US EPA ARCHIVE DOCUMENT

451381-02

STUDY TITLE

Tolerance Enforcement Method for Parent RH-7281 and Its Two Acid Metabolites, RH-1452 and RH-1455, in Potato Peel Waste

DATA REQUIREMENT

Guideline 830.1340

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STUDY COMPLETED ON

June 1, 2000

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Rohm and Haas Technical Report No. 34-00-49

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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).

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GLP Compliance Statement

As per 40 CFR 160.3 method development is not required to be conducted in compliance with GLP. However, the work was conducted in the environment of United States Environmental Protection Agency FIFRA Good Laboratory Practices.

The recovery data from the field residue analysis were obtained under GLP.

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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 Agricultural Research Division and has been validated as a true and accurate representation of the data collected.

QA activities for this study:

Date	Activity	Date Reported
05/04/-05/08/2000	Audit of Preliminary Method	05/08/2000
05/30/2000	Audit of Final Report	05/30/2000

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1. Summary

This tolerance enforcement method determines RH-7281 and its two acid metabolites, RH-1452 and RH-1455, in potato peel waste. This method is a supplement to the tolerance enforcement method for RH-7281 and its two acid metabolites, RH-1452 and RH-1455, in Potato and Potato Processed Fractions (TR 34-98-142).

Since the peel method is very similar to the potato RAC method which was independently validated and radiovalidated, no independent laboratory validation and radiovalidation will be conducted.

As described in the report, RH-7281, RH-1452, and RH-1455 residues are extracted from the matrix by blending with acetonitrile/2% $NaHCO_3$. RH-7281 is separated from RH-1452 and RH-1455 by liquid-liquid partition and then further purified by Florisil solid phase extraction (SPE) and Alumina-B SPE. RH-1452 and RH-1455 are partially purified by liquid-liquid partition and then are methylated to their methyl esters (RH-7391 and RH-7399, respectively). The esters are further purified by Florisil column chromatography.

Quantitation is performed by gas-liquid chromatography using electron capture detection (GC/ECD). The limit of quantitation (LOQ) is 0.020 ppm for all three compounds as established by actual fortifications at this level. The mean recoveries over the range 0.02 to 0.10 ppm are:

	RH-7281	RH-1452	RH-1455
Recovery	90.23±15.10%	90.19±20.24%	87.74±22.26%
Samples	n = 27	n = 27	n = 27

2. Introduction

RH-7281 is a fungicide recently developed by Rohm and Haas Company and is targeted to be used on a variety of crops. This tolerance enforcement method was developed to monitor the residue levels of the parent compound and its metabolites in potato peel waste to establish tolerances for registration of the compound.

This report compiles data from the preliminary analytical method [Ref.1] and data from residue trials [Ref.2].

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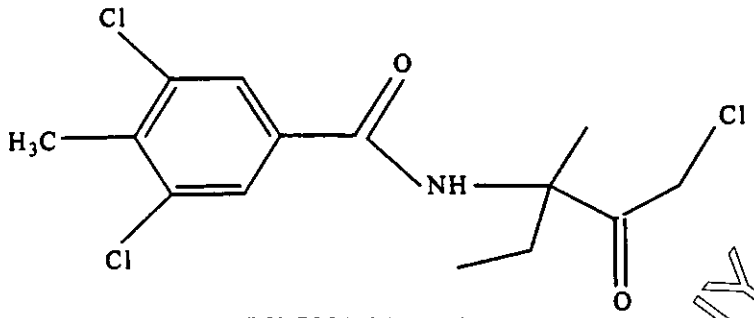
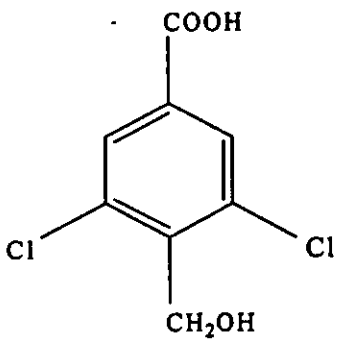
3. Experimental Compounds

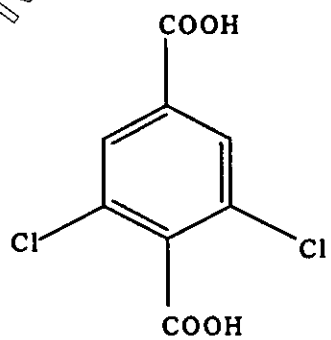
3.1 Structure of the Reference Substances



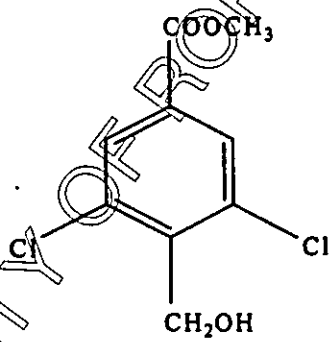
RH-7281, MW = 335



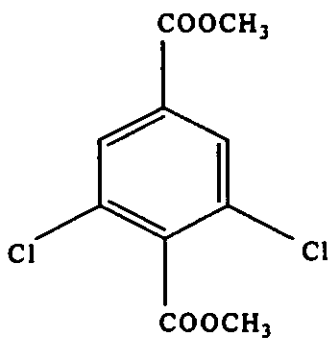
RH-1452, MW = 220



RH-1455, MW = 234



RH-7391, MW = 234



RH-7399, MW = 262

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3.2 Data for the Reference Substances

RH-7281:

CA Name:	3,5-dichloro-N-(3-chloro-1-ethyl-1-methyl-2-oxopropyl)-4-methylbenzamide
CAS Number:	156052-68-5
R&H ID	RH-117281*
Appearance	White Solid

RH-1452:

CA Name:	3,5-dichloro-4-hydroxymethylbenzoic acid
CAS Number:	N/A
R&H ID	RH-141452*
Appearance	White Solid

RH-1455:

CA Name:	2,6-dichloro-1,4-benzenedicarboxylic acid
CAS Number:	N/A
R&H ID	RH-141455*
Appearance	White Solid

RH-7391 (methyl ester of RH-1452):

CA Name:	Methyl, 3,5-dichloro-4-hydroxymethylbenzoate
CAS Number:	N/A
R&H ID	RH-147391*
Appearance	White Solid

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RH-7399 (methyl ester of RH-1455):

CA Name:	Dimethyl-2,6-dichloro-terephthalate
CAS Number:	N/A
R&H ID	RH-147399*
Appearance	White Solid

* Full R&H numbers. The designations typically used in studies are the four digit abbreviations: RH-7281, RH-1452, RH-1455, RH-7391, and RH-7399.

4. Chemicals/Prepared Solutions

4.1 Chemicals

Chemicals	Grade	Supplier*
Acetonitrile(ACN)	HPLC	Baker
Alumina B Cartridge	LC-Alumina B (6 mL)	Supelco #57085
Celite	A.C.S.	Baker
Cotton, Sterile	Absorbent	Johnson & Johnson
Diazald™	99.9%	Aldrich
Di(ethylene glycol) Ethyl Ether	HPLC	Aldrich
Ethyl Acetate	HPLC	Baker
Ethyl Ether	HPLC	Aldrich
Florisil Cartridge	LC-Florisil (20 mL)	Supelco #57131
Florisil Powder	60-100 Mesh	U.S. Silica
Hexane	HPLC	Baker
Hydrogen Chloride(HCl)	1N, HPLC	VWR
RH-1452	Analytical Standard	Rohm & Haas Co.
RH-1455	Analytical Standard	Rohm & Haas Co.
RH-7281	Analytical Standard	Rohm & Haas Co

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RH-7391	Analytical Standard	Rohm & Haas Co.
RH-7399	Analytical Standard	Rohm & Haas Co.
Sodium Bicarbonate($NaHCO_3$)	A.C.S.	Fisher Scientific
Sodium Hydroxide(NaOH, aq.)	50%, w/w	Fisher Scientific
Sodium Sulfate(Na_2SO_4)	Anhydrous Granular	EM Science
Toluene	HPLC	Baker
Water	Milli-Q	Millipore System

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

4.2 Prepared Solutions

Solutions for Sample Extraction:

2% (w/v) $NaHCO_3$ solution was prepared by dissolving 20 g of $NaHCO_3$ in 1000 mL of Milli-Q water in a 1000 mL flat bottom flask.

80/20 (v/v) ACN/2% $NaHCO_3$ solution was prepared mixing 800 mL of ACN and 200 mL of 2% $NaHCO_3$ in a 1000 mL flat bottom flask.

Solution for Florisil and Alumina-B SPE of RH-7281:

80/20 (v/v) hexane/ethyl acetate was prepared by mixing 800 mL of hexane with 200 mL ethyl acetate in a 1000 mL flat bottom flask.

Solutions for Florisil Column Chromatography of RH-7391 and RH-7399:

80/20 (v/v) toluene/hexane solution was prepared by mixing 800 mL of toluene and 200 mL of hexane in a 1000 mL flat bottom flask.

80/15/5 (v/v) toluene//hexane/ethyl acetate solution was prepared by mixing 800 mL of toluene, 150 mL of hexane, and 50 mL of ethyl acetate in a 1000 mL flat bottom flask.

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4.3 Column Chromatography Packing Materials

Activated Florisil: Approximately 1 kg of Florisil powder was placed in a beaker and baked in an oven at 150°C for two hours. The activated Florisil was transferred to a desiccator and cooled to room temperature. The material was transferred in 500 mL plastic bottles and stored in a desiccator.

5. Equipment

Equipment	Description	Source*
Blender	Model 34BL97	Waring Co.
Buchner Funnel	200 mL	Coors
Chromatographic Column	14.5 mm ID	ACE Glass
Erlenmayer	300 mL	Pyrex
Filter Paper	# 4	Whatman
Filtration Flask	500 mL	Kimax
Flat Bottom Flask, 24/40 ST	1000 mL	Fisher Scientific
Food Processor	Model #84145	Hobart
Impinger	30 mL	Aldrich
pH paper	0-14	Brinkman
Rotary Evaporator	Model R114	Brinkman
Round Bottom Flasks, 24/40 ST	100, 300, and 500 mL	Fisher Scientific
Separatory Funnels	500 mL	Fisher Scientific
Sonicator	Model 2210	Branson
Visprep SPE Vacuum Manifold	Manifold	Supelco
Volumetric Flask	100 mL and 500 mL	Kimax

Standard laboratory equipment, balance, beakers, test tubes, etc.

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

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6. Method

6.1 Method Flow Diagram

5.0 g of potato peel waste samples

⇓

Blending Extraction with 80/20 ACN/2% $NaHCO_3$

⇓

--------- Liquid-Liquid Partition ----------

↓	↓
RH-7281	*RH-1452+ RH-1455*
↓	↓
Florisil Solid Phase Extraction	Methylation
↓	↓
Alumina-B Solid Phase Extraction	Florisil Column Chromatography
↓	↓
Quantitation (RH-7281)	Quantitation (RH-7391 + RH-7399)

6.2 Sample Preparation

Prepare frozen potato peel samples by chopping the samples in a food processor with dry ice and allow dry ice to sublime overnight in a freezer. Store the processed sample in a freezer.

6.3 Sample Extraction

Weigh 5.0 g of a homogenized potato peel sample in a disposable weighing dish. Transfer the sample to a blender jar. If desired, spike fortification samples with a known amount of RH-7281, RH-1452, and RH-1455 in ethyl acetate (1.0 ppm mix of the three). Allow the solvent to evaporate from the sample in a fume hood for about 20 minutes. Add about 2 g of Celite to the blender jar.

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Add 100 mL of 80/20 acetonitrile/2% $NaHCO_3$ (extraction solution) to the blender jar. Blend the slurry for 1 minute at medium speed. Filter the slurry through #4 Whatman filter paper in a Buchner funnel into a 500 mL vacuum filtration flask. Rinse the blender jar and the filter cake with 20 mL of the extraction solution. Combine the filtrates (approximately 120 mL) in a 500 mL (24/40 ST) round bottom flask and concentrate to about 30 mL (**Solution A**) on a rotovap at about 40°C under diminished pressure (about 50 mm Hg).

6.4 Liquid-Liquid Partition to Separate RH-7281

Transfer **Solution A** in the 500 mL round bottom flask to a 500 mL separatory funnel. Rinse the flask with 80 mL of 2% $NaHCO_3$ solution and 75 mL of ethyl acetate sequentially and add both to the separatory funnel. Shake the funnel for 1 minute with frequently venting and allow the two phases to separate.

Transfer the aqueous phase (lower layer) to the original Solution A flask and the organic phase to another 500 mL (24/40 ST) round bottom flask. Transfer the aqueous phase back to the separatory funnel and extract with another 75 mL of ethyl acetate in the same manner. Transfer the aqueous phase (**Solution B,** containing RH-1452 and RH-1455) to the original Solution A flask. Proceed with **Solution B** to section 6.7 for liquid-liquid partition of RH-1452 and RH-1455.

Combine both organic layers and concentrate to dryness on a rotovap at about 40 °C under diminished pressure (about 50 mm Hg). Dissolve the residues in 10 mL of hexane with sonication for 10 seconds (**Solution C**, containing RH-7281). Proceed with **Solution C** to section 6.5 for RH-7281 clean up.

6.5 Florisil Solid Phase Extraction for RH-7281*

Insert a Florisil SPE cartridge into a solid phase extraction manifold. Condition the cartridge with 5 mL of ethyl acetate followed by 10 mL of hexane. Apply **Solution C** (from section 6.4) to the column and elute to the top of the bed. Rinse the 500 mL round bottom flask with 10 mL of hexane and apply the rinse to the column. Discard all the effluents obtained thus far.

Elute RH-7281 from the column with 30 mL of 80/20 hexane/ethyl acetate into a test tube. Transfer the eluate to a 100 mL (24/40 ST) round bottom flask and concentrate to dryness on a rotovap at 40°C. Dissolve the residues in 5.0 mL of hexane (**Solution D**) with sonication for 10 seconds and proceed to section 6.6.

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6.6 Alumina-B Solid Phase Extraction for RH-7281*

Insert an Alumina-B solid phase extraction cartridge into a solid phase extraction manifold. Condition the cartridge with 3 mL of ethyl acetate followed by 5 mL of hexane. Apply **Solution D** (from section 6.5) to the cartridge and elute to the top of the bed. Rinse the 100 mL round bottom flask with 5 mL of hexane and apply the wash to the cartridge. Discard the effluent at this stage.

Elute RH-7281 from the cartridge with 20 mL of 80/20 hexane/ethyl acetate solution into a test tube. Transfer the eluate from the test tube to a 100 mL (24/40 ST) round bottom flask. Wash the tube with about 1 mL of 80/20 hexane/ethyl acetate and add to the 100 mL round bottom flask. Concentrate the eluate to dryness on a rotovap at about 40 °C under diminished pressure (about 50 m Hg). Dissolve the residues in 5.0 mL of ethyl acetate with sonication for 10 seconds and proceed to quantitation of RH-7281 (section 7).

6.7 Liquid-Liquid Extraction to Recover RH-1452 and RH-1455

Add 30 mL of 1.0 N HCl solution to **Solution B** (from section 6.4) and measure the pH of the solution. If necessary, add more 1.0 N HCl solution to ensure the pH of the solution is ≤ 1. Transfer the solution to a 500 mL separatory funnel. Rinse the 500 mL flask with 75 mL of ethyl acetate and add to the separatory funnel. Shake the funnel for 30 seconds with frequent venting and allow two phases to separate. Transfer the aqueous phase (lower layer) to the original Solution B flask. Transfer the organic phase to a 300 mL Erlenmeyer flask containing about 5 g of Na_2SO_4 and let it sit for about 2 minutes with occasional swirling. Decant the solution to a 300 mL (24/40 ST) round bottom flask.

Transfer the aqueous phase back to the seperatory funnel and extract the aqueous phase with another 75 mL of ethyl acetate in the same manner. Discard the aqueous phase to waste. Transfer the organic phase to the same 300 mL Erlenmeyer flask containing the sodium sulfate desiccant and let it sit for about 2 minutes with occasional swirling. Decant the solution and combine it with the first organic phase in the same 300 mL round bottom flask.

Concentrate the solution to dryness on a rotovap at about 40 °C under diminished pressure (continue the evaporation for at least 1 minute after the last drop of liquid disappears to ensure dryness). Dissolve the residue with 1 mL of ethyl acetate and sonicate for 10 seconds. Add 1.0 mL of ethyl ether (**Solution E**) and proceed to section 6.8.

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6.8 Methylation of RH-1452 and RH-1455

Methylate **Solution E** (from section 6.7) with diazomethane by following the methylation procedure described in Appendix. When the methylation is complete, concentrate the solution to dryness on a rotovap at about 40°C under diminished pressure (about 50 m Hg) and re-dissolve the methyl esters of RH-1452 (RH-7391) and RH-1455 (RH-7399) in 20 mL of hexane (**Solution F**) with sonication for 10 seconds.

Diazomethane is found to be an effective reagent for methylating RH-1452 and RH-1455. Only carboxylic acid groups are methylated to their methyl esters while the hydroxyl group in RH-1452 is left unreacted.

6.9 Florisil Column Chromatography for RH-7391 and RH-7399*

Insert a small cotton plug into a 14.5 mm ID x 25 cm length glass chromatographic column and dry pack the column with 15 g of activated Florisil. Add about 2 g of sodium sulphate to the column. Load **Solution F** (from section 6.8) to the column and elute to the top of the bed. Rinse the 100 mL flask with 50 mL of 80/20 toluene/hexane and apply to the column. Discard the effluents at this stage. Elute RH-7391 and RH-7399 from the column with 100 mL of 80/15/5 toluene/hexane/ethyl acetate into a 300 mL (24/40 ST) round bottom flask.

Concentrate the eluate to dryness on a rotovap at 40 °C. Dissolve the residues in 5.0 mL of ethyl acetate with sonication for 10 seconds. Proceed to section 7 for quantitation of RH-7391 and RH-7399.

*The analyte(s) elution profile should be checked for each new lot of Florisil and Alumina-B cartridge. Minimum volume of solvent should be used to elute most of target analyte(s) from the column or cartridge. An example procedure for testing the elution profile for a new lot of Alumina-B cartridge is as follows:

1). Using 10 mL of 0.10 µg/mL RH-7281 in ***hexane*** standard, follow the elution schemes as outlined under section 6.6.

2). Collect the pre-elution cuts as well as the target elution cut. Wash the cartridge with 20 mL of ethyl acetate and collect this post-elution cut.

3). Concentrate the pre-elution cuts, the target cut, and the post-elution cut to dryness.

4). Dissolve the cuts in 10 mL of hexane and inject them as outlined in the GC/ECD quantitation section.

5). If the target cut contains a minimum of 85% of RH-7281, it may be considered acceptable.

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

a). For case where the pre-wash cut contains significant standard; either the pre-wash amount or eluting solvent percentage may be decreased.

b). For cases where the post-wash cut contains significant standard; the percentage of the more polar component in the eluting solvent may be increased.

7. Quantitation

RH-7281 and the two esters (RH-7391 and RH-7399) are analyzed separately to minimize the matrix effects.

A Rtx-5 column is used for the primary detection of RH-7281 and a Rtx-225 column is used for the primary detection of RH-7391 and RH-7399, both by GC/ECD.

RH-7281 is confirmed by GC/MSD using a Rtx-5 column and RH-7391 and RH-7399 are confirmed by GC/ECD using a Rtx-35 column.

7.1 Instrumentation and Conditions

7.1.1 Primary Detection

Quantatition of RH-7281:

Injector: HP 5890 II
On-column mode
Injection temp.: 150°C
Injection volume: 1.0 µL

Column: Rtx-5 (0.53 mm ID x 30 m; 0.25µm film)
Carrier gas: Nitrogen
Head Pressure: 3.2 psi
Flow rate: 4.0 mL/min.

Oven temperature program:
Initial temp. = 80°C, hold for 1 min.
Ramp 1 at 20°C/min.,
Final temp. 1 = 250°C, hold for 5 min.
Ramp 2 at 30°C/min.,
Final temp. 2 = 280°C, hold for 3 min.

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Detector: Temp. = 300°C
Mode: Constant flow (column + makeup = 70 mL/min.)
Makeup Gas = Nitrogen

Typical Retention Time: RH-7281: ~11 min.

Quantitation of RH-7391 and RH-7399:

Injector: HP 6890
On-column mode
Injection temp.: 180°C
Injection volume: 1.0 µL

Column: Rtx-225 (0.32 mm ID x 30 m; 0.25 µm film)
Carrier gas: Helium
Head Pressure: 16.9 psi
Flow rate: 4.0 mL/min.

Oven temperature program:
Initial temp. = 60°C, hold for 1 min.
Ramp 1 at 20°C/min.,
Final temp. 1 = 200°C, hold for 10 min.
Ramp 2 at 50°C/min.,
Final temp. 2 = 220°C, hold for 3 min.

Detector: Temp. = 280°C
Mode: Constant flow (column + makeup = 60 mL/min.)
Makeup Gas = Nitrogen

Typical Retention Time: RH-7399: ~ 9 min.
RH-7391: ~10 min.

7.1.2. Confirmatory Detection

HP6890/5973 GC/MSD (HP-5MS) for RH-7281

Injector: HP6890 auto-sampler
Splitless mode
Injection temp.: 150°C
Injection volume: 1.0 µL

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Column: HP-5MS (0.25 mm ID x 30 m; 0.25 µm film)
Carrier gas: Helium
Head Pressure: 9.2 psi
Flow rate: 1.0 mL/min.

Oven temperature program:
Initial temp. = 80°C, hold for 1 min.
Ramp1 at 20°C/min.,
Final temp. 1 = 250°C, hold for 5 min.
Ramp 2 at 30°C/min.,
Final temp. 2 = 280°C, hold for 3 min.

SIM: m/z 258 (qauntitation ion)
m/z 187
m/z 189

Typical Retention Time: RH-7281: ~11 min.

HP 6890 GC/ECD (Rtx-35) for RH-7391 and RH-7399:

Injector: HP 6890
On-column mode
Injection temp.: 150°C
Injection volume: 1.0 µL

Column: Rtx-35 (0.32 mm ID x 30 m; 0.25 µm film)
Carrier gas: Helium
Head Pressure: 9.9 psi
Flow rate: 2.0 mL/min.

Oven temperature program:
Initial temp. = 60°C, hold for 1 min.
Ramp at 10°C/min.,
Final temp. = 220°C, hold for 5 min.

Detector: Temp. = 300°C
Mode: Constant flow (column + makeup = 60 mL/min.)
Makeup Gas = Nitrogen

Typical Retention Time: RH-7399: ~16.1 min.
RH-7391: ~16.4 min.

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7.2 Preparation of Standard Solutions

7.2.1 Stock Standard Solutions

Weigh ~10.0 mg each of RH-7281, RH-1452, RH-1455, RH-7391 or RH-7399 analytical standards on an analytical balance. Transfer each material into individual 100 mL volumetric flasks, correct for purity, and add adequate volume of ethyl acetate to make 100 ppm stock solutions. Store in a refrigerator.

7.2.2 Intermediate Standard and Spiking Solutions

Make the 1.0 µg/mL intermediate standard solutions from the above stock solutions by diluting 1.0 mL of each stock solution to 100 mL with ethyl acetate. Make the spiking solution (mixed three) by taking 1.0 mL each of 100 µg/mL stock solutions of RH-7281, RH-1452, and RH-1455 and dilute to 100 mL with ethyl acetate. Store all solutions in a refrigerator.

7.2.3 Working Standards

Make the working standard solutions by diluting the 1.0 µg/mL intermediate standard solution with ethyl acetate :

Concentration	Aliquot size and source	Final Volume
0.15 µg/mL	15 mL of 1.0 µg/mL	100 mL
0.10 µg/mL	10 mL of 1.0 µg/mL	100 mL
0.050 µg/mL	5.0 mL of 1.0 µg/mL	100 mL
0.010 µg/mL	1.0 mL of 1.0 µg/mL	100 mL

Store working standard solutions in a refrigerator and prepare fresh every six months.

7.3 Preparation of Standard Curves

A minimum of four standard solutions are prepared in a desired concentration range. Standards and samples are preferably quantitated by peak area, although height may be used. A linear regression is used to fit the instrument response into the equation: $Y = mX + b$. A quadratic regression can also be used: $Y = nX^2 + mX + b$. The concentrations of RH-7281, RH-1452, and RH-1455 in potato peel samples are then determined from the standard curves.

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7.4 Fortification Recovery

Control samples are fortified with a known amount of RH-7281, RH-1452, and RH -1455 prior to extraction. Percent recovery is calculated by measuring the peak area or peak height, calculating the µg/mL found from the standard curves as shown in Equation 1:

$$\frac{\text{(Found (µg/mL) x Final Vol. (mL)}}{\text{Fortification Amount (µg)}} \times 100\% = \text{\% Recovery} \qquad \text{Eq.1}$$

Although control samples are spiked on a weight basis with the acid metabolites, detection and quantitation are made on the corresponding ester derivatives. Because of this, a correction for the change in molecular weight must be made in order to accurately calculate analyte recoveries based on the acids. A correction factor of 1.06 is used to adjust for the difference in the molecular weight between RH-1452 and its methyl ester RH-7391. Similarly, a factor of 1.12 is used to adjust the RH-1455 assay for the higher molecular weight of its methyl ester RH-7399. The correction factors are calculated as follows:

RH-7391 (mw = 234) / RH-1452 (mw = 220) = 1.06

RH-7399 (mw = 262) / RH-1455 (mw = 234) = 1.12

The correction can also be made by constructing standard curves of the esters to directly reflect the concentration of the acids. For example, 0.106 µg/mL of RH-7391 standard corresponds to 0.100 µg/mL of RH-1452.

7.5 Sample Analysis

Injection volumes are the same for samples and standards. If necessary, the samples are diluted to an appropriate volume to give a final concentration within the standard curve range. Either peak heights or areas are measured and the concentration of each component is determined from the standard curve.

The residue concentration in ppm is determined as follows:

$$\frac{\text{Concentration (µg/mL) x Final Volume (mL)}}{\text{Sample Weight (g)}} = \text{µg/g} = \text{ppm} \qquad \text{Eq.2}$$

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7.6 Sample Calculation

A typical calculation for the recovery of 0.10 ppm fortification of potato peel (Tables 1 and 2; Figures 33 and 50) is demonstrated as follows:

Calculation for RH-7281:

$$\frac{0.074778\ (\mu g/mL) \times 5.0\ (mL)}{0.50\ \mu g} \times 100\% = 74.8\%$$

Calculation for RH-1452:

$$\frac{0.097000\ (\mu g/mL) \times 5.0\ (mL)}{0.50\ \mu g \times 1.06} \times 100\% = 91.5\%$$

Calculation for RH-1455:

$$\frac{0.081986\ (\mu g/mL) \times 5.0\ (mL)}{0.50\ \mu g \times 1.12} \times 100\% = 73.2\%$$

The above values were calculated using a Rohm and Haas Data System(Reference #2).

7.7 Expression of Total Residue Level

In analysis of field trial samples, total residue level is usually reported. The conversion of concentration of the metabolite to the equivalent concentration of parent is calculated as follows:

$$ppm_{\text{parent equivalent for RH-1452}} = ppm_{RH\text{-}1452} \times 335/220 = 1.52 \times ppm_{RH\text{-}1452} \qquad \text{Eq.3}$$

$$ppm_{\text{parent equivalent for RH-1455}} = ppm_{RH\text{-}1455} \times 335/234 = 1.43 \times ppm_{RH\text{-}1455} \qquad \text{Eq.4}$$

Thus, the total parent equivalent residue level in the samples is defined as:

$$ppm_{\text{parent equivalent}} = ppm_{RH\text{-}7281} + 1.52 \times ppm_{RH\text{-}1452} + 1.43 \times ppm_{RH\text{-}1455} \qquad \text{Eq.5}$$

7.8 Time Required to Run a Set of Samples

The time required to run a set of 6 samples is about 24 hours including data acquisition.

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8. Results and Discussion

Both GC/ECD and GC/MSD have acceptable sensitivity for RH-7281, RH-7391, and RH-7399. Either linear responses or quadratic responses can be used in the concentration range of 0.01 - 0.15 µg/mL depending on the regression coefficient (Figures 1 - 22).

As shown in Figures 23-25, 36 and Figures 40-42, 53, no significant interference peaks were found at the same retention times of RH-7281, RH-7391, and RH-7399. In some cases, small interference peaks (at the level of LOD) were detected (Figures 24 and 41), due probably to contamination.

The limits of quantitation (LOQ) for RH-7281, RH-7391, and RH-7399 are all established at 0.02 ppm by analysis of fortifications at that target level (Tables 1 and 2).

Within the concentration range of 0.02 - 0.10 ppm, the mean recovery of RH-7281 from fortified potato peel samples is 90.23±15.10% (n = 27). The mean recovery of RH-1452 is 90.19±20.24% (n = 27) and the mean recovery of RH-1455 is 87.74±22.26% (n = 27).

The detection of RH-7281, RH-7391, and RH-7399 is confirmed by using either a different detection method (MSD for RH-7281) or a different stationary phase of the analytical column (Rtx-35 for RH-7391 and RH-7399). The results are demonstrated in Tables 1 and 2; Figures 6-10 and 36-39 (RH-7281); Figures 17-22 and 53-56 (RH-7391 and RH-7399).

The GC inlet system including the insert and guard column must be clean and conditioned to minimize the degradation of RH-7281 and the broadening of RH-7391 peak. A temperature program, e.g. increasing injection temperature 30 °C below the maximum column bleeding temperature for 1 min. after the elution of the analyte peaks, is found effective to remove residues that remain in the inlet system.

Removal of 0.50 m of the analytical column after a few analytical sets (about 100 samples) may be needed to improve the chromatography. If poor chromatography still persists after these changes, a new analytical column may be required.

Typical chromatograms of standards, calibration curve, potato peel control, fortified samples, and trial samples are shown in Figures 1 to 56.

9. References

1. TR 34-00-37. "Preliminary Residue Analytical Method for Parent RH-7281 and Its Two Acid Metabolites, RH-1452 and RH-1455, in Potato Peel Waste", Ipin Guo, Kenneth Kurilla, Charles Hofmann, May 12, 2000.

2. TR 34-00-48. "Analysis of Potato Peel for Residues of Parent RH-117,821 and Metabolites RH-141,452 and RH-141,455", Deborah Graves, May 31, 2000.

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10. Project Information

Study Title: Tolerance Enforcement Method for Parent RH-7281 and Its Two Acid Metabolites, RH-1452 and RH-1455, in Potato Peel Waste

Project Number: 1401.15

Sponsor and Testing Facility:

Rohm and Haas Company
Research Laboratory
727 Norristown Road
Spring House, PA 19477-0904

Dates:

Work Initiated: February 2000
Work Completed: May 2000

Technical Personnel:

Ipin Guo, Ken Kurilla, Charles Hofmann

Notebook References:

IG-4(#70535)

Archival Information:

Research notebooks will be archived in the library. Original report and raw data will be in the Rohm and Haas Archives.

