US EPA ARCHIVE DOCUMENT

STUDY TITLE

Tolerance Enforcement Method for RH-117,281 in Grapes and Processed Fractions

DATA REQUIREMENT

Guideline 830.1340

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STUDY COMPLETED ON

September 24, 1998

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Rohm and Haas Technical Report No. 34-98-150

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10(D) (1) (a), (b), or (c).

Company: Rohm and Haas Company

Company Agent: Janet Ollinger Date: **November 30, 1998**

Signature: J Ollinger - Product Registration Mgr.

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GLP COMPLIANCE STATEMENT

As per 40 CFR 160.3, method development is not required to be conducted in compliance with Good Laboratory Practice regulations. However, the method was developed in the spirit of GLP as defined by US EPA in 40 CFR 160.

Recovery data from magnitude of the residue studies are included here. These studies were conducted in full compliance with GLP.

Method validation studies at MKL and ETL, and radiovalidation at Rohm and Haas Co. were conducted in full compliance with GLP, with the following exception:

1. Two different versions of the final approved protocol for radiovalidation were distributed. The error was detected prior to the experiment and the appropriate version was used by the principal investigator. The differences were mainly clarification items. This error did not result in any deviations.

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STUDY CERTIFICATION

This report is a true and accurate representation of the data collected.

9.24.98

E. L. Burdge Date
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QUALITY ASSURANCE STATEMENT

This report and the original raw data have been reviewed by the Quality Assurance Unit of the Rohm and Haas Company and has been validated as a true and accurate representation of the data collected.

QA activities for this study:

Date	Activity	QA Unit	Date Reported
method validation at ETL (protocol 34P-96-92)			
10/29/96	protocol review	RandH Co	10/29/96
11/21,25/96	in-process inspection	ETL	12/04/96
method validation at MKL (protocol 34P-96-93)			
10/29/96	protocol review	RandH Co	10/29/96
11/25/96	in-process inspection	MKL	01/14/97
12/02/96	raw data review	MKL	01/14/97
12/05/96	in-process inspection	MKL	01/14/97
02/05/98	final review of data package	MKL	02/05/98
radiovalidation at Rohm and Haas Co. (protocol 34P-97-15)			
4/21&7/10/76	protocol reviews	RandH Co	4/21&7/10/76
01/12/98	in-process inspection	RandH Co	01/12/98
final report (TR34-98-150)			
09/21/98	final report audit	RandH Co	09/21/98

Ronda S. Krick 9/24/98

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Rohm and Haas Company

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34P-96-92 Protocol for Preliminary Grape Method Validation Using GC/MSD (Enviro-Test Laboratories)

34P-96-93 Protocol for Preliminary Grape Method Validation Using GC/ECD (McKenzie Laboratories)

34P-97-15 Radiovalidation of Preliminary Residue Analytical Method forParent RH-117,281 in Grapes (TR 34-96-128)

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1. Summary

This report describes the tolerance enforcement method for analyzing RH-117,281 in grape raw agricultural commodity (RAC) and the processed fractions, grape juice and raisin. This report is the compilation of three previously issued preliminary residue methods (TR 34-96-128, TR 34-97-136, and TR 34-97-164).

RH-117,281 residues in grape are extracted by chopping and liquid-liquid extraction. The extracts are cleaned-up using carbon solid phase extraction (SPE) and alumina-B SPE. RH-117,281 residues in grape juice are extracted by liquid-liquid extraction and the extracts are cleaned-up using alumina B SPE. RH-117,281 residues in raisin are extracted by chopping and matrix solid phase dispersion with Bondesil ODS silica. The extracts are cleaned-up using Florisil column chromatography.

Quantitation of RH-117,281 was performed by gas-liquid chromatography using electron capture detection (GC/ECD). Residues were confirmed using gas-liquid chromatography with mass selective detection (GC/MSD). The limit of quantitation (LOQ) is 0.01 ppm, established by actual fortifications at this level. The limit of detection (LOD) is 0.003 ppm.

The overall method recoveries are summarized in the following table. The grape method was successfully radiovalidated using ^{14}C field aged residues.

Matrix	Recovery (%) GC/ECD
Grape[a]	101±13.5
Grape Juice[a]	90.4±12.5
Raisin[b]	83.8±10.5

[a] Overall recovery data includes recovery results at the LOQ.
[b] Overall recovery data does not include recovery results at the LOQ (See section 9 page 24)

2. Introduction

RH-117,281 is a fungicide recently developed at Rohm and Haas Company and targeted for use on a variety of crops. In order to file for the commercial registration, an analytical residue method is required to enable enforcement of the tolerance of RH-117,281 on grape and processed fractions.

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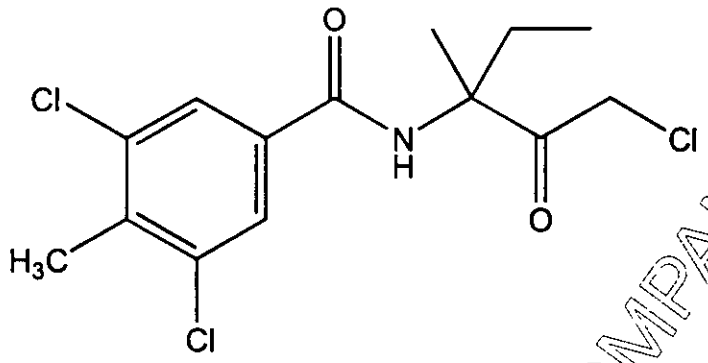
3. Experimental Compound

The structure of RH-117,281 is shown below:



CA Name:	3,5-dichloro-N-(3-chloro-1-ethyl-1-methyl-2-oxopropyl)-4-methylbenzamide
Proposed Generic Name:	zoxamide
CAS Number:	156052-68-5

R&H I.D.*	Lot #	Purity	Appearance	Expiration Date
RH-117,281	ELM1157	97.9%	White Solid	Nov. 11, 2001

*The full R&H number is RH-117,821. The designation typically used in studies is the four digit abbreviation: RH-7281.

4. Chemicals and Supplies

Chemicals	Grade	Supplier*
Alumina B cartridges	LC-Alumina B® (6 ml)	Supelco (cat # 5-7085)
Basic Aluminum oxide	Alumina B	JT Baker
Bondesil® ODS Silica	Analytical	Varian (cat # 1221-3012)
Carbon cartridges	Envi-Carb® (6 ml)	Supelco (cat # 5-7094)
Dichloromethane	HPLC	JT Baker

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Dichloromethane	HPLC	JT Baker
Ethyl Acetate	HPLC	JT Baker
Florisil®	60-100 mesh	JT Baker (cat # 3372-07)
Hexane	HPLC	JT Baker
Methanol	HPLC	JT Baker
Petroleum ether	Optima®	Fisher Scientific
Sodium chloride (NaCl)	ACS	JT Baker
Sodium sulfate (Na_2SO_4)	Analytical, anhydrous	Fisher Scientific
Water	Milli-Q®	Milli-Q Water Purification System

* Other manufacturer brands may be substituted if shown to be suitable.

Prepared Solutions and Chromatography

1.) Aqueous sodium chloride solutions:
   a) 10% (w/v) NaCl was prepared by dissolving 100 g NaCl in 1000 ml Milli-Q water in a 1000 ml flat bottom flask.
   b) 0.1 N NaCl was prepared by dissolving 5.85 g NaCl in a final volume of 1000 ml Milli-Q water in a 1000 ml flat bottom flask.

2.) Aqueous methanol solution
   80/20 (v/v) methanol/water solution was prepared by mixing 800 ml methanol and 200 ml Milli-Q water in a 1000 ml flat bottom flask

3.) Ethyl Acetate/Hexane solutions:

Ethyl Acetate (ml)	Hexane (ml)	Final Proportion of Ethyl Acetate/Hexane
30	970	3/97
100	900	10/90
150	850	15/85
300	700	30/70
400	600	40/60

Note: all solutions were prepared in a 1000 ml flat bottom flask using graduated cylinders to measure volumes.

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4). Preparation of Deactivated Florisil

About 100 g of Florisil was placed on an aluminum tray and baked in an oven at 150°C for four hours. The activated Florisil was transferred to a glass bottle, capped, and cooled to room temperature. Five ml of water was added to the activated Florisil, resulting in 5% (v/w) water . The deactivated Florisil was homogenized on a shaker for two hours. The material was stored in a sealed dessicator prior to use.

5. Equipment

Equipment	Description	Source*
Blender	Model 34BL97	Waring
Bottle, glass	2000 ml	EMSCO
Buchner Funnel	200 ml	Coors
Cotton	Non-absorbent	Johnson & Johnson
Dessicator	Dry Keeper®	Sanplatec
Filter Paper	#1, #3, #4	Whatman
Filter Flask	500 ml	Kimax
Flat Bottom Flask, 24/40 ST	1000 ml	Fisher Scientific
Food Chopper	Model 8181	Hobart
Glass Chromatography Column	25 cm L x 11mm I.D.	Ace Glass
Glass Mortar and Pestle	4 oz.	Pyrex
Glass Vials	25 ml	Fisher Scientific
Glass Wool		VWR Scientific
Graduated Cylinder	25, 100, 250, 500, 1000 ml	Fisher Scientific
Polypropylene Centrifuge Bottle	250 ml	Fisher Scientific
Rotary Evaporator (Rotovap)	Model R114	Brinkman
Round Bottom Flask, 24/40 ST	250, 300, 500 ml	Fisher Scientific
Separatory Funnel	500 ml	Fisher Scientific
Shaker	Gyratory	Arthur Thomas Co.
Sieve, USA Std.	#16 (14 mesh)	WS Tyler
Sonicator	Model 2210	Branson
SPE Extraction Tube w/ Frit	60 ml	Supelco
SPE Manifold	Visiprep®	Supelco
Volumetric Flask	100, 500 ml	Kimax

Standard laboratory equipment, balance, beakers, *etc.*

*Other manufacturer brands may be substituted if shown to be suitable.

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6 Methods

6.1 Method for Grape RAC

6.1.1 Method Flow Diagram

Grape Sample (10 g)
↓
Extraction (80% MeOH/Water)
↓
Liquid-Liquid Partition (Ethyl Acetate/Water)
↓
Carbon Solid Phase Extraction
↓
Alumina B Solid Phase Extraction
↓
GC/ECD Quantitation

6.1.2 Sample Preparation

Process grapes using a Hobart food chopper with dry ice. Allow the dry ice to sublime overnight in a freezer. Store the processed samples in a freezer.

6.1.3 Sample Extraction

Weigh 10 g of processed grape sample in a 250 ml polypropylene centrifuge bottle. At this time, sample can be fortified by spiking with a known amount of RH-7281. If so, allow any solvent to evaporate in a fume hood for ~ one hour until the sample is free of solvent.

Add 80 ml of 80/20 MeOH/water to the centrifuge bottle and transfer the slurry to a blender. Rinse the bottle with an additional 20 ml of the extraction solution and add the rinsate to the blender. Blend the slurry for three minutes at a medium speed. Filter the slurry through a Buchner funnel mounted with #3 or #4 Whatman filter paper into a 500 ml filtration flask. Rinse the blender and the filter cake with another 50 ml of methanol. Combine the filtrates (about 150 ml) in a 500 ml 24/40 ST round bottom flask and concentrate to approximately 30 ml on a rotary evaporator (rotovap) at ~40 °C under diminished pressure (~50 mm Hg).

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6.1.4 Liquid-Liquid Partition

Add the extract to a 500 ml separatory funnel containing 200 ml of 0.1 N NaCl solution and 100 ml of dichloromethane. Shake the funnel with frequent venting for 1 minute and allow the two phases to separate. Transfer the organic phase (lower layer) to a 24/40 ST 500 ml round bottom flask and partition the aqueous phase with another 100 ml of dichloromethane in the same manner. Discard the aqueous phase. Combine both organic layers in the same 500 ml flask and concentrate to dryness on a rotovap at ~40°C under diminished pressure (~50 mm Hg). Dissolve the residue with 20 ml of hexane and sonicate the sample for 10 seconds.

6.1.5 Carbon Solid Phase Extraction

Insert carbon cartridges into a solid phase extraction manifold. Top the carbon cartridge with ~1.0 cm of anhydrous sodium sulfate and rinse the cartridge with 10 ml of hexane. Discard the effluent. Apply the 20 ml of residue solution in the 500 ml flask to the cartridge. Rinse the flask with 20 ml of hexane twice and apply the rinsates to the column. Collect the eluate (~60 total volume) in a 250 ml flask. (RH-7281 is eluted from the column with hexane).

6.1.6 Alumina-B Solid Phase Extraction (See Note on Page 17 for the Elution Profile Check)

Insert Alumina-B cartridges into a solid phase extraction manifold. Wash the cartridge with 5 ml of ethyl acetate and then 5 ml of hexane. Apply the hexane solution containing the residue from section 6.1.5 to the cartridge. Rinse the flask twice with 10 ml of hexane each and apply the rinsates to the cartridge. Wash the loaded cartridge with 25 ml each of petroleum ether and 25 ml of 3/97 (v/v) ethyl acetate/hexane solution. Discard the effluent at this stage. Elute RH-7281 from the cartridge with 25 ml 15/85 (v/v) ethyl acetate/hexane solution into a 250 ml 24/40 ST round bottom flask. Concentrate the eluate to dryness on a rotovap at about 40°C under diminished pressure (~50 mm Hg). Dissolve the residue in 10 ml of hexane and sonicate it for 10 seconds. The sample is now ready for quantitation (section 7).

6.1.7 Time to Run a Sample Set

The time required to run one sample set of eight samples through the entire method is approximately 16 hours including GC analysis.

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6.2 Method for Grape Juice

6.2.1 Method Flow Diagram

Grape Juice Sample (10 g)
↓
Liquid-Liquid Partition (Ethyl Acetate/NaCl Solution)
↓
Alumina-B Column Chromatography
↓
GC/ECD Quantitation

6.2.2 Sample Preparation

Grape juice is used directly without processing.

6.2.3 Extraction and Liquid-Liquid Partition

Measure out 10 g (~10ml) of grape juice into a 25 ml glass vial. Spike samples with RH-7281 at this point, if desired. Mix the fortified sample by manually shaking for a few seconds. Transfer the sample to a 500 ml separatory funnel. Add 150 ml of 10% (w/v) NaCl solution then add 75 ml of ethyl acetate. Rinse the vial three times with 25 ml of ethyl acetate and add to the funnel. Shake the funnel with frequent venting for one minute and allow the two phases to separate. Transfer the organic phase (top layer) to a 500 ml 24/40 ST round bottom flask and the aqueous phase to another separatory funnel. Extract the aqueous layer with another 75 ml of ethyl acetate in the same manner. Discard the aqueous phase. Combine both organic layers in the same 500 ml 24/40 ST round bottom flask and concentrate to dryness on a rotovap at ~ 40°C under diminished pressure (~ 50 mm Hg). Dissolve the residue in 60 ml of hexane and sonicate it for one minute.

6.2.4 Alumina-B Column Chromatography (See Note on Page 17 for the Elution Profile Check)

Place a piece of glass wool or cotton in a glass chromatography column (25 cm L x 11 mm I.D). Dry pack the column by adding 15 ml (~ 6 cm in height) of Alumina-B powder while tapping the column to ensure good packing. Add 1 cm of anhydrous sodium sulfate to the top of the packing. Wash the column with 25 ml of hexane. Apply hexane solution containing the residue sample (from section 6.2.3) to the column. Rinse the round bottom flask with 20 ml of hexane and apply it to the column when the solution

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level is ~ 1 mm from the top of the packing. Do not allow the column to run dry. Wash the column with 25 ml of petroleum ether and then 100 ml of 10/90 (v/v) ethyl acetate/hexane. Discard all effluents.

Apply 150 ml of 40/60 (v/v) ethyl acetate/hexane to the column and collect the final eluate in a 300 ml 24/40 ST round bottom flask. Concentrate the sample to complete dryness at 40°C and diminished pressure (~50 mm of Hg). Dissolve the residue in 10 ml of ethyl acetate and sonicate it for 10 seconds. The sample is now ready for quantitation (section 7).

6.2.5 Time to Run a Sample Set

The time required to run one sample set of eight samples through the entire method is approximately 14 hours including GC analysis.

6.3 Method for Raisin

6.3.1 Method Flow Diagram

Raisin Sample (5 g)
↓
Matrix Solid Phase Dispersion Extraction
↓
Florisil Column Chromatography
↓
GC/ECD Quantitation

6.3.2 Sample Preparation

Homogenize the raisins in a Hobart food chopper with dry ice. Sublime the dry ice from the sample in a freezer overnight and store the processed sample in a freezer.

6.3.3 Matrix Solid Phase Dispersion Extraction (MSPD)

Measure out 5 g of frozen raisin homogenate into a glass mortar. Spike samples with RH-7281 at this point if desired. Add 10g of Bondesil ODS silica packing. Thoroughly grind the sample with the glass pestle for approximately two minutes. Place the samples in a fume hood for eight hours to allow the sample to air dry. Use a pestle to loosen the dried powder from the mortar. Grind the powder until it is uniform. Pass the powder

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through a 14 mesh sieve onto a large circle of filter paper and pour the powder into an SPE tube with a polyethylene frit in the bottom. Add the solid particulate that remained in the sieve to the column. Tap the tube lightly, then lightly pack the column with a glass syringe plunger. Place a second polyethylene frit on top of the powder.

Wash the mortar and pestle with 5 ml of hexane and add this wash to the tube. Allow the tube to sit for ~ 5 minutes to allow the solvent to evaporate. Place the tubes on the Visiprep SPE manifold unit and wash the column with 25 ml of hexane, discarding the effluent. Add 50 ml of 10/90 (v/v) ethyl acetate/hexane to the tube and collect the eluate. At the end of the elution, turn on the vacuum slightly to drain all the eluate from the column. Place the eluate in a 250 ml 24/40 ST round bottom flask and concentrate it to dryness on a rotovap at 40°C under diminished pressure (~ 50 mm Hg). Dissolve the residue in 10 ml of hexane and sonicate for one minute.

6.3.4 Florisil Column Chromatography (See Note below for the Elution Profile Check)

Wet pack a glass column, 25 cm x 11 mm I.D. by placing a piece of cotton or glass wool in the bottom of the column and add 25 ml of hexane to the closed column. After this, slowly add 15 ml of 5% deactivated Florisil (~ 15 cm in height), while tapping the column to ensure good packing. Add 1 cm of sodium sulfate to the top of the column. Apply the residue sample to the column. Wash the round bottom flask with 20 ml of hexane and apply this to the column. Wash the column with 25 ml of 10% ethyl acetate/ hexane, discarding all effluents to this point.

Apply 50 ml of 30/70 ethyl acetate /hexane and collect the eluate in a 250 ml 24/40 ST round bottom flask. Concentrate the eluate to complete dryness using a rotovap at 40°C under diminished pressure (~ 50 mm Hg). Dissolve the residue in 10 ml ethyl acetate and sonicate it for 10 seconds. The sample is now ready for quantitation (section 7).

6.3.5 Time to Run a Sample Set

The time required to run one sample set of eight samples through the entire method is approximately 20 hours including GC analysis.

Note: Elution Profile Check

The elution profile should be checked for each new lot of Alumina-B, Bondesil, Carbon or Florisil to minimize the effect of lot to lot variability. A general procedure is described as follows:

1). Add 10 ml of 0 10 µg/ml RH-7281 standard to the column or cartridge.

2). Follow the elution schemes as outlined for each individual procedure (see 6 1 5, 6.1.6, 6.2 4, 6.3.3 or 6.3 4) and collect the pre-elution cut as well as the target elution cut

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3) Wash the column with 30 ml of ethyl acetate and collect the post-elution cut.

4). Take the pre-elution, the target, and the post-elution cuts each to dryness and redissolve each of the cuts in 10 ml of ethyl acetate. Inject them as outlined for GC/ECD quantitation (7.1).

5) If the target cut contains a minimum of 85% of RH-7281, the lot can be considered acceptable

6). If the 85% recovery criteria for standards is not met, the following adjustment in the elution scheme should be made:

a) For cases where the pre-wash cut contains a significant amount of standard, the percentage of ethyl acetate in either the pre-wash or eluting solvent can be decreased

b) For cases where the post-wash cut contains significant standard, the target elution volume may be increased or the percentage of ethyl acetate in the eluting solvent can be increased.

7 Quantitation

The primary quantitation method was gas chromatography with electron capture detection (GC/ECD). The confirmatory method was gas chromatography with mass selective detection (GC/MSD).

7.1 Instrumentation and conditions

7.1.1 Instrumentation for Primary Quantitation (GC/ECD)

Varian Model 3500

Injector:	Varian Model 8100 Auto-Sampler	
	On-column mode	
	Guard column:	Rtx-1 (0.53 mm I.D. x 1 m; 0.25µm film)
		Restek (catalogue # 10122)
	Injection temp.:	150°C
	Injection volume:	1.0 µl
Column:	Rtx-1 (0.25mm I.D. x 15 m; 0.25µm film)	
	Restek (cat. # 10120)	
	Carrier gas:	Nitrogen
	Head Pressure:	6.0 psi
	Flow rate:	0.7 ml/min.

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Detector temp:300°C

Oven temperature program:
Initial temp. = 90 °C, hold for 1 min.
Ramp at 20 °C/min.,
Final temp. = 250 °C, hold for 3 min.

Typical Retention Time for RH-7281: about 11 min.

7.1.2 Instrumentation for Confirmatory Quantitation (GC/MSD)

GC/MSD Instrument: Hewlett Packard Model HP5890/5972

Injector: Hewlett Packard Model HP18593 auto-sampler
On-column mode
Guard column: Rtx-1 (0.53mm I.D. x 1.0 m; 0.25µm film)
Restek (cat. # 10122)
Injection temp.: 150 °C
Injection volume: 2.0 µl

Column: Rtx-1 (0.25mm I.D. x 30 m; 0.25 µm film)
Restek (cat # 10123)
Carrier gas: Helium
Head Pressure: 12 psi
Flow rate: 1.0 ml/min.

Detector temperature: 300 °C

Oven temperature program:
Initial temp. = 90 °C, hold for 1 min.
Ramp at 20 °C/min.,
Final temp. = 250 °C, hold for 3 min.

Monitored ions: m/z 187, 189, and 258

Typical Retention Time for RH-7281: about 10 min.

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7.2 Preparation of Standard Curves

Prepare a 100 µg/ml standard stock solution by weighing 10.0 mg of RH-7281 into a 100 ml volumetric flask. Bring up to volume with ethyl acetate.

Prepare a 1.0 µg/ml stock solution by pipetting 1.0 ml of 100 µg/ml stock solution into a 100 ml volumetric flask and bringing to volume with ethyl acetate.

Prepare standard solutions of 0.010, 0.020, 0.050, 0.10, and 0.50 µg/ml by diluting the 1.0 µg/ml stock solution with ethyl acetate as shown below.

Concentration	Amount Taken	Dilution To
0.5 µg/ml	50 ml of 1.0 µg/ml	100 ml
0.10 µg/ml	10 ml of 1.0 µg/ml	100 ml
0.05 µg/ml	5 ml of 1.0 µg/ml	100 ml
0.02 µg/ml	20 ml of 0.1 µg/ml	100 ml
0.01 µg/ml	10 ml of 0.1 µg/ml	100 ml

The standards should be remade every six months as a precautionary measure to ensure reliability. The standards should be stored in the refrigerator. If there is major variability in the standards, they should be remade.

