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STUDY TITLE

**Tolerance Enforcement Method for Parent RH-117,281
in Tomato RAC and Processed Fractions**

DATA REQUIREMENT

Guideline 860.1340

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GLP COMPLIANCE STATEMENT

Since method development is excluded from Good Laboratory Practice (GLP) regulations as defined by the US EPA in 40 CFR 160.3, the development of the method described in the present report was not conducted under GLP, however, the method was developed in a GLP environment. Residue data included in this report were collected in compliance with 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 and has been validated as a true and accurate representation of the data collected.

QA Activities for this study.

Date	Activity	Date Reported
8/2/99	audit of preliminary tomato method	8/3/99
10/21, 11/10/99	audit of preliminary tomato paste and puree method	10/21, 11/10/99
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1. Summary

This report describes the tolerance enforcement method for analyzing residues of parent RH-117,281 in tomato RAC (raw agricultural commodity) and the processed fractions, tomato puree and tomato paste. This report is the compilation of two previously issued preliminary residue methods (Ref 1 and 2) with additional data from field residue trials (Ref 3)

RH-117,281 residues in tomato RAC are extracted with acetonitrile. The extracts are cleaned-up using liquid-liquid partitioning followed by carbon solid phase extraction (SPE) and alumina-B SPE. RH-117,281 residues are extracted from tomato puree and paste by acetonitrile coupled with Matrix Solid Phase Dispersion (MSPD). The extracts are cleaned-up using carbon SPE and alumina-B SPE. In all cases, quantitation of RH-117,281 is performed by capillary gas-liquid chromatography using electron capture detection (GC/ECD). Residues were confirmed using capillary gas-liquid chromatography with mass selective detection (GC/MSD). The limit of quantitation (LOQ) is 0.010 ppm, established by actual fortifications at this level. The limit of detection (LOD) is estimated to be 0.003 ppm. The mean recoveries of RH-117,281 from tomato RAC, puree and paste using GC/ECD quantitation are summarized in the following table:

	Tomato RAC	Tomato Puree	Tomato Paste
Mean Recovery (%)	93.85	90.69	94.12
Standard Deviation (%)	10.95	13.65	9.33
Relative Std Dev (%)	11.71	15.05	9.91
Number of Samples	25	26	26

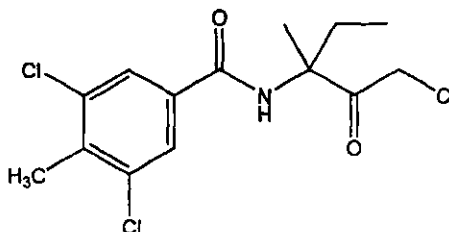
2. Introduction

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

3. Experimental Compound

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

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CA Name 3,5-dichloro-N-(3-chloro-1-ethyl-1-methyl-2-oxopropyl)-4-methylbenzamide

Proposed Generic Name zoxamide

CAS Number 156052-68-5

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

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

4. Chemicals and Supplies

Chemicals	Grade	Supplier ¹	Catalogue Number
Acetonitrile	HPLC	JT Baker	9017-33
Alumina B cartridges	LC-Alumina B (6 ml)	Supelco	5-7085
Aluminum Oxide	Brockman I Basic Powder	Baker	0539-01
C ₁₈ Silica (Bondesil)	Envirelut	Varian	1221-4016
Carbon cartridges	Enviro-Carb (6 ml)	Supelco	5-7094
Ethyl Acetate	HPLC	JT Baker	9282-03
Hexane	Ultra-Resi-Analyzed or Optima	JT Baker Fisher Scientific	9262-02 H303-1
Sodium Bicarbonate (NaHCO ₃)	A.C.S. Reagent	JT Baker	3506-01
Sodium Sulfate (Na ₂ SO ₄)	A.C.S. Reagent anhydrous	Aldrich or EM Science	23,931-3 SX0760-1
Water ²	Milli-Q®	Millipore	

- ^a Other manufacturer brands may be substituted if shown to be suitable
^b Prepared in-house using Milli-Q[®] Water Purification System

Preparation of Solutions

1) 0.1 M sodium bicarbonate solution: dissolve 8.3 g NaHCO₃ in a final volume of 1000 mL Milli-Q[®] water using a 1000 mL volumetric flask

2) Ethyl Acetate/Hexane solutions

Ethyl Acetate (mL)	Hexane (mL)	Final Proportion of Ethyl Acetate/Hexane
100	900	10/90
300	700	30/70

Note: all solutions were prepared using graduated cylinders to separately measure volumes and were stored in 1000 mL bottles.

5. Equipment

In addition to standard laboratory equipment (balance, pipets, spatulas, etc.) the following specific items were used:

Equipment	Description	Source ^a
Blender	Model 31BL92	Waring
Blender jar	Model 8470 (500 mL, glass)	Eberbach
Bottle, glass	1000 mL (Media Storage)	Wheaton
Bottle, centrifuge	250 mL	VWR
Centrifuge	Model AccuSpin FR	Beckman
Centrifuge rotor	AH-4	Beckman
Cylinder, graduated	25, 50, 100, 250, 500, 1000mL	VWR
Filter Paper	#4 (7 cm diameter)	Whatman
Flask, Erlenmeyer	1000 mL (24/40 ST)	Kimax
Flask, Filter	250 mL	Kimax
Flask, Pear-shaped Recovery	100, 200, 500 mL (24/40 ST)	Ace Glass
Flask, Round Bottom	100, 500 mL (24/40 ST)	Kimax
Flask, Volumetric	100, 500, 1000 mL	Kimax
Food Chopper	Model 8181	Hobart
Frit, Polyethylene	Cat. # 57184	Supelco
Funnel, Buchner	200 mL	Coors
Funnel, Separatory	500 mL	Kimax
Jar, glass	2 ounce	Emsco

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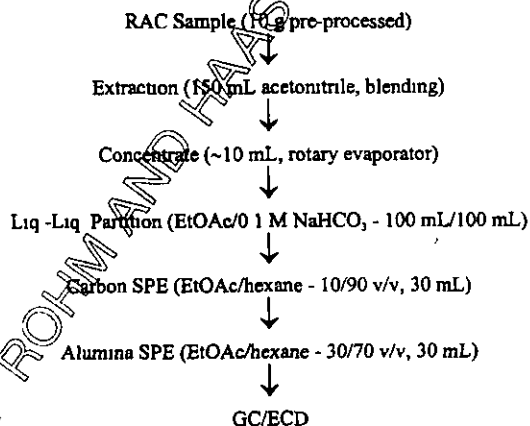
Mortar and pestle	4 ounce	Fisher
Oven, vacuum	Model 1400E	VWR
Pipet, volumetric	10 mL	Kimax
Pipetman®	Model P-200, P-1000	Rainin
Reservoirs, SPE	60 mL, Cat. # 57022	Supelco
Rotary Evaporator (Rotovap)	Models EL 131 or B-168	Büchi
Sonicator	Models 2210 or 210	Branson
SPE Manifold	Visiprep™	Supelco
Tissuemizer	Model TR-10	Fekmar

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

6. Methods

6.1 Method for Tomato RAC

6.1.1 Method Flow Diagram



6.1.2 Sample Preparation

Prepare tomatoes by chopping the fruit with dry ice using a food chopper. Allow the dry ice to sublime overnight in a freezer and store the processed samples in a freezer.

6.1.3 Sample Extraction

The tomato samples can be extracted by blending or homogenization

Blending Weigh 10 g of processed tomato sample and place it in a glass blender jar. If the sample is to be fortified, place the blender jar in a ventilated hood and add a known amount of RH-7281 standard (in hexane) to the tomato sample. Keep the sample in the hood for 1 hour to allow the hexane to evaporate. Add 100 mL of acetonitrile to the blender jar and blend the sample for ~60 sec at the low speed setting. Using a Buchner funnel, filter the homogenate through Whatman #4 filter paper into a 250 mL filter flask. Rinse the homogenizing vessel with an additional 50 mL acetonitrile. Transfer the filtrate to a 500 mL round-bottom flask and concentrate to ~10 mL using a rotary evaporator ($\leq 30^{\circ}$ C, 50-100 mm Hg).

Homogenization Weigh 10 g of processed tomato sample and place it in a 250 mL centrifuge bottle. If the sample is to be fortified, place the bottle in a ventilated hood and add a known amount of RH-7281 standard (in hexane) to the tomato sample. Keep the sample in the hood for 1 hour to allow the hexane to evaporate. Add 50 mL acetonitrile to the bottle and homogenize for ~60 sec using a Nisslemizer®. Centrifuge the sample at 4,000 rpm for 10 min at $\sim 10^{\circ}$ C. Using a Buchner funnel, filter the supernatant through Whatman #4 filter paper into a 250 mL filter flask. Repeat the homogenization and filtration procedures twice with 50 mL acetonitrile each time. Transfer the filtrate to a 500 mL round-bottom flask and concentrate to ~10 mL using a rotary evaporator ($\leq 30^{\circ}$ C, 50-100 mm Hg).

6.1.4 Liquid-Liquid Partitioning

Add 100 mL of 0.1 M sodium bicarbonate solution to the sample in the round-bottom flask and then transfer the sample to a 500 mL separatory funnel. Rinse the flask with 100 mL ethyl acetate and add to the separatory funnel. Shake the separatory funnel for ~1 min with frequent venting. After the phases have separated, drain the lower, aqueous phase into the original round-bottom flask and decant the upper, organic phase into a new 500 mL pear-shaped recovery flask. Re-extract the aqueous phase with 100 mL ethyl acetate as above. Drain and discard the aqueous phase. Combine the organic phases and take to dryness using a rotary evaporator ($\leq 50^{\circ}$ C, 50-100 mm Hg). Redissolve the residue in 10 mL hexane using sonication for 10-20 sec.

6.1.5 Carbon Solid Phase Extraction (SPE)

To each carbon SPE cartridge, add a small amount of anhydrous sodium sulfate (~0.5 cm) and pre-rinse the cartridge with 5 mL ethyl acetate followed by two rinses of 5 mL hexane. Do not let the cartridge run dry. Discard the rinsates. Apply the 10 mL residue in hexane (section 6.1.4) and collect the effluent. Rinse the recovery flask twice with 15 mL 10/90 (v/v) ethyl acetate/hexane each time and apply the rinsates to the cartridge. Collect the effluents in a 200 mL pear-shaped recovery flask (see Note on page 14). Take

the sample to dryness using a rotary evaporator ($\leq 50^{\circ}\text{C}$, 50-100 mm Hg) Dissolve the residue in 10 mL hexane using sonication for 10-20 sec

6.1.6 Alumina B Solid Phase Extraction

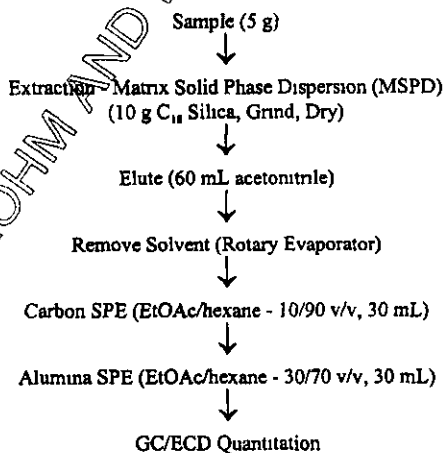
Pre-rinse each alumina B SPE cartridge with 5 mL ethyl acetate followed by two 5 mL rinses with hexane. Do not let the cartridge run dry. Discard the rinsates. Apply the 10 mL residue in hexane from section 6.1.5 and discard the effluent. Rinse the recovery flask with 10 mL hexane and apply to the cartridge, again discarding the effluent. Finally, elute RH-7281 from the cartridge using 30 mL of 30/70 (v/v) ethyl acetate/hexane and collect the eluate in a 100 mL pear-shaped recovery flask (see Note page 14). Take the sample to dryness using a rotary evaporator ($\leq 50^{\circ}\text{C}$, 50-100 mm Hg). Redissolve the residue in 10 mL hexane using sonication for 10-20 sec. The sample is now ready for quantitation (see section 7).

6.1.7 Time to Run a Sample Set

The time required to run one sample set of 6 samples through the entire method is approximately 17 hours including GC analysis.

6.2 Method for tomato puree and tomato paste

6.2.1 Method Flow Diagram



6 2 2 Sample Preparation

Tomato puree and tomato paste are used directly without processing

6 2 3 Extraction Matrix Solid Phase Dispersion

Weigh 5 g of tomato puree or tomato paste into a mortar. If the sample is to be fortified, place the mortar in a ventilated hood and add a known amount of RPH 7281 standard (in hexane) to the tomato sample. Keep the sample in the hood for 1 hour to allow the hexane to evaporate. Add 10 g of C₁₈ silica and grind with pestle for approximately one minute or so until the sample is homogeneous. Dry the sample in a vacuum oven under full vacuum for 40 minutes at ~50°C. Break-up the clumped, dried matrix using a pestle and spatula.

Pack a 60 mL SPE reservoir as follows:

- 1 Place a polyethylene frit in the bottom
- 2 Add 5 g of aluminum oxide
- 3 Place a frit on top of the aluminum oxide
- 4 Add the C₁₈ silica/puree or paste matrix contained in the mortar
- 5 Place a frit on top of C₁₈ silica/puree or paste matrix and then pack tube
- 6 Place the tube on a Vistsprep™ unit

Add 60 mL acetonitrile to the mortar as a rinse then add this rinse to the packed SPE tube. Elute the sample and collect the acetonitrile eluate in a 2 ounce (~60 mL) glass jar. Transfer the eluate to a 100 mL round bottom flask and rinse the jar with an additional 5 mL of acetonitrile. Take the sample to dryness using a rotary evaporator (~50°C, 50-100 mm Hg). Redissolve the residue in 10 mL hexane using sonication (10-20 sec).

6 2 4 Carbon Solid Phase Extraction

To each carbon SPE cartridge, add a small amount of anhydrous sodium sulfate (~0.5 cm) and pre-rinse the cartridge with 5 mL ethyl acetate followed by two rinses of 5 mL hexane. Do not let the cartridge run dry. Discard the rinsates. Apply the 10 mL residue in hexane (section 6 2 3) to the carbon cartridges and collect the effluent. Rinse the round bottom flask with 2 x 15 mL of 10/90 (v/v) of ethyl acetate /hexane and pass each rinse through the carbon cartridge. Collect the effluents in a 100 mL round bottom flask and take the sample to dryness using a rotary evaporator (~50°C, 50-100 mm Hg). Redissolve the residue in 10 mL hexane using sonication (10-20 sec).

6.2.5 Alumina-B Solid Phase Extraction

Pre-rinse each alumina B SPE cartridge with 5 mL ethyl acetate followed by two 5 mL rinses with hexane. Do not let the cartridge run dry. Discard the rinsates. Apply the 10 mL residue in hexane from section 6.2.4 and discard the effluent. Rinse the round bottom flask with 10 mL hexane and apply to the cartridge, again discarding the effluent. Add 30 mL of 30/70 (v/v) of ethyl acetate/hexane to the empty round bottom and sonicate for ~10 sec. Add this to the alumina B cartridge and collect the eluate containing RH-7281 in a 100 mL round bottom flask (see Note below). Take the sample to dryness using a rotary evaporator (~50°C, 50-100 mm Hg). Redissolve the residue in 5 mL hexane using sonication for 10-20 sec. The sample is now ready for quantitation (see section 7).

6.2.6 Time to Run a Sample Set

The time required to run one sample set of 6 samples through the entire method is approximately 15 hours including GC analysis.

