solutions.
3. Any modifications to the analytical method.
4. All chromatograms and instrumental conditions.
5. Analytical data tables.
6. Sample extraction and analysis dates.
7. List of study personnel, signatures and initials.
8. Laboratory notebook(s) and/or worksheets.
9. Chronological collection of study correspondence and phone logs.
10. Sample tracking sheet.
11. All other data necessary for the reconstruction of the study.

STATISTICAL METHODS:

The overall average and standard deviation for the fortified recoveries will be calculated and included in the final report.

REPORTING OF RESULTS:

A validation report should be written which presents the results from this validation study and shows the recovery values, the mean recovery and the standard deviation of all the recoveries.

PROTOCOL AMENDMENTS AND DEVIATIONS:

All changes in or revisions to the approved protocol and the reasons for the changes shall be documented in the form of a protocol amendment or deviation. Each protocol amendment or deviation shall be signed and dated by the Study Director and appropriate Group Leader, and maintained with the protocol. Any protocol amendment or deviation shall include the reason for change and its effect on the outcome of the study.

QUALITY ASSURANCE:

Quality assurance of the in-life phase of the study shall be the responsibility of the QA Unit of ABC Laboratories, Inc. and shall be carried out in accordance with applicable GLP regulations and Standard Operating Procedures of the Test Site. The QA Unit of ABC Laboratories, Inc. must provide timely written reports of all inspections to the Study Director and American Cyanamid Company Management. A statement signed by the Quality Assurance manager or designee, listing the phases inspected and the inspection dates, will be included in the analytical phase report. The Quality Assurance Unit of American Cyanamid Company will review the protocol and the final report and will provide an additional Statement of Quality Assurance.

GLP COMPLIANCE:

This study will be performed in compliance with the Good Laboratory Practice Standards as specified in 40 CFR Part 160. The Principal Analyst will sign a Statement of Compliance in regard to that portion of the study conducted by him. A Statement of Compliance or non-compliance with Good Laboratory Practices will be signed by the Study Director and Group Leader at the conclusion of the study.

APPROVALS:

Residue Chemistry Group Leader:

P. Miller — 3-19-97 (Date)

Study Director:

H. Nejad — 3/19/97 (Date)

ATTACHMENT I

PR Notice 96-1

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Pesticide Regulation (PR) NOTICE 96-1
February 7, 1996

NOTICE TO MANUFACTURERS, FORMULATORS, PRODUCERS AND REGISTRANTS OF PESTICIDES PRODUCTS

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

ATTENTION: **Persons Responsible for Federal Registration of Pesticides or Submission of Petitions for Tolerances or Exemptions from Tolerances for Pesticides**

SUBJECT: **TOLERANCE ENFORCEMENT METHODS - INDEPENDENT LABORATORY VALIDATION BY PETITIONER**

Since the issuance of PR Notice 88-5 (7/15/88), EPA scientists have reviewed many Independent Laboratory Validation (ILV) trials and provided guidance on conducting these trials to registrants of pesticide products. This notice is intended to clarify the requirements for submission of an Independent Laboratory Validation to accompany new pesticide analytical methods and does not contain additional data requirements. This notice supersedes PR Notice 88-5.

I. BACKGROUND

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) requires the registration of all pesticides. If a proposed use of a pesticide results in residues in or on raw agricultural commodities or processed foods/feeds, a tolerance (or an exemption from a tolerance) is required under the Federal Food, Drug, and Cosmetic Act (FFDCA). Any organization seeking a tolerance must petition the Agency to establish the tolerance.

EPA regulations in 40 CFR §158.240, 180.7 and 180.34 require petitioners for pesticide tolerances to furnish adequate residue analytical methods to determine the total toxic residue for pesticides in or on raw agricultural commodities, and as appropriate, in processed foods/feeds. The total toxic residue includes the parent pesticide and its degradation products, metabolites (free or bound), and impurities which are of toxicological concern. These methods enable the Agency to establish tolerances after determining the maximum pesticide residues of concern which could be present in or on treated raw agricultural commodities or processed foods/feeds. The analytical methods are subsequently used by the Food and Drug Administration, U.S. Department of Agriculture and individual States for tolerance enforcement. Guidance on analytical method data requirements is provided in the Pesticide Assessment Guidelines (Subdivision O, Reference 171-4). EPA's review of

pesticide petitions for tolerances includes validation of new methods in EPA laboratories to ensure their suitability for enforcement purposes. An enforcement method must be suitable for use in Federal and State laboratories throughout the country. Moreover, sufficient information must be submitted about the analytical method to permit a competent analyst to apply it successfully.

Prior to the issuance of PR Notice 88-5, EPA method validation trials were often impeded by poorly written and incomplete descriptions of the analytical procedures submitted by petitioners. This created delays in evaluating petitions and unnecessarily tied up Agency laboratory resources while the methods were rewritten and consultations occurred between EPA representatives and the method developers. Since the issuance of PR Notice 88-5, residue analytical methods submitted to the Agency which require Agency method validation have dramatically improved. However, the Agency recognizes that some clarification of the Independent Laboratory Validation trial data requirements is needed. The intent of this notice is to clarify the requirements for submission of an Independent Laboratory Validation trial to accompany new pesticide analytical methods.

II. DISCUSSION

A. When An Independent Laboratory Validation (ILV) Trial Is Required:

Results of an ILV study must accompany the following types of submissions:

1. The first tolerance petition (including those for temporary tolerances) for residues of a pesticide in/on a raw agricultural commodity, or a processed food/feed.

2. Any new tolerance for residues of a pesticide with previously established tolerances if a new method is proposed for enforcement or if the previously approved enforcement method has been significantly modified to accommodate the new commodity. If the registrant is uncertain whether a method change is "significant", the Agency should be consulted.

B. When An Independent Laboratory Validation (ILV) Trial Is Not Required:

1. Results of an ILV trial are usually not required for an enforcement analytical method which the Agency deems superior to the currently accepted enforcement method.

2. An ILV trial is not normally required for confirmatory methods. However, at the discretion of the Agency, an ILV trial may be required for these methods on a case-by-case basis.

C. Criteria for Selection of Independent Laboratory

The registrant should select an independent laboratory based on the same performance standards as for any other scientific project. The laboratory facility chosen to conduct the ILV trial may be a State enforcement agency laboratory, a university laboratory, or a privately owned laboratory which may include one in the registrant's organization. In order to provide an unbiased evaluation the laboratory chosen to conduct the ILV trial must not have participated in the development of the original method and must not use the same equipment, instruments and supplies. Furthermore, personnel conducting the ILV trial should not report to a study director who was involved in development, validation or subsequent use of the method.

D. Requirements for Independent Laboratory Validation Trial

The Independent Laboratory Validation trials must be conducted under FIFRA Good Laboratory Practice standards as specified in 40 CFR §160. The method must be performed as written with no significant modifications. A successful ILV trial will require adequate results for the total toxic residue on at least one set of samples. The laboratory conducting the ILV will be allowed to test up to three sets of samples by the method on a given commodity. The number of sets is limited to three in order to provide an impartial evaluation by an analyst inexperienced with this particular method. If additional commodities are analyzed by the same method, they will be considered to be separate ILV trials.

A set of samples consists of two control samples, two control samples fortified at the proposed tolerance, and two control samples fortified at the Limit of Quantitation (LOQ). If the tolerance is proposed at the LOQ, the second fortification level should be twice the LOQ. At the discretion of the registrant, one additional fortification at another level may be included in the set of samples.

The laboratory conducting the ILV trial may contact the developers or previous users of the method prior to running the first set of samples; however, all communications must be logged and reported to EPA. Under no circumstances should personnel familiar with the method visit the independent laboratory to observe or offer help. If minor changes are made to the original

method resulting in improved performance as a result of the ILV trial, a new trial for the amended method will not be necessary. Any subsequent additions or modifications to the original method shall be incorporated into the method write-up that is sent to the EPA for validation.

If tolerances for several commodities are proposed, and one method is to be used for all of the commodities a rationale for the selection of the test commodity should be provided. If the same method is used for both plant and animal commodities, then separate ILV trials should be run on both plant and animal matrices. If after three sets, the validation is not successful, a new method must be submitted for another ILV trial.

An ILV trial will be considered successful if the results of the study satisfy the requirements in Subdivision O of the Residue Chemistry Guidelines, i.e., the recovery rates should be 70-120% and interference should be negligible compared to the proposed tolerance level.

E. Information to be Reported to the Agency

If the ILV trial is successful, the following should be submitted by the petitioner.

1. Address and contact person for the independent laboratory.

2. Description of the analytical method.

3. All recovery and control values for all commodities that were obtained during all ILV trials.

4. Representative chromatograms for all ILV trials performed.

5. Description of the instruments used and operating parameters.

6. Description of any problems encountered.

7. Any steps considered critical, i.e., steps where little variation is allowable or directions must be precisely followed.

8. The number of person-hours required to complete one set of samples.

9. The number of calendar days required for one set of samples.

10. Any contact between the independent laboratory and the method developers or others familiar with the method, including the reasons for the contact, any changes in the method that resulted, and the time of this communication with respect to the progress of the ILV trial (i.e. after the first set, during the second set, etc).

F. The Agency will Continue to Conduct Method Validation

If the Agency determines that the petitioner has submitted a successful ILV trial, the method will be validated by the Agency.

III. Effective Date

All submissions received by the Agency after 2/7/97 must include the results of an Independent Laboratory Validation trial conducted according to this Notice. Submissions received prior to that date need to adhere to the requirements set forth in PR Notice 88-5.

IV. For Further Information

Persons wishing further information on this notice may contact:

Dallas P. Wright, Jr.
U.S. Environmental Protection Agency
Analytical Chemistry Laboratory
Building 306, Room 113, ARC-East
Beltsville, MD 20705

Telephone Number: 301-504-8225

OR

Francis D. Griffith, Jr.
U.S. Environmental Protection Agency
Health Effects Division (7509C)
401 M Street, S.W.
Washington, DC 20460

Telephone Number: 703-305-5826

Daniel M. Barolo, Director
Office of Pesticide Programs

CYANAMID

RESIDUE SUPPORT STUDY PROTOCOL AMENDMENT

Distribution: ES File
Quality Assurance Unit
Laboratory Personnel
Those Signing Protocol
Residue Chemistry Manager

PROTOCOL NUMBER: XD96PT03

AMENDMENT NUMBER: 01

TITLE: Independent Laboratory Validation of GC Determinative and GC/MS Confirmatory Method M 2686 for the Determination of CL 303,630 Residues in Various Fruits (such as Stone Fruits, Pome Fruits, Strawberries, and Grapes)

AMENDMENT(S) TO BE MADE:

In part 2 of the experimental design, 3/18/97 is the correct date for the draft method M 2686.

REASON FOR AMENDMENT:

Entry error

IMPACT ON STUDY:

There will be no impact on the study.

APPROVALS:

Residue Chemistry I
Sr. Group Leader: Phillip Miller — Date: 05/14/97

Study Director: Huns Nejad — Date: 05/14/97

BASF

Distribution: Residue Science File
Quality Assurance Unit
Laboratory Personnel
Those Signing Protocol

PROTOCOL AMENDMENT

Study No.: **XD96PT03**

Amendment No.: **02**

Description of Amendment

To change all references in the protocol that are affected by the acquisition of American Cyanamid by BASF.

The name of the sponsor is changed to:	☑ BASF Agro Research P.O. Box 400 Princeton, NJ 08543-0400	☐ Remains the same
The name of the test facility is changed to:	☑ BASF Agro Research P.O. Box 400 Princeton, NJ 08543-0400	☐ Remains the same
The name of the test site is changed to:	☐ BASF Agro Research P.O. Box 400 Princeton, NJ 08543-0400	☑ Remains the same

In addition, other references are changed as follows:

American Cyanamid and Cyanamid become BASF.
GAPRD and CARC become BASF Agro Research.

Reason for Amendment:

To update the respective corporate identities following the acquisition of American Cyanamid by BASF.

Impact on Study:

None.

SIGNATURE:

Huns Nejad — 6/4/01

Huns Nejad (Study Director) — Date

Richard Braddock — 6/4/01

Richard Braddock (Sponsor Representative) — Date

APPENDIX B

BASF Method M 2686

CYANAMID

AMERICAN CYANAMID COMPANY
AGRICULTURAL PRODUCTS RESEARCH DIVISION
ENVIRONMENTAL SCIENCES
P.O. BOX 400
PRINCETON, NEW JERSEY 08543-0400

Recommended Method of Analysis - M 2686

CL 303,630 (chlorphenapyr): GC Determinative and GC/MS Confirmatory Method for the Determination of CL 303,630 Residues in Various Fruits (such as Stone Fruits, Pome Fruits, Strawberries and Grapes)

A. Principle

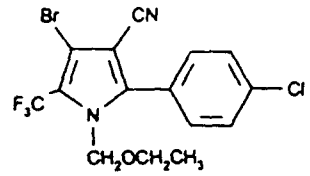
Residues of CL 303,630 are extracted from samples with methanol/Milli-Q water and purified using solid phase extraction techniques. Quantitation of CL 303,630 residues is accomplished by fused silica capillary gas chromatography equipped with an electron capture detector. Results are calculated as CL 303,630 by the direct comparison of peak heights to those of external standards. The validated sensitivity (LOQ, limit of quantitation) of this method is 0.05 ppm for each commodity.

CL 303,630 residues can be confirmed by using GC/mass spectrometry (chemical ionization mode) and selected ion monitoring at m/z 347^- and 349^-.

B. Reagents (Items from other manufacturers may be used, if they are proven to be functionally equivalent.)

1. Analytical Standard: CL 303,630, analytical grade of known purity. Obtained from American Cyanamid Company, Agricultural Products Research Division, P.O. Box 400, Princeton, New Jersey, 08543-0400.

CL 303,630: 4-bromo-2-(4-chlorophenyl)-1-(ethoxy-methyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile.



M. W. = 407.6

2. Solvents: B & J Brand High Purity Solvents, Baxter Healthcare Corporation, Muskegon, MI.

   a. Methanol
   b. Methylene Chloride
   c. Hexane
   d. Acetone

3. Water, Deionized: Water passed through Millipore's Milli-Q Plus Ultra Pure Water System. Use this water for all steps.

4. Solution: Extraction Solvent (15% Water in Methanol): Dilute 150 mL of Milli-Q water (B.3) to 1 liter with methanol in a 1 liter volumetric flask and mix well.

C. Apparatus (Items from other manufacturers may be used, provided they are **proven** to be functionally equivalent.)

1. Balance: Analytical, Sartorius, precision ± 0.05 mg.

2. Laboratory Glassware: General laboratory.

3. Flash Evaporator: Buchler Instruments, Model RE-0121C equipped with a heated water bath maintained at approximately 30°C, and a dry ice solvent trap.

4. Gas Chromatograph: A Hewlett Packard Model 5890 Series II instrument equipped with an inlet system for a capillary column and a Nickel-63 High Temperature Electron Capture detector.

5. Fused Silica Capillary Column: 15 m x 0.53 mm nominal I.D., SPB-20 bonded phase with film thickness of 1.0 micron, Supelco, Inc., Bellefonte, PA, Catalog Number 2-5332.

6. Recorder: Hewlett Packard, HP 3396A Integrator. (Must use real ink type recorders for GLP chromatogram long term storage.)

7. Plastic Syringe, Disposable: Luer-Lok, 30 mL capacity, Catalog Number 309662, Becton Dickinson & Co., Franklin Lakes, NJ.

8. Adaptors: IST (International Sorbent Technology) Isolute PTFE Column Adaptors, Distributed by Jones Chromatography, Lakewood, CO, Catalog Number 120-1100. (NOTE: Some adaptors from other suppliers may contain interfering contaminants which may give an EC response.)

9. Visiprep Solid Phase Extraction Manifold: Visiprep 24, Catalog Number 5-7250, distributed by Supelco, Inc., Bellefonte, PA.

10. Solid Phase Extraction Cartridges:

    a. International Sorbent Technology (IST) Isolute C-18 Cartridges (1g/6 mL), Catalog Number 220-0100-C, distributed by Jones Chromatography, Lakewood, CO.

    b. International Sorbent Technology (IST) Isolute Silica Cartridge (1g/6 mL), Catalog Number 460-0100-C, distributed by Jones Chromatography, Lakewood, CO.

11. Reservoir, Empty: 70-mL capacity, Catalog Number 120-1008 distributed by Jones Chromatography, Lakewood, CO.

12. Waring Blender: Model 31BL46 with 1-quart capacity glass blender jar, Waring Products Division, Dynamic Corp. of America, New Hartford, CT.

13. PowerStat Variable Auto Transformer: Input 120V, Output 0-140V, Catalog Number 62546-455, VWR Scientific Products Corporation, McGaw Park, IL .

14. Filter Paper: 9-cm diameter, glass fiber, 934-AH, Whatman Laboratory Division, Springfield Mill, Maidstone, Kent, England.

15. Filtering Flasks: 500-mL capacity, Corning Glass Works.

16. Filtering Funnel: Buchner, porcelain, 9-cm diameter.

17. Microliter Syringe: Hamilton, Model 701, 10-µL capacity.

18. Food Chopper: Hobart, Model 84185-D.

19. Mass Spectrometer: Finnigan Mat SSQ710.

20. Gas Chromatograph: Varian Model 3400.

21. GC Column: 15m x 0.25 mm, 0.25 micron DB-5MS film (J&W Scientific, Cat. No 122-5512).

D. Preparation of Standard Solutions (Prepare monthly, store in amber bottles in refrigerator.)

1. Stock Solution

Weigh accurately a known amount (approximately 10 mg) of CL 303,630 into a 100-mL volumetric flask. Dilute to the mark with acetone and mix well. Calculate and record the exact concentration of CL 303,630.

NOTE: Resulting concentration of the standard stock solution must be corrected for standard purity.

2. Fortification and Gas Chromatographic Solutions

a. Pipet into a 100-mL volumetric flask an appropriate amount of the Standard Stock Solution to deliver 1000 µg of CL 303,630. Dilute to the mark with acetone and mix well. This solution contains 10 µg/mL of CL 303,630.

b. Pipet into a 100-mL volumetric flask a 10-mL aliquot of the 10 µg/mL standard solution. Dilute to the mark with acetone and mix well. This solution contains 1 µg/mL of CL 303,630.

c. Pipet into a 100-mL volumetric flask a 10-mL aliquot of the 1 µg/mL standard solution. Dilute to the mark with hexane and mix well. This solution contains 0.1 µg/mL of CL 303,630.

d. Pipet into separate 100-mL volumetric flasks 2-, 1-, 0.5- and 0.25-mL aliquots of the 1 µg/mL standard solution. Dilute to the mark with hexane and mix well. These solutions contain 0.02, 0.01, 0.005 and 0.0025 µg/mL respectively, of CL 303,630 and are used for the linearity check. Use the 0.01 µg/mL solution as the working standard for both GC determinative and GC/MS Confirmatory analyses.

E. Gas Chromatographic Conditions

Operating conditions described below are provided as a guide to establish actual operating conditions and should be adjusted as necessary to obtain CL 303,630 peak shape and resolution from background peaks equivalent to or better than those shown in Figures 1 to 6.

1. Instrument: Hewlett Packard Model 5890 Series II gas chromatograph.

2. Detector: Nickel-63 High Temperature Electron Capture Detector.

3. Column: Fused silica capillary, 15 m x 0.53 mm I.D., SPB-20 with film thickness 1.0 micron.

4. Integrator: HP 3396A.

5. Instrument Conditions:

a.	Carrier Gas (Helium)	9.3 mL/min
b.	Make-up Gas for Capillary Column (95% Argon/5% Methane)	67 mL/min
c.	Chart Speed	0.5 cm/min
d.	Retention Time	approx. 6.1 minutes
e.	Run Time	approx. 10 minutes
f.	Injection Volume	1 µL
g.	Injector Temperature	260°C
h.	Detector Temperature	350°C
i.	Initial Column Temperature	195°C
j.	Initial Time (Hold)	0 minutes
k.	Temperature Rate	7°C per minute
l.	Final Temperature	240°C (Hold for 5 min)
m.	Purge Time	0.50 min
n.	Purge Flow	15 mL/min

If late-eluting peaks which might interfere with subsequent injections are a problem, then the following gradient programming is recommended.

a.	Initial Temperature	195°C
b.	Initial Time (Hold)	0 minutes
c.	Temperature Rate	7°C per minute
d.	Final Temperature	290°C
e.	Purge Time	0.50 min
f.	Hold	290°C for 5 minutes
g.	Injector Temperature	290°C

F. Linearity Check

1. Adjust the GC conditions to obtain a peak response of approximately 30-40% FSD (full-scale deflection) for a 10-pg injection of working standard (0.01 µg/mL).