A minimum of four standard solutions are prepared in a desired concentration range and injected in duplicate for GC/ECD or GC/MSD quantitation. Standards are preferably quantitated by peak area, although height may be used. Linear regression is used to fit the instrument response (Y) to concentration (X) using the equation: $Y = mX + b$. A quadratic regression can also be used to fit the instrument response (Y) to concentration (X) using the equation: $Y = aX^2 + bX + c$. The concentration of RH-7281 in grape, juice, and raisin is then determined from the standard curve.

7.3 Fortification Recoveries

Percent recovery is calculated by measuring the peak area or peak height and calculating the µg/ml found from the standard curve, as shown in Equation 1:

$$\frac{\text{Residue Found (µg/ml)} \times \text{Final Vol. (ml)}}{\text{Fortification Amount Added (µg)}} \times 100\% = \%\ \text{Recovery} \quad \text{(Eq. 1)}$$

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7.4 Sample Analysis

If necessary, the samples are diluted to an appropriate volume to give a final concentration within the standard curve range. The residue concentration is determined as follows:

$$\frac{[\text{Residue Found } (\mu g/ml) \times \text{Final Vol. } (ml)]}{\text{Sample Weight } (g)} = \mu g/g = \text{ppm} \qquad \text{(Eq. 2)}$$

7.5 Sample Calculations

Grape: A typical calculation for the recovery of 0.050 ppm (µg/g) fortification of grape (Table 7 and Figure 12) is demonstrated as follows: the peak area of RH-7281 is 55.62, which, from the standard calibration curve, corresponds to 0.0400 µg/ml. For a 10 g sample fortified with 0.5 µg RH-7281 and with a final sample volume of 10 ml, recovery is calculated as in Eq. 3.

$$\frac{[0.0400\ \mu g/ml \times 10ml]}{0.50\mu g} \times 100\% = 80.0\ \% \qquad \text{(Eq. 3)}$$

Grape Juice: A typical calculation for the recovery of 0.050 ppm (µg/g) fortification of white grape juice (Table 8 and Figure 20) is demonstrated as follows: the peak area of RH-7281 is 53.001, which, from the standard calibration curve, corresponds to 0.0488 µg/ml. For a 10 g sample fortified with 0.5 µg RH-7281 and with a final sample volume of 10 ml, recovery is calculated as in Eq. 4.

$$\frac{[0.0488\mu g/ml \times 10ml]}{0.5\mu g} \times 100\% = 97.6\ \% \qquad \text{(Eq. 4)}$$

Raisin: A typical calculation for the recovery of 0.050 ppm (µg/g) fortification of raisin (Table 8 and Figure 28) is demonstrated as follows: the peak area of RH-7281 is 89.858, which, from the standard calibration curve, corresponds to 0.0452 µg/ml. For a 10 g sample fortified with 0.5 µg RH-7281 and with a final sample volume of 10 ml, recovery is calculated as in Eq. 5.

$$\frac{[0.0452\mu g/ml \times 10ml]}{0.5\mu g} \times 100\% = 90.4\ \% \qquad \text{(Eq. 5)}$$

Treated Samples: A typical calculation for determining the quantity of RH-7281 residue in a field-treated sampe of grape RAC (Figure 13) is demonstrated as follows: the peak

area of RH-7281 is 75958.1 which, from the standard calibration curve and accounting for the 5x dilution, corresponds to 0.312557 µg/ml. For a 10 g grape sample and a 10 ml final sample volume, the concentration of RH-7281 residues is calculated as in Eq 6.

$$\frac{[0.313\ \mu g/ml] \times 10\ ml}{10\ g} = 0.313\ \mu g/g = \text{ppm} \qquad \text{(Eq. 6)}$$

8 ^{14}C Radiovalidation

Samples from the ^{14}C-RH-7281 grape metabolism study (TR 34-98-49) identified as RAC: 95-0082-07 (control sample) and RAC: 95-0082-12 (treated sample) were analyzed using the tolerance analytical method for parent RH-7281 in grapes (TR 34-96-128) as described in radiovalidation protocol 34P-97-15. The radiovalidation procedure validated the ability of the tolerance method to measure RH-7281 in field aged residues by comparing the amount of RH-7281 detected by the analytical method to the amount of RH-7281 found in grapes from a metabolism study.

Briefly, two replicates of the control samples (RAC: 95-0082-07) and 10 replicates of the treated samples (RAC: 95-0082-12) were combusted and the radioactivity of the starting sample was determined. The treated sample in triplicates were then processed using the preliminary grape residue analytical method. Aliquots of the extracts and sub-samples of the filter cake were also taken for counting/combustion in order to determine the extraction efficiency. The radioactivity of the final extract was counted and the concentration of RH-7281 was determined by GC/ECD.

Radiovalidation of the analytical method using grape RAC matrix demonstrated that the residue of RH-7281 present in a sample from a grape metabolism study was adequately measured. The results from the radiovalidation study are presented in Tables 1-3.

The average extraction efficiency was 99.0 % (Table 1) and the average concentration of RH-7281 recovered was 0.296 ppm (Table 2). The total radioactive residues (TRR) found using the tolerance method (0.534 ppm; Table 3) is 72.7 % of the average TRR value of 0.735 ppm obtained from the grape metabolism study. The average amount of RH-7281 found using the tolerance method (0.296 ppm) was 69.0 % of the value (0.429 ppm; Table 3) obtained in the metabolism study. The reason for these discrepancies is attributed to the fact that different amounts of radioactivity were present in the initial grape samples because, in both the grape metabolism and radiovalidation studies, the amounts of RH-7281 recovered represented similar fractions of the TRR (58.4% vs 55.4%, respectively; Table 3). Consequently, the amounts of RH-7281 found in the radiovalidation study were divided by 72.7 % to adjust for the different TRR values. The results summarized in the following table show that the accountability of the tolerance enforcement method is 95.1 %.

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	RH-7281 Found in:		
	Metabolism Study (ppm)	Radiovalidation (ppm)	Accountability (%)
Mean	0.429	0.408	95.1
Std. Deviation		0.060	14.1
Number		3	3

9 Results and Discussion

Both primary (GC/ECD) and confirmatory (GC/MSD) quantitation procedures have acceptable sensitivity for RH-7281. Representative chromatograms are shown in Figures 1-29 (GC/ECD) and in Figures 30-44 (GC/MSD). The limit of quantitation (LOQ) for grape and processed grape matrices is established at 0.01 ppm for both GC/ECD and GC/MSD by analysis of fortifications at that target level (Tables 4-6). The limit of detection is 0.003 ppm. Using the data taken from Tables 4-9, the overall method recoveries of RH-7281 from the 3 matrices using both GC/ECD and GC/MSD quantitation is summarized in the following table.

Matrix	Statistic	Recovery (%) GC/ECD	Recovery (%) GC/MSD
Grape[a]	Mean	101	92.6
	Standard Deviation	13.5	15.6
	Number of Samples	40	64
	Minimum Recovery	74.9	63.2
	Maximum Recovery	133	126
Grape Juice[a]	Mean	90.4	91.0
	Standard Deviation	12.5	16.8
	Number of Samples	36	34
	Minimum Recovery	69.7	60.0
	Maximum Recovery	125	132
Raisin[b]	Mean	83.8	82.2
	Standard Deviation	10.5	11.4
	Number of Samples	24	26
	Minimum Recovery	62.5	55.1
	Maximum Recovery	106	96.5

[a] Overall recovery data includes recovery results at the LOQ.
[b] Overall recovery data does not include recovery results at the LOQ

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The mean and standard deviation values for recovery at the LOQ of 0.01 ppm for the grape and grape juice matrices were similar to the mean and standard deviation values for all fortification level recoveries and, thus, were included in calculating the overall method recoveries. However, LOQ fortifications of RH-7281 in raisin gave a higher mean and standard deviation compared to fortification level recoveries and, consequently, were not used in calculating the overall method recovery for this matrix. Not using the LOQ fortification in calculating overall method recovery is not uncommon because these more variable data tend to unnecessarily skew the overall recovery.

There was no interference found in the grape and grape juice controls at the same retention time as RH-7281 using either GC/ECD or GC/MSD (Figures 7-9, 15-17, 36, and 39). There was no interference found in the raisin controls using GC/MSD; however, there was a peak shoulder that appeared in some of the GC/ECD runs. The presence of this peak shoulder was attributed to column contamination because it appeared in both standards, samples, and washes. The peak shoulder did not appear after the Rtx-1 column was replaced. The raisin control samples for GC/ECD and GC/MSD are shown in Figures 24, 25, 26 and 41.

The GC/ECD and GC/MSD inlet system, including the insert and guard column, must be cleaned and conditioned in order to minimize degradation of RH-7281. A temperature program, for example, increasing injection temperature to 290 °C (50°C/min.) after the elution of the parent peak, was found effective in removing residues that remained in the inlet system. Removal of 5 - 10 cm of the guard column after a few analytical sets (about 100 samples) may be needed to improve chromatography.

The GC/ECD and GC/MSD injection port should be cleaned regularly (after about 100 injections) by disassembling the port and sonicating it in methanol for about two minutes. If chromatography does not improve, a new 0.53 mm x 1 m guard column should be installed with 5 - 10 cm also removed from the analytical column. If poor chromatography still persists after these changes, a new analytical column may be required.

Ethyl acetate is recommended as the wash solvent for the injection port of the GC/ECD and GC/MSD. The syringe in the auto-sampler should be checked for a plug prior to each analytical run.

Radiovalidation of the tolerance enforcement method using the grape RAC matrix demonstrated that the residue of RH-7281 present in a sample from a grape metabolism study was adequately measured. Overall, accountability at 95.1 % is excellent.

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10. References

TR 34-96-128	Preliminary Residue Analytical Method for Parent RH-7281 in Grape RAC
TR 32-97-136	Preliminary Residue Analytical Method for Parent RH-7281 in Grape Juice
TR 34-97-164	Preliminary Residue Analytical Method for Parent RH-7281 in Raisins
TR 34-98-49	^{14}C-RH-117,281: Nature of the Residue in Fruiting Grape Plants
34P-96-92	Protocol for Preliminary Grape Method Validation Using GC/MSD (Enviro-Test Laboratories)
34P-96-93	Protocol for Preliminary Grape Method Validation Using GC/ECD (McKenzie Laboratories)
34P-97-15	Radiovalidation of Preliminary Residue Analytical Method for Parent RH-117,281 in Grapes (TR 34-96-128)
34P-97-18A	Protocol for 1997 Grape RAC Residue Trial Sample Analysis (Enviro-Test Laboratories)
34P-97-18B	Protocol for Grape Processed Fraction Trial Sample Analysis (McKenzie Laboratories)
34P-98-02A	Protocol for 1998 Grape RAC Residue Trial Sample Analysis (Enviro-Test Laboratories)

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11. Project Information

Title:	Tolerance Enforcement Method for RH-117,281 in Grapes and Processed Fractions
Project Number:	1401.15
Sponsor:	Rohm and Haas Company Research Laboratories 727 Norristown Road Spring House, PA 19477-0904
Testing Laboratories:	Rohm and Haas Company Research Laboratories 727 Norristown Road Spring House, PA 19477-0904 McKenzie Laboratories 3725 E. Atlanta Ave, Suite One Phoenix, AZ 85040 Enviro-Test Laboratories 9936-67 Avenue Edmonton, Alberta T6E 0P5 Canada
Dates:	Work Initiated: March, 1996 Work Completed: July, 1998
Technical Personnel:	Ernest L. Burdge, Margaret A. Kendi, Ipin Guo
Notebook References:	RH-IG-001 (R&H #65461) MAK1 (R&H #66571) GLP-56 GLP-65
Data Archives:	Research notebooks are stored in the Rohm and Haas Library, raw data are stored in the Rohm and Haas GLP Records Archive Facility. Raw data associated with Protocol 34P-96-92 are stored with the ETL Residue Trial Sample Analysis (TR 34-98-154).
Peer Review:	Michael A. Koals

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Table 1 Combustion and Liquid Scintillation Counting Results for Analysis of ^{14}C-RH-7281 in a Grape Metabolism Sample (RAR 95-0082-12)

Statistic	TRR (dpm)[a]	TRR Extracted (dpm)	Bound Residue (dpm)	Average Material Balance[b]	Average Extraction Efficiency[c]
Mean	237,000	234,703	19,467	107%	99.0%
Std Dev	39,600	18,367	8,153		
No. Samples	10	3	3		

[a] Total radioactive residues (TRR) data from combustion run #1207 for a 10g sample
[b] Material Balance = [TRR extracted + Bound Residue]/TRR x 100
[c] Extraction Efficiency = [TRR Extracted/TRR] x 100

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Table 2 Analysis of ^{14}C-RH-7281 in a Grape Metabolism Sample (RAR 95-0082-12) Using Grape Analytical Method TR 34-96-128

	RH-7281 (ppm found)[a]	Adjusted RH-7281[b]
	0.249	0.343
	0.304	0.418
	0.336	0.462
Mean	0.296	0.408
Std. Deviation	0.044	0.060

[a] Obtained using GC/ECD quantitation

[b] Adjusted RH-7281 = [RH-7281 ppm found]/72.7 %

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Table 3 Comparison of Grape Metabolism Results to Analytical Method Results for Sample RAR 95-0082-12

	Grape Metabolism[a] (Average Recovery)	Tolerance Method (Average Recovery)
TRR (ppm found)	0.735	0.534[b]
RH-7281 (ppm found)	0.429	0.296
RH-7281 (% of TRR)	58.4	55.4

[a] Data taken from TR 34-98-49

[b] Value obtained using specific activity of 14C-RH-7281 (44,401 dpm/µg)
0.534 ppm (µg/g) = [23,700 dpm/g]/44,401 dpm/µg

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Table 4 Fortification Recovery Data for Grape RAC at LOQ

Fortification (µg/g)	Data Source[a]	Recovery (%) GC/ECD	Data Source[a]	Recovery (%) GC/MSD
Green Grapes				
0.010	TR 34-96-128	116	TR 34-96-128	122
0.010	TR 34-96-128	105	TR 34-96-128	102
0.010	TR 34-96-128	133	TR 34-96-128	118
0.010	GG-MKZ-002-1	112	01/21/97 (GG-ETL-002-1)	118
0.010	GG-MKZ-003-1	116	01/21/97 (GG-ETL-003-1)	110
0.010	GG-MKZ-002-2	103	01/22/97 (GG-ETL-002-2)	87.0
0.010	GG-MKZ-003-2	99.0	01/22/97 (GG-ETL-003-2)	94.7
0.010	07/27/98	90.3		
0.0108			10/19/97	118
0.0109	03/31/98	102		
0.0109			04/03/98	81.1
0.0109			04/03/98	92.3
Red Grapes				
0.010	TR 34-96-128	74.9	TR 34-96-128	110
0.010	TR 34-96-128	86.7	TR 34-96-128	86.8
0.010	TR 34-96-128	103	TR 34-96-128	95.6
0.010	RG-MKZ-002-1	109	01/23/97 (RG-ETL-003-1)	100
0.010	RG-MKZ-003-1	111	01/23/97 (RG-ETL-002-1)	117
0.010	RG-MKZ-002-2	124	01/27/97 (RG-ETL-002-2)	92.2
0.010	RG-MKZ-003-2	111	01/27/97 (RG-ETL-003-2)	96.6
0.0108			07/24/97	126
0.0108			08/01/97	86.7
0.0108			08/15/98	25.2[b]
0.0109			04/01/98	96.3
0.0109			04/01/98	91.6
Mean		106		102

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Std. Deviation	14.2	13.6
No. of Samples	16	21

[a] TR 34-96-128: Preliminary Residue Analytical Method for Parent RH-7281 in Grapes; Sample ID numbers containing the letters MKZ were analyzed by McKenzie Laboratories (protocol 34P-96-93); Numbers containing the letters ETL were analyzed by Enviro-Test Laboratories (protocol 34P-96-92)
Dates represent standard curve numbers reported in NRDS files (protocols 34P-96-92, 34P-97-18A, 34P-98-02A; Rohm and Haas Company).

[b] Recovery value not included in statistical analysis

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Table 5 Fortification Recovery Data for Grape Juice at LOQ

Fortification (µg/g)	Data Source[a]	Recovery (%) GC/ECD	Data Source[a]	Recovery (%) GC/MSD
Purple Juice				
0.010	TR 34-97-136	90.9	TR 34-97-136	100
0.010	TR 34-97-136	80.5	TR 34-97-136	96.5
0.010	TR 34-97-136	102	TR 34-97-136	87.0
0.010	TR 34-97-136	83.6	TR 34-97-136	93.6
0.010	TR 34-97-136	96.8	TR 34-97-136	96.0
White Juice				
0.010	TR 34-97-136	109	TR 34-97-136	132
0.010	TR 34-97-136	98.9	TR 34-97-136	79.5
0.010	TR 34-97-136	107	TR 34-97-136	93.6
0.010	TR 34-97-136	109	TR 34-97-136	77.3
0.010	TR 34-97-136	113	TR 34-97-136	107
0.010	07/16/98	125		
Mean		101		96.3
Std. Deviation		13.2		15.4
No. of Samples		11		10

[a] TR 34-97-136: Preliminary Residue Analytical Method for Parent RH-7281 in Grape Juice

Dates represent standard curve numbers reported in NRDS files (protocol 34P-97-18B; Rohm and Haas Company).

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Table 6 Fortification Recovery Data for Raisin at LOQ

Fortification (µg/g)	Data Source[a]	Recovery (%) GC/ECD	Data Source[a]	Recovery (%) GC/MSD
Raisin				
0.010	TR 34-97-164	143	TR 34-97-164	87.5
0.010	TR 34-97-164	108	TR 34-97-164	78.7
0.010	TR 34-97-164	98.4	TR 34-97-164	110
Golden Raisin				
0.010	TR 34-97-164	120	TR 34-97-164	94.8
0.010	TR 34-97-164	87.2	TR 34-97-164	56.2
0.010	TR 34-97-164	93.0	TR 34-97-164	122
0.010			07/23/98	109
Mean		108		94.0
Std. Deviation		20.6		22.3
No. of Samples		6		7

[a] TR 34-97-164: Preliminary Residue Analytical Method for Parent RH-7281 in Raisins

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Table 7 Fortification Recovery for Grape RAC

Fortification (µg/g)	Data Source[a]	Recovery (%) GC/ECD	Data Source[a]	Recovery (%) GC/MSD
Green Grapes				
0.050	TR 34-96-128	108	TR 34-96-128	108
0.050	TR 34-96-128	101	TR 34-96-128	102
0.050	GG-MKZ-004-1	79.0	01/21/97 (GG-ETL-004-1)	83.2
0.050	GG-MKZ-004-2	95.0	01/22/97 (GG-ETL-004-2)	69.6
0.050				
0.054			7/8/97	74.4
0.054			10/15/97	90.4
0.10	TR 34-96-128	109	TR 34-96-128	92.5
0.10	TR 34-96-128	116	TR 34-96-128	102
0.10	GG-MKZ-005-1	87.0	01/21/97 (GG-ETL-005-1)	105
0.10	GG-MKZ-005-2	100	01/22/97 (GG-ETL-005-2)	80.6
0.15	TR 34-96-128	109	TR 34-96-128	92.8
0.15	TR 34-96-128	114	TR 34-96-128	97.5
0.15	GG-MKZ-006-1	90.0	01/21/97 (GG-ETL-006-1)	106
0.15				
0.15	GG-MKZ-006-2	91.0	01/22/97 (GG-ETL-006-2)	90.8
0.15				
0.216			07/08/97	97.2
0.216			09/12/97	88.4
0.216			09/17/97	83.3
0.216			10/19/97	80.1
0.296			04/03/98	91.2
1.98			04/03/98A	84.3
3.00	07/27/98	95.8		
4.94			03/31/98	81.0
Red Grapes				
0.020	TR 34-96-128	96.6	TR 34-96-128	87.9
0.050	TR 34-96-128	98.4	TR 34-96-128	74.5
0.050	TR 34-96-128	80.0	TR 34-96-128	120
0.050	RG-MKZ-004-1	84.0	01/23/97	68.8

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			(RG-ETL-004-1)	
0.050	RG-MKZ-004-2	123	01/27/97	82.4
			(RG-ETL-004-2)	
0.054			07/22/97	113
0.054			07/28/97	110
0.054			08/18/97	63.2
0.10	TR 34-96-128	83.0	TR 34-96-128	83.3
0.10	RG-MKZ-005-1	93.0	01/23/97	71.0
			(RG-ETL-005-1)	
0.10	RG-MKZ-005-2	97.0	01/27/97	76.4
			(RG-ETL-005-2)	
0.108			08/14/97	112
0.108			10/16/97	68.7
0.15	TR 34-96-128	80.2	TR 34-96-128	78.6
0.15	RG-MKZ-006-1	97.0	1/23/97	84.4
			(RG-ETL-006-1)	
0.15	RG-MKZ-006-2	105	1/27/97	76.0
			(RG-ETL-006-2)	
0.216			08/20/97	70.6
0.216			10/19/97	80.1
1.98			04/01/98	92.4
0.216			7/24/97A	73.2
0.216			09/09/97	81.3
2.96			04/01/98	118
Mean		97.2		88.0
Std. Deviation		12.0		14.6
No. of Samples		24		43

[a] TR 34-96-128: Preliminary Residue Analytical Method for Parent RH-7281 in Grapes; Sample ID numbers containing the letters MKZ were analyzed by McKenzie Laboratories (protocol 34P-96-93); Numbers containing the letters ETL were analyzed by Enviro-Test Laboratories (protocol 34P-96-92)

Dates represent standard curve numbers reported in NRDS files (protocols 34P-96-92, 34P-97-18A, 34P-98-02A; Rohm and Haas Company).

