No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 19(d)(1)(A), (B), or (C).

COMPANY: Rohm and Haas Company

COMPANY AGENT: Ann M. Tillman

DATE: June 6, 1994

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 spirit of Good Laboratory Practices as defined by the United States Environmental Protection Agency.

R. G. Deakins
Research Scientist
Rohm and Haas Company

S. S. Stavinski
Applicant/Submitter
Rohm and Haas Company

Quality Assurance Statement

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

R. S. Krick
QA Analyst
Rohm and Haas Company
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I. Summary

This method has evolved from a previously issued method LM 34-93-170 "Preliminary HPLC Method for RH-5992 in Apples". The preliminary method contained optional clean-up steps and a higher limit of quantitation.

RH-5992 residues are extracted from apples by blending with acidic methanol/water. The extract is partially purified by liquid-liquid partitions and final purification is accomplished by basic alumina column chromatography.

Quantitation is by high performance liquid chromatography with UV detection. The limit of quantitation (LOQ) was demonstrated at 0.02 ppm. Average fortification recovery was 81.3 +/- 11.5 %.

II. Introduction

RH - 5992 is an insecticide being developed for registration in the United States and Canada as well as other nations throughout the world. In order to obtain commercial registration an analytical residue method for apples is required to obtain residue data for the setting and enforcement of tolerances. The structure of RH-5992 is shown below:

![Chemical structure of RH-5992]

Rohm and Haas Number: RH-5992

Chemical Name:
N-(1,1-dimethyllethy)-N'- (4-ethylbenzoyl)-3,5-dimethylbenzohydrazide

CAS Number: 112410-23-8

Common Name: Tebufenozide
III. Method

A. CHEMICALS / SUPPLIES / SOLUTIONS

<table>
<thead>
<tr>
<th>CHEMICALS / SUPPLIES</th>
<th>SUPPLIER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celite</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Dry Ice</td>
<td></td>
</tr>
<tr>
<td>Hydrochloric Acid (concentrated)</td>
<td>Mallinckrodt, Fisher</td>
</tr>
<tr>
<td>Methanol, HPLC reagent</td>
<td>Baker, Baxter</td>
</tr>
<tr>
<td>Hexane, HPLC reagent</td>
<td>Baker, BDH</td>
</tr>
<tr>
<td>RH-5992 Analytical Standard</td>
<td>Rohm &amp; Haas Company</td>
</tr>
<tr>
<td>Sodium Chloride ACS</td>
<td>Fisher Scientific, BDH</td>
</tr>
<tr>
<td>Sodium Sulfate</td>
<td>Fisher Scientific, BDH</td>
</tr>
<tr>
<td>Water HPLC</td>
<td>Waters HPLC grader or Milli-Q</td>
</tr>
<tr>
<td>Methylene Chloride, HPLC reagent</td>
<td>Baker</td>
</tr>
<tr>
<td>0.45 micron filters, Acrodisc</td>
<td>VWR</td>
</tr>
</tbody>
</table>

COLUMN CHROMATOGRAPHY CLEAN UP

| Aluminum oxide, Basic                     | Baker # 0539-01 |

ALTERNATIVE CLEAN-UP:

| Florisil (5%) Deactivated 60-100 Mesh | U.S Silica, Fisher |

SOLUTIONS

| Hydrochloric Acid 0.1 N Aqueous          |                          |
| Extraction Solvent: 1 part 0.1 N HCl (aq) + 9 parts Methanol (v/v) |                          |
| Sodium Chloride 10% water solution      |                          |
| 5% Ethyl Acetate / Hexane               |                          |
| 10% Ethyl Acetate / Hexane              |                          |
| 20% Ethyl Acetate / Hexane              |                          |
| Mobile Phase 67% MeOH/ Water            |                          |
### B. EQUIPMENT

<table>
<thead>
<tr>
<th>EQUIPMENT</th>
<th>Brand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blender, Explosion Proof</td>
<td>Waring</td>
</tr>
<tr>
<td>Buchner Funnels and Vacuum flasks</td>
<td>Fisher</td>
</tr>
<tr>
<td>Round Bottom Flasks</td>
<td>Pyrex</td>
</tr>
<tr>
<td>Rotary Evaporator</td>
<td>Buchi, Janke &amp; Kunkel</td>
</tr>
<tr>
<td>Hobart Food Chopper</td>
<td>Hobart Manufacturing Co.</td>
</tr>
<tr>
<td>Separatory Funnel</td>
<td>Pyrex</td>
</tr>
<tr>
<td>Standard Laboratory Equipment:</td>
<td></td>
</tr>
<tr>
<td>balances, beakers, etc.</td>
<td>Metler, Pyrex, Sertorius</td>
</tr>
<tr>
<td>Chromatographic Columns</td>
<td>ACE Glass</td>
</tr>
<tr>
<td>14.5 mm ID by 25 mm</td>
<td>Cat. # 5907-10</td>
</tr>
<tr>
<td>Alternative Extractions:</td>
<td></td>
</tr>
<tr>
<td>Polytron</td>
<td>Kinematica CH-6010</td>
</tr>
<tr>
<td>250 ml polypropylene centrifuge bottles</td>
<td>Various manufacturers</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>Sorvall RC2-B</td>
</tr>
<tr>
<td>Chromatographic column</td>
<td></td>
</tr>
<tr>
<td>30 x 2.5 (id ) cm</td>
<td>Various manufacturers</td>
</tr>
</tbody>
</table>

**NOTE:** Other manufacturer brands may be substituted if they can be shown to be suitable.
C. Instrumentation

Samples were analyzed using a Waters Associates (Millipore) High Performance Liquid Chromatograph configured as follows:

<table>
<thead>
<tr>
<th>Auto Sample</th>
<th>WISP 710B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pump I</td>
<td>Model 510 (only one pump required)</td>
</tr>
<tr>
<td></td>
<td>Chromatography Pump</td>
</tr>
<tr>
<td>Detector:</td>
<td>Spectra-Physics UV 2000 Detector (UV.)</td>
</tr>
<tr>
<td>Column Heater</td>
<td>Water YCM</td>
</tr>
<tr>
<td>System Control</td>
<td>I. NEC Powermate 2</td>
</tr>
<tr>
<td></td>
<td>II System Interface Module</td>
</tr>
<tr>
<td>Software</td>
<td>Maxima 820</td>
</tr>
<tr>
<td></td>
<td>(Dynamic Solutions, Division of Millipore)</td>
</tr>
<tr>
<td>Data System</td>
<td>Hardware: Hewlett Packard Series 9000</td>
</tr>
<tr>
<td></td>
<td>Model 300</td>
</tr>
<tr>
<td></td>
<td>Software: 4400 Chromatography Software</td>
</tr>
<tr>
<td></td>
<td>(Nelson Analytical, Inc)</td>
</tr>
</tbody>
</table>

Any other suitable system may be used for analysis after verifying system suitability.