2. Inject 1 µL of the standard solutions containing 0.0025, 0.005, 0.01 and 0.02 µg/mL.

3. Determine the response factor (ratio) for all injections by dividing the peak response by the amount (pg) injected. Calculate the average response ratio. A deviation of any standard response ratio by more than 15% from the average response ratio indicates instrumental or standard difficulties which must be corrected before proceeding with the analyses.

4. Linearity checks should be performed at least weekly during the analysis of samples from every field residue study and when the chromatographic system has been adjusted or serviced.

G. Sample Preparation

1. Pulverize sufficient dry ice in a food chopper (Hobart, Model 84185-D) to chill the bowl and blade thoroughly.

2. Add the prefrozen samples in small portions to enable reduction to fine particle size. It may be necessary to add small portions of dry ice during the chopping procedure to ensure that the samples remain in a frozen state.

3. Mix the sample well to obtain a good homogeneous mixture and pack an aliquot in several suitable containers.

4. Allow the frozen samples to stand in a freezer overnight for the dry ice to dissipate completely.

5. Keep all samples frozen until ready for analysis.

H. Recovery Test

The validity of the procedure should always be demonstrated by recovery tests before the analysis of unknown samples is attempted. As a quality control measure, at least one concurrent recovery should be run with each set of samples.

1. Weigh a 10- or 20-g sample of finely chopped commodities (see extraction steps for exact procedure) into an appropriate container and transfer to a 1-quart blender jar.

2. Add by pipet a volume of standard fortification solution appropriate to the fortification level to be tested.

3. Continue with the extraction and cleanup steps.

I. Extraction (all commodities)

1. Weigh 10 or 20 grams of the desired sample commodity according to Table I. Weigh into an appropriate container and transfer to a 1-quart blender jar.

Table I

Commodity	Weight Taken, g	Aliquot for Analysis, mL	Final Volume, mL
Stone Fruits: (Plums, Prunes and Cherries)	20	15	10
Peaches:	10	75	25
Pome Fruits: (Pears and Apples)	20	75	25
Strawberries:	20	15	10
Grapes:	20	15	10

2. Add 300 mL of extraction solvent to the blender jar and blend for approximately five minutes at moderate-high speed without splashing solvent out of the blender jar. Control blender speed with a Powerstat Variable Auto Transformer, if necessary.

3. Pass 5-10 mL of extraction solvent (Reagent B.4.) through a 9-cm glass-fiber filter paper held on a Buchner funnel using vacuum and a 500-mL filter flask. Discard the solution. Filter the extract from step I.2. using mild vacuum, collecting the filtrate in the 500-mL filtration flask.

4. Pipet a 15- or 75-mL sample aliquot (see Table I) into a 100- or 200-mL beaker. Add 20 mL of Milli-Q water to the 15-mL aliquot and 75 mL of Milli-Q water to the 75 mL aliquot. Mix thoroughly by gentle swirling. Proceed with step J.1 for all commodities except peaches.

5. For peaches pipet a 75-mL sample aliquot into a 250-mL separatory funnel. Add 30 mL of Milli-Q water to the separatory funnel. Partition the sample by vigorously shaking for 30 seconds with 100 mL of hexane. Repeat this step two times, collect and combine the upper clear hexane layers in a 500-mL evaporation flask. (The lower aqueous layer as well as the boundary layer should be collected in a beaker and then transferred quantitatively to the separatory funnel. Only the upper clean hexane layer is transferred to the 500-mL evaporation flask.) Evaporate the hexane layers to dryness using a flash evaporator. Dissolve the residue in 75 mL of 15% Milli-Q water/methanol followed by 75 mL of Milli-Q water. Proceed to step J.1.

J. Solid Phase Extraction Cleanup

1. Prepare an IST C-18 cartridge using a Visiprep Solid Phase Extraction Manifold. Wash the cartridge with 5 mL of methanol followed by 10 mL of Milli-Q water.

2. Connect a 70-mL non-fritted reservoir onto the top of the C-18 cartridge using an adaptor. Using vacuum, pull the sample from step I.4 or I.5 through the C-18 cartridge at an approximate rate of 1-2 drops/sec. Using vacuum, air dry the C-18 cartridge for 30 seconds (do not exceed).

3. Remove the 70-mL reservoir and C-18 cartridge from the Visiprep Solid Phase Extraction Manifold. Connect a 30-mL disposable syringe onto the top of the C-18 cartridge. Add 10 mL of hexane to the syringe and push the solution dropwise into a 100-mL evaporation flask using vacuum (1 drop/sec). Evaporate the hexane to dryness using a flash evaporator. (Use 15-30 mL of methanol to aid in water removal, if needed.)

4. Dissolve the residue in hexane according to Table I in Section I. For peaches proceed to step J. 5.

5. For peaches, dissolve the residue in 10 mL of methylene chloride. Prepare an IST silica gel cartridge using a Visiprep Solid Phase Extraction Manifold. Wash the cartridge with 5 mL of methylene chloride. Remove the cartridge from the Visiprep Solid Phase Extraction Manifold. Connect a 30-mL disposable syringe onto the top of the silica cartridge using an adaptor. Using vacuum, pass the 10-mL extract through the silica cartridge at the rate of 1-2 drops/second and collect in a 100-mL evaporation flask. Rinse the flask with 5 mL of methylene chloride and pass through the silica cartridge and collect in the same 100-mL evaporation flask. Evaporate the methylene chloride to dryness using a flash evaporator. Add 15 mL of methanol to the flask and evaporate to dryness using a flash evaporator.

6. Dissolve the residue in 25 mL of hexane for GC analysis.

K. Gas Chromatographic Analysis

1. After obtaining a stable GC response as described in Section F, inject a 1-µL aliquot of the working standard (0.01 µg/mL), followed by a maximum of two sample injections and another 1-µL standard injection.

2. Compare the sample peak height with that obtained from a 10-pg injection of the GC working standard (1 µL of the 0.01 µg/mL working standard).

3. If the sample peak is higher than the highest linearity standard injected, dilute to an appropriate volume with hexane and reinject.

4. Make a standard injection after each sample or after every second sample and use the average peak height of the standard injection before and after the sample injection for the calculation.

L. Calculations

For each sample calculation, use the sample peak height and the average peak height measurement of the external standard obtained before and after the sample injection as follows:

$$PPM = \frac{R(SAMP) \times V1 \times V3 \times C(STD) \times V5 \times D.F.}{R(STD) \times W \times V2 \times V4}$$

$$\% \text{ RECOVERY} = \frac{PPM \text{ FOUND} \times 100}{FV \times FC/W} = \frac{PPM \text{ FOUND}}{PPM \text{ ADDED}} \times 100$$

Where:

R(SAMP) = Peak height of the sample in millimeters

R(STD) = Average peak height of the working standards in millimeters

W = Weight of sample taken for analysis in grams (10 or 20 g)

V1 = Volume of extraction solvent used in milliliters (300 mL)

V2 = Aliquot of the extract taken for analysis in milliliters (15 or 75 mL)

V3 = Volume of hexane added to dissolve final residues for chromatographic analysis in milliliters (10 mL or 25 mL)

V4 = Volume of sample solution injected in microliters (1 µL)

V5 = Volume of working standard solution injected in microliters (1 µL)

C(STD) = Concentration of working standard solution injected in micrograms per milliliter (0.01 µg/mL)

D.F. = Dilution factor

FV = Fortification volume in milliliters

FC = Fortification concentration (of standard solution added) in micrograms per milliliter

Typical chromatograms for determining CL 303,630 residues in various fruit commodities are shown in Figures 1 through 6.

M. GC/MS Confirmatory Analysis

1. Sample Preparation for GC/MS Confirmation: Any commodities requiring mass spectrometric confirmation are directly amenable to mass spectrometric analysis.

2. GC/MS Standard Solution: Use the 0.01 µg/mL (10 ng/mL) standard solution.

3. GC/MS Instrumentation: (Items from other manufacturers may be used provided they have been proven to be functionally equivalent.)

   a. Mass Spectrometer: Finnigan MAT SSQ710

   b. Gas Chromatograph: Varian Model 3400

   c. GC Column: 5 m x 0.25 mm I.D., 0.25 micron DB-5MS film (J & W Scientific, Custom Number 115786)

4. GC/MS Conditions:

a.	GC Column Oven Temperature	60°C for 0.5 min; 20°C/min to 280°C, hold at 280°C for 5 min.
b.	Injector Temperature	280°C (split valve open at 0.5 min.)
c.	Transfer Line Temperature	250°C

d.	Column Head Pressure	5 psi H_2
e.	Source Temperature	150°C
f.	CI Reagent Gas	Methane at 8200 mT (indicated)
g.	Conversion Dynode	+15 kV
h.	Electron Multiplier	-1200 Volts
i.	Preamplifier Range (Full Scan)	1 E-07 amps/volt
j.	Preamplifier Range (SIM)	1 E-08 amps/volt
k.	Ions Monitored	m/z 347⁻, 349⁻
l.	Retention Time of Analyte	approximately 6.2 min.
m.	Mode Used	Negative Ion, Chemical Ionization
n.	Full Scan Range	300 to 400 m/z
o.	Scan Time	0.5 sec
p.	Injection Volume	1 µL

The conditions above are specific for the instruments on which they were determined. Conditions will vary from instrument to instrument and should be adjusted to give sensitivity and adequate resolution of well defined peaks at approximately the retention time listed in M.4.1. Prior to analysis, the mass spectrometer should be tuned to give proper resolution and peak shape on an appropriate reference material and the data system should be calibrated.

5. GC/MS Confirmatory Analysis:

   a. Using parameters detailed in M.4, a 100-pg on-column injection of the analyte (1 µL of 0.1 µg/mL) should give a background-subtracted mass spectrum similar to that shown in Figure 7. Determine the mass centroids of the ions at m/z 347⁻ and 349⁻ and set the mass spectrometer for selected ion monitoring of these ions with a +/- 0.2 dalton scan window and a dwell time of 250 msec/ion (0.5 sec/scan).

   b. Inject 1-µL aliquots of the working standard (M.2) until a reasonably constant response is obtained (Figure 8).

   c. Follow the injection sequence working standard, Sample Number 1, Sample Number 2, working standard, Sample Number 3, Sample Number 4, working standard.

   d. If the response of the working standard decreases to an unacceptable level during the analysis, instrumental parameters should be adjusted to restore adequate sensitivity. If such adjustments are made, inject duplicate aliquots of the working standard to determine the new response values of the standard.

6. Data Treatment: The sample is confirmed as containing residues of CL 303,630 when:

a. The retention time of the presumed analyte in the sample is within 15 seconds of the averaged retention times of the analyte peaks in the bracketing standards.

b. The ions indicative of the analyte in the sample maximize at retention times which are within 5 seconds of each other.

c. The ratio of the m/z 347⁻ ion to the m/z 349⁻ ion in the sample agrees with that observed for the standard.

d. The quantitation by GC/MS is comparable to the residue found by the determinative method.

Typical mass chromatograms for mass spectrum, standard, control and fortified samples are shown in Figures 9 through 18.

APPROVALS:

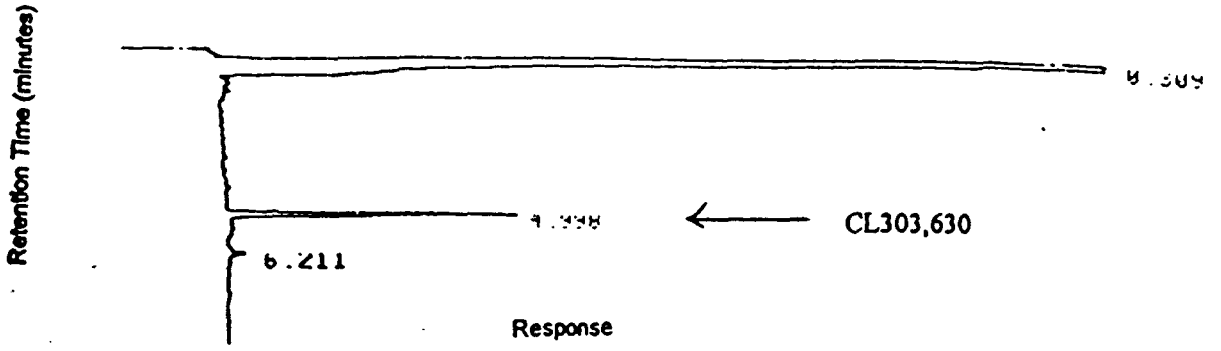
Phillip Miller for PM. 06/11/97
Date
Sr. Group Leader
Residue Chemistry

Tom Cavalier for B.B. 6-11-97
Brion W. Babbitt Date
Author

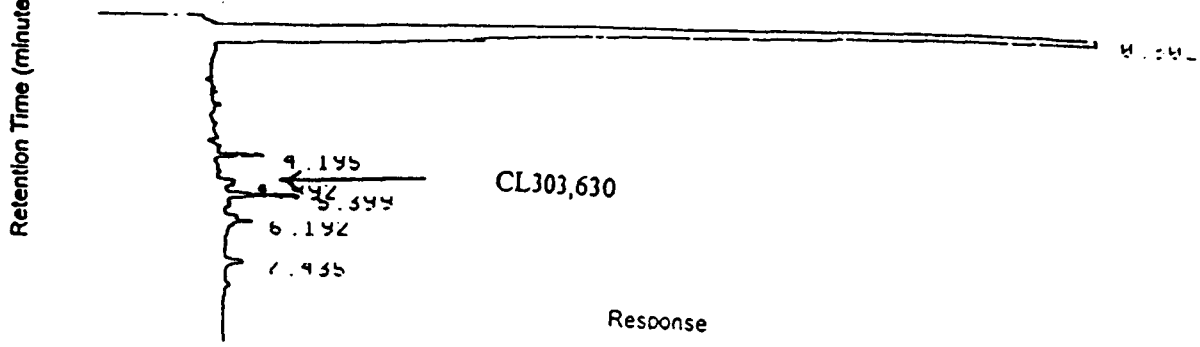
Huns Nejad 6/11/97
Huns Nejad Date
Author

/ct

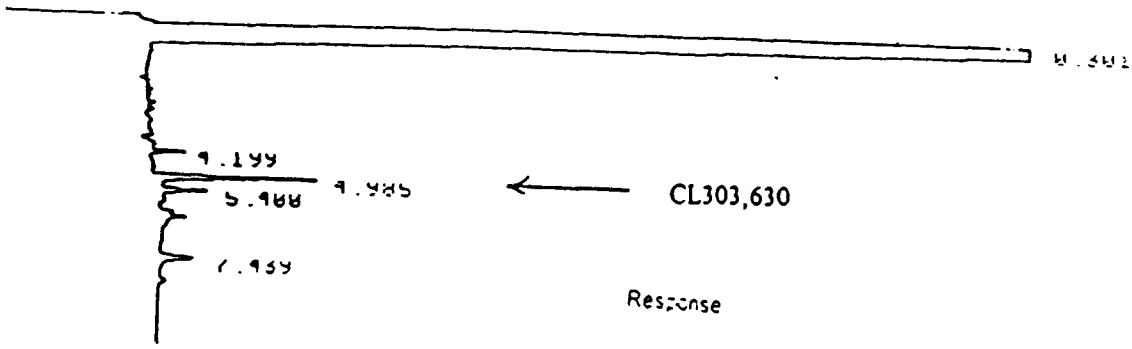
Figure 1: Typical Chromatograms for the Determination of CL 303,630 Residues in Peaches



CL 303,630 Standard, (0.01 µg/mL), 10 pg Injected

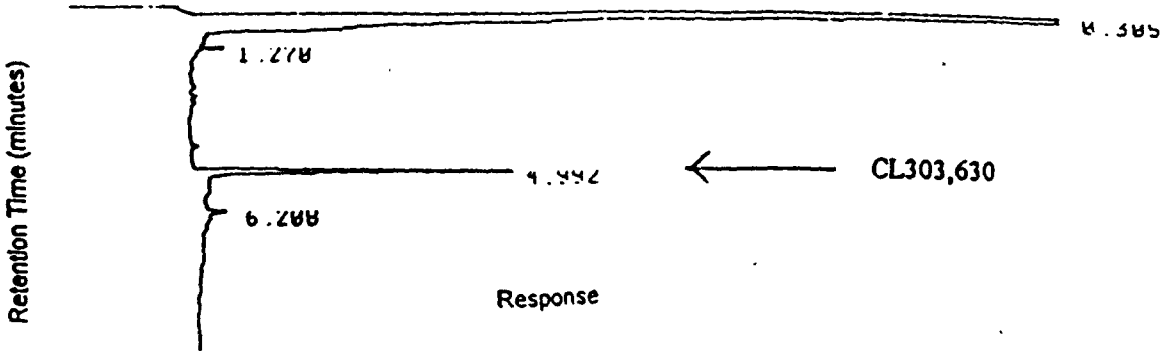


Control Peaches, 100 µg Injected, 0.005 ppm Apparent CL 303,630 Found

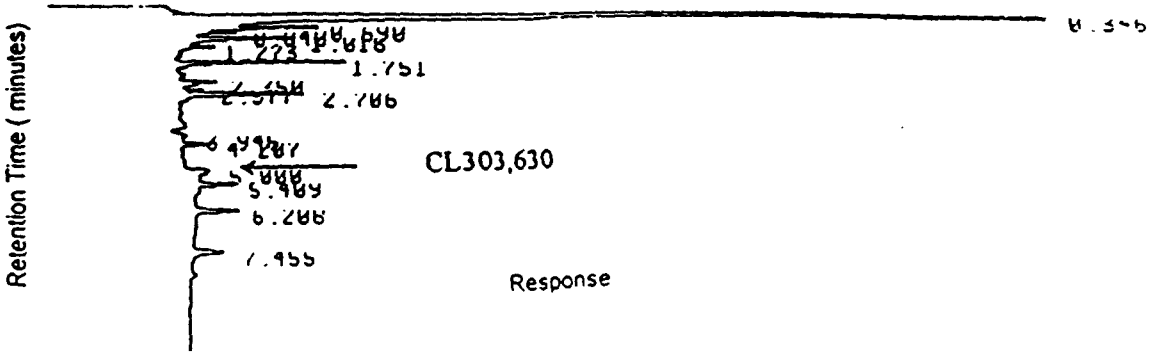


Control Peaches, Fortified with CL 303,630 at 0.05 ppm, 100 µg Injected, 0.057 ppm Found, 114% Recovered

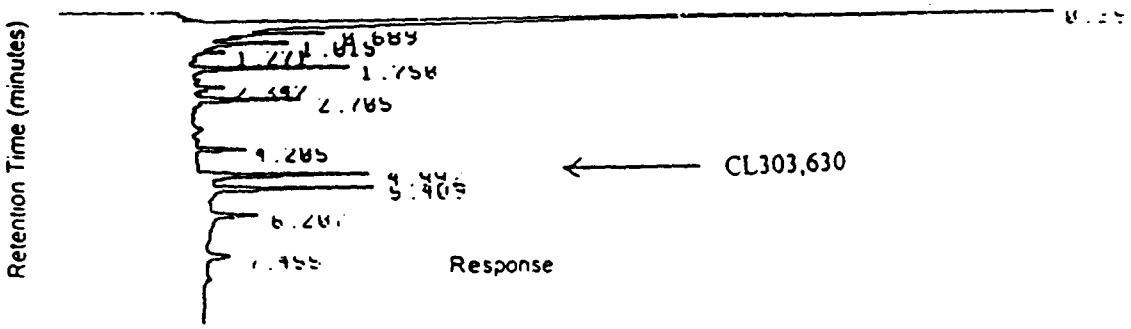
Figure 2: Typical Chromatograms for the Determination of CL 303,630 Residues in Plums



