STUDY TITLE

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

DATA REQUIREMENT

Guideline 830.1340

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STUDY COMPLETED ON

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SPONSOR

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LABORATORY PROJECT ID

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

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

Company: Rohm and Haas Company

Company Agent: Janet Ollinger, Ph.D. Date: June 1, 2000

Signature: [Signature]
Product Registration Manager
GLP Compliance Statement

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

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

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Quality Assurance Statement

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

QA activities for this study:

<table>
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<th>Activity</th>
<th>Date Reported</th>
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<td>Audit of Preliminary Method</td>
<td>05/08/2000</td>
</tr>
<tr>
<td>05/30/2000</td>
<td>Audit of Final Report</td>
<td>05/30/2000</td>
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</table>

Rhonda S. Krick  
QA Analyst  
Rohm & Haas Company  

Date
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1. Summary

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

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

As described in the report, RH-7281, RH-1452, and RH-1455 residues are extracted from the matrix by blending with acetonitrile/2% NaHCO₃. RH-7281 is separated from RH-1452 and RH-1455 by liquid-liquid partition and then further purified by Florisil solid phase extraction (SPE) and Alumina-B SPE. RH-1452 and RH-1455 are partially purified by liquid-liquid partition and then are methylated to their methyl esters (RH-7391 and RH-7399, respectively). The esters are further purified by Florisil column chromatography.

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Recovery</th>
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<tbody>
<tr>
<td>RH-7281</td>
<td>90.23±15.10%</td>
</tr>
<tr>
<td>RH-1452</td>
<td>90.19±20.24%</td>
</tr>
<tr>
<td>RH-1455</td>
<td>87.74±22.26%</td>
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</table>

<table>
<thead>
<tr>
<th>Samples</th>
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<tbody>
<tr>
<td>n = 27</td>
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2. Introduction

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

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

3.1 Structure of the Reference Substances

\[ \text{RH-7281, MW = 335} \]

\[ \text{RH-1452, MW = 220} \]

\[ \text{RH-1455, MW = 234} \]

\[ \text{RH-7391, MW = 234} \]

\[ \text{RH-7399, MW = 262} \]
3.2 Data for the Reference Substances

**RH-7281:**
- CA Name: 3,5-dichloro-N-(3-chloro-1-ethyl-1-methyl-2-oxopropyl)-4-methylbenzamide
- CAS Number: 156052-68-5
- R&H ID: RH-117281*
- Appearance: White Solid

**RH-1452:**
- CA Name: 3,5-dichloro-4-hydroxymethylbenzoic acid
- CAS Number: N/A
- R&H ID: RH-141452*
- Appearance: White Solid

**RH-1455:**
- CA Name: 2,6-dichloro-1,4-benzenedicarboxylic acid
- CAS Number: N/A
- R&H ID: RH-141455*
- Appearance: White Solid

**RH-7391 (methyl ester of RH-1452):**
- CA Name: Methyl, 3,5-dichloro-4-hydroxymethylbenzoate
- CAS Number: N/A
- R&H ID: RH-147391*
- Appearance: White Solid
RH-7399 (methyl ester of RH-1455):

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

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

4. Chemicals/Prepared Solutions

4.1 Chemicals

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

Solutions for Sample Extraction:

2% (w/v) NaHCO₃ solution was prepared by dissolving 20 g of NaHCO₃ in 1000 mL of Milli-Q water in a 1000 mL flat bottom flask.

80/20 (v/v) ACN/2% NaHCO₃ solution was prepared mixing 800 mL of ACN and 200 mL of 2% NaHCO₃ in a 1000 mL flat bottom flask.

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

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

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

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

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

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

5. Equipment

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

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

6.1 Method Flow Diagram

5.0 g of potato peel waste samples

\[ \downarrow \]

Blending Extraction with 80/20 ACN/2% NaHCO₃

\[ \downarrow \]

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

\[ \downarrow \]

RH-7281

\[ \downarrow \]

RH-1452+ RH-1455

\[ \downarrow \]

Florisil Solid Phase Extraction

\[ \downarrow \]

Methylation

\[ \downarrow \]

Alumina-B Solid Phase Extraction

\[ \downarrow \]

Florisil Column Chromatography

\[ \downarrow \]

Quantitation (RH-7281)

\[ \downarrow \]

Quantitation (RH-7391 + RH-7399)