HPLC column:
- Adsorbsphere C-18 5 micron (Alltech Applied Science)
- Length: 25 cm
- I.D: 4.6 mm

Conditions
- Flow: 2 ml/min
- Column Temperature: 55 degrees C
- Wavelength: (235 to 260 nm, 0.1 AUFS)
- Injection size: 25-50 ul

Any single wavelength between 235 mm and 260 mm may be used. The Spectra-Physics UV 2000 detector was used at 240 nm.

Mobile Phase: 67% MeOH/water

Other instrumentation (Waters 600 E) and columns (SupercoLC18) have been used. Any other combination of C-18 column and instrumentation brand could be used as long as a 0.05 ug/ml (or lower) standard can be quantified and no interferences exist. A wavelength of 254 nm and a flow of 1.6 ml/min. has also been used successfully. Variation of HPLC parameters are allowed to optimize any HPLC system.
D. Preparation of Standards

(1) Stock solution

Weigh a known amount of analytical standard between 10 and 100 μg into a tared 50 ml beaker. Dissolve the standard in methanol, dissolving with several small portions, then carefully transfer the solution to a 100 ml volumetric flask and bring to volume. This stock solution is between 100 μg/ml and 1000 μg/ml depending on the actual weight and purity of the standard taken. Store frozen at -10 +/- 8 °C Remake at one year intervals.

(2) Intermediate Standard

A 10 μg/ml intermediate standard is made by taking an accurate volume of stock solution (1) to a precise volume with the appropriate HPLC solvent. For example, 10 ml of a 100 μg/ml stock solution (100 % MeOH) diluted to 100 ml with 63 % MeOH/water would yield a 10 μg/ml solution in 67 % MeOH/water.

Working Standards

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Take</th>
<th>Dilute to (with mobile phase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(3) 1.0 μg/ml</td>
<td>10 ml of (2)*</td>
<td>100 ml</td>
</tr>
<tr>
<td>(4) 0.5 μg/ml</td>
<td>5 ml of (2)</td>
<td>100 ml</td>
</tr>
<tr>
<td>(5) 0.1 μg/ml</td>
<td>10 ml of (3)</td>
<td>100 ml</td>
</tr>
<tr>
<td>(6) 0.05 μg/ml</td>
<td>5 ml of (3)</td>
<td>100 ml</td>
</tr>
<tr>
<td>(7) 0.02 μg/ml</td>
<td>2 ml of (3)</td>
<td>100 ml</td>
</tr>
<tr>
<td>(8) 0.01 μg/ml</td>
<td>1 ml of (3)</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

All intermediate and working standards should be kept refrigerated and be remade at 6 month intervals.

* For these working standards, the intermediate solution (2) is assumed to be exactly 10 μg/ml.
E. Analytical Procedure

1. Sample Processing
   Apple samples are homogenized with a Hobart Food Chopper or other suitable food processor. The food chopper is first pre-chilled with dry ice. Apples are quartered and added to the food chopper bowl with dry ice. The samples are thoroughly chopped and homogenized and then collected in plastic containers. The dry ice is allowed to sublime in a freezer overnight and the samples are stored frozen at -15 +/- 10 °C until analysis.

2. Diagram
   A flow diagram of the method is shown below. The individual steps are detailed in the following diagram.

   Step 1  Extraction 20 g with acid/water/methanol
   Step 2  Hexane Partition
   Step 3  Methylene Chloride Partition
   Step 4  Concentration
   Step 5  Column Chromatography Clean-Up step
   Step 6  Quantitation HPLC

3. Detailed Method

   The original method development work was done at Rohm & Haas Company, which then issued preliminary method LM 34-93-170 "Preliminary HPLC method for RH-5592 in Apples". A Canadian analytical laboratory, Envero-Test Laboratories¹, then validated the method under Rohm & Haas protocol 34P-94-03, making minor changes in the method after consulting with the author. Further method development was done at Rohm & Haas to verify the changes and demonstrate a lower limit of quantitation (LOQ). The detailed method that follows includes a recommended procedure with variations which were used successfully. A comparison of results over time as the method has evolved show excellent agreement (see Section V Results and Discussion). The purpose of presenting a recommended procedure with variations that have been verified, is to increase the ruggedness of the method and permit allowable modifications at different laboratories based on a laboratory's analytical equipment, reagents and preference.

¹ Envero-Test Laboratories, 9936-67 Avenue, Edmonton, Alberta T6E,0P5, Canada.
STEP 1  **Recommended Extraction, Filtration**
Weigh 20 grams (+/- 0.1 grams) of processed apple into a blender jar. Add about 5 grams of Celite filter aid and 100 ml of extraction solvent (MeOH/0.1 N HCl aqueous 9:1 v/v). Blend at medium speed for about two minutes. Filter with a Buchner funnel by vacuum, rinsing the blender jar with several small portions of extraction solvent totaling 50 ml. Add the rinses over the filter cake. Total volume of the filtrate will be approximately 150 ml. Transfer the filtrate to a 500 ml separatory funnel.

**ALTERNATIVE EXTRACTION; Polytron, Centrifuge:**
Weigh 25 grams (+/- 0.1 g) into a 250 ml polypropylene centrifuge bottle (Celite not added.) Add 100 mls of extraction solvent and blend with a polytron tissue homogenizer for about two minutes. Centrifuge at 5000 rpm and collect the supernatant in a 1000 ml separatory funnel. Add 20 mls of extraction solvent to the pellet, shake for about 1 minute and centrifuge, as described above. Collect the supernatant in the same 1000 ml separatory funnel.

STEP 2  **Hexane Partition**
Add 15 mls of 10% sodium chloride solution to the extract in the separatory funnel. Partition the extract with 30 mls of hexane. Discard the hexane (upper phase). For highly colored extracts, a second partition with hexane may be used.