CL 303,630 Standard, (0.01 µg/mL), 10 pg Injected

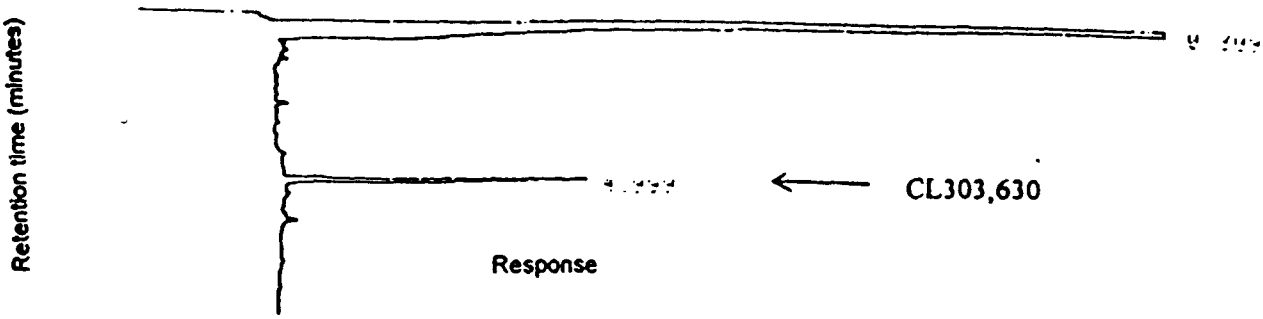


Control Plums, 100 µg Injected, 0.007 ppm Apparent CL 303,630 Found

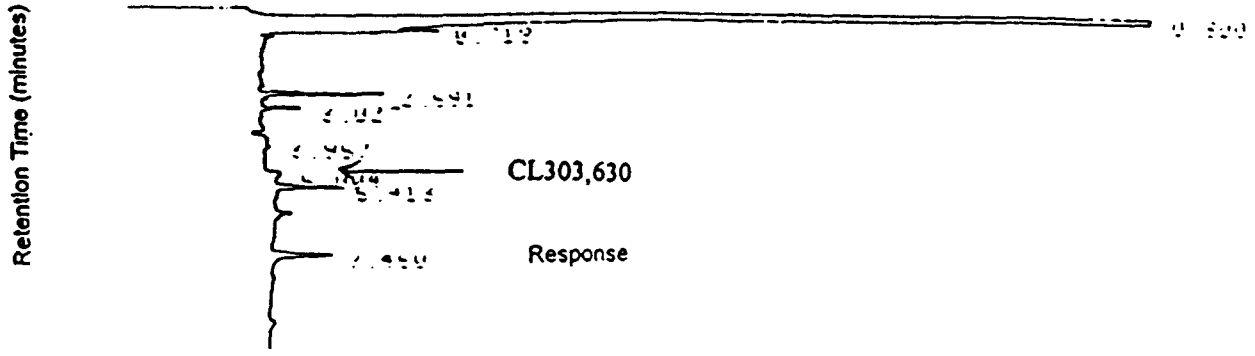


Control Plums, Fortified with CL 303,630 at 0.05 ppm, 100 µg Injected, 0.055 ppm Found, 108% Recovered

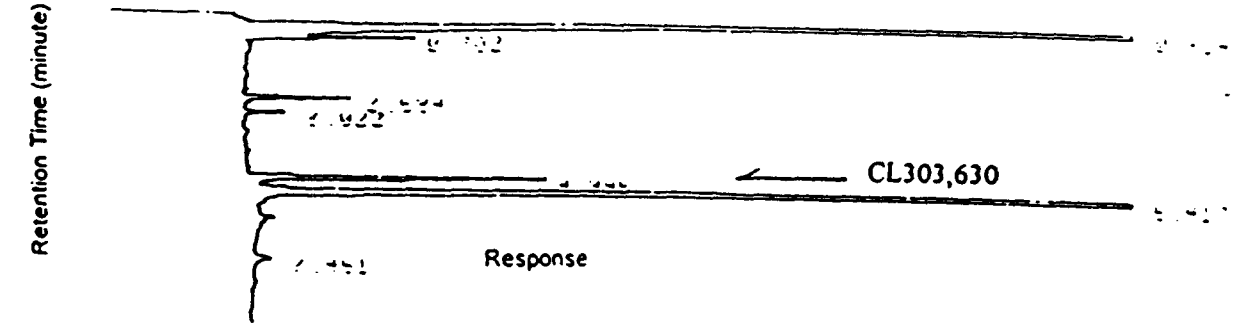
Figure 3: Typical Chromatograms for the Determination of CL 303,630 Residues in Green Pears



CL 303,630 Standard, (0.01 µg/mL), 10 pg Injected

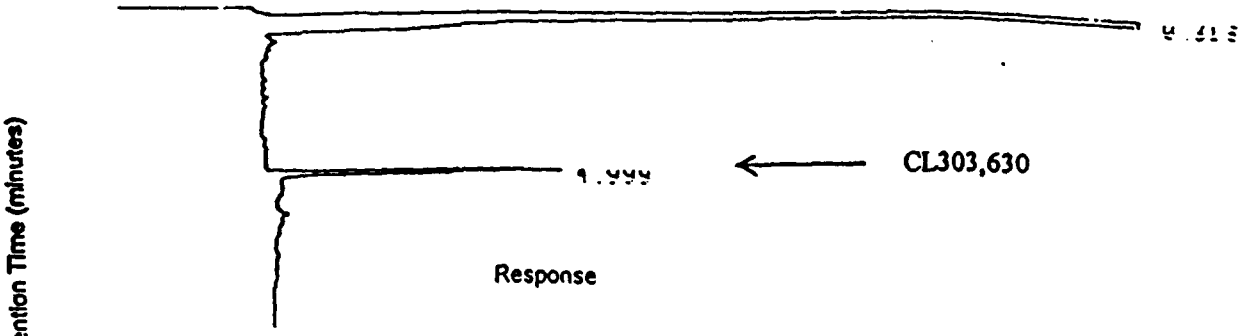


Control Green Pears, 200 µg Injected, <0.002 ppm Apparent CL 303,630 Residue Found

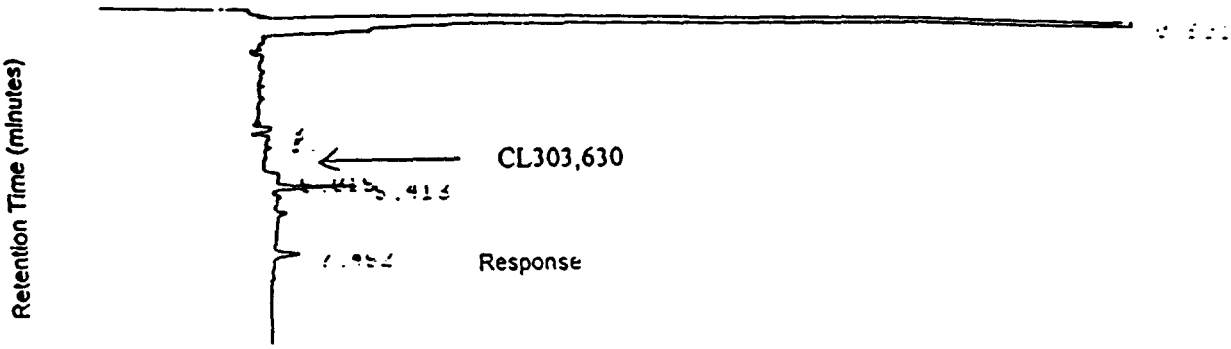


Control Green Pears, Fortified with CL 303,630 at 0.05 ppm, 200 µg Injected, 0.051 ppm Found, 102% Recovered

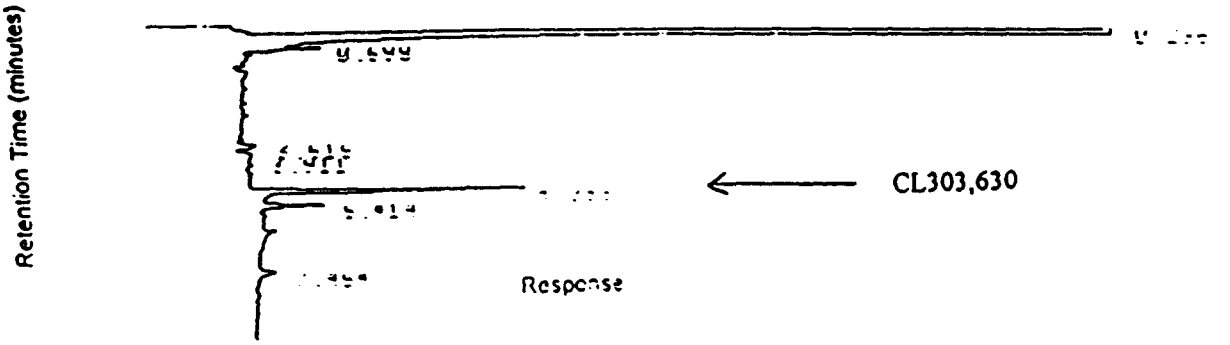
Figure 4: Typical Chromatograms for the Determination of CL 303,630 Residues in Red Apples



CL 303,630 Standard, (0.01 µg/mL), 10 pg Injected

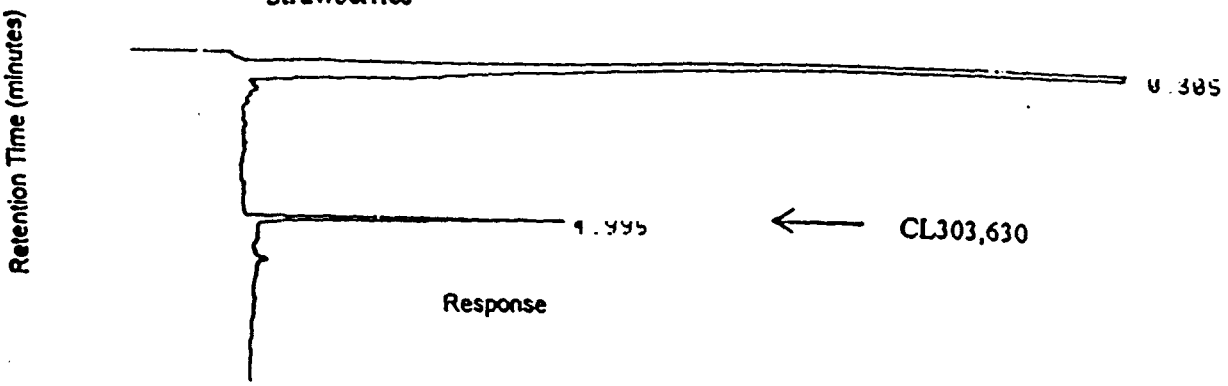


Control Apples, 200 µg Injected, 0.0014 ppm Apparent CL 303,630 Found

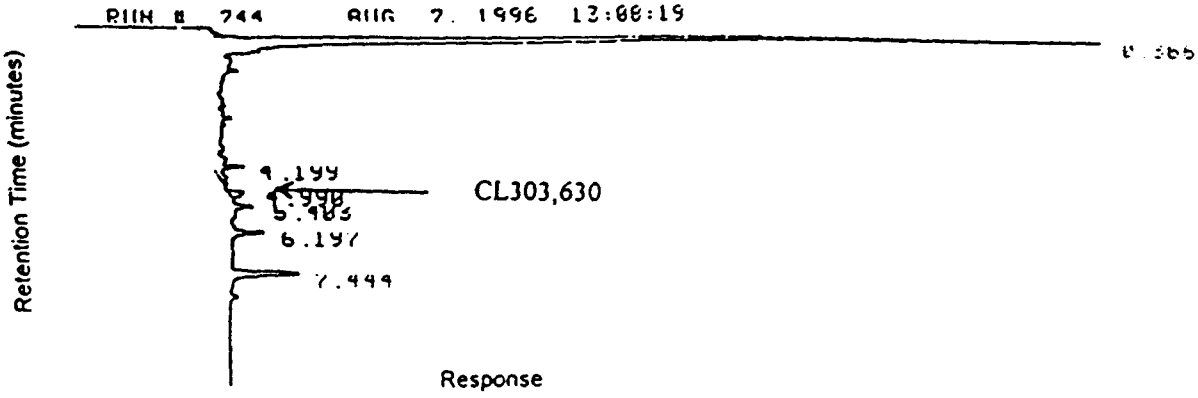


Control Apples, Fortified with CL 303,630 at 0.05 ppm, 200 µg Injected, 0.049 ppm Found, 98 % Recovered

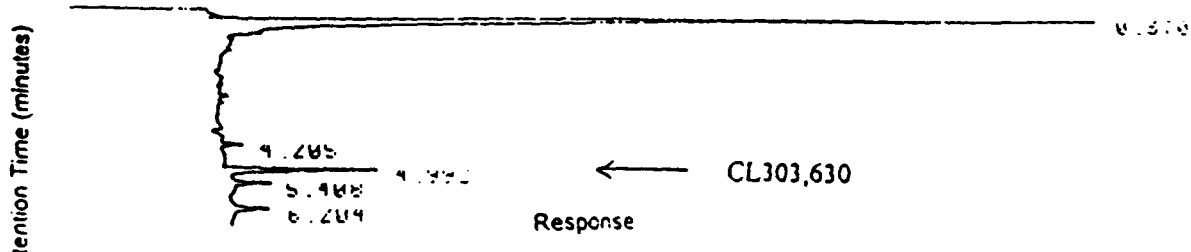
Figure 5: Typical Chromatograms for the Determination of CL 303,630 Residues in Strawberries



CL 303,630 Standard, (0.01 µg/mL), 10 pg Injected

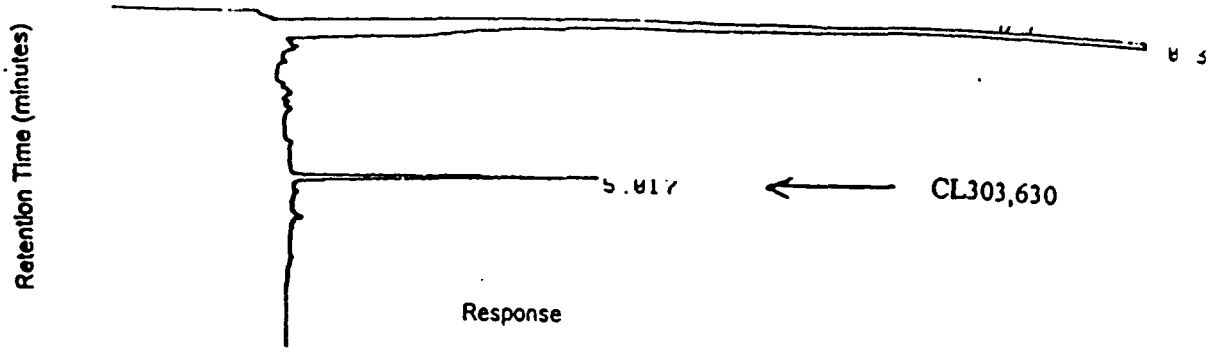


Control Strawberries, 100 µg Injected, 0.006 ppm Apparent CL 303,630 Residues Found

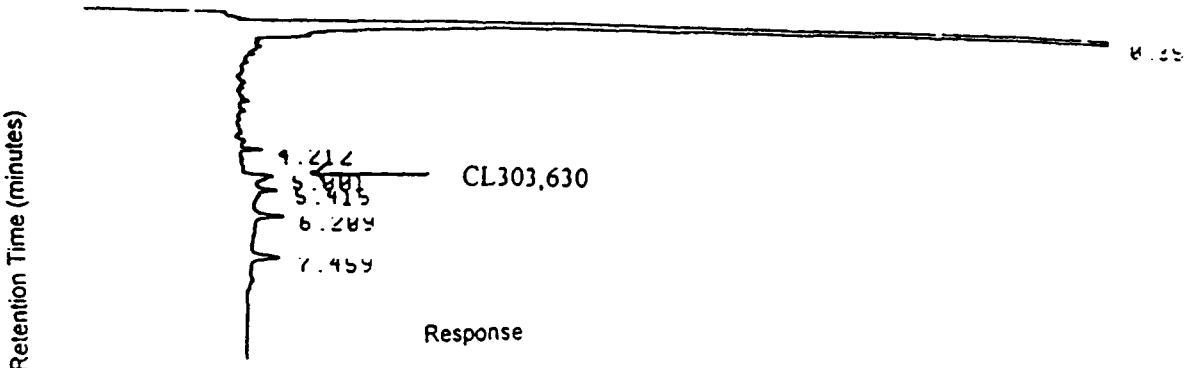


Control Strawberries, Fortified with CL 303,630 at 0.05 ppm, 100 µg Injected, 0.05 ppm Found, 100% Recovered

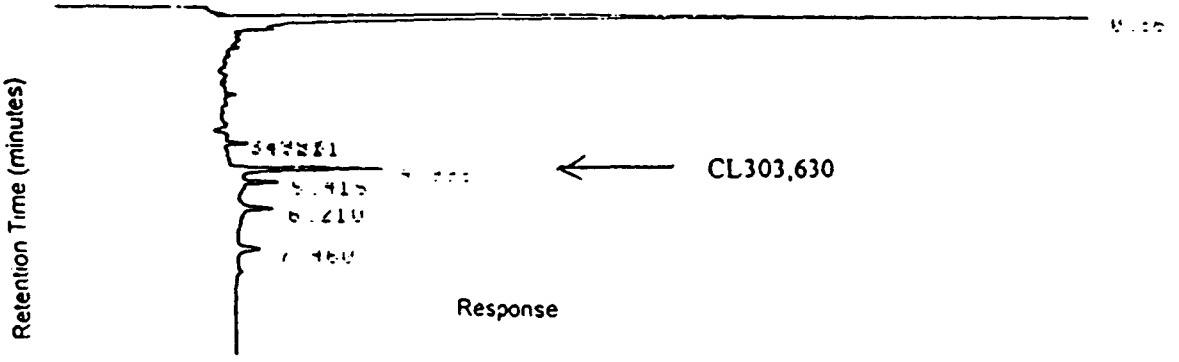
Figure 6: Typical Chromatograms for the Determination of CL 303,630 Residues in White Grapes



CL 303,630 Standard, (0.01 µg/mL), 10 pg Injected



Control White Grapes, 100 µg Injected, 0.0068 ppm Apparent CL 303,630 Residue Found



Control White Grapes, Fortified with CL 303,630 at 0.05 ppm, 100 µg Injected, 0.049 ppm Found, 98% Recovered

Figure 7: Typical Full Scan Mass Spectrum from the Injection of 100 pg of CL 303,630 Standard

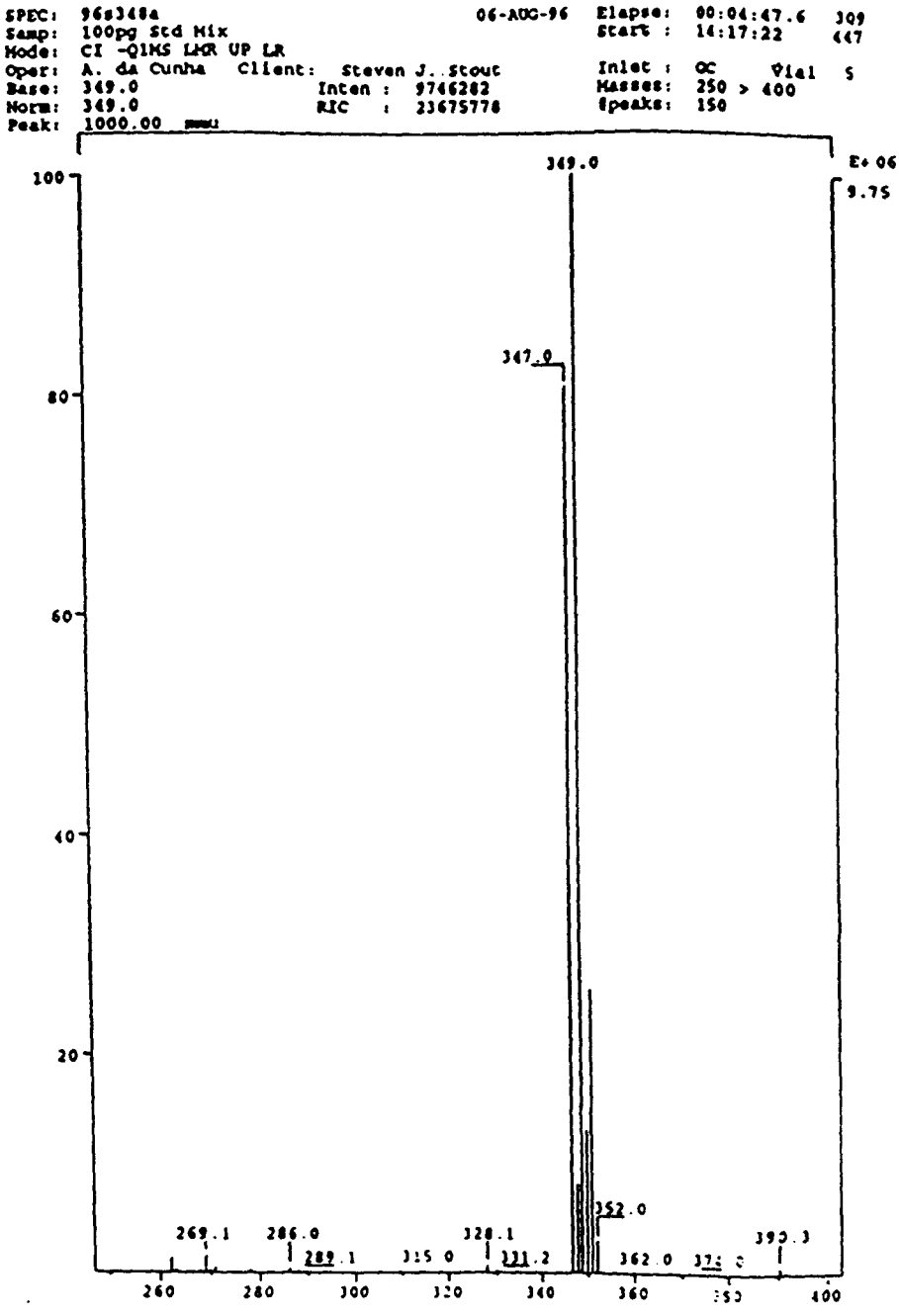


Figure 8: Typical Mass Chromatograms from the Injection of 1 µL of Working Standard (M.2.)

