

US EPA ARCHIVE DOCUMENT

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**TITLE**

RH-5992 Apple Residue Analytical Method

**DATA REQUIREMENT**

Guideline 171-4

**AUTHORS**

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**STUDY COMPLETION DATE**

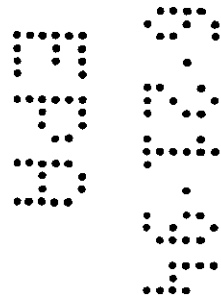
March 22, 1994

**PERFORMING LABORATORY**

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**LABORATORY PROJECT ID**

Rohm and Haas Technical Report No TR 34-94-38



REPORT NO. 34-94-38

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)

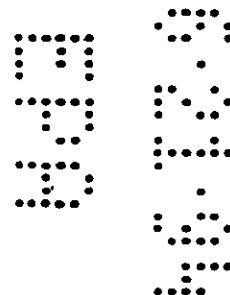
COMPANY Rohm and Haas Company

COMPANY AGENT Ann M. Tillman

DATE June 6, 1994

  
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.

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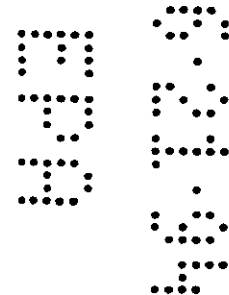
**Quality Assurance Statement**

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* 3/22/94  
R. S. Krick Date  
QA Analyst  
Rohm and Haas Company

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## I. Summary

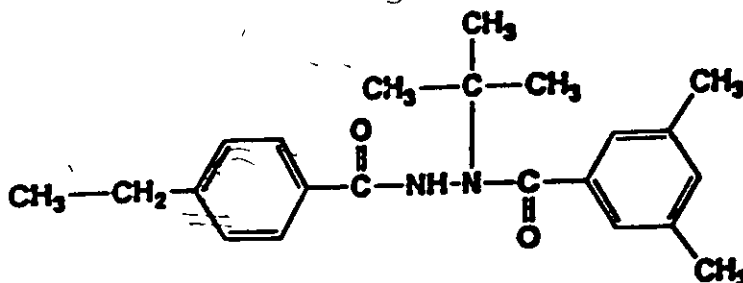
This method has evolved from a previously issued method LM 34-93-170 "Preliminary HPLC Method for RH-5992 in Apples". The preliminary method contained optional clean-up steps and a higher limit of quantitation.

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:



Rohm and Haas Number: RH-5992

Chemical Name:

N-(1,1-dimethylethyl)-N'-(4-ethylbenzoyl)-3,5-dimethylbenzohydrazide

CAS Number: 112410-23-8

Common Name: Tebufenozide

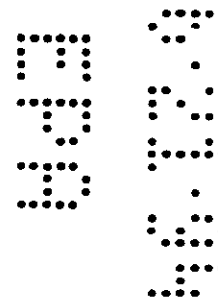
## III. Method

## A. CHEMICAL / SUPPLIES / SOLUTIONS

CHEMICALS / SUPPLIES	SUPPLIER
Celite	Fisher Scientific
Dry Ice	
Hydrochloric Acid (concentrated)	Maillanckrodt, Fisher
Methanol, HPLC reagent	Baker, Baxter
Hexane, HPLC reagent	Baker, BDH
RH-5992 Analytical Standard	Rohm & Haas Company
Sodium Chloride ACS	Fisher Scientific, BDH
Sodium Sulfate	Fisher Scientific, BDH
Water HPLC	Waters HPLC grade or Milli-Q
Methylene Chloride, HPLC reagent	Baker
0.45 micron filters, Acrodisc	VWR

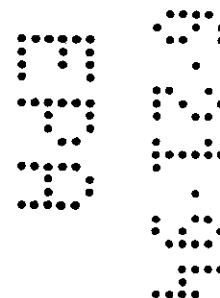
COLUMN CHROMATOGRAPHY CLEAN UP	
Aluminum oxide, Basic	Baker # 0539-01
ALTERNATIVE CLEAN-UP:	
Florisil ( 5 % ) Deactivated 60-100 Mesh	U.S. Silica, Fisher

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**

<b>EQUIPMENT</b>	
<b>Blender, Explosion Proof</b>	<b>Waring</b>
<b>Buchner Funnels and Vacuum flasks</b>	<b>Fisher</b>
<b>Round Bottom Flasks</b>	<b>Pyrex</b>
<b>Rotary Evaporator</b>	<b>Buchi, Janke &amp; Kunkel</b>
<b>Hobart Food Chopper</b>	<b>Hobart Manufacturing Co.</b>
<b>Separatory Funnels</b>	<b>Pyrex</b>
<b>Standard Laboratory Equipment: balances, beakers, etc.</b>	<b>Mettler, Pyrex, Sartorius</b>
<b>Chromatographic Columns 14.5 mm ID by 25 mm</b>	<b>ACE Glass Cat. # 5907-10</b>
<b>Alternative Extractions:</b>	
<b>Polytron 250 ml polypropylene centrifuge bottles</b>	<b>Kinematica CH-6010 Various manufacturers</b>
<b>Centrifuge</b>	<b>Sorvall RC2-B</b>
<b>Chromatographic column 30 x 2.5 (id) cm</b>	<b>Various manufacturers</b>
<b>NOTE: Other manufacturer brands may be substituted if they can be shown to be suitable.</b>	





## C. Instrumentation

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

Auto Sample	WISP 710B
Pump I	Model 510 (only one pump required) Chromatography Pump
Detector:	Spectra-Physics UV 2000 Detector (UV.)
Column Heater	Water YCM
System Control	I. NEC Powermate 2 II System Interface Module
Software	Maxima 820 (Dynamic Solutions, Division of Millipore)
Data System	Hardware: Hewlett Packard Series 9000 Model 300 Software : 4400 Chromatography Software (Nelson Analytical, Inc)
Any other suitable system may be used for analysis after verifying system suitability	
HPLC column;	Adsorbosphere 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 wave length between 235 nm and 260 nm 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 ( Supelco LC18 ) 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.

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**D. Preparation of Standards****(1) Stock solution**

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

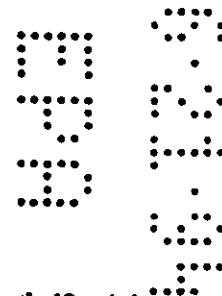
**(2) Intermediate Standard**

A 10 ug/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 ug/ml stock solution (100 % MeOH) diluted to 100 ml with 63 % MeOH/water would yield a 10 ug/ml solution in 67 % MeOH/water.

**Working Standards**

	<u>Concentration</u>	<u>Take</u>	<u>Dilute to (with mobile phase)</u>
(3)	1 0 ug/ml	10 ml of (2)*	100 ml
(4)	0 5 ug/ml	5 ml of (2)	100 ml
(5)	0 1 ug/ml	10 ml of (3)	100 ml
(6)	0 05 ug/ml	5 ml of (3)	100 ml
(7)	0 02 ug/ml	2 ml of (3)	100 ml
(8)	0 01 ug/ml	1 ml of (3)	100 ml

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 ug/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  $-15 \pm 10^\circ \text{C}$  until analysis.

### 2. Diagram

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

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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-5992 in Apples". A Canadian analytical laboratory, Enviro-Test Laboratories<sup>1</sup>, 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 7 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 laboratories' analytical equipment, reagents and preference.

<sup>1</sup> Enviro-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 80 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<sup>2</sup> 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

<sup>2</sup> 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 StepBasic Alumina Column Clean-Up

Place a small plug of glass wool or cotton into the bottom of 14.5 mm (id) X 25 cm (ht) 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. Rinse 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 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 3.5 ml vial with methylene chloride and brought to a 2.0 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  $\mu\text{g/ml}$  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  $\mu\text{l/ml}$  to 2.0  $\mu\text{l/ml}$ . In order to quantify at lower sensitivities, a range of 0.01  $\mu\text{g/ml}$  to 0.5  $\mu\text{g/ml}$  would be acceptable, if less sensitivity is desired, a range of 0.05  $\mu\text{g/ml}$  to 2.0  $\mu\text{g/ml}$  would be acceptable. Inject samples at the same volume (between 50-150  $\mu\text{l}$ ) 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 have at least two sets of standards. A standard curve is constructed from each day's standards.

Equation 1 residue concentration

$$\frac{\text{component concentration } (\mu\text{g/ml}) \times \text{final volume (ml)}}{\text{sample weight(g)}} = \text{ppm}$$

**B. Fortification recovery**

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

Equation 2: fortification recovery

$$\frac{[(\text{found } (\mu\text{g/ml}) \times \text{final vol. (ml)}) - \text{ctl. correction } (\mu\text{g})]}{\text{fortification amount } (\mu\text{g})} = \% \text{ Recovery}$$

**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  $81.3 \pm 11.5\%$ . 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 Florsil 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 IStatistical RecoveryAll Recoveries

<u>Count</u>	<u>Mean</u>	<u>Standard Deviation</u>	<u>Minimum</u>	<u>Maximum</u>
25	81.3	11.5	64	108

Recovery By Fortification Level

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

Table IIComparison of Results (ppm found)

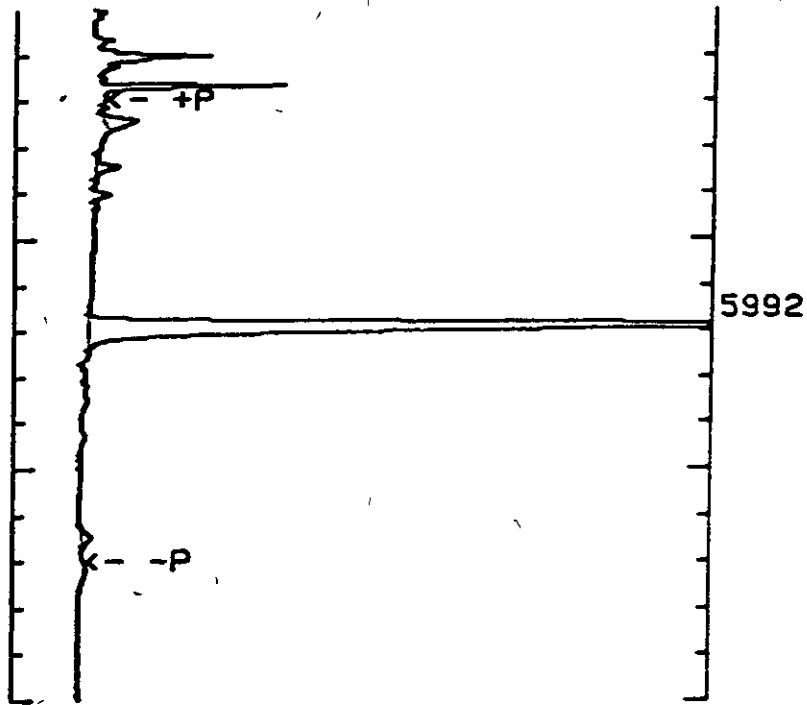
<u>Sample</u>	<u>Rohm and Haas (3/92)</u>	<u>Enviro-Test (2/94)</u>
91-0094-006	0.786	1.2
91-0074-001	0.44	0.53 0.41
91-0074-004	0.496	0.49 0.37



**APPENDIX I**

Data file: 0311942                      Type STANDARD  
 =====  
 Sample Name: APPLE    Cal. Curve: 3/11/94  
 Date: 11 Mar 1994 12:36    Method 5992J8a                      Analyst . JC  
 Interface: 713                      Cycles: 2                      Channel: A  
 =====  
 Instrument: LC-87-12                      Column: ADSORBOSPHERE C18  
 Particle Size (um): 5UM                      Column Length (cm) 25CM                      Column I.D. (mm): 4.6MM  
 Mobile Phase: 67DIOMEOL/WATER  
 Gradient Time (min): 15                      Grad. Slope (#): 6                      Flowrate (mL/min): 2ML/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)



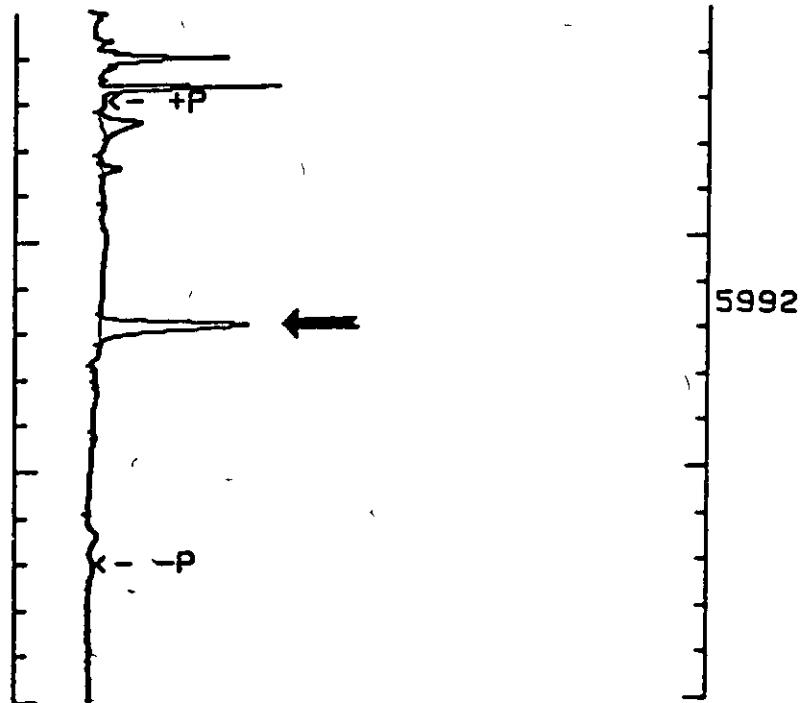
Retention Time	Compound Name	PPM Injected	Area	Height
6.89	5992	0.500	1.350E+02	5.390E+03

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STANDARDS  
 ROHM AND HAAS HPLC SYSTEM

Date files: 0311943                      Type: STANDARD  
 -----  
 Sample Name: APPLE    Cal. Curve: 3/11/94  
 Date: 11 Mar 1994 12 53    Method 5992J8a                      Analyst JC  
 Interface: 713                      Cycle# 3                      Channel# A  
 -----  
 Instrument: LC-87-12                      Column: ADSORBOSPHERE C18  
 Particle Size (um): 5UM    Column Length (cm): 25CM    Column I.D. (mm): 4.608  
 Mobile Phase: 67%MECN/WATER  
 Gradient Time (min): 15    Grad Slope (beta): 6    Flowrate (mL/min): 2ML/M  
 Back Pressure (psi):                      Temperature (deg): 55  
 Detector 1: uv2000                      Detector 2:

Plot times: 0 to 15 minutes  
 Plot range: 50 millivolts ( 85 mv offset)



Retention Time	Compound Name	PPM Injected	Area	Height
6.86	5992	0 100	2 560E+01	1 070E+03

FILE 3397

Data file: 0311944                      Type: STANDARD

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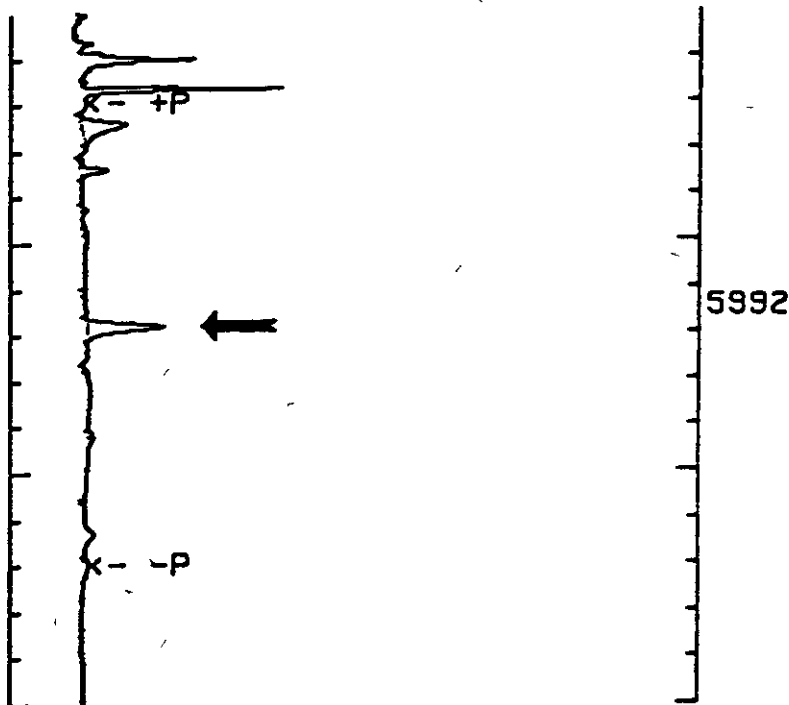
Sample Name: APPLE    Cal Curve: 3/11/94  
 Date: 11 Mar 1994 13 09    Method: 5992J8m                      Analyst: JC  
 Interface: 713                      Cycle#: 4                      Channel#: A

---

Instrument: LC-87-12                      Column: ADSORBOSPHERE C18  
 Particle Size (um): 5UM    Column Length (cm): 25CM    Column ID (mm): 4.6MM  
 Mobile Phase: 67%MEOH/WATER  
 Gradient Time (min): 15    Grad. Slope (#): 6    Flowrate (mL/min): 2ML/M  
 Back Pressure (psi):                      Temperature (deg): 55  
 Detector 1: uv2000                      Detector 2:

---

Plot times: 0 to 15 minutes  
 Plot range: 50 millivolts ( .33 mv offset)



Retention Time	Compound Name	PPM Injected	Area	Height
6.81	5992	0.050	1.260E+01	5.520E+02

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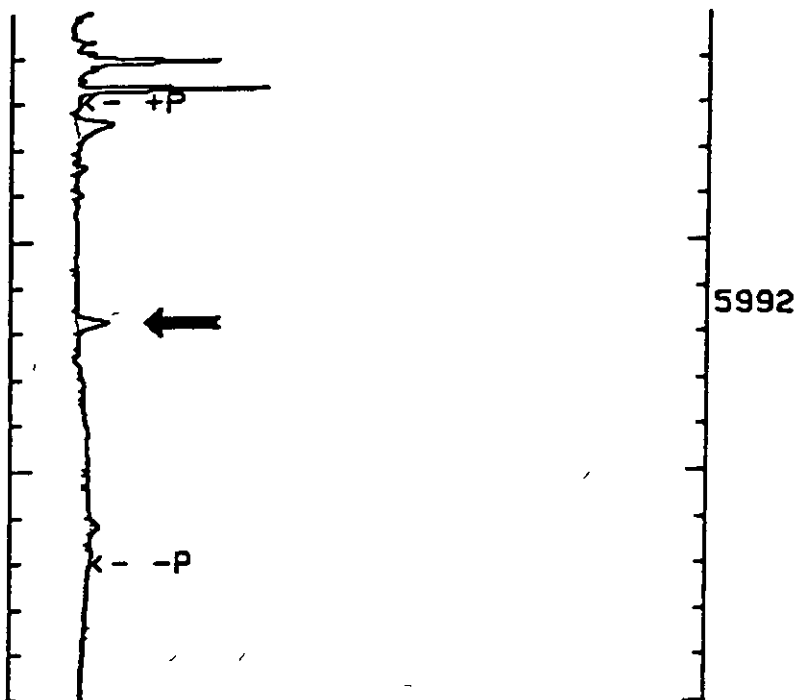
Data file. 0311945

Type: STANDARD

Sample Name APPLE Cal Curve 3/11/94  
 Date: 11 Mar 1994 13 26 Method 5992J8m Analyst JC  
 Interface: 713 Cycle#: 5 Channel#: A

Instrument LC 87-12 Column ADSORBUSPHERE C18  
 Particle Size (um): 5UM Column Length (cm): 25CM Column I D (mm) 4.6MM  
 Mobile Phase: 67%MEOH/WATER  
 Gradient Time (min): 15 Grad Slope (#): 6 Flowrate (uL/min): 200/11  
 Back Pressure (psi): Temperature (deg): 55  
 Detector 1: uv2000 Detector 2:

Plot times. 0 to 15 minutes  
 Plot range 50 millivolts (- 056 mv offset)



Retention Time	Compound Name	PPM Injected	Area	Height
6.78	5992	0.020	5.160E+00	2.290E+02

(32)





Data file 03119414 Type: STANDARD

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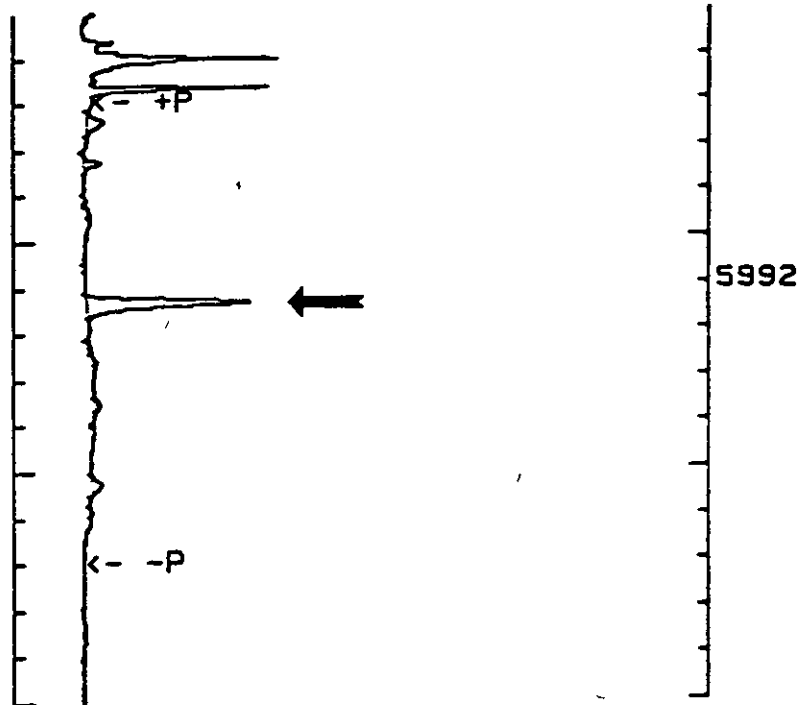
Sample Name APPLE Cal Curve 3/11/96  
 Date 11 Mar 1994 16 02 Method 5992.JBM Analyst JC  
 Interface 713 Cycle# 14 Channel#: A

---

Instrument LC 87-12 Column: ADSORBOSPHIC 2E C18  
 Particle Size (um) 5UM Column Length (cm) 25CM Column I.D. (mm): 4.6MM  
 Mobile Phase: 67%MEOH/WATER  
 Gradient Time (min): 15 Grad Slope (#) 6 Flowrate (mL/min): 2ML/M  
 Back Pressure (psi): Temperature (deg): 55  
 Detector 1: uv2000 Detector 2:

---

Plot times: 0 to 15 minutes  
 Plot range: 50 millivolts ( 55 mv offset)



Retention Time	Compound Name	PPM Injected	Area	Height
6.34	5992	0.00	2.630E+01	1.180E+03

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Data file Q3119415 Type STANDARD

---

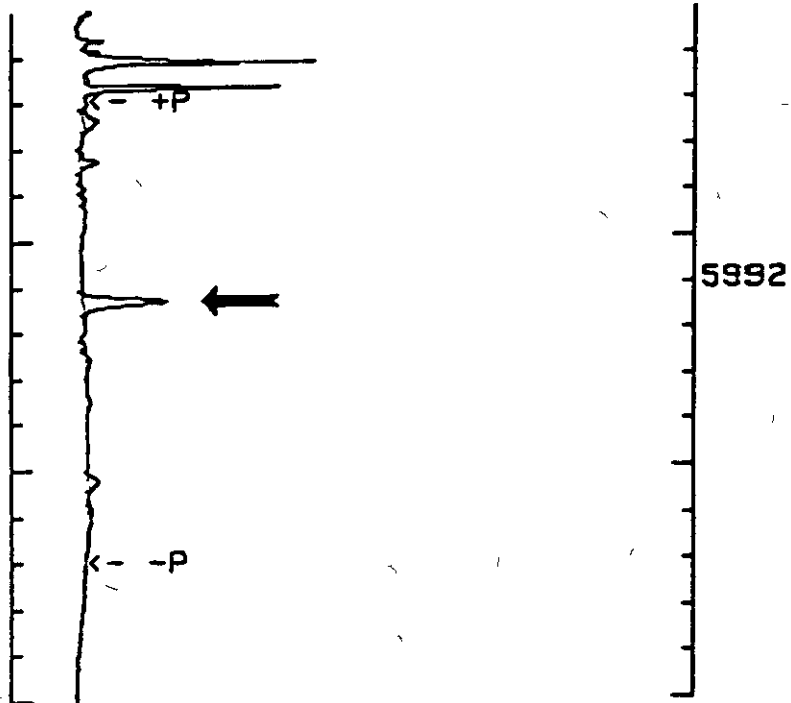
Sample Name APPLE Cal Curve: 3/11/96  
 Date 11 Mar 1996 16 26 Method 5992J8m Analyst . JC  
 Interface 713 Cycle# 15 Channel#: A

---

Instrument LC 87-12 Column. ADSORBOSPHERE C18  
 Particle Size (um): 5UM Column Length (cm). 25CM Column I.D. (mm): 4.6MM  
 Mobile Phase: 67%MEOH/WATER  
 Gradient Time (min) 15 Grad Slope (#). 6 Flowrate (mL/min): 2ML/MIN  
 Back Pressure (psi): Temperature (deg) 55  
 Detector 1 uv2000 Detector 2

---

Plot times: 0 to 15 minutes  
 Plot range: 50 millivolts ( 68 mv offset)

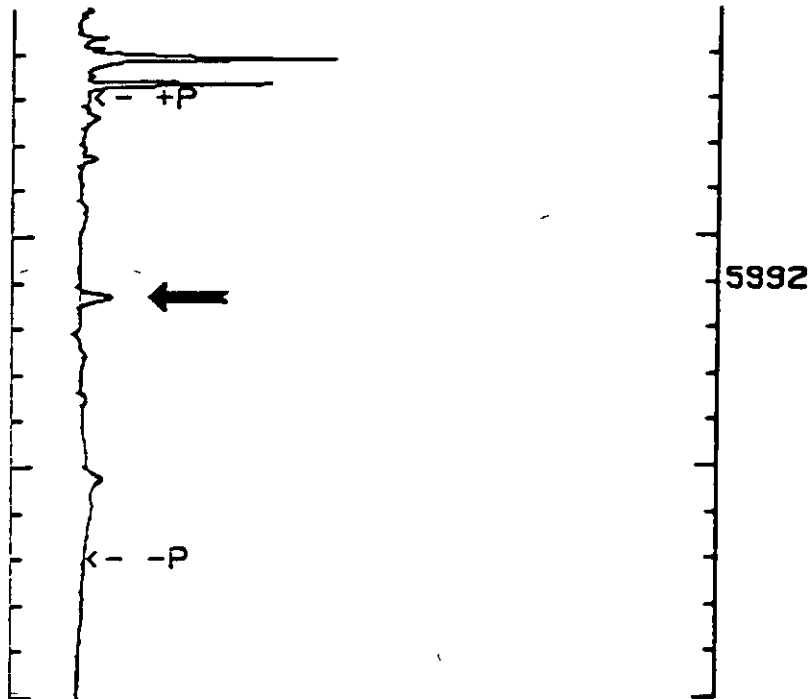


Retention Time	Compound Name	PPM Injected	Area	Height
6.32	5992	0.050	1.290E+01	5.890E+02

3.37 2.11 77

Data file 03119416 Type STANDARD  
 Sample Name APPLE Cal Curve: 3/11/96  
 Date 11 Mar 1996 16 44 Method 5992J8a Analyst JC  
 Interface 713 Cycle# 16 Channel#: A  
 Instrument LC 87 12 Column ADSORBOSPHERE C18  
 Particle Size (um). SUM Column Length (cm) 25CM Column I.D. (mm): 4.6MM  
 Mobile Phase: 67%HEXON/WATER  
 Gradient Time (min): 15 Grad Slope (#) 6 Flowrate (mL/min): 2ML/M  
 Back Pressure (psi)- Temperature (deg) 55  
 Detector 1 uv2000 Detector 2

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

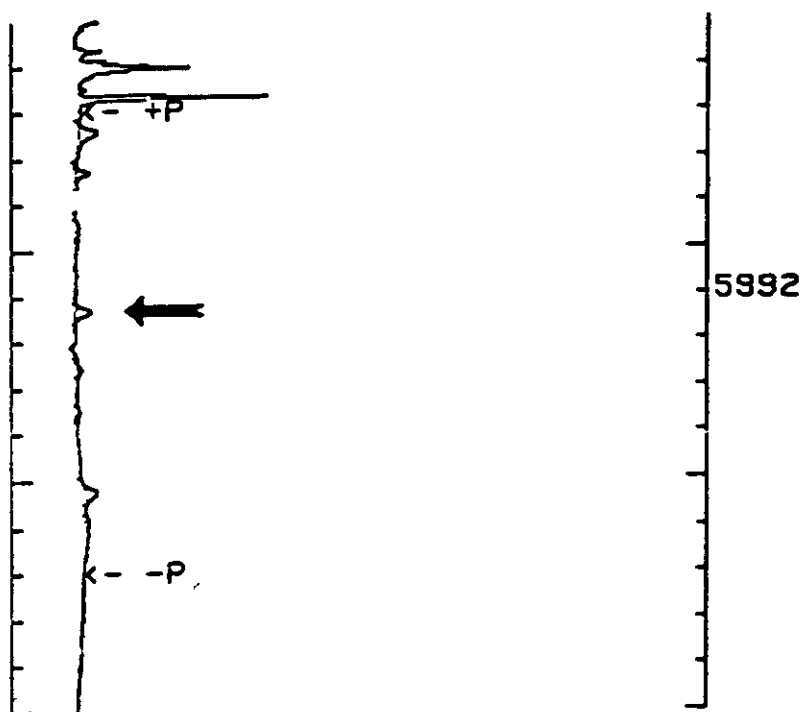


Retention Time	Compound Name	PPM Injected	Area	Height
6.32	5992	0.020	4.960E+00	2.310E+02

BEST AVAILABLE COPY

Date file 03119417 Type STANDARD  
 -----  
 Sample Name APPLE Cal. Curve 3/11/94  
 Date: 11 Mar 1994 16 48 Method 5992J8a Analyst JF  
 Interface: 713 Cycle# 17 Channel# A  
 -----  
 Instrument LC-87-12 Column: ADSORBOSPHERE C18  
 Particle Size (um) 5µm 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 (psi): Temperature (deg) 55  
 Detector 1 uv2000 Detector 2:

Plot times: 0 to 15 minutes  
 Plot range: 50 millivolts ( 73 mv offset)



Retention Time	Compound Name	PPM Injected	Area	Height
6.32	.992	0.010	2.700E+00	1.220E+02

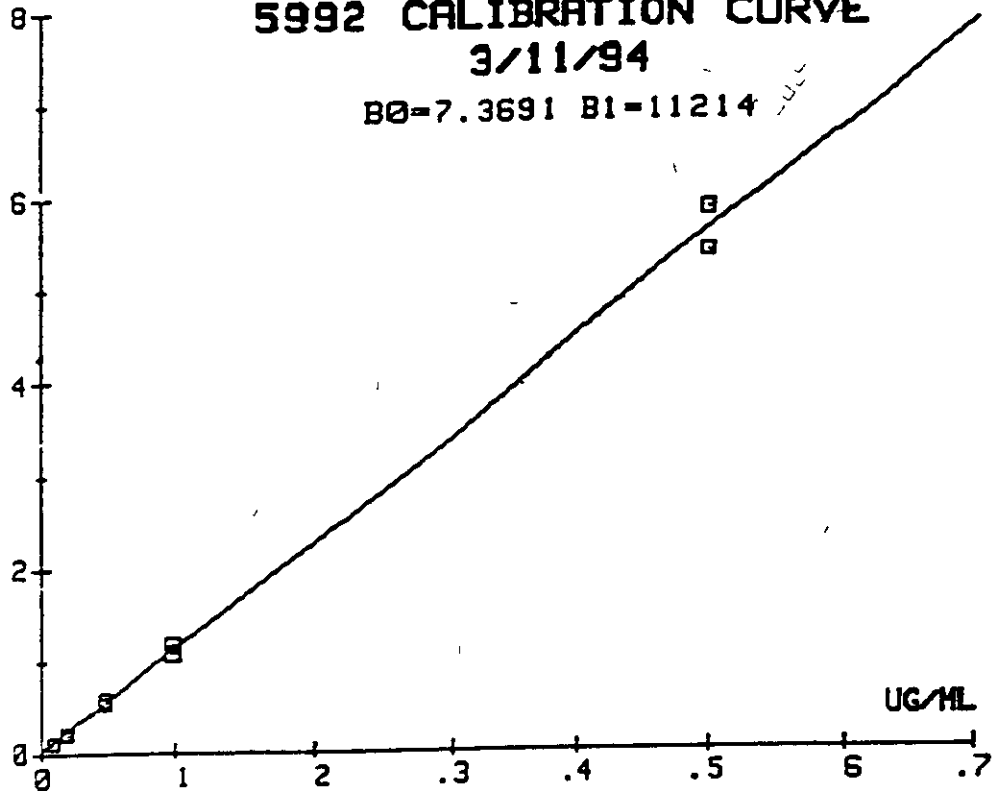
1994

HEIGHT / 1,000

**5992 CALIBRATION CURVE**

**3/11/94**

**B0=7.3691 B1=11214**



Concentrations in report are calculated from equation:  
 $HEIGHT = B0 + B1(UG/ML)$   
obtained by least-squares fit of standard injection data.

**BEST AVAILABLE COPY**

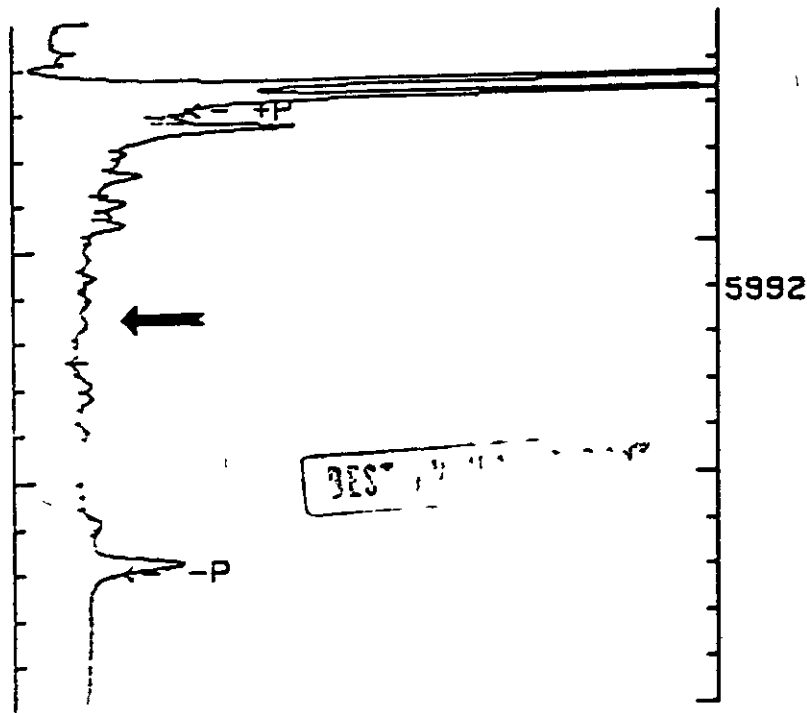
Data file: Q311967  
 Method file: 5992J8m  
 Type: SAMPLE

RAR number 94 0000  
 Sample No. 001  
 Component: APPLE

Sample Name: APPLE  
 Date: 11 Mar 1996 14.44  
 Interface: 713  
 Method: 5992J8m  
 Cycle#: 7  
 Cal Curve: 3/11/96  
 Analyst: JC  
 Channel#: A

Instrument: LC 87-12  
 Particle Size (um): 5UM  
 Mobile Phase: 67%HEXON/WATER  
 Gradient Time (min): 15  
 Back Pressure (psi):  
 Detector 1: uv2000  
 Column: ADSORBOSPHERE C18  
 Column Length (cm): 25CM  
 Column I.D. (mm): 4.6MM  
 Grad. Slope (B): 6  
 Temperature (deg): 55  
 Detector 2:

Plot times: 0 to 15 minutes  
 Plot range: 50 millivolts (-1.7 mv offset)



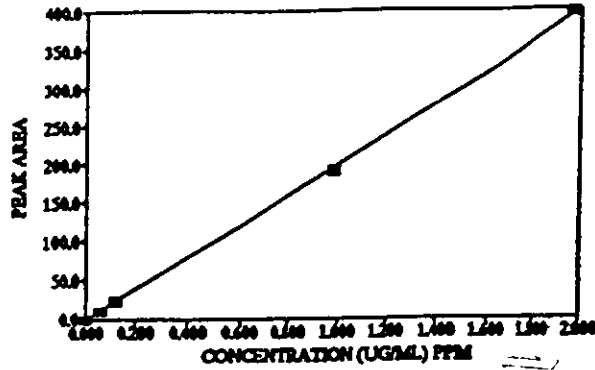
Ret Time	Compound Name	Peak Area	Peak Height	ug/ml Found	Volume (ml)	Samp Wt.	Recov Fact.	PPM
5.50	5992	0.000E+00	0.000E+00	0.00	10.0	20.0	1.000	0.00

**APPLE CONTROL  
 BASIC ALUMINA CLEAN-UP  
 DESIGNATED AS SAMPLE 1 IN APPENDIX II (METHOD  
 CONFIRMATION)**





RH-5992  
FEBRUARY 294



COMPOUND: RH-5992				FEB. 294			
LINEARITY CURVE DATA				SAMPLE DATA* VALIDATION DAY 1			
CH/OM LD	CONC (PPM) X	PK AREA Y	Corrected Y	CHROM LD	SAMPLE ID. (E4-01-140-09A)	SAMPLE AREA	*SAMPLE CONC. (PPM)
-	0.00	0	0.0	4	CTL1	0	0.00
1	0.059	10	12	5	CTL2	0	0.00
2	0.118	21	24	6	SPK+1A	27	0.054
3	0.983	193	196	7	SPK+1B	26	0.052
8	0.118	24	24	10	SPK+2A	113	0.23
9	0.983	190	196	11	**SPK+2B	118	0.24
14	1.97	397	393	12	**SPK+3A	317	0.64
17	0.983	192	196	13	SPK+3B	327	0.66
18	1.97	396	393	15	SPK+3A	162	0.65
				16	SPK+3B	168	0.67

\*\*Spike 2B and 3B to be re-run due to integrator malfunction.  
Sample used was 91-0074-001(E4-01-140-09A)

Ref. RH5992VL.WQ?

Regression Output:

Constant	0.00
Std Err of Y Est	3.51
R Squared	1.000
No. of Observations	9
Degrees of Freedom	8
X Coefficient(s)	200
Std Err of Coef.	1.873

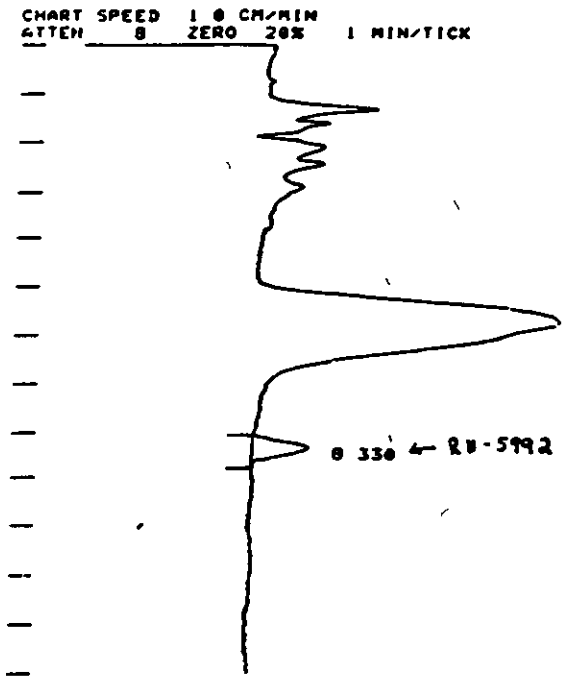
**BEST AVAILABLE COPY**

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

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TITLE: RH 5992 14 23 2 FEB 94  
 SERIAL NO 1 SAMPLE: 0.059UG ML METHOD: 5992  

PK#	PK# NAME	RESULT	TIME (MIN)	AREA COUNTS	SEP CODE
1		100.0000	0.330	10234	88
TOTALS		100.0000		10234	

MULTIPLIER 1.00000  
 SAVED FILE 5992230

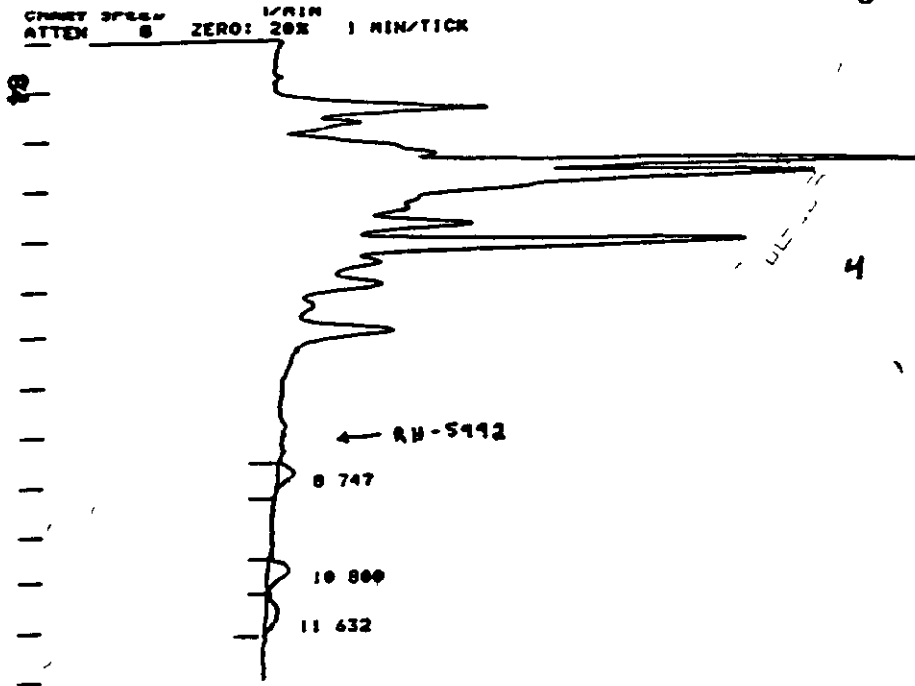
BEST AVAILABLE COPY

NOTES:  
 LC 18 801800001 TEMP. 55°C  
 WAVELENGTH 254. 150UL INJ  
 1.0ML/MIN 653NPH/WATER  
 VALIDATION DAY 1

Instrument: Waters 600E  
 Column: Supelco LC 18  
 Particle size (µm): 5  
 Column Length (cm): 25  
 Column I.D.: 4.6 mm

Mobile Phase: 65% MeOH/water  
 Flowrate (µL/min.): 1.6  
 Temperature: 55°C  
 Detector: Water 486, Tunable Absorbance Detector UV, 1 254  
 Auto sampler: VISP 712  
 Injection Volume (µL): 150

STANDARDS  
 ENVIRO-TEST LABS HPLC SYSTEM



Control Sample  
 25g  
 10 ml EV  
 15.15 2 FEB 94

PEAK NO	PEAK NAME	RESULT	TIME (MIN)	AREA COUNTS	SEP CODE
1		27 3219	8.747	2983	BB
2		43 4420	10.800	4743	BV
3		29 2361	11.632	3192	VB
TOTALS		100 0000		10918	

MULTIPLIER: 1 00000  
 SAVED FILE 5992233

NOTES:  
 LC 10 801800001 TEAP 55°C  
 WAVELENGTH 254. 150UL INJ  
 1 6UL/MIN 652PROM/WATER  
 VALIDATION DAY 1

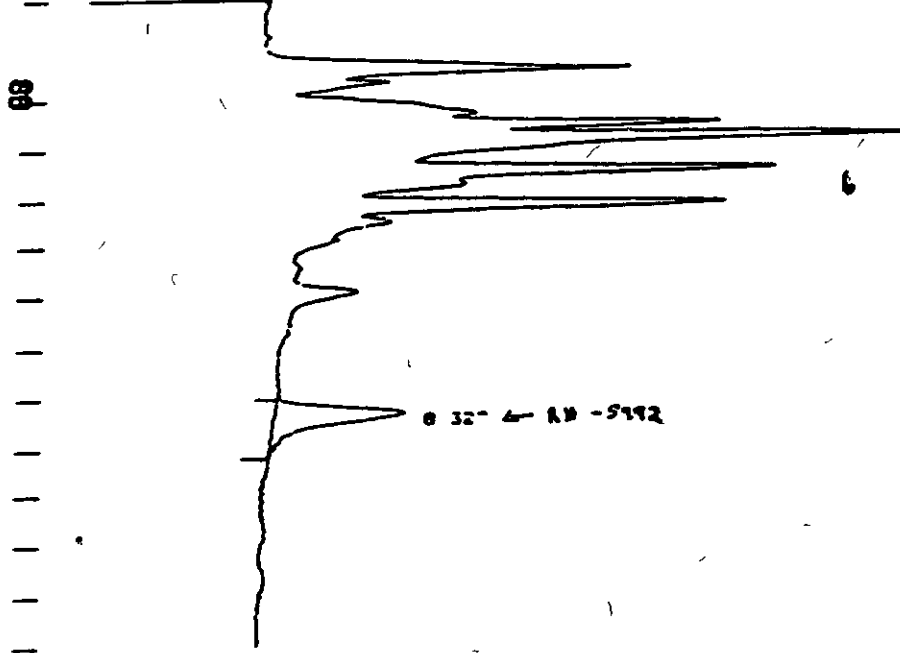
2501 15000 21

Instrument: Waters 600E  
 Column: Supelco LC 18  
 Particle size (um): 5  
 Column Length (cm): 25  
 Column i.d.: 4.6 mm

Mobile Phase: 65% MeOH/water  
 Flowrate (mL/min.): 1.6  
 Temperature: 55°C  
 Detector: Water 484, Tunable Absorbance Detector W 1 254  
 Auto sampler: WISP 712  
 Injection Volume (ul): 150

APPLE CONTROL  
 FLORISIL CLEAN-UP

CHART SPEED 1.0 CM/MIN  
ATTEN 8 ZERO 20% 1 MIN/TICK



*Spots saved 147 ug*  
*{ 25g*  
*10 ml PV*

91-0074-001  
 15.50 2 FEB 74  
 TITLE RM 5992  
 CHANNEL NO 1 SAMPLE SP11M-F2-10 METHOD 5992  
 PEAK NO 1  
 PEAK NAME 1  
 RESULT 100 0000  
 TIME (MIN) 0.327  
 AREA COUNTS 27248  
 SEP CODE 88  
 TOTALS 100 0000 27248

MULTIPLIER 1 00000  
SAVED FILE 5992235

NOTES:  
LC 18 801000001 TEMP 55°C  
WAVELENGTH 254. 150UL INJ  
1.0ML/MIN 653NOM/WATER  
VALIDATION DAY 1

**BEST AVAILABLE COPY**

Instrument Waters 600E  
Column Supelco LC 18  
Particle size (µm): 5  
Column Length (cm): 25  
Column I.D. 4.6 mm

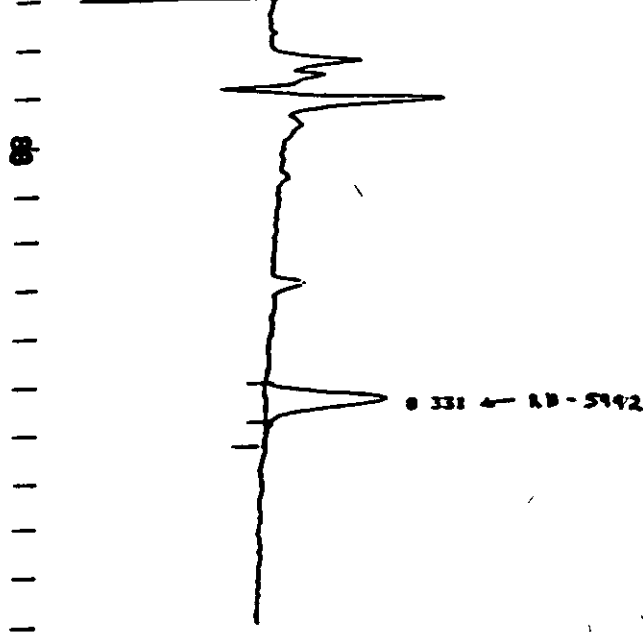
Mobile Phase: 65% MeOH/water  
Flowrate (ml/min.): 1.6  
Temperature: 55°C  
Detector: Water 484, Tunable Absorbance Detector UV, 1 254  
Auto sampler VISP 712  
Injection Volume (µl): 150

**APPLE FORTIFICATION 0.059 PPM  
FLORISIL CLEAN-UP**

TR-34-94-38

8 35

CHART SPEED 1.0 CM/MIN  
ATTEN 8 ZERO 20% 1 MIN/TICK



TITLE: RN 5992 16 25 2 FEB 94  
 ANAL NO 1 SAMPLE: 0.118UG/RL METHOD 5992  

PEAK NO	PEAK NAME	RESULT	TIME (MIN)	AREA COUNTS	SEP CODE
1		100.0000	0.331	23031	88
TOTALS		100.0000		23031	

MULTIPLIER: 1.00000  
 SAVED FILE 5992237

NOTES:  
 LC IS 001000000 TE AP. 50°C  
 WAVELENGTH 254. 150UL INJ.  
 1 G/L/MIN 653NEON/WATER  
 VALIDATION DAY 1

8837 - 5.12

Instrument: Waters 600E  
 Column: Supelco LC 18  
 Particle size (µm): 5  
 Column Length (cm): 25  
 Column I.D.: 4.6 mm

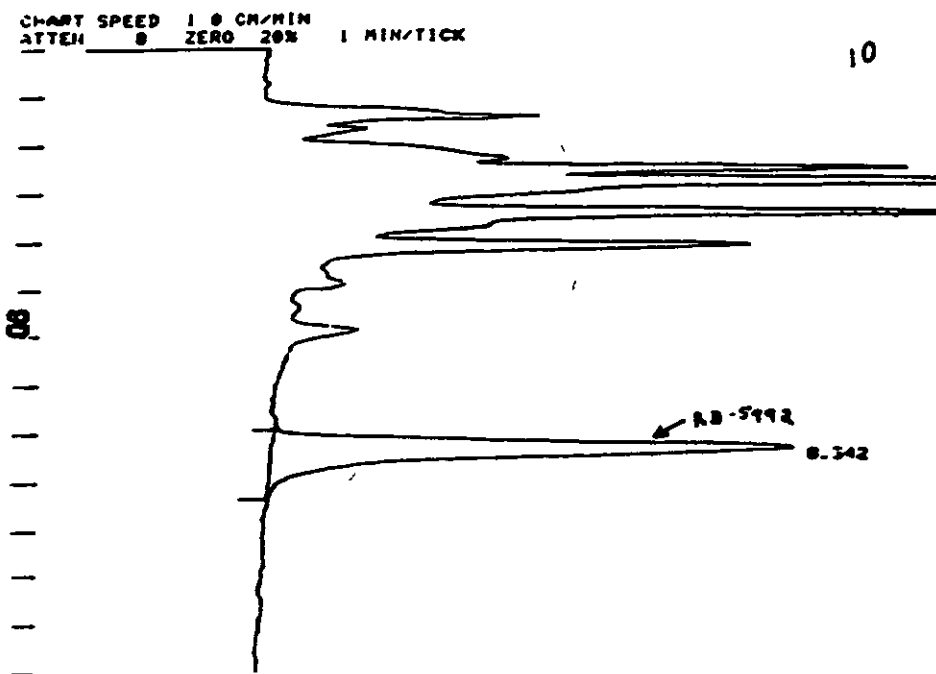
Mobile Phase: 653 NeOH/water  
 Flowrate (µL/min.): 1.6  
 Temperature: 55°C  
 Detector: Water 484, Tunable Absorbance Detector UV, 254  
 Auto sampler: WISP 712  
 Injection Volume (µL): 150

APPLE FORTIFICATION 0.28 PPM  
 FLORISIL CLEAN-UP

TR-34-94-38

Report # RHC03.REP

36



10

*Spk Serial = 6.11.94*  
*25g*  
*10ml PV*

91 10 24-001  
 24-01 NC 004  
 16:00 2 FEB 04

TITLE: RM 0000  
 CHANNEL NO 1 SAMPLE SPK2A-F2-10 METHOD 5992

PEAK NO	PEAK NAME	RESULT	TIME (MIN)	AREA COUNTS	SEP CODE
1		100.0000	0.342	113011	BB
TOTALS		100.0000		113011	

MULTIPLIER 1 00000  
 SAVED FILE 5992239

**BEST AVAILABLE COPY**

NOTES:  
 LC 18 20100001 1040 55°C  
 WAVELENGTH 254. 150UL INJ  
 1 µL/MIN 45%MEOH/WATER  
 VALIDATION DAY 1

Instrument: Waters 600E	Mobile Phase: 45% MeOH/water
Column: Supelco LC 18	Flowrate (µL/min.): 1.6
Particle size (µm): 5	Temperature: 55°C
Column Length (cm): 25	Detector: Meter 484, Turbidity Absorbance Detector UV, 1 254
Column I.D.: 4.6 mm	Auto sampler: WISP 712
	Injection Volume (µL): 150

**APPLE FORTIFICATION 0.79 PPM  
 FLORISIL CLEAN-UP**

**APPENDIX II**

**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 C 18 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 I.

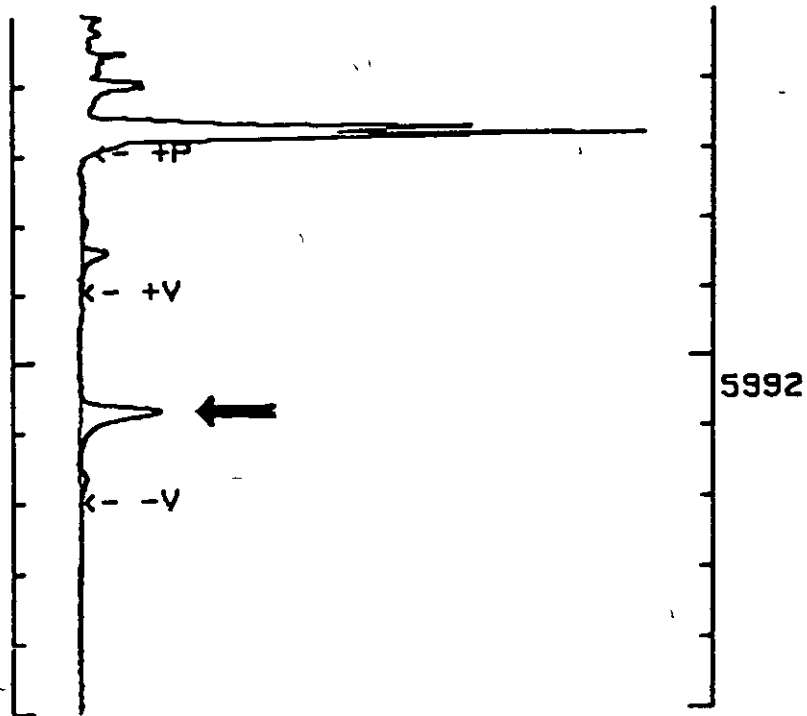
Data file: J314942

Type: STANDARD

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

Instrument: LC-87-12 Column: LC-8P  
 Particle Size (um): 5UM 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/M  
 Back Pressure (psi): Temperature (deg): 55  
 Detector 1: uv2000 Detector 2:

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



Retention Time	Compound Name	PPM Injected	Area	Height
5.72	5992	0.100	2.530E+01	9.010E+02

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TR-34-94-38

40

Data file: J34943

Type: STANDARD

Sample Name:

Cal. Curve 3/14/94

Date: 14 Mar 1994 17 28

Method: 5992JCM

Operator: JC

Interface: 713

Cycle#: 3

Channel#: A

Instrument: LC-82-12

Column: LC DP

Particle Size (um): 5UM

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/M

Back Pressure (psi):

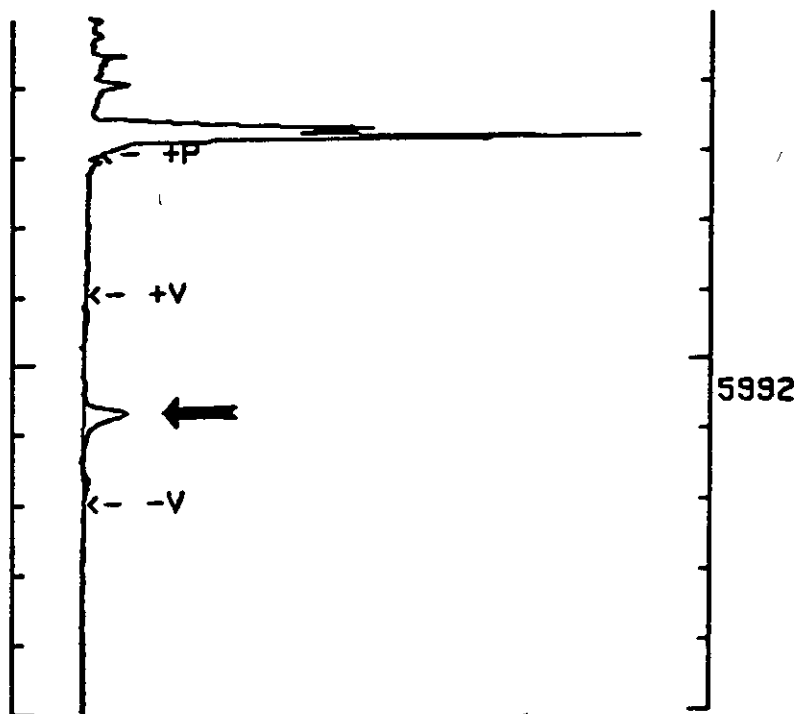
Temperature (deg): 55

Detector 1: w2000

Detector 2:

Plot times: 0 to 10 minutes

Plot range: 75 millivolts ( 41 mv offset)



Retention Time	Compound Name	PPM Injected	Area	Height
5.71	5992	0.050	1.310E+01	4.650E+02

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Date file: J314944

Type: STANDARD

Sample Name:

Cal. Curve: 3/14/94

Date: 14 Mar 1994 17:39

Method: 5992JCM

Operator: JC

Interface: 713

Cycle#: 4

Channel#: A

Instrument: LC-87-12

Column: LC-0P

Particle Size (um): 5UM

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/M

Back Pressure (psi):

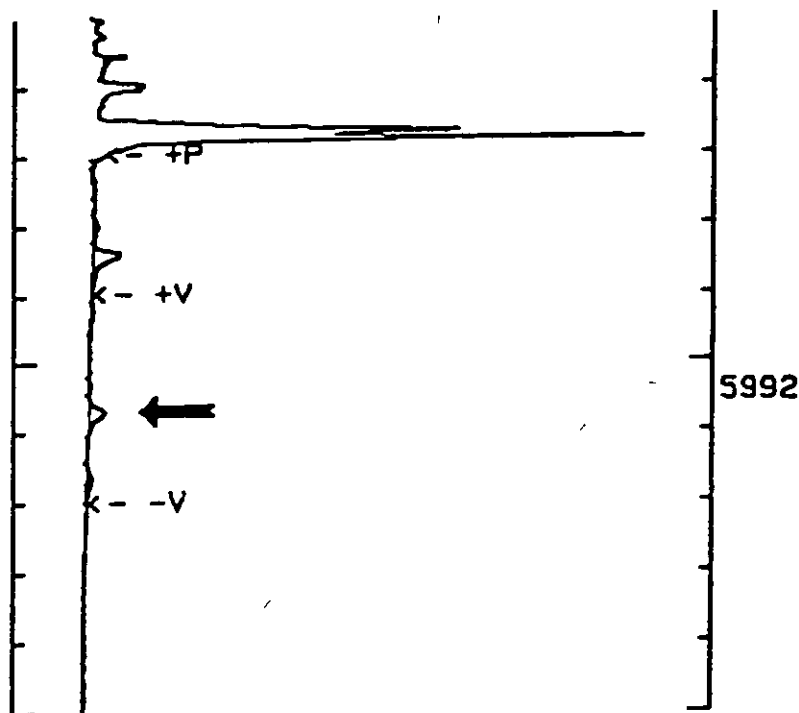
Temperature (deg): 55

Detector 1: uv2000

Detector 2:

Plot times: 0 to 10 minutes

Plot range: 75 millivolts (41 mV offset)



Retention Time	Compound Name	PPM Injected	Area	Height
5.70	5992	0.020	4.270E+00	1.790E+02

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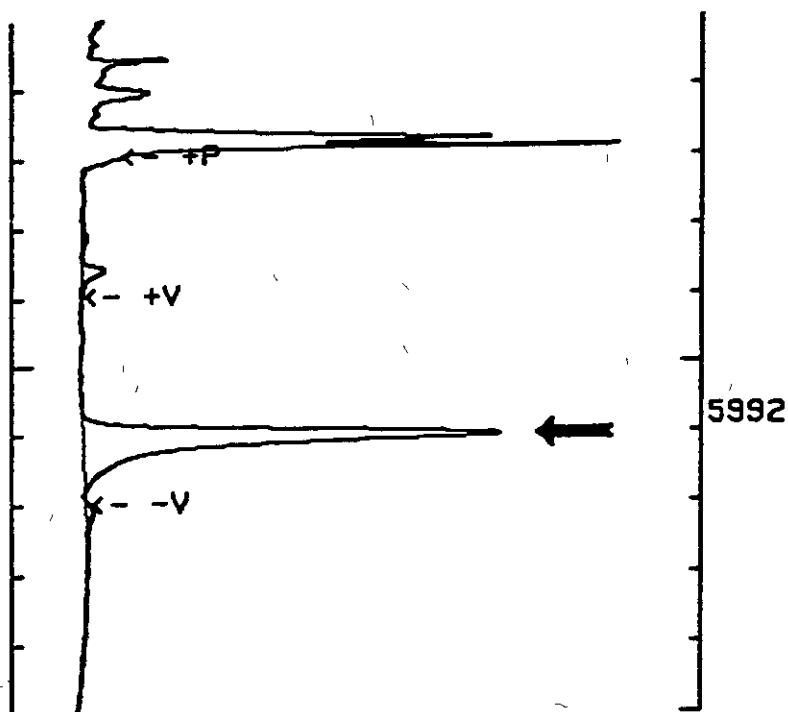
Data file: J3149411

Type: STANDARD

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

Instrument: LC 87-12 Column: LC DP  
Particle Size (um): 5UM Column Length (cm): 25CM Column I.D. (mm): 4.6MM  
Mobile Phase: 50% ACM/WATER  
Gradient Time (min): Grad. Slope (#): 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 ( 4.2 mv offset)



Retention Time	Compound Name	PPM Injected	Area	Height
6.02	5992	0.500	1.390E+02	4.510E+03

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TR-34-94-38

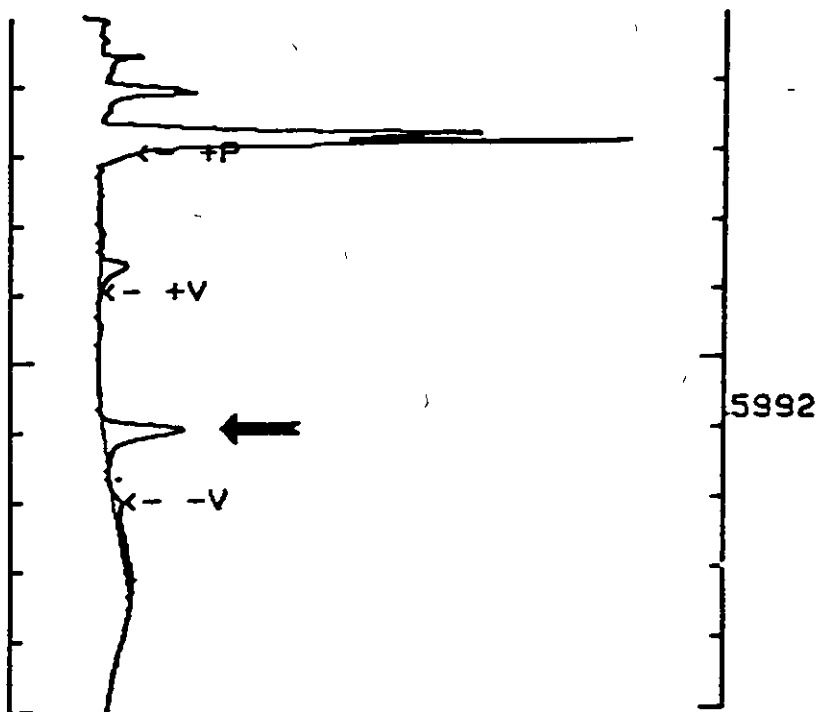
44

Data file: J3149412 Type: STANDARD

Sample Name: Cal. Curve: 3/14/94  
Date: 14 Mar 1994 19 11 Method: 5992JCM Operator: JC  
Interface: 713 Cycle#: 12 Channel#: A

Instrument: LC 87-12 Column: LC-0P  
Particle Size (um): SUN 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/M  
Back Pressure (psi): Temperature (deg): 55  
Detector 1: uv2000 Detector 2:

Plot times: 0 to 10 minutes  
Plot range: 75 millivolts ( 4.5 mv offset)



Retention Time	Compound Name	PPM Injected	Area	Height
5.97	5992	0.100	2.380E+01	8.500E+02

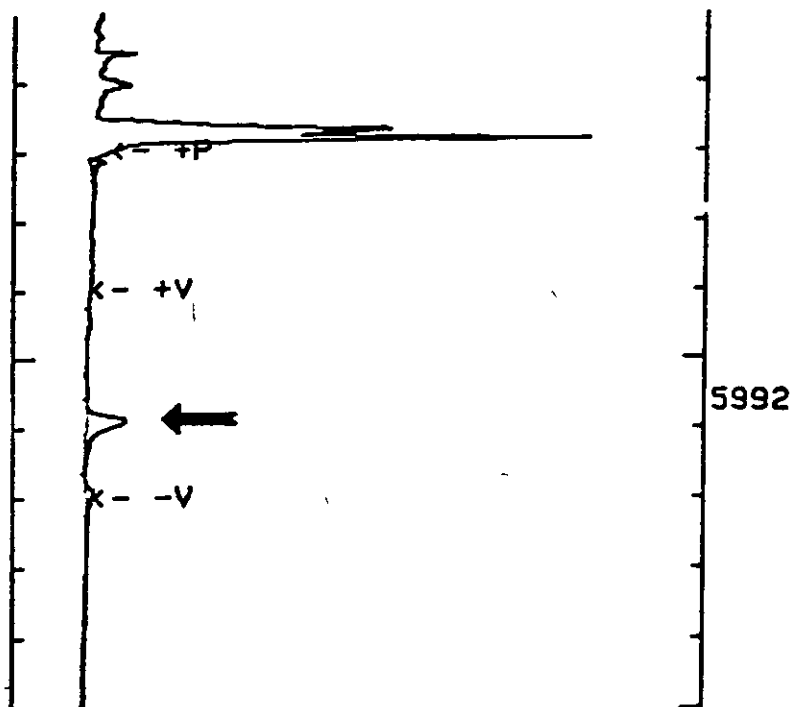
BEST AVAILABLE COPY

Data files: J3149413 Type: STANDARD

Sample Name: Cal. Curve: 3/14/94  
 Date: 14 Mar 1994 19 23 Method: 5992JCa Operator: JC  
 Interface: 713 Cycled: 13 Channel#: A

Instrument: LC-87-12 Column: LC-8P  
 Particle Size (um): 5UM Column Length (cm): 25CM Column I.D. (mm) 4.6MM  
 Mobile Phase: 50% ACN/WATER  
 Gradient Time (min): Grad. Slope (β): 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 ( 4 mv offset)



Retention Time	Compound Name	PPM Injected	Area	Height
5.88	5992	0.050	1.310E+01	4.470E+02

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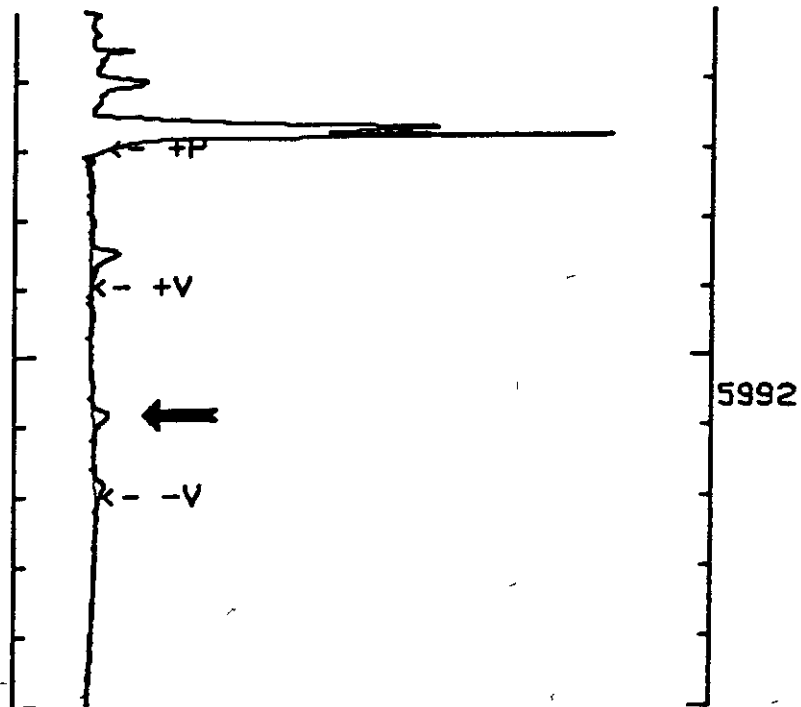
Data file: J3149414

Type: STANDARD

Sample Name: Cal. Curve: 3/14/96  
Date: 14 Mar 1996 19 36 Method: 5992JCM Operator: JC  
Interface: 713 Cycle#: 14 Channel#: A

Instrument: LC-87-12 Column: LC-8P  
Particle Size (um): 5UM 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/M  
Back Pressure (psi): Temperature (deg): 55  
Detector 1: uv2000 Detector 2:

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



Retention Time	Compound Name	PPM Injected	Area	Height
5.85	5992	0.020	4.220E+00	1.600E+02

BEST AVAILABLE COPY

TR-34-94-38

47

Date file: J3149415

Type: STANDARD

Sample Name:

Cal. Curve: 3/14/96

Date: 14 Mar 1996 19 46

Method: 5992JCh

Operator: JC

Interface: 713

Cycle#: 15

Channel#: A

Instrument: LC-87-12

Column: LC-8P

Particle Size (um): 5UM

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/M

Back Pressure (psi):

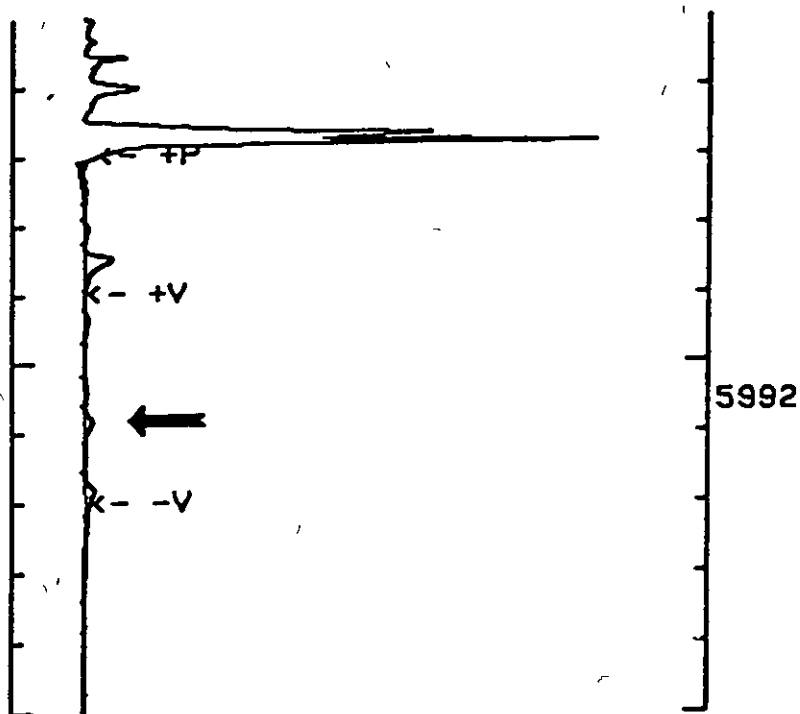
Temperature (deg): 55

Detector 1: uv2000

Detector 2:

Plot times: 0 to 10 minutes

Plot range: 75 millivolts ( 3 mv offset)



Retention Time	Compound Name	PPM Injected	Area	Height
5.82	5992	0.010	2.260E+00	9.530E+01

BEST

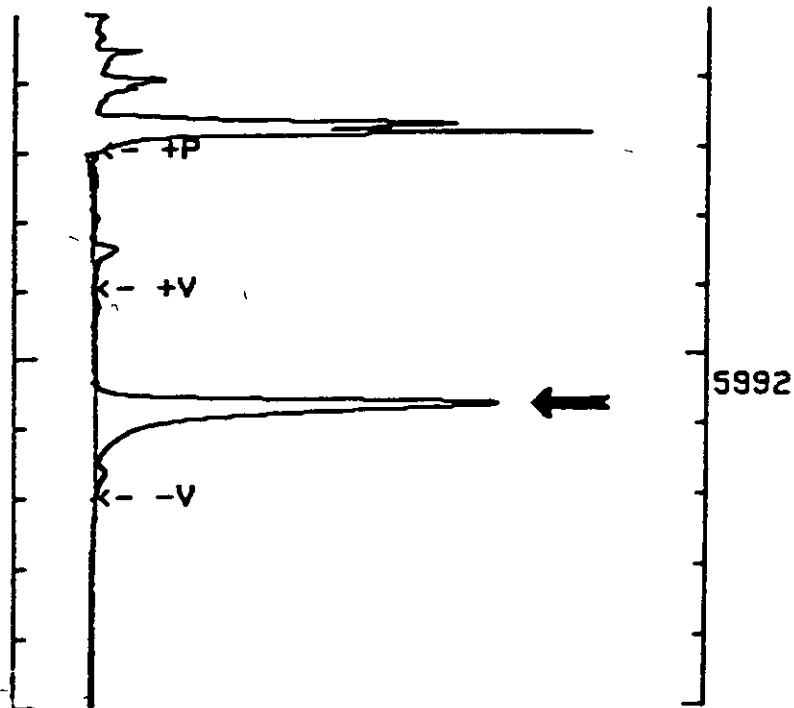


Data file: J3149621 Type: STANDARD

Sample Name: Cal. Curve: 3/14/94  
 Date: 14 Mar 1996 20 55 Method: 5992.JCM Operator: JC  
 Interface: 713 Cycle#: 21 Channel#: A

Instrument: LC-87-12 Column: LC-8P  
 Particle Size (um): 5UM Column Length (cm): 25CM Column ID (mm): 4.6MM  
 Mobile Phase: 50% ACN/WATER  
 Gradient Time (min): Grad. Slope (°): 6 Flowrate (mL/min): 2RL/M  
 Back Pressure (psi): Temperature (deg): 55  
 Detector 1: uv2000 Detector 2:

Plot times: 0 to 10 minutes  
 Plot range: 75 millivolts ( 4.4 mv offset)



Retention Time	Compound Name	PPM Injected	Area	Height
5.69	5992	0.500	1.320E+02	4.350E+03

BEST AVAILABLE COPY

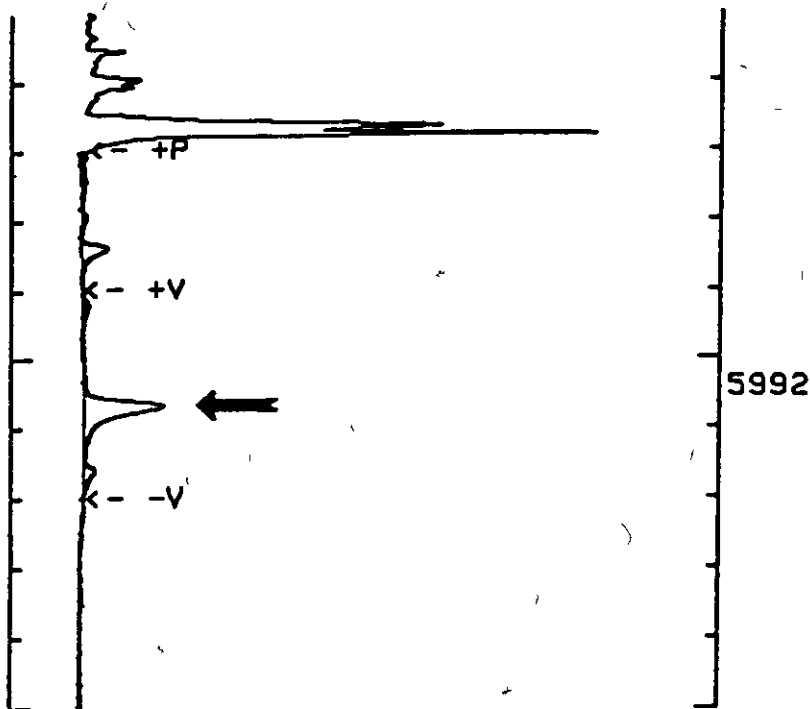
Date file: J3149422

Type: STANDARD

Sample Name: Cal. Curve: 3/14/94  
 Date 14 Mar 1994 21 06 Method: 5992JCM Operator: JC  
 Interface: 713 Cycles: 22 Channel: A

Instrument: LC-87-12 Column: LC-0P  
 Particle Size (um): SUN 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/M  
 Back Pressure (psi): Temperature (deg): 55  
 Detector 1: uv2000 Detector 2:

Plot times: 0 to 10 minutes  
 Plot range: 75 millivolts ( 3.7 mv offset)



Retention Time	Compound Name	PPM Injected	Area	Height
5.67	5992	0.100	2.610E+01	8.630E+02

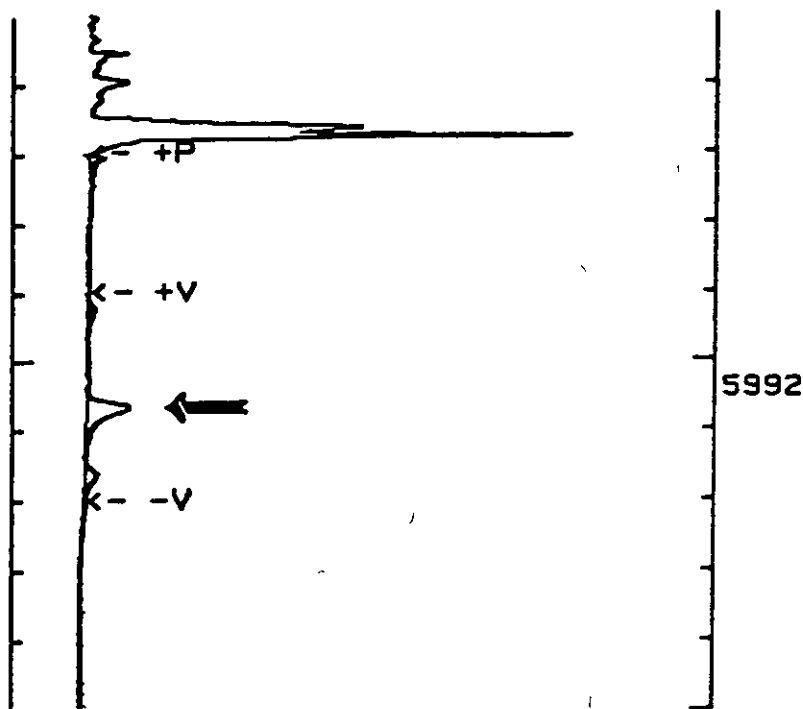
5992

Data file: J3149423 Type: STANDARD

Sample Name: Cal. Curve: 3/14/96  
 Date: 16 Mar 1996 21:18 Method: 5992JCM Operator: JC  
 Interface: 713 Cycle#: 25 Channel#: A

Instrument: LC-87-12 Column: LC-8P  
 Particle Size (um): SUN 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/M  
 Back Pressure (psi): Temperature (deg): 55  
 Detector 1: uv2000 Detector 2:

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



Retention Time	Compound Name	PPM Injected	Area	Height
5.66	5992	0.050	1.330E+01	4.420E+02

BEST AVAILABLE COPY

Data file: J3149426

Type: STANDARD

Sample Name:

Cal. Curve: 3/14/94

Date: 14 Mar 1994 21.30

Method: 5992JCM

Operator: JC

Interface: 713

Cycle#: 24

Channel# A

Instrument: LC-87-12

Column: LC-8P

Particle Size (um): SUN

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/M

Back Pressure (psi):

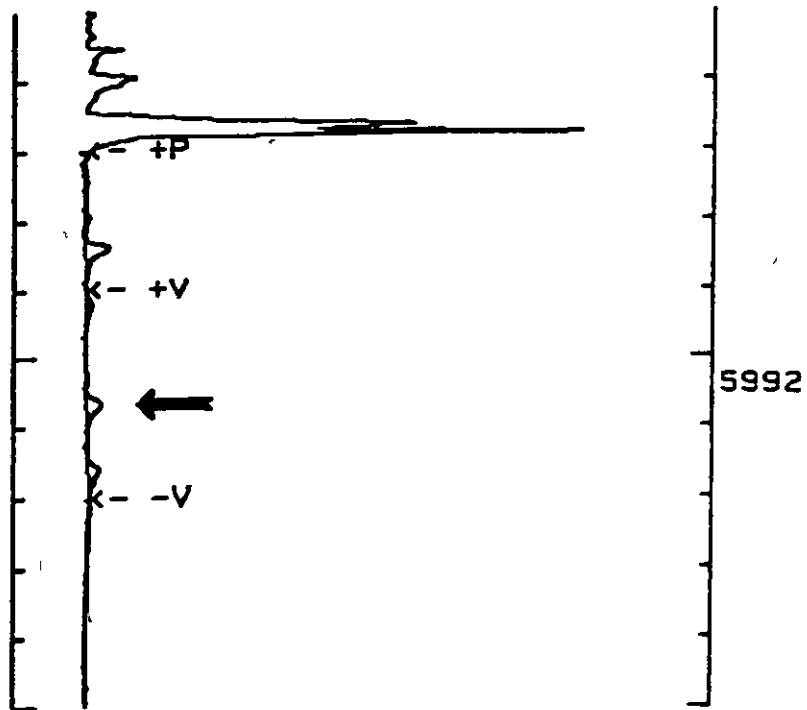
Temperature (deg): 55

Detector 1: uv2000

Detector 2:

Plot times: 0 to 10 minutes

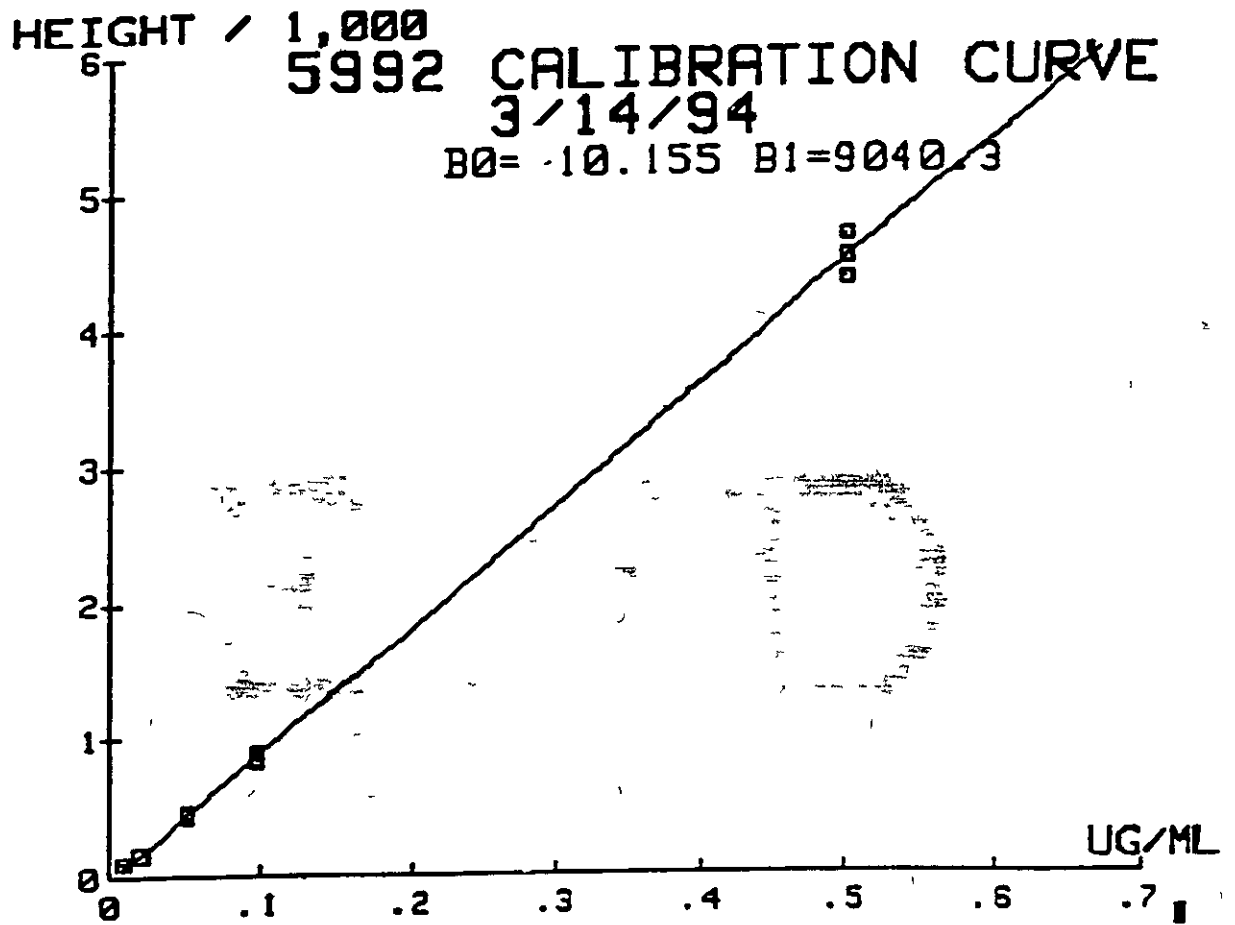
Plot range: 75 millivolts ( 3.1 mv offset)



Retention Time	Compound Name	PPM Injected	Area	Height
5.65	5992	0.020	4.400E+00	1.660E+02

355





Concentrations in report are calculated from equation:  
 $HEIGHT = B0 + B1(UG/ML)$   
obtained by least-squares fit of standard injection data.

55

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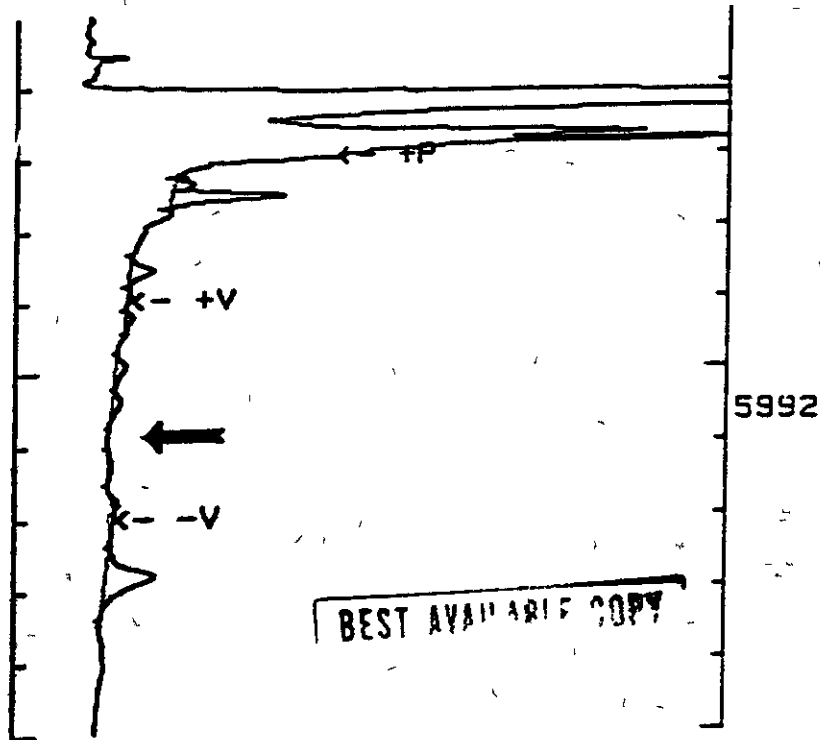
Data file: J3149416  
Method file: 5992JCM  
Type: SAMPLE

RAR numbers:  
Sample No:  
Component: APPLE

Sample Name: Cal. Curve: 3/14/96  
Date: 14 Mar 1996 19:57 Method: 5992JCM Operator: JC  
Interface: 713 Cycle#: 16 Channel#: A

Instrument: LC-67-12 Column: LC-6P  
Particle Size (um): 5UM Column Length (cm): 25CM Column I.D. (mm): 4.6MM  
Mobile Phase: 50% ACN/WATER  
Gradient Time (min): Grad. Slope (B): 6 Flowrate (mL/min): 2ML/M  
Back Pressure (psi): Temperature (deg): 55  
Detector 1: u2000 Detector 2:

Plot times: 0 to 10 minutes  
Plot range: 75 millivolts ( 2.5 mv offset)



Ret. Time	Compound Name	Peak Area	Peak Height	ug/ml Found	Volume (ml)	Samp Wt.	Recov. Fact.	PPM
5.86	5992	0.000E+00	0.00E+00	0.00	10.0	20.0	1.00	0.00

SAMPLE 1 RE-INJECTION FOR CONFIRMATION

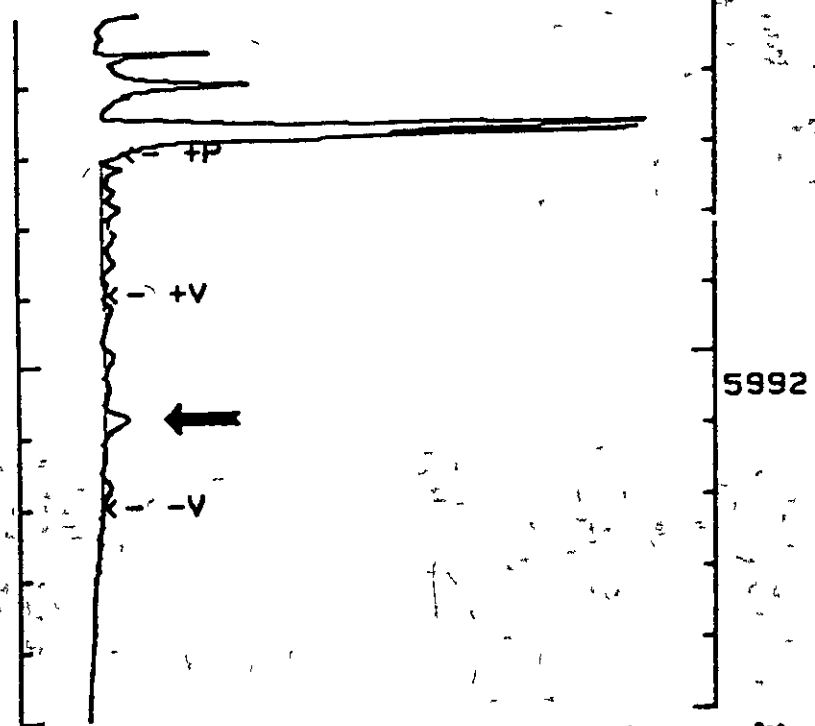
Date file: J3149417  
 Method file: 5992JCM  
 Type: FORTIFICATION

BAR number:  
 Sample No:  
 Component: APPLE

Sample Name: \_\_\_\_\_ Cal. Curve: 3/14/94  
 Date: 14 Mar 1994 20 09 Method: 5992JCM Operator: JC  
 Interfaces: 713 Cycles: 17 Channel: A

Instrument: LC-87-12 Column: LC-8P  
 Particle Size (um): 5UM 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/M  
 Back Pressure (psi): \_\_\_\_\_ Temperature (deg): 55  
 Detector 1: uv2000 Detector 2: \_\_\_\_\_

Plot times: 0 to 10 minutes  
 Plot range: 75 millivolts ( 5 mv offset)



Ret. Time	Compound Name	Peak Area	Peak Height	ug/ml Found	Volume (ml)	Cl Cor	ug Added	Pct Recov
5.76	5992	6.400E+00	254E+03	0.0292	10.0	0.000	0.400	73.0

SAMPLE 2 RE-INJECTION FOR CONFIRMATION



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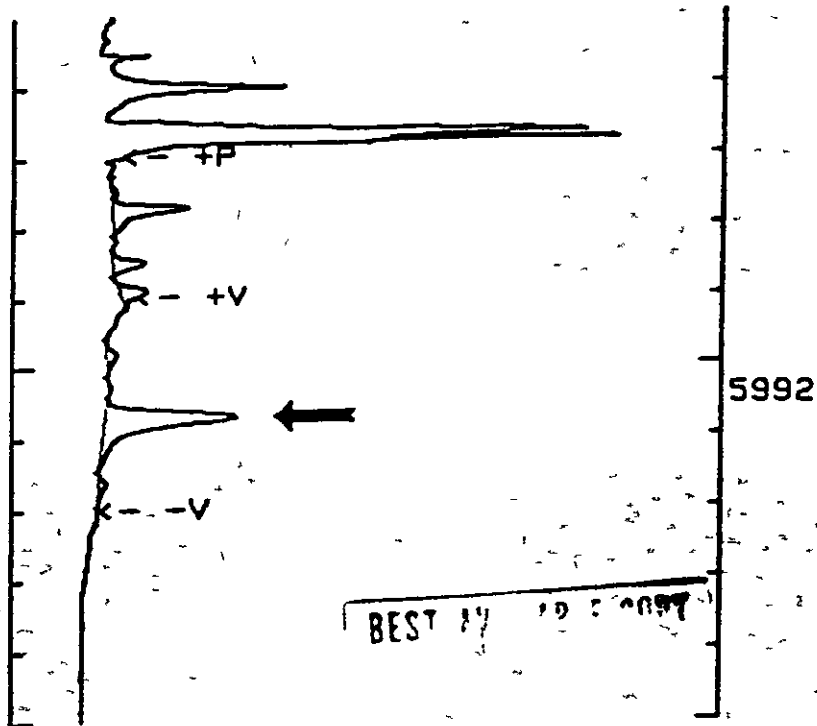
Date file: J3149420  
Method file: 5992JCM  
Type: FORTIFICATION

RAR number:  
Sample No:  
Component: APPLE

Sample Name: Cal. Curves: 3/14/96  
Date: 14 Mar 1996 20:43 Method: 5992JCM Operator: JC  
Interfaces: 713 Cycles: 20 Channel: A

Instrument: LC 57-12 Column: LC-DP  
Particle Size (um): 5UM 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/M  
Back Pressure (psi): Temperature (deg): 53  
Detector 1: uv2000 Detector 2:

Plot times: 0 to 10 minutes  
Plot range: 75 millivolts ( 5.5 mv offset)



Ret. Time	Compound Name	Peak Area	Peak Height	ug/ml Found	Volume (ml)	Cl. Corr.	ug Added	Pct Recov
5.70	5992	4.050E+01	1.30E+04	0.145	10.0	0.000	2.00	72.5

SAMPLE 3 RE-INJECTION FOR CONFIRMATION

Data file: J314941

Type: STANDARD

Sample Name:

Cal. Curve: 3/14/94

Date: 14 Mar 1994 17 05

Method: 5992JCa

Operator: JC

Interface: 713

Cycles: 1

Channel: A

Instrument: LC-87-12

Column: LC-09

Particle Size (um): 5UM

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/M

Back Pressure (psi):

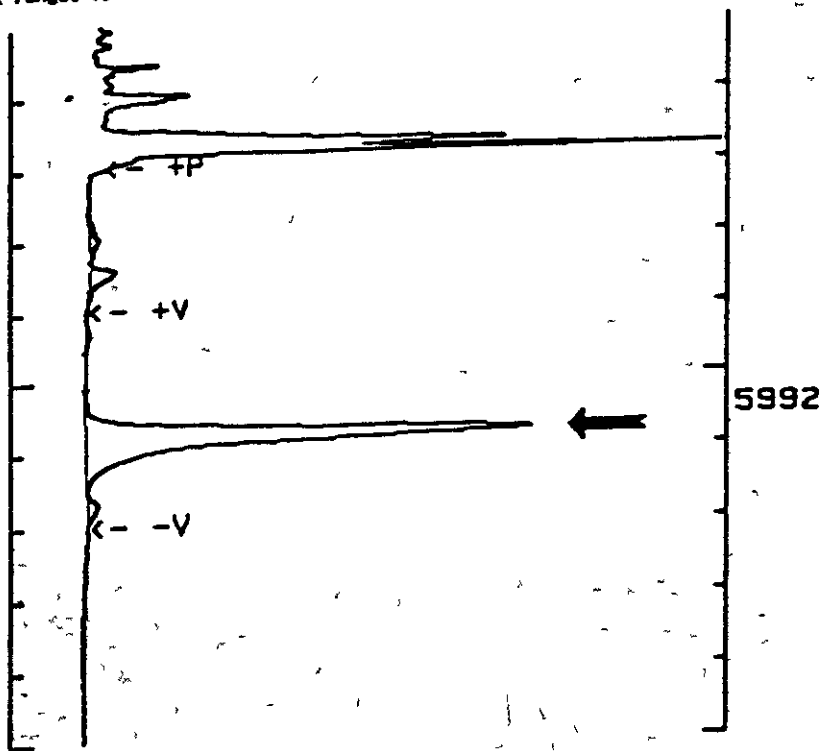
Temperature (deg): 55

Detector 1: uv2000

Detector 2:

Plot times: 0 to 10 minutes

Plot range: 75 millivolts ( 3.9 mv offset)



Retention Time	Compound Name	PPM Injected	Area	Height
5.75	5992	0.500	1.340E+02	4.680E+03