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

DATA REQUIREMENT
Guideline 860.1340

AUTHORS
Ernest Burdge, Kenneth Kurilla, Ipein Guts, Charles Hofmann

STUDY COMPLETED ON
December 3, 1999

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

Enviro-Test Laboratories (ETL)
9936 - 67 Avenue
Edmonton, Alberta T6E 0P5
Canada

LABORATORY PROJECT ID
Rohm and Haas Technical Report No. 34-99-111
STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

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

Company: Rohm and Haas Company

Company Agent: Janet Ollinger, Ph.D.  Date: December 16, 1999

Signature: [Signature]
Product Registration Manager
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.

Ernest L. Burdige 12-3-99
Author
Rohm and Haas Company

Stanley S. Stavinski 01-7-99
Sponsor
Rohm and Haas Company

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.

<table>
<thead>
<tr>
<th>Date</th>
<th>Activity</th>
<th>Date Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/2/99</td>
<td>audit of preliminary tomato method</td>
<td>8/3/99</td>
</tr>
<tr>
<td>10/21, 10/24/99</td>
<td>audit of preliminary tomato paste and puree method</td>
<td>10/21, 11/10/99</td>
</tr>
</tbody>
</table>

Rhonda S. Knick 12/3/99
Quality Assurance
Rohm and Haas Company
Table of Contents

1 Summary
2 Introduction
3 Experimental Compound
4 Chemicals and Supplies
5 Equipment
6 Methods
   6.1 Method for Tomato RAC
      6.1.1 Method Flow Diagram
      6.1.2 Sample Preparation
      6.1.3 Sample Extraction
      6.1.4 Liquid-Liquid Partition
      6.1.5 Carbon Solid Phase Extraction
      6.1.6 Alumina-B Solid Phase Extraction
      6.1.7 Time to Run a Sample Set
6.2 Method for Tomato Puree and Tomato Paste
   6.2.1 Method Flow Diagram
   6.2.2 Sample Preparation
   6.2.3 Extraction Matrix Solid Phase Dispersion
   6.2.4 Carbon Solid Phase Extraction
   6.2.5 Alumina-B Solid Phase Extraction
   6.2.6 Time to Run a Sample Set
7 Quantitation
   7.1 Instrumentation and Conditions
      7.1.1 Primary Quantitation
      7.1.2 Confirmatory Quantitation
   7.2 Preparation of Standard Curves
   7.3 Fortification Recovery
   7.4 Sample Analysis
   7.5 Sample Calculations
8 Radiolabeling
9 Results and Discussion
10 References
11 Project Information

Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1</td>
<td>Fortification Recovery Results for RH-7281 in Tomato RAC</td>
<td>22</td>
</tr>
<tr>
<td>Table 2</td>
<td>Fortification Recovery Results for RH-7281 in Tomato Puree</td>
<td>24</td>
</tr>
<tr>
<td>Table 3</td>
<td>Fortification Recovery Results for RH-7281 in Tomato Paste</td>
<td>25</td>
</tr>
</tbody>
</table>
Figures

GC/ECD

Figure 1  0.005 ppm Standard RH-7281  26
Figure 2  0.010 ppm Standard RH-7281  27
Figure 3  0.020 ppm Standard RH-7281  28
Figure 4  0.050 ppm Standard RH-7281  29
Figure 5  0.10 ppm Standard RH-7281  30
Figure 6  Calibration Curve for RH-7281  31
Figure 7  Control Tomato RAC  32
Figure 8  Control Tomato RAC  33
Figure 9  Control Tomato RAC  34
Figure 10  Fortification of Tomato RAC at 0.010 ppm  35
Figure 11  Fortification of Tomato RAC at 0.050 ppm  36
Figure 12  Fortification of Tomato RAC at 0.10 ppm  37
Figure 13  Treated Tomato RAC (RAR 98-0095, Sample #2)  38
Figure 14  Treated Tomato RAC (RAR 98-0095, Sample #3)  39
Figure 15  Treated Tomato RAC (RAR 98-0095, Sample #3)  40
Figure 16  Control Tomato Puree  41
Figure 17  Control Tomato Puree  42
Figure 18  Control Tomato Puree  43
Figure 19  Fortification of Tomato Puree at 0.010 ppm  44
Figure 20  Fortification of Tomato Puree at 0.050 ppm  45
Figure 21  Fortification of Tomato Puree at 0.10 ppm  46
Figure 22  Treated Tomato Puree (RAR 98-0101; Sample 5)  47
Figure 23  Control Tomato Puree  48
Figure 24  Control Tomato Puree  49
Figure 25  Control Tomato Puree  50
Figure 26  Fortification of Tomato Puree at 0.010 ppm  51
Figure 27  Fortification of Tomato Puree at 0.050 ppm  52
Figure 28  Fortification of Tomato Puree at 0.10 ppm  53
Figure 29  Treated Tomato Puree (RAR 98-0101, Sample 5)  54