Peer Reviewer:

Danny Choo

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Table 1 Recovery Data of RH-7281

Fort. Level (µg/g)	Reference	Recovery, % Primary	Confirm.	Date mm/dd/yy
0.02	TR 34-00-37	131		03/10/2000
0.02	TR 34-00-37	124		03/10/2000
0.02	TR 34-00-37	102		03/17/2000
0.02	TR 34-00-37	90.4		03/17/2000
0.02	TR 34-00-37	76.8		03/24/2000
0.02	TR 34-00-37	77.1		03/24/2000
0.02	TR 34-00-37	89.5	114	04/03/2000
0.02	TR 34-00-37	91.7	105	04/03/2000
0.02	TR 34-00-37	88.1		04/18/2000
0.02	TR 34-00-37	78.7		04/18/2000
0.02	TR 34-00-48	79.3		05/15/2000
0.05	TR 34-00-37	95.7		03/10/2000
0.05	TR 34-00-37	90.0		03/10/2000
0.05	TR 34-00-37	74.3		03/17/2000
0.05	TR 34-00-37	76.2		03/17/2000
0.05	TR 34-00-37	78.6		03/24/2000
0.05	TR 34-00-37	68.2		03/24/2000
0.05	TR 34-00-37	106	106	04/03/2000
0.05	TR 34-00-37	94.6	96.2	04/03/2000
0.05	TR 34-00-37	105		04/18/2000
0.05	TR 34-00-37	98.5		04/18/2000
0.10	TR 34-00-37	99.4		03/10/2000
0.10	TR 34-00-37	72.8		03/17/2000
0.10	TR 34-00-37	84.8		03/24/2000
0.10	TR 34-00-37	90.6	88.4	04/03/2000
0.10	TR 34-00-37	98.1		04/18/2000
0.10	TR 34-00-48	74.8		05/15/2000
Mean		90.23	101.92	
SD		15.10	9.84	
n =		27	5	

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Table 2 Recovery Data of RH-1452 and RH-1455

Fort Level (µg/g)	Reference	RH1452(%) Primary	RH1452(%) Confirm	RH-1455(%) Primary	RH-1455(%) Confirm.	Date mm/dd/yy
0 02	TR 34-00-37	85.6		106		03/10/2000
0.02	TR 34-00-37	83.6		103		03/10/2000
0 02	TR 34-00-37	161		135		03/17/2000
0 02	TR 34-00-37	118		92.0		03/17/2000
0 02	TR 34-00-37	81.2		59.4		03/24/2000
0.02	TR 34-00-37	74.3		52.9		03/24/2000
0 02	TR 34-00-37	92 2		127		04/03/2000
0 02	TR 34-00-37	103		63.7		04/03/2000
0.02	TR 34-00-37	76.0	88.5	126	138	04/18/2000
0 02	TR 34-00-37	82.9	86.0	107	117	04/18/2000
0.02	TR 34-00-48	79.2		72.1		05/15/2000
0.05	TR 34-00-37	103		98.7		03/10/2000
0.05	TR 34-00-37	101		88.3		03/10/2000
0.05	TR 34-00-37	61.9		79.2		03/17/2000
0.05	TR 34-00-37	60.5		84.8		03/17/2000
0.05	TR 34-00-37	81 9		69.5		03/24/2000
0 05	TR 34-00-37	116		86.2		03/24/2000
0.05	TR 34-00-37	83.2		67.7		04/03/2000
0 05	TR 34-00-37	94.3		67 9		04/03/2000
0.05	TR 34-00-37	97 9	90.4	107	108	04/18/2000
0.05	TR 34-00-37	97.9	94.8	108	116	04/18/2000
0.10	TR 34-00-37	63.4		56.3		03/10/2000
0.10	TR 34-00-37	73.0		74.3		03/17/2000
0.10	TR 34-00-37	89.3		77.9		03/24/2000
0 10	TR 34-00-37	94.6		82.8		04/03/2000
0.10	TR 34-00-37	88 6	94 4	103	103	04/18/2000
0.10	TR 34-00-48	91.5		73.2		05/15/2000
Mean		90.19	90.82	87.74	116.40	
SD		20.24	3.79	22.26	13.39	
n =		27	5	27	5	

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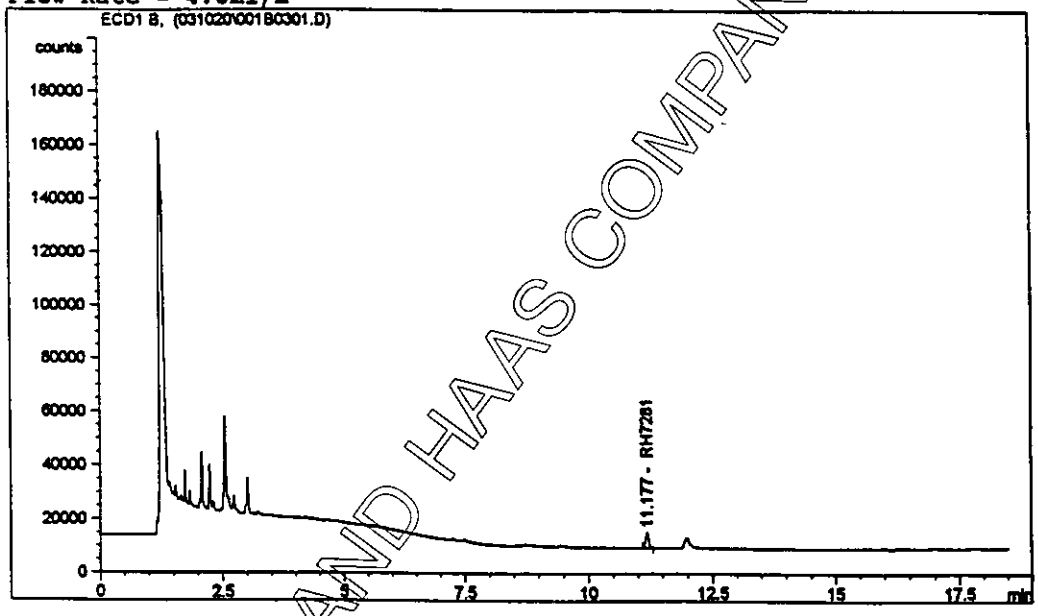
Data File C:\HPCHEM\2\DATA\031020\001B0301.D Sample Name: std1

0.01ppm RH7281

Injection Date : 3/10/2000 4:37:13 PM Seq. Line : 3
Sample Name : std1 Vial : 1
Acq. Operator : ig Inj : 1
Inj Volume : 1 µl

Sequence File : C:\HPCHEM\2\SEQUENCE\IG031020.S
Acq. Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 3/3/2000 2:11:59 PM by ig
Analysis Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 5/9/2000 12:22:42 PM by ig
RH7281 Potato Peel Waste METHOD,
Rtx-5, 30m, 0.53mm, 0.5um film
Flow Rate = 4.0ml/m



Default Calibration Report (other requested info not available)

External Standard Report (no recalibration)

Sorted By : Signal
Calib. Data Modified : 5/9/2000 12:22:35 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 B,

RetTime [min]	Type	Area counts*s	Amt/Area	Amount [ng/ul]	Grp	Name
11.177	BBA	2.72881e4	4.02528e-7	1.09842e-2		RH7281

Instrument 2 5/9/2000 12:24:48 PM ig Page 1 of 2

Figure 1. 0.010 µg/ml Standard RH-7281 (GC/ECD).

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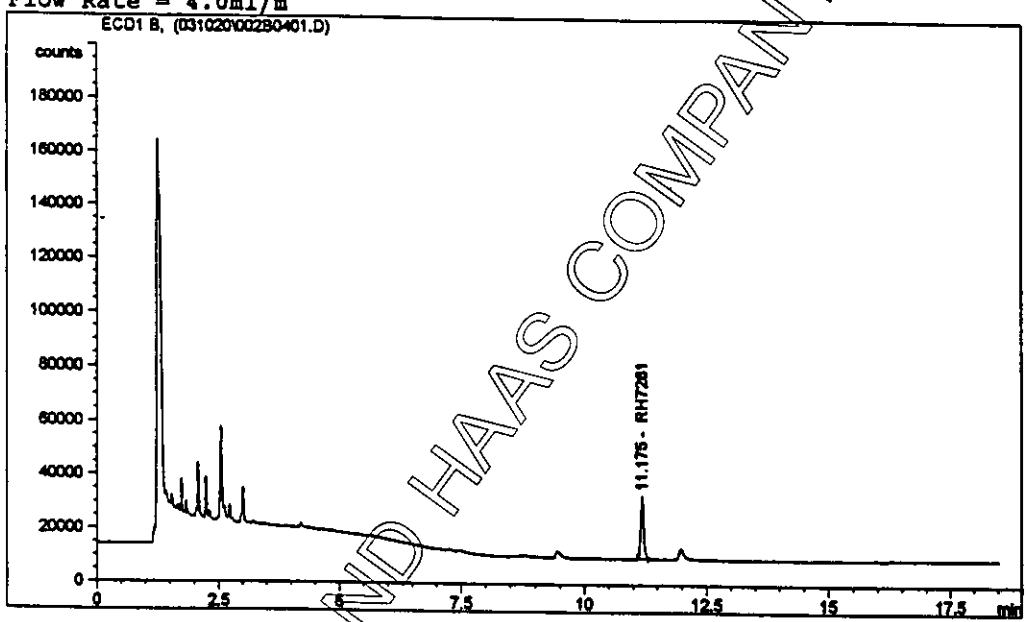
Data File C:\HPCHEM\2\DATA\031020\002B0401.D Sample Name: std2

0.05ppm RH7281

Injection Date : 3/10/2000 5:00:52 PM Seq. Line : 4
Sample Name : std2 Vial : 2
Acq. Operator : ig Inj : 1
Inj Volume : 1 µl

Sequence File : C:\HPCHEM\2\SEQUENCE\IG031020.S
Acq. Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 3/3/2000 2:11:59 PM by ig
Analysis Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 5/9/2000 12:22:42 PM by ig
RH7281 Potato Peel Waste METHOD,
Rtx-5, 30m, 0.53mm, 0.5um film
Flow Rate = 4.0ml/m



Default Calibration Report (other requested info not available)

External Standard Report (no recalibration)

Sorted By : Signal
Calib. Data Modified : 5/9/2000 12:22:35 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 B,

RetTime [min]	Type	Area counts*s	Amt/Area	Amount [ng/ul]	Grp	Name
11.175	BBA	1.09140e5	4.23650e-7	4.62373e-2		RH7281

Totals : 4.62373e-2

Instrument 2 5/9/2000 12:24:51 PM ig Page 1 of 2

Figure 2. 0.050 µg/ml Standard RH-7281 (GC/ECD).

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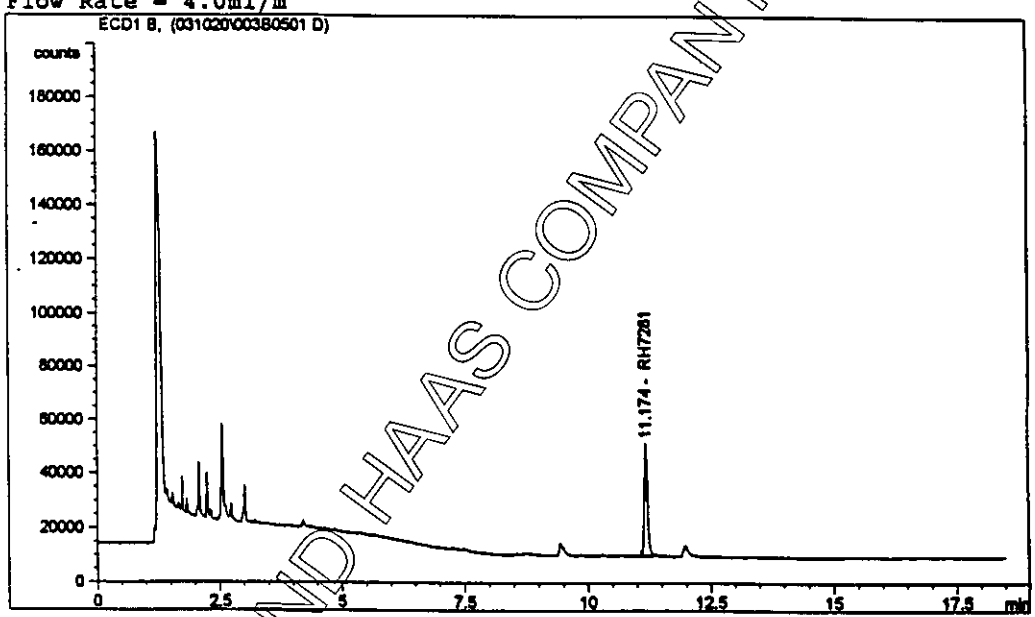
Data File C:\HPCHEM\2\DATA\031020\003B0501.D Sample Name: std3

0.10ppm RH7281

Injection Date : 3/10/2000 5:24:24 PM Seq. Line : 5
Sample Name : std3 Vial : 3
Acq. Operator : ig Inj : 1
Inj Volume : 1 µl

Sequence File : C:\HPCHEM\2\SEQUENCE\IG031020.S
Acq. Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 3/3/2000 2:11:59 PM by ig
Analysis Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 5/9/2000 12:22:42 PM by ig
RH7281 Potato Peel Waste METHOD,
Rtx-5, 30m, 0.53mm, 0.5um film
Flow Rate = 4.0ml/m



Default Calibration Report (other requested info not available)

External Standard Report (no recalibration)

Sorted By : Signal
Calib. Data Modified : 5/9/2000 12:22:35 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 B,

RetTime [min]	Type	Area counts*s	Amt/Area	Amount [ng/ul]	Grp	Name
11.174	BBA	1.92409e5	4.50833e-7	8.67442e-2		RH7281

Totals : 8.67442e-2

Instrument 2 5/9/2000 12:24:54 PM ig Page 1 of 2

Figure 3. 0.10 µg/ml Standard RH-7281 (GC/ECD).

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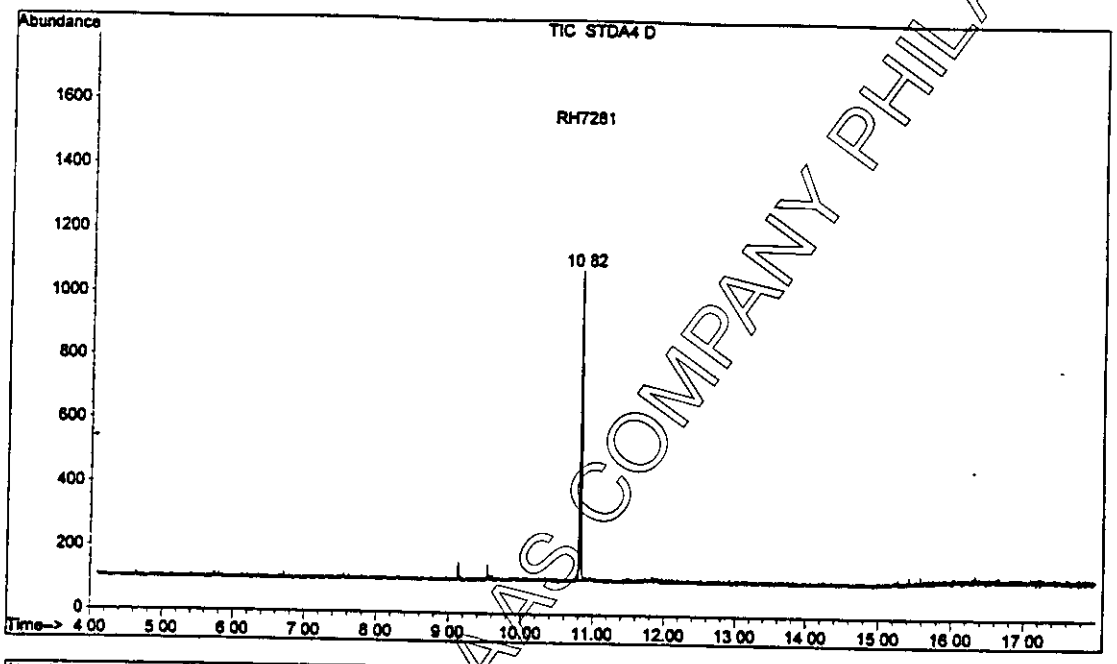
```
File        : C:\HPCHEM\1\DATA\032920\STDA4.D
Operator    : ig
Acquired    : 29 Mar 00  17:53     using AcqMethod 7281SIM
Instrument  :    5973
Sample Name: std 0.15ppm
Misc Info   :
Vial Number: 4
```



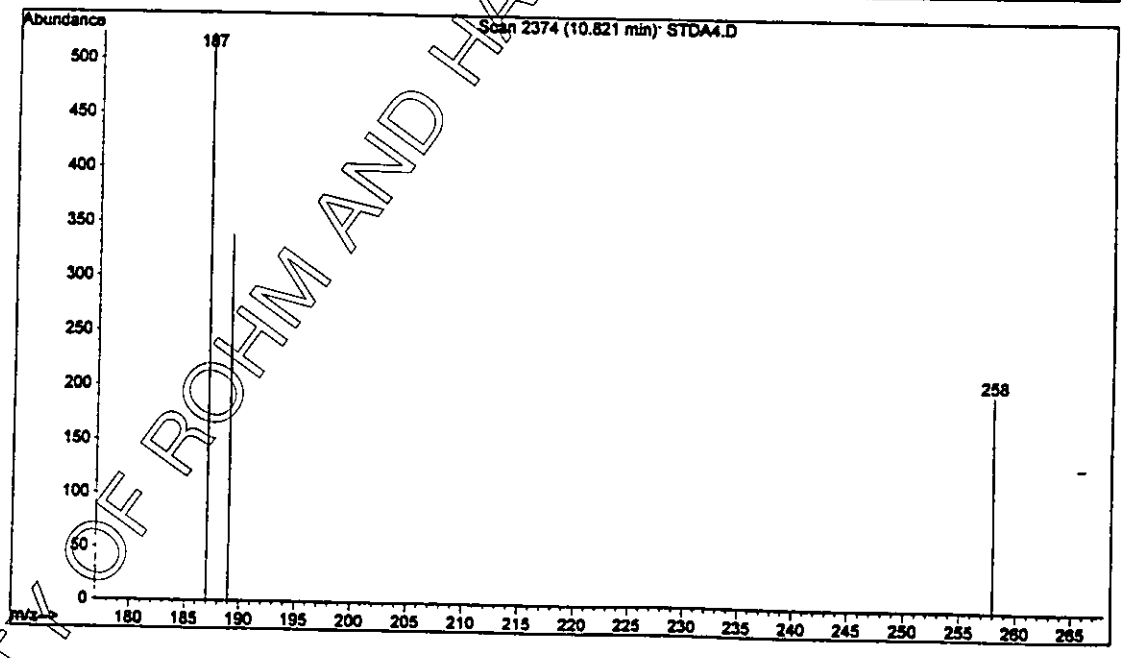


Figure 9. 0.15 µg/ml Standard RH-7281 (GC/MSD, Peak Area = 2194).

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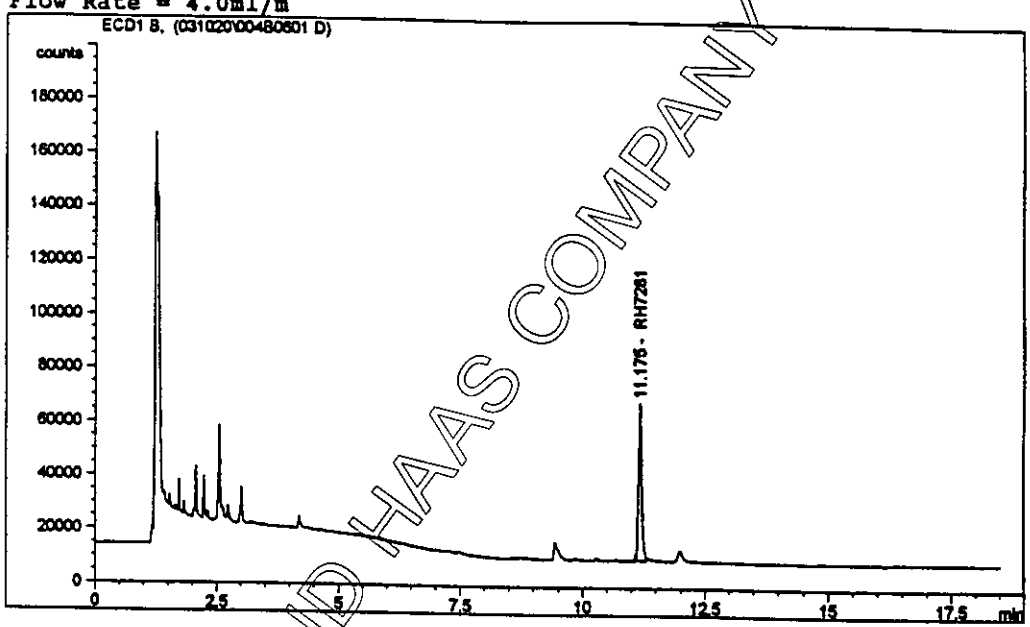
Data File C:\HPCHEM\2\DATA\031020\00480601.D Sample Name: std4

0.15ppm RH7281

Injection Date : 3/10/2000 5:48:00 PM
Sample Name : std4
Acq. Operator : ig

Seq. Line : 6
Vial : 4
Inj : 1
Inj Volume : 1 µl

Sequence File : C:\HPCHEM\2\SEQUENCE\IG031020.S
Acq. Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 3/3/2000 2:11:59 PM by ig
Analysis Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 5/9/2000 12:22:42 PM by ig
RH7281 Potato Peel Waste METHOD,
Rtx-5, 30m, 0.53mm, 0.5um film
Flow Rate = 4.0ml/m



Default Calibration Report (other requested info not available)

External Standard Report (no recalibration)

Sorted By : Signal
Calib. Data Modified : 5/9/2000 12:22:35 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 B,

RetTime [min]	Type	Area counts*s	Amt/Area	Amount [ng/ul]	Grp	Name
11.175	BBA	2.70596e5	4.85023e-7	1.31245e-1		RH7281
Totals :				1.31245e-1		

Instrument 2 5/9/2000 12:24:57 PM ig

Page 1 of 2

Figure 4. 0.15 µg/ml Standard RH-7281 (GC/ECD).

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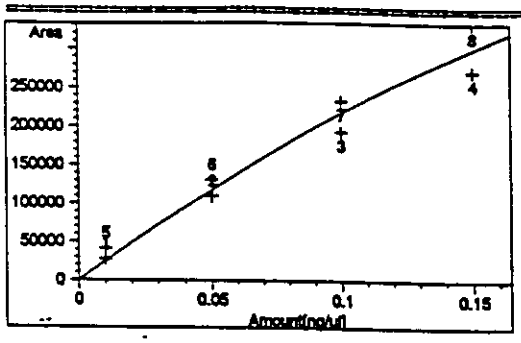
Method C:\HPCHEM\2\METHODS\PEEL-P.M

Calibration Curves



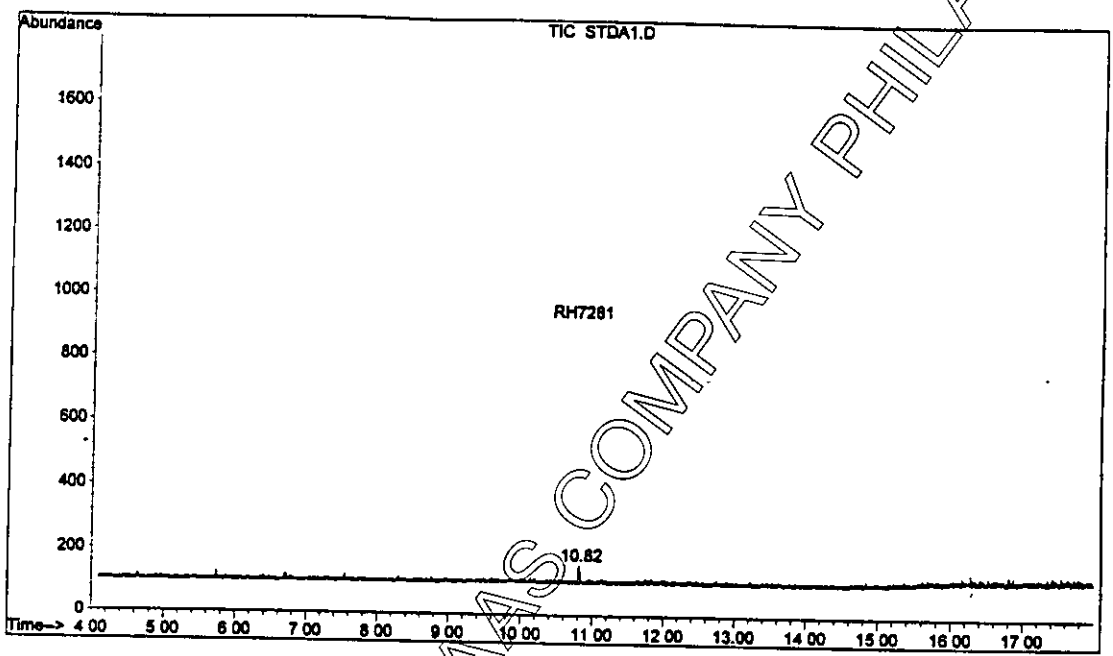
RH7281 at exp. RT: 11.176
ECD1 B,
Correlation: 0.99472
Residual Std. Dev.: 21449.61478
Formula: $y = ax^2 + bx$
a: -3.51352e6
b: 2.52289e6
x: Amount[ng/ul]
y: Area

Figure 5. Calibration Curve of RH-7281 (GC/ECD).

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78

```
File        : C:\HPCHEM\1\DATA\032920\STDA1.D
Operator    : ig
Acquired    : 29 Mar 00  16:33     using AcqMethod 7281SIM
Instrument  :    5973
Sample Name: std 0.01ppm
Misc Info   :
Vial Number: 1
```



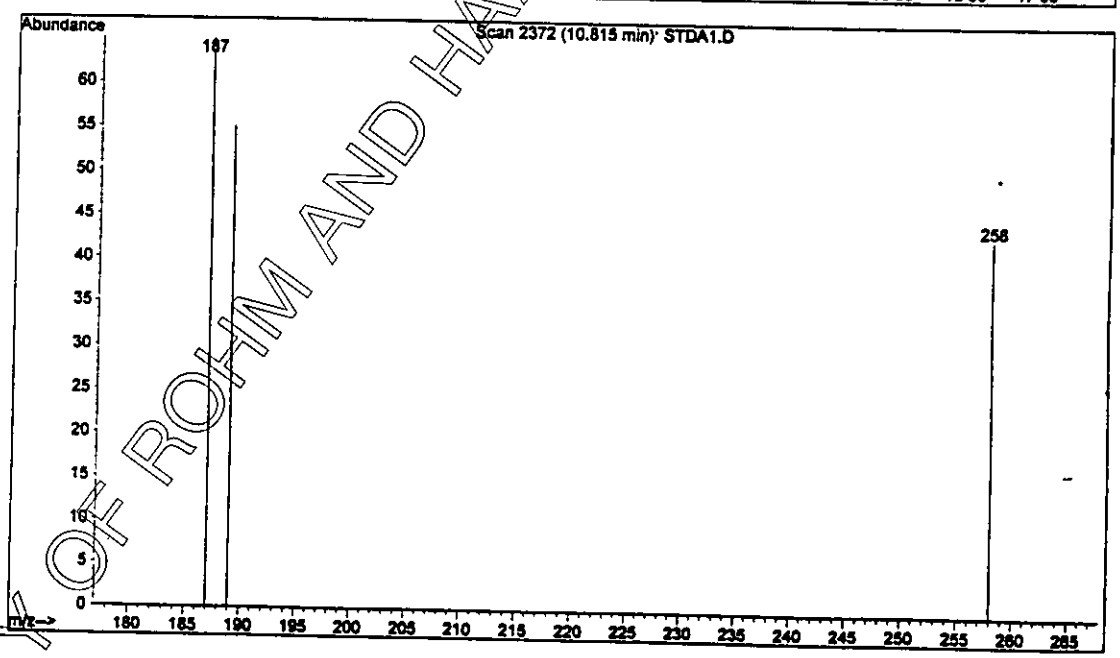
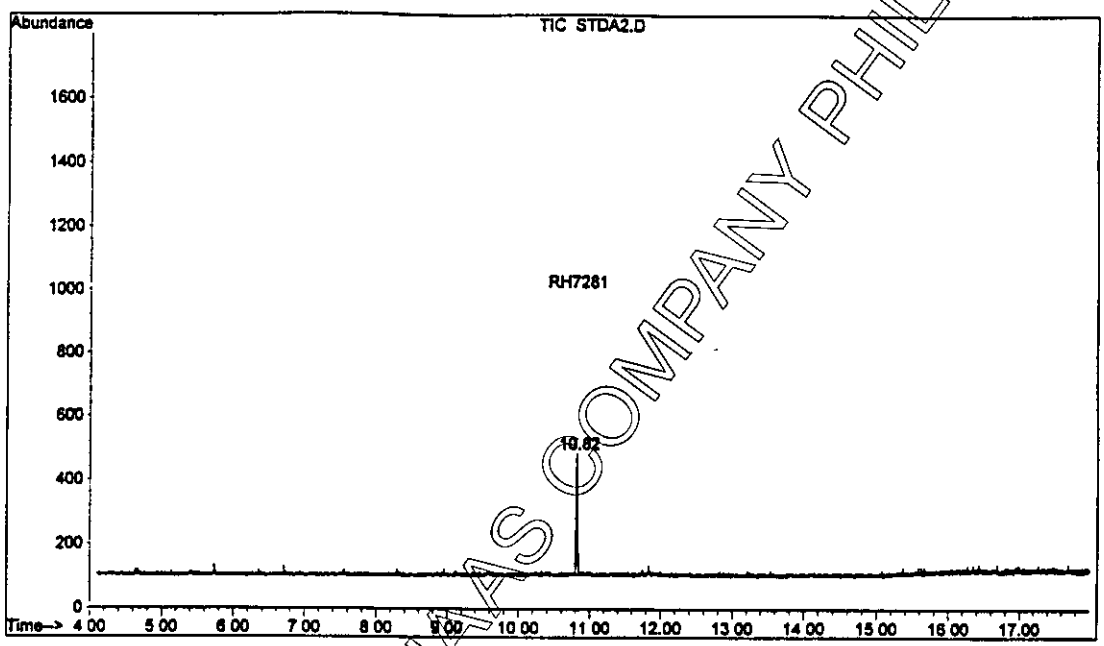


Figure 6. 0.010 µg/ml Standard RH-7281 (GC/MSD, Peak Area = 129).

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

```
File        : C:\HPCHEM\1\DATA\032920\STDA2.D
Operator    : ig
Acquired    : 29 Mar 00  17:00     using AcqMethod 7281SIM
Instrument  :    5973
Sample Name: std 0.05ppm
Misc Info   :
Vial Number: 2
```



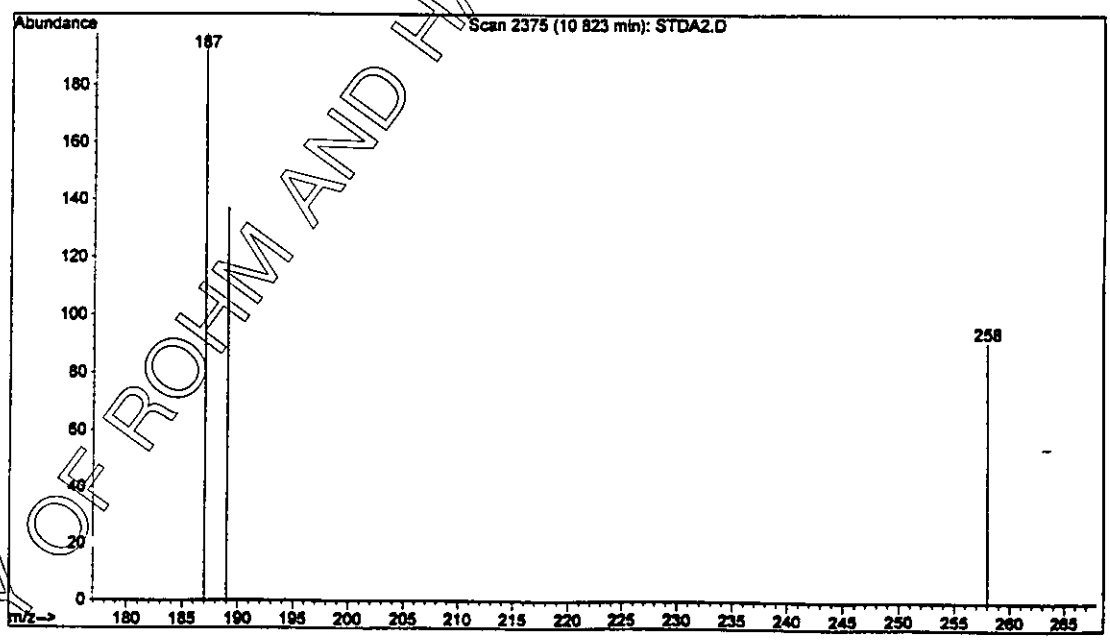
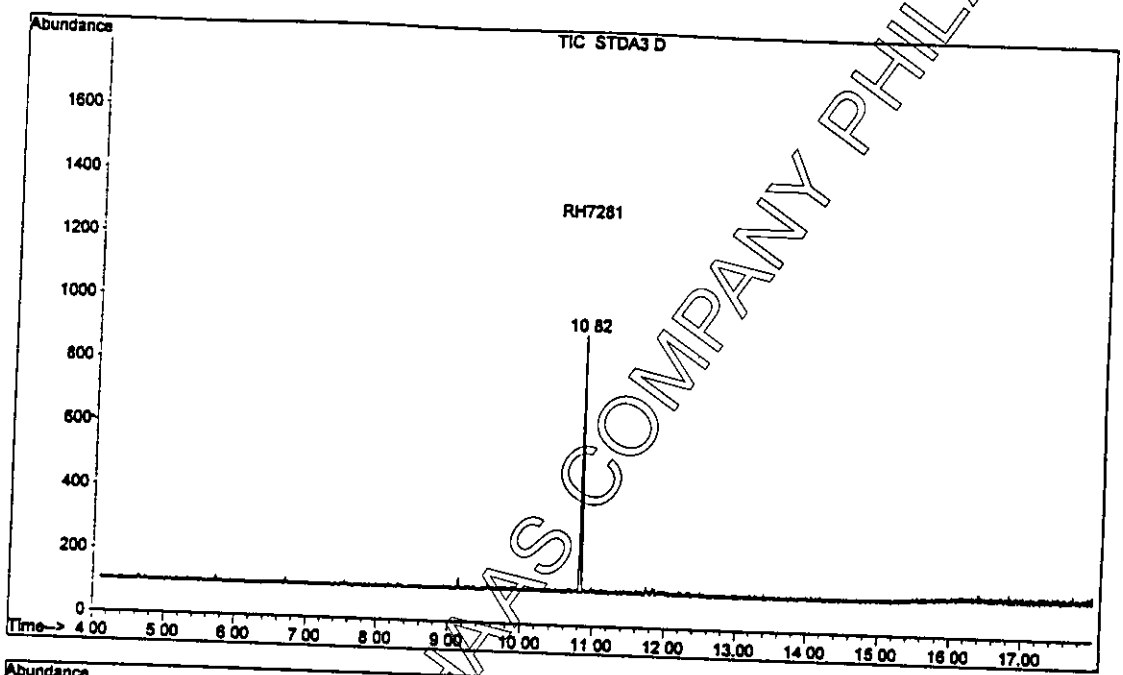


Figure 7. 0.050 µg/ml Standard RH-7281 (GC/MSD, Peak Area = 864).