STEP 3  **Methylene Chloride Partition**
Add an additional 150 mls of 10% sodium chloride solution to the extract. Partition the extract with a 2 X 100 ml portions of methylene chloride (lower phase), shaking vigorously for about one minute for each partition. Drain each methylene chloride fraction through approximately 15 grams of sodium sulfate in a glass funnel plugged with glass wool, into a 500 ml round bottom flask.

STEP 4  **Concentration**
Concentrate, at ambient temperature, the methylene chloride to dryness under vacuum with a rotary evaporator. For the initial work with apples, the samples were taken up in mobile phase and quantified by HPLC. The following clean-up step is recommended.

---

2 This is a minimum amount to add. Up to 300 mls of sodium chloride solution may be added but that amount requires the use of a 1000 ml separatory funnel.
**STEP 5**  

**Recommended Clean-up Step**

**Basic Alumina Column Clean-Up**

Place a small plug of glass wool or cotton into the bottom of 14.5 mm (id) X 25 cm (ut) chromatography column. Measure 15 ml of basic alumina (packed) and pour into the column. Add about 1 cm of sodium sulfate to the top of the column bed and then wash the column with 25 ml of hexane. Drain the hexane wash to about 2 mm above the column bed, do not let the column run dry until the final elution.

Dissolve the residue from step 4 in 25 ml of 5% ethyl acetate/hexane. Apply the sample to the column. Rince the sample container with 10 ml of 5% ethyl acetate/hexane and add to the column after the initial 25 ml has drained to about 2 mm above the column bed. Discard these eluents. Wash the column with 50 ml of 10% ethyl acetate/hexane. Discard the eluent.

Elute the RH-5992 residues from the column with 150 ml of 20% ethyl acetate/hexane, collecting the eluent in a 300 ml round bottom flask. Concentrate the eluent to dryness with a rotary evaporator (45-55°C). Take up the residue in an appropriate amount of mobile phase (usually 10 ml) and put solution through a 0.45 μ filter disk, if necessary, prior to HPLC injection.

**Alternative Clean-up Step.**

**Concentration**

The sample was concentrated to about 1-2 ml using a roto-evaporator at 40°C. The extract was quantitatively transferred to a 35 ml vial with methylene chloride and brought to a 20 ml final volume using a nitrogen evaporator.

**Florisil Cleanup**

A glass column (30 cm X 2.5 cm, with a 250 ml reservoir) was dry packed with 25g of 5% deactivated Florisil. The column was preconditioned with 25 ml of hexane before 1 ml of the 2 ml final volume extract was applied onto the column. The column was eluted with 50 ml of 5% ethyl acetate/hexane as a wash, which was discarded. The RH-5992 was eluted with 150 ml of 15% ethyl acetate/hexane into a 250 ml round bottom flask.

*Note: For either clean-up chosen, it is necessary to standardize the absorbent (alumina or Florisil) in the following manner, before analyzing samples.*
A. Using a standard between 2 and 10 μg/ml of RH-5992, follow the elution schemes as outlined under the procedure

B. Collect the pre-elution cuts, as well as the target elution cuts

C. Collect a post-cut consisting of a small volume of solvent, which is at least equal in solvent strength to the target elution solvent

D. Concentrate the pre-cut, target-cut and post-cut to dryness.

E. Dissolve the cuts in an appropriate amount of mobile phase and inject them as outlined under HPLC quantitation.

F. If the target cut contains minimum of 85% of the RH-5992 material, it may be considered acceptable

G. If the 85% recovery criteria for standard is not met, the analyst may adjust the elution scheme as follows:

1) For cases where the prewash cut contains significant standard, either the prewash amount or eluting solvent percentage may be decreased.

2) For cases where the post wash cut contains significant standard, the target elution volume may be increased or the eluting solvent percentage may be increased.

IV. High Performance Liquid Chromatography Quantitation

A. Standards

A minimum of four standard solutions are prepared in the concentration range of 0.01 μl/ml to 2.0 μl/ml. In order to quantify at lower sensitivities, a range of 0.01 μg/ml to 0.5 μg/ml would be acceptable, if less sensitivity is desired, a range of 0.05 μg/ml to 2.0 μg/ml would be acceptable. Inject samples at the same volume (between 50-150 μl) as RH-5992 standards. If necessary, the samples are diluted to an appropriate volume to give a response within the standard curve range.

Standards and samples are preferably quantitated by peak height, although peak area may be used. An HPLC run should be have at least two sets of standards. A standard curve is constructed from each day’s standards.

Equation 1  

\[ \text{component concentration (μg/ml)} \times \text{final volume (ml)} = \text{ppm} \]

\[ \text{sample weight(g)} \]
B. Fortification recovery

Control samples are fortified with known amounts of RH-5992 prior to extraction.

Equation 2: fortification recovery

\[
\frac{(\text{found (µg/ml) X final vol. (ml)} - \text{ctl. correction (µg)})}{\text{fortification amount (µg)}} = \% \text{ Recovery}
\]

V. Results and Discussion

The statistical data for the recovery of fortifications are summarized in Table I following this discussion. Overall recovery for 25 fortifications was 81.3±11.5%. The limit of quantitation (LOQ) was demonstrated down to 0.02 ppm (although 1 fortification at 0.01 ppm was tried). This LOQ, 0.02 ppm, should be sufficient considering the level of residue found in treated samples.

Some treated apple samples were analyzed in 1992 as part of the method development process. These same samples were analyzed in 1994 at Enviro-Test Labs with the preliminary method (LM-34-93-170) and the Florasol clean-up which is included as the alternative clean-up in this method. Table II lists the results of the initial and Enviro-Test Labs results. Appendix I has examples of chromatography. Appendix II contains the confirmation detection of residues.
Table I

Statistical Recovery

All Recoveries

<table>
<thead>
<tr>
<th>Count</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>81.3</td>
<td>11.5</td>
<td>64</td>
<td>108</td>
</tr>
</tbody>
</table>