GC/MSD

Figure 30  0.010 ppm Standard RH-7281  55
Figure 31  0.020 ppm Standard RH-7281  56
Figure 32  0.050 ppm Standard RH-7281  57
Figure 33  0.10 ppm Standard RH-7281  58
Figure 34  Calibration Curve for RH-7281  59
Figure 35  Control Tomato RAC  60
Figure 36  Fortification of Tomato RAC at 0.010 ppm  61
Figure 37  Treated Tomato RAC (RAR 98-0095, Sample #2)  62
Appendices

Appendix I  Radiovalidation of Residue Analytical Method  67
1. Summary

This report describes the tolerance enforcement method for analyzing residues of patent 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 fortification tests 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:

<table>
<thead>
<tr>
<th></th>
<th>Tomato RAC</th>
<th>Tomato Puree</th>
<th>Tomato Paste</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Recovery (%)</td>
<td>95.89</td>
<td>90.69</td>
<td>94.12</td>
</tr>
<tr>
<td>Standard Deviation (%)</td>
<td>10.09</td>
<td>13.65</td>
<td>9.33</td>
</tr>
<tr>
<td>Relative Std. Dev (%)</td>
<td>10.71</td>
<td>15.05</td>
<td>9.91</td>
</tr>
<tr>
<td>Number of Samples</td>
<td>25</td>
<td>26</td>
<td>26</td>
</tr>
</tbody>
</table>

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

Proposed Generic Name: Zoxamide

CAS Number: 156052-68-5

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

*The full R&H number is RH-117,281. The designation typically used in studies is the four digit abbreviation RH-7281.

4. Chemicals and Supplies

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Grade</th>
<th>Supplier</th>
<th>Catalogue Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>HPLC</td>
<td>JT Baker</td>
<td>9017-33</td>
</tr>
<tr>
<td>Alumina B cartridges</td>
<td>LC-Alumina B (6 ml)</td>
<td>Supelco</td>
<td>5-7085</td>
</tr>
<tr>
<td>Aluminum Oxide</td>
<td>Brockman I</td>
<td>Baker</td>
<td>0539-01</td>
</tr>
<tr>
<td>Basic Powder</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₆ Silica (Bondesil)</td>
<td>Envirosil</td>
<td>Varian</td>
<td>1221-4016</td>
</tr>
<tr>
<td>Carbon cartridges</td>
<td>Envi-Carb (6 ml)</td>
<td>Supelco</td>
<td>5-7094</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>HPLC</td>
<td>JT Baker</td>
<td>9282-03</td>
</tr>
<tr>
<td>Hexane</td>
<td>Ultra-Res-Analyzed or Optima</td>
<td>JT Baker</td>
<td>9262-02</td>
</tr>
<tr>
<td>Sodium Bicarbonate (NaHCO₃)</td>
<td>A.C.S Reagent</td>
<td>Fisher Scientific</td>
<td>H303-1</td>
</tr>
<tr>
<td>Sodium Sulfate (Na₂SO₄)</td>
<td>A.C.S Reagent</td>
<td>Aldrich</td>
<td>23,931-3</td>
</tr>
<tr>
<td>Water*</td>
<td>Milli-Q®</td>
<td>Millipore</td>
<td>SX0760-1</td>
</tr>
</tbody>
</table>

*Water should be used at a temperature of 4°C or less.
* Other manufacturer brands may be substituted if shown to be suitable
* 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

<table>
<thead>
<tr>
<th>Ethyl Acetate (mL)</th>
<th>Hexane (mL)</th>
<th>Ethyl Acetate/Hexane</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>900</td>
<td>10/90</td>
</tr>
<tr>
<td>300</td>
<td>700</td>
<td>30/70</td>
</tr>
</tbody>
</table>

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:

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

6.1 Method for Tomato RAC

6.1.1 Method Flow Diagram

RAC Sample (1g, pre-processed)

↓

Extraction (550 mL aceonitrile, blending)

↓

Concentration (-10 mL, rotary evaporator)

↓

Liq -Liq Partition (EtOAc/0.1 M NaHCO₃ - 100 mL/100 mL)

↓

Carbon SPE (EtOAc/hexane - 10/90 v/v, 30 mL)

↓

Alumina SPE (EtOAc/hexane - 30/70 v/v, 30 mL)

↓

GC/ECD

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

*Other manufacturer brands may be substituted if shown to be suitable.
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 acetone 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 acetone. Transfer the filtrate to a 500 mL round-bottom flask and concentrate to ~10 mL using a rotary evaporator (~50°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 acetone to the bottle and homogenize for ~60 sec using a Virtis® Centrifuge. Centrifuge the sample at 4,000 rpm for 10 min at ~10°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 acetone each time. Transfer the filtrate to a 500 mL round-bottom flask and concentrate to ~10 mL using a rotary evaporator (~50°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 vortexing. 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 to dry and discard the aqueous phase. Combine the organic phases and take to dryness using a rotary evaporator (~50°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-wash 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 eluates in a 200 mL pear-shaped recovery flask (see Note on page 14). Take
the sample to dryness using a rotary evaporator (≤50°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 eluent. Rinse the recovery flask with 10 mL hexane and apply to the cartridge, again discarding the eluent.