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

```
File        : C:\HPCHEM\1\DATA\032920\STDA3.D
Operator    : ig
Acquired    : 29 Mar 00  17:26      using AcqMethod 7281SIM
Instrument  :    5973
Sample Name: std 0.10ppm
Misc Info   :
Vial Number: 3
```



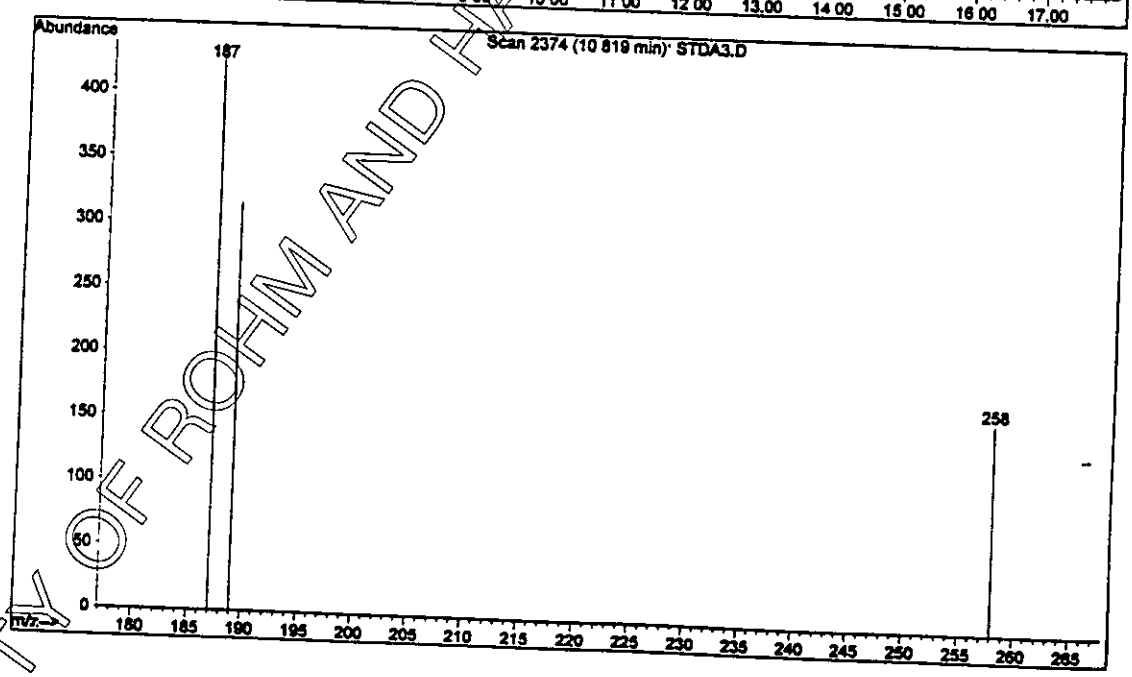
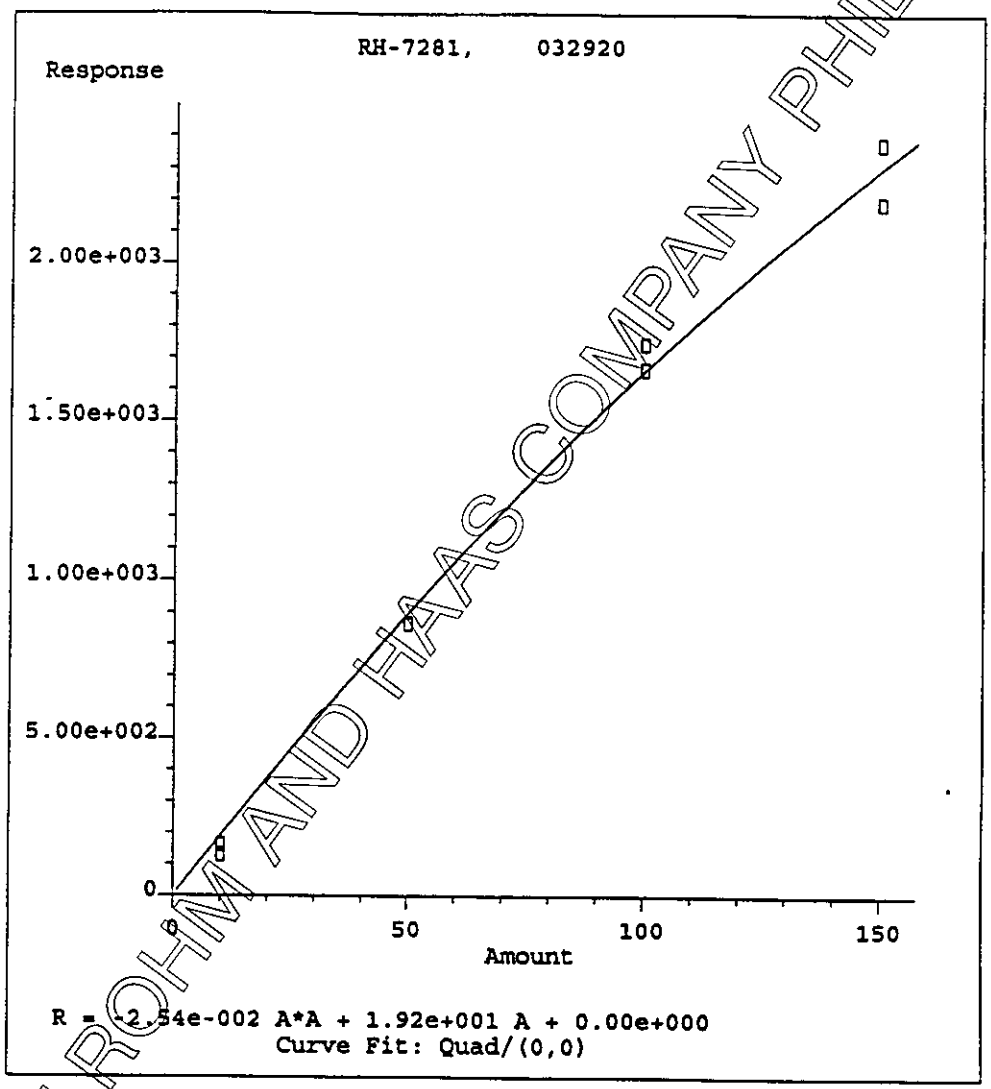


Figure 8. 0.10 µg/ml Standard RH-7281 (GC/MSD, Peak Area = 1745).

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78



Method Name: C:\HPCHEM\1\METHODS\7281SIM.M
Calibration Table Last Updated: Thu Mar 30 08:08:58 2000

Figure 10. Calibration Curve of RH-7281 (GC/MSD).

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78

Quantitation Report

Data File : c:\hpchem\2\data\031320\STD1.D
Acq On : 3-13-00 5:50:11 PM
Sample : 0.01 m2
Misc :

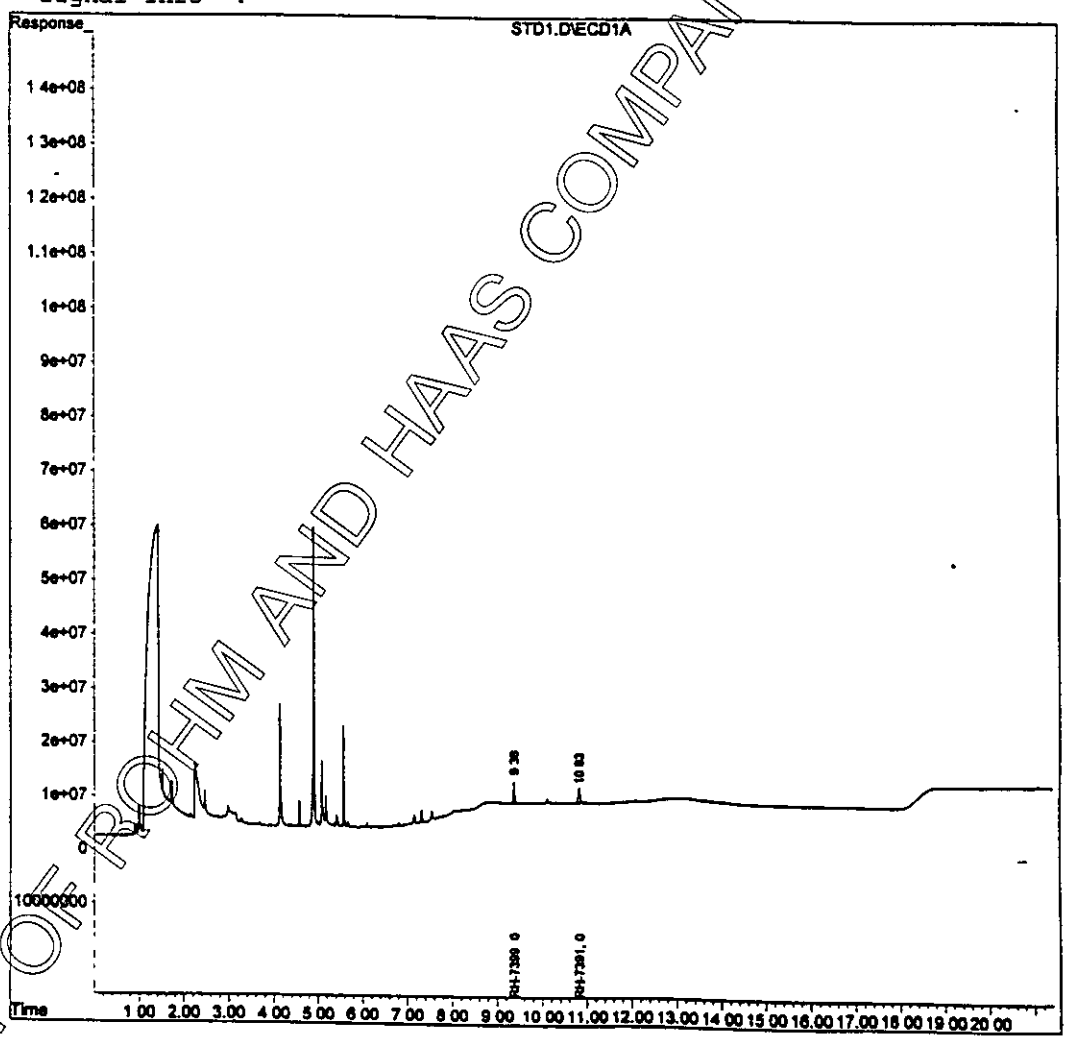
Vial: 1
Operator:
Inst : GC ECD
Multiplr: 1.00
Sample Amount: 0.00

IntFile : EVENTS.E

Quant Time: Mar 14 7:59 19100 Quant Results File: PEEL-M.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-M.M (Chemstation Integrator)
Title : 02-29-00
Last Update : Tue Mar 14 07:54:35 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-M.M

Volume Inj. :
Signal Phase :
Signal Info :



STD1.D PEEL-M.M Tue Mar 14 07:59:19 2000 Page 2

Figure 11. 0.010 µg/ml Standard RH-7391 and RH-7399 (GC/ECD, Rtx-225)
Peak Area$_{RH-7391}$ = 79582668, Peak Area$_{RH-7399}$ = 72697150.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Quantitation Report

Data File : c:\hpchem\2\data\031320\STD2.D
Acq On : 3-13-00 6:17:23 PM
Sample : 0.05 m2
Misc :

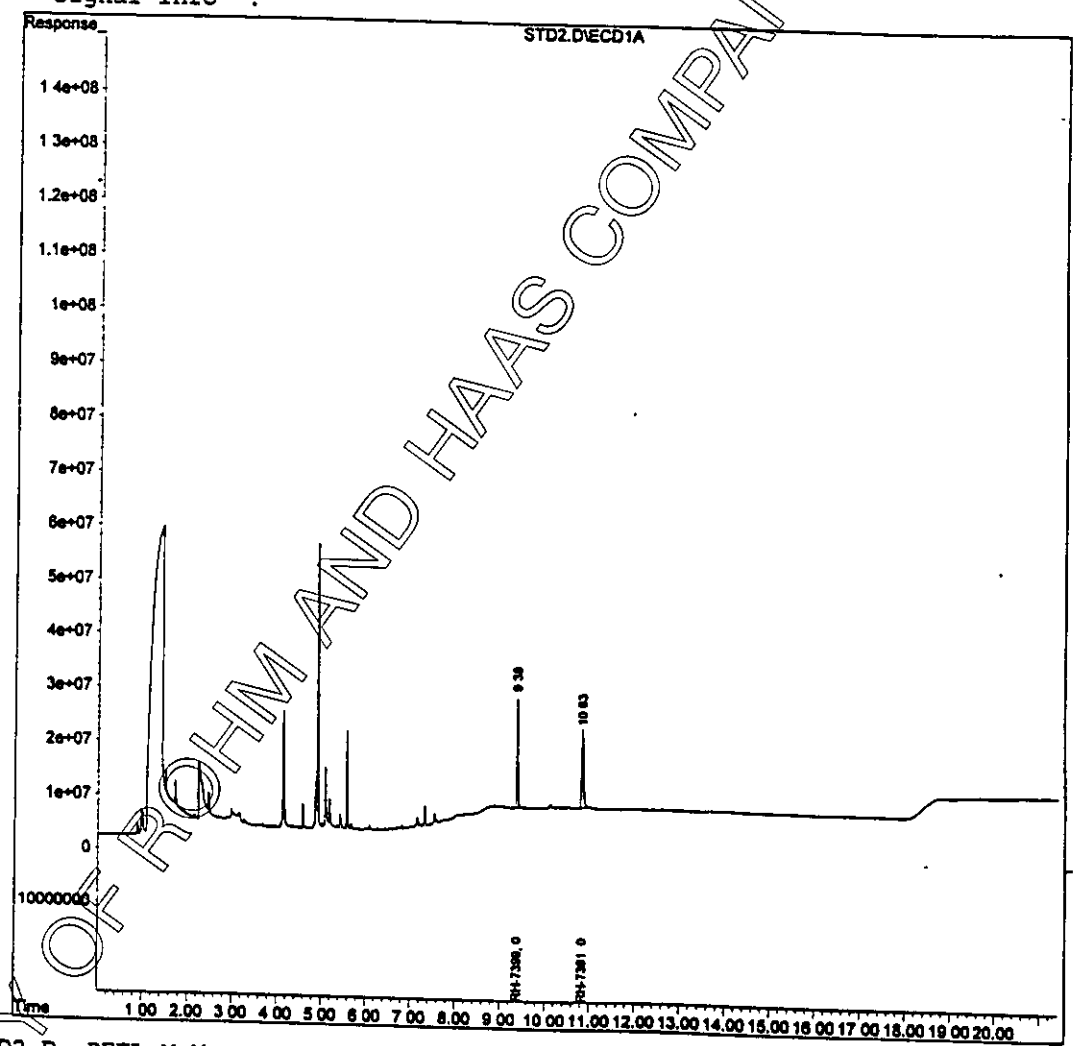
Vial: 2
Operator:
Inst : GC/ECD
Multiplr: 1.00
Sample Amount: 0.00

IntFile : EVENTS.E

Quant Time: Mar 14 7:59 19100 Quant Results File: PEEL-M.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-M.M (Chemstation Integrator)
Title : 02-29-00
Last Update : Tue Mar 14 07:54:35 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-M.M

Volume Inj. :
Signal Phase :
Signal Info :



STD2.D PEEL-M.M Tue Mar 14 08:00:03 2000

Page 2

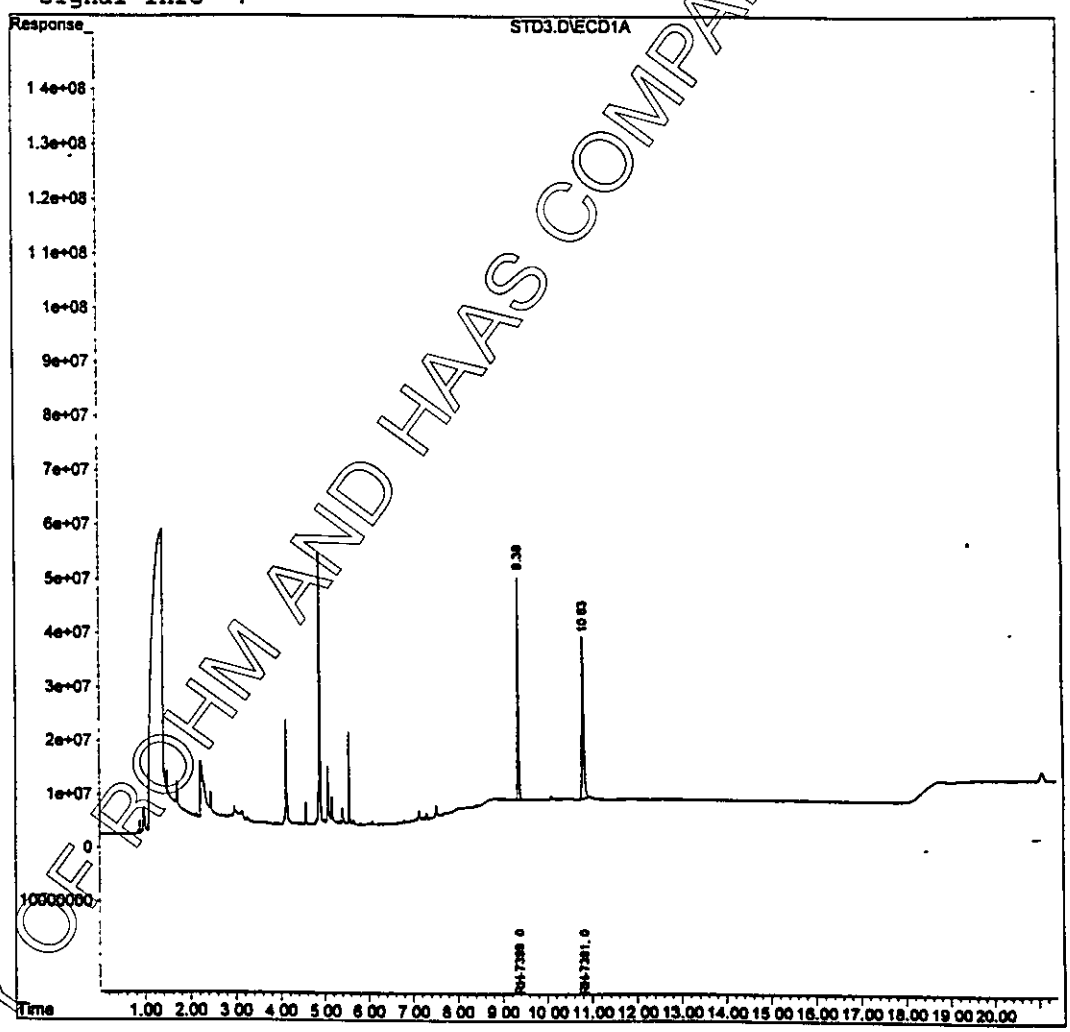
Figure 12. 0.050 µg/ml Standard RH-7391 and RH-7399 (GC/ECD, Rtx-225)
Peak Area $_{RH-7391}$ = 424704907, Peak Area $_{RH-7399}$ = 370760445.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78

Quantitation Report

```
Data File : c:\hpchem\2\data\031320\STD3.D                    Vial: 3
Acq On    : 3-13-00 6:44:36 PM                              Operator:
Sample    : 0.10 m2                                         Inst :  GC/ECD
Misc      :                                                 Multiplr: 1.00
                                                          Sample Amount: 0.00
IntFile   : EVENTS.E

Quant Time: Mar 14  8:00 19100  Quant Results File: PEEL-M.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-M.M (Chemstation Integrator)
Title        : 02-29-00
Last Update  : Tue Mar 14 07:54:35 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-M.M

Volume Inj.  :
Signal Phase :
Signal Info  :
```



STD3.D PEEL-M.M Tue Mar 14 08:00:45 2000 Page 2

Figure 13. 0.10 µg/ml Standard RH-7391 and RH-7399 (GC/ECD, Rtx-225)
Peak Area $_{RH\text{-}7391}$ = 869576680, Peak Area $_{RH\text{-}7399}$ = 754744397.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Quantitation Report

Data File : c:\hpchem\2\data\031320\STD4.D
Acq On : 3-13-00 7:11:49 PM
Sample : 0.15 m2
Misc :

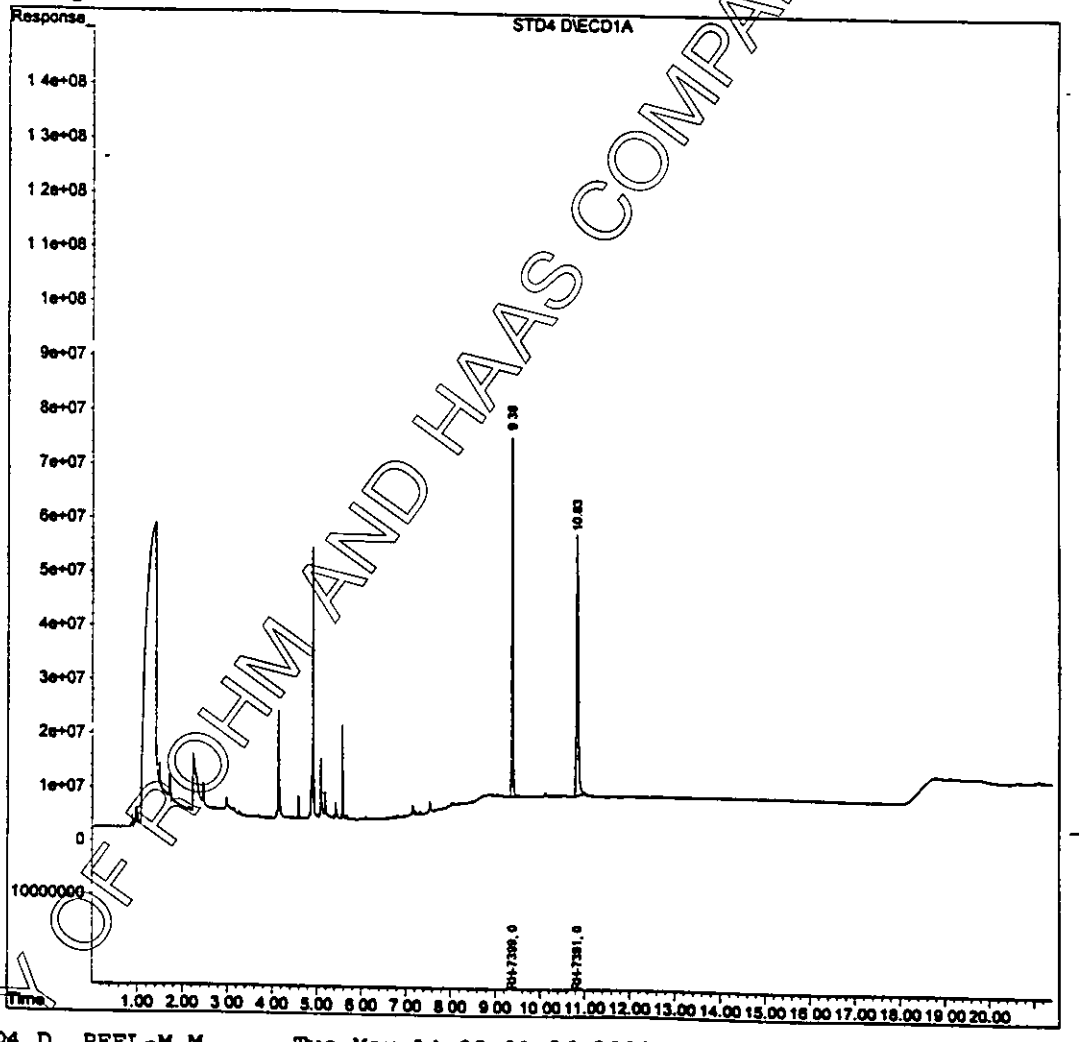
Vial: 4
Operator:
Inst : GC ECD
Multiplr: 1.00
Sample Amount: 0.00

IntFile : EVENTS.E

Quant Time: Mar 14 8:01 19100 Quant Results File: PEEL-M.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-M.M (Chemstation Integrator)
Title : 02-29-00
Last Update : Tue Mar 14 07:54:35 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-M.M

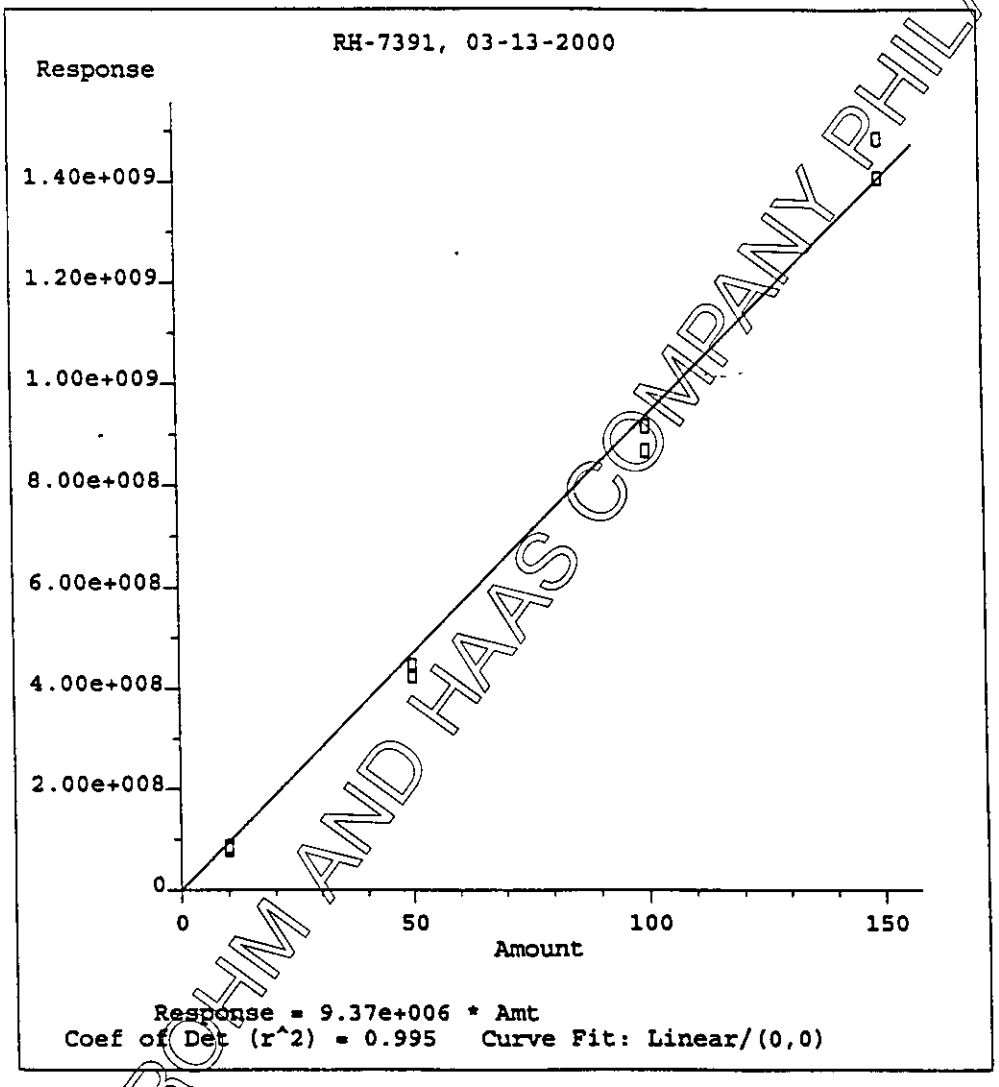
Volume Inj. :
Signal Phase :
Signal Info :



STD4.D PEEL-M.M Tue Mar 14 08:01:26 2000 Page 2

Figure 14. 0.15 µg/ml Standard RH-7391 and RH-7399 (GC/ECD, Rtx-225)
Peak Area $_{RH-7391}$ = 1407692926, Peak Area $_{RH-7399}$ = 1221064799.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

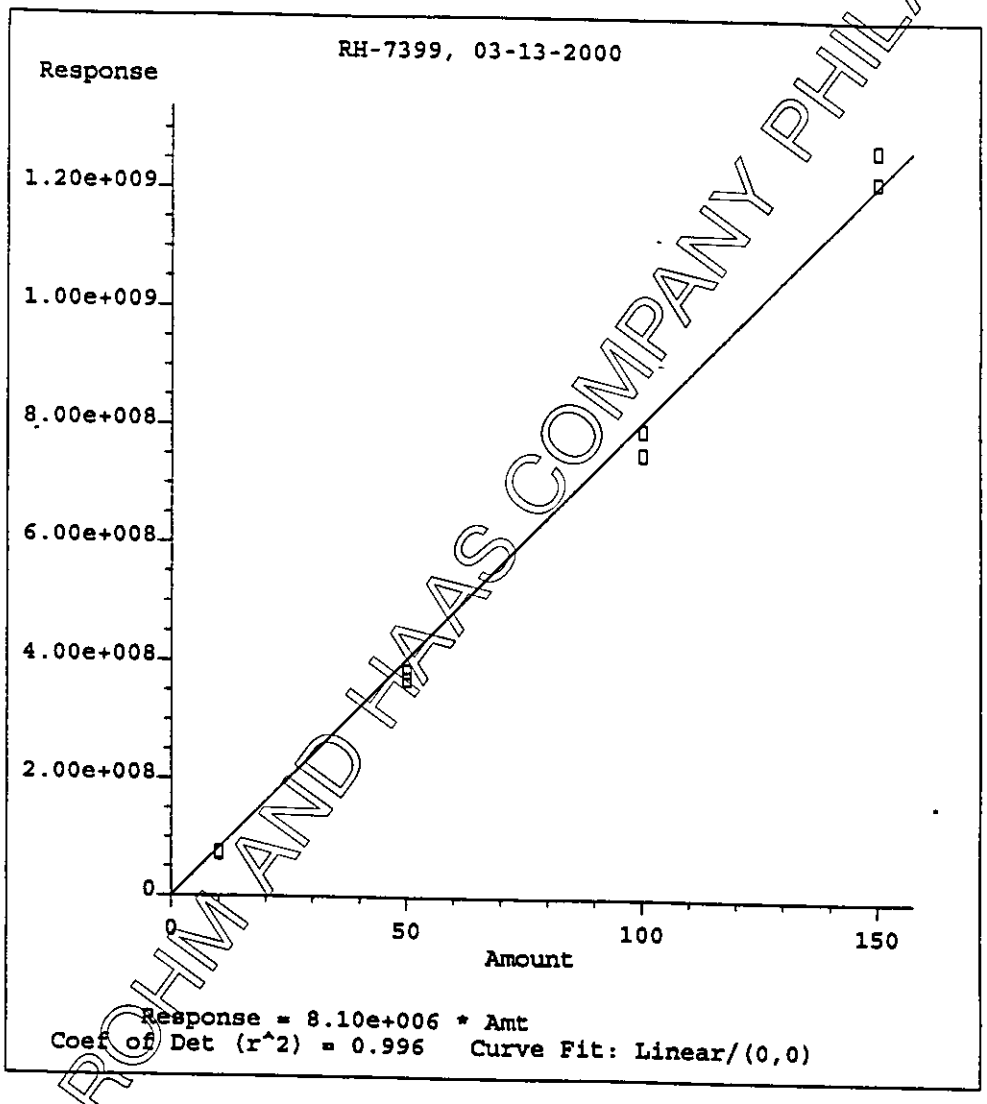


Method Name: C:\HPCHEM\2\METHODS\PEEL-M.M
Calibration Table Last Updated: Tue Mar 14 07:47:28 2000

Figure 15. Calibration Curve of RH-7391 (GC/ECD, Rtx-225).

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78



Method Name: C:\HPCHEM\2\METHODS\PEEL-M.M
Calibration Table Last Updated: Tue Mar 14 07:47:28 2000

Figure 16. Calibration Curve of RH-7399 (GC/ECD, Rtx-225).

