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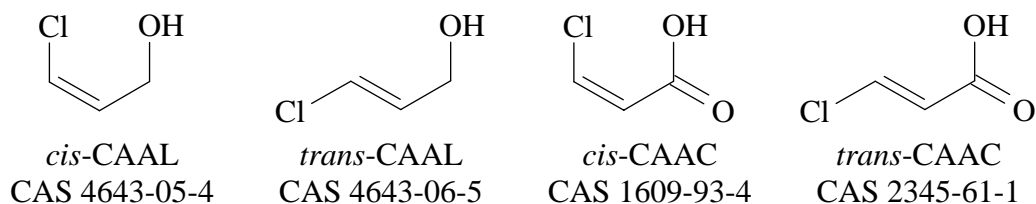


Determination of 3-Chloroallyl Alcohol and 3-Chloroacrylic Acid in Grapes
by Capillary Gas Chromatography with Mass Spectrometric Detection

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1. SCOPE

This method is applicable for the quantitative determination of residues of *cis*-3-chloroallyl alcohol (CAAL), *trans*-CAAL, *cis*-3-chloroacrylic acid (CAAC), and *trans*-CAAC in grapes. The method was validated over the concentration range of 3.0 to 30 ng/g with a limit of quantitation of 3.0 ng/g.



2. PRINCIPLE

Homogenized grape tissue is made basic with sodium hydroxide and residues of CAAL are extracted with methyl *tert*-butyl ether (MTBE). The MTBE extract is purified using silica gel and graphitized carbon solid-phase extraction (SPE) columns. CAAL residues are not retained on the SPE columns. CAAL residues are concentrated in hexane using a Snyder distillation column. The 4-penten-1-ol internal standard is added and residues of CAAL are derivatized, using pyridine and isobutyl chloroformate, to their corresponding *cis*- and *trans*-3-chloroallyl isobutyl carbonates (CAIBC). Excess derivatization reagent is neutralized by the addition of methanol and a dilute sodium hydroxide solution. The hexane is concentrated to approximately 0.5 mL and residues of CAAL are determined by gas chromatography with mass spectrometric detection (GC/MS).

The grape tissue is made acidic with hydrochloric acid and residues of CAAC are extracted with MTBE. The MTBE extract is dried over anhydrous magnesium sulfate and purified using a graphitized carbon SPE column. CAAC residues are not retained on the SPE column. Isooctane and the ethoxyacetic acid internal standard are added, and the sample is concentrated to approximately 0.5 mL. Residues of

CAAC are derivatized, using *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA), to their corresponding *cis*- and *trans*-3-chloroacrylic acid *tert*-butyldimethylsilyl esters (CAAC TBDMSE) and determined by GC/MS.

3. SAFETY PRECAUTIONS

- 3.1. Each analyst must be acquainted with the potential hazards of the reagents, products, and solvents used in this method before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: MATERIAL SAFETY DATA SHEETS, LITERATURE, AND OTHER RELATED DATA. Safety information on non-Dow AgroSciences LLC products should be obtained from the container label or from the supplier. Disposal of reagents, reactants, and solvents must be in compliance with local, state, and federal laws and regulations.
- 3.2. Acetic acid, acetone, hexane, isooctane, methanol, methyl *tert*-butyl ether (MTBE), and 1-propanol are flammable and should be used in well-ventilated areas away from ignition sources.
- 3.3. Acetic acid, solutions of hydrochloric acid, and solutions of sodium hydroxide are corrosive and can cause severe burns. It is imperative that proper eye and personal protective equipment be used when handling these compounds.
- 3.4. *cis*- and *trans*-3-Chloroallyl alcohol and *cis*- and *trans*-3-chloroacrylic acid are corrosive and lachrymators. It is imperative that proper eye and personal protective equipment be used when handling these compounds. Handling of neat material should be carried out in a fume hood.
- 3.5. Isobutyl chloroformate is highly toxic, irritating to eyes, respiratory system and skin. It is imperative that proper eye and personal protective equipment be used when handling this reagent. Handling of neat material should be carried out in a fume hood.
- 3.6. *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA) is irritating to eyes, respiratory system and skin. It is imperative that proper eye and personal protective equipment be used when handling this reagent. Handling of neat material should be carried out in a fume hood.

4. EQUIPMENT (Note 12.1.)

- 4.1. Balance, analytical, Model AE200, Mettler-Toledo, Inc., Hightstown, NJ 08520.
- 4.2. Balance, top loading, Model P-1200, Mettler-Toledo, Inc.

- 4.3. Centrifuge, with rotors to accommodate 45-mL vials and 16 x 100 mm culture tubes, Model Centra-GP8, International Equipment Company, Needham Heights, MA 02194.
- 4.4. Evaporator, N-Evap, Model 111, Organomation Associates, Inc., South Berlin, MA 01549.
- 4.5. Gas chromatograph, Model 5890 Series II, Hewlett-Packard, Wilmington, DE 19808.
- 4.6. Hammer mill, Model 2001, equipped with a 3/16-inch screen, AGVISE Laboratories, Northwood, ND 58267.
- 4.7. Heater, dry bath incubator, catalog number 11-718-2, Fisher Scientific, Pittsburgh, PA 15219.
- 4.8. Heater, dry bath incubator (aluminum) heating block, catalog number 11-718-16, Fisher Scientific.
- 4.9. Hot plate, Thermolyne extra-capacity, catalog number 11-496-5A, Fisher Scientific.
- 4.10. Injector, automatic, Model 7673, Hewlett-Packard.
- 4.11. Mass selective detector, Model 5971A, Hewlett-Packard, Palo Alto, CA 94304.
- 4.12. Mass selective detector data system, Model G1701AA, Hewlett-Packard.
- 4.13. Shaker, variable speed reciprocating with box carrier, Model 6000, Eberbach Corporation, Ann Arbor, MI 48106.
- 4.14. Vacuum manifold, Model spe-21, Mallinckrodt Baker, Inc., Phillipsburg, NJ 08865.
- 4.15. Vial crimper, catalog number 8710-0979, Hewlett-Packard.
- 4.16. Vortex mixer, Model K-550-G, Scientific Industries, Inc., Bohemia, NY 11716.
5. GLASSWARE AND MATERIALS (Note 12.1.)
 - 5.1. Boiling stones, PTFE, catalog number 09-191-20, Fisher Scientific.
 - 5.2. Column, capillary gas chromatography, DB-17 liquid phase, 20 m x 0.18 mm i.d., 0.3- μ m film thickness, catalog number 121-1723, J & W Scientific, Folsom, CA 95630.

- 5.3. Column, graphitized carbon SPE, 0.25-g packing, catalog number 57092, Supelco, Inc., Bellefonte, PA 16823.
- 5.4. Column, silica gel SPE, 1.0-g packing, catalog number 7086-07, Mallinckrodt Baker, Inc.
- 5.5. Column, Snyder distilling, 19/22 joint, catalog number 569001-0319, Kontes, Vineland, NJ 08360.
- 5.6. Column, SPE adapter, PTFE, catalog number 120-1100, Jones Chromatography, Inc., Lakewood, CO 80228.
- 5.7. Culture tube, 16 x 100 mm screw-cap with PTFE-lined cap, catalog number 14-930-10B, Fisher Scientific.
- 5.8. Filter, carrier gas, UOP mat/sen Purifier with 1/8" compression fittings, catalog number 2-2680-U, Supelco, Bellefonte, PA 16823.
- 5.9. Flask, Erlenmeyer, 50-mL with 19/22 joint, catalog number 296510-0050, Kontes.
- 5.10. Inlet sleeve, double gooseneck splitless, catalog number 5181-3315, Hewlett-Packard.
- 5.11. Pipetter, Drummond microdispenser, 50- and 100- μ L capacity, catalog numbers 21-170-15C and 21-170-15D, Fisher Scientific.
- 5.12. Pipetter bore, for Drummond microdispenser, 50- and 100- μ L bore, catalog numbers 21-169D, and 21-169F, Fisher Scientific.
- 5.13. Syringe, 100-, 250-, and 500- μ L capacity, catalog numbers 81000, 81100, and 81217, Hamilton Co., Reno, NV 89520.
- 5.14. Vial, autosampler, 2-mL, catalog number C4011-1, National Scientific Co., Lawrenceville, GA 30243.
- 5.15. Vial, 45-mL, catalog number 03-339-5D, Fisher Scientific.
- 5.16. Vial cap, with liner, for 45-mL vial, catalog number 02-883-5G, Fisher Scientific.
- 5.17. Vial cap, for autosampler vial, catalog number C4000-54B, National Scientific Company.
- 5.18. Vial insert, limited volume (200- μ L), for 2-mL autosampler vial, catalog number C4011-631, National Scientific Co., Lawrenceville, GA 30243.

6. REAGENTS, STANDARDS, AND PREPARED SOLUTIONS (Note 12.1.)
 - 6.1. Reagents
 - 6.1.1. Acetic acid, HPLC grade, catalog number A35-500, Fisher Scientific.
 - 6.1.2. Acetone, OmniSolv grade, catalog number AX0110-1, EM Science, Cincinnati, OH 45212.
 - 6.1.3. Hexane, OmniSolv grade, catalog number HX0296-1, EM Science.
 - 6.1.4. Helium, gas, 99.995% purity, BOC Gases, Murray Hill, NJ 07974.
 - 6.1.5. Hydrochloric acid, 6.0 N, traceable to NIST, catalog number LC15370-2, Fisher Scientific.
 - 6.1.6. Isobutyl chloroformate, 98%, catalog number 17,798-9, Aldrich Chemical Company, Milwaukee, WI 53233.
 - 6.1.7. Isooctane (2,2,4-trimethylpentane), Optima grade, catalog number O301-4, Fisher Scientific.
 - 6.1.8. Magnesium sulfate anhydrous, certified, catalog number M65-500, Fisher Scientific.
 - 6.1.9. Methanol, HPLC grade, catalog number A452-4, Fisher Scientific.
 - 6.1.10. *N*-Methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA), catalog number 48920, Pierce, Rockford, IL 61105.
 - 6.1.11. Methyl *tert*-butyl ether, HPLC grade, catalog number E127-4, Fisher Scientific.
 - 6.1.12. Nitrogen, gas, 99.99% purity, BOC Gases.
 - 6.1.13. 1-Propanol, 99.5+%, HPLC grade, catalog number 29,328-8, Aldrich Chemical Company.
 - 6.1.14. Pyridine, HPLC grade, catalog number 27,040-7, Aldrich Chemical Company.
 - 6.1.15. Sodium chloride, ACS reagent grade, catalog number S640-500, Fisher Scientific.
 - 6.1.16. Sodium hydroxide, 0.1 N solution, certified grade, catalog number SS276-1, Fisher Scientific.
 - 6.1.17. Sodium hydroxide, 1.0 N solution, certified grade, catalog number SS266-1, Fisher Scientific.

6.2. Standards

- 6.2.1. Ethoxyacetic acid, 98%, catalog number 13,711-1, Aldrich Chemical Company, Milwaukee, WI 53233.
- 6.2.2. *cis*-3-Chloroacrylic acid, Dow AgroSciences LLC, Indianapolis, IN 46268.
- 6.2.3. *trans*-3-Chloroacrylic acid, Dow AgroSciences LLC.
- 6.2.4. *cis*-3-Chloroallyl alcohol, Dow AgroSciences LLC.
- 6.2.5. *trans*-3-Chloroallyl alcohol, Dow AgroSciences LLC.
Obtain from Test Substances Coordinator, Dow AgroSciences LLC, 9330 Zionsville Road, Building 306/A1, Indianapolis, IN 46268.
- 6.2.6. 4-Penten-1-ol, 99%, catalog number 11,127-9, Aldrich Chemical Company.

7. PREPARATION OF STANDARDS

7.1. Preparation of *cis*- and *trans*-Isomers of CAAL and CAAC, 4-Penten-1-ol, and Ethoxyacetic Acid Standard Stock Solutions

- 7.1.1. Tare a 100-mL volumetric flask and PTFE stopper. Using a 100- μ L gas tight volumetric syringe, transfer 86 μ L (Note 12.2.) of the *cis*-CAAL analytical standard into the flask and stopper the flask. Record the weight and dilute to volume with acetone to obtain a 1000- μ g/mL stock solution of *cis*-CAAL.
- 7.1.2. Tare a 100-mL volumetric flask and PTFE stopper. Using a 100- μ L gas tight volumetric syringe, transfer 86 μ L (Note 12.2.) of the *trans*-CAAL analytical standard into the flask and stopper the flask. Record the weight and dilute to volume with acetone to obtain a 1000- μ g/mL stock solution of *trans*-CAAL.
- 7.1.3. Weigh 0.1000 g of the *cis*-CAAC analytical standard and quantitatively transfer into a 100-mL volumetric flask. Add 100 μ L of glacial acetic acid to the flask and dilute to volume with acetone to obtain a 1000- μ g/mL stock solution of *cis*-CAAC.
- 7.1.4. Weigh 0.1000 g of the *trans*-CAAC analytical standard and quantitatively transfer into a 100-mL volumetric flask. Add 100 μ L of glacial acetic acid to the flask and dilute to volume with acetone to obtain a 1000- μ g/mL stock solution of *trans*-CAAC.
- 7.1.5. Tare a 100-mL volumetric flask and PTFE stopper. Using a 250- μ L gas tight volumetric syringe, transfer 120 μ L (Note 12.2.) of the 4-penten-1-ol standard into the flask and stopper the flask. Record the weight and dilute to volume with acetone to obtain a 1000- μ g/mL stock solution of 4-penten-1-ol.

7.1.6. Tare a 100-mL volumetric flask and PTFE stopper. Using a 100- μ L gas tight volumetric syringe, transfer 91 μ L (Note 12.2.) of the ethoxyacetic acid standard into the flask and stopper the flask. Record the weight, add 100 μ L of glacial acetic acid and dilute to volume with acetone to obtain a 1000- μ g/mL stock solution of ethoxyacetic acid.