Finally, elute RH-7281 from the cartridge using 30 mL of 30/70 (v/v) ethyl acetate/hexane and collect the eluates in a 100 mL pear-shaped recovery flask (see Note page 14). Take the sample to dryness using a rotary evaporator (≤50°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 tomatoes puree and tomato paste

6.2.1 Method Flow Diagram

Sample (5 g) → Extraction - Matrix Solid Phase Dispersion (MSPD) (10 g C18 Silica, Grnd, Dry) → Elute (60 mL acetetitrile) → Remove Solvent (Rotary Evaporator) → Carbon SPE (EtOAc/hexane - 10/90 v/v, 30 mL) → Alumina SPE (EtOAc/hexane - 30/70 v/v, 30 mL) → GC/MS Quantitation.
6.2.2 Sample Preparation

Tomato purée and tomato paste are used directly without processing.

6.2.3 Extraction Matrix Solid Phase Dispersion

Weigh 5 g of tomato purée or tomato paste into a mortar. If the sample is to be fortified, place the mortar in a ventilized hood and add a known amount of RbF-281 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/pure or paste matrix contained in the mortar.
5. Place a frit on top of C18 silica/pure or paste matrix and then pack tube.
6. Place the tube on a Vissprep™ unit.

Add 60 mL acetonitrile to the mortar and 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 rinsate 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 eluent. Rinse the round bottom flask with 10 mL hexane and apply to the cartridge, again discarding the eluent. Add 30 mL of 30/70 (v/v) of ethyl acetate/hexane to the empty round bottom flask and elute 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 mg/mL RH-7281 standard in hexane to the column or cartridge.

2) Follow the elution protocol as outlined for each individual procedure (see sections 6.1.5, 6.1.6, 6.2.4, or 6.2.7) 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

<table>
<thead>
<tr>
<th>Quantitation Method</th>
<th>Tomato RAC</th>
<th>Puree and Paste</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument</td>
<td>GC/ECD</td>
<td>GC/ECD</td>
</tr>
<tr>
<td>Injection: mode</td>
<td>on-column</td>
<td>on-column</td>
</tr>
<tr>
<td>temp. (°C)</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>volume (µL)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Column: analytical</td>
<td>HP-5MS</td>
<td>HP-5MS</td>
</tr>
<tr>
<td>25 mm ID x 30 m</td>
<td>0.25 µm film</td>
<td>0.25 mm ID x 30 m</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>helium</td>
<td>helium</td>
</tr>
<tr>
<td>Head pressure (psig)</td>
<td>35</td>
<td>13.5</td>
</tr>
<tr>
<td>Flow rate (mL/min)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Detector temp. (°C)</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Oven Temp. Program:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial temp. (°C)</td>
<td>80 (hold 1 min)</td>
<td>80 (hold 1 min)</td>
</tr>
<tr>
<td>Ramp 1 (°C/min)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Temp. 1 (°C)</td>
<td>260 (hold 8 min)</td>
<td>250 (hold 5 min)</td>
</tr>
<tr>
<td>Ramp 2 (°C/min)</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Temp. 2 (°C)</td>
<td>290 (hold 1 min)</td>
<td>280 (hold for 3 min)</td>
</tr>
<tr>
<td>Retention time (min)**</td>
<td>-11-12</td>
<td>-11-12</td>
</tr>
</tbody>
</table>

* 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°C at 35°C/min and hold 7 min. at 260°C, then retention time of RH-728I will be 8-9 min.)
7.1.2 Confirmatory Quantitation

<table>
<thead>
<tr>
<th>Quantitation Method</th>
<th>Tomato RAC</th>
<th>Puree and Paste</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument</td>
<td>GC/MSD</td>
<td>GC/MSD</td>
</tr>
<tr>
<td>Injection mode</td>
<td>splitless</td>
<td>splitless</td>
</tr>
<tr>
<td>temp. (°C)</td>
<td>180</td>
<td>180</td>
</tr>
<tr>
<td>volume (μL)</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Column: analytical</td>
<td>HP-5MS</td>
<td>HP-5MS</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>helium</td>
<td>helium</td>
</tr>
<tr>
<td>Head pressure (psi)</td>
<td>10.4</td>
<td>12.0</td>
</tr>
<tr>
<td>Flow rate (mL/min.)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Detector temp. (°C)</td>
<td>Transfer Line 280</td>
<td>Transfer Line 280</td>
</tr>
<tr>
<td>Oven Temp. Program:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial temp. (°C)</td>
<td>100 (hold 1 min)</td>
<td>80 (hold 1 min)</td>
</tr>
<tr>
<td>Ramp 1 (°C/min.)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Temp. 1 (°C)</td>
<td>250 (hold 3 min)</td>
<td>250 (hold 8 min)</td>
</tr>
<tr>
<td>Ramp 2 (°C/min.)</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>Temp. 2 (°C)</td>
<td>280 (hold 3 min)</td>
<td>280 (hold for 1 min)</td>
</tr>
<tr>
<td>Monitored ions (m/z)</td>
<td>187, 189, 258*</td>
<td>187, 189, 258*</td>
</tr>
<tr>
<td>Retention time (min.)**</td>
<td>~11-12</td>
<td>~11-12</td>
</tr>
</tbody>
</table>

* Quantitation ion. 305, 405 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.
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.

