

Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pesticide Name: Triazamate (RH-7988)

MRID #: 443505-43

Matrix: Soil

Analysis: LC/MS/MS

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443505-43

STUDY TITLE

Analytical Method for the Determination of RH-7988 and Its Metabolites
RH-0422, RH-7280, RH-3008, RH-0131, and RH-9983 in Soil By High Performance
Liquid Chromatography With MS/MS (Daughter Ion) Detection

DATA REQUIREMENTS

OPPTS 860.1340

AUTHORS

Nadine Bergin, Walter Zogorski, Enaksha Wickremesinha,
Nila Nanavati, and M. Michael Arjmand

STUDY COMPLETED ON

July 1, 1997

PERFORMING LABORATORY

Centre Analytical Laboratories, Inc.
3048 Research Drive
State College, PA 16801

LABORATORY PROJECT ID

Rohm and Haas Technical Report No. TR 34-97-60

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

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

Company: Rohm and Haas Company
Company Agent: Ann M. Tillman, Ph.D.
Title: Product Registration Manager
Signature: *Ann M. Tillman* Date: 7/7/97

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GLP COMPLIANCE STATEMENT

This method development report is an exploratory "study" as defined in 40 CFR 160.3. It is, thus, excluded from GLP compliance. The work was, however, conducted in conformance with applicable standard operating procedures and general GLP regulations. Much of the data presented in this report was excerpted from GLP studies.

Nadine Bergin July 197
Nadine Bergin Date
Author
Rohm & Haas

S. S. Stavinski July 197
S. S. Stavinski, Ph.D. Date
Sponsor
Rohm & Haas

S. S. Stavinski July 197
S. S. Stavinski, Ph.D. Date
Applicant/Submitter
Rohm & Haas

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Rohm and Haas Company, and have been verified as true and accurate representations of the data collected. Excerpted data has also been reviewed by QA units at Centre Analytical Laboratories.

H. E. Aller July 1, 1997
H. E. Aller Date
QA Officer
Rohm and Haas Company

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References

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2. Supplement to Aerobic and Anaerobic Metabolism of RH-7988, Identification of Unknown Metabolites, Rohm and Haas Co. TR#34-92-79, MRID 42935050
3. An Anaerobic Soil Metabolism Study with [¹⁴C]RH-7988, Rohm and Haas Co. TR# 34-96-172
4. Anaerobic Soil Metabolism Study with [¹⁴C]RH-7988, Rohm and Haas Co., TR #34-96-173
5. Preliminary Analytical Method for the Determination of RH-7988 and Its Metabolites RH-0422, RH-7280, RH-3008, and RH-0131 in Soil by High Pressure Liquid Chromatography With MS/MS (Daughter Ion) Detection, Rohm and Haas Co. TR # 34-96-96
6. Radiovalidation of the Soil Residue Analytical Method for RH-7988, TR # 34-97-019 (concurrent submission)
7. RH-7988 Field Dissipation Study in New York, Rohm and Haas Co. TR # 34-97-006 (concurrent submission)

1. Introduction

RH-7988 is the active ingredient of Aphistar, also known as triazamate or Aphistar 50WSP Systemic Aphicide, which has been developed for the selective control of aphids in apples, pears, and leafy vegetables, etc. Metabolism studies of RH-7988 (RH-57988) in soil (references 1, 2, 3, and 4) have shown the residue to contain the parent molecule and five metabolites, referred to here as RH-0422 (RH-70422), RH-7280 (RH-87280), RH-0131 (RH-100131), RH-3008 (RH-123008), and RH-9983 (RH-89983). The structures of these are shown in Figure 1.

The analytical method described in this report determines the amount of RH-7988 and its five metabolites in soil, using a multi-analyte method, where all of the analytes are extracted together and quantified during a single run. The method is summarized in the flow diagram depicted in page 9. The method was developed based upon the preliminary method (reference 5) which analyzed RH-7988 and four soil metabolites (RH-0422, RH-7280, RH-0131, and RH-3008). The current method has been modified slightly to allow for the determination of the fifth metabolite, RH-9983. The accuracy of this analytical method is estimated based on the recovery of known levels of RH-7988 and its metabolites (RH-0422, RH-7280, RH-0131, RH-3008, and RH-9983) fortified in control soil samples.

This report also contains summary data from a radiovalidation of this method (reference 6).

2. Summary

This report details the residue analytical method for RH-7988 and five soil metabolites (RH-0422, RH-7280, RH-0131, RH-3008, and RH-9983) using high performance liquid chromatography with MS/MS detection. The preliminary analytical method for the analysis of RH-7988 and its soil metabolites is detailed in TR 34-96-90 (reference 5). In this preliminary method, RH-7988 and only four metabolites (RH-0422, RH-7280, RH-0131, and RH-3008) were analyzed. During subsequent metabolism studies, a fifth soil metabolite, RH-9983, was identified. The current analytical method detailed in this report analyzes residues of RH-7988, the four soil metabolites (RH-0422, RH-7280, RH-3008, and RH-0131), and also the recently-identified RH-9983, in a single run.

Residues of the test substance (RH-7988) and its soil metabolites (RH-0422, RH-7280, RH-0131, RH-3008, and RH-9983) are extracted from soil by homogenizing and shaking with methanol/formic acid. The extract is concentrated, resuspended in an ammonium formate buffer, and passed through a Sephadex G-50 gel filtration column. For LC/MS/MS analysis, methanol is added to the eluant containing RH-7988 and its metabolites. A 15 cm x 4.6 mm

Keystone Prism HPLC column is used to separate the analytes, using a gradient elution profile. An ion spray interface is used to introduce the HPLC eluant to the mass spectrometer. The analytes are detected in the triple quadrupole mode (MS/MS) by monitoring characteristic daughter ions resulting from passage of their parent molecular ions through the first quadrupole (Q1) into the collision cell (Q2), where fragmentation of the parent ions occur following collision with argon gas, with the resulting fragments separated in the second mass analyzing quadrupole (Q3). The structures of the analytes are given in Figure 1.

The limit of quantification (the lowest fortification specified by the method which gives adequate recovery according to EPA guidelines) for LC/MS/MS analysis is 0.010 ppm for RH-7988 and RH-0422, and 0.020 ppm for RH-7280, RH-0131, RH-3008, and RH-9983.

The limit of detection (LOD) by LC/MS/MS (equivalent to 30% of the limit of quantification) is 0.003 ppm for RH-7988 and RH-0422, and 0.006 ppm for RH-7280, RH-0131, RH-3008, and RH-9983.

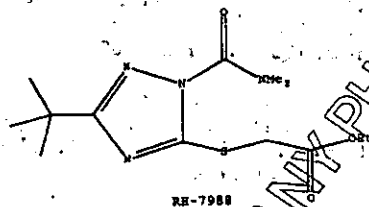
Average fortification recoveries for this study were compiled from the preliminary method (reference 5) analysis of RH-7988 and four metabolites (does not analyze for RH-9983), and from fortifications analyzed during a field dissipation study conducted in New York state (reference 7). (This field dissipation study includes recoveries from the analysis of RH-7988 and the four original soil metabolites (not including RH-9983); RH-9983 analyzed as a single analyte; and from the analysis of RH-7988 and the five soil metabolites (including RH-9983)). The test site was similar to a typical apple orchard, and was composed of Loamy sand soil that was collected to at depths up to 36" inches.

Fortification levels of 0.01, 0.025, and 0.05 ppm each of RH-7988 and RH 0422, and 0.02, 0.05, and 0.1 ppm each of RH-7280, RH-0131, RH-3008, and RH-9983 were used. The mean fortification recovery numbers (n=78) were 85.19 ± 10.50, 89.63 ± 9.69, 87.18 ± 12.26, 75.82 ± 11.32, and 87.05 ± 9.76 percent for RH-7988, RH-0422, RH-0131, RH-7280, and RH-3008 respectively. The mean fortification recovery for RH-9983 (n= 66) was 85.73 ± 13.18 percent. Table 6 provides a summary of the fortification recoveries.

A radiovalidation study of the method was conducted and the resulting data verifies that the method described in this report has been radiovalidated (reference 6). Detailed information about this radiovalidation study is provided in the summary.

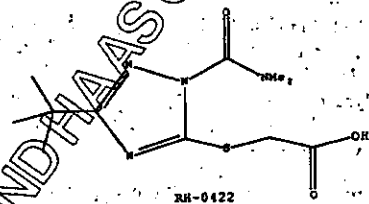
3. Experimental Compounds

Figure 1. Molecular Structures of RH-7988 and Metabolites
RH-7988 (RH-57988)



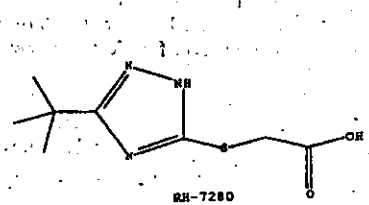
Chemical name = 1-[(dimethylaminocarbonyl)-3-(1,1-dimethylethyl)-1H-1,2,4-triazol-5-yl]thioacetic acid ethyl ester
 Common name = triazamate
 CAS # 112143-82-5

Soil Metabolites:
RH-0422 (RH-70422)



Chemical name = 1-[(dimethylaminocarbonyl)-3-(1,1-dimethylethyl)-1H-1,2,4-triazol-5-yl]thioacetic acid

RH-7280 (RH-87280)

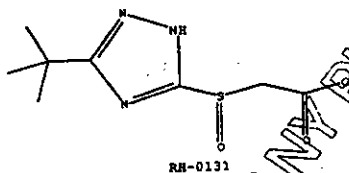


Chemical name = 3-[(1,1-dimethylethyl)-1H-1,2,4-triazol-5-yl]thioacetic acid
 (cont'd)

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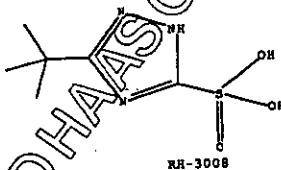
Figure 1. Molecular Structures of RH-7988 and Metabolites, cont'd

RH-0131 (RH-100131)



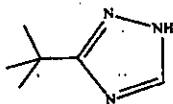
Chemical name = 3-[(1,1-dimethylethyl)-1H-1,2,4-triazol-5-yl]-S-oxo-thioacetic acid

RH-3008 (RH-123008)



Chemical name = 3-[(1,1-dimethylethyl)-1H-1,2,4-triazol-5-yl]sulfonic acid

RH-9983 (RH-89983)



Chemical name = 3-(1,1-Dimethylethyl)-1H-1,2,4-triazole

4. Chemicals and Supplies4.1 Chemicals

	Grade	Source
Methanol	HPLC	Baker
Formic Acid	Reagent	EM Science
Ammonium Formate	Reagent	Aldrich Chemical
Sephadex G-50		Sigma
Water	HPLC	Baker
Acetonitrile	HPLC	Baker
Poly(propylene glycol) [PPG] 2000	Reagent	Aldrich
Poly(propylene glycol) [PPG] 1000	Reagent	Aldrich
Poly(propylene glycol) [PPG] 425	Reagent	Aldrich

4.2 Standards*

	Source
RH-57988	Rohm & Haas
RH-70422	Rohm & Haas
RH-87280	Rohm & Haas
RH-100131	Rohm & Haas
RH-123008	Rohm & Haas
RH-89983	Rohm & Haas

* These numbers are the full RH-numbers for the compounds, however, they are frequently referred to by the abbreviated four-digit designations RH-7988, RH-0422, RH-7280, RH-0131, RH-3008, and RH-9983.