7.2. Preparation of 4-Penten-1-ol and Ethoxyacetic Acid Internal Standard Solutions

7.2.1. Pipet a 1.0-mL aliquot of the 4-penten-1-ol stock solution from Step 7.1.5 into a 100-mL volumetric flask. Add 100 μ L of 1-propanol and dilute with hexane to obtain a 10- μ g/mL solution of 4-penten-1-ol.

7.2.2. Pipet a 1.0-mL aliquot of the ethoxyacetic acid stock solution from Step 7.1.6 into a 100-mL volumetric flask. Add 100 μ L of glacial acetic acid and dilute with isooctane to obtain a 10- μ g/mL solution of ethoxyacetic acid.

7.2.3. Pipet a 3.0-mL aliquot of the 10- μ g/mL solution of 4-penten-1-ol from Step 7.2.1 into a 200-mL volumetric flask. Add 200 μ L of 1-propanol and dilute with hexane to obtain a 150-ng/mL solution of 4-penten-1-ol.

7.2.4. Pipet a 3.0-mL aliquot of the 10- μ g/mL solution of ethoxyacetic acid from Step 7.2.2 into a 200-mL volumetric flask. Add 200 μ L of glacial acetic acid, 8 mL of acetone, and dilute with isooctane to obtain a 150-ng/mL solution of ethoxyacetic acid.

7.3. Preparation of *cis*- and *trans*-CAAL Calibration Standards

7.3.1. Pipet a 1.0-mL aliquot of the *cis*- and *trans*-CAAL stock solutions from Steps 7.1.1 and 7.1.2 into a 1000-mL volumetric flask. Add 1.0 mL of 1-propanol and dilute with hexane to obtain a 1.0- μ g/mL solution of *cis*- and *trans*-CAAL.

7.3.2. Pipet a 1.0-mL aliquot of the *cis*- and *trans*-CAAL stock solutions from Steps 7.1.1 and 7.1.2 into a 100-mL volumetric flask. Add 100 μ L of 1-propanol and dilute with hexane to obtain a 10- μ g/mL solution of *cis*- and *trans*-CAAL.

7.3.3. Prepare solutions for calibration of *cis*- and *trans*-CAAL by diluting 200 μ L of 1-propanol, 6 mL of the 10 μ g/mL solution of 4-penten-1-ol from Step 7.2.1, and the appropriate aliquot of the solution from Step 7.3.1 or 7.3.2 with hexane as follows:

| Initial Standard Concentration | Aliquot of Initial Std. | Final Soln. Volume | Solution Final Concentration | Equivalent. Sample Conc. ^a |
|--------------------------------|-------------------------|--------------------|------------------------------|---------------------------------------|
| µg/mL | mL | mL | ng/mL | ng/g |
| 1.0 | 3.0 | 200 | 15 | 1.5 |
| 1.0 | 6.0 | 200 | 30 | 3.0 |
| 1.0 | 15 | 200 | 75 | 7.5 |
| 10 | 3.0 | 200 | 150 | 15.0 |
| 10 | 6.0 | 200 | 300 | 30.0 |

^a The equivalent sample concentration is based on taking a 5-g grape sample to a final sample volume of 0.5 mL.

7.4. Preparation of *cis*- and *trans*-CAAC Calibration Standards

7.4.1. Pipet a 1.0-mL aliquot of the *cis*- and *trans*-CAAC stock solutions from Steps 7.1.3 and 7.1.4 into a 1000-mL volumetric flask. Add 1.0 mL of glacial acetic acid, 40 mL of acetone, and dilute with isoctane to obtain a 1.0-µg/mL solution of *cis*- and *trans*- CAAC.

7.4.2. Pipet a 1.0-mL aliquot of the *cis*- and *trans*-CAAC stock solutions from Steps 7.1.3 and 7.1.4 into a 100-mL volumetric flask. Add 100 µL of glacial acetic acid, 2 mL of acetone, and dilute with isoctane to obtain a 10-µg/mL solution of *cis*- and *trans*-CAAC.

7.4.3. Prepare solutions for calibration of *cis*- and *trans*-CAAC by diluting 200 µL of glacial acetic acid, 8 mL of acetone, 6 mL of the 10 µg/mL solution of ethoxyacetic acid from Step 7.2.2, and the appropriate aliquot of the solution from Step 7.4.1 or 7.4.2 with hexane as follows:

| Initial Standard Concentration | Aliquot of Initial Std. | Final Soln. Volume | Solution Final Concentration | Equivalent. Sample Conc. ^a |
|--------------------------------|-------------------------|--------------------|------------------------------|---------------------------------------|
| µg/mL | mL | mL | ng/mL | ng/g |
| 1.0 | 3.0 | 200 | 15 | 1.5 |
| 1.0 | 6.0 | 200 | 30 | 3.0 |
| 1.0 | 15 | 200 | 75 | 7.5 |
| 10 | 3.0 | 200 | 150 | 15.0 |
| 10 | 6.0 | 200 | 300 | 30.0 |

^a The equivalent sample concentration is based on taking a 5-g grape sample to a final sample volume of 0.5 mL.

7.5. Preparation of *cis*- and *trans*-CAAL Fortification Standards

7.5.1. Prepare solutions for fortification of samples by diluting the 10-µg/mL solution from Section 7.3.2 with acetone as follows:

| Aliquot of 10- μ g/mL Std. | Final Soln. Volume | Solution Final Concentration | Equivalent. Sample Conc. ^a |
|-----------------------------------|-----------------------|---------------------------------|------------------------------------------|
| mL | mL | ng/mL | ng/g |
| 0.90 | 100 | 90 | 0.90 |
| 3.0 | 100 | 300 | 3.0 |
| 6.0 | 100 | 600 | 6.0 |
| 15.0 | 50 | 3000 | 30.0 |

^a The equivalent sample concentration is based on fortification of a 5-g grape sample with 50- μ L of the appropriate fortification solution.

7.6. Preparation of *cis*- and *trans*-CAAC Fortification Standards

7.6.1. Prepare solutions for fortification of samples by diluting the 10- μ g/mL solution from Section 7.4.2 with acetone as follows:

| Aliquot of 10- μ g/mL Std. | Add Glacial Acetic Acid | Final Soln. Volume | Solution Final Concentration | Equivalent. Sample Conc. ^a |
|-----------------------------------|----------------------------|-----------------------|---------------------------------|------------------------------------------|
| mL | μ L | mL | ng/mL | ng/g |
| 0.90 | 100 | 100 | 90 | 0.90 |
| 3.0 | 100 | 100 | 300 | 3.0 |
| 6.0 | 100 | 100 | 600 | 6.0 |
| 15.0 | 50 | 50 | 3000 | 30.0 |

^a The equivalent sample concentration is based on fortification of a 5-g grape sample with 50- μ L of the appropriate fortification solution.

8. GAS CHROMATOGRAPHY/MASS SPECTROMETRY

8.1. Column

Install the splitless column inlet sleeve (Section 5.10.) and the capillary column (Section 5.2.) in the split/splitless injection port of the gas chromatograph following the manufacturer's recommended procedures.

8.2. Typical Operating Conditions

Instrumentation: Hewlett-Packard Model 5890 series II gas chromatograph
Hewlett-Packard Model 7673 autoinjector
Hewlett-Packard Model 5971A mass selective detector
Hewlett-Packard Model G1701AA data system

Column: J&W Scientific fused silica capillary
Durabond-17 liquid phase

20 m x 0.18 mm i.d.
0.3- μ m film thickness

Temperatures:

Column 65 °C for 1.0 min
65 °C to 150 °C at 7 °C/min
150 °C to 280 °C at 25 °C/min

Injector 270 °C
Interface 300 °C

Carrier Gas: helium

Head Pressure 70 kPa

Injection Mode: splitless

Purge Delay 0.7 min
Splitter Flow 50 mL/min
Septum Purge 1.0 mL/min

Injection Volume: 2 μ L

Detector Mode: electron impact ionization with selected ion monitoring

Calibration Program maximum sensitivity autotune
Electron Multiplier set at 200 volts above autotune

Ions Monitored for CAAL Determination:

cis- and *trans*-CAAL as
CAIBC m/z 136 (quantitation)
 m/z 75 (confirmation)
 m/z 138 (confirmation)

4-Pentenyl IBC m/z 68 (internal standard)

Dwell Time: 50 msec

Ions Monitored for CAAC Determination:

cis- and *trans*-CAAC as
CAAC TBDMSE m/z 163 (quantitation)
 m/z 93 (confirmation)
 m/z 165 (confirmation)

Ethoxyacetic acid
TBDMSE m/z 161 (internal standard)

Dwell Time: 50 msec

Typical mass spectra of *cis*- and *trans*-isomers of CAIBC and CAAC TBDMSE are shown in Figures 1 and 2, respectively. Typical mass spectra of the internal standards 4-pentenyl isobutyl carbonate and ethoxyacetic acid t-butyldimethylsilyl ester are shown in Figure 3.

8.3. Calibration Curves

Typical calibration curves for the determination of residues of *cis*- and *trans*-CAAL in grapes are shown in Figures 4 and 5, respectively. Typical calibration curves for the determination of residues of *cis*- and *trans*-CAAC in grapes are shown in Figures 6 and 7, respectively.

8.4. Typical Chromatograms

Typical chromatograms of a standard, control sample, and a 3.0-ng/g recovery sample for the determination of residues of *cis*- and *trans*-CAAL in grapes are shown in Figures 8-10, respectively. Typical chromatograms of a standard, control sample, and a 3.0-ng/g recovery sample for the determination of residues of *cis*- and *trans*-CAAC in grapes are shown in Figures 11-13, respectively.

9. DETERMINATION OF CAAL and CAAC RESIDUES IN GRAPES

9.1. Method Validation

Verify the analytical procedure given in Sections 9.3 through 9.5 by analyzing the following with each sample set:

At least one reagent blank.

At least two control samples.

At least two control samples fortified at 3.0 ng/g (limit of quantitation).

At least one control sample fortified at 6.0 ng/g.

At least one control sample fortified at 30 ng/g.

9.2. Sample Preparation

9.2.1. Prepare the samples for analysis by freezing with liquid nitrogen and then grinding or chopping using a hammer mill with a 3/16-inch screen size.

9.3. Sample Analysis

9.3.1. Weigh 5.0 g of prepared grape sample (Section 9.2.1.) into a series of 45-mL vials.

9.3.2. For preparing fortified samples, use at least one of the samples as a control and fortify the remaining samples by adding 50- μ L of the appropriate spiking solutions from Sections 7.5.1 and 7.6.1 to obtain concentrations ranging from 0.9 to 30 ng/g.

- 9.3.3. Add approximately 5 g of sodium chloride and 3 mL of 1.0 N sodium hydroxide.
- 9.3.4. Add 5 mL of MTBE and seal the vial with a cap (Section 5.16.).
- 9.3.5. Shake the vial for 5 minutes using a reciprocating shaker set at approximately 180 excursions/minute.
- 9.3.6. Centrifuge the vial for 5 minutes at 2500 rpm (Note 12.3.).
- 9.3.7. Place a graphitized carbon SPE column (Section 5.3.) on the vacuum manifold box.
- 9.3.8. Place a silica gel SPE column (Section 5.4.) on top of the graphitized carbon column using a PTFE SPE column adapter (Section 5.6.).
- 9.3.9. Condition the SPE columns by adding 5 mL of MTBE to the silica gel SPE column reservoir. With the aid of vacuum, pull the MTBE through the columns at a flow rate of approximately 2 mL/min.
- 9.3.10. Position a 45-mL vial in the vacuum manifold box to collect the eluate from Steps 9.3.11 through 9.3.13.
- 9.3.11. Using a disposable Pasteur pipet, transfer the top MTBE layer of the sample from Step 9.3.6 into the silica gel SPE column reservoir. With the aid of vacuum, pull the sample through the columns at a flow rate of approximately 2 mL/min.
- 9.3.12. Repeat Steps 9.3.4 through 9.3.6 and Step 9.3.11. With the aid of vacuum, pull the sample through the columns at a flow rate of approximately 2 mL/min.
- 9.3.13. Repeat Step 9.3.12.
- 9.3.14. Remove the 45-mL vial from the vacuum manifold box and save for Step 9.4 (Continuation of Sample Analysis for CAAL).
- 9.3.15. Add 1 mL of 6.0 N hydrochloric acid to the vial from Step 9.3.11.
- 9.3.16. Add 4 mL of MTBE and seal the vial with a cap.
- 9.3.17. Shake the vial for 5 minutes using a reciprocating shaker set at approximately 180 excursions/minute.
- 9.3.18. Centrifuge the vial for 5 minutes at 2500 rpm.
- 9.3.19. Place a graphitized carbon SPE column on the vacuum manifold box.

- 9.3.20. Add approximately 0.5 g of anhydrous magnesium sulfate to the SPE column reservoir.
- 9.3.21. Condition the SPE column by adding 5 mL of MTBE to the column reservoir. With the aid of vacuum, pull the MTBE through the column at a flow rate of approximately 2 mL/min (Note 12.4.).
- 9.3.22. Position a 16 x 100 mm screw-cap culture tube in the vacuum manifold box to collect the eluate from Steps 9.3.23 through 9.3.25.
- 9.3.23. Using a disposable Pasteur pipet, transfer the top MTBE layer of the sample from Step 9.3.18 into the SPE column reservoir. With the aid of vacuum, pull the sample through the column at a flow rate of approximately 2 mL/min.
- 9.3.24. Repeat Steps 9.3.16 through 9.3.18 and Step 9.3.23. With the aid of vacuum, pull the sample through the column at a flow rate of approximately 2 mL/min.
- 9.3.25. Add 10 μ L of acetic acid and 1 mL of MTBE to the SPE column reservoir. With the aid of vacuum, pull the MTBE through the column at a flow rate of approximately 2 mL/min.
- 9.3.26. Remove the 16 x 100 mm screw-cap culture tube from the vacuum manifold box and save for Step 9.5 (Continuation of Sample Analysis for CAAC).
- 9.4. Continuation of Sample Analysis for CAAL
 - 9.4.1. Transfer the sample from the vial in Step 9.3.14 into a 50-mL Erlenmeyer flask (Section 5.9.). Rinse the vial with 2 mL of MTBE and add the rinse to the flask.
 - 9.4.2. Add 6 mL of hexane, 10 μ L of 1-propanol, and approximately 3 PTFE boiling chips (Section 5.1.) to the flask.
 - 9.4.3. Attach a Snyder column (Section 5.5.) to the flask.
 - 9.4.4. In a fume hood, heat the contents of the flask to a steady boil using a hot plate set at approximately 100 °C.
 - 9.4.5. Allow the sample to concentrate until a portion of the bottom center of the flask shows signs of dryness.
 - 9.4.6. Remove the flask from the hot plate and add 1 mL of hexane to the flask through the top of the Snyder column. Allow the flask to equilibrate to ambient temperature.