<table>
<thead>
<tr>
<th>Stock Solution (μg/mL)</th>
<th>Volume (mL) of Stock Solution</th>
<th>Final Concentration (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td>5.0</td>
<td>0.005</td>
</tr>
<tr>
<td>0.10</td>
<td>10</td>
<td>0.010</td>
</tr>
<tr>
<td>0.10</td>
<td>20</td>
<td>0.020</td>
</tr>
<tr>
<td>1.0</td>
<td>5.0</td>
<td>0.050</td>
</tr>
<tr>
<td>1.0</td>
<td>10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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 (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:

\[
\text{Recovery (Eq. 1)} = \frac{(\text{Final (μg/mL) x Final Vol. (mL)}) - (\text{Control (μg) x 100%)}}{\text{Fortification Amount (μg)}}
\]
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:

\[\text{Residue Found (µg/mL) x Final Vol. (mL)} = \frac{\mu g}{g} = \text{ppm}\]

Sample Weight (g)

(Eq 2)

7.5 Sample Calculations

A typical calculation for the recovery of a 0.050 ppm (µg/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 (µg/mL). The recovery for a 10.0 g sample fortified with 0.500 µg RH-7281, a final sample volume of 100 mL, and no residue in the control is calculated as below:

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

A typical calculation for determining RH-7281 residue in field-aged tomatoes (Figure 13) is demonstrated as follows: the peak height of RH-7281 is 343,492, which, from the standard calibration curve, corresponds to 0.03126 ppm (µg/mL). For a 10 g field-aged sample in a final sample volume of 250 mL, the amount of RH-7281 residue is calculated using Eq 2.

\[
\frac{0.03126 \mu g/mL \times 250 mL}{10.0 g} = 0.0875 \mu g/g = 0.0875 \text{ ppm}
\]

8. Radiovalidation

Samples from a NC 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 D) are summarized in the following table.

<table>
<thead>
<tr>
<th>RH-7281 Residue ppm</th>
<th>Analytical Method</th>
<th>Metabolism Study (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.092 ± 0.001</td>
<td>0.116 ± 0.062</td>
<td></td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th></th>
<th>Tomato RAC</th>
<th>Tomato Puree</th>
<th>Tomato Paste</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOQ (ppm)</td>
<td>0.010</td>
<td>0.010</td>
<td>0.010</td>
</tr>
<tr>
<td>LOD (ppm)</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>Mean Recovery (%)</td>
<td>93.83</td>
<td>90.69</td>
<td>94.12</td>
</tr>
<tr>
<td>Standard Deviation (%)</td>
<td>10.05</td>
<td>13.65</td>
<td>9.33</td>
</tr>
<tr>
<td>Relative Std Dev (%)</td>
<td>10.71</td>
<td>15.05</td>
<td>9.91</td>
</tr>
<tr>
<td>Number of Samples</td>
<td>26</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Maximum Recovery (%)</td>
<td>78.0</td>
<td>69.9</td>
<td>76.8</td>
</tr>
<tr>
<td>Minimum Recovery (%)</td>
<td>78.0</td>
<td>69.9</td>
<td>76.8</td>
</tr>
</tbody>
</table>

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, 23-24) 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 250°C (360°C/mm.) 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 sonicingating it in methanol for several minutes. Finally, if chromatography does not improve, install a new analytical column.
10. References


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
Spring House, PA 19477-0904

Enviro-Test Laboratories
9936-67 Avenue
Edmonton, Alberta
T6E 0P5 Canada

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

Technical Personnel:
Brett 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
Table 1. Fortification Recovery Results for RH-7281 in Tomato RAC

<table>
<thead>
<tr>
<th>Fortification (ppm)</th>
<th>Primary Quantitation</th>
<th>Confirmantry Quantitation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Source of Data *</td>
<td>GC/ECD (% Recovery)</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-68</td>
<td>99.3</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-68</td>
<td>103</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-68</td>
<td>93.6</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-68</td>
<td>99.2</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-68</td>
<td>79.4</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-68</td>
<td>94.6</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-68</td>
<td>88.2</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-68</td>
<td>89.6</td>
</tr>
<tr>
<td>0.020</td>
<td>TR34-99-68</td>
<td>94.4</td>
</tr>
<tr>
<td>0.020</td>
<td>TR34-99-68</td>
<td>103</td>
</tr>
<tr>
<td>0.020</td>
<td>TR34-99-68</td>
<td>125</td>
</tr>
<tr>
<td>0.020</td>
<td>TR34-99-68</td>
<td>104</td>
</tr>
<tr>
<td>0.020</td>
<td>TR34-99-68</td>
<td>86.4</td>
</tr>
<tr>
<td>0.050</td>
<td>TR34-99-68</td>
<td>89.2</td>
</tr>
<tr>
<td>0.050</td>
<td>TR34-99-68</td>
<td>98.4</td>
</tr>
<tr>
<td>0.050</td>
<td>TR34-99-68</td>
<td>104</td>
</tr>
<tr>
<td>0.050</td>
<td>TR34-99-68</td>
<td>92.9</td>
</tr>
<tr>
<td>0.050</td>
<td>TR34-99-68</td>
<td>86.6</td>
</tr>
<tr>
<td>-----</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>0.10</td>
<td>101</td>
<td>96.8</td>
</tr>
<tr>
<td>0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean</th>
<th>93.83</th>
<th>96.40</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>10.05</td>
<td>7.49</td>
</tr>
<tr>
<td>RSD</td>
<td>10.71</td>
<td>7.77</td>
</tr>
<tr>
<td>N</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

