



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

ENVIRONMENTAL CHEMISTRY SECTION
BUILDING 1105—JOHN C. STENNIS SPACE CENTER
STENNIS SPACE CENTER, MISSISSIPPI 39529-6000
TELEPHONE (601) 688-3216

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MEMORANDUM

SUBJECT: ECM Evaluation Report for Nicosulfuron and Rimsulfuron
(ECM 0020S1-S4) 129008
129009

FROM: Aubry E. Dupuy, Jr., Section Chief
BEAD/ACB/Environmental Chemistry Section
Aubry E. Dupuy, Jr.

THRU: Donald A. Marlow, Branch Chief
BEAD/Analytical Chemistry Branch *DM*

TO: Henry M. Jacoby, Branch Chief (7507C)
EFED/Environmental Fate and Groundwater Branch

The EFED has requested environmental chemistry method evaluations (ECMEs) for a number of compounds on the various FIFRA priority lists. The enclosed ECM report, "Evaluation of a Method for the Determination of Nicosulfuron, Rimsulfuron, and Related Degradates (IN-V9367 and IN-70941) in Soil (ECM 0020S1-S4)", is submitted in response to a request by EFGWB for this evaluation.

The Registrant's method was evaluated by our contractor. The method met most of the performance DQOs for the compounds, although poor precision was observed for one metabolite, IN-70941, at the LOQ. If you have questions, please call me at (601) 688-3212 or Bob Maxey at (601) 688-1225.

Attachment

cc: Bob Maxey/ECS



2003713

MRID #430231-02

EVALUATION OF A METHOD FOR THE DETERMINATION OF NICOSULFURON,
RIMSULFURON, AND RELATED DEGRADATES (IN-V9367 AND IN-70941)
IN SOIL (ECM0020S1-S4)


(Contains FIFRA-CBI)

FINAL REPORT

Prepared By:
Science Applications International Corporation

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U.S. EPA Office of Pesticide Programs
Stennis Space Center, MS

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Mark Roby
SAIC Task Manager/Study Director


Date

Table of Contents

<u>Section</u>	<u>Page</u>
1.0 Summary and Conclusions	1
Laboratory Evaluation	1
Major Difficulties	2
Conclusions	2
Comments	3
2.0 Analytical Results	4
3.0 Experimental Details	8
Method Summary	8
Procedural Notes and Accommodations to Variables	9
Calculations	10
Chemical Structure Diagrams	12
Appendix A - Calibration Data	A-1
Appendix B - Representative Chromatograms	B-1

1.0 SUMMARY AND CONCLUSIONS

This report describes testing of a registrant's method (MRID# 430231-02) for the determination of nicosulfuron and its degradate, IN-V9367, and rimsulfuron and its degradate, IN-70941, in a soil matrix. Method testing was conducted at the laboratory of SAIC's subcontractor, Agricultural & Priority Pollutants Laboratories, Inc. (APPL). The laboratory evaluation, major difficulties, experimental conclusions and comments are presented in this section.

1.1 Laboratory Evaluation

The MRID package containing the registrant's method provides for the determination of nicosulfuron, rimsulfuron, and related degradates, IN-V9367 and IN-70941 using LC/MS with a thermospray interface. A total of five ions, characteristic of the target analytes, were used for quantitation.

1.1.1 Nicosulfuron (DPX-V9360)

Nicosulfuron recoveries ranged from 78.0% to 86.0% with a mean recovery of 81.1% and a relative standard deviation (RSD) of 4.4% in soils fortified at 0.02 mg/Kg (LOQ). For soils fortified at 0.2 mg/Kg (10xLOQ), the recoveries ranged from 75.0% to 96.0% with a mean recovery of 84.1% and an RSD of 11.0%. The instrument response to nicosulfuron in soils fortified at the method detection limit (MDL) exceeded noise by a factor greater than three (S/N = 5). The MDL for testing purposes was 0.0067 mg/Kg. The nicosulfuron retention times from the fortified samples fell within the retention time windows established from the nicosulfuron calibration standard retention times. No peaks were found within the nicosulfuron retention time window in either the reagent blank or the matrix blank.

1.1.2 IN-V9367

Recoveries for the nicosulfuron degradate IN-V9367 ranged from 100% to 115% with a mean recovery of 108% and an RSD of 6.0% for soils fortified at 0.02 mg/Kg (LOQ). For soils fortified at 0.2 mg/Kg (10xLOQ), the recoveries ranged from 83.3% to 118% with a mean recovery of 97.3% and an RSD of 16.9%. The instrument response to IN-V9367 in samples fortified at the method detection limit (MDL) exceeded noise by a factor greater than three (S/N = 9). The MDL for testing purposes was 0.0067 mg/Kg. The IN-V9367 retention times from the fortified samples fell within the retention time windows established from the IN-V9367 calibration standard retention times. No peaks were found within the IN-V9367 retention time window in either the reagent blank or the matrix blank.

1.1.3 Rimsulfuron (DPX-E9636)

Rimsulfuron recoveries ranged from 73.5% to 81.5% with a mean recovery of 78.8% and an RSD of 4.5% for soils fortified at 0.02 mg/Kg (LOQ). For soils fortified at 0.2 mg/Kg (10xLOQ), the recoveries ranged from 60.9% to 81.1% with a mean recovery of 72.0% and an RSD of 12.5%. The instrument response to rimsulfuron in soils fortified at the method detection limit (MDL) exceeded noise by a factor greater than three (S/N = 5). The MDL for testing purposes was 0.0067 mg/Kg. The rimsulfuron retention times from the fortified samples fell within the retention time windows established from the rimsulfuron calibration standard retention times. No peaks were found within the rimsulfuron retention time window in either the reagent blank or the matrix blank.

1.1.4 IN-70941

Recoveries of metabolite IN-70941 ranged from 60.6% to 103% with a mean recovery of 87.0% and an RSD of 21.7% for soils fortified at 0.02 mg/Kg (LOQ). For soils fortified at 0.2 mg/Kg (10xLOQ), the recoveries ranged from 84.5% to 135% with a mean recovery of 114% and an RSD of 18.6%. The instrument response to IN-70941 in samples fortified at the method detection limit (MDL) exceeded noise by a factor greater than three (S/N = 18). The MDL for the testing purposes was 0.0067 mg/Kg. The IN-70941 retention times from the fortified samples fell within the retention time windows established from the IN-70941 calibration standard retention times. No peaks were found within the IN-70941 retention time window in either the reagent blank or the matrix blank.

1.2 Major Difficulties

The calibration procedures used in testing this method differed from the registrants procedure in two ways:

- (1) The calibration included a concentration level (0.015 $\mu\text{g/mL}$) lower than the lowest level in the registrants method (0.03 $\mu\text{g/mL}$). The lower concentration standard was included to ensure all measurements from samples fortified at the LOQ were bracketed in compliance with the OPP standard operating procedure for calibration.
- (2) Bracketing sample pairs with calibration standards at concentrations lower and higher than the expected sample concentration was not done. Instead, an initial four-point calibration (0.015 $\mu\text{g/mL}$, 0.03 $\mu\text{g/mL}$, 0.2 $\mu\text{g/mL}$, and 0.4 $\mu\text{g/mL}$) was run followed by samples with calibration check standards inserted between each set of fortification levels.

In following this calibration procedure, difficulties were encountered in maintaining calibration over an extended period of time. Therefore, each fortification level was analyzed at separate times. For example, samples fortified at the LOQ were extracted, the instrument calibrated, and the extract measured on a different day from samples fortified at 10 X LOQ. Given the tendency for thermospray LC/MS response to drift, continuous updating of the calibration by interspersing standards with samples throughout the run sequence or by internal standards would be advisable.

1.3 Conclusions

Method performance met project recovery and precision objectives (70-120% recovery, RSD \leq 20%) for nicosulfuron and its degradate, IN-V9367, and for rimsulfuron. The method also met acceptance criteria for the rimsulfuron degradate, IN-70941, at the 10xLOQ fortification level. Poor precision (RSD = 21.7%) was observed for IN-70941 at the LOQ, although this value did not exceed the value reported by the registrant.

For samples fortified at 0.02 mg/Kg (LOQ), the mean recovery results obtained in this study were generally lower than those reported by the registrant with the exception of IN-V9367. In terms of recovery RSD, the results obtained in this study were considerably lower than those reported by the registrant with the exception of IN-70941 which was similar to the registrants reported result. The results reported by the registrant are listed below:

<u>Compound</u>	<u>Range, %</u>	<u>Mean Recovery</u>	<u>RSD</u>	<u>n</u>
IN-V9367	76 - 109	91%	15%	6
IN-70941	82 - 148	101%	22%	6
DPX-V9360	65 - 124	90%	24%	6
DPX-E9636	69 - 115	85%	20%	6

1.4 Comments

A potential difficulty occurs in the preparation of the extract concentrate for analysis. The registrant's method cautions that the calibration standards solutions contain less than 10% acetonitrile to avoid possible solvent mismatch effects on the early eluting IN-V9367. While this is easy to achieve for the calibration standards it is very difficult to achieve for the sample extracts. Thus, the sample extracts would be subject to solvent mismatch effects but the calibration standards would not. It is not clear to what extent this complication had on the results of this study. The sample extract ion traces show much more baseline disturbance in the region of the IN-V9367 retention time than do the corresponding calibration standard ion traces. Integration of the IN-V9367 peak from the sample extracts was much more difficult and variable than for the other target analytes. This potential difficulty may be resolved by modifying the solvent program so that IN-V9367 elutes later (longer retention time).