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78

Quantitation Report

Data File : c:\hpchem\2\data\050120\STD1.D
Acq On : 5-1-00 5:02:10 PM
Sample : 0.01 m2
Misc :

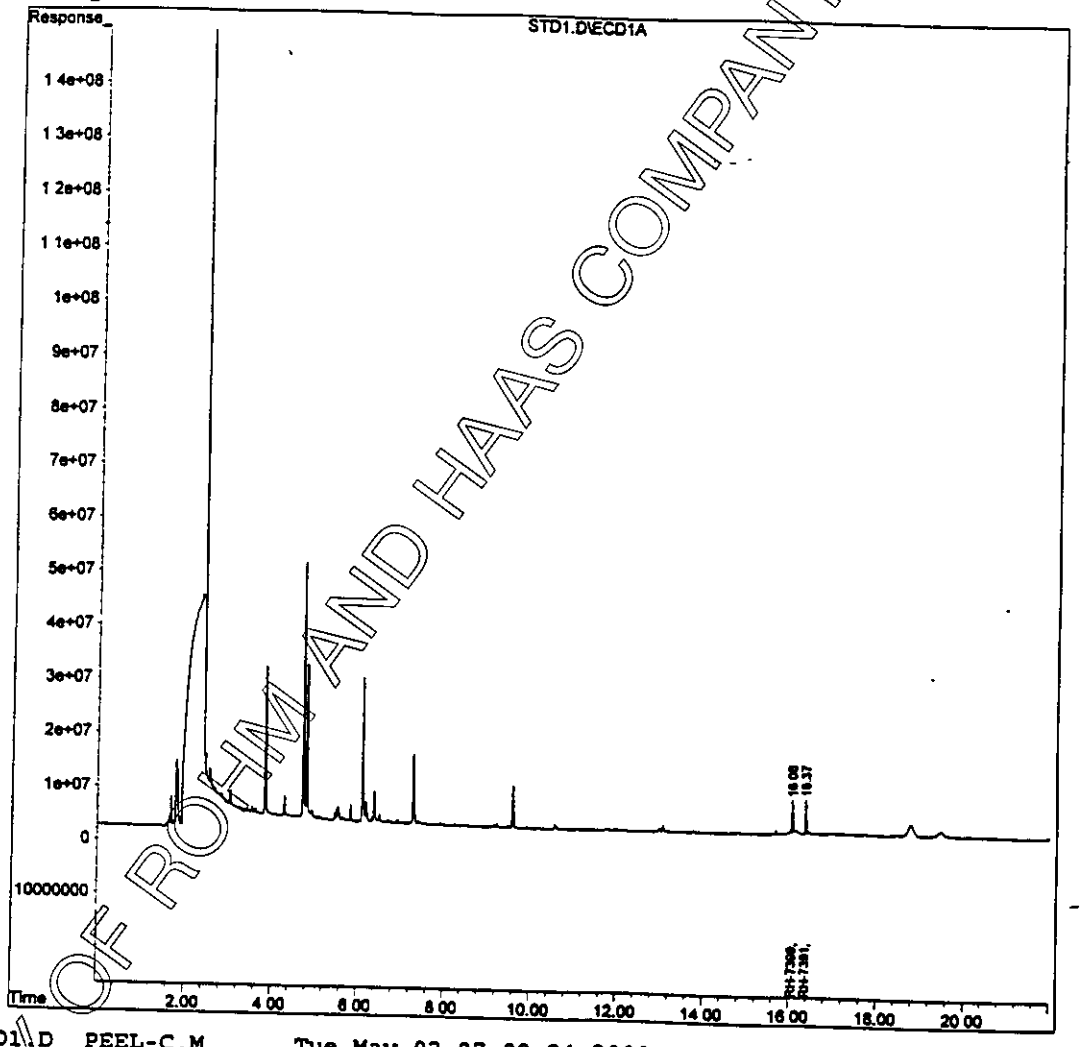
Vial: 1
Operator:
Inst : GC ECD
Multiplr: 1.00
Sample Amount: 0.00

IntFile : EVENTS.E

Quant Time: May 2 7:02 19100 Quant Results File: PEEL-C.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-C.M (Chemstation Integrator)
Title : 02-29-00
Last Update : Tue May 02 06:47:05 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-C.M

Volume Inj. :
Signal Phase :
Signal Info :



STD1.D PEEL-C.M Tue May 02 07:02:24 2000 Page 2

Figure 17. 0.010 µg/ml Standard RH-7391 and RH-7399 (GC/ECD, Rtx-35)
Peak Area$_{RH-7391}$ =105206321, Peak Area$_{RH-7399}$ =102601052.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Quantitation Report

Data File : c:\hpchem\2\data\050120\STD2.D
Acq On : 5-1-00 5:32:04 PM
Sample : 0.05 m2
Misc :

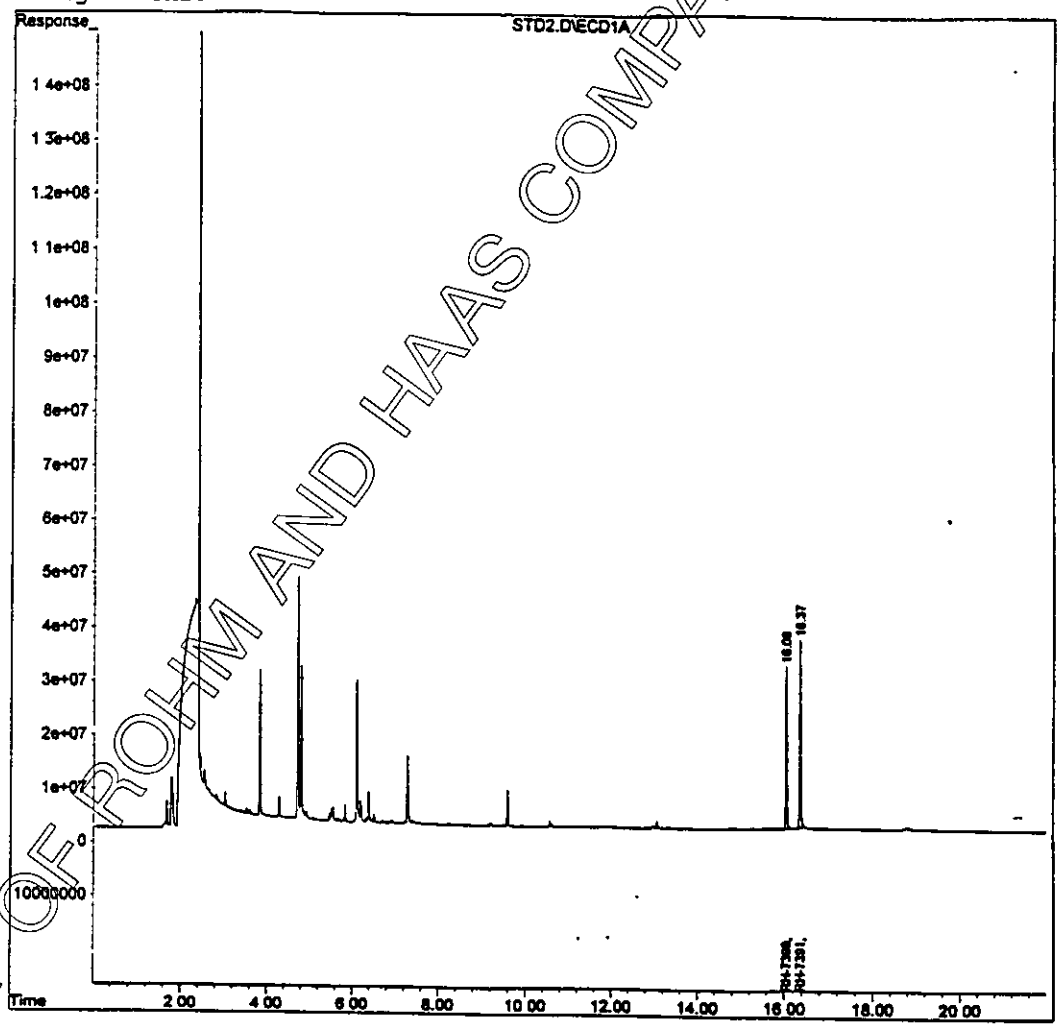
Vial: 2
Operator:
Inst : GC ECD
Multiplr: 1.00
Sample Amount: 0.00

IntFile : EVENTS.E

Quant Time: May 2 7:02 19100 Quant Results File: PEEL-C.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-C.M (Chemstation Integrator)
Title : 02-29-00
Last Update : Tue May 02 06:47:05 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-C.M

Volume Inj. :
Signal Phase :
Signal Info :



STD2.D PEEL-C.M Tue May 02 07:03:06 2000 Page 2

Figure 18. 0.050 µg/ml Standard RH-7391 and RH-7399 (GC/ECD, Rtx-35)
Peak Area $_{RH\text{-}7391}$ =626408524, Peak Area $_{RH\text{-}7399}$ =516386376.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78

Quantitation Report

Data File : c:\hpchem\2\data\050120\STD3.D
Acq On : 5-1-00 6:02:00 PM
Sample : 0.10 m2
Misc :

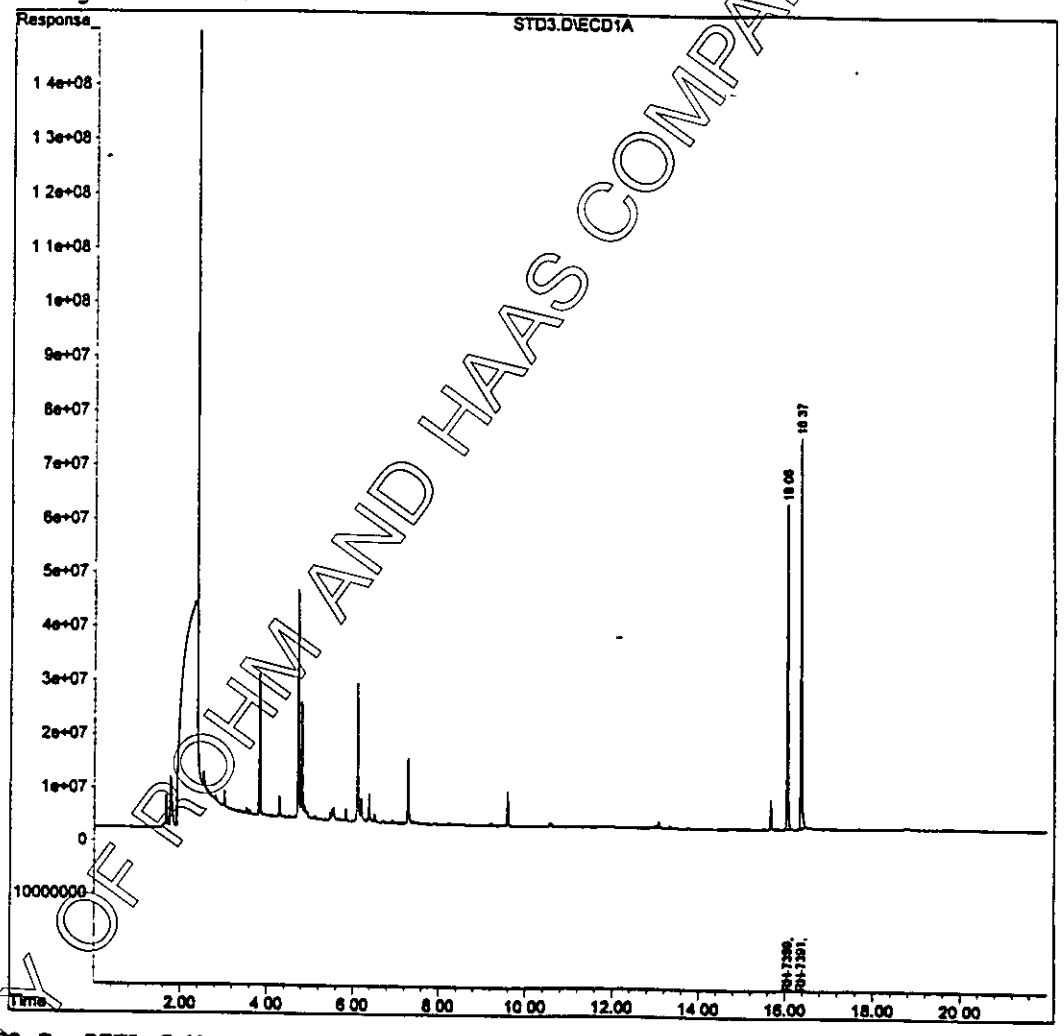
Vial: 3
Operator:
Inst : GC ECD
Multiplr: 1.00
Sample Amount: 0.00

IntFile : EVENTS.E

Quant Time: May 2 7:03 19100 Quant Results File: PEEL-C.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-C.M (Chemstation Integrator)
Title : 02-29-00
Last Update : Tue May 02 06:47:05 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-C.M

Volume Inj. :
Signal Phase :
Signal Info :



STD3.D PEEL-C.M Tue May 02 07:03:48 2000 Page 2

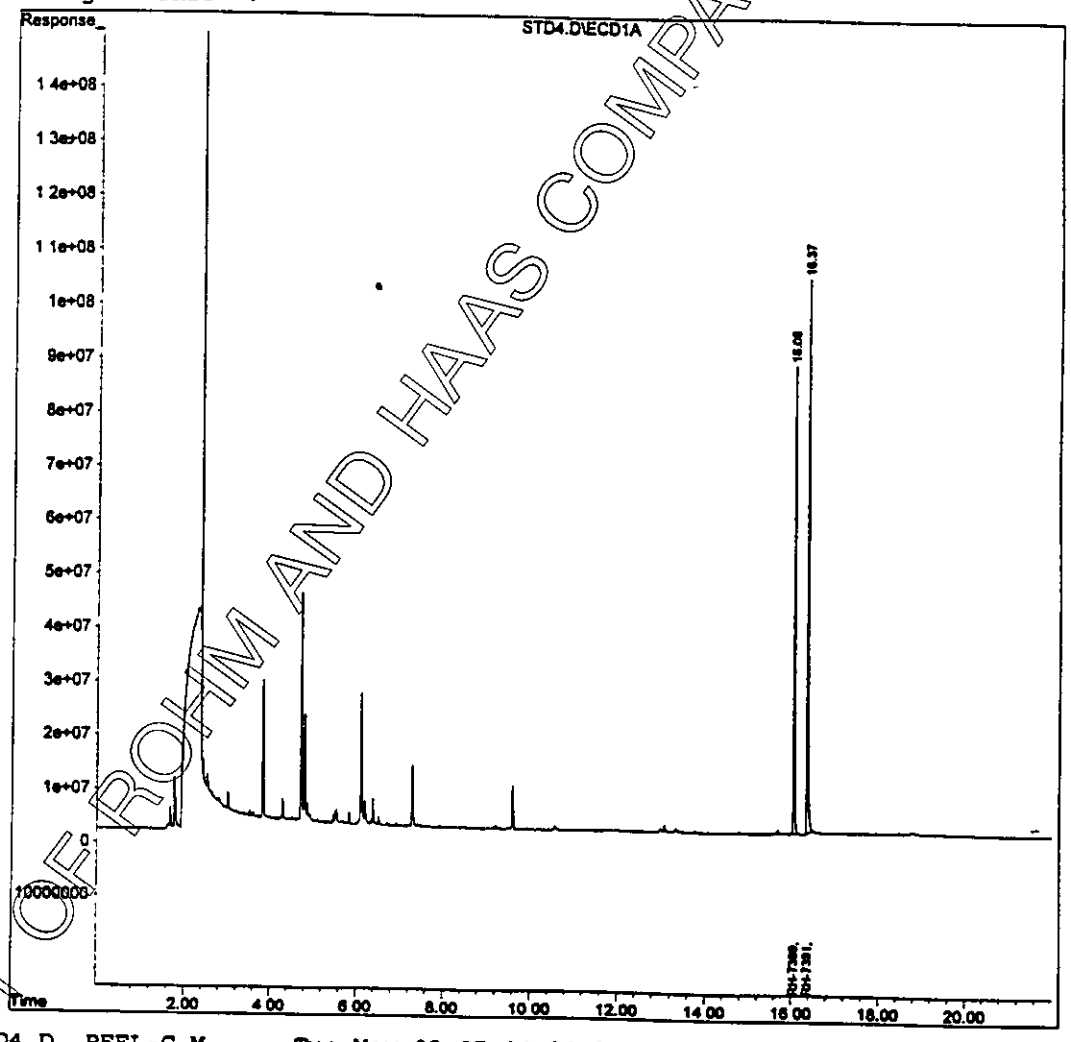
Figure 19. 0.10 µg/ml Standard RH-7391 and RH-7399 (GC/ECD, Rtx-35)
Peak Area $_{RH-7391}$ =1299297577 , Peak Area $_{RH-7399}$ =1035717308.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78

Quantitation Report

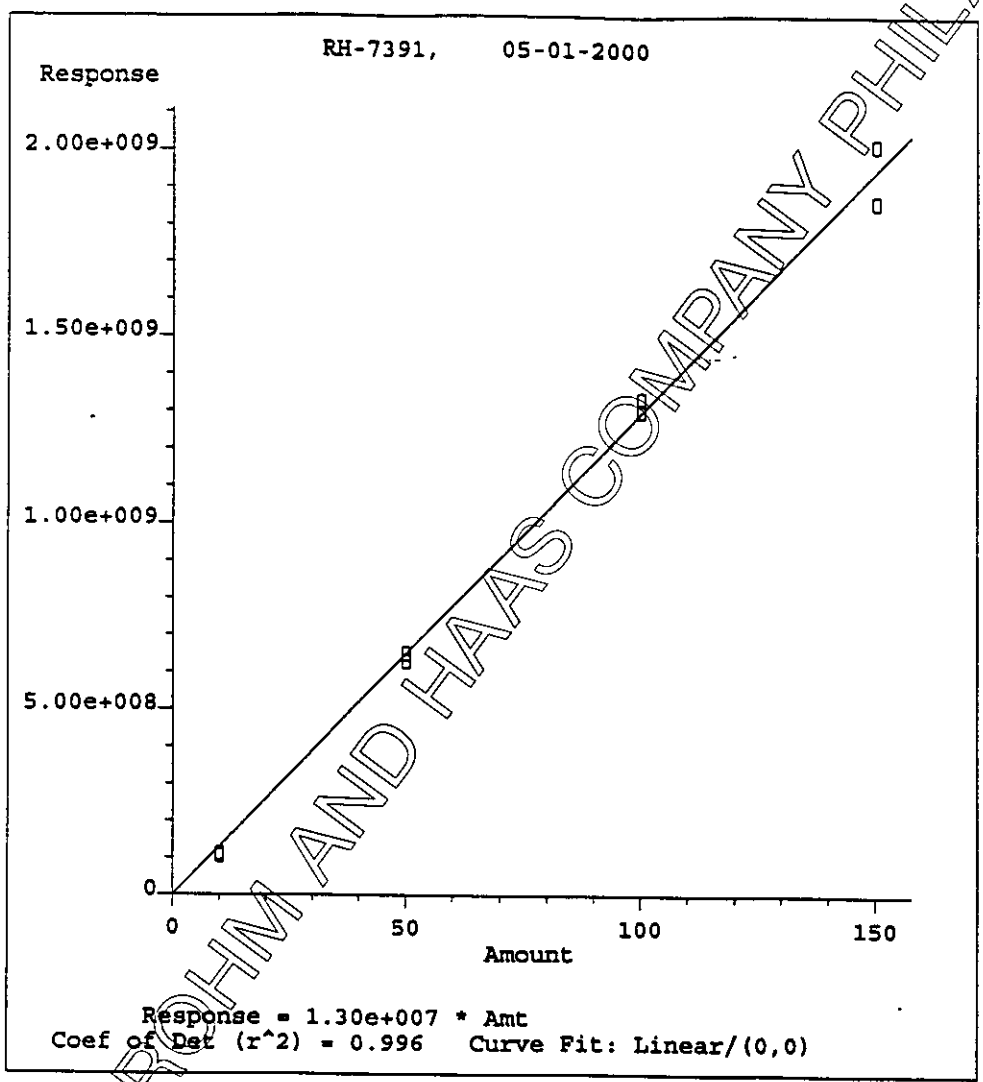
```
Data File : c:\hpchem\2\data\050120\STD4.D                    Vial: 4
Acq On    : 5-1-00 6:31:55 PM                              Operator:
Sample    : 0.15 m2                                        Inst    : GC/ECD
Misc      :                                                Multiplr: 1.00
                                                       Sample Amount: 0.00
IntFile   : EVENTS.E

Quant Time: May  2  7:04 19100  Quant Results File: PEEL-C.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-C.M (Chemstation Integrator)
Title        : 02-29-00
Last Update  : Tue May 02 06:47:05 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-C.M

Volume Inj.  :
Signal Phase :
Signal Info  :
```



STD4.D PEEL-C.M Tue May 02 07:04:30 2000 Page 2

Figure 20. 0.15 µg/ml Standard RH-7391 and RH-7399 (GC/ECD, Rtx-35)
Peak Area $_{RH\text{-}7391}$ = 1858354530, Peak Area $_{RH\text{-}7399}$ =144959168.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

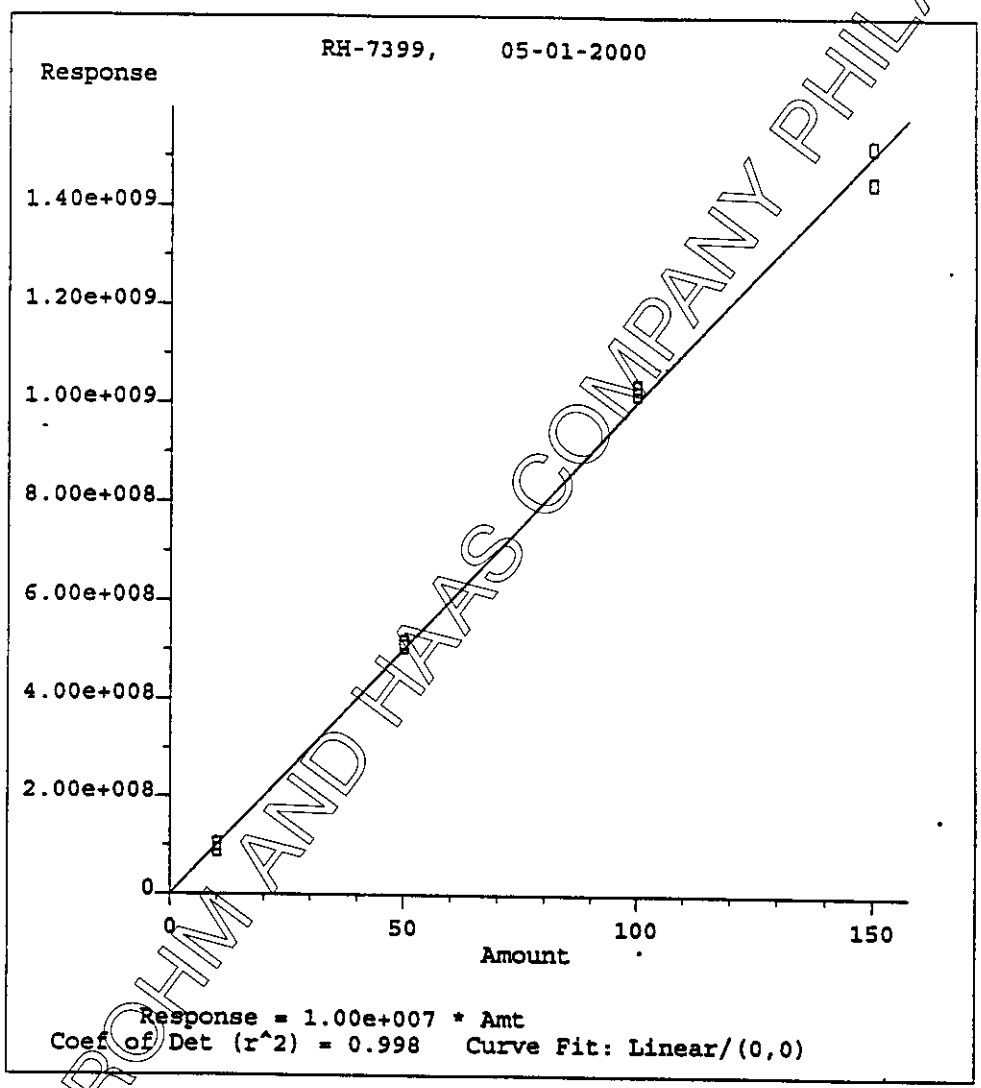


Method Name: C:\HPCHEM\2\METHODS\PEEL-M.M
Calibration Table Last Updated: Tue May 02 06:47:05 2000

Figure 21. Calibration Curve of RH-7391 (GC/ECD, Rtx-35).

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78



Method Name: C:\HPCHEM\2\METHODS\PEEL-M.M
Calibration Table Last Updated: Tue May 02 06:47:05 2000

Figure 22: Calibration Curve of RH-7399 (GC/ECD, Rtx-35).

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78

Data File C:\HPCHEM\2\DATA\031020\006B0801.D

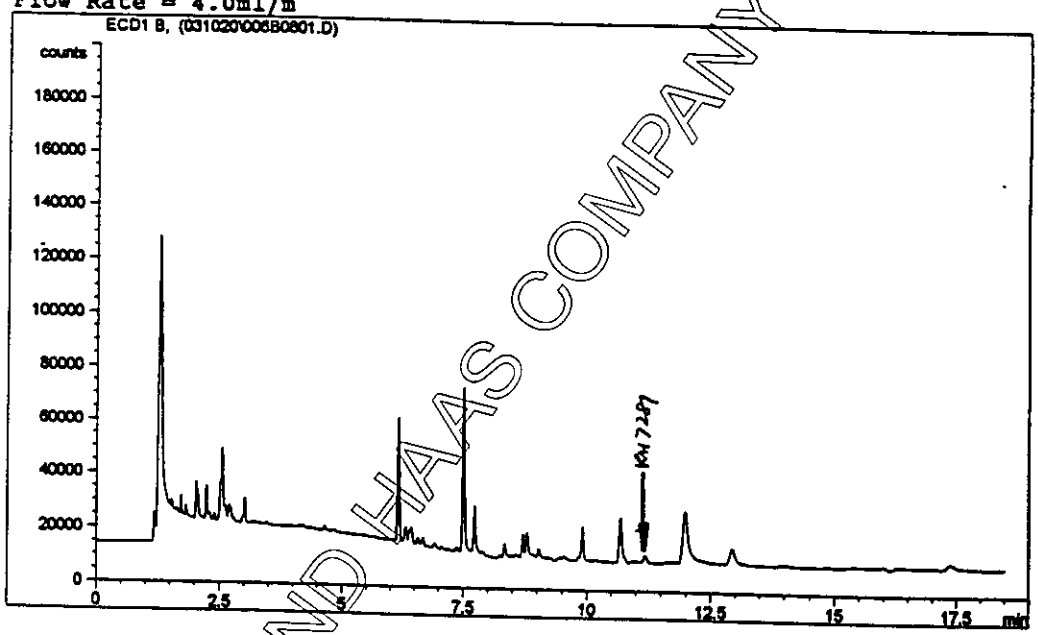
Sample Name: p1-001

peel control

Injection Date : 3/10/2000 6:35:15 PM
Sample Name : p1-001
Acq. Operator : ig

Seq. Line : 8
Vial : 6
Inj : 1
Inj Volume : 1 µl

Sequence File : C:\HPCHEM\2\SEQUENCE\IG031020.S
Acq. Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 3/3/2000 2:11:59 PM by ig
Analysis Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 5/9/2000 12:22:42 PM by ig
RH7281 Potato Peel Waste METHOD,
Rtx-5, 30m, 0.53mm, 0.5um film
Flow Rate = 4.0ml/m



External Standard Report

Sorted By : Signal
Calib. Data Modified : 5/9/2000 12:22:35 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 B,

RetTime [min]	Type	Area counts*s	Amt/Area	Amount [ng/ul]	Grp	Name
11.176		-	-	-		RH7281
Totals :				0.00000		

Results obtained with enhanced integrator!

Instrument 2 5/9/2000 12:25:04 PM ig

Page 1 of 2

Figure 23. Control Potato for RH-7281 Analysis (GC/ECD).
RH-7281 Found = 0 ppm

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78

Data File C:\HPCHEM\2\DATA\032720\006B0801.D Sample Name: p3-001

peel control

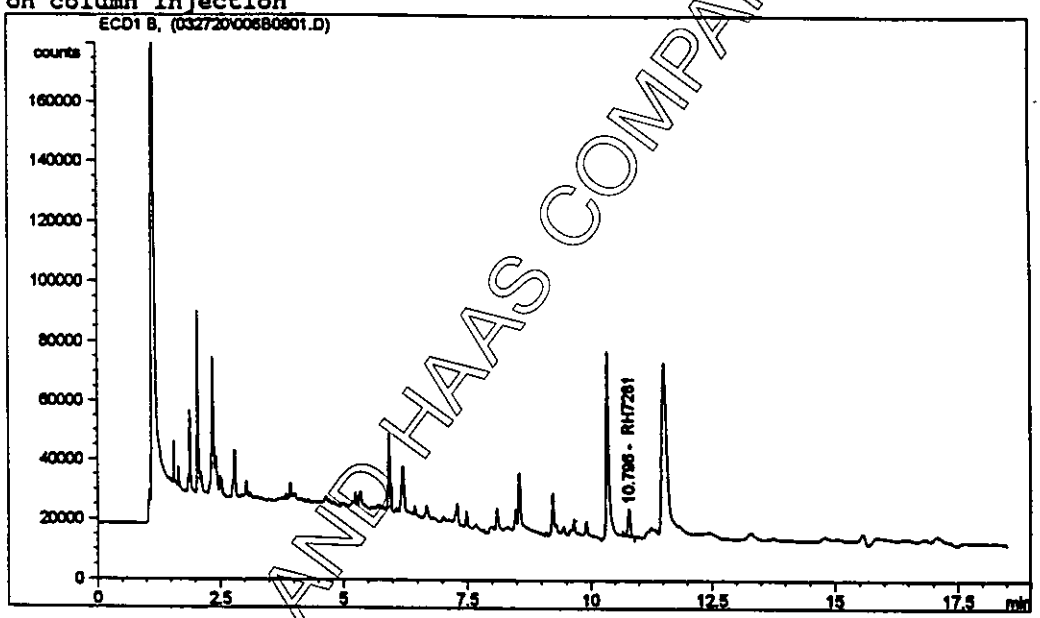
Injection Date : 3/27/2000 7:08:44 PM
Sample Name : p3-001
Acq. Operator : ig

Seq. Line : 8
Vial : 6
Inj : 1
Inj Volume : 1 µl

Acq. Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 3/27/2000 9:14:19 AM by ig
Analysis Method : C:\HPCHEM\2\METHODS\PULSOR.M
Last changed : 5/24/2000 1:14:40 PM by dc
(modified after loading)

RH7281 tomato puree

Column flow, N2, 4.0 ml/min
RTX-5MS (0.53mm I.D. x 30m; 0.5um film)
on column injection



External Standard Report

Sorted By : Signal
Calib. Data Modified : 5/23/2000 3:02:16 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 B,

RetTime [min]	Type	Area counts*s	Amt/Area	Amount [ug/ml]	Grp	Name
10.796	VB	3.49344e4	2.73048e-7	9.53877e-3		RH7281

Totals : 9.53877e-3

Instrument 2 5/24/2000 1:14:48 PM dc Page 1 of 2

Figure 24. Control Potato for RH-7281 Analysis (GC/ECD, 03/24/2000).
RH-7281 Found = 0.00954 ppm

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

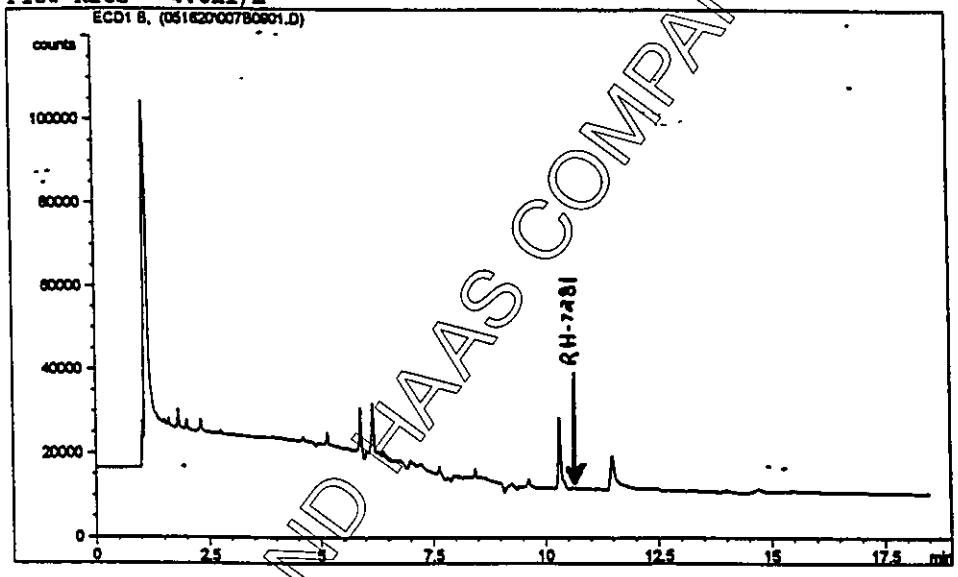
FORM 4685 REV 10/78

Data File C:\HPCHEM\2\DATA\051620\007B0901.D Sample Name: p-002

Control Peel 97-0078-1

Injection Date : 5/16/2000 6:50:01 PM Seq. Line : 9
Sample Name : p-002 Vial : 7
Acq. Operator : ig Inj : 1
Inj Volume : 1 µl

Sequence File : C:\HPCHEM\2\SEQUENCE\IG051620.S
Acq. Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 5/13/2000 3:59:01 PM by ig
Analysis Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 5/26/2000 1:38:56 PM by dc
RH7281 Potato Peel Waste METHOD,
Rtx-5, 30m, 0.53mm, 0.5um film
Flow Rate = 4.0ml/m



External Standard Report

Sorted By : Signal
Calib. Data Modified : 5/26/2000 1:38:47 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 B,

RetTime [min]	Type	Area counts*s	Amt/Area	Amount [ng/ul]	Grp	Name
10.758		-	-	-		RH7281

Totals : 0.00000

Results obtained with enhanced integrator!