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Table 8 Fortification Recovery Results for Grape Juice

Fortification (µg/g)	Data Source[a]	Recovery (%) GC/ECD	Data Source[a]	Recovery (%) GC/MSD
Purple Juice				
0.020	TR 34-97-136	76.2	TR 34-97-136	85.6
0.020	TR 34-97-136	81.0	TR 34-97-136	81.5
0.020	TR 34-97-136	69.9	TR 34-97-136	61.4
0.050	TR 34-97-136	84.4	TR 34-97-136	80.6
0.050	TR 34-97-136	81.0	TR 34-97-136	82.4
0.050	TR 34-97-136	69.7	TR 34-97-136	67.3
0.10	TR 34-97-136	90.2	TR 34-97-136	60.0
0.10	TR 34-97-136	91.5	TR 34-97-136	80.6
0.10	TR 34-97-136	80.9	TR 34-97-136	91.3
0.15	TR 34-97-136	98.1	TR 34-97-136	68.4
0.15	TR 34-97-136	87.0	TR 34-97-136	82.3
0.15	TR 34-97-136	71.4	TR 34-97-136	70.4
White Juice				
0.020	TR 34-97-136	97.5	TR 34-97-136	105
0.020	TR 34-97-136	101	TR 34-97-136	99.0
0.020	TR 34-97-136	91.5	TR 34-97-136	102
0.050	TR 34-97-136	89.4	TR 34-97-136	85.9
0.050	TR 34-97-136	89.0	TR 34-97-136	92.4
0.050	TR 34-97-136	97.5	TR 34-97-136	97.4
0.10	TR 34-97-136	83.1	TR 34-97-136	107
0.10	TR 34-97-136	78.5	TR 34-97-136	96.2
0.10	TR 34-97-136	80.9	TR 34-97-136	99.0
0.15	TR 34-97-136	85.1	TR 34-97-136	130
0.15	TR 34-97-136	84.5	TR 34-97-136	88.1
0.15	TR 34-97-136	88.6	TR 34-97-136	118
3.0	07/16/98	92.1		
Mean		85.6		88.8
Std. Deviation		8.6		17.1
No. of Samples		25		24

[a] TR 34-97-136: Preliminary Residue Analytical Method for Parent RH-7281 in Grape Juice
Dates represent standard curve numbers reported in NRDS files (protocol 34P-97-18B; Rohm and Haas Company)

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Table 9 Fortification Recovery Results for Raisin

Fortification (µg/g)	Data Source[a]	Recovery (%) GC/ECD	Data Source[a]	Recovery (%) GC/MSD
Raisin				
0.050	TR 34-97-164	85.8	TR 34-97-164	81.6
0.050	TR 34-97-164	92.5	TR 34-97-164	62.5
0.050	TR 34-97-164	90.4	TR 34-97-164	92.2
0.10	TR 34-97-164	85.6	TR 34-97-164	83.0
0.10	TR 34-97-164	103	TR 34-97-164	70.5
0.10	TR 34-97-164	83.8	TR 34-97-164	95.3
0.50	TR 34-97-164	69.8	TR 34-97-164	85.8
0.50	TR 34-97-164	96.3	TR 34-97-164	78.3
0.50	TR 34-97-164	84.7	TR 34-97-164	84.9
1.0	TR 34-97-164	79.6	TR 34-97-164	88.2
1.0	TR 34-97-164	106	TR 34-97-164	80.4
1.0	TR 34-97-164	90.1	TR 34-97-164	90.3
Golden Raisin				
0.050	TR 34-97-164	90.7	TR 34-97-164	76.4
0.050	TR 34-97-164	80.7	TR 34-97-164	78.7
0.050	TR 34-97-164	75.3	TR 34-97-164	74.2
0.10	TR 34-97-164	91.7	TR 34-97-164	77.3
0.10	TR 34-97-164	78.3	TR 34-97-164	83.2
0.10	TR 34-97-164	76.3	TR 34-97-164	86.6
0.50	TR 34-97-164	62.5	TR 34-97-164	76.1
0.50	TR 34-97-164	69.9	TR 34-97-164	55.1
0.50	TR 34-97-164	77.0	TR 34-97-164	89.4
1.0	TR 34-97-164	90.1	TR 34-97-164	81.0
1.0	TR 34-97-164	74.0	TR 34-97-164	75.9
1.0	TR 34-97-164	76.8	TR 34-97-164	77.7
3.0			07/23/98	96.5
3.0			07/25/98	115
Mean		83.8		82.2
Std. Deviation		10.5		11.4
No. of Samples		24		26

[a] TR 34-97-164: Preliminary Residue Analytical Method for Parent RH-7281 in Grape Juice
Dates represent standard curve numbers reported in NRDS files (protocol 34P-97-18B; Rohm and Haas Company).

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```
Acq. Method        : GC7281.M                          Seq. Line : -
Acq. Operator      : Ipin                                   Vial : 1
Injection Date     : 7/26/96 5:33:46 PM                      Inj : -
Sample Name        : Grape Method                   Inj Volume : Unknown

Analysis Method : C:\HPCHEM\1\METHODS\GC7231.M
                  (modified after loading)
GC Method
```

Current Chromatogram(s)

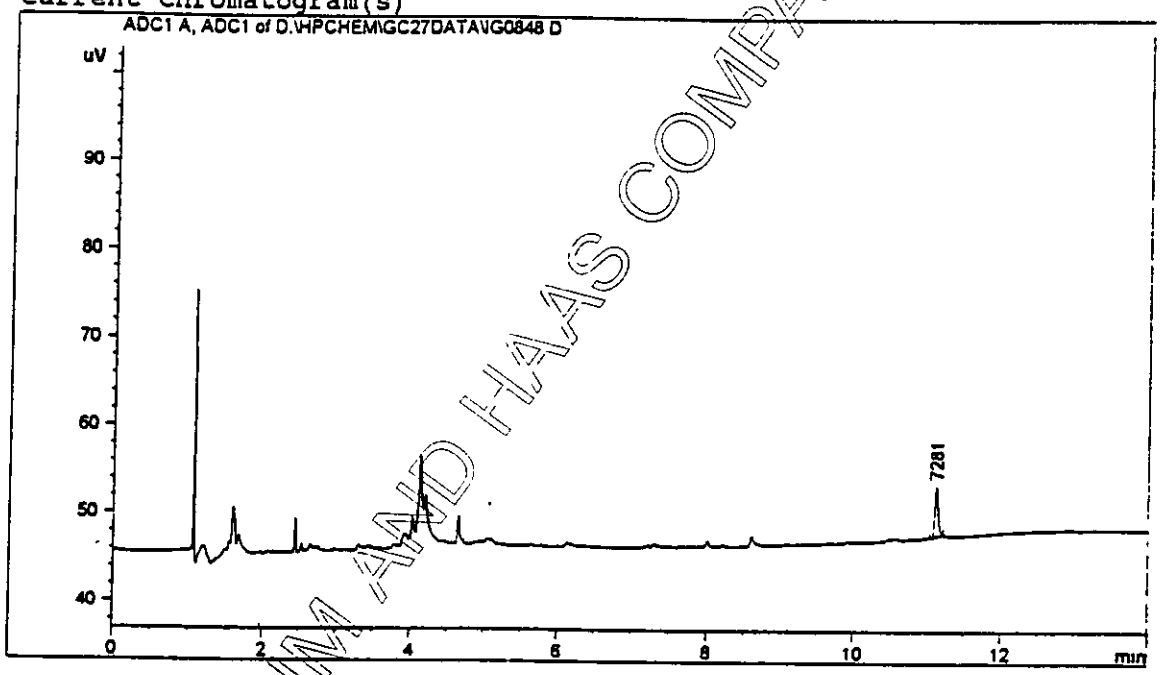

Figure 1 0.010 µg/ml RH-7281 Standard (GC/ECD)
Peak Area = 19.21

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```
==========================================================================
Acq. Method      : GC7281.M                          Seq. Line :  -
Acq. Operator    : Ipin                                   Vial :  1
Injection Date   : 7/26/96 5:52:22 PM                      Inj :  -
Sample Name      : Grape Method                     Inj Volume : Unknown

Analysis Method : C:\HPCHEM\1\METHODS\GC7281.M
                  (modified after loading)
GC Method
```

Current Chromatogram(s)

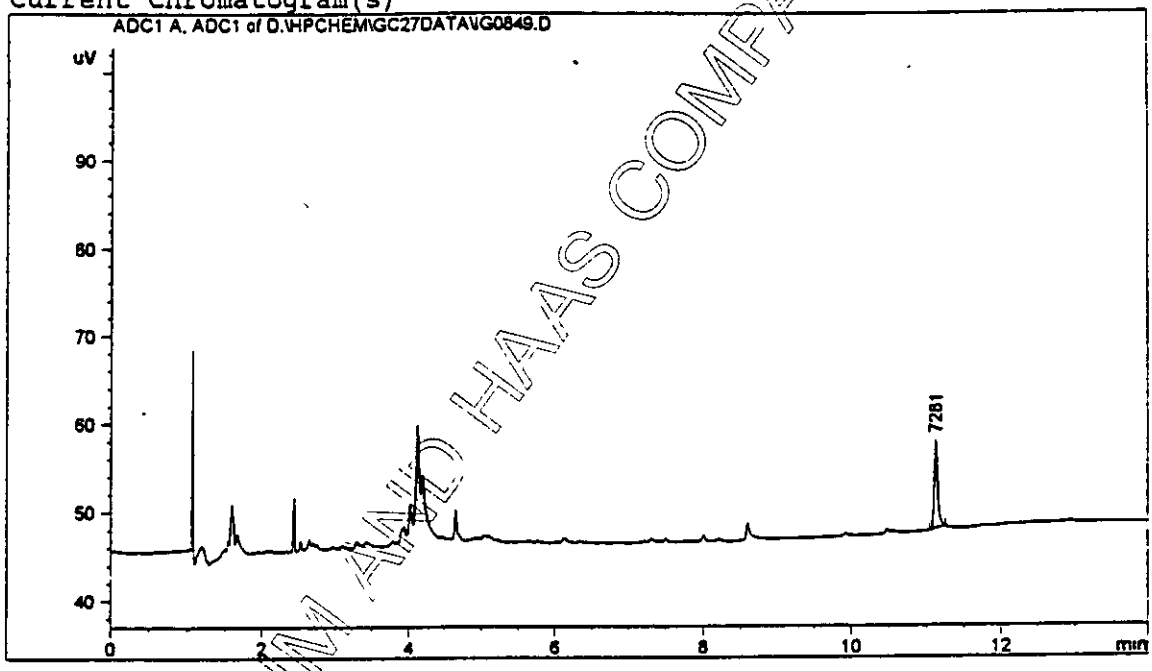

Figure 2 0.020 µg/ml RH-7281 Standard (GC/ECD)
Peak Area = 34.17

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```
==================================================================
Acq. Method      : GC7281.M                          Seq. Line :  -
Acq. Operator    : Ipin                                   Vial :  1
Injection Date   : 7/26/96 6:11:00 PM                      Inj :  -
Sample Name      : Grape Method                     Inj Volume : Unknown

Analysis Method : C:\HPCHEM\1\METHODS\GC7281.M
                  (modified after loading)
GC Method
```

Current Chromatogram(s)

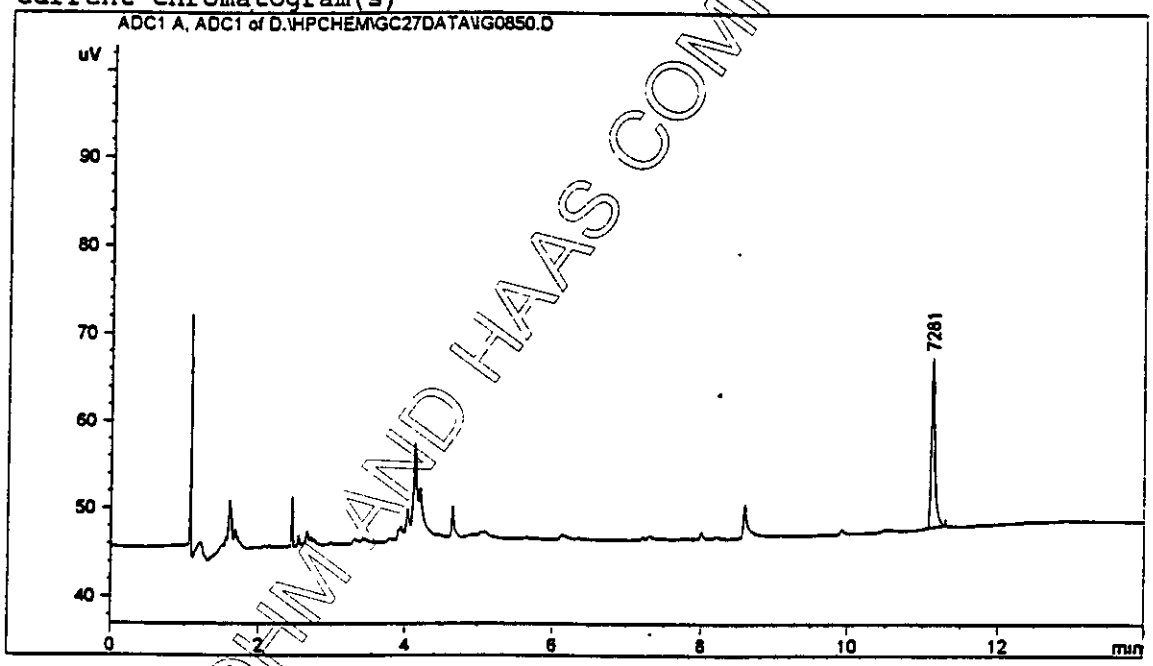

Figure 3 0.050 µg/ml RH-7281 Standard (GC/ECD)
Peak Area = 66.66

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

```
==========================================================================
Acq. Method       : GC7281.M                          Seq. Line :  -
Acq. Operator     : Ipin                                 Vial :  1
Injection Date    : 7/26/96 6:29:37 PM                    Inj :  -
Sample Name       : Grape Method                     Inj Volume : Unknown

Analysis Method : C:\HPCHEM\1\METHODS\GC7281.M
                  (modified after loading)
GC Method
```

Current Chromatogram(s)

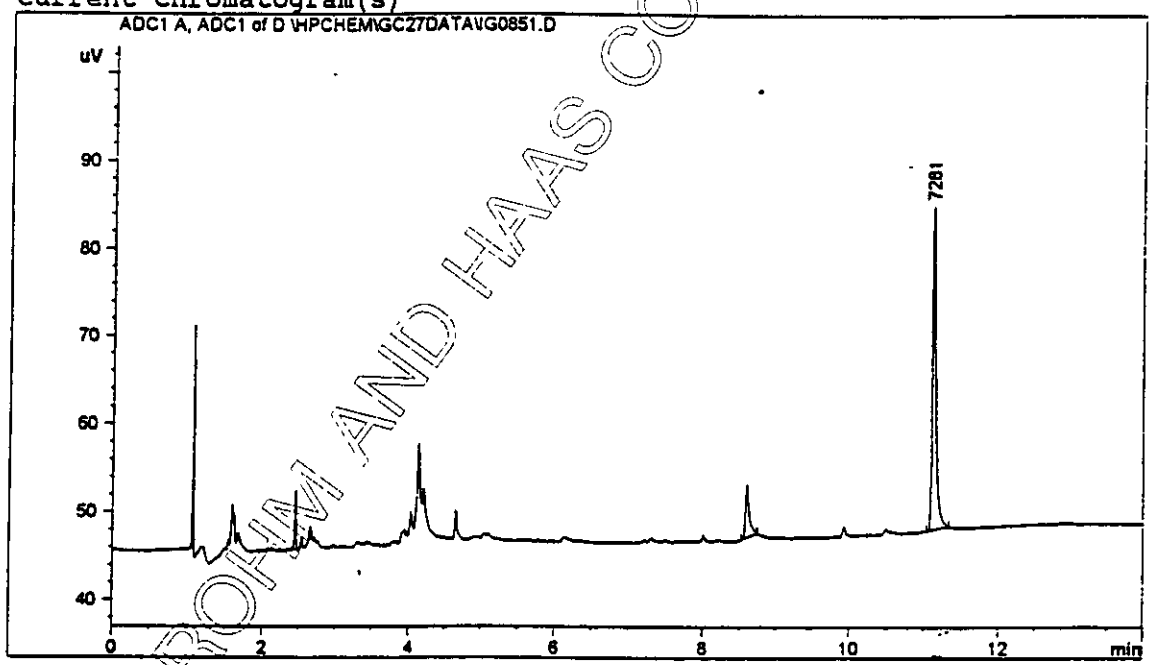

Figure 4 0.10 µg/ml RH-7281 Standard (GC/ECD)
Peak Area = 125.1

```
Acq. Method        : GC7281.M                          Seq. Line :  -
Acq. Operator      : Ipin                                   Vial :  1
Injection Date     : 7/26/96 6:48:13 PM                      Inj :  -
Sample Name        : Grape Method                     Inj Volume : Unknown

Analysis Method : C:\HPCHEM\1\METHODS\GC7281.M
                  (modified after loading)
GC Method
```

Current Chromatogram(s)

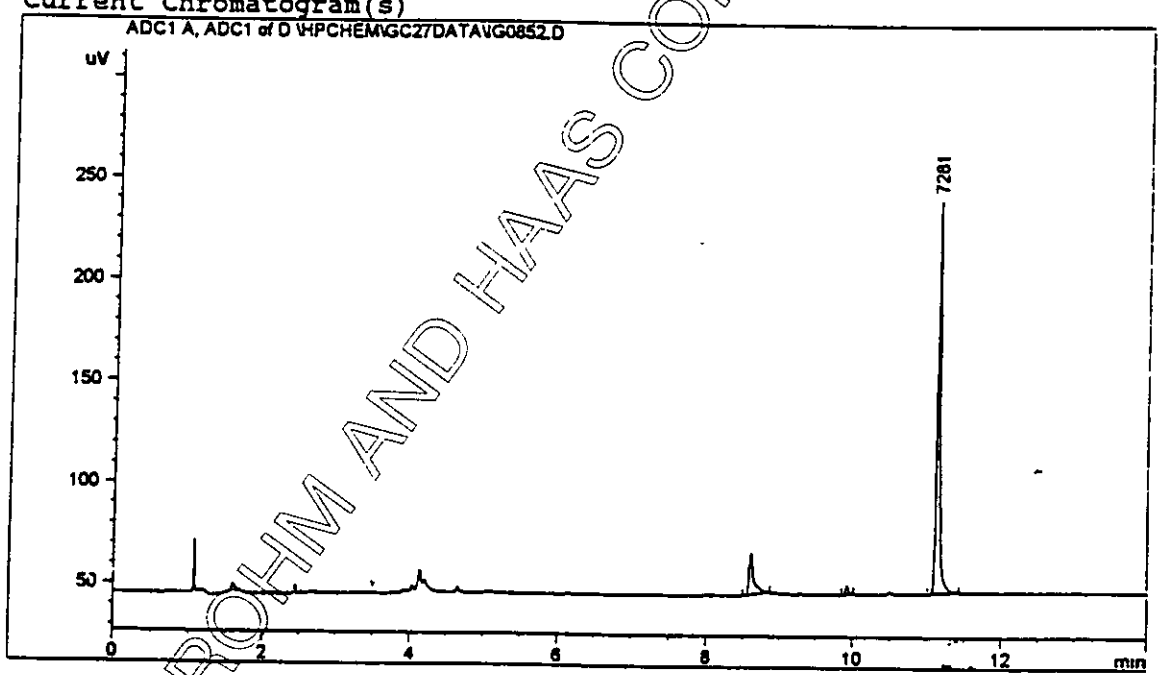

Figure 5 0.50 µg/ml RH-7281 Standard (GC/ECD)
Peak Area = 539.9

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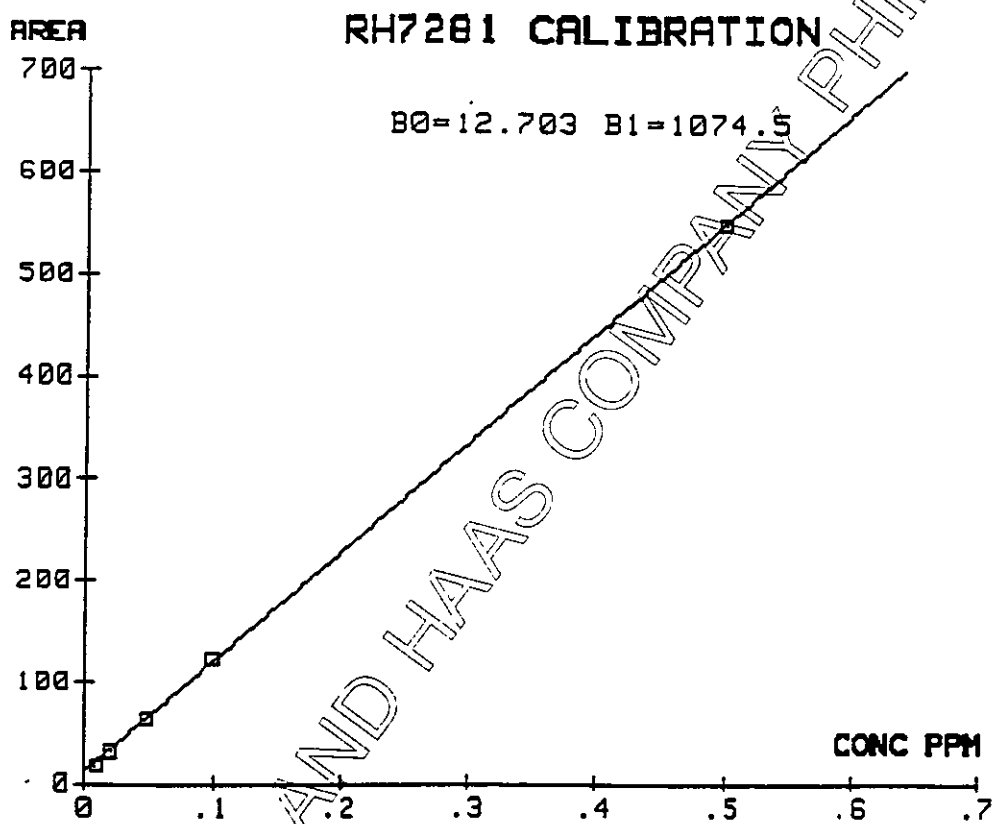

Concentrations in report are calculated from equation:
AREA = B0 + B1(CONC PPM)
obtained by least-squares fit of standard injection data.

Figure 6 Calibration Curve for RH-7281 (GC/ECD)
$r^2 = 0.9991$

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PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

```
Acq. Method        : GC7281.M                          Seq. Line :  -
Acq. Operator      : Ipin                                   Vial :  1
Injection Date     : 7/25/96 12:00:50 PM                     Inj :  -
Sample Name        : Grape Method                     Inj Volume : Unknown