This method was tested using a Hewlett Packard 5988 Thermospray LC/MS instead of the Finnigan 4600 quadrupole with Vestec thermospray interface used by the registrant.

The time required for completing one set of 4 samples (4 replicates at a given fortification level), 4 calibration standards, and associated QC samples (matrix blank, instrument blanks, and calibration check) was approximately 2 working days. Sample preparation requires one day. Samples can be analyzed on the LC/MS overnight and data reduced the following day.

2.0 ANALYTICAL RESULTS

This section presents method testing results. Summary tables are presented along with individual results from each sample at each spiking level. The mean, standard deviation, and relative standard deviation are calculated in terms of percent recovery and in terms of measured concentration.

2.1 Data Summary

2.1.1 Nicosulfuron (DPX-V9360)

Spike Level (mg/Kg)	Mean Rec.	SD ¹ Rec.	RSD ² Rec.	Mean Conc.	SD ² Conc.
LOQ (0.02)	81.1	3.6	4.4	0.016	0.0007
10xLOQ (0.2)	84.1	9.2	11.0	0.168	0.0185

¹ SD = Standard Deviation

² RSD = Relative Standard Deviation

2.1.2 IN-V9367

Spike Level (mg/Kg)	Mean Rec.	SD ¹ Rec.	RSD ² Rec.	Mean Conc.	SD ² Conc.
LOQ (0.02)	108	6.4	6.0	0.022	0.0012
10xLOQ (0.2)	97.3	16.4	16.9	0.195	0.0334

¹ SD = Standard Deviation

² RSD = Relative Standard Deviation

2.1.3 Rimsulfuron (DPX-E9636)

Spike Level (mg/Kg)	Mean Rec.	SD ¹ Rec.	RSD ² Rec.	Mean Conc.	SD ² Conc.
LOQ (0.02)	78.8	3.6	4.5	0.016	0.0007
10xLOQ (0.2)	72.0	9.0	12.5	0.144	0.0177

¹ SD = Standard Deviation

² RSD = Relative Standard Deviation

2.1.4 IN-70941

Spike Level (mg/Kg)	Mean Rec.	SD ¹ Rec.	RSD ² Rec.	Mean Conc.	SD ² Conc.
LOQ (0.02)	87.0	18.9	21.7	0.017	0.0038
10xLOQ (0.2)	114	21.3	18.6	0.229	0.0426

¹ SD = Standard Deviation

² RSD = Relative Standard Deviation

2.2 Individual Results for Soil Samples

2.2.1 Individual Results for Nicosulfuron (DPX-V9360) Fortified at 0.02 mg/Kg

Sample Number	Retention Time (min)	Concentration Found (mg/Kg)	Concentration of Fortified Sample (mg/Kg)	Percent Recovery
1 Nicosulfuron	13.24	0.0158	0.02	79.0
2 Nicosulfuron	13.24	0.0163	0.02	81.5
3 Nicosulfuron	13.24	0.0156	0.02	78.0
4 Nicosulfuron	13.24	0.0172	0.02	86.0

2.2.2 Individual Results for Nicosulfuron (DPX-V9360) Fortified at 0.2 mg/Kg

Sample Number	Retention Time (min)	Concentration Found (mg/Kg)	Concentration of Fortified Sample (mg/Kg)	Percent Recovery
1 Nicosulfuron	13.41	0.192	0.2	96.0
2 Nicosulfuron	13.41	0.158	0.2	79.0
3 Nicosulfuron	13.41	0.173	0.2	86.5
4 Nicosulfuron	13.41	0.150	0.2	75.0

2.2.3 Individual Results for IN-V9367 Fortified at 0.02 mg/Kg (LOQ)

Sample Number	Retention Time (min)	Concentration Found (mg/Kg)	Concentration of Fortified Sample (mg/Kg)	Percent Recovery
1 IN-V9367	7.41	0.0210	0.02	105
2 IN-V9367	7.33	0.0220	0.02	110
3 IN-V9367	7.33	0.0230	0.02	115
4 IN-V9367	7.41	0.0201	0.02	100

2.2.4 Individual Results for IN-V9367 Fortified at 0.2 mg/Kg (10xLOQ)

Sample Number	Retention Time (min)	Concentration Found (mg/Kg)	Concentration of Fortified Sample (mg/Kg)	Percent Recovery
1 IN-V9367	12.78	0.237	0.2	118
2 IN-V9367	12.78	0.170	0.2	84.9
3 IN-V9367	12.78	0.206	0.2	103
4 IN-V9367	12.78	0.166	0.2	83.3

2.2.5 Individual Results for Rimsulfuron (DPX-E9636) Fortified at 0.02 mg/Kg

Sample Number	Retention Time (min)	Concentration Found (mg/Kg)	Concentration of Fortified Sample (mg/Kg)	Percent Recovery
1 Rimsulfuron	16.66	0.0163	0.02	81.5
2 Rimsulfuron	16.66	0.0160	0.02	80.0
3 Rimsulfuron	16.66	0.0160	0.02	80.0
4 Rimsulfuron	16.66	0.0147	0.02	73.5

2.2.6 Individual Results for Rimsulfuron (DPX-E9636) Fortified at 0.2 mg/Kg

Sample Number	Retention Time (min)	Concentration Found (mg/Kg)	Concentration of Fortified Sample (mg/Kg)	Percent Recovery
1 Rimsulfuron	16.91	0.154	0.2	77.2
2 Rimsulfuron	16.83	0.138	0.2	68.8
3 Rimsulfuron	16.83	0.162	0.2	81.1
4 Rimsulfuron	16.83	0.122	0.2	60.9

2.2.7 Individual Results for IN-70941 Fortified at 0.02 mg/Kg (LOQ)

Sample Number	Retention Time (min)	Concentration Found (mg/Kg)	Concentration of Fortified Sample (mg/Kg)	Percent Recovery
1 IN-70941	12.24	0.0173	0.02	86.4
2 IN-70941	12.24	0.0197	0.02	98.3
3 IN-70941	12.24	0.0206	0.02	103
4 IN-70941	12.24	0.0121	0.02	60.6

2.2.8 Individual Results for IN-70941 Fortified at 0.2 mg/Kg (10xLOQ)

Sample Number	Retention Time (min)	Concentration Found (mg/Kg)	Concentration of Fortified Sample (mg/Kg)	Percent Recovery
1 IN-70941	12.33	0.238	0.2	119
2 IN-70941	12.33	0.238	0.2	119
3 IN-70941	12.33	0.270	0.2	135
4 IN-70941	12.33	0.169	0.2	84.5

3.0 Experimental Details

A brief summary of the analytical method, notes on the analytical procedure/accommodations to variables, and example calculations are presented in this section.

3.1 Method Summary

A soil matrix was fortified with nicosulfuron (DPX-V9360) and its degradate, IN-V9367, rimsulfuron (DPX-E9636) and its degradate, IN-70941, at three different concentrations corresponding to the MDL, the LOQ, and ten times the LOQ. The fortification levels were: 0.0067 mg/Kg, 0.02 mg/Kg, and 0.2 mg/Kg. Four replicates at each concentration were prepared and analyzed according to the described procedure. Sample concentrations were calculated using a mean calibration factor determined from a four-point external standardization. The concentration of the four calibration standards were: 0.015- μ g/mL, 0.030- μ g/mL, 0.20- μ g/mL, and 0.40- μ g/mL. The low-point calibration standard corresponds to the one-half the LOQ. The method protocol is described in brief below.

3.1.1 Extraction Method

A 10 g sample was extracted with 3 X 10 mL acetonitrile/water (8:2, v:v) using sonication. Each extraction was centrifuged for 15 minutes at ca. 1000 rpm and the liquid phase decanted into a graduated centrifuge tube. The combined extracts were centrifuged and the total volume recorded. A 5 mL aliquot of the supernatant was concentrated to ca. 0.5 mL using a nitrogen blowdown apparatus at ambient temperature. The concentrate volume was adjusted to 1 mL with water, mixed, centrifuged and an aliquot of the supernatant transferred to an autosampler vial for analysis.