Figure 25. Control Potato for RH-7281 Analysis (GC/ECD, 05/15/2000 RH-7281 Found = 0 ppm

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78

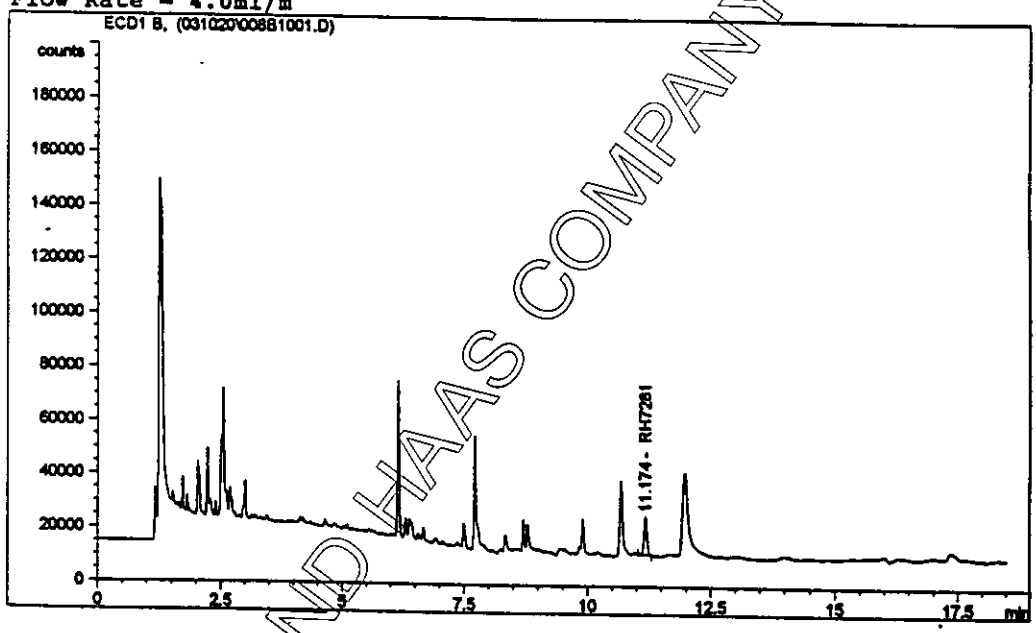
Data File C:\HPCHEM\2\DATA\031020\008B1001.D Sample Name: p1-003

peel fort. 0.02ppm

Injection Date : 3/10/2000 7:22:25 PM
Sample Name : p1-003
Acq. Operator : ig

Seq. Line : 10
Vial : 8
Inj : 1
Inj Volume : 1 µl

Sequence File : C:\HPCHEM\2\SEQUENCE\IG031020.S
Acq. Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 3/3/2000 2:11:59 PM by ig
Analysis Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 5/9/2000 12:22:42 PM by ig
RH7281 Potato Peel Waste METHOD,
Rtx-5, 30m, 0.53mm, 0.5um film
Flow Rate = 4.0ml/m



External Standard Report

Sorted By : Signal
Calib. Data Modified : 5/9/2000 12:22:35 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 B,

RetTime [min]	Type	Area counts*s	Amt/Area	Amount [ng/ul]	Grp	Name
11.174	PBA	6.03334e4	4.10532e-7	2.47688e-2		RH7281

Totals : 2.47688e-2

Results obtained with enhanced integrator!

Instrument 2 5/9/2000 12:25:10 PM ig Page 1 of 2

Figure 26. Fortification of RH-7281 at 0.020 ppm (GC/ECD, 03/10/2000)
RH-7281 Found = 0.02477 ppm, Recovery = 124%.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Data File C:\HPCHEM\2\DATA\032720\008B1001.D

Sample Name: p3-003

peel fort. 0.02ppm

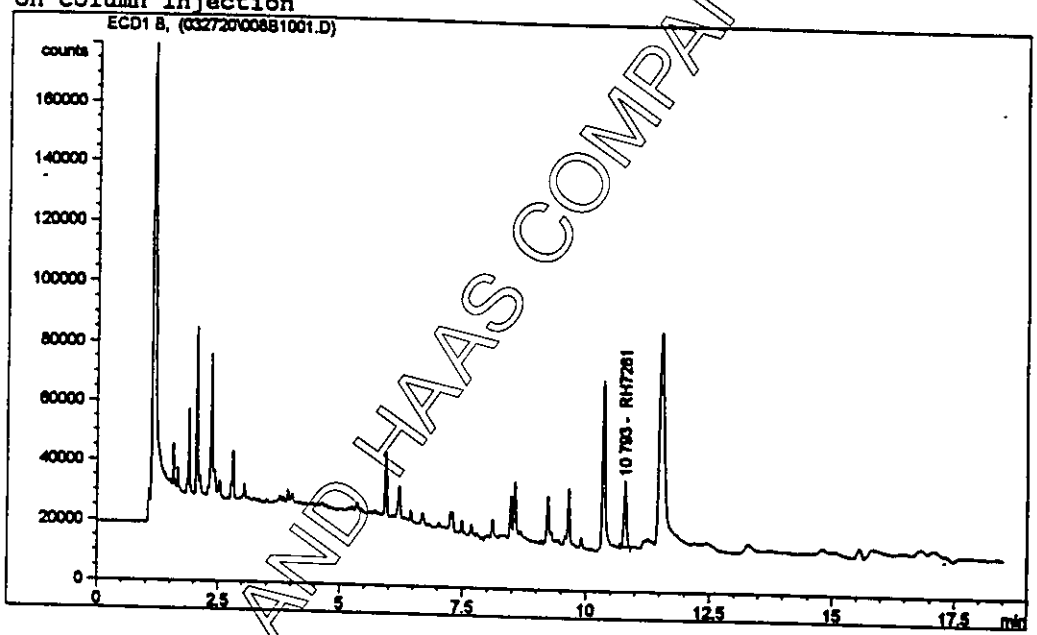
Injection Date : 3/27/2000 7:56:03 PM
Sample Name : p3-003
Acq. Operator : ig

Seq. Line : 10
Vial : 8
Inj : 1
Inj Volume : 1 µl

Acq. Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 3/27/2000 9:14:19 AM by ig
Analysis Method : C:\HPCHEM\2\METHODS\PULSOR.M
Last changed : 5/23/2000 3:07:10 PM by dc
(modified after loading)

RH7281 tomato puree

Column flow, N2, 4.0 ml/min
RTX-5MS (0.53mm I.D. x 30m; 0.5um film)
on column injection



External Standard Report

Sorted By : Signal
Calib. Data Modified : 5/23/2000 3:02:16 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 B,

RetTime [min]	Type	Area counts*s	Amt/Area	Amount [ug/ml]	Grp	Name
10.793	PB	8.46440e4	2.80536e-7	2.37457e-2		RH7281

Totals : 2.37457e-2

Instrument 2 5/23/2000 3:07:26 PM dc

Page 1 of 2

Figure 27. Fortification of RH-7281 at 0.020 ppm (GC/ECD, 03/24/2000)
RH-7281 Found = 0.02375 ppm, Recovery = 77.1%.

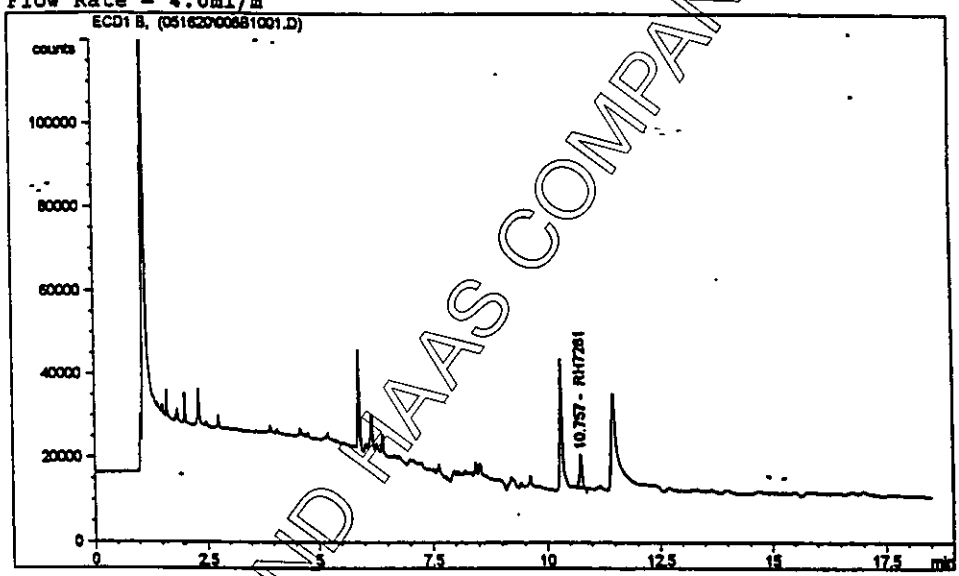
PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78

Data File C:\HPCHEM\2\DATA\051620\008B1001.D Sample Name: p-003

Fortification at 0.02ppm of 97-0078-1

```
Injection Date  : 5/16/2000 7:13:39 PM          Seq. Line :  10
Sample Name     : p-003                              Vial :   8
Acq. Operator   : ig                                  Inj :   1
                                                Inj Volume : 1 µl
Sequence File   : C:\HPCHEM\2\SEQUENCE\IG051620.S
Acq. Method     : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed    : 5/13/2000 3:59:01 PM by ig
Analysis Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed    : 5/26/2000 1:38:56 PM by dc
RH7281 Potato Peel Waste METHOD,
Rtx-5, 30m, 0.53mm, 0.5um film
Flow Rate = 4.0ml/m
```



External Standard Report

```
Sorted By              :      Signal
Calib. Data Modified   :      5/26/2000 1:38:47 PM
Multiplier             :      1.0000
Dilution               :      1.0000

Signal 1: ECD1 B,

RetTime  Type     Area      Amt/Area    Amount    Grp  Name
 [min]          counts*s                [ng/ul]
-------|------|----------|----------|----------|--|------------------
 10.757 BB     3.23099e4  4.91065e-7 1.58663e-2    RH7281

Totals :                              1.58663e-2

 Results obtained with enhanced integrator!
```

Figure 28. Fortification of RH-7281 at 0.020 ppm (GC/ECD, 05/15/2000)
RH-7281 Found = 0.01587 ppm, Recovery = 79.3%

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Data File C:\HPCHEM\2\DATA\031020\010B1201.D

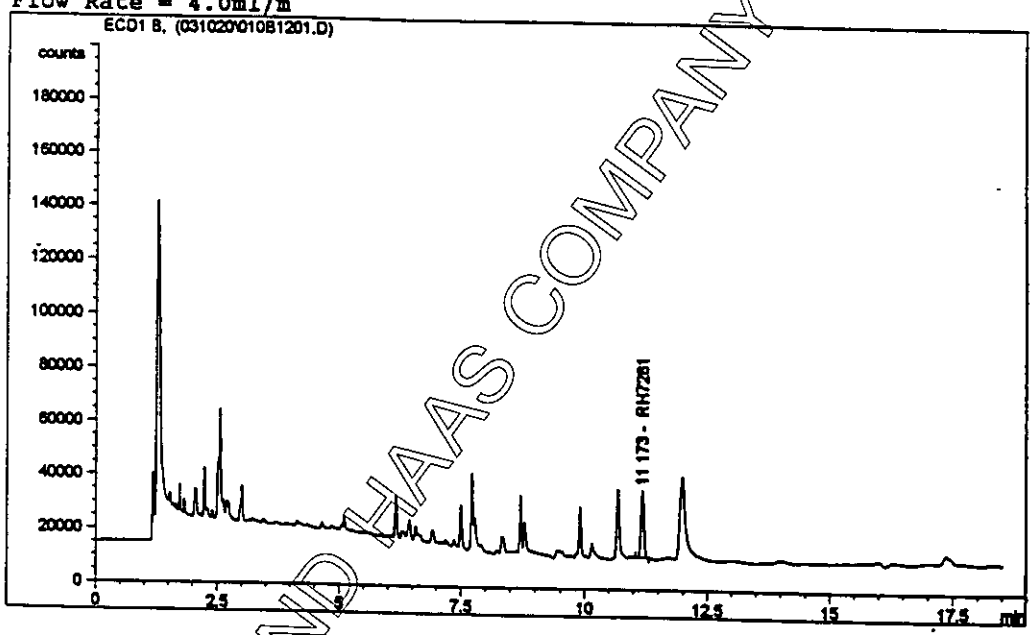
Sample Name: p1-005

peel fort. 0.05ppm

Injection Date : 3/10/2000 8:09:36 PM
Sample Name : p1-005
Acq. Operator : ig

Seq. Line : 12
Vial : 10
Inj : 1
Inj Volume : 1 µl

Sequence File : C:\HPCHEM\2\SEQUENCE\IG031020.S
Acq. Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 3/3/2000 2:11:59 PM by ig
Analysis Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 5/9/2000 12:22:42 PM by ig
RH7281 Potato Peel Waste METHOD,
Rtx-5, 30m, 0.53mm, 0.5um film
Flow Rate = 4.0ml/m



External Standard Report

Sorted By : Signal
Calib. Data Modified : 5/9/2000 12:22:35 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 B,

RetTime [min]	Type	Area counts*s	Amt/Area	Amount [ng/ul]	Grp	Name
11.173	BBA	1.06432e5	4.22877e-7	4.50078e-2		RH7281
Totals :				4.50078e-2		

Results obtained with enhanced integrator!

Instrument 2 5/9/2000 12:25:16 PM ig

Page 1 of 2

Figure 29. Fortification of RH-7281 at 0.050 ppm (GC/ECD, 03/10/2000)
RH-7281 Found = 0.04501 ppm, Recovery = 90.0%.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78

Data File C:\HPCHEM\2\DATA\032720\010B1201.D

Sample Name: p3-005

peel fort. 0.05ppm

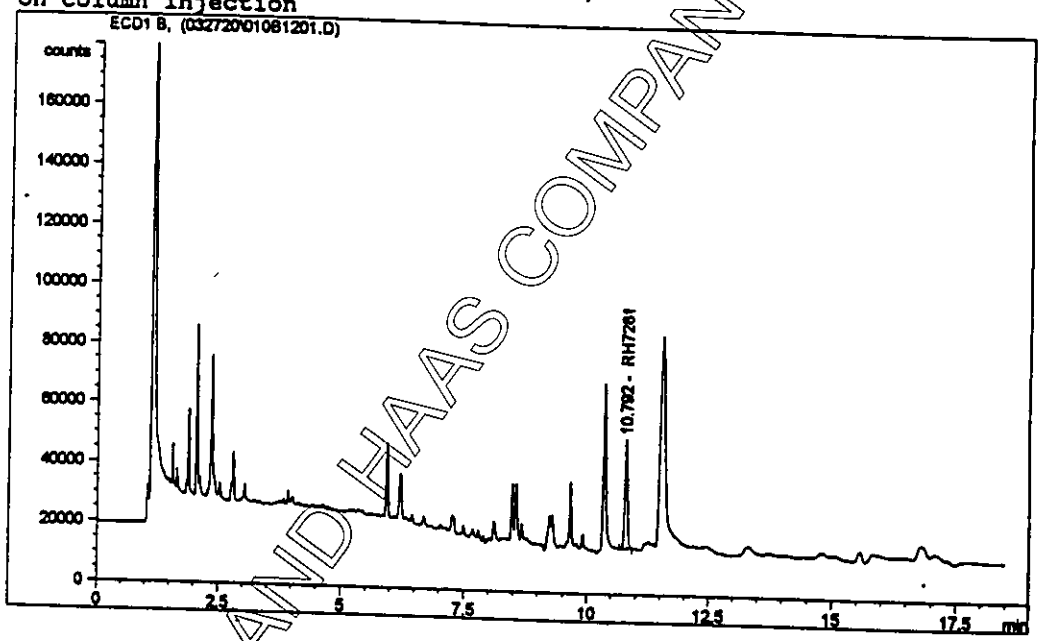
Injection Date : 3/27/2000 8:43:31 PM
Sample Name : p3-005
Acq. Operator : ig

Seq. Line : 12
Vial : 10
Inj : 1
Inj Volume : 1 µl

Acq. Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 3/27/2000 9:14:19 AM by ig
Analysis Method : C:\HPCHEM\2\METHODS\PULSOR.M
Last changed : 5/23/2000 3:07:10 PM by dc
(modified after loading)

RH7281 tomato puree

Column flow, N2, 4.0 ml/min
RTX-5MS (0.53mm I.D. x 30m; 0.5um film)
on column injection



External Standard Report

Sorted By : Signal
Calib. Data Modified : 5/23/2000 3:02:16 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 B,

RetTime [min]	Type	Area counts*s	Amt/Area	Amount [ug/ml]	Grp	Name
10.792	VB	1.40407e5	2.90041e-7	4.07238e-2		RH7281
Totals :				4.07238e-2		

Instrument 2 5/23/2000 3:07:44 PM dc

Page 1 of 2

Figure 30. Fortification of RH-7281 at 0.050 ppm (GC/ECD, 03/24/2000)
RH-7281 Found = 0.04072 ppm, Recovery = 68.2%.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

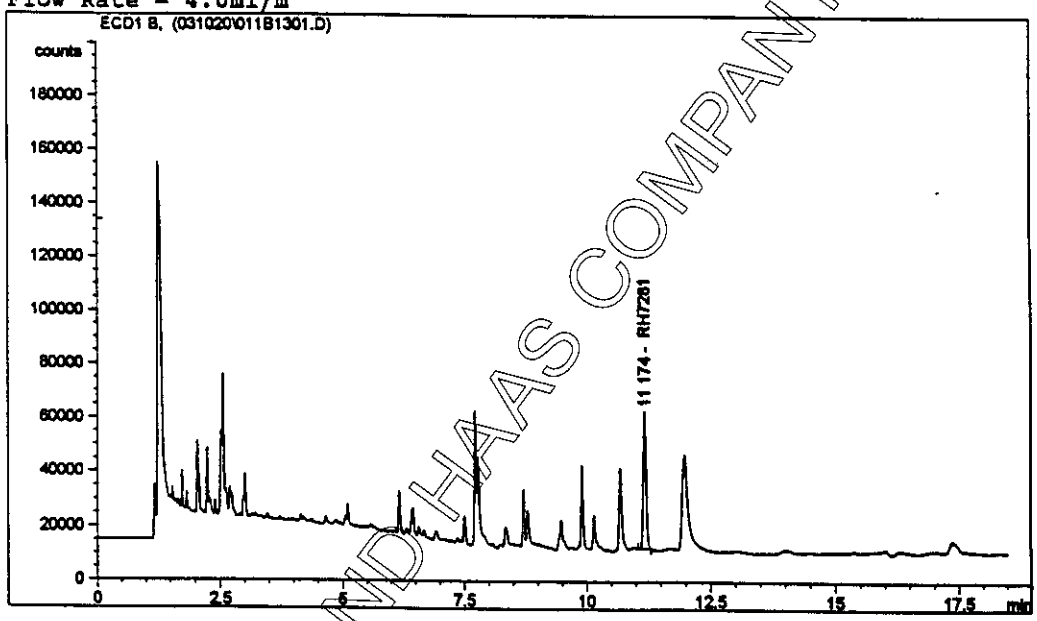
Data File C:\HPCHEM\2\DATA\031020\011B1301.D Sample Name: p1-006

peel fort. 0.10ppm

Injection Date : 3/10/2000 8:33:13 PM
Sample Name : p1-006
Acq. Operator : ig

Seq. Line : 13
Vial : 11
Inj : 1
Inj Volume : 1 µl

Sequence File : C:\HPCHEM\2\SEQUENCE\IG031020.S
Acq. Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 3/3/2000 2:11:59 PM by ig
Analysis Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 5/9/2000 12:22:42 PM by ig
RH7281 Potato Peel Waste METHOD,
Rtx-5, 30m, 0.53mm, 0.5um film
Flow Rate = 4.0ml/m



External Standard Report

Sorted By : Signal
Calib. Data Modified : 5/9/2000 12:22:35 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 B,

RetTime [min]	Type	Area counts*s	Amt/Area	Amount [ng/ul]	Grp	Name
11.174	BBA	2.15996e5	4.60030e-7	9.93644e-2		RH7281
Totals :				9.93644e-2		

Results obtained with enhanced integrator!

Instrument 2 5/9/2000 12:25:19 PM ig Page 1 of 2

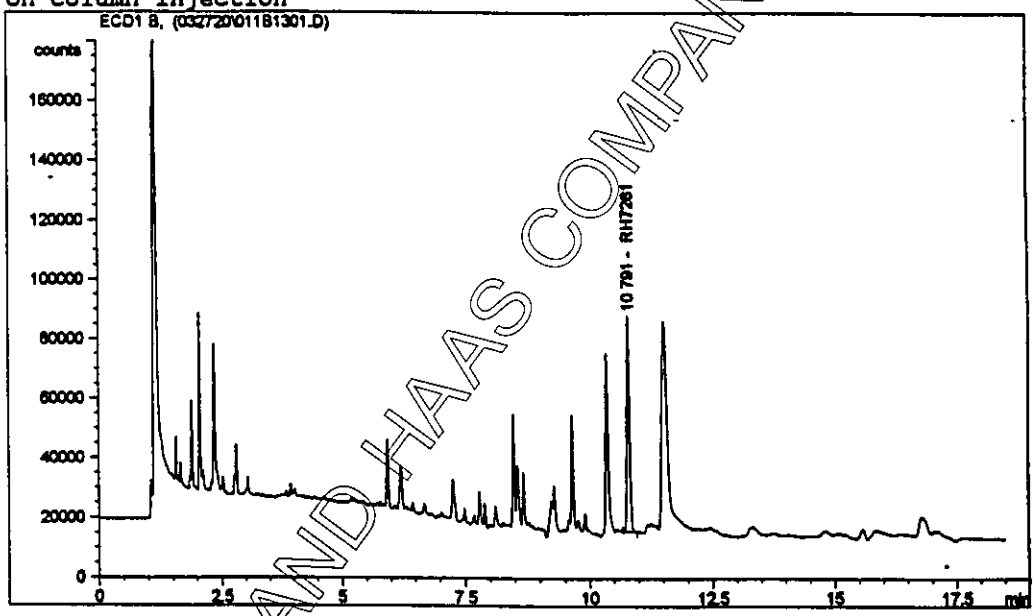
Figure 31. Fortification of RH-7281 at 0.10 ppm (GC/ECD, 03/10/2000)
RH-7281 Found = 0.09936 ppm, Recovery = 99.4 %.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Data File C:\HPCHEM\2\DATA\032720\011B1301.D Sample Name: p3-006

peel fort. 0.10ppm

```
Injection Date  : 3/27/2000 9:07:17 PM          Seq. Line :  13
Sample Name     : p3-006                            Vial :  11
Acq. Operator   : ig                                 Inj :   1
                                              Inj Volume : 1 µl
Acq. Method     : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed    : 3/27/2000 9:14:19 AM by ig
Analysis Method : C:\HPCHEM\2\METHODS\PULSOR.M
Last changed    : 5/23/2000 3:07:10 PM by dc
                  (modified after loading)
RH7281 tomato puree

Column flow, N2, 4.0 ml/min
RTX-5MS (0.53mm I.D. x 30m; 0.5um film)
on column injection
```



External Standard Report

```
Sorted By             :      Signal
Calib. Data Modified  :      5/23/2000 3:02:16 PM
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: ECD1 B,
```

RetTime [min]	Type	Area counts*s	Amt/Area	Amount [ug/ml]	Grp	Name
10.791	VV	2.82721e5	3.22520e-7	9.11831e-2		RH7281

Totals : 9.11831e-2

Instrument 2 5/23/2000 3:08:09 PM dc Page 1 of 2

Figure 32. Fortification of RH-7281 at 0.10 ppm (GC/ECD, 03/24/2000)
RH-7281 Found = 0.09118 ppm, Recovery = 84.8 %.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

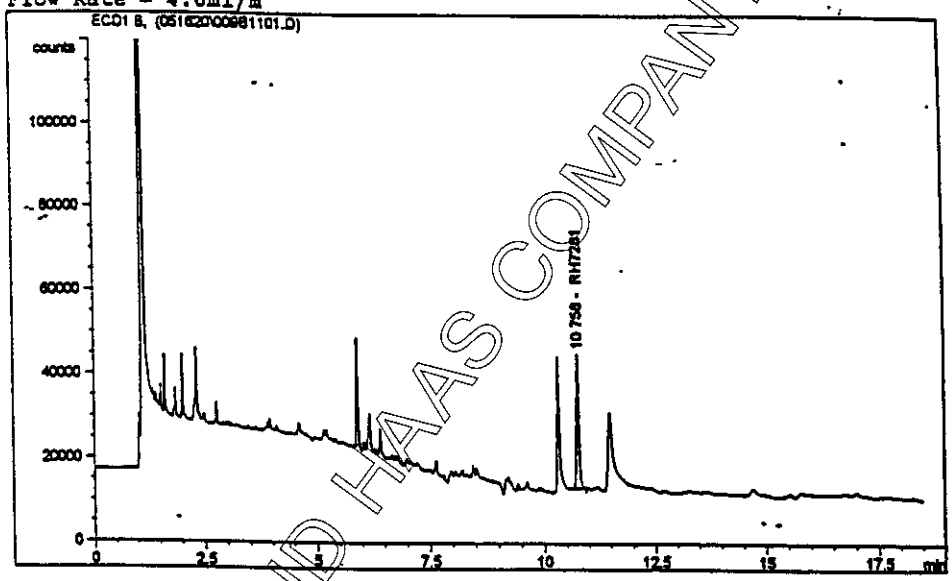
Data File C:\HPCHEM\2\DATA\051620\009B1101.D Sample Name: p-004

Fortification at 0.10 ppm of 97-0078-1

Injection Date : 5/16/2000 7:37:16 PM
Sample Name : p-004
Acq. Operator : ig

Seq. Line : 11
Vial : 9
Inj : 1
Inj Volume : 1 µl

Sequence File : C:\HPCHEM\2\SEQUENCE\IG051620.S
Acq. Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 5/13/2000 3:59:01 PM by ig
Analysis Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 5/26/2000 1:38:56 PM by dc
RH7281 Potato Peel Waste METHOD,
Rtx-5, 30m, 0.53mm, 0.5um film
Flow Rate = 4.0ml/m



External Standard Report

Sorted By : Signal
Calib. Data Modified : 5/26/2000 1:38:47 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 B,

RetTime [min]	Type	Area counts*s	Amt/Area	Amount [ng/ul]	Grp	Name
10.758	BB	1.34137e5	5.58158e-7	7.48696e-2		RH7281
Totals :				7.48696e-2		

Results obtained with enhanced integrator!

Instrument 2 5/26/2000 1:39:57 PM dc Page 1 of 2

Figure 33. Fortification of RH-7281 at 0.10 ppm (GC/ECD, 05/15/2000)
RH-7281 Found = 0.074778 ppm, Recovery 74.8% (as calculated by NRDS)

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

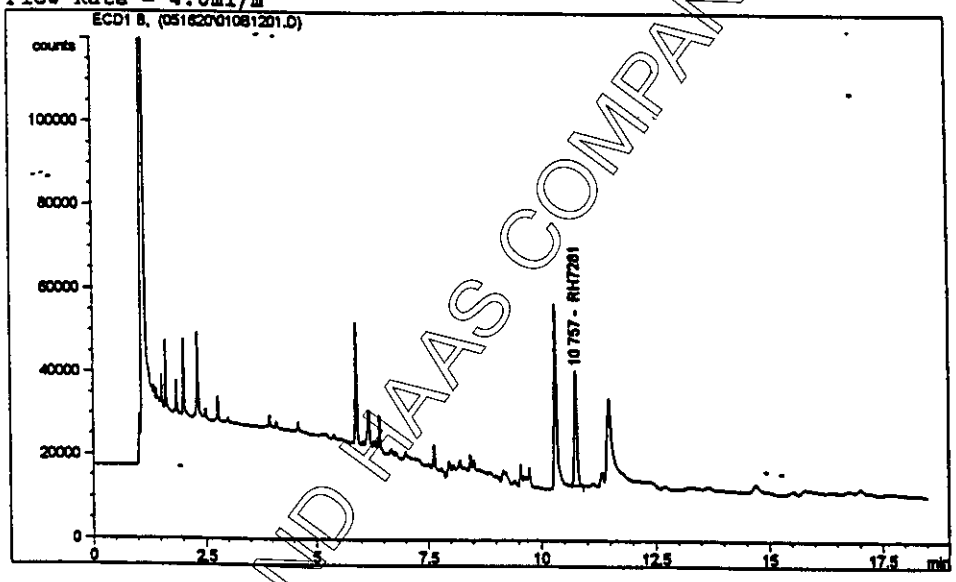
FORM 4685 REV 10/78

Data File C:\HPCHEM\2\DATA\051620\010B1201.D Sample Name: p-005

Trial Sample Rep 1 97-0078-5

Injection Date : 5/16/2000 8:00:55 PM Seq. Line : 12
Sample Name : p-005 Vial : 10
Acq. Operator : ig Inj : 1
Inj Volume : 1 µl

Sequence File : C:\HPCHEM\2\SEQUENCE\IG051620.S
Acq. Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 5/13/2000 3:59:01 PM by ig
Analysis Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 5/26/2000 1:38:56 PM by dc
RH7281 Potato Peel Waste METHOD,
Rtx-5, 30m, 0.53mm, 0.5um film
Flow Rate = 4.0ml/m



External Standard Report

Sorted By : Signal
Calib. Data Modified : 5/26/2000 1:38:47 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 B,

RetTime [min]	Type	Area counts*s	Amt/Area	Amount [ng/ul]	Grp	Name
10.757	BB	1.13882e5	5.41617e-7	6.16806e-2		RH7281

Totals : 6.16806e-2

Results obtained with enhanced integrator!

Figure 34. Trial Sample Replicate 1 for Determination of RH-7281 (GC/ECD, 97-0078), RH-7281 Found = 0.0617 ppm

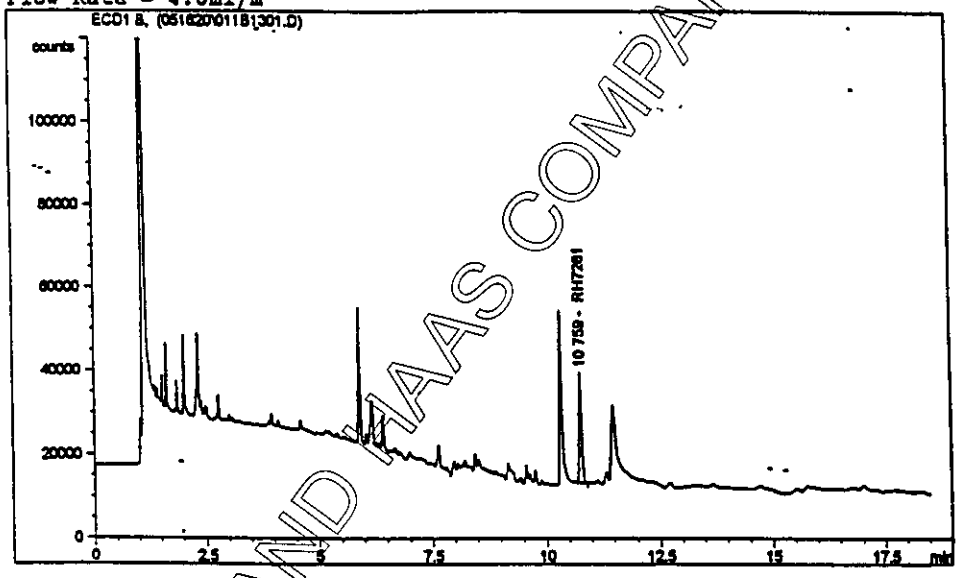
PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Data File C:\HPCHEM\2\DATA\051620\011B1301.D Sample Name: p-006

Trial Sample Rep 2 97-0078-5

Injection Date : 5/16/2000 8:24:38 PM Seq. Line : 13
Sample Name : p-006 Vial : 11
Acq. Operator : ig Inj : 1
Inj Volume : 1 µl

Sequence File : C:\HPCHEM\2\SEQUENCE\IG051620.S
Acq. Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 5/13/2000 3:59:01 PM by ig
Analysis Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 5/26/2000 1:38:56 PM by dc
RH7281 Potato Peel Waste METHOD,
Rtx-5, 30m, 0.53mm, 0.5um film
Flow Rate = 4.0ml/m



External Standard Report

Sorted By : Signal
Calib. Data Modified : 5/26/2000 1:38:47 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 B,

RetTime [min]	Type	Area counts*s	Amt/Area	Amount [ng/ul]	Grp	Name
10.759	BB	1.07732e5	5.36997e-7	5.78519e-2		RH7281

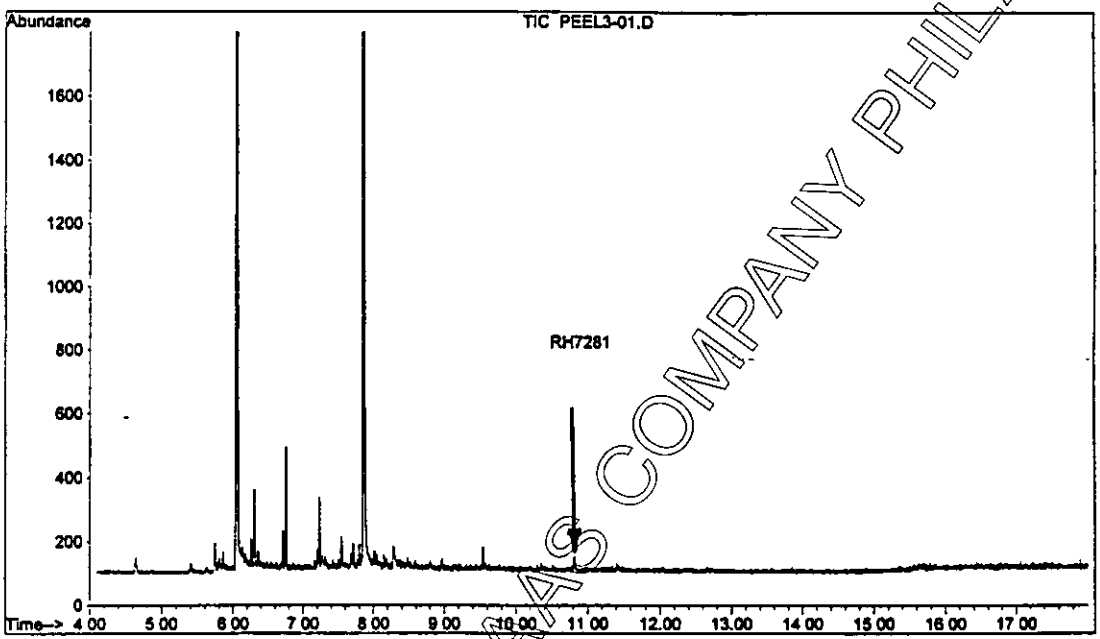
Totals : 5.78519e-2

Results obtained with enhanced integrator!