Analysis Method    : C:\HPCHEM\1\METHODS\GC7281.M
                     (modified after loading)
GC Method
```

Current Chromatogram(s)

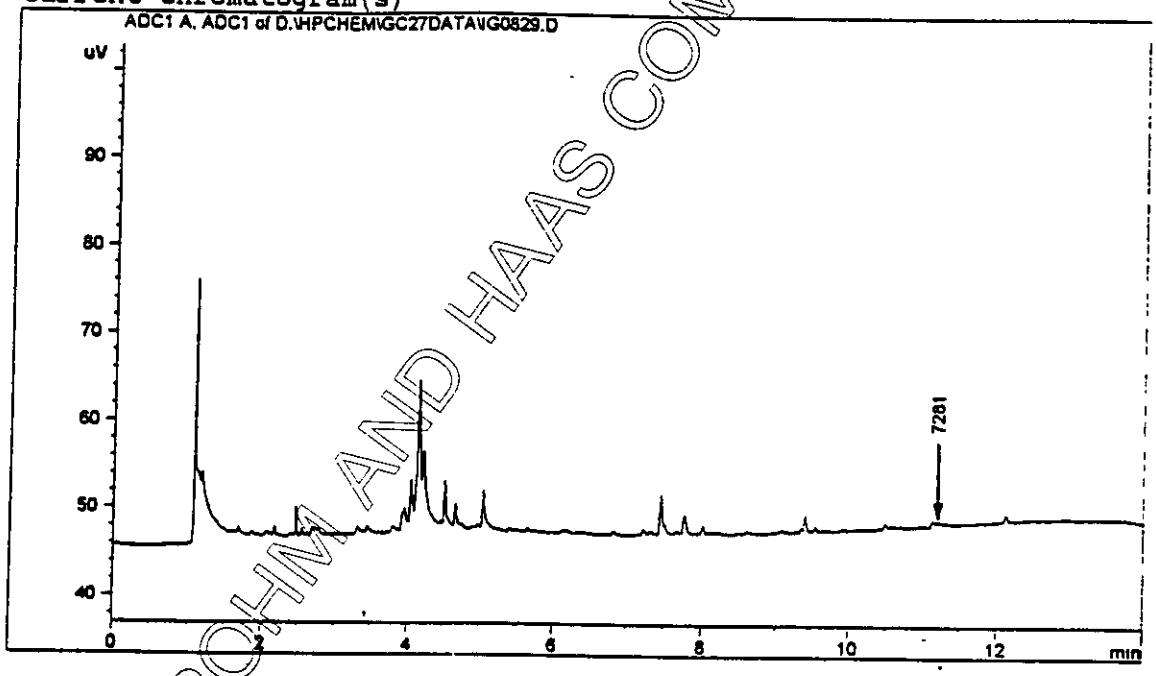


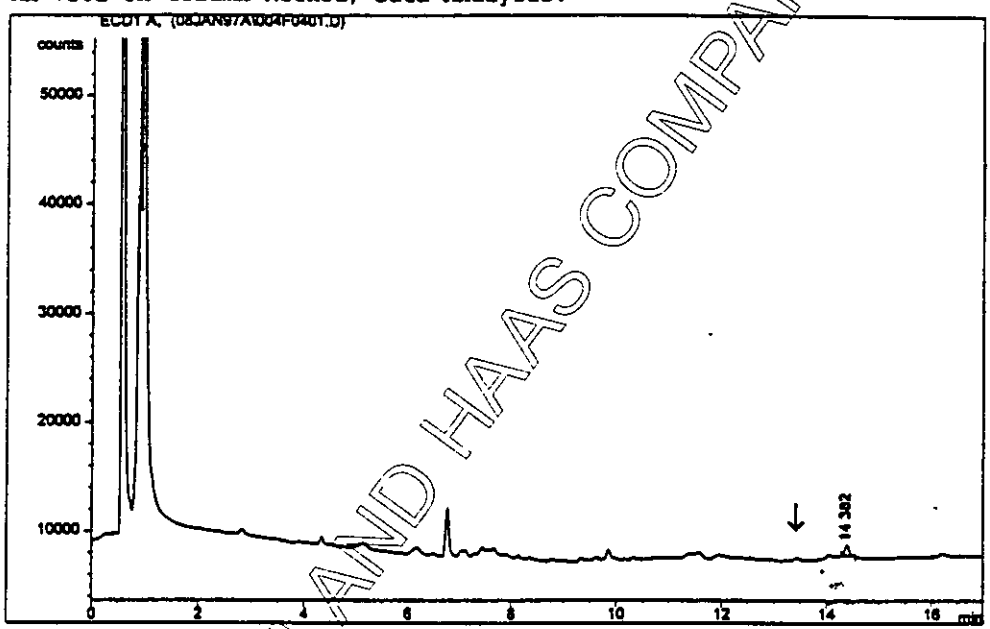
Figure 7 Control Grape (GC/ECD)
Peak Area = 0

Data File C:\HPCHEM\MKL00401\DATA\7281\08JAN97A\004F0401.D 2-Control

Grapes
RG-MKZ-001-1
10.0 g in 10.0 mL

Injection Date : 1/8/97 7:43:56 PM Seq. Line : 4
Sample Name : 2-Control Vial : 4
Acq. Operator : MT Inj : 1
Inj Volume : 1 µl

Acq. Method : C:\HPCHEM\MKL00401\METHODS\RH7281OC.M
Last changed : 1/8/97 9:21:31 AM by MT
Analysis Method : C:\HPCHEM\MKL00401\METHODS\7281ANLY.M
Last changed : 1/9/97 7:38:39 AM by MT 34P-96-93 Grapes MV2
RH-7281 On-Column Method, Data Analysis.



External Standard Report

Sorted By : Retention Time
Calib. Data Modified : Thursday, January 09, 1997 7:33:27 AM
Multiplier : 1.0000
Dilution : 1.0000
Uncalibrated Peaks : compound name not specified

Signal 1: ECD1 A,

Figure 8 Control Grape (GC/ECD)
Peak Area = 0

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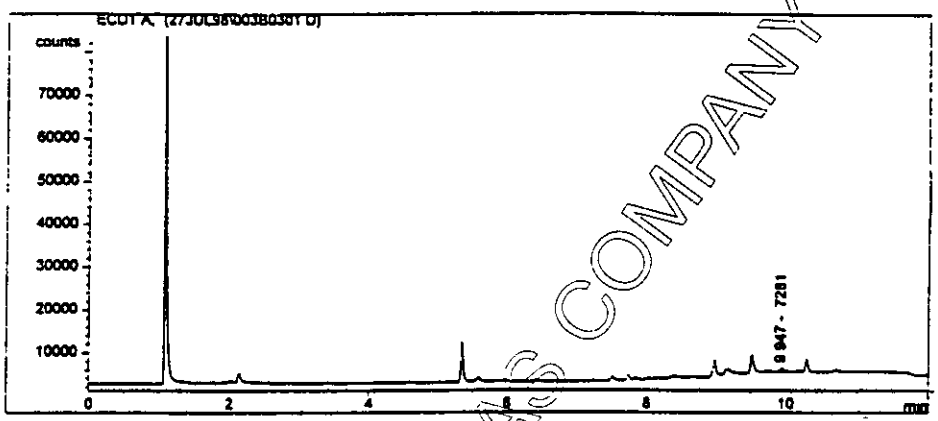
```
Data File C:\HPCHEM\MKL00590\DATA\7281\27JUL98\00380301.D          Control GR#1
    34P-97-18B 7281 SET#GR1
    Control Grape#1
     Final volume 10 ml  in Hexane
     ① RAR# : 96-0207 ; sample weight = 10.0 g
    ==================================================================
    Injection Date  : 7/27/98 4:43:00 PM            Seq. Line :   3
    Sample Name     : Control GR#1                       Vial :   3
    Acq. Operator   : LH   34P-97-18B                     Inj :   1
                                                   Inj Volume : 1 µl
    Acq. Method     : C:\HPCHEM\MKL00590\METHODS\7281ME.M
    Last changed    : 7/24/98 8:23:23 AM by LH   34P-97-18B
    Analysis Method : C:\HPCHEM\MKL00590\METHODS\7281ANL.M
    Last changed    : 8/24/98 1:00:25 PM by LH   34P-97-18B
    RH7281 ON-COLUMN INJECTION Method
    ==================================================================
```




```
    ==================================================================
                          External Standard Report
    ==================================================================

    Sorted By              :      Retention Time
    Calib. Data Modified   :      Monday, August 24, 1998 12:59:13 PM
    Multiplier             :      1.0000
    Dilution               :      1.0000

    Signal 1: ECD1 A,

    RetTime Sig Type     Area      Amt/Area    Amount    Grp    Name
     [min]             counts*s               [µg/mL]
    -------|--|------|----------|----------|----------|--|--------------
      9.947  1 PP     3542.41602    0.00000    0.00000    7281

    Totals :                                   0.00000

     Results obtained with enhanced integrator!
    1 Warnings or Errors :

    Warning : Negative results set to zero (cal. curve intercept), (7281)

    ==================================================================
```

Figure 9 Control Grape (GC/ECD)

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

```
Acq. Method        : GC7281.M                          Seq. Line : -
Acq. Operator      : Ipin                                   Vial : 1
Injection Date     : 7/25/96 1:15:22 PM                      Inj : -
Sample Name        : Grape Method                   Inj Volume : Unknown

Analysis Method    : C:\HPCHEM\1\METHODS\GC7281.M
                     (modified after loading)
GC Method
```

Current Chromatogram(s)

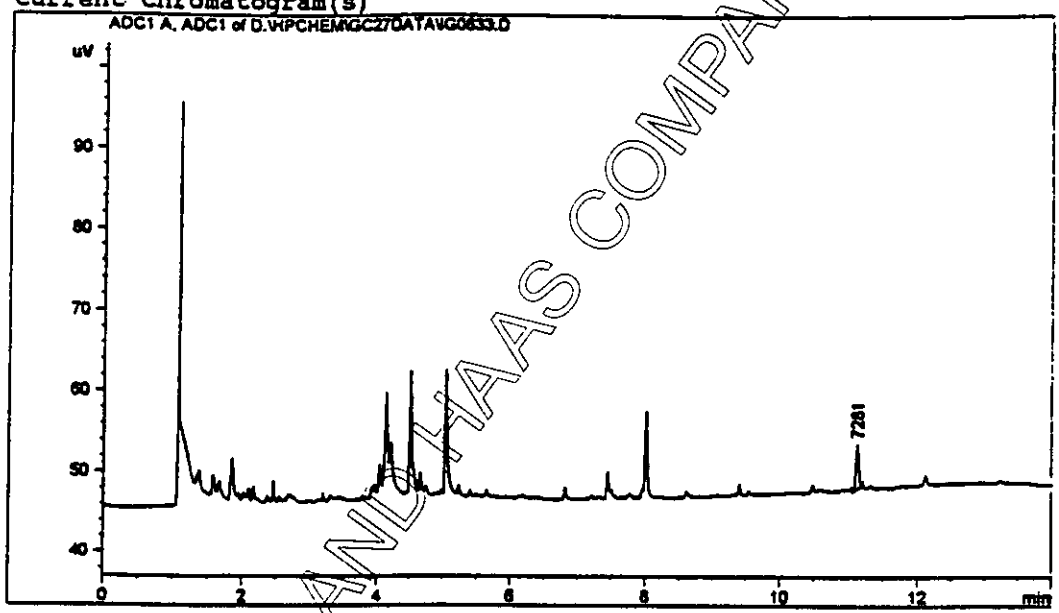


Figure 10 Fortification Grape at 0.010 ppm (GC/ECD)
Peak Area = 23.89; Found: 0.0104 ppm
Recovery = 104 %

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

```
Acq. Method      : GC7281.M                          Seq. Line :  -
Acq. Operator    : Ipin                                  Vial :  1
Injection Date   : 7/26/96 2:27:47 PM                     Inj :  -
Sample Name      : Grape Method                     Inj Volume : Unknown

Analysis Method  : C:\HPCHEM\1\METHODS\GC7281.M
                   (modified after loading)
GC Method
```

Current Chromatogram(s)

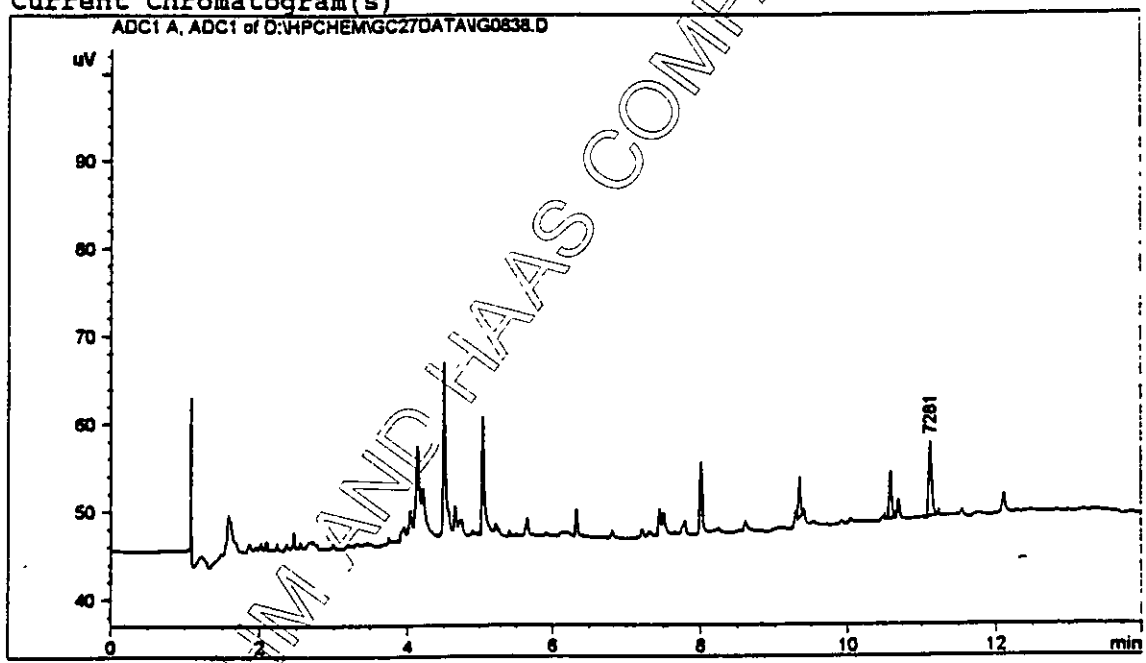


Figure 11 Fortification Grape at 0.020 ppm (GC/ECD)
Peak Area = 33.44; Found: 0.0193 ppm
Recovery = 96.6 %

```
Acq. Method      : GC7281.M                          Seq. Line : -
Acq. Operator    : Ipin                                   Vial : 1
Injection Date   : 7/26/96 2:46:20 PM                      Inj : -
Sample Name      : Grape Method                   Inj Volume : Unknown

Analysis Method  : C:\HPCHEM\1\METHODS\GC7281.M
                   (modified after loading)
GC Method
```

Current Chromatogram(s)

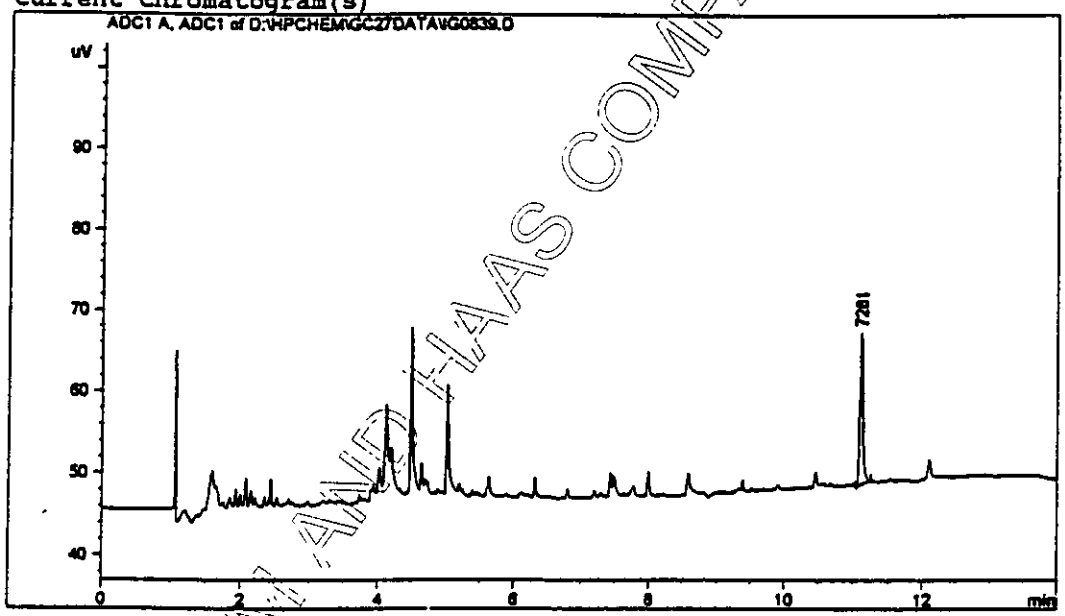
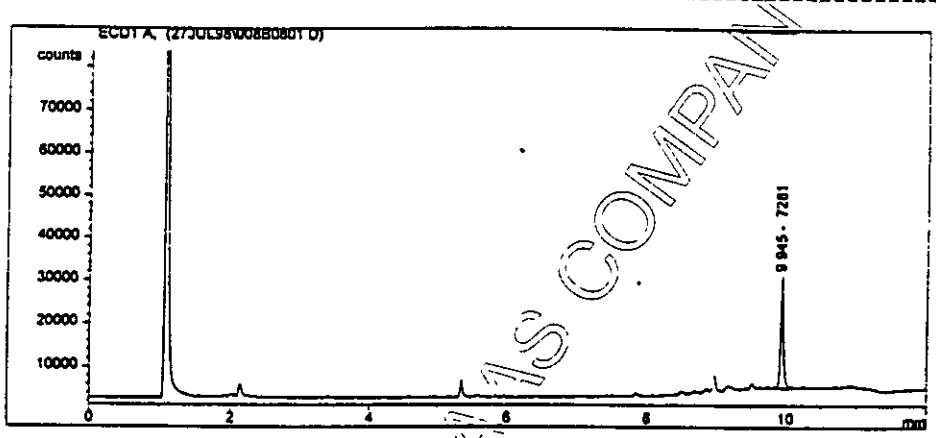


Figure 12 Fortification Grape at 0.050 ppm (GC/ECD)
Peak Area = 55.62; Found: 0.0400 ppm
Recovery = 80.0 %

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Data File C:\HPCHEM\MKL00590\DATA\7281\27JUL98\008B0801.D Sample Name: GR#2
34P-97-18B 7281 SET#GR1
Grape#2 x5 dil (A) LH 25 Aug 98
Final volume 10 ml in Hexane
(1) RAR #: 96-0207; Sample Weight = 10.0 g

Injection Date : 7/27/98 6:11:40 PM Seq. Line : 8
Sample Name : GR#2 Vial : 8
Acq. Operator : LH 34P-97-18B Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\HPCHEM\MKL00590\METHODS\7281ME.M
Last changed : 7/24/98 8:23:23 AM by LH 34P-97-18B
Analysis Method : C:\HPCHEM\MKL00590\METHODS\7281ANL.M
Last changed : 8/24/98 1.00:25 PM by LH 34P-97-18B
RH7281 ON-COLUMN INJECTION Method



External Standard Report

Sorted By : Retention Time
Calib. Data Modified : Monday, August 24, 1998 12:59:13 PM
Multiplier : 1.0000
Dilution : 5.0000

Signal 1: ECD1 A,

RetTime [min]	Sig	Type	Area counts*s	Amt/Area	Amount [µg/mL]	Grp	Name
9.945	1	BBA	7.59581e4	8.22972e-7	3.12557e-1		7281

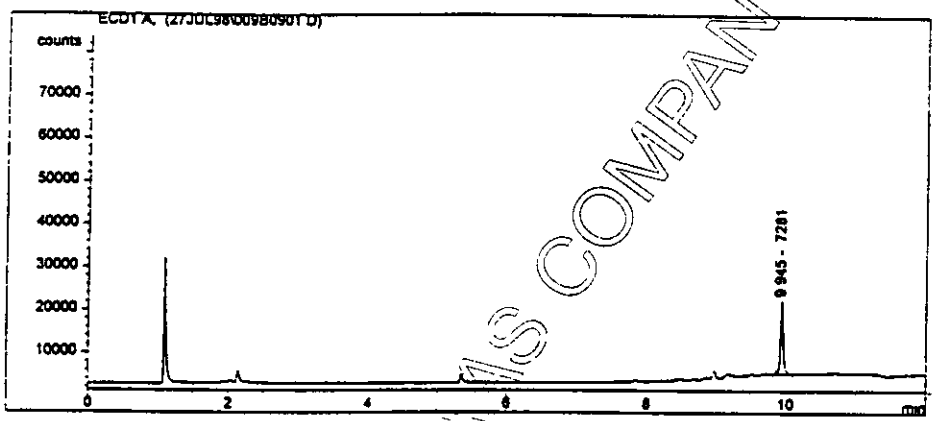
Totals : 3.12557e-1

Results obtained with enhanced integrator!

Figure 13 Treated Grape Sample #2 (RAR 96-0207) (GC/ECD)
1.25 lb/A; Analysis 7/27/98; Found: 0.313 ppm

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

```
Data File C:\HPCHEM\MKL00590\DATA\7281\27JUL98\009B0901.D      Sample Name: GR#3
    34P-97-18B 7281 SET#GR1
     Grape#3  x10 dil
     Final volume 10 ml  in Hexane
        ① RAR #: 96-0207; Sample weight = 10.0 g.
    ==================================================================
    Injection Date  : 7/27/98 6:29:23 PM            Seq. Line :   9
    Sample Name     : GR#3                               Vial :   9
    Acq. Operator   : LH   34P-97-18B                     Inj :   1
                                                   Inj Volume : 1 µl
    Acq. Method     : C:\HPCHEM\MKL00590\METHODS\7281ME.M
    Last changed    : 7/24/98 8.23·23 AM by LH   34P-97-18B
    Analysis Method : C:\HPCHEM\MKL00590\METHODS\7281ANL.M
    Last changed    : 8/24/98 1:00:25 PM by LH   34P-97-18B
    RH7281 ON-COLUMN INJECTION Method
    ==================================================================
```




```
    ==================================================================
                          External Standard Report
    ==================================================================

    Sorted By              :   Retention Time
    Calib. Data Modified   :   Monday, August 24, 1998 12:59:13 PM
    Multiplier             :   1.0000
    Dilution               :  10.0000

    Signal 1: ECD1 A,

    RetTime Sig Type    Area      Amt/Area    Amount    Grp   Name
     [min]            counts*s                [µg/mL]
    -------|--|------|----------|----------|----------|--|--------------
      9.945  1 PBA    4.94318e4  7.95296e-7 3.93129e-1    7281