3.1.2 Analysis Method

The sample extract concentrates were analyzed by LC/MS with thermospray interface using a Hewlett Packard 5988 Thermospray LC/MS. Chromatographic conditions were similar to those provided by the registrant except where noted. For any deviation from the registrant's conditions, the registrant's condition is given in parentheses after the actual experimental condition used in these studies:

Column:	Zorbax 5 micron ODS, 250 X 4.6 mm (Whatman Partisil C8, 250 X 4.6 mm)		
Flow Rate:	0.9 mL/minute, (1.0 mL/minute)		
Column Temperature:	ambient, (not specified)		
Injection Volume:	200 μ L		
Post-column Addition:	0.5M ammonium acetate at 0.1 mL/min., (0.3 mL/min.)		
Mobile Phase Program:	<u>Time (minutes)</u>	<u>% acetonitrile</u>	<u>% 0.1M acetic acid</u>
	0	0	100
	5	30	70
	12	45	55
	15	45	55
	20	100	0
Probe Temperature:	114 °C, (probe and instrument specific)		
Source Temperature:	276 °C, (325 °C)		
Ionization Mode:	Thermospray Positive Ion		
Mass Calibration:	Polypropylene glycol		

Selected Ions Monitored: m/z 156, 199, 230, 247, 325

Quantitation Ions: IN-V9367, m/z 230 + 247
 IN-70941, m/z 325
 nicosulfuron (DPX-V9360), m/z 156 + 199 + 230 + 247
 rimsulfuron (DPX-E9636), m/z 156 + 199 + 325

3.1.3 Standards Information

Nicosulfuron (DPX-V9360)

US EPA Pesticides Repository
 Lot No. AEG1
 Neat, 99.2% purity
 Received 5/7/93, Opened 5/10/93
 Sent to APPL, 5/8/95

IN-V9367

Provided by OPP
 from DuPont
 Ref. No. E62739-063
 Neat, 99.5% purity
 Received 5/4/95
 Sent to APPL, 5/8/95

Rimsulfuron (DPX-E9636)

US EPA Pesticides Repository
 Lot No. AHM1
 Neat, 99.7% purity
 Received 5/7/93, Opened 5/8/93
 Sent to APPL, 5/8/95

IN-70941

Provided by OPP
 from DuPont
 Code No. E58306-49
 Neat, 99.5% purity
 Received 5/4/95
 Sent to APPL, 5/8/95

Matrix Information: ECM Program Soil
 California Batch #1 (5/5/95)
 Sent to APPL 6/14/95

3.2 Procedural Notes and Accommodations to Variables

A Hewlett Packard 5988 Thermospray LC/MS was used for extract analysis instead of the Finnigan 4600 quadrupole with Vestec thermospray interface.

A Hewlett Packard 1090L was used for post column addition instead of the Kratos 400 pump.

A Zorbax 5 micron ODS column was used instead of the Whatman Partisil C8.

Soil samples were extracted using a Tekmar Sonic Disruptor Model 600 with 0.5 inch tip instead of the vortex mixer and ultrasonic bath described in the registrant's procedure.

No filtration was used. Instead extracts were centrifuged before aliquots were taken.

Samples were not further processed once the extract concentrates were brought up to a final volume of 1.0 mL.

The calibration procedures used in testing this method differed from the registrant's procedure. In testing the method, an initial four point calibration at 0.015, 0.03, 0.2, and 0.4 $\mu\text{g}/\text{mL}$ was used to generate a mean calibration factor which was in turn used to quantitate analyte response. The lowest calibration level was included to ensure that all measurements from samples fortified at the LOQ were bracketed. The registrant's calibration procedure established response linearity using a three-point calibration at 0.03, 0.2, and 0.4 $\mu\text{g}/\text{mL}$. Analyte response was then quantitated using the mean response of calibration standards bracketing sample pairs in the run sequence. The

concentrations of the calibration standard were selected to be at concentrations lower and higher than the expected sample concentration.

3.3 Calculations

Example calculations are presented for calibration factor, mean calibration factor, extract concentration, and sample concentration. The formula used to calculate the percent relative standard deviation (RSD) is also given.

3.3.1 Calibration Factor (chromatogram #L8A14)

$$\text{Calibration Factor (CF)} = \frac{\text{area counts}}{\text{concentration}}$$

From the 0.03- $\mu\text{g/mL}$ calibration standard: for rimsulfuron, area counts = 71551:

$$CF_{\text{rimsulfuron}} = \frac{71551}{0.03} = 2385033$$

3.3.2 Mean Calibration Factor (chromatograms # LZ814 through L8C14)

$$CF_{\text{mean}} = \frac{(CF_1 + CF_2 + \dots + CF_n)}{n}$$

where n = the number of calibration points.

The four-point rimsulfuron soil calibration data are as follows:

n	Concentration ($\mu\text{g/mL}$)	Area Counts	CF
1	0.015	40295	2686333
2	0.030	71551	2385033
3	0.20	428254	2141270
4	0.40	899342	2248355

$$CF_{\text{mean}} = \frac{(2686333 + 2385033 + 2141270 + 2248355)}{4} = 2365248$$

3.3.3 Extract Concentration (chromatogram #L8F14)

$$\text{Concentration}_{\text{extract}} = \frac{\text{area counts}}{CF_{\text{mean}}}$$

For replicate 1 (LOQ), rimsulfuron area counts = 80435, and $CF_{\text{mean}} = 2365248$:

$$\text{Rimsulfuron Concentration}_{\text{extract}} = \frac{80435}{2365248} = 0.0340 \mu\text{g/mL}$$

3.3.4 Sample Concentration (chromatogram #L8F14)

$$\text{Concentration}_{\text{sample}} = \frac{\text{Conc}_{\text{extract}} \times V_f \times V_t}{\text{Initial Weight}_{\text{sample}} \times V_a}$$

where V_f = final extract volume, V_t = total extract volume, and V_a = aliquot volume. For replicate 1 (LOQ), rimsulfuron concentration_{extract} = 0.034 $\mu\text{g/mL}$, $V_f = 1.0 \text{ mL}$, $V_t = 23.5 \text{ mL}$, $V_a = 5.0 \text{ mL}$, and initial weight_{sample} = 10 g:

$$\text{Conc.}_{\text{sample}} = \frac{0.034 \mu\text{g/mL} \times 1.0 \text{ mL} \times 23.5 \text{ mL}}{10 \text{ g} \times 5.0 \text{ mL}} = 0.0160 \mu\text{g/g}$$

3.3.5 Relative Standard Deviation (RSD) is calculated by dividing the standard deviation of the measured concentration of the analytes by the mean concentration recovered and multiplying by 100%.

$$\text{Mean} = \bar{x} = \frac{\sum x_i}{n}$$

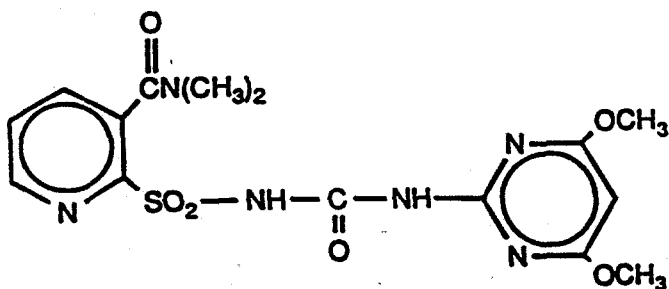
$$\text{std. dev.} = s = \sqrt{\frac{\sum (\bar{x} - x_i)^2}{n - 1}}$$

$$\text{Precision as RSD} = \frac{s}{\bar{x}} \times 100 \%$$

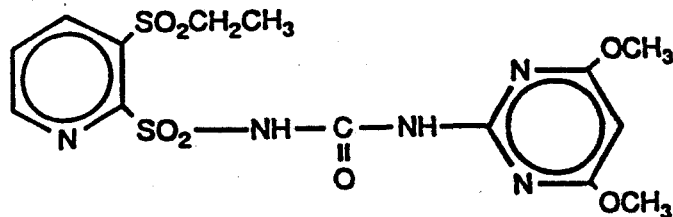
3.3.6 Percent Difference (PD) is calculated by subtracting the mean calibration factor of the initial calibration from the calibration factor of a calibration check standard, dividing the result by the mean calibration factor from the initial calibration, and multiplying by 100%.

$$\text{Percent Difference} = \frac{CF_{\text{calcheck}} - \overline{CF}_{\text{initial}}}{\overline{CF}_{\text{initial}}} \times 100 \%$$

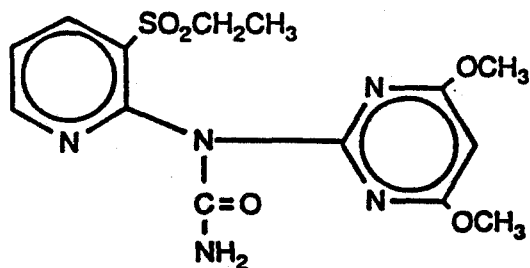
3.4 Chemical Structure Diagrams of Nicosulfuron (DPX-V9360), Rimsulfuron (DPX-E9636), IN-V9367, and IN-70941



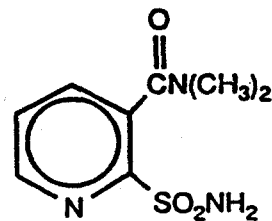
Nicosulfuron (DPX-V9360)



Rimsulfuron (DPX-E9636)



IN-70941



IN-V9367

Appendix A - Calibration Data

Initial calibration curve and calibration check standards data for the samples are presented below.