* TR 34-99-68 (Ref 1), "Dates" represent standard curve numbers reported on Rohm and Haas NRDS files (Ref 3)
Table 2. Fortification Recovery Results for RH-7281 in Tomato Puree

<table>
<thead>
<tr>
<th>Fortification (ppm)</th>
<th>Primary Quantitation</th>
<th>Confiratory Quantitation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Source of Data *</td>
<td>GC/ECD (% Recovery)</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-95</td>
<td>88.1</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-95</td>
<td>90.9</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-95</td>
<td>75.8</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-95</td>
<td>84.4</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-95</td>
<td>109</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-95</td>
<td>113</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-95</td>
<td>106</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-95</td>
<td>110</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-95</td>
<td>69.8</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-95</td>
<td>70.1</td>
</tr>
<tr>
<td>0.010</td>
<td>07/09/99</td>
<td>69.9</td>
</tr>
<tr>
<td>0.010</td>
<td>07/09/99</td>
<td>69.9</td>
</tr>
<tr>
<td>0.020</td>
<td>TR34-99-95</td>
<td>91.9</td>
</tr>
<tr>
<td>0.020</td>
<td>TR34-99-95</td>
<td>83.8</td>
</tr>
<tr>
<td>0.020</td>
<td>TR34-99-95</td>
<td>103</td>
</tr>
<tr>
<td>0.020</td>
<td>TR34-99-95</td>
<td>110</td>
</tr>
<tr>
<td>0.020</td>
<td>TR34-99-95</td>
<td>82.8</td>
</tr>
<tr>
<td>0.020</td>
<td>07/09/99</td>
<td>82.9</td>
</tr>
<tr>
<td>0.050</td>
<td>TR34-99-95</td>
<td>85.0</td>
</tr>
<tr>
<td>0.050</td>
<td>TR34-99-95</td>
<td>83.2</td>
</tr>
<tr>
<td>0.050</td>
<td>TR34-99-95</td>
<td>94.6</td>
</tr>
<tr>
<td>0.10</td>
<td>TR34-99-95</td>
<td>102</td>
</tr>
<tr>
<td>0.10</td>
<td>TR34-99-95</td>
<td>95.2</td>
</tr>
<tr>
<td>0.10</td>
<td>TR34-99-95</td>
<td>84.9</td>
</tr>
<tr>
<td>0.10</td>
<td>TR34-99-95</td>
<td>98.7</td>
</tr>
<tr>
<td>0.10</td>
<td>TR34-99-95</td>
<td>104</td>
</tr>
</tbody>
</table>

Mean: 90.69
Standard Deviation: 13.65
Relative Std. Dev: 15.05
Number of Samples: 26

* TR34-99-95 (Ref 2), "Data" represent standard curve numbers reported in Rohm and Haas NRDS files (Ref 3)
### Table 3. Fortification Recovery Results for RH-7281 in Tomato Paste

<table>
<thead>
<tr>
<th>Fortification (ppm)</th>
<th>Primary Quantitation</th>
<th>Confirmatory Quantitation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Source of Data *</td>
<td>CGECD (% Recovery)</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-95</td>
<td>91.4</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-95</td>
<td>93.2</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-95</td>
<td>88.1</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-95</td>
<td>88.9</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-95</td>
<td>90.8</td>
</tr>
<tr>
<td>0.010</td>
<td>TR34-99-95</td>
<td>97.9</td>
</tr>
<tr>
<td>0.010</td>
<td>OB/10/99</td>
<td>97.3</td>
</tr>
<tr>
<td>0.020</td>
<td>TR34-99-95</td>
<td>87.4</td>
</tr>
<tr>
<td>0.020</td>
<td>TR34-99-95</td>
<td>87.4</td>
</tr>
<tr>
<td>0.020</td>
<td>TR34-99-95</td>
<td>86.6</td>
</tr>
<tr>
<td>0.020</td>
<td>TR34-99-95</td>
<td>87.3</td>
</tr>
<tr>
<td>0.020</td>
<td>TR34-99-95</td>
<td>95.2</td>
</tr>
<tr>
<td>0.050</td>
<td>TR34-99-95</td>
<td>87.3</td>
</tr>
<tr>
<td>0.050</td>
<td>TR34-99-95</td>
<td>90.2</td>
</tr>
<tr>
<td>0.050</td>
<td>TR34-99-95</td>
<td>95.1</td>
</tr>
<tr>
<td>0.10</td>
<td>TR34-99-95</td>
<td>97.8</td>
</tr>
<tr>
<td>0.10</td>
<td>TR34-99-95</td>
<td>93.3</td>
</tr>
<tr>
<td>0.10</td>
<td>TR34-99-95</td>
<td>91.3</td>
</tr>
<tr>
<td>0.10</td>
<td>TR34-99-95</td>
<td>87.2</td>
</tr>
<tr>
<td>0.10</td>
<td>TR34-99-95</td>
<td>117</td>
</tr>
</tbody>
</table>