    Totals :                                 3.93129e-1

     Results obtained with enhanced integrator!
    ==================================================================
```

Figure 14 Treated Grape Sample #3 (RAR 96-0207) (GC/ECD)
2.5 lb/A; Analysis 7/27/98; Found: 0.393 ppm

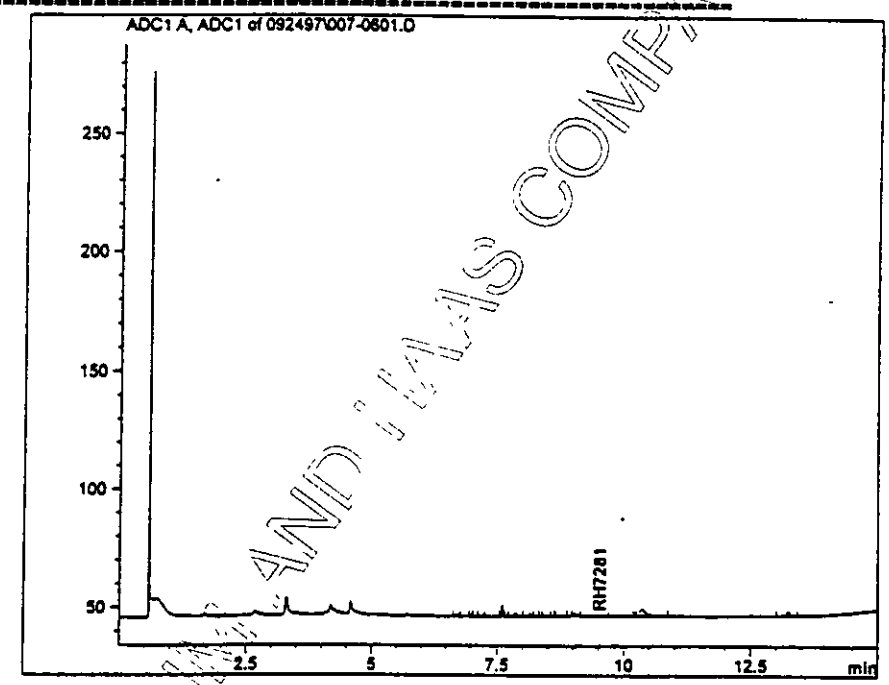
PROPERTY OF PONTI AND ASSOCIATES COMPANY PRIVATE PROPRIETARY

Data file: 007-0601.D RAR Number: -
Method file: GC7281G.M Sample No: 2
Type: Control Component: white juice

Sample Name: Cal. Curve: 9-24-97
Date: 9/24/97 5:09:26 PM Method: GC7281G.M Analyst: mak
Interface 301\

Instrument: Varian GC3500 #1 Column RXT-1
Particle Size(um): 5 Col.Length(cm): 15 Col.I.D.(mm) 0.25

Start Temp-Time (deg-min) 100/1 Ramp Hold (deg-min)
Program Rate (deg/min) 20 End Time-Temp (deg-min) 250/3
Prog Slope (# or Linear) linear Inj Port Temp: 150
Flowrate/Gas: 1.0/Nitrogen Split Ratio: none
Det 1-Type/Temp: ECD/300 Injection Volume 1



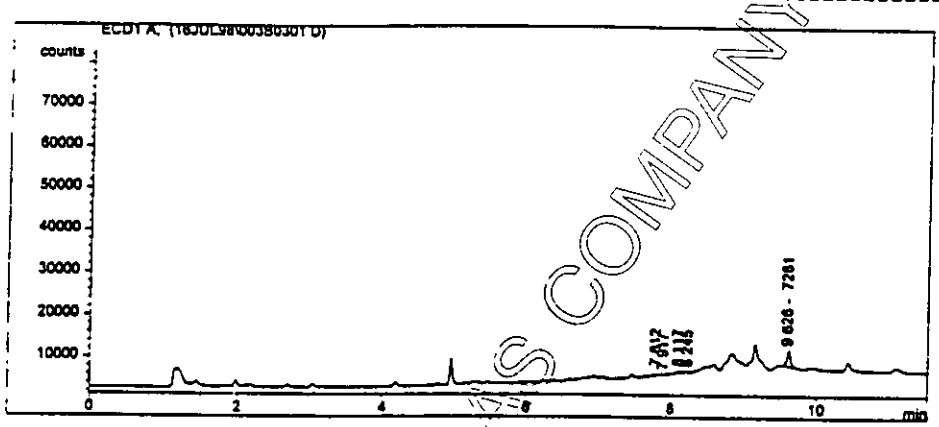
Ret. Time	Cpd. Name	Peak Area	Peak Height	Volume (ml)	Samp. Wt.
9.52	RH7281	0.690	0.155	10	10

Figure 15 Control Grape Juice (GC/ECD)

PROPERTY OF PROCTER AND GAMBLE COMPANY PHILADELPHIA

Data File C:\HPCHEM\MKL00590\DATA\7281\16JUL98\003B0301.D Control GJC#1
34P-97-18B 7281 SET#GJ1
control GJClarified#1
Final volume 10 ml
① RAR #: 96-0207; Sample weight = ~~1.0g~~ @ 20 Aug 98 JMW 10.0g

Injection Date : 7/16/98 7:44:55 PM Seq. Line : 3
Sample Name : Control GJC#1 Vial : 3
Acq. Operator : LH 34P-97-18B Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\HPCHEM\MKL00590\METHODS\7281ME.M
Last changed : 7/16/98 10 21:58 AM by LH 34P-97-18B
Analysis Method : C \HPCHEM\MKL00590\METHODS\7281ANL.M
Last changed : 7/17/98 7:51:55 AM by LH 34P-97-18B
RH7281 ON-COLUMN INJECTION Method



External Standard Report

Sorted By : Retention Time
Calib. Data Modified : 7/17/98 7:51:50 AM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 A,
Uncalibrated Peaks : compound name not specified

RetTime [min]	Sig	Type	Area counts*s	Amt/Area	Amount [µg/mL]	Grp	Name
7.812	1	PV	3685.27783	0.00000	0.00000	?	
7.917	1	VP	213.04701	0.00000	0.00000	?	
8.117	1	VV	2782.67017	0.00000	0.00000	?	
8.245	1	VBA	913.24359	0.00000	0.00000	?	
9.626	1	BBA	1.33906e4	7.26711e-7	9.73112e-3	7281	

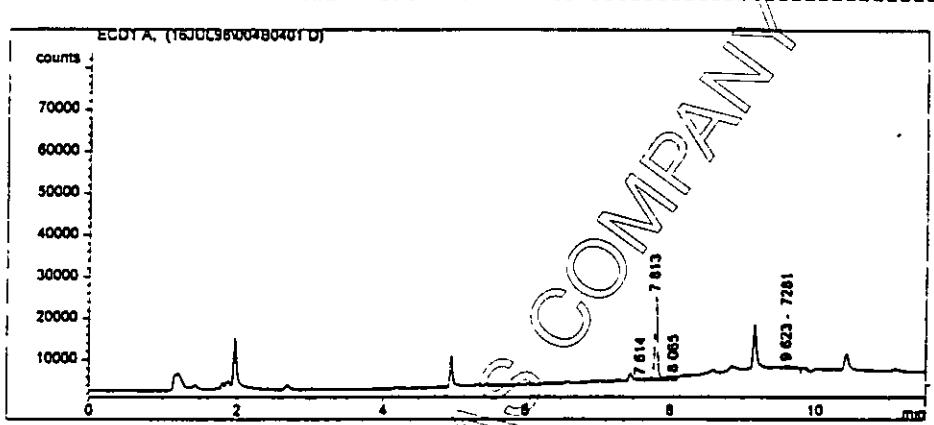
Totals : 9.73112e-3

Figure 16 Control Grape Juice (GC/ECD)

THE PROPERTY OF OHM AND HAAS COMPANY PHILADELPHIA

Data File C:\HPCHEM\MKL00590\DATA\7281\16JUL98\004B0401.D UTC GJUC#1
34P-97-18B 7281 SET#GJ1
~~control GJClarified#1~~ UTC GJUnclarified#1
Final volume 10 ml
(A) LH17J-193
(1) RR#: 96-0207, Sample weight = 10.0g

Injection Date : 7/16/98 8:01.54 PM Seq. Line : 4
Sample Name : UTC GJUC#1 Vial : 4
Acq. Operator : LH 34P-97-18B Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\HPCHEM\MKL00590\METHODS\7281ME.M
Last changed : 7/16/98 10.21:58 AM by LH 34P-97-18B
Analysis Method : C:\HPCHEM\MKL00590\METHODS\7281ANL.M
Last changed : 7/17/98 7:51:55 AM by LH 34P-97-18B
RH7281 ON-COLUMN INJECTION Method



External Standard Report

Sorted By : Retention Time
Calib. Data Modified : 7/17/98 7:51:50 AM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 A,
Uncalibrated Peaks : compound name not specified

RetTime [min]	Sig	Type	Area counts*s	Amt/Area	Amount [µg/mL]	Grp	Name
7.614	1	BV	178.01035	0.00000	0.00000	?	
7.813	1	VV	5.59489e4	0.00000	0.00000	?	
8.065	1	VV	4864.11914	0.00000	0.00000	?	
9.623	1	BBA	3441.46558	7.24770e-7	2.49427e-3		7281

Totals : 2.49427e-3

Results obtained with enhanced integrator!

Figure 17 Control Grape Juice (GC/ECD)

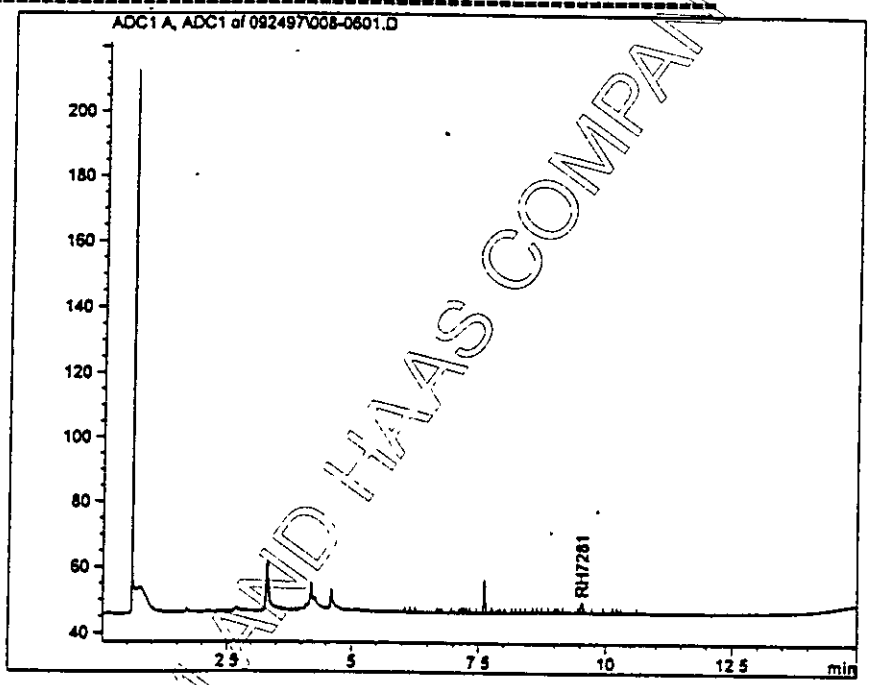
PROPERTY OF FRANK AND FRANK COMPANY PHILADELPHIA

Data file: 008-0601.D RAR Number: -
Method file: GC7281G.M Sample No: 3
Type: Fortification Component: white juice

Sample Name: Cal. Curve: 9-24-97
Date: 9/24/97 5.37 34 PM Method: GC7281G.M Analyst: mak
Interface 301\

Instrument: Varian GC3500 #1 Column RXT-1
Particle Size(um): 5 Col.Length(cm): 15 Col.I.D.(mm) 0.25

Start Temp-Time (deg-min) 100/1 Ramp Hold (deg-min)
Program Rate (deg/min) 20 End Time-Temp (deg-min) 250/3
Prog Slope (# or Linear) linear Inj Port Temp: 150
Flowrate/Gas: 1.0/Nitrogen Split Ratio: none
Det 1-Type/Temp: ECD/300 Injection Volume 1



Ret. Time	Cpd. Name	Peak Area	Peak Height	Volume (ml)	Samp. Wt.	ug Added
9.53	RH7281	12.704	3.297	10	10	0.100

Figure 18 Fortification Grape Juice at 0.010 ppm (GC/ECD)
Found: 0.0113 ppm Recovery = 113 %

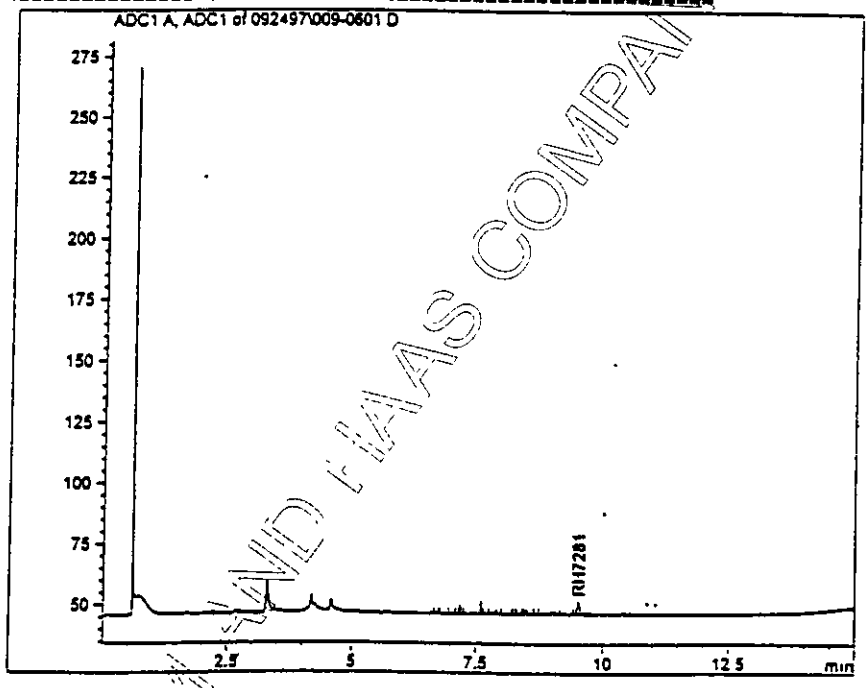
PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Data file: 009-0601.D RAR Number: -
Method file: GC7281G.M Sample No: 4
Type: Fortification Component: white juice

Sample Name: Cal. Curve: 9-24-97
Date: 9/24/97 6:05:45 PM Method: GC7281G.M Analyst: mak
Interface 301\

Instrument: Varian GC3500 #1 Column RXT-1
Particle Size(um): 5 Col.Length(cm): 15 Col.I.D.(mm) 0.25

Start Temp-Time (deg-min) 100/1 Ramp Hold (deg-min)
Program Rate (deg/min) 20 End Time-Temp (deg-min) 250/3
Prog Slope (# or Linear) linear Inj Port Temp: 150
Flowrate/Gas: 1.0/Nitrogen Split Ratio: none
Det 1-Type/Temp: ECD/300 Injection Volume 1



Ret. Time	Cpd. Name	Peak Area	Peak Height	Volume (ml)	Samp. Wt.	ug. Added
9.52	RH7261	20.526	5.432	10	10	0.200

Figure 19 Fortification Grape Juice at 0.020 ppm (GC/ECD)
Found: 0.0183 ppm Recovery = 91.5 %

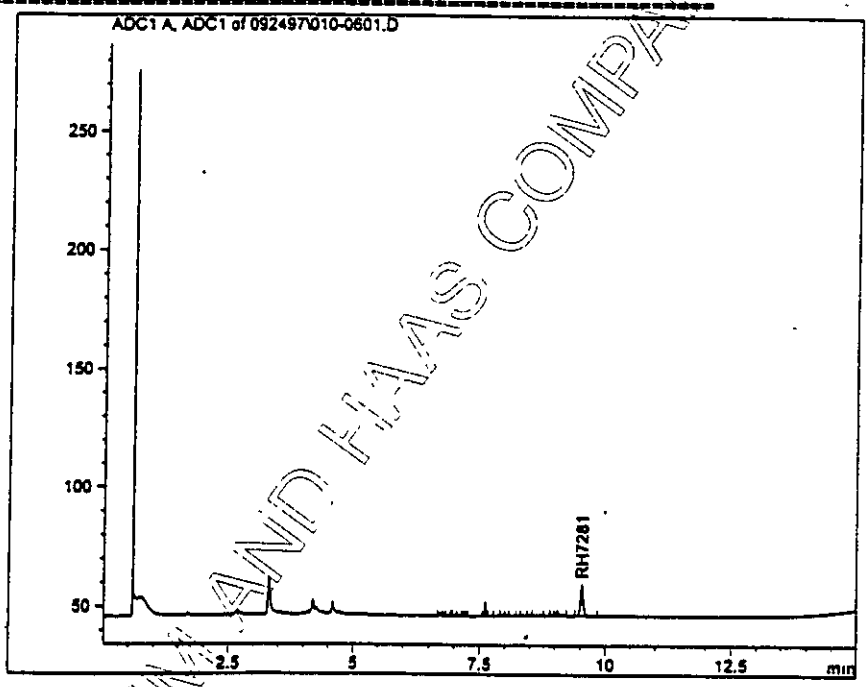
PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Data file: 010-0601.D RAR Number: -
Method file: GC7281G.M Sample No: 5
Type: Fortification Component: white juice

Sample Name: Cal. Curve· 9-24-97
Date: 9/24/97 6:33:53 PM Method: GC7281G.M Analyst: mak
Interface 301\

Instrument: Varian GC3500 #1 Column RXT-1
Particle Size(um): 5 Col.Length(cm): 15 Col.I.D.(mm) 0.25

Start Temp-Time (deg-min) 100/1 Ramp Hold (deg-min)
Program Rate (deg/min) 20 End Time-Temp (deg-min) 250/3
Prog Slope (# or Linear) linear Inj Port Temp: 150
Flowrate/Gas: 1.0/Nitrogen Split Ratio: none
Det 1-Type/Temp: ECD/300 Injection Volume 1



Ret. Time	Cpd. Name	Peak Area	Peak Height	Volume (ml)	Samp. Wt.	ug-Added
9.53	RH7281	53.001	13.661	10	10	0.500

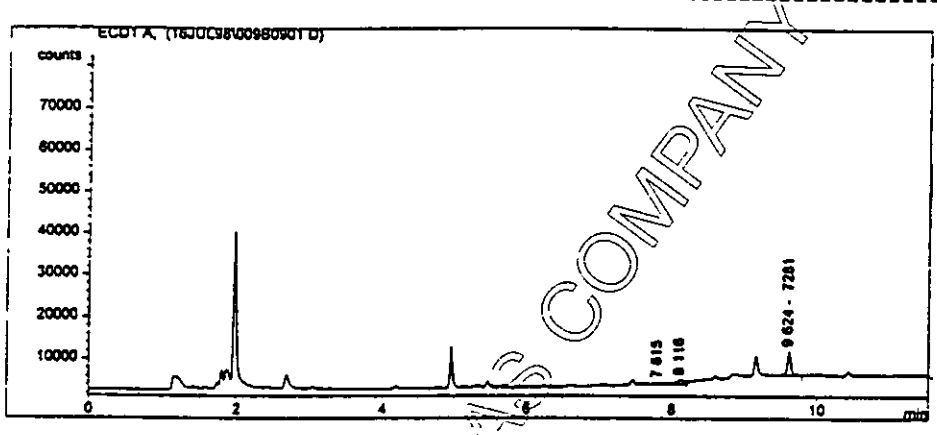
Figure 20 Fortification Grape Juice at 0.050 ppm (GC/ECD
Found: 0.0488 ppm Recovery = 97.6 %

PROPERTY OF ROHM AND HAAS COMPANY, PHILADELPHIA

Data File C.\HPCHEM\MKL00590\DATA\7281\16JUL98\009B0901.D Sample Name: GJC#2
34P-97-18B 7281 SET#GJ1
~~GJClarified#2~~ GJclarified #2 (SP) 7/17/98
Final volume 10 ml

① RAR#: 96-0207; Sample weight = 10 Dg

Injection Date : 7/16/98 9:26:23 PM Seq. Line : 9
Sample Name : GJC#2 Vial : 9
Acq. Operator : LH 34P-97-18B Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\HPCHEM\MKL00590\METHODS\7281ME.M
Last changed : 7/16/98 10:21:58 AM by LH 34P-97-18B
Analysis Method : C:\HPCHEM\MKL00590\METHODS\7281ANL.M
Last changed : 7/17/98 7:51:55 AM by LH 34P-97-18B
RH7281 ON-COLUMN INJECTION Method



External Standard Report

Sorted By : Retention Time
Calib. Data Modified : 7/17/98 7:51:50 AM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 A,
Uncalibrated Peaks : compound name not specified

RetTime [min]	Sig	Type	Area counts*s	Amt/Area	Amount [µg/mL]	Grp	Name
7.815	1	BV	3210.74805	0.00000	0.00000	?	
8.116	1	VV	1.06604e4	0.00000	0.00000	?	
9.624	1	BBA	1.97129e4	7.26926e-7	1.43298e-2	7281	
Totals :					1.43298e-2		

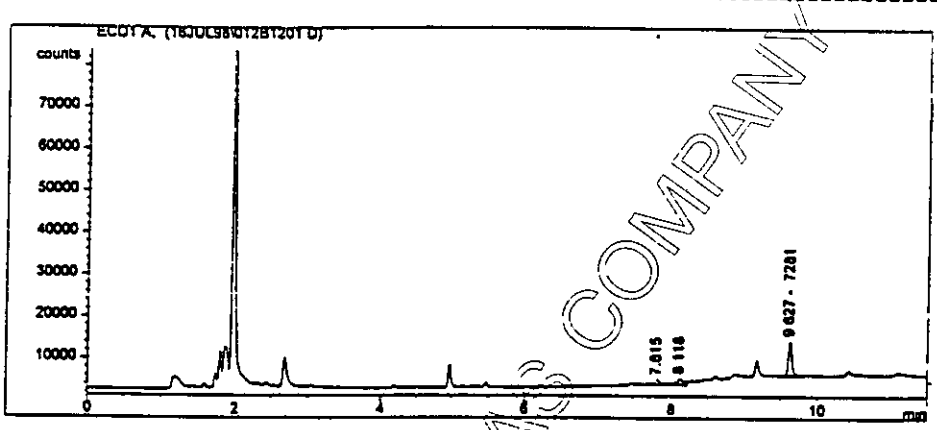
Results obtained with enhanced integrator!

Figure 21 Treated Grape Juice (Clarified) Sample #2 (RAR 96-0207)
1.25 lb/A; Analysis 7/16/98; Found: 0.0143 ppm (GC/ECD)

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Data File C:\HPCHEM\MKL00590\DATA\7281\16JUL98\012B1201.D Sample Name: GJC#3
34P-97-18B 7281 SET#GJ1
~~GJClarified#3~~ GJclarified#3
Final volume 10 ml
(SP) W17 J-193
(1) RAR#: 96-0207; Sample weight = 10.0g

Injection Date : 7/16/98 10:16:57 PM Seq. Line : 12
Sample Name : GJC#3 Vial : 12
Acq. Operator : LH 34P-97-18B Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\HPCHEM\MKL00590\METHODS\7281ME.M
Last changed : 7/16/98 10:21:58 AM by LH 34P-97-18B
Analysis Method : C:\HPCHEM\MKL00590\METHODS\7281ANL.M
Last changed : 7/17/98 7:51:55 AM by LH 34P-97-18B
RH7281 ON-COLUMN INJECTION Method



External Standard Report

Sorted By : Retention Time
Calib. Data Modified : 7/17/98 7:51:50 AM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 A,
Uncalibrated Peaks : compound name not specified

RetTime [min]	Sig	Type	Area counts*s	Amt/Area	Amount [µg/mL]	Grp	Name
7.815	1	BV	5631.65332	0.00000	0.00000	?	
8.118	1	VV	1.17955e4	0.00000	0.00000	?	
9.627	1	BBA	2.90445e4	7.27073e-7	2.11175e-2		7281
Totals :					2.11175e-2		

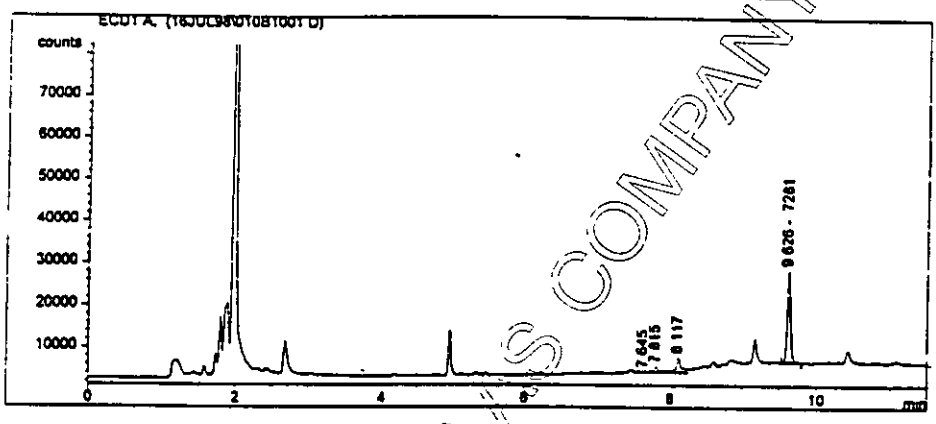
Results obtained with enhanced integrator!

Figure 22 Treated Grape Juice (Clarified) Sample #3 (RAR 96-0207)
2.5 lb/A; Analysis 7/16/98; Found: 0.0211 ppm (GC/ECD)

ROHM AND HAAS COMPANY PHILADELPHIA

Data File C \HPCHEM\MKL00590\DATA\7281\16JUL98\010B1001.D Sample Name: GJUC#2
34P-97-18B 7281 SET#GJ1
~~GJUClarified#2~~ GJUnclarified#2
Final volume 10 ml
(38) LH17J-148
① RAR#: 96-0207 ; Sample weight = 10.0g

Injection Date : 7/16/98 9:43:14 PM Seq. Line : 10
Sample Name : GJUC#2 Vial : 10
Acq. Operator : LH 34P-97-18B Inj : 1
Inj Volume : 1 µl
Acq. Method : C:\HPCHEM\MKL00590\METHODS\7281ME.M
Last changed : 7/16/98 10:21:58 AM by LH 34P-97-18B
Analysis Method : C:\HPCHEM\MKL00590\METHODS\7281ANL.M
Last changed : 7/17/98 7:51:55 AM by LH 34P-97-18B
RH7281 ON-COLUMN INJECTION Method



External Standard Report

Sorted By : Retention Time
Calib. Data Modified : 7/17/98 7:51:50 AM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 A,
Uncalibrated Peaks : compound name not specified

RetTime [min]	Sig	Type	Area counts*s	Amt/Area	Amount [µg/mL]	Grp	Name
7.645	1	BP	236.28250	0.00000	0.00000	?	
7.815	1	VV	5694.57520	0.00000	0.00000	?	
8.117	1	VV	1.96397e4	0.00000	0.00000	?	
9.626	1	BBA	6.88160e4	7.27252e-7	5.00465e-2		7281

Totals : 5.00465e-2

Results obtained with enhanced integrator!

Figure 23 Treated Grape Juice (Unclarified) Sample #2 (RAR 96-0207) 1.25 lb/A; Analysis 7/16/98; Found: 0.0500 ppm (GC/ECD)

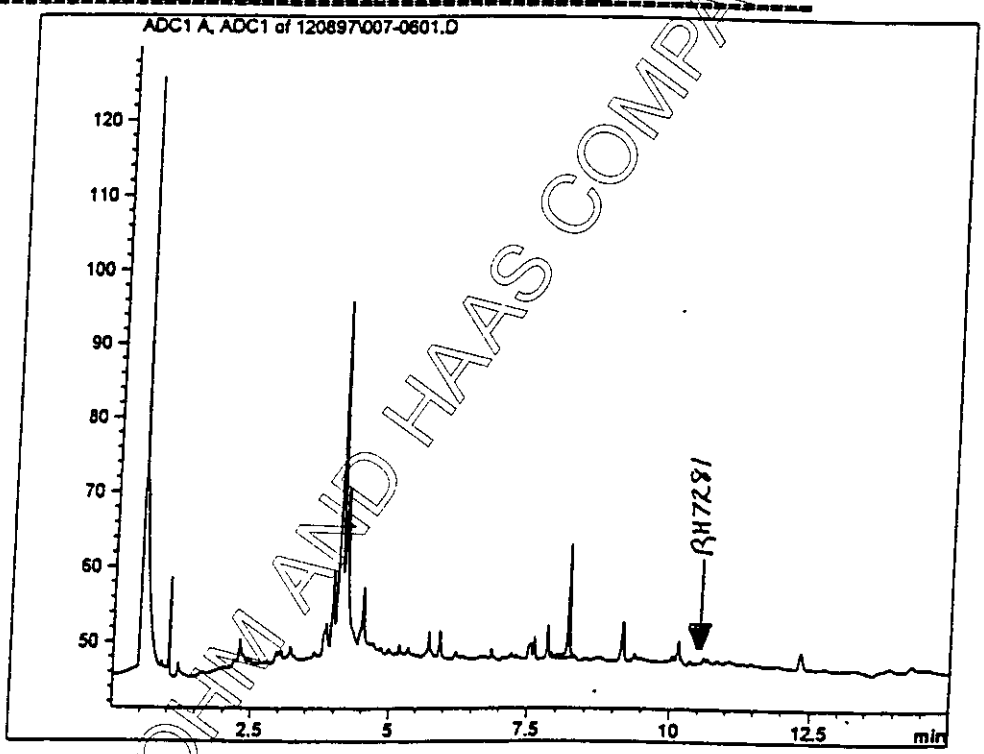
PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Data file: 007-0601.D RAR Number: -
Method file: GC7281G.M Sample No: 2
Type: Control Component: golden raisin

Sample Name: Cal. Curve: ~~raisin dilute~~ 12-1-97 MAK 1-9-98
Date: 12/8/97 6:28:49 PM Method: GC7281G.M Analyst: mak
Interface 301\

Instrument: Varian GC3500 #1 Column RXT-1
Particle Size(um): 5 Col.Length(cm): 15 Col.I.D.(mm) 0.25

Start Temp-Time (deg-min) 100/1 Ramp Hold (deg-min)
Program Rate (deg/min) 20 End Time-Temp (deg-min) 250/3
Prog Slope (# or Linear) linear Inj Port Temp: 150
Flowrate/Gas: 1.0/Nitrogen Split Ratio: none
Det 1-Type/Temp: ECD/300 Injection Volume 1



Ret. Time	Cpd. Name	Peak Area	Peak Height	Volume (ml)	Samp. Wt.
0.00	RH7281	0.000	0.000	10	5

Figure 24 Control Raisin (GC/ECD)

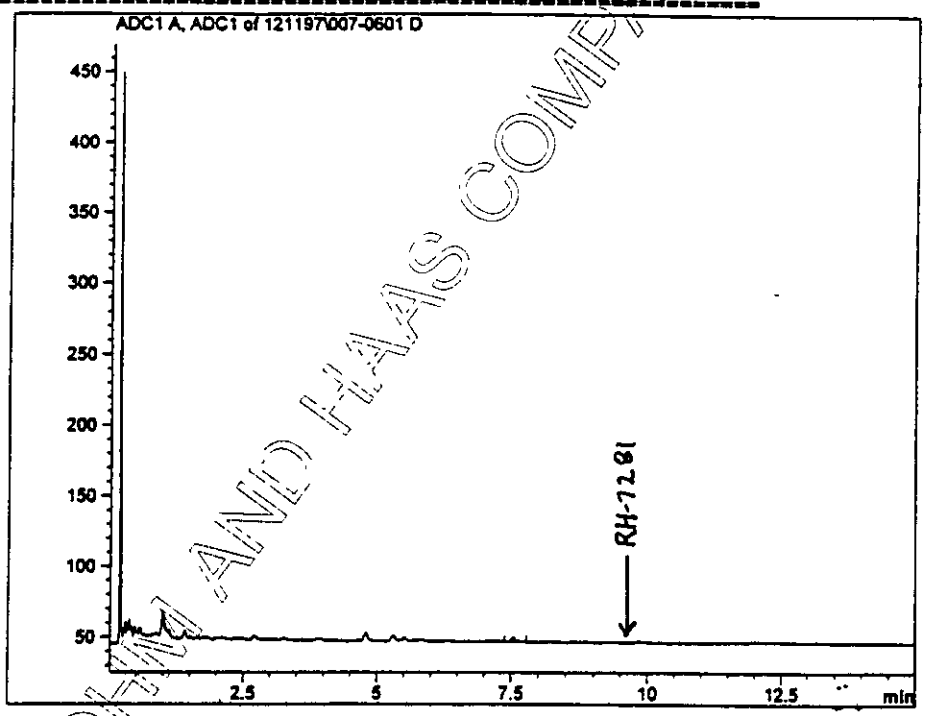
PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Data file: 007-0601.D RAR Number: -
Method file: GC7281G.M Sample No: 2
Type: Control Component: raisin

Sample Name: Cal. Curve: ~~raisin~~ 12-11-96 MAK 2-9-97
Date: 12/12/97 6:13:06 PM Method: GC7281G.M Analyst: mak
Interface 301\

Instrument: Varian GC3500 #2 Column RXT-1
Particle Size(um): 5 Col.Length(cm): 15 Col.I.D.(mm) 0.25

Start Temp-Time (deg-min) 100/1 Ramp Hold (deg-min)
Program Rate (deg/min) 20 End Time-Temp (deg-min) 250/3
Prog Slope (# or Linear) linear Inj Port Temp: 150
Flowrate/Gas: 1.0/Nitrogen Split Ratio: none
Det 1-Type/Temp: ECD/300 Injection Volume 1

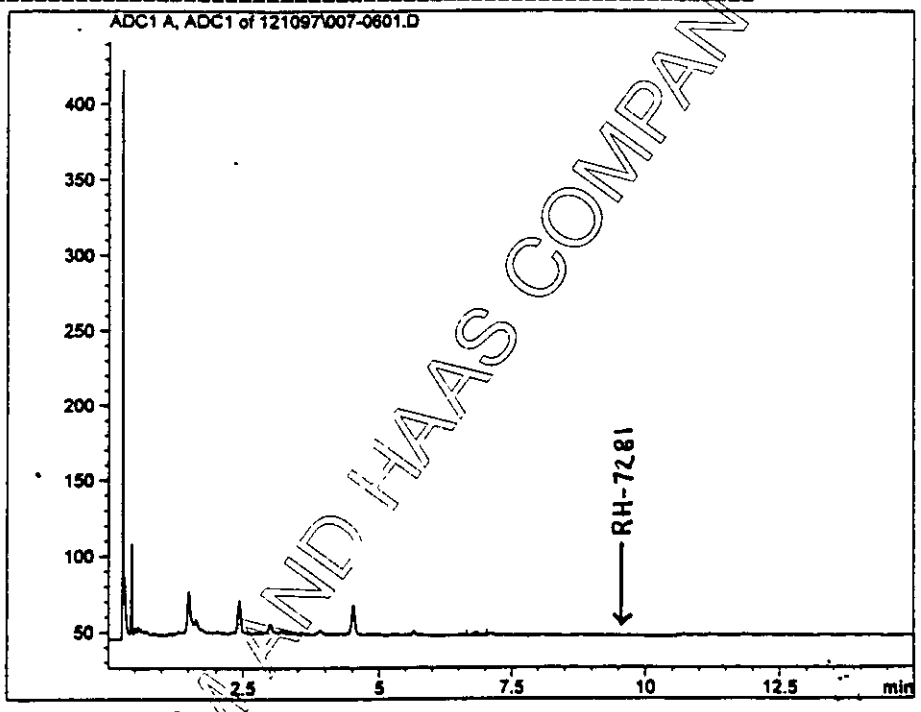


Ret. Time	Cpd. Name	Peak Area	Peak Height	Volume (ml)	Samp. Wt.
0.00	RH7281	0.000	0.000	10	5

Figure 25 Control Raisin (GC/ECD)

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