A.1 Nicosulfuron (DPX-V9360) Calibration Data

A.1.1 Initial Calibration Data for Nicosulfuron (Samples Fortified at 0.02 mg/Kg)

Retention Time (min)	Area Counts	Concentration ($\mu\text{g/mL}$)	Calibration Factor
13.33	68625	0.015	4575000
13.24	149128	0.03	4970933
13.33	706625	0.20	3533125
13.24	1582229	0.40	3955572

Mean Calibration Factor = 4258658

Standard Deviation = 639184

Relative Standard Deviation = 15.0

A.1.2 Calibration Check Data for Nicosulfuron (Samples Fortified at 0.02 mg/Kg)

Calibration Check	Retention Time (min)	Area Counts	Concentration ($\mu\text{g/mL}$)	Calibration Factor	Percent Difference
1	13.24	133702	0.03	4456733	4.6

A.1.3 Initial Calibration Data for Nicosulfuron (Samples Fortified at 0.2 mg/Kg)

Retention Time (min)	Area Counts	Concentration ($\mu\text{g/mL}$)	Calibration Factor
13.41	86016	0.015	5734400
13.41	129158	0.03	4305267
13.41	1036346	0.20	5181730
13.41	2013698	0.40	5034245

Mean Calibration Factor = 5063910

Standard Deviation = 588744

Relative Standard Deviation = 11.6

A.1.4 Calibration Check Data for Nicosulfuron (Samples Fortified at 0.2 mg/Kg)

Calibration Check	Retention Time (min)	Area Counts	Concentration ($\mu\text{g/mL}$)	Calibration Factor	Percent Difference
1	13.41	868559	0.2	4342795	-14.6

A.2 IN-V9367 Calibration Data

A.2.1 Initial Calibration Data for IN-V9367 (Samples Fortified at 0.02 mg/Kg)

Retention Time (min)	Area Counts	Concentration ($\mu\text{g/mL}$)	Calibration Factor
7.41	137353	0.015	9156867
7.41	258636	0.03	8621200
7.41	1645823	0.20	8229115
7.41	3213786	0.40	8034465

Mean Calibration Factor = 8510412

Standard Deviation = 495255

Relative Standard Deviation = 5.8

A.2.2 Calibration Check Data for IN-V9367 (Samples Fortified at 0.02 mg/Kg)

Calibration Check	Retention Time (min)	Area Counts	Concentration ($\mu\text{g/mL}$)	Calibration Factor	Percent Difference
1	7.41	271472	0.03	9049067	6.3

A.2.3 Initial Calibration Data for IN-V9367 (Samples Fortified at 0.2 mg/Kg)

Retention Time (min)	Area Counts	Concentration ($\mu\text{g/mL}$)	Calibration Factor
7.58	572422	0.015	38161467
7.58	692483	0.03	23082767
7.58	2418591	0.20	12092955
7.58	5292764	0.40	13231910

Mean Calibration Factor = 21642275

Standard Deviation = 12067622

Relative Standard Deviation = 55.8

Note: Because a non-linear response was obtained (CF RSD = 55.8), a single point calibration using the calibration factor associated with the 0.2 µg/mL standard (12092955) was used to calculate IN-V9367 concentrations in samples fortified at 0.2 mg/Kg.

A.2.4 Calibration Check Data for IN-V9367 (Samples Fortified at 0.2 mg/Kg)

Calibration Check	Retention Time (min)	Area Counts	Concentration (µg/mL)	Calibration Factor	Percent Difference
1	7.49	1976099	0.2	9880495	-18.3

A.3 Rimsulfuron Calibration Data

A.3.1 Initial Calibration Data for Rimsulfuron (Samples Fortified at 0.02 mg/Kg)

Retention Time (min)	Area Counts	Concentration (µg/mL)	Calibration Factor
16.74	40295	0.015	2686333
16.74	71551	0.03	2385033
16.74	428254	0.20	2141270
16.74	899342	0.40	2248355

Mean Calibration Factor = 2365248

Standard Deviation = 236162

Relative Standard Deviation = 10.0

A.3.2 Calibration Check Data for Rimsulfuron (Samples Fortified at 0.02 mg/Kg)

Calibration Check	Retention Time (min)	Area Counts	Concentration (µg/mL)	Calibration Factor	Percent Difference
1	16.58	57708	0.03	1923600	-18.7

A.3.3 Initial Calibration Data for Rimsulfuron (Samples Fortified at 0.2 mg/Kg)

Retention Time (min)	Area Counts	Concentration (µg/mL)	Calibration Factor
16.91	71981	0.015	4798733
16.91	128901	0.03	4296700
16.91	838382	0.20	4191910
16.91	1725523	0.40	4313807

Mean Calibration Factor = 4400288
 Standard Deviation = 271041
 Relative Standard Deviation = 6.2

Note: Even though the initial calibration was linear, the calibration check standard gave a calibration factor differing from the initial calibration factor by -22.0%. Therefore, a single point calibration using the calibration factor from the 0.2 µg/mL standard (4191910) was used to calculate rimsulfuron concentrations in the sample extracts. The calibration check standard gave a calibration factor differing from the single point calibration factor by less than 20% (-18.3%).

A.3.4 Calibration Check Data for Rimsulfuron (Samples Fortified at 0.2 mg/Kg)

Calibration Check	Retention Time (min)	Area Counts	Concentration (µg/mL)	Calibration Factor	Percent Difference
1	16.83	685268	0.2	3426340	-18.3

A.4 IN-70941 Calibration Data

A.4.1 Initial Calibration Data for IN-70941 (Samples Fortified at 0.02 mg/Kg)

Retention Time (min)	Area Counts	Concentration (µg/mL)	Calibration Factor
12.24	44874	0.015	2991600
12.24	90509	0.03	3016967
12.24	604706	0.20	3023530
12.24	1298229	0.40	3245572

Mean Calibration Factor = 3069417
 Standard Deviation = 118241
 Relative Standard Deviation = 3.8

Note: The calibration check standard gave a calibration factor differing from the initial mean calibration factor by -26.9%. Therefore, a linear regression treatment of the calibration data was used to calculate IN-70941 concentrations in the sample extracts. The calibration check standard gave a measured concentration differing from the expected concentration (0.03 µg/mL) by less than 20% (-17.3%). The linear regression treatment of the calibration data gave the following parameters:

slope = 3422859
 intercept = -13332
 correlation coefficient = 0.9994

A.4.2 Calibration Check Data for IN-70941 (Samples Fortified at 0.02 mg/Kg)

Calibration Check	Retention Time (min)	Area Counts	Std. Conc. ($\mu\text{g/mL}$)	Measured Concentration ($\mu\text{g/mL}$)	Percent Difference
1	12.24	67286	0.03	0.0248	-17.3

A.4.3 Initial Calibration Data for IN-70941 (Samples Fortified at 0.2 mg/Kg)

Retention Time (min)	Area Counts	Concentration ($\mu\text{g/mL}$)	Calibration Factor
12.41	100275	0.015	6685000
12.41	216366	0.03	7212200
12.41	1227596	0.20	6137980
12.41	3530194	0.40	8825485

Mean Calibration Factor = 7215166

Standard Deviation = 1159675

Relative Standard Deviation = 16.1

A.4.4 Calibration Check Data for IN-70941 (Samples Fortified at 0.2 mg/Kg)

Calibration Check	Retention Time (min)	Area Counts	Concentration ($\mu\text{g/mL}$)	Calibration Factor	Percent Difference
1	12.33	1327234	0.2	6636170	-8.0

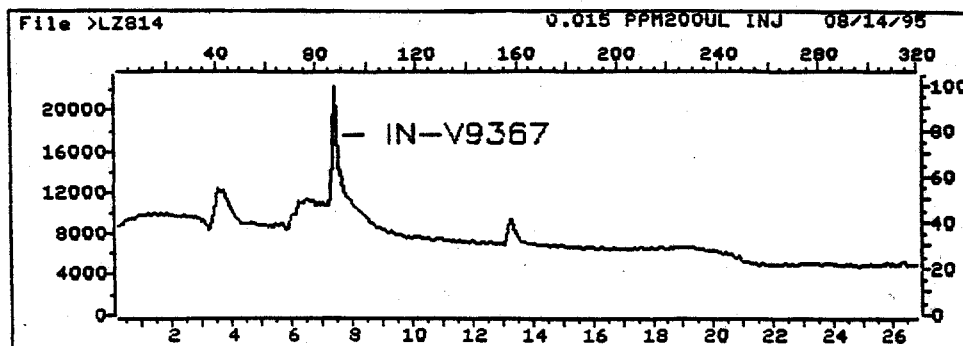
Appendix B - Representative Chromatograms

This section contains representative soil chromatograms of the calibration standards, reagent blank, matrix blank, and spiked samples at each fortification level in the following order:

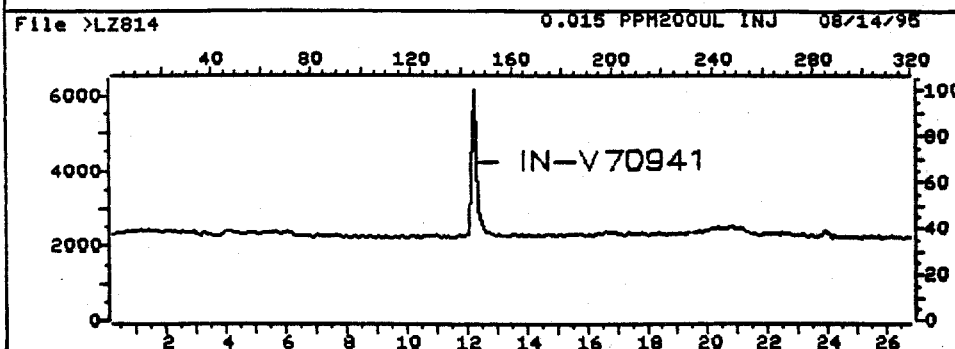
- Calibration Standards (0.015, 0.03, 0.2, and 0.4 $\mu\text{g/mL}$)
- Reagent Blank
- Matrix Blank
- Soil at the MDL (0.0067 mg/Kg)
- Soil at the LOQ (0.02 mg/Kg)
- Soil at 10xLOQ (0.2 mg/Kg)