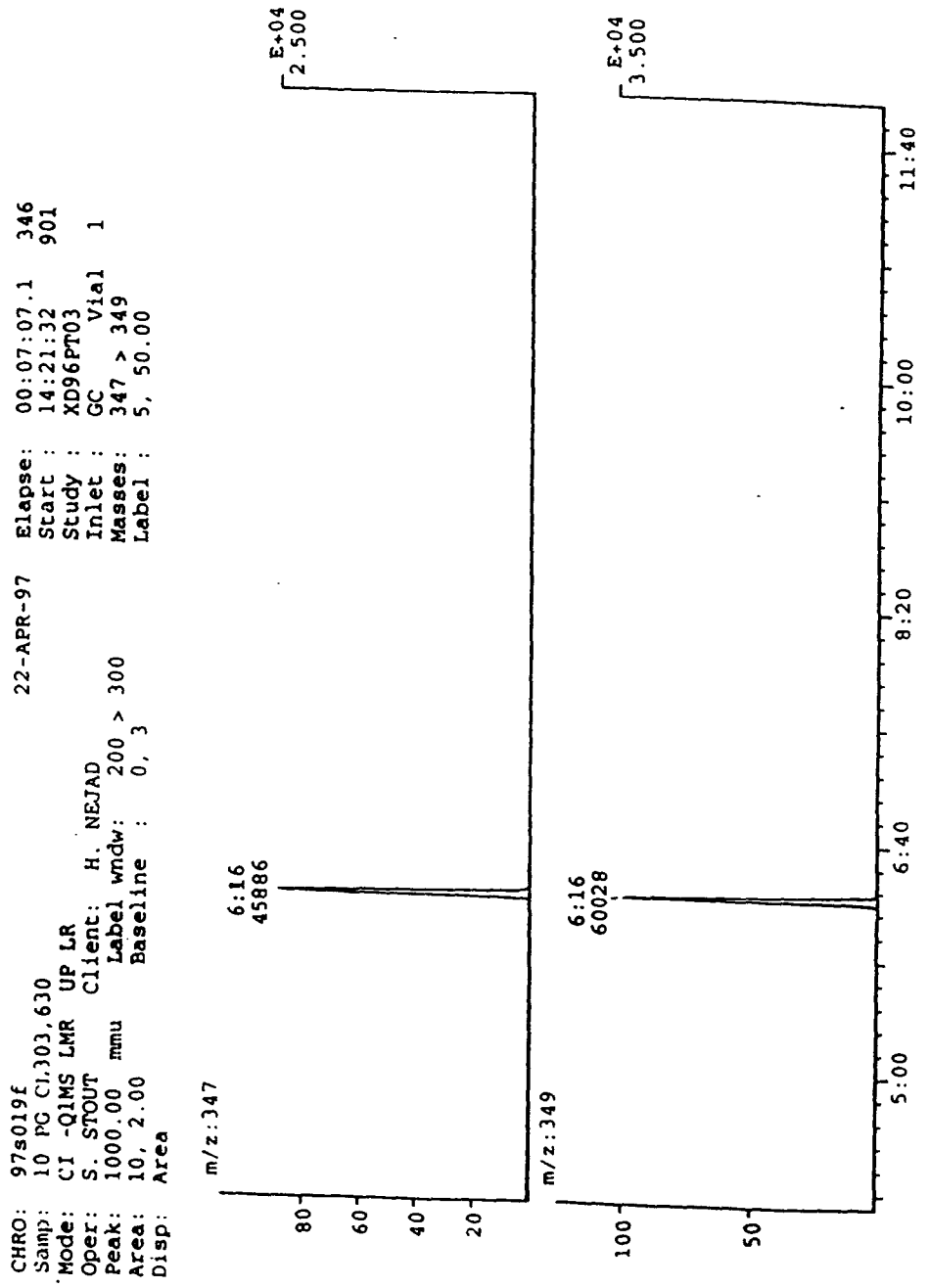


Figure 9: Typical Mass Chromatograms from the Injection of a Control Bing Cherry Sample

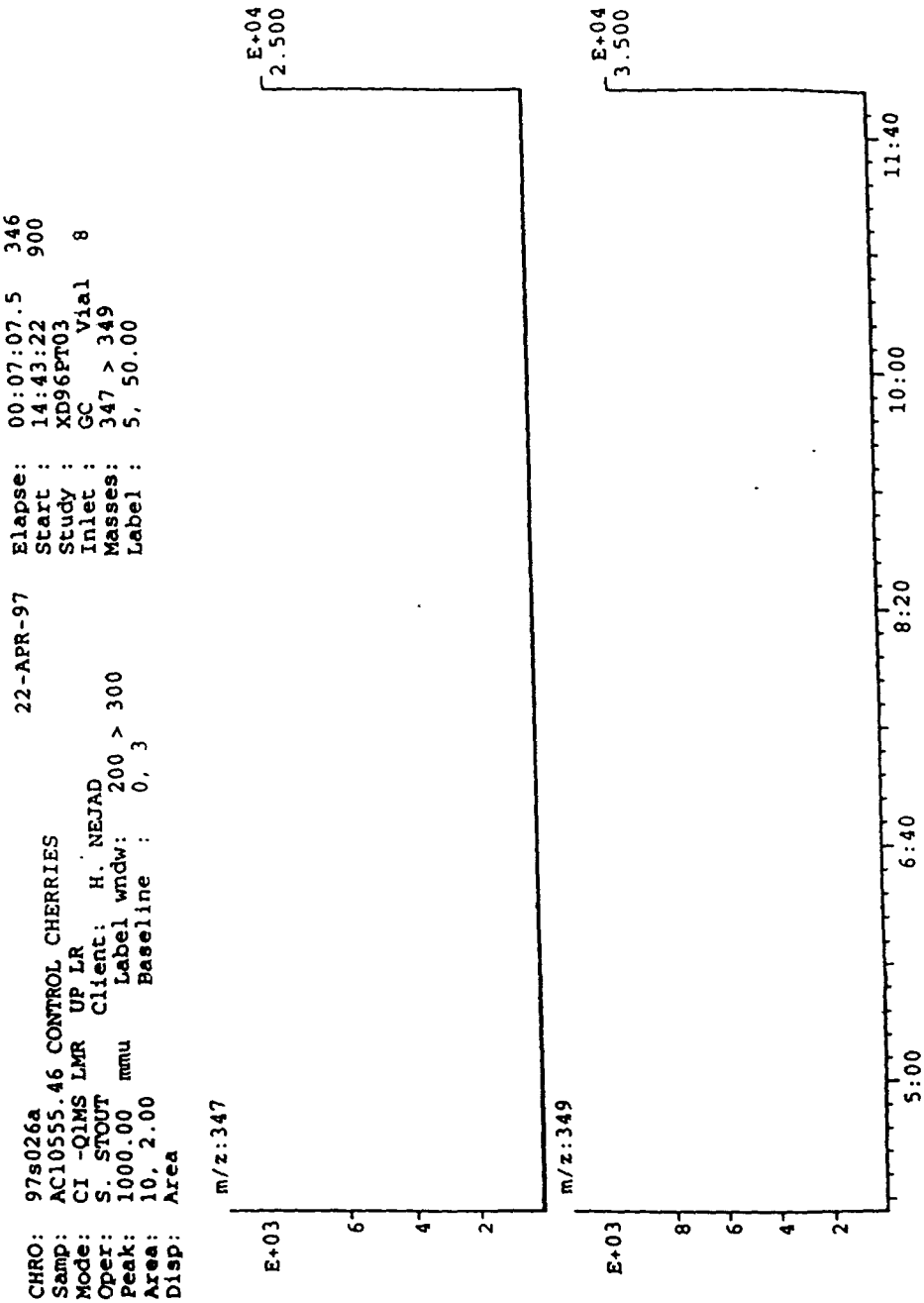
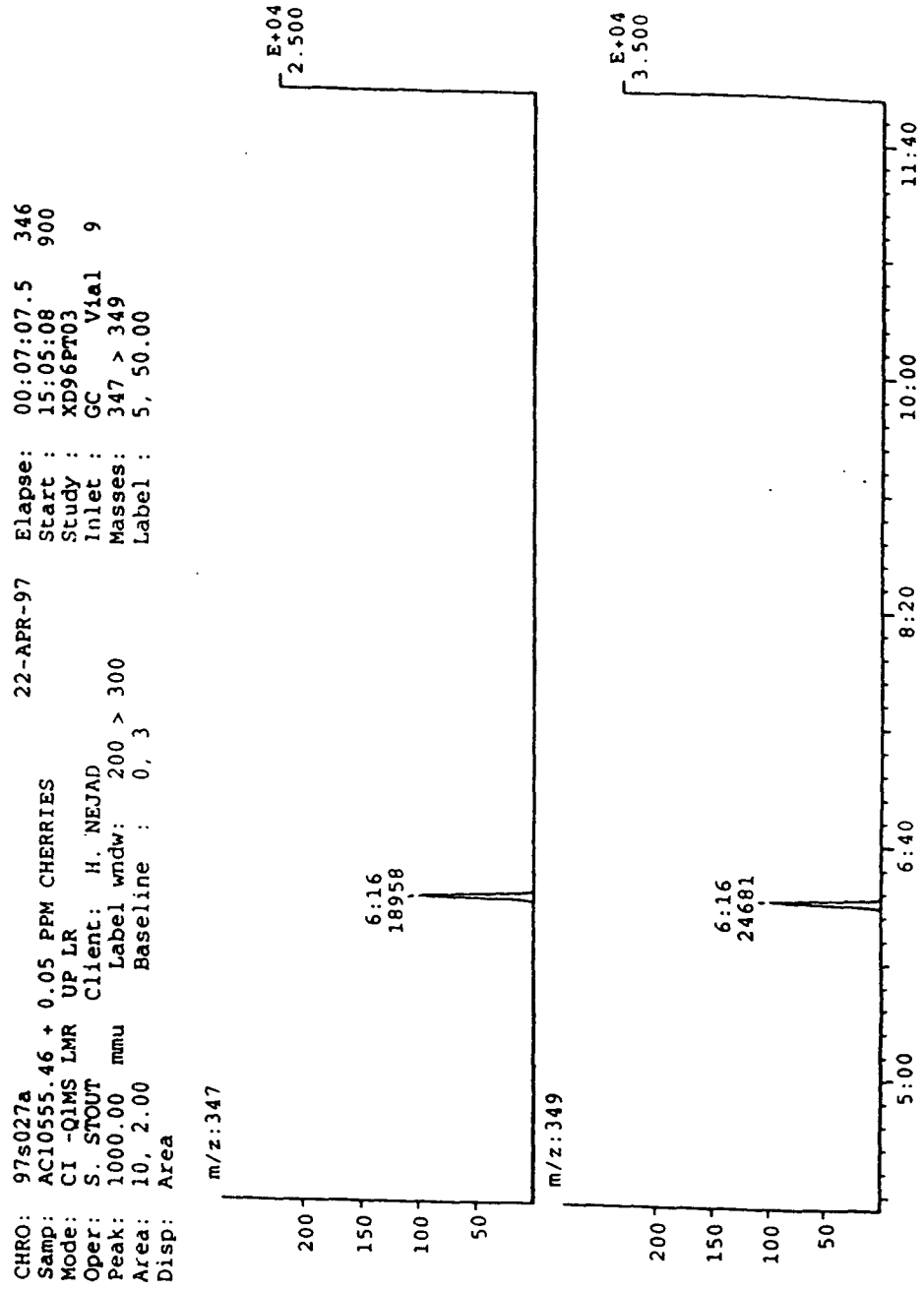


Figure 10: Typical Mass Chromatograms from the Injection of a 0.05 ppm Fortified Bing Cherry Sample



M 2686, Page 22 of 30

Figure 11: Typical Mass Chromatograms from the Injection of a Control Red Apple Sample

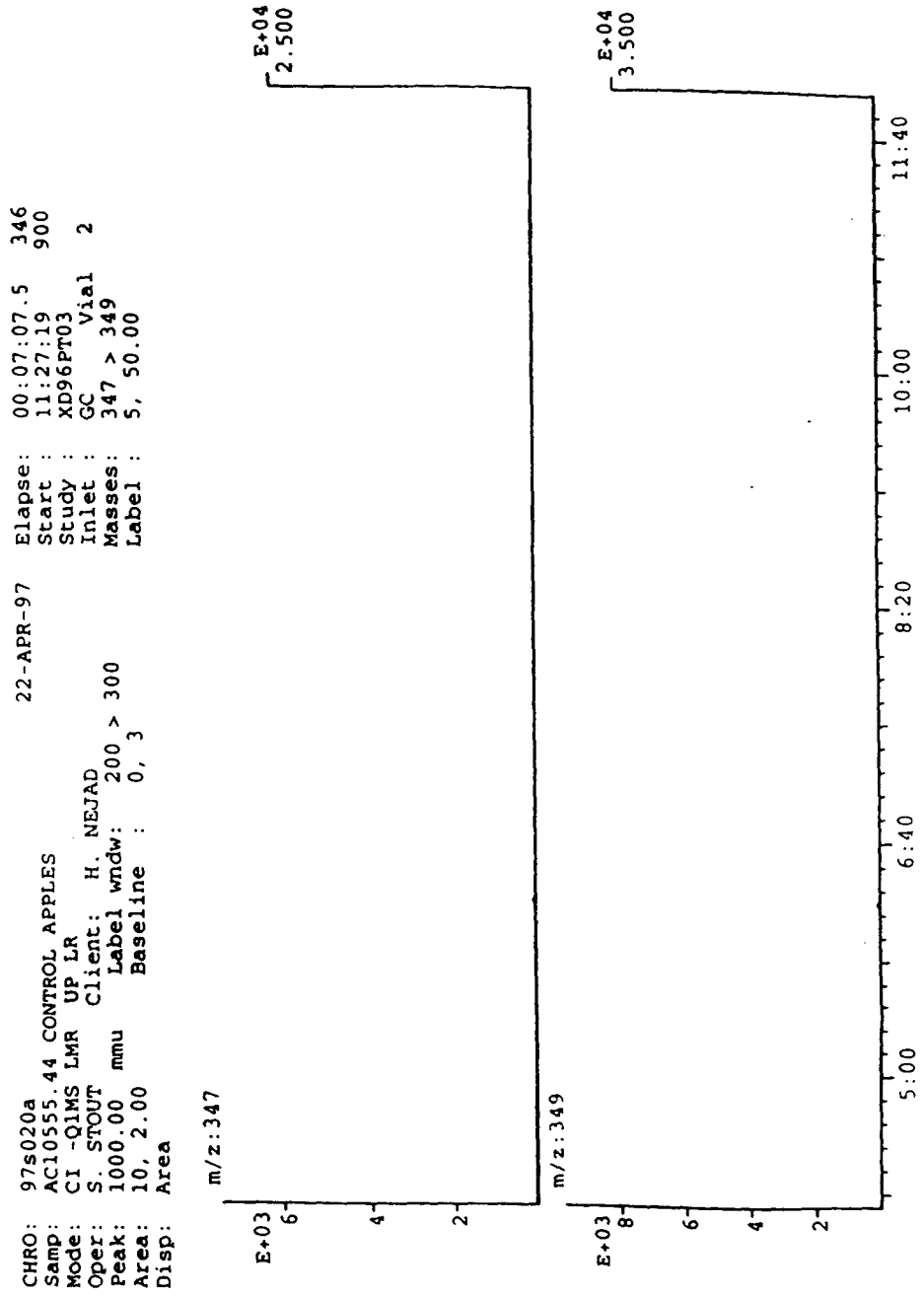


Figure 12: Typical Mass Chromatograms from the Injection of a 0.05 ppm Fortified Red Apple Sample

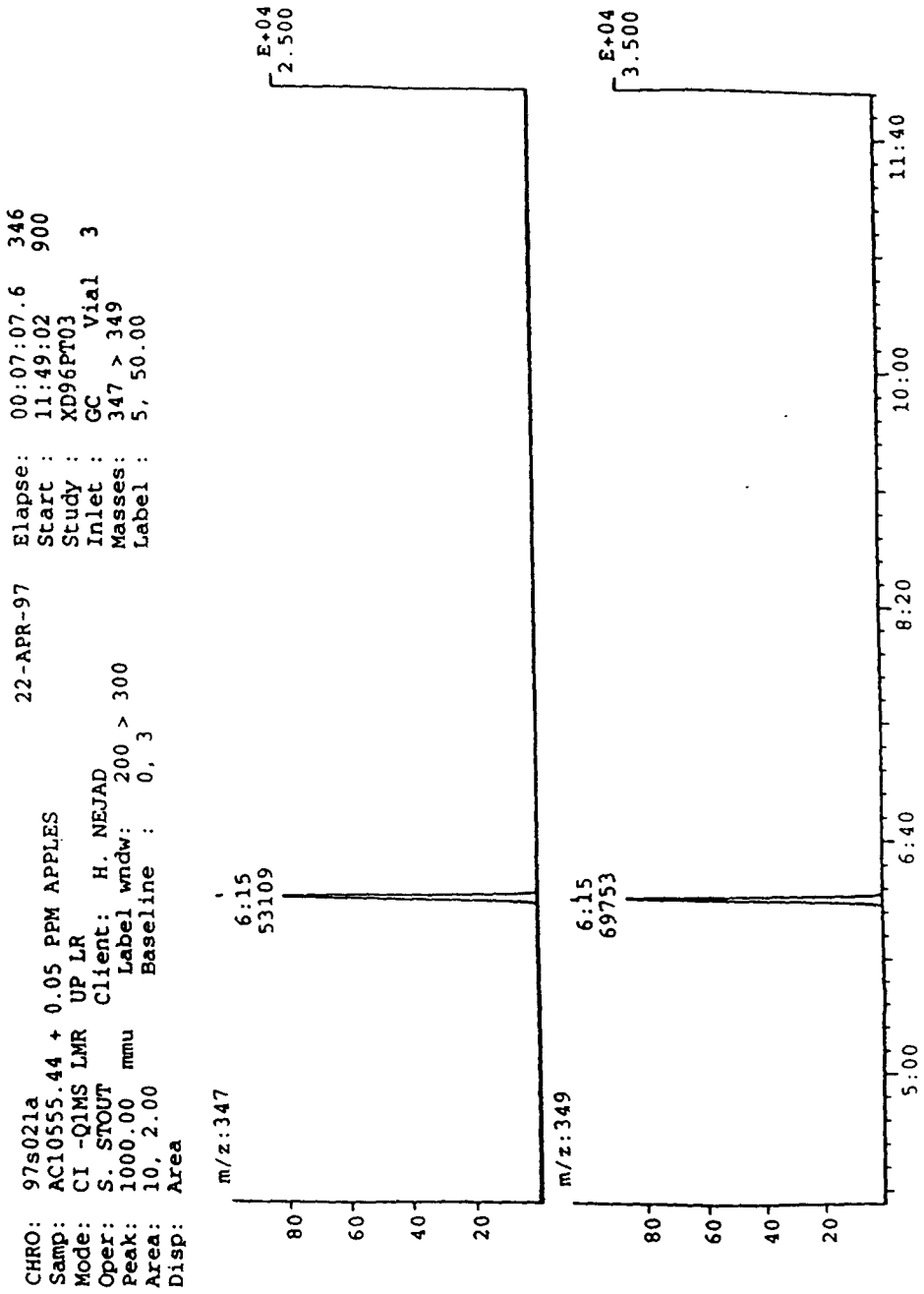


Figure 13: Typical Mass Chromatograms from the Injection of a Control Grape Sample