Figure 35. Trial Sample Replicate 2 for Determination of RH-7281 (GC/ECD, 97-0078), RH-7281 Found = 0.0579 ppm

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

```
File        : C:\HPCHEM\1\DATA\032920\PEEL3-01.D
Operator    : ig
Acquired    : 29 Mar 00  18:46     using AcqMethod 7281SIM
Instrument  :    5973
Sample Name: control, potato peel
Misc Info   :
Vial Number: 6
```



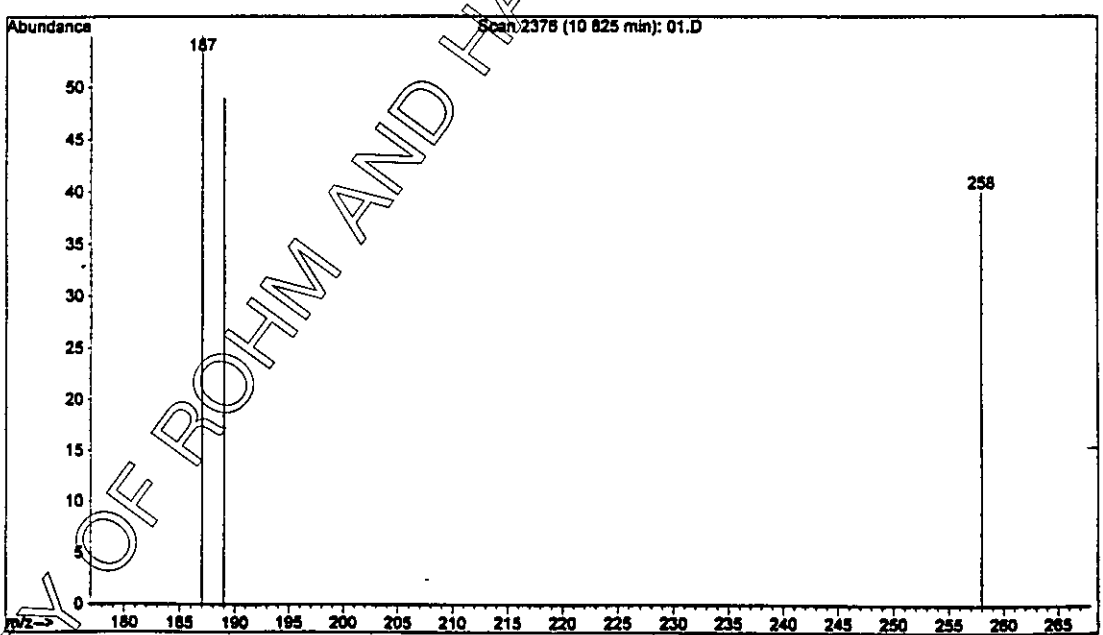
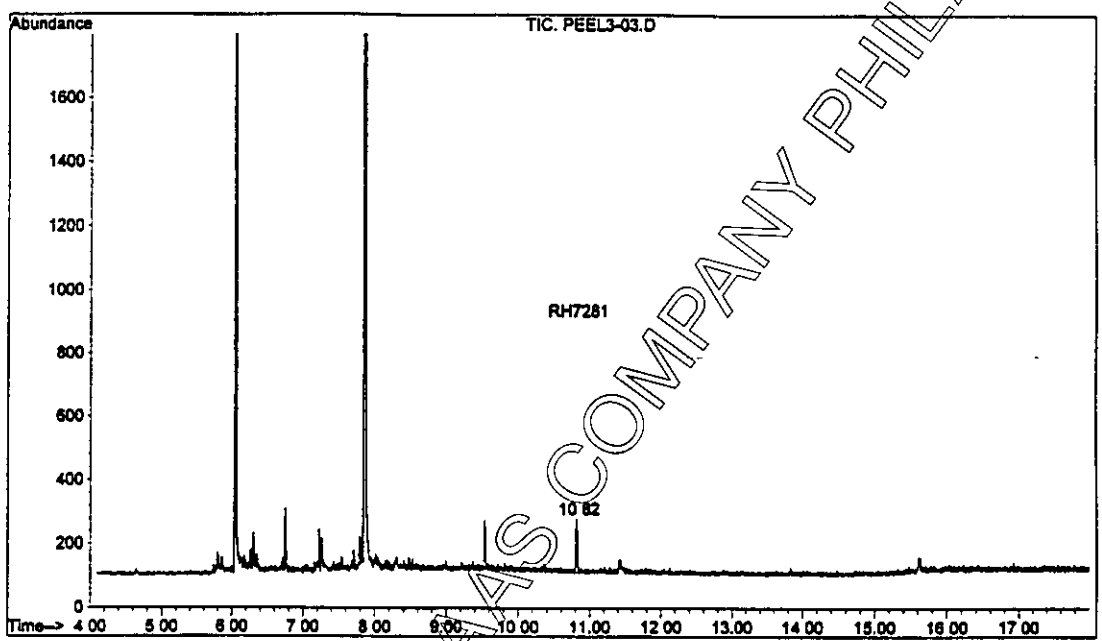


Figure 36. Control of Potato for RH-7281 Analysis (GC/MSD)
RH-7281 Found = 0 ppm.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

```
File        : C:\HPCHEM\1\DATA\032920\PEEL3-03.D
Operator    : ig
Acquired    : 29 Mar 00  19:39     using AcqMethod 7281SIM
Instrument  :    5973
Sample Name: 0.02ppm spike
Misc Info   :
Vial Number: 8
```



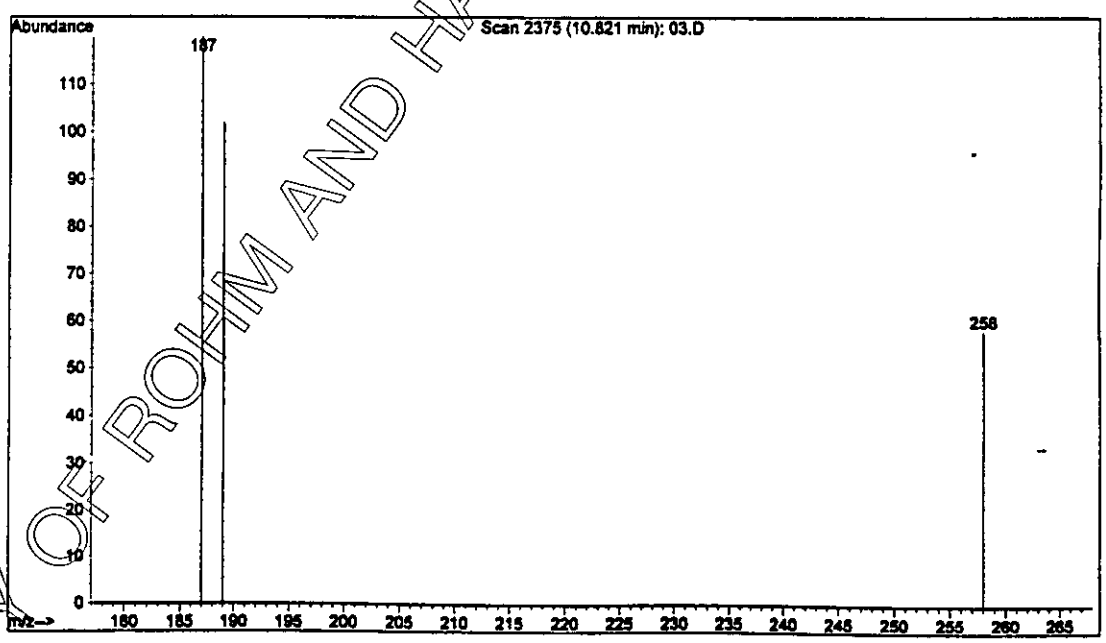
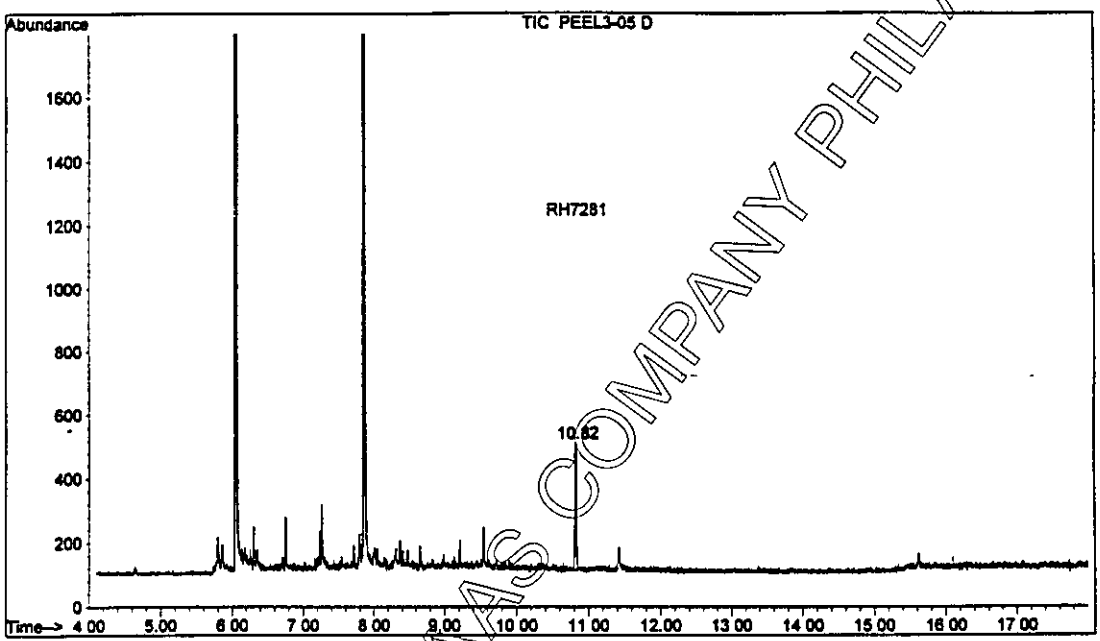


Figure 37. Fortification of RH-7281 at 0.02 ppm (GC/MSD)
RH-7281 Found = 0.02089 ppm, Recovery = 104%.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

```
File        : C:\HPCHEM\1\DATA\032920\PEEL3-05.D
Operator    : ig
Acquired    : 29 Mar 00  20:33     using AcqMethod 7281SIM
Instrument  :    5973
Sample Name: 0.05ppm spike
Misc Info   :
Vial Number: 10
```



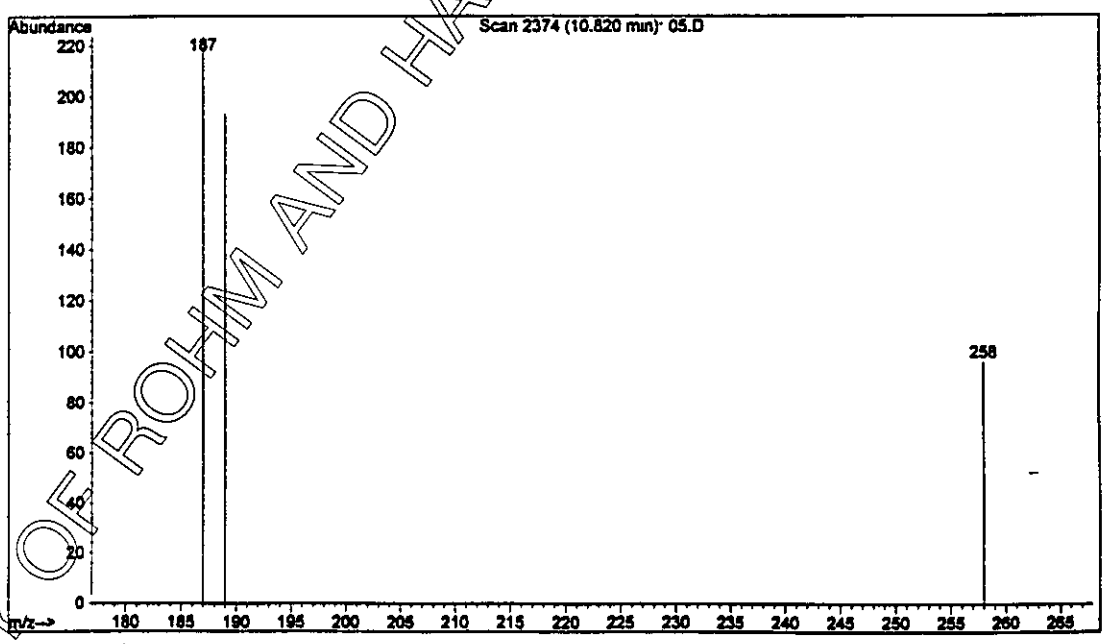
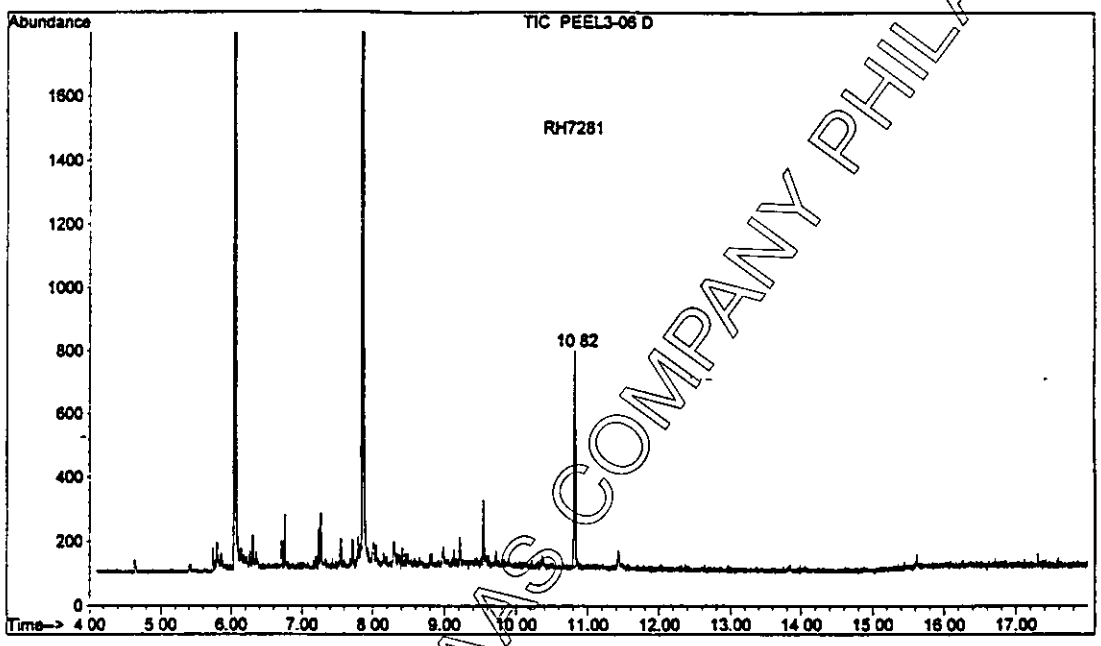


Figure 38. Fortification of RH-7281 at 0.050 ppm (GC/MSD)
RH-7281 Found = 0.04814 ppm, Recovery = 96.3 %.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

```
File        : C:\HPCHEM\1\DATA\032920\PEEL3-06.D
Operator    : ig
Acquired    : 29 Mar 00  20:59     using AcqMethod 7281SIM
Instrument  :    5973
Sample Name: 0.10ppm spike
Misc Info   :
Vial Number: 11
```



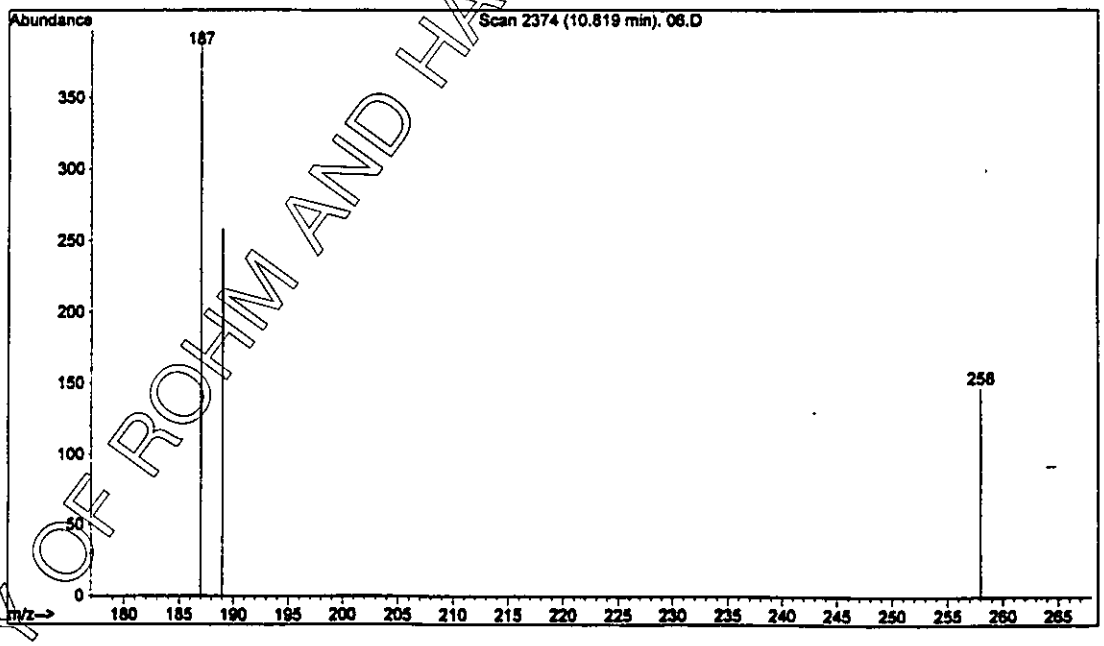


Figure 39. Fortification of RH-7281 at 0.10 ppm (GC/MSD)
RH-7281 Found = 0.08841 ppm, Recovery = 88.4%.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Quantitation Report

Data File : c:\hpchem\2\data\031320\M1-001.D
Acq On : 3-13-00 8:06:14 PM
Sample : contrl
Misc :

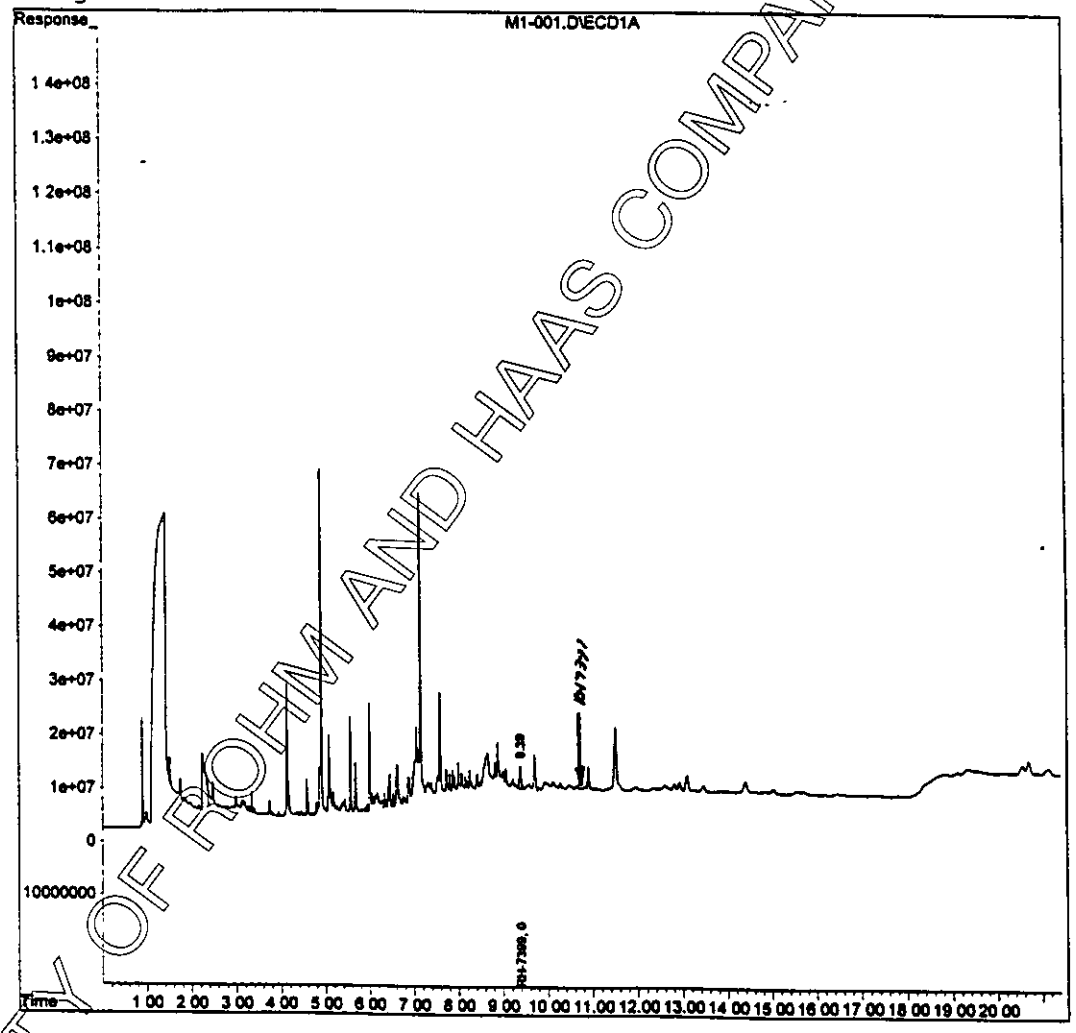
Vial: 6
Operator:
Inst : GC ECD
Multiplr: 1.00
Sample Amount: 0.00

IntFile : EVENTS.E

Quant Time: Mar 14 8:02 19100 Quant Results File: PEEL-M.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-M.M (Chemstation Integrator)
Title : 02-29-00
Last Update : Tue Mar 14 07:54:35 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-M.M

Volume Inj. :
Signal Phase :
Signal Info :



M1-001.D PEEL-M.M Tue Mar 14 08:02:50 2000 Page 2

Figure 40. Control Potato for RH-1452 and RH-1455 Analysis (Rtx-225, 03/10/2000) RH-7391 Found = 0 ppm, RH-7399 Found = 0.015 ppm.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78

Quantitation Report

Data File : C:\HPCHEM\2\DATA\032720\M3-001.D
Acq.On : 3-27-00 19:47:50
Sample : contrl
Misc :

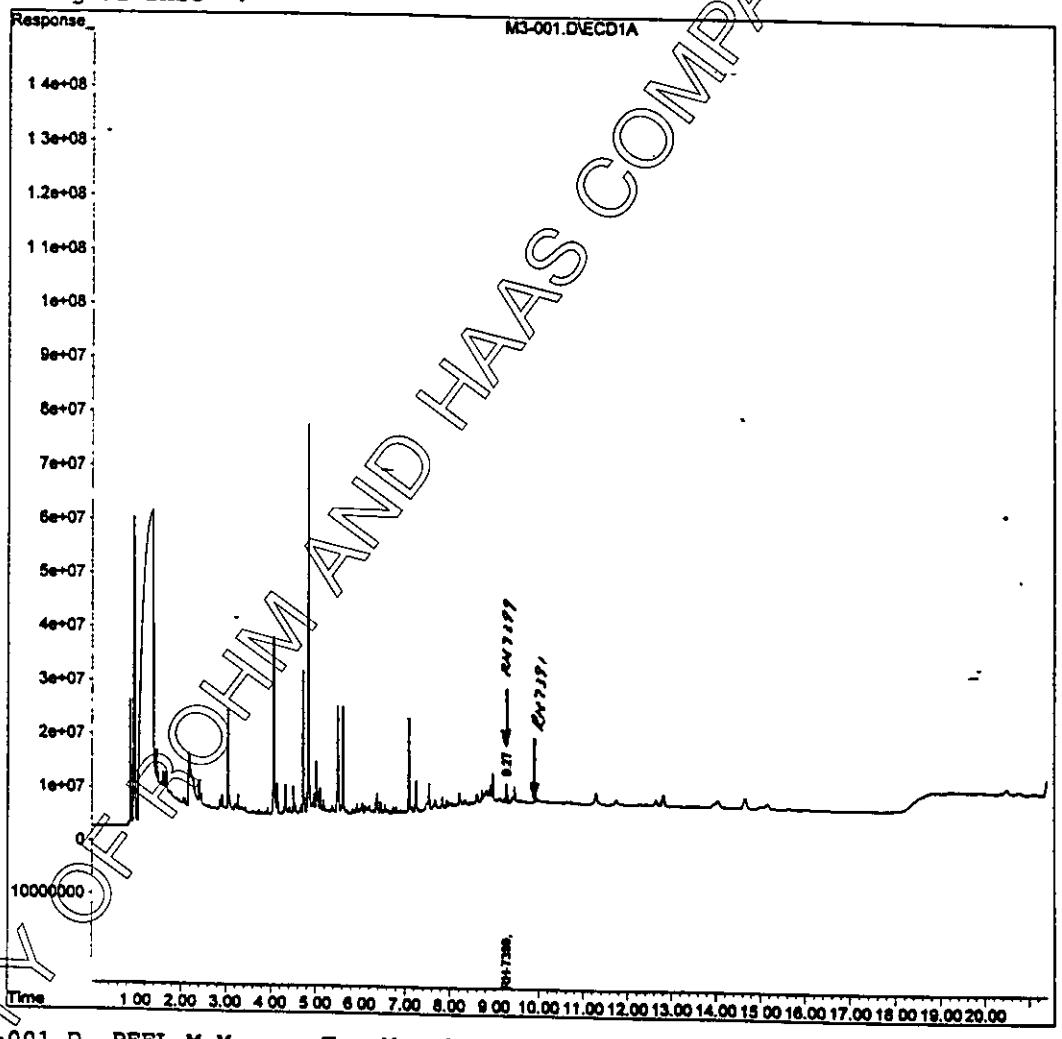
Vial: 6
Operator:
Inst : GC ECD
Multiplr: 1.00
Sample Amount: 0.00

IntFile : EVENTS.E

Quant Time: Mar 28 8:56 19100 Quant Results File: PEEL-M.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-M.M (Chemstation Integrator)
Title : 02-29-00
Last Update : Tue Mar 28 07:42:23 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-M.M

Volume Inj. :
Signal Phase :
Signal Info :



M3-001.D PEEL-M.M Tue May 23 13:54:37 2000 Page 2

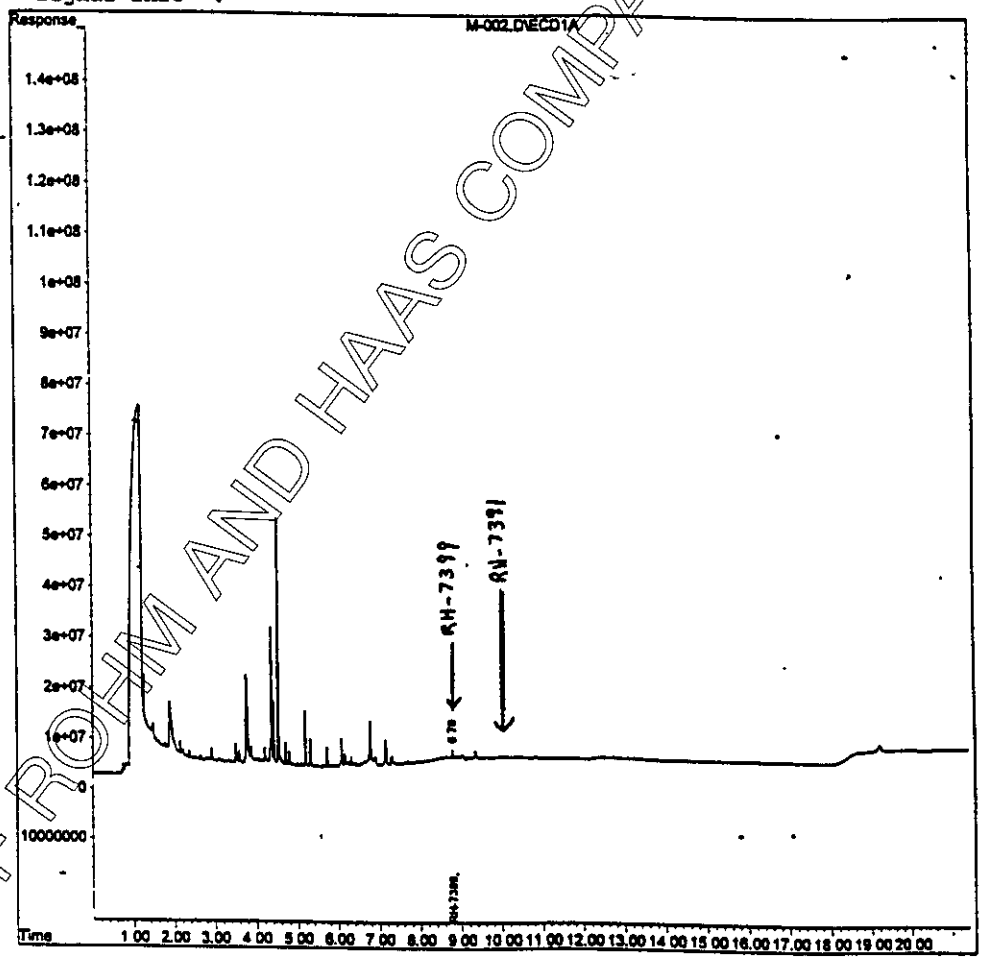
Figure 41. Control Potato for RH-1452 and RH-1455 Analysis (Rtx-225, 03/24/2000) RH-7391 Found = 0 ppm, RH-7399 Found = 0.0096 ppm.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78

Quantitation Report

```
Data File : c:\hpchem\2\data\051620\M-002.D                    Vial: 7
Acq On    : 5-16-00 6:56:27 PM                              Operator: ig
Sample    : Control Peel                                    Inst    : GC ECD
Misc      :                                                 Multiplr: 1.00
                                                          Sample Amount: 0.00
IntFile   : EVENTS.E

Quant Time: May 30 13:21 19100  Quant Results File: PEEL-M.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-M.M (Chemstation Integrator)
Title        : 02-29-00
Last Update  : Tue May 30 12:50:34 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-M.M

Volume Inj.  :
Signal Phase :
Signal Info  :
```



M-002.D PEEL-M.M Tue May 30 13:21:29 2000 Page 2

Figure 42. Control Potato for RH-1452 and RH-1455 Analysis (Rtx-225, 05/15/2000) RH-7391 Found = 0 ppm, RH-7399 Found = 0 ppm.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Quantitation Report

Data File : c:\hpchem\2\data\031320\M1-003.D
Acq On : 3-13-00 9:00:32 PM
Sample : 0.02ppm fort
Misc :