Note: Elution Profile Check

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

- 1) Add 10 mL of 0.10 µg/mL RH-7281 standard in hexane to the column or cartridge.
- 2) Follow the elution schemes as outlined for each individual procedure (see sections 6.1.5, 6.1.6, 6.2.4, or 6.2.5) and collect the pre-elution (Alumina B) and target elution cuts.
- 3) Wash the column with 30 mL of ethyl acetate and collect the post-elution cut.
- 4) Take each of the cuts to dryness and redissolve in 10 mL of ethyl acetate. Inject them as outlined for GC/ECD quantitation (section 7).
- 5) If the target cut contains a minimum of 85% of RH-7281, the lot can be considered acceptable.
- 6) If the post-wash cut contains a more than 15% of RH-7281, the target elution volume or the percentage of ethyl acetate in the eluting solvent should be modified. If the pre-elution cut of Alumina B contains more than 15% of RH-7281, the lot should be considered unacceptable.

7. Quantitation

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

7.1 Instrumentation and conditions

See comments on GC maintenance in Section 9

7.1.1 Primary Quantitation

	Tomato RAC	Puree and Paste
Quantitation Method	GC/ECD	GC/ECD
Instrument	HP* 6890 μ ECD	HP 6890 μ ECD
Injection: mode	on-column	on-column
temp. ($^{\circ}$C)	150	150
volume (μL)	1.0	1.0
Column: analytical	HP-5MS 0.25 mm ID x 30 m 0.25 μ m film	HP-5MS 0.25 mm ID x 30 m 0.25 μ m film
Carrier gas	helium	helium
Head pressure (psi)	13.5	13.5
Flow rate (mL/min.)	1.0	1.0
Detector temp. ($^{\circ}$C)	300	300
Oven Temp. Program:		
Initial temp. ($^{\circ}$C)	80 (hold 1 min)	80 (hold 1 min)
Ramp 1 ($^{\circ}$C/min.)	20	20
Temp. 1 ($^{\circ}$C)	260 (hold 8 min.)	250 (hold 5 min)
Ramp 2 ($^{\circ}$C/min.)	30	30
Temp. 2 ($^{\circ}$C)	290 (hold 1 min)	280 (hold for 3 min)
Retention time (min.)**		
RH-7281	-11-12	-11-12

* HP: Hewlett Packard

** Retention time might vary depending on column maintenance (see comments in Section 9) or the oven temperature program (for example, if ramp oven to 260 $^{\circ}$ C at 35 $^{\circ}$ C/min. and hold 7 min. at 260 $^{\circ}$ C, then retention time of RH-7281 will be ~8-9 min)

7.1.2 Confirmatory Quantitation

	Tomato RAC	Puree and Paste
Quantitation Method	GC/MSD	GC/MSD
Instrument	HP 6890 HP 5973 MSD	HP 6890 HP 5973 MSD
Injection: mode	splitless	splitless
temp. (°C)	180	180
volume (µL)	1.0	1.0
Column: analytical	HP-5MS 0.25 mm ID x 30 m 0.25 µm film	HP-5MS 0.25 mm ID x 30 m 0.25 µm film
Carrier gas	helium	Helium
Head pressure (psi)	10.4	12.0
Flow rate (mL/min.)	1.0	1.0
Detector temp. (°C)	Transfer Line 280 Quad 150 Ion Source 230	Transfer Line 280 Quad 150 Ion Source 230
Oven Temp. Program:		
Initial temp. (°C)	100 (hold 1 min)	80 (hold 1 min)
Ramp 1 (°C/min.)	20	20
Temp. 1 (°C)	250 (hold 5 min)	250 (hold 8 min)
Ramp 2 (°C/min.)	30	30
Temp. 2 (°C)	280 (hold 3 min)	280 (hold for 1 min)
Monitored ions (m/z)	187, 189, 258*	187, 189, 258*
Retention time(min.)**		
RH-7281	~11-12	~11-12

* Quantitation ion 258 m/z

** Retention time might vary depending on column maintenance (see comments in Section 9)

Note Brands of analytical instruments from other manufacturers might be suitable, however, no attempt was made to evaluate such instruments

7.2 Preparation of Standard Calibration Curves

Prepare a 100 µg/mL standard stock solution by weighing 10 mg of RH-7281 (correct for purity of standard) into a 100 mL volumetric flask. Bring up to volume with ethyl acetate.

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

Prepare a 0.10 µg/mL stock solution by pipetting 10 mL of 1.0 µg/mL into a 100 mL volumetric flask and bringing to volume with hexane.

Prepare standard solutions of 0.005, 0.010, 0.020, 0.050, and 0.10 µg/mL by pipetting the appropriate volumes of 0.10 µg/mL and 1.0 µg/mL stock solutions shown in the following table into a 100 mL volumetric flask and bringing to volume with hexane.

Stock Solution (µg/mL)	Volume (mL) of Stock Solution	Final Concentration (µg/mL)
0.10	5.0	0.005
0.10	10	0.010
0.10	20	0.020
1.0	5.0	0.050
1.0	10	0.10

The standards should be stored in a refrigerator and remade every six months as a precautionary measure to ensure reliability (Lab Memo 34-99-12)

A minimum of four standard solutions are prepared in a desired concentration range and injected in duplicate for GC/ECD or GC/MSD quantitation. Standards are quantitated by peak height or peak area, ensuring that the integration method is consistent for all standards and samples analyzed during a GC run. To obtain a standard calibration curve, the instrument responses (e.g. peak heights) for the standards are fit to the concentrations of standard using linear or quadratic regression. The concentration of RH-7281 in the sample is then determined from the regression equation for the standard calibration curve.

7.3 Fortification Recovery

Percent recovery is calculated by measuring the peak height (or peak area, see section 7.2), calculating the µg/mL (ppm) found from the regression equation for the standard calibration curve, and correcting for background in the control sample as shown in Equation 1.

$$\frac{[\text{Found } (\mu\text{g/mL}) \times \text{Final Vol. (mL)}] - [\text{Control } (\mu\text{g})]}{\text{Fortification Amount } (\mu\text{g})} \times 100\% = \% \text{ Recovery (Eq 1)}$$

7.4 Sample Analysis

If necessary, the samples are diluted to an appropriate volume to give a final concentration within the standard curve range, preferably in the range of 0.010-0.050 ppm. The residue concentration is determined as follows:

$$\frac{\text{Residue Found } (\mu\text{g/mL}) \times \text{Final Vol. (mL)}}{\text{Sample Weight (g)}} = \mu\text{g/g} = \text{ppm} \quad (\text{Eq. 2})$$

7.5 Sample Calculations

A typical calculation for the recovery of a 0.050 ppm ($\mu\text{g/g}$) fortification of tomato (Table 1 and Figure 11) is demonstrated as follows: the peak height of RH-7281 is 33,546,477 which, from the standard calibration curve, corresponds to 0.04918 ppm ($\mu\text{g/mL}$). The recovery for a 10.0 g sample fortified with 0.500 μg RH-7281, a final sample volume of 10.0 mL, and no residue in the control is calculated as below:

$$\frac{[0.04918 \mu\text{g/mL} \times 10.0 \text{ mL}] - [0 \mu\text{g}]}{0.500 \mu\text{g}} \times 100\% = 98.4\%$$

A typical calculation for determining RH-7281 residue in field-aged tomatoes (Figure 13) is demonstrated as follows: the peak area of RH-7281 is 343,492, which, from the standard calibration curve, corresponds to 0.03126 ppm ($\mu\text{g/mL}$). For a 10 g field-aged sample in a final sample volume of 28.0 mL, the amount of RH-7281 residue is calculated using Eq. 2.

$$\frac{0.03126 \mu\text{g/mL} \times 28.0 \text{ mL}}{10.0 \text{ g}} = 0.0875 \mu\text{g/g} = 0.0875 \text{ ppm}$$

8. Radiovalidation

Samples from a ^{14}C -RH-7281 tomato metabolism study (Ref. 4) were analyzed using the residue analytical method for parent RH-7281 in tomato (Ref. 1). The results (see Appendix 1) are summarized in the following table.

<u>RH-7281 Residue</u>	<u>Analytical Method</u>	<u>Metabolism Study</u>
ppm	0.092 ± 0.001	0.116 ± 0.062

Radiovalidation of the analytical method demonstrated that the residues of RH-7281 present in a tomato metabolism sample were adequately measured by the analytical method.

9. Results and Discussion

Both primary (GC/ECD) and confirmatory (GC/MSD) quantitation procedures have acceptable sensitivity for RH-7281. Representative chromatograms of the primary quantitation method are shown in Figures 1-29 and those for the confirmatory method are shown in Figures 30-41. Fortification recoveries for tomato RAC, puree, and paste are shown in Tables 1, 2, and 3, respectively. The limit of quantitation (LOQ), limit of detection (LOD), and the overall method recoveries of RH-7281 using the primary quantitation method (GC/ECD) are summarized for each matrix in the following table.

	Tomato RAC	Tomato Puree	Tomato Paste
LOQ (ppm)	0.010	0.010	0.010
LOD (ppm)	0.003	0.003	0.003
Mean Recovery (%)	93.83	90.69	94.12
Standard Deviation (%)	10.05	13.65	9.33
Relative Std Dev (%)	10.71	15.05	9.91
Number of Samples	26	26	26
Maximum Recovery (%)	113	113	117
Minimum Recovery (%)	78.0	69.9	76.8

There was no interference found in control tomato samples at the same retention time as RH-7281 using GC/ECD (Figures 7-9, 16-18, 23-25) or GC/MSD (Figures 35, 38, 40).

Proper maintenance of the GC/ECD and GC/MSD injection and inlet systems is critically important to the chromatography of RH-7281. For example, increasing the injection temperature to 290°C (30°C/min.) after the elution of the RH-7281 peak removed residues that remained in the inlet system. Also, removal of 5 - 10 cm of the analytical column after a few analytical sets (about 100 samples) can improve chromatography. It should be noted, however, that changing the length of the analytical column can slightly alter the retention time of RH-7281. The injection port should also be cleaned regularly (after about 100 injections) by disassembling the port and sonicating it in methanol for several minutes. Finally, if chromatography does not improve, install a new analytical column.

10. References

- 1 Burdge, E., et al Aug. 1999. Preliminary Residue Analytical Method for Parent RH-117,281 in Tomato (TR 34-99-68)
- 2 Kunilla, K , et al Nov 1999 Preliminary Residue Analytical Method for Parent RH-117,281 in Tomato Puree and Paste (TR 34-99-65)
- 3 Graves, D (Concurrent Submission). RH-117,281 Fungicide Field Residue Trials in Tomatoes (TR 34-99-161)
- 4 Sharma, A (Concurrent Submission) RH-117,281 Metabolism in Fruiting Tomato (TR 34-99-159)
- 5 Guo, I March 1999. Storage Stability of the Standard Solutions: RH-7281, RH-0721, RH-1452, RH-1455, RH-7399, and RH-7399 (Lab Memo 34-99-12)

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11. Project Information

Title Tolerance Enforcement Method for Parent
RH-117,281 in Tomato RAC and Processed Fractions

Project Number 1401.15

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

Testing Laboratories Rohm and Haas Company
Research Laboratories
727 Norristown Road
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9936-67 Avenue
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T6E 0P5 Canada

Dates Work Initiated: March, 1999
Work Completed: November, 1999

Technical Personnel. Ernest Burdge, Ken Kurilla, Ipin Guo,
and Charles Hofmann

Notebook References: RH# 67681 (ELB-27) - pg 72
RH# 70011 (ELB-28) - pg 62

Data Archives. Research notebooks are stored in the Rohm and Haas
Library and raw data are stored in the Rohm and Haas
GLP Records Archive Facility

Peer Review: Dennis Martin

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Table 1. Fortification Recovery Results for RH-7281 in Tomato RAC

Fortification (ppm)	Source of Data ^a	Primary Quantitation	Source of Data ^a	Confirmatory Quantitation
		GC/ECD (% Recovery)		GC/MSD (% Recovery)
0.010	TR34-99-68	99.5		
0.010	TR34-99-68	103		
0.010	TR34-99-68	93.6		
0.010	TR34-99-68	99.2		
0.010	TR34-99-68	79.4		
0.010	TR34-99-68	94.6		
0.010	TR34-99-68	91.7		
0.010	TR34-99-68	88.2		
0.010	TR34-99-68	90.6	TR34-99-68	98.7
0.010	TR34-99-68	94.4	TR34-99-68	105
0.010			10/12/99	111
0.010			10/18/99	98.6
0.010			10/19/99	90.0
0.010			10/25/99	97.6
0.010			10/26/99	96.9
0.010			10/26/99A	96.2
0.010			10/27/99	99.3
0.010			10/28/99	92.9
0.010			10/28/99A	90.0
0.020	TR34-99-68	103		
0.020	TR34-99-68	125		
0.020	TR34-99-68	104		
0.020	TR34-99-68	86.4		
0.020	TR34-99-68	89.2	TR34-99-68	95.7
0.050	TR34-99-68	98.4		
0.050	TR34-99-68	104		
0.050	TR34-99-68	92.9		
0.050	TR34-99-68	86.6		
0.050	TR34-99-68	88.9	TR34-99-68	91.7
0.050			10/25/99	97.4
0.050			10/28/99	85.6
0.050			10/28/99A	84.3
0.10	TR34-99-68	95.6		
0.10	TR34-99-68	96.9		
0.10	TR34-99-68	83.6		
0.10	TR34-99-68	79.1		
0.10	TR34-99-68	78.0	TR34-99-68	79.0

TR 34-99-111

23

0.10		10/12/99	101
0.10		10/26/99	96.8
0.10		10/27/99	101
0.20		10/19/99	101
0.20		10/26/99A	89.4
0.30		10/18/99	10
1.0		11/08/99	96.9
1.5		11/08/99	104
<hr/>			
Mean	93.83		96.40
Standard Deviation	10.05		7.49
Relative Std. Dev	10.71		7.77
Number of Samples	25		25

* TR 34-99-68 (Ref 1), "Dates" represent standard curve numbers reported in Rohm and Haas NRDS files (Ref 3)

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Table 2. Fortification Recovery Results for RH-7281 in Tomato Puree

Fortification (ppm)	Source of Data *	Primary Quantitation	Confirmatory Quantitation
		GC/ECD (% Recovery)	GC/MSD (% Recovery)
0.010	TR34-99-95	88.1	
0.010	TR34-99-95	90.9	
0.010	TR34-99-95	75.8	TR34-99-95 98.8
0.010	TR34-99-95	84.4	TR34-99-95 103
0.010	TR34-99-95	109	
0.010	TR34-99-95	113	
0.010	TR34-99-95	106	
0.010	TR34-99-95	110	
0.010	TR34-99-95	69.8	
0.010	TR34-99-95	70.1	
0.010	07/09/99	69.9	
0.010	07/09/99	69.9	
0.020	TR34-99-95	91.8	
0.020	TR34-99-95	83.8	TR34-99-95 87.6
0.020	TR34-99-95	103	
0.020	TR34-99-95	110	
0.020	TR34-99-95	82.8	
0.020	07/09/99	82.9	
0.050	TR34-99-95	85.0	
0.050	TR34-99-95	83.2	TR34-99-95 95.5
0.050	TR34-99-95	94.6	
0.050	TR34-99-95	102	
0.10	TR34-99-95	95.2	
0.10	TR34-99-95	84.9	TR34-99-95 91.1
0.10	TR34-99-95	98.7	
0.10	TR34-99-95	104	
	Mean	90.69	95.20
	Standard Deviation	13.65	6.09
	Relative Std Dev	15.05	6.40
	Number of Samples	26	5

* TR 34-99-95 (Ref 2), "Dates" represent standard curve numbers reported in Rohm and Haas NRDS files (Ref 3)