6.2 Sample Preparation

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

6.3 Sample Extraction

Weigh 5.0 g of a homogenized potato peel sample in a disposable weighing dish. Transfer the sample to a blender jar. If desired, spike fortification samples with a known amount of RH-7281, RH-1452, and RH-1455 in ethyl acetate (1.0 ppm mix of the three). Allow the solvent to evaporate from the sample in a fume hood for about 20 minutes. Add about 2 g of Celite to the blender jar.
Add 100 mL of 80/20 acetonitrile/2% NaHCO₃ (extraction solution) to the blender jar. Blend the slurry for 1 minute at medium speed. Filter the slurry through #4 Whatman filter paper in a Buchner funnel into a 500 mL vacuum filtration flask. Rinse the blender jar and the filter cake with 20 mL of the extraction solution. Combine the filtrates (approximately 120 mL) in a 500 mL (24/40 ST) round bottom flask and concentrate to about 30 mL (Solution A) on a rotovap at about 40°C under diminished pressure (about 50 mm Hg).

6.4 Liquid-Liquid Partition to Separate RH-7281

Transfer Solution A in the 500 mL round bottom flask to a 500 mL separatory funnel. Rinse the flask with 80 mL of 2% NaHCO₃ solution and 75 mL of ethyl acetate sequentially and add both to the separatory funnel. Shake the funnel for 1 minute with frequently venting and allow the two phases to separate.

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

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

6.5 Florisil Solid Phase Extraction for RH-7281*

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

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

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

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

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

Add 30 mL of 1.0 N HCl solution to Solution B (from section 6.4) and measure the pH of the solution. If necessary, add more 1.0 N HCl solution to ensure the pH of the solution is ≤ 1. Transfer the solution to a 500 mL separatory funnel. Rinse the 500 mL flask with 75 mL of ethyl acetate and add to the separatory funnel. Shake the funnel for 30 seconds with frequent venting and allow two phases to separate. Transfer the aqueous phase (lower layer) to the original Solution B flask. Transfer the organic phase to a 300 mL Erlenmeyer flask containing about 5 g of Na2SO4 and let it sit for about 2 minutes with occasional swirling. Decant the solution to a 300 mL (24/40 ST) round bottom flask.

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

Concentrate the solution to dryness on a rotovap at about 40 °C under diminished pressure (continue the evaporation for at least 1 minute after the last drop of liquid disappears to ensure dryness). Dissolve the residue with 1 mL of ethyl acetate and sonicate for 10 seconds. Add 1.0 mL of ethyl ether (Solution E) and proceed to section 6.8.
6.8 Methylation of RH-1452 and RH-1455

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

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

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

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

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

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

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

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

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

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

5). If the target cut contains a minimum of 85% of RH-7281, it may be considered acceptable.
6. If the 85% of recovery criteria is not met, the following adjustment in elution scheme should be made:

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

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

7. Quantitation

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

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

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

7.1 Instrumentation and Conditions

7.1.1 Primary Detection

Quantitation of RH-7281:

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

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

Oven temperature program:
   Initial temp. = 80°C, hold for 1 min.
   Ramp 1 at 20°C/min.,
   Final temp. 1 = 250°C, hold for 5 min.
   Ramp 2 at 30°C/min.,
   Final temp. 2 = 280°C, hold for 3 min.
Detector: Temp. = 300°C  
Mode: Constant flow (column + makeup = 70 mL/min.)  
Makeup Gas = Nitrogen

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

Quantitation of RH-7391 and RH-7399:

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

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

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

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

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

7.1.2. Confirmatory Detection

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

Injector: HP6890 auto-sampler  
Splitless mode  
Injection temp.: 150°C  
Injection volume: 1.0 μL
Column: HP-5MS (0.25 mm ID x 30 m; 0.25 μm film)
  Carrier gas: Helium
  Head Pressure: 9.2 psi
  Flow rate: 1.0 mL/min.