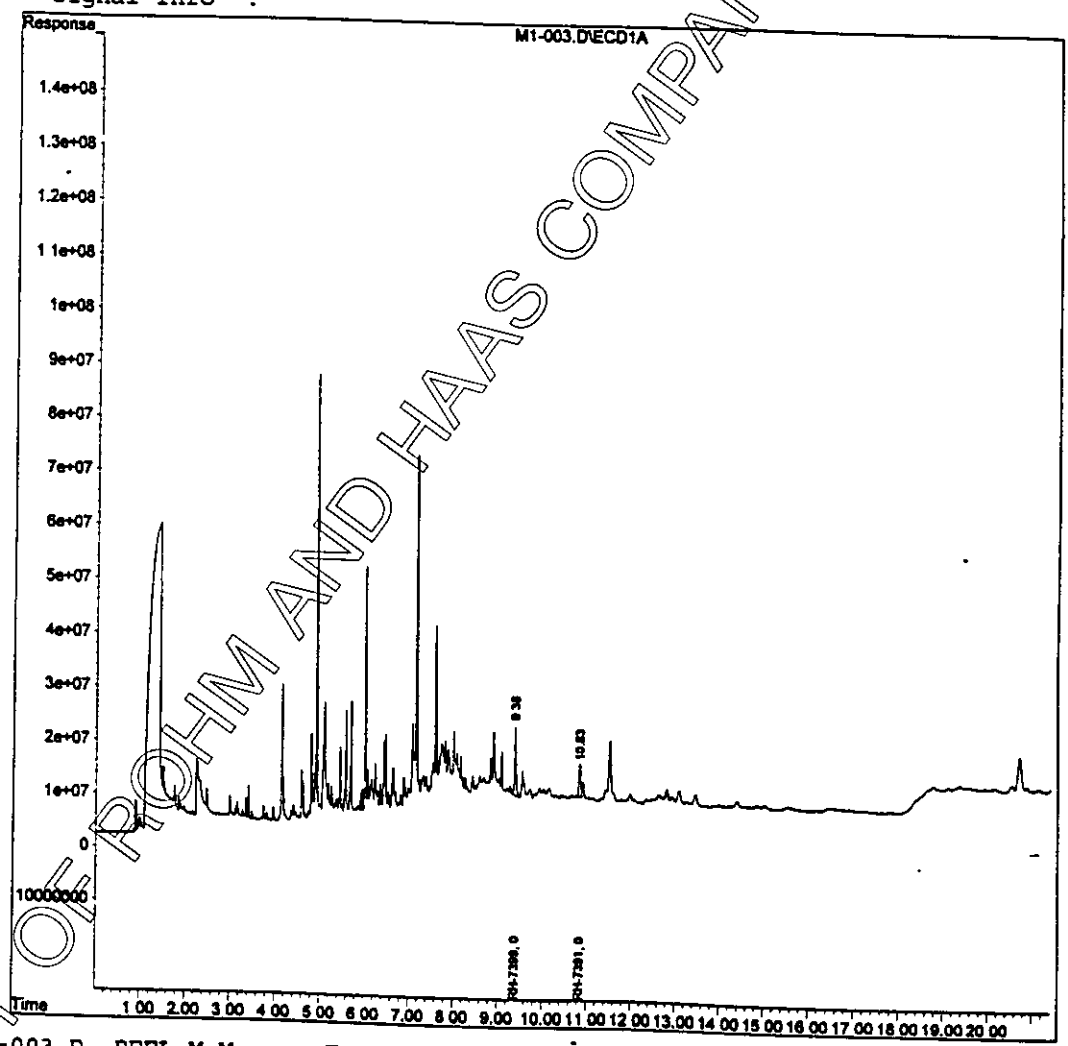
Vial: 8
Operator:
Inst : GC ECD
Multiplr: 1.00
Sample Amount: 0.00

IntFile : EVENTS.E

Quant Time: Mar 14 8:04 19100 Quant Results File: PEEL-M.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-M.M (Chemstation Integrator)
Title : 02-29-00
Last Update : Tue Mar 14 07:54:35 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-M.M

Volume Inj. :
Signal Phase :
Signal Info :



M1-003.D PEEL-M.M Tue Mar 14 08:04:14 2000

Page 2

Figure 43. Fortification of RH-1452 and RH-1455 at 0.020 ppm (Rtx-225, 03/10/2000)
RH-7391 Found = 0.01772 ppm, Recovery $_{RH-1452}$ = 83.6%.
RH-7399 Found = 0.03877 ppm, Recovery $_{RH-1455}$ = 103%.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Quantitation Report

Data File : C:\HPCHEM\2\DATA\032720\M3-003.D
Acq On : 3-27-00 20:42:41
Sample : 0.02ppm fort
Misc :

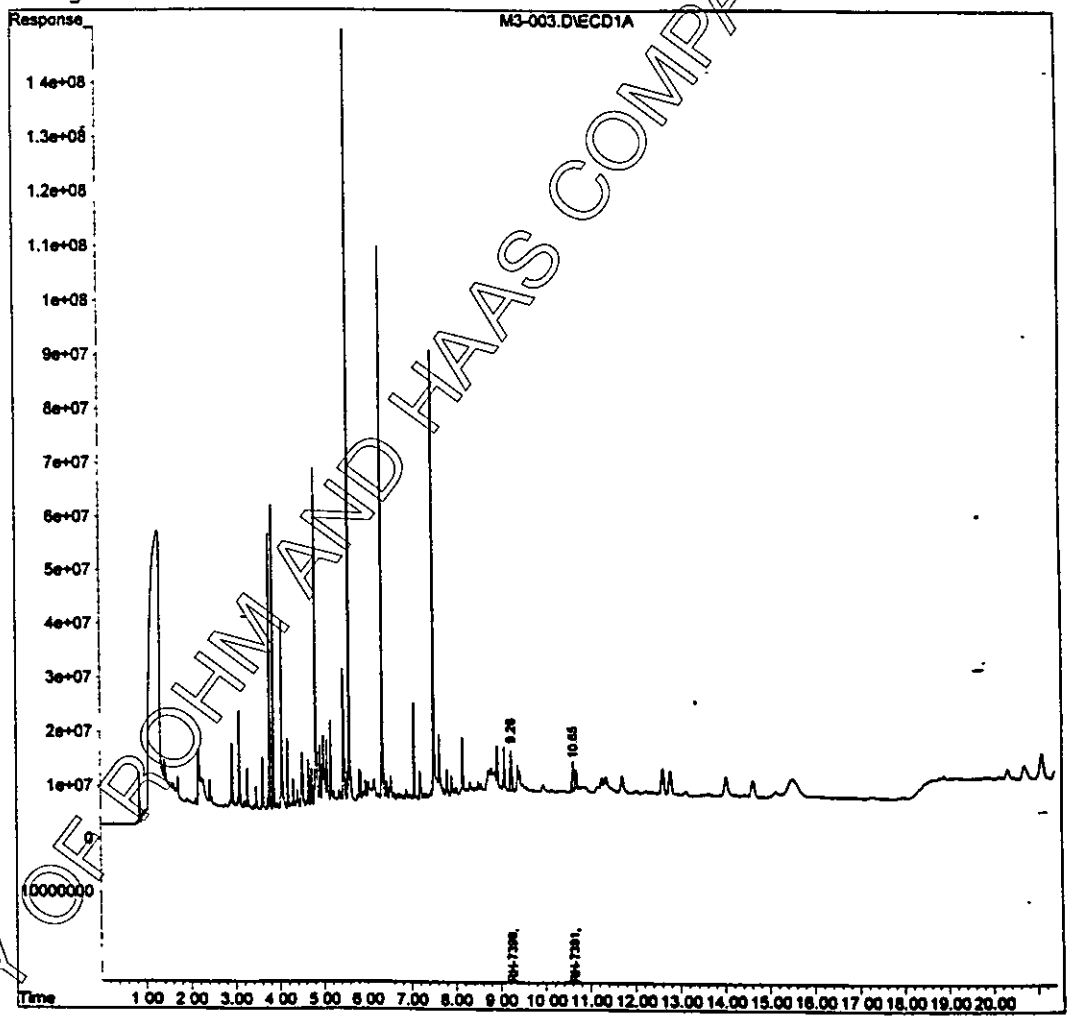
Vial: 8
Operator: -
Inst : GC ECD
Multiplr: 1.00
Sample Amount: 0.00

IntFile : EVENTS.E

Quant Time: Mar 28 8:58 19100 Quant Results File: PEEL-M.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-M.M (Chemstation Integrator)
Title : 02-29-00
Last Update : Tue Mar 28 07:42:23 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-M.M

Volume Inj. :
Signal Phase :
Signal Info :



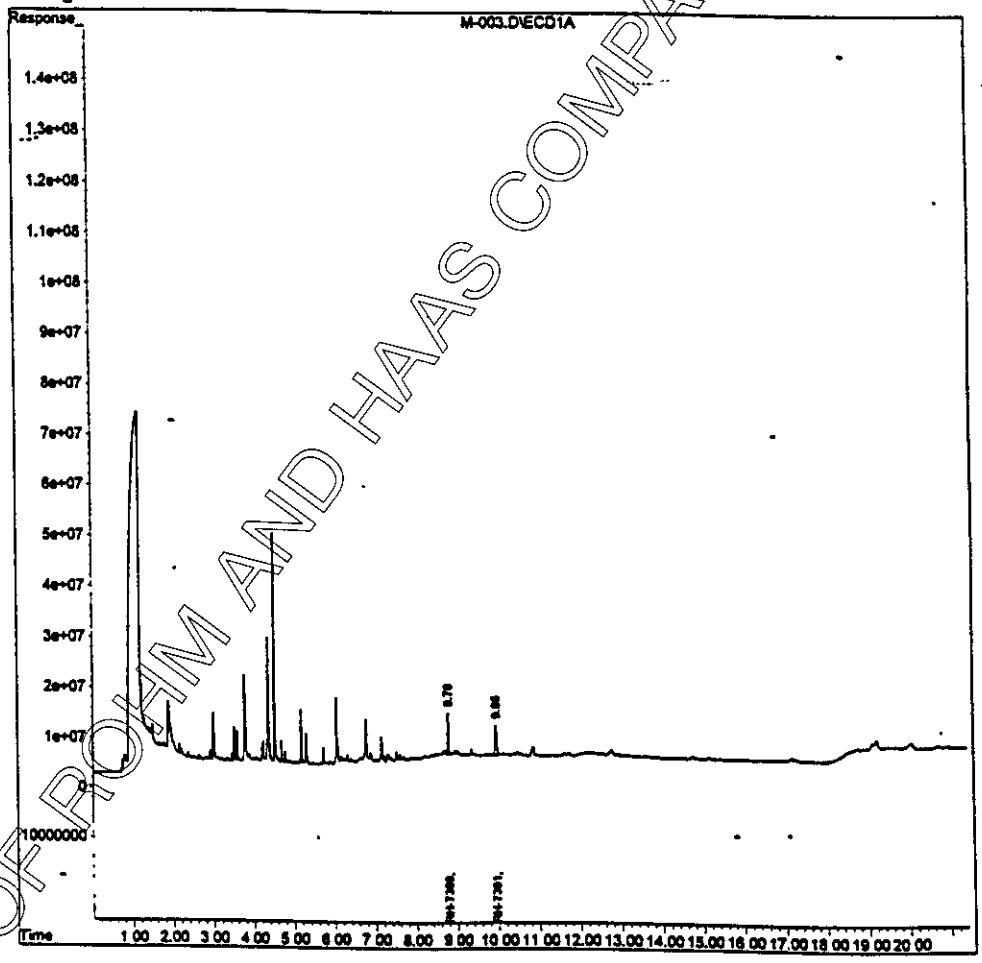
M3-003.D PEEL-M.M Tue May 23 13:58:30 2000 Page 2

Figure 44. Fortification of RH-1452 and RH-1455 at 0.020 ppm (Rtx-225, 03/24/2000)
RH-7391 Found = 0.01575 ppm, Recovery $_{RH-1452}$ = 74.3%.
RH-7399 Found = 0.02144 ppm, Recovery $_{RH-1455}$ = 52.9%.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Quantitation Report

```
Data File : c:\hpchem\2\data\051620\M-003.D                Vial: 8
Acq On    : 5-16-00 7:23:49 PM                          Operator: ig
Sample    : 0.02ppm fort                                   Inst   : GC ECD
Misc      :                                              Multiplr: 1.00
                                                      Sample Amount: 0.00
IntFile   : EVENTS.E

Quant Time: May 30 13:22 19100  Quant Results File: PEEL-M.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-M.M (Chemstation Integrator)
Title        : 02-29-00
Last Update  : Tue May 30 12:50:34 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-M.M

Volume Inj.  :
Signal Phase :
Signal Info  :
```



M-003.D PEEL-M.M Tue May 30 13:22:23 2000 Page 2

Figure 45. Fortification of RH-1452 and RH-1455 at 0.020 ppm (Rtx-225, 05/15/2000)
RH-7391 Found = 0.01673 ppm, Recovery $_{RH\text{-}1452}$ = 79.2%
RH 7399 Found = 0.01618 ppm, Recovery $_{RH\ 1455}$ = 72.1%

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Quantitation Report

Data File : c:\hpchem\2\data\031320\M1-005.D
Acq On : 3-13-00 9:54:51 PM
Sample : 0.05ppm fort
Misc :

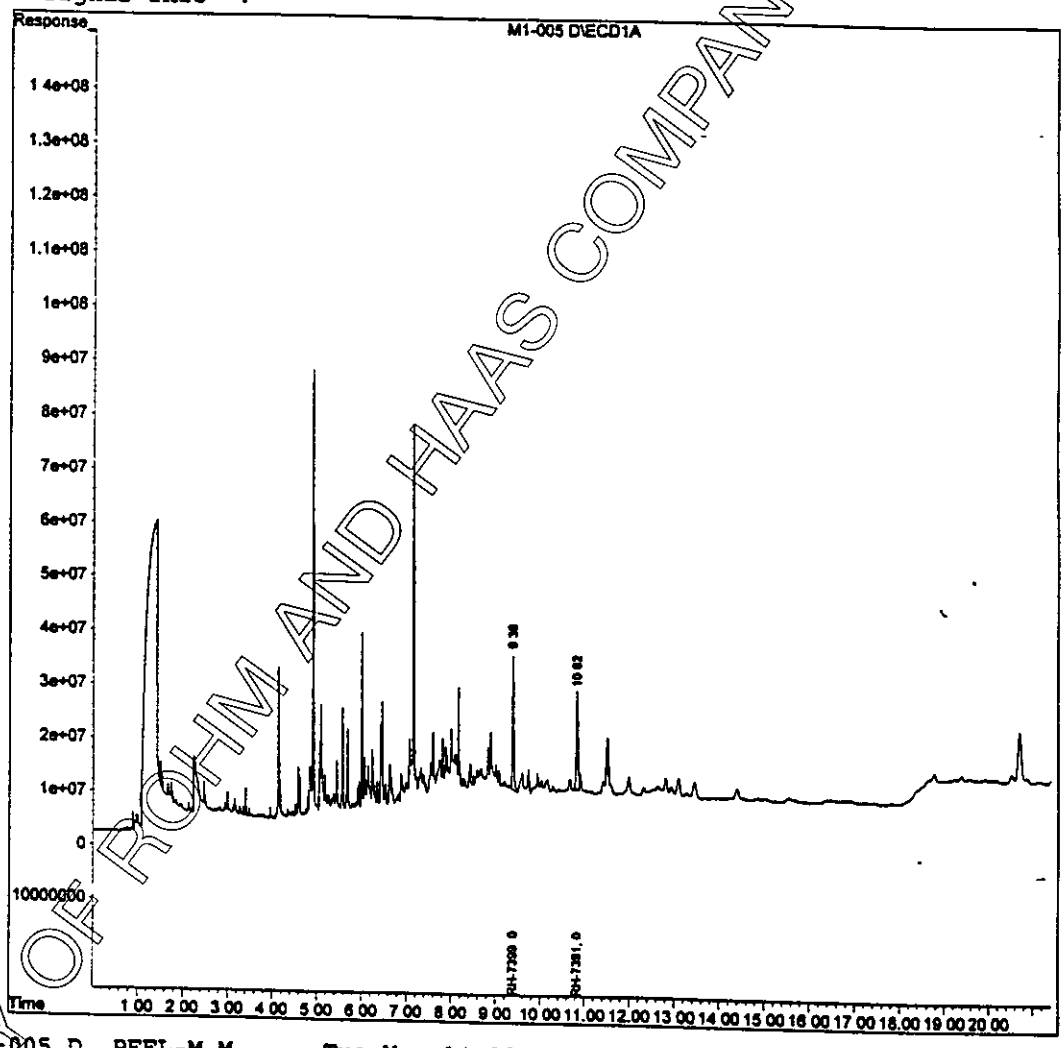
IntFile : EVENTS.E

Vial: 10
Operator:
Inst : GC ECD
Multiplr: 1.00
Sample Amount: 0.00

Quant Time: Mar 14 8:05 19100 Quant Results File: PEEL-M.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-M.M (Chemstation Integrator)
Title : 02-29-00
Last Update : Tue Mar 14 07:54:35 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-M.M

Volume Inj. :
Signal Phase :
Signal Info :



M1-005.D PEEL-M.M Tue Mar 14 08:05:42 2000 Page 2

Figure 46. Fortification of RH-1452 and RH-1455 at 0.050 ppm (Rtx-225, 03/10/2000)
RH-7391 Found = 0.05359 ppm, Recovery $_{RH-1452}$ = 101%.
RH-7399 Found = 0.06447 ppm, Recovery $_{RH-1455}$ = 88.3%.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Quantitation Report

Data File : C:\HPCHEM\2\DATA\032720\M3-005.D
Acq On : 3-27-00 21:37:31
Sample : 0.05ppm fort
Misc :

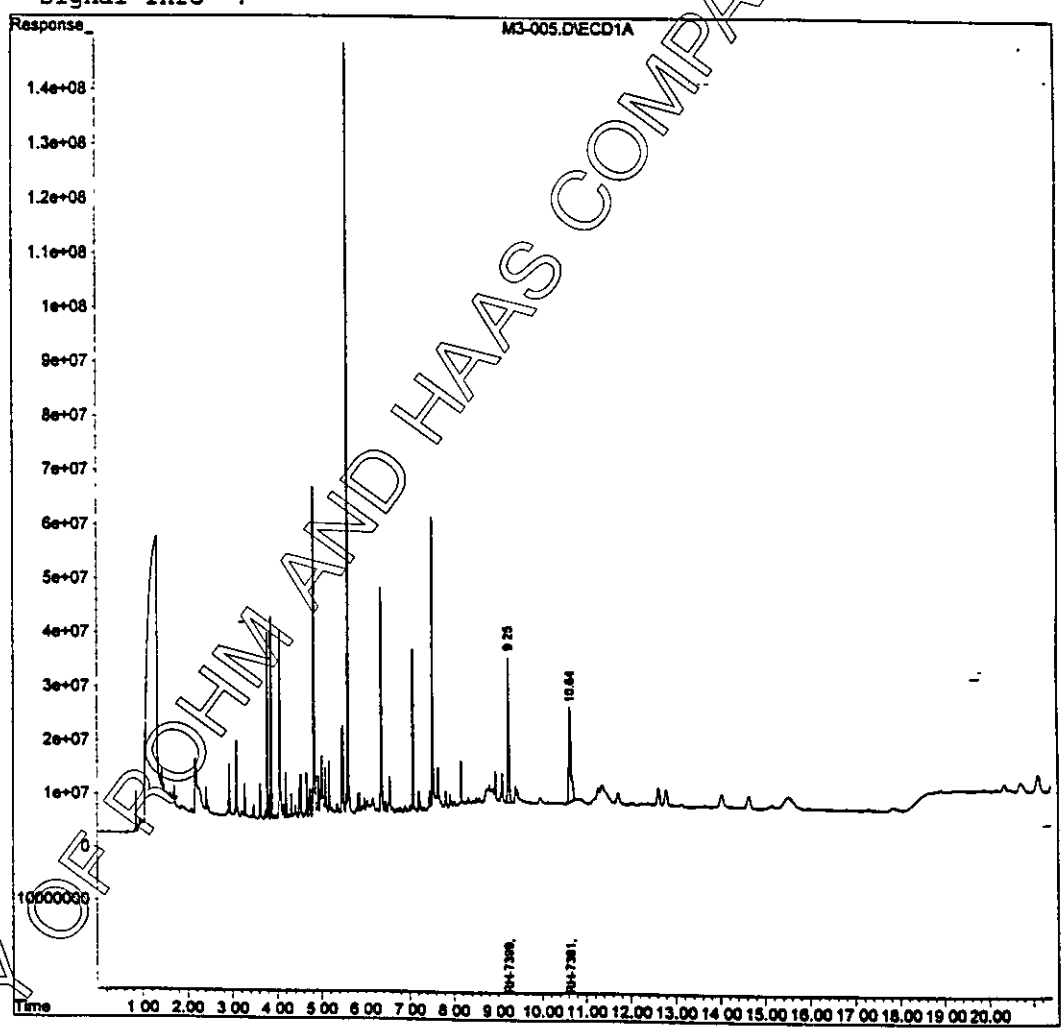
Vial: 10
Operator:
Inst : GC ECD
Multiplr: 1.00
Sample Amount: 0.00

IntFile : EVENTS.E

Quant Time: Mar 28 8:59 19100 Quant Results File: PEEL-M.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-M.M (Chemstation Integrator)
Title : 02-29-00
Last Update : Tue Mar 28 07:42:23 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-M.M

Volume Inj. :
Signal Phase :
Signal Info :



M3-005.D PEEL-M.M Tue May 23 13:57:31 2000 Page 2

Figure 47. Fortification of RH-1452 and RH-1455 at 0.050 ppm (Rtx-225, 03/24/2000)
RH-7391 Found = 0.06123 ppm, Recovery $_{RH-1452}$ = 116%.
RH-7399 Found = 0.05786 ppm, Recovery $_{RH-1455}$ = 86.2%.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78

Quantitation Report

Data File : c:\hpchem\2\data\031320\M1-006.D
Acq On : 3-13-00 10:21:59 PM
Sample : 0.10ppm fort
Misc :

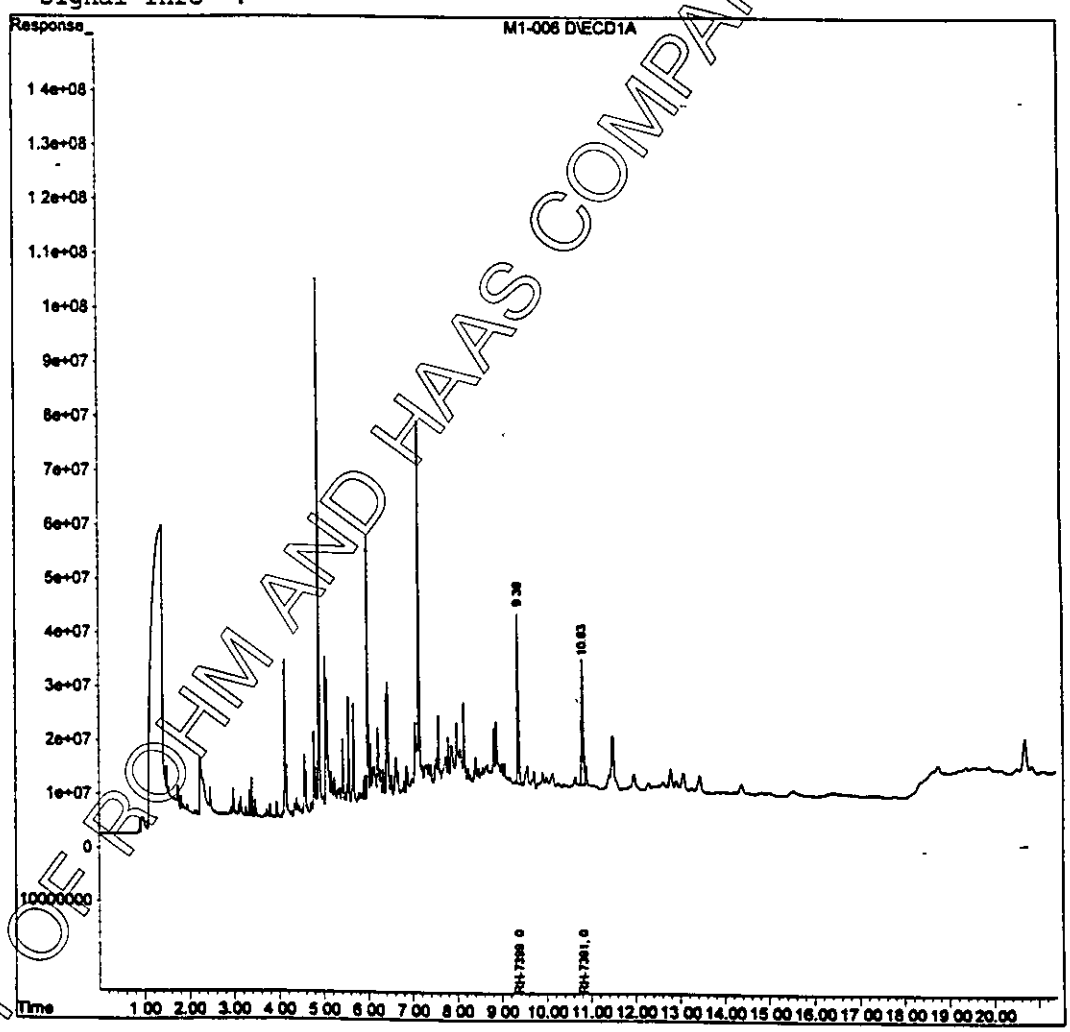
Vial: 11
Operator:
Inst : GC ECD
Multiplr: 1.00
Sample Amount: 0.00

IntFile : EVENTS.E

Quant Time: Mar 14 8:06 19100 Quant Results File: PEEL-M.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-M.M (Chemstation Integrator)
Title : 02-29-00
Last Update : Tue Mar 14 07:54:35 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-M.M

Volume Inj. :
Signal Phase :
Signal Info :



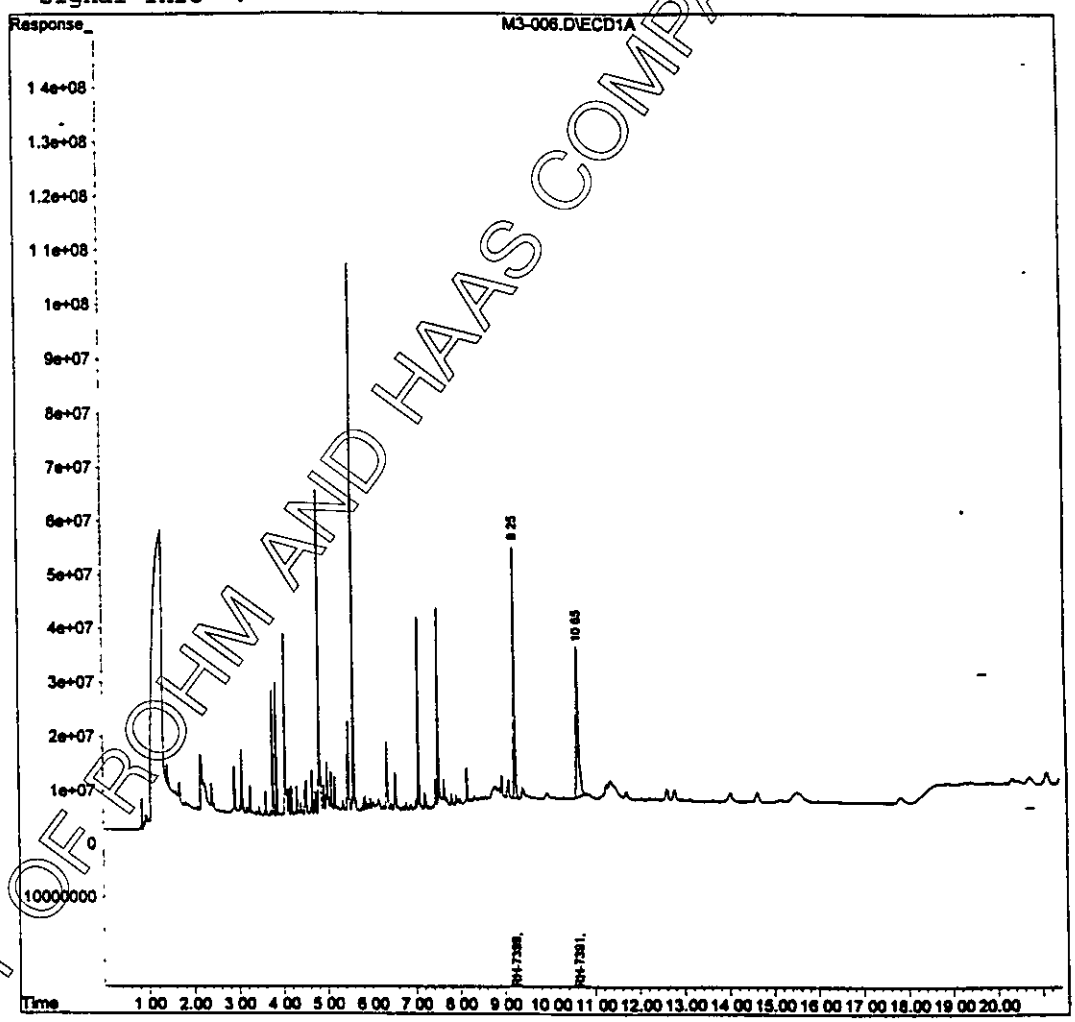
M1-006.D PEEL-M.M Tue Mar 14 08:06:25 2000 Page 2

Figure 48. Fortification of RH-1452 and RH-1455 at 0.10 ppm (Rtx-225, 03/10/2000)
RH-7391 Found = 0.06718 ppm, Recovery $_{RH\text{-}1452}$ = 63.4%.
RH-7399 Found = 0.07801 ppm, Recovery $_{RH\text{-}1455}$ = 56.3%.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Quantitation Report

```
Data File : C:\HPCHEM\2\DATA\032720\M3-006.D              Vial: 11
Acq On    : 3-28-00 8:27:27                          Operator:
Sample    : 0.10ppm fort                                Inst     : GC ECD
Misc      :                                             Multiplr: 1.00
                                                    Sample Amount: 0.00
IntFile   : EVENTS.E

Quant Time: Mar 28  9:00 19100  Quant Results File: PEEL-M.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-M.M (Chemstation Integrator)
Title        : 02-29-00
Last Update  : Tue Mar 28 07:42:23 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-M.M

Volume Inj.  :
Signal Phase :
Signal Info  :
```



M3-006.D PEEL-M.M Tue May 23 13:56:53 2000 Page 2

Figure 49. Fortification of RH-1452 and RH-1455 at 0.10 ppm (Rtx-225, 03/24/2000)
RH-7391 Found = 0.09463 ppm, Recovery $_{RH-1452}$ = 89.3%.
RH-7399 Found = 0.09684 ppm, Recovery $_{RH-1455}$ = 77.9%.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78

Quantitation Report

Data File : c:\hpchem\2\data\051620\M-004.D
Acq On : 5-16-00 7:51:16 PM
Sample : 0.10ppm fortification
Misc :

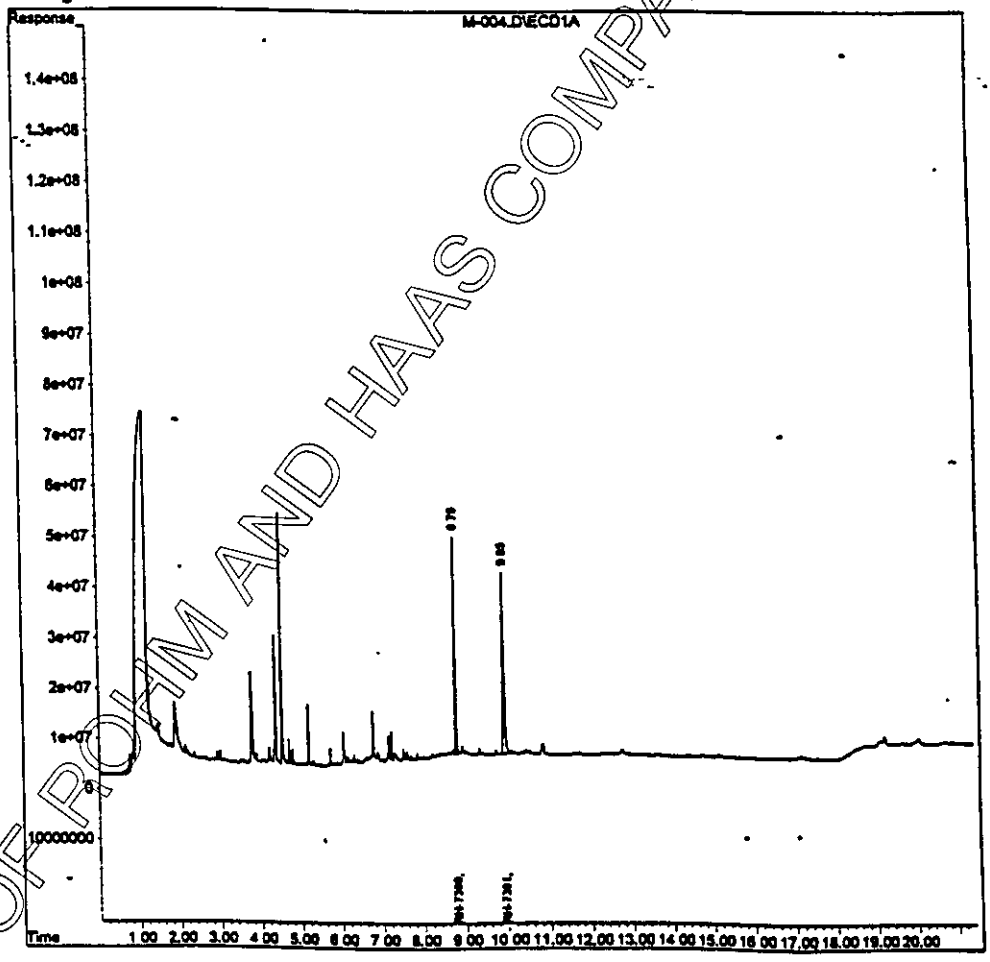
Vial: 9
Operator: ig
Inst : GC ECD
Multiplr: 1.00
Sample Amount: 0.00