*TR 34-99-95 (Ref.2), "Data" represent standard curve numbers reported in Rohm and Haas NRD5 files (Ref. 3)
Figure 1  0.005 ppm RH-7281 Standard (GC/ECD)
Figure 2  0.010 ppm RH-7281 Standard  (GC/ECD)
Figure 3  0.020 ppm RH-7281 Standard (GC/ECD)
Figure 4  0.050 ppm RN-7281 Standard  (GC/ECD)
TR 34-99-111

Quantitation Report

Date file: c:\\.User\\Profile\\Local Settings\\Start Menu\\Programs\\Common\C:\\\METHODS\\ENVIS.M (Chemstation Interpret)

Quant Time: Jul 26 15:02 1999 Quant Results File: ENVIS.RES

Quant Method: C:\\METHODS\\ENVIS.M (Chemstation Interpret)

Title: 04-07-99

Last Update: Mon Jul 26 14:44:02 1999

Response file: Multiple Level Calibration

Data File: EVENTS.\n
Volume (ng):

Signal Phase:

Signal Inte:

Compound R.T. Response:

Target Compound

RH-7281 03-29-99 13.32 71462837

Figure 5 0.10 ppm RH-7281 Standard (GC/ECD)
Figure 6 Calibration Curve for RH-7281 (GC/ECD)
Quantitation Report

Date File: 01\_llipchev\_dgate\_w203399\_CRT\_CONTROL.C

Vial: 9

Acq On: 3-20-99 9:13:13 PM

Sample: Control 0233999

Operator: Inc

Inc: 0.00

Mul: 1.00

Sample Amount: 0.20

Infill: EVENTS I

Quant Time: Jul 26 15:03 1999

Quant Results File: ENHIE RSE

Quant Method: C:\MYCHEM\my METHODS\ENHIE M (Chemstation Integration)

Title: 08-09-99

Last Update: Jul 26 14:44:02 1999

Response Via: Multiple Level Calibration

DataAcq Path: ENHIE.M

Volume Inj: 

Signal Phase: 

Signal Info: 

---

Figure 7 Control Tomato RAC (GC/ECD)
Figure 8  Control Tomato RAC (GC/ECD)
Figure 9  Control Tomato RAC (GC/ECD)
TR 34-99-111

Quantitation Report

Data File: c:\\\data\032899\SAMPLE.D

Vial: 8

File: EVENTS

Quant Time: Jul 26 15:04 1999

Quant Results File: ENTRIE.RSS

Quant Method: C:\\METHODS\ENTRIE.M (Chromatogram Integration)

Title: 06-07-99

Last Update: Jun 26 14:44:02 1999

Response File: ENTRIE.M

Database File: ENTRIE.RSS

Volume Inj.: Signal Phase:

Signal Info:

<table>
<thead>
<tr>
<th>Compound</th>
<th>R.T</th>
<th>Responses</th>
<th>Conc Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>RH-7281</td>
<td>12.31</td>
<td>6786648</td>
<td>9.990 ppm</td>
</tr>
</tbody>
</table>

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

Recovery = 99.5%
Quantitation Report

Data File: c:\upchem\3\data\E012999\SAMPLE.D
Vial: 11
Sample: 0.05 ppm fort. E012999
File: 0.05 ppm fort. E012999
Sample Amount: 0.05

Infile: EVENTS.D

Quant Time: Jul 26 15:06 1999
Quant Results File: EVENTS.D

Quant Method: C:\UPCHEM\1\METHODS\EVENTS.D (Chromatogram Integrator)
Title: 04-30-99
Last Update: Mon Jul 26 16:44:03 1999
Response Type: Multiple Level Calibration
Detection Method: EVENTS.D

Volume Inj: 
Signal Phase: 
Signal Info: 

---

Figure 11 Fortification of Tomato RAC at 0.050 ppm RH-7281 (GC/ECD)
Recovery = 98.4%
Figure 12  Fortification of Tomato RAC at 0.10 ppm RH-7281 (GC/ECD)
Recovery = 95.5 %
Figure 13 Treated Tomato RAC (RAR 98-0091; Sample #2) (GC/ECD)
Found: 0.0875 ppm
Figure 14  Treated Tomato RAC (RAR 98-0091; Sample #3)  (GC/ECD)
Found: 0.108 ppm
Figure 15  Treated Tomato RAC (RAR 98-0090; Sample #3) (GC/ECD)  
Found: 0.193 ppm
Quantitation Report

Data File: c:\\\data\53299\S-501.D
Acq On: 6-22-99 7:38.27 PM
Sample: control, tomato puree
IntFile: EVENTS.E