- 9.4.7. Remove the Snyder column from the flask. Using a disposable Pasteur pipet, transfer the sample to a 16 x 100 mm screw-cap culture tube. Rinse the flask with 2 mL of hexane and transfer the rinse to the culture tube.
- 9.4.8. Add 1.0 mL of the 150 ng/mL 4-penten-1-ol internal standard solution in hexane (Section 7.2.3.).
- 9.4.9. Transfer a 500- μ L aliquot of each of the calibration standards from Section 7.3.3 into a series of 16 x 100 mm screw-cap culture tubes.
- 9.4.10. Derivatize the sample from Step 9.4.8 and calibration standards from Step 9.4.9 as follows:
- 9.4.11. Add 50 μ L of pyridine and 50 μ L of isobutyl chloroformate.
- 9.4.12. Seal the tube with a PTFE-lined cap.
- 9.4.13. Place the tube in an aluminum block heater set at 70 °C and allow 15 minutes for derivatization.
- 9.4.14. Remove the tube from the aluminum block heater and allow to cool for approximately 5 minutes.
- 9.4.15. Add 50 μ L of methanol and seal the tube with a PTFE-lined cap. Vortex the tube for approximately 5 seconds to mix.
- 9.4.16. Add 1 mL of 0.1 N sodium hydroxide and seal the tube with a PTFE-lined cap.
- 9.4.17. Shake the tube for approximately 3 minutes using a reciprocating shaker set at approximately 180 excursions/minute.
- 9.4.18. Centrifuge the tube for approximately 3 minutes at 2000 rpm.
- 9.4.19. For samples only, concentrate the top hexane layer to approximately 0.5 mL under nitrogen at ambient temperature. Use one of the calibration standards as a visual comparison to approximate a final volume of 0.5 mL in the samples (Note 12.5.).
- 9.4.20. Place a limited volume insert (Section 5.18.) into an autosampler vial (Section 5.14.). Using a disposable Pasteur pipet, transfer an aliquot of the top hexane layer from the sample or calibration standard into the limited volume insert. Seal the vial with a cap and crimper.
- 9.4.21. Analyze the calibration standards and samples by capillary GC/MS as described in Section 8.2, monitoring the ions specified for CAAL determination. Determine the suitability of the chromatographic system using the following performance criteria:

- a. Standard curve linearity: Determine that the correlation coefficient equals or exceeds 0.995 for the least squares equation which describes the detector response as a function of standard curve concentration. If power regression is used, the power exponent should be between 0.90-1.10.
- b. Peak resolution: Visually determine that sufficient resolution has been achieved for the analyte and internal standard relative to background interferences.
- c. Appearance of chromatograms: Visually determine that the chromatograms resemble those shown in Figures 8-10 with respect to peak response, baseline noise, and background interference. Visually determine that a minimum signal-to-noise ratio of 10:1 has been attained for the 30-ng/mL calibration standard equivalent to 3.0-ng/g *cis*- and *trans*-CAAL in grapes.

9.5. Continuation of Sample Analysis for CAAC

- 9.5.1. Transfer a 500- μ L aliquot of each of the calibration standards from Section 7.4.3 into a series of 16 x 100 mm screw-cap culture tubes. Seal each vial with a PTFE-lined cap.
- 9.5.2. Add 1.0 mL of the 150 ng/mL ethoxyacetic acid internal standard solution (Section 7.2.4.) to the vial from Step 9.3.26.
- 9.5.3. Concentrate the contents of the tube to approximately 0.5 mL under nitrogen at ambient temperature. Use one of the calibration standards from Step 9.5.1 as a visual comparison to approximate a final volume of 0.5 mL in the sample (Note 12.5.).
- 9.5.4. Derivatize the sample from Step 9.5.3 and calibration standards from Step 9.5.1 as follows:
- 9.5.5. Add 50 μ L of MTBSTFA and seal the tubes with a PTFE-lined cap. Vortex the tubes briefly to mix. Allow 15 minutes for derivatization at ambient temperature, then centrifuge the tubes for approximately 5 minutes at 2500 rpm.
- 9.5.6. Place a limited volume insert into an autosampler vial. Using a disposable Pasteur pipet, transfer an aliquot of the sample or calibration standard from Section 9.5.5. Seal the vial with a cap and crimper.
- 9.5.7. Analyze the calibration standards and samples by capillary GC/MS as described in Section 8.2, monitoring the ions designated for the determination of CAAC. Determine the suitability of the chromatographic system using the following performance criteria:

- a. Standard curve linearity: Determine that the correlation coefficient equals or exceeds 0.995 for the least squares equation which describes the detector response as a function of standard curve concentration. If power regression is used, the power exponent should be between 0.90-1.10.
- b. Peak resolution: Visually determine that sufficient resolution has been achieved for the analyte and internal standard relative to background interferences.
- c. Appearance of chromatograms: Visually determine that the chromatograms resemble those shown in Figures 11-13 with respect to peak response, baseline noise, and background interference. Visually determine that a minimum signal-to-noise ratio of 10:1 has been attained for the 30-ng/mL calibration standard equivalent to 3.0-ng/g *cis*- and *trans*-CAAC in grapes.

10. CALCULATIONS

10.1. Calculation of CAAL and CAAC Standard Calibration Curves

- 10.1.1. Determine the peak areas for the analytes and internal standards resulting from completion of Step 9.4.21 for CAAL and Step 9.5.7 for CAAC as indicated below (Note 12.6.).

| | |
|-------------------------------------------------------|-----------------------------------------------------------------------|
| <i>cis</i> - and <i>trans</i> -CAAL as CAIBC | <i>m/z</i> 136 (quantitation) <i>m/z</i> 75 and 138 (confirmation) |
| 4-Pentenyl IBC | <i>m/z</i> 68 (internal standard) |
| <i>cis</i> - and <i>trans</i> -CAAC as CAAC TBDMSE | <i>m/z</i> 163 (quantitation) <i>m/z</i> 93 and 165 (confirmation) |
| Ethoxyacetic acid TBDMSE | <i>m/z</i> 161 (internal standard) |

- 10.1.2. For each standard, calculate the quantitation ratio.

For example, using the data for *cis*-CAAL from Figure 8 and *cis*-CAAC from Figure 11:

$$\begin{aligned} \text{Quantitation Ratio } \textit{cis}\text{-CAAL} &= \frac{\textit{m/z} 136 \text{ peak area of } \textit{cis}\text{-CAIBC}}{\textit{m/z} 68 \text{ peak area of 4-Pentenyl IBC}} \\ \text{Quantitation Ratio } \textit{cis}\text{-CAAL} &= \frac{4232}{341936} = 0.01238 \end{aligned}$$

$$\text{cis-CAAC Quantitation Ratio} = \frac{m/z 163 \text{ peak area of cis-CAAC TBDMSE}}{m/z 161 \text{ peak area of Ethoxyacetic acid TBDMSE}}$$

$$\text{cis-CAAC Quantitation Ratio} = \frac{42609}{180114} = 0.2366$$

10.1.3. Prepare standard curves by plotting the sample equivalent concentration on the abscissa (*x*-axis) and the respective quantitation ratio on the ordinate (*y*-axis) as shown in Figures 4-7. Using regression analysis, determine the equation for the curve with respect to the abscissa.

$$Y = \text{constant} \times X^{(\text{exponent})}$$

$$X = \left[\frac{Y}{\text{constant}} \right]^{1/\text{exponent}}$$

$$\text{cis-CAAL Concentration (ng/g)} = \left[\frac{\text{cis-CAAL Quantitation Ratio}}{\text{constant}} \right]^{1/\text{exponent}}$$

10.2. Calculation of Percent Recovery

10.2.1. Determine the gross concentration in each recovery sample by substituting the quantitation ratio obtained into the appropriate equation and solving for the concentration.

For example, using power regression with the *cis*-CAAL data from Figures 4 and 10:

$$\text{cis-CAAL Concentration (gross ng/g)} = \left[\frac{\text{cis-CAAL Quantitation Ratio}}{0.004133} \right]^{1/0.9981}$$

$$\text{cis-CAAL Quantitation Ratio} = \frac{3489}{314769}$$

$$\text{cis-CAAL Concentration (gross ng/g)} = \left[\frac{\left(\frac{3489}{314769} \right)}{0.004133} \right]^{1/0.9981} = 2.687$$

- 10.2.2. Determine the net concentration in each recovery sample by subtracting the concentration in the control sample from that of the gross concentration in the recovery sample.

For example, using the *cis*-CAAL data from Figures 9 and 10:

$$\text{Net } cis\text{-CAAL (ng/g)} = [\text{Gross} - \text{Control}] \text{ } cis\text{-CAAL (ng/g)}$$

$$\begin{aligned}\text{Net } cis\text{-CAAL (ng/g)} &= [2.687 - 0.000] \text{ ng/g} \\ &= 2.687 \text{ ng/g}\end{aligned}$$

- 10.2.3. Determine the percent recovery by dividing the net concentration of each recovery sample by the theoretical concentration added.

$$\text{Recovery} = \frac{\text{Concentration Found}}{\text{Concentration Added}} \times 100\%$$

$$\text{Recovery} = \frac{2.687 \text{ ng/g}}{3.0 \text{ ng/g}} \times 100\%$$

$$\text{Recovery} = 90\%$$

10.3. Determination of Residues of *cis*- and *trans*-Isomers of CAAL and CAAC from Grapes

- 10.3.1. Determine the gross concentration in each grape sample by substituting the quantitation ratio obtained into the appropriate equation for the standard calibration curve, and calculating the net residue concentration as described in Sections 10.1 and 10.2.

- 10.3.2. For those analyses that require correction for method recovery, use the average recovery of all the recovery samples from a given sample set to correct for method efficiency.

$$cis\text{-CAAL Conc. (corrected ng/g)} = cis\text{-CAAL Conc. (gross ng/g)} \times \left(\frac{100}{\text{Average Percent Recovery}} \right)$$

11. RESULTS AND DISCUSSION

11.1. Method Validation

11.1.1. Recovery Levels and Precision

A method validation study was conducted to determine the recovery levels and the precision of the method for the determination of residues of *cis*- and *trans*-isomers of CAAL and CAAC from grapes. The results are summarized in Tables 1-4.

Recovery values of *cis*-CAAL, *trans*-CAAL, *cis*-CAAC, and *trans*-CAAC from grape samples fortified over the concentration range from 3.0 to 30 ng/g averaged 83±4%, 89±4%, 95±2%, and 94±1%, respectively.

11.1.2. Standard Curve Linearity

For the power least squares regression equations describing the detector response as a function of the standard calibration curve concentrations, correlation coefficients (r^2) were greater than 0.995 for all of the calibration curve determinations during the method validation, while the power exponents were between 0.9763 and 1.014.

11.1.3. Calculated Limits of Quantitation and Detection

Following established guidelines (Section 13.1.), the limits of quantitation (LOQ) and detection (LOD) were calculated using the standard deviation from the 3.0-ng/g recovery results. The LOQ was calculated as ten times the standard deviation (10s), and the LOD was calculated as three times the standard deviation (3s) of the results for the analysis of 8 samples. The results are summarized in Tables 1-4.

The calculated LOQs for *cis*- and *trans*-CAAL were 0.99 and 0.98 ng/g which supports a method LOQ of 3.0 ng/g. The calculated LODs were 0.3 and 0.29 ng/g.

The calculated LOQs for *cis*- and *trans*-CAAC were 0.44 and 0.09 ng/g which supports a method LOQ of 3.0 ng/g. The calculated LODs were 0.13 and 0.03 ng/g.

11.2. Confirmation of Residue Identity

Confirmation of the presence of *cis*- and *trans*-isomers of CAAL and CAAC is by comparison of the retention time (gas chromatography) as well as the peak area ratios resulting from selected ion monitoring (mass spectrometry) for standards and samples (Note 12.6.).

Using the peak area results determined in Section 10.1.1 for the 3.0- through 30-ng/g grape equivalent calibration standards, calculate the confirmation ratios for the *cis*- and *trans*-isomers of CAAL and CAAC. Determine the average confirmation ratio for each analyte and use these values to confirm the presence of *cis*- and *trans*-isomers of CAAL and CAAC in each of the samples.

$$\text{CAAL Confirmation Ratio 1} = \frac{m/z\ 136\ \text{peak area of CAIBC}}{m/z\ 75\ \text{peak area of CAIBC}}$$

$$\text{CAAL Confirmation Ratio 2} = \frac{m/z\ 138\ \text{peak area of CAIBC}}{m/z\ 75\ \text{peak area of CAIBC}}$$

$$\text{CAAL Confirmation Ratio 3} = \frac{m/z\ 138\ \text{peak area of CAIBC}}{m/z\ 136\ \text{peak area of CAIBC}}$$

$$\text{CAAC Confirmation Ratio 1} = \frac{m/z\ 165\ \text{peak area of CAAC TBDMSE}}{m/z\ 163\ \text{peak area of CAAC TBDMSE}}$$

$$\text{CAAC Confirmation Ratio 2} = \frac{m/z\ 93\ \text{peak area of CAAC TBDMSE}}{m/z\ 163\ \text{peak area of CAAC TBDMSE}}$$

$$\text{CAAC Confirmation Ratio 3} = \frac{m/z\ 93\ \text{peak area of CAAC TBDMSE}}{m/z\ 165\ \text{peak area of CAAC TBDMSE}}$$

For example, using the data for the *cis*-CAAL standard from Figure 8:

$$\begin{aligned} \text{cis-CAAL Confirmation Ratio 1} &= \frac{4232}{13932} \\ &= 0.3038 \end{aligned}$$

Confirmation of the presence of each analyte is indicated when the confirmation ratios for the samples are within the range of $\pm 20\%$ of the average confirmation ratios found for the respective standards.

11.3. Assay Time

A typical analytical set would consist of a minimum of five standards encompassing the expected range of sample concentrations, a reagent blank, a control (a non-fortified sample), a minimum of four fortified controls (two of which must be at the LOQ), and 9 samples. This typical analytical set can be prepared in approximately 12 hours, followed by the chromatographic analysis.

Acceptable “stopping points” in the method are as follows:

- 11.3.1. Method activity may be stopped after completion of Step 9.3.17. Vials from Steps 9.3.17 (CAAC) and 9.3.14 (CAAL) may be stored refrigerated for several days prior to method continuation (Note 12.7.).

- 11.3.2. Method activity may be stopped after completion of Step 9.3.26. Tubes from Step 9.3.26 (CAAC) and vials from Step 9.3.14 (CAAL) may be stored refrigerated for several days prior to method continuation (Note 12.7.).
- 11.3.2. Method activity may be stopped after completion of Step 9.4.8. Tubes from Steps 9.4.8 (CAAL) and 9.3.26 (CAAC) may be stored refrigerated for several days prior to method continuation (Note 12.7.).

12. NOTES

- 12.1. Equipment, glassware, materials, reagents, and chemicals considered to be equivalent to those specified may be substituted with the understanding that their performance must be confirmed by appropriate tests. Common laboratory supplies are assumed to be readily available and are, therefore, not listed.
- 12.2. Some of the standards used in this method are liquid at room temperature. The volumes given are intended to simplify the task of weighing out 0.1000 g of the respective standard.
- 12.3. It is likely that an emulsion will be observed after centrifugation in the first MTBE extract of the basified grape sample. If this occurs, shake the sample vigorously by hand for a few seconds to break the emulsion then centrifuge for 5 minutes at 2500 rpm.
- 12.4. Minimal use of vacuum is needed to perform Steps 9.3.21 through 9.3.25. Once flow through the column is initiated by application of vacuum, little to no vacuum is needed to maintain the flow.
- 12.5. The nitrogen flow for purposes of concentrating samples in Steps 9.4.18 and 9.5.3 should be adjusted such that the surface of the sample is dimpled. Avoid excessive turbulence and swirling of the sample to prevent potential loss of analyte.
- 12.6. Matrix interferences in the grape samples might prevent the ability to utilize the m/z 93 ion response for confirmation of *cis*-CAAC (See Figures 12 and 13).
- 12.7. All vials and tubes should be sealed with caps prior to refrigerated storage.

13. REFERENCES

- 13.1. Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G. *Anal. Chem.* **1983**, *55*, 2210-2218.

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Table 1. Recovery of *cis*-3-Chloroallyl Alcohol from Grapes

| Sample Identification | Date of Analysis | <i>cis</i> -CAAL, ng/g | | Percent Recovery | Statistical Calculations ^a |
|-----------------------|------------------|------------------------|-----------------|------------------|---------------------------------------|
| | | Added | Found | | |
| 28790301 | 11-Dec-1999 | 0.00 | ND ^b | -- | |
| 28790301 | 11-Dec-1999 | 0.00 | ND | -- | |
| 28790301 | 14-Dec-1999 | 0.00 | ND | -- | |
| 28790301 | 14-Dec-1999 | 0.00 | ND | -- | |
| 28790301 | 11-Dec-1999 | 0.90 | 0.980 | NA ^c | |
| 28790301 | 14-Dec-1999 | 0.90 | 0.815 | NA | |
| 28790301 | 11-Dec-1999 | 3.0 | 2.539 | 85 | |
| 28790301 | 13-Dec-1999 | 3.0 | 2.687 | 90 | |
| 28790301 | 13-Dec-1999 | 3.0 | 2.556 | 85 | |
| 28790301 | 13-Dec-1999 | 3.0 | 2.466 | 82 | $\bar{x} = 2.54$ |
| 28790301 | 14-Dec-1999 | 3.0 | 2.347 | 78 | $s = 0.099$ |
| 28790301 | 14-Dec-1999 | 3.0 | 2.584 | 86 | $(3s)^d = 0.30$ |
| 28790301 | 14-Dec-1999 | 3.0 | 2.589 | 86 | $(10s)^e = 0.99$ |
| 28790301 | 14-Dec-1999 | 3.0 | 2.528 | 84 | RSD = 4% |
| 28790301 | 13-Dec-1999 | 6.0 | 5.043 | 84 | |
| 28790301 | 13-Dec-1999 | 6.0 | 5.075 | 85 | $\bar{x} = 5.06$ |
| 28790301 | 14-Dec-1999 | 6.0 | 4.938 | 82 | $s = 0.096$ |
| 28790301 | 14-Dec-1999 | 6.0 | 5.170 | 86 | RSD = 2% |
| 28790301 | 13-Dec-1999 | 30 | 23.35 | 78 | $\bar{x} = 23.7$ |
| 28790301 | 13-Dec-1999 | 30 | 24.48 | 82 | $s = 1.07$ |
| 28790301 | 14-Dec-1999 | 30 | 24.59 | 82 | RSD = 5% |
| 28790301 | 14-Dec-1999 | 30 | 22.32 | 74 | |
| | | | | $\bar{x} = 83$ | |
| | | | | $s = 4$ | |
| | | | | $n = 16$ | |

^a Statistical calculations include average, standard deviation, and relative standard deviation for each fortification level.

^b Not detected.

^c Not applicable.

^d Calculated Limit of Detection.

^e Calculated Limit of Quantitation.

Table 2. Recovery of *trans*-3-Chloroallyl Alcohol from Grapes

| Sample Identification | Date of Analysis | <i>trans</i> -CAAL, ng/g | | Percent Recovery | Statistical Calculations ^a |
|-----------------------|------------------|--------------------------|-----------------|------------------|---------------------------------------|
| | | Added | Found | | |
| 28790301 | 11-Dec-1999 | 0.00 | ND ^b | -- | |
| 28790301 | 11-Dec-1999 | 0.00 | ND | -- | |
| 28790301 | 14-Dec-1999 | 0.00 | ND | -- | |
| 28790301 | 14-Dec-1999 | 0.00 | ND | -- | |
| 28790301 | 11-Dec-1999 | 0.90 | 0.939 | NA ^c | |
| 28790301 | 14-Dec-1999 | 0.90 | 0.951 | NA | |
| 28790301 | 11-Dec-1999 | 3.0 | 2.585 | 86 | |
| 28790301 | 13-Dec-1999 | 3.0 | 2.742 | 91 | |
| 28790301 | 13-Dec-1999 | 3.0 | 2.614 | 87 | |
| 28790301 | 13-Dec-1999 | 3.0 | 2.635 | 88 | $\bar{x} = 2.71$ |
| 28790301 | 14-Dec-1999 | 3.0 | 2.672 | 89 | $s = 0.098$ |
| 28790301 | 14-Dec-1999 | 3.0 | 2.850 | 95 | $(3s)^d = 0.29$ |
| 28790301 | 14-Dec-1999 | 3.0 | 2.793 | 93 | $(10s)^e = 0.98$ |
| 28790301 | 14-Dec-1999 | 3.0 | 2.798 | 93 | RSD = 4% |
| 28790301 | 13-Dec-1999 | 6.0 | 5.197 | 87 | |
| 28790301 | 13-Dec-1999 | 6.0 | 5.404 | 90 | $\bar{x} = 5.36$ |
| 28790301 | 14-Dec-1999 | 6.0 | 5.271 | 88 | $s = 0.17$ |
| 28790301 | 14-Dec-1999 | 6.0 | 5.589 | 93 | RSD = 3% |
| 28790301 | 13-Dec-1999 | 30 | 25.19 | 84 | $\bar{x} = 25.6$ |
| 28790301 | 13-Dec-1999 | 30 | 26.48 | 88 | $s = 0.93$ |
| 28790301 | 14-Dec-1999 | 30 | 26.19 | 87 | RSD = 4% |
| 28790301 | 14-Dec-1999 | 30 | 24.45 | 81 | |
| | | | | $\bar{x} = 89$ | |
| | | | | $s = 4$ | |
| | | | | $n = 16$ | |

^a Statistical calculations include average, standard deviation, and relative standard deviation for each fortification level.

^b Not detected.

^c Not applicable.

^d Calculated Limit of Detection.

^e Calculated Limit of Quantitation.

Table 3. Recovery of *cis*-3-Chloroacrylic Acid from Grapes

| Sample Identification | Date of Analysis | <i>cis</i> -CAAC, ng/g | | Percent Recovery | Statistical Calculations ^a |
|-----------------------|------------------|------------------------|-----------------|------------------|---------------------------------------|
| | | Added | Found | | |
| 28790301 | 13-Dec-1999 | 0.00 | ND ^b | -- | |
| 28790301 | 13-Dec-1999 | 0.00 | ND | -- | |
| 28790301 | 14-Dec-1999 | 0.00 | ND | -- | |
| 28790301 | 14-Dec-1999 | 0.00 | ND | -- | |
| 28790301 | 13-Dec-1999 | 0.90 | 0.947 | NA ^c | |
| 28790301 | 14-Dec-1999 | 0.90 | 0.959 | NA | |
| 28790301 | 13-Dec-1999 | 3.0 | 2.931 | 98 | |
| 28790301 | 13-Dec-1999 | 3.0 | 2.863 | 95 | |
| 28790301 | 13-Dec-1999 | 3.0 | 2.929 | 98 | |
| 28790301 | 13-Dec-1999 | 3.0 | 2.812 | 94 | $\bar{x} = 2.88$ |
| 28790301 | 14-Dec-1999 | 3.0 | 2.889 | 96 | $s = 0.044$ |
| 28790301 | 15-Dec-1999 | 3.0 | 2.827 | 94 | $(3s)^d = 0.13$ |
| 28790301 | 15-Dec-1999 | 3.0 | 2.874 | 96 | $(10s)^e = 0.44$ |
| 28790301 | 15-Dec-1999 | 3.0 | 2.912 | 97 | RSD = 2% |
| 28790301 | 13-Dec-1999 | 6.0 | 5.697 | 95 | |
| 28790301 | 13-Dec-1999 | 6.0 | 5.572 | 93 | $\bar{x} = 5.68$ |
| 28790301 | 15-Dec-1999 | 6.0 | 5.773 | 96 | $s = 0.083$ |
| 28790301 | 15-Dec-1999 | 6.0 | 5.661 | 94 | RSD = 2% |
| 28790301 | 14-Dec-1999 | 30 | 28.36 | 95 | $\bar{x} = 28.3$ |
| 28790301 | 14-Dec-1999 | 30 | 27.97 | 93 | $s = 0.31$ |
| 28790301 | 15-Dec-1999 | 30 | 28.32 | 94 | RSD = 1% |
| 28790301 | 15-Dec-1999 | 30 | 28.73 | 96 | |
| | | | | $\bar{x} = 95$ | |
| | | | | $s = 1$ | |
| | | | | $n = 16$ | |

^a Statistical calculations include average, standard deviation, and relative standard deviation for each fortification level.

^b Not detected.

^c Not applicable.

^d Calculated Limit of Detection.

^e Calculated Limit of Quantitation.

Table 4. Recovery of *trans*-3-Chloroacrylic Acid from Grapes

| Sample Identification | Date of Analysis | <i>trans</i> -CAAC, ng/g | | Percent Recovery | Statistical Calculations ^a |
|-----------------------|------------------|--------------------------|-----------------|------------------|---------------------------------------|
| | | Added | Found | | |
| 28790301 | 13-Dec-1999 | 0.00 | ND ^b | -- | |
| 28790301 | 13-Dec-1999 | 0.00 | ND | -- | |
| 28790301 | 14-Dec-1999 | 0.00 | ND | -- | |
| 28790301 | 14-Dec-1999 | 0.00 | ND | -- | |
| 28790301 | 13-Dec-1999 | 0.90 | 0.914 | NA ^c | |
| 28790301 | 14-Dec-1999 | 0.90 | 0.894 | NA | |
| 28790301 | 13-Dec-1999 | 3.0 | 2.817 | 94 | |
| 28790301 | 13-Dec-1999 | 3.0 | 2.827 | 94 | |
| 28790301 | 13-Dec-1999 | 3.0 | 2.824 | 94 | |
| 28790301 | 13-Dec-1999 | 3.0 | 2.828 | 94 | $\bar{x} = 2.82$ |
| 28790301 | 14-Dec-1999 | 3.0 | 2.810 | 94 | $s = 0.009$ |
| 28790301 | 15-Dec-1999 | 3.0 | 2.834 | 94 | $(3s)^d = 0.03$ |
| 28790301 | 15-Dec-1999 | 3.0 | 2.813 | 94 | $(10s)^e = 0.09$ |
| 28790301 | 15-Dec-1999 | 3.0 | 2.811 | 94 | RSD = 0.3% |
| 28790301 | 13-Dec-1999 | 6.0 | 5.680 | 95 | |
| 28790301 | 13-Dec-1999 | 6.0 | 5.488 | 91 | $\bar{x} = 5.58$ |
| 28790301 | 15-Dec-1999 | 6.0 | 5.655 | 94 | $s = 0.097$ |
| 28790301 | 15-Dec-1999 | 6.0 | 5.513 | 92 | RSD = 2% |
| 28790301 | 14-Dec-1999 | 30 | 28.29 | 94 | $\bar{x} = 28.2$ |
| 28790301 | 14-Dec-1999 | 30 | 27.59 | 92 | $s = 0.45$ |
| 28790301 | 15-Dec-1999 | 30 | 28.16 | 94 | RSD = 2% |
| 28790301 | 15-Dec-1999 | 30 | 28.67 | 96 | |
| | | | | $\bar{x} = 94$ | |
| | | | | $s = 1$ | |
| | | | | $n = 16$ | |

^a Statistical calculations include average, standard deviation, and relative standard deviation for each fortification level.

^b Not detected.

^c Not applicable.

^d Calculated Limit of Detection.

^e Calculated Limit of Quantitation.

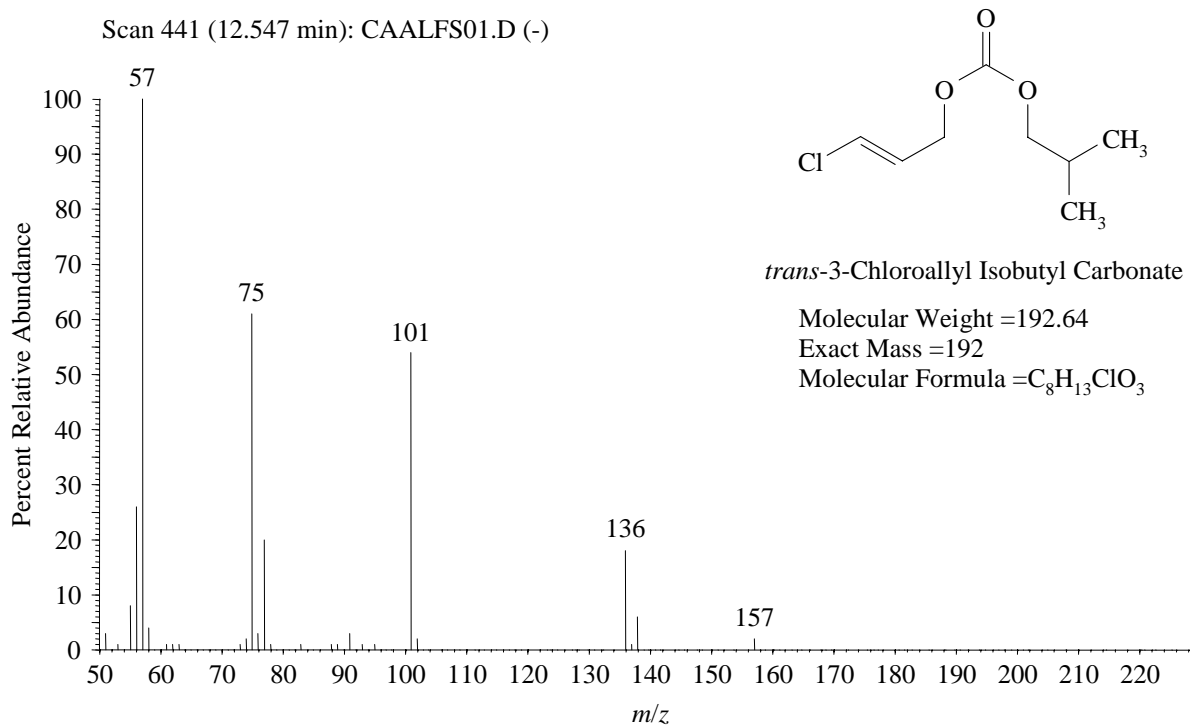
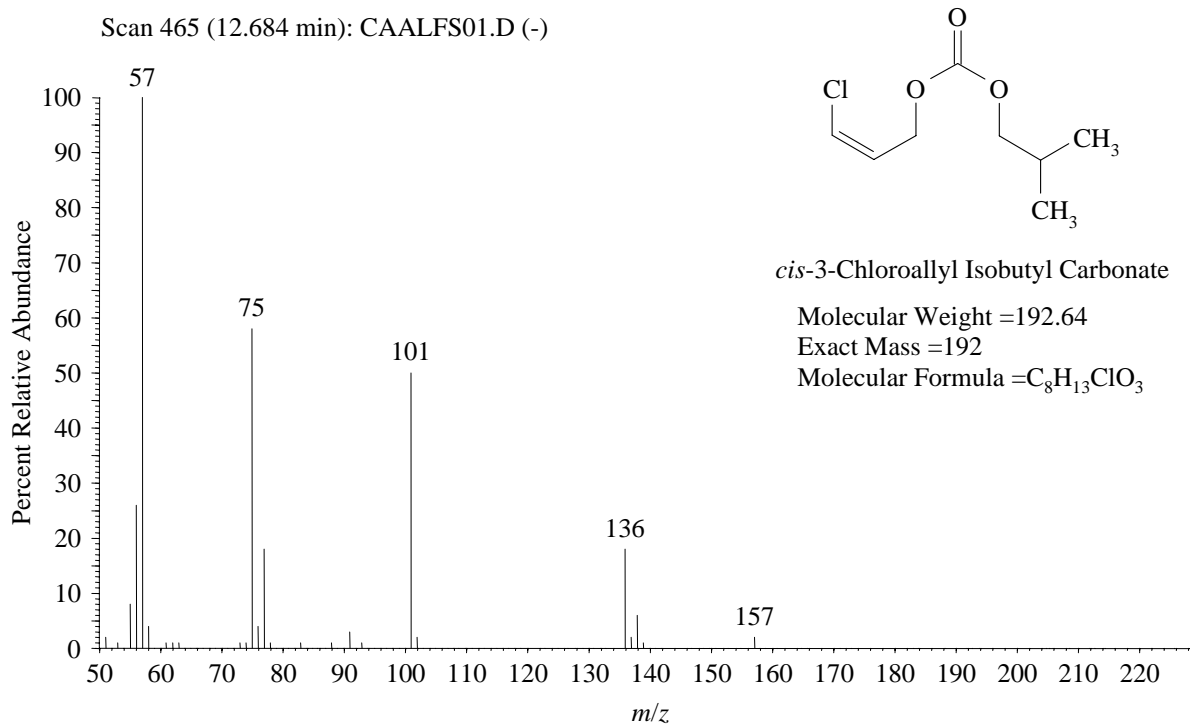


Figure 1. Mass Spectra of the *cis*- and *trans*-3-Chloroallyl Isobutyl Carbonate (CAIBC)

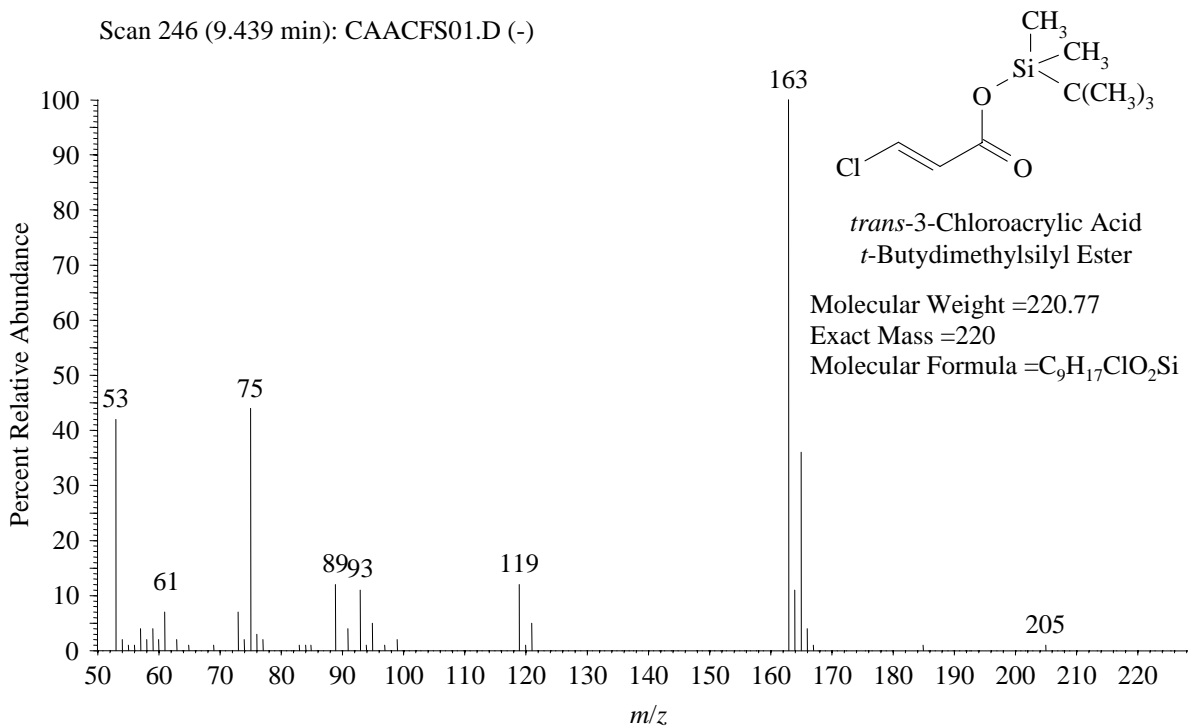
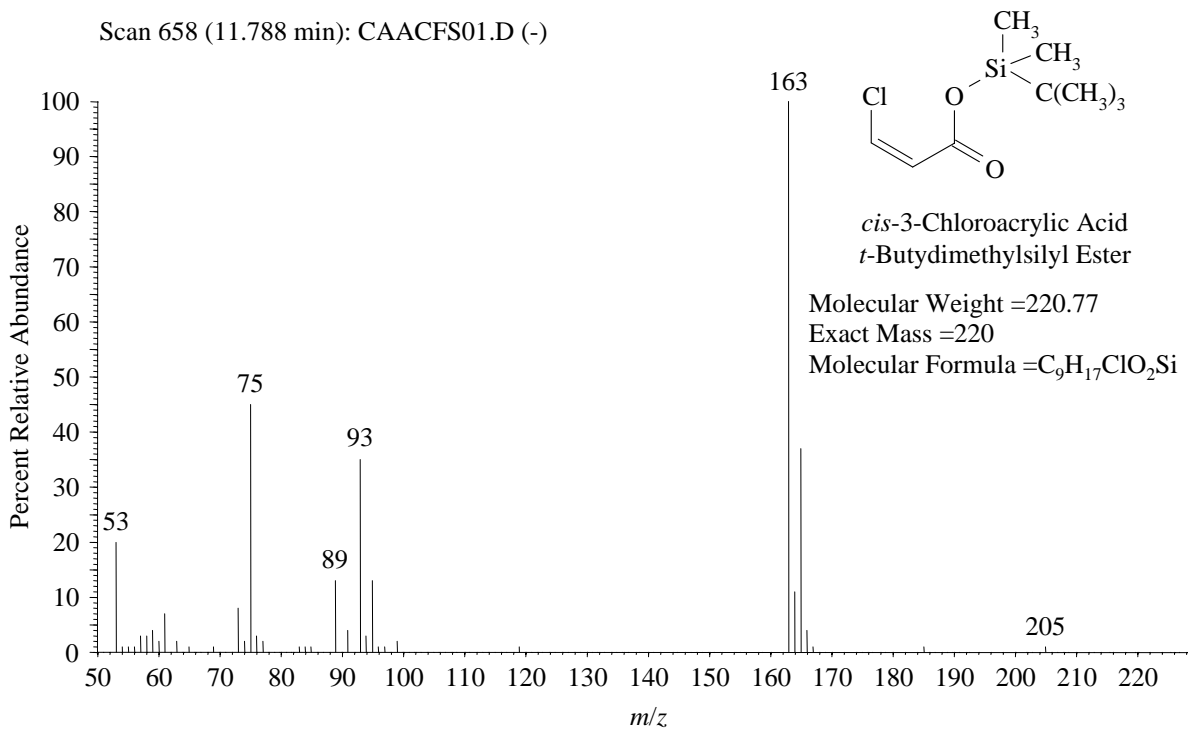


Figure 2. Mass Spectra of the *cis*- and *trans*-3-Chloroacrylic Acid *tert*-Butyldimethylsilyl Ester (CAAC TBDMSE)

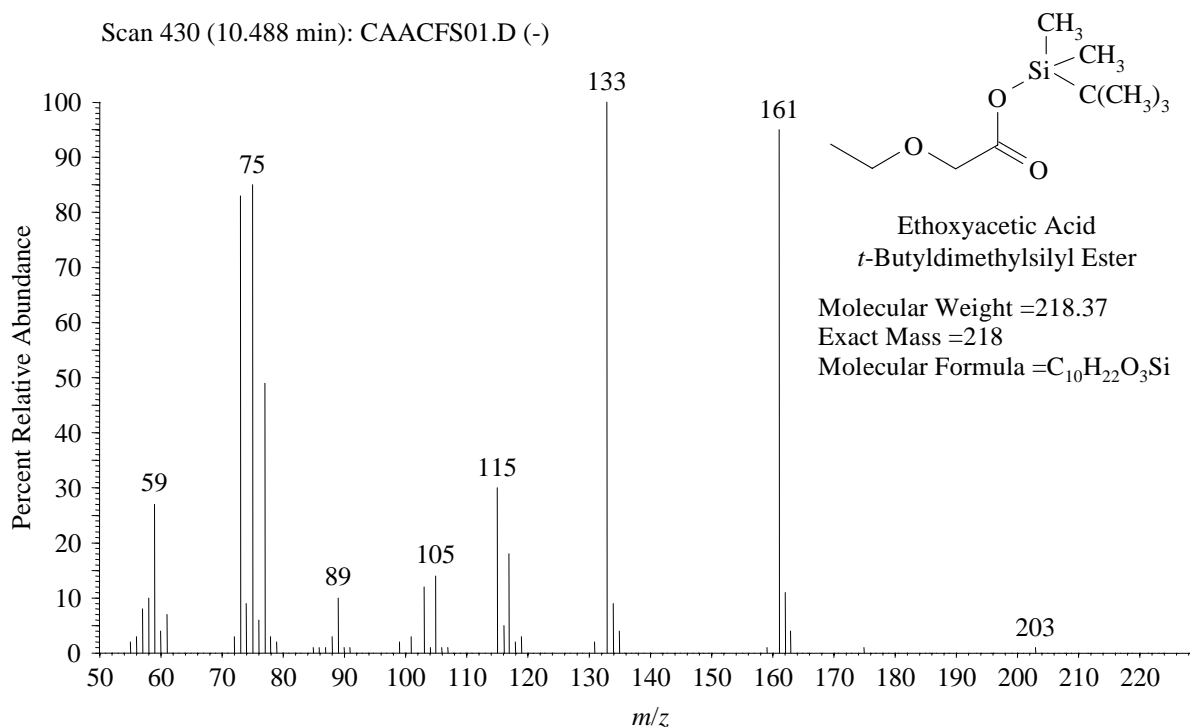
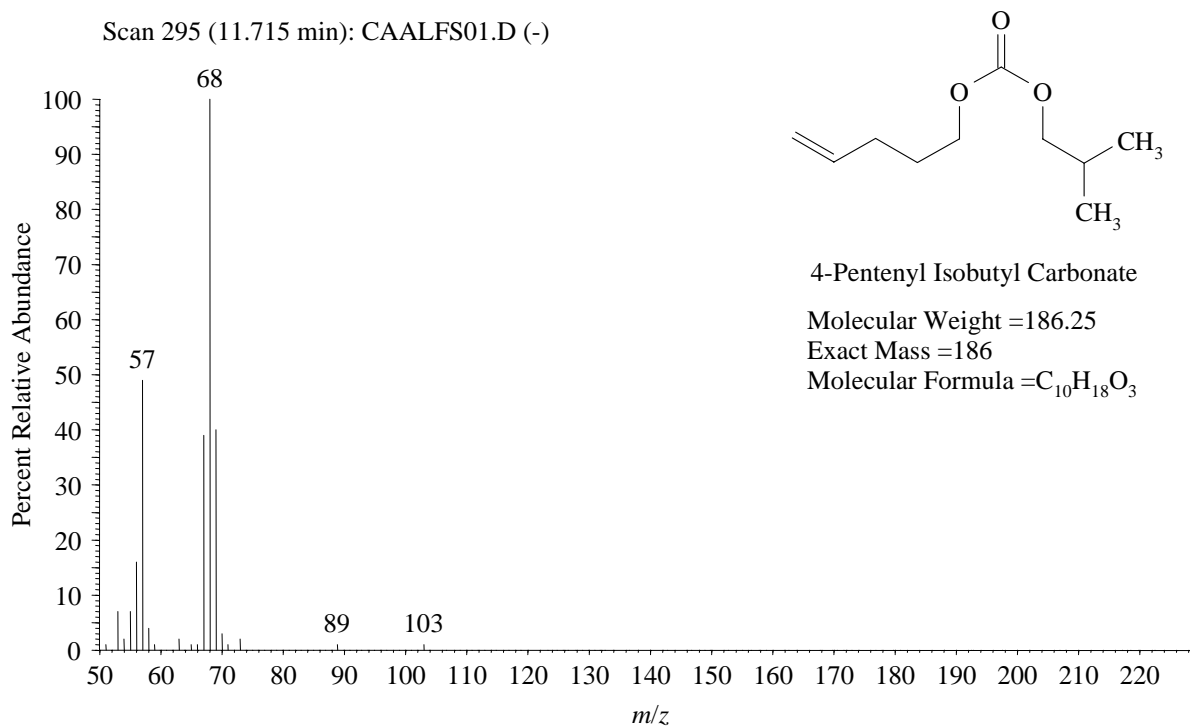
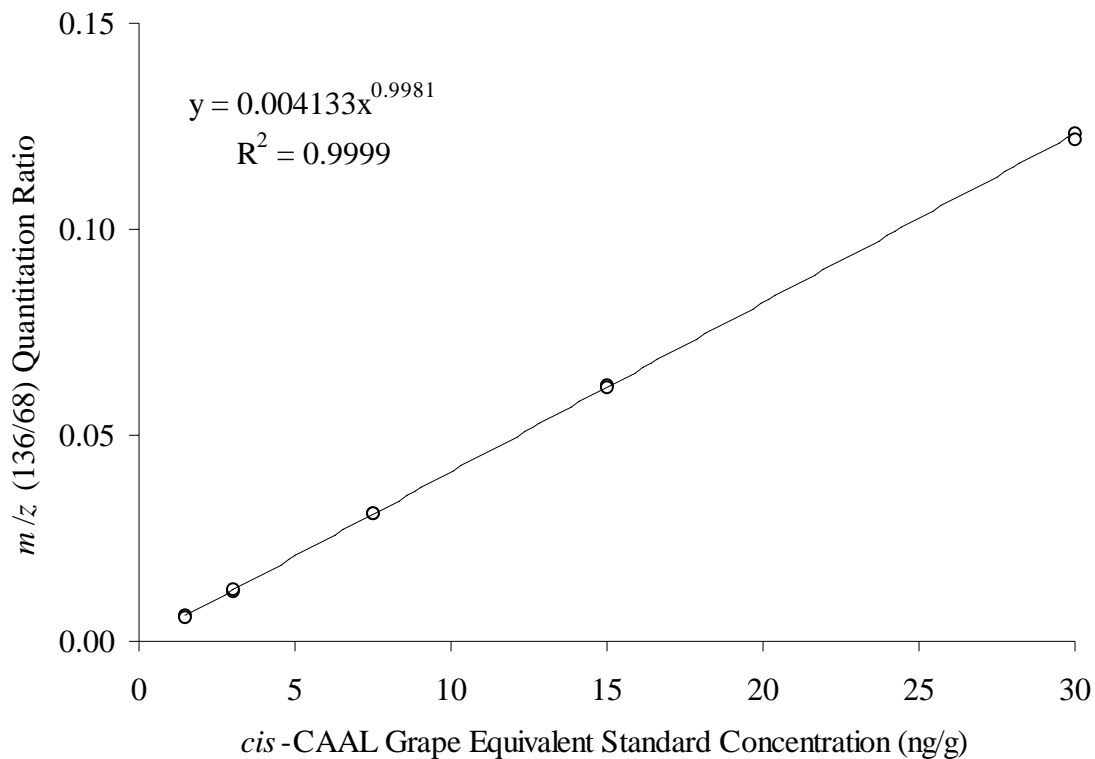


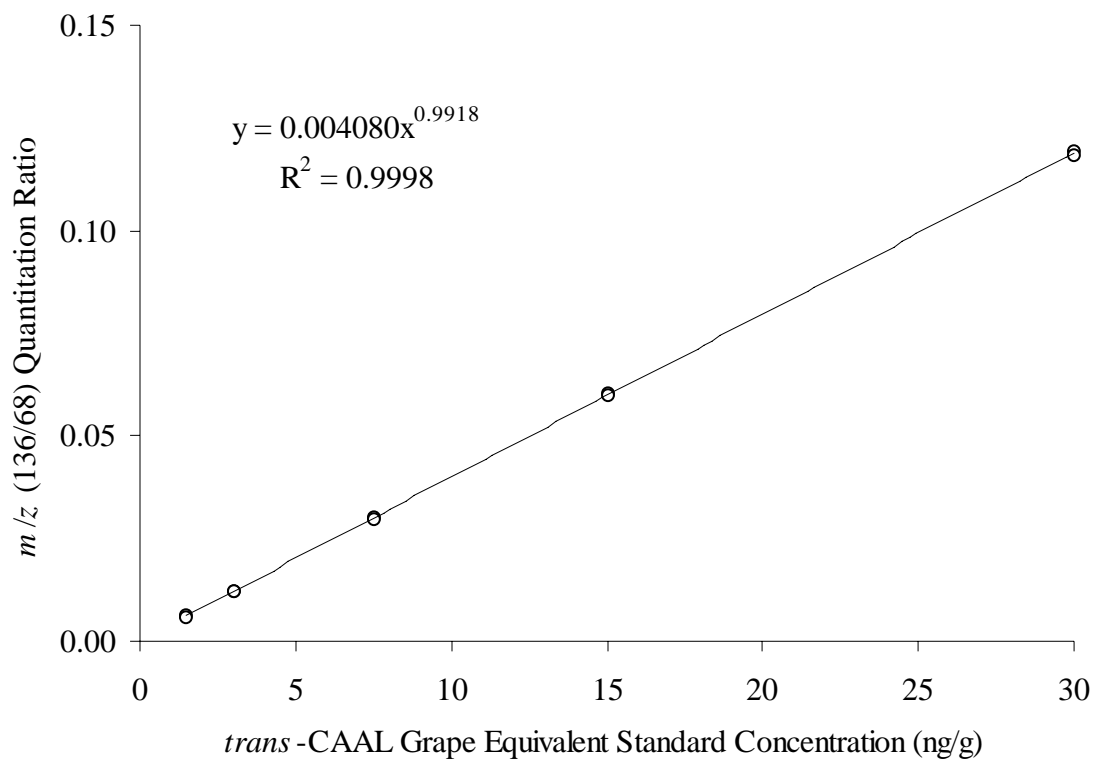
Figure 3. Mass Spectra of the Internal Standards 4-Pentenyl Isobutyl Carbonate and Ethoxyacetic Acid *tert*-Butyldimethylsilyl Ester



| <i>cis</i> -CAAL Sample Equivalent Concentration | Selected Ion | | <i>cis</i> -CAAL Quantitation Ratio |
|--------------------------------------------------------|-------------------------------------|----------------------------------------|----------------------------------------|
| | Peak Area Response | | |
| ng/g | <i>cis</i> -CAIBC <i>m/z</i> 136 | 4-Pentenyl IBC <i>m/z</i> 68 (I.S.) | <i>m/z</i> [136/68 (I.S.)] |
| 1.5 | 1920 | 306551 | 0.00626 |
| 3.0 | 4232 | 341936 | 0.01238 |
| 7.5 | 10195 | 327970 | 0.03109 |
| 15 | 20185 | 325140 | 0.06208 |
| 30 | 43561 | 353769 | 0.1231 |
| 1.5 | 2039 | 337819 | 0.00604 |
| 3.0 | 5081 | 406559 | 0.01250 |
| 7.5 | 11544 | 372089 | 0.03102 |
| 15 | 20459 | 332313 | 0.06157 |
| 30 | 45626 | 375112 | 0.1216 |

Power Regression Equation: $X = (Y/0.004133)^{(1/0.9981)}$
 Coefficient of Determination (r^2): 0.9999

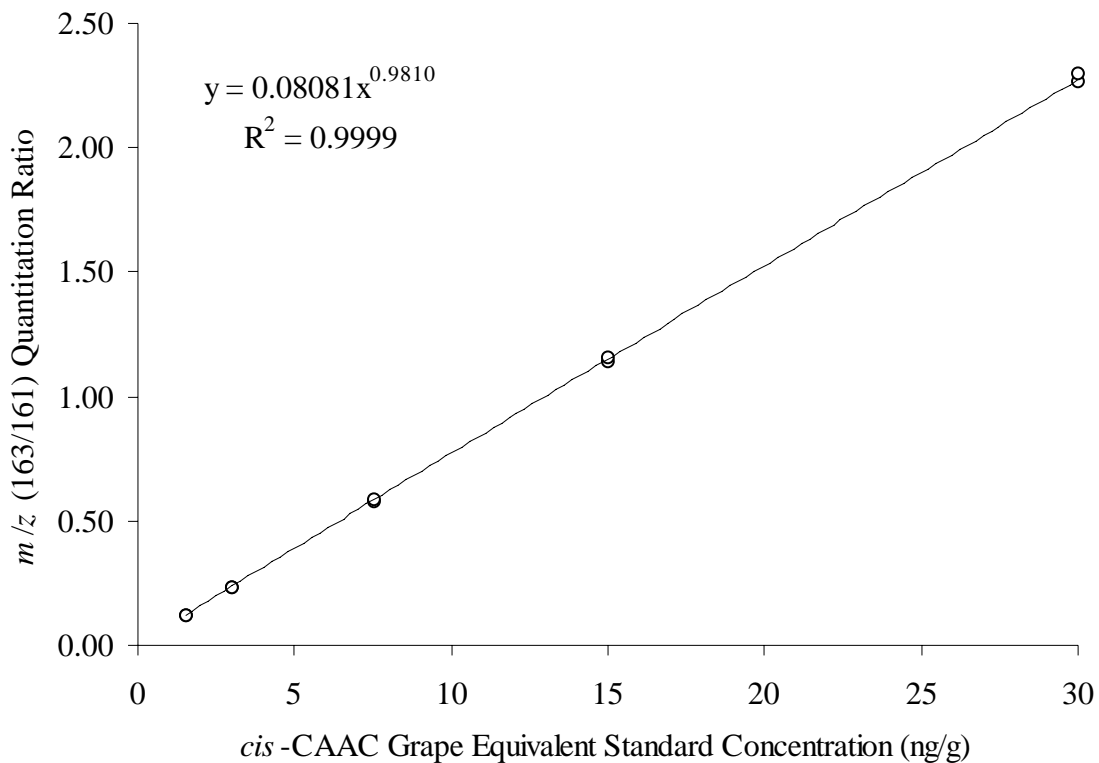
Figure 4. Typical Calibration Curve for the Determination of Residues of *cis*-3-Chloroallyl Alcohol from Grapes



| <i>trans</i> -CAAL Sample Equivalent Concentration | Selected Ion Peak Area Response | | <i>trans</i> -CAAL Quantitation Ratio |
|----------------------------------------------------------|---------------------------------------|----------------------------------------|------------------------------------------|
| | <i>trans</i> -CAIBC <i>m/z</i> 136 | 4-Pentenyl IBC <i>m/z</i> 68 (I.S.) | |
| ng/g | <i>m/z</i> 136 | <i>m/z</i> 68 (I.S.) | <i>m/z</i> [136/68 (I.S.)] |
| 1.5 | 1927 | 306551 | 0.00629 |
| 3.0 | 4102 | 341936 | 0.01200 |
| 7.5 | 9978 | 327970 | 0.03042 |
| 15 | 19607 | 325140 | 0.06030 |
| 30 | 42215 | 353769 | 0.1193 |
| 1.5 | 2015 | 337819 | 0.00596 |
| 3.0 | 4915 | 406559 | 0.1209 |
| 7.5 | 11135 | 372089 | 0.02993 |
| 15 | 19833 | 332313 | 0.05968 |
| 30 | 44402 | 375112 | 0.1184 |

Power Regression Equation: $X = (Y/0.004080)^{(1/0.9918)}$
 Coefficient of Determination (r^2): 0.9998

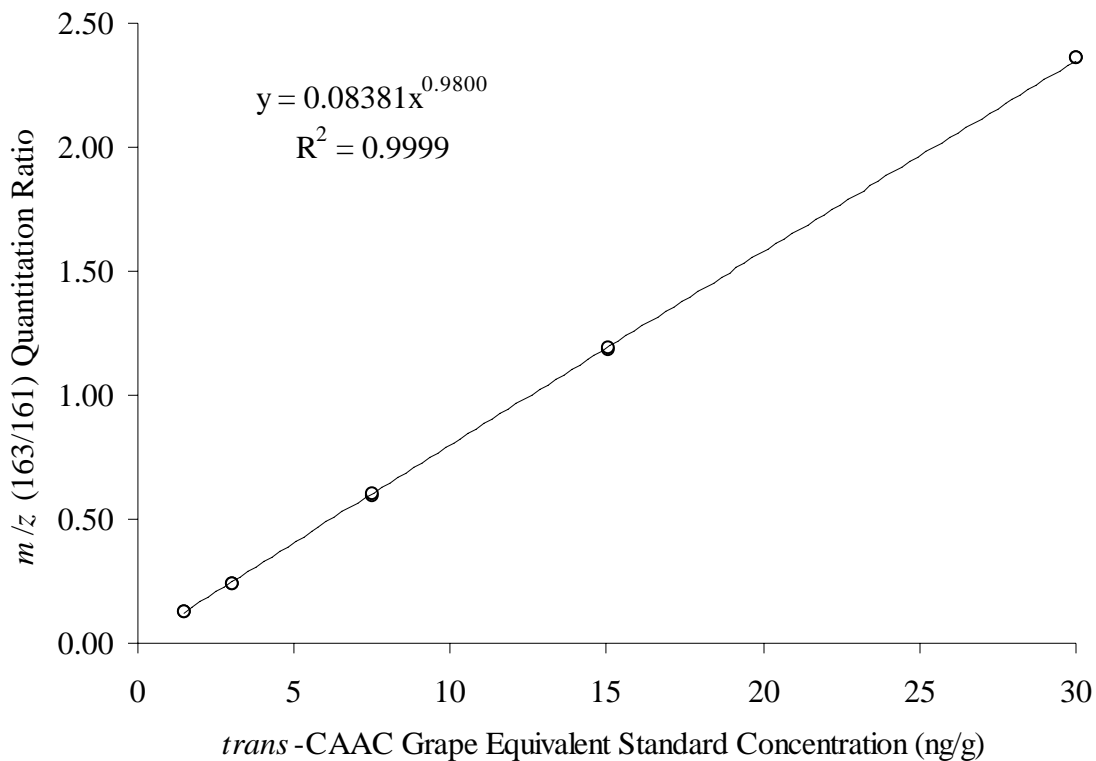
Figure 5. Typical Calibration Curve for the Determination of Residues of *trans*-3-Chloroallyl Alcohol from Grapes



| <i>cis</i> -CAAC Sample Equivalent Concentration | Selected Ion Peak Area Response | | <i>cis</i> -CAAC Quantitation Ratio |
|--------------------------------------------------|-------------------------------------------|---------------------------------------------------|-------------------------------------|
| ng/g | <i>cis</i> -CAAC TBDMSE <i>m/z</i> 163 | Ethoxyacetic acid TBDMSE <i>m/z</i> 161 (I.S.) | <i>m/z</i> [163/161 (I.S.)] |
| 1.5 | 21762 | 179003 | 0.1216 |
| 3.0 | 41262 | 175982 | 0.2345 |
| 7.5 | 104928 | 182033 | 0.5764 |
| 15 | 203481 | 178295 | 1.141 |
| 30 | 391101 | 172351 | 2.269 |
| 1.5 | 22591 | 186626 | 0.1210 |
| 3.0 | 42609 | 180114 | 0.2366 |
| 7.5 | 113101 | 193495 | 0.5845 |
| 15 | 213982 | 184434 | 1.160 |
| 30 | 433856 | 188854 | 2.297 |

Power Regression Equation: $X = (Y/0.08081)^{1/0.9810}$
 Coefficient of Determination (r^2): 0.9999

Figure 6. Typical Calibration Curve for the Determination of Residues of *cis*-3-Chloroacrylic Acid from Grapes

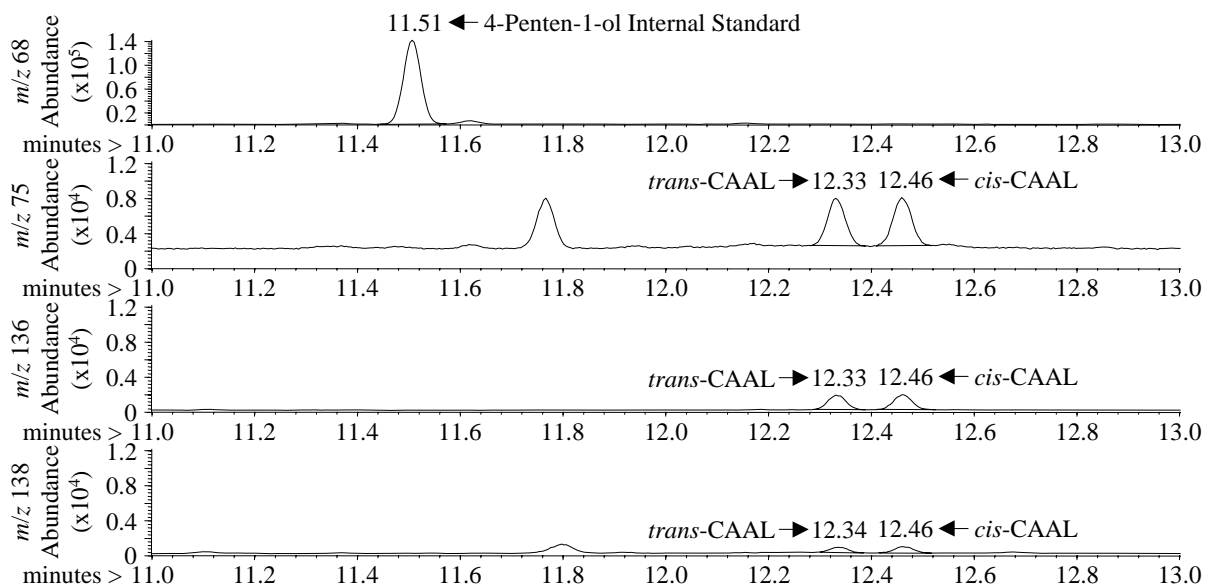


| <i>trans</i> -CAAC Sample Equivalent Concentration | Selected Ion Peak Area Response | | <i>trans</i> -CAAC Quantitation Ratio |
|----------------------------------------------------|-----------------------------------|-----------------------------------------|---------------------------------------|
| ng/g | <i>trans</i> -CAAC TBDMSE m/z 163 | Ethoxyacetic acid TBDMSE m/z 161 (I.S.) | m/z [163/161 (I.S.)] |
| 1.5 | 22629 | 179003 | 0.1264 |
| 3.0 | 42670 | 175982 | 0.2425 |
| 7.5 | 109122 | 182033 | 0.5995 |
| 15 | 211468 | 178295 | 1.186 |
| 30 | 406728 | 172351 | 2.360 |
| 1.5 | 23393 | 186626 | 0.1253 |
| 3.0 | 44095 | 180114 | 0.2448 |
| 7.5 | 116760 | 193495 | 0.6034 |
| 15 | 220051 | 184434 | 1.193 |
| 30 | 446180 | 188854 | 2.363 |

Power Regression Equation: $X = (Y/0.08381)^{(1/0.9800)}$
 Coefficient of Determination (r^2): 0.9999

Figure 7. Typical Calibration Curve for the Determination of Residues of *trans*-3-Chloroacrylic Acid from Grapes

CAAL_003.D - 30 ng/mL cis- and trans-CAAL Standard - 13 Dec 1999
 Equivalent to 3.0 ng/g in Grapes



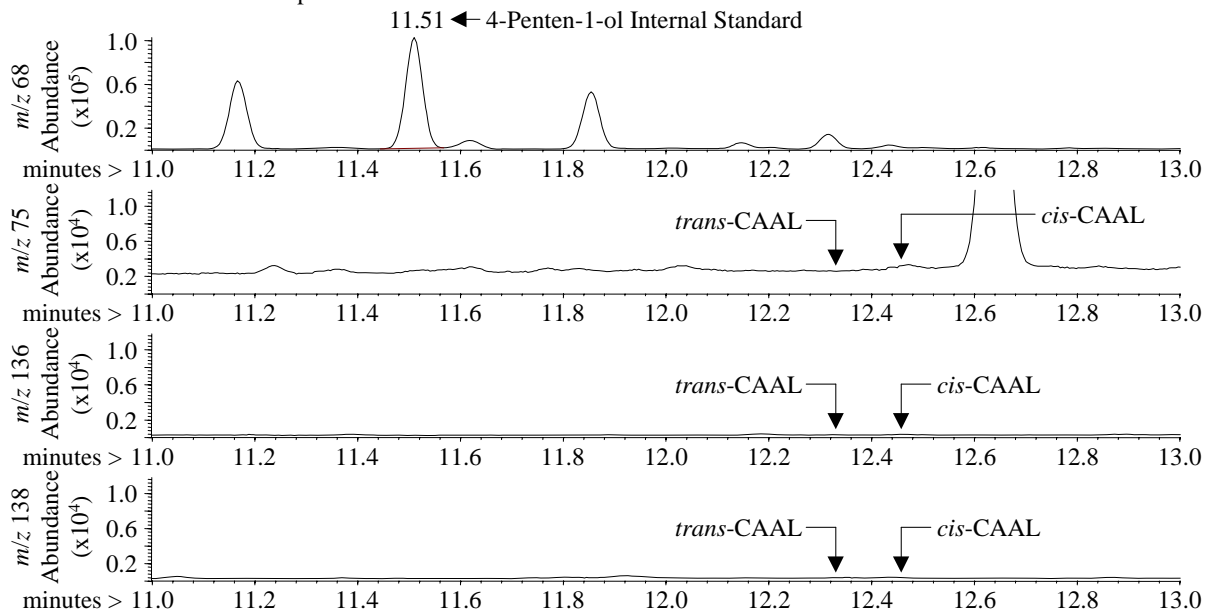
m/z 68 Internal Standard Peak Area: 341936

| | <i>cis</i> - | <i>trans</i> - |
|---------------------------------------|--------------|----------------|
| CAAL m/z 75 Peak Area: | 13932 | 13278 |
| CAAL m/z 136 Peak Area: | 4232 | 4102 |
| CAAL m/z 138 Peak Area: | 1861 | 1481 |
| CAAL m/z (136/68) Quantitation Ratio: | 0.01238 | 0.01200 |
| Gross CAAL Found (ng/g): | NA | NA |
| Percent Recovery: | NA | NA |

| | Confirmation Ratio | | |
|-----------------------------------------|--------------------|--------------|---------------|
| | m/z (136/75) | m/z (138/75) | m/z (138/136) |
| <i>cis</i> - CAAL in Standard | 0.3038 | 0.1336 | 0.4397 |
| Average of <i>cis</i> -CAAL Standards | 0.3153 | 0.1169 | 0.3715 |
| Absolute Percent Difference | 3.6% | 14% | 18% |
| <i>trans</i> - CAAL in Standard | 0.3089 | 0.1118 | 0.3618 |
| Average of <i>trans</i> -CAAL Standards | 0.3152 | 0.1075 | 0.3411 |
| Absolute Percent Difference | 2.0% | 4.0% | 6.1% |

Figure 8. Typical Selected Ion Chromatograms from a 30-ng/mL Standard Equivalent to 3.0 ng/g of *cis*- and *trans*-3-Chloroallyl Alcohol in Grapes

CAAL_010.D - 990067/Set 1/Sample 3 - 11 Dec 1999
 28790301 Grape Control

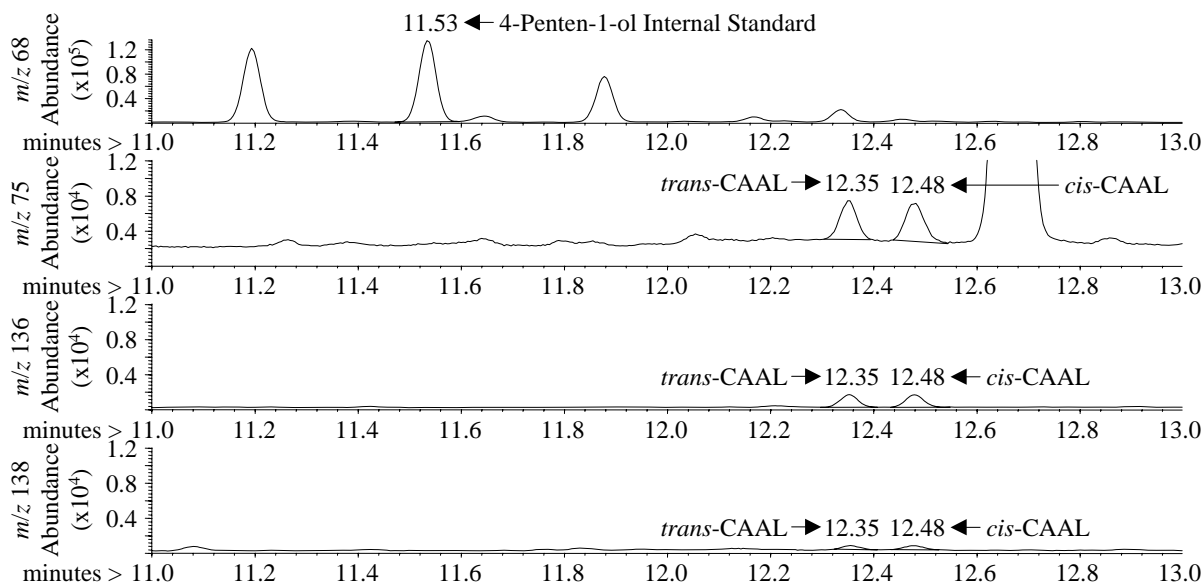


m/z 68 Internal Standard Peak Area: 237536

| | <i>cis</i> - | <i>trans</i> - |
|---------------------------------------|--------------|----------------|
| CAAL m/z 75 Peak Area: | ND | ND |
| CAAL m/z 136 Peak Area: | ND | ND |
| CAAL m/z 138 Peak Area: | ND | ND |
| CAAL m/z (136/68) Quantitation Ratio: | NA | NA |
| Gross CAAL Found (ng/g): | NA | NA |
| Percent Recovery: | NA | NA |

Figure 9. Typical Selected Ion Chromatograms from a Control Grape Sample for the Determination of *cis*- and *trans*-3-Chloroallyl Alcohol

CAAL_013.D - 990067/Set 1/Sample 6 - 13 Dec 1999
 28790301 Grape Control Fortified at 3.0 ng/g with *cis*- and *trans*-CAAL



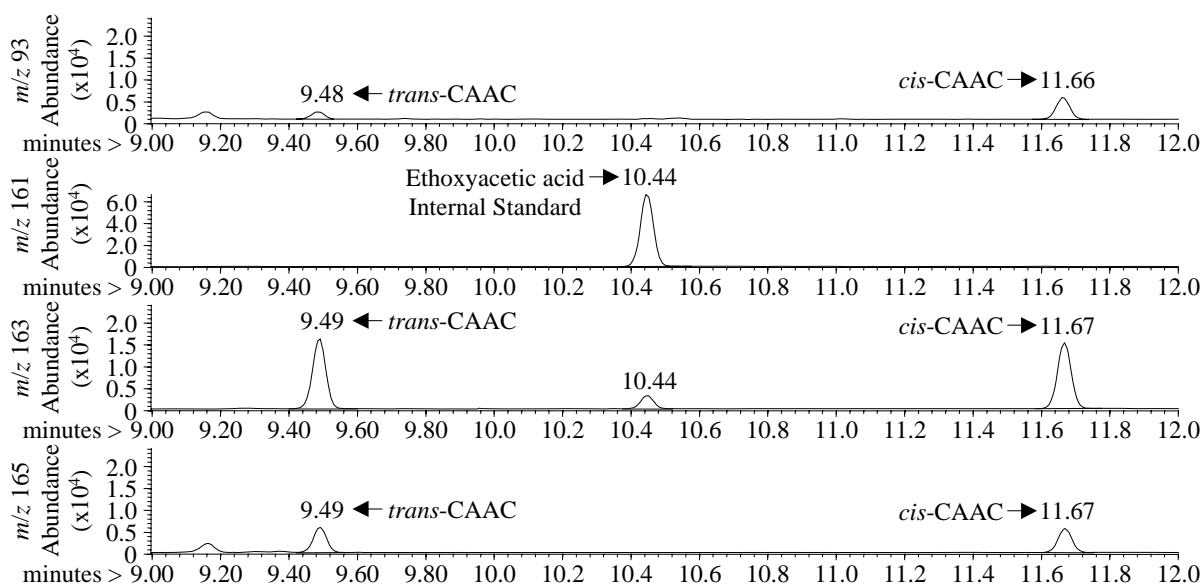
m/z 68 Internal Standard Peak Area: 314769

| | <i>cis</i> - | <i>trans</i> - |
|---------------------------------------|--------------|----------------|
| CAAL m/z 75 Peak Area: | 11281 | 11097 |
| CAAL m/z 136 Peak Area: | 3489 | 3493 |
| CAAL m/z 138 Peak Area: | 1272 | 1053 |
| CAAL m/z (136/68) Quantitation Ratio: | 0.01108 | 0.01110 |
| Gross CAAL Found (ng/g): | 2.687 | 2.742 |
| Percent Recovery: | 90 | 91 |

| | Confirmation Ratio | | |
|-----------------------------------------|--------------------|--------------|---------------|
| | m/z (136/75) | m/z (138/75) | m/z (138/136) |
| <i>cis</i> - CAAL in Standard | 0.3093 | 0.1128 | 0.3646 |
| Average of <i>cis</i> -CAAL Standards | 0.3153 | 0.1169 | 0.3715 |
| Absolute Percent Difference | 1.9% | 3.5% | 1.9% |
| <i>trans</i> - CAAL in Standard | 0.3148 | 0.0949 | 0.3015 |
| Average of <i>trans</i> -CAAL Standards | 0.3152 | 0.1075 | 0.3411 |
| Absolute Percent Difference | 0.1% | 12% | 12% |

Figure 10. Typical Selected Ion Chromatograms from a Control Grape Sample Fortified at 3.0 ng/g with *cis*- and *trans*-3-Chloroallyl Alcohol

CAAC_022.D - 30 ng/mL cis- and trans-CAAC Calibration Standard - 14 Dec 1999
 Equivalent to 3.0 ng/g in Grapes



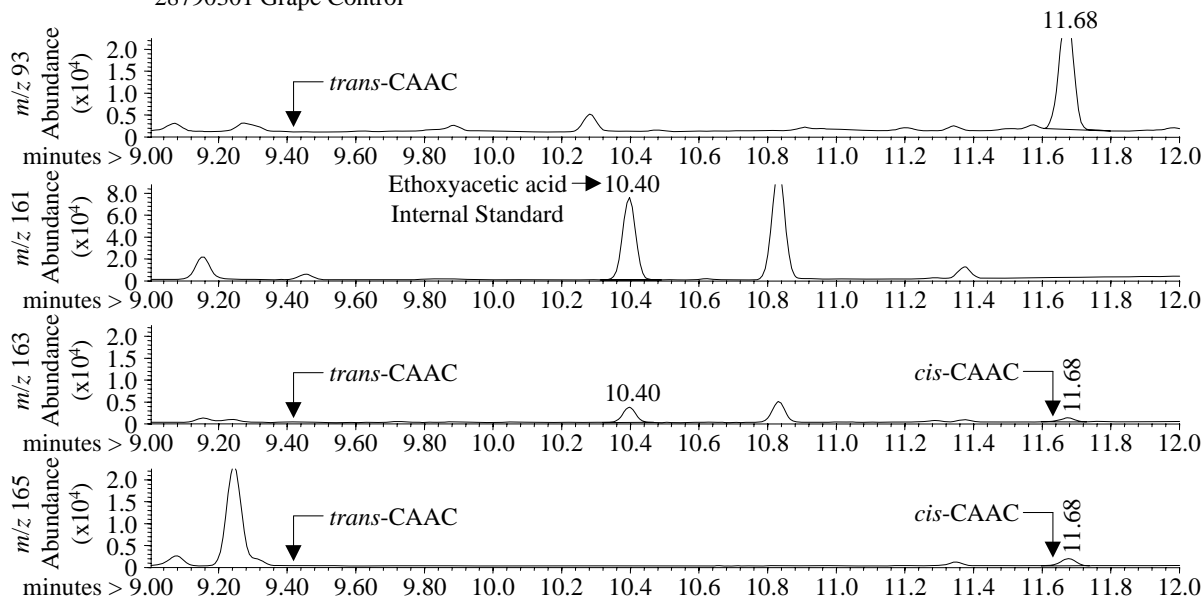
m/z 161 Internal Standard Peak Area: 180114

| | <i>cis</i> - | <i>trans</i> - |
|-----------------------------------------------|--------------|----------------|
| CAAC <i>m/z</i> 93 Peak Area: | 14011 | 4456 |
| CAAC <i>m/z</i> 163 Peak Area: | 42609 | 44095 |
| CAAC <i>m/z</i> 165 Peak Area: | 15600 | 15881 |
| CAAC <i>m/z</i> (163/161) Quantitation Ratio: | 0.2366 | 0.2448 |
| Gross CAAC Found (ng/g): | NA | NA |
| Percent Recovery: | NA | NA |

| | Confirmation Ratio | | |
|-----------------------------------------|----------------------|---------------------|---------------------|
| | <i>m/z</i> (165/163) | <i>m/z</i> (93/163) | <i>m/z</i> (93/165) |
| <i>cis</i> - CAAC in Standard | 0.3661 | 0.3288 | 0.8981 |
| Average of <i>cis</i> -CAAC Standards | 0.3650 | 0.3306 | 0.9058 |
| Absolute Percent Difference | 0.3% | 0.5% | 0.9% |
| <i>trans</i> - CAAC in Standard | 0.3602 | 0.1011 | 0.2806 |
| Average of <i>trans</i> -CAAC Standards | 0.3625 | 0.1016 | 0.2803 |
| Absolute Percent Difference | 0.6% | 0.5% | 0.1% |

Figure 11. Typical Chromatogram of a 30-ng/mL Standard Equivalent to 3.0 ng/g of *cis*- and *trans*-3-Chloroacrylic Acid in Grapes

CAAC_009.D - 990067/Set 1/Sample 2 - 13 Dec 1999
 28790301 Grape Control

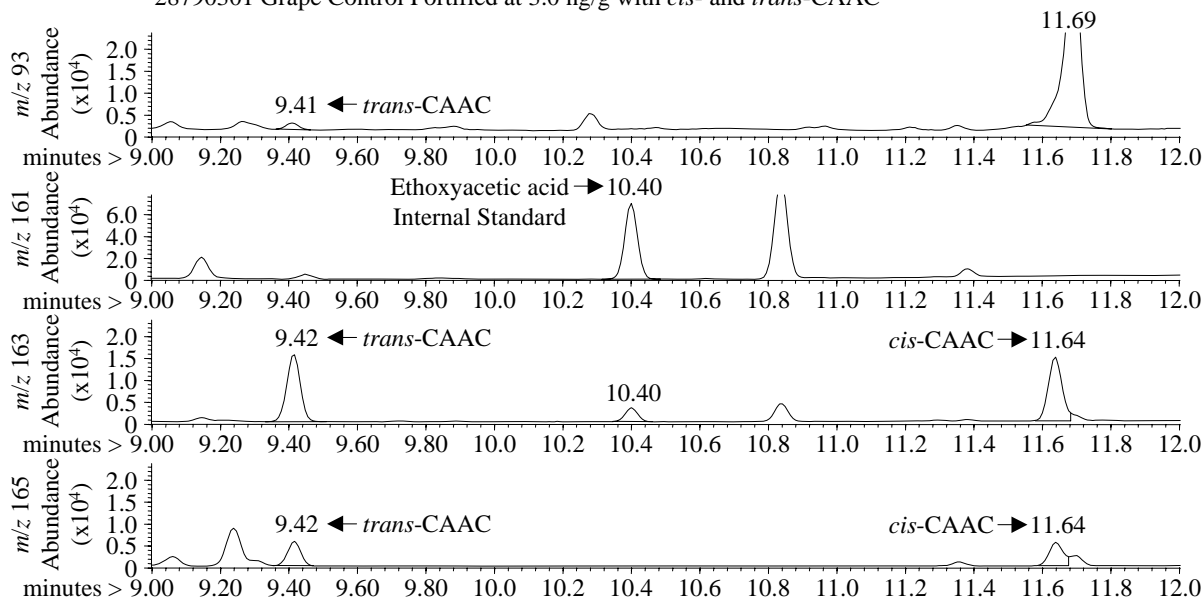


m/z 161 Internal Standard Peak Area: 201780

| | <i>cis</i> - | <i>trans</i> - |
|-----------------------------------------------|--------------|----------------|
| CAAC <i>m/z</i> 93 Peak Area: | Note 12.7 | ND |
| CAAC <i>m/z</i> 163 Peak Area: | ND | ND |
| CAAC <i>m/z</i> 165 Peak Area: | ND | ND |
| CAAC <i>m/z</i> (163/161) Quantitation Ratio: | NA | NA |
| Gross CAAC Found (ng/g): | NA | NA |
| Percent Recovery: | NA | NA |

Figure 12. Typical Selected Ion Chromatograms from a Control Grape Sample for the Determination of *cis*- and *trans*-3-Chloroacrylic Acid

CAAC_013.D - 990067/Set 1/Sample 6 - 13 Dec 1999
 28790301 Grape Control Fortified at 3.0 ng/g with *cis*- and *trans*-CAAC



m/z 161 Internal Standard Peak Area: 182390

| | <i>cis</i> - | <i>trans</i> - |
|-----------------------------------------------|--------------|----------------|
| CAAC <i>m/z</i> 93 Peak Area: | Note 12.7 | 3775 |
| CAAC <i>m/z</i> 163 Peak Area: | 41360 | 42321 |
| CAAC <i>m/z</i> 165 Peak Area: | 16099 | 14858 |
| CAAC <i>m/z</i> (163/161) Quantitation Ratio: | 0.2268 | 0.2320 |
| Gross CAAC Found (ng/g): | 2.863 | 2.827 |
| Percent Recovery: | 95 | 94 |

| | Confirmation Ratio | | |
|-----------------------------------------|----------------------|---------------------|---------------------|
| | <i>m/z</i> (165/163) | <i>m/z</i> (93/163) | <i>m/z</i> (93/165) |
| <i>cis</i> - CAAC in Standard | 0.3892 | interference | interference |
| Average of <i>cis</i> -CAAC Standards | 0.3650 | | |
| Absolute Percent Difference | 6.6 | | |
| <i>trans</i> - CAAC in Standard | 0.3511 | 0.0892 | 0.2541 |
| Average of <i>trans</i> -CAAC Standards | 0.3625 | 0.1016 | 0.2803 |
| Absolute Percent Difference | 3.1 | 12 | 9.4 |

Figure 13. Typical Selected Ion Chromatograms from a Control Grape Sample Fortified at 3.0 ng/g with *cis*- and *trans*-3-Chloroacrylic Acid