Table 3. Fortification Recovery Results for RH-7281 in Tomato Paste

Fortification (ppm)	Source of Data *	Primary Quantitation	Confirmatory Quantitation
		GC/ECD (% Recovery)	GC/MSD (% Recovery)
0.010	TR34-99-95	91.4	TR34-99-95 97.2
0.010	TR34-99-95	93.2	TR34-99-95 107
0.010	TR34-99-95	88.1	
0.010	TR34-99-95	82.7	
0.010	TR34-99-95	88.9	
0.010	TR34-99-95	115	
0.010	TR34-99-95	90.8	
0.010	TR34-99-95	76.8	
0.010	TR34-99-95	97.9	
0.010	TR34-99-95	107	
0.010	08/10/99	97.3	
0.010	08/10/99	107	
0.020	TR34-99-95	87.4	TR34-99-95 97.8
0.020	TR34-99-95	85.7	
0.020	TR34-99-95	87.6	
0.020	TR34-99-95	94.6	
0.020	TR34-99-95	95.4	
0.020	08/10/99	95.2	
0.050	TR34-99-95	87.3	TR34-99-95 91.8
0.050	TR34-99-95	90.2	
0.050	TR34-99-95	95.1	
0.050	TR34-99-95	97.8	
0.10	TR34-99-95	93.5	TR34-99-95 93.5
0.10	TR34-99-95	91.3	
0.10	TR34-99-95	87.2	
0.10	TR34-99-95	117	
	Mean	94.12	97.46
	Standard Deviation	9.33	5.89
	Relative Std Dev	9.91	6.04
	Number of Samples	26	5

* TR 34-99-95 (Ref.2), "Dates" represent standard curve numbers reported in Rohm and Haas NRDS files (Ref.3)

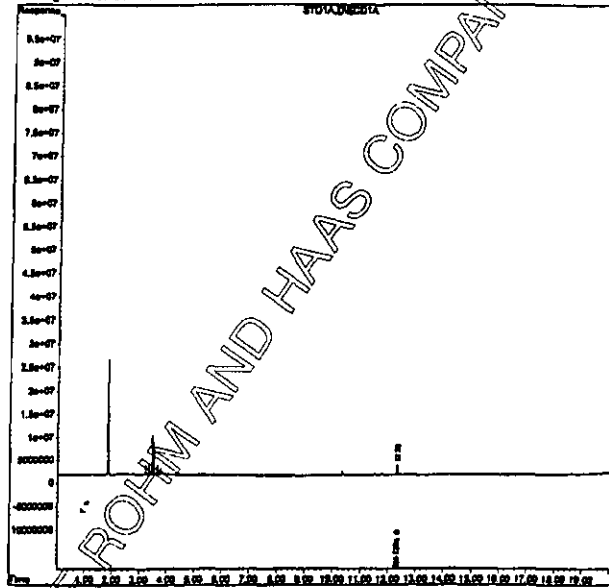
Quantitation Report

Data File : c:\hpcchem\2\data\0022999\STD1A.D Vial 2
 Acq On : 3-29-99 6:28:44 PM Operator:
 Sample : 0.005 ppm Inst GC ECD
 Misc : Multiplr- 1.00
 Sample Amount 0.00
 IntFile : EVENTS.E

Quant Time: Jul 26 14 59 1999 Quant Results File: ERNIE RES

Quant Method : C:\HPCHEM\2\METHODS\ERNIE.M (Chemstation IntegSoft)
 Title : 04-07-99
 Last Update : Mon Jul 26 14:44:02 1999
 Response via : Multiple Level Calibration
 DataAcq Meth : ERNIE.M

Volume Inj. :
 Signal Phase :
 Signal Info :



Compound	R.T	Response
Target Compounds		
1) RH-7281 03-29-99	12.33	2238008

Figure 1 0.005 ppm RH-7281 Standard (GC/ECD)

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Quantitation Report

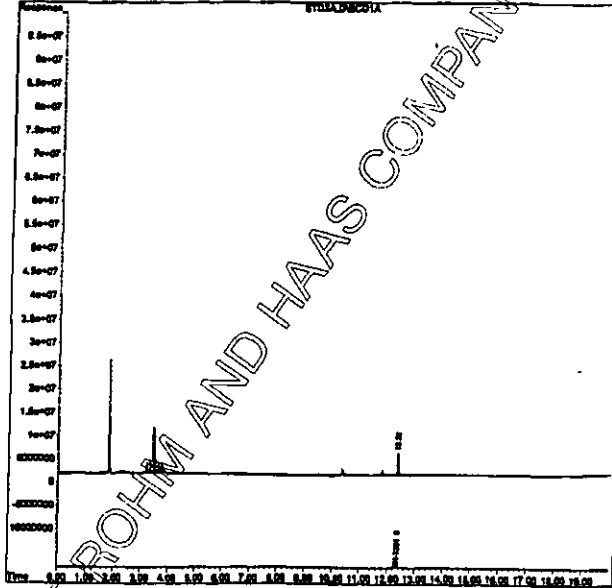
Data File : c:\hpcchem\2\data\032999\STD2A.D Vial: 3
 Acq On : 3-29-99 6:56:10 PM Operator:
 Sample : 0.010 ppm Inst : GC ECD
 Misc : Multiplr: 1.00
 Sample Amount: 0.00

IntFile : EVENTS.E

Quant Time: Jul 26 15 00 1999 Quant Results File: ERNIE RES

Quant Method : C:\HPCHEM\2\METHODS\ERNIE.M (Chemstation Integrator)
 Title : 04-07-99
 Last Update : Mon Jul 26 14:44:02 1999
 Response via : Multiple Level Calibration
 DataAcq Meth : ERNIE.M

Volume Inj :
 Signal Phase :
 Signal Info :



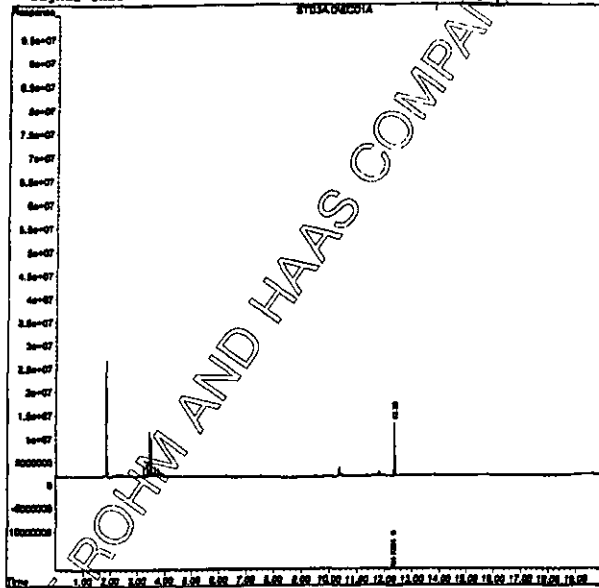
Compound	R.T.	Response
Target Compounds		
1) RH-7281 03-29-99	12.72	4950653

Figure 2 0.010 ppm RH-7281 Standard (GC/ECD)

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Quantitation Report

Data File : c:\hpcchem\2\data\022999\STDJA.D Vial: 4
Acq On : 3-29-99 7:23:35 PM Operator:
Sample : 0.020 ppm Inst : GC ECD
Misc : Multiplr: 1.00
Sample Amount: 0.00
IntFile : EVENTS.E
Quant Time: Jul 26 19:00 1999 Quant Results File: ERMIE.RES
Quant Method : C:\HPCHEM\2\METHODS\ERMIE.M (Chemstation Integration)
Title : 04-07-99
Last Update : Mon Jul 26 14:44:02 1999
Response via : Multiple Level Calibration
DataAcq Meth : ERMIE.M
Volume Inj :
Signal Phase :
Signal Info :



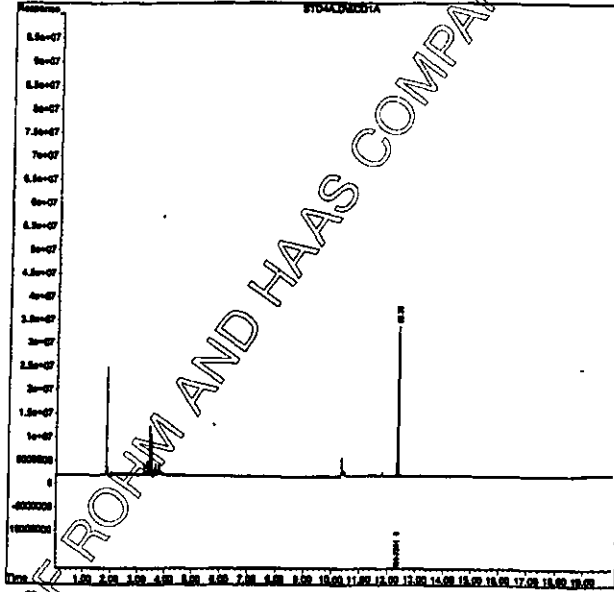
Compound	R.T	Response
Target Compounds		
1) RH-7281 03-29-99	12.32	11466457

Figure 3 0.020 ppm RH-7281 Standard (GC/ECD)

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Quantitation Report

Data File : c:\hpchem\2\data\032999\STD4A.D Vial: 5
Acq On : 3-29-99 7:51:01 PM Operator:
Sample : 0.050 ppm Inst : GC ECD
Misc : Multiplr: 1.00 Sample Amount: 0.04
InFile : EVENTS.E ;
Quant Time: Jul 26 15:01 1999 Quant Results File: ERNIE.RES
Quant Method : C:\HPCHEM\2\METHODS\ERNIE.M (Chemstation Integrator)
Title : 04-07-99
Last Update : Mon Jul 26 16:44:02 1999
Response via : Multiple Level Calibration
DataAcq Meth : ERNIE.M
Volume Inj. :
Signal Phase :
Signal Info



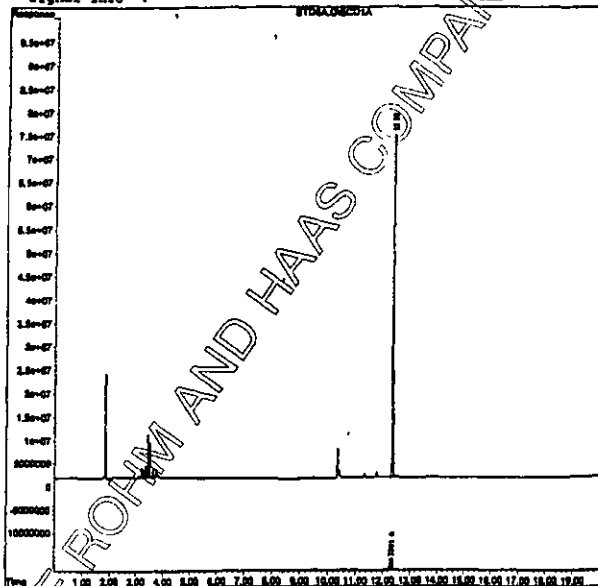
Compound	R T	Response
Target Compounds		
1) RH-7281 03-29-99	12.22	32055661

Figure 4 0.050 ppm RH-7281 Standard (GC/ECD)

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Quantitation Report

Data File : c:\hpcchem\2\data\032999\STOSA.D Vial: 6
 Acq On : 3-29-99 0:18:26 PM Operator:
 Sample : 0.100 ppm Inst: GC ECD
 Misc : Multiplr: 1.00
 Sample Amount: 0.00
 IntFile : EVENTS E
 Quant Time: Jul 26 13:02 1999 Quant Results File: ERNIE.RES
 Quant Method : C:\HPCHEM\2\METHODS\ERNIE.M (Chemstation Integrator)
 Title : 04-07-99
 Last Update : Mon Jul 26 14:44:02 1999
 Response via : Multiple Level Calibration
 DataAcq Meth : ERNIE M
 Volume Inj. :
 Signal Phase :
 Signal Info :



Compound	R T.	Response
1) Target Compounds		
RH-7281 03-29-99	12.32	73421637

Figure 5 0.10 ppm RH-7281 Standard (GC/ECD)

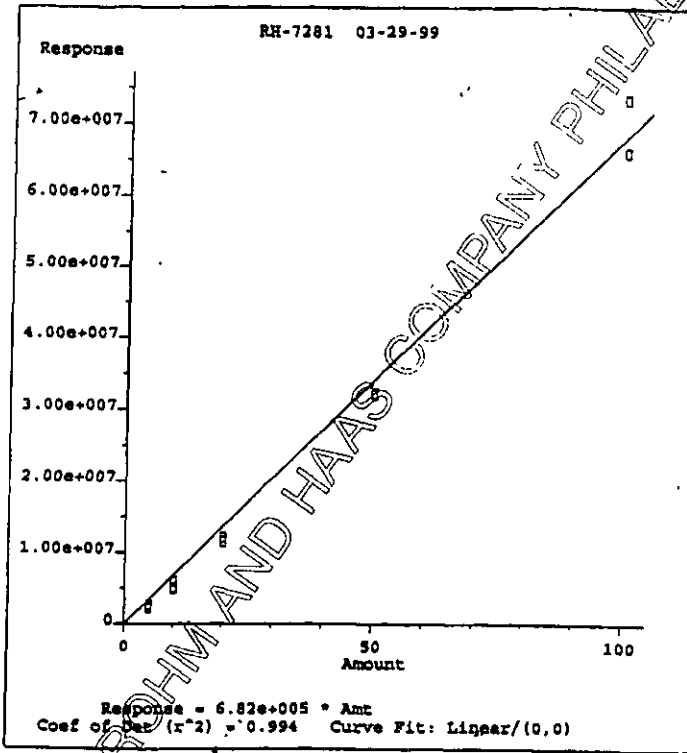


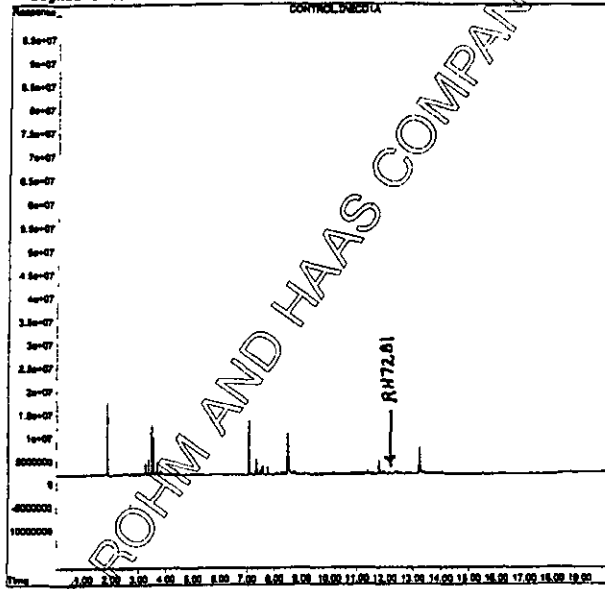
Figure 6 Calibration Curve for RH-7281 (GC/ECD)

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Quantitation Report

Data File : c:\hpcem\2\data\032999\CONTROL.D Vial: 7
 Acq On : 3-29-99 9:13:15 PM Operator:
 Sample : Control 032999 Inst GC ECD
 Misc : Multiplr 1.00
 Sample Amount: 0.00
 IntFile : EVENTS E
 Quant Time: Jul 26 15:03 1999 Quant Results File: ERNIE RES
 Quant Method C:\HPCHEM\2\METHODS\ERNIE.M (Chemstation Integrator)
 Title : 04-07-99
 Last Update : Mon Jul 26 14:44:02 1999
 Response via Multiple Level Calibration
 DataAcq Mech : ERNIE.M

Volume Inj :
 Signal Phase :
 Signal Info :



Compound	R T.	Response	Conc Units
1) Target Compounds			
RH-7261 03-29-99	0.00	0	N.D. ppb

Figure 7 Control Tomato RAC (GC/ECD)