Quant Time: Jun 24 8:00 1999
Quant Results File: RH7281.RDS

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

Figure 16
Control Tomato Puree (GC/ECD)
Figure 17  Control Tomato Puree  (GC/ECD)
Quantitation Report

Date File: c:\pchem\data\#2599\J-921 D

Aug On: 6-23-99 6:00:38 PM

Sample: control tomato puree

Note: Sample Amount: 0.00

Infile: EVENTS.B

Quant Time: Jun 23 8:41 1999 Quant Results File: KNT7281 R38

Quant Method: C:\USC\PC\METHODS\KNT7281 M (Chemstation Integrator)

Last Update: Mon Jun 28 00:16:44 1999

Response via: Multiple Level Calibration

DataAcq Meth: KNT7281 M

Volume Inj:

Signal Phase:

Signal Info:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ret Time</th>
<th>Response</th>
<th>Conc Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNT-7281</td>
<td>0.00</td>
<td>0.00</td>
<td>0 M D. ppb</td>
</tr>
</tbody>
</table>

Figure 18: Control Tomato Puree (GC/ECD)
Quantitation Report

Data File: c:\hpchem\data\042399\5-552.D
Acq Date: 6-22-99 8:03:20 AM
Sample: 0.01ppm spike, tomato puree

Int File: EVENTS.E

Quant Time: Jun 24 6:59 1999
Quant Results File: 57281.062

Quant Method: C:\HPCHM\METHODS\57281.M (Chromatogram Integrator)
Title: 04-07-99
Last Update: Thu Jun 24 07:50:50 1999
Response via: Multiple Level Calibration
Data Acq Meth: 57281 M

Figure 19  Fortification of Tomato Puree at 0.010 ppm RH-7281 (GC/ECD)
Peak Area = 8,737.167  Recovery = 88.1 %
Figure 20  Fortification of Tomato Puree at 0.050 ppm RH-7281 (GC/ECD)
Peak Area = 42,125,405  Recovery = 85.0 %
Quantitation Report

Data File : c:\hpchem\3\data\643298\5-586.D
Vial : 12
Acq On : 6-22-99 9:38:44 PM
Operator : k.r.
Sample : 0 10ppm spike, tomato puree
Insn : Tasc 0.3 ppm
Sample Amount : Sample
Misc :

InFile : EVENTS.E

Quant Time: Jun 24 6:32 1999 Quant Results File: RH7281.EXE
Quant Method : C:\UPCHEM\3\METHODS\RH7281.M (Chemstation Integrator)
Title : 06-27-99
Last Update : Thu Jun 24 07:50:50 1999
Response via : Multiple Level Calibration
DataAcq Meth : RH7281 M

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

Data File: c:\bench\data\070999\8156-5 D
Acq On: 7-9-99 9:18:26 PM
Sample: RAS98-0101
Method: 
Sample Amount: 0.09

Int File: EVENTS.X
Quan Time: Jul 12 8:49 1999 Quant Results File: EVENT1.RES

Quan Method: \CHEK\MATLAB\RT781.M (ChemStation Integration)
Title: 01-30-99
Last Update: Mon Jul 12 08:36:07 1999
Response type: Multiple Level Calibration
Data type: RT781.M

Volume Inf:
Signal Phase:
Signal Info:

Target Compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>R.T.</th>
<th>Response</th>
<th>Conc Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAR-7581</td>
<td>07-09-99 (075888)</td>
<td>12.25</td>
<td>7608888888</td>
</tr>
</tbody>
</table>

Figure 22  Treated Tomato Purre (RAR 98-0101, Sample #5) (GC/ECD)
Found: 0.0442 ppm
Figure 23  Control Tomato Paste  (GC/ECD)
Peak Area = 0
Quantitation Report

Data File: c:\tmp\test1\data\880499\3-701.D
Vial: 7
Acq On: 8-4-99 5:20:40 PM
Operator: kk
Sample: control, tomato paste
Instr: GC ECD
Mode: Multiple 1 00
Sample Amount: 0 00

Quant Time: Aug 5 8:53 1999 Quants Results File: ERT7281.RKS

Quant Method: C:\WPCBOD\VISTEX\ERT7261.M (Chemstation Integration)
Title: 04-07-99
Last Update: Thu Aug 05 04:26:25 1999
Responses via: Multiple Level Calibration
DataLog file: ERT7261.M

Volume IJC: Signal Phases
Signal Info:

<table>
<thead>
<tr>
<th>Compound</th>
<th>B.T.</th>
<th>Response</th>
<th>Conc Units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Target Compounds

- ER-7281 (880499) 0.00 0 N.D. ppb

Figure 24  Control Tomato Paste  (GC/ECD)
Figure 25  Control Tomato Paste  (GC/ECD)
Quantitation Report

Data File: c:\pchem\data\673099\S-683.D
Acq On: 7-30-99 4:37:46 PM
Sample: 0.01 ppm spike, tomato paste
Micr: 

InNFile: EVENTS.R
QuanC Time: Aug 2 7:48 1999 QuanC Results File RH7281.M
QuanC Method: C:\PCHAS\2\METRICAS\RH7281.M (Chemstation Integrator)
File: 
Last Update: The 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:\hpchem\data\070999\S-268.D
Acq On: 7-10-99 7:58:11 PM
Sample: 0.05 ppm spike, tomato paste
Inc: 
MISC: Multipl 1.00