4.3 Equipment and Supplies

	Source
Balance	Mettler
Centrifuge bottles, 150 mL	Nalgene
Hobart Food Processor	Hobart
Mechanical shaker	Eberbach
Bench-top centrifuge (model HN-II)	IEC
Filter for Mobile Phase Preparation (Nylon 66 membrane 0.45 μ m x 47 mm)	Supelco
Round-bottom flask, 250 mL	Pyrex
Centrifuge Tube, 50 mL, Pear Shaped	Kimble
Rotary Evaporator	Buchi
Graduated Centrifuge Tube, 15 mL	Kontes
PD-10 Empty Disposable Column	Supelco
Visiprep Vacuum Manifold	Supelco

(cont'd)

Sonicator
Standard Lab Equipment
(beakers, pipets, volumetric flasks, etc.)

Fisher Scientific

Note: Equivalent materials may be substituted for those specified in this method. These substitutions, however, must be demonstrated to produce satisfactory results.

4.4 Solutions

Solution A:

0.1% formic acid in 6 mM ammonium formate.
Dissolve 0.378 g ammonium formate in 1 L of type I water.
Add 1 mL formic acid.
The pH of this solution is approx.

Solution B:

0.1% formic acid in acetonitrile (mobile phase "A").
Add 1 mL of formic acid to 1 liter of acetonitrile.

Solution C:

200 mM ammonium formate.
Dissolve 2.3 g ammonium formate in approximately 800 mL
Type I water.
Adjust volume to 1 liter.

Solution D:

0.3% formic acid in 4 mM ammonium formate (mobile phase "B").
Add 20 mL of solution C to 980 mL of type I water.
Add 3 ml formic acid.

LC/MS/MS Tuning Solution (PPG)

Add: 0.40 g of PPG 2000 solution,
0.10 g of PPG 1000 solution,
14 mg of PPG 425 solution,
126 mg ammonium formate,
1 mL acetonitrile, and adjust volume to 1L with 50%
methanol in water

4.5 Preparation of Standards and Fortification Solutions

Analytical standards are prepared for two purposes. They are used for fortifying untreated samples to determine analytical recovery and also to calibrate the response of the detector used in the analysis.

The absolute volumes of the standards may be varied by the analyst as long as the correct proportions of solute to solvent are maintained.

1. Stock Solution

To prepare stock standard solutions of 100 µg/mL, weigh 10 mg of analytical standard (corrected for purity) and bring up to 100 mL with methanol in 100 mL volumetric flask. Prepare separate solutions for each of the five analytes. Store all stock standard solutions in a freezer at -20°C or colder, in glass bottles with teflon-lined caps only. These stock standard solutions should be replaced within one year from the date of preparation.

2. Fortification Solutions

To prepare a mixed fortification solution of 0.5 µg/mL of RH-7988 and RH-0422 and 1.0 µg/mL each of RH-7280, RH-0131, and RH-3008, add 0.5 mL each from the 100 µg/mL stock solutions of RH-7988 and RH-0422 and 1.0 mL each from the 100 µg/mL stock solutions of RH-7280, RH-0131, RH-3008, and RH-9983 into a single 100 mL volumetric flask, and adjust volume to 100 mL with methanol/ Solution A (40:60) v/v.

Note: 1 mL of this solution spiked in 10 g of soil is equivalent to 0.05 ppm of RH-7988 and RH-0422 and 0.1 ppm of RH-7280, RH-0131, RH-3008, and RH-9983. Smaller volumes of this solution can be used to prepare lower fortification levels.

Store all fortification standard solutions in a refrigerator at 2 to 6°C for a maximum period of three months from the date of preparation, after which, make new standards using the stock solutions.

3. Calibration Standards

A 5-point LC/MS/MS calibration standard is prepared in methanol/solution A (40:60) via serial dilution of the fortification solution (0.5 µg/mL each of RH-7988 and RH-0422 and 1.0 µg/mL each of RH-7280, RH-0131, RH-3008, and RH-9983). Typical, standard concentrations used are 1.25, 2.5, 5, 10, and 25 ng/mL each of RH-7988 and RH-0422 and 2.5, 5, 10, 20, and 50 ng/mL each of RH-7280, RH-0131, RH-3008, and RH-9983.

The following is a typical example:

Initial Conc. (µg/mL)*	Volume (mL)	Diluted to (mL)	Final Conc. (µg/mL)*
0.5/1.0	10	100	0.05/0.1
0.05/0.1	50	100	0.025/0.05
0.025/0.05	40	100	0.01/0.02
0.01/0.02	50	100	0.005/0.01
0.005/0.01	50	100	0.0025/0.005
0.0025/0.005	50	100	0.00125/0.0025

* RH-7988 and RH-0422 / RH-7280, RH-0131, RH-3008, RH-9983

Store all calibration standard solutions in a refrigerator at 2 to 6°C for a maximum period of three months from the date of preparation, after which, make new standards using the stock solutions.

5. Instrumentation

5.1 LC/MS/MS

Mass Spectrometer:

PE Sciex API-III, serial no. 124930381
 Ionspray Liquid Introduction Interface
 Interface heater temperature: 65°C
 Air Nebulizer gas flow: 0.8 L/min
 N₂ curtain gas flow: 0.8 L/min
 Harvard infusion pump
 Apple Macintosh Quadra 950

Computer:

Software: (1) Macintosh system 7.1
 (2) PE Sciex Tune 2.5
 (3) PE Sciex RAD 2.6
 (4) PE Sciex Macspec 3.3
 (5) PE Sciex MacQuan 1.4

HPLC: Shimadzu SIL-10A Auto Injector
 Shimadzu LC-10AD gradient pumps
 Shimadzu CTO-10A column oven
 Shimadzu SCL-10A system controller

Column: Prism RP, 15 cm x 4.6 mm, 5 μ m
 (Keystone Scientific Inc., part. no. 155-321)

Column Temperature: 35°C

Mobile Phase A: 0.1% formic acid in acetonitrile

Mobile Phase B: 0.3% formic acid in 4 mM ammonium formate

Flow Rate: 1.0 mL/min

Split Ratio: ~15:1

Injected Volume: 100 μ L

Mobile Phase Program:

Time (min)	%A	%B
0	20	80
10	50	50
18	95	5
21	95	5
22	20	80
27	stop	

Total Run Time 27 minutes

Analyte Retention Times:

Analyte	Retention Time (min)
RH-9983	2.6
RH-0131	5.2
RH-7280	5.4
RH-3008	8.9
RH-0422	11.7
RH-7988	14.6

5.2 Calibration and Standardization:

Calibrate and tune the mass spectrometer on a daily basis prior to analyzing samples. Both mass analyzing quadrupoles (Q1 and Q3) have

to be calibrated each time. This is done by infusing a standard solution of poly(propylene glycol) [PPG] into the mass spectrometer using the ion spray interface, while monitoring positive ions. A typical mass calibration tune with PPG for both quadrupoles (Q1 and Q3) is shown in Figure 2.

5.3 Analysis

Analysis is performed using five "periods", each represented by a separate state file. These state files (corresponding to the first, second, third, fourth, and fifth period) are optimized for the analysis of RH-9983, RH-7280 and RH-0131 (one period), RH-3008, RH-0422, and RH-7988, respectively.

Analytes (1 to 2.5 µg/mL dissolved in 40% acetonitrile containing 4 mM ammonium formate and 0.1% formic acid) are also infused using the ion spray interface to optimize parameters for both parent and daughter ions. The parent ion scans (MS) and the their daughter ion profiles (MS/MS) for all five analytes are shown in Figures 3 to 7.

The analytes are detected in the triple quadrupole mode (MS/MS) by monitoring characteristic daughter ions resulting from passage of their parent molecular ions through the first quadrupole (Q1) into the collision cell (Q2), where fragmentation of the parent ions occur, with the resulting fragments separated in the second mass analyzing quadrupole (Q3). The mass-charge ratios of the parent ions and the monitored daughter ions are given below.

Analyte	Ionization Mode	Parent Ion	Daughter Ion	Retention
		Monitored (m/z)	Monitored (m/z)	Time (min)
RH-3008	negative ion	204	124	8.9
RH-7280	negative ion	214	155	5.4
RH-0131	negative ion	230	171	5.2
RH-0422	positive ion	287	198	11.7
RH-7988	positive ion	315	198	14.6
RH-9983	positive ion	126	70	2.6

Note: Slight variations in the retention times could occur with each batch of mobile phase.