```
Data file;  007-0601.D     RAR Number:   -
Method file:  GC7281G.M       Sample No:  2
Type: Control              Component: golden raisin
==========================================================
Sample Name:              Cal. Curve: raisin 12-10-97 mak 2-5-98
Date:  12/10/97 7:20:33 PM Method:  GC7281G.M Analyst:  mak
Interface  301\
==========================================================
Instrument:  Varian GC3500 #2          Column RXT-1
Particle Size(um):  5 Col.Length(cm):  15 Col.I.D.(mm) 0.25
==========================================================
Start Temp-Time (deg-min)    100/1    Ramp Hold (deg-min)
Program Rate (deg/min)    20  End Time-Temp (deg-min)    250/3
Prog Slope (# or Linear)    linear      Inj Port Temp:    150
Flowrate/Gas:    1.0/Nitrogen      Split Ratio:    none
Det 1-Type/Temp:    ECD/300     Injection Volume    1
==========================================================
```



Ret. Time	Cpd. Name	Peak Area	Peak Height	Volume (ml)	Samp. Wt.
0.00	RH7281	0.000	0.000	10	5

Figure 26 Control Raisin (GC/ECD)

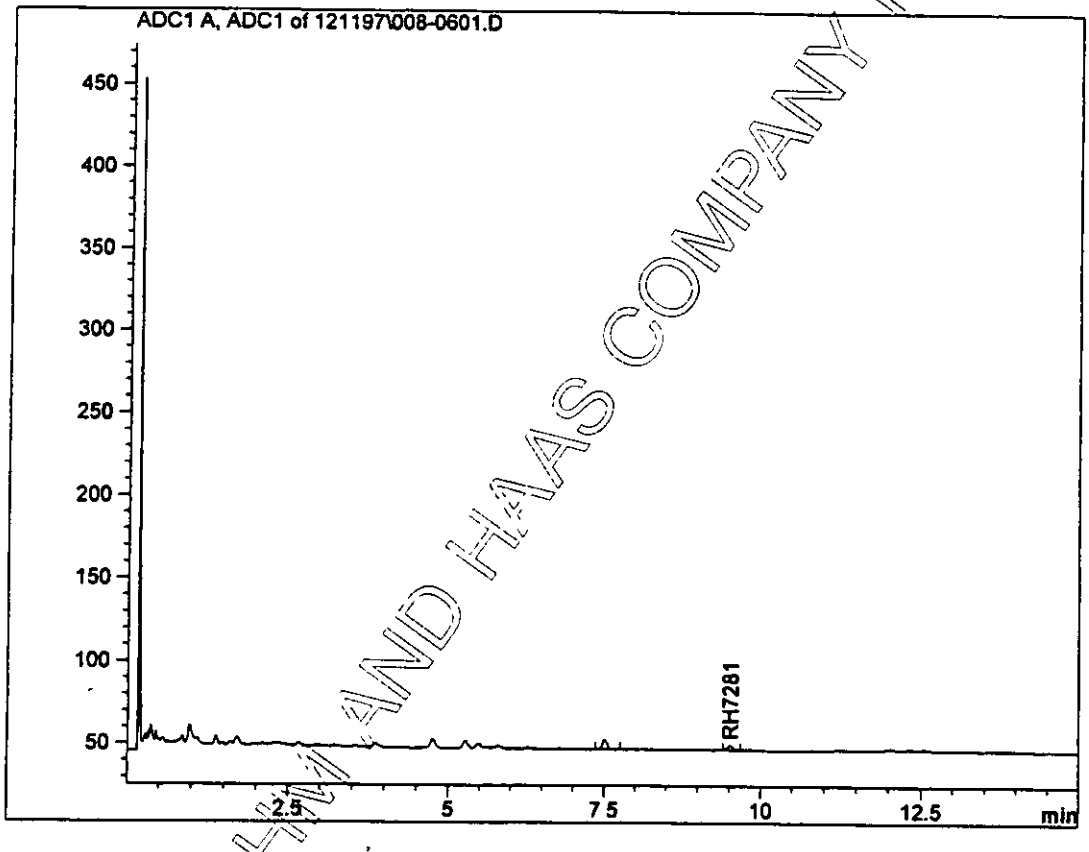
PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Data file: 008-0601.D RAR Number: -
Method file: GC7281G.M Sample No: 3
Type: Fortification Component: raisin

Sample Name: Cal. Curve. raisin 12-11-97 MMK 2-9-98
Date. 12/12/97 6:36:43 PM Method. GC7281G.M Analyst: mak
Interface 301\

Instrument: Varian GC3500 #2 Column RXT-1
Particle Size(um): 5 Col.Length(cm): 15 Col.I.D.(mm) 0.25

Start Temp-Time (deg-min) 100/1 Ramp Hold (deg-min)
Program Rate (deg/min) 20 End Time-Temp (deg-min) 250/3
Prog Slope (# or Linear) linear Inj Port Temp: 150
Flowrate/Gas: 1.0/Nitrogen Split Ratio: none
Det 1-Type/Temp: ECD/300 Injection Volume 1



Ret. Time	Cpd. Name	Peak Area	Peak Height	Volume (ml)	Samp. Wt.	ug Added
9.52	RH7281	19.888	2.989	10	5	0.100

Figure 27 Fortification Raisin at 0.010 ppm (GC/ECD)
Found: 0.0098 ppm Recovery = 98.4 %

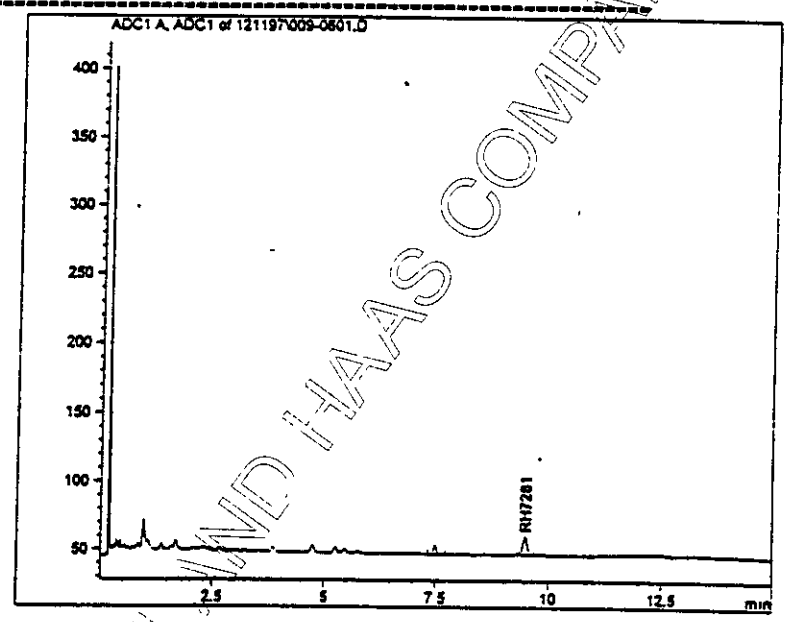
PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Data file: 009-0601.D RAR Number: -
Method file: GC7281G.M Sample No: 4
Type: Fortification Component: raisin

Sample Name: Cal. Curve: ~~raisin~~ 12-11-97 - MW2 2-4-98
Date: 12/12/97 7:00:23 PM Method: GC7281G.M Analyst: mak
Interface 301\

Instrument: Varian GC3500 #2 Column RXT-1
Particle Size(um): 5 Col.Length(cm): 15 Col.I.D.(mm) 0.25

Start Temp-Time (deg-min) 100/1 Ramp Hold (deg-min)
Program Rate (deg/min) 20 End Time-Temp (deg-min) 250/3
Prog Slope (# or Linear) linear Inj Port Temp: 150
Flowrate/Gas: 1.0/Nitrogen Split Ratio: none
Det 1-Type/Temp: ECD/300 Injection Volume 1



Ret. Time	Cpd. Name	Peak Area	Peak Height	Volume (ml)	Samp. Wt.	ug Added
9.50	RH7281	89.858	13.779	10	5	0.500

Figure 28 Fortification Raisin at 0.050 ppm (GC/ECD)
Found: 0.0098 ppm Recovery = 90.4 %

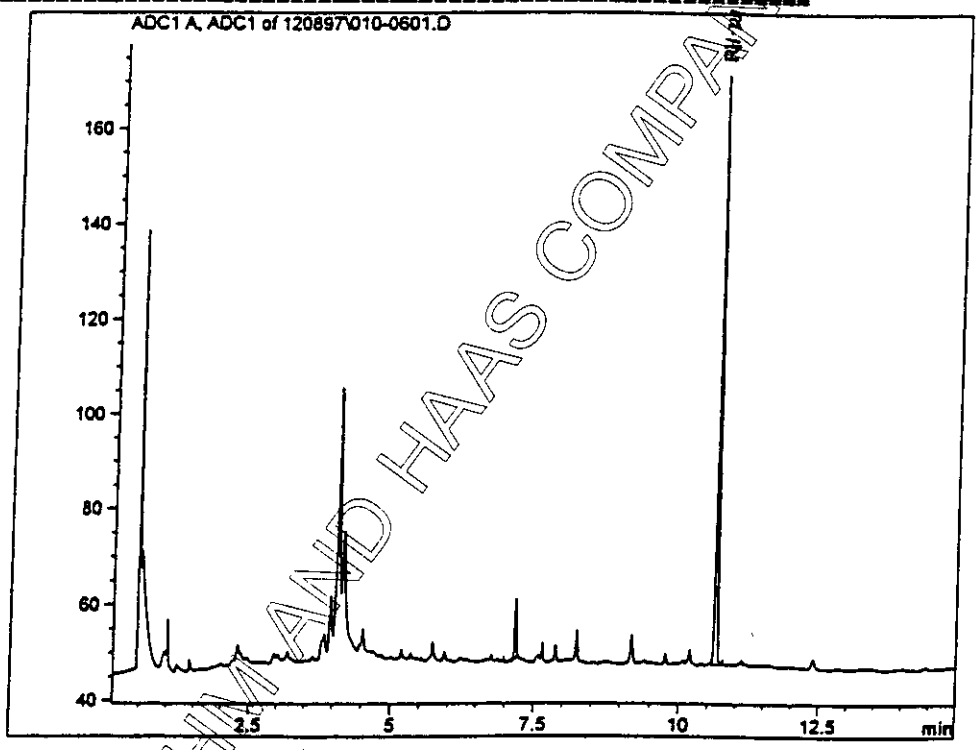
PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Data file: 010-0601.D RAR Number: -
Method file: GC7281G.M Sample No: 5
Type: Fortification Component: golden raisin

Sample Name: Cal. Curve: ~~raisin dilute~~ 12-8-97 MAK 2-4-98
Date: 12/8/97 7:57:44 PM Method: GC7281G.M Analyst: mak
Interface 301\

Instrument: Varian GC3500 #1 Column RXT-1
Particle Size(um): 5 Col.Length(cm): 15 Col.I.D.(mm) 0.25

Start Temp-Time (deg-min) 100/1 Ramp Hold (deg-min)
Program Rate (deg/min) 20 End Time-Temp (deg-min) 250/3
Prog Slope (# or Linear) linear Inj Port Temp: 150
Flowrate/Gas: 1.0/Nitrogen Split Ratio: none
Det 1-Type/Temp: ECD/300 Injection Volume 1



Ret. Time	Cpd. Name	Peak Area	Peak Height	Volume (ml)	Samp. Wt.	ug Added
10.63	RH7281	277.610	125.828	10	5	1.000

Figure 29 Fortification Raisin at 0.10 ppm (GC/ECD)
Found: 0.0763 ppm Recovery = 76.3 %

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