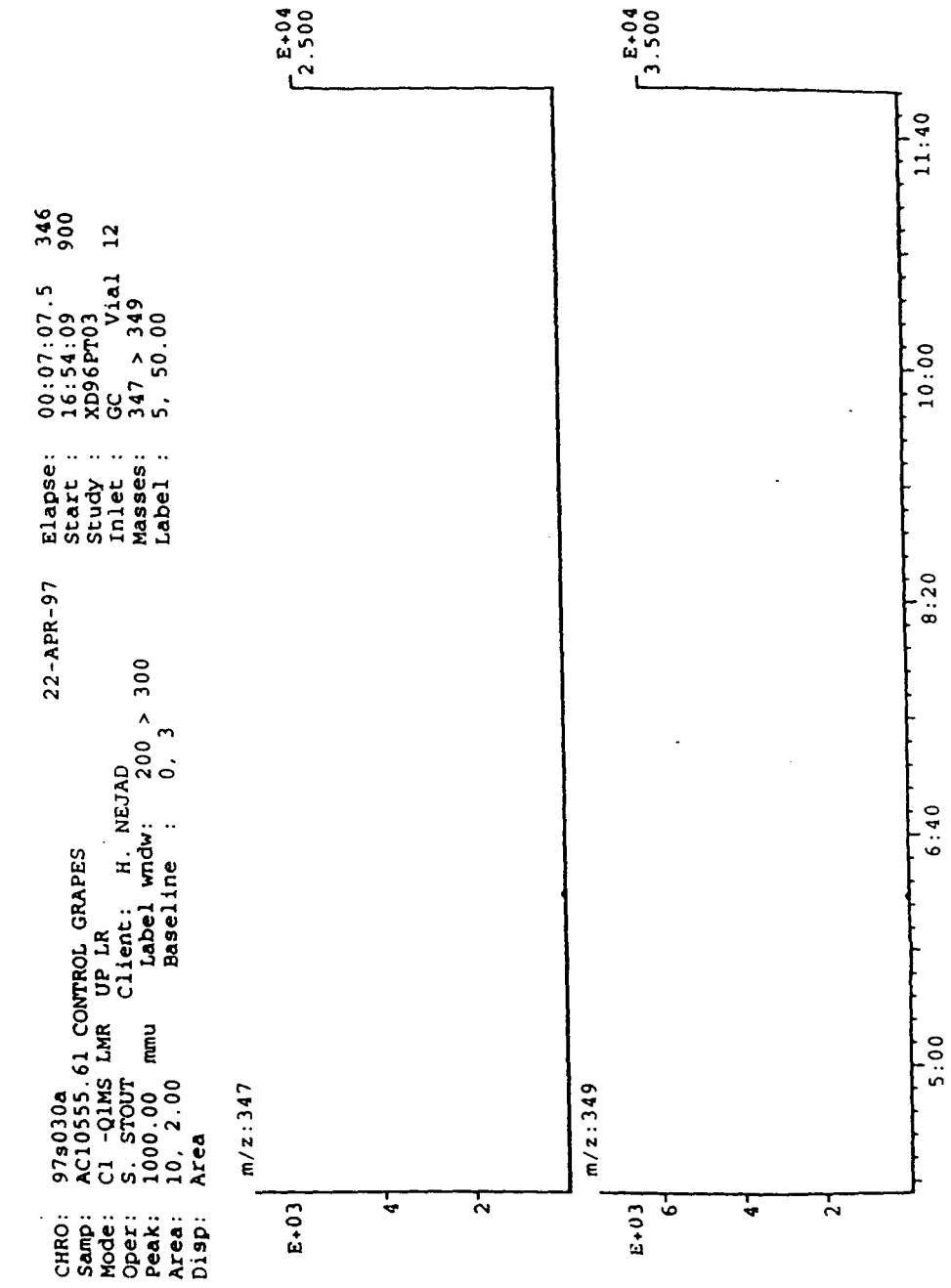
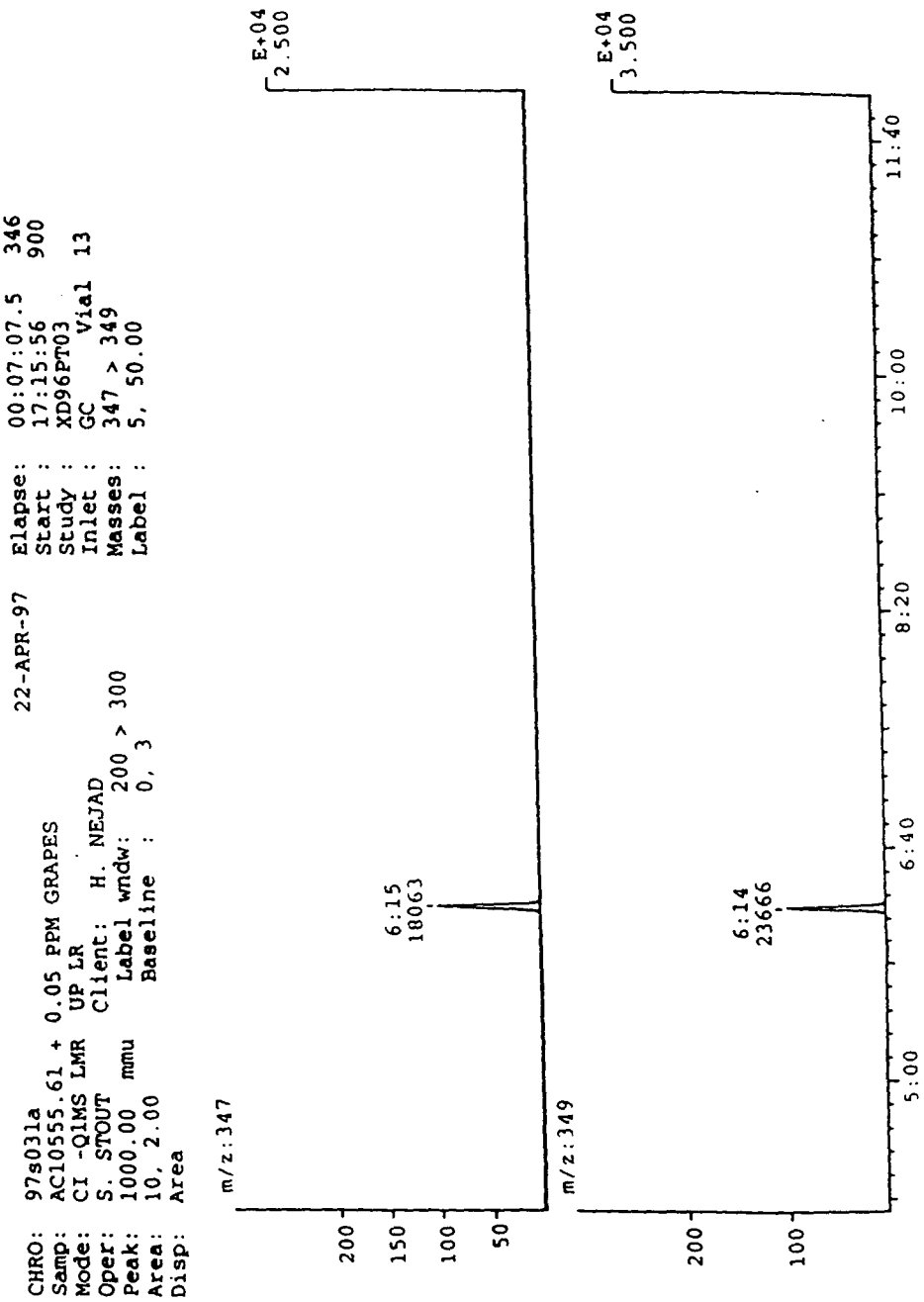


Figure 14: Typical Mass Chromatograms from the Injection of a 0.05 ppm Fortified Grape Sample



M 2686, Page 26 of 30

Figure 15: Typical Mass Chromatograms from the Injection of a Control Strawberry Sample

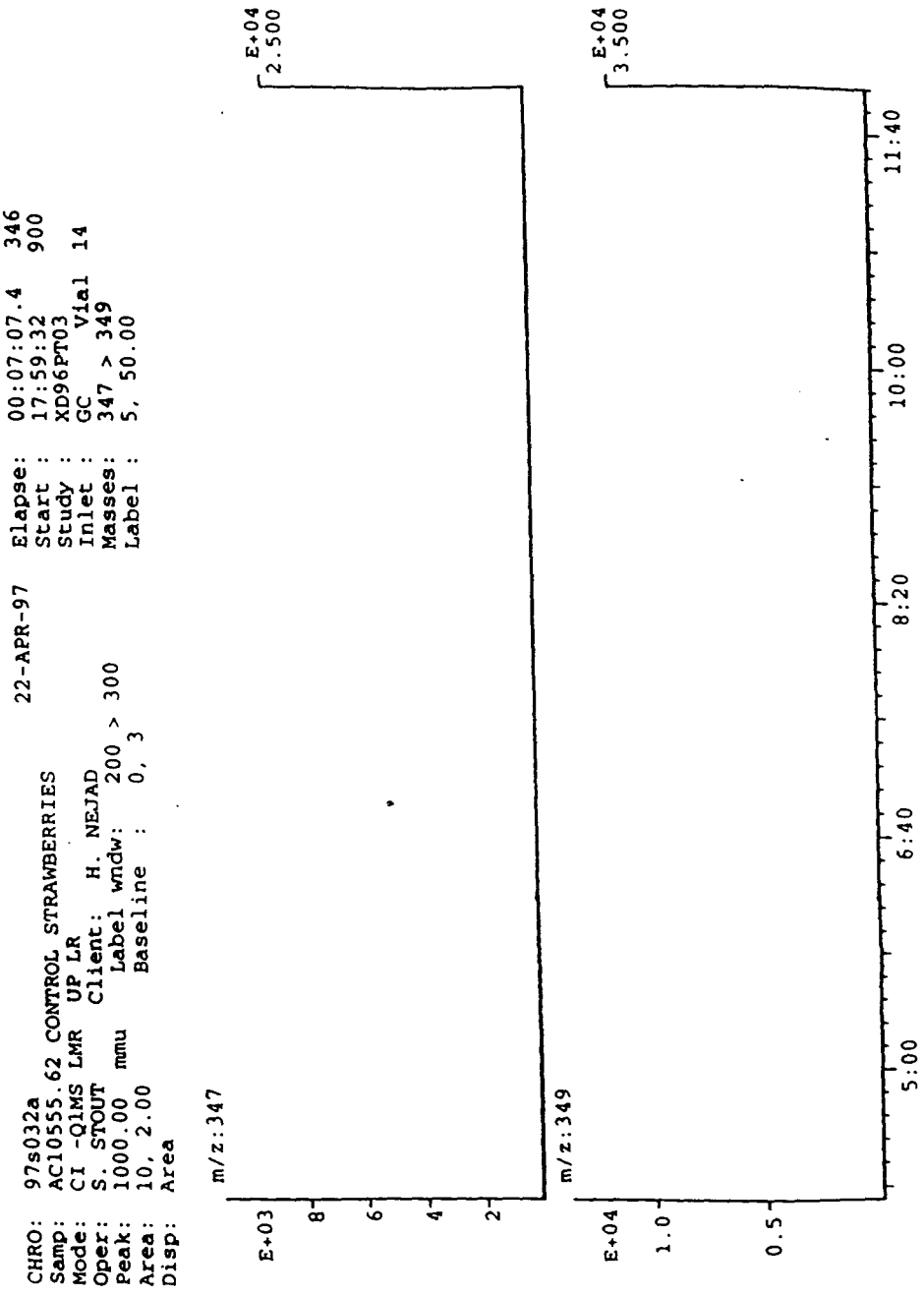


Figure 16: Typical Mass Chromatograms from the Injection of a 0.05ppm Fortified Strawberry Sample

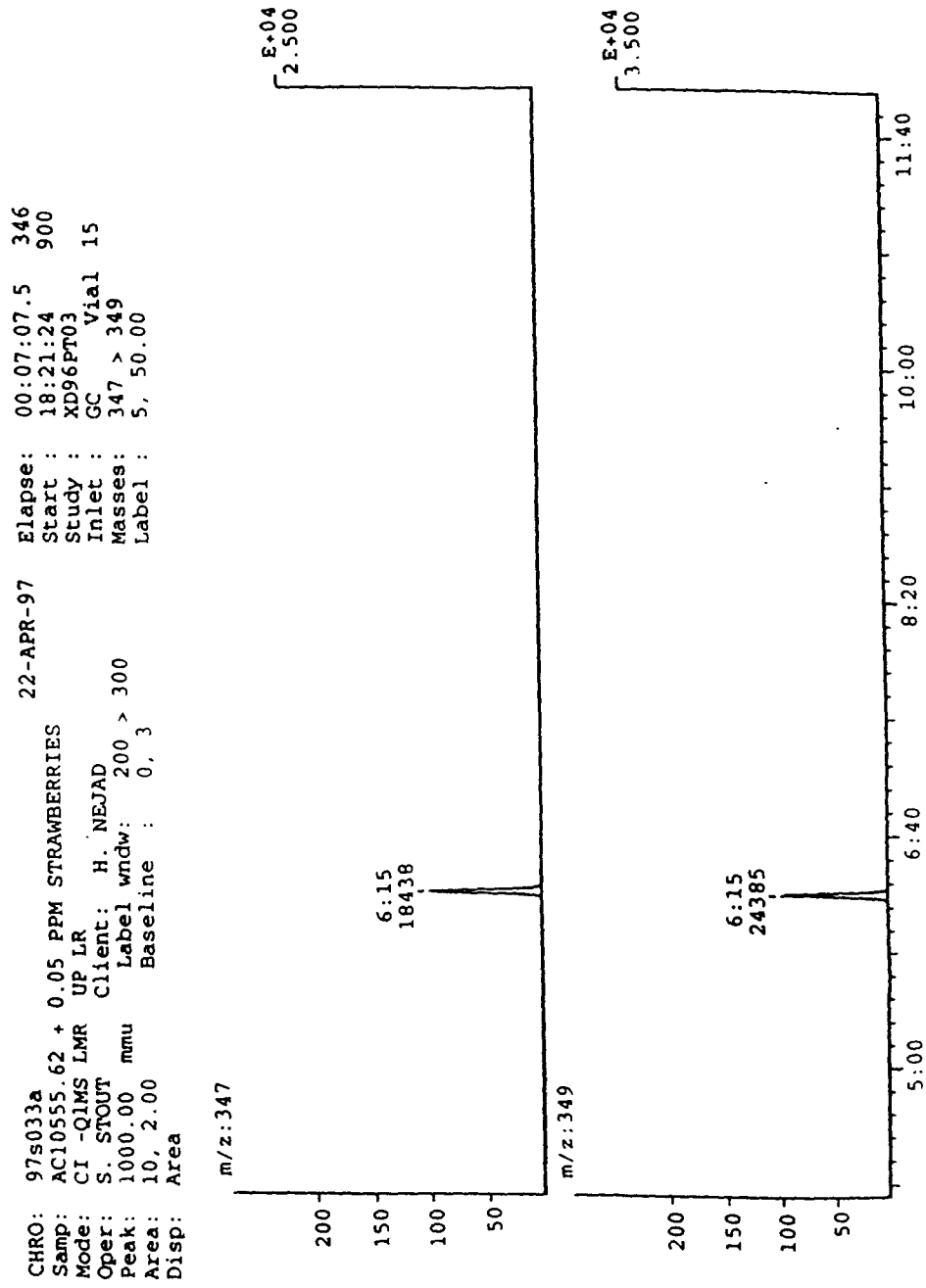


Figure 17: Typical Mass Chromatograms from the Injection of a Control Peach Sample

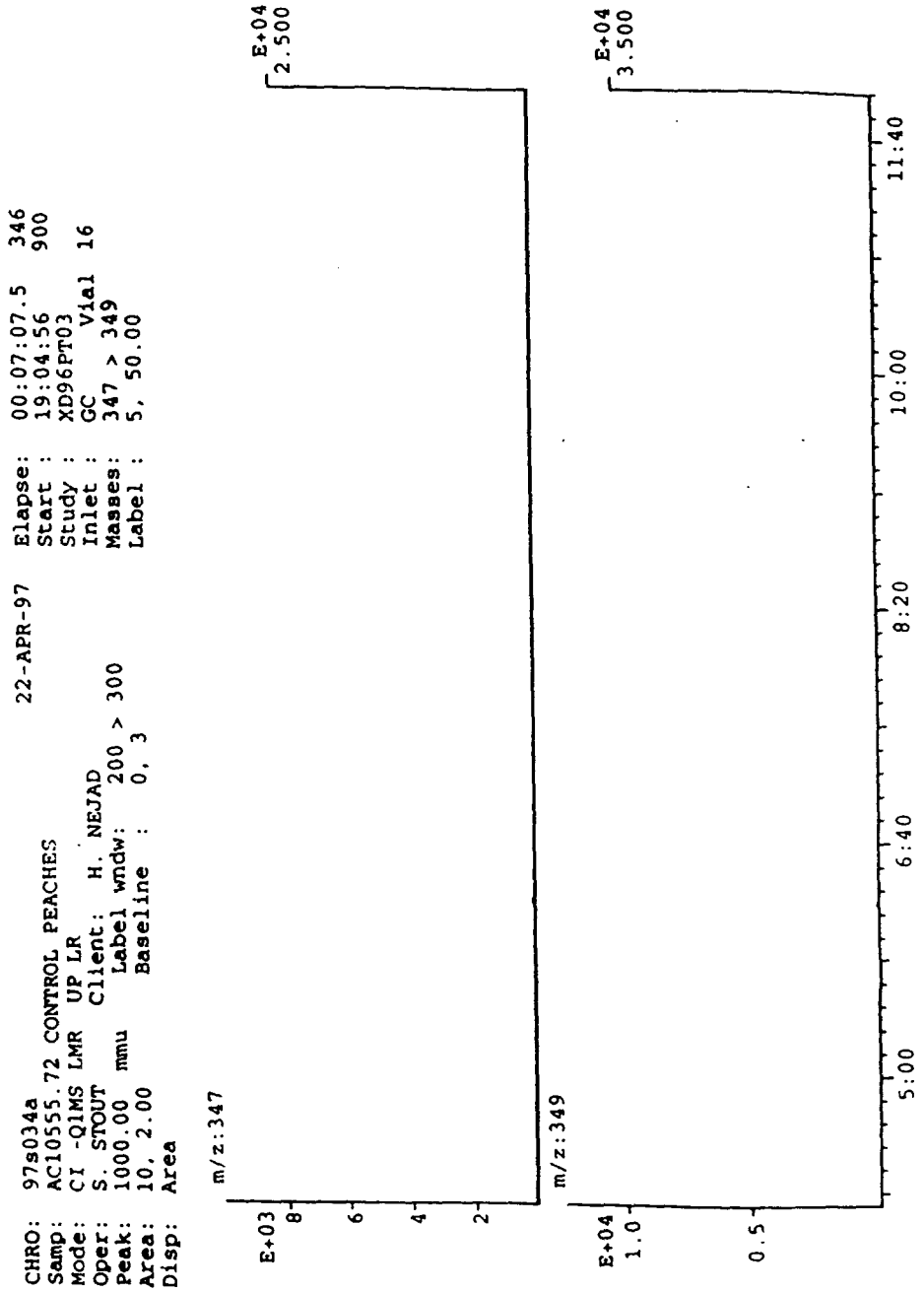
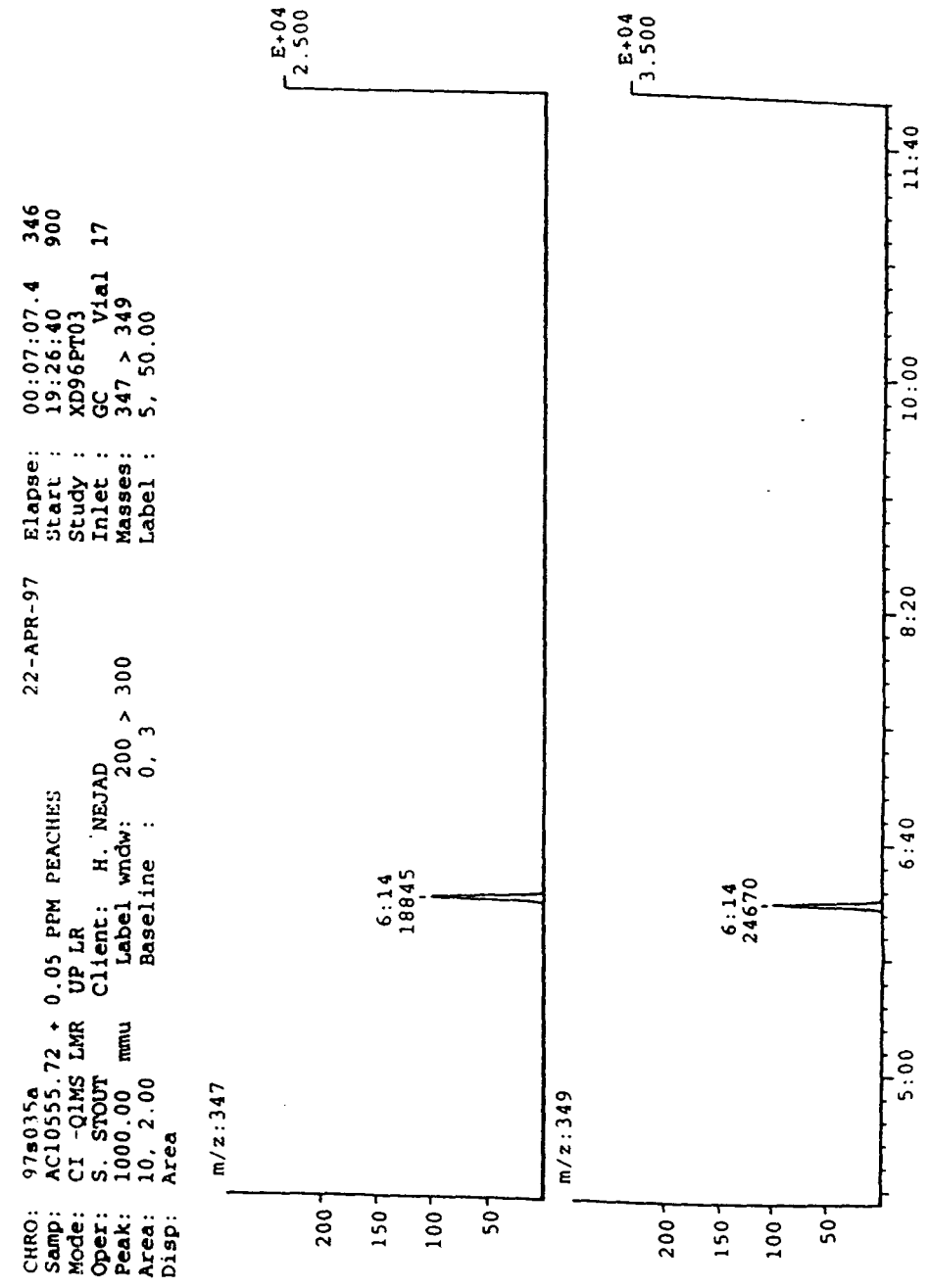