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Quantitation Report

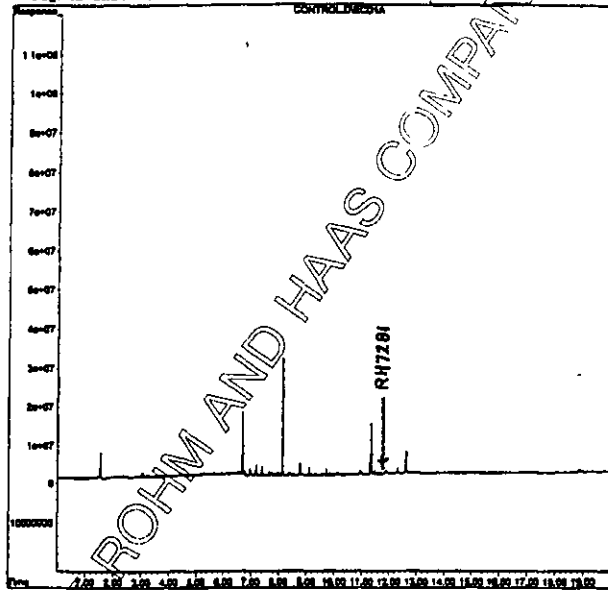
Data File : c:\hpcchem\2\data\040799\CONTROL.D Vial: 7
 Acq On : 4-7-99 8:25:24 PM Operator:
 Sample : Control E040599 (81) Inst : GC ECD
 Misc : Multiplr: 1.00
 Sample Amount: 0.00

IntFile : EVENTS.E

Quant Time: Jul 27 10:09 1999 Quant Results File: ERMIE.RES

Quant Method : C:\HPCHEM\2\METHODS\ERMIE.M (Chemstation Integrator)
 Title : 04-07-99
 Last Update : Tue Jul 27 09 49 59 1999
 Response via : Multiple Level Calibration
 DataAcq Mech : ERMIE.M

Volume Inj :
 Signal Phase :
 Signal Info :



Compound	R.T.	Response	Conc Units
Target Compounds			
1) 7281 04-07-99	0.00	0	N.D. ppb

Figure 8 Control Tomato RAC (GC/ECD)

Quantitation Report

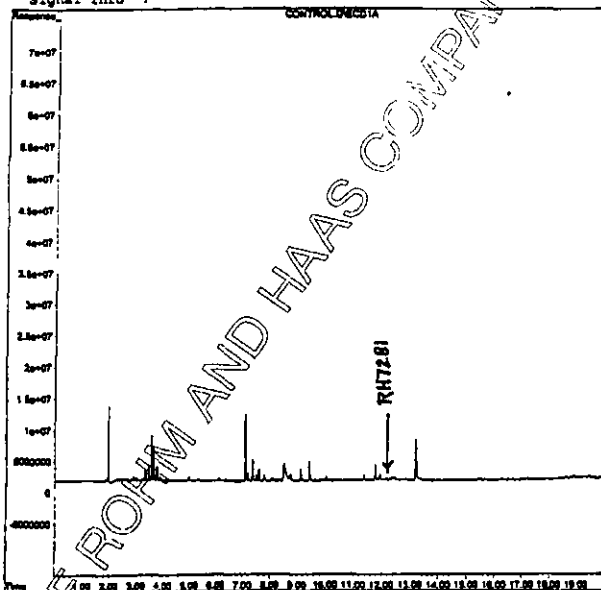
Data File : c:\hpcbem\2\data\040699\CONTROL.D Vial: 7
 Acq On : 4-3-99 7 40:51 PM Operator:
 Sample : Control 0031199 Inst : GC ECD
 Misc : Multiplr: 1.00
 Sample Amount: 0.00

IncrFile EVENTS E

Quant Time: Jul 26 15:39 1999 Quant Results File. ERNIE RES

Quant Method : C:\HPCHEM\2\METHODS\ERNIE.M (Chemstation Integrator)
 Title : 04-07-99
 Last Update : Mon Jul 26 15:27:16 1999
 Response via : Multiple Level Calibration
 DataAcq Meth : ERNIE M

Volume Inj :
 Signal Phase :
 Signal Info :



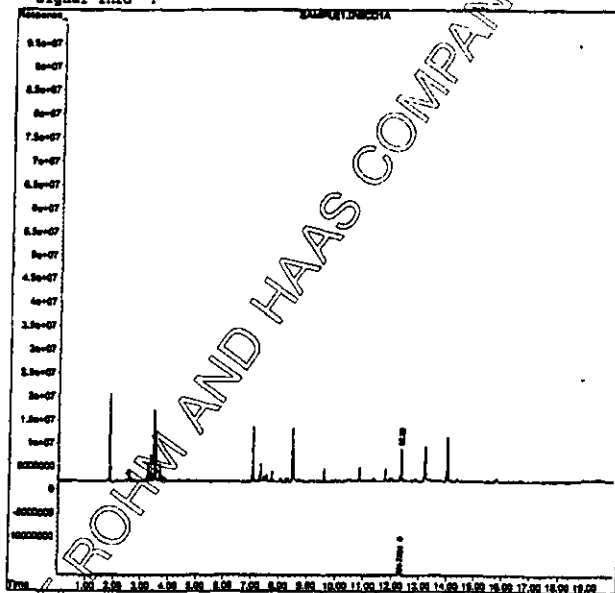
Compound	RT	Response	Conc Units
Target Compounds			
1) RH-7281 04-06-99	0.00	0	N D. ppb

Figure 9 Control Tomato RAC (GC/ECD)

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Quantitation Report

Data File : c:\hpcchem\2\data\032999\SAMPLE1.D Vial: 8
 Acq On : 3-29-99 9 40:38 PM Operator:
 Sample : 0.01 ppm fort. RH7281 Inst : GC ECD
 Misc : Multiplr: 1.00
 Sample Amount: 0.00
 InctFile : EVENTS E
 Quant Time: Jul 26 15:04 1999 Quant Results File: ERMIE.RES
 Quant Method : C:\HPCHEM\2\METHODS\ERMIE.M (Chemstation Integrator)
 Title : 04-07-99
 Last Update : Mon Jul 26 14:44:02 1999
 Response via : Multiple Level Calibration
 DataAcq Meth : ERMIE.M
 Volume Inj. :
 Signal Phase :
 Signal Info :



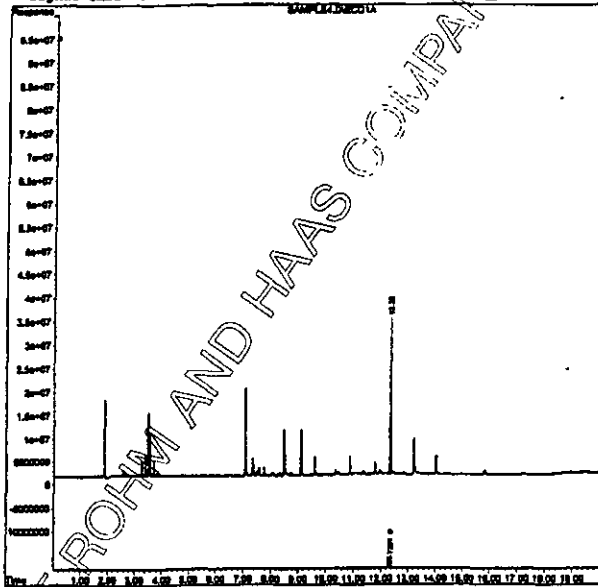
Compound	R.T	Response	Conc Units
Target Compounds			
RH-7281 03-29-99	12.33	6786483	9.950 ppb

Figure 10 Fortification of Tomato RAC at 0.010 ppm RH-7281 (GC/ECD)
 Recovery = 99.5 %

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Quantitation Report

Data File : c:\hpc\chem\2\data\032999\SAMPLE4.D Vial: 11
 Acq On : 3-29-99 11:02:43 PM Operator:
 Sample : 0.05 ppm fort. E032999 Inst : GC ECD
 Misc : Multiplr: 1.00
 Sample Amount: 0.05
 IntFile : EVENTS E
 Quant Time: Jul 26 15:06 1999 Quant Results File: ERNIE RES
 Quant Method : C:\HPCHEM\2\METHODS\ERNIE.M (Chemstation Integrator)
 Title : 04-07-99
 Last Update : Mon Jul 26 14:44:02 1999
 Response via : Multiple Level Calibration
 DataAcq Meth : ERNIE.M
 Volume Inj. :
 Signal Phase :
 Signal Info :



Compound	R.T.	Response	Conc Units
Target Compounds			
1) RH-7281 03-29-99	12.332	33546477	49.183 ppb

Figure 11 Fortification of Tomato RAC at 0.050 ppm RH-7281 (GC/ECD)
 Recovery = 98.4 %

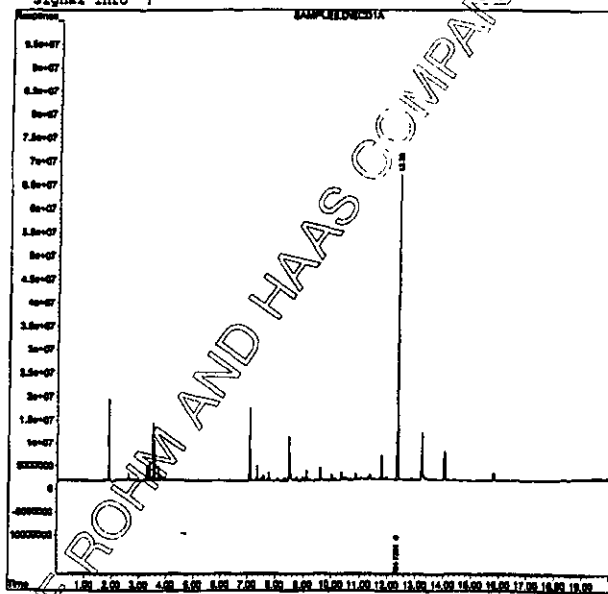
Quantitation Report

Data File : c:\hpchem\2\data\032999\SAMPLES.D Vial: 12
 Acq On : 3-29-99 11:30:04 PM Operator:
 Sample : 0 10 ppm fort RH72999 Inst : GC ECD
 Misc : Multiplr: 1.00
 IncFile : EVENTS.E Sample Amount: 0.00

Quant Time: Jul 26 15:06 1999 Quant Results File: ERNIE.RES

Quant Method : C:\HPCHM\2\METHODS\ERNIE.M (Chemstation Integrator)
 Title : 04-07-99
 Last Update : Mon Jul 26 14:44:02 1999
 Response via : Multiple Level Calibration
 DataAcq Meth : ERNIE.M

Volume Inj. :
 Signal Phase :
 Signal Info :



Compound	R.T.	Response	Conc Units
Target Compounds			
RH-7281 03-29-99	12.33	65229782	95.635 ppb

Figure 12 Fortification of Tomato RAC at 0 10 ppm RH-7281 (GC/ECD)
 Recovery = 95.6 %

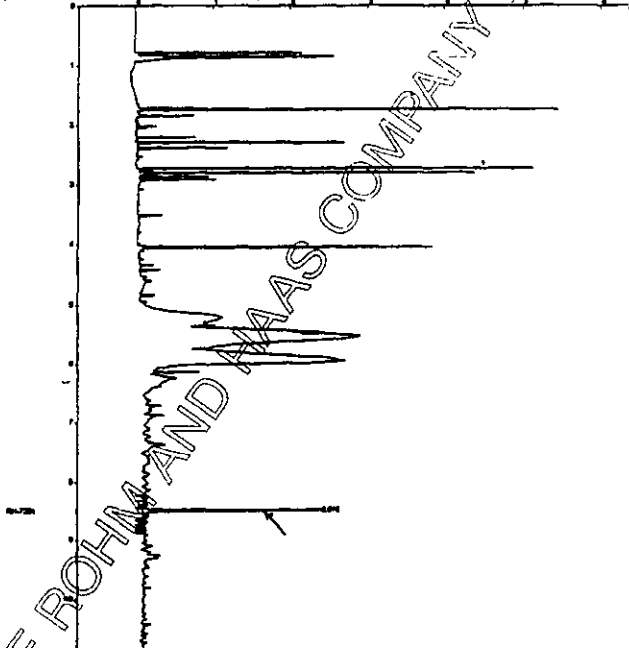
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Title :
 Run File : c:\star\module18\mb066 run
 Method File : C:\STAR\MODULE18\RH7291.MTH
 Sample ID : R909876-23A, 28mL
 Client ID : 98-0091-8
 Injection Date: 25-NOV-99 8:13 PM Calculation Date: 26-NOV-99 9:14 AM

Operator : NS Detector Type : ADCS (10 Volts)
 Workstation : Bus Address : 18
 Instrument : 3500 ECD Sample Rate : 10.00 Hz
 Channel : B - B Run Time : 14.002 min

***** Star Chromatography Workstation ***** Version 4.5 *****

Chart Speed = 1.72 cm/min Attenuation = 3000 Zero Offset = 100
 Start Time = 0.000 min End Time = 11.000 min Min / Max = 1.00



Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)
1	RH-7291	100.0000	8.510	-0.240	343492
Totals:		100.0000		-0.240	343492

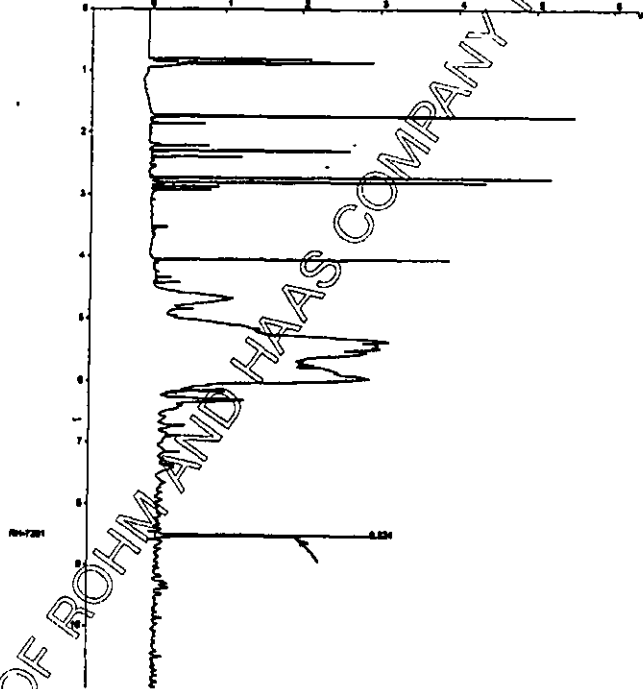
Figure 13 Treated Tomato RAC (RAR 98-0091; Sample #2) (GC/ECD)
 Found: 0.0875 ppm

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TR 34-99-111

39

Title :
Run File : C:\STAR\WORKST18\RB067.MW
Method File : C:\STAR\WORKST18\RH7281.MW
Sample ID : 890876-24A.28ML
Client XP : 98-0091-3
Injection Date: 25-NOV-99 8:33 PM Calculation Date: 26-NOV-99 1:40 PM
Operator : KB Detector Type: ADCS (10 Volts)
Workstation : Bus Address : 18
Instrument : 3500 ECD Sample Rate : 10.00 Hz
Channel : S - S Run Time : 14.003 min
***** Star Chromatography Workstation ***** Version 4.5 *****
Chart Speed = 1.00 cm/min Attenuation = 3000 Zero Offset = 100
Start Time = 0.000 min End Time = 11.000 min Min. Pick = 1.00



Peak No	Peak Name	Result (%)	Ret. Time (min)	Time Offset (min)	Area (counts)
1	RH-7281	100.0000	8.524	-0.076	423309
Totals:		100.0000		-0.076	423309