Recovery By Fortification Level

<table>
<thead>
<tr>
<th>ppm Level</th>
<th>Count</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>1</td>
<td>70</td>
<td>-</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>0.02</td>
<td>3</td>
<td>73.3</td>
<td>9.5</td>
<td>66</td>
<td>84</td>
</tr>
<tr>
<td>0.059</td>
<td>6</td>
<td>87.8</td>
<td>14.2</td>
<td>68</td>
<td>108</td>
</tr>
<tr>
<td>0.1</td>
<td>2</td>
<td>69.5</td>
<td>4.9</td>
<td>66</td>
<td>73</td>
</tr>
<tr>
<td>0.25</td>
<td>1</td>
<td>75</td>
<td>-</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>0.28</td>
<td>6</td>
<td>80.2</td>
<td>11.7</td>
<td>64</td>
<td>96</td>
</tr>
<tr>
<td>0.79</td>
<td>6</td>
<td>86.8</td>
<td>6.7</td>
<td>79</td>
<td>96</td>
</tr>
</tbody>
</table>

Table II

Comparison of Results (ppm found)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Rohm and Haas (3/92)</th>
<th>Enviro-Test (2/94)</th>
</tr>
</thead>
<tbody>
<tr>
<td>91-0094-006</td>
<td>0.786</td>
<td>1.2</td>
</tr>
<tr>
<td>91-0074-001</td>
<td>0.44</td>
<td>0.53</td>
</tr>
<tr>
<td>91-0074-004</td>
<td>0.496</td>
<td>0.49</td>
</tr>
</tbody>
</table>
APPENDIX I
TR-34-94-38

Date file: 0311942  Type: STANDARD

Interface: 713  Cycled: 2  Channel: A

Instrument: LC-67-12  Column: AD500BROSPHERE C18
Particle Size (um): SUN  Column Length (cm): 25C  Column I.D. (mm): 4.6MM
Mobile Phase: 675/HEX/WATER
Gradient Time (min): 15  Grad. Slope (#), 6  Flowrate (mL/min): 20L/M
Back Pressure (psi): Temperature (deg): 55
Detector 1: UV2000  Detector 2:

Plot times: 0 to 15 minutes
Plot range: 50 millivolts (1 mV offset)

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Compound</th>
<th>PPM</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.89</td>
<td>5992</td>
<td>0.500</td>
<td>1.350E+02</td>
<td>5.300E+05</td>
</tr>
</tbody>
</table>

STANDARDS
ROHM AND HAAS HPLC SYSTEM

BEST AVAILABLE COPY
Data file: 0311943  Type: STANDARD

Date: 11 Mar 1994 12 53  Method 5992.8Aa  Analyst JC
Interfaces F13  Cycle: 3  Channel: A

Instrument: LC-87-12  Column: ADSORBOSPHERE C18
Particle Size (um): 5.0  Column Length (cm): 25 CM  Column I.D. (mm): 4.6 mm
Mobile Phase: 6%HEON/84%  Gradient Time (min): 15  Grad Slope (%): 6
Flowrate (mL/min): 24.7  M
Back Pressure (psi):  Rail Temperature (deg): 55
Detector 1: UV2000  Detector 2:

Plot times: 0 to 15 minutes
Plot range: 50 millivolts (±5 mv offset)

Retention  Compound  PPM
Time  Name  Injected  Area  Height
4.06  5992  0 100  2 560E+01  1 670E+03

5992
Sample Name: APPLE
Data: 11 Mar 1994 13:09
Method: 5992:23Dm
Cal Curve: 3/11/94
Cycl/Cycle: 4
Channel(s): A

Instrument: LC-97-12
Column: ADSORBOSHERE C18
Particle Size (µm): 5µm
Column Length (cm): 25 cm
Column ID (mm): 4.6 mm
Running Phase: NITROGEN
Gradient Time (min): 15
Grad. Slope (#): 6
Flowrate (mL/min): 3mL/min
Back Pressure (psi): 65
Temperature (deg): 55
Detector 1: uv2000
Detector 2:

Plot times: 0 to 15 minutes
Plot ranges: 50 millivolt (±33 mv offset)

Retention Time | Compound | PPM
---|---|---
6.01 | 5992 | 0.050 | 260E-01 | 5 520E-02
Data file: 0311945  Type: STANDARD

Sample Name: APPLE  Cal Curve: 3/11/94
Date: 11 Mar 1994 13 26  Method: 5992/IBM  Analyst: JC
Interface: 713  Cycled: 5  Channel#: A

Instrument: LC 87-12  Column: AEGISORB SPHERE C18
Particle Size (μm): 5  Column Length (cm): 25CM  Column I.D. (mm): 4.6MM
Mobile Phase: 67% MEON/WATER
Gradient Time (min): 15  Grad. Slope (δ): 6  Flowrate (mL/min): 2.0mL
Back Pressure (psi):  55  Temperature (deg): 55
Detector 1: uv2000  Detector 2:

Plot times: 0 to 15 minutes
Plot range: 50 millivolts (-0.56 mv offset)

<table>
<thead>
<tr>
<th>Time</th>
<th>Compound</th>
<th>PPM</th>
<th>Injected</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.78</td>
<td>5992</td>
<td>0.020</td>
<td>5.160E+00</td>
<td>2.290E+02</td>
<td></td>
</tr>
</tbody>
</table>
Data file: 0311946  Type: STANDARD
Date: 11 Mar 9994  13:42  Method: 5992/8m  Analyst: JC
Interface: 713  Cycles: 6  Channel: A

Instrument: LC 67-12  Columns: ADSORBOSPHERE C18
Particle Size (um): 5.0  Column Length (cm): 25CM  Column I.D. (mm): 4.6MM/
Mobile Phase: 67%NEON/WATER
Gradient Time (min): 15  Grad. Slope (#): 6  Flowrate (mL/min): 2ML/M
Back Pressure (ps):  Temperature (deg): 55
Detector 1: uv2000  Detector 2:

Plot times: 0 to 15 minutes
Plot range: 50 millivolts (-26 mv offset)

Retention  Compound  PPM
Time  Name  Injected  Area  Height
6 069  5992  0 010  2 450E+00  1 220E+02

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Data file: 03119413

Sample Name: APPLE
Date: 11 Mar 1994 15:58
Method: S992J8m
Cal Curve: 5/11/94
Interc: 713
Cycle#: 13
Channel#: A

Instruments: LC-87-12
Column: ADSORBOSPHERE C18
Particle Size (um): 5.0
Column Length (cm): 25
Mobile Phase: 67% TFA/WATER
Gradient Time (min): 15
Grad. Slope (#): 6
Flowrate (mL/min): 2.0
Back Pressure (psi): 15
Temperature (deg): 55
Detector 1: uv2000
Detector 2:

Plot times: 0 to 15 minutes
Plot range: 50 millivolts (20 mv offset)