Figure 18: Typical Mass Chromatograms from the Injection of a 0.05 ppm Fortified Peach Sample



APPENDIX C

ABC Laboratories, Inc. Report #43899

Laboratories

Columbia, Missouri

STUDY SPONSOR

American Cyanamid Company
Attn: Huns Nejad
Agricultural Products Research Division
P.O. Box 400
Clarksville and Quakerbridge Road
Princeton, New Jersey 08543-0400

PROTOCOL

XD96PT03

STUDY TITLE

Independent Laboratory Validation of GC Determinative and GC/MS Confirmatory Method M 2686 for the Determination of CL 303,630 Residues in Various Fruits (such as Stone Fruits, Pome Fruits, Strawberries, and Grapes)

DATA REQUIREMENT

Subdivision O, 171-4 Residue Analytical Method and PR Notice 96-1

AUTHOR

Richard F. Kennedy
Principal Analytical Investigator

ANALYTICAL REPORT FINALIZED ON

July 30, 1997

PERFORMING LABORATORY

ABC Laboratories, Inc.
Analytical Chemistry and Field Studies
7200 E. ABC Lane
Columbia, Missouri 65202-8015

ABC LABORATORIES' PROJECT ID

Final Analytical Report #43899

ABC Laboratories California
32380 Avenue 10
Madera, CA 93638 USA
Tel: 209/675-0689 Fax: 209/675-0684

ABC Laboratories Missouri
7200 E. ABC Lane
Columbia, MO 65202 USA
Tel: 573/474-8579 Fax: 573/443-9033

ABC Laboratories Europe
38 Castleroe Road
Coleraine, N. Ireland BT51 3RL
Tel: 44 (01265) 320639 Fax: 44 (01265) 320653

STUDY COMPLIANCE STATEMENT

Study Compliance Statement for ABC Laboratories' Final Analytical Report #43899, "Independent Laboratory Validation of GC Determinative and GC/MS Confirmatory Method M 2686 for the Determination of CL 303,630 Residues in Various Fruits (such as Stone Fruits, Pome Fruits, Strawberries, and Grapes)," for American Cyanamid Company, Princeton, New Jersey.

ABC's Principal Analytical Investigator for the above test confirms that the study was conducted in compliance with the U.S. EPA Good Laboratory Practice Standards; Pesticide Programs (40 CFR 160).

All original raw data have been sent to American Cyanamid Company with the final analytical report and a copy of the raw data and final analytical report have been retained at ABC Laboratories, Inc.

Richard F. Kennedy 7/30/97

Richard F. Kennedy Date
ABC Laboratories'
Principal Analytical Investigator

APPROVAL PAGE

Submitted by:

ABC Laboratories, Inc.
7200 E. ABC Lane
Columbia, Missouri 65202-8015
(573) 474-8579

Prepared by:

Richard F. Kennedy 7/30/97
Richard F. Kennedy Date
Principal Analytical Investigator/Research Scientist
Analytical Chemistry and Field Studies

Approved by:

Nancy Shikles 7/30/97
Nancy Shikles Date
Senior Quality Assurance Specialist

Del A. Koch 30 Jul 97
Del A. Koch Date
Manager
Analytical Chemistry and Field Studies

PROJECT PERSONNEL

The Principal Analytical Investigator for this project at ABC Laboratories, Inc., was Richard F. Kennedy, Research Scientist, Analytical Chemistry and Field Studies. Coordinating the study for American Cyanamid Company was Huns Nejad as Study Director. The following personnel from ABC Laboratories, Inc., were associated with various phases of the study:

Name	Title
Richard F. Kennedy	Research Scientist
Leanne Forbis	Scientist
Carol Doerge	Technical Scientist
J. Craig Harris	Associate Technician

Review of the report, including raw data, was conducted by:

Nancy G. Shikles	Senior Quality Assurance Specialist

Sample receiving and storage was conducted by:

Martha A. Pezold	Senior Technician
Thomas P. Sanders	Associate Technician
Deborah Kerr	Assistant Technician

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PROJECT PURPOSE

The purpose of this study was to conduct an independent laboratory validation of American Cyanamid Company Method M 2686, Draft Dated 03/18/97, for determining CL 303,630 residues in various fruits (such as stone fruits, pome fruits, strawberries, and grapes). The validation study was carried out according to American Cyanamid Company Protocol Number XD96PT03 and PR Notice 96-1.

Control peaches, plums, prunes, cherries, pears, apples, strawberries, and grapes were analyzed in duplicate without fortification and in duplicate after fortification with analytical standards. The fortification levels specified in protocol XD96PT03 were 0.0500, 0.100, and 0.500 ppm.

Overall, sixty-four analyses were conducted in this study that represented a validation trial. For each fruit type, eight analyses were conducted consisting of two unfortified samples and six fortified samples. The average recovery and standard deviation of the forty-eight analyses of fortified fruit was 94 ± 9.0%.

PROJECT HISTORY

The Study Director for this project, Huns Nejad, signed protocol XD96PT03 on March 19, 1997. The experimental start date for the analytical phase of the study was April 1, 1997. The analytical phase was completed April 16, 1997.

STANDARD REFERENCE MATERIAL

American Cyanamid Company supplied the standard reference material used in the validation study. The standard was labeled as follows:

Compound	Lot #	Date of Expiration	ABC Ref. #	Purity	Date Received
CL 303,630	AC9389-90	13-Apr-01	PS-9828	99.7%	25-Mar-97

The reference standard was kept in a refrigerator. American Cyanamid Company performed the characterization of the reference material and maintains the documentation. Stock solutions and dilutions were prepared as presented in the raw data for this study. These solutions were kept refrigerated when not in use.

TEST SYSTEM

American Cyanamid Company supplied the control fruit that were used in this study. The samples were identified as follows:

Control Peaches:	AC 10555.72
Control Plums:	AC 10555.48
Control Prunes:	AC 10555.49
Control Cherries:	AC 10555.46
Control Pears:	AC 9088.63
Control Apples:	AC 10555.44
Control Strawberries:	AC 10555.62
Control Grapes:	AC 10555.61

The containers of each fruit were received at ABC Laboratories on March 20, 1997. Samples were received in good condition, frozen on dry ice. All samples were stored in a freezer.

METHOD OF ANALYSIS

The method of analysis for determining CL 303,630 residues in fruit is described in American Cyanamid Company Method M 2686, Draft Dated 03/18/97. Minor modifications to the gas chromatograph conditions were made for some commodities because of late eluting peaks. The validated sensitivity of Method M 2686 or limit of quantitation (LOQ) is 0.0500 ppm. The LOQ is the minimum concentration of analyte that has been proven to be quantified from sample matrix.

INSTRUMENTATION

The following equipment, which is equivalent to that described in American Cyanamid Company Method M 2686, was used for the gas chromatographic (GC) determinations:

1. Instrument: Hewlett Packard (HP) 5890 Series II Gas Chromatograph with a HP 7673 Automatic Sampler

2. Detector: Nickel-63 High Temperature Electron Capture Detector

3. Column: Fused silica capillary, 15 m x 0.53 mm I.D., SPB-20 with film thickness 1.0 micron.

4. Data Acquisition System: VG Data Systems Ltd. VAX MULTICHROM

VALIDATION RESULTS AND CALCULATIONS

The results of the method validation study for the determination of CL 303,630 residues in fruit are presented in Tables I through VIII. Tables I through VIII are intended to facilitate tracking data contained in the raw data for this study. The results in Tables I through VIII can be recalculated by using the variables provided for each sample and the equations shown at the top of each table page. A description of each variable and the calculation formulae are presented in this report and in Section L of American Cyanamid Company Method M 2686.

Figure 1 through 8 shows typical chromatograms of a 0.0100 μg/mL working standard, a control, and a 0.0500 ppm fortification level for each fruit type.

The first trial of the validation study showed acceptable recovery for the peaches, plums, prunes, cherries, pears, apples, strawberries, and grapes. The overall average and standard deviation of the fortified recovery samples (N=48) in the first trial was 94 $\pm$ 9.0%.

For the analysis of a set of eight fruit samples by American Cyanamid Company Method M 2686, three lab analysts were used. To extract the eight samples and prepare them for GC analysis, a total of 20 person-hours were used. Three hours (short program) or eight hours (long program) were then needed to inject eight samples plus nine standard solutions on the GC equipped for unattended analysis. After GC analysis, two hours were needed to process the chromatograms, and to calculate and check the results captured on computer. Overall, 1.25 calendar days were needed to complete the analysis of eight samples using this method.

After receiving the protocol and method, the sponsor was contacted by FAX to confirm the receipt of the method and protocol. The sponsor was also contacted by phone to convey that the study should start on the next Friday. After all eight fruit samples for the first trial were extracted and injected on GC, the sponsor was contacted and the results were discussed. The results from the first trial were sent to the sponsor. Correspondence records are contained in the raw data for this study.

CALCULATION FORMULAE AND NOTES FOR DETAILED ANALYTICAL DATA TABLES

$$App.\ PPM\ Found = \frac{R(Samp)\ x\ (V1)\ x\ (V3)\ x\ C(Std)\ x\ (V5)\ x\ (DF)}{Avg.R(Std)\ x\ (W)\ x\ (V2)\ x\ (V4)}$$

$$\%\ Recovery = \frac{App.\ PPM\ Found\ x\ 100}{FV\ x\ FC/W}$$

Where:

R(samp) = Peak response of sample.
Avg.R(Std) = Average peak response of working standards, R(Std1), and R(Std2).
V1 = Volume in mL of extracting solvent.
V2 = Volume in mL of aliquot taken for analysis.
V3 = Volume in mL of final solution used for analysis.
V4 = Volume in μL of sample solution injected.
V5 = Volume in μL of standard solution injected.
W = Weight of sample taken for analysis in grams.
C(Std) = Concentration in μg/mL of standard solution.
DF = Dilution factor, if needed, of final solution.
FV = Fortification volume in mL.
FC = Fortification concentration (of standard solution added) in μg/mL.

Notes:

(1) For control samples, an apparent residue value was calculated using actual peak response. Even though the calculated residue value may be lower than the validated sensitivity of the method, the value is shown to give an indication of the detection limit of the method.

(2) Scientific notation was used for final results (i.e. 1.00E-01 = 0.100; 1.00E-02 = 0.0100)

(3) Recoveries were not corrected for amount found in control samples.

Table I: Detailed Recovery Data for the Determination of CL 303,630 Residues in Peaches (Trial 1)

American Cyanamid Protocol Number: XD96PT03
Sample Type: Peaches
Product: Pirate
Analyte: CL 303,630
Method Used: M 2686
Response Measured: Peak Height in Microvolts
N.A.= Not Applicable

$$\text{(1) App. PPM Found} = \frac{R(Samp) \times V1 \times V3 \times C(Std) \times V5 \times DF}{Avg.R(Std) \times W \times V2 \times V4}$$

$$\%\ \text{Recovery} = \frac{\text{App. PPM Found} \times 100}{\text{PPM Added}} = \frac{\text{App. PPM Found} \times 100}{FV \times FC/W}$$

Cust. ID XD96PT03	ABC ID 43899-	Chrom. ID	FV mL	FC µg/mL	PPM Added	W gm	V1 mL	V2 mL	V3 mL	DF	Date Extracted	Date Analyzed	C(Std) µg/mL	R(Std 1) Units	R(Std 2) Units	Avg. R(Std) Units	R(Samp) Units	Apparent PPM Found	% Rec.
AC 10555.72	57	LP6	N.A.	N.A.	N.A.	10.0	300	75.0	25.0	1.00	4/14/97	4/14/97	0.0100	45505	44299	44902	1144	2.55E-03	
AC 10555.72	58	LP7	N.A.	N.A.	N.A.	10.0	300	75.0	25.0	1.00	4/14/97	4/14/97	0.0100	45505	44299	44902	1144*	<2.55E-03	
AC 10555.72	59	LP9	0.500	1.00	0.0500	10.0	300	75.0	25.0	1.00	4/14/97	4/14/97	0.0100	44299	45342	44821	22929	5.12E-02	102
AC 10555.72	60	LP10	0.500	1.00	0.0500	10.0	300	75.0	25.0	1.00	4/14/97	4/14/97	0.0100	44299	45342	44821	32063	7.15E-02	143
AC 10555.72	61	LP12	1.00	1.00	0.100	10.0	300	75.0	25.0	1.00	4/14/97	4/14/97	0.0100	45342	46071	45707	36616	8.01E-02	80
AC 10555.72	62	LP13	1.00	1.00	0.100	10.0	300	75.0	25.0	1.00	4/14/97	4/14/97	0.0100	45342	46071	45707	36779	8.05E-02	81
AC 10555.72	63	LQ6	0.500	10.0	0.500	10.0	300	75.0	25.0	5.00	4/14/97	4/15/97	0.0100	44784	45110	44947	44094	4.91E-01	98
AC 10555.72	64	LQ7	0.500	10.0	0.500	10.0	300	75.0	25.0	5.00	4/14/97	4/15/97	0.0100	44784	45110	44947	44100	4.91E-01	98

(1) V4 = V5 = 1 microliter and thus cancel out of the calculation.
(*) The R(Samp) value substituted here is the estimated minimum response that the instrument can reliably detect.

Prepared by: Richard F. Kennedy Date: 6/25/97 Checked by: Dianne J. Fortris Date: 6-25-97

Table II: Detailed Recovery Data for the Determination of CL 303,630 Residues in Plums (Trial 1)

American Cyanamid Protocol Number: XD96PT03
Sample Type: Plums
Product: Pirate
Analyte: CL 303,630
Method Used: M 2686
Response Measured: Peak Height in Microvolts
N.A.= Not Applicable

$$(1)\ \text{App. PPM Found} = \frac{R(Samp) \times V1 \times V3 \times C(Std) \times V5 \times DF}{Avg.R(Std) \times W \times V2 \times V4}$$

$$\%\ \text{Recovery} = \frac{\text{App. PPM Found} \times 100}{\text{PPM Added}} = \frac{\text{App. PPM Found} \times 100}{FV \times FC/W}$$

Cust. ID XD96PT03	ABC ID 43899-	Chrom. ID	FV mL	FC µg/mL	PPM Added	W gm	V1 mL	V2 mL	V3 mL	DF	Date Extracted	Date Analyzed	C(Std) µg/mL	R(Std 1) Units	R(Std 2) Units	Avg. R(Std) Units	R(Samp) Units	Apparent PPM Found	% Rec.
AC 10555.48	9	LD6	N.A.	N.A.	N.A.	20.0	300	15.0	10.0	1.00	4/4/97	4/4/97	0.0100	43415	43139	43277	1144*	<2.64E-03	
AC 10555.48	10	LD7	N.A.	N.A.	N.A.	20.0	300	15.0	10.0	1.00	4/4/97	4/4/97	0.0100	43415	43139	43277	1144*	<2.64E-03	
AC 10555.48	11	LD9	1.00	1.00	0.0500	20.0	300	15.0	10.0	1.00	4/4/97	4/4/97	0.0100	43139	42858	42999	21357	4.97E-02	99
AC 10555.48	12	LD10	1.00	1.00	0.0500	20.0	300	15.0	10.0	1.00	4/4/97	4/4/97	0.0100	43139	42858	42999	21294	4.95E-02	99
AC 10555.48	13	LD12	2.00	1.00	0.100	20.0	300	15.0	10.0	1.00	4/4/97	4/4/97	0.0100	42858	42991	42925	38929	9.07E-02	91
AC 10555.48	14	LD13	2.00	1.00	0.100	20.0	300	15.0	10.0	1.00	4/4/97	4/4/97	0.0100	42858	42991	42925	39374	9.17E-02	92
AC 10555.48	15	LD15	1.00	10.0	0.500	20.0	300	15.0	10.0	5.00	4/4/97	4/4/97	0.0100	42991	41903	42447	39582	4.66E-01	93
AC 10555.48	16	LD16	1.00	10.0	0.500	20.0	300	15.0	10.0	5.00	4/4/97	4/4/97	0.0100	42991	41903	42447	38979	4.59E-01	92

(1) V4 = V5 = 1 microliter and thus cancel out of the calculation.
(*) The R(Samp) value substituted here is the estimated minimum response that the instrument can reliably detect.

Prepared by: Richard F. Kennedy Date: 4/22/97 Checked by: Lanne J. Forbis Date: 4-22-97

Table III: Detailed Recovery Data for the Determination of CL 303,630 Residues in Prunes (Trial 1)

American Cyanamid Protocol Number: XD96PT03
Sample Type: Prunes
Product: Pirate
Analyte: CL 303,630
Method Used: M 2686
Response Measured: Peak Height in Microvolts
N.A.= Not Applicable

$$(1)\ \text{App. PPM Found} = \frac{R(Samp) \times V1 \times V3 \times C(Std) \times V5 \times DF}{Avg.R(Std) \times W \times V2 \times V4}$$

$$\%\ \text{Recovery} = \frac{\text{App. PPM Found} \times 100}{\text{PPM Added}} = \frac{\text{App. PPM Found} \times 100}{FV \times FC/W}$$

Cust. ID XD96PT03	ABC ID 43899-	Chrom. ID	FV mL	FC µg/mL	PPM Added	W gm	V1 mL	V2 mL	V3 mL	DF	Date Extracted	Date Analyzed	C(Std) µg/mL	R(Std 1) Units	R(Std 2) Units	Avg. R(Std) Units	R(Samp) Units	Apparent PPM Found	% Rec.
AC 10555.49	17	LF6	N.A.	N.A.	N.A.	20.0	300	15.0	10.0	1.00	4/7/97	4/8/97	0.0100	39461	39245	39353	1144*	<2.91E-03	
AC 10555.49	18	LF7	N.A.	N.A.	N.A.	20.0	300	15.0	10.0	1.00	4/7/97	4/8/97	0.0100	39461	39245	39353	1144*	<2.91E-03	
AC 10555.49	19	LF9	1.00	1.00	0.0500	20.0	300	15.0	10.0	1.00	4/7/97	4/8/97	0.0100	39245	40087	39666	17840	4.50E-02	90
AC 10555.49	20	LF10	1.00	1.00	0.0500	20.0	300	15.0	10.0	1.00	4/7/97	4/8/97	0.0100	39245	40087	39666	17441	4.40E-02	88
AC 10555.49	21	LF12	2.00	1.00	0.100	20.0	300	15.0	10.0	1.00	4/7/97	4/8/97	0.0100	40087	40655	40371	33859	8.39E-02	84
AC 10555.49	22	LF13	2.00	1.00	0.100	20.0	300	15.0	10.0	1.00	4/7/97	4/8/97	0.0100	40087	40655	40371	35034	8.68E-02	87
AC 10555.49	23	LF15	1.00	10.0	0.500	20.0	300	15.0	10.0	5.00	4/7/97	4/8/97	0.0100	40655	39714	40185	36736	4.57E-01	91
AC 10555.49	24	LF16	1.00	10.0	0.500	20.0	300	15.0	10.0	5.00	4/7/97	4/8/97	0.0100	40655	39714	40185	36639	4.56E-01	91

(1) V4 = V5 = 1 microliter and thus cancel out of the calculation.
(*) The R(Samp) value substituted here is the estimated minimum response that the instrument can reliably detect.