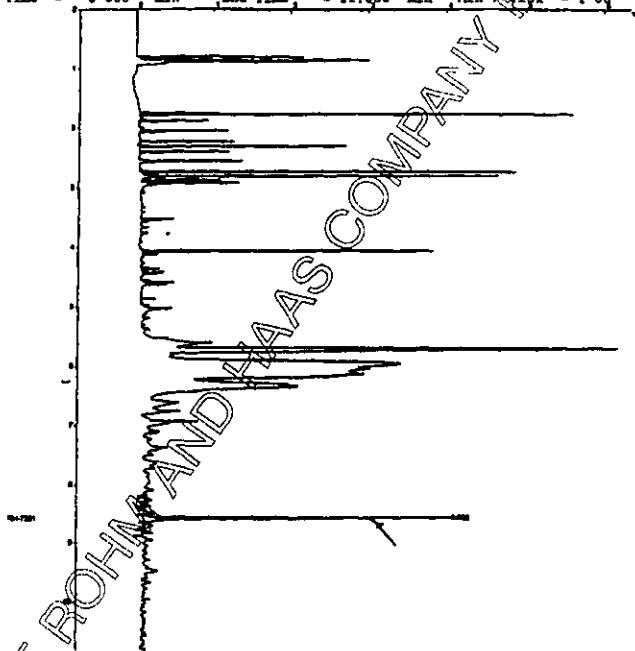
Figure 14 Treated Tomato RAC (RAR 98-0091; Sample #3) (GC/ECD)
Found: 0.108 ppm

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Title :
Run File : c:\star\module10\hb042.run
Method File : C:\STAR\MODULE10\RG7281.MTH
Sample ID : E9C9676-21A.28mL
Client ID : 98-0090-3
Injection Date: 25-NOV-99 6:54 PM Calculation Date: 26-NOV-99 9:13 AM
Operator : NB Detector Type: ADCB (10 Volts)
Workstation: Bus Address : 18
Instrument : 3500 ECD Sample Rate : 10.00 Hz
Channel : B = B Run Time : 14.002 min

***** Star Chromatography Workstation ***** Version 4.5 *****
Chart Speed = 1.72 cm/min Attenuation = 3000 Zero Offset = 10%
Start Time = 0.000 min End Time = 11.000 min Min Pick = 1.00



Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)
1	RH-7281	100.0000	8.585	-0.165	754803
Totals:		100.0000		-0.165	754803

Figure 15 Treated Tomato RAC (RAR 98-0090; Sample #3) (GC/ECD)
Found: 0.193 ppm

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Quantitation Report

Data File : c:\hpcchem\2\data\062299\5-581.D
Acq On : 6-22-99 7:39:27 PM
Sample : control, tomato puree
Misc :

Vial: 7
Operator: k
Inst : GC-MS
Multiplr: 1000
Sample Amount: 0.00

IntFile : EVENTS.E

Quant Time: Jun 24 8:08 1999 Quant Results File: RH7281.R25

Quant Method C:\HPCHEM\2\METHODS\RH7281.M (Chemstation Integrator)
Title : 04-07-99
Last Update : Thu Jun 24 07:50 50 1999
Response via Multiple Level Calibration
DataAcq Meth RH7281.M

Volume Inj. :
Signal Phase :
Signal Info :

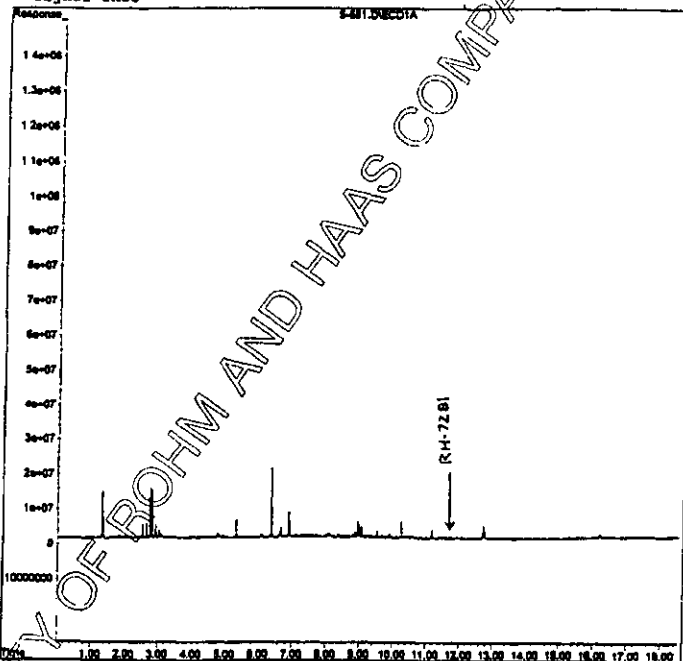


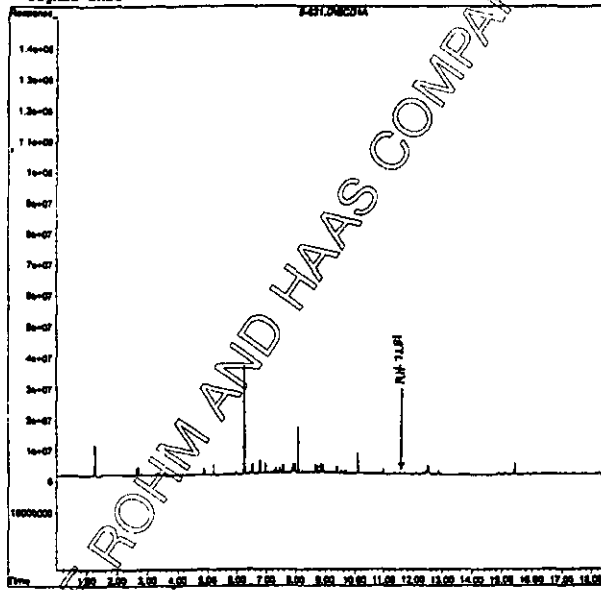
Figure 16 Control Tomato Puree (GC/ECD)

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Quantitation Report

Data File : c:\hpcchem\2\data\070499\5-631.D Vial: 7
Acq On : 7-4-99 6:53:30 PM Operator:
Sample : control, tomato puree Inst : GC ECD
Misc : Misc Multiplr: 1.00
Sample Amount 0.00
In:File : EVENTS.E
Quant Time Jul 7 8:42 1999 Quant Results File RH7281.RES
Quant Method : C:\HPCHEM\2\METHODS\RH7281.M (Chemstation Integrator)
Title 04-20-99
Last Update Wed Jul 07 08 23:12 1999
Response via Multiple Level Calibration
DataAcq Meth : RH7281.M

Volume Inj
Signal Phase
Signal Info



Compound	R.T.	Response	Conc Units
Target Compounds			
RH-7281 07-04-99 (063099)	0.00	0	N D. ppb

Figure 17 Control Tomato Puree (GC/ECD)

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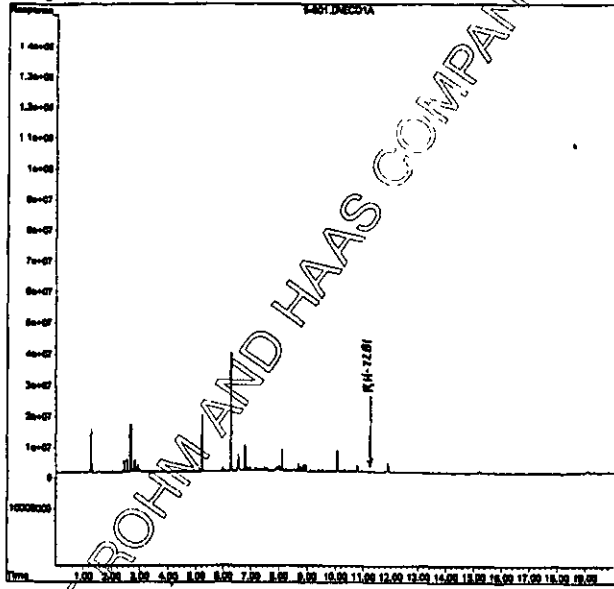
Quantitation Report

Data File c:\hpcchem\2\data\062599\5-601.D Vial: 7
Acq On 6-25-99 6:08:38 PM Operator: k.k
Sample : control, tomato puree Inst : GC ECD
Misc : Multiplr: 1.00
IntFile : EVENTS.E Sample Amount: 0.00

Quant Time: Jun 28 8 41 1999 Quant Results File: RH7281.RES

Quant Method : C:\HPCHEM\2\METHODS\RH7281.M (Chemstation Integrator)
Title : 04-07-99
Last Update : Mon Jun 28 08:16:44 1999
Response via : Multiple Level Calibration
DataAcq Meth : RH7281.M

Volume Inj :
Signal Phase :
Signal Info :



Compound	R.T.	Response	Conc Units
Target Compounds			
1) RH-7281 062599(62399)	0.00	0	N.D. ppb

Figure 18 Control Tomato Puree (GC/ECD)

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Quantitation Report

Data File : c:\hpcchem\2\data\062299\5-582.D
Acq On : 6-22-99 8:03:20 PM
Sample : 0.01ppm spike, tomato puree
Misc :
IntFile : EVENTS E
Vial: 8
Operator: k k
Inst : GC ECD
Multiplr: 1.00
Sample Amount: 0.50
Quant Time: Jun 24 8.09 1999 Quant Results File: RH7281.RES
Quant Method : C:\HPCHEM\2\METHODS\RH7281.M (Chemstation Integrator)
Title : 04-07-99
Last Update : Thu Jun 24 07:50:50 1999
Response via : Multiple Level Calibration
DataAcq Mech : RH7281 M
Volume Inj. :
Signal Phase :
Signal Info :

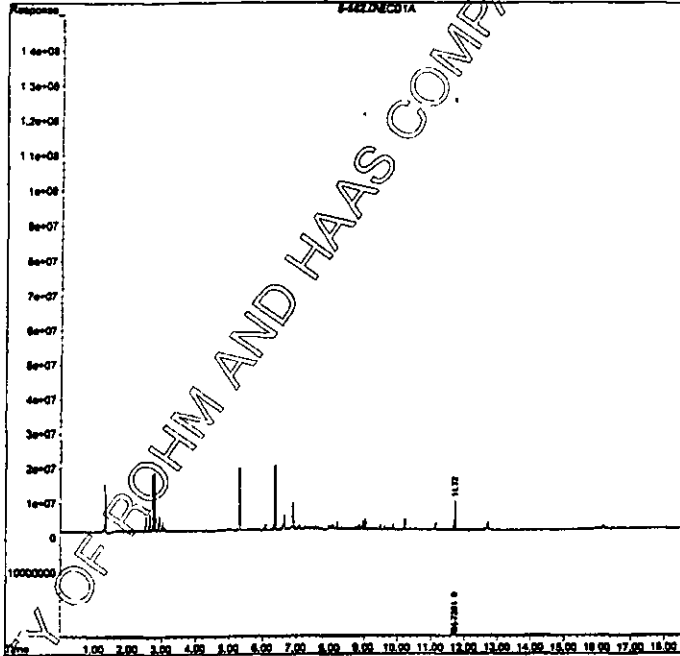


Figure 19 Fortification of Tomato Puree at 0.010 ppm RH-7281 (GC/ECD)
Peak Area = 8,737,167 Recovery = 88.1 %

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Quantitation Report

Data File : c:\hpchem\2\data\062299\5-585.D
Acq On : 6-22-99 9:14:55 PM
Sample : 0.05ppm spike, tomato puree
Misc :

Vial: 11
Operator: k.k
Inst : GC ECD
Multiplr: 1.00
Sample Amount: 1.00

IntFile : EVENTS.E

Quant Time: Jun 24 8 11 1999 Quant Results File: RH7281.C25

Quant Method : C:\HPCHEM\2\METHODS\RH7281.M (ChemStation Integrator)
Title : 04-07-99
Last Update : Thu Jun 24 07:50:50 1999
Response via : Multiple Level Calibration
DataAcq Meth : RH7281.M

Volume Inj :
Signal Phase :
Signal Info :

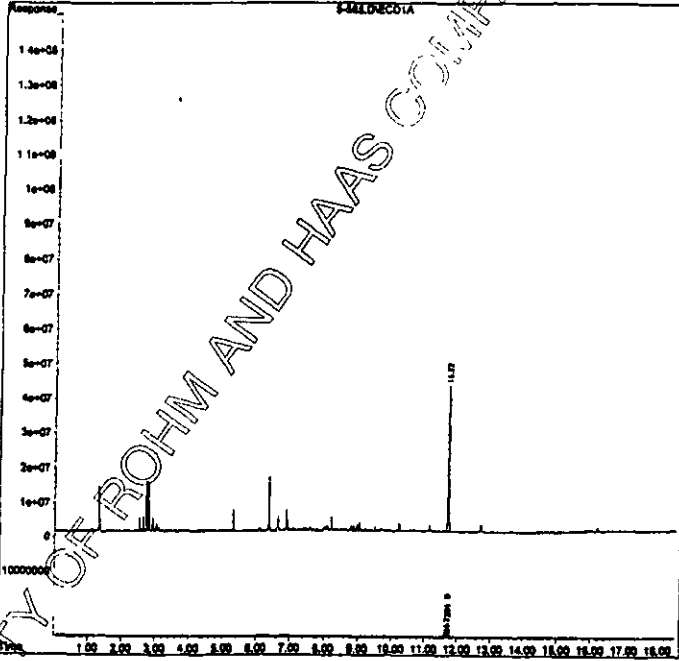


Figure 20 Fortification of Tomato Puree at 0.050 ppm RH-7281 (GC/ECD)
Peak Area = 42,125,405 Recovery = 85.0 %

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Quantitation Report

Data File . c:\hpcchem\2\data\062299\5-586.D
Acq On : 6-22-99 9:38:44 PM
Sample : 0 10ppm spikes, tomato puree
Misc :

Vial: 12
Operator k.k
Inst GC-800
Multipl: 1.00
Sample Amount: 0.05

IntFile : EVENTS.E

Quant Time: Jun 24 8:12 1999 Quant Results File: RH7281.RES

Quant Method . C:\HPCHEM\2\METHODS\RH7281.M (Chemstation Integrator)
Title : 04-07-99
Last Update : Thu Jun 24 07:50:50 1999
Response via : Multiple Level Calibration
DataAcq Meth : RH7281.M

Volume Inj. :
Signal Phase :
Signal Info :

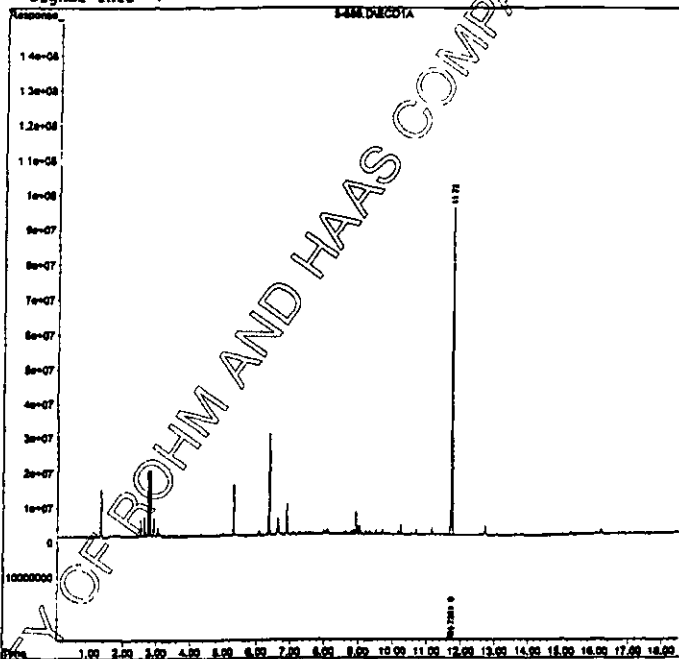


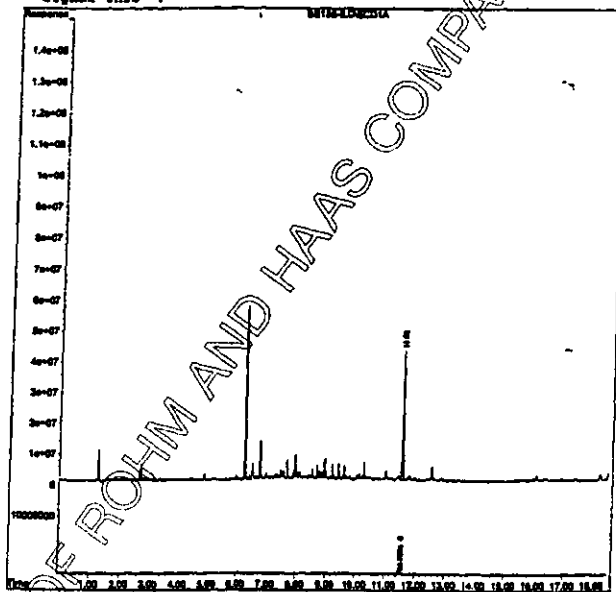
Figure 21 Fortification of Tomato Puree at 0 10 ppm RH-7281 (GC/ECD)
Peak Area = 94,372,064 Recovery = 95.2 %