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Compound</th>
<th>PPM</th>
<th>Injected</th>
<th>Area</th>
<th>Height</th>
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</thead>
<tbody>
<tr>
<td>6.36</td>
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<td>300</td>
<td>1340E+02</td>
<td>5.84E+03</td>
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</table>

5992
Sample Name: APPLE
Date: 11 Mar 1994
Method: 5992
Analyzer: JC
Cycled: 14
Chrom: A
Instrument: LC 67-12
Column: AGSDORBUSPHAC A C18
Particle Size (μm): 5
Column Length (cm): 25CM
Column I.D. (mm): 4.6MM
Mobile Phase: 67% MEK/WATER
Gradient Time (min): 15
Grad Slope (%): 6
Flowrate (ml/min): 20L/M
Back Pressure (psig): 55
Temperature (deg): 55
Detector 1: uv2000
Detector 2:
Plot time: 0 to 15 minutes
Plot range: 50 millivolts (55 mv offset)

Retention Time | Compound | PPM |
--- | --- | --- |
5 | 5992 | 0.00 | 2.530E+01 | 1.390E+03

Best Available Copy
**Data file:** Q3119415  
**Type:** STANDARD

---

**Sample Name:** APPLE  
**Col Curve:** 3/11/94

**Date:** 11 Mar 1994  
**Method:** 5992JRes  
**Analyst:** AC  
**Interface:** 713  
**Cycle:** 15  
**Channel:** A

---

**Instrument:** LC 67-12  
**Column:** ADSORBOSPHERE C18  
**Particle Size (μm):** 50  
**Column Length (cm):** 25  
**Column ID (mm):** 4.6

**Mobile Phase:** 67%NEON/WATER  
**Gradient Time (min):** 15  
**Grad Slope (#):** 6  
**Flowrate (mL/min):** 2ML/IN

**Back Pressure (psi):**  
**Temperature (deg):** 55

**Detector 1:** UV2000  
**Detector 2:**

---

**Plot times:** 0 to 15 minutes  
**Plot range:** 50 millivolts (60 mv offset)

---

<table>
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<th>Compound</th>
<th>PPM</th>
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<tbody>
<tr>
<td>5992</td>
<td>0 050</td>
<td>1.29OE+01</td>
<td>5 89OE+02</td>
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</table>
TR 34 94-38

Data file 03119416 Type STANDARD
Sample Name APPLE Cal Curve: 3/11/94
Date 11 Mar 1994 16:44 Method 5992j8m Analyst JC
Interface 713 Cycles 16 Channel#: A

Instrument LC 6712 Column AODORBOSPHENE C18
Particle Size (um) 5.0 Column Length (cm) 25CM Column I.D. (mm): 4.6 mm
Mobile Phase: 67%TMEON/WATER
Gradient Time (min): 15 Grad Slope (#): 6 Flowrate (mL/min): 2.0 mL/min
Back Pressure (psi): Temperature (deg): 55
Detector 1 UV2000 Detector -

Plot times 0 to 15 minutes
Plot range 50 millivolts ( 81 mv offset)

<table>
<thead>
<tr>
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<th>PPM</th>
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<tr>
<td>Time</td>
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<td>Injected</td>
</tr>
<tr>
<td>6.32</td>
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<td>0.020</td>
</tr>
</tbody>
</table>

BEST AVAILABLE COPY
Data file: 03119417  Type: STANDARD

Date: 11 Mar 1994 16:48  Method: 5992J/Nm  Analyst: JF
Interface: 713  Cycles: 17  Channel#: A

Instrument: LC-87-12  Column: ADSORBOSPHERE C18
Particle Size (μm): 5  Column Length (cm): 250  Column I.D. (mm): 4.6
Mobile Phase: 67% MEON/WATER
Gradient Time (min): 15  Grad Slope (°): 6  Flowrate (mL/min): 300/30
Back Pressure (psig): 55  Temperature (deg): 55
Detector 1: 2000  Detector 2:

Plot time: 0 to 15 minutes
Plot range: 50 milliV (±3 mm offset)

Retention | Compound | PPM
Time | Name | Injected | Area | Height
--- | --- | --- | --- | ---
3.32 | 5992 | 0.010 | 2700E+00 | 1.220E+02
Concentrations in report are calculated from equation:
HEIGHT = B0 + B1(UG/ML)
obtained by least-squares fit of standard injection data.
APPLE CONTROL
BASIC ALUMINA CLEAN-UP
DESIGNATED AS SAMPLE 1 IN APPENDIX II (METHOD CONFIRMATION)
APPLE FORTIFICATION 0.02 PPM
BASIC ALUMINA CLEAN-UP
DESIGNATED AS SAMPLE 2 IN APPENDIX II (METHOD CONFIRMATION)
Data file: 03119412  EAR number: 94-0000
Method file: 5992.7Ab  Sample No: 001
Type: FORTIFICATION  Component: APPLE

Sample Name: APPLE  Cal. Curve: 3/17/94
Date: 11 Mar 1994  15:56  Methods: 5992.7Ab  Analyst: JC
Interface: 713  Cycle: 12  Channels: A

Instrument: LC-67-12  Column: ABDZORBOSPHAER CH
Particle Size (um): 5  Column Length (cm): 25 cm  Column I.D. (mm): 4.6 mm
Mobile Phase: 67% MECH/WATER
Gradient Time (min): 15  Grad. Slope (#): 6  Flowrate (mL/min): 2.0 mL/min
Back Pressure (psig): 0  Temperature (deg): 55
Detector 1: UV/2000  Detector 2: 

Plot times: 0 to 15 minutes
Plot range: 50 millivolts (-70 mv offset)

<table>
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<tr>
<th>Ret Time</th>
<th>Compound</th>
<th>Peak Area</th>
<th>Peak Height</th>
<th>ug/mL</th>
<th>Volume Ctl</th>
<th>ug Ctl</th>
<th>ug Found</th>
<th>Pct Recovery</th>
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</thead>
<tbody>
<tr>
<td>5.35</td>
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<td>4320000</td>
<td>.163E+04</td>
<td>0.143</td>
<td>10.0</td>
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APPLE FORTIFICATION 0.1 PPM
BASIC ALUMINA CLEAN-UP
DESIGNATED AS SAMPLE 3 IN APPENDIX II (METHOD CONFIRMATION)
RH-5992
FEBRUARY 2/94