Calibration Standard
0.015 $\mu\text{g/mL}$
200 μL injection volume

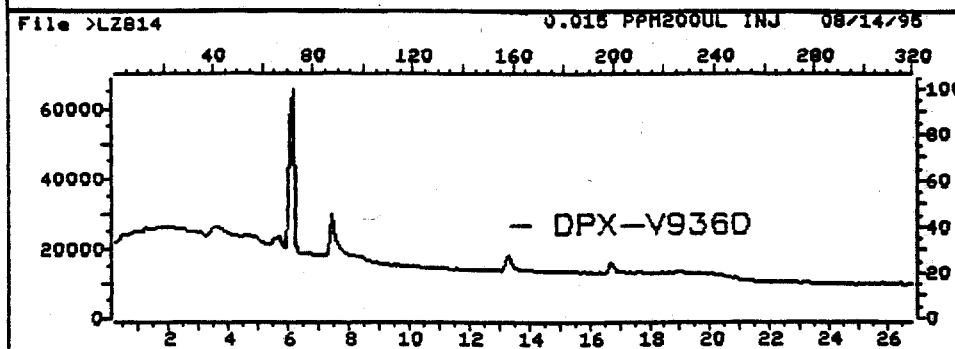
$m/z = 230 + 247$



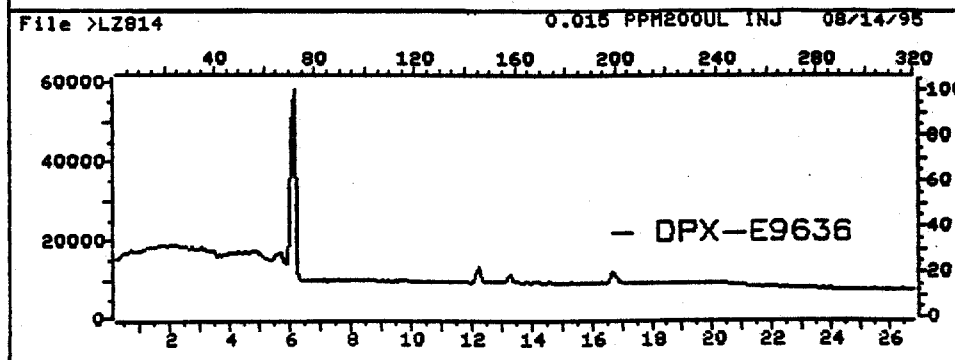
$m/z = 325$



$m/z = 156 + 199 + 230 + 247$

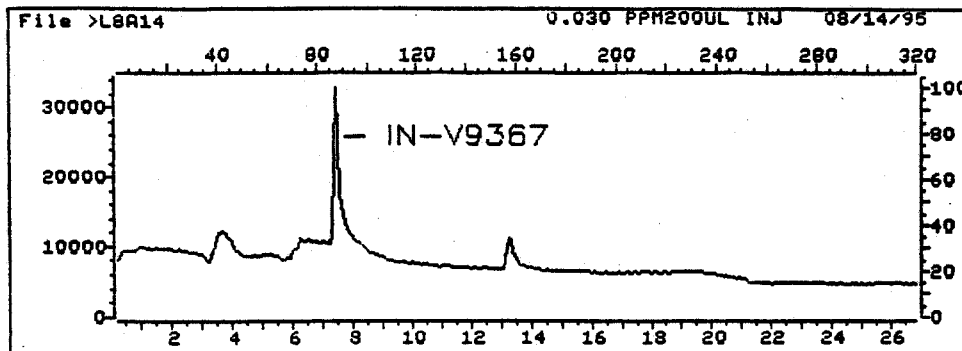


$m/z = 156 + 199 + 325$

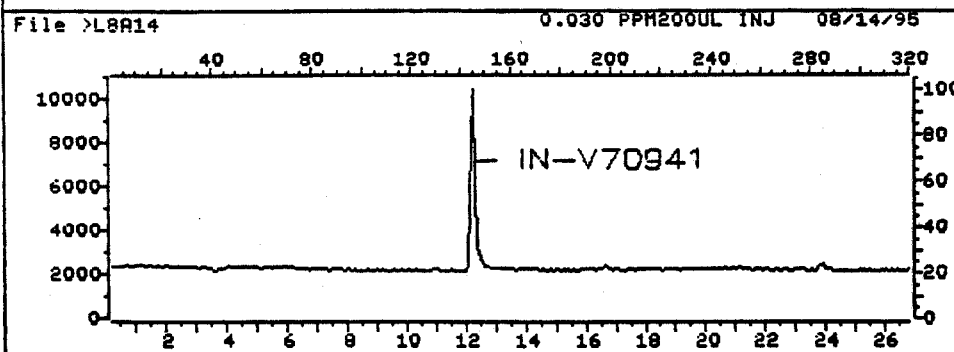


Calibration Standard
0.03 µg/mL
200 µL injection volume

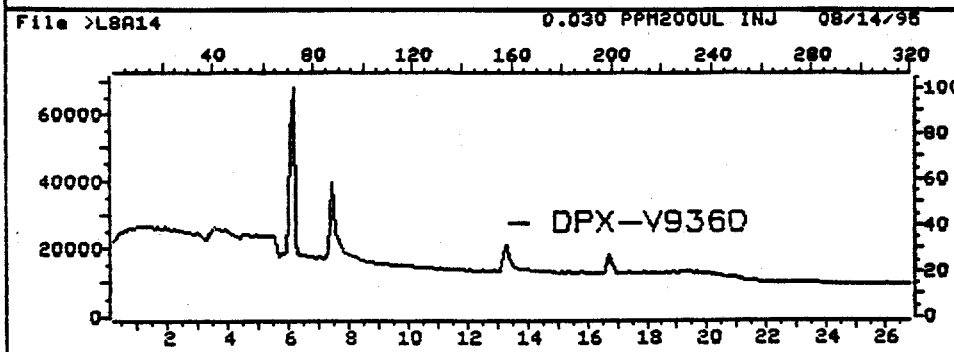
m/z = 230 + 247



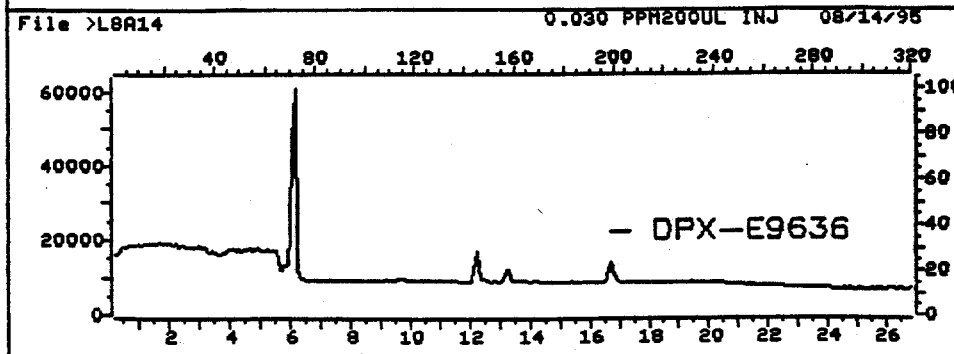
m/z = 325



m/z = 156 + 199 + 230 + 247

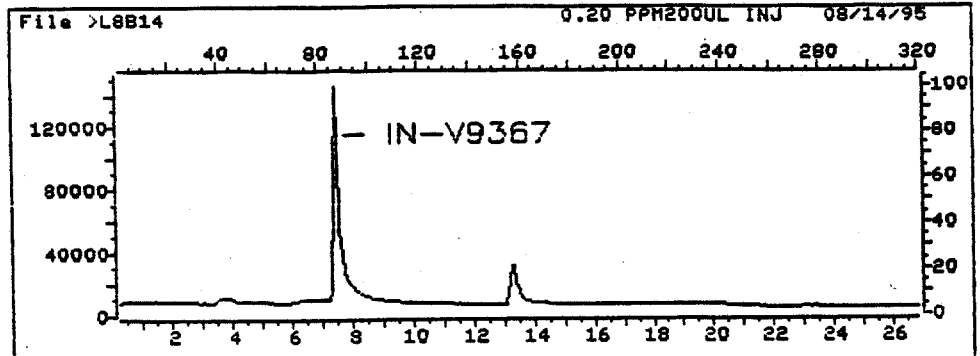


m/z = 156 + 199 + 325

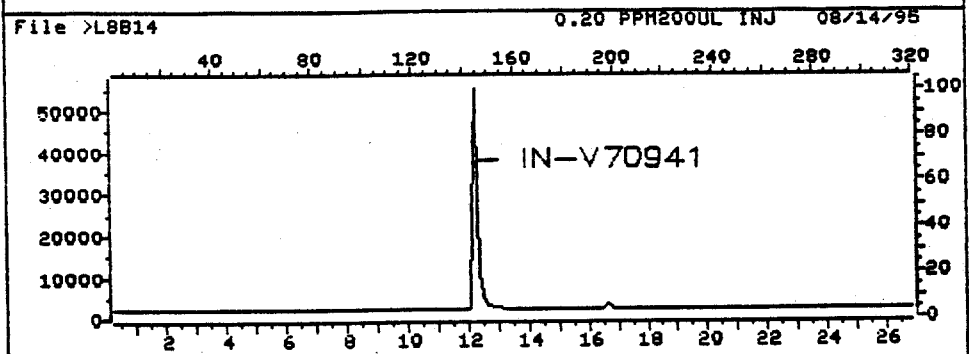


Calibration Standard
0.2 $\mu\text{g/mL}$
200 μL injection volume

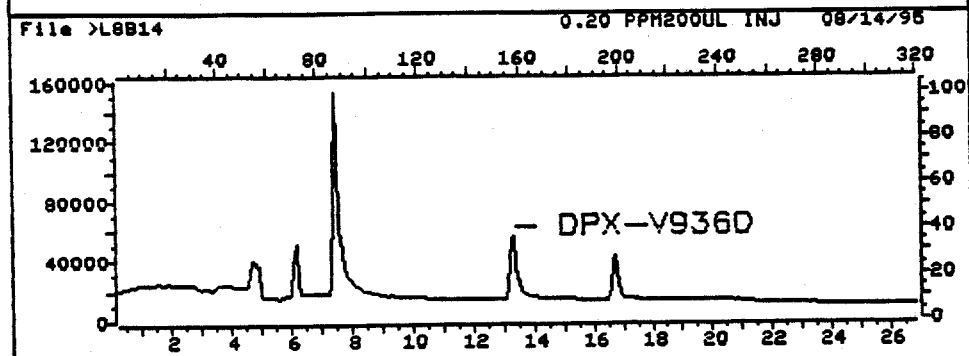
$m/z = 230 + 247$



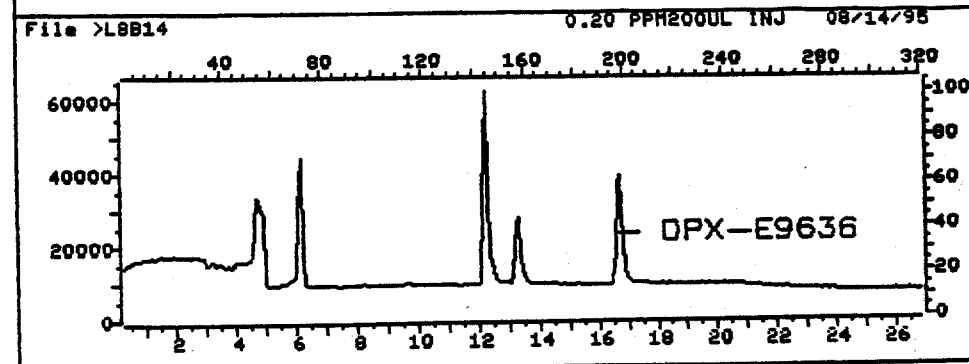