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Quantitation Report

Data File : c:\hpchem\1\data\070999\8815e-s D Vial: 13
Acq On : 7-9-99 9:09:30 PM Operator: KK
Sample : RAR98-0101 Inst : GC ECD
Misc : Multiplr: 1.00
IntFile : EVENTS.E Sample Amount: 0.08
Quant Time Jul 12 8:49 1999 Quant Results File: RM7281 RES
Quant Method : C:\HPCHEM\1\METHODS\RM7281.M (Chemstation Integrator)
Title : 04-20-99
Last Update : Mon Jul 12 08:26:07 1999
Response via : Multiple Level Calibration
DataAcq Meth : RM7281.M
Volume Inj :
Signal Phase :
Signal Info :



Compound	R.T.	Response	Conc Units
Target Compounds			
RM-7281 07-09-99 (070899)	11.55	760381868	44.162 ppb

Figure 22 Treated Tomato Puree (RAR 98-0101, Sample #5) (GC/ECD)
Found: 0.0442 ppm

Quantitation Report

Data File c:\hpcchem\2\data\073099\5-681.D
Acq On : 7-30-99 6 11:50 PM
Sample : control, tomato paste
Misc :

Vial: 7
Operator: kk
Inst : GC ECD
Multiplier: 1.00
Sample Amount: 0.200

IntFile EVENTS.E

Quant Time: Aug 2 7:47 1999 Quant Results File RH7281.RSS

Quant Method : C:\HPCHEM\2\METHODS\RH7281.M (Chemstation Integrator)
Title 04-07-99
Last Update : Thu Jul 29 08:35:42 1999
Response via : Multiple Level Calibration
DataAcq Meth : RH7281.M

Volume Inj
Signal Phase
Signal Info

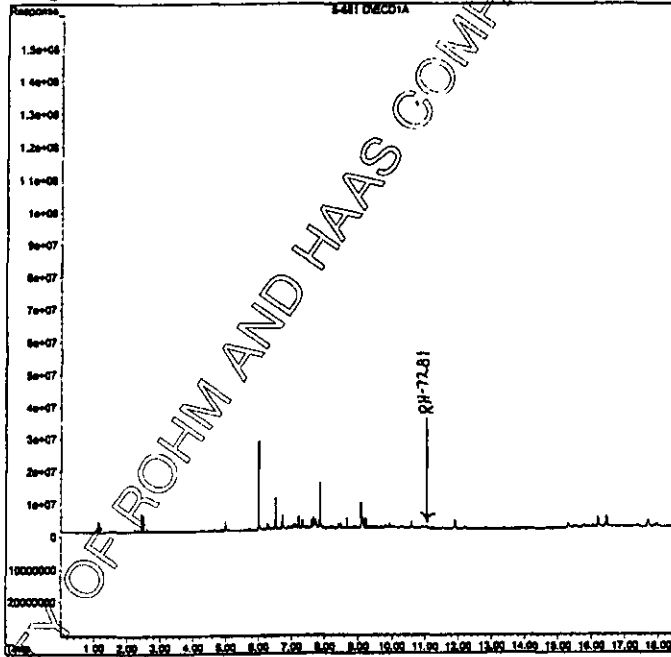


Figure 23 Control Tomato Paste (GC/ECD)
Peak Area = 0

Quantitation Report

Data File : c:\hpchem\2\data\080499\5-701.D
 Acq On : 8-4-99 5:20:40 PM
 Sample : control, tomato paste
 Misc :

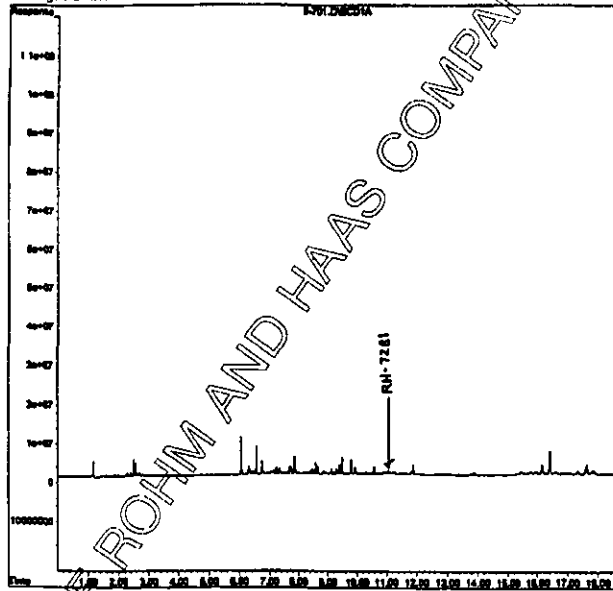
Vial: 7
 Operator: kh
 Inst: GC ECD
 Multiplr: 1.00
 Sample Amount: 0.00

IntFile : EVENTS.E

Quant Time: Aug 5 8:52 1999 Quant Results File: RH7281.RES

Quant Method : C:\HPCHEM\2\METHODS\RH7281.M (Chemstation Integrator)
 Title : 04-07-99
 Last Update : Thu Aug 05 08:20:15 1999
 Response via : Multiple Level Calibration
 DataAcq Meth : RH7281.M

Volume Inj. :
 Signal Phase :
 Signal Info :



Compound	R.T.	Response	Conc Units
1) RH-7281 (080499)	0.00	0	N.D. ppb

Figure 24 Control Tomato Paste (GC/ECD)

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Quantitation Report

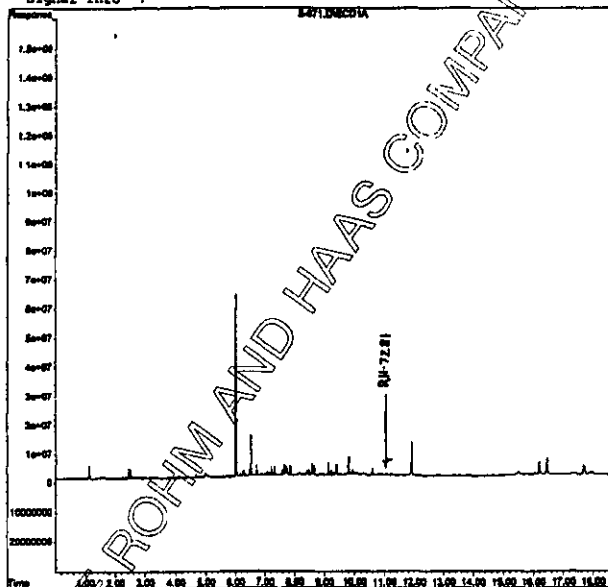
Data File : c:\hpchem\2\data\072899\5-671.D Vial: 7
 Acq On : 7-28-99 7:29:26 PM Operator: kk
 Sample : control. tomato paste Inst : GC ECD
 Misc : Multiplic: 1.00
 Sample Amount: 0.00

IntFile : EVENTS X

Quant Time Jul 29 8:47 1999 Quant Results File: RH7281 RES

Quant Method : C:\HPCHEM\2\METHODS\RH7281.M (Chemstation Integrator)
 Title : 04-07-99
 Last Update : Thu Jul 29 08:35:42 1999
 Response via : Multiple Level Calibration
 DataAcq Meth : RH7281.M

Volume Inj :
 Signal Phase :
 Signal Info :



Compound	R.T.	Response	Conc Units
Target Compounds			
RH-7281 (072899)	0.00	0	N.D. ppb

Figure 25 Control Tomato Paste (GC/ECD)

Quantitation Report

Data File : c:\hpcchem\2\data\073099\5-682.D
Acq On : 7-30-99 6:37:49 PM
Sample : 0.01ppm spike, tomato paste
Misc :

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

IntFile : EVENTS.E

Quant Time: Aug 2 7:48 1999 Quant Results File RH7281.EEG

Quant Method : C:\HPCHEM\2\METHODS\RH7281.M (Chemstation Integrator)
Title : 04-07-99
Last Update : Thu Jul 29 08:35:42 1999
Response via : Multiple Level Calibration
DataAcq Meth : RH7281.M

Volume Inj :
Signal Phase
Signal Info :

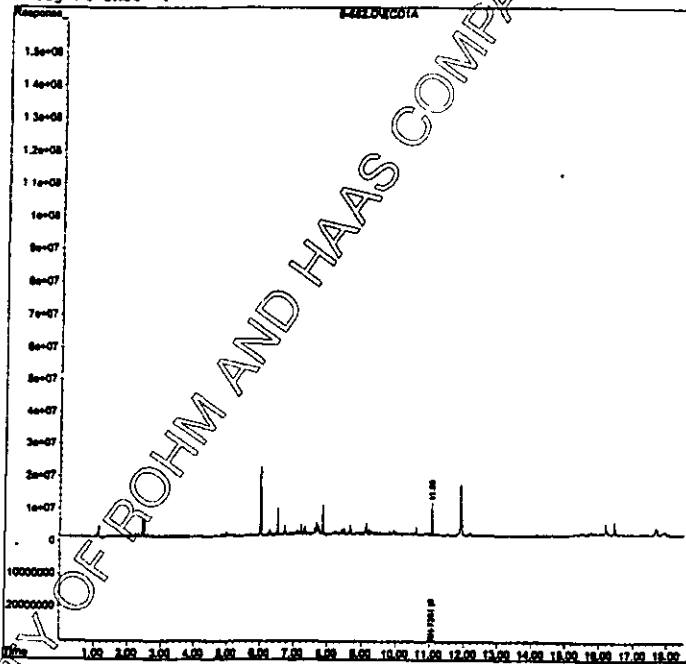


Figure 26

Fortification of Tomato Paste at 0.010 ppm RH-7281 (GC/ECD)
Peak Area = 162,587,067 Recovery = 88.1 %

Quantitation Report

Data File : c:\hpcchem\2\data\073099\5-685.D
Acq On : 7-30-99 7:55:41 PM
Sample : 0.05ppm spike, tomato paste
Misc :

Vial 11
Operator kk
Inst GC/ECD
Multiplr 1.00
Sample Amount 5.00

IntFile : EVENTS.E

Quant Time Aug 2 7:50 1999 Quant Results File RH7281.RSA

Quant Method : C:\HPCHEM\2\METHODS\RH7281.M (Chemstation Integrator)
Title : 04-07-99
Last Update : Thu Jul 29 08:35 42 1999
Response via : Multiple Level Calibration
DataAcq Meth : RH7281.M

Volume Inj :
Signal Phase :
Signal Info :

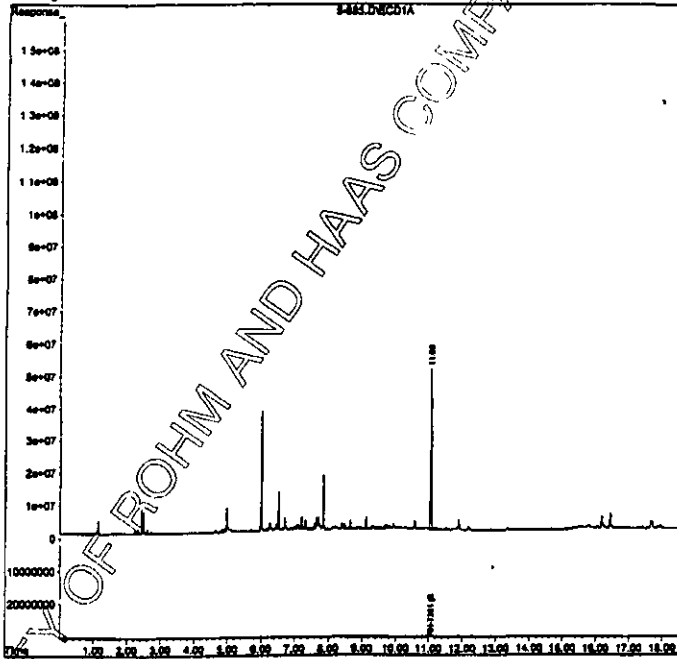


Figure 27 Fortification of Tomato Paste at 0.050 ppm RH-7281 (GC/ECD)
Peak Area = 832,435,186 Recovery = 90.2 %

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Quantitation Report

Data File : c:\hpcchem\2\data\073099\5-686.D
Acq On : 7-30-99 8:21:39 PM
Sample : 0.10ppm spike, tomato paste
Misc :
Vial: 12
Operator: kh
Inst : GC/ECD
Multipl: 2.00
Sample Amount: 0.20
IntrFile : EVENTS.E
Quant Time: Aug 2 7:51 1999 Quant Results File: RH7281.RES
Quant Method : C:\HPCHEM\2\METHODS\RH7281.M (Chemstation Integrator)
Title : 04-07-99
Last Update : Thu Jul 29 08 35:42 1999
Response via : Multiple Level Calibration
DataAcq Meth : RH7281.M
Volume Inj. :
Signal Phase :
Signal Info :

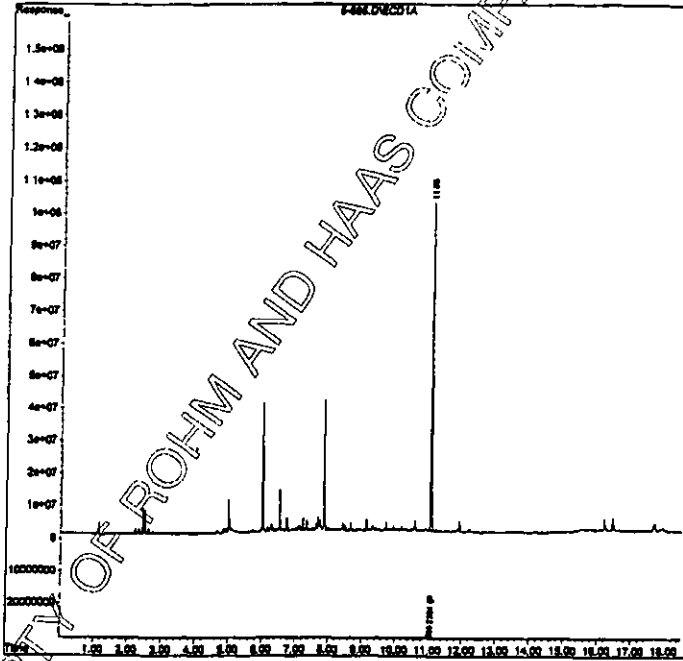


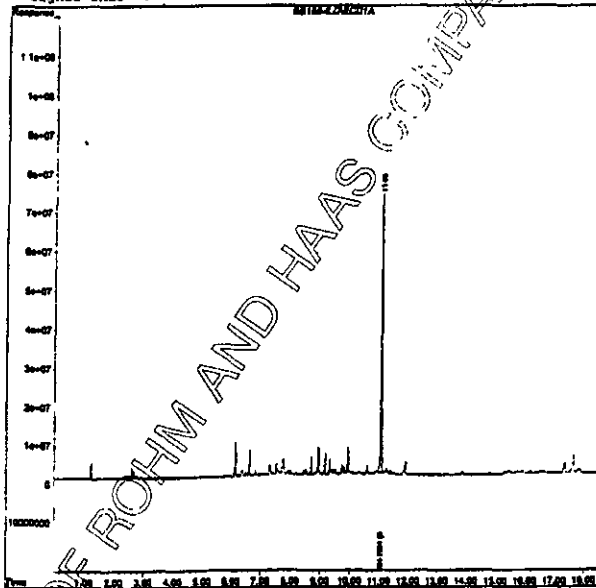
Figure 28 Fortification of Tomato Paste at 0.10 ppm RH-7281 (GC/ECD)
Peak Area = 1,685,556,886 Recovery = 91.3 %