![Graph showing linearity curve data](image)

### COMPND: RH-5992

#### LINEARITY CURVE DATA

<table>
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<tr>
<th>CHROM.</th>
<th>CONC (PPM)</th>
<th>PK. AREA</th>
<th>Corrected PK. AREA</th>
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<td>1</td>
<td>0.00</td>
<td>0</td>
<td>0.00</td>
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<tr>
<td>2</td>
<td>0.059</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>0.118</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>0.183</td>
<td>99</td>
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<td>136</td>
</tr>
<tr>
<td>6</td>
<td>0.118</td>
<td>24</td>
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</tr>
<tr>
<td>7</td>
<td>0.183</td>
<td>99</td>
<td>136</td>
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<tr>
<td>8</td>
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<tr>
<td>9</td>
<td>0.183</td>
<td>99</td>
<td>136</td>
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<tr>
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</tr>
<tr>
<td>16</td>
<td>0.183</td>
<td>99</td>
<td>136</td>
</tr>
</tbody>
</table>

**Sample Data: Validation Day 1**

<table>
<thead>
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<th>CHROM.</th>
<th>SAMPLE LD.</th>
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<th>SAMPLE</th>
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<td>(E4-E1-140-09A)</td>
<td>AREA</td>
<td>CONC (PPM)</td>
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<tr>
<td>4</td>
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<td>5</td>
<td>CTRL2</td>
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<td>0.00</td>
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<tr>
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<td>7</td>
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<td>0.052</td>
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<td>8</td>
<td>SPK+2A</td>
<td>113</td>
<td>0.23</td>
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<tr>
<td>9</td>
<td><strong>SPK+2B</strong></td>
<td>118</td>
<td>0.24</td>
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<tr>
<td>10</td>
<td><strong>SPK+3A</strong></td>
<td>317</td>
<td>0.64</td>
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<td>12</td>
<td><strong>SPK+3B</strong></td>
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<td>13</td>
<td><strong>SPK+3B</strong></td>
<td>168</td>
<td>0.67</td>
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</tbody>
</table>

**Note:** Spike 2B and 3B to be re-run due to integrator malfunction.

Sample used was 91-0074-001(E4-E1-140-09A)

Ref. RH5992V1LWQ?

### Regression Output

- Constant: 0.00
- Std Err of Y Est: 3.51
- R Squared: 1.000
- No. of Observations: 9
- Degrees of Freedom: 8

<table>
<thead>
<tr>
<th>X Coefficient(s)</th>
<th>Std Err of Coef.</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
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</tbody>
</table>

Based on a 25g sample and 10 mL final volume which takes into account the aliquot factor.

See Residue Analysis Worksheets for more complete data.
TR-34-94-38

CHART SPEED 1.0 CM/MIN
ATTACH 0 ZERO 26% 1 MIN/TICK

0 1 2 3 4 5 6 7 8 9 10 11

TITLE RH 5992
METHOD NO 5992
SAMPLE 2 0.59UG ML

RESULT 100.0000
TIME 1.335E-06
AREA 102
QUANT 10254

MULTIPLIER 1 00000

S:VED FILE 5992235

NOTES:
LIT 18 #1000001 T 99.5 55°C
WAVELENGTH 254, 15ML, 1M1
1 uL/MIN 65MCM/ML/WATER

VALIDATION DAY 1

Instrument: Waters 600E
Column: Supelco LC 18
Column Length (cm) 25
Column I.D. 4.6 mm
Mobile Phase: ACN/H2O/water
Flowrate (ml/min): 1.6
Particle size (pm): 5
Temperature: 55°C
Detector: UV
Auto sampler: VISP 712
Injection volume (uL): 150

STANDARDS
Enviro-Test Labs HPLC System
TR-34.94.33

TITLE: RH 5992

CHANNEL NO 1

SAMPLE CTCL-22-10

PEAK NO NAME RESULT TIME AREA SEPC

1 72 3219 2.747 2903 8B
2 44 4220 18 900 4743 5V
3 29 2261 11 432 3192 V8

TOTALS 180 0000 16918

MULTIPLIER: 10000

SAVED FILE 5992223

NOTES:
LC 18 40000001 TEMP 55°C
WAVELNGTH 254, 150UL. INJ
1 ML/IN 05%REOH/WATER
VALIDATION DAY 1

Instruments: Waters 600E
Columns: Superior LC 18
Porlta size (mm): 5
Column Length (cm): 25
Column i.D.: 4.6 mm

Mobile Phase: 65% ReOH/WATER
Flowrate (ML/Min): 1.6
Temperature: 55°C
Detector: Water 486, Variable Absorbance Detector UV 254
Auto samplers WISP 752
Injection Volume (ML): 150

APPLE CONTROL
FLORISIL CLEAN-UP
APPLE FORTIFICATION 0.059 PPM
FLORISIL CLEAN-UP
APPLE FORTIFICATION 0.28 PPM
FLORISIL CLEAN-UP
APPLE FORTIFICATION 0.79 PPM
FLORISIL CLEAN-UP
CONFIRMATION DETECTION of RESIDUES

In order to confirm that residues found by the method are due to RH-5992 and not due to interferences, a different HPLC method has been developed. In the confirmatory detection method, both the mobile phase and column used are different. The mobile phase is 50% Acetonitrile / Water and the column is a Supelco LC - DP (phenyl). This column's separation chemistry is very different from a C18 column. The chromatography system and other conditions used are the same as the conditions used for the C18 column as stated on page 8 of this report and the chromatogram instrument header on the following examples. The control and two fortifications shown are re-injections of C18 column injections that have been marked in Appendix L.
TR-34-94-38

Data file:  J31042
Type:  STANDARD

Sample Name:  
Cal. Curve:  3/14/94
Date:  14 Mar 1994  17:16  Method:  5992L0m
Operator:  JC
Interface:  713
Cycles:  2
Channels:  A

Instrument:  LC-67-12
Column:  LC-0P
Particle Size (µm):  5U
Column Length (cm):  25cm
Column I.D. (mm):  4.6mm
Mobile Phase:  50% ACN/WATER
Gradient Time (min):  
Grad. Slope (°):  6
Flowrate (mL/min):  2mL/min
Back Pressure (psi):  
Temperature (deg):  55
Detector 1:  uv/200
Detector 2:

Plot times:  0 to 10 minutes
Plot range:  75 millivolts (± 4 mv offset)