$m/z = 325$



$m/z = 156 + 199 + 230 + 247$

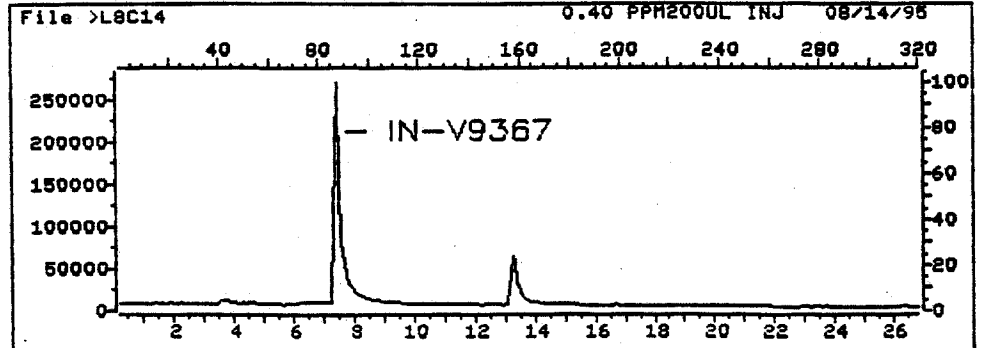


$m/z = 156 + 199 + 325$

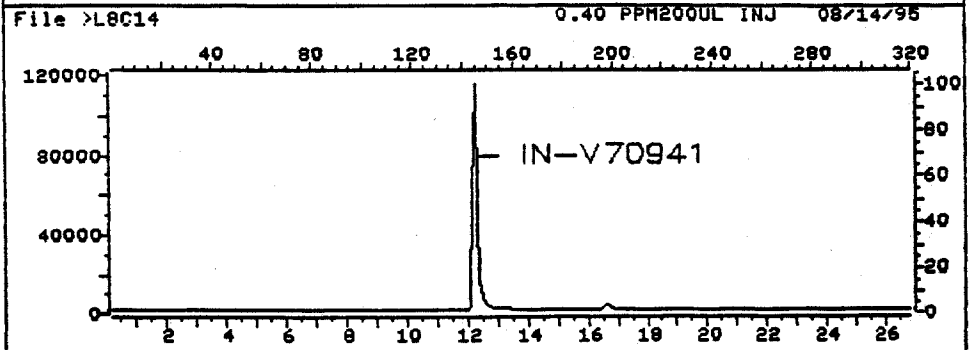


Calibration Standard
0.4 $\mu\text{g/mL}$
200 μL injection volume

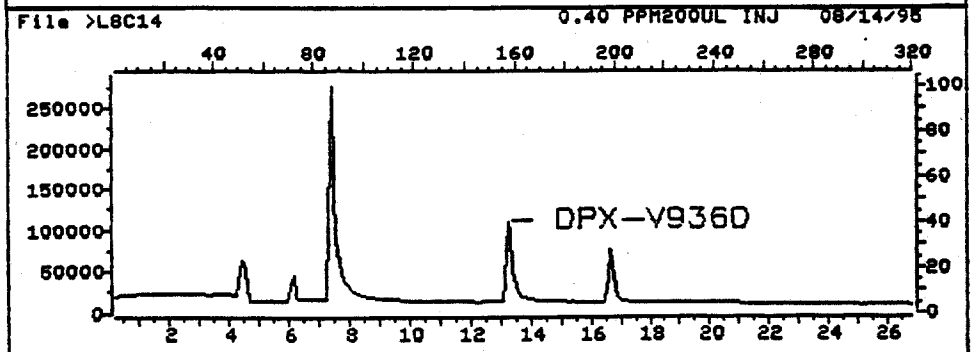
$m/z = 230 + 247$



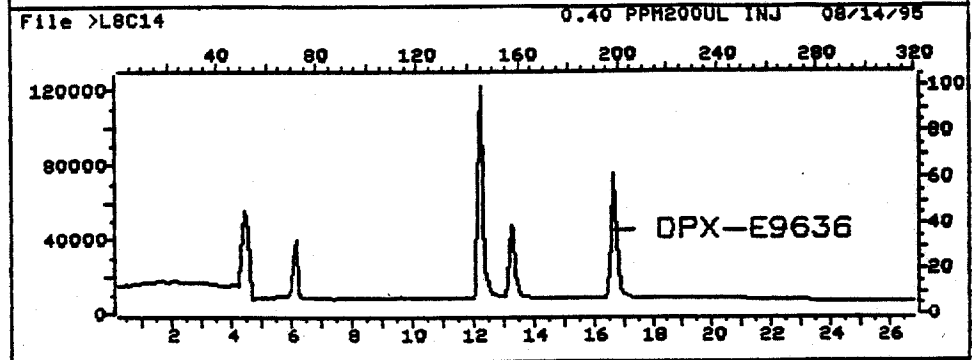
$m/z = 325$



$m/z = 156 + 199 + 230 + 247$

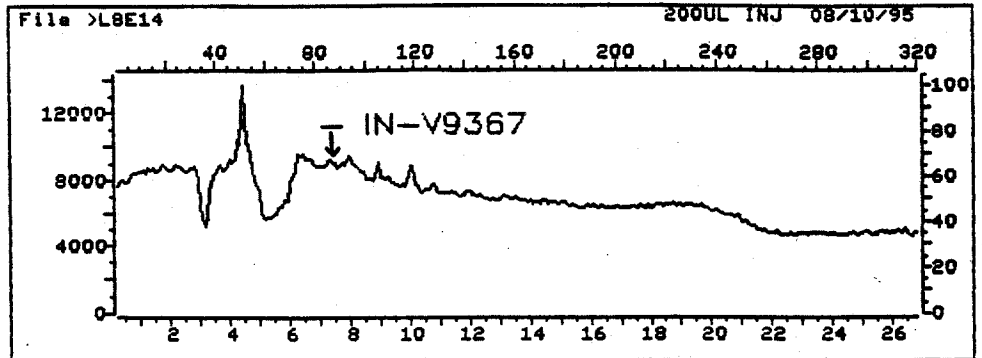


$m/z = 156 + 199 + 325$

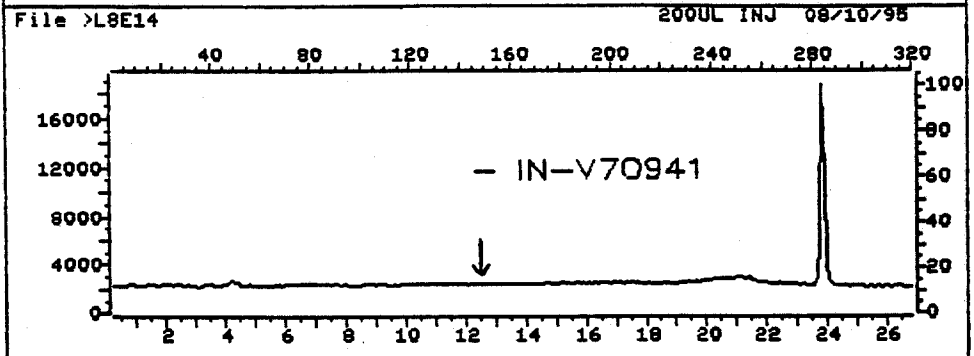


Reagent Blank
1 mL final volume
200 μ L injection volume

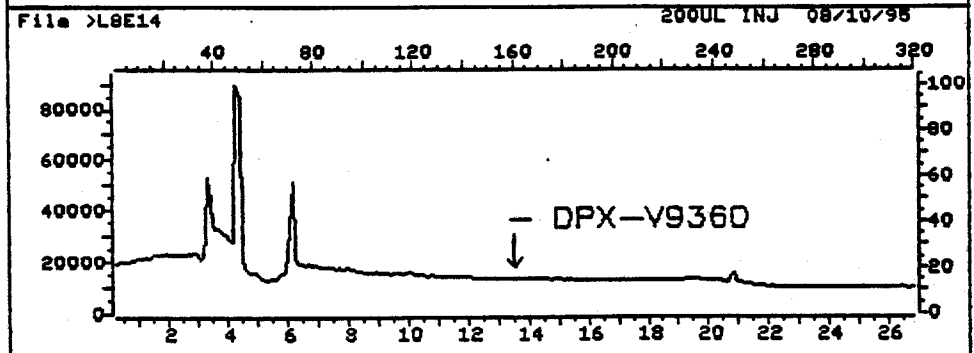
$m/z = 230 + 247$



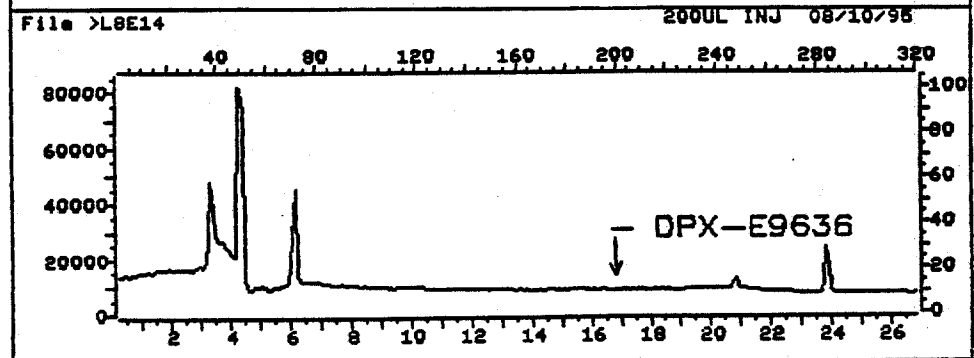
$m/z = 325$



$m/z = 156 + 199 + 230 + 247$

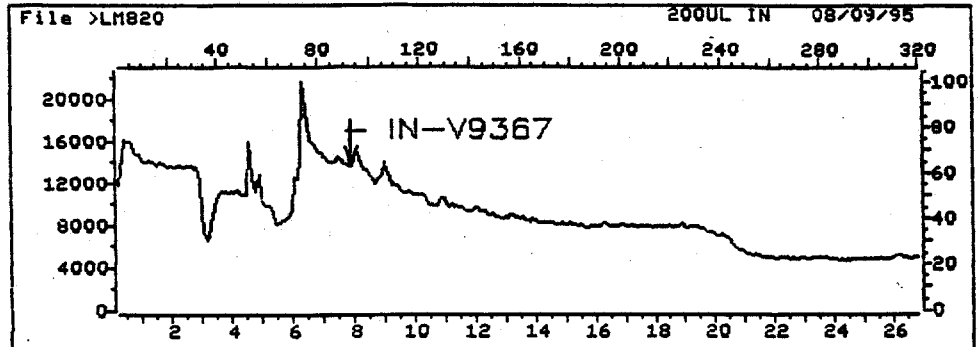


$m/z = 156 + 199 + 325$

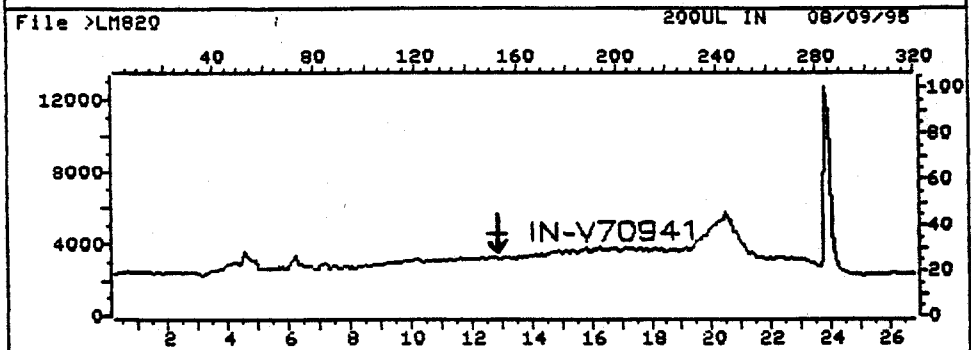