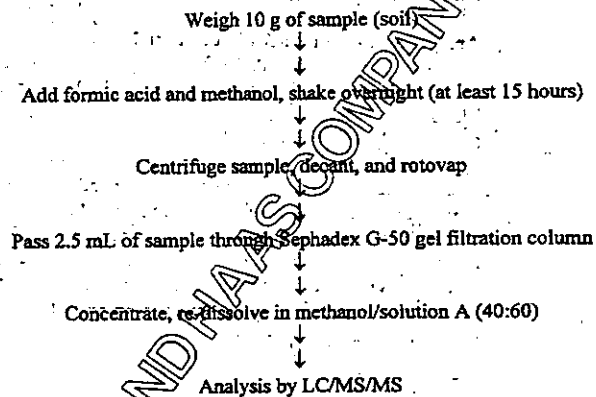
Typical state file parameters for PPG and for the three periods of analyte monitoring are given in Table 1. Data acquisition is performed by the RAD software program. Typical RAD data acquisition parameters are given in Table 2.

6. Method

6.1 Flow Diagram

The flow diagram of the method is given below, followed by a detailed description of each step.

Method Flow Diagram



6.2 Sample Processing

Chop the frozen soil samples into smaller pieces with a cleaver and rubber mallet or by any other appropriate means. Remove stones and debris as found. Add the smaller pieces of frozen soil into a Hobart Food Chopper bowl which has been pre-chilled with ice. Thoroughly chop the combined soil and homogenize with dry ice. Allow the dry ice to sublime in a freezer overnight and store frozen at -10°C or colder, until analysis.

6.3 Extraction

1. Weigh 10 g of homogenized soil into a 150 mL centrifuge bottle.
2. Fortifications should be made at this point.
3. Add 10 mL of 0.5% formic acid in water.
4. Let stand for 2-3 minutes.
5. Add 90 mL methanol.
6. Cap tightly and shake overnight (at least 15 hours) in a mechanical shaker.
7. Centrifuge at approximately 2000 rpm in a bench-top model centrifuge (swing bucket rotor) for 10 to 15 minutes.
8. Carefully decant the supernatant into a 250 mL round-bottom flask.
9. Add 50 mL 0.05% formic acid in methanol to the extracted soil and sonicate for 10 minutes. Centrifuge and add the supernatant to the same round-bottom flask.
10. Concentrate down to approximately 1 to 2 mL in a rotary evaporator under vacuum, at 35 to 40°C.
11. Decant carefully into a graduated 15 mL centrifuge tube.
12. Add 3 to 5 mL of solution A to the 250-mL round bottom flask, swirl and add this solution to the graduated centrifuge tube. Repeat until the volume is 10 mL. Mix well using a Pasteur pipet.

6.4 Gel filtration (clean-up)

6.4.1 Preparation of Sephadex G-50 Gel Filtration Column

- Weigh 10 g of Sephadex G-50 in a 200 mL beaker.
2. Add 125 ml Type I water. Allow the gel to swell for at least 3 hours.
 3. Place a frit at the bottom of the PD-10 column and attach on to a solid phase extraction device.
 4. Add 11 to 12 mL of the swollen gel per column.
 5. Allow the extra water to drain.
 6. Make sure that the column is packed up to the mark on the PD-10 column; add more gel if needed. No vacuum is necessary for packing the column.
 7. Position another frit at the top of the column. The columns should not run dry.

8. To store columns, cap the tops and the bottoms. Caps are also provided with the columns. Store columns at room temperature for immediate use, or store in refrigerator for use within 1 to 2 weeks.

6.4.2 Gel Filtration

1. Condition the Sephadex G-50 columns by adding 10 mL of solution A and drain to the top of the frit. Use of vacuum might be needed.
2. Add 2.5 mL of the sample (make sure the 10 mL sample is mixed well) and allow it to drain to the top of the frit.
3. Wash the column with 3.5 mL of solution A. Discard the wash.
4. Elute the compounds with 6 mL of solution A and collect.
5. Add 4 mL of methanol and to make the final volume 10 mL.

6.5 Analysis by LC/MS/MS

Inject a 100 μ L aliquot of each six-component calibration standard in the range of 0.0025 to 0.025 μ g/mL each of RH-7988 and RH-0422 and 0.005 to 0.025 μ g/mL each of RH-7280, RH-0131, RH-3008, and RH-9983 into the LC/MS/MS. Standard curves (for each analyte) are constructed by linear regression using the MacQuan software for each set of analysis.

Inject a 100 μ L aliquot of each sample/fortification/control into the LC/MS/MS. The concentration of each sample/fortification/blank is determined from the standard curve, based on the peak area of each analyte.

If necessary, dilute the samples to give a response within the standard curve range.

Note: The extraction results in a dilution factor of 2 (2.5 mL of the 10 mL extracted is passed through the Sephadex column and the eluant volume is adjusted to 5 mL). Therefore, the final volume will be 20 mL.

6.6 Time required for Analysis

A set of eight samples (including blanks, fortifications and actual samples) can be carried through the entire analytical procedure.

within two working days, a total time of 16 hours. This excludes the "overnight" shaking of samples. The LC/MS/MS run time for a set is approximately eight hours.

7. Calculations

Within an analytical set, all extracts (samples, method blanks, and procedural recovery spikes) were bracketed with a five level calibration curve. Generally, two to four extracts were run between standards.

Standard curves for LC/MS/MS analysis were built with MacQuan program (Version 1.3).

The RH-7988, RH-0422, RH-3008, RH-7280, RH-0133, and RH-9983 residue concentrations are determined as follows:

7.1 Fortification Recovery

For samples fortified with known amounts of analytes prior to extraction, measure the peak area (this is done by MacQuan software), determine the concentrations ($\mu\text{g/mL}$) from the standard curves, and calculate the percent recovery from Equation 1.

Equation 1

$$\left(\frac{[\mu\text{g/mL Found} \times \text{Total Sample Volume (mL)}] - \text{Control correction}}{\text{Fortification } (\mu\text{g})} \right) \times 100 = \% \text{ Recovery}$$

NOTE:

Total sample vol. (mL) = Total volume before clean-up (mL) x dilution factor after clean-up

7.2 Component Residue Concentration

Determine the component residue concentration as follows:

Equation 2

$$\frac{\mu\text{g/mL Found} \times \text{Total Sample Volume (mL)}}{\text{Sample Weight (g)}} = \text{ppm}$$

NOTE:

Total sample vol. (mL) = Total volume before clean-up (mL) x dilution factor after clean-up

8. Results and Discussion

8.1 LC/MS/MS Analysis

Recovery data are summarized in Tables 3 to 6. Table 3 summarizes fortification recoveries for RH-7988 and four metabolites (RH-0422, RH-7280, RH-3008, and RH-0131) which have been compiled from TR 34-96-90, and TR 34-97-006 (references 5 and 7). These results include the recovery numbers from 56 fortified control soil samples. The average recoveries from the analyses were 85.60 ± 8.42 , 88.76 ± 8.79 , 88.75 ± 11.82 , 72.39 ± 11.05 , and 84.66 ± 9.51 percent for RH-7988, RH-0422, RH-0131, RH-7280, and RH-3008 respectively.

Table 4 displays the fortification recovery data based on the analysis of RH-9983 as a single analyte. The average recovery based on 44 fortified samples was 86.20 ± 12.61 %.

After a method was developed to include RH-9983 in the analysis, RH-7988 and all five metabolites were analyzed together in a single run. Twenty-two control samples were fortified with standard solutions containing RH-7988 and the five soil metabolites and were then analyzed. These results are found in Table 5. The average recoveries for RH-7988, RH-0422, RH-0131, RH-7280, RH-3008, and RH-9983 were $84.16 \pm 15.79\%$, 91.85 ± 12.00 %, 83.18 ± 13.37 %, 84.57 ± 12.00 %, 93.15 ± 10.40 %, and $84.78 \pm 14.32\%$ respectively.

The results of these separate analyses were used to calculate a combined weighted average recovery percentage for each compound. These results are summarized in Table 6.

The limit of quantification (LOQ) based on the lowest actual fortification data was 0.01 ppm each for RH-7988 and RH-0422 and 0.02 ppm each for RH-3008, RH-7280, RH-9983 and RH-0131.

The limit of detection (LOD) by LC/MS/MS (equivalent to 30% of the limit of quantification) is 0.003 ppm each of RH-7988 and RH-0422, and 0.006 ppm each of RH-7280, RH-0131, RH-3008, and RH-9983.

Figures 9 to 14 illustrate representative calibration curves for RH-7988, RH-0422, RH-3008, RH-7280, RH-0131, and RH-9983 respectively. Figures 15 to 19 show representative ion extract chromatograms for the four calibration standards used in constructing the calibration curves. Figures 20 to 22 represent ion extract chromatograms for control soil and fortified controls of soil samples.

A slight matrix effect was observed for the analytes RH-3008 and RH-0131. This was characterized by "wider" peaks compared to the calibration standards. However, the peak areas were not affected.

Appendix A contains a print-out of a Sample Data Set After Calibration and Integration by PE SCIEX MacQuan Software Program.

8.2 Radiovalidation Study

A radiovalidation of RH-7988 and its metabolites in soil was conducted (reference 6) using sandy loam and silty clay loam samples generated by Ricerca, Inc. in the course of aerobic and anaerobic soil metabolism studies. The samples for radiovalidation were fortified and incubated according to the procedures described in the reports by Ricerca. Samples were harvested at specific intervals and then frozen in their entirety. These samples were sent by Ricerca, Inc. Painesville, Ohio to Centre Analytical for radiovalidation work. Four soil samples (three from the aerobic metabolism study and one from the anaerobic metabolism study) were analyzed by the ^{14}C methods as well as the residue analytical method described in TR 34-96-90 (reference 5).

For ^{14}C analysis of the soil samples, the total radioactive residues (TRR) in the soils were determined by combustion followed by LSC analysis. Samples were extracted by shaking overnight in acidic methanol. The total extracted radioactivity was determined by LSC analysis. The bound

radioactivity (post extraction solids; PES) was also determined by combustion followed by LSC analysis. The extractable residues were quantitated by HPLC, by collecting and counting radioactivity in second fractions. Identities of the analytes were further confirmed by co-chromatography on TLC. All of these metabolites had been independently identified by Ricerca by co-chromatography as well as mass spectroscopy (references 5 and 8).

The mass recovery of radiolabel from the 14-day sandy loam soil (CAL ID 964470) and the 14-day silty clay loam soil samples (CAL ID 964472) was 102.3% and 99.9% respectively (reference 6). The mass recovery of radiolabel from the 2-month sandy loam soil sample (CAL ID 964471) was 83.9%. The mass recovery from the 30-day silty clay loam anaerobic soil sample (CAL ID 965704) was 91.0%. The mass recovery from the 14-day sandy loam soil (CAL ID 964470) during the analysis of RH-9983 was 93.6%. The residue (ppm) of each component found by ^{14}C methods are shown in Table 7.

Quantitative confirmatory analysis was also performed at Centre Analytical (reference 6) using the mass spectrometer following separation of the analytes by HPLC, according to method TR 34-96-90 (Reference 1). Characteristic daughter ions resulting from passage of their parent molecular ions through the first quadrupole (Q1) into the collision cell (Q2), where fragmentations of the parent ions occur following collision with argon gas, were separated in the second mass analyzing quadrupole (Q3). Quantitation was based on the analytes detected in the triple quadrupole mode (MS/MS). The residues of each component determined by this method are also shown in Table 7. The amounts of most samples as a percent of the quantity found by ^{14}C analysis were found to be 63-137%. The percentage found by cold analysis for two analytes, RH-0131 and RH-0422, in soil sample CAL ID 964471 was 186% and 218%, respectively, and these numbers were based on very low residue levels (<0.03 ppm).

8.3 Radiovalidation Conclusions

Similar results were obtained from both the ^{14}C analysis and the cold residue analysis method (Method TR #34-96-90). Method TR # 34-96-90 (modified to analyze for RH-9983) was radiovalidated for the analytes RH-0422, RH-7280, RH-0131, RH-3008, and RH-9983. Further, the ^{14}C analyses indicate that no significant loss was observed at the extraction step in this method.

The radiovalidation study that was done validated the preliminary analysis method described in TR # 34-96-90. This preliminary method has only been modified slightly to include the analysis of RH-9983 and provide additional recovery numbers. The finalized method is described in this report, therefore, it is also radiovalidated.

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Table 1. Typical State File Values for PPG Calibration and for the Five Periods of Analyte Monitoring.

Parameter	Q1 PPG Calibration	Q3 PPG Calibration	MS/MS Period 1	MS/MS Period 2	MS/MS Period 3	MS/MS Period 4	MS/MS Period 5
DI	*	*	*	*	*	*	*
ISV	5000	5000	5000	-3800	-3800	5000	5000
IN	650	650	650	-650	-650	650	650
OR	50	50	60	-55	-80	40	40
RO	30	30	30	-30	30	30	30
M1	1000	1000	200	200	200	200	200
RE1	126.6	126.6	126.6	126.6	126.6	126.6	126.6
DMI	0.13	0.13	0.14	0.14	0.14	0.14	0.14
R1	28.5	28.5	27	-28	-27.2	25	25
L7	-49	28	17	13	-14	17	17
R2	-60	29	10	-10	-10	14	14
M3	1000	1000	200	200	200	200	200
RE3	124.1	122.0	122.0	122.0	122.0	122.0	122.0
DM3	0.11	0.13	0.13	0.13	0.13	0.13	0.13
RX	-10	-28	-10	-6	-6	9	9
R3	-33	28	-1	-1	-6	5	5
L9	-250	-250	100	205	205	-195	-195
FP	-250	-250	-100	220	220	-205	-205
MU	-3600	-3600	-3600	-3600	3600	-3600	-3600
CC	10	10	1	1	1	1	1
CGT	Off	Off	345	345	345	345	345

* value not applicable for the ion spray interface.

Note : State file values will often be changed slightly on a daily basis during instrument optimization procedures.

**The method has been modified from TR 34-96-90 to accommodate the analysis of 6 analytes. This table reflects those modifications.

Table 2. Typical RAD Data Acquisition Parameters.

	Period 1	Period 2	Period 3	Period 4	Period 5
Scan type	MRM	MRM	MRM	MRM	MRM
Delay	0.05	0.05	0.05	0.05	0.05
Acquire	3.5 min.	3 min.	3 min.	3 min.	3 min.
Scan rate	0.77	0.33	0.33	0.67	0.67
Dwell time	650 ms	1000 ms	1500 ms	750 ms	750 ms
Pause time	0.02 ms	0.02 ms	0.02 ms	0.02 ms	0.02 ms
Q1 mass	126	214	230	287	315
Q3 mass	70	155	171	124	198

NOTE: The delay time, acquire time, scan rate, and dwell time are given as an example, and may need to be optimized based on any changes in the retention times, chromatography, and instrument sensitivity/performance.

**The method has been modified from TR 34-96-90 to accommodate the analysis of 6 analytes. This table reflects those modifications.

TABLE 3. Percent Recovery of RH-7988, RH-0422, RH-0131, RH-7280, and RH-3008 in Soil (5-Analyte LC/MS/MS Detection)

Sample ID	Fortification** (ppm)	Recovery RH-7988 (%)	Recovery RH-0422 (%)	Recovery RH-3008 (%)	Recovery RH-7280 (%)	Recovery RH-0131 (%)
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(Data from TR # 34-96-90)

Analyzed 3/18/96						
Spk A	0.05	89.8	94.2	88.1	84.4	78.2
Spk B	0.05	94.2	95.5	90.0	79.6	85.8
Analyzed 3/20/96						
Spk A	0.01	89.6	78.2	81.4	83.8	82.2
Spk B	0.01	91.3	85.5	84.5	87.0	80.8
Spk C	0.025	84.6	84.7	75.3	72.4	72.1
Spk D	0.025	83.9	94.0	81.5	71.5	70.9
Analyzed 3/21/96						
Spk A	0.01	84.3	82.5	73.8	75.0	77.0
Spk B	0.01	85.1	84.9	82.5	78.8	84.5
Spk C	0.025	91.8	92.1	83.3	104.6	111.5
Spk D	0.025	95.8	97.1	93.1	100.1	111.7
Spk E	0.05	85.4	88.7	90.5	88.0	102.7
Spk F	0.05	89.0	96.6	82.3	70.2	81.9
Analyzed 3/24/96						
Spk A	0.01	85.9	90.2	88.9	86.6	88.0
Spk B	0.01	85.9	82.6	86.9	85.0	86.3
Spk C	0.025	88.8	89.6	89.5	77.3	75.5
Spk D	0.025	90.7	83.2	89.4	80.5	82.7
Spk E	0.05	78.2	84.7	78.4	70.9	68.4
Spk F	0.05	77.9	84.7	80.5	68.0	66.6
Analyzed 3/25/96						
Spk A	0.01	84.4	101.3	105.9	86.9	92.4
Spk B	0.01	82.0	103.7	90.6	62.5	57.7
Spk C	0.025	91.7	94.8	92.2	64.2	73.1
Spk D	0.025	88.0	87.0	91.8	82.8	84.5
Spk E	0.05	94.9	94.4	92.7	67.2	92.1
Spk F	0.05	88.2	105.2	102.2	70.1	92.3

NEW YORK SOIL
(Data from TR # 34-97-006)

Analyzed 10/11/96						
Spk A	0.01/0.02	77.1	86.9	73.2	63.8	80.7
Spk B	0.05/0.10	82.1	84.7	89.6	59.9	88.3
Analyzed 8/25/96						
Spk A	0.01/0.02	79.4	87.9	90.8	67.0	86.9
Spk B	0.05/0.10	81.8	98.5	91.1	75.2	97.0
Analyzed 9/28/96						
Spk A	0.01/0.02	86.3	88.7	92.2	65.9	100.3
Spk B	0.05/0.10	68.8	84.1	86.7	58.9	94.7
Analyzed 9/30/96						
Spk A	0.01/0.02	84.6	89.8	96.5	65.3	103.8
Spk B	0.05/0.10	77.0	88.0	92.3	67.8	99.3

(cont'd)

(Table 3 cont'd)

Sample ID	Fortification (ppm)	Recovery RH-7988 (%)	Recovery RH-0422 (%)	Recovery RH-3008 (%)	Recovery RH-7280 (%)	Recovery RH-0131 (%)
Analyzed 10/2/96						
Spk A	0.01/0.02	75.2	99.2	78.4	69.8	86.9
Spk B	0.05/0.10	96.5	98.1	80.5	64.9	103.3
Analyzed 10/3/96, 1/3/97						
Spk A	0.01/0.02	84.2	85.1	70.4	69.4	95.7*
Spk B	0.05/0.10	82.6	91.5	100.3	81.0	79.5*
*re-analysis 1/3/97						
Analyzed 10/14/96						
Spk A	0.01/0.02	107.1	102.0	97.6	88.3	101.4
Spk B	0.05/0.10	80.6	92.6	88.4	66.7	100.6
Analyzed 10/8/96						
Spk A	0.01/0.02	94.6*	102.8	82.1	82.3	99.7
Spk B	0.05/0.10	66.7*	67.1	72.8	55.1	81.9
*re-analysis 1/3/97						
Analyzed 10/9/96						
Spk A	0.01/0.02	90.4	97.9	81.1	53.2	105.2
Spk B	0.05/0.10	86.4	80.9	86.4	65.7	103.1
Analyzed 10/10/96						
Spk A	0.01/0.02	91.8	103.5	92.4	68.7	94.4
Spk B	0.05/0.10	78.7	81.0	80.1	57.2	86.3
Analyzed 10/15/96						
Spk A	0.01/0.02	79.1	75.2	81.6	74.9	96.1
Spk B	0.05/0.10	71.6	70.9	71.3	65.1	74.8
Analyzed 10/17/96						
Spk A	0.01/0.02	92.2	88.2	82.4*	84.4	101.5
Spk B	0.05/0.10	70.1	72.7	66.7*	68.2	78.6
*re-analysis 1/8/97						
Analyzed 10/18/96						
Spk A	0.01/0.02	88.3	88.5	89.1	79.3	97.2
Spk B	0.05/0.10	84.6	87.7	73.8	54.7	86.2
Analyzed 10/22/96						
Spk A	0.01/0.02	107.2	94.8	75.4	65.4*	93.8
Spk B	0.05/0.10	74.3	70.4	82.5	53.4*	90.3
*re-analysis 1/8/97						
Analyzed 12/12/96						
Spk A	0.01/0.02	78.8	83.1	94.9	75.7	99.8
Spk B	0.05/0.10	76.6	81.2	80.3	85.7	97.3
Analyzed 10/28/96						
Spk A	0.01/0.02	88.9	83.1	64.8*	77.5	93.4
Spk B	0.05/0.10	78.7	78.8	73.8*	58.5	74.0
*re-analysis 1/9/97						
Average		85.60	88.76	84.66	72.39	88.75
Std. Deviation		8.42	8.79	9.51	11.05	11.82
n		56	56	56	56	56

For each spike, the first fortification number is the ppm level for RH-7988 and RH-0422.
The second number indicates the fortification of RH-0131, RH-7280, and RH-3008

TABLE 4. Percent Recovery of RH-9983 in Soil (Analyzed as a Single Analyte)

(Data from TR # 34-97-006) NEW YORK SOIL

Analysis Date	Fortification (ppm)	Recovery (%)
1/20/97	0.02	93.9
1/20/97	0.02	95.2
1/23/97	0.02	106.6
1/26/97	0.02	92.6
1/27/97	0.02	61.3
1/28/97	0.02	88.2
1/29/97	0.02	80.4
1/30/97	0.02	88.8
1/31/97	0.02	99.7
2/3/97	0.02	86.8
2/4/97	0.02	85.8
2/5/97	0.02	92.4
2/10/97	0.02	110.1
2/11/97	0.02	99.6
2/12/97	0.02	90.1
2/13/97	0.02	85.8
2/14/97	0.02	102.0
2/17/97	0.02	85.1
3/11/97	0.02	91.3
3/11/97	0.02	60.8
3/18/97	0.02	91.3
5/4/97	0.02	91.9
1/20/97	0.1	86.0
1/20/97	0.1	80.1
1/23/97	0.1	90.6
1/25/97	0.1	61.1
1/27/97	0.1	62.0
1/28/97	0.1	51.0
1/29/97	0.1	82.9
1/30/97	0.1	85.0
1/31/97	0.1	87.1
2/3/97	0.1	85.8
2/4/97	0.1	105.5
2/5/97	0.1	88.6
2/10/97	0.1	91.2
2/11/97	0.1	96.6
2/12/97	0.1	77.2
2/13/97	0.1	83.1
2/14/97	0.1	81.8
2/17/97	0.1	85.8
3/11/97	0.1	93.2
3/11/97	0.1	81.7
3/18/97	0.1	91.6
5/4/97	0.1	74.0
Average		86.20
Std. Deviation		12.61
n		44

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TABLE 5. Percent Recovery of RH-7988, RH-0422, RH-0131, RH-7280, RH-3008, and RH-9983 in Soil (6-Analyte LC/MS/MS Detection)

(Data from TR # 34-97-006) NEW YORK SOIL
Fortification Recoveries in Soil

Fort.Level (ppm)	Recovery RH-7988	Recovery RH-0422	Recovery RH-0131	Recovery RH-7280	Recovery RH-3008	Recovery RH-9983
0.01*0.02	75.0	89.4	69.7	72.2	83.1	74.7
0.01*0.03	113.9	129.1	124.9	112.4	128.9	122.6
0.01*0.02	74.8	98.0	91.4	98.1	107.2	93.2
0.01*0.02	80.9	86.5	82.6	86.9	89.3	87.1
0.01*0.03	103.9	106.8	102.9	105.3	109.8	100.8
0.01*0.02	93.6	94.2	98.4	88.9	105.0	89.2
0.01*0.02	89.7	94.1	83.9	92.4	91.4	80.3
0.01*0.02	72.6	88.3	72.5	70.8	90.0	82.5
0.01*0.02	85.4	89.6	73.2	78.8	90.7	78.9
0.01*0.02	86.6	103.6	85.2	90.1	94.4	87.1
0.01*0.02	71.3	87.4	81.1	82.6	89.2	74.0
0.05*0.1	108.0	103.1	87.7	104.9	100.2	104.3
0.05*0.2	73.5	90.4	88.9	82.4	90.5	90.9
0.05*0.1	80.2	86.3	88.9	86.3	85.2	80.6
0.05*0.1	39.3	100.5	85.1	76.5	91.5	45.9
0.05*0.2	104.7	91.1	81.7	77.1	92.4	91.4
0.05*0.1	83.9	85.6	83.1	82.6	93.8	79.8
0.05*0.1	70.5	78.6	75.1	81.8	78.9	80.4
0.05*0.1	83.4	74.4	72.7	74.6	80.1	75.9
0.05*0.1	84.2	76.4	62.4	70.1	87.0	75.7
0.05*0.1	87.3	86.8	73.5	71.3	88.1	80.3
0.05*0.1	88.9	74.4	70.6	75.0	92.3	89.5
Average	84.16	91.85	83.48	84.57	93.15	84.78
Std. Dev.	15.78	12.00	13.37	12.00	10.40	14.32
n	22	22	22	22	22	22

**For each spike, the first fortification number is the ppm level for RH-7988 and RH-0422. The second number indicates the fortification of RH-0131, RH-7280, RH-3008, RH-9983

TABLE 6. Average Fortification Recoveries for Six Analytes in Soil

	RH-7988 (%)	RH-0422 (%)	RH-0131 (%)	RH-7280 (%)	RH-3008 (%)	RH-9983 (%)
Analysis 1						
5 Analytes *						
(Table 3)						
<i>Recovery (%)</i>	85.60	88.76	88.75	72.39	84.66	
<i>Std. Deviation</i>	8.42	8.79	11.82	11.05	9.51	
<i>n</i>	56	56	56	56	56	
Analysis 2						
RH-9983 Single **						
(Table 4)						
<i>Recovery (%)</i>						86.20
<i>Std. Deviation</i>						12.61
<i>n</i>						44
Analysis 3						
6 Analytes ***						
(Table 5)						
<i>Recovery (%)</i>	84.16	91.86	83.18	84.57	93.15	84.78
<i>Std. Deviation</i>	15.79	12.00	13.37	12.00	10.40	14.32
<i>n</i>	22	22	22	22	22	22
<i>Combined Average</i>	85.19	89.63	87.18	75.82	87.05	85.73
<i>Standard Deviation</i>	10.50	9.69	12.26	11.32	9.76	13.18
<i>n (total)****</i>	78	78	78	78	78	66

- * Analysis was done to determine the residues of RH-7988, RH-0422, RH-0131, RH-7280, RH-3008
 ** RH-9983 was fortified and analyzed as a single analyte
 *** All six analytes were analyzed together in a single run

**** Note: The Combined Average and Standard deviation are calculated using a weighted average based on the average for each analysis and then weighted for the number of samples in that particular analysis. See sample calculation below:

e.g. $RH-7988 = \frac{(85.60 \cdot 56) + (84.16 \cdot 22)}{(56 + 22)} = 85.19$

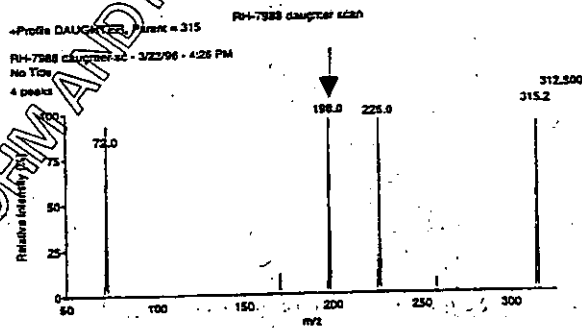
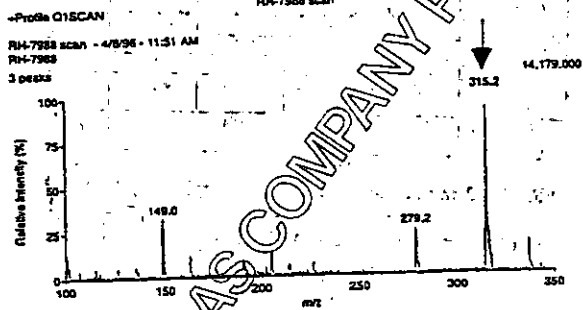
TABLE 7. Summary Table of ¹⁴C and Non-¹⁴C Residue Analysis**

Sample ID	CAL ID	Analysis	PPM of Analyte					
			RH-7988	RH-0423	RH-0131	RH-7280	RH-3008	RH-9983
RAR NO.		Method						
96-0018-019	964470	¹⁴ C	0.003	0.133	0.085	0.015	0.025	0.019
96-0018-020	964471	¹⁴ C	0.002	0.005	0.017	0.007	0.042	*
96-0018-019	964472	¹⁴ C	0.001	0.059	0.056	0.031	0.008	*
96-0018-114 (soil)	965704	¹⁴ C	0.000	0.066	0.006	0.025	0.000	*
96-0018-114 (aqueous)	965704	¹⁴ C	0.000	0.114	0.009	0.035	0.001	*
96-0018-019	964470	TR 34-96-90	<0.01	0.140	0.070	<0.02	<0.02	0.020
96-0018-020	964471	TR 34-96-90	0.01	0.012	0.032	<0.02	0.047	*
96-0018-019	964472	TR 34-96-90	<0.01	0.055	0.035	<0.02	<0.02	*
96-0018-114 (soil)	965704	TR 34-96-90	<0.01	0.072	<0.02	>0.025	<0.02	*
96-0018-114 (aqueous)	965704	TR 34-96-90	<0.01	0.156	<0.02	0.039	<0.02	*

*RH-9983 was not analyzed in these samples.

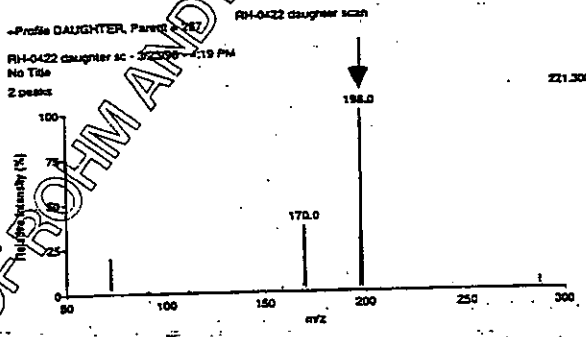
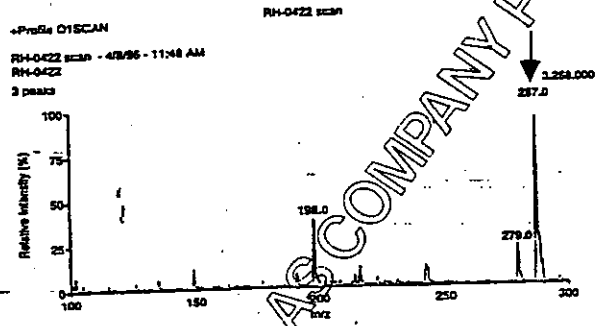
**Note: Data is from TR # 34-97-019 (Reference 6)

Figure 2 Parent Ion Scan (top) and Daughter Ions Profile (bottom) of RH-7988



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Figure 3 Parent Ion Scan (top) and Daughter Ions Profile (bottom) of RH-0422



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Figure 4 Parent Ion Scan (top) and Daughter Ions Profile (bottom) of RH-3008

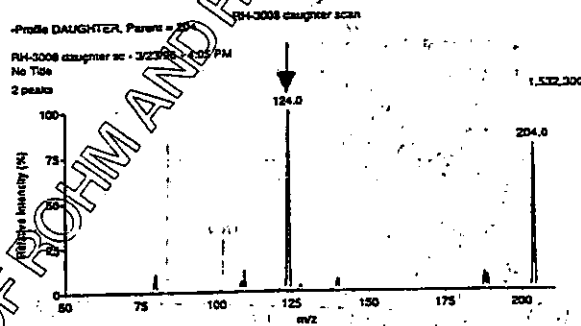
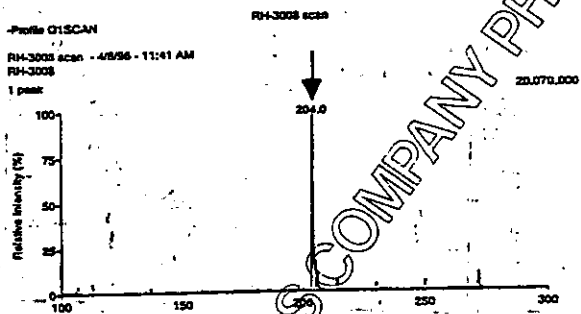
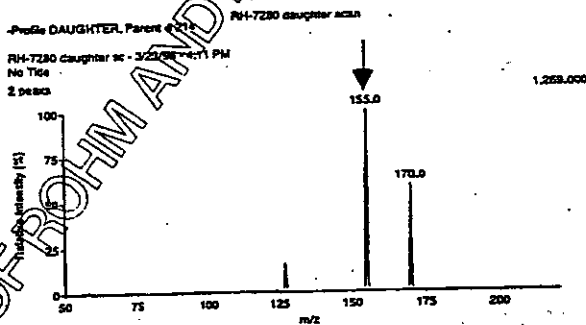
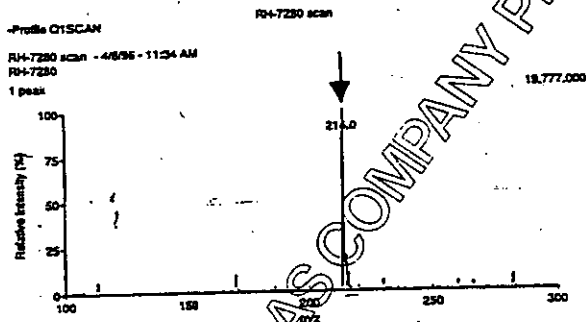


Figure 5 Parent Ion Scan (top) and Daughter Ions Profile (bottom) of RH-7280

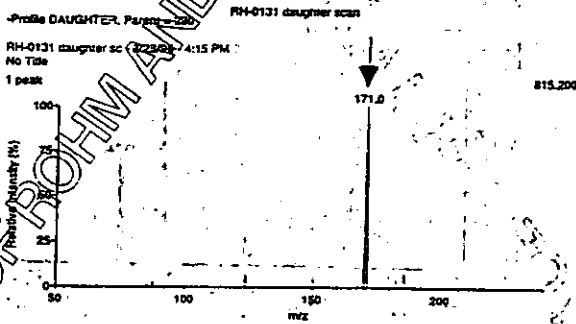
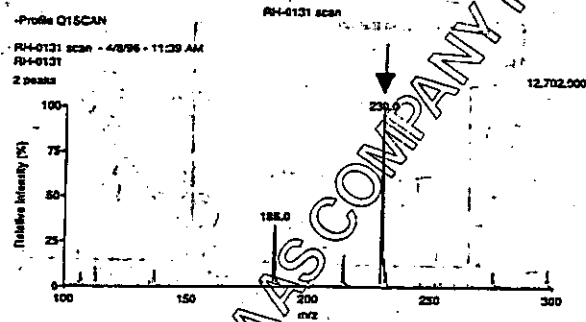


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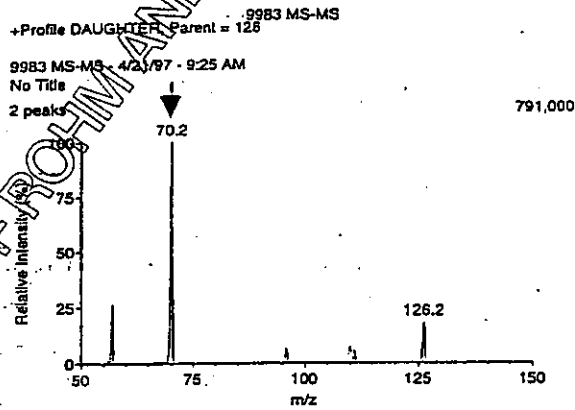
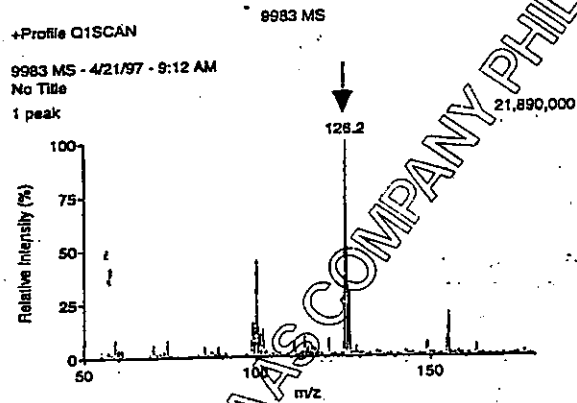
CLN 42 7

Figure 6 Parent Ion Scan (top) and Daughter Ions Profile (bottom) of RH-0131



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Figure 7 Parent Ion Scan (top) and Daughter Ions Profile (bottom)
of RH-9983



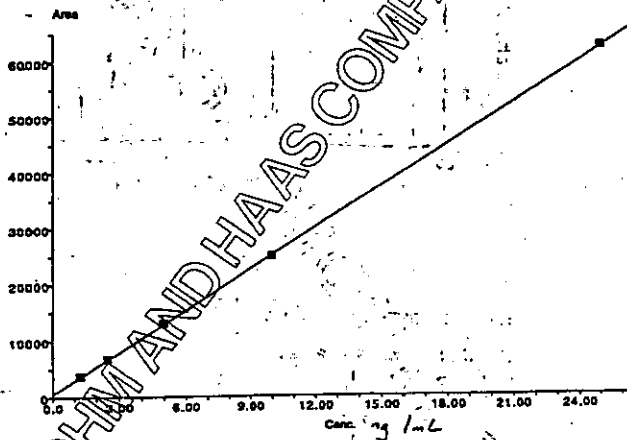
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Figure 8 Calibration Curve for RH-7988 (range 0.00125 to 0.025 µg/mL)

MacQues, version 1.4-PPU
Printed: Mon, Apr 7, 1987 08:12
Calibration File: CalbFile Path: Mac HD:022-211 NY - ZPH 033187 04-07
Comments: CAL study 602-211, RH 34P-83-28A, Batch 033187, 24-30 inch depth, Total vol. = 40,000 mL, vol. = 100 mL

RH-7988 315.0-198.0 No Internal Standard
Linear
Intercept = 562.039124
Slope = 2438.438477
Correlation Coeff. = 0.996280



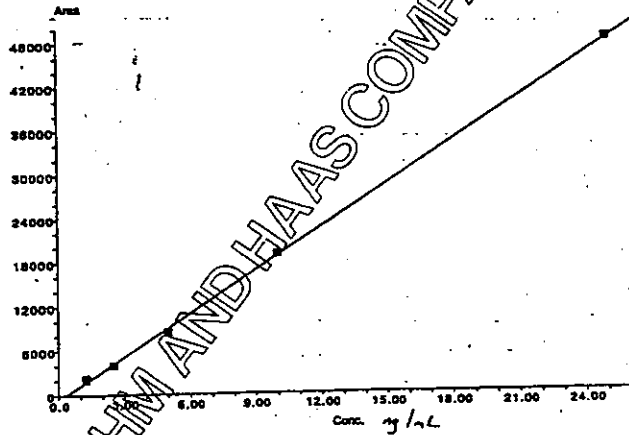
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Figure 9 Calibration Curve for RH-0422 (range 0.00125 to 0.025 µg/mL)

MacQuant, version 1.4-PPU
Printed: Mon, Apr 7, 1987 08:12
Calibration File: CalbFile Path: Mac HD:002-011 NY - 2:PH 002197 (24-30)
Comment: CAL study 002-011, PH 247-80-25A, Batch 002197, 24-80 inch depth, Total vol. = 40 mL, 100 mg/L = 100 µL

RH-0422 267.0 -> 196.0 No Internal Standard
Linear
Intercept = -646.459900
Slope = 1932.019653
Correlation Coef. = 0.999792

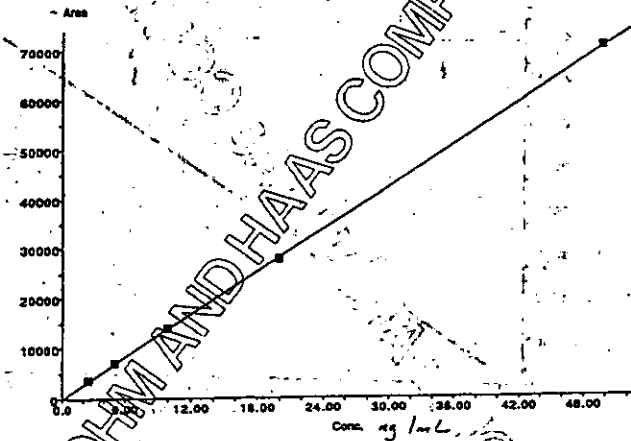


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Figure 10 Calibration Curve for RH-3008 (range 0.0025 to 0.05 µg/mL)

MacComp, version 14-PPU
Printed: Mon, Apr 7, 1987 08:13
Calibration File: CalbFile Path: Mac 140302-211 NY - ERM 033187 (24-30)
Concentration: CM, study 002-211, RH 34P-63-28A, Batch 033187, 34-00 inch depth, Total vol. = 40 ml, 100 µL

RH-3008 204.0→124.0 No Internal Standard
Linear
Intercept = -74.842509
Slope = 1409.147735
Correlation Coef. = 0.999970



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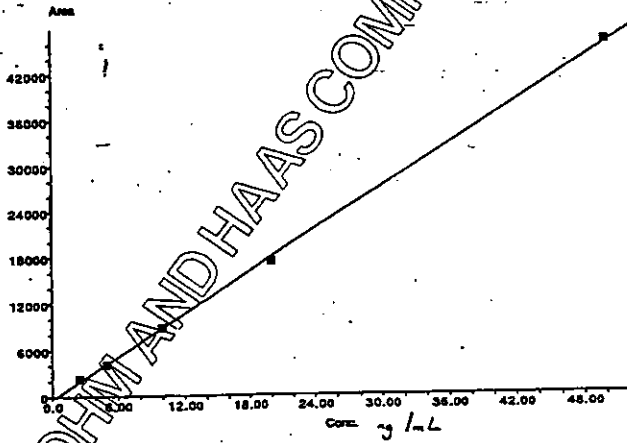
Figure 11 Calibration Curve for RH-7280 (range 0.0025 to 0.05 µg/mL)

MacQuay, section LA-PPU
Printed: Mon, Apr 7, 1987 08:12
Calibration File: C:\MSD\02-211\HY - 25\4 032187 04-02
Comments: CAL. study 002-211, PH 247-89-25A, Batch 032187, 24-80 inch depth, Total vol. = 400 µL, Inj. vol. = 100 µL

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RH-7280 214.0-155.0 No Internal Standard
Linear

Intercept = -375.06008
Slope = 818.450684
Correlation Coef. = 0.999748



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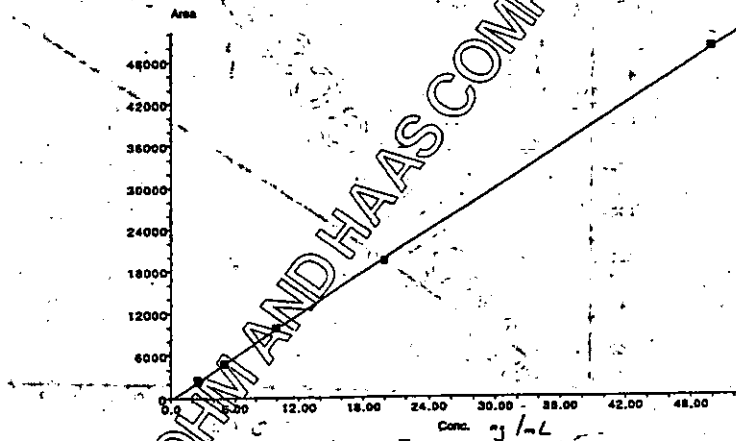
Figure 12 Calibration Curve for RH-0131 (range 0.0025 to 0.05 µg/mL)

MacQuant, Version 1.4-FPU
Printed: Mon, Apr 7, 1987 08:12
Calibration File: CalFile; Print: Mac H2000-011 NY - 2701 023187 (24-30);
Concentration: CAL study 002-011, RH-0131-03-25A, Batch 023187, 24-30 inch depth, Total vol. = 40 mL, Inj. vol. = 100 µL

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RH-0131 230.0 → 171.0 No Internal Standard

Linear
Intercept = -237.346309
Slope = 898.060354
Correlation Coef. = 0.999228



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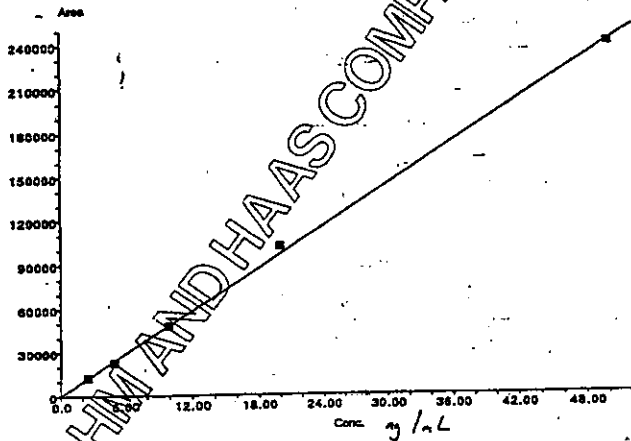
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Figure 13 Calibration Curve for RH-9983 (range 0.0025 to 0.05 $\mu\text{g/mL}$)

MacQm, version 1.4-FPU
Printed: Mon, Apr 7, 1997 09:13
Calibration File: CalcFile Path: Mac HD:002-211 NY - ERW 033197 04-02
Comments: CAL study 002-211, RH 34P-63-28A, Batch 033197, 24-30 inch depth, Total vol. = 40, 100 μL = 100 μL .

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RH-9983 128.0 -> 70.0 No Internal Standard
Linear
Intercept = 531.549253
Slope = 4792.257324
Correlation Coef. = 0.999589



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Figure 14 Chromatogram of Calibration Standards Representing
0.0025 µg/mL each of RH-7280, RH-0131, RH-3008 and RH-9983, and
0.00125 µg/mL each of RH-0422 and RH-7988

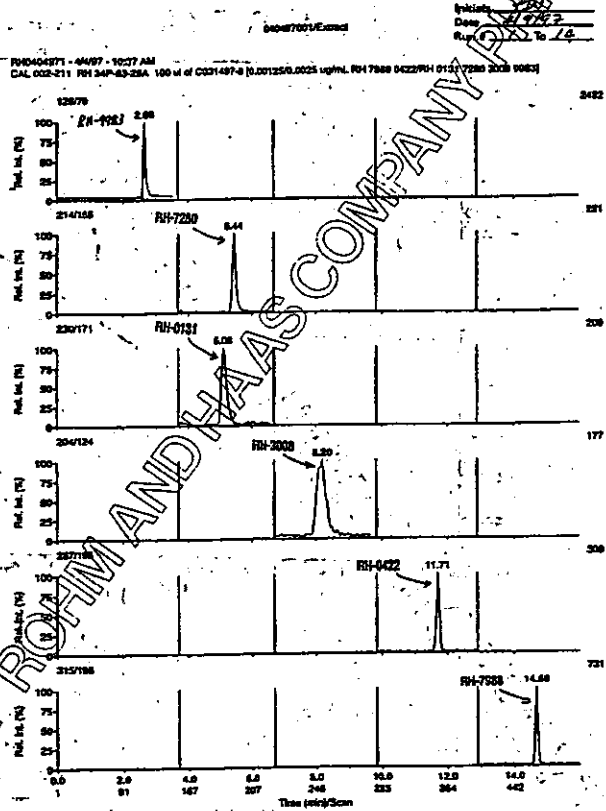
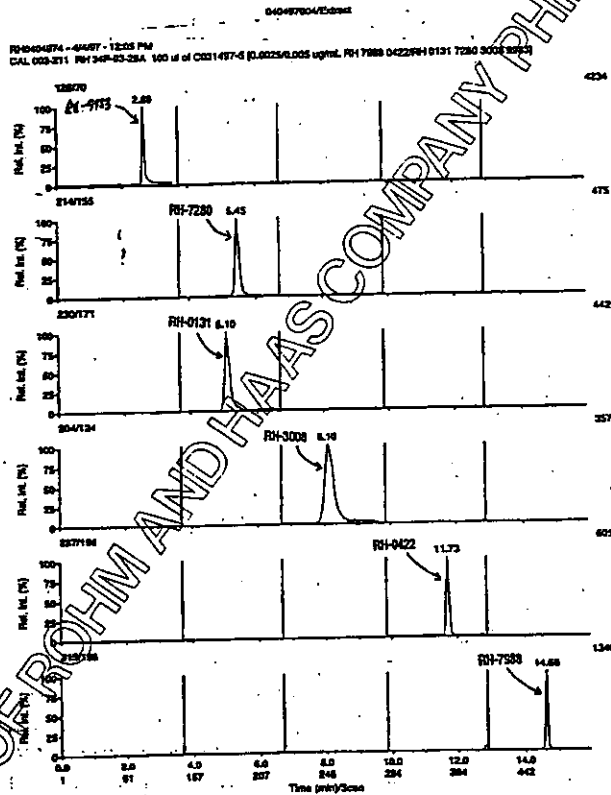
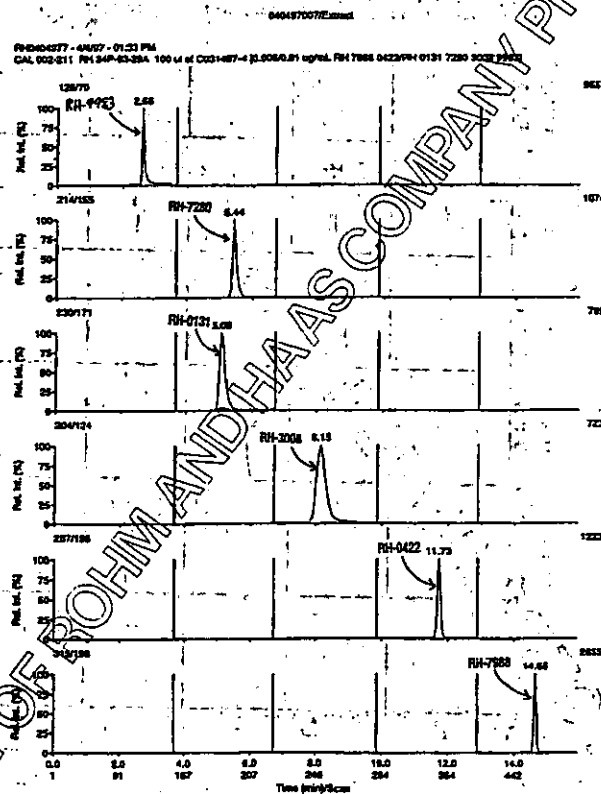


Figure 15 Chromatogram of Calibration Standards Representing
0.005 µg/mL each of RH-7280, RH-0131, RH-3008 and RH-9983, and
0.0025 µg/mL each of RH-0422 and RH-7988



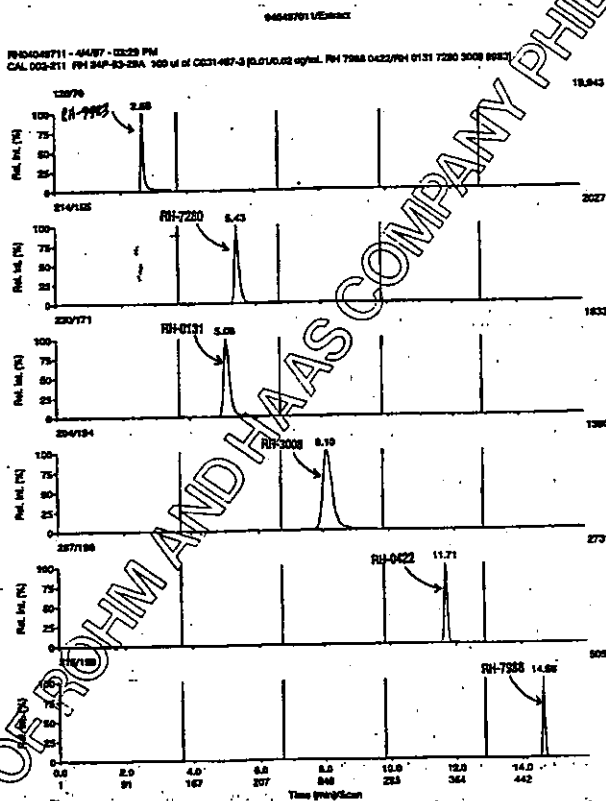
TR 34-97-60

Figure 16 Chromatogram of Calibration Standards Representing 0.01 $\mu\text{g}/\text{mL}$ each of RH-7280, RH-0131, RH-3008 and RH-9983; and 0.005 $\mu\text{g}/\text{mL}$ each of RH-0422 and RH-7988



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Figure 17 Chromatogram of Calibration Standards Representing
0.02 µg/mL each of RH-7280, RH-0131, RH-3008, and RH-9983, and
0.01 µg/mL each of RH-0422 and RH-7988



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Figure 18 Chromatogram of Calibration Standards Representing 0.05 µg/mL each of RH-7280, RH-0131, RH-3008, and RH-9983, and 0.025 µg/mL each of RH-0422 and RH-7988

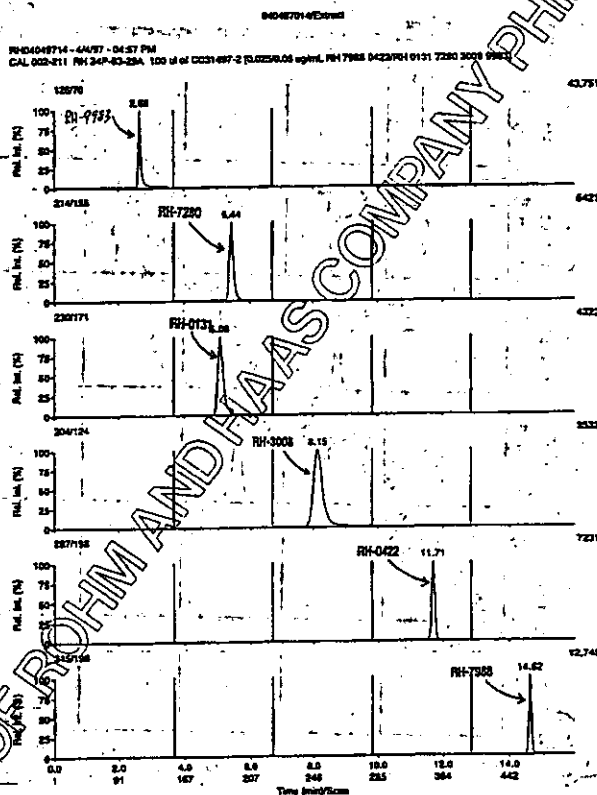


Figure 19 Chromatogram of a Control Soil Extract

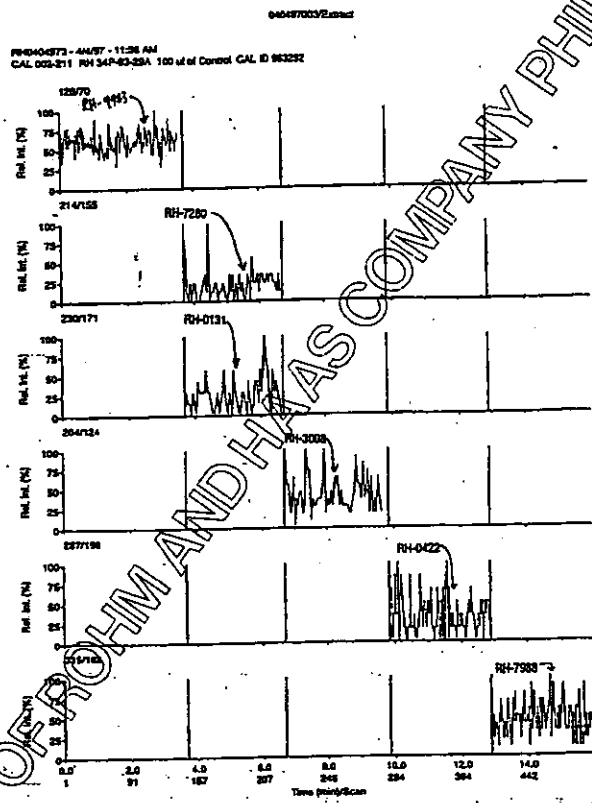
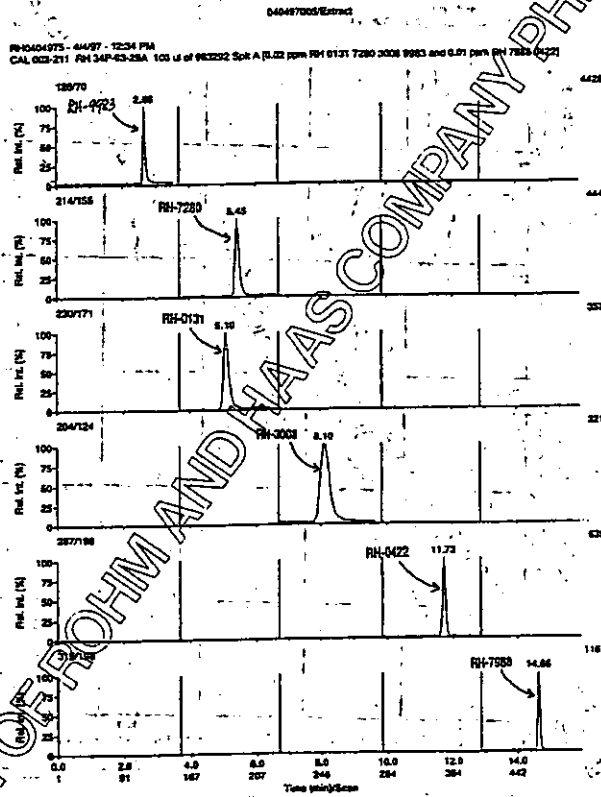


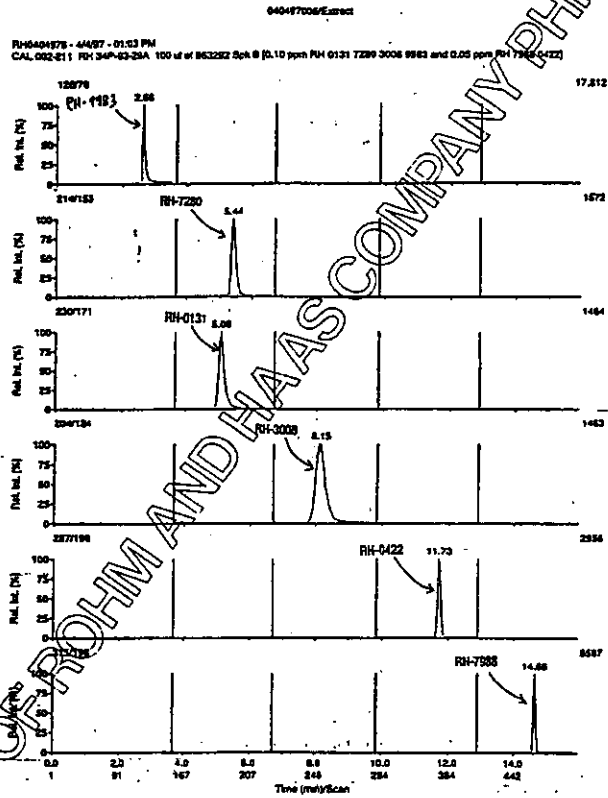
Figure 20 Chromatogram of a Soil Sample Fortified with 0.02 ppm each of RH-7280, RH-0131, RH-3008, and RH-9983, and 0.01 ppm each of RH-0422 and RH-7988



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Figure 21 Chromatogram of a Soil Sample Fortified with 0.1 ppm each of RH-7280, RH-0131, RH-3008, and RH-9983, and 0.05 ppm each of RH-0422 and RH-7988



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Appendix Print-Outs of a Sample Data Set After Calibration and
Integration by PE SCIEX MacQuan Software Program

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