<table>
<thead>
<tr>
<th>Retention Time (min)</th>
<th>Compound Name</th>
<th>PPM</th>
<th>Injected</th>
<th>Area</th>
<th>Height</th>
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<tbody>
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<td>0</td>
<td>100</td>
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<td>530E-01</td>
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BEST AVAILABLE COPY
Sample Name: 034943  
Type: STANDARD  
Cal. Curve 3/14/94  
Date: 14 Mar 1994 17:28  
Method: 5992  
Operator: JC  
Interfaces: 713  
Channels: A  
Cycles: 3  
Instrument: LC-EF-12  
Column: LC DP  
Particle Size (um): SOL  
Column Length (cm): 25CM  
Column I.D. (mm): 4.6MM  
Mobile Phase: 50% ACN/WATER  
Gradient Time (min):  
Grad. Slope (F): 6  
Flowrate (mL/min): 20ML/M  
Back Pressure (psi):  
Temperature (deg): 55  
Detector 1: UV2000  
Detector 2:  
Plot times: 0 to 10 minutes  
Plot range: 75 millivolts (+41 mv offset)  

<table>
<thead>
<tr>
<th>Retention Time (min)</th>
<th>Compound</th>
<th>Name</th>
<th>PPM</th>
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<th>Height</th>
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<tbody>
<tr>
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<td>4.650E+02</td>
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</tr>
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</table>
**TR-34-94-38**

**Date file:** J314944
**Types:** STANDARD

---

**Sample Name:**
**Date:** 14 Mar 1994 17 39
**Method:** 5992JCM
**Operator:** JC
**Interface:** 713
**Cycle:** 6
**Channel:** A

---

**Instrument:** LC-87-12
**Column:** LC-8P
**Particle Size (μm):** SUN
**Column Length (cm):** 25CN
**Column I.D. (mm):** 6.640
**Mobile Phase:** 50% ACN/WATER

---

**Gradient Time (min):**
**Grad. Slope (°):** 6
**Flowrate (mL/min):** 2.0
**Back Pressure (psig):**
**Temperature (deg):** 55
**Detector 1:** uv/2000
**Detector 2:**

---

**Plot times:** 0 to 10 minutes
**Plot range:** 75 millivolts (4.1 mv offset)

---

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Compound Name</th>
<th>PPM</th>
<th>Injected Area</th>
<th>Height</th>
</tr>
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<tbody>
<tr>
<td>5 70</td>
<td>5992</td>
<td>0.02</td>
<td>4 270E+00</td>
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</table>
Sample Name:  5992
Date:  16 Mar 1994  17:52  Method:  S5992/0Am  Operator:  JC
Interface:  713  Cycle#:  5  Channel#:  4

Instrument:  LC-87-12  Column:  LC-DP
Particle Size (um):  550  Column Length (cm):  250A  Column I.D. (mm):  4.6MM
Mobile Phase:  50% ACN/WATER
Gradient Time (min):  6  Flowrate (mL/min):  2mL/M
Back Pressure (psi):  Temperature (deg):  55
Detector 1:  uv2000  Detector 2:

Plot times:  0 to 10 minutes
Plot range:  75 millivolts (3.5 mv offset)

<table>
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<th>Height</th>
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<tbody>
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<td>5.69</td>
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<td>9.22E+01</td>
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</table>

\[ \text{Plot diagram showing peaks at different retention times} \]
Data file: J3149411
Type: STANDARD

Sample Name: Cal Curve: 3/16/94
Date: 14 Mar 1994 19:00  Method: 5992JOCa Operator: JC
Interface: 713  Cycled: 11  Channel: A

Instrument: LC 67-12  Column: LC OP
Particle Size (um): 5LM  Column Length (cm): 25CM  Column I.D. (mm): 4.6MM
Mobile Phase: 50% ACN/WATER
Gradient Time (min): 6  Flowrate (mL/min): 2mL/min
Back Pressure (psi): 6  Temperature (deg): 55
Detector 1: UV2000  Detector 2:

Plot time: 0 to 10 minutes
Plot range: 75 millivolts (4.2 mv offset)

<table>
<thead>
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<th>Retention</th>
<th>Compound</th>
<th>PPM</th>
<th>Area</th>
<th>Height</th>
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<tr>
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<td>4.51E+03</td>
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Sample Name:  
Date: 16 Mar 1996 19 11  
Method: 5992 ACN  
Operator: JC  
Interface: 713  
Column: LC-10P  
Particle Size (mm): 5M  
Column Length (cm): 25CM  
Column I.D. (mm): 4.6MM  
Mobile Phase: 50% ACN/WATER  
Gradient Time (min): 6  
Flowrate (mL/min): 2mL/min  
Back Pressure (psi): 55  
Detector 1: UV/200  
Detector 2:  

Plot times: 0 to 10 minutes  
Plot range: 75 millivolts (4.3 mv offset)  

Retention Time | Compound Name | PPM Injected | Area | Height  
--- | --- | --- | --- | ---  
5.97 | 5992 | 0.100 | 2.350E+01 | 8.500E+02
Data files: 33149413
Type: STANDARD

Sample Name: Cal. Curve: 3/16/94
Interfaces: 71C Cycled: 13 Channel#2: C

Instrument: LC-87-12 Column: LC-801P
Particle Size (um): 5.0 Column Length (cm): 250H Column I.D. (mm): 4.6MM
Mobile Phases: 50% ACN/WATER
Gradient Time (min): Grad. Slope (#): 6 Flowrate (ml/min): 2ml/min
Back Pressure (psi): Temperature (deg): 55
Detector 1: 47000 Detector 2:

PLOT TIMES: 0 to 10 minutes
Plot range: 75 millivolts (4 mv offset)

<table>
<thead>
<tr>
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<th>PPM Injected</th>
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<th>Height</th>
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BEST 4/14/94
**Data file**: 3149416  
**Types**: STANDARD

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<th>Cal. Curve: 3/16/94</th>
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<td>Date: 14 Mar 1994</td>
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<tr>
<td>Interface: 713</td>
<td>Operator: JC</td>
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<tr>
<td>Cycled 14</td>
<td>ChannelA: A</td>
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**Instruments**: LC-87-12  
**Columns**: LC-80P  
**Particle Size (μm)**: 5UM  
**Column Length (cm)**: 25CM  
**Column I.D. (mm)**: 6.0MM  
**Mobile Phase**: 50% ACN/WATER  
**Gradient Time (min)**: 6  
**Grad. Slope (%)**: 6  
**Flowrate (mL/min)**: 2ML/Min  
**Back Pressure (psi)**: 55  
**Temperature (deg)**: 55  
**Detector 1**: un2000  
**Detector 2**:  