Prepared by: Richard F. Kennedy Date: 4/22/97 Checked by: Leanne J. Forbis Date: 4-22-97

Table IV: Detailed Recovery Data for the Determination of CL 303,630 Residues in Cherries (Trial 1)

American Cyanamid Protocol Number: XD96PT03
Sample Type: Cherries
Product: Pirate
Analyte: CL 303,630
Method Used: M 2686
Response Measured: Peak Height in Microvolts
N.A.= Not Applicable

$$(1)\ \text{App. PPM Found} = \frac{R(Samp) \times V1 \times V3 \times C(Std) \times V5 \times DF}{Avg.R(Std) \times W \times V2 \times V4}$$

$$\%\ \text{Recovery} = \frac{\text{App. PPM Found} \times 100}{\text{PPM Added}} = \frac{\text{App. PPM Found} \times 100}{FV \times FC/W}$$

Cust. ID XD96PT03	ABC ID 43899-	Chrom. ID	FV mL	FC µg/mL	PPM Added	W gm	V1 mL	V2 mL	V3 mL	DF	Date Extracted	Date Analyzed	C(Std) µg/mL	R(Std 1) Units	R(Std 2) Units	Avg. R(Std) Units	R(Samp) Units	Apparent PPM Found	% Rec.
AC 10555.46	25	LG6	N.A.	N.A.	N.A.	20.0	300	15.0	10.0	1.00	4/8/97	4/8/97	0.0100	41166	38635	39901	1144*	<2.87E-03	
AC 10555.46	26	LG7	N.A.	N.A.	N.A.	20.0	300	15.0	10.0	1.00	4/8/97	4/8/97	0.0100	41166	38635	39901	1144*	<2.87E-03	
AC 10555.46	27	LG9	1.00	1.00	0.0500	20.0	300	15.0	10.0	1.00	4/8/97	4/8/97	0.0100	38635	39218	38927	19623	5.04E-02	101
AC 10555.46	28	LG10	1.00	1.00	0.0500	20.0	300	15.0	10.0	1.00	4/8/97	4/9/97	0.0100	38635	39218	38927	18613	4.78E-02	96
AC 10555.46	29	LG12	2.00	1.00	0.100	20.0	300	15.0	10.0	1.00	4/8/97	4/9/97	0.0100	39218	38625	38922	34094	8.76E-02	88
AC 10555.46	30	LG13	2.00	1.00	0.100	20.0	300	15.0	10.0	1.00	4/8/97	4/9/97	0.0100	39218	38625	38922	31934	8.20E-02	82
AC 10555.46	31	LG15	1.00	10.0	0.500	20.0	300	15.0	10.0	5.00	4/8/97	4/9/97	0.0100	38625	39645	39135	34243	4.37E-01	87
AC 10555.46	32	LG16	1.00	10.0	0.500	20.0	300	15.0	10.0	5.00	4/8/97	4/9/97	0.0100	38625	39645	39135	33935	4.34E-01	87

(1) V4 = V5 = 1 microliter and thus cancel out of the calculation.
(*) The R(Samp) value substituted here is the estimated minimum response that the instrument can reliably detect.

Prepared by: Richard F. Kennedy Date: 4/22/97 Checked by: Leanne J. Forbis Date: 4-22-97

Table V: Detailed Recovery Data for the Determination of CL 303,630 Residues in Pears (Trial 1)

American Cyanamid Protocol Number: XD96PT03
Sample Type: Pears
Product: Pirate
Analyte: CL 303,630
Method Used: M 2686
Response Measured: Peak Height in Microvolts
N.A.= Not Applicable

$$(1)\ \text{App. PPM Found} = \frac{R(Samp) \times V1 \times V3 \times C(Std) \times V5 \times DF}{Avg.R(Std) \times W \times V2 \times V4}$$

$$\%\ \text{Recovery} = \frac{\text{App. PPM Found} \times 100}{\text{PPM Added}} = \frac{\text{App. PPM Found} \times 100}{FV \times FC/W}$$

Cust. ID XD96PT03	ABC ID 43899-	Chrom. ID	FV mL	FC µg/mL	PPM Added	W gm	V1 mL	V2 mL	V3 mL	DF	Date Extracted	Date Analyzed	C(Std) µg/mL	R(Std 1) Units	R(Std 2) Units	Avg. R(Std) Units	R(Samp) Units	Apparent PPM Found	% Rec.
AC 9088.63	33	L16	N.A.	N.A.	N.A.	20.0	300	75.0	25.0	1.00	4/9/97	4/9/97	0.0100	40357	40573	40465	1144*	<1.41E-03	
AC 9088.63	34	L17	N.A.	N.A.	N.A.	20.0	300	75.0	25.0	1.00	4/9/97	4/9/97	0.0100	40357	40573	40465	1144*	<1.41E-03	
AC 9088.63	35	L19	1.00	1.00	0.0500	20.0	300	75.0	25.0	1.00	4/9/97	4/9/97	0.0100	40573	40213	40393	38258	4.74E-02	95
AC 9088.63	36	L110	1.00	1.00	0.0500	20.0	300	75.0	25.0	1.00	4/9/97	4/9/97	0.0100	40573	40213	40393	39136	4.84E-02	97
AC 9088.63	37	L112	2.00	1.00	0.100	20.0	300	75.0	25.0	2.00	4/9/97	4/9/97	0.0100	40213	40650	40432	37906	9.38E-02	94
AC 9088.63	38	L113	2.00	1.00	0.100	20.0	300	75.0	25.0	2.00	4/9/97	4/9/97	0.0100	40213	40650	40432	37819	9.35E-02	94
AC 9088.63	39	L115	1.00	10.0	0.500	20.0	300	75.0	25.0	10.0	4/9/97	4/9/97	0.0100	40650	40849	40750	37736	4.63E-01	93
AC 9088.63	40	L116	1.00	10.0	0.500	20.0	300	75.0	25.0	10.0	4/9/97	4/9/97	0.0100	40650	40849	40750	37783	4.64E-01	93

(1) V4 = V5 = 1 microliter and thus cancel out of the calculation.
(*) The R(Samp) value substituted here is the estimated minimum response that the instrument can reliably detect.

Prepared by: Richard E. Kennedy Date: 4/22/97 Checked by: Lanne J. Forbis Date: 4-22-97

Table VI: Detailed Recovery Data for the Determination of CL 303,630 Residues in Apples (Trial 1)

American Cyanamid Protocol Number: XD96PT03
Sample Type: Apples
Product: Pirate
Analyte: CL 303,630
Method Used: M 2686
Response Measured: Peak Height in Microvolts
N.A.= Not Applicable

$$(1)\ \text{App. PPM Found} = \frac{R(Samp) \times V1 \times V3 \times C(Std) \times V5 \times DF}{Avg.R(Std) \times W \times V2 \times V4}$$

$$\%\ \text{Recovery} = \frac{\text{App. PPM Found} \times 100}{\text{PPM Added}} = \frac{\text{App. PPM Found} \times 100}{FV \times FC/W}$$

Cust. ID XD96PT03	ABC ID 43899-	Chrom. ID	FV mL	FC µg/mL	PPM Added	W gm	V1 mL	V2 mL	V3 mL	DF	Date Extracted	Date Analyzed	C(Std) µg/mL	R(Std 1) Units	R(Std 2) Units	Avg. R(Std) Units	R(Samp) Units	Apparent PPM Found	% Rec.
AC 10555.44	1	LC6	N.A.	N.A.	N.A.	20.0	300	75.0	25.0	1.00	4/3/97	4/3/97	0.0100	43784	42456	43120	1144*	<1.33E-03	
AC 10555.44	2	LC7	N.A.	N.A.	N.A.	20.0	300	75.0	25.0	1.00	4/3/97	4/3/97	0.0100	43784	42456	43120	1144*	<1.33E-03	
AC 10555.44	3	LC9	1.00	1.00	0.0500	20.0	300	75.0	25.0	1.00	4/3/97	4/3/97	0.0100	42456	43061	42759	40484	4.73E-02	95
AC 10555.44	4	LC10	1.00	1.00	0.0500	20.0	300	75.0	25.0	1.00	4/3/97	4/3/97	0.0100	42456	43061	42759	41009	4.80E-02	96
AC 10555.44	5	LC12	2.00	1.00	0.100	20.0	300	75.0	25.0	2.00	4/3/97	4/3/97	0.0100	43061	43292	43177	39009	9.03E-02	90
AC 10555.44	6	LC13	2.00	1.00	0.100	20.0	300	75.0	25.0	2.00	4/3/97	4/3/97	0.0100	43061	43292	43177	39523	9.15E-02	92
AC 10555.44	7	LC15	1.00	10.0	0.500	20.0	300	75.0	25.0	10.0	4/3/97	4/3/97	0.0100	43292	40952	42122	39515	4.69E-01	94
AC 10555.44	8	LC16	1.00	10.0	0.500	20.0	300	75.0	25.0	10.0	4/3/97	4/3/97	0.0100	43292	40952	42122	39504	4.69E-01	94

(1) V4 = V5 = 1 microliter and thus cancel out of the calculation.
(*) The R(Samp) value substituted here is the estimated minimum response that the instrument can reliably detect.

Prepared by: Richard F. Kennedy Date: 4/22/97 Checked by: Lianne J. Forbis Date: 4-22-97

Table VII: Detailed Recovery Data for the Determination of CL 303,630 Residues in Strawberries (Trial 1)

American Cyanamid Protocol Number: XD96PT03
Sample Type: Strawberries
Product: Pirate
Analyte: CL 303,630
Method Used: M 2686
Response Measured: Peak Height in Microvolts
N.A.= Not Applicable

(1) App. PPM Found = $\frac{R(Samp) \times V1 \times V3 \times C(Std) \times V5 \times DF}{Avg.R(Std) \times W \times V2 \times V4}$

% Recovery = $\frac{\text{App. PPM Found} \times 100}{\text{PPM Added}} = \frac{\text{App. PPM Found} \times 100}{FV \times FC/W}$

Cust. ID XD96PT03	ABC ID 43899-	Chrom. ID	FV mL	FC µg/mL	PPM Added	W gm	V1 mL	V2 mL	V3 mL	DF	Date Extracted	Date Analyzed	C(Std) µg/mL	R(Std 1) Units	R(Std 2) Units	Avg. R(Std) Units	R(Samp) Units	Apparent PPM Found	% Rec.
AC 10555.62	49	LK6	N.A.	N.A.	N.A.	20.0	300	15.0	10.0	1.00	4/11/97	4/11/97	0.0100	42520	45686	44103	1144*	<2.59E-03	
AC 10555.62	50	LK7	N.A.	N.A.	N.A.	20.0	300	15.0	10.0	1.00	4/11/97	4/11/97	0.0100	42520	45686	44103	1144*	<2.59E-03	
AC 10555.62	51	LK9	1.00	1.00	0.0500	20.0	300	15.0	10.0	1.00	4/11/97	4/11/97	0.0100	45686	45868	45777	21652	4.73E-02	95
AC 10555.62	52	LK10	1.00	1.00	0.0500	20.0	300	15.0	10.0	1.00	4/11/97	4/11/97	0.0100	45686	45868	45777	23211	5.07E-02	101
AC 10555.62	53	LK12	2.00	1.00	0.100	20.0	300	15.0	10.0	1.00	4/11/97	4/11/97	0.0100	45868	45256	45562	40949	8.99E-02	90
AC 10555.62	54	LK13	2.00	1.00	0.100	20.0	300	15.0	10.0	1.00	4/11/97	4/11/97	0.0100	45868	45256	45562	42072	9.23E-02	92
AC 10555.62	55	LK15	1.00	10.0	0.500	20.0	300	15.0	10.0	5.00	4/11/97	4/11/97	0.0100	45256	45775	45516	42356	4.65E-01	93
AC 10555.62	56	LK16	1.00	10.0	0.500	20.0	300	15.0	10.0	5.00	4/11/97	4/11/97	0.0100	45256	45775	45516	43667	4.80E-01	96

(1) V4 = V5 = 1 microliter and thus cancel out of the calculation.
(*) The R(Samp) value substituted here is the estimated minimum response that the instrument can reliably detect.

Prepared by: Richard F. Kennedy Date: 4/22/97 Checked by: Dianne J. Forbis Date: 4-21-97

Table VIII: Detailed Recovery Data for the Determination of CL 303,630 Residues in Grapes (Trial 1)

American Cyanamid Protocol Number: XD96PT03
Sample Type: Grapes
Product: Pirate
Analyte: CL 303,630
Method Used: M 2686
Response Measured: Peak Height in Microvolts
N.A.= Not Applicable

$$(1)\ \text{App. PPM Found} = \frac{R(Samp) \times V1 \times V3 \times C(Std) \times V5 \times DF}{Avg.R(Std) \times W \times V2 \times V4}$$

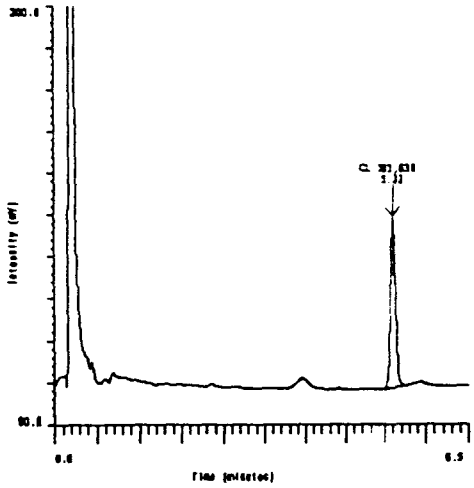
$$\%\ \text{Recovery} = \frac{\text{App. PPM Found} \times 100}{\text{PPM Added}} = \frac{\text{App. PPM Found} \times 100}{FV \times FC/W}$$

Cust. ID XD96PT03	ABC ID 43899-	Chrom. ID	FV mL	FC µg/mL	PPM Added	W gm	V1 mL	V2 mL	V3 mL	DF	Date Extracted	Date Analyzed	C(Std) µg/mL	R(Std 1) Units	R(Std 2) Units	Avg. R(Std) Units	R(Samp) Units	Apparent PPM Found	% Rec.
AC 10555.61	41	LJ6	N.A.	N.A.	N.A.	20.0	300	15.0	10.0	1.00	4/10/97	4/10/97	0.0100	40016	39775	39896	1144*	<2.87E-03	
AC 10555.61	42	LJ7	N.A.	N.A.	N.A.	20.0	300	15.0	10.0	1.00	4/10/97	4/10/97	0.0100	40016	39775	39896	1144*	<2.87E-03	
AC 10555.61	43	LJ9	1.00	1.00	0.0500	20.0	300	15.0	10.0	1.00	4/10/97	4/10/97	0.0100	39775	39808	39792	20743	5.21E-02	104
AC 10555.61	44	LJ10	1.00	1.00	0.0500	20.0	300	15.0	10.0	1.00	4/10/97	4/10/97	0.0100	39775	39808	39792	20352	5.11E-02	102
AC 10555.61	45	LJ12	2.00	1.00	0.100	20.0	300	15.0	10.0	1.00	4/10/97	4/10/97	0.0100	39808	40555	40182	36258	9.02E-02	90
AC 10555.61	46	LJ13	2.00	1.00	0.100	20.0	300	15.0	10.0	1.00	4/10/97	4/10/97	0.0100	39808	40555	40182	36616	9.11E-02	91
AC 10555.61	47	LJ15	1.00	10.0	0.500	20.0	300	15.0	10.0	5.00	4/10/97	4/10/97	0.0100	40555	40303	40429	36772	4.55E-01	91
AC 10555.61	48	LJ16	1.00	10.0	0.500	20.0	300	15.0	10.0	5.00	4/10/97	4/10/97	0.0100	40555	40303	40429	37588	4.65E-01	93

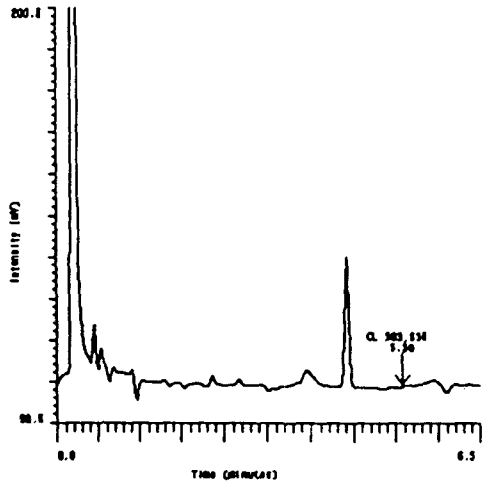
(1) V4 = V5 = 1 microliter and thus cancel out of the calculation.
(*) The R(Samp) value substituted here is the estimated minimum response that the instrument can reliably detect.

Prepared by: Richard F. Kennedy Date: 4/22/97 Checked by: Dianne J. Forbis Date: 4-22-97

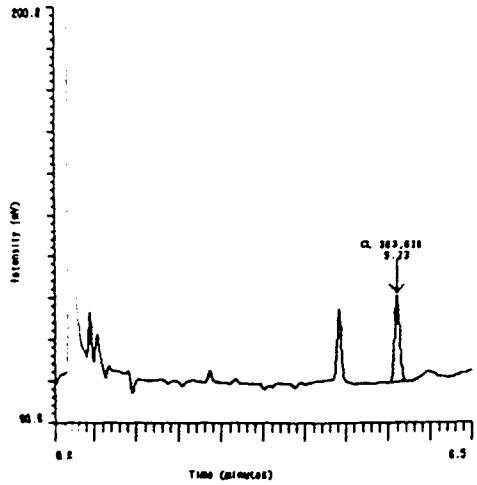
Figure 1. Typical Chromatograms for the Determination of CL 303,630 Residues in Peaches.



CL 303,630 standard, 0.0100 μg/mL,
10 pg injected

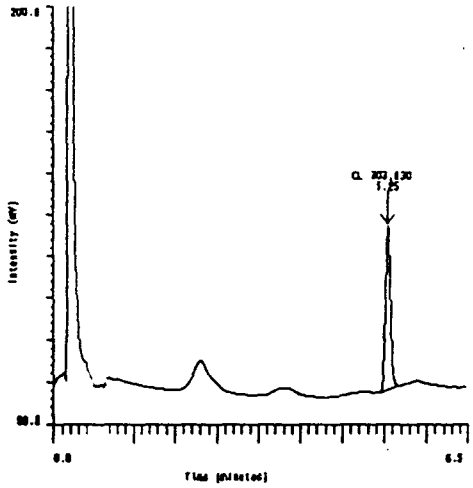


Control Peaches, 100 μg Injected
<0.00255 ppm Apparent CL 303,630
Residues Found

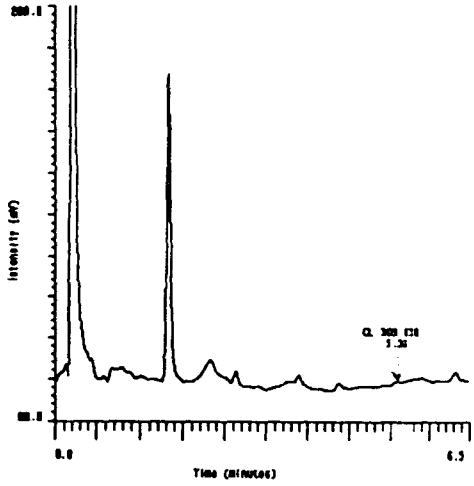


Control Peaches
Fortified with CL 303,630 at 0.05 ppm
100 μg Injected, 0.0512 ppm Found
102% Recovered

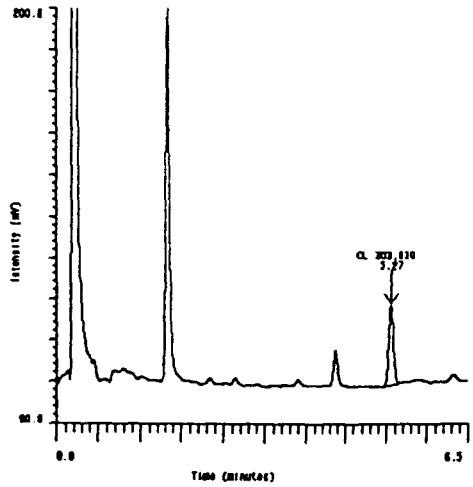
Figure 2. Typical Chromatograms for the Determination of CL 303,630 Residues in Plums.