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

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

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

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

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

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

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

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

Typical Retention Time: RH-7399: ~16.1 min.
  RH-7391: ~16.4 min.
7.2 Preparation of Standard Solutions

7.2.1 Stock Standard Solutions

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

7.2.2 Intermediate Standard and Spiking Solutions

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

7.2.3 Working Standards

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

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<th>Aliquot size and source</th>
<th>Final Volume</th>
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<td>0.050 µg/mL</td>
<td>5.0 mL of 1.0 µg/mL</td>
<td>100 mL</td>
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<td>0.010 µg/mL</td>
<td>1.0 mL of 1.0 µg/mL</td>
<td>100 mL</td>
</tr>
</tbody>
</table>

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

7.3 Preparation of Standard Curves

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

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

\[
\frac{\text{Found (μg/mL)} \times \text{Final Vol. (mL)}}{\text{Fortification Amount (μg)}} \times 100\% = \% \text{ Recovery} \quad \text{Eq.1}
\]

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

\[
\frac{\text{RH-7391 (mw = 234)}}{\text{RH-1452 (mw = 220)}} = 1.06
\]

\[
\frac{\text{RH-7399 (mw = 262)}}{\text{RH-1455 (mw = 234)}} = 1.12
\]

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

7.5 Sample Analysis

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

The residue concentration in ppm is determined as follows:

\[
\frac{\text{Concentration (μg/mL)} \times \text{Final Volume (mL)}}{\text{Sample Weight (g)}} \mu g/g = \text{ppm} \quad \text{Eq.2}
\]
7.6 Sample Calculation

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

*Calculation for RH-7281:*

\[
\frac{0.074778 \text{ (µg/mL)} \times 5.0 \text{ (mL)}}{0.50 \text{ µg}} \times 100\% = 74.8\%
\]

*Calculation for RH-1452:*

\[
\frac{0.097000 \text{ (µg/mL)} \times 5.0 \text{ (mL)}}{0.50 \text{ µg} \times 1.06} \times 100\% = 91.5\%
\]

*Calculation for RH-1455:*

\[
\frac{0.081986 \text{ (µg/mL)} \times 5.0 \text{ (mL)}}{0.50 \text{ µg} \times 1.12} \times 100\% = 73.2\%
\]

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

7.7 Expression of Total Residue Level

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

\[\text{ppm}_{\text{parent equivalent for RH-1452}} = \text{ppm}_{\text{RH-1452}} \times 335/220 = 1.52 \times \text{ppm}_{\text{RH-1452}} \quad \text{Eq.3}\]

\[\text{ppm}_{\text{parent equivalent for RH-1455}} = \text{ppm}_{\text{RH-1455}} \times 335/234 = 1.43 \times \text{ppm}_{\text{RH-1455}} \quad \text{Eq.4}\]

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

\[\text{ppm}_{\text{parent equivalent}} = \text{ppm}_{\text{RH-7281}} + 1.52 \times \text{ppm}_{\text{RH-1452}} + 1.43 \times \text{ppm}_{\text{RH-1455}} \quad \text{Eq.5}\]

7.8 Time Required to Run a Set of Samples

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

Both GC/ECD and GC/MSD have acceptable sensitivity for RH-7281, RH-7391, and RH-7399. Either linear responses or quadratic responses can be used in the concentration range of 0.01 - 0.15 µg/mL depending on the regression coefficient (Figures 2 and 22).

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

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

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

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

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

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

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

9. References


10. Project Information

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

Project Number: 1401.15

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

Dates:
Work Initiated: February 2000
Work Completed: May 2000

Technical Personnel:
Ipin Guo, Ken Kurilla, Charles Hofmann

Notebook References:
IG-4(#70535)

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

Peer Reviewer:
Danny Choo
Table 1  Recovery Data of RH-7281

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Mean 90.23  101.92

SD 15.10  9.84

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

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Sample Name : std1  
Vial : 1
Acq. Operator : ig  
Inj : 1
Inj Volume : 1 ul
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Acq. Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 3/3/2000 2:11:59 PM by ig
Analysis Method : C:\HPCHEM\2\METHODS\PEEL-P.M

RH7281 Potato Peel Waste METHOD,
Rtx-5, 30m, 0.53mm, 0.5um film
Flow Rate = 4.0mL/m

Default Calibration Report (other requested info not available)
External Standard Report (no recalibration)

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal : ECD1 B,

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Figure 1. 0.010 μg/ml Standard RH-7281 (GC/ECD).
Data File C:\HPCHEM2\DATA\031020\002B0401.D
Sample Name: std2
Flow Rate = 4.0 ml/min

0.05ppm RH7281
Injection Date : 3/10/2000 5:00:52 PM
Seq. Line : 4
Sample Name : std2
Vial : 7
Acq. Operator : ig
Inj : 1
Inj Volume : 1.0 µl

Sequence File : C:\HPCHEM2\SEQUENC\IG031020.S
Acq. Method : C:\HPCHEM2\METHODS\PEEL-P.M
Last changed : 3/3/2000 2:11:59 PM by ig
Analysis Method : C:\HPCHEM2\METHODS\PEEL-P.M

RH7281 Potato Peel Waste METHOD,
Rtx-5, 30m, 0.53mm, 0.5um film

Default Calibration Report (other requested info not available)

External Standard Report (no recalibration)

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal A: ECD1 B,

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

Totals : 4.62373e-2

Instrument 2 5/9/2000 12:24:51 PM in

Figure 2. 0.050 µg/ml Standard RH-7281 (GC/ECD).
Figure 3. 0.10 µg/ml Standard RH-7281 (GC/ECD).
Figure 9. 0.15 µg/ml Standard RH-7281 (GC/MSD, Peak Area = 2194).
0.15 ppm RH7281

Injection Date: 3/10/2000 5:48:00 PM  
Sample Name: std4  
Acq. Operator: ig  
Seq. Line: 6  
Vial: 4  
Inj:  
Inj Volume: 1 μl

Sequence File: C:\HPChem\2\SEQUENCE\IG031020.S  
Acq. Method: C:\HPChem\2\METHODS\PEEL-P.M  
Last changed: 3/3/2000 2:11:59 PM by ig  
Analysis Method: C:\HPChem\2\METHODS\PEEL-P.M  

RH7281 Potato Peel Waste METHOD,  
Rtx-5, 30m, 0.53mm, 0.5um film  
Flow Rate = 4.0ml/min

Default Calibration Report (other requested info not available)

External Standard Report (no recalibration)

Sorted By: Signal  
Multiplier: 1.0000  
Dilution: 1.0000

Signal 1: ECD1 B,

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<td>4.85023e-7</td>
<td>1.31245e-1</td>
<td>RH7281</td>
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Totals: 1.31245e-1

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

Figure 4. 0.15 μg/ml Standard RH-7281 (GC/ECD).
Figure 5. Calibration Curve of RH-7281 (GC/ECD).
Figure 6.  0.010 μg/ml Standard RH-7281 (GC/MSD, Peak Area = 129).
0.050 µg/ml Standard RH-7281 (GC/MSD, Peak Area = 864).
Figure 8. 0.10 μg/ml Standard RH-7281 (GC/MSD, Peak Area = 1745).
Figure 10. Calibration Curve of RH-7281 (GC/MSD).
Figure 11. 0.010 µg/ml Standard RH-7391 and RH-7399 (GC/ECD, Rtx-225)
Peak Area $_{RH-7391} = 79582668$, Peak Area $_{RH-7399} = 72697150$. 
Figure 12. 0.050 µg/ml Standard RH-7391 and RH-7399 (GC/ECD, Rtx-225)
Peak Area $_{\text{RH-7391}} = 424704907$, Peak Area $_{\text{RH-7399}} = 370760445$. 
Figure 13. 0.10 µg/ml Standard RH-7391 and RH-7399 (GC/ECD, Rtx-225)
Peak Area RH-7391 = 869576680, Peak Area RH-7399 = 754744397.
Figure 14. 0.15 µg/ml Standard RH-7391 and RH-7399 (GC/ECD, Rtx-225)  
Peak Area $\text{RH-7391} = 1407692926$,  
Peak Area $\text{RH-7399} = 1221064799$.  

40
Response = 9.37e+006 * Amt
Coef of Det (r^2) = 0.995  Curve Fit: Linear/(0,0)

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

Figure 15.  Calibration Curve of RH-7391 (GC/ECD, Rtx-225).
Figure 16. Calibration Curve of RH-7399 (GC/ECD, Rtx-225).
Figure 17.  0.010 μg/ml Standard RH-7391 and RH-7399 (GC/ECD, Rtx-35)
Peak Area_{RH-7391} = 105206321,  Peak Area_{RH-7399} = 102601052.
Quantitation Report

Data File: c:\hpchem\2\data\050120\STD2.D  Vial: 2
Acq On: 5-1-00 5:32:04 PM  Operator: --
Sample: 0.05 m2  Inst: GC ECD
Misc:  
Sample Amount: 0.00

IntFile: EVENTS.E

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

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

Volume Inj.:
Signal Phase:
Signal Info:

Figure 18. 0.050 µg/ml Standard RH-7391 and RH-7399 (GC/ECD, Rx-35)
Peak Area_{RH-7391} =626408524,  Peak Area_{RH-7399} =516386376.
Figure 19. 0.10 μg/ml Standard RH-7391 and RH-7399 (GC/ECD, Rtx-35)
Peak Area RH-7391 = 1299297577, Peak Area RH-7399 = 1035717308.
Figure 20. 0.15 µg/ml Standard RH-7391 and RH-7399 (GC/ECD, Rtx-35)
Peak Area RH-7391 = 1858354530, Peak Area RH-7399 =144959168.
Figure 21. Calibration Curve of RH-7391 (GC/ECD, Rtx-35).
Figure 22: Calibration Curve of RH-7399 (GC/ECD, Rtx-35).
peel control

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

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

External Standard Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal : ECD1 B,
RetTime Type Area Amt/Area Amount Grp Name
(min) [counts/s] [ng/ul]
11.176 ||-----------------||-------------------||----------------|

Totals : 0.00000

Results obtained with enhanced integrator!

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

Figure 23. Control Potato for RH-7281 Analysis (GC/ECD).
RH-7281 Found = 0 ppm
Data File C:\HPCHEM2\DATA\032720\006B0801.D

Sample Name: p3-001

Injection Date : 3/27/2000 7:08:44 PM
Sample Name : p3-001
Acq. Operator : ig
Acq. Method : C:\HPCHEM2\METHODS\PEEL-P.M
Last changed : 3/27/2000 9:14:19 AM by ig
Analysis Method : C:\HPCHEM2\METHODS\PULSOR.M
Last changed : 5/24/2000 1:14:40 PM by dc

RH7281 tomato puree

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

External Standard Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 B

Retention Type Area Amt/Area Amount Grp Name
[Min] counts' s [µg/ml]
----------------- ------- --------- ------- ------- ----------------------
10.796 VB 3.49344e4 2.73048e-7 9.53877e-3 RH7281

Totals : 9.53877e-3

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

Page 1 of 2

Figure 24. Control Potato for RH-7281 Analysis (GC/ECD, 03/24/2000).
RH-7281 Found = 0.00954 ppm
Figure 25. Control Potato for RH-7281 Analysis (GC/ECD, 05/15/2000) RH-7281 Found = 0 ppm
Figure 26. Fortification of RH-7281 at 0.020 ppm (GC/ECD, 03/10/2000)
RH-7281 Found = 0.02477 ppm, Recovery = 124%.
Data File C:\HPCHEM2\DATA\032720\008B1001.D 

Sample Name: p3-003 

Injection Date : 3/27/2000 7:56:03 PM 
Seq. Line : 10 
Acq. Operator : ig 
Vial : 8 
Analysis Method : C:\HPCHEM2\METHODS\PEEL-P.M 
Inj : 1 
Last changed : 3/27/2000 9:14:19 AM by ig 
Inj Volume : 1 μl 
Last changed : 5/23/2000 3:07:10 PM by dc 
(modified after loading) 

RH7281 tomato puree 

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

Sorted By : Signal 
Calib. Date Modified : 5/22/2000 3:02:16 PM 
Multiplier : 1.0000 
Dilution : 1.0000 

Signal 1: ECD1 B, 

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

Totals : 2.37457e-2 


Figure 27. Fortification of RH-7281 at 0.020 ppm (GC/ECD, 03/24/2000) 
RH-7281 Found = 0.02375 ppm, Recovery = 77.1%.
Data File C:\HPCHEM\DATA\051620\008B1001.D

Fortification at 0.02ppm of 97-0078-1

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

---

External Standard Report

Sorted By: Signal
Multiplier: 1.0000
Dilution: 1.0000

Signal: ECD1 B,

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<tr>
<td>10.757</td>
<td>BB</td>
<td>3.23059e4</td>
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<td>1.58663e-2</td>
<td>RH7281</td>
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Totals: 1.58663e-2

Results obtained with enhanced integrator!

Figure 28. Fortification of RH-7281 at 0.020 ppm (GC/ECD, 05/15/2000)
RH-7281 Found = 0.01587 ppm, Recovery = 79.3%
Figure 29. Fortification of RH-7281 at 0.050 ppm (GC/ECD, 03/10/2000)
RH-7281 Found = 0.04501 ppm, Recovery = 90.0%.
Data File C:\HPChEMLDATA\032720\01081201.D

peel fort. 0.05 ppm

Injection Date: 3/27/2000 8:43:31 PM
Sample Name: p3-005
Acq. Operator: ig
Acq. Method: C:\HPChEML\METHODSPeel-P.M
Last changed: 3/27/2000 9:14:19 AM by ig
Analysis Method: C:\HPChEML\METHODSPULSOR.M
Last changed: 5/23/2000 3:07:10 PM by dc
(modified after loading)

RH7281 tomato puree

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

External Standard Report

Sorted By: Signal
Multiplier: 1.0000
Dilution: 1.0000

Signal 1: ECD1 B,

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

Totals: 4.07238e-2


Figure 30. Fortification of RH-7281 at 0.050 ppm (GC/ECD, 03/24/2000)
RH-7281 Found = 0.04072 ppm, Recovery = 68.2%.
Data File C:\HPCHEM\2\DATA\031020\011B1301.D

peel fort. 0.10 ppm

Injection Date : 3/10/2000 8:33:13 PM  Seq. Line : 13
Sample Name : p1-006  Vial : 11
Acq. Operator : ig  Inj : 1
Inj Volume : 1 µl
Sequence File : C:\HPCHEM\2\SEQUENCE\IG0311020.S
Acq. Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 3/3/2000 2:11:59 PM by ig
Analysis Method : C:\HPCHEM\2\METHODS\PEEL-P.M
RH7281 Potato Peel Waste METHOD,
Rtx-5, 30m, 0.53mm, 0.5um film
Flow Rate = 4.0mL/min

---

External Standard Report
---

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal is ECD1 B,

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<td>RH7281</td>
<td></td>
</tr>
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Totals :

9.93644e-2

Results obtained with enhanced integrator!

Figure 31. Fortification of RH-7281 at 0.10 ppm (GC/ECD, 03/10/2000)
RH-7281 Found = 0.09936 ppm, Recovery = 99.4 %.
Data File C:\HPCHEM2\DATA\032720\011B1301.D
Sample Name: P1-006

peel fort. 0.10ppm

Sample Name : p1-006 Vial : 11
Acq. Operator : ig Inj : 11

Inj Volume : 1.0 µl

Acq. Method : C:\HPCHEM2\METHODS\PEEL-P.M
Last changed : 3/27/2000 9:14:19 AM by ig
Analysis Method : C:\HPCHEM2\METHODS\PULSOR.M
Last changed : 5/23/2000 3:07:10 PM by dc
(modified after loading)

RH7281 tomato puree

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

EXTERNAL STANDARD REPORT

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ECD1 B,

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<th>Amount</th>
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<td>[a.u.]</td>
<td>[ug/ml]</td>
<td></td>
<td></td>
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<td>10.791</td>
<td>VV</td>
<td>2.82721e5</td>
<td>3.22520e-7</td>
<td>9.11831e-2</td>
<td>RH7281</td>
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Totals : 9.11831e-2


Figure 32. Fortification of RH-7281 at 0.10 ppm (GC/ECD, 03/24/2000)
RH-7281 Found = 0.09118 ppm, Recovery = 84.8%.
Data File C:\HPCHEM\2\DATA\051620\009B1101.D

Fortification at 0.10 ppm 

Injection Date : 5/16/2000 7:37:16 PM
Sample Name : P-004
Acq. Operator : Ig
Seq. Line : 11
Inj Volume : 1 µl
Vial : 9

Sequence File : C:\HPCHEM\2\SEQUENCE\IG051620.S
Acq. Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 5/13/2000 3:59:01 PM by Ig
Analysis Method : C:\HPCHEM\2\METHODS\PEEL-P.M
Last changed : 5/26/2000 1:38:56 PM by dc

RH7281 Potato Peel Waste METHOD,
RTX-5, 30m, 0.53mm, 0.5µm film
Flow Rate = 4.0ml/min

External Standard Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal : ECD1 B,

Retention Type Area Amt/Area Amount Grp Name
[min] counts [ng/µl]
--- --- --- --- --- ---
10.758 BB 1.34177e5 5.58158e-7 7.48696e-2 RH7281

Totals : 7.48696e-2

Results obtained with enhanced integrator!


Figure 33. Fortification of RH-7281 at 0.10 ppm (GC/ECD, 05/15/2000)
RH-7281 Found = 0.074778 ppm, Recovery 74.8% (as calculated by NRDS)
Data File C:\HPCH\DATA\051620\02081801.D
Sample Name: P-005

Trial Sample Rep 1 97-0078-5

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

Sequence File : C:\HPCH\SEQUENCE\IG051620.S
Acq. Method : C:\HPCH\METHODS\PEEL.P.M
Last changed : 5/13/2000 3:59:01 PM by ig
Analysis Method : C:\HPCH\METHODS\PEEL.P.M
Last changed : 5/26/2000 1:38:56 PM by dc

RH7281 Potato Peel Waste METHOD,
Rtx-5, 10μm, 0.53mm, 0.5μm film
Flow Rate = 4.00mL/min

---

External Standard Report

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

Signal Type: ECD1 B,

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<th>Amt/Area</th>
<th>Amount [ng/μL]</th>
<th>Grp</th>
<th>Name</th>
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<td>BB</td>
<td>113882e5</td>
<td>5.41617e-7</td>
<td>6.16806e-2</td>
<td>RH7281</td>
<td></td>
</tr>
</tbody>
</table>

Totals :
6.16806e-2

Results obtained with enhanced integrator!

---

Figure 34. Trial Sample Replicate 1 for Determination of RH-7281 (GC/ECD, 97-0078), RH-7281 Found = 0.0617 ppm
Figure 35. Trial Sample Replicate 2 for Determination of RH-7281 (GC/ECD, 97-0078), RH-7281 Found = 0.0579 ppm
Figure 36. Control of Potato for RH-7281 Analysis (GC/MSD)
RH-7281 Found = 0 ppm.
Figure 37. Fortification of RH-7281 at 0.02 ppm (GC/MSD)
RH-7281 Found = 0.02089 ppm, Recovery = 104%.
Figure 38. Fortification of RH-7281 at 0.050 ppm (GC/MSD)
RH-7281 Found = 0.04814 ppm, Recovery = 96.3 %.
Figure 39. Fortification of RH-7281 at 0.10 ppm (GC/MSD)
RH-7281 Found = 0.08841 ppm, Recovery = 88.4%.
Figure 40. Control Potato for RH-1452 and RH-1455 Analysis (Rtx-225, 03/10/2000)
RH-7391 Found = 0 ppm, RH-7399 Found = 0.015 ppm.
Figure 41. Control Potato for RH-1452 and RH-1455 Analysis (Rtx-225, 03/24/2000)

RH-7391 Found = 0 ppm, RH-7399 Found = 0.0096 ppm.
Figure 42. Control Potato for RH-1452 and RH-1455 Analysis (Rtx-225, 05/15/2000)  
RH-7391 Found = 0 ppm,  
RH-7399 Found = 0 ppm.
Figure 43. Fortification of RH-1452 and RH-1455 at 0.020 ppm (Rtx-225, 03/10/2000)
RH-7391 Found = 0.01772 ppm, Recovery RH-1452 = 83.6%.
RH-7399 Found = 0.03877 ppm, Recovery RH-1455 = 103%.
Quantitation Report

Data File: C:\HPCHEM\2\DATA\032720\M3-003.D  Vial: 8
Acq On: 3-27-00 20:42:41  Operator: 
Sample: 0.02 ppm fort
Misc: 
IntFile: EVENTS.E

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

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

Volume Inj: 
Signal Phase: 
Signal Info: 

Figure 44. Fortification of RH-1452 and RH-1455 at 0.020 ppm (Rtx-225, 03/24/2000)
RH-7391 Found = 0.01575 ppm,  Recovery RH-1452 = 74.3%.
RH-7399 Found = 0.02144 ppm,  Recovery RH-1455 = 52.9%.
Figure 45. Fortification of RH-1452 and RH-1455 at 0.020 ppm (Rtx-225, 05/15/2000)

RH-7391 Found = 0.01673 ppm, 
RH 7399 Found = 0.01618 ppm,

Recovery RH-1452 = 79.2%
Recovery RH-1455 = 72.1%
Figure 46. Fortification of RH-1452 and RH-1455 at 0.050 ppm (Rtx-225, 03/10/2000)

RH-7391 Found = 0.05359 ppm, Recovery RH-1452 = 101%.
RH-7399 Found = 0.06447 ppm, Recovery RH-1455 = 88.3%.
Figure 47. Fortification of RH-1452 and RH-1455 at 0.050 ppm (Rtx-225, 03/24/2000)

RH-7391 Found = 0.06123 ppm,  
Recovery RH-1452 = 116%.

RH-7399 Found = 0.05786 ppm,  
Recovery RH-1455 = 86.2%.
Figure 48. Fortification of RH-1452 and RH-1455 at 0.10 ppm (Rtx-225, 03/10/2000)

RH-7391 Found = 0.06718 ppm, Recovery RH-1452 = 63.4%.
RH-7399 Found = 0.07801 ppm, Recovery RH-1455 = 56.3%.
Quantitation Report

Data File: C:\HPCHEM\2\DATA\032720\M3-006.D
Acq Off: 3-28-00 8:27:27
Sample: 0.10ppm fort
Misc:

IntFile: EVENTS.E

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

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

Volume Inj.: 
Signal Phase:
Signal Info:

Figure 49. Fortification of RH-1452 and RH-1455 at 0.10 ppm (Rtx-225, 03/24/2000)
RH-7391 Found = 0.09463 ppm, Recovery RH-1452 = 89.3%.
RH-7399 Found = 0.09684 ppm, Recovery RH-1455 = 77.9%.
Figure 50. Fortification of RH-1452 and RH-1455 at 0.10 ppm (Rtx-225, 05/15/2000)
RH-7391 Found = 0.09700 ppm,    Recovery RH-1452 = 91.5%
RH-7399 Found = 0.081986 ppm,    Recovery RH-1455 = 73.2%
Quantitation Report

- Data File: c:\hpchem\2\data\051620\M-005.D
- Acq On: 5-16-00 8:18:39 PM
- Sample: Trial Sample Rep 1
- Misc: 
  - IntFile: EVENTS.E
  - Quant Time: May 30 13:23 19100
  - Quant Results File: PEEL-M.RES
  - Quant Method: C:\\HPCHEM2\METHODS\PEEL-M.M (Chemstation Integrator)
  - Title: 02-29-00
  - Last Update: Tue May 30 12:50:34 2000
  - Response via: Multiple Level Calibration
  - DataAcq Meth: PEEL-M.M

Volume Inj: 
Signal Phase: 
Signal Info: 

Figure 51 Trial Sample Replicate 1 for Determination of RH-1452 and RH-1455 (Rtx- 225, 97-0078), RH-1452 Found < LOD, RH-1455 Found < LOD.
Figure 52  Trial Sample Replicate 2 for Determination of RH-1452 and RH-1455 (Rtx-225, 97-0078),

RH-1452 Found = 0 ppm
RH-1455 Found = 0.0260 ppm
Figure 53. Control Peel for RH-1452 and RH-1455 Analysis (Rtx-35)
RH-7391 Found = 0 ppm, RH-7399 Found = 0.010 ppm,
Figure 54. Fortification of RH-1452 and RH-1455 at 0.020 ppm (Rtx-35)
RH-7391 Found = 0.01824 ppm, Recovery RH-1452 = 86.0%.
RH-7399 Found = 0.03643 ppm, Recovery RH-1455 = 117%.
Quantitation Report

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

IntFile: EVENTS.E

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

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

Volume Inj:
Signal Phase:
Signal Info:

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

Vial: 11
Operator: 
Inst.: GC ECD
Multiplier: X.00
Sample Amount: 0.00

IntFile: EVENTS.E

Quant Time: May 2 7:09 19100

Quant Results File: PEEL-C.RES

Quant Method: C:\HPCHEM\2\METHODS\PEEL-C.M (Chemstation Integrator)
Title: 02-29-00
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Figure 56. Fortification of RH-1452 and RH-1455 at 0.10 ppm (Rtx-35)
RH-7391 Found = 0.1001 ppm, Recovery RH-1452 = 94.4%
RH-7399 Found = 0.1250 ppm, Recovery RH-1455 = 103%
METHYLATION PROCEDURE

1. Introduction

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

2. Apparatus and Materials

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

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

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

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

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

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

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

3.6 The Diazald charge in the apparatus is sufficient to generate enough diazomethane to methylate 5 to 8 samples.
Technical Report 34-00-49

4. Safety

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

4.2 Impinger with visible glass flaws must not be used.

Figure 1. Diazomethane Generator

- Vent
- N₂
- Sample in

Ethyl ether, 10 mL
Di(ethylene glycol) ethyl ether, 5 mL
50% NaOH, 5 mL
Diazald, 0.5g
EPA Addendum For Residue Analytical Method
PP# 9F5058
Zoxamide on Potatoes and Potato Peels

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

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

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

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

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