Quan File: EVENTS.E
Quan Time: Aug 2 9:50 1999 Quan Results File RH7281.D
Quan Method: C:\HPCHEM\METHODS\RH7281.M (Chemstation Integrator)
Title: 04-07-99
Last Update: Thu Jul 29 08:35 42 1999
Response via: Multiple Level Calibration
DataAcq Mech: RH7281.M

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

File: c:\hpchem\Vdata\61088\68199-4.D

Aug On: 8-10-99 8:32:43 PM
Sample: Paste Residue Sample 98-0101
Vial: 13

Operator: Th
Inst: GC ECD
Multiple: 1.000
Sample Amount: 0.00

Quant Time: Aug 11 9:32 1999
Quant Results File: RNT281.RES

Quant Method: C:\BCPROM\\\\METID\RNT281.M (Chemstation Integrator)
Title: EA-07-99
Last Update: Aug 11 09:14:27 1999
Response: Multiple Level Calibration

Sample Inj: Signal Phase: Signal Info:

---

Figure 29: Treated Tomato Paste (RAR 98-0101; Sample #5) (GC/ECD)
Found: 0.0918 ppm
TR 34-99-111

File: C:\REPORTS\DATA\25599\STD1.D
Operator: 8 May 99 2:33 using Agilent ChemStation
Instrument: 5972
Sample Name: STD 0.01ppb
Vial Number: 17

R.T. Glue Response: Cons Units Dev(MHz)

Byron Monitoring Compounds

Target Compounds
Peak RH-7281, 05-04-99

| Value | 10.97 | 9.98 | 9.73 ppm 100 |

Figure 30 0.010 ppm RH-7281 Standard (GC/MSD)
Figure 31 0.020 ppm RH-7281 Standard (GC/MSD)
Figure 32  0.050 ppm RH-7281 Standard  (GC/MSD)
Figure 33  0 10 ppm RH-7281 Standard (GC/MSD)
TR 34-99-111

Figure 34 Calibration Curve for RH-7281 (GC/MSD)
Figure 35  Control Tomato RAC  (GOMSD)
Figure 36  Fortification of Tomato RAC at 0.010 ppm RH-7281 (GC/ECD)
Recovery = 98.7 %
Figure 37  Treated Tomato RAC (RAR 98-0095, Sample #2) (GC/MSD)
Found. 0 180 ppm
Figure 38  Control Tomato Puree  (GC/MSD)
Peak Area = 0
Figure 39  Fortification of Tomato Puree at 0.010 ppm RH-7281 (GC/MSD)
Peak Area = 488  
Recovery = 98.8 %
File: C:\HPChem\DATA\372999\5-671 D
Operator: 
Acquired: 29 Jul 93 12:49 using Agilent method 720SIM
Instrument: 5973
Sample Name: control, tomato paste
Vial Info: 
Vial Number: 6

**Figure 40** Control Tomato Paste (GC/MSD)
Peak Area = 0
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/MSD (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 ± 0.073 (i.e., ±53.2%) or 0.116 ± 0.062. The average residue level of RH-7281 found by the analytical method was 0.099 ± 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: *       | Extraction Results:  
|-------------------------------|-----------------------------|-----------------------------
| Total Radioactive Residues (TRR) | 0.263 ± 0.030 μg/g         | Extracted                   |
| Relative Standard Deviation   | 2.3%                        | Total Recovered Radioactivity | 11,600 ± 400 dpm/g         |
|                               |                             | Extracted TRR               | 0.259 ± 0.009 μg/g         |
|                               |                             | Extraction Efficiency *      | 96.6 %                      |
|                               |                             | Material Balance *          | 98.5 %                      |

* Data from Ref. 4 (Table 2)
  TRR (Total Radioactive Residues)
  number of samples = 15
  specific radioactivity = 4,200 dpm/μg

* number of samples = 2

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

* 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)

<table>
<thead>
<tr>
<th>RH-7281</th>
<th>Analytical Method</th>
<th>Metabolism Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(GC/ECD)</td>
<td>(LC)</td>
</tr>
<tr>
<td>Fortification Recovery (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.650 ppm</td>
<td>91.5</td>
<td></td>
</tr>
<tr>
<td>0.10 ppm</td>
<td>24.4</td>
<td></td>
</tr>
<tr>
<td>Mean (%)</td>
<td>93.0</td>
<td></td>
</tr>
<tr>
<td>Residue found</td>
<td>0.091</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.092 ± 0.001</td>
<td>0.116 ±</td>
</tr>
<tr>
<td>Mean (ppm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residue corrected ppm</td>
<td>0.092 ± 0.001</td>
<td>0.139 ± 0.073</td>
</tr>
</tbody>
</table>

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

* value calculated from data in Ref 4 (Table 4) as follows
  LC Results
  RH-7281 peak = 420 ppm
  injected on LC = 720 ppm
  % of dpm in RH-7281 = 54.85%

RH-7281 residues in RSD% 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

* 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.