CL 303,630 standard, 0.0100 μg/mL,
10 pg injected

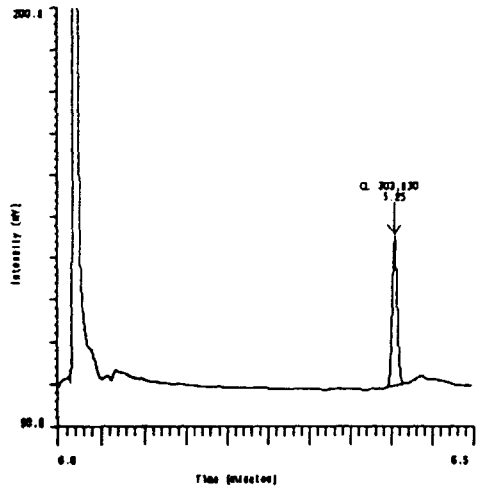


Control Plums, 100 μg Injected
<0.00264 ppm Apparent CL 303,630
Residues Found

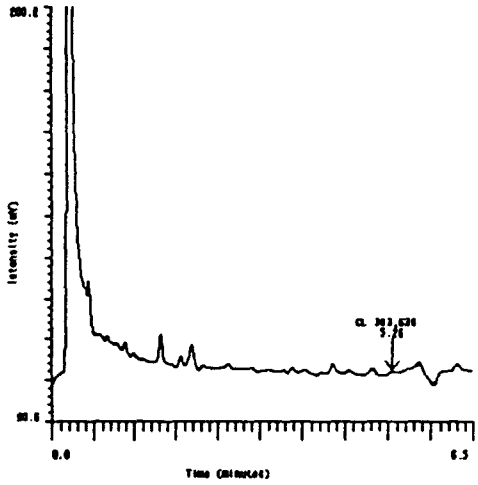


Control Plums
Fortified with CL 303,630 at 0.05 ppm
100 μg Injected, 0.0497 ppm Found
99% Recovered

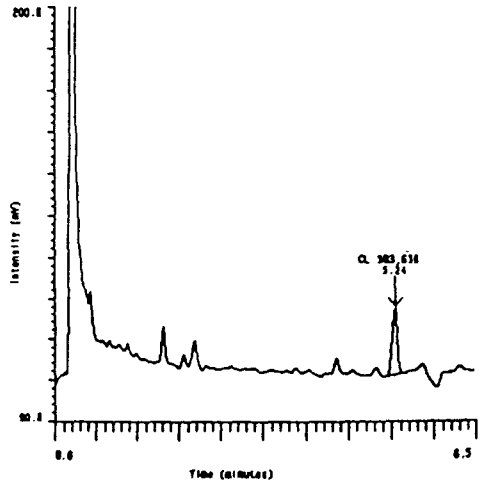
Figure 3. Typical Chromatograms for the Determination of CL 303,630 Residues in Prunes.



CL 303,630 standard, 0.0100 μg/mL,
10 pg injected

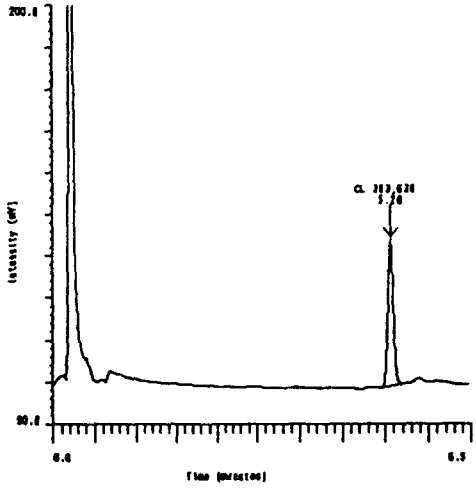


Control Prunes, 100 μg Injected
<0.00291 ppm Apparent CL 303,630
Residues Found

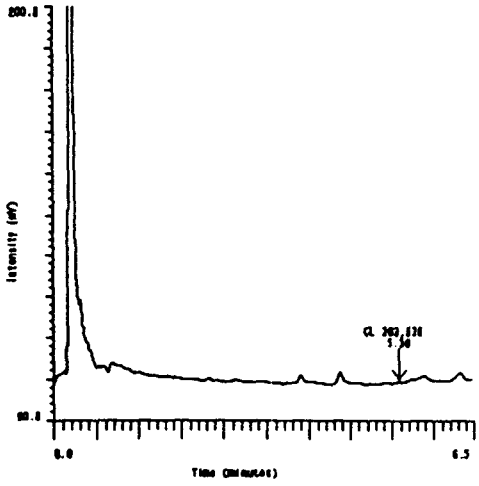


Control Prunes
Fortified with CL 303,630 at 0.05 ppm
100 μg Injected, 0.0450 ppm Found
90% Recovered

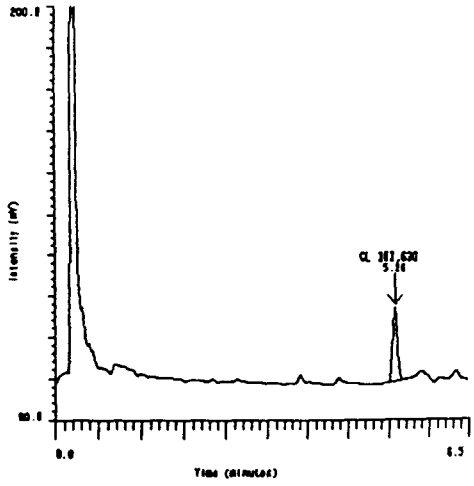
Figure 4. Typical Chromatograms for the Determination of CL 303,630 Residues in Cherries.



CL 303,630 standard, 0.0100 μg/mL,
10 pg injected

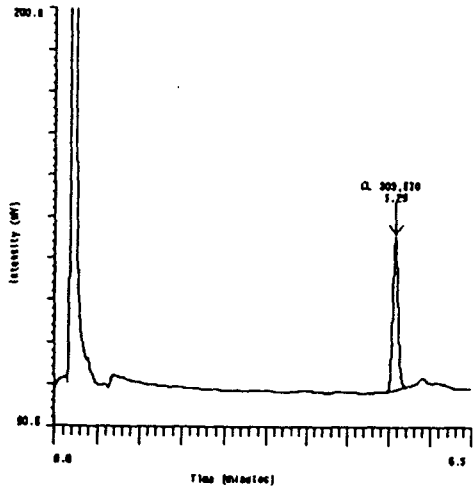


Control Cherries, 100 μg Injected
<0.00287 ppm Apparent CL 303,630
Residues Found

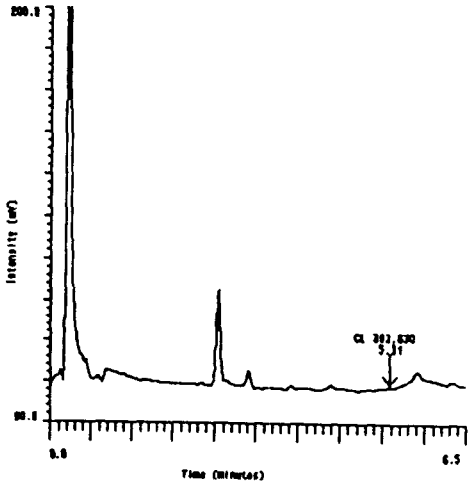


Control Cherries
Fortified with CL 303,630 at 0.05 ppm
100 μg Injected, 0.0504 ppm Found
101% Recovered

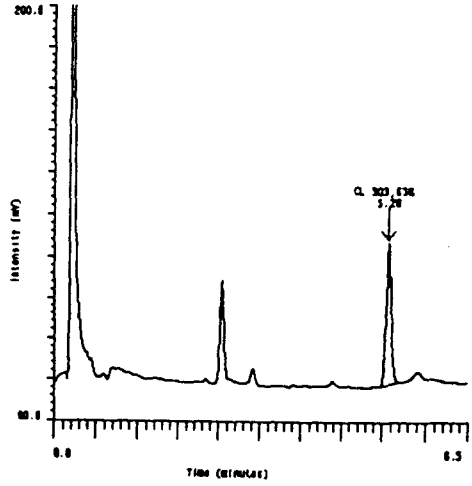
Figure 5. Typical Chromatograms for the Determination of CL 303,630 Residues in Pears.



CL 303,630 standard, 0.0100 μg/mL,
10 pg injected

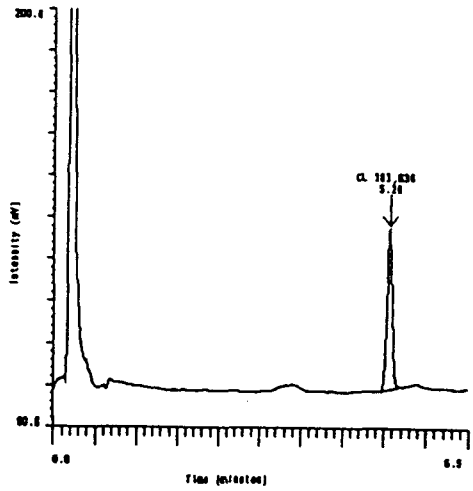


Control Pears, 200 μg Injected
<0.00141 ppm Apparent CL 303,630
Residues Found

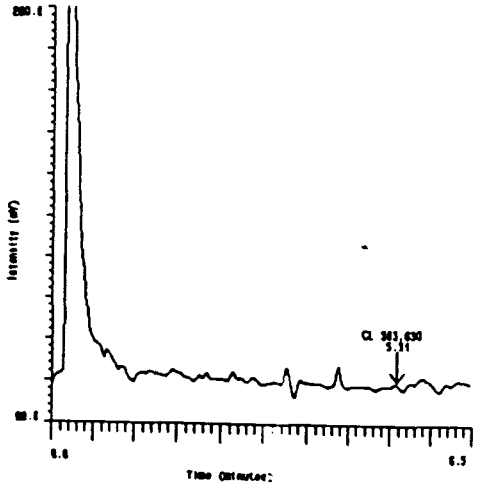


Control Pears
Fortified with CL 303,630 at 0.05 ppm
200 μg Injected, 0.0474 ppm Found
95% Recovered

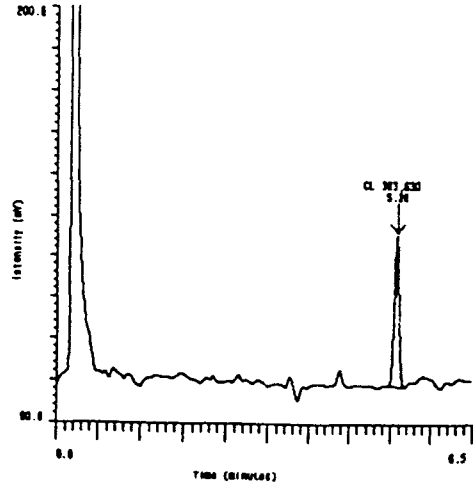
Figure 6. Typical Chromatograms for the Determination of CL 303,630 Residues in Apples.



CL 303,630 standard, 0.0100 μg/mL,
10 pg injected

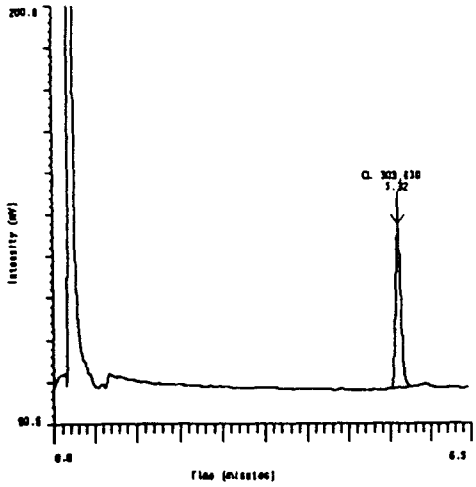


Control Apples, 200 μg Injected
<0.00133 ppm Apparent CL 303,630
Residues Found

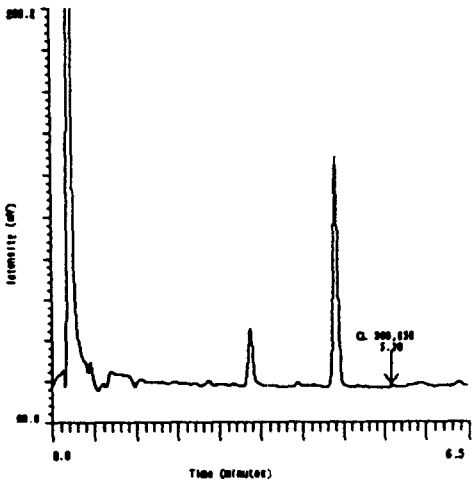


Control Apples
Fortified with CL 303,630 at 0.05 ppm
200 μg Injected, 0.0473 ppm Found
95% Recovered

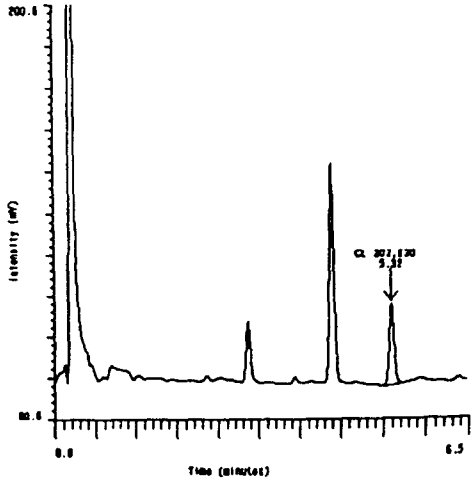
Figure 7. Typical Chromatograms for the Determination of CL 303,630 Residues in Strawberries.



CL 303,630 standard, 0.0100 μg/mL,
10 pg injected

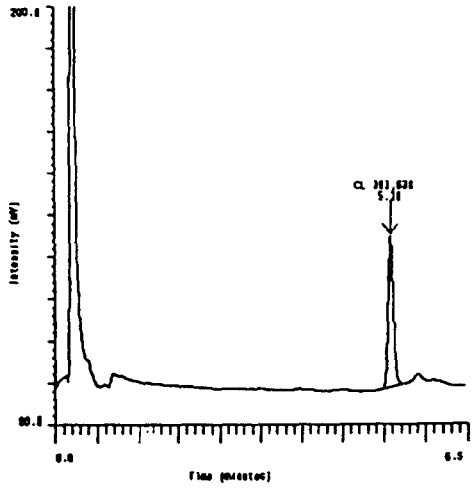


Control Strawberries, 100 μg Injected
<0.00259 ppm Apparent CL 303,630
Residues Found

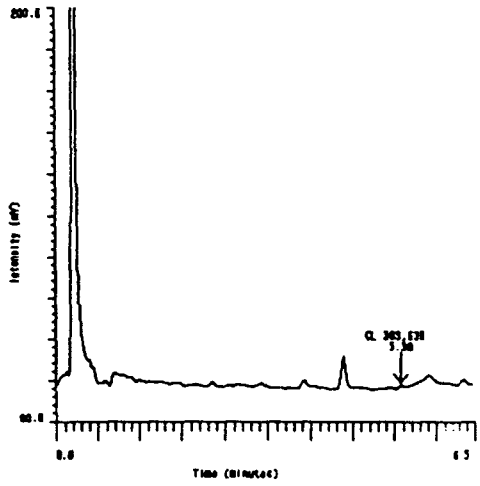


Control Strawberries
Fortified with CL 303,630 at 0.05 ppm
100 μg Injected, 0.0473 ppm Found
95% Recovered

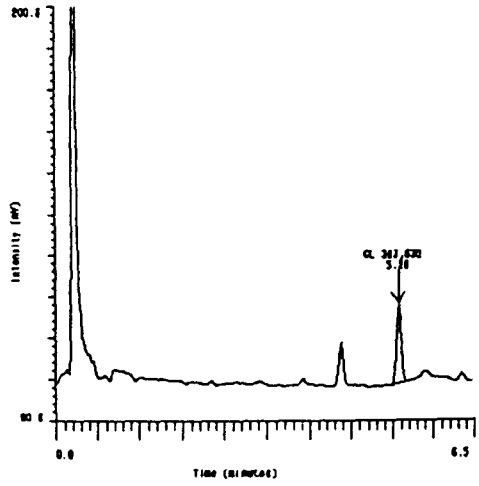
Figure 8. Typical Chromatograms for the Determination of CL 303,630 Residues in Grapes.



CL 303,630 standard, 0.0100 μg/mL,
10 pg injected



Control Grapes, 100 μg Injected
<0.00287 ppm Apparent CL 303,630
Residues Found



Control Grapes
Fortified with CL 303,630 at 0.05 ppm
100 μg Injected, 0.0521 ppm Found
104% Recovered

QUALITY ASSURANCE STATEMENT

ABC Laboratories' Quality Assurance Unit reviewed study #43899, "Independent Laboratory Validation of GC Determinative and GC/MS Confirmatory Method M 2686 for the Determination of CL 303,630 Residues in Various Fruits (such as Stone Fruits, Pome Fruits, Strawberries, and Grapes)," for American Cyanamid Company, Princeton, New Jersey. The following inspections/audits were conducted on this study:

Date of Inspection*	Phase Inspected	Date Reported To Management**	Date Reported To Study Director ***
03/21/97	Protocol	03/24/97	03/24/97
04/03/97	Weighing and Fortifications	04/04/97	04/04/97
04/21/97 and 04/22/97	Draft Analytical Report	04/23/97	4/30/97
07/08/97	Final Analytical Report	07/08/97	07/30/97

*Findings were reported to the Principal Analytical Investigator on the day of the inspection, except draft report reported on 04/22/97.
**Date reported to ABC Laboratories' management.
***Date to study director's management.

The undersigned conducted the final report audit. The audits indicate the report is an accurate reflection as it was conducted by ABC Laboratories, Inc.

Nancy G. Shikles 7/30/97
Nancy G. Shikles Date
Senior Quality Assurance Specialist

APPENDIX D

Communication Log

Summary of Highlights of Communication between BASF and ABC Laboratories, Inc.

3/19/97	Huns Nejad, Study Director for BASF, sent R. Frank Kennedy, Principal Analyst for ABC Laboratories, Inc., a shipment of various frozen control fruit commodities.
3/19/97	Huns Nejad sent Frank Kennedy a copy of Protocol XD96PT03 along with a copy of method M 2686, draft dated 3/18/97. Frank Kennedy was informed that a shipment of analytical standard was going to be sent.
3/24/97	Frank Kennedy sent Huns Nejad a facsimile as proof of receipt of Protocol XD96PT03 and Method M 2686, draft dated 3/18/97.
4/15/97	Frank Kennedy called Huns Nejad to inform him that he had successfully validated Method M 2686, draft dated 3/18/97. He reported recoveries between 80% and 105% with the exception of 143% for peaches. He also indicated that minor modifications were made to the GC conditions. Huns Nejad requested copies of the preliminary results.
4/15/97	Preliminary results were sent to Huns Nejad.
4/16/97	Sample extracts, along with a 0.01 µg/mL standard, were sent to Huns Nejad for mass spectrometric confirmation.
4/17/97	Frank Kennedy sent Huns Nejad Sample Tracking Forms, GC Instrument Parameters, chromatography and Calculation reports for various sample sets.
4/18/97	Sample extracts and standard sent for mass spectrometric confirmation, were received on 4/18/97.
4/18/97	Huns Nejad received copies of preliminary raw data from ABC Laboratories, Inc.
4/21/97	Revised detailed analytical data tables were sent to Huns Nejad.
4/22/97	Additional revised detailed analytical data tables were sent to Huns Nejad.

Summary of Highlights of Communication Between BASF and ABC Laboratories, Inc. (continued)

4/23/97	A copy of the Method Approval Form for Method M 2686 was sent to Frank Kennedy.
4/29/97	A facsimile was sent was Frank Kennedy informing Huns Nejad that the Method Approval Form was received.
5/2/97	Draft report #43899 was received from Frank Kennedy.
5/14/97	Huns Nejad called Frank Kennedy to discuss draft report # 43899. Huns Nejad indicated that Amendment #1 would be issued.
5/14/97	A copy of Amendment #1 was sent to Frank Kennedy.
5/15/97	A facsimile was sent to Huns Nejad informing him that the changes to the draft report, requested on 5/14/97, had been made.
6/12/97	A copy of the final version of Method M 2686 was sent to Frank Kennedy along with instructions to finalize the analytical report.
7/30/97	ABC Laboratories, Inc. finalized the analytical report #43899.

Dupe

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUN 11 1996

OFFICE OF
ENFORCEMENT AND
COMPLIANCE ASSURANCE

Mr. Phil Buckler
Director, Safety & Quality Assurance
Analytical Bio-Chemistry Labs, Inc.
7200 East ABC Lane
P. O. Box 1097
Columbia, MO 65205

Dear Mr. Buckler:

Re: Good Laboratory Practice Standards Inspection of April 9-11, 1996

This letter is formal notification of the results of the April 9-11, 1996 inspection, conducted by representatives of the Environmental Protection Agency (EPA), pursuant to Sections 8 and 9 of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA).

The purpose of this inspection was to assess your facility's compliance efforts with regard to the FIFRA Good Laboratory Practice Standards (GLPS) at 40 C.F.R. Part 160 and the quality and integrity of your data submitted pursuant to Section 4 of FIFRA.

Based solely on a review of the information gathered during the April 9-11, 1996 inspection of your facility, we have concluded that an enforcement action will not be issued.

The enclosed copy of the inspection and data audit report includes a description of the findings made by the inspection team.

The Agency determination in this matter in no way constitutes an endorsement of your laboratory by EPA, nor does this letter imply EPA approval of your facility and operations which could be used in any advertising or marketing activities.

Thank you for your cooperation during the inspection. If you have questions concerning the assessment of your facility, data quality and integrity, please contact Francisca E. Liem, Chief, Laboratory Data Integrity Branch at (202) 564-2365.

Sincerely,

for Rick Colbert, Director
Agriculture and Ecosystems Division
Office of Compliance

Enclosure

cc: