



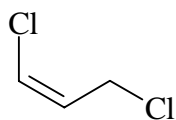
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Determination of Residues of 1,3-Dichloropropene in Grapes by
Capillary Gas Chromatography with Mass Selective Detection

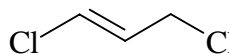
L. T. Yeh, G. E. Schelle and S. C. Dolder
Global Environmental Chemistry Laboratory—Indianapolis Lab
Dow AgroSciences LLC
Indianapolis, Indiana 46268-1054

1. SCOPE

This method is applicable for the quantitative determination of *cis*-1,3-dichloropropene (*cis*-1,3-D) and *trans*-1,3-dichloropropene (*trans*-1,3-D) in grapes. The method measures each isomer in grapes over the concentration range of 0.003-0.5 µg/g with a validated limit of quantitation of 0.003 µg/g.



cis-1,3-D
CAS No. 10061-01-5



trans-1,3-D
CAS No. 10061-02-6

2. PRINCIPLE

The 1,3-dichloropropene isomers are extracted from grapes by shaking the homogenized grape tissue with a solution of hexane containing 2-bromo-1-chloropropane as an internal standard. The sample is then centrifuged to separate the organic extract from water and the macerated grapes. An aliquot of the hexane extract is analyzed by capillary gas chromatography with mass selective detection.

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 Hexane, methanol, and 1,3-D are flammable and volatile and should be used in well-ventilated areas away from ignition sources. It is imperative that proper eye and personal protection equipment be worn when handling these chemicals.

4. EQUIPMENT (Note. 12.1)

4.1 Balance, analytical, Model AE100, Mettler Instrument Corporation, Hightstown, NJ 08520.

4.2 Balance, analytical, Model PM600, Mettler Instrument Corporation.

4.3 Centrifuge, Model CU-5000, Damon/Iec Divison, Needham Heights, MA 02194.

4.4 Gas chromatograph, Model 5890A Series II, Hewlett-Packard, Wilmington, DE 19808.

4.5 Hammermill, Model 2001, equipped with a 3/16-inch screen, AGVISE Laboratory, Northwood, ND 58267.

4.6 Injector, automatic, Model 7673, Hewlett-Packard.

4.7 Mass selective detector, Model 5971A, Hewlett-Packard, Palo Alto, CA 94304.

4.8 Mass selective detector data system, Model G1701AA, Hewlett-Packard.

4.9 Pipetter, adjustable, Eppendorf, 10-100 μ L, catalog number 05-402-48, Fisher Scientific, Pittsburgh, PA 15275.

4.10 Pipetter, adjustable, Eppendorf, 50-200 μ L, catalog number 05-402-49, Fisher Scientific.

4.11 Shaker, Model 6010, Eberbach Corporation, Ann Arbor, MI 48106.

5. GLASSWARE AND MATERIALS (Note. 12.1)
 - 5.1 Column, capillary gas chromatography, DB-VRX liquid phase, 30 m x 0.25 mm i.d., 1.4- μ m film thickness, catalog number 122-1534, J & W Scientific, Folsom, CA 95630.
 - 5.2 Filter, charcoal, catalog number 7972, Chrompack, Inc., Raritan, NJ 08869.
 - 5.3 Filter, moisture, catalog number 7971, Chrompack, Inc.
 - 5.4 Filter, oxygen, catalog number 7970, Chrompack, Inc.
 - 5.5 Liner, splitless inlet, catalog number 5181-3315, Agilent Technologies, Wilmington, DE, 19808.
 - 5.6 Syringe, 10- μ L, Model 701N, Hamilton Company, Reno, NV 89520.
 - 5.7 Syringe, gastight, 50 μ L, catalog number 2-6258-U, Supelco, Bellefonte, PA 16823.
 - 5.8 Syringe, gastight, 100 μ L, catalog number 2-6259, Supelco.
 - 5.9 Vial, 40-mL, catalog number 03-338-26A, Fisher Scientific.
 - 5.10 Vial, autosampler, 2-mL, catalog number C4000-1, National Scientific Company, Lawrenceville, GA 30243.
 - 5.11 Vial cap, for autosampler vial, catalog number C4000-54B, National Scientific Company.
6. REAGENTS AND CHEMICALS
 - 6.1 Reagents
 - 6.1.1 2-Bromo-1-chloropropane, 95%, catalog number 23,127-4, Aldrich, Milwaukee, WI 53201.
 - 6.1.2 Hexane, OmniSolv grade, catalog number HX0296-1, EM Science, Gibbstown, NJ 08027.
 - 6.1.3 Methanol, HPLC grade, catalog number 3041, Mallinkrodt Baker, Inc., Paris, KY 40361.
 - 6.1.4 Standards
 - a. *cis*-1,3-dichloropropene.
 - b. *trans*-1,3-dichloropropene.

Obtain from Test Substance Coordinator, Dow AgroSciences LLC, 9330 Zionsville Road, Building 306/A1, Indianapolis, IN 46268-1054.

7. PREPARATION OF STANDARD SOLUTIONS (Notes 12.2 and 12.3)

7.1 Preparation of Extraction Solvent and Internal Standard Solution

7.1.1 Weigh approximately 0.1 g of 2-bromo-1-chloropropane into a 50-mL volumetric flask containing approximately 20 mL of hexane. Dilute to volume with hexane to obtain a 2000 µg/mL stock solution

7.1.2 Pipette 10.0 mL of the 2000 µg/mL stock solution prepared in Section 7.1.1 into a 100-mL volumetric flask and dilute to volume with hexane to obtain a solution containing 200 µg/mL of 2-bromo-1-chloropropane.

7.1.3 Pipette 2.0 mL of the 200-µg/mL solution prepared in Section 7.1.2 into a 2000-mL volumetric flask and dilute to volume with hexane to obtain a solution containing 0.2 µg/mL of 2-bromo-1-chloropropane. This internal standard solution (ISTD) will be used to prepare calibration standards and also be used as the extraction solvent.

7.2 Preparation of Calibration Standard Solutions

7.2.1 Accurately weigh 0.1 g of *cis*-1,3-D and 0.1 g of *trans*-1,3-D analytical standards into separate 50-mL volumetric flasks containing approximately 25 mL of hexane and dilute both standards to volume with hexane. Transfer a 25.0-mL aliquot of each standard solution into a 4-oz (120 mL) Qorpak bottle with a PTFE-lined closure. This will be the stock solution for *cis*-1,3-D and *trans*-1,3-D at a concentration of 1000 µg/mL for each compound.

7.2.2 Make a series of dilutions from the stock solution (Section 7.2.1) with ISTD solution (Section 7.1.3.) to obtain calibration standard solutions as follows:

<u>Initial Conc.</u> µg/mL	<u>Aliquot</u> mL	<u>Final Volume</u> mL	<u>Final Conc.</u> µg/mL
1000	1.0	100	10.0 ^a
10.0	25.0	250	1.0 ^a
1.0	50.0	100	0.50
1.0	10.0	100	0.10
1.0	5.0	100	0.050
1.0	1.0	100	0.010
1.0	0.50	100	0.0050
1.0	0.25	100	0.0025
1.0	0.10	100	0.0010

^a These solutions are prepared for further dilution and are not used to establish the calibration curve.

7.3 Preparation of Fortification Solution

7.3.1 Accurately weigh 0.1 g of *cis*-1,3-D and 0.1 g of *trans*-1,3-D analytical standards into separate 10-mL volumetric flasks containing approximately 5 mL of methanol. Dilute both standards to volume with methanol and combine these standard solutions by pouring them into a 4-oz (120 mL) Qorpak bottle with a PTFE-lined closure. This will be the stock solution for *cis*-1,3-D and *trans*-1,3-D at a concentration of 5000 µg/mL for each compound.

7.3.2 Make a series of dilutions of the stock solution (Section 7.3.1) with methanol to obtain fortification solutions as follows:

<u>Initial Conc.</u> µg/mL	<u>Aliquot</u> mL	<u>Final Volume</u> mL	<u>Final Conc.</u> µg/mL	<u>Equivalent Sample Conc.</u> µg/g ^a
5000	10.0	100	500.0	0.5
500	10.0	100	50.0	0.05
500	2.0	100	10.0	0.01
50	6.0	100	3.0	0.003
50	3.0	100	1.5	0.0015

^a The equivalent sample concentration is the concentration that would result from fortifying 10 g grapes with 10 µL of the appropriate fortification solution.

8. GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MSD)

8.1 Column

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

8.2 Typical Operating Conditions (Note 12.4)

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
DB-VRX liquid phase
30 m x 0.25 mm i.d.
1.4-µm film thickness

Temperatures:	Cryogenic cooling using liquid N ₂
Column	25 °C for 1.0 min 35 °C to 130 °C at 10 °C/min 130 °C to 220 °C at 60 °C/min 220 °C for 1.0 min
Injector	200 °C
Interface	230 °C
Carrier Gas:	Helium
Constant Flow	N/A
Head Pressure	42 kPa
Linear Velocity	Approximately 33.7 cm/sec with vacuum compensation on.
Injection Mode:	splitless
Purge Delay	0.9 min
Splitter Flow	35 mL/min
Septum Purge	1.0 mL/min
Injection Volume:	1 µL
Detector Mode:	electron impact ionization with selected ion monitoring
Calibration Program	maximum sensitivity autotune
Electron Multiplier	1918 volt (300 volts above autotune)
Ions Monitored:	
<i>cis</i> -1,3-D	<i>m/z</i> 75 (quantitation) and <i>m/z</i> 110, 112 (for confirmation)
<i>trans</i> -1,3-D	<i>m/z</i> 75 (quantitation) and <i>m/z</i> 110, 112 (for confirmation)
ISTD	<i>m/z</i> 77 (internal standard)
Dwell Time	100 msec

Typical mass spectra of *cis*-1,3-D, *trans*-1,3-D, and 2-bromo-1-chloropropane are shown in Figures 1 and 2.

Typical calibration curves for the determination of *cis*-1,3-D and *trans*-1,3-D in grapes are shown in Figures 3 and 4, respectively.

Typical chromatograms for standard, extracts of grape control, and extracts of grape control fortified at limit of quantitation are presented in Figures 5-10.

9. DETERMINATION OF RECOVERY OF 1,3-D FROM GRAPES

9.1 Method Validation

Unless otherwise specified, a sample set should contain the following samples:

At least one reagent blank.

At least one control.

At least two controls fortified at the limit of quantitation.

At least two controls fortified at the expected sample concentration.

9.2 Sample Preparation

Prepare the samples for analysis by freezing the grapes with liquid nitrogen and then grinding or chopping with a hammermill equipped with a 3/16-inch screen size.

9.3 Sample Analysis

9.3.1 Weigh approximately 10 g of homogenized grapes into a 40-mL vial.

9.3.2 For recovery samples, inject 10 μ L of the appropriate fortification solution from Section 7.3.2 into the control grapes. To avoid loss of 1,3-D due to evaporation, inject the solution onto the grapes at the bottom of the vial.

9.3.3 Immediately add 10 mL of the extraction solution containing the ISTD prepared in Section 7.1.3. Seal the vial with a PTFE-lined closure.

9.3.4 Shake the mixture for a minimum of 15 minutes at approximately 180 excursions/minute.

9.3.5 Centrifuge the sample for 3 minutes at approximately 2400 rpm.

9.3.6 Transfer a portion of the organic layer (upper) into an autosampler vial.

9.3.7 Analyze the calibration standards and sample extract by GC/MSD as described in Section 8.2.

9.3.8 Determine the suitability of the chromatographic system.

- 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 interference.
- c. Appearance of chromatograms: Visually determine that the chromatograms resemble those shown in Figures 5-10 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 0.0025- $\mu\text{g}/\text{mL}$ calibration standard equivalent to 0.0025 $\mu\text{g}/\text{g}$ *cis*- and *trans*-1,3-D in grapes.

10. CALCULATIONS

10.1 Calculation of Percent Recovery

- 10.1.1 Inject a series of calibration standards as described in Section 8.2 and determine the peak areas for the analytes and the internal standard as indicated below.

<i>cis</i> -1,3-D	m/z 75 (quantitation)
<i>trans</i> -1,3-D	m/z 75 (quantitation)
2-Bromo-1-chloropropane	m/z 77 (internal standard)

- 10.1.2 For each standard, calculate each analyte's quantitation ratio. For example, using the data for *cis*-1,3-D in Figure 9:

$$\text{Quantitation ratio} = \frac{\text{peak area of quantitation ion}}{\text{peak area of intrnal standard ion}}$$

$$\text{Quantitation ratio} = \frac{\text{peak area at } m/z \text{ 75}}{\text{peak area at } m/z \text{ at 77}}$$

$$\text{Quantitation ratio} = \frac{685}{43688}$$

$$\text{Quantitation ratio} = 0.01568$$

- 10.1.3 Prepare a standard curve by plotting the concentration of the analyte on the abscissa (x-axis) and the respective quantitation ratio on the ordinate (y-axis) as shown in Figures 3 and 4. Using power regression analysis, determine the equation for the curve with respect to the abscissa.

For example, using power regression (13.1) with the *cis*-1,3-D data from Figure 3:

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

$$cis\text{-}1,3\text{-D conc.}(\mu\text{g/mL}) = \left(\frac{cis\text{-}1,3\text{-D quantitation ratio}}{\text{constant}} \right)^{1/\text{exponent}}$$

$$cis\text{-}1,3\text{-D conc.} (\mu\text{g/mL}) = \left(\frac{cis\text{-}1,3\text{-D quantitation ratio}}{5.0595} \right)^{1/0.9729}$$

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 above equation and solving for the concentration.

For example, using the data for *cis*-1,3-D from Figure 9:

$$cis\text{-}1,3\text{-D conc.} \quad = \quad \left(\frac{cis\text{-}1,3\text{-D quantitation ratio}}{5.0595} \right)^{1/0.9729}$$

(gross $\mu\text{g/mL}$)

$$cis\text{-}1,3\text{-D conc.} \quad = \quad \left(\frac{0.01568}{5.0595} \right)^{1/0.9729}$$

(gross $\mu\text{g/mL}$)

$$cis\text{-}1,3\text{-D conc.} \quad = \quad 0.0026 \mu\text{g/mL}$$

(gross $\mu\text{g/mL}$)

Convert the concentration of $\mu\text{g/mL}$ to $\mu\text{g/g}$ as follows:

$$cis\text{-}1,3\text{-D conc.} \quad = \quad 0.0026 \mu\text{g/mL} \times \frac{10 \text{ mL}}{10 \text{ g}}$$

$$\text{(gross } \mu\text{g/g)} \quad = \quad 0.0026 \mu\text{g/g}$$

- 10.2.2 Determine the net concentration in each recovery sample by subtracting the apparent *cis*-1,3-D concentration in the control sample from that of the gross *cis*-1,3-D concentration in the recovery sample.

For example, using the *cis*-1,3-D data from Figures 6 and 8:

$$\begin{array}{l} \textit{cis}\text{-1,3-D conc.} \\ \text{(net } \mu\text{g/g)} \end{array} = \begin{array}{l} \textit{cis}\text{-1,3-D conc.} \\ \text{(gross } \mu\text{g/g)} \end{array} - \begin{array}{l} \textit{cis}\text{-1,3-D conc.} \\ \text{(control } \mu\text{g/g)} \end{array}$$

$$\begin{array}{l} \textit{cis}\text{-1,3-D conc.} \\ \text{(net } \mu\text{g/g)} \end{array} = 0.0026 \mu\text{g/g} - 0.0 \mu\text{g/g}$$

$$\begin{array}{l} \textit{cis}\text{-1,3-D conc.} \\ \text{(net)} \end{array} = 0.0026 \mu\text{g/g}$$

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

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

$$\text{Recovery} = \frac{0.0026 \mu\text{g/g}}{0.0030 \mu\text{g/g}} \times 100\%$$

$$\text{Recovery} = 88\%$$

10.3 Determination of *cis*-1,3-D and *trans*-1,3-D in Grapes

- 10.3.1 Determine the gross concentration of each analyte in each treated sample by substituting the quantitation ratio obtained into the equation for the standard calibration curve, and calculating the uncorrected residue result as described in Sections 10.2.1 and 10.2.2.
- 10.3.2 For those analytes 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.

For example, using the *cis*-1,3-D data from Figure 9 and the average recovery from Table 1 for the sample analyzed on 19-Jan-2000:

$$\begin{array}{l} \textit{cis}\text{-1,3-D conc.} \\ \text{(corrected } \mu\text{g/mL)} \end{array} = \begin{array}{l} \textit{cis}\text{-1,3-D conc.} \\ \text{(gross } \mu\text{g/mL)} \end{array} \times \left(\frac{100}{\text{Average \% recovery}} \right)$$

$$\begin{array}{l} \textit{cis}\text{-1,3-D conc.} \\ \text{(corrected } \mu\text{g/mL)} \end{array} = 0.0026 \mu\text{g/g} \times \frac{100}{94}$$

$$\begin{array}{l} \textit{cis}\text{-1,3-D conc.} \\ \text{(corrected gross)} \end{array} = 0.0028 \mu\text{g/g}$$

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 *cis*-1,3-D and *trans*-1,3-D in grapes. The results are summarized in Tables 1-2.

The average recoveries for *cis*-1,3-D and *trans*-1,3-D in grapes fortified over the concentration range of 0.0030 to 0.50 µg/g were 92±9% and 90±10%, respectively. All of the individual recovery samples in the case of each of the analytes were between 70 and 120%.

11.1.2 Standard Curve Linearity

For the power least squares regression analysis, the correlation coefficients (r^2) were greater than 0.995 for each of the analytes for all of the calibration curve determinations during the method validation, while the power exponents were between 0.9986 and 0.9995. The results indicate linearity of the detector response as a function of the standard calibration curve.

11.1.3 Calculated Limits of Quantitation and Detection

Following established guidelines (13.2), the limits of quantitation (LOQ) and detection (LOD) were calculated for *cis*-1,3-D and *trans*-1,3-D using the standard deviation from the 0.003-µg/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 of the analysis of eight samples for each analyte. The results are summarized in Tables 1-2.

The calculated LOQ was 0.0012 for both *cis*-1,3-D and *trans*-1,3-D. All of which are lower than the targeted method LOQ of 0.0030 µg/g for each analyte. Since the lowest level of fortification for recovery samples was 0.0030-µg/g for each analyte, the method LOQ is considered to be 0.0030 µg/g.

The calculated LOD was 0.00036 µg/g for both *cis*-1,3-D and *trans*-1,3-D.

11.2 Confirmation of Residue Identity

The presence of *cis*-1,3-D and *trans*-1,3-D are confirmed by comparing the retention time (gas chromatography) as well as the peak area ratios resulting from selected ion monitoring (mass spectrometry) of standards with samples. If confirmation is required, the mass spectra of *cis*-1,3-D and *trans*-1,3-D (Figure 1) contain additional ions, m/z at

- 110 or 112, that may be used for confirmation.
- 11.2.1 Inject the series of calibration standards described in Step 7.2.2 using instrumental conditions described in Section 8.2 and determine the peak areas for *cis*-1,3-D and *trans*-1,3-D at *m/z* 75 (quantitation) and *m/z* 110 and 112 (for confirmation).
- 11.2.2 For each standard, calculate the confirmation ratio. Calculate the average of the confirmation ratios of all the standards. Use the average confirmation ratio to confirm the presence of *cis*-1,3-D and *trans*-1,3-D in grape samples.

For example, using the data for the *cis*-1,3-D standard from Figure 5:

$$\text{Confirmation Ratio} = \frac{\text{peak area of confirmation ion}}{\text{peak area of quantitation ion}}$$

$$\text{Confirmation Ratio} = \frac{\text{peak area at } m/z \text{ 110}}{\text{peak area at } m/z \text{ 75}}$$

$$\text{Confirmation Ratio} = \frac{124}{630}$$

$$\text{Confirmation Ratio} = 0.20$$

Confirmation of the presence of *cis*-1,3-D is indicated when the confirmation ratio for the sample is within the range of $\pm 20\%$ of the average found for the standards.

11.3 Assay Time and Stopping Points

A typical analytical run would consist of a minimum of seven standards encompassing the expected range of sample concentrations, a reagent blank, a control (a non-fortified sample), a minimum of three fortified controls (two of which must be at the LOQ), and 22 samples. This typical analytical set can be prepared in approximately 3 hours, followed by the chromatographic analysis.

Sample preparation time is considered to be minimal and no “stopping points” are recommended.

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 To avoid loss of 1,3-D through evaporation, always add solvent into the container before adding pure 1,3-D standards.

12.3 Section 7 provides suggested concentrations for calibration standard preparation. Other dilution schemes may be followed.

12.4 Operating conditions may be modified to obtain optimal separation.

13. REFERENCES

13.1 Freund, J. E.; Williams, F. J. *Dictionary/Outline of Basic Statistics*; Dover: New York, 1991; p 170.

13.2 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*-1,3-D from Grapes

Sample Number	Date of Analysis	<i>cis</i> -1,3-D, $\mu\text{g/g}$		Recovery ^b %	Statistical Calculations, $\mu\text{g/g}$
		Added	Found		
SN28790301 ^a	13-Jan-2000	0.000	0.000	--	
SN28790301	19-Jan-2000	0.000	0.000	--	
SN28790301	13-Jan-2000	0.0030	0.0024	79	
SN28790301	13-Jan-2000	0.0030	0.0026	86	
SN28790301	13-Jan-2000	0.0030	0.0028	94	
SN28790301	19-Jan-2000	0.0030	0.0025	84	$\bar{x} = 0.0026$
SN28790301	19-Jan-2000	0.0030	0.0026	88	$s = 0.00012$
SN28790301	19-Jan-2000	0.0030	0.0026	87	$3s^c = 0.00036$
SN28790301	19-Jan-2000	0.0030	0.0026	88	$10s^d = 0.0012$
SN28790301	19-Jan-2000	0.0030	0.0027	90	RSD = 5%
SN28790301	13-Jan-2000	0.010	0.0093	93	$\bar{x} = 0.0089$
SN28790301	13-Jan-2000	0.010	0.0088	88	$s = 0.00040$
SN28790301	13-Jan-2000	0.010	0.0086	86	RSD = 4%
SN28790301	19-Jan-2000	0.050	0.048	96	$\bar{x} = 0.046$
SN28790301	19-Jan-2000	0.050	0.044	87	$s = 0.0025$
SN28790301	19-Jan-2000	0.050	0.046	91	RSD = 5%
SN28790301	19-Jan-2000	0.50	0.53	105	$\bar{x} = 0.54$
SN28790301	19-Jan-2000	0.50	0.53	106	$s = 0.025$
SN28790301	19-Jan-2000	0.50	0.57	114	RSD = 5%
				$\bar{x} =$	92
				$s =$	9
				$n =$	17

^a Grapes were purchased from a local grocery store.

^b Data transferred from Excel. The calculation might be slightly different for some of the values due to rounding.

^c Calculated limit of detection.

^d Calculated limit of quantitation.

Table 2. Recovery of *trans*-1,3-D from Grapes

Sample Number	Date of Analysis	<i>trans</i> -1,3-D, µg/g		Recovery ^b %	Statistical Calculations, µg/g
		Added	Found		
SN2879030 ^a	13-Jan-2000	0.000	0.000	--	
SN28790301	19-Jan-2000	0.000	0.000	--	
SN28790301	13-Jan-2000	0.0030	0.0023	77	
SN28790301	13-Jan-2000	0.0030	0.0025	83	
SN28790301	13-Jan-2000	0.0030	0.0027	89	
SN28790301	19-Jan-2000	0.0030	0.0026	87	$\bar{x} = 0.0025$
SN28790301	19-Jan-2000	0.0030	0.0025	84	$s = 0.00013$
SN28790301	19-Jan-2000	0.0030	0.0023	77	$3s^c = 0.00039$
SN28790301	19-Jan-2000	0.0030	0.0025	83	$10s^d = 0.0013$
SN28790301	19-Jan-2000	0.0030	0.0026	86	RSD = 5%
SN28790301	13-Jan-2000	0.010	0.0092	92	$\bar{x} = 0.0088$
SN28790301	13-Jan-2000	0.010	0.0086	86	$s = 0.00030$
SN28790301	13-Jan-2000	0.010	0.0087	87	RSD = 4%
SN28790301	19-Jan-2000	0.050	0.047	93	$\bar{x} = 0.045$
SN28790301	19-Jan-2000	0.050	0.042	84	$s = 0.0030$
SN28790301	19-Jan-2000	0.050	0.047	94	RSD = 6%
SN28790301	19-Jan-2000	0.50	0.53	105	$\bar{x} = 0.54$
SN28790301	19-Jan-2000	0.50	0.53	107	$s = 0.020$
SN28790301	19-Jan-2000	0.50	0.57	113	RSD = 4%
				$\bar{x} = 90$	
				$s = 10$	
				$n = 17$	

^a Grapes were purchased from a local grocery store.

^b Data transferred from Excel. The calculation might be slightly different for some of the values due to rounding.

^c Calculated limit of detection.

^d Calculated limit of quantitation.

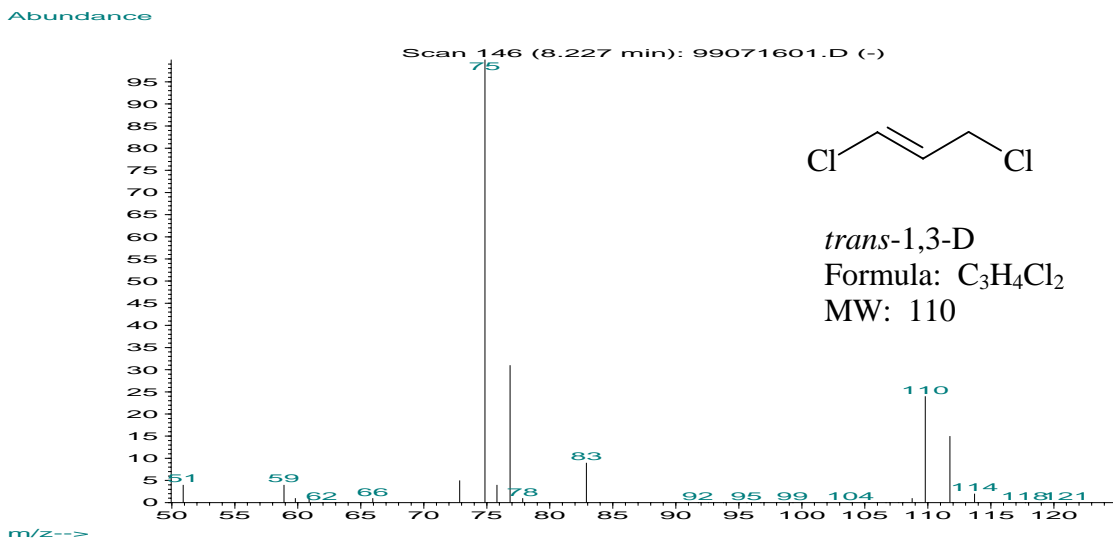
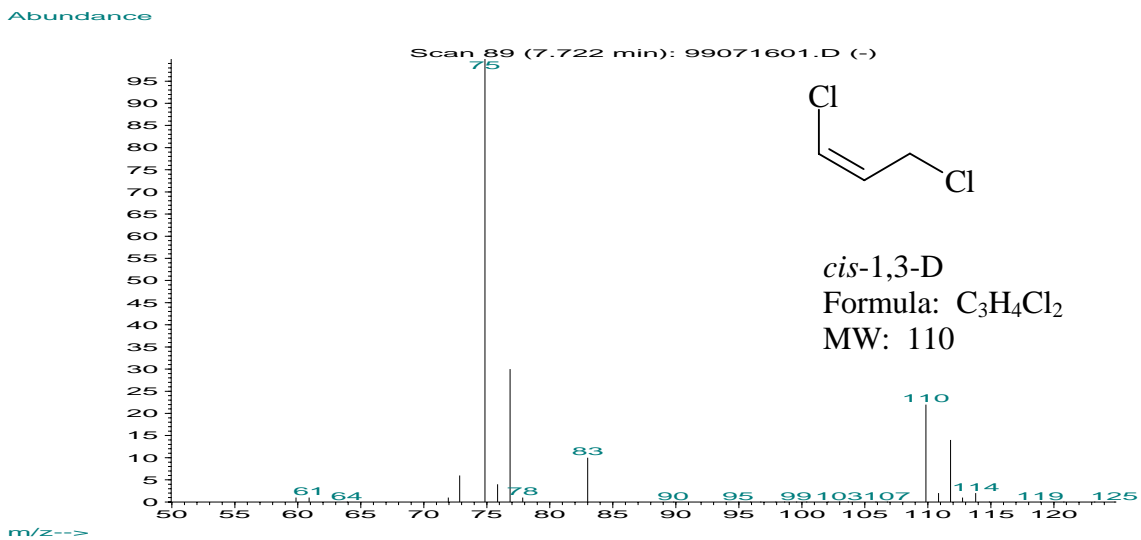


Figure 1. Mass Spectra for *cis*-1,3-D and *trans*-1,3-D

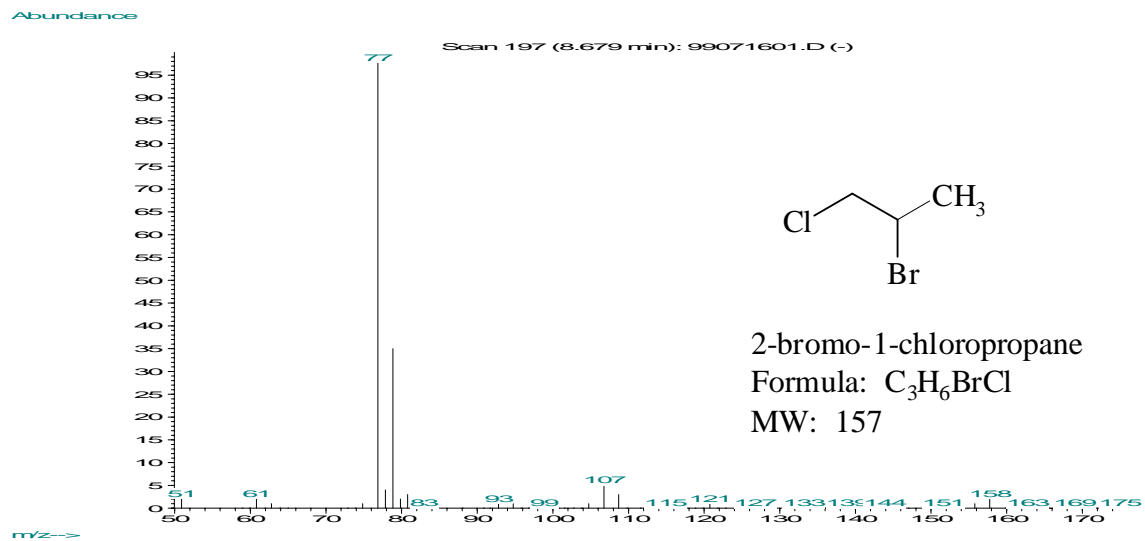
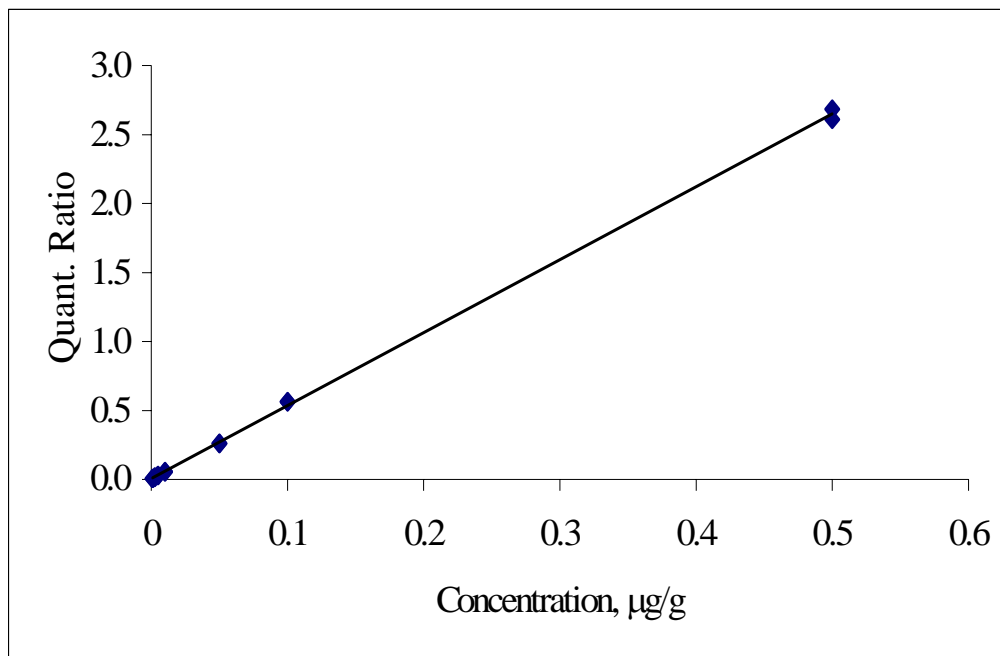


Figure 2. Mass Spectra for 2-bromo-1-chloropropane

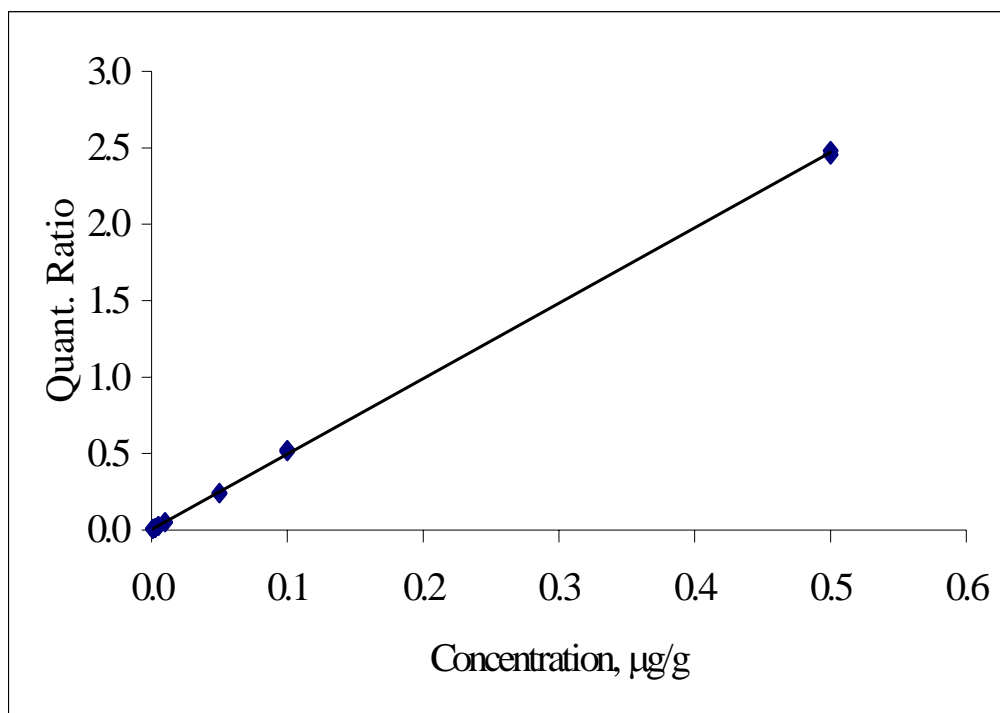


<i>cis</i> -1,3-D Concentration µg/mL	Equivalent Sample Conc. µg/g	<i>cis</i> -1,3-D/ISTD Quantitation Ratio <i>m/z</i> 75 / <i>m/z</i> 77	
		Start of Sequence	End of Sequence
0.0010	0.0010	0.0064	0.0064
0.0025	0.0025	0.0148	0.0158
0.0050	0.0050	0.0284	0.0275
0.010	0.010	0.0567	0.0528
0.050	0.050	0.259	0.262
0.10	0.10	0.561	0.564
0.50	0.50	2.610	2.683

Power Regression Equation: $X = (Y/5.0595)^{(1/0.9729)}$

Correlation Coefficient (r^2): 0.9995

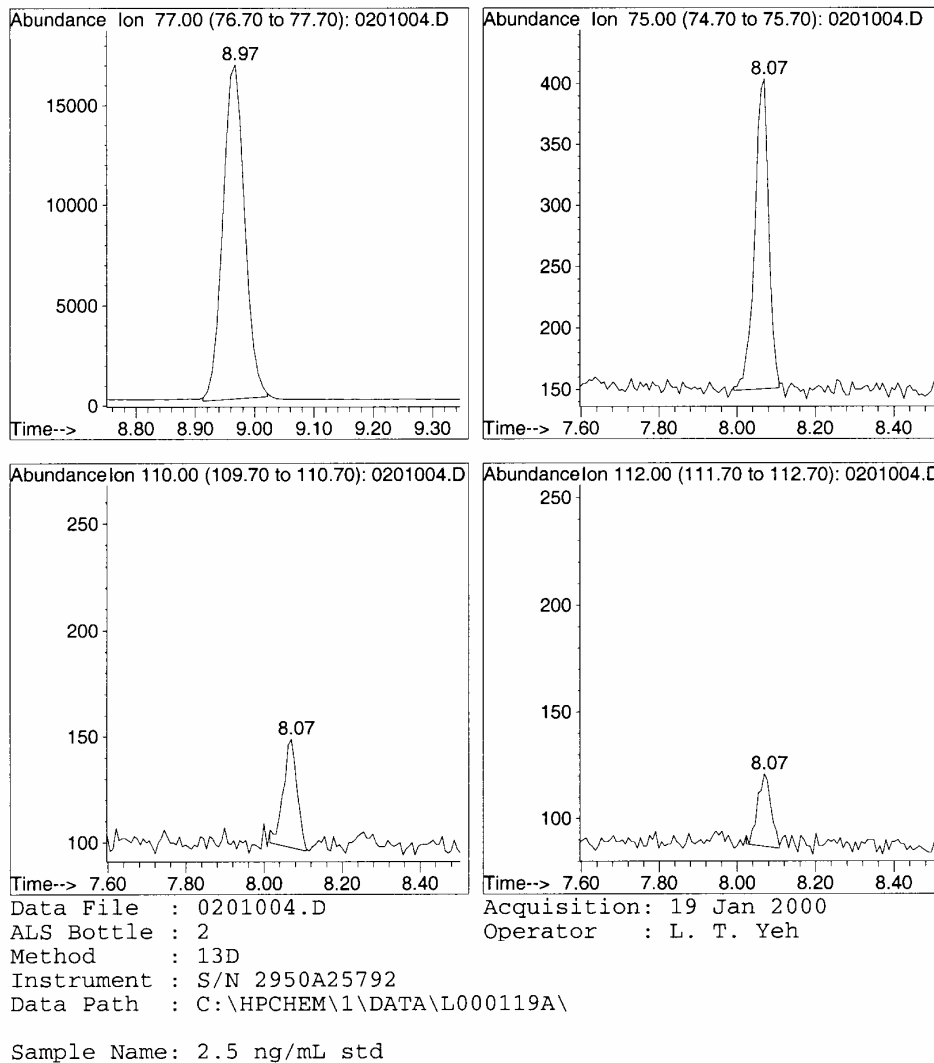
Figure 3. Typical Calibration Curve for the Determination of *cis*-1,3-D in Grapes



<i>trans</i> -1,3-D Concentration µg/mL	Equivalent Sample Conc. µg/g	<i>trans</i> -1,3-D /ISTD Quantitation Ratio <i>m/z</i> 75 / <i>m/z</i> 77	
		Start of Sequence	End of Sequence
0.0010	0.0010	0.0061	0.0067
0.0025	0.0025	0.0136	0.0143
0.0050	0.0050	0.0255	0.0258
0.010	0.010	0.0524	0.0501
0.050	0.050	0.244	0.240
0.10	0.10	0.515	0.523
0.50	0.50	2.454	2.480

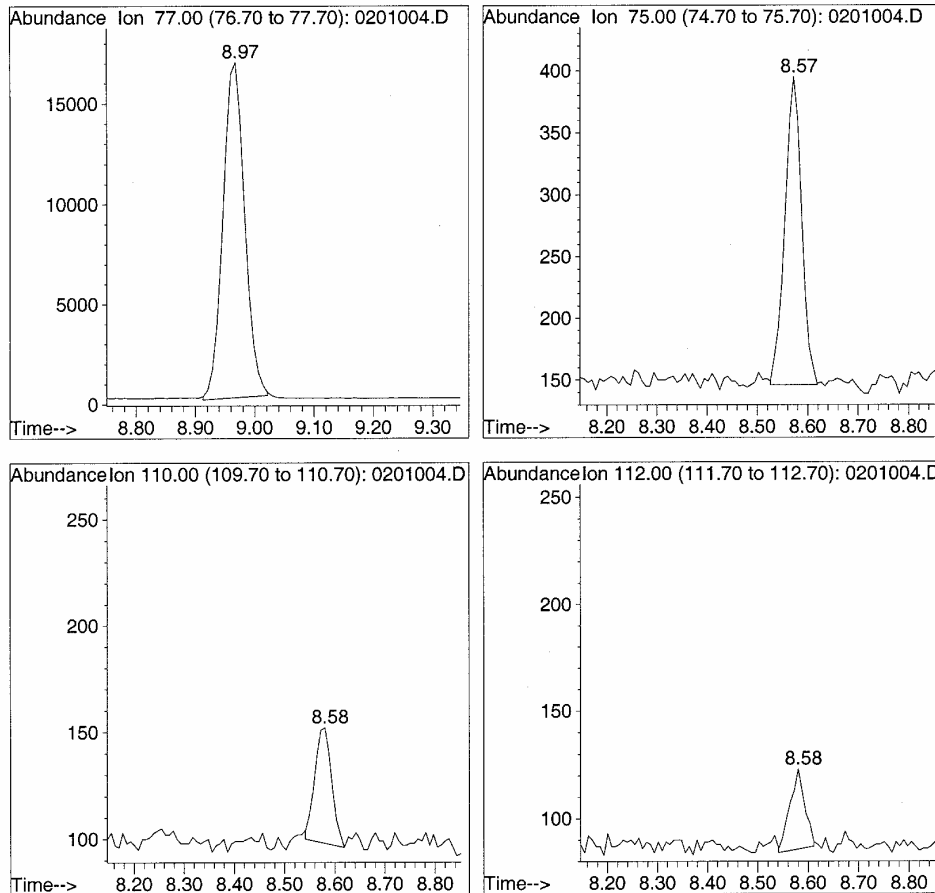
Power Regression Equation: $X = (Y/4.6361)^{(1/0.9681)}$
 Correlation Coefficient (r^2): 0.9991

Figure 4. Typical Calibration Curve for the Determination of *trans*-1,3-D in Grapes



Compound	Ion	Retention Time	Peak Area
ISTD	77	8.97	42480
cis-1,3-Dichloropropene	75	8.07	630
	110	8.07	124
	112	8.07	80

Figure 5. Typical Chromatogram of a 0.0025- μ g/mL *cis*-1,3-D Standard Equivalent to 0.0025 μ g/g of *cis*-1,3-D in Grapes

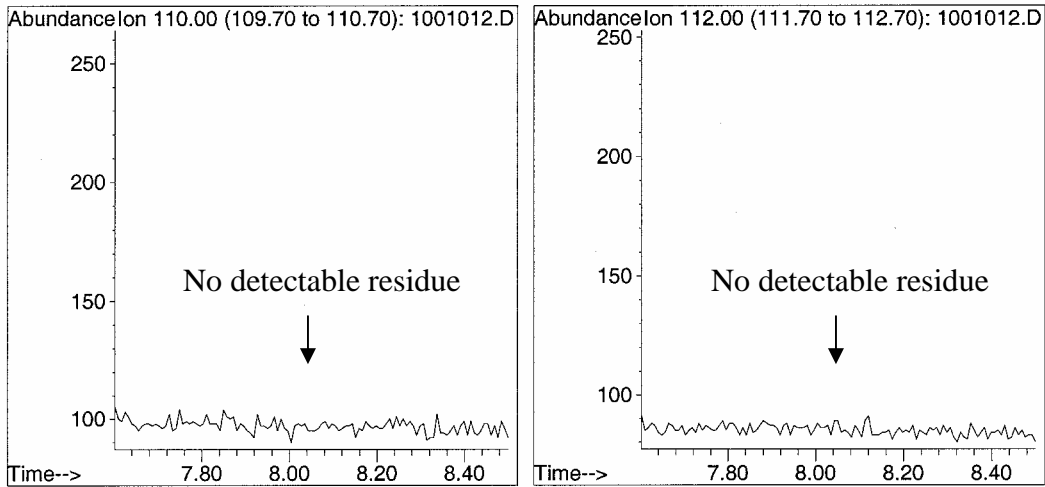
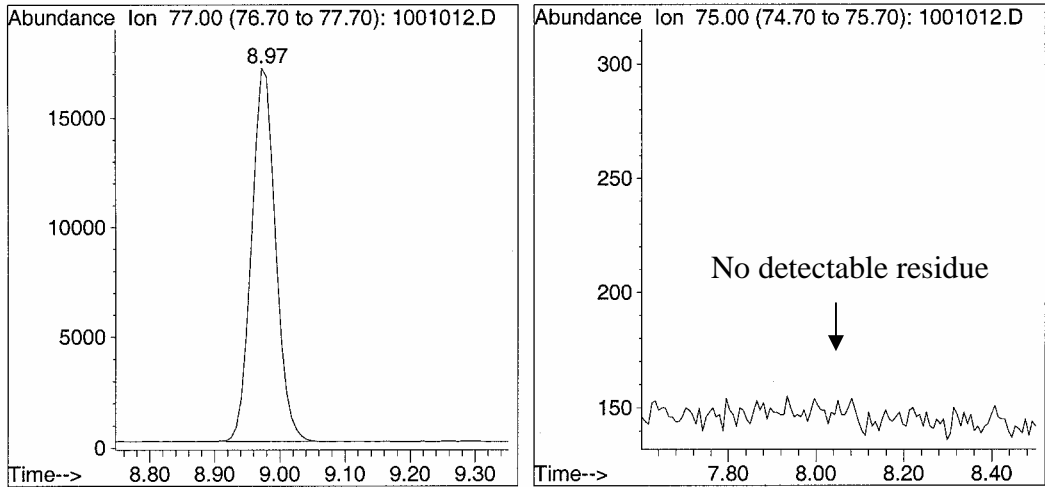


Data File : 0201004.D
 ALS Bottle : 2
 Method : 13D
 Instrument : S/N 2950A25792
 Data Path : C:\HPCHEM\1\DATA\L000119A\
 Acquisition: 19 Jan 2000
 Operator : L. T. Yeh

Sample Name: 2.5 ng/mL std

Compound	Ion	Retention Time	Peak Area
ISTD	77	8.97	42480
trans-1,3-Dichloropropene	75	8.57	576
	110	8.58	124
	112	8.58	80

Figure 6. Typical Chromatogram of a 0.0025- μ g/mL *trans*-1,3-D Standard Equivalent to 0.0025 μ g/g of *trans*-1,3-D in Grapes

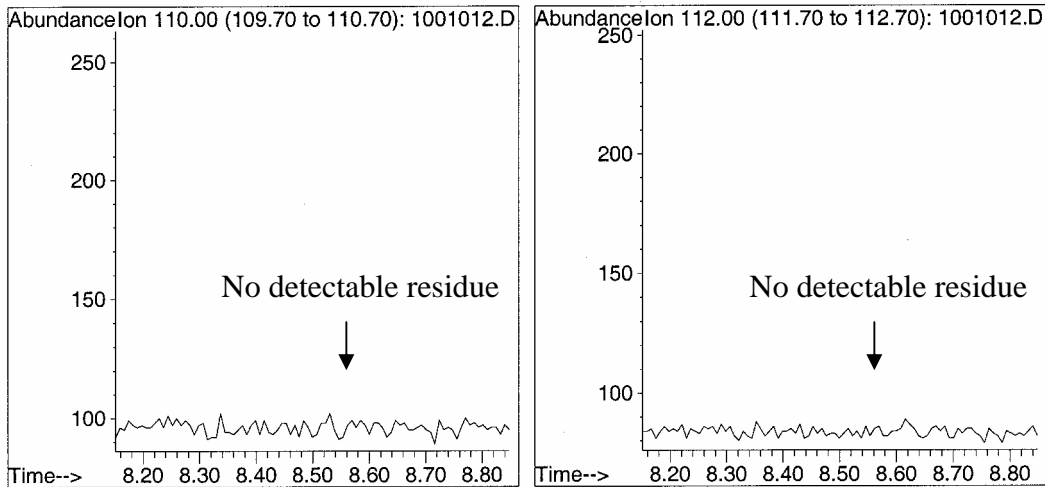
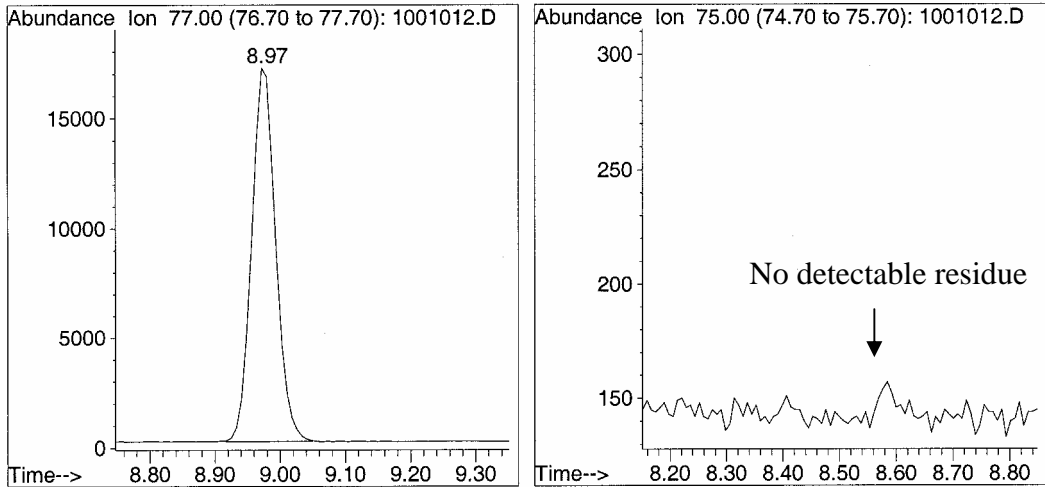


Data File : 1001012.D
 ALS Bottle : 10
 Method : 13D
 Instrument : S/N 2950A25792
 Data Path : C:\HPCHEM\1\DATA\L000119A\
 Acquisition: 19 Jan 2000
 Operator : L. T. Yeh

Sample Name: Control red grape

Compound	Ion	Retention Time	Peak Area
ISTD	77	8.97	43430
cis-1,3-Dichloropropene	75	Not Found	Not Found
	110	Not Found	Not Found
	112	Not Found	Not Found

Figure 7. Typical Chromatogram of Control Grapes Containing no Detectable Residue of cis-1,3-D