```
File        : C:\HPCHEM\1\DATA\072796\STND1.D
Operator    :
Acquired    : 27 Jul 96   7:26 pm using AcqMethod 7281SIM
Instrument  :    GC/MS
Sample Name: 0.01 ppm
Misc Info   :
Vial Number: 2
```

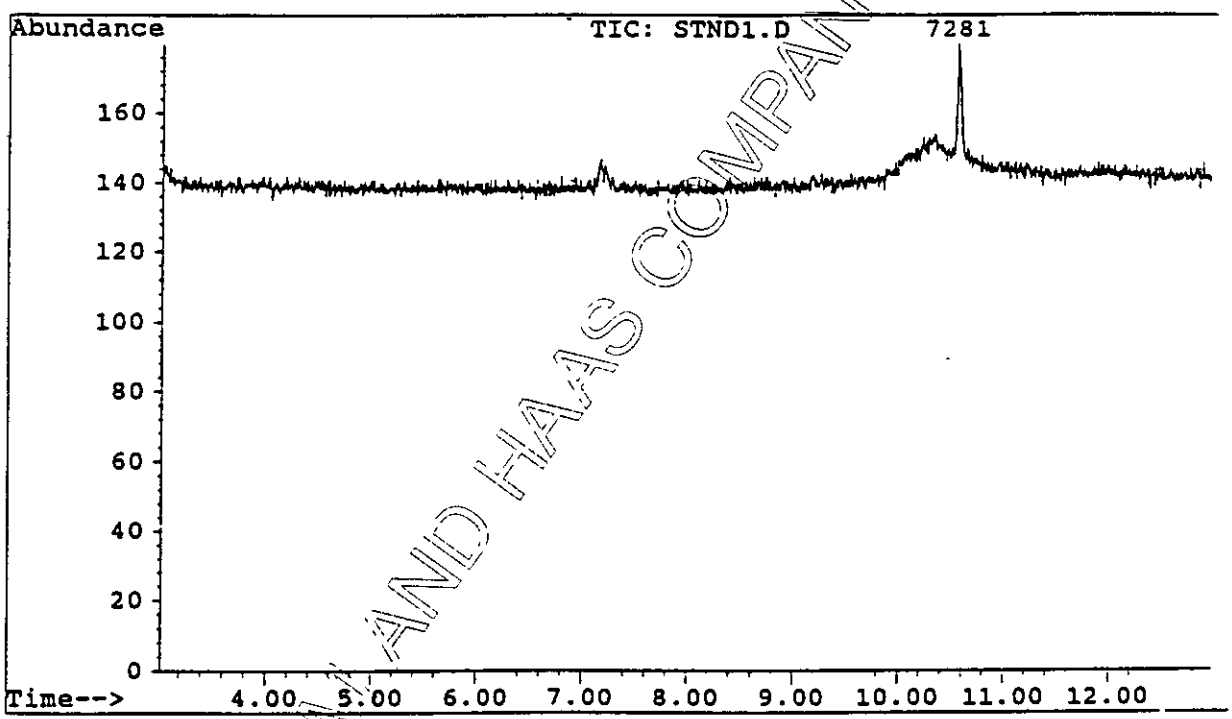


Figure 30 0.010 µg/ml RH-7281 Standard (GC/MSD)
Peak Area = 801.1

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

```
File        : C:\HPCHEM\1\DATA\072796\STND2.D
Operator    :
Acquired    : 27 Jul 96   7:45 pm using AcqMethod 7281SIM
Instrument  :    GC/MS
Sample Name: 0.02 ppm
Misc Info   :
Vial Number: 3
```

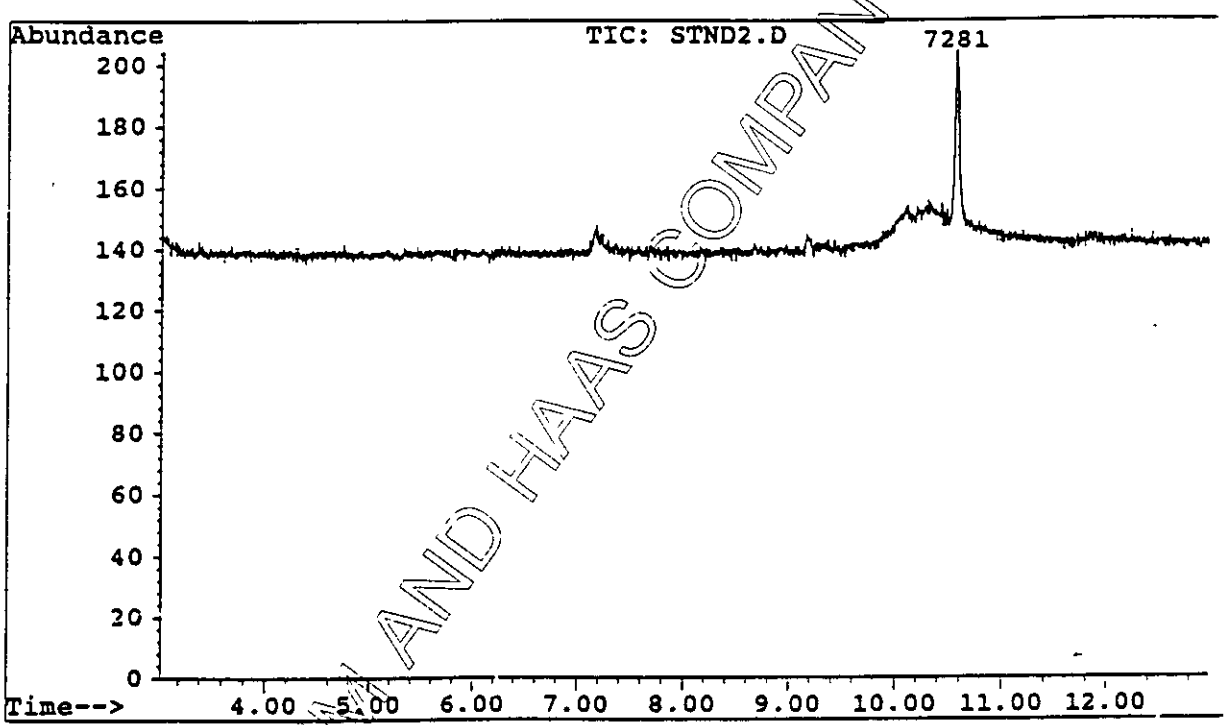


Figure 31 0.020 µg/ml RH-7281 Standard (GC/MSD)
Peak Area = 1727

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

```
File        : C:\HPCHEM\1\DATA\072796\STND3.D
Operator    :
Acquired    : 27 Jul 96   8:04 pm using AcqMethod 7281SIM
Instrument  :    GC/MS
Sample Name: 0.05 ppm
Misc Info   :
Vial Number: 4
```

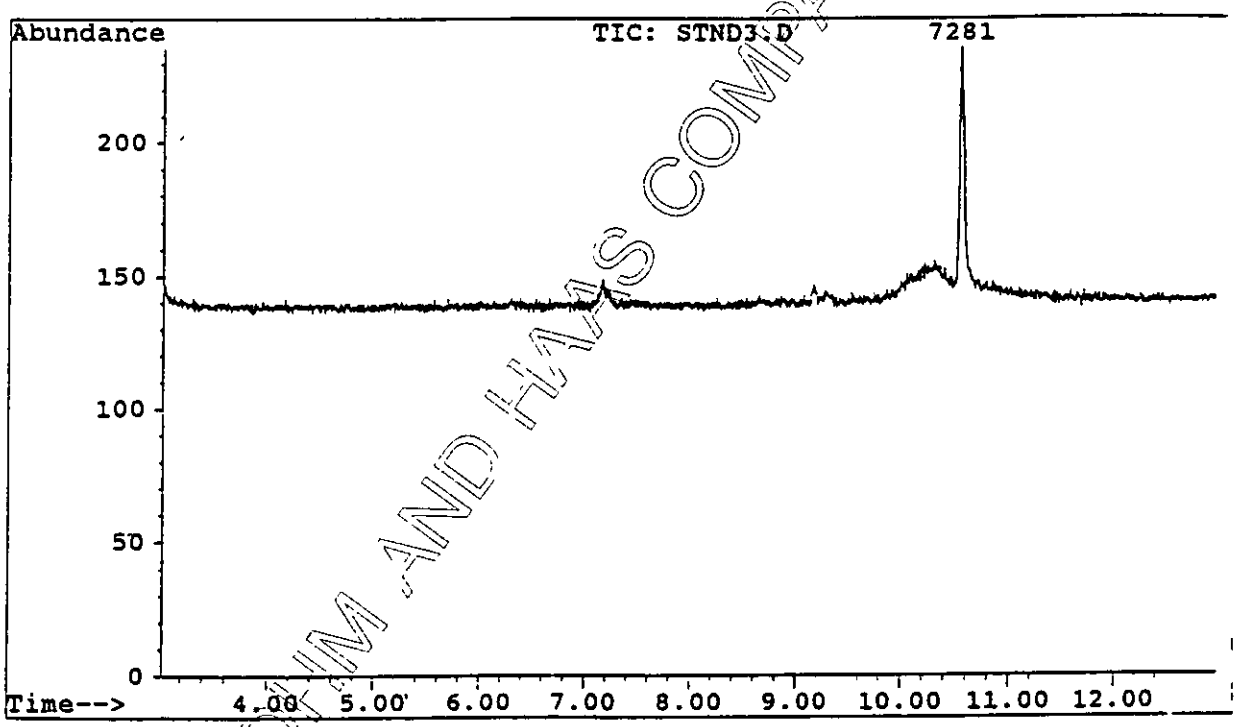


Figure 32 0.050 µg/ml RH-7281 Standard (GC/MSD)
Peak Area = 2804

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

File : C:\HPCHEM\1\DATA\072796\STND4.D
Operator :
Acquired : 27 Jul 96 8:23 pm using AcqMethod 7281SIM
Instrument : GC/MS
Sample Name: 0.10 ppm
Misc Info :
Vial Number: 5

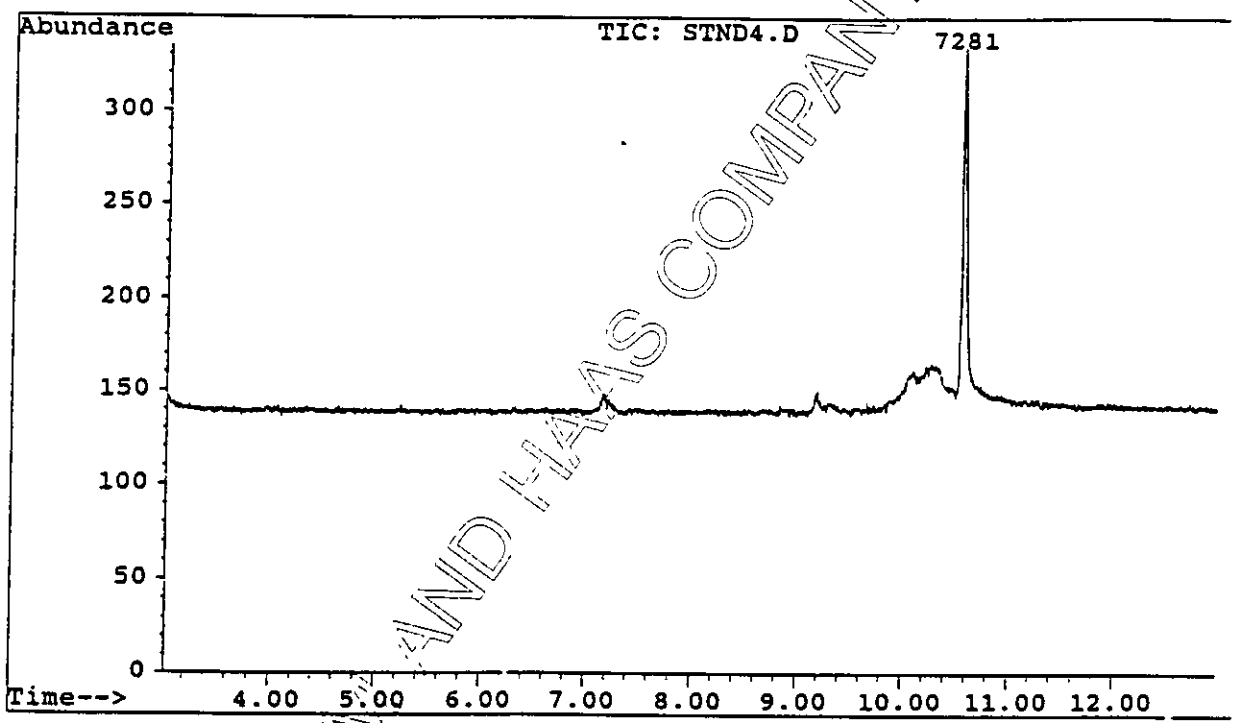


Figure 33 0.10 µg/ml RH-7281 Standard (GC/MSD)
Peak Area = 5121

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

```
File        : C:\HPCHEM\1\DATA\072796\STND5.D
Operator    :
Acquired    : 27 Jul 96   8:42 pm using AcqMethod 7281SIM
Instrument  :    GC/MS
Sample Name: 0.50 ppm
Misc Info   :
Vial Number: 6
```

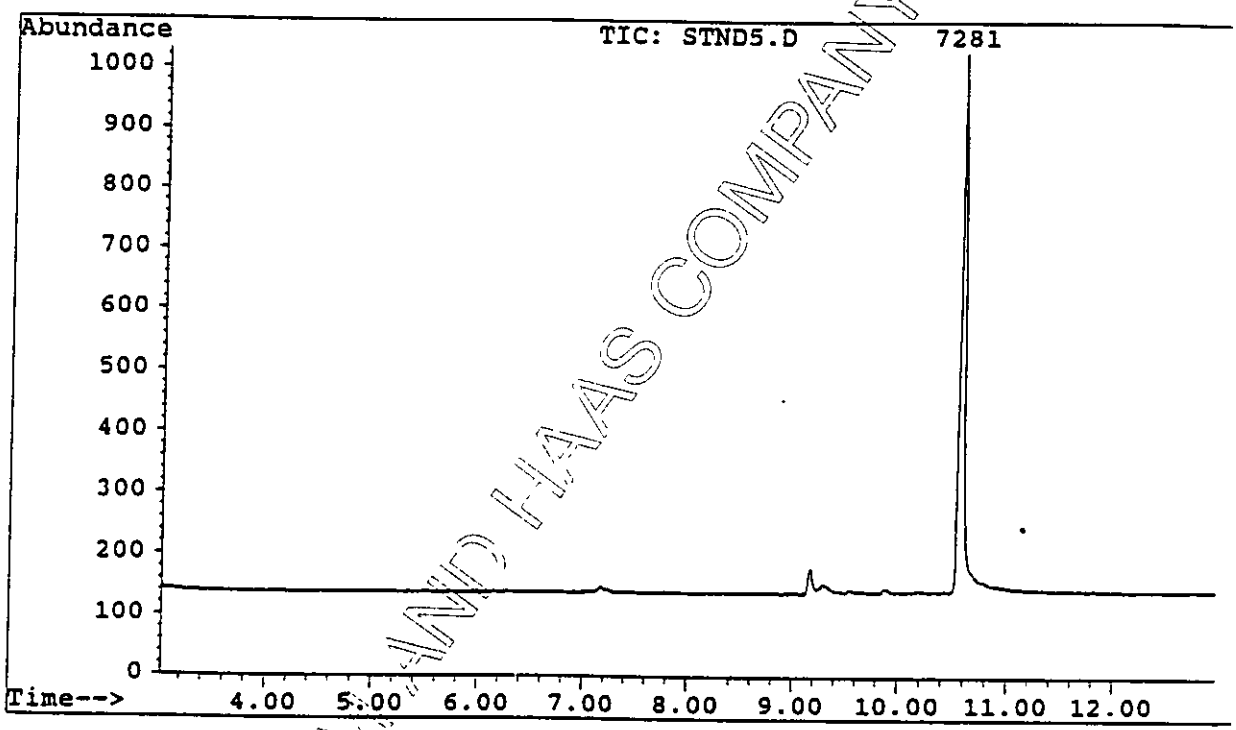


Figure 34 0.50 µg/ml RH-7281 Standard (GC/MSD)
Peak Area = 25410

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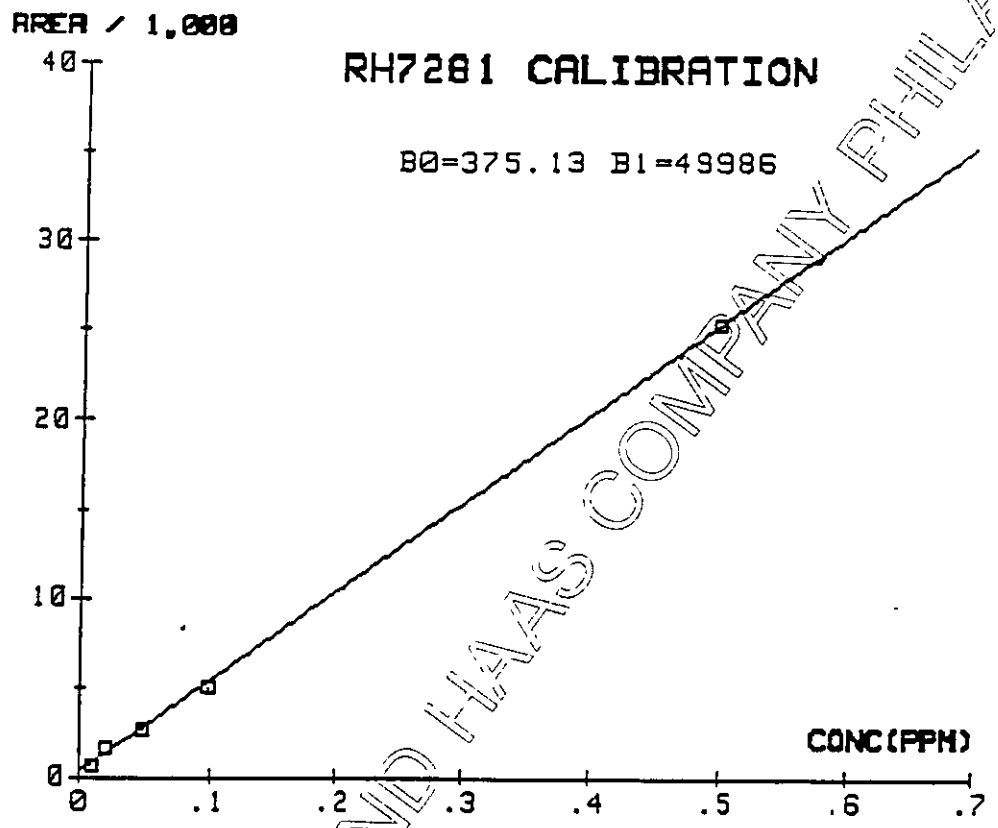

Concentrations in report are calculated from equation:
AREA = B0 + B1(CONC(PPM))
obtained by least-squares fit of standard injection data.

Figure 35 Standard Curve for RH-7281 Standard (GC/MSD)
$r^2 = 0.9996$

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```
File        : C:\HPCHEM\1\DATA\072596\CONTR1.D
Operator    :
Acquired    : 25 Jul 96  10:14 pm using AcqMethod 7281SIM
Instrument  :    GC/MS
Sample Name: ctrl
Misc Info   :
Vial Number: 8
```

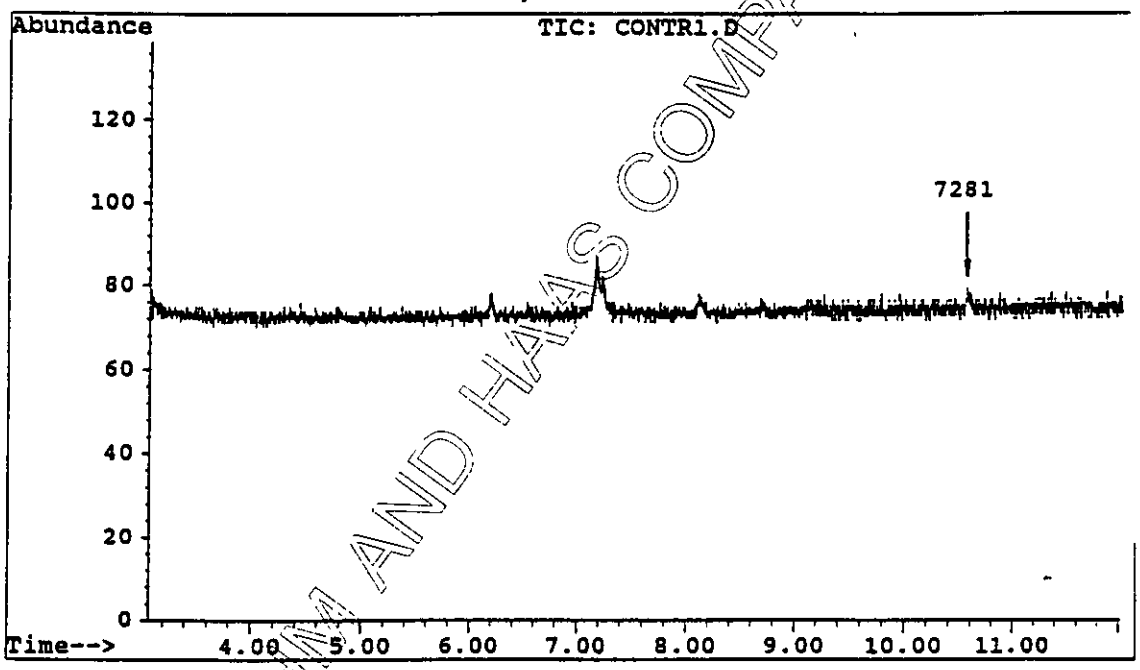

Figure 36 Control Grape (GC/MSD)
Peak Area = 0

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File : C:\HPCHEM\1\DATA\072596\OS3.D
Operator :
Acquired : 25 Jul 96 9:36 pm using AcqMethod 7281SIM
Instrument : GC/MS
Sample Name: OS3
Misc Info :
Vial Number: 6

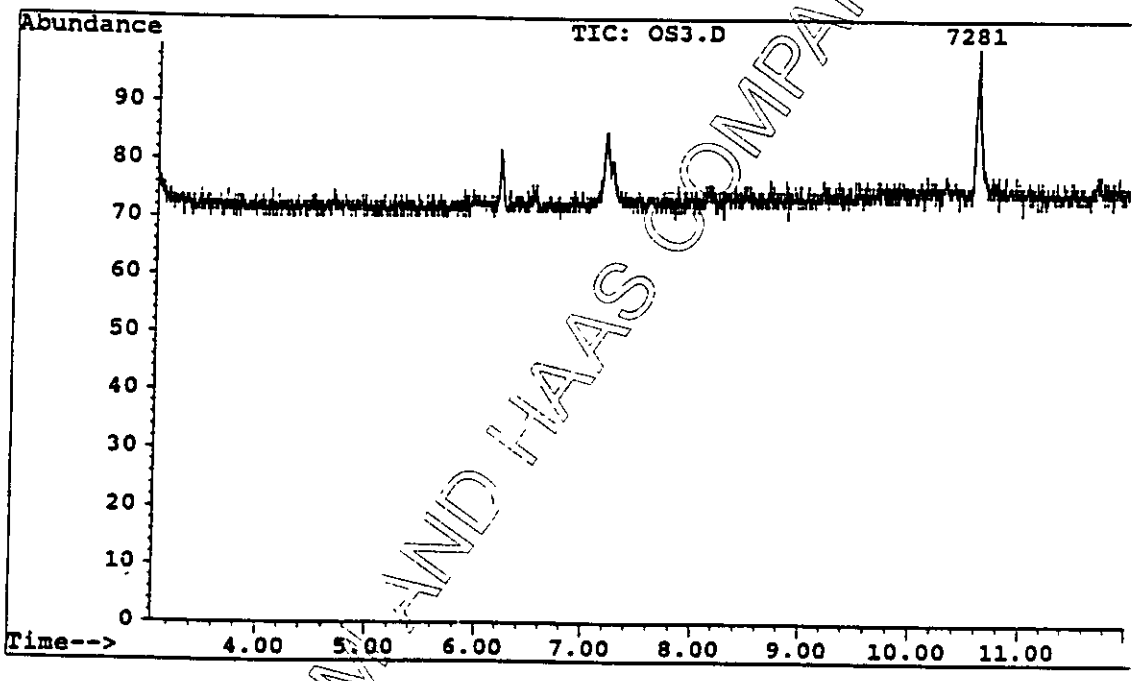

Figure 37 Fortification Grape at 0.010 ppm (GC/MSD)
Found: 0.0096 ppm Recovery = 95.6 %

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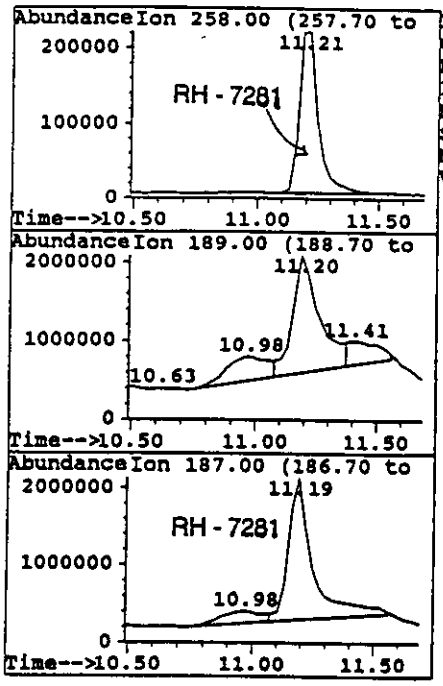
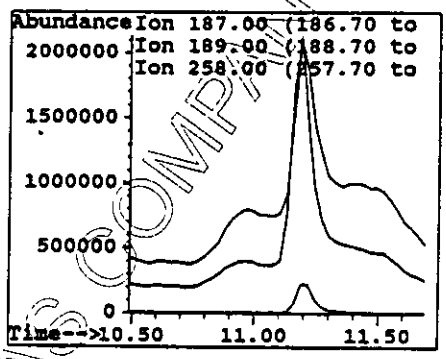


File: C:\HPCHEM\1\DATA\TN070897\1101008
Operator: T.N
Date Acquired: 8 Jul 97 6:54 pm
Instrument: 5971c
Method File: RH7281
Sample Name: E705623-02A, 2.0m
Misc Info: Analysis of RH-72
Vial Number : 11.