IntFile : EVENTS.E

Quant Time: May 30 13:22 19100 Quant Results File: PEEL-M.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-M.M (Chemstation Integrator)
Title : 02-29-00
Last Update : Tue May 30 12:50:34 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-M.M

Volume Inj. :
Signal Phase :
Signal Info :



M-004.D PEEL-M.M Tue May 30 13:23:06 2000 Page 2

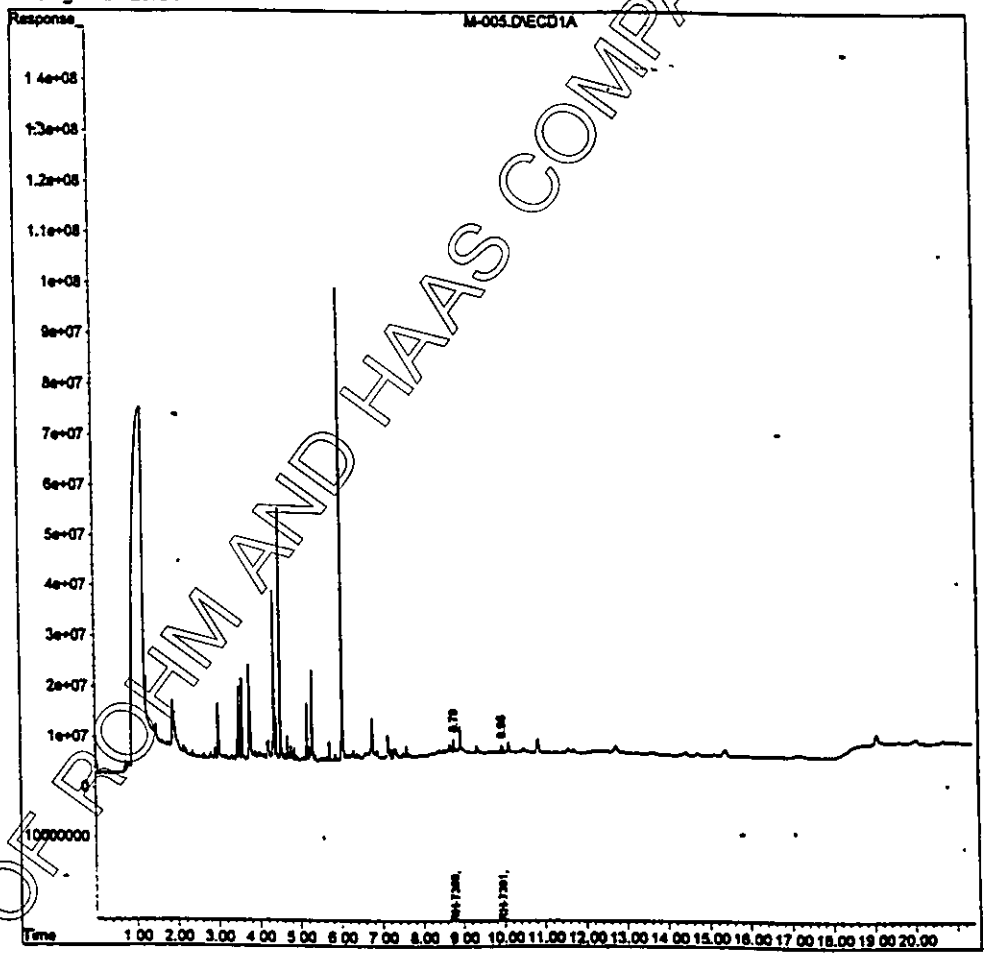
Figure 50. Fortification of RH-1452 and RH-1455 at 0.10 ppm (Rtx-225, 05/15/2000)
RH-7391 Found = 0.09700 ppm, Recovery $_{RH\text{-}1452}$ = 91.5%
RH-7399 Found = 0.081986 ppm, Recovery $_{RH\text{-}1455}$ = 73.2%

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78

Quantitation Report

```
Data File : c:\hpchem\2\data\051620\M-005.D                    Vial: 10
Acq On    : 5-16-00 8:18:39 PM                              Operator: ig
Sample    : Trial Sample Rep 1                              Inst    : GC ECD
Misc      :                                                 Multiplr: 1.00
                                                       Sample Amount: 0.00
IntFile   : EVENTS.E

Quant Time: May 30 13:23 19100  Quant Results File: PEEL-M.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-M.M (Chemstation Integrator)
Title        : 02-29-00
Last Update  : Tue May 30 12:50:34 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-M.M

Volume Inj.  :
Signal Phase :
Signal Info  :
```



M-005.D PEEL-M.M Tue May 30 13:23:48 2000 Page 2

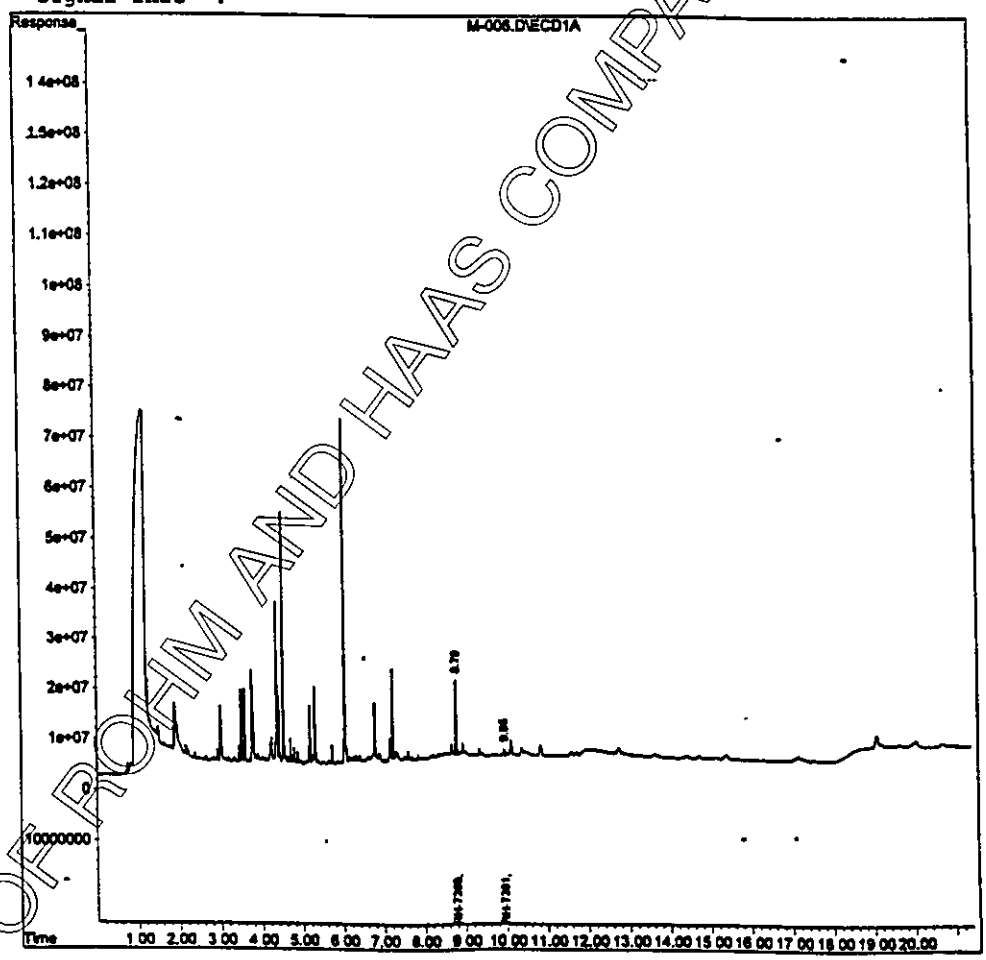
Figure 51 Trial Sample Replicate 1 for Determination of RH-1452 and RH-1455 (Rtx- 225, 97-0078), RH-1452 Found<LOD, RH-1455 Found < LOD.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78

Quantitation Report

```
Data File : c:\hpchem\2\data\051620\M-006.D                Vial: 11
Acq On    : 5-16-00 8:46:02 PM                           Operator: ig
Sample    : Trial Sample Rep 2                              Inst    : GC ECD
Misc      :                                              Multiplr: 1.00
                                                      Sample Amount: 0.00
IntFile   : EVENTS.E

Quant Time: May 30 13:24 19100  Quant Results File: PEEL-M.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-M.M (Chemstation Integrator)
Title        : 02-29-00
Last Update  : Tue May 30 12:50:34 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-M.M

Volume Inj.  :
Signal Phase :
Signal Info  :
```



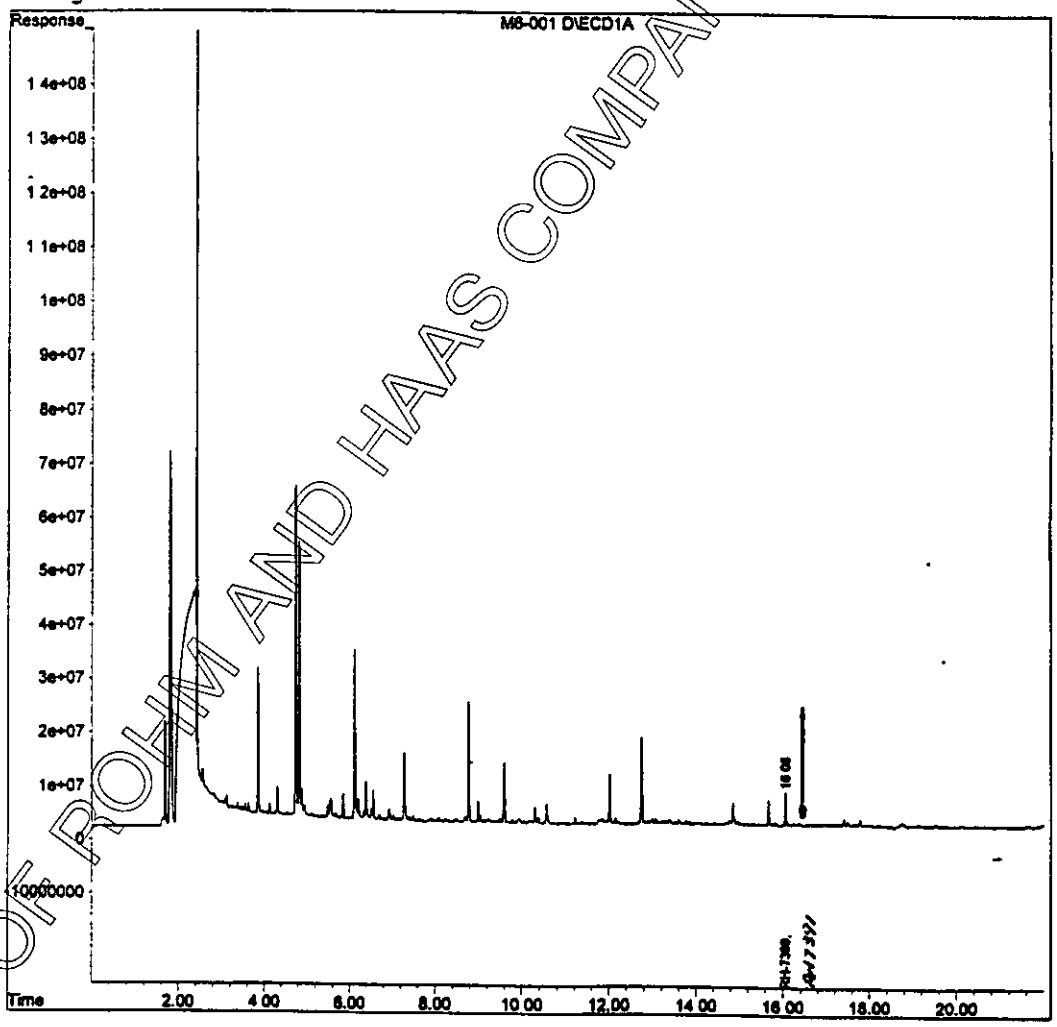
M-006.D PEEL-M.M Tue May 30 13:24:30 2000 Page 2

Figure 52 Trial Sample Replicate 2 for Determination of RH-1452 and RH-1455 (Rtx-225, 97-0078), RH-1452 Found = 0 ppm
RH-1455 Found = 0.0260 ppm

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Quantitation Report

```
Data File : c:\hpchem\2\data\050120\M6-001.D          Vial: 6
Acq On    : 5-1-00 7:31:44 PM                       Operator:
Sample    : contrl                                  Inst     : GC/ECD
Misc      :                                         Multiplr: 1.00
                                                    Sample Amount: 0.00
IntFile   : EVENTS.E

Quant Time: May  2  7:05 19100  Quant Results File: PEEL-C.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-C.M (Chemstation Integrator)
Title        : 02-29-00
Last Update  : Tue May 02 06:47:05 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-C.M

Volume Inj.  :
Signal Phase :
Signal Info  :
```



M6-001.D PEEL-C.M Tue May 02 07:05:54 2000 Page 2

Figure 53. Control Peel for RH-1452 and RH-1455 Analysis (Rtx-35)
RH-7391 Found = 0 ppm, RH-7399 Found = 0.010 ppm,

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78

Quantitation Report

Data File : c:\hpchem\2\data\050120\M6-003.D
Acq On : 5-1-00 8:31:27 PM
Sample : 0.02ppm fort
Misc :

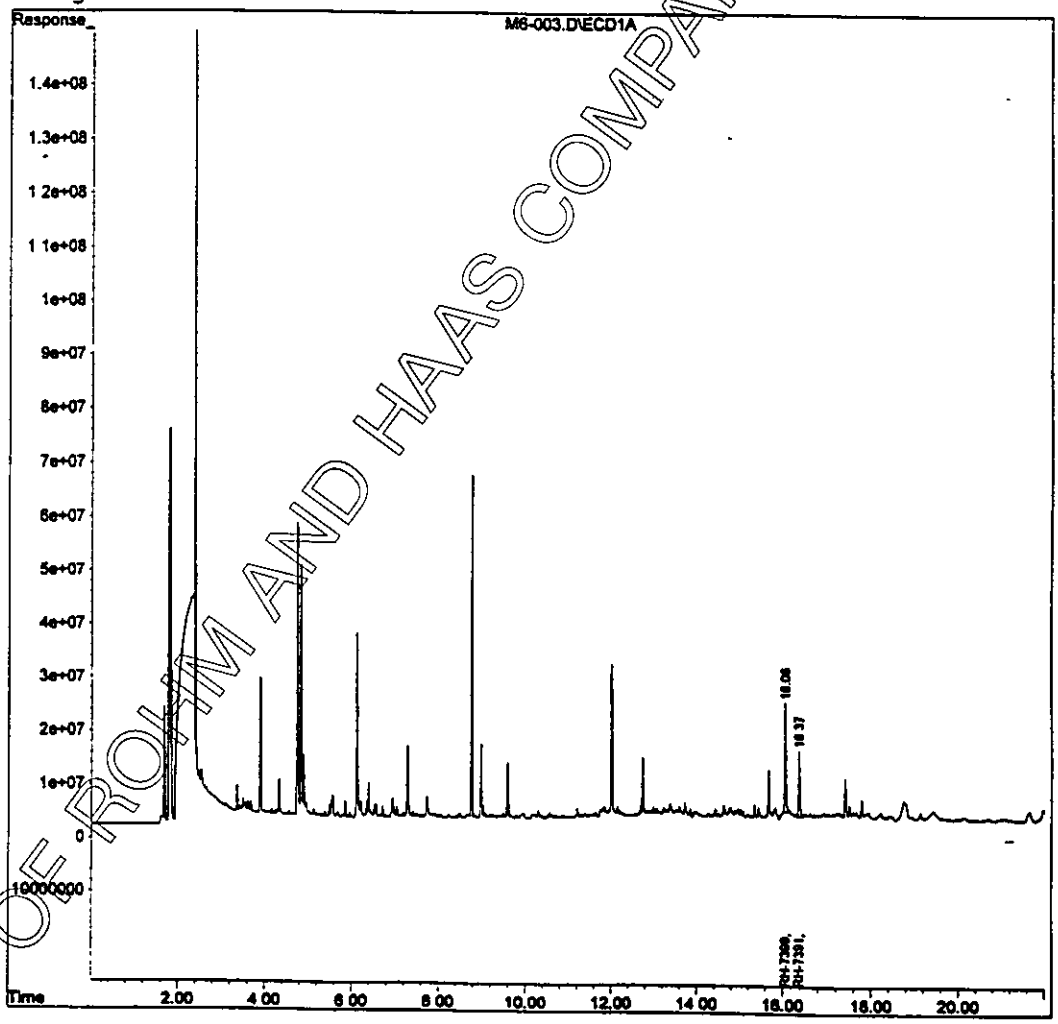
Vial: 8
Operator:
Inst : GC-ECD
Multiplr: 1.00
Sample Amount: 0.00

IntFile : EVENTS.E

Quant Time: May 2 7:07 19100 Quant Results File: PEEL-C.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-C.M (Chemstation Integrator)
Title : 02-29-00
Last Update : Tue May 02 06:47:05 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-C.M

Volume Inj. :
Signal Phase :
Signal Info :



M6-003.D PEEL-C.M Tue May 02 07:07:20 2000 Page 2

Figure 54. Fortification of RH-1452 and RH-1455 at 0.020 ppm (Rtx-35)
RH-7391 Found = 0.01824 ppm, Recovery $_{RH-1452}$ = 86.0%.
RH-7399 Found = 0.03643 ppm, Recovery $_{RH-1455}$ = 117%.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

Quantitation Report

Data File : c:\hpchem\2\data\050120\M6-004.D
Acq On : 5-1-00 9:01:20 PM
Sample : 0.05ppm fort
Misc :

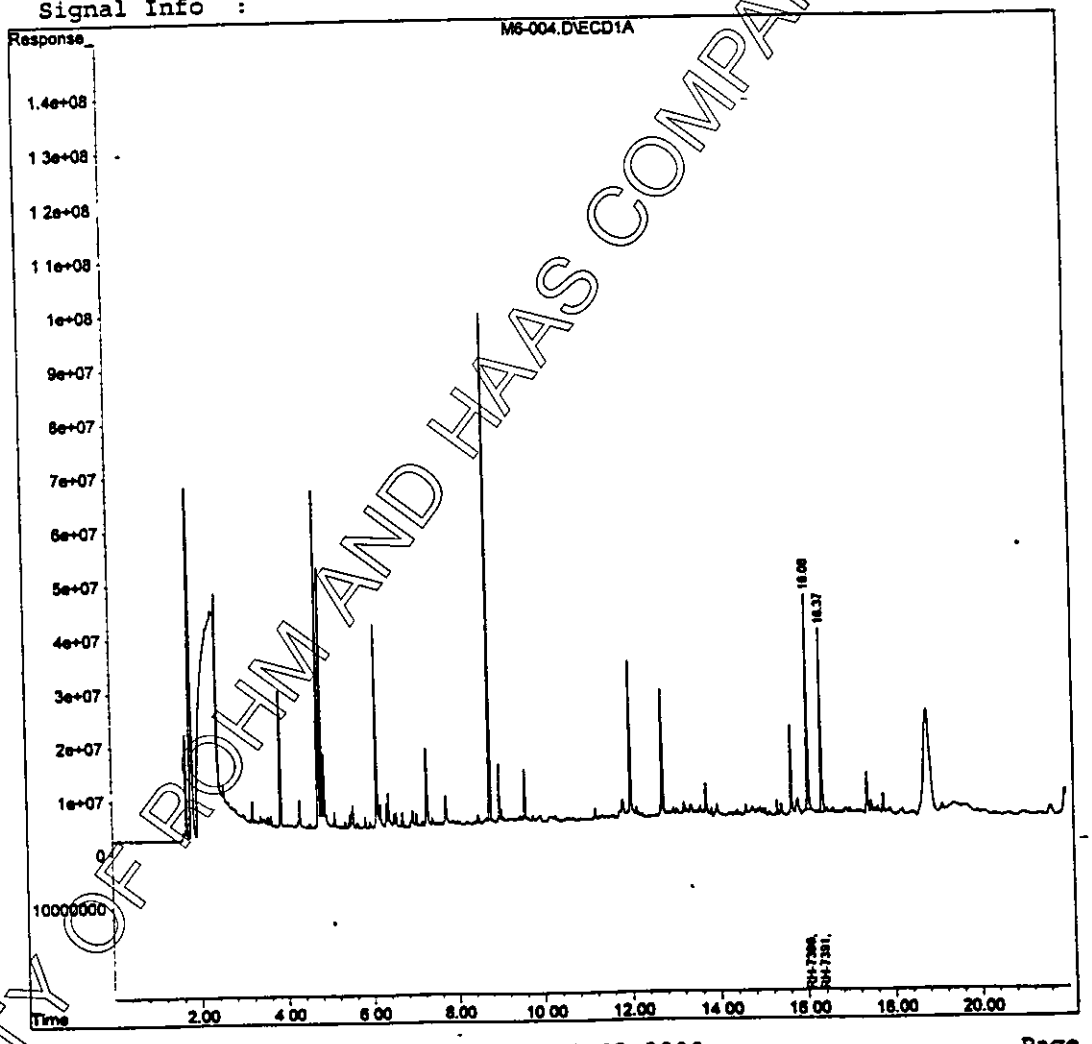
Vial: 9
Operator:
Inst : GC ECD
Multiplr: 1.00
Sample Amount: 0.00

IntFile : EVENTS.E

Quant Time: May 2 7:07 19100 Quant Results File: PEEL-C.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-C.M (Chemstation Integrator)
Title : 02-29-00
Last Update : Tue May 02 06:47:05 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-C.M

Volume Inj. :
Signal Phase :
Signal Info :



M6-004.D PEEL-C.M Tue May 02 07:08:03 2000 Page 2

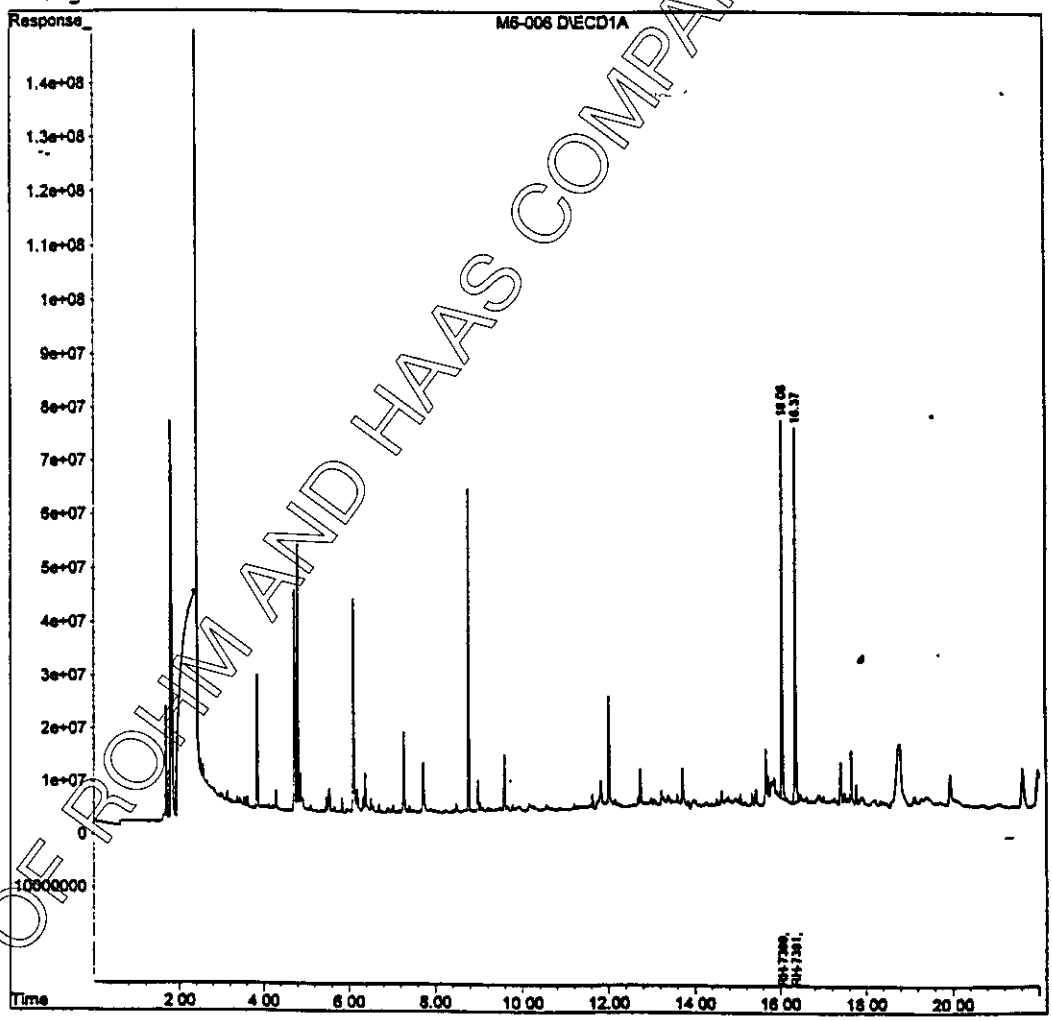
Figure 55. Fortification of RH-1452 and RH-1455 at 0.050 ppm (Rtx-35)
RH-7391 Found = 0.04792 ppm, Recovery $_{RH-1452}$ = 90.4%.
RH-7399 Found = 0.07053 ppm, Recovery $_{RH-1455}$ = 108%.

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78

Quantitation Report

```
Data File : c:\hpchem\2\data\050120\M6-006.D          Vial: 11
Acq On    : 5-1-00 10:01:02 PM                     Operator:
Sample    : 0.10ppm fort                           Inst    : GC-ECD
Misc      :                                        Multiplr: 1.00
                                                 Sample Amount: 0.00
IntFile   : EVENTS.E

Quant Time: May  2  7:09 19100  Quant Results File: PEEL-C.RES

Quant Method : C:\HPCHEM\2\METHODS\PEEL-C.M (Chemstation Integrator)
Title        : 02-29-00
Last Update  : Tue May 02 06:47:05 2000
Response via : Multiple Level Calibration
DataAcq Meth : PEEL-C.M

Volume Inj.  :
Signal Phase :
Signal Info  :
```



M6-006.D PEEL-C.M Tue May 02 07:09:28 2000 Page 2

Figure 56. Fortification of RH-1452 and RH-1455 at 0.10 ppm (Rtx-35)
RH-7391 Found = 0.1001 ppm, Recovery $_{RH-1452}$ = 94.4%
RH-7399 Found = 0.1250 ppm, Recovery $_{RH-1455}$ = 103%

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

METHYLATION PROCEDURE

1. Introduction

The following procedure describes a methylation method by which organic acids are esterified with gaseous diazomethane that is generated simultaneously from a diazomethane generator. This procedure avoids the problems associated with the storage of solutions of diazomethane.

2. Apparatus and Materials

2.1 Equipment

2.1.1. Impinger assembly, 30 mL (Aldrich)
2.1.2. Tygon tubing, 0.25" I.D.
2.1.3. Round bottom flask, 100 mL, 24/40 ST
2.1.4. Rubber stopper (24/40 ST) with two holes (0.5 mm I.D.)
2.1.5. Needles, 10 cm x 0.5 mm I.D.
2.1.6. Volumetric pipettes, 5 and 10 mL
2.1.7. Stand and clamps
2.1.8. Glass culture tube, 20 mL

2.2 Chemicals and Reagents

2.2.1. Diazald (N-methyl-N-nitroso-*p*-toluenesulphonamide), Aldrich 99% or equivalent
2.2.2. Di(ethylene glycol) ethyl ether, ACS reagent grade
2.2.3. Diethyl ether, methanol, pesticide grade or equivalent
2.2.4. Sodium hydroxide (50% wt/v, in water), ACS reagent grade
2.2.5. House Nitrogen

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

FORM 4685 REV 10/78

3. Sample Methylation

3.1 Assemble the diazomethane generator as shown in Figure 1. Remove the bottom tube of the impinger and add to it 0.5 - 1.0 g of Diazald, 5 - 10 mL of diethyl ether, and ~5 mL of di(ethylene glycol) ethyl ether. Gently swirl the tube until the Diazald is completely dissolved and then fit the tube to the apparatus. Immerse the outlet needle in 10 mL of diethyl ether contained in a glass culture tube.

3.2 Turn on the nitrogen and adjust the flow rate to approximately 3 bubbles per second in the impinger tube. The flow rate through the diethyl ether in the culture tube is checked visually to ensure that there are no leaks in the system.

3.3 Remove the bottom tube of the impinger and add ~5 mL of 50% sodium hydroxide. Fit the tube to the apparatus. The flow rate through the diethyl ether in the culture tube should now be greater than the flow into the impinger as gaseous diazomethane is being produced. Monitor the color of the diethyl ether in the culture tube until it turns yellow.

3.4 Replace the culture tube containing the diethyl ether with the 250 mL round bottom flask containing the sample. Pass diazomethane through this solution until a straw yellow color appears and then for an additional 5 min. Remove the flask and allow the sample to stabilize for at least 5 min. If the yellow color does not persist, methylate the sample again.

3.5 Evaporate the methylated sample to dryness on a rotovap at 40°C. The yellow color (excess diazomethane) should disappear gradually while vacuum is applied.

3.6 The Diazald charge in the apparatus is sufficient to generate enough diazomethane to methylate 5 to 8 samples.

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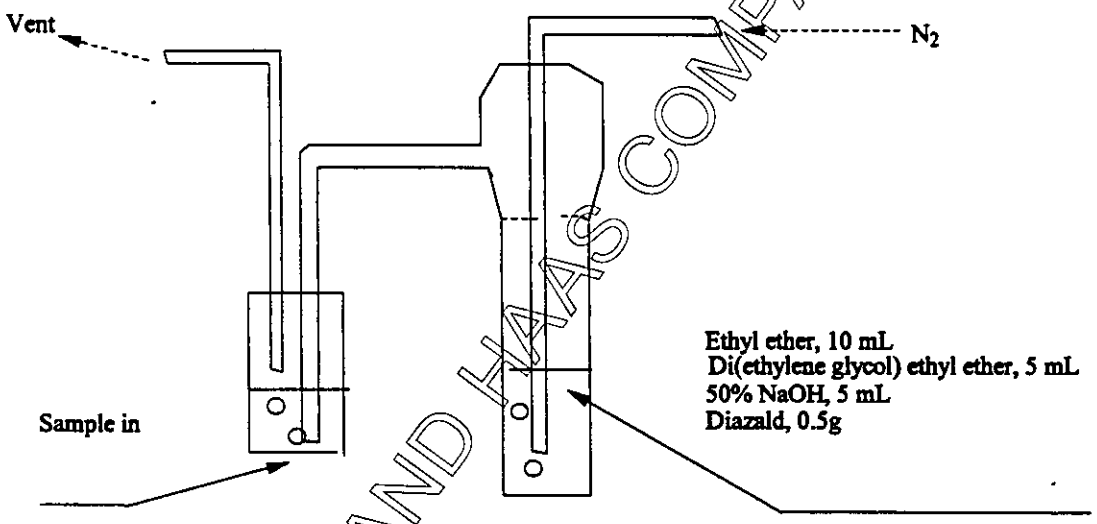
4. Safety

4.1 Diazomethane is toxic and may be carcinogenic. The procedure must be performed in a fume hood.

4.2 Impinger with visible glass flaws must not be used.

Figure 1. Diazomethane Generator



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

1) The 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 - 120°C for 1 minute; 10°C/min. to 260°C and hold 4 minutes, 30°C/min. to 280°C and hold 6 minutes.

2) The methylating (diazomethane) procedure needs to be modified because the necessary yellow color of diazomethane was not being sufficiently formed with the procedure as written. The ACB suggests onre way to modify the procedue is as follows:

Diazomethane was generated by adding 3.0 g of diazald to 5 mL of diethylene glycol, followed by 3 mL of 60% (w/v) potassium hydroxide. Nitrogen was slowly bubbled through the mixture into a tube containing 30 mL toluene After the yellow color was obtained, a 2 mL aliquot of diazomethane/toluene solution was added to each sample.