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Quantitation Report

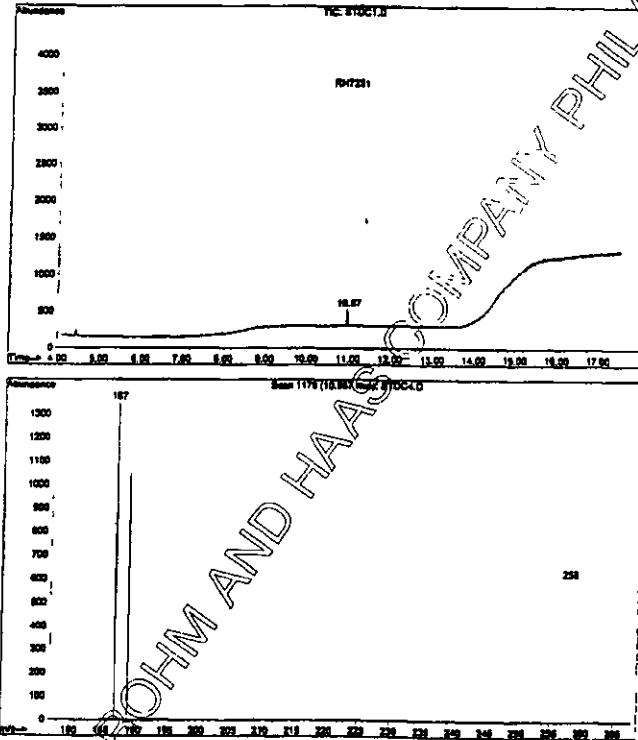
Data File : c:\hpcchem\2\data\081099\88159-6.D Vial: 12
Acq On : 8-10-99 8:22:43 PM Operator: kk
Sample : Paste Residua sample 98-0101 Inst : GC ECD
Misc : Multiplr: 1.00
Sample Amount: 0.00
IntFile : EVENTS E
Quant Time Aug 11 8:35 1999 Quant Results File: RH7281.RES
Quant Method : C:\HPCHEM\2\METHODS\RH7281.M (Chemstation Interpreter)
Title : 04-07-99
Last Update : Wed Aug 11 08:14:17 1999
Response via : Multiple Level Calibration
DataAcq Mech : RH7281 M
Volume Inj. :
Signal Phase :
Signal Info :



Compound	R T.	Response
Target Compounds		
RH-7281 (081099)	11.06	1340562000

Figure 29 Treated Tomato Paste (RAR 98-0101; Sample #5) (GC/ECD)
Found. 0.0918 ppm

File : C:\RPHRM\1\DATA\050699\STD1.D
 Operator :
 Acquired : 9 May 99 2:32 using AcqMethod 7281SIM
 Instrument : 5973
 Sample Name : STD 0.01PPM
 Misc Info :
 Vial Number : 17



System Monitoring Compounds	R T	Q Ion	Response	Conc	Units	Dev(Min)	Ovalue
Target Compounds							
1> RH-7281, 05-04-99	10.97	258	909	6.73	ppb		100

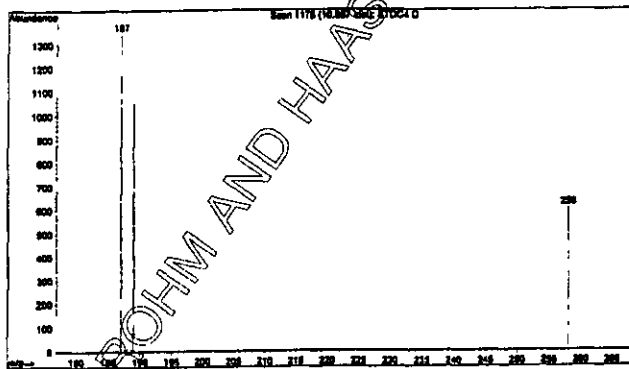
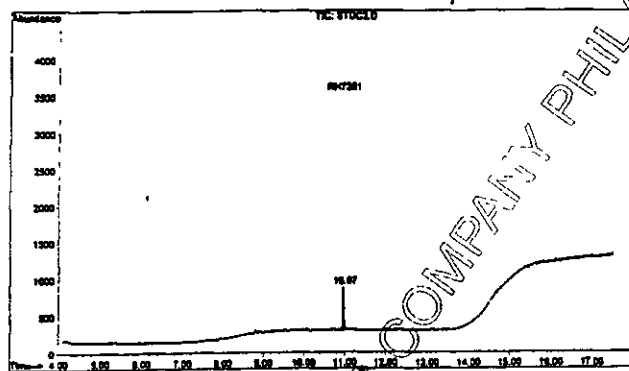
Figure 30 0.010 ppm RH-7281 Standard (GC/MSD)

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File : C:\EPCHER\1\DATA\050899\STD01.D
Operator :
Acquired : 9 May 99 2:53 using AcqMethod 7281SIM
Instrument : 5973
Sample Name STD 0 02PPM
Misc Info :
Vial Number: 18



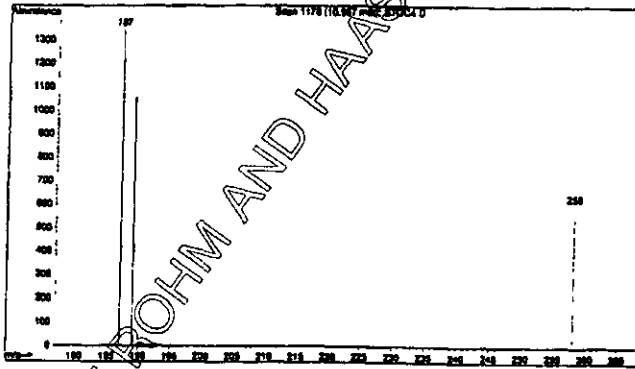
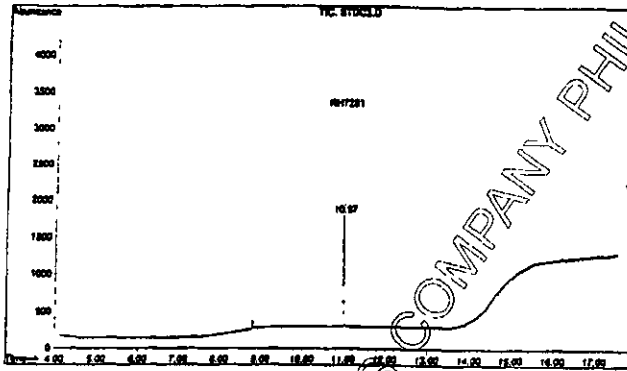
System Monitoring Compounds	R.T.	QIon	Response	Conc	Units	Dev (Min)
Target Compounds						Qvalue
1) RH-7281, 05-04-99	10.97	258	2082	19.99	ppb	100

Figure 31 0.020 ppm RH-7281 Standard (GC/MSD)

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File : C:\EPCHEN\1\DATA\050899\STDC3.D
Operator :
Acquired : 9 May 99 3:15 using AcqMethod 7281SIM
Instrument : 5973
Sample Name STD 0.05PPM
Misc Info :
Vial Number: 19



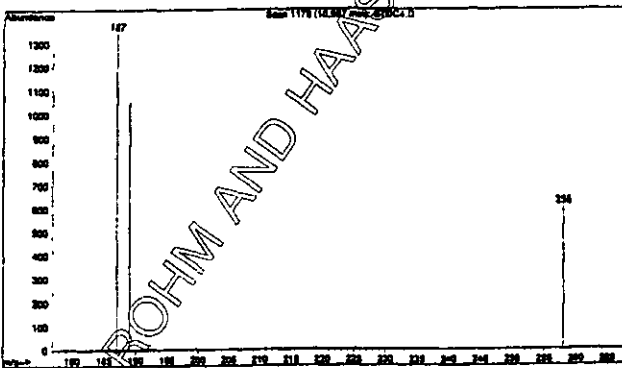
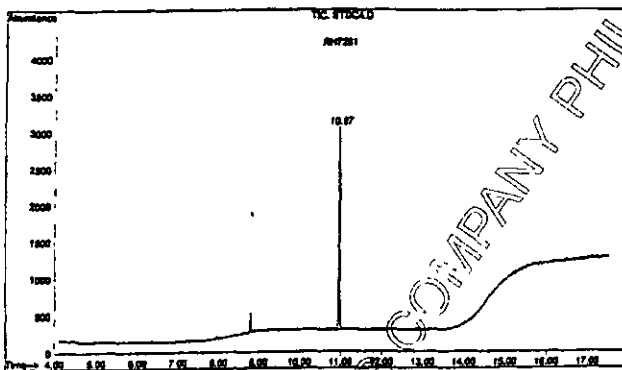
System Monitoring Compounds	R.T	QIon	Response	Conc	Units	Dev (Min)
Target Compounds						Qvalue
1) RH-7281, 05-04-99	10.97	258	5477	52.56	ppb	100

Figure 32 0.050 ppm RH-7281 Standard (GC/MSD)

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File : C:\RPCHEM\1\DATA\050899\STDC4.D
Operator :
Acquired : 9 May 99 3:36 using AcqMethod 726161M
Instrument : 5973
Sample Name: STD 0.10PPM
Misc Info :
Vial Number: 20



----- R.T. Qion Response Conc Units Dev(Min)

System Monitoring Compounds

Target Compounds	R.T.	Qion	Response	Conc	Units	Dev(Min)
1) RH-7281, 05-04-99	10.97	258	9793	93.98	ppb	100

Figure 33 0.10 ppm RH-7281 Standard (GC/MSD)

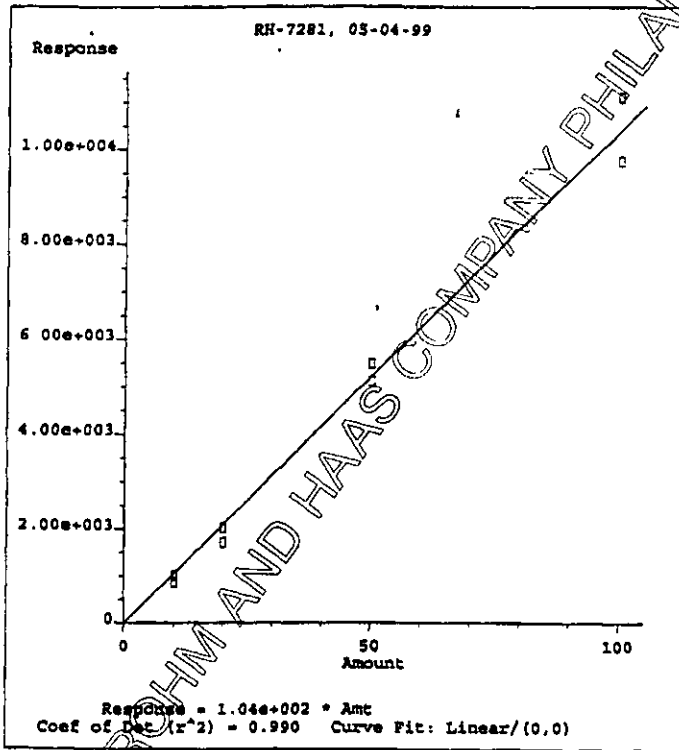
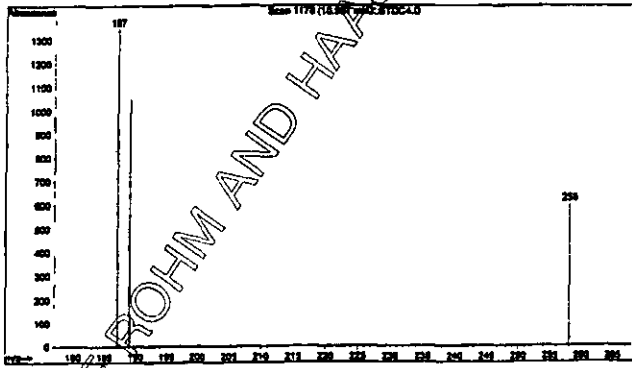
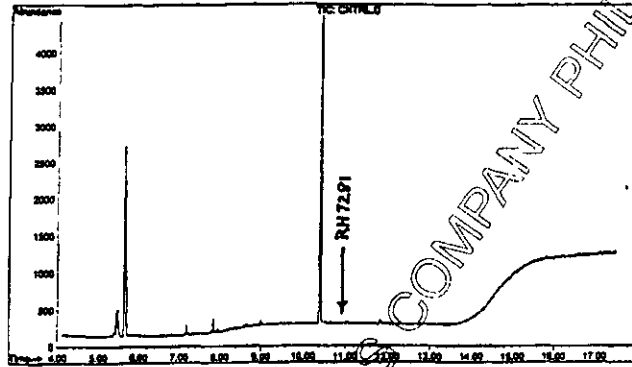


Figure 34 Calibration Curve for RH-7281 (GC/MSD)

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File : C:\HPCHEM\1\DATA\050499\CTRL.D
 Operator :
 Acquired : 9 May 99 4:19 using AcqMethod 728181M
 Instrument : 8973
 Sample Name : TOMATO CONTROL K050499
 Misc Info :
 Vial Number: 21



System Monitoring Compounds	R T	Q Ion	Response	Conc	Units	Dev (Min)	Qvalue
Target Compounds							
1) RH-7281, 05-04-99	0.00	358					W.D

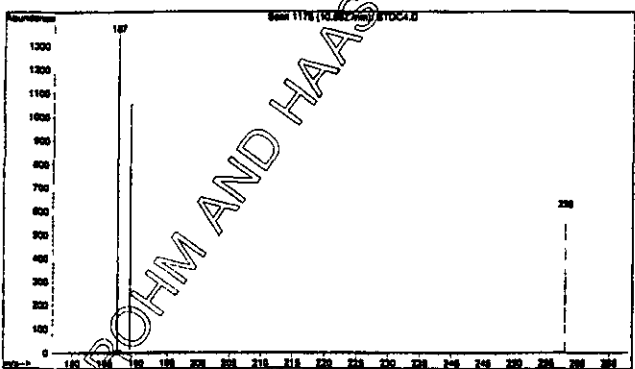
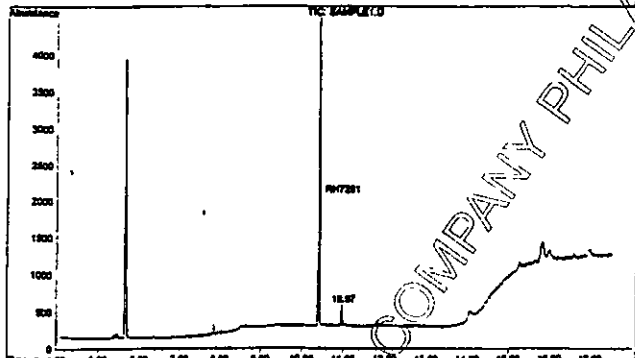
Figure 35 Control Tomato RAC (GC/MSD)