**Plot times**: 0 to 10 minutes  
**Plot ranges**: 75 millivolts (3.6 mv offset)

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Compound Name</th>
<th>PPM</th>
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TR-34-94-38

Data file: 33149415  Type: STANDARD

Sample Name:  Cal. Curve: 3/14/94
Sample: 7113  Cycles: 13  Channels: A

Instrument: LC-87-12  Columns: LC-OP
Particle Size (um): 50  Column Length (cm): 25CH  Column ID (um): 4.6mm
Mobile Phase: 50% ACN/WATER
Gradient Time (min):  Grad. Slope (#): 6  Flowrate (ml/min): 2NL/M
Back Pressure (psi):  Temperature (deg): 55
Detector 1: UV200  Detector 2:

Plot times: 0 to 10 minutes
Plot ranges: 75 millivolts (3 mv offset)

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Compound Name</th>
<th>PPM</th>
<th>Injected</th>
<th>Area</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.82</td>
<td>5992</td>
<td>0.010</td>
<td>2.260E+00</td>
<td>9.350E+01</td>
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</tr>
</tbody>
</table>

BEST
Data file: J3140421  
Types: STANDARD

Sample Name:  
Date: 14 Mar 1994 20:55  
Method: S992A5O  
Operator: JC

Interface: 713  
Cycled: 21  
Channel# A

Instrument: LC-ESP-12  
Column: LC-OP

Particle Size (um): 50  
Column Length (cm): 25  
Column I.D. (mm): 4.6

Mobile Phase: 50% ACN/WATER

Gradient Time (min): 6  
Grad. Slope (%): 6  
Flowrate (mL/min): 20L/H

Back Pressure (psi): 93  
Temperature (deg): 53

Detector 1: UV2000  
Detector 2:  

Plot times: 0 to 10 minutes  
Plot range: 0.06 to 0.18 millivolts (4.4 mV offset)

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Compound Name</th>
<th>PPM</th>
<th>Area</th>
<th>Height</th>
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</thead>
<tbody>
<tr>
<td>5.60</td>
<td>S992</td>
<td>0.50</td>
<td>1.32E+02</td>
<td>4.35E+03</td>
</tr>
</tbody>
</table>

BEST AVAILABLE COPY
Sample Name: J3140422
Date: 14 Mar 1994 21:06
Methods: 5992:LC
Operators: JC
Interface: 713
Columns: Z2
Channels: A

Instruments: LC-67-12
Column Length (cm): 250
Column ID (mm): 4.6
Mobile Phases: 50% ACN/WATER
Temperature (deg): 55
Detector 1: U/V2000
Detector 2: detector 21

Plot Time: 0 to 10 minutes
Plot Range: 75 millivolts (3.7 mV offset)

<table>
<thead>
<tr>
<th>Time</th>
<th>Compound</th>
<th>PPM</th>
<th>Time</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
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<td>5992</td>
<td>0.100</td>
<td>2.61E+01</td>
<td>8.63E+02</td>
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</tbody>
</table>
Sample Name: JS16P423  Type: STANDARD

Interfaces: 713  Cycles: 28  Channels: A

Instrument: LC-67-12  Column: LC-6P
Particle Size (um): 5.0  Column Length (cm): 250
Mobile Phase: 50% ACN/WATER
Gradient Time (min): 3.0  Flowrate (mL/min): 2.4
Back Pressure (psi): 20  Temperature (deg): 55
Detector 1: 432000  Detector 2:

Plot time: 0 to 10 minutes
Plot range: 75 millivolts (3.8 mv offset)

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Compound Name</th>
<th>PPM Injected</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
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<td>5992</td>
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<td>1.33E+01</td>
<td>4.02E+02</td>
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</tbody>
</table>
Sample Name: J3149426
Types: STANDARD

Date: 14 Mar 1994 21:30
Methods: 5992JChem
Operator: JC
Interfaces: 713
Cycles: 24
Channel: A

Instrument: LC-57-12
Column: LC-6P
Particle Size (µm): 5.0
Column Length (cm): 250
Column I.D. (mm): 4.6
Mobile Phase: 50% ACN/WATER
Gradient Time (min): 6
Gradient Slope (d): 6
Fluxrate (mL/min): 2.0
Back Pressure (psi): 55
Temperature (deg): 55
Detector 1: uV5000
Detector 2:

Plot times: 0 to 10 minutes
Plot range: 75 millivolts (3.1 mV offset)

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Compound</th>
<th>PPM</th>
<th>Injected Area</th>
<th>Integrated Height</th>
</tr>
</thead>
<tbody>
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<td>5.65</td>
<td>5992</td>
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<td>400E+00</td>
<td>1.60E+02</td>
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</tbody>
</table>

- P
- V

5992
Data file: J3494423  Type: STANDARD

Sample Name: Cal. Curve 3/16/94
Interface: 713  Cycle: 25  Channel#: A

Instrument: LC-87-12  Columns: LC DP
Particle Size (nm): 5.5μm  Column Length (cm): 25cm  Column I.D. (mm): 4.6mm
Mobile Phase: 50% ACN/WATER
Gradient Time (min): Grad. Slope (%): 6  Flowrate (mL/min): 2mL/min
Back Pressure (psi): Temperature (deg): 55
Detector 1: UV2200  Detector 2:

Plot times: 0 to 10 minutes
Plot range: 75 millivolts (3.9 mV offset)

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Compound Name</th>
<th>PPM Injected</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.66</td>
<td>5992</td>
<td>0.010</td>
<td>1.91E+00</td>
<td>8.13E+01</td>
</tr>
</tbody>
</table>
5992 CALIBRATION CURVE
3/14/94

B0 = -10.155  B1 = 9040.3

Concentrations in report are calculated from equation:

\[ \text{HEIGHT} = B0 + B1(\text{UG/ML}) \]

obtained by least-squares fit of standard injection data.
SAMPLE 1 RE-INJECTION FOR CONFIRMATION
SAMPLE 2 RE-INJECTION FOR CONFIRMATION
SAMPLE 3 RE-INJECTION FOR CONFIRMATION