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STUDY TITLE

RH-3866 Total Residue Analytical Method for Apple and Grape

non-CBI

DATA REQUIREMENT

171-4 Residue Analytical Method

AUTHOR

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STUDY COMPLETED ON

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PERFORMIMG LABORATORY

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LABORATORY PROJECT I. D.

Technical Report No. 310-84-27

See Addendum 2

FORM 208

310-84-27

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No Claim of confidentiality is made for any information contained in this study on the basis od its falling within the scope of FIFRA 10(d)(1)(A), (B), or (C).

Company: Rohm and Haas Company

Company Agent: Michael A. Morelli Date: February 12, 1987

Product Registration Manager: Michael A. Morelli

GOOD LABORATORY PRACTICE STATEMENT

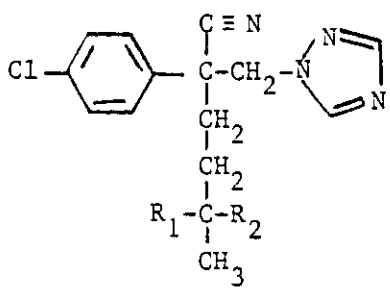
No GLP statement is required for this type of study under 40CFR160.

SUBJECT: RH-3866 Total Residue Analytical Method for Apple and Grape

1. Introduction

This report details the total residue analytical method for residues of RH-3866, RH-9090, RH-9090 conjugates, and RH-9089 in apple and grape. Residues are soxhlet extracted from the apple/grape overnight with 0.5 N HCl/MeOH. The extraction converts RH-9090 conjugate residues to RH-9090. Extracted RH-9089 is converted to RH-9090 by $NaBH_4$ reduction. The extract is partially purified by a pet ether partition, two methylene chloride partitions, Chelex 100-Fe^{+++} affinity chromatography, and florisil column chromatography. RH-3866 and RH-9090 quantitation is performed by GLC on OV101-ultrabond with N/P and ECD detectors, respectively.

2. Experimental Compounds



	R_1	R_2
RH-3866	H	H
RH-9090	H	OH
RH-9089	-	=O
RH-9090-conjugates	H	-O-glucose O-malonyl-glucose

3. Chemicals

Item	Grade	Source
1. Boiling stones	Reagent	Hengar Co.
2. Chelex 100, 50-100 mesh	Reagent	Bio-Rad
3. Ferric chloride	Reagent	Mallinckrodt
4. Florisil, 60-100 mesh	Reagent	Floridin Co.
5. Hydrochloric acid	Reagent	Mallinckrodt
6. Hydrogen	HP	Air Products
7. Litmus paper	---	Micro Essential Lab
8. Methanol	Pesticide	Burdick & Jackson
9. Methylene chloride	Pesticide	Burdick & Jackson
10. 2% OV101-ultrabond 20 M	mesh 100-120	Supelco
11. P-10 gas	HP	Air Products
12. Petroleum ether	Pesticide	Burdick & Jackson
13. RH-3866	Standard	Rohm and Haas
14. RH-9090	Standard	Rohm and Haas
15. RH-9089	Standard	Rohm and Haas
16. RH-9090 conjugates	Standard	Rohm and Haas
17. Sodium borohydride	Reagent	Baker
18. Sodium chloride	Reagent	Fisher
19. Sodium hydroxide, 50% W/W	Reagent	Fisher
20. Sodium sulfate, anhydrous granular	Reagent	Mallinckrodt
21. Toluene	Pesticide	Burdick & Jackson
22. Triazole	Reagent	Sigma
23. Water	HPLC	Milli-Q Purification System

4. Apparatus

Columns -	Glass 250 mm length, 10.5 mm I.D., Teflon stopcock, 200 ml reservoir, Kontes
-	Glass 250 mm length, 25 mm I.D., Teflon needle delivery valve, 500 ml reservoir, Fischer-Porter
-	GLC, glass, 6' x 2 mm I.D., Supelco.
Extraction Thimbles -	Cellulose, 43 x 123 mm, Brinkman
Flasks -	Erlenmeyer, 500 ml, 2000 ml, Arthur H. Thomas Co. Round bottom, 500 ml, 300 ml, Arthur H. Thomas Co. Volumetric, 100 ml, 50 ml, Arthur H. Thomas Co.
Funnels -	Powder, 10 cm
-	Separatory, 500 ml, Arthur H. Thomas Co.
Gas Chromatographs -	Tracor Model 560, ^{63}Ni electron capture detector
-	Varian 3400, N/P detector
Graduated Cylinders -	1000 ml, 250 ml, 100 ml, Arthur H. Thomas Co.
Hobart Food Processor -	Model 84145, Hobart Corp.
Nelson Data Reduction System -	Hewlett-Packard Model 2000 PC,
-	Nelson Analytical Software, Nelson, Inc.
Pipets -	10 ml, 5 ml, 1 ml, glass, disposable, Arthur H. Thomas Co.
Rotary Evaporator -	Büchi Rotovapor R, Brinkmann
Soxhlet Extractor -	Model K-586000, size 23, Kontes.

5. Method

5.1 Sample Processing

Whole grapes and quartered apples are chopped in a Hobart food processor with dry ice. The dry ice is removed by sublimation in a freezer at -10 °C.

5.2 Soxhlet Extraction

Weigh a representative 10 g sample of apple/grape into an extraction thimble and place in a soxhlet extraction apparatus. Add several boiling stones and 270 ml of 0.5 N HCl/MeOH into a 500 ml round bottom flask, attach to the soxhlet extractor, and reflux for 16 hours. After cooling, measure final volume. RH-9090 conjugates will be hydrolyzed during extraction to RH-9090.

5.3 Sodium Borohydride Reduction

Make the extract basic (Litmus paper) by addition of 6-7 ml of 50% (w/w) sodium hydroxide. Add 200 mg sodium borohydride, swirl, and let stand at room temperature for 20 minutes. RH-9089 will be reduced to RH-9090.

5.4 Partitioning

Add 2% aqueous sodium chloride solution to give a 1:5 water:methanol ratio. Transfer to a 500 ml separatory funnel containing 100 ml of petroleum ether. Vigorously shake the separatory funnel for 30 sec. After phase separation, draw off the lower water-methanol layer into a 500 ml separatory funnel containing 150 ml methylene chloride. Add 2% aqueous sodium chloride to the separatory funnel to give a final 1:1 water-methanol ratio. Shake vigorously for 1 min. After phase separation, collect the lower methylene chloride layer. Repeat the partitioning step exactly as described with a second 150 ml portion of methylene chloride. Combine both methylene chloride fractions in a 500 ml 24/40 ₮ round bottom flask. Evaporate to dryness on a rotary evaporator at 50 °C under diminished pressure.

5.5 Chelex 100-Fe^{+++} Column Chromatography

Place 600 g of Bio-Rad Chelex 100 in a 2 liter Erlenmeyer flask. Add 1000 ml of Milli-Q water, swirl gently, and decant to remove fine particulates. Add 50 ml of a saturated ferric chloride solution to the aqueous suspension, swirl gently, and decant. Repeat to ensure complete chelation of Fe^{+++} to the resin. Wash the resin twice with 1000 ml portions of 1:4 (v/v) methanol and water. Discard all washes.

Slurry pack a 25 cm x 2.5 cm I.D. glass column to a height of approximately 8 cm with the iron-activated Chelex 100. Equilibrate the column with 100 ml of 1:4 (v/v) methanol-water at a flow rate of 10 ml/min. RH-3866 and RH-9090 form a strong affinity complex with Fe^{+++}. To elute them from the Chelex 100-Fe^{+++} column, a strong competitive ligand must be used, triazole.

Dissolve the residue from the methylene chloride partitioning step, Section 5.4, in 15 ml 1:4 (v/v) methanol-water. Apply this solution to the Chelex 100-Fe^{+++} column and elute to the top of the resin bed at a flow rate of 4 ml/min. Wash the 500 ml round bottom flask with a second 15 ml aliquot. Apply this aliquot to the column and elute exactly as described above. Rinse flask with 50 ml of 1:4 (v/v) methanol-water and then add to the column and again elute to the top of the resin bed. Discard all fractions collected.

Wash the 500 ml round bottom flask with 170 ml of 0.5 N triazole in 1:1 (v/v) methanol-water. Apply to the Chelex 100-Fe^{+++} column and elute at a flow rate of 10 ml/min until the column is dry. Collect the eluant. Add 5 g sodium chloride to the eluant.

5.6 Methylene Chloride Partition

Quantitatively transfer the Chelex 100-Fe^{+++} eluant, Section 5.5, to a 500 ml separatory funnel containing 150 ml methylene chloride. Shake the separatory funnel vigorously for 30 sec. After phase separation, drain the lower methylene chloride phase into a 500 ml separatory funnel containing 50 ml water. Vigorously shake the 500 ml separatory funnel for 30 sec. After phase separation, collect the lower methylene chloride layer in a 500 ml 24/40 Ŧ round bottom flask. This second partition removes small quantities of triazole remaining in the methylene chloride. Extract and wash the eluant again with 150 ml of methylene chloride exactly as described above. Combine both methylene chloride fractions and evaporate to dryness by rotary evaporator at 50 °C under diminished pressure.

5.7 Florisil Column Chromatography

Activate the Florisil (60-100 m) by heating for 3 hr. in a 220 °C oven. Remove from oven and store in a tightly capped glass jar until cool. Add 5% water by weight and equilibrate by tumbling the jar for two hours. Keep tightly capped until column preparation.

Pack a 25 cm x 10.5 mm I.D. glass column with 13 ml (packed) of the 5% water deactivated Florisil. Top the column with 1 g of anhydrous granular sodium sulfate.

Redissolve the residue from the second methylene chloride partition, Section 5.6, in 25 ml of toluene. Quantitatively transfer the toluene solution to the column and elute to the top of the sodium sulfate layer. Wash the 500 ml round bottom flask with 10 ml toluene, add the wash to the column, and elute to the top of the sodium sulfate layer. Add 35 ml of 1% methanol/toluene (v/v) to the column and elute to the top of the bed. Discard all washes. Elute the RH-3866 with 100 ml of 1% methanol/toluene and collect the eluant in a 250 ml 24/40 Ŧ round bottom flask. Evaporate the sample by rotary evaporator at 50 °C under diminished pressure. Redissolve the residue in 10 ml of 1% methanol/toluene (v/v). The sample is ready for GLC analysis of RH-3866.

Add 35 ml of 3% methanol/toluene (v/v) to the column and continue elution until the liquid has reached the top of the sodium sulfate layer. Discard all washes. Elute the RH-9090 with 100 ml of 7.5% methanol/toluene (v/v) and collect the eluant in a 250 ml 24/40 Ŧ round bottom flask. Evaporate to dryness by rotary evaporator at 50 °C under diminished pressure. Add 10 ml of 5% methanol/toluene (v/v) to dissolve the residue. The sample is now ready for GLC analysis of RH-9090.

5.8 GLC Quantitation

5.8.1 Instrument and Conditions	RH-3866	RH-9090
GLC:	Varian 3400	Tracor 560
Detector:	Varian N/P	Tracor ^{63}Ni ECD
Column:	6' coiled glass, 2 mm I.D.	6' coiled glass, 2 mm I.D.
Packing:	2% OV101-ultrabond, 100/120 mesh	2% OV101-ultrabond, 100/120 mesh

5.8.1 (con't.)

Instrument and Conditions	RH-3866	RH-9090
Carrier Gas:	Helium	P-10 (10% methane/Argon)
Inlet Pressure:	80 PSI	50 PSI
Column Flow Rate:	40 ml/min.	40 ml/min.
Detector Purge Flow Rate:	---	20 ml/min.
Column Temperature:	217 °C	240 °C
Injector Temperature:	275 °C	270 °C
Detector Temperature:	300 °C	360 °C

5.8.2 Data Reduction

Data was collected and integrated by a Nelson Analytical 4400 series data system consisting of Nelson Analytical Chromatography Software and a Hewlett-Packard Model 2000 modular computer.

5.8.3 Preparation of Standard Curves

Standard solutions of RH-3866 or RH-9090 in 1% methanol/toluene (v/v) were prepared by serial dilution in the concentration range of 1.0 µg/ml - 0.01 µg/ml. Twelve µl of RH-3866 or thirteen µl of RH-9090 of each standard are injected, and the resulting peak areas are measured. A standard curve of the peak areas measured vs. concentration (µg/ml) is constructed. The standard curve is linear within the concentration range. Standard curves are prepared for each analysis day.

5.8.4 Quantitation

Twelve microliters of the RH-3866 sample is injected into the GLC. If necessary, the sample is diluted to an appropriate volume to give a response within the standard curve range. The peak area is computed and the concentration is determined by the data reduction system as follows:

$$\frac{\text{Total Volume (ml)} \times \text{Concentration (µg/ml)} \times 100}{\text{Average Recovery (\%)}} = \text{Total µg}$$

$$\frac{\text{Total µg}}{\text{Sample Weight (g)}} = \text{ppm}$$

Quantitation of RH-9090 is performed in an identical manner, except 13 µl of the sample is injected.

Sample chromatographs of controls, fortification, and treated samples of apple and grape are illustrated in Figures 1-12.

5.8.5 Fortification Recovery

For samples fortified with known amounts of standards prior to extraction, compute the peak area and calculate % Recovery as follows:

$$\text{\% Recovery} = \frac{\text{(µg/ml found)} \times \text{Final Sample Volume (ml)} \times 100}{\text{Fortification (µg)}}$$

5.9 Confirmatory Column Analysis

Confirmatory analysis for RH-3866 is performed on the Tracor 560 with the Tracor ^{63}Ni ECD using identical instrument conditions as listed in Section 5.8.1, except a 220 °C oven temperature.

Confirmatory analysis for RH-9090 is performed on the Varian 3400 with N/P detector using the same instrument settings as listed in Section 5.8.1, except a 240 °C oven temperature.

Sample chromatographs of the confirmatory analysis are illustrated in Figure 13-16.

Table 1 contains detailed analytical confirmatory data.

An alternate confirmatory column is 2% OV-17 + 1% OV-210 on 100-120 mesh Supelcoport.

5.10 Extraction Efficiency

The extraction efficiency of total ^{14}C residues from grapes/apples field treated with RH-3866-^{14}C was determined. The grapes/apples were combusted and radioassayed. They were then soxhlet extracted and the extract was radioassayed. The extraction efficiency for grapes was 95%. The extraction efficiency for apples was 96%.

Table 2 contains the summary radioassay data, and Appendix 1 contains the detailed radioassay data.

5.11 Recoveries

Recovery of RH-3866 was 92.1 ± 17.2%. Recovery of RH-9090 was 83 ± 20.4%.

5.12 Sensitivity

Sensitivity of the method for RH-3866 and RH-9090 was 0.01 ppm, determined by actual fortifications at this level.

Table 1

Confirmatory Analysis

Sample	RH-3866 ppm Figures	RH-3866 ppm Method	RH-3866 ppm Confirmatory	RH-9090 ppm Figures	RH-9090 ppm Method	RH-9090 ppm Confirmatory
Grape 84-0357-003	3 + 13	0.218	0.223	6 + 16	0.071	0.058

Table 2

Extraction Efficiency

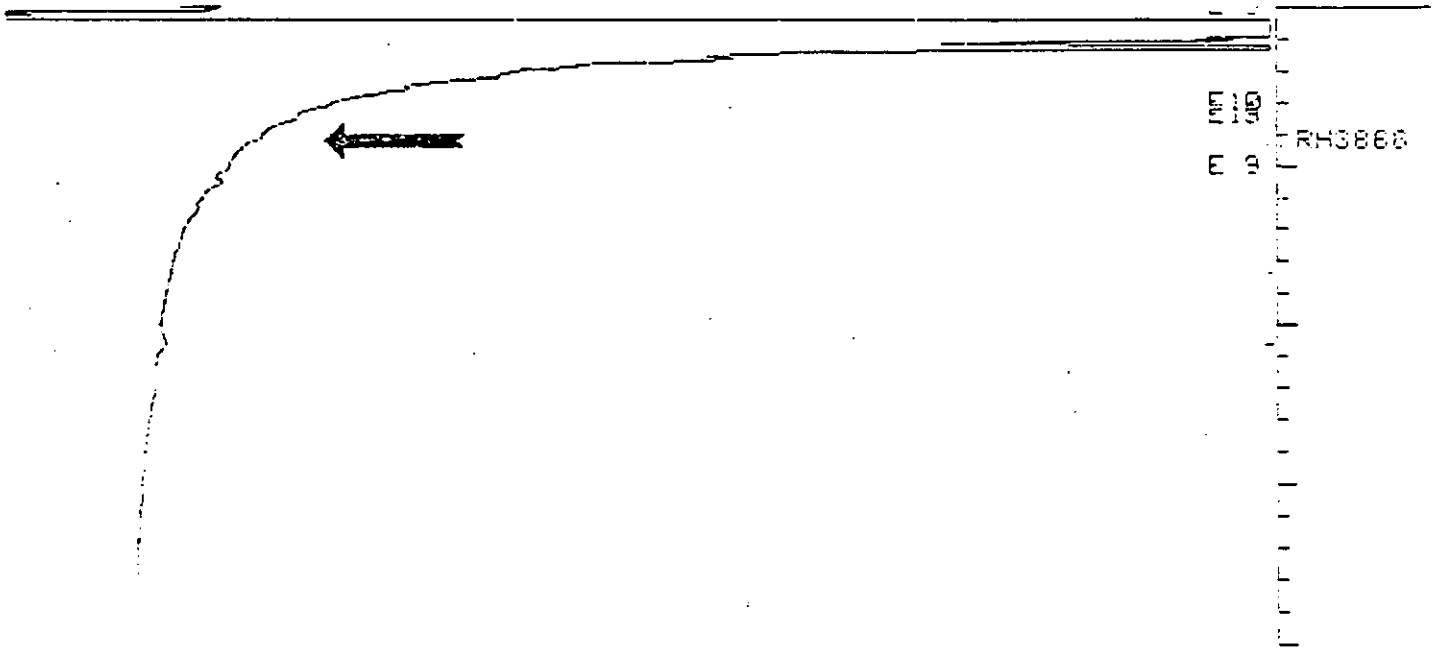
	Combustion dpm/10 g	Radioassay dpm Extracted	% Extraction Efficiency
Apple	49,100	47,300	96
Grape	42,400	40,300	95

Figure 1 Grape-Control RH-3866

DATA FILE: S11048420
METHOD FILE: STAN_6
TYPE: SAMPLE

RAR NUMBER: 84-0357
SAMPLE NO: 009

Start time= 0.00 Stop time= 20.00 minutes Offset= -2 mv
Full Range = 30 millivolts



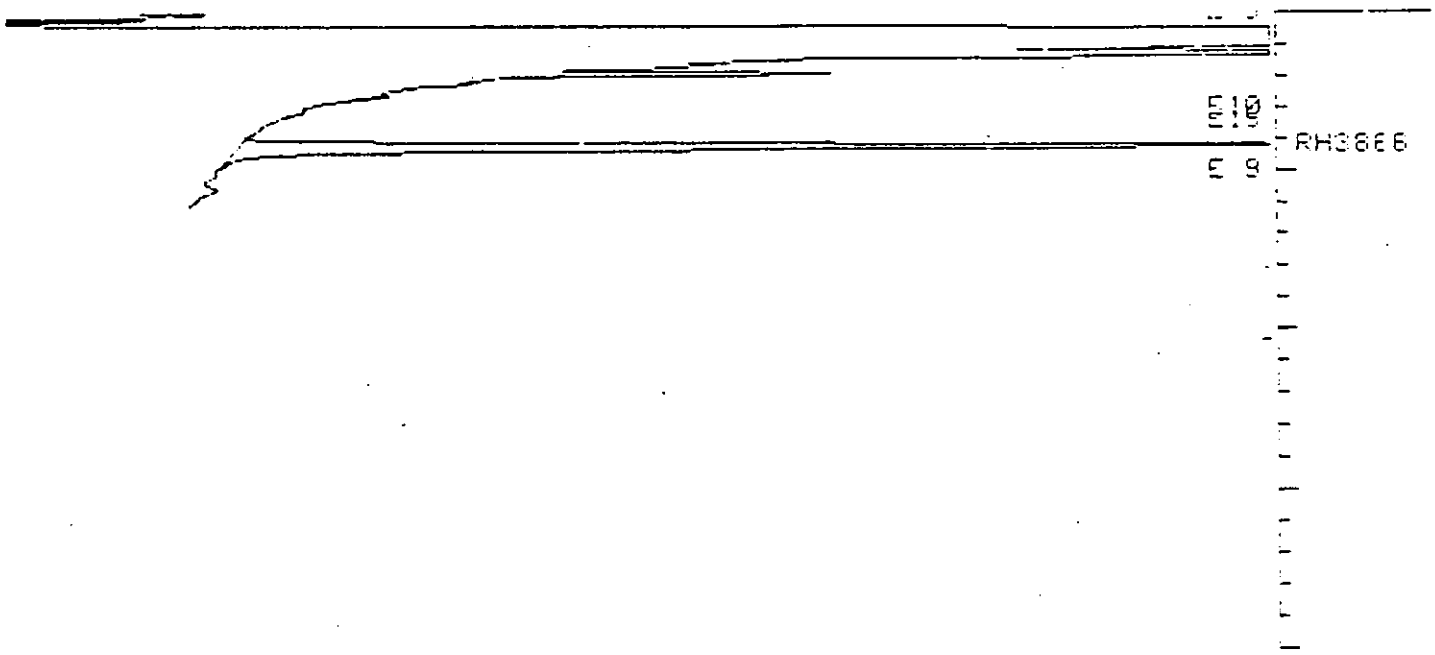
RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	SAMP WT.	RECOV FACT.	PPM
4.20	RH3866	0.0	0.0	0.0000	10.00	10.0	.900	0.0000

Figure 2 Grape-Fortification RH-3866

DATA FILE: S11048421
METHOD FILE: STAN_E
TYPE: FORTIFICATION

RAR NUMBER: 84-0357
SAMPLE NO: 009

Start time= 0.00 Stop time= 20.00 minutes Offset= -3 mv
Full Range = 30 millivolts



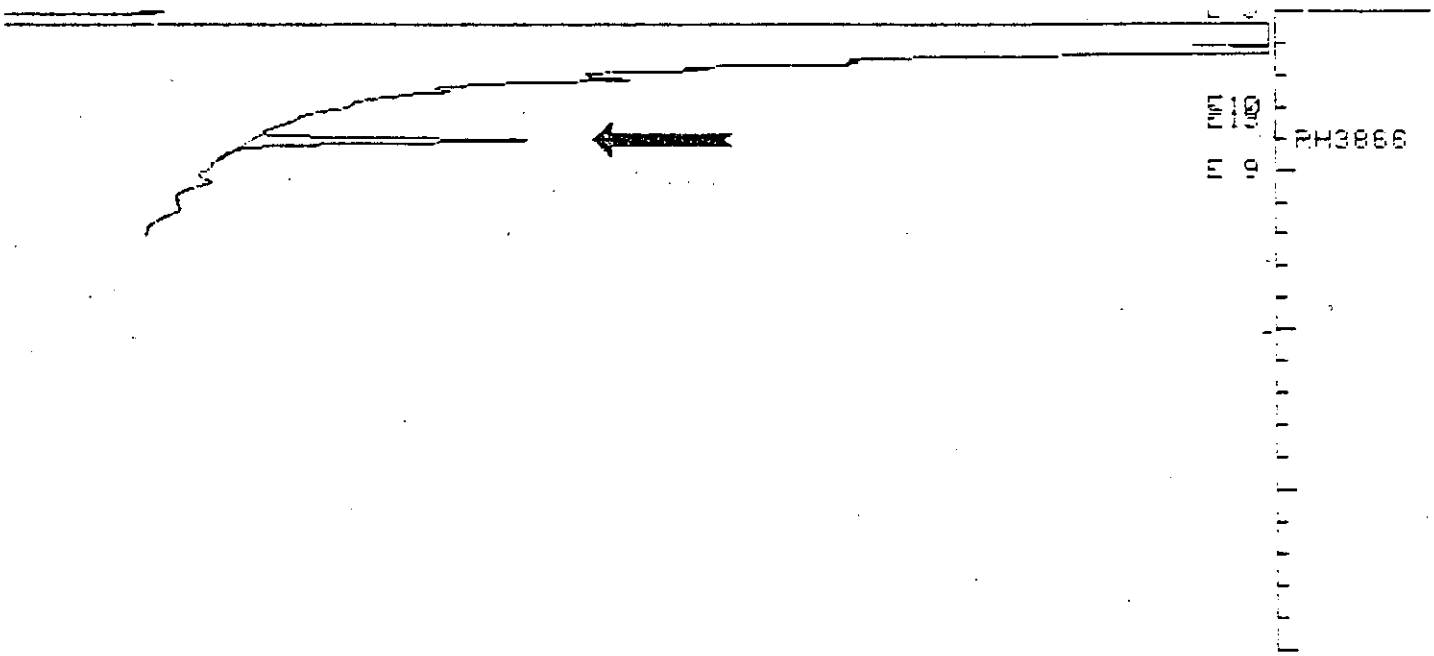
RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	CTL CORR.	UG ADDED	PCT RECOV
4.22	RH3866	3235.3	2583.2	.8561	10.00	0.000	10.000	85.6

Figure 3 Grape-Treated RH-3866

DATA FILE: S11048428
METHOD FILE: STAN_6
TYPE: SAMPLE

RAR NUMBER: 84-0357
SAMPLE NO: 003

Start time= 0.00 Stop time= 20.00 minutes Offset= -4 mv
Full Range = 30 millivolts



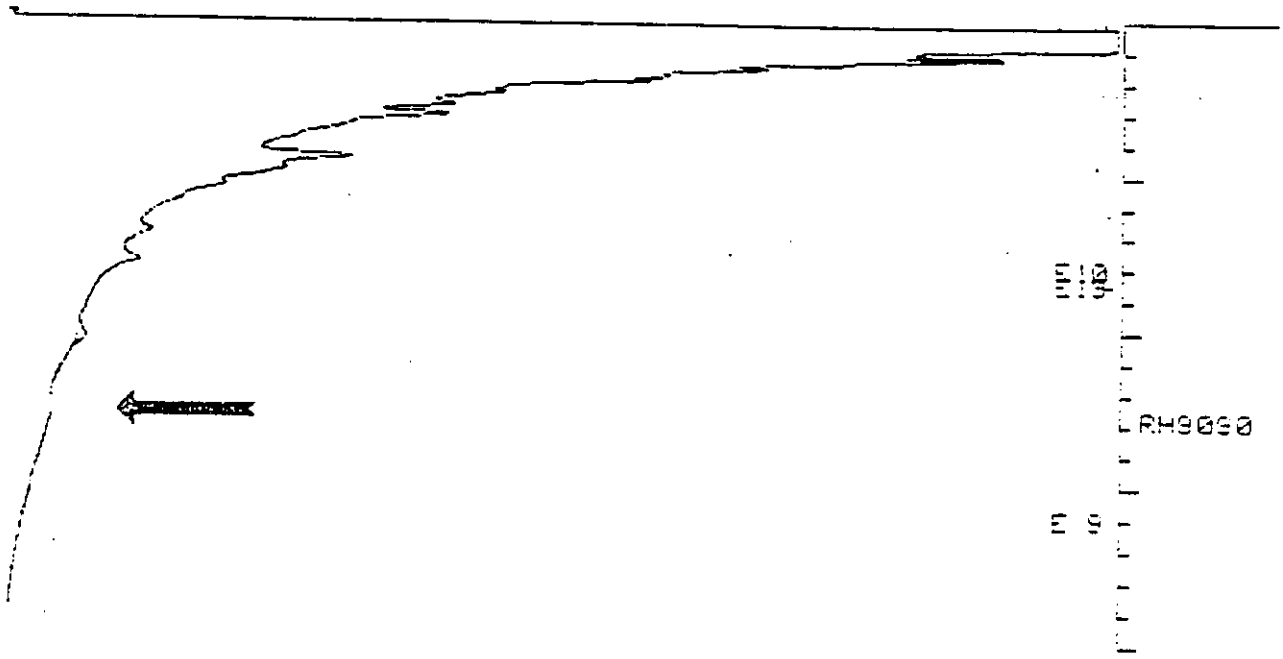
RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	SAMP WT.	RECOV FACT.	PPM
4.05	RH3866	740.0	629.6	.1958	10.00	10.0	.900	.2176

Figure 4 Grape-Control RH-9090

DATA FILE: S0709847
METHOD FILE: STAN_2
TYPE: SAMPLE

RAR NUMBER: 84-0229
SAMPLE NO: 005

Start time= 0.00 Stop time= 20.00 minutes Offset= -4 mv
Full Range = 10 millivolts



RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	SAMP WT.	RECOV FACT.	PPM
12.80	RH9090	0.0	0.0	0.0000	10.00	10.0	.700	0.0000

Figure 5 Grape-Fortification RH-9090

DATA FILE: S11078412
METHOD FILE: STAN_2
TYPE: FORTIFICATION

RAR NUMBER: 84-0357
SAMPLE NO: 009

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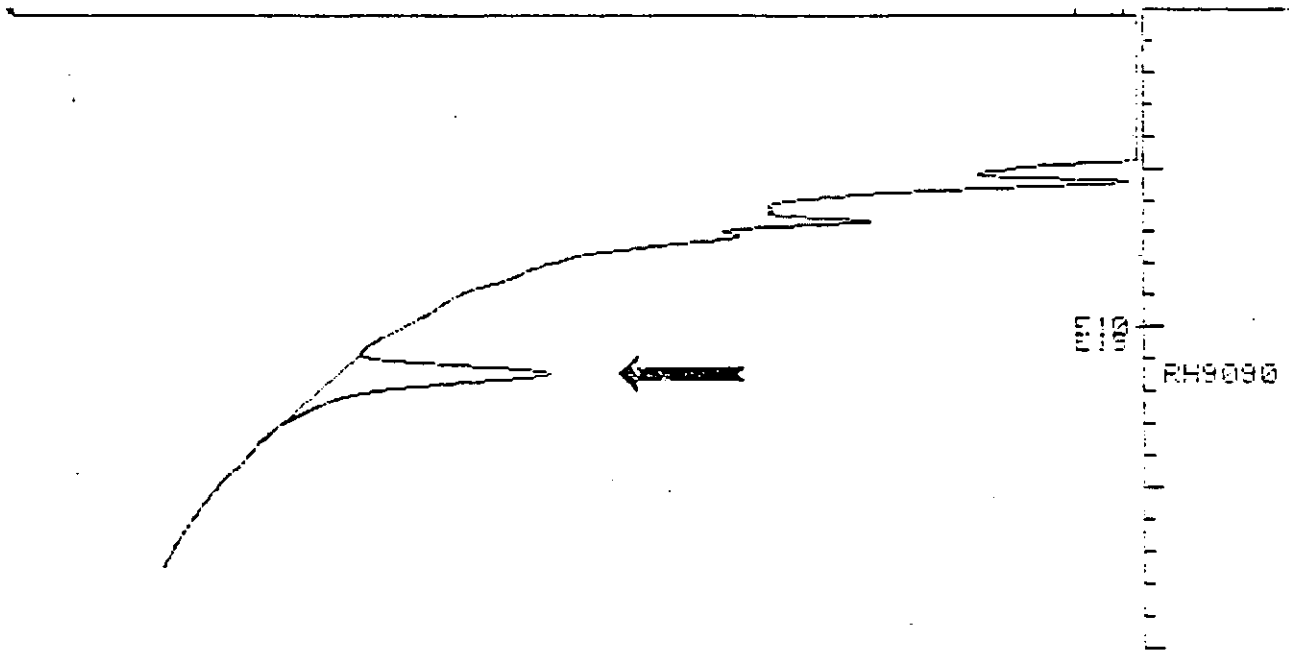
Full Range = 5 millivolts



RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	CTL CORR.	UG ADDED	PCT RECOV
11.45	RH9090	340.4	79.0	.1338	10.00	.053	2.000	64.3

Figure 6 Grape-Treated RH-9090

DATA FILE: S11078416
METHOD FILE: STAN_2
TYPE: SAMPLE

RAR NUMBER: 84-0357
SAMPLE NO: 003

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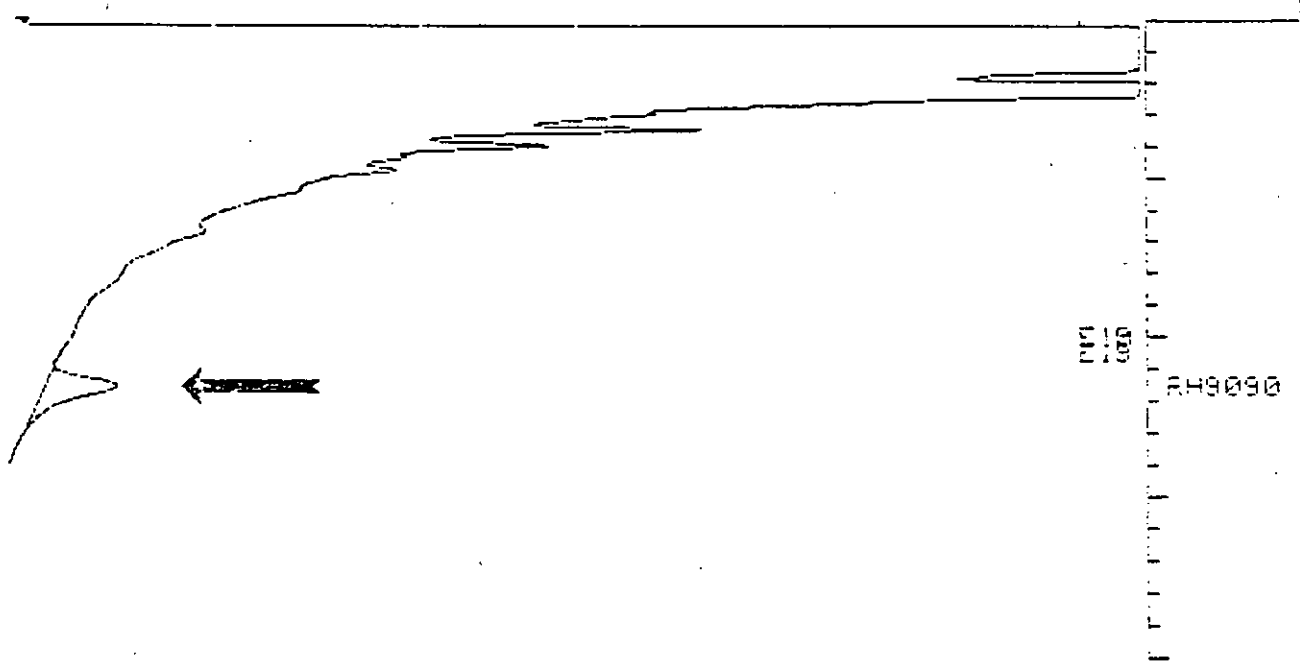


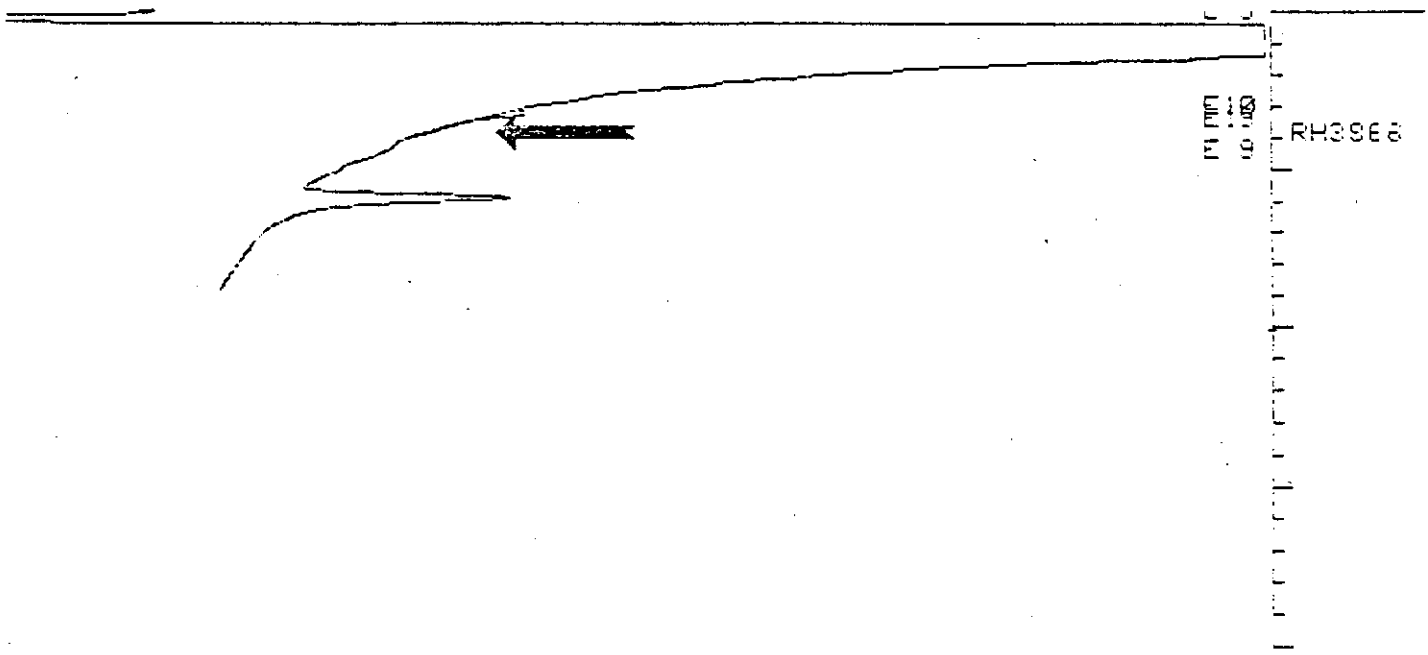
RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	SAMP WT.	RECOV FACT.	PPM
11.52	RH9090	127.0	27.5	.0499	10.00	10.0	.700	.0713

Figure 7 Apple-Control RH-3866

DATA FILE: S1030845
METHOD FILE: STAN_6
TYPE: SAMPLE

RAR NUMBER: 84-0274
SAMPLE NO: 019

Start time= 0.00 Stop time= 20.00 minutes Offset= -3 mv
Full Range = 25 millivolts



RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	SAMP WT.	RECOV FACT.	PPM
3.85	RH3866	0.0	0.0	0.0000	10.00	10.0	.900	0.0000

Figure 8 Apple-Fortification RH-3866

DATA FILE: S1030847
METHOD FILE: STAN_6
TYPE: FORTIFICATION

RAR NUMBER: 84-0274
SAMPLE NO: 019

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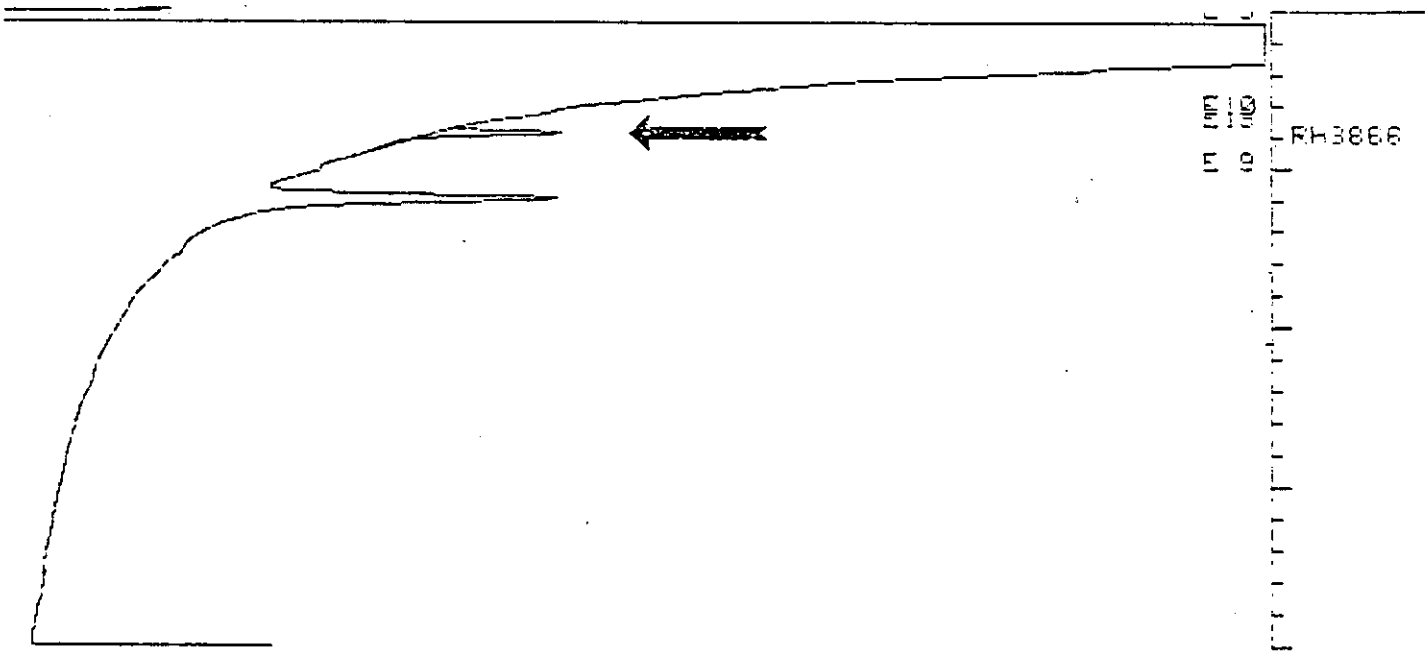
Full Range = 25 millivolts



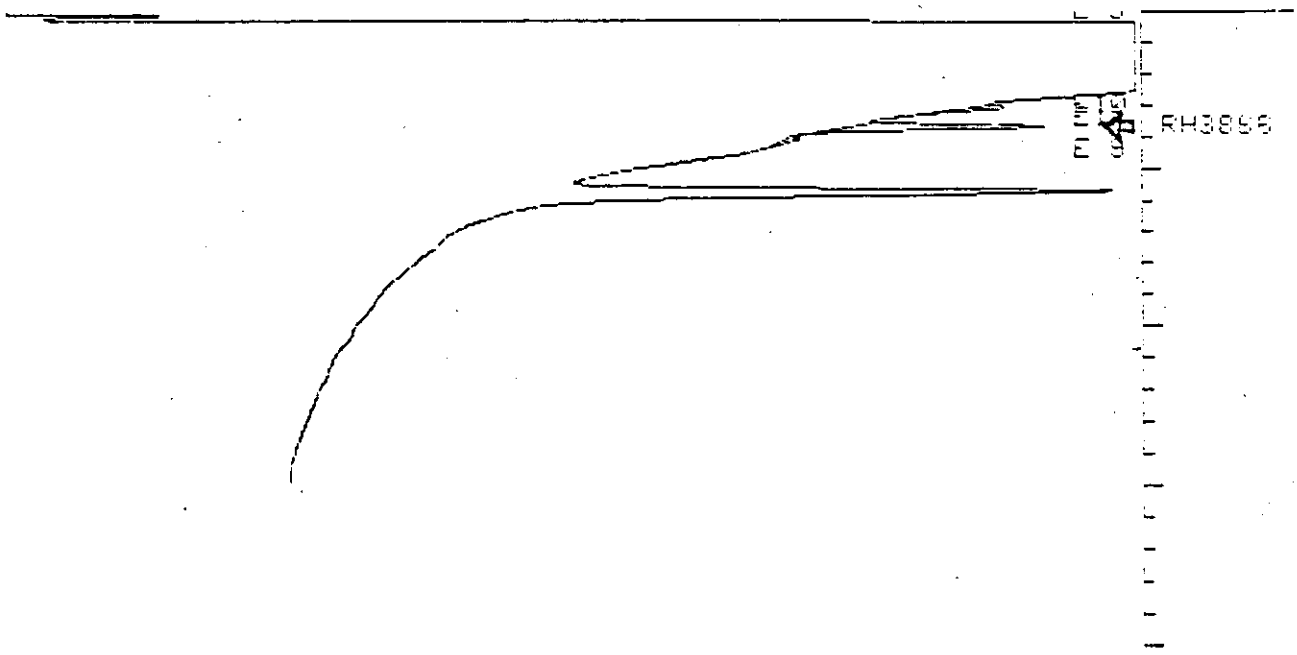
RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	CTL CORR.	UG ADDED	PCT RECOV
3.90	RH3866	228.5	233.6	.1071	10.00	0.000	1.000	107.1

Figure 9 Apple-Treated RH-3866

DATA FILE: S10308410
METHOD FILE: STAN_6
TYPE: SAMPLE

RAR NUMBER: 84-0274
SAMPLE NO: 008

Start time= 0.00 Stop time= 20.00 minutes Offset= 2 mv
Full Range = 25 millivolts



RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	SAMP WT.	RECOV FACT.	PPM
3.68	RH3866	435.9	422.6	.2043	10.00	10.0	.900	.2270

Figure 10 Apple Control RH-9090

DATA FILE: S1027844
METHOD FILE: STAN_2
TYPE: SAMPLE

RAR NUMBER: 8440300
SAMPLE NO: 004

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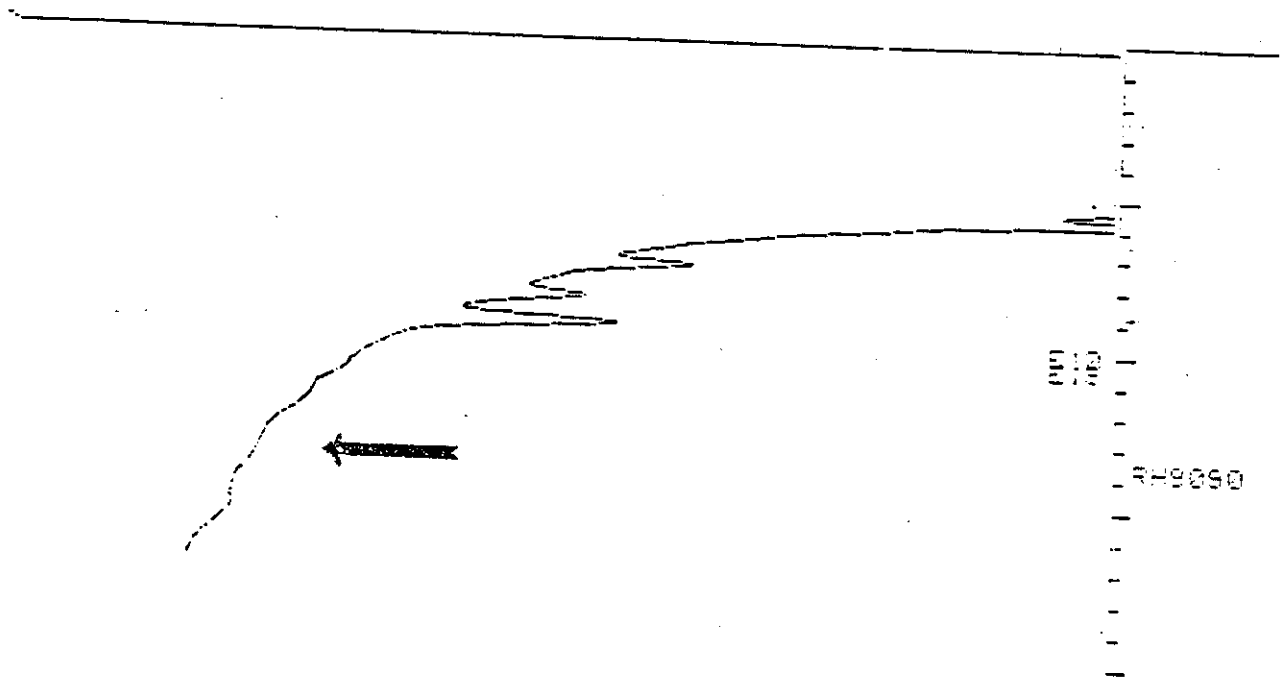
Full Range = 5 millivolts



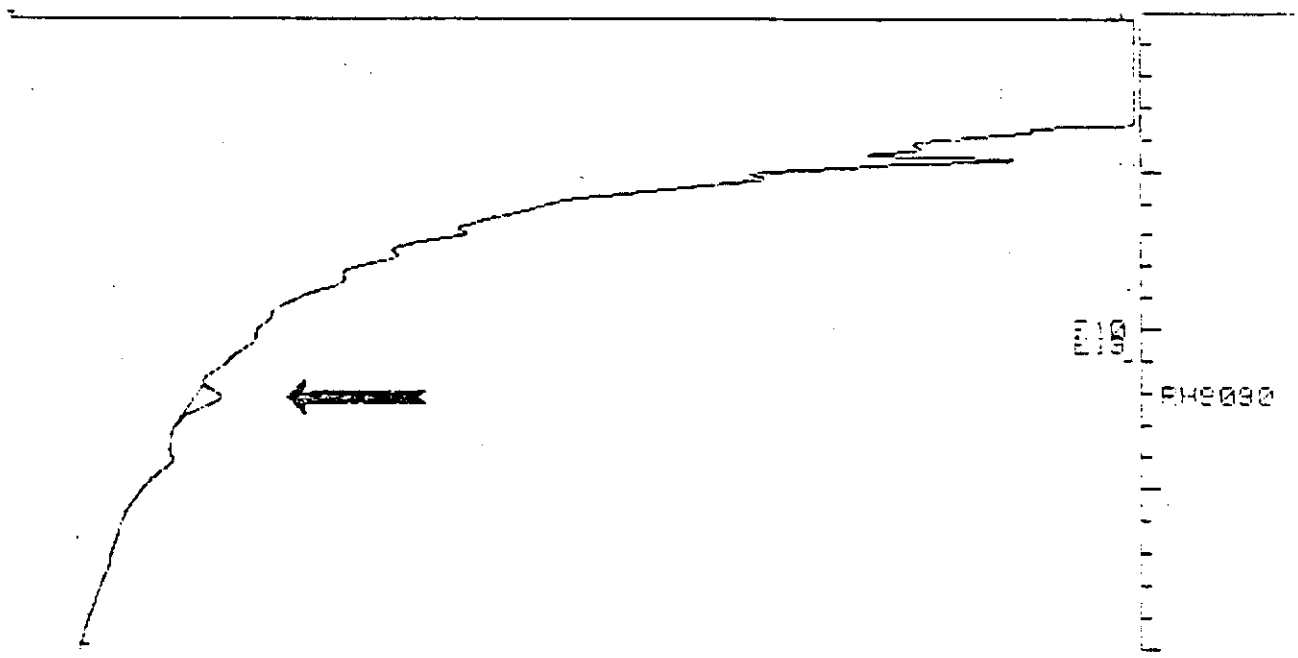
RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	SAMP WT.	RECOV FACT.	PPM
13.68	RH9090	0.0	0.0	0.0000	10.00	10.0	.700	0.0000

Figure 11 Apple Fortification RH-9090

DATA FILE: S1031847
METHOD FILE: STAN_2
TYPE: FORTIFICATION

RAR NUMBER: 84-0274
SAMPLE NO: 019

Start time= 0.00 Stop time= 20.00 minutes Offset= -5 mv
Full Range = 5 millivolts



RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	CTL CORR.	UG ADDED	PCT RECOV
12.10	RH9090	34.1	9.6	.0200	10.00	.087	.100	113.0

Figure 12 Apple Treated RH-9090

DATA FILE: S10318411
METHOD FILE: STAN_2
TYPE: SAMPLE

RAR NUMBER: 84-0274
SAMPLE NO: 009

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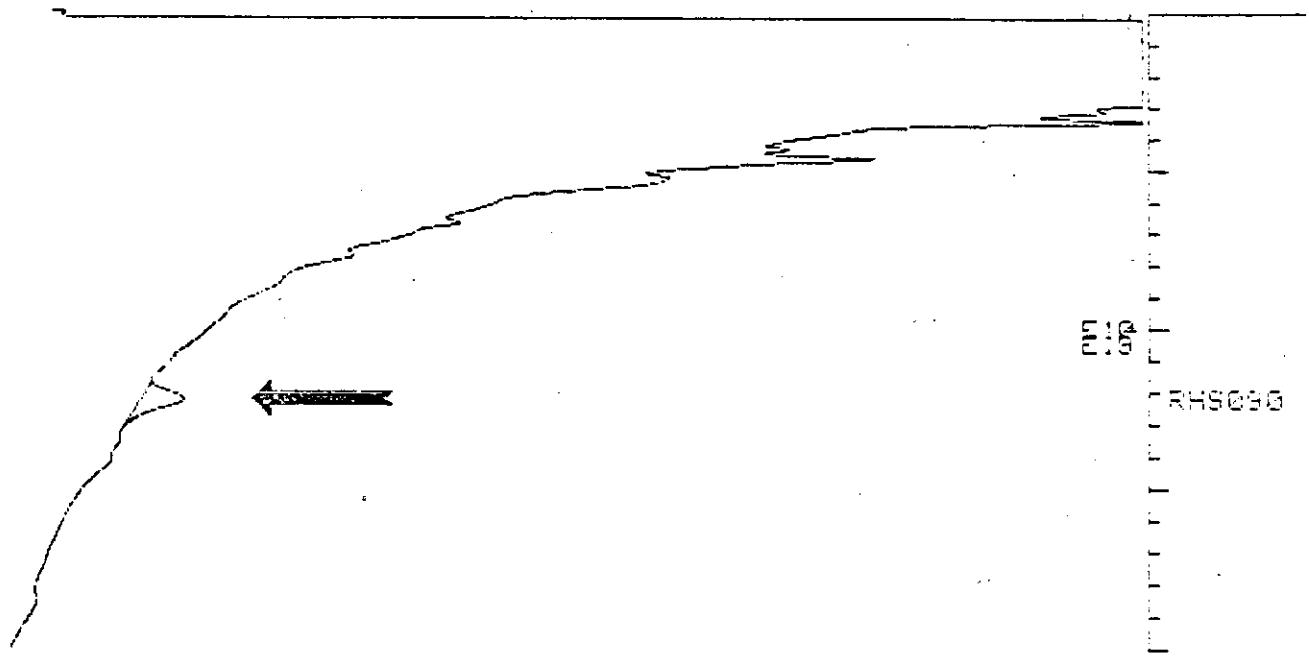
Full Range = 5 millivolts



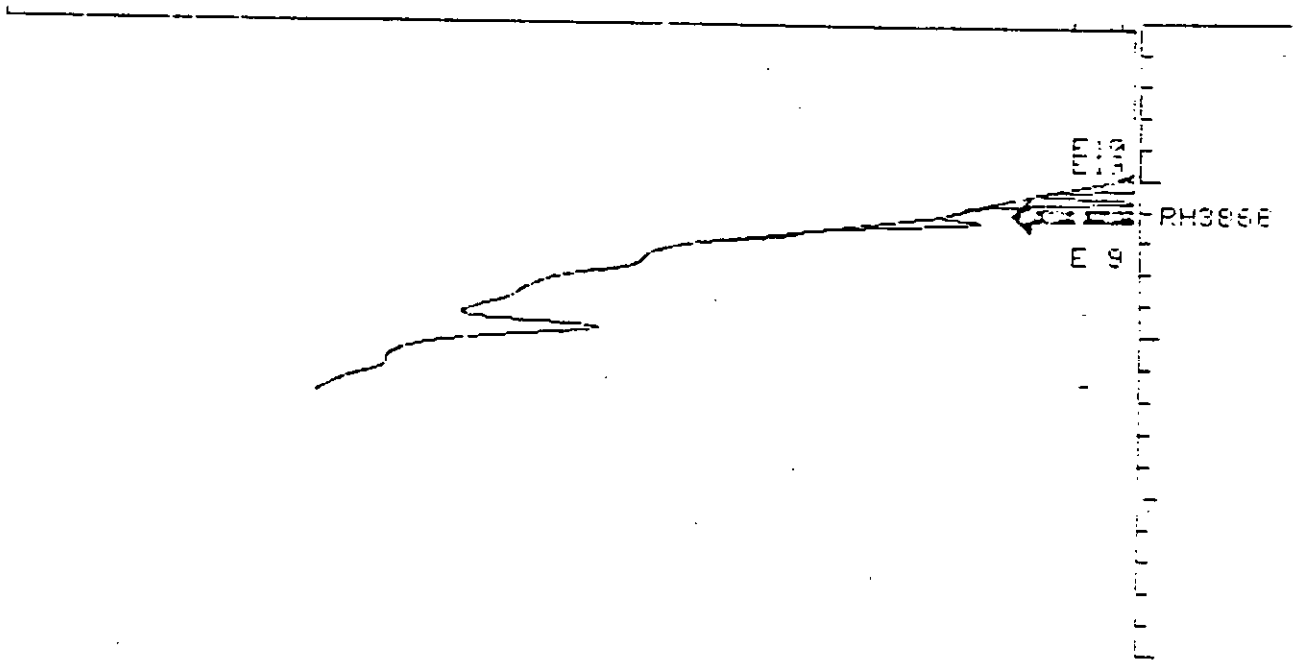
RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	SAMP WT.	RECOV FACT.	PPM
12.20	RH9090	62.9	16.5	.0369	10.00	10.0	.700	.0527

Figure 13 Confirmatory Analysis
Grape-Control RH-3866

DATA FILE: S11088411
METHOD FILE: STAN_6
TYPE: SAMPLE

RAR NUMBER: 84-0357
SAMPLE NO: CON

Start time= 0.00 Stop time= 20.00 minutes Offset= -2 mv
Full Range = 30 millivolts



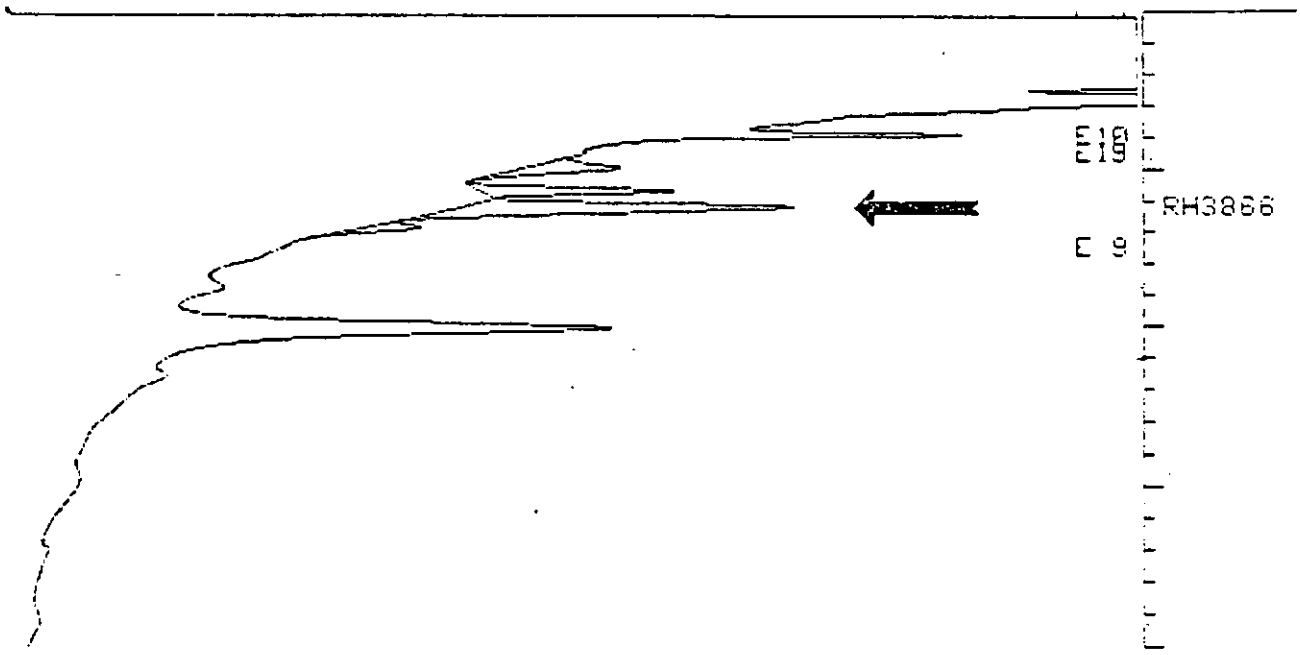
RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	SAMP WT.	RECOV FACT.	PPM
6.15	RH3866	0.0	0.0	0.0000	10.00	10.0	.900	0.0000

Figure 14 Confirmatory Analysis
Grape-Treated RH-3866

DATA FILE: S11088410
METHOD FILE: STAN_6
TYPE: SAMPLE

RAR NUMBER: 84-0357
SAMPLE NO: 003

Start time= 0.00 Stop time= 20.00 minutes Offset= -5 mv
Full Range = 7 millivolts



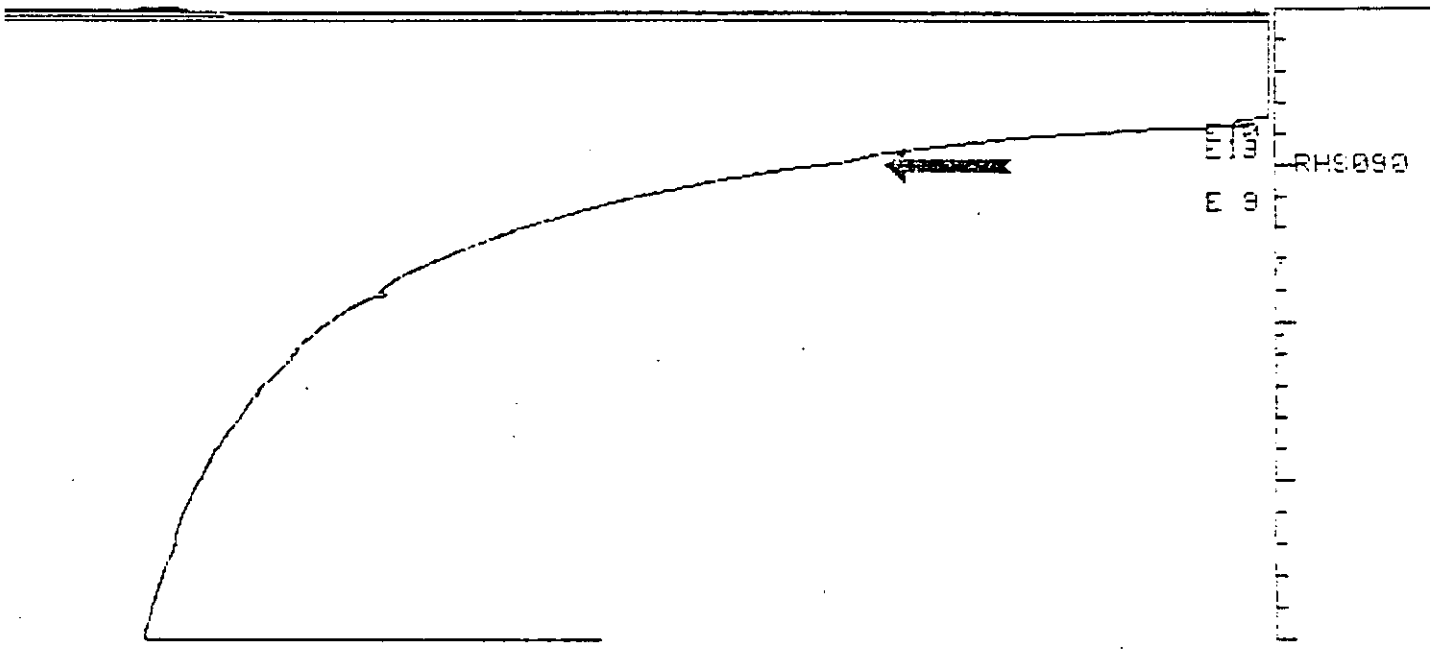
RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	SAMP WT.	RECOV FACT.	PPM
6.22	RH3866	361.0	185.9	.2003	10.00	10.0	.900	.2226

Figure 15 Confirmatory Analysis
Grape-Control RH-9090

DATA FILE: S1110843
METHOD FILE: STAN_2
TYPE: SAMPLE

RAR NUMBER: 84-0357
SAMPLE NO: CON.

Start time= 0.00 Stop time= 20.00 minutes Offset= -5 mv
Full Range = 10 millivolts



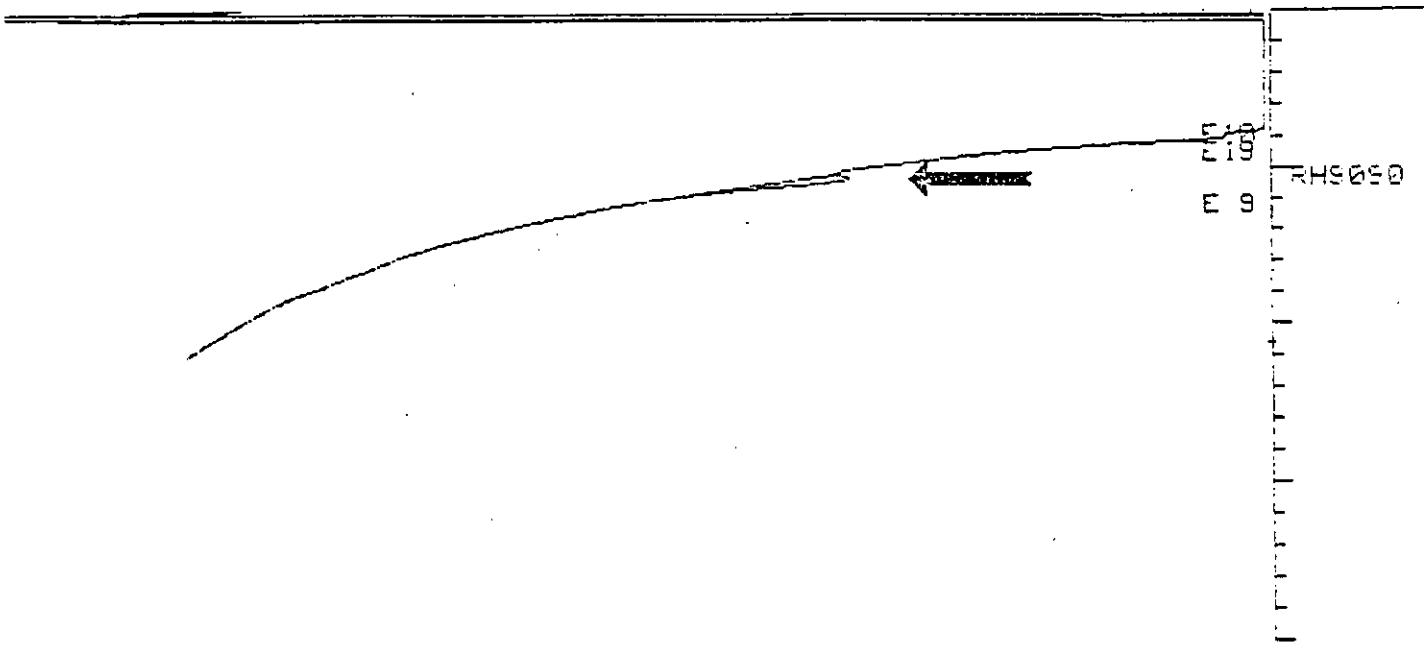
RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	SAMP WT.	RECOV FACT.	PPM
5.00	RH9090	0.0	0.0	0.0000	10.00	10.0	.700	0.0000

Figure 16 Confirmatory Analysis
Grape-Treated RH-9090

DATA FILE: S1110848
METHOD FILE: STAN_2
TYPE: SAMPLE

RAR NUMBER: 84-0357
SAMPLE NO: 003

Start time= 0.00 Stop time= 20.00 minutes Offset= -6 mv
Full Range = 10 millivolts



RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	SAMP WT.	RECOV FACT.	PPM
5.35	RH9090	82.7	37.1	.0407	10.00	10.0	.700	.0581

Appendix 1

Detailed Combustion and Radioassay Data

RH-3866-C14 METHOD DEVELOPMENT
GRAPE COMBUSTION

GROUP NAME: SS500W29 RUN NUMBER: 4474

STORED CALIBRATION PARAMETERS
A= 46.2031 B= 165.599 C= -171.118 CURVE=7
COMBUSTION SPIKE 52600 DPM
SAMPLE COUNTS MUST BE AT LEAST 3 CPM ABOVE BACKGROUND TO BE SIGNIFICANT.

CONTROL SAMPLES

SAMPLE IDENT.	AVERAGE SAMPLE CPM RED	GREEN	BLUE	SAMP. WGT. (GMS.)
GRAPE-C	30.6	29.2	0.0	0.120
GRAPE-C	28.5	27.5	0.0	0.144
GRAPE-C	26.5	23.1	0.0	0.180
AVERAGE	28.5	26.6	0.0	
STD. DEV.	2.1	3.2	0.0	
NO. SAMPLE	(3)	(3)	(3)	

TREATED SAMPLES

SAMPLE IDENT.	AVG SAMP. CPM	AVG ESCR	COUNT. EFF. (PCT.)	SAMPLE* DPM	SAMP. WGT. (GMS.)	DPM** PER GM	TOTAL WGT. (GMS.)	TOTAL DPM
SUBGROUP 1. DATA TAKEN FROM G CHANNEL.								
GRAPE-1	431.0	0.39863	85.0	475.6	0.173	2843.0	0.173	491.8
GRAPE-1	611.5	0.40647	85.2	686.2	0.182	3898.9	0.182	709.6
GRAPE-1	844.7	0.41170	85.4	958.2	0.211	4696.0	0.211	990.9
GRAPE-1	1040.5	0.40530	85.2	1189.8	0.276	4457.9	0.276	1230.4

* CORRECTED FOR BACKGROUND AND ESCR COUNTING EFFICIENCY
** CORRECTED FOR COMBUSTION EFFICIENCY (96.7 PCT.)

RH-3866-C14 METHOD DEVELOPMENT
GRAPE COMBUSTION

TREATED SAMPLES

SAMPLE IDENT.	AVG SAMP. CPM	AVG ESCR	COUNT. EFF. (PCT.)	SAMPLE* DPM	SAMP. WGT. (GMS.)	DPM** PER GM	TOTAL WGT. (GMS.)	TOTAL DPM
SUBGROUP	(CONTINUED)							
GRAPE-1	1120.4	0.40340	85.2	1284.4	0.194	6846.4	0.194	1328.2
GRAPE-1	941.6	0.40287	85.1	1074.6	0.335	3317.2	0.335	1111.3
GRAPE-1	617.0	0.38823	84.7	697.0	0.200	3604.0	0.200	720.8
AVERAGE	801.0					4237.6		940.4
STD. DEV.	254.2					1314.8		307.9
NO. SAMPLES	(7)					(7)		(7)

* CORRECTED FOR BACKGROUND AND ESCR COUNTING EFFICIENCY
** CORRECTED FOR COMBUSTION EFFICIENCY (96.7 PCT.)

COMBUSTION SPIKES

SAMPLE IDENT.	AVG SAMP. CPM	AVG ESCR	COUNT. EFF. (PCT.)	SAMPLE* DPM	COMB. EFF. (PCT)
GRAPE-S	43149.6	0.4200	85.6	50395.2	95.8
GRAPE-S	43580.1	0.4114	85.4	51018.4	97.0
GRAPE-S	43795.0	0.4166	85.5	51183.9	97.3
AVERAGE					96.7
STD. DEV.					0.8
NO. SAMPLES					(3)

* CORRECTED FOR BACKGROUND AND ESCR COUNTING EFFICIENCY

********** -END OF JOB- **********

RH-3366-C14 METHOD DEVELOPMENT
GRAPE-C14

GROUP NAME: S500W29B RUN NUMBER: 4472

SPECIFIC ACTIVITY FOR RH-3366-C14 METH 21000 DPM PER MICROGRAM
STORED CALIBRATION PARAMETERS
A= 49.6104 B= 93.6759 C= -51.1520 CURVE=7
SAMPLE COUNTS MUST BE AT LEAST 4 CPM ABOVE BACKGROUND TO BE SIGNIFICANT.

BACKGROUND CONTROL PRESET AT 30.0 CPM

TREATED SAMPLES

SAMPLE IDENT.	AVG SAMP. CPM	AVG ESCR	COUNT. EFF. (PCT.)	SAMPLE* DPM	SAMP. VOL. (MLS.)	DPM PER ML	TOTAL VOL. (MLS.)	TOTAL DPM	RESIDUE (FPM)
SUBGROUP 1. DATA TAKEN FROM R CHANNEL.									
1-EXTRACT	94.7	0.53557	85.1	76.1	0.200	380.3	110.000	41833.0	0.0181
1-EXTRACT	84.3	0.59637	87.3	62.2	0.200	311.2	110.000	34237.1	0.0148
AVERAGE	89.5					345.8		38035.1	0.0165
STD. DEV.	7.4					48.8		5371.1	0.0023
NO. SAMPLES	(2)					(2)		(2)	(2)
SUBGROUP 2. DATA TAKEN FROM R CHANNEL.									
2-EXTRACT	79.1	0.64827	88.0	55.2	0.200	276.1	160.000	44183.9	0.0131
2-EXTRACT	75.5	0.64057	88.6	51.3	0.200	256.5	160.000	41040.9	0.0122
AVERAGE	77.3					266.3		42612.4	0.0127
STD. DEV.	2.5					13.9		2222.4	0.0007
NO. SAMPLES	(2)					(2)		(2)	(2)

* CORRECTED FOR BACKGROUND AND ESCR COUNTING EFFICIENCY

RH-3866-C14 METHOD DEVELOPMENT
APPLE-C14 COMBUSTION
RH-3866-C14 TREATED

GROUP NAME: S0500W3U RUN NUMBER: 4542

STORED CALIBRATION PARAMETERS
A= 41.6600 B= 100.714 C= -196.371 CURVE=7
COMBUSTION SPIKE 52000 DPM
SAMPLE COUNTS MUST BE AT LEAST 3 DPM ABOVE BACKGROUND TO BE SIGNIFICANT.

CONTROL SAMPLES

SAMPLE IDENT.	AVERAGE SAMPLE CPM RED	GREEN	BLUE	SAMP. WGT. (GMS.)
APPLE-C	38.4	36.7	0.0	0.157
APPLE-C	36.7	33.3	0.0	0.150
APPLE-C	34.2	32.0	0.0	0.146
AVERAGE	36.4	34.0	0.0	
STD. DEV.	2.1	2.4	0.0	
NO. SAMPLE	(3)	(3)	(3)	

TREATED SAMPLES

SAMPLE IDENT.	AVG SAMP. CPM	AVG ECCR	COUNT. EFF. (PCT.)	SAMPLE* DPM	SAMP. WGT. (GMS.)	DPM** PER GM	TOTAL WGT. (GMS.)	TOTAL DPM
SUBGROUP 1 . DATA TAKEN FROM C CHANNEL.								
APPLE-T	1047.3	0.46470	87.0	1165.3	0.194	6441.0	0.194	1249.7
APPLE-T	529.1	0.47197	87.0	569.2	0.215	2809.1	0.215	610.4
APPLE-T	768.5	0.47027	87.0	844.4	0.193	4491.9	0.193	905.5
APPLE-T	1188.3	0.46627	87.0	1321.6	0.206	6890.4	0.206	1417.4
APPLE-T	541.5	0.46053	86.9	583.6	0.174	3397.2	0.174	625.9
APPLE-T	537.2	0.47133	87.0	578.5	0.180	3299.8	0.180	620.4
APPLE-T	829.7	0.45887	86.9	916.0	0.148	6187.4	0.148	982.3
AVERAGE	776.6					4912.5		916.0
STD. DEV.	263.1					1725.5		324.6
NO. SAMPLES	(7)					(7)		(7)

* CORRECTED FOR BACKGROUND AND ECCR COUNTING EFFICIENCY
** CORRECTED FOR COMBUSTION EFFICIENCY (98.2 PCT.)

RH-3866-C14 METHOD DEVELOPMENT
C14-APPLE EXTRACTION EFFICIENC

GROUP NAME: S500W30Z RUN NUMBER: 4554

SPECIFIC ACTIVITY FOR RH-3866-C14 METH 21000 DPM PER MICROGRAM
STORED CALIBRATION PARAMETERS
A= 44.0242 B= 93.2730 C= -44.9827 CURVE=7
SAMPLE COUNTS MUST BE AT LEAST 3 CPM ABOVE BACKGROUND TO BE SIGNIFICANT.

BACKGROUND CONTROL PRESET AT 30.0 CPM

TREATED SAMPLES

SAMPLE IDENT.	AVG SAMP. CPM	AVG ESCR	COUNT. EFF. (PCT.)	SAMPLE* DPM	SAMP. VOL. (MLS.)	DPM PER ML	TOTAL VOL. (MLS.)	TOTAL DPM	RESIDUE (PPM)
SUBGROUP 1 . DATA TAKEN FROM R CHANNEL.									
1-APPLEC14	88.0	0.62160	84.6	68.5	0.250	274.2	170.000	46607.2	0.0131
1-APPLEC14	99.8	0.61617	84.4	82.7	0.250	330.7	170.000	56225.1	0.0157
1-APPLEC14	81.4	0.64797	85.6	60.1	0.250	240.3	170.000	40843.4	0.0114
1-APPLEC14	86.9	0.62333	84.7	67.2	0.250	268.9	170.000	45715.1	0.0128
AVERAGE	89.0					278.5		47347.7	0.0133
STD. DEV.	7.7					37.9		6437.6	0.0018
NO. SAMPLES	(4)					(4)		(4)	(4)

ROHM AND HAAS COMPANY
INDEPENDENCE MALL WEST
PHILADELPHIA, PENNSYLVANIA 19105

MRID 165036

STUDY TITLE

Addendum to RH-3866 Total Residue Analytical Method for Grape and Apple (TR 310-84-27)

DATA REQUIREMENT

§ 171-4 Residue Analytical Method

AUTHOR

S. S. Stavinski, C. K. Brackett, T. F. Burnett, R. O. Deakyne

STUDY COMPLETED ON

July 8, 1986

PERFORMING LABORATORY

Rohm and Haas Company
Research Laboratories
Spring House, PA 19477

LABORATORY PROJECT I.D.

31H-86-15

31H-86-15

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: Michael A. Morelli Date: February 12, 1987

Product Registration Manager Michael A. Morelli

GOOD LABORATORY PRACTICE STATEMENT

No GLP statement is required for this type of study under 40CFR160.

Lab Memo
No. 31H-86-15

Agricultural Products
Environmental Sciences

July 8, 1986

To: Dr. W. S. Hurt

From: S. S. Stavinski, C. K. Brackett, T. F. Burnett, and R. O. Deakyne

Subject: Addendum to RH-3866 Total Residue Analytical Method for Grape and Apple (TR 310-84-27)

Modifications to the RH-3866 total residue analytical method (TR 310-84-27) have been made to improve sample cleanup and RH-9090 quantitation. These improvements also allow the total method to be applied to a wider range of substrates, including difficult apple and grape processed fractions.

Modifications to the RH-3866 total residue analytical method (TR 310-84-27) are given below:

Section 5.5 Chelex 100-Fe^{+++} Column Chromatography

Prepare the Chelex 100-Fe^{+++} fresh daily resin by adding a 0.5% $FeCL_3$ solution (0.5 g $FeCl_3$/100 mL water) to 400 mL of hydrated Chelex 100. Wash twice with 1000 mL of 1:4 (v/v) methanol water and prepare column as described.

Section 5.6 Methylene Chloride Partition

Partition the Chelex 100-Fe^{+++} eluant twice with 150 mL methylene chloride. Combine both methylene chloride fractions and partition three times with 100 mL of water. Dry methylene chloride over anhydrous sodium sulfate, collect the methylene chloride phase and evaporate to dryness at 50°C under diminished pressure.

Section 5.7 Bio-Sil A Column Chromatography

Substitute Bio-Sil A for Florisil. Bio-Sil A, 100-200 mesh, is purchased from Bio-Rad Laboratories. Activate the Bio-Sil A by heating at 200°C for 48 hours. Remove and store dessicated in 8 oz. tightly capped glass vials.

Proceed as described in Section 5.7 but use the Bio-Sil A Elution pattern:

Bio-Sil-A Elution Pattern

Redissolve residue in 25 mL of 2% (v/v) acetone/toluene.
Wash with 10 mL 2% (v/v) acetone/toluene.
Wash with 40 mL 20% (v/v) acetone/toluene.
Elute RH-3866 fraction with 75 mL 25% (v/v) acetone/toluene.
Wash with 15 mL 35% (v/v) acetone/toluene.
Elute RH-9090 fraction with 75 mL 50% (v/v) acetone/toluene.

Standardize the elution pattern when a new batch of Bio-Sil A is prepared to ascertain that no batch dependent changes occur.

Section 5.8 GLC Quantitations

Substitute the following GLC conditions for RH-9090 quantitation:

GLC Hewlett Packard 5890
Detector: Hewlett Packard ^{63}Ni ECD
Column: J&W Scientific Megabore DB-17, 30 meter
Carrier Gas: P-10 (10% methane/Argon)
Injector Temperature: 200°C
Detector Temperature: 325°C
Inlet Pressure: 80 PSI
Purge Flow: 60 mL/min
Column Flow: 8.7 mL/min
Initial Temperature: 200°C
Initial Hold Time: 5 min
Program Rate: 10°C/min
Final Temperature: 250°C
Final Hold Time: 20 min
Total Run Time: 30 min
Injection Volume: 3 ul

Illustrative chromatographs of apple and grape samples processed by the modified method are attached. Figures 1-5 illustrate chromatographs of RH-3866 standard, grape control, grape fortified, apple control, and apple fortified samples, respectively. Figures 6-10 illustrate chromatographs of RH-9090 standard, grape control, grape fortified, apple control, and apple fortified samples, respectively.

C. K. Brackett

R. O. Deakyne

T. F. Burnett

S. S. Stavinski

/maw
Attachment

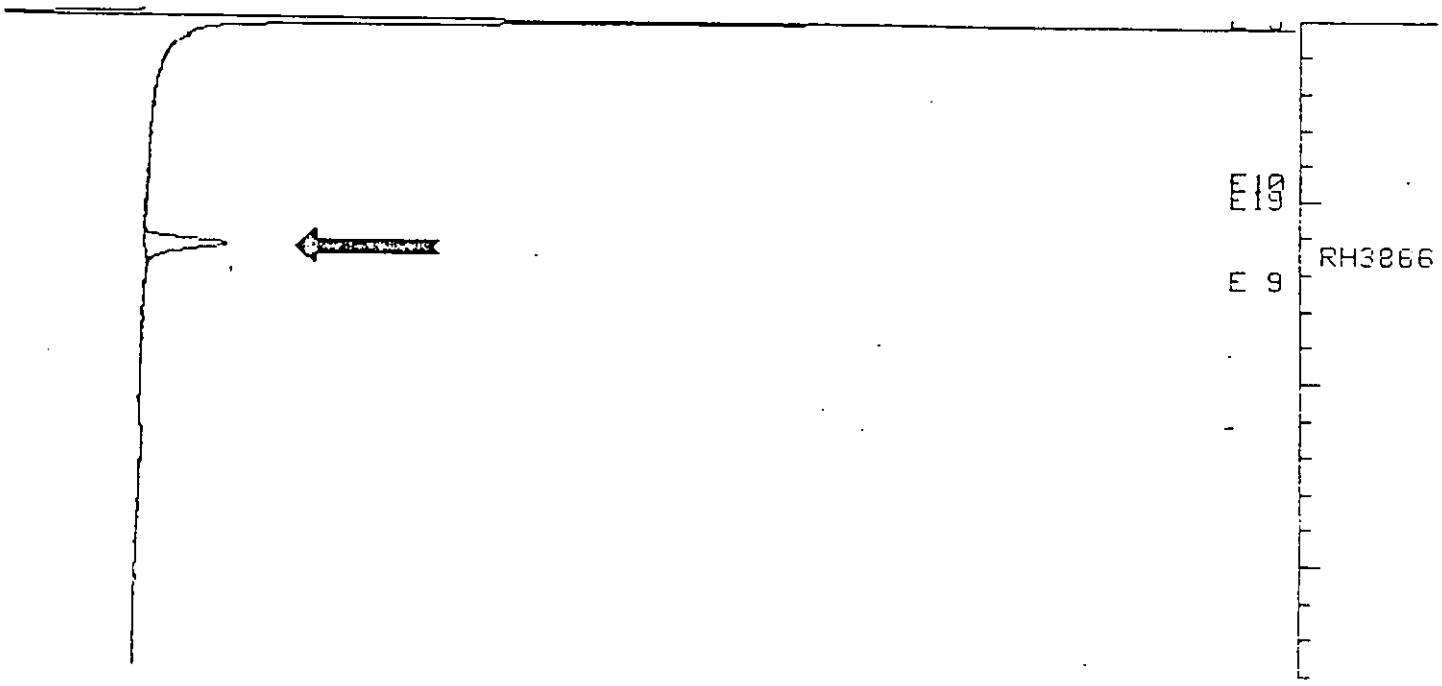
* Cover memo only

Figure 1

RH-3866 Standard

DATA FILE: S07028616
METHOD FILE: STAN_3866
TYPE: STANDARD

Start time= 0.00 Stop time= 18.00 minutes Offset= -3 mv
Full Range = 20 millivolts



RETENTION TIME	COMPOUND NAME	PPM: INJECTED	AREA	HEIGHT
6.48	RH3866	.1000	255.60	123.26

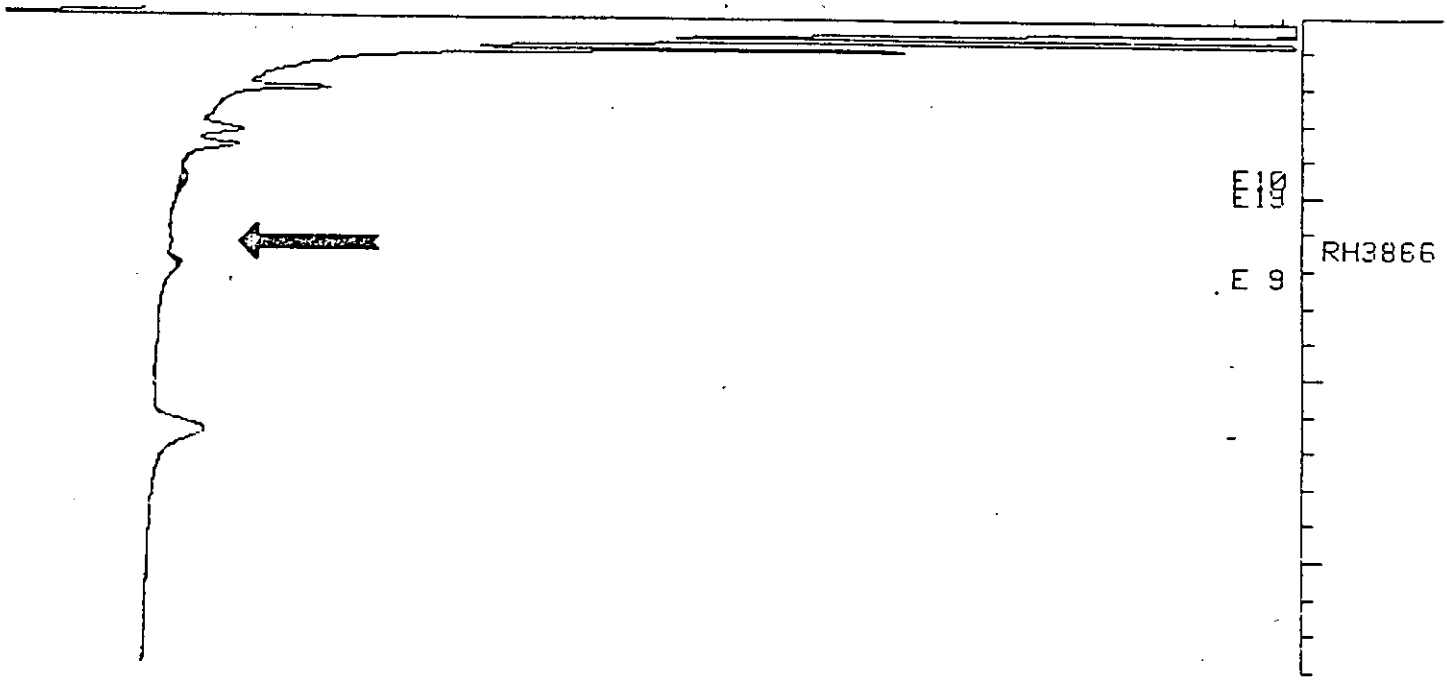
Figure 2

RH-3866 Grape Control

DATA FILE: S07028628
METHOD FILE: STAN_3866
TYPE: SAMPLE

RAR NUMBER: 85-0373
SAMPLE NO: 008

Start time= 0.00 Stop time= 18.00 minutes Offset= -4 mv
Full Range = 20 millivolts



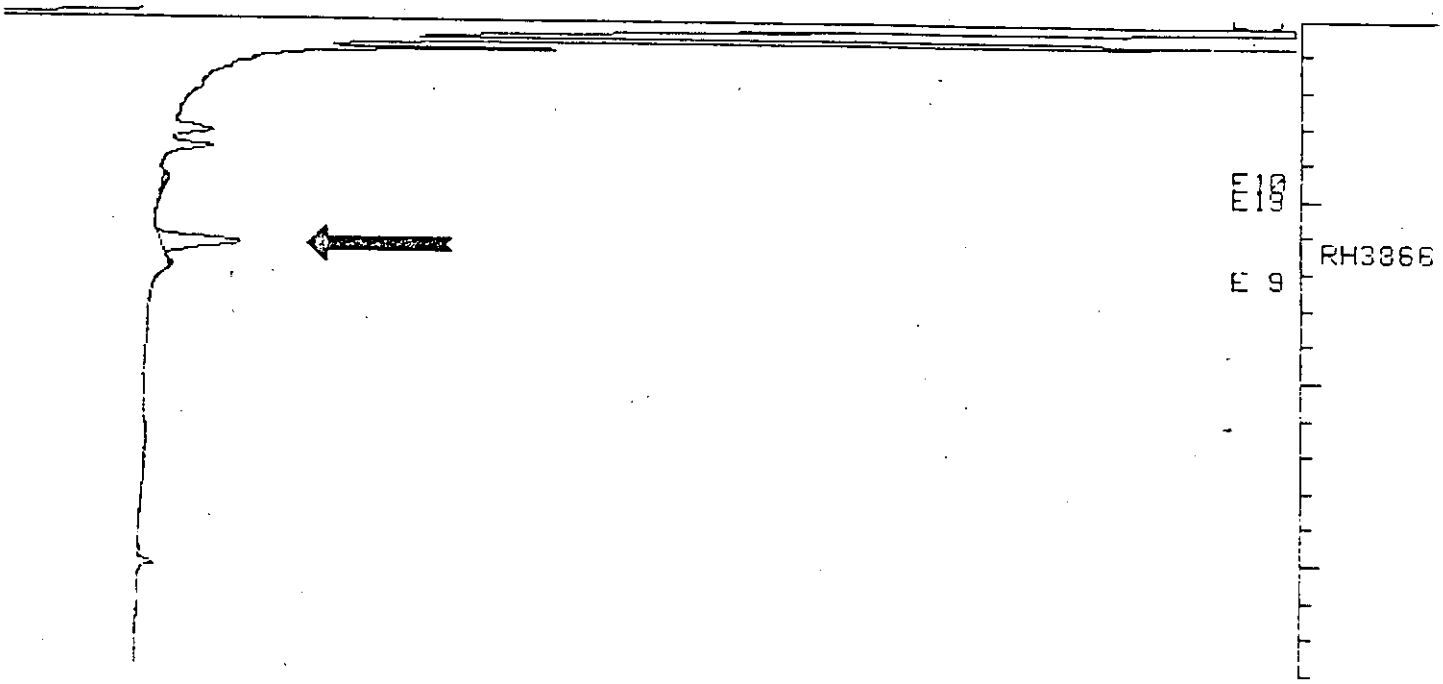
RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	SAMP WT.	RECOV FACT.	PPM
6.45	RH3866	0.0	0.0	0.0000	10.00	10.0	.900	0.0000

Figure 3
RH-3866 Grape Fortification

DATA FILE: S07028629
METHOD FILE: STAN_3866
TYPE: FORTIFICATION

RAR NUMBER: 85-0373
SAMPLE NO: 008

Start time= 0.00 Stop time= 18.00 minutes Offset= -4 mv
Full Range = 20 millivolts



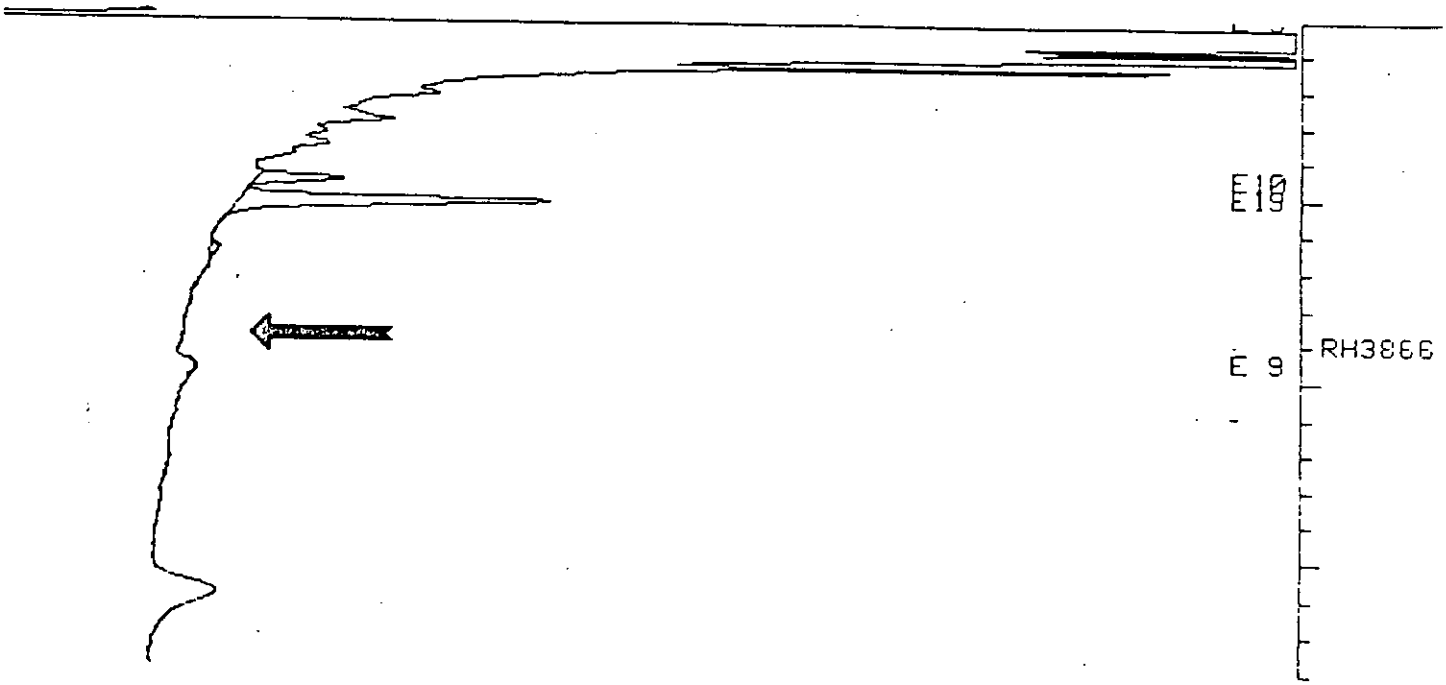
RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	CTL CORR.	UG ADDED	PCT RECOV
6.45	RH3866	229.2	120.2	.0880	10.00	0.000	1.000	88.0

Figure 4
RH-3866 Apple Control

DATA FILE: S0624867
METHOD FILE: STAN_3866
TYPE: SAMPLE

RAR NUMBER: 85-0490
SAMPLE NO: 004

Start time= 0.00 Stop time= 18.00 minutes Offset= -3 mv
Full Range = 20 millivolts



RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	SAMP WT.	RECOV FACT.	PPM
8.92	RH3866	0.0	0.0	0.0000	10.00	10.0	.900	0.0000

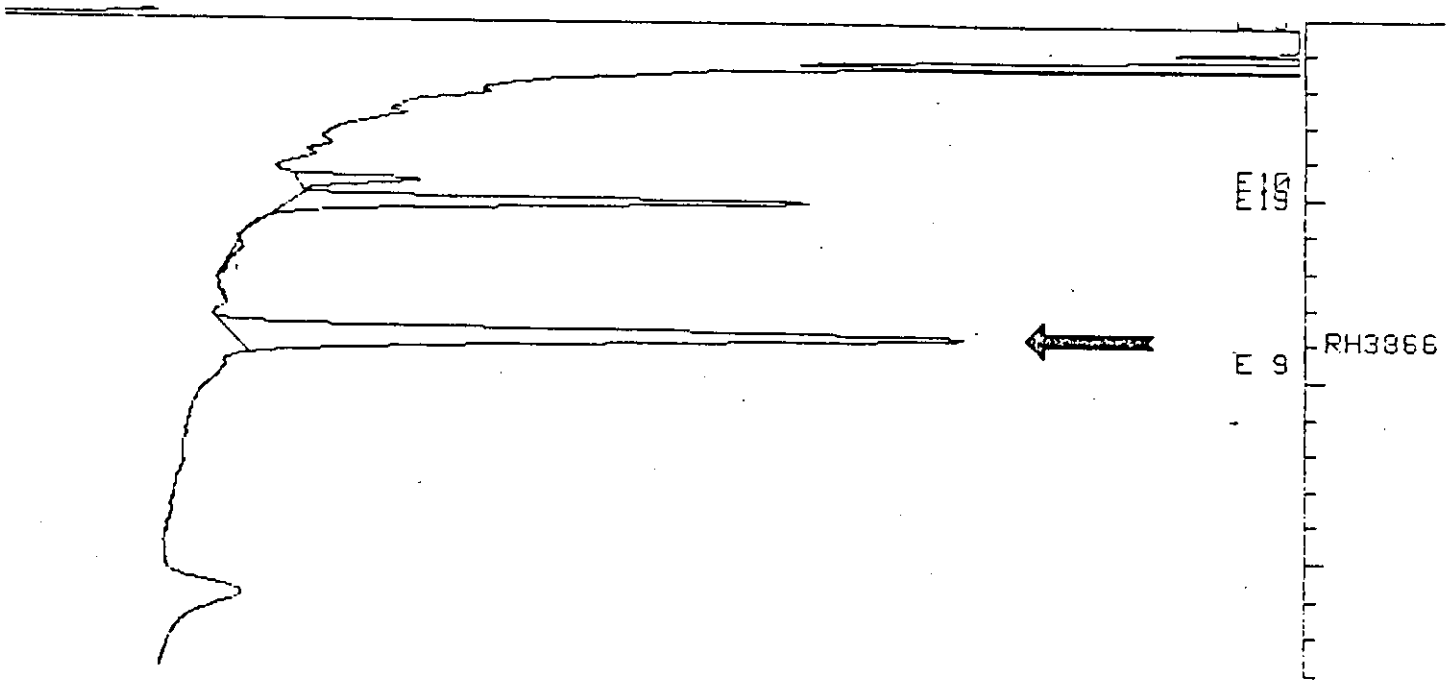
Figure 5

Apple Fortification

DATA FILE: S0624865
METHOD FILE: STAN_3866
TYPE: FORTIFICATION

RAR NUMBER: 85-0490
SAMPLE NO: 004

Start time= 0.00 Stop time= 18.00 minutes Offset= -4 mv
Full Range = 20 millivolts



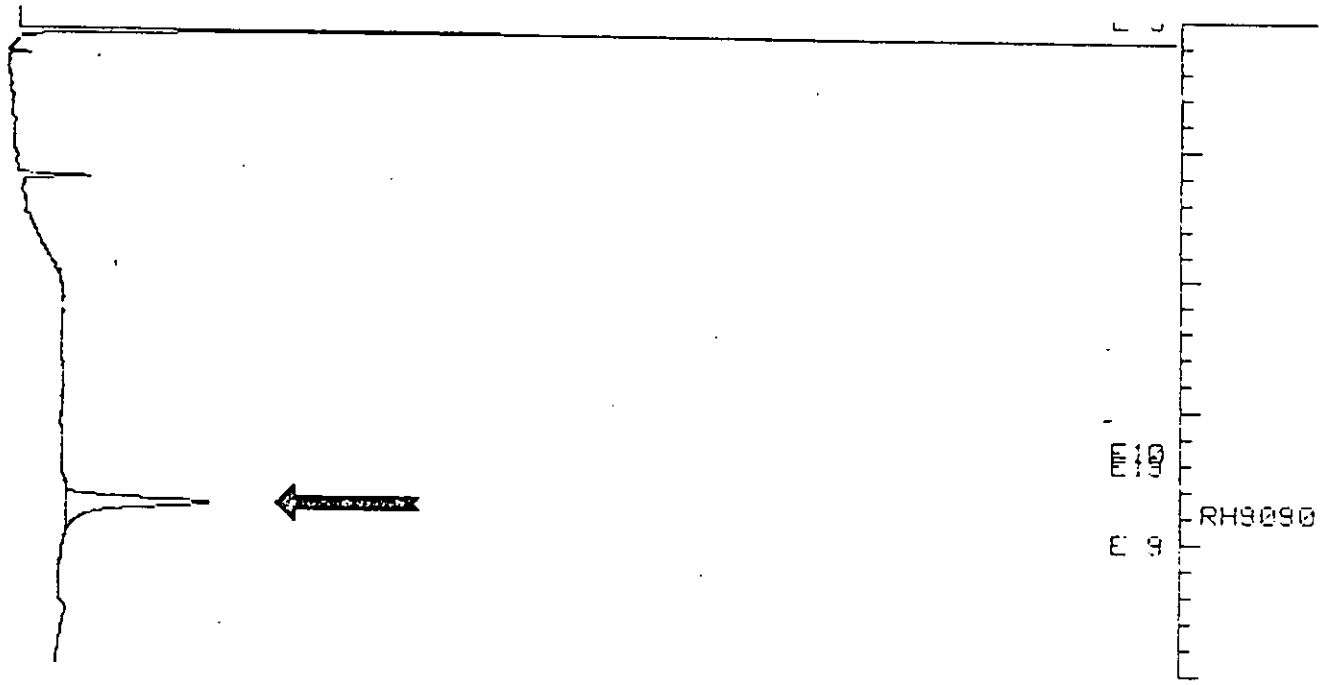
RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	CTL CORR.	UG ADDED	PCT RECOV
8.92	RH3866	2635.3	1108.3	.6394	10.00	0.000	8.000	79.9

Figure 6

RH-9090 Standard

DATA FILE: S0625865
METHOD FILE: STAN_HP17
TYPE: STANDARD

Start time= 0.00 Stop time= 25.00 minutes Offset= -6 mv
Full Range = 100 millivolts



RETENTION TIME	COMPOUND NAME	PPM INJECTED	AREA	HEIGHT
18.85	RH9090	.1000	2830.28	1044.60

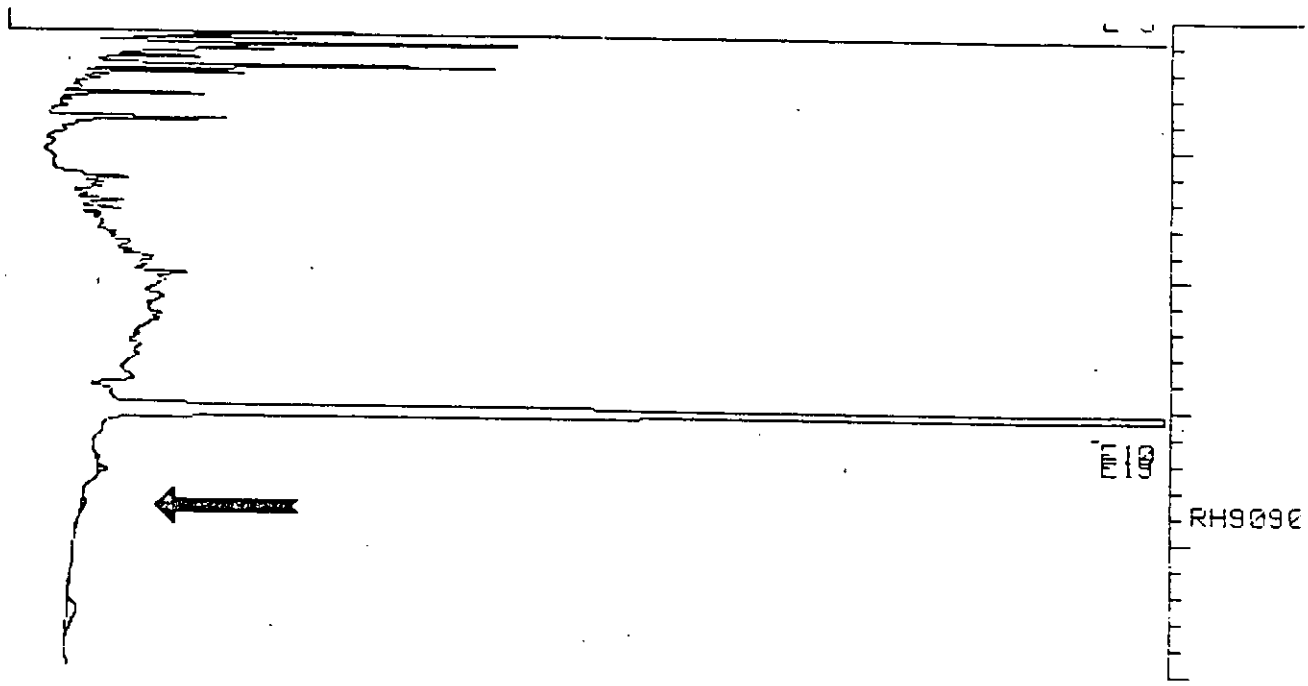
Figure 7

RH-9090 Grape Control

DATA FILE: S0701868
METHOD FILE: STAN_HP17
TYPE: SAMPLE

RAR NUMBER: 85-0373
SAMPLE NO: 008

Start time= 0.00 Stop time= 25.00 minutes Offset= -6 mv
Full Range = 100 millivolts



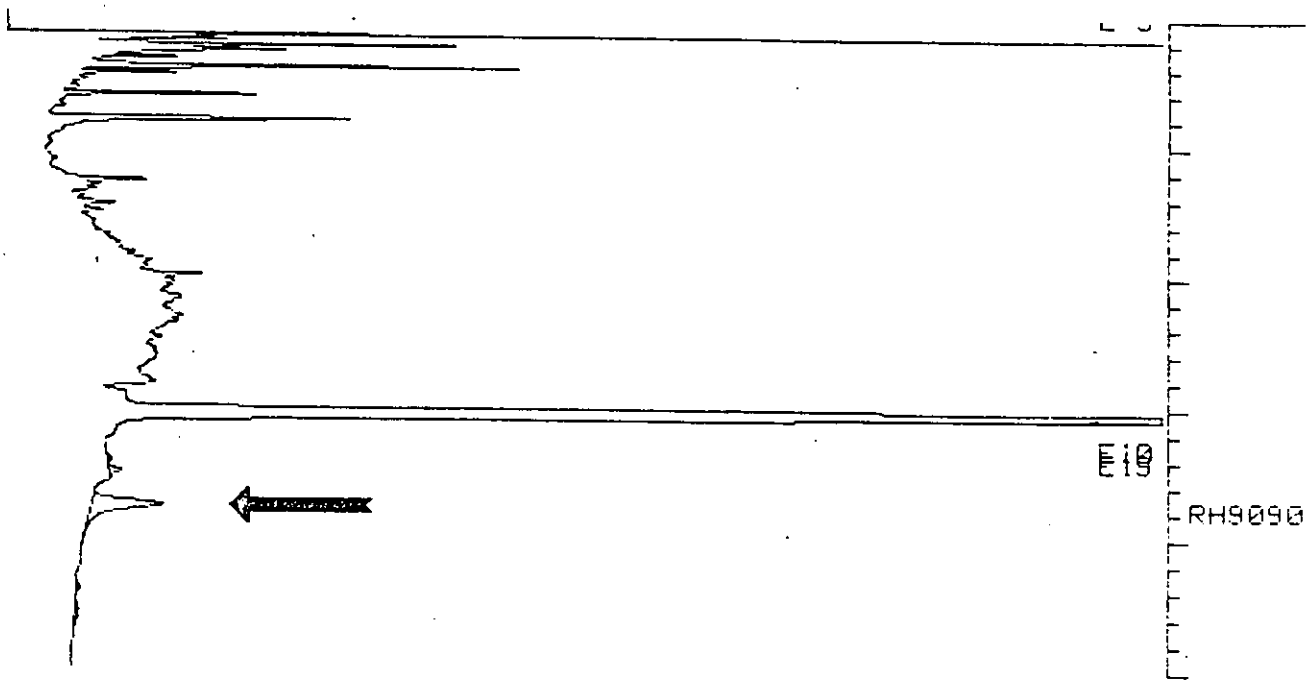
RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	SAMP WT.	RECOV FACT.	PPM
18.87	RH9090	96.6	39.1	0.0000	10.00	10.0	.700	0.0000

RH-9090 Grape Fortification

DATA FILE: S0701869
METHOD FILE: STAN_HP17
TYPE: FORTIFICATION

RAR NUMBER: 85-0373
SAMPLE NO: 008

Start time= 0.00 Stop time= 25.00 minutes Offset= -6 mv
Full Range = 100 millivolts



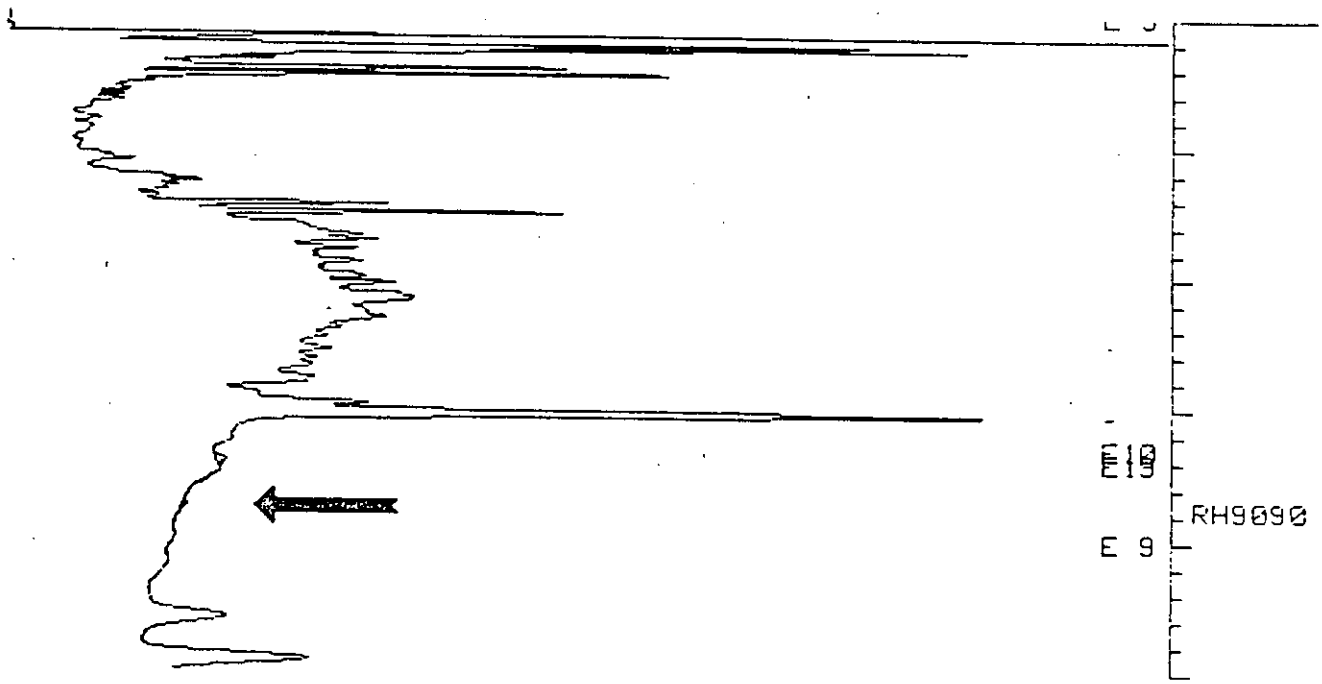
RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	CTL CORR.	UG ADDED	PCT RECOV
18.83	RH9090	1341.2	517.7	.0397	10.00	.029	.500	73.7

RH-9090 Apple Control

DATA FILE: S0625869
METHOD FILE: STAN_HP17
TYPE: SAMPLE

RAR NUMBER: 85-0490
SAMPLE NO: 004

Start time= 0.00 Stop time= 25.00 minutes Offset= -7 mv
Full Range = 100 millivolts



RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	SAMP WT.	RECOV FACT.	PPM
18.78	RH9090	73.0	35.8	0.0000	10.00	10.0	.700	0.0000

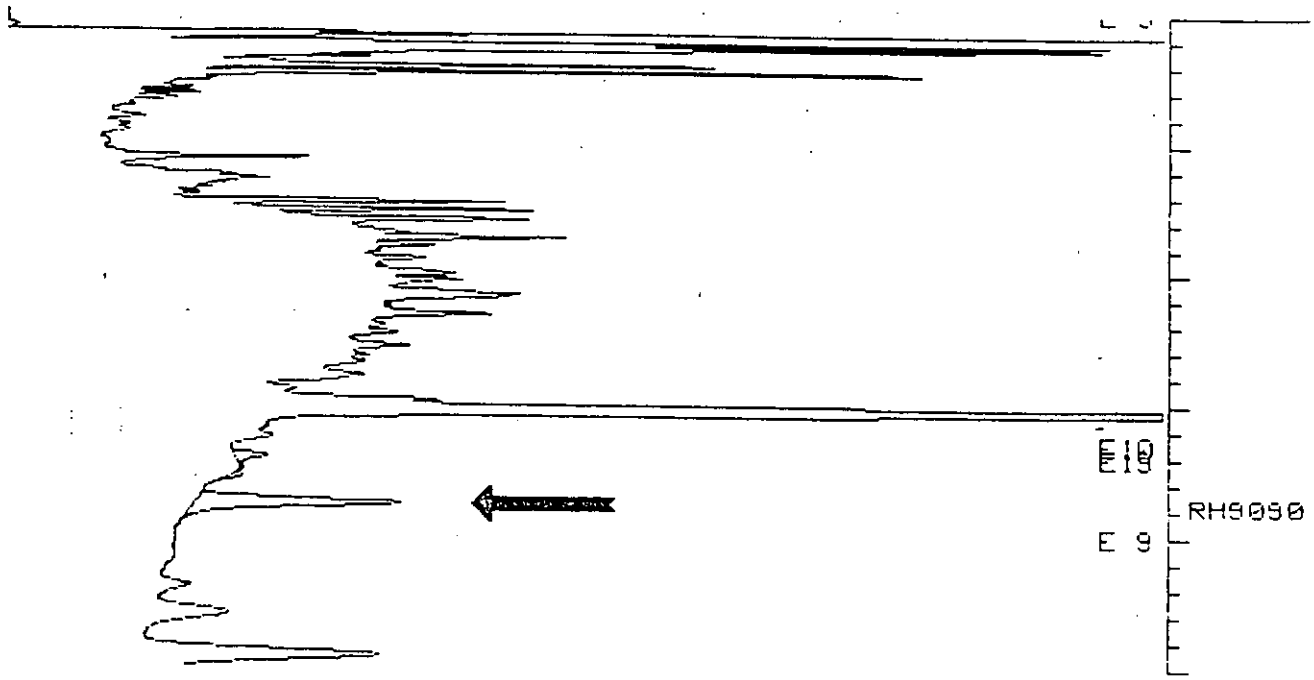
Figure 10

Apple Fortification

DATA FILE: S0625868
METHOD FILE: STAN_HP17
TYPE: FORTIFICATION

RAR NUMBER: 85-0490
SAMPLE NO: 004

Start time= 0.00 Stop time= 25.00 minutes Offset= -6 mv
Full Range = 100 millivolts



RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	CTL CORR.	UG ADDED	PCT RECOV
18.82	RH9090	3411.0	1509.5	.1193	10.00	.026	1.700	68.6

PB-166
40402-02

404092-02

Study Title

Addendum 2 to RH-3866 Total Residue Analytical Method for Grape and Apple (Technical Report No. 310-84-27)

Data Requirement

Guideline 171-4

Authors

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Laboratory Project ID

Lab Memo Number 31S-87-46. Addendum to Technical Report No. 310-84-27

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Project Reference Data

TITLE: Addendum 2 to RH-3866 Total Residue Analytical Method for Grape and Apple (Technical Report No. 310-84-27)

AUTHORS: R. O. Deakyne, C. K. Brackett, L. S. Mazza and S. S. Stavinski

STUDY DIRECTOR: S. S. Stavinski

DATES: Work Started: 1987
Work Completed: 1987

NAME OF INSTITUTION: Rohm and Haas Company
727 Norristown Road
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LOCATION OF WORK: Same

Residue, Metabolism
Environmental Fate

August 3, 1987

Subject: Addendum 2 to RH-3866 Total Residue Analytical Method for Grape and Apple (Technical Report No. 310-84-27)

Objective: Modifications to the RH-3866 total residue analytical method (Technical Report No. 310-84-27) have been made to improve sample cleanup and RH-9090 quantitation.

Summary: Modifications to the method include a methanol pre-wash of the Chelex-100 to eliminate reagent artifacts and the elimination of Triazole as an eluant. The water wash of methylene chloride in the second partition step has also been deleted.

Experimental Details:

Section 5.5

Preparation of Chelex 100-Fe^{+++} Column for Chromatography

Place 600 grams of Bio-Rad Chelex-100 in a 2 liter Erlenmeyer Flask. Add 1 liter of methanol, swirl, let settle and decant. Repeat for a total of 4 methanol washes. Repeat process with four 1-liter Milli-Q water washes. This pre-wash process reduces artifacts on GLC chromatography due to Chelex-100.

Chelex 100-Fe^{+++} Column Chromatography

Prepare the Chelex 100-Fe^{+++} resin fresh daily by adding a 0.5% $FeCl_3$ solution (0.5 g $FeCl_3$/100 mL water) to 400 mL of hydrated Chelex 100. Wash twice with 1000 mL of 1:4 (v/v) methanol-water and prepare column as described.

Slurry pack a 25 cm x 2.5 cm I.D. glass column to a height of approximately 8 cm with the iron-activated Chelex-100. Equilibrate the column with 100 mL of 1:4 (v/v) methanol-water at a flow rate of 10 mL/min.

Dissolve the residue from the methylene chloride partitioning step, 5.4, in 15 mL 1:4 (v/v) methanol-water. Apply this solution to the Chelex 100-Fe^{+++} column and elute to the top of the resin bed at a flow rate of 4 mL/min. Wash the 500 ml round bottom flask with a second 15 mL aliquot. Apply this aliquot to the column and elute exactly as described above.

Rinse flask with 25 mL of 1:4 (v/v) methanol-water and then add to the column and again elute to the top of the resin bed. Discard all fractions collected.

Wash the 500 mL round bottom flask with 200 mL of 1:1 (v/v) methanol-water. Apply to the Chelex 100-Fe^{+++} column and elute at a flow rate of 10 mL/min, collecting the eluant, until the column is dry. Add 5 g sodium chloride to the eluant.

Section 5.6

Methylene Chloride Partition

Partition the Chelex 100-Fe^{+++} eluant with two 150 mL aliquots of methylene chloride in a 500 mL separatory funnel, shaking vigorously for 30 seconds each time. Dry methylene chloride over anhydrous sodium sulfate, combining the extracts in a 500 mL round bottom flask. Evaporate the methylene chloride to dryness at 50°C under reduced pressure by rotary evaporator.

Section 5.7

Bio-Sil A Column Chromatography

Substitute Bio-Sil A for Florisil. Bio-Sil A, 100-200 mesh, is purchased from Bio-Rad Laboratories. Activate the Bio-Sil A by heating at 200°C for 48 hours. Remove and store dessicated in 8 oz. tightly capped glass vials.

Proceed as described in Section 5.7 but use the Bio-Sil A Elution pattern:

Redissolve residue in 25 mL of 2% (v/v) acetone/toluene.
Wash with 10 mL 2% (v/v) acetone/toluene.
Wash with 40 mL 20% (v/v) acetone/toluene.
Elute RH-3866 fraction with 100 mL of 25% (v/v) acetone/toluene.
Wash with 15 mL 35% (v/v) acetone/toluene.
Elute RH-9090 fraction with 100 mL 50% (v/v) acetone/toluene.

Standardize the elution pattern when a new batch of Bio-Sil A is prepared to ascertain that no batch dependent changes occur.

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Section 5.8

GLC Quantitations

Substitute the following GLC conditions:

GLC Hewlett Packard 5890

Detector: Hewlett Packard ^{63}Ni ECD

Column: J&W Scientific Megabore DB-17, 30 meter

Carrier Gas: P-10 (10% methane/Argon)

Injector Temperature: 200°C

Detector Temperature: 325°C

Inlet Pressure: 80 PSI

Purge Flow: 60 mL/min

Column Flow: 8.7 mL/min

Initial Temperature: 200°C

Initial Hold Time: 5 min

Program Rate: 10°C/min

Final Temperature: 250°C

Final Hold Time: 20 min

Total Run Time: 30 min

Injection Volume: 3 ul

Results and Discussion:

Modifications to the Total Residue Analytical Method were made to improve the quality of chromatograms and the recovery of RH-9090. The recovery of samples included with the addendum averaged 94% ± 4% for RH-3866 and 80% ± 4% for RH-9090.

Illustrative chromatographs of apple and grape samples processed by the modified method are attached.

The list of figures is shown below:

Figure 1	RH-3866 Standard Curve
Figure 2	RH-3866 Standards
Figure 3	RH-3866 Standards
Figure 4	Apple Control Sample
Figure 5	Apple Fortification RH-3866
Figure 6	Apple Fortification RH-3866
Figure 7	Apple Fortification RH-3866
Figure 8	RH-3866 Standard Curve
Figure 9	RH-3866 Standards
Figure 10	RH-3866 Standards
Figure 11	RH-3866 Standards
Figure 12	RH-3866 Standards
Figure 13	RH-3866 Standards
Figure 14	Apple Fortification RH-3866
Figure 15	Grape Fortification RH-3866
Figure 16	RH-9090 Standard Curve
Figure 17	RH-9090 Standards
Figure 18	RH-9090 Standards
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Figure 20	Apple Control Sample
Figure 21	Apple Fortification RH-9090
Figure 22	Apple Fortification RH-9090
Figure 23	Apple Fortification RH-9090
Figure 24	Apple Fortification RH-9090
Figure 25	Grape Fortification RH-9090

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Figure I

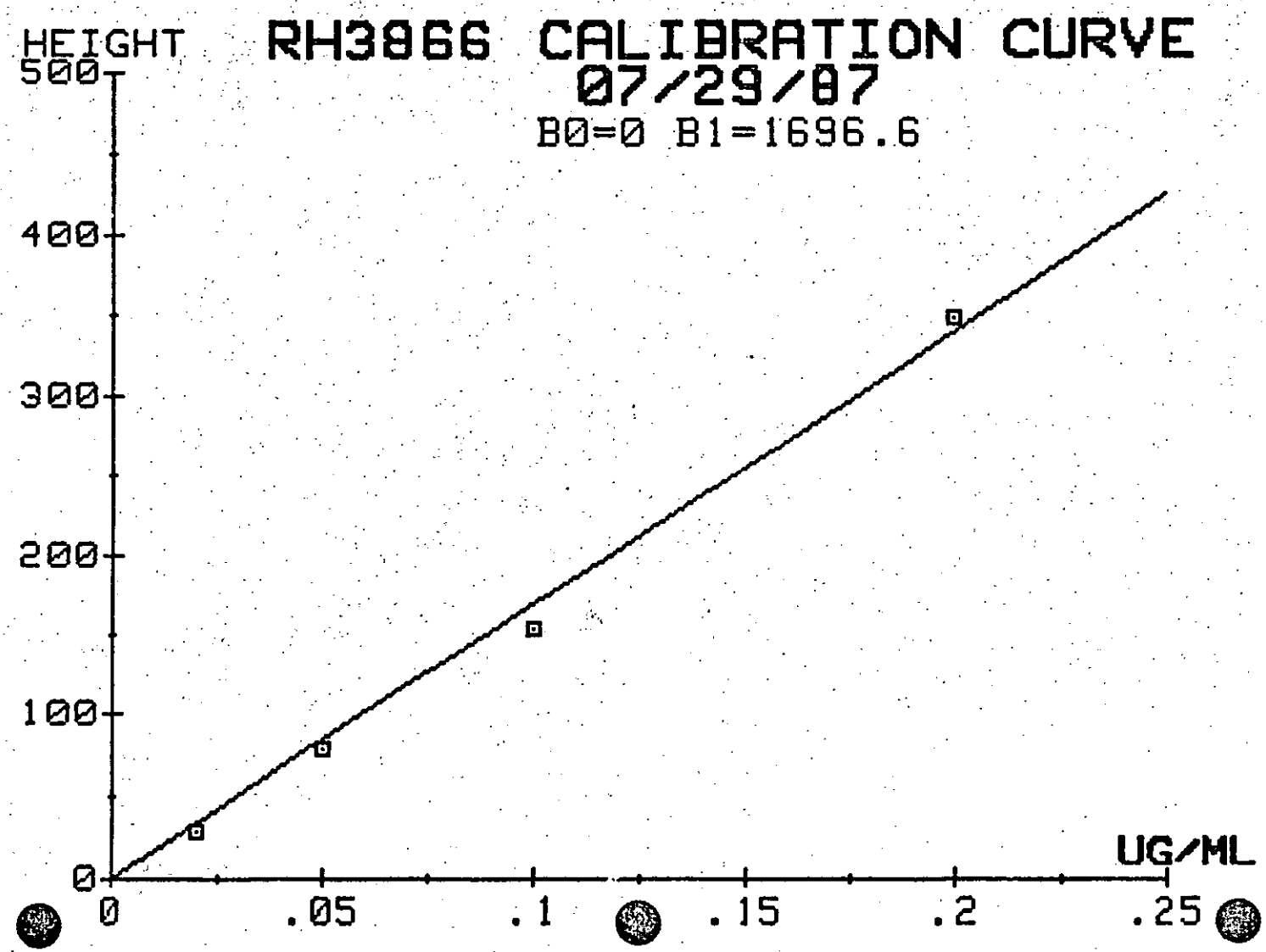

RH3866 CALIBRATION CURVE
07/29/87
B0=0 B1=1696.6
HEIGHT
500
400
300
200
100
0
UG/ML
0
.05
.1
.15
.2
.25

Figure 2

DATA FILE: D0726879
METHOD FILE: STAN_3866
TYPE: STANDARD

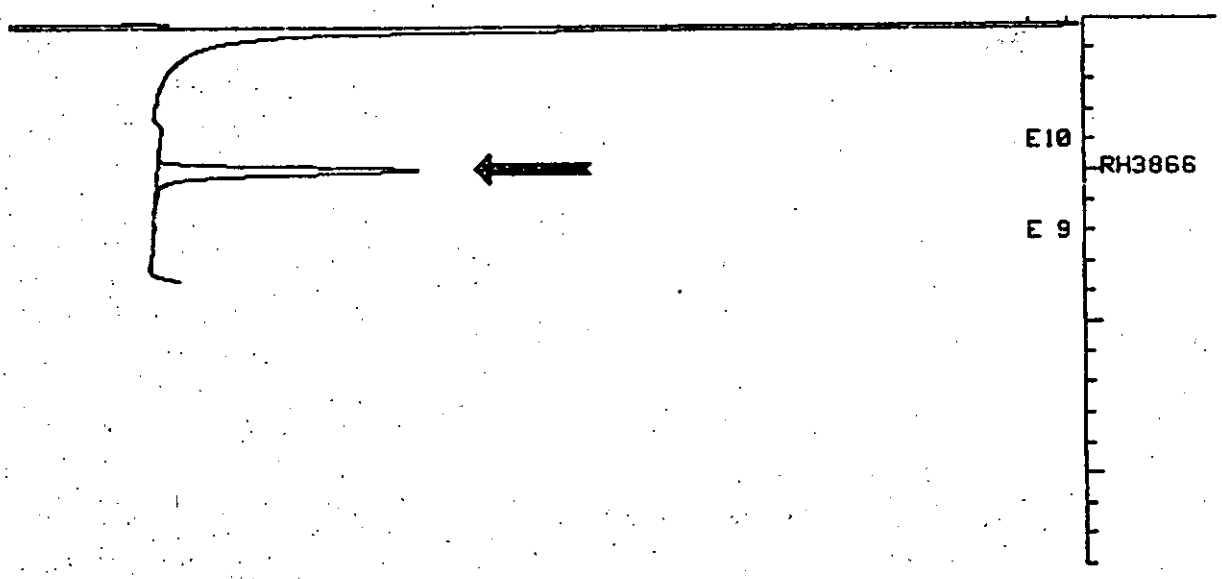
Start time= 0.00 Stop time= 18.00 minutes Offset= -4 mv
Full Range = 15 millivolts

RETENTION TIME	COMPOUND NAME	PPM INJECTED	AREA	HEIGHT
4.91	RH3866	.0200	.60	30.66

Figure 3

DATA FILE: D0726871
METHOD FILE: STAN_3866
TYPE: STANDARD

Start time= 0.00 Stop time= 18.00 minutes Offset= -4 mv
Full Range = 15 millivolts



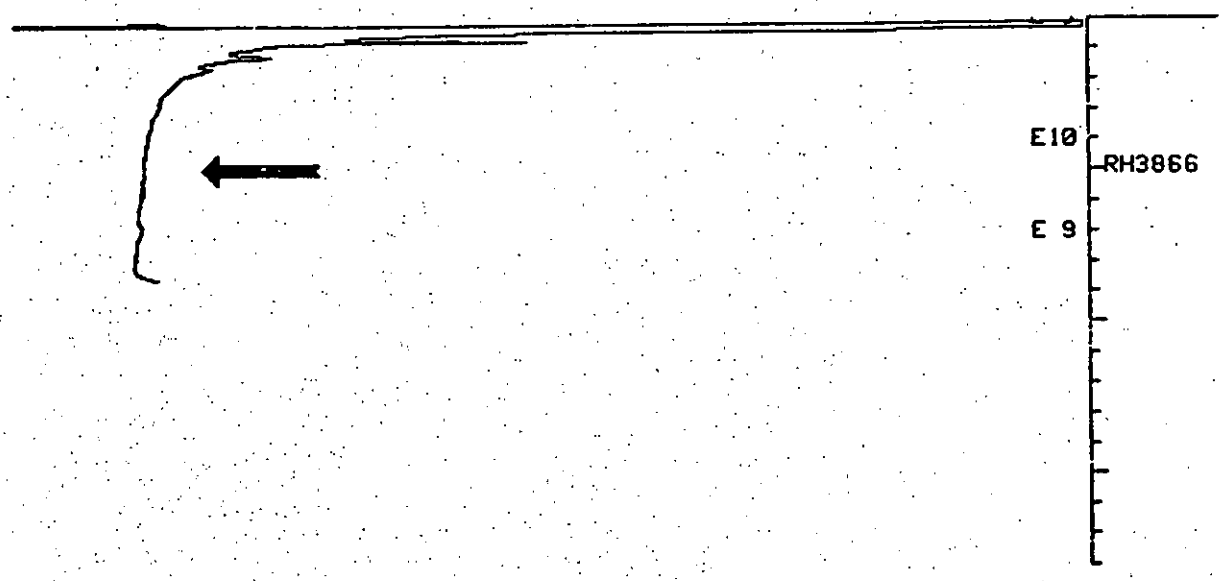
RETENTION TIME	COMPOUND NAME	PPM INJECTED	AREA	HEIGHT
4.91	RH3866	.2000	6.44	348.20

Figure 4

DATA FILE: D07268712
METHOD FILE: STAN_3866
TYPE: SAMPLE

RAR NUMBER: APPLE
SAMPLE NO: CNTL5

Start time= 0.00 Stop time= 18.00 minutes Offset= -4 mv
Full Range = 15 millivolts



RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	SAMP WT.	RECOV FACT.	PPM
4.91	RH3866	0.0	0.0	0.0000	40.00	10.0	1.000	0.0000

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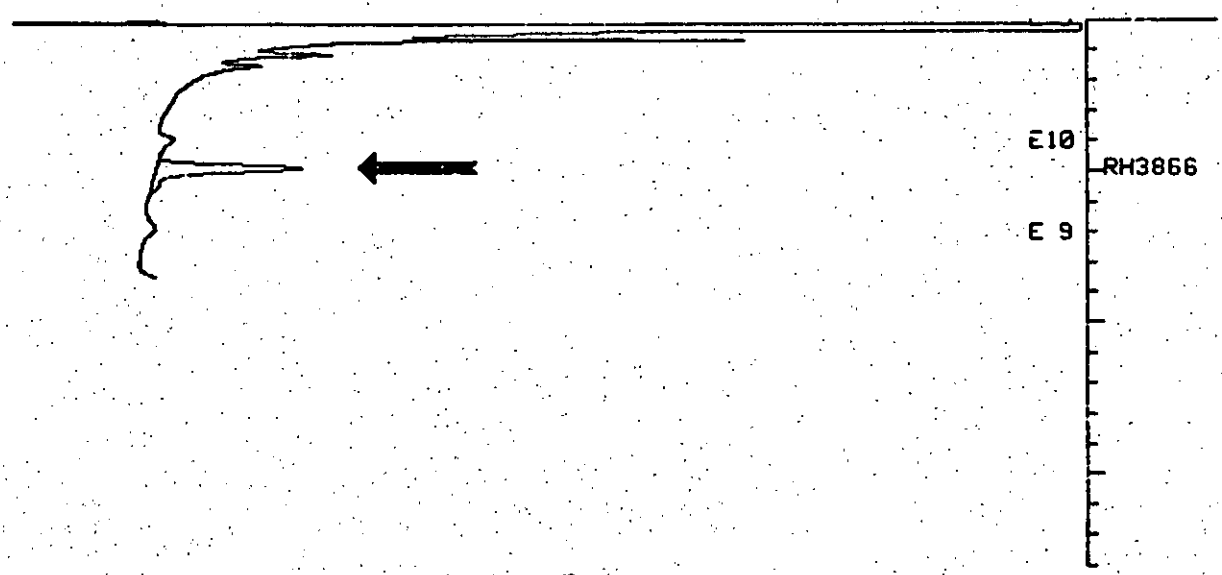
Figure 5

DATA FILE: D07268714
METHOD FILE: STAN_3866
TYPE: FORTIFICATION

RAR NUMBER: APPLE
SAMPLE NO: 1

Start time= 0.00 Stop time= 18.00 minutes Offset= -4 mv
Full Range = 15 millivolts



RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	CTL CORR.	UG ADDED	PCT RECOV
4.91	RH3866	3.7	197.3	.1163	40.00	0.000	4.810	96.7

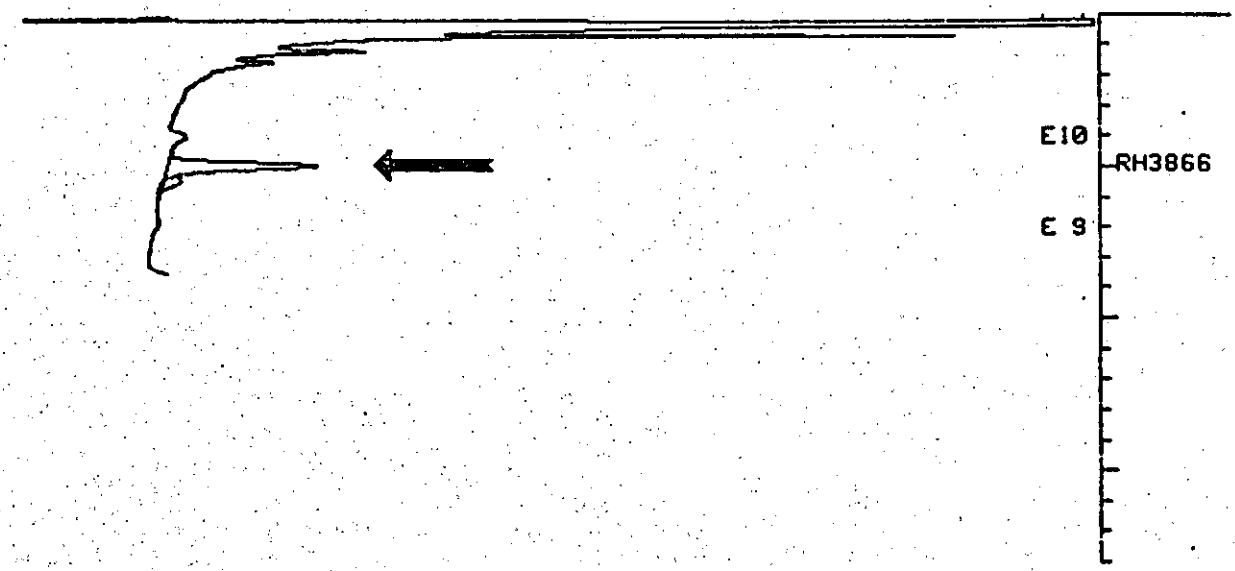
12

Figure 6

DATA FILE: D07268716
METHOD FILE: STAN_3866
TYPE: FORTIFICATION

RAR NUMBER: APPLE
SAMPLE NO: 2

Start time= 0.00 Stop time= 18.00 minutes Offset= -4 mv
Full Range = 15 millivolts



RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	CTL CORR.	UG ADDED	PCT RECOV
4.91	RH3866	3.5	202.2	.1192	40.00	0.000	4.810	99.1

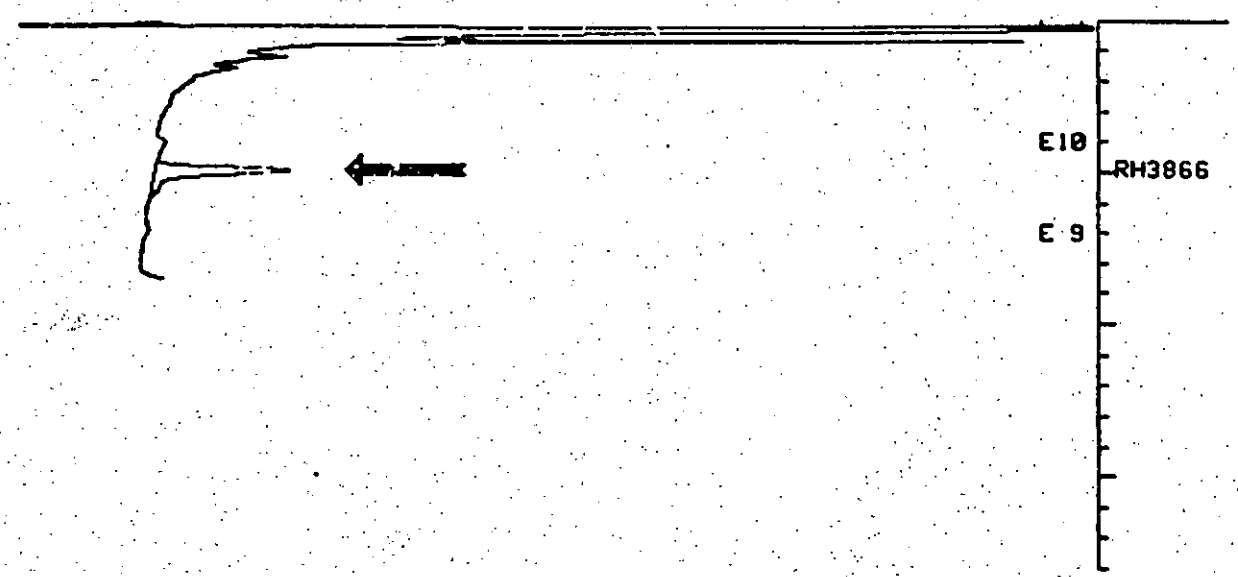
B

Figure 7

DATA FILE: D07268718
METHOD FILE: STAN 3866
TYPE: FOR VERIFICATION

RAR NUMBER: APPLE
SAMPLE NO: 3

Start time= 0.00 Stop time= 18.00 minutes Offset= -4 mv
Full Range = 15 millivolts



RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	CTL CORR.	UG ADDED	PCT RECOV
4.91	RH3866	3.5	185.6	.1094	40.00	0.000	4.810	91.0

Figure 8

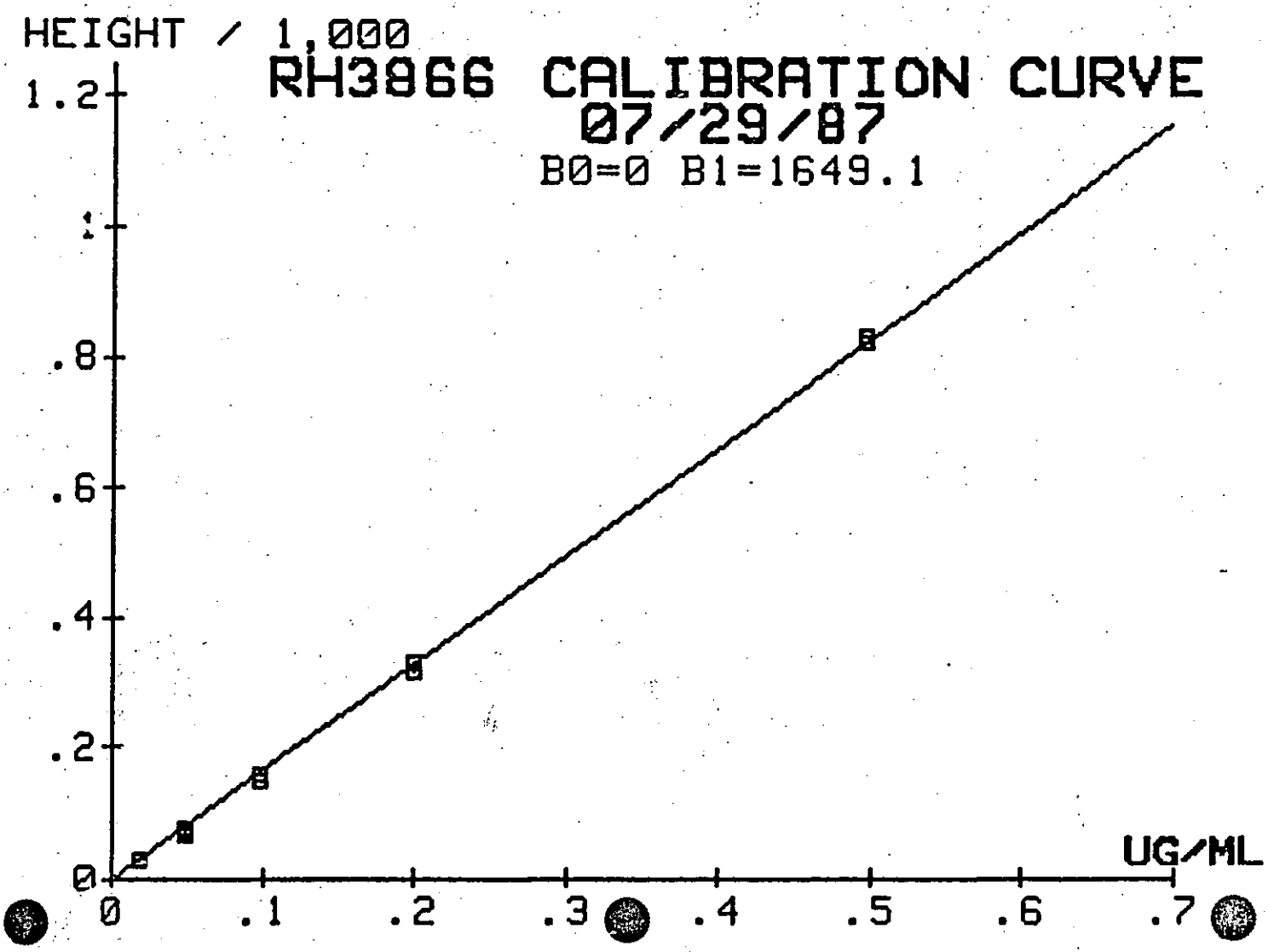
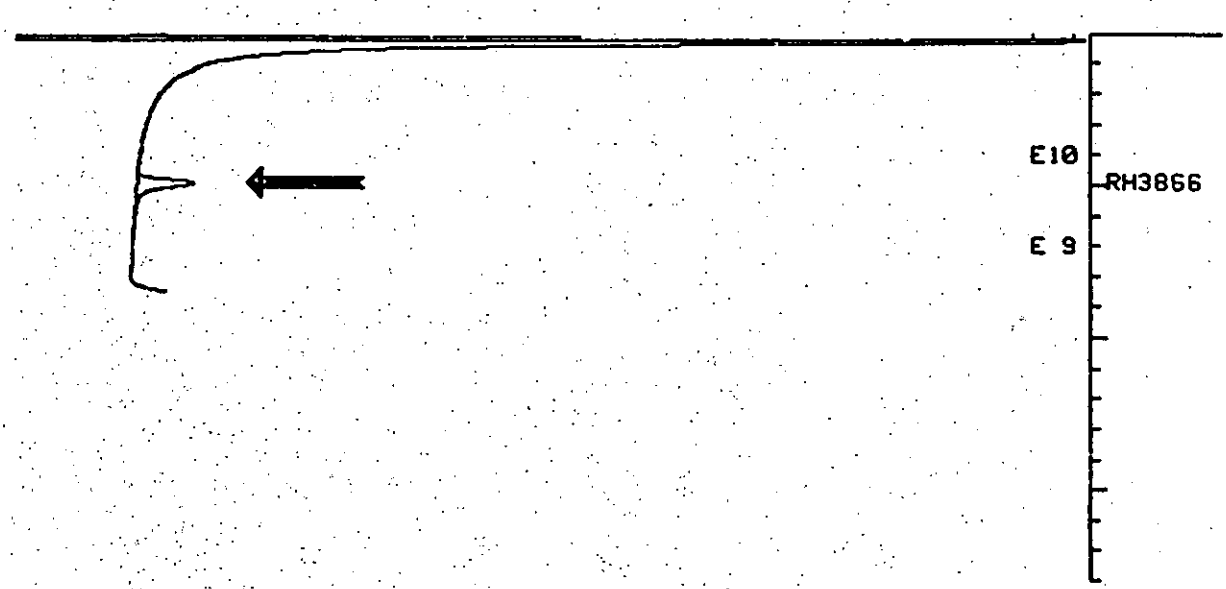

HEIGHT / 1,000
RH3866 CALIBRATION CURVE
07/29/87
B0=0 B1=1649.1
1.2
1
.8
.6
.4
.2
0
0
.1
.2
.3
.4
.5
.6
.7
UG/ML

Figure 9

DATA FILE: B07288710
METHOD FILE: STAN_3866
TYPE: STANDARD

Start time= 0.00 Stop time= 18.00 minutes Offset= -4 mv
Full Range = 15 millivolts

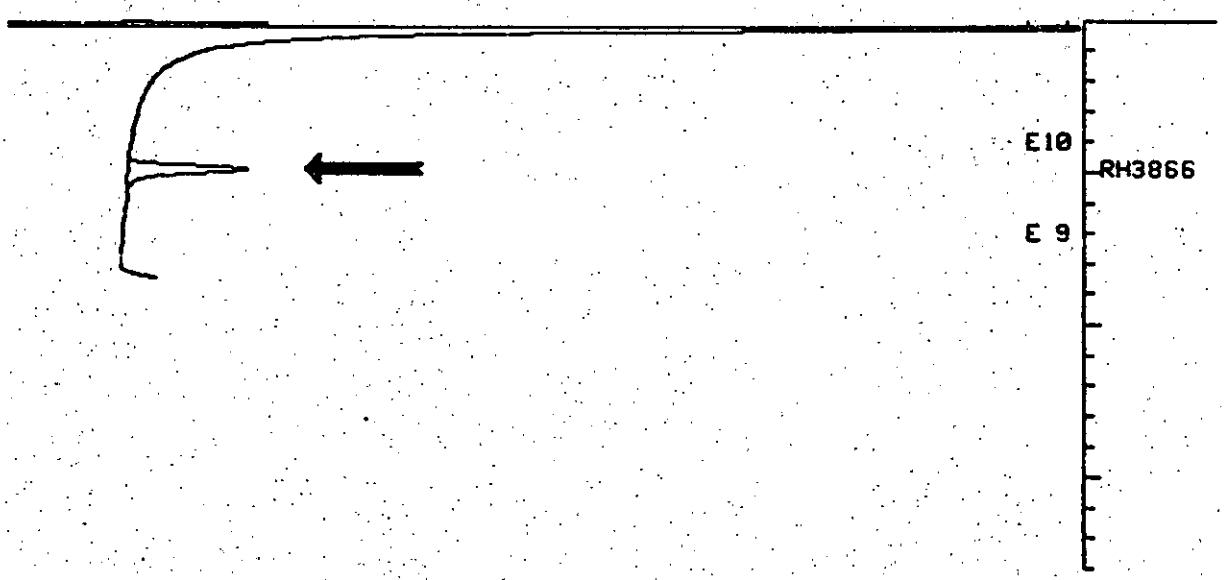


RETENTION TIME	COMPOUND NAME	PPM INJECTED	AREA	HEIGHT
4.91	RH3866	.0500	1.50	74.19

Figure 10

DATA FILE: B0728879
METHOD FILE: STAN_3866
TYPE: STANDARD

Start time= 0.00 Stop time= 18.00 minutes Offset= -4 mv
Full Range = 15 millivolts



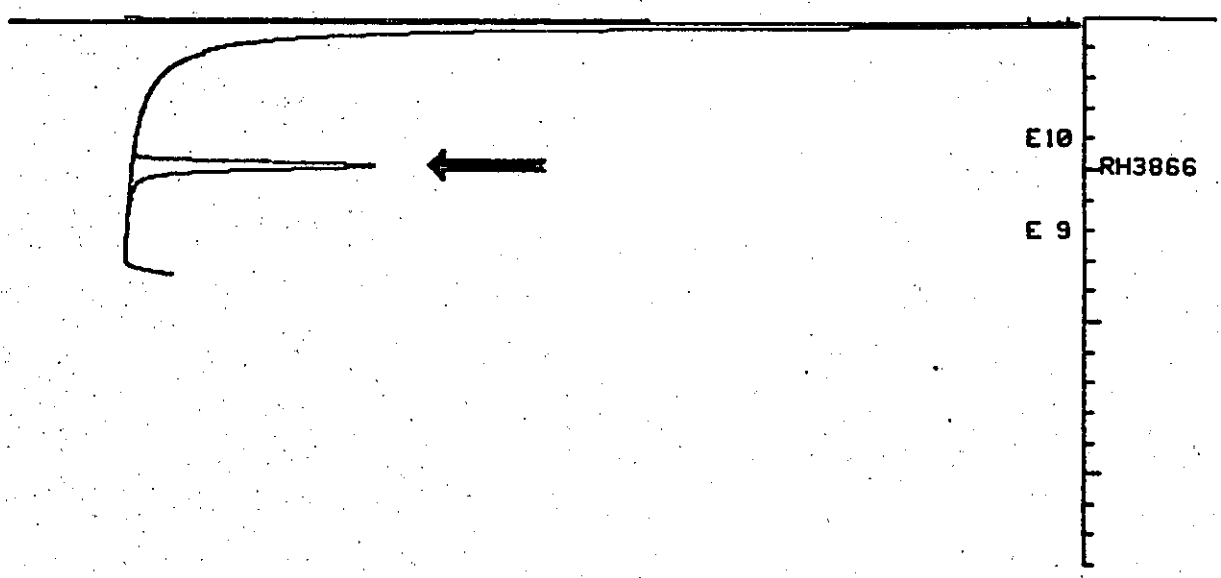
RETENTION TIME	COMPOUND NAME	PPM INJECTED	AREA	HEIGHT
4.91	RH3866	.1000	3.06	160.24

11

Figure 11

DATA FILE: B0728878
METHOD FILE: STAN_3866
TYPE: STANDARD

Start time= 0.00 Stop time= 18.00 minutes Offset= -4 mv
Full Range = 15 millivolts



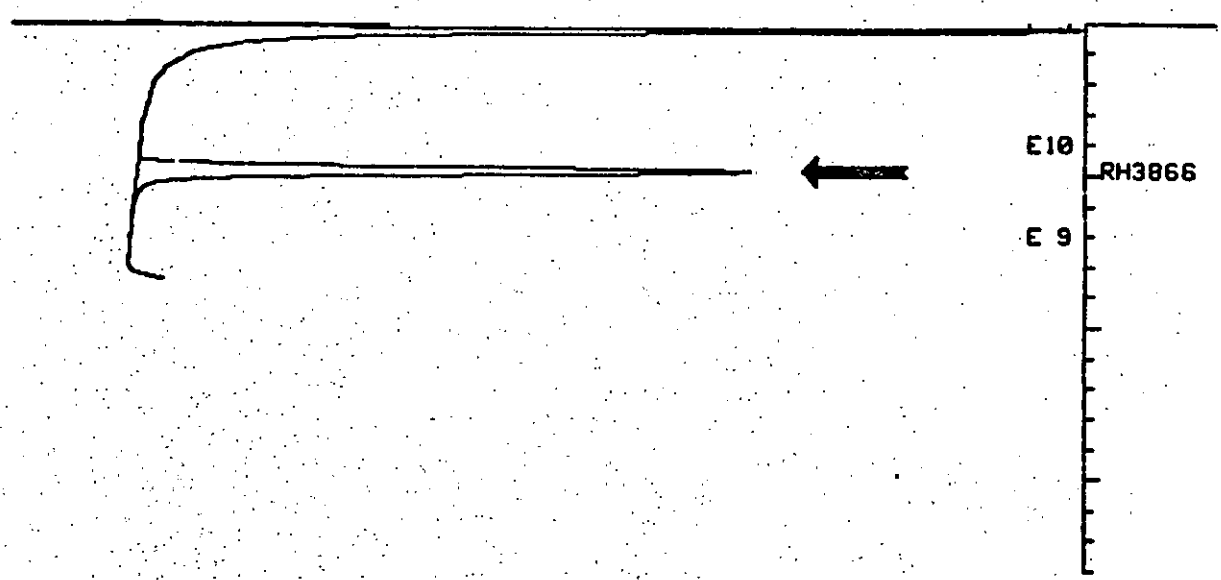
RETENTION TIME	COMPOUND NAME	PPM INJECTED	AREA	HEIGHT
4.91	RH3866	.2000	6.33	319.56

18

Figure 12

DATA FILE: B0728877'
METHOD FILE: STAN_3866
TYPE: STANDARD

Start time= 0.00 Stop time= 18.00 minutes Offset= -4 mv
Full Range = 15 millivolts

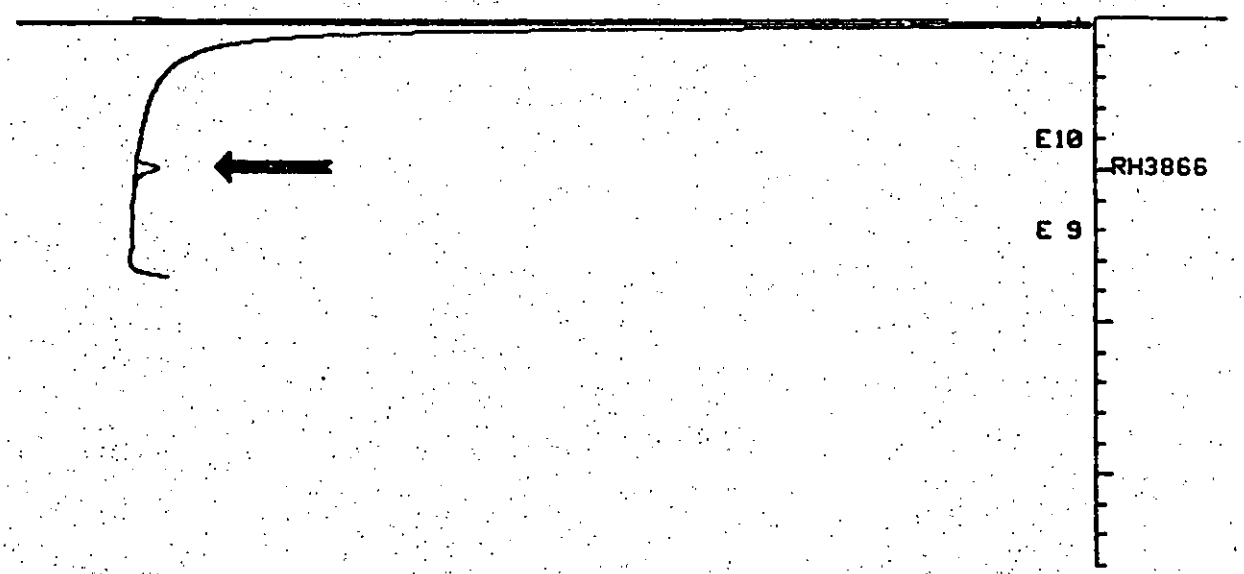


RETENTION TIME	COMPOUND NAME	PPM INJECTED	AREA	HEIGHT
4.88	RH3866	.5000	15.38	834.59

Figure 13

DATA FILE: B07288711
METHOD FILE: STAN_3866
TYPE: STANDARD

Start time= 0.00 Stop time= 18.00 minutes Offset= -4 mv
Full Range = 15 millivolts



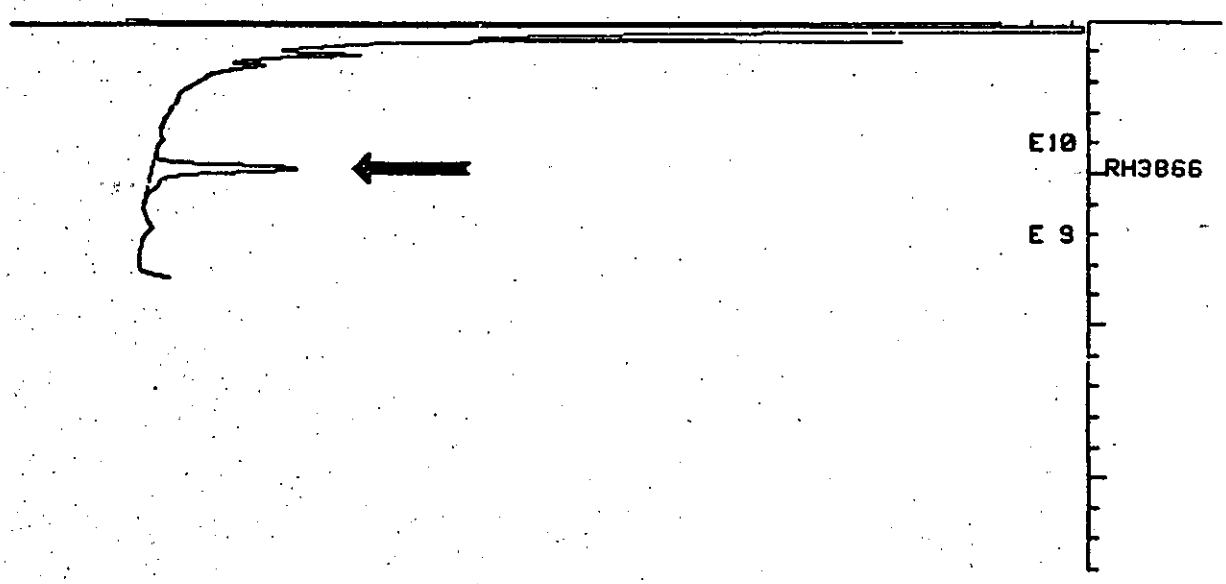
RETENTION TIME	COMPOUND NAME	PPM INJECTED	AREA	HEIGHT
4.91	RH3866	.0200	.62	29.86

Figure 14

DATA FILE: B07288728
METHOD FILE: STAN_3866
TYPE: FORTIFICATION

RAR NUMBER: APPLE
SAMPLE NO: 4

Start time= 0.00 Stop time= 18.00 minutes Offset= -4 mv
Full Range = 15 millivolts



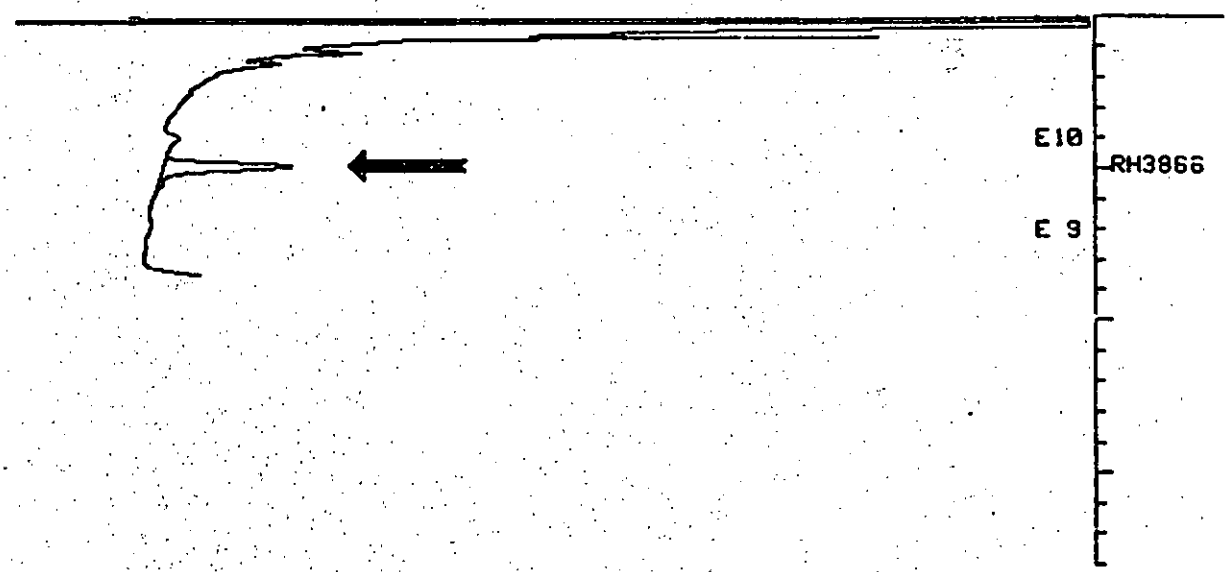
RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	CTL CORR.	UG ADDED	PCT RECOV
4.88	RH3866	3.8	188.7	.1144	40.00	0.000	4.810	95.1

Figure 15

DATA FILE: B07288729
METHOD FILE: STAN_3866
TYPE: FORTIFICATION

RAR NUMBER: GRAPE
SAMPLE NO: 6

Start time= 0.00 Stop time= 18.00 minutes Offset= -4 mv
Full Range = 15 millivolts



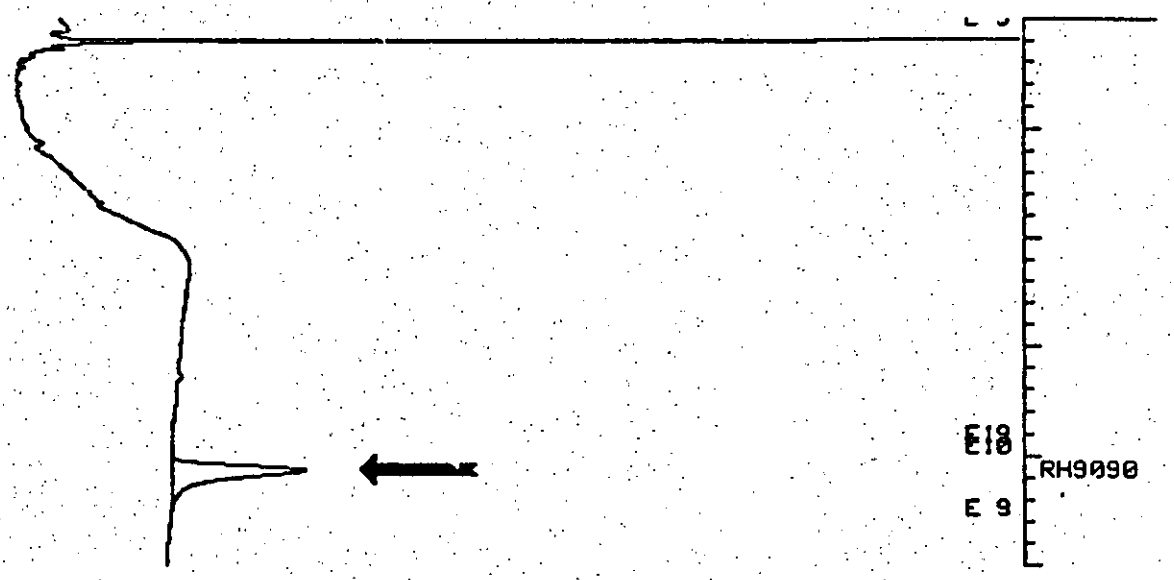
RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	CTL CORR.	UG ADDED	PCT RECOV
4.91	RH3866	3.3	175.2	.1062	40.00	0.000	4.810	88.3

22

Figure 18

DATA FILE: L07268713
METHOD FILE: STAN_HP17
TYPE: STANDARD

Start time= 0.00 Stop time= 25.00 minutes Offset= -45 mv
Full Range = 150 millivolts

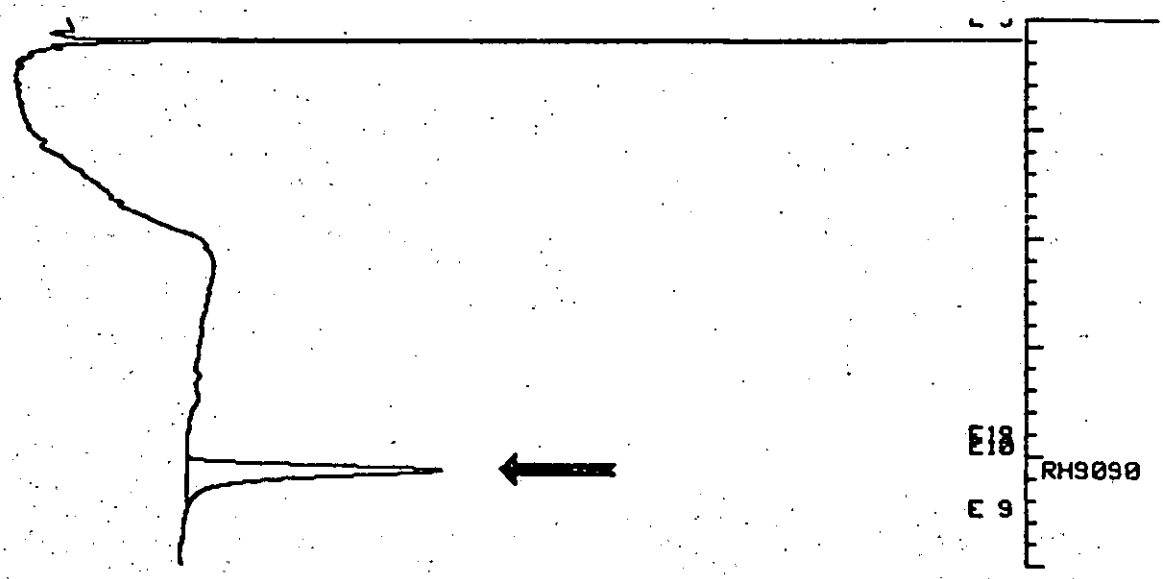


RETENTION TIME	COMPOUND NAME	PPM INJECTED	AREA	HEIGHT
20.61	RH9090	.1000	70.44	1764.00

Figure 17

DATA FILE: L07268712
METHOD FILE: STAN_HP17
TYPE: STANDARD

Start time= 0.00 Stop time= 25.00 minutes Offset= -48 mv
Full Range = 150 millivolts



RETENTION TIME	COMPOUND NAME	PPM INJECTED	AREA	HEIGHT
20.61	RH9090	.2000	132.55	3420.16

24

Figure 16

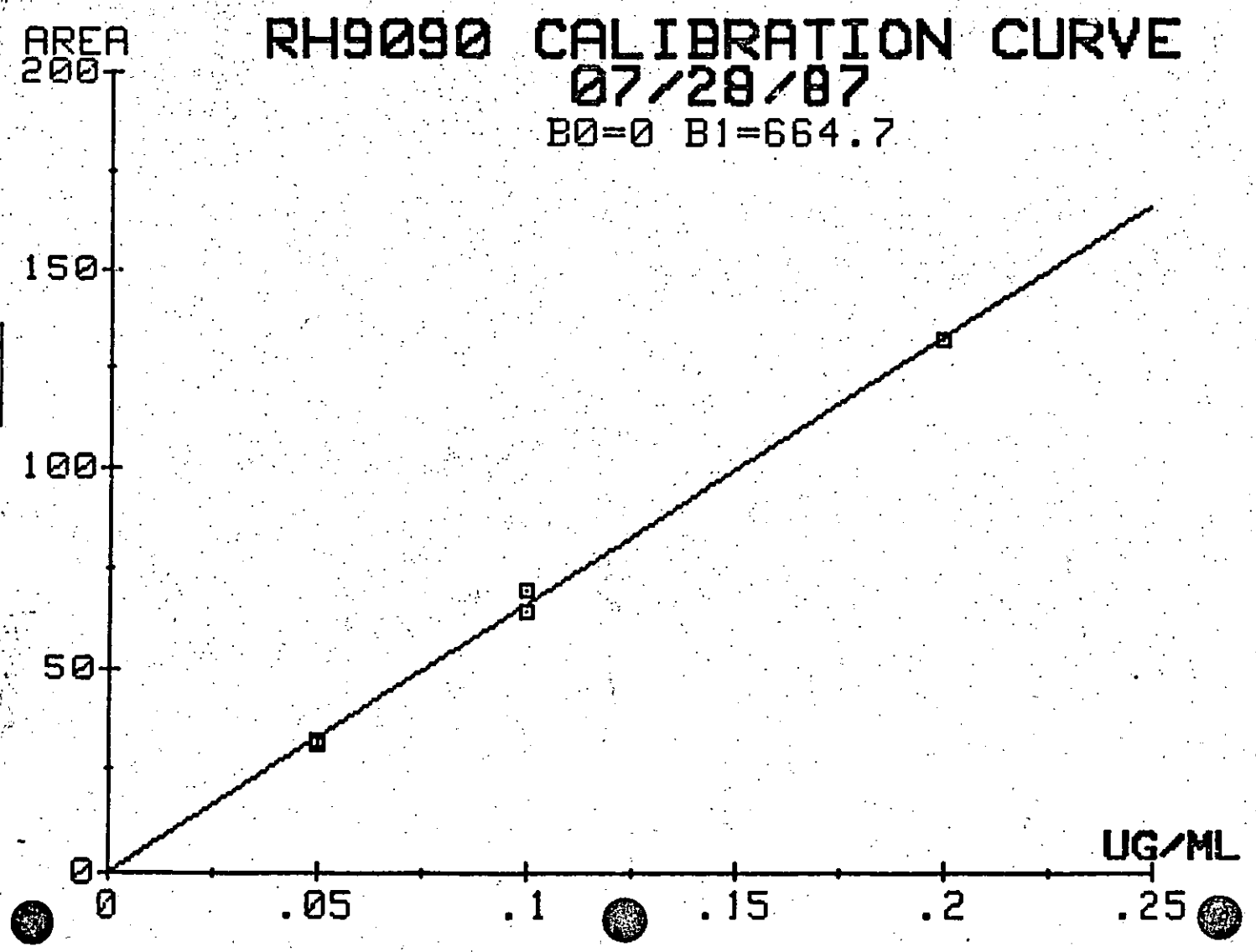
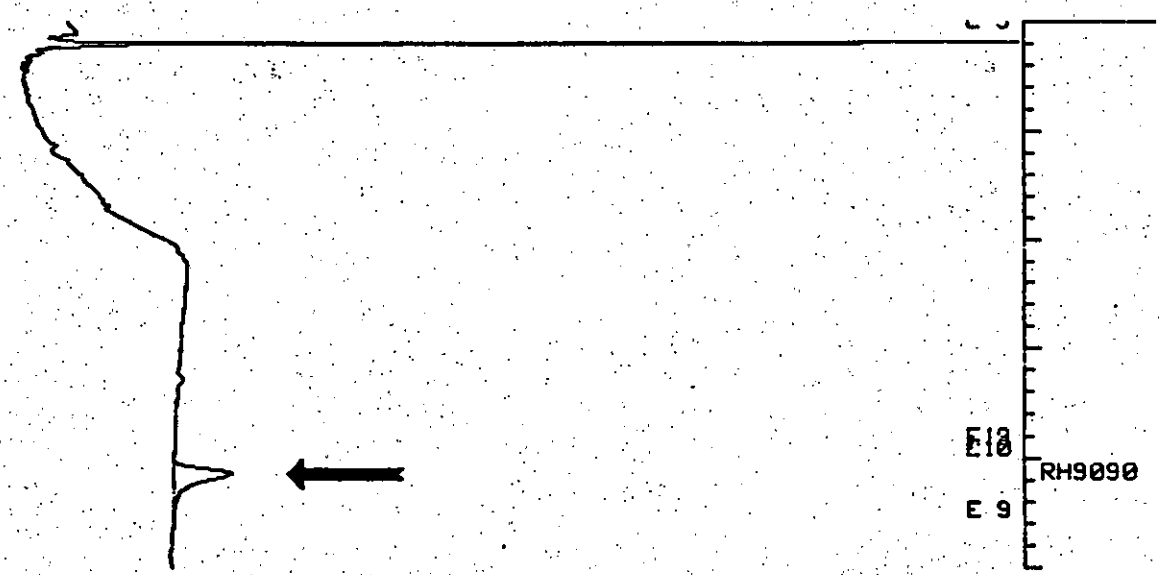

RH9090 CALIBRATION CURVE
07/28/87
B0=0 B1=664.7
AREA
200
150
100
50
0
0
.05
.1
.15
.2
.25
UG/ML

Figure 19

DATA FILE: 'L07268714
METHOD FILE: STAN_HP17
TYPE: STANDARD

Start time= 0.00 Stop time= 25.00 minutes Offset= -43 mv
Full Range = 150 millivolts



RETENTION TIME	COMPOUND NAME	PPM INJECTED	AREA	HEIGHT
20.64	RH9090	.0500	31.86	771.31

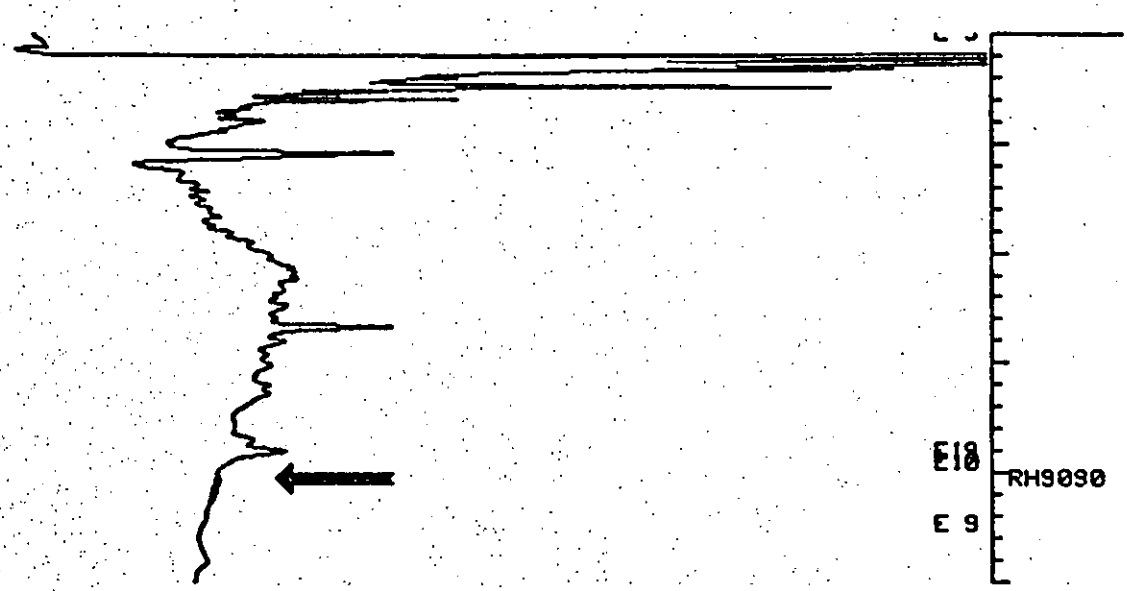
76

Figure 20

DATA FILE: L0726876
METHOD FILE: STAN_HP17
TYPE: SAMPLE

RAR NUMBER: APPLE
SAMPLE NO: 5 CTL

Start time= 0.00 Stop time= 25.00 minutes Offset= -47 mv
Full Range = 150 millivolts



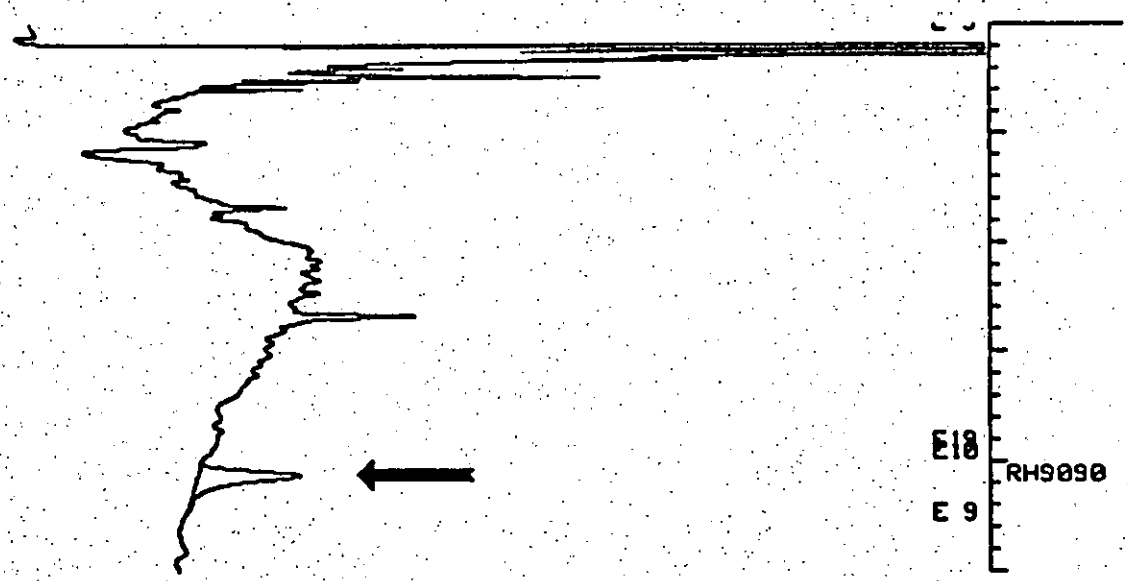
RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	SAMP WT.	RECOV FACT.	PPM
20.24	RH9090	4.5	52.0	0.0000	10.00	10.0	.700	0.0000

Figure 21

DATA FILE: L0726877
METHOD FILE: STAN_HP17
TYPE: FORTIFICATION

RAR NUMBER: APPLE
SAMPLE NO: 1

Start time= 0.00 Stop time= 25.00 minutes Offset= -49 mv
Full Range = 150 millivolts



RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	CTL CORR.	UG ADDED	PCT RECOV
20.61	RH9090	51.0	1369.7	.0767	10.00	0.000	1.000	76.7

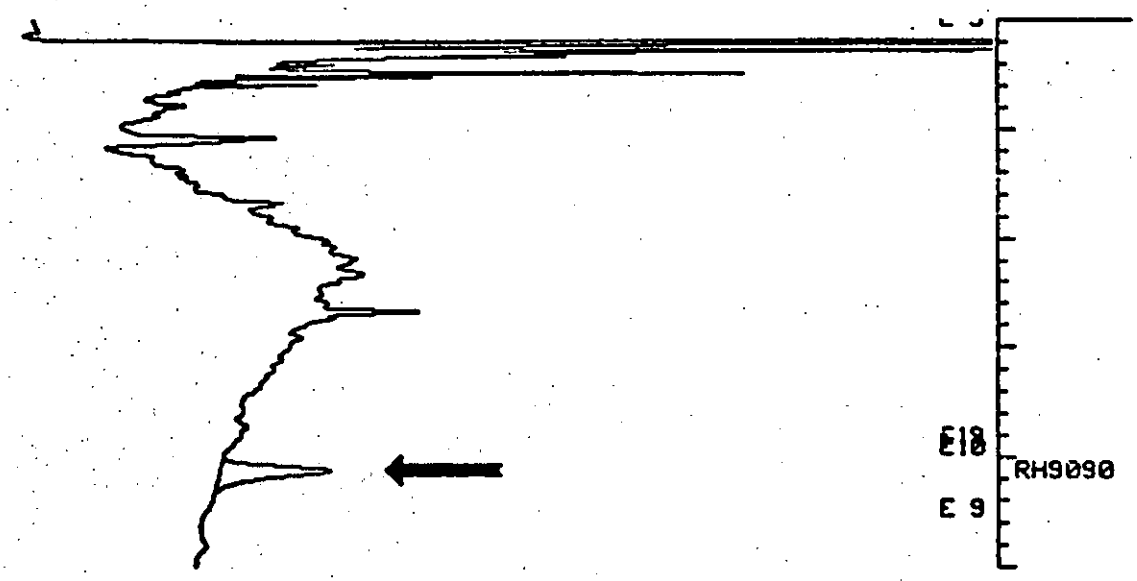
26

Figure 22

DATA FILE: L0726878
METHOD FILE: STAN_HP17
TYPE: FORTIFICATION

RAR NUMBER: APPLE
SAMPLE NO: 2

Start time= 0.00 Stop time= 25.00 minutes Offset= -49 mv
Full Range = 150 millivolts



RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	CTL CORR.	UG ADDED	PCT RECOV
20.57	RH9090	53.3	1475.7	.0802	10.00	0.000	1.000	80.2

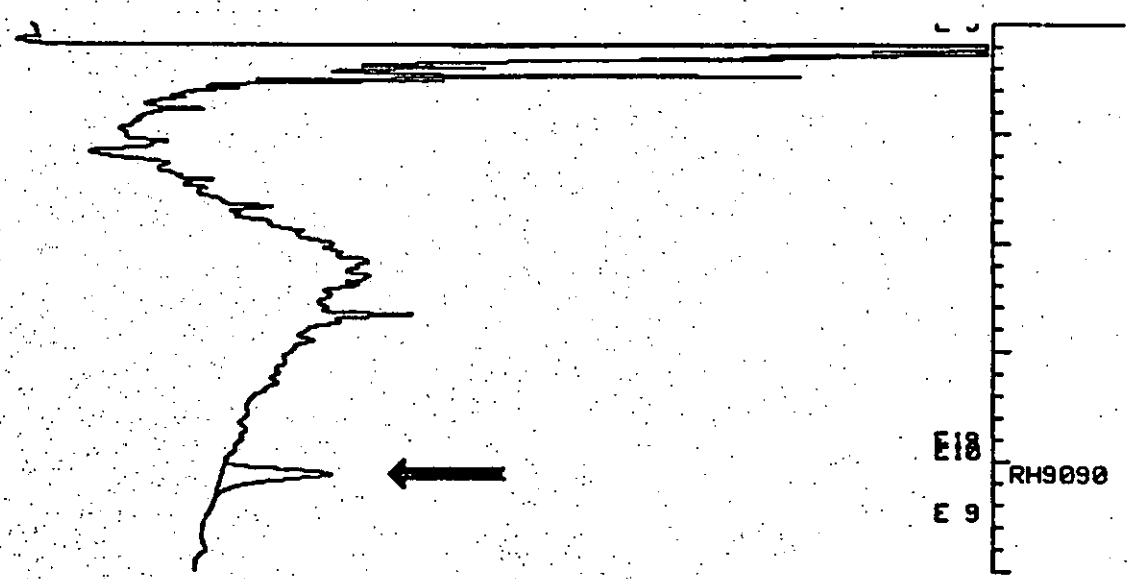
29

Figure 23

DATA FILE: L0726879
METHOD FILE: STAN_HP17
TYPE: FORTIFICATION

RAR NUMBER: APPLE
SAMPLE NO: 3

Start time= 0.00 Stop time= 25.00 minutes Offset= -48 mv
Full Range = 150 millivolts



RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	CTL CORR.	UG ADDED	PCT RECOV
20.57	RH9090	52.2	1448.9	.0786	10.00	0.000	1.000	78.6

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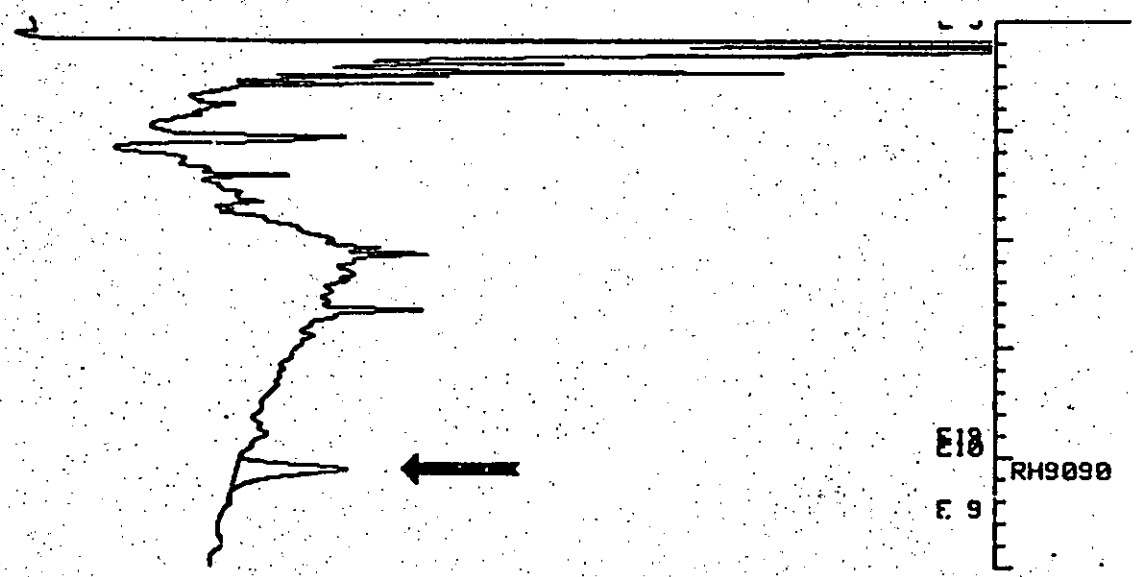
Figure 24

DATA FILE: L07268710
METHOD FILE: STAN_HP17
TYPE: FORTIFICATION

RAR NUMBER: APPLE
SAMPLE NO: 4

Start time= 0.00 Stop time= 25.00 minutes Offset= -48 mv
Full Range = 150 millivolts



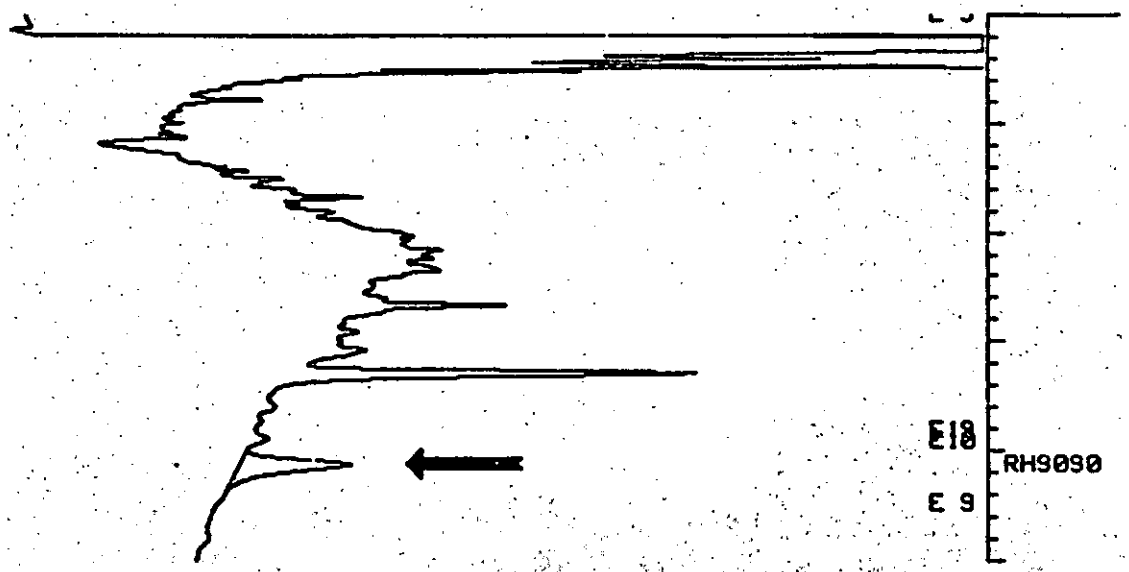
RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	CTL CORR.	UG ADDED	PCT RECOV
20.61	RH9090	52.0	1460.2	.0783	10.00	0.000	1.000	78.3

Figure 25

DATA FILE: L07268711
METHOD FILE: STAN_HP17
TYPE: FORTIFICATION

RAR NUMBER: GRAPE
SAMPLE NO: 6

Start time= 0.00 Stop time= 25.00 minutes Offset= -48 mv
Full Range = 150 millivolts



RET. TIME	COMPOUND NAME	PEAK AREA	PEAK HEIGHT	UG/ML	VOLUME (ML)	CTL CORR.	UG ADDED	PCT RECOV
20:61	RH9090	58.5	1481.0	.0879	10.00	0.000	1.000	87.9

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