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File : C:\RPCHEM\1\DATA\050099\SAMPLE1.D
Operator :
Acquired : 9 May 99 4:41 using AcqMethod 7201SIM
Instrument : 8973
Sample Name 0.01PPM FORT. E050499
Misc Info :
Vial Number: 22

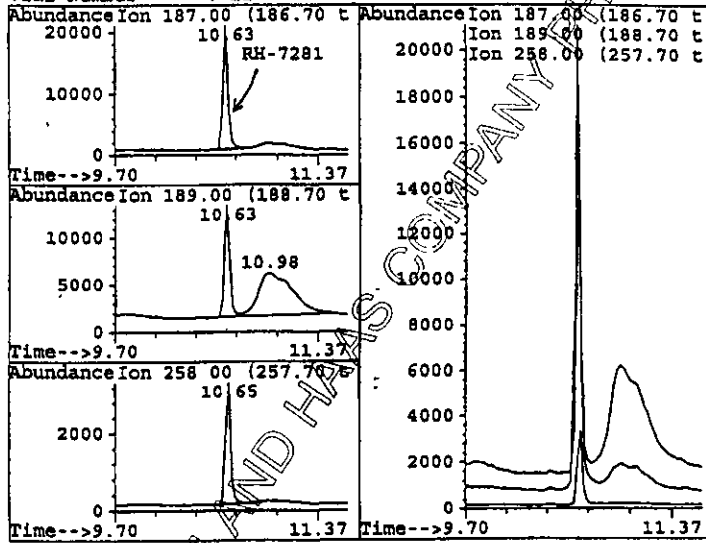


System Monitoring Compounds	R.T	QIon	Response	Conc	Units	Dev(Min)	Qvalue
1) RH-7281, 05-04-99	10.97	258	1028	9.87	ppb	100	

Figure 36 Fortification of Tomato RAC at 0.010 ppm RH-7281 (GC/ECD)
Recovery = 98.7 %

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Sample name: E9-09-676-35A, 10.0mL FV
 Misc Info: 98-0095-2
 File name : C:\HPCHEM\1\DATA\NB102699\1101009.D
 Date Acquired : 26 Oct 99 3:25 pm
 Operator : NB
 Instrument : 5972 - In
 Method File : RH7281S
 Vial Number : 11



Target Ion	m/z	RT (min.)	Area	Height	Type	Width
	187	10.63	563530	17805	BV	0.053

Q Ion	m/z = 189	RT (min.)	Area	Height	Type	Width
		10.63	354238	10966	BV	0.054
		10.98	851842	4413	VBA	0.265

Q2 Ion	m/z = 258	RT (min.)	Area	Height	Type	Width
		10.65	95845	2922	BB	0.093

Figure 37 Treated Tomato RAC (RAR 98-0095, Sample #2) (GC/MSD)
 Found. 0.180 ppm

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

File : C:\HPCHEM\1\DATA\072899\5-601.D
Operator :
Acquired : 28 Jul 99 11:03 using AcqMethod 7281SIM
Instrument : S973
Sample Name : control, tomato puree
Misc Info :
Vial Number: 6

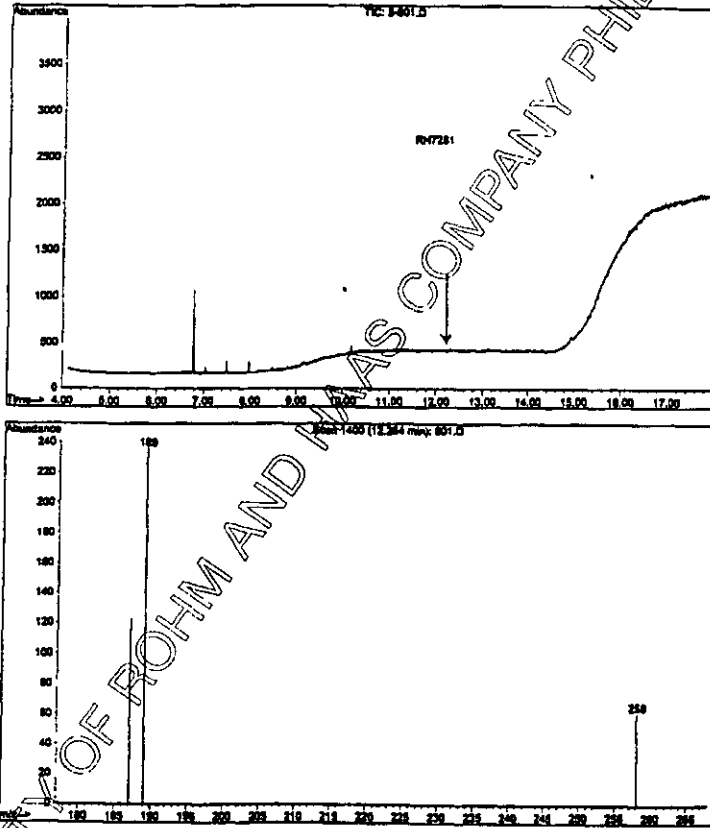


Figure 38 Control Tomato Puree (GC/MSD)
Peak Area = 0

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

File : C:\HPCHEM\1\DATA\072899\5-602.D
Operator :
Acquired : 28 Jul 99 12.22 using AcqMethod 7281SIM
Instrument : 5971
Sample Name: 0.01ppm spike, tomato puree
Misc Info :
Vial Number: 7

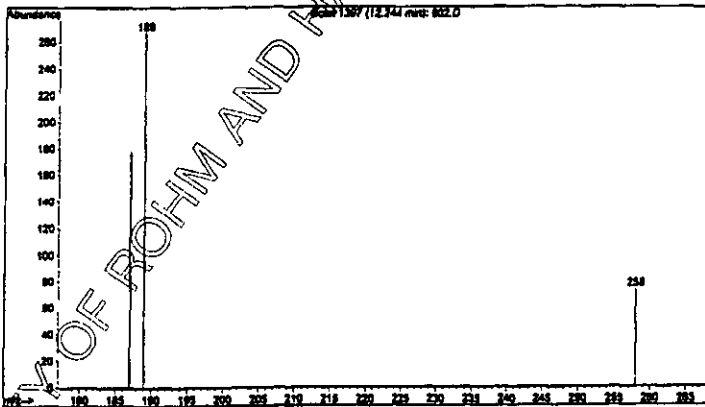
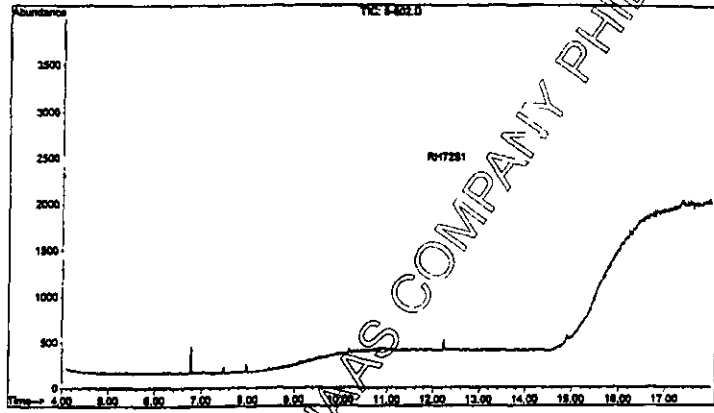


Figure 39 Fortification of Tomato Puree at 0.010 ppm RH-7281 (GC/MSD)
Peak Area = 488 Recovery = 98.8 %

PROPERTY OF ROHM AND HAAS COMPANY PHILADELPHIA

TR 34-99-111

File : C:\HPCHEM\1\DATA\072999\5-671.D
Operator :
Acquired : 29 Jul 99 12:49 using AcqMethod 7281SIM
Instrument : 5973
Sample Name : control, tomato paste
Misc Info :
Vial Number : 6

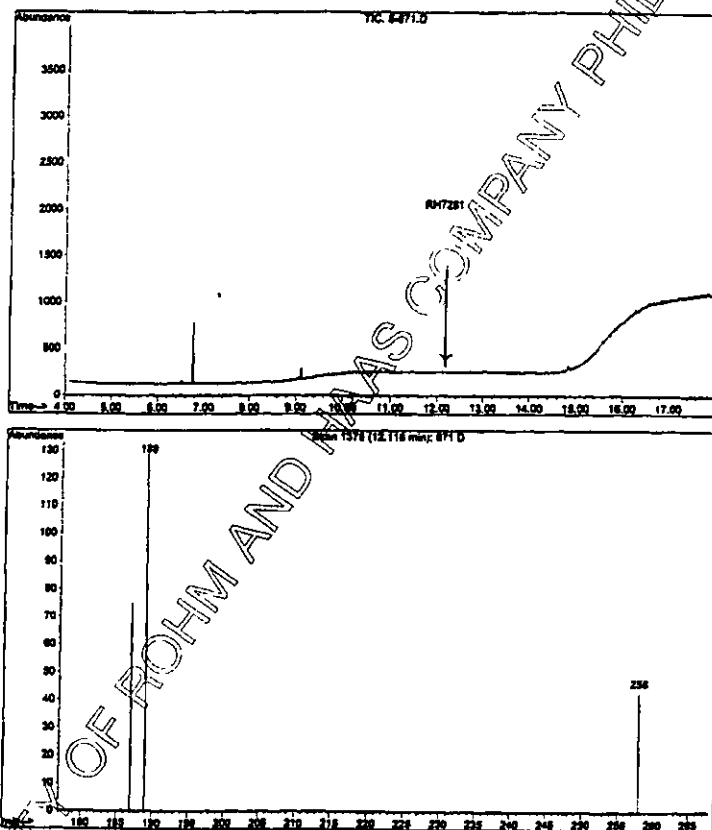


Figure 40 Control Tomato Paste (GC/MSD)
Peak Area = 0

TR 34-99-111

File : C:\HPCHEM\1\DATA\072999\5-672.D
Operator :
Acquired : 29 Jul 99 13:15 using AcqMethod 7281SIM
Instrument : 5973
Sample Name: 0.01ppm spike, tomato paste
Misc Info :
Vial Number: 7

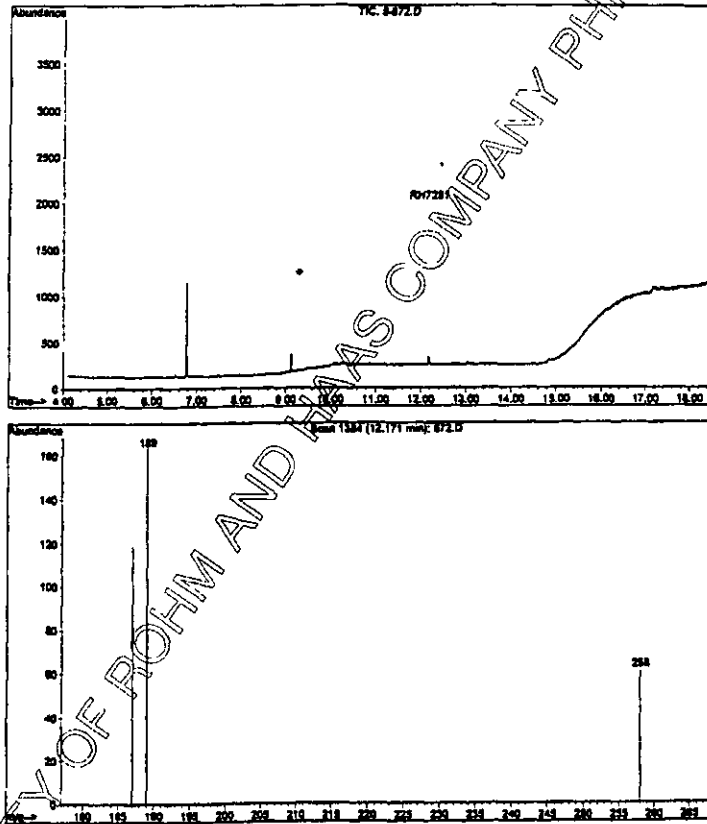


Figure 41 Fortification of Tomato Paste at 0.010 ppm RH-7281 (GC/MSD)
Peak Area = 357 Recovery = 97.2 %

APPENDIX I

Radiovalidation

The radiovalidation experiment validated the ability of the analytical method to measure RH-7281 in field aged residues by comparing the amount of RH-7281 detected by the analytical method to the amount of RH-7281 measured in a tomato metabolism study

Briefly, the control and treated samples were extracted and cleaned up using the residue analytical method. Simultaneously, two levels (0.050 and 0.10 ppm) of RH-7281 fortification of the control sample were processed by the analytical method as a measure of method recovery. The levels of radioactivity were measured in subsamples of the initial extracts and filter cakes (bound radioactivity) in order to determine the material balance and extraction efficiency of the method. The concentration of RH-7281 in the final extracts was quantitated by GC/ECD (primary quantitation method). The results are presented in Tables 1 and 2 below.

The residue analytical method extracted 96.6% of the radioactivity in the tomato sample (Table 1) and the average recovery of the 2 fortifications was 93.0% (Table 2). These data demonstrate that the residues of RH-7281 were accurately measured by the analytical method.

The level of RH-7281 found in the metabolism study (Table 2) was 0.139 ppm (normalized) or 0.116 ppm (uncorrected). Since there was significant variability (53.2% relative standard deviation) in the total radioactive residues (TRR) of the tomato sample (Table 1), the subsample analyzed in the metabolism study could have a residue of 0.139 \pm 0.073 (i.e. \pm 53.2%) or 0.116 \pm 0.062. The average residue level of RH-7281 found by the analytical method was 0.099 \pm 0.001 ppm which is within the variability of the metabolism value. Based on these results, the residue analytical method was successfully radiovalidated.

Table 1 Efficiency of Extraction and Material Balance of Radioactivity from Tomato RAC

Combustion Results^a	
Total Radioactive Residues (TRR)	0.263 ± 0.010 µg/g
Relative Standard Deviation	3.2%
Extraction Results^b	
Extracted	1,200 ± 283 dpm/g
Bound	403 ± 117 dpm/g
Total Recovered Radioactivity	11,600 ± 400 dpm/g
Extracted TRR	0.259 ± 0.009 µg/g
Extraction Efficiency ^c	96.6 %
Material Balance ^d	98.5 %

^a Data from Ref. 4 (Table 2)
 TRR (Total Radioactive Residues)
 number of samples = 15
 specific radioactivity = 44,800 dpm/µg

^b number of samples = 2

^c Extraction Efficiency = (Extracted/Total Recovered Radioactivity) x 100

^d Material Balance = [Extracted TRR/Combustion TRR] x 100

Table 2 Quantitation of RH-7281 in Tomato Metabolism Samples Using the Tomato Residue Analytical Method (TR34-99-68)

RH-7281	Analytical Method (GC/ECD)	Metabolism Study (LC)
Fortification Recovery (%)		
0.050 ppm	91.5	
0.10 ppm	94.4	
Mean (%)	93.0	
Residue found		
	0.093	
	0.092	
Mean (ppm)	0.092 ± 0.001	0.116 ^b
Residue corrected	ppm	0.139 ± 0.073 ^c

^a corrected for average fortification recovery of 93.0 %
ppm corrected = (ppm found)/0.93 = 0.092 ppm/0.93

^b value calculated from data in Ref 4 (Table 4) as follows

LC Results:

RH-7281 peak = 4240 dpm
injected on LC = 7730 dpm
% of dpm in RH-7281 = 54.85%

RH-7281 residues in EtOAc fraction:

EtOAc = 73% of TRR = 73% x 0.29 µg/g = 0.212 µg/g (ppm)
RH-7281 = 54.85% of EtOAc = 54.85% of 0.212 ppm = 0.116 ppm

^c value reported in Ref 4 (Table 4) ± 53.2 % (± RSD from combustion results)

**EPA Addendum For Residue Analytical Method
PP# 0F6093
Zoxamide on Tomatoes and Cucumbers**

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