Ion 258.00 (257.70 to 258.70): 1101008.D
E705623-02A, 2.0mL, 34P-97-18A (005)

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	11.214	VBA	0.084	11875683	11.071	11.558

Ion 189.00 (188.70 to 189.70): 1101008.D
E705623-02A, 2.0mL, 34P-97-18A (005)

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	10.629	BV	0.083	744650	10.558	10.737
2	10.984	PV	0.168	32268106	10.737	11.077
3	11.204	VV	0.113	112654017	11.077	11.373
4	11.414	VBA	0.169	29393179	11.373	11.558

Ion 187.00 (186.70 to 187.70): 1101008.D
E705623-02A, 2.0mL, 34P-97-18A (005)

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	10.976	PV	0.175	16392665	10.729	11.070
2	11.195	VBA	0.108	130166782	11.070	11.558

Figure 38 Treated Grape Sample #005 (RAR 96-0207) (GC/MSD) 1.25 lb/A; Analysis 7/8/97; Found: 0.241 ppm

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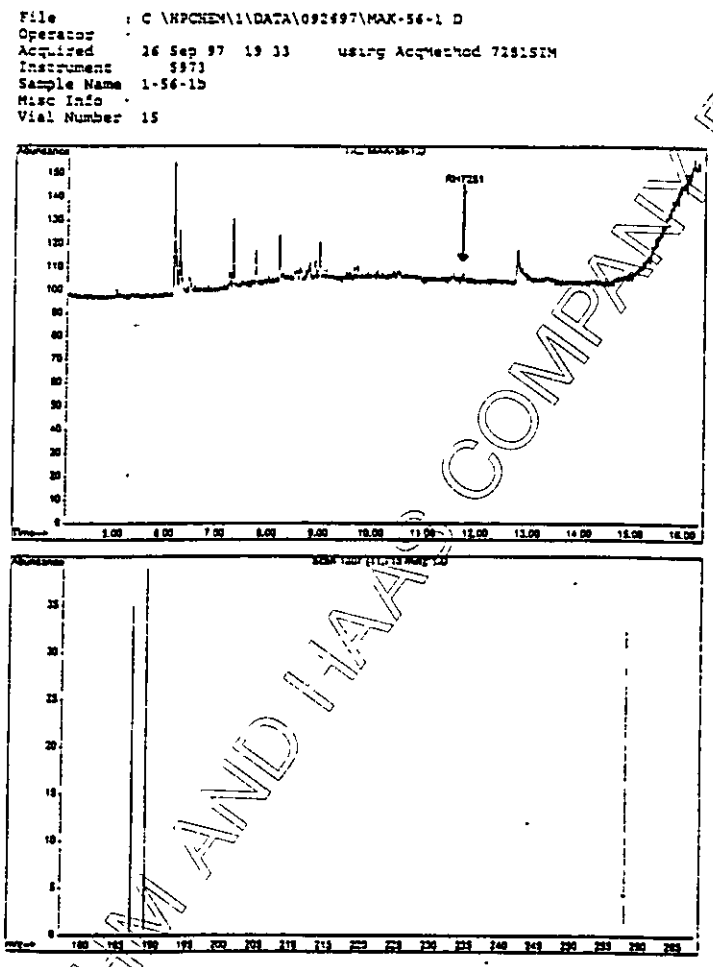

Figure 39 Control Grape Juice (GC/MSD)

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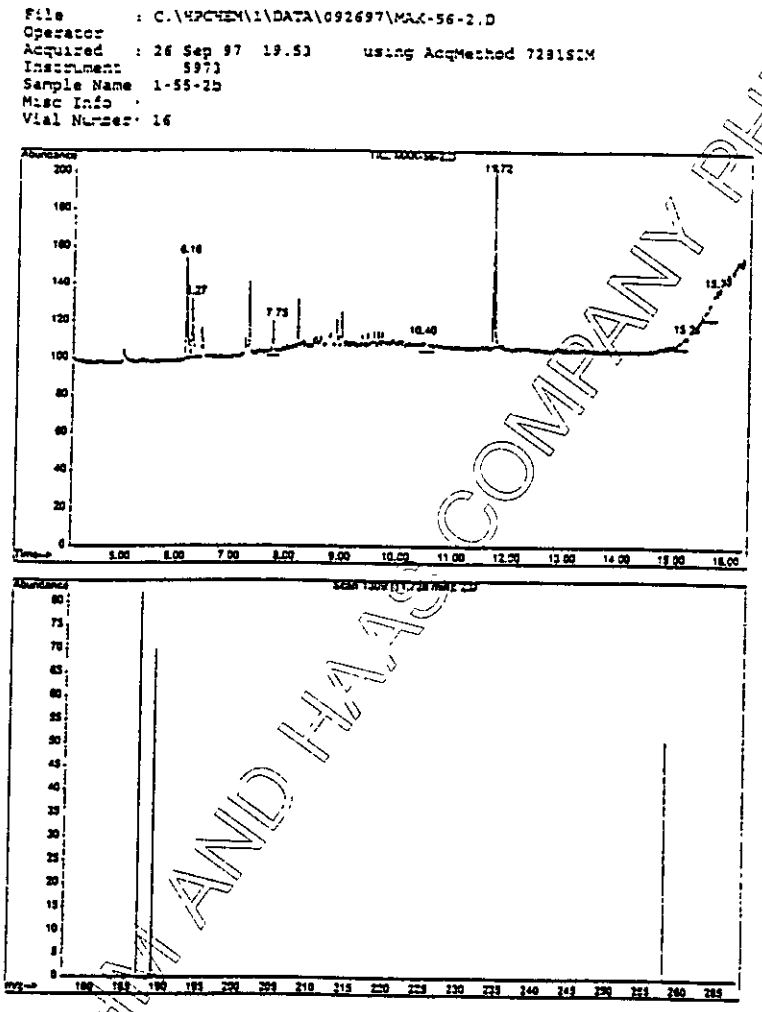


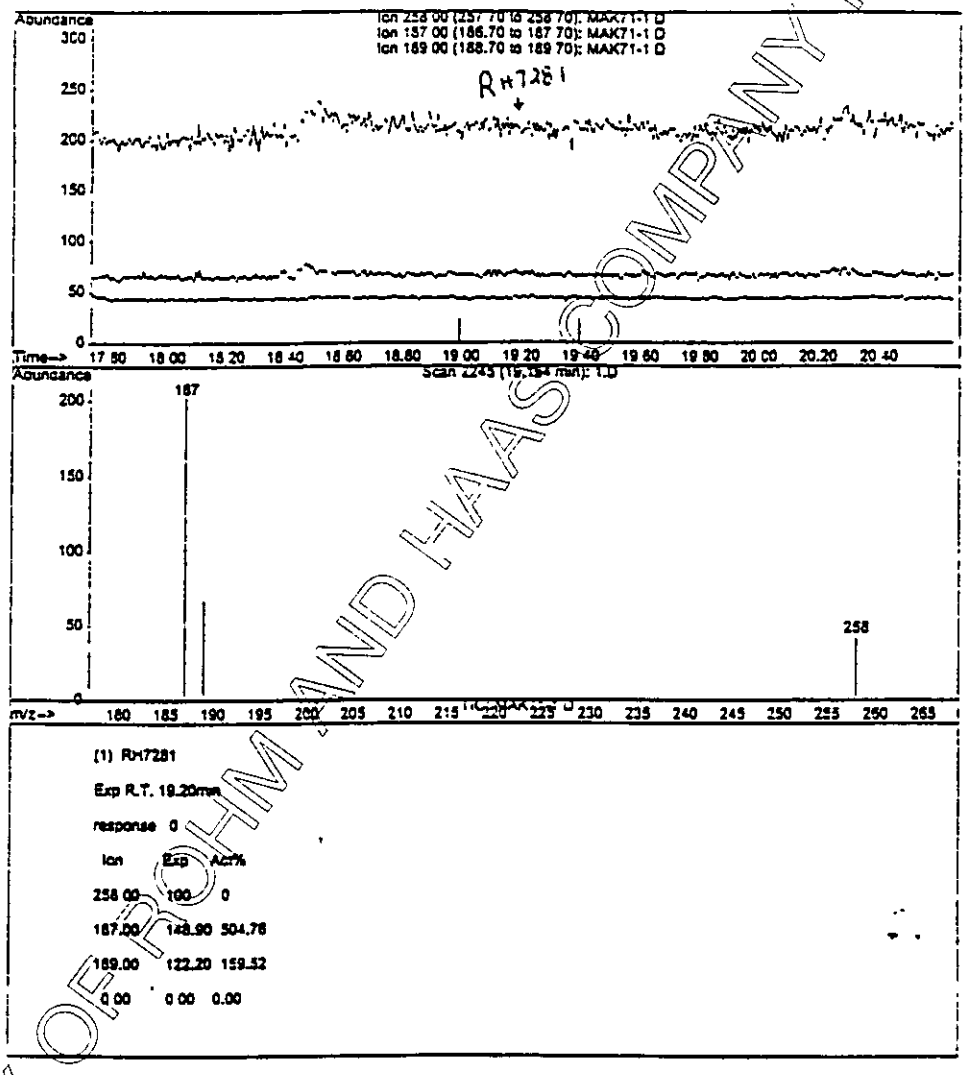
Figure 40 Fortification Grape Juice at 0.010 ppm (GC/MSD)
Found: 0.0107 ppm Recovery = 107 %

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Quantitation Report

Data File : C:\HPCHEM\1\DATA\121497\MAK71-1.D Vial: 7
Acq On : 15 Dec 97 11.30 Operator:
Sample : mak71-1 Inst : 5973
Misc : Multiplr. 1.00
Sample Amount: 0.00

MS Integration Params: rteint.p
Method : C:\HPCHEM\1\METHODS\7281.M (RTE Integrator)
Title : RH7281
Last Update : Wed Dec 17 10:19:40 1997
Response via : Initial Calibration



MAK71-1.D 7281.M Mon Dec 29 14:00:15 1997

Figure 41 Control Raisin (GC/MSD)

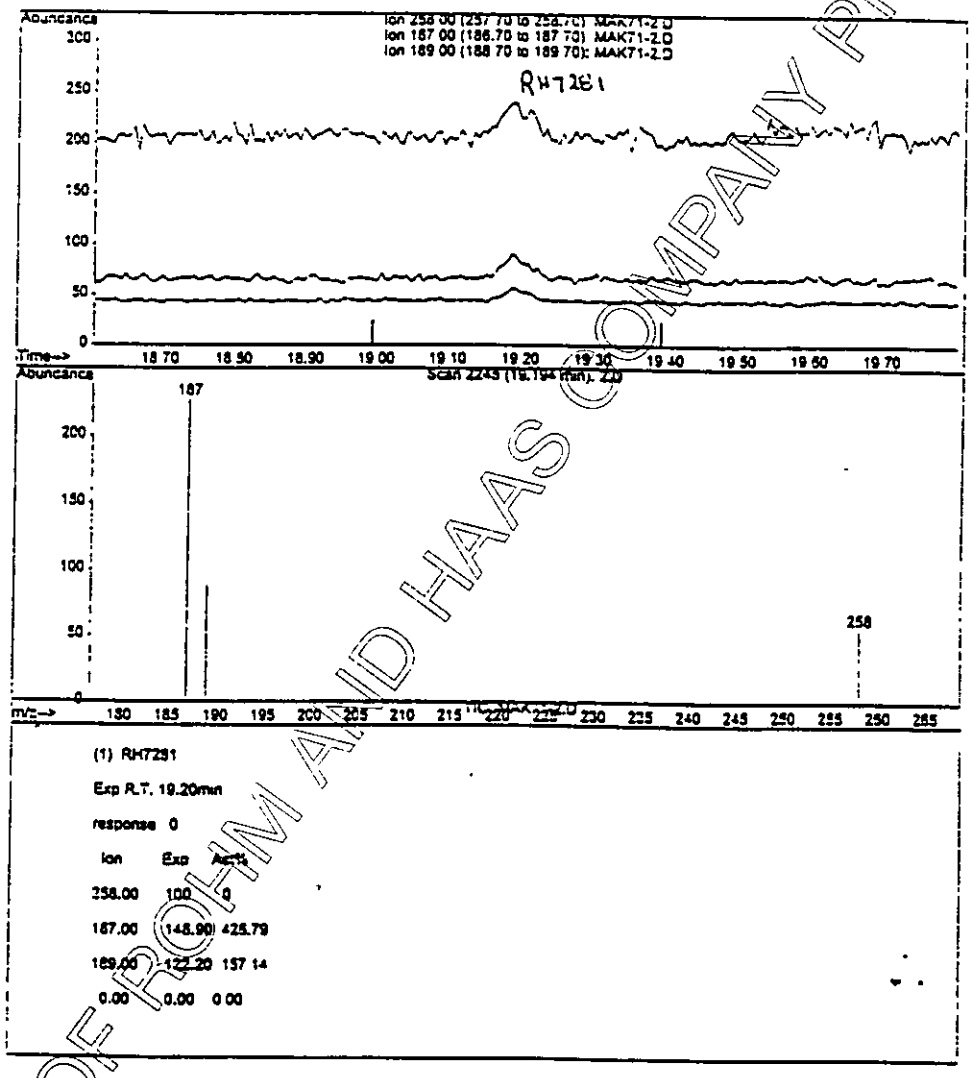
PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Quantitation Report

Data File : C:\HPCHEM\1\DATA\121497\MAK71-2.D
Acq On : 15 Dec 97 12:00
Sample : mak71-2
Misc :

Vial: 8
Operator:
Inst : 5973
Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: rteint.p
Method : C:\HPCHEM\1\METHODS\7281.M (RTE Integrator)
Title : RH7281
Last Update : Wed Dec 17 10:19.40 1997
Response via : Initial Calibration



MAK71-2.D 7281.M Mon Dec 29 14:00:58 1997

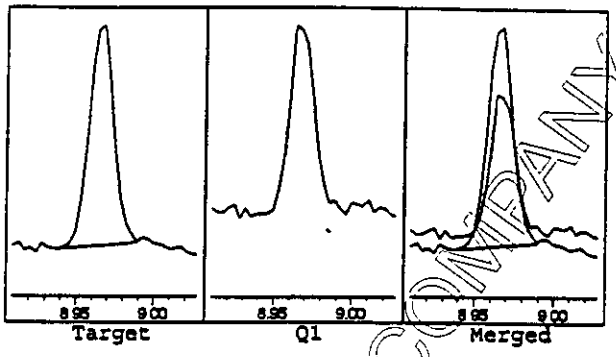
Figure 42 Fortification Raisin at 0.010 ppm (GC/MSD)
Found: 0.0079 ppm Recovery = 78.7 %

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

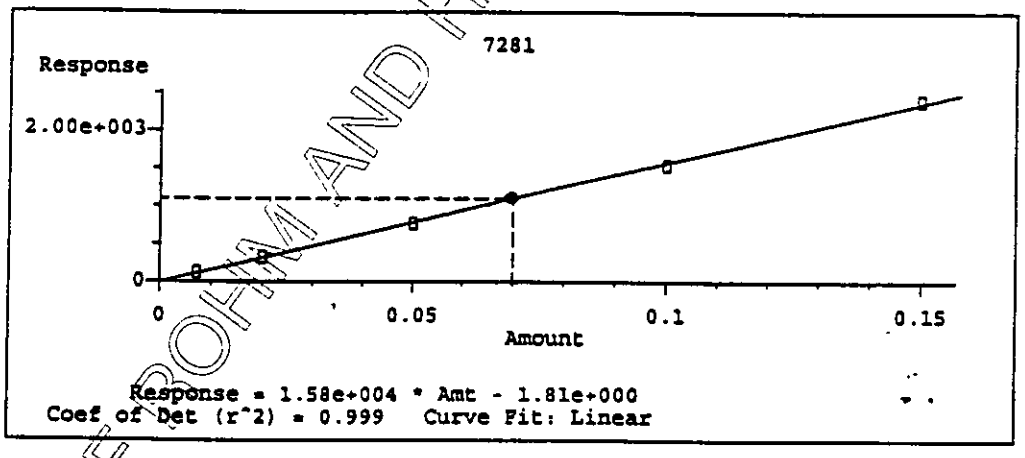
7281

File : C:\HPCHEM\1\DATA\7281G\GRAPEJ\S072598A\S0725A05.D
Operator : LH
Acquired : 25 Jul 1998 3:15 pm using AcqMethod 7281R
Sample Name: Raisin#2 X5 dil
Misc Info : RA1 5ML
Vial Number: 21
CurrentMeth: C:\HPCHEM\1\METHODS\7281R.M
① RAR#: 96-0207; Sample weight = ~~10.0g~~ ② 5.0g

Compound: 7281
Ret Time: 8.97
Concentration: 0.0695 µg/mL
Pk # and Type: 1 m



	Signal	Ratios	Limits		RT	Limits	Resp	Integ Type
Tgt	187.00	100.0%			8.97	8.82	1095	rteint
Q1	189.00	65.3	44.1-	84.1	8.96	to	715	rteint
Q2	0.00	0.0	0.0-	0.0	0.00	9.12	0	sys def
Q3	0.00	0.0	0.0-	0.0	0.00		0	sys def



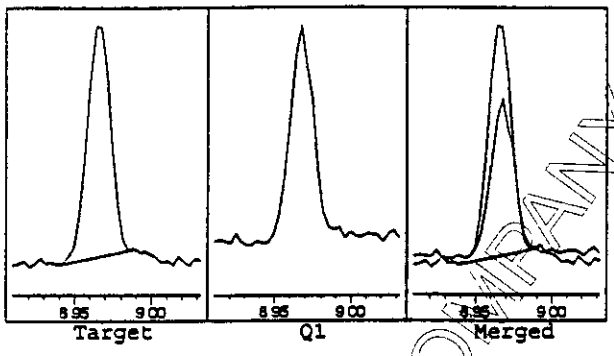
S0725A05.D Tue Jul 28 12:56:36 1998 Page 2

Figure 43 Treated Sample #2 (x5 Diluted) (RAR 96-0207) (GC/MSD) 1.25 lb/A; Analysis 7/22/98; Found: 0.348 ppm

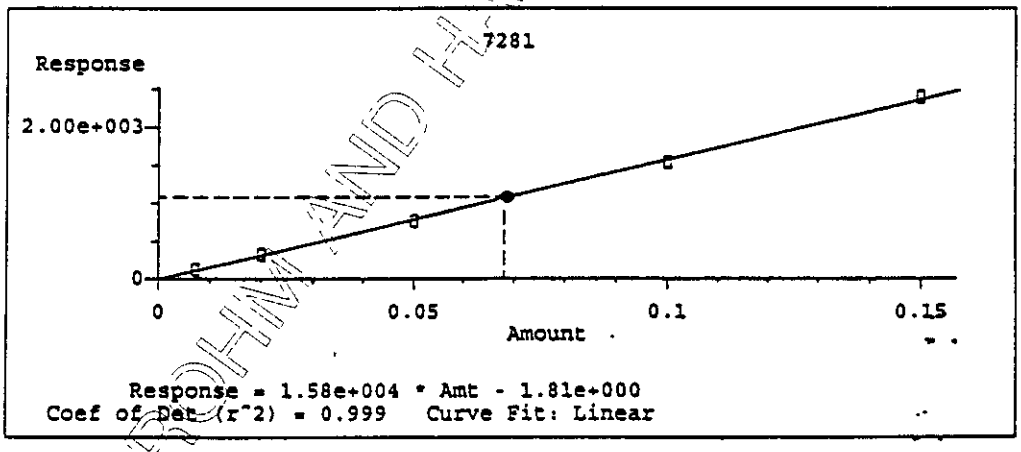
PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

File : C:\HPCHEM\1\DATA\7281G\GRAPEJ\S072598A\S0725A06.D
Operator : LH
Acquired : 25 Jul 1998 3:29 pm using AcqMethod 7281R
Sample Name: Raisin#3 x10 dil
Misc Info : RA1 5ML
Vial Number: 22
CurrentMeth: C:\HPCHEM\1\METHODS\7281R.M
① RAR# 96-0207 ; Sample weight = ~~10.6g~~ ② 5.0g

Compound: 7281
Ret Time: 8.96
Concentration: 0.0684 µg/mL
Pk # and Type: 1 m



	Signal	Ratios	Limits		RT	Limits	Resp	Integ Type
Tgt	187.00	100.0%			8.96	8.82	1078	rteint
Q1	189.00	68.5	44.1-	84.1	8.97	to	738	rteint
Q2	0.00	0.0	0.0-	0.0	0.00	9.12	0	sys def
Q3	0.00	0.0	0.0-	0.0	0.00		0	sys def



S0725A06.D Tue Jul 28 12:48:09 1998 Page 2

Figure 44 Treated Sample #3 (x10 Diluted) (RAR 96-0207) (GC/MSD) 2.5 lb/A; Analysis 7/25/98; Found: 0.684 ppm

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EPA Addendum For Residue Analytical Method
PP#9F5058
Zoxamide on Grapes and Potatoes

1) ACB made minor modifications to the GC-ECD instrument parameters:

GC-ECD - HP 6890 GC, equipped with an ECD Micro.
Injector Temperature - 150°C
Detector Temperature - 300°C
Column Flow - 1 mL/min. He
Column. RTX-5 30 m x 0.25 mm id and 0.25 um film thickness
Make-up gas: 95% Argon/5% Methane at 60 mL/min.

Temperature Program - 80°C for 1 minute, 20°C/min. to 260°C and hold 8 minutes; 30°C/min. to 280°C and hold 1 minute.