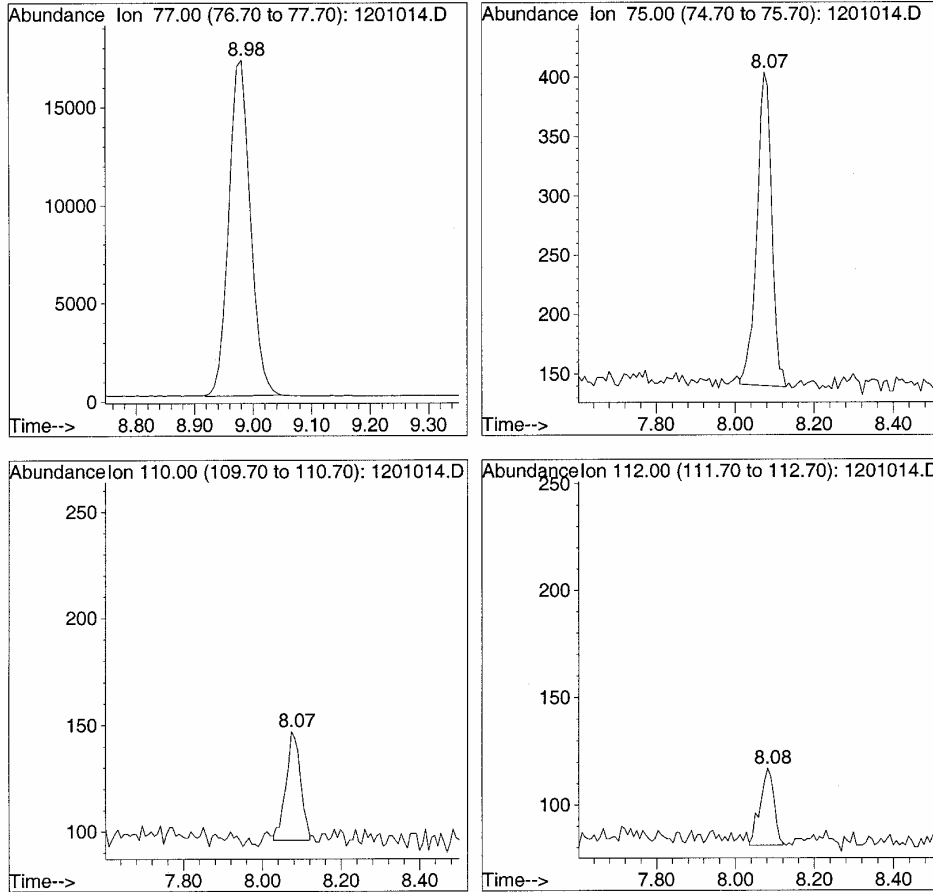


Data File : 1001012.D
 ALS Bottle : 10
 Method : 13D
 Instrument : S/N 2950A25792
 Data Path : C:\HPCHEM\1\DATA\L000119A\
 Acquisition: 19 Jan 2000
 Operator : L. T. Yeh

Sample Name: Control red grape

<u>Compound</u>	<u>Ion</u>	<u>Retention Time</u>	<u>Peak Area</u>
ISTD	77	8.97	43430
trans-1,3-Dichloropropene	75	Not Found	Not Found
	110	Not Found	Not Found
	112	Not Found	Not Found

Figure 8. Typical Chromatogram of Control Grapes Containing no Detectable Residue of trans-1,3-D

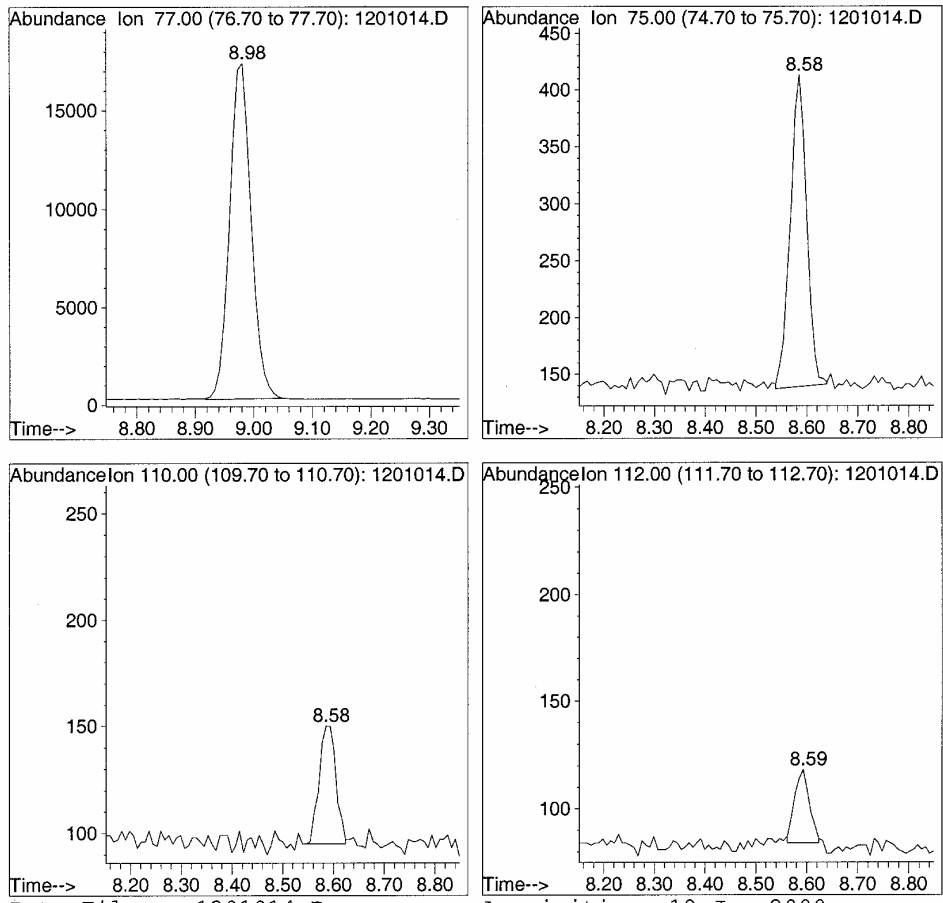


Data File : 1201014.D
 ALS Bottle : 12
 Method : 13D
 Instrument : S/N 2950A25792
 Data Path : C:\HPCHEM\1\DATA\L000119A\
 Acquisition: 19 Jan 2000
 Operator : L. T. Yeh

Sample Name: Control grape + 3.0 ng/g - 5

Compound	Ion	Retention Time	Peak Area
ISTD	77	8.98	43688
cis-1,3-Dichloropropene	75	8.07	685
	110	8.07	131
	112	8.08	88

Figure 9. Typical Chromatogram of Control Grapes Fortified at 0.0030 $\mu\text{g/g}$ (LOQ) with cis-1,3-D



Data File : 1201014.D
 ALS Bottle : 12
 Method : 13D
 Instrument : S/N 2950A25792
 Data Path : C:\HPCHEM\1\DATA\L000119A\
 Acquisition: 19 Jan 2000
 Operator : L. T. Yeh

Sample Name: Control grape + 3.0 ng/g - 5

Compound	Ion	Retention Time	Peak Area
ISTD	77	8.98	43688
trans-1,3-Dichloropropene	75	8.58	616
	110	8.58	127
	112	8.59	69

Figure 10. Typical Chromatogram of Control Grapes Fortified at 0.0030 µg/g (LOQ) with trans-1,3-D