Matrix Blank
1 mL final volume
200 μ L injection volume

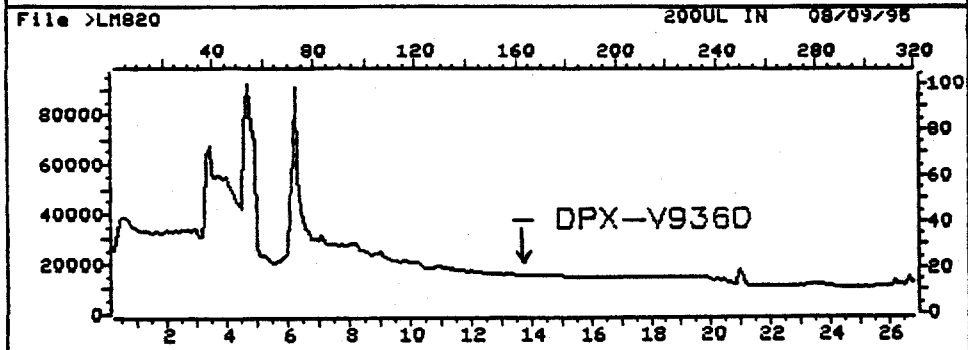
$m/z = 230 + 247$



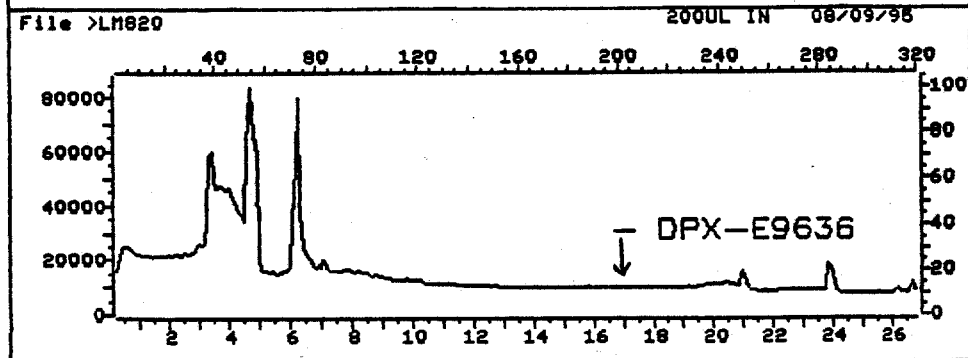
$m/z = 325$



$m/z = 156 + 199 + 230 + 247$

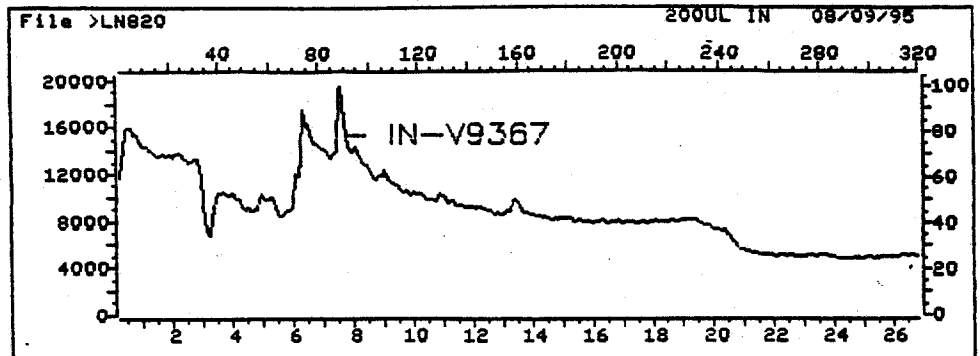


$m/z = 156 + 199 + 325$

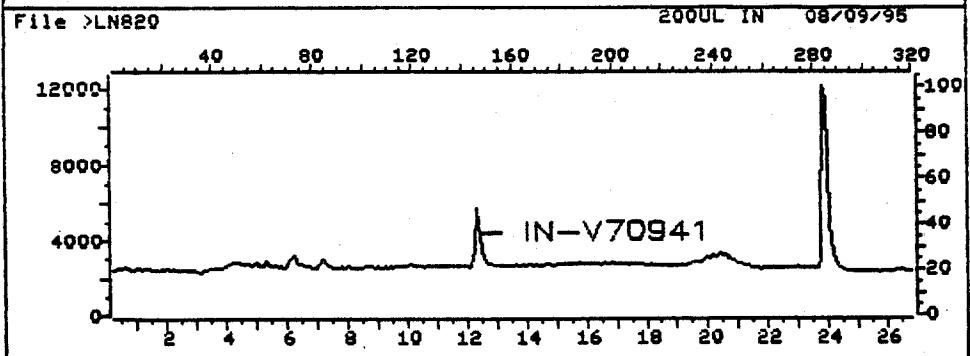


Soil at the MDL
0.0067 mg/Kg
1 mL final volume
200 μ L injection volume

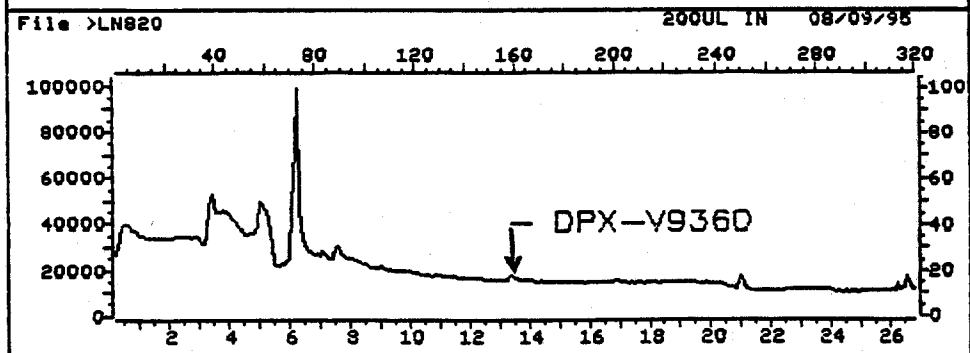
$m/z = 230 + 247$



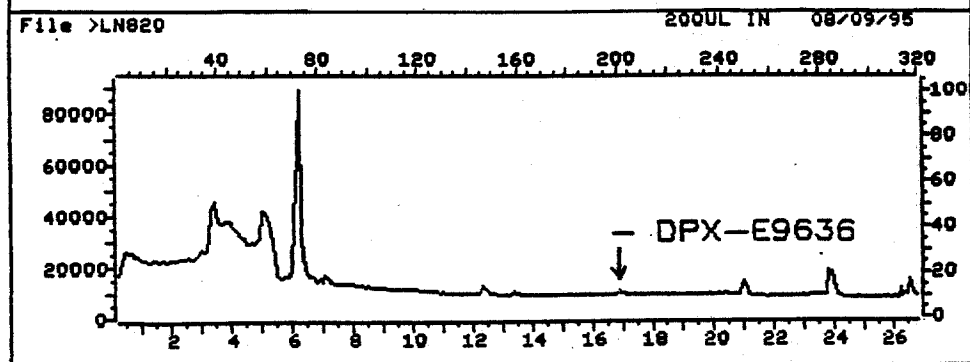
$m/z = 325$



$m/z = 156 + 199 + 230 + 247$

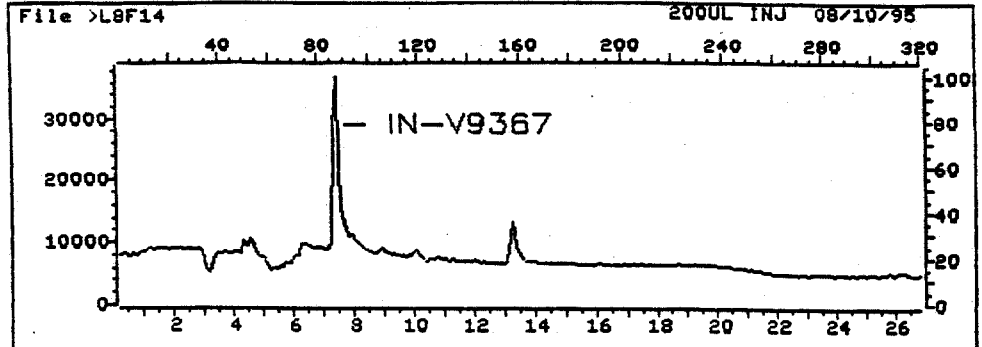


$m/z = 156 + 199 + 325$

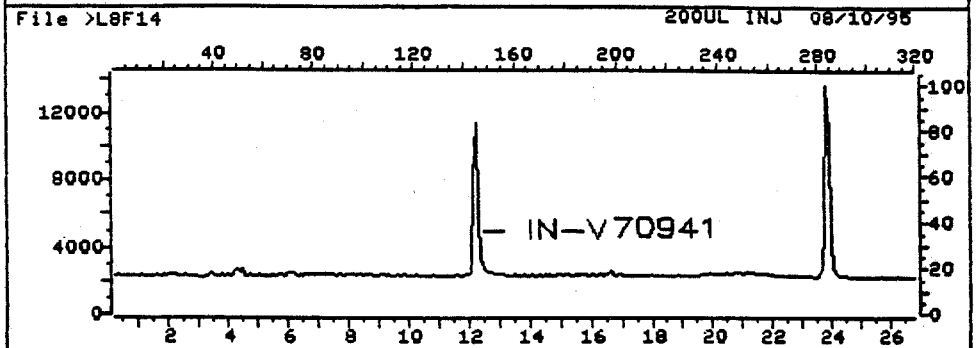


Soil at the LOQ
0.02 mg/Kg
1 mL final volume
200 μ L injection volume

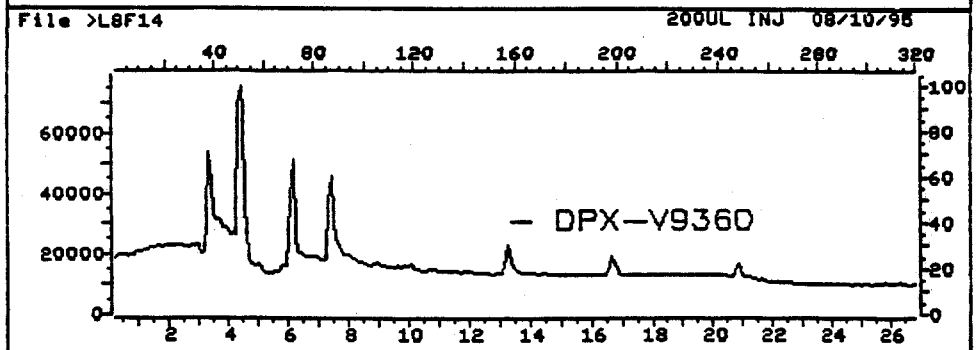
$m/z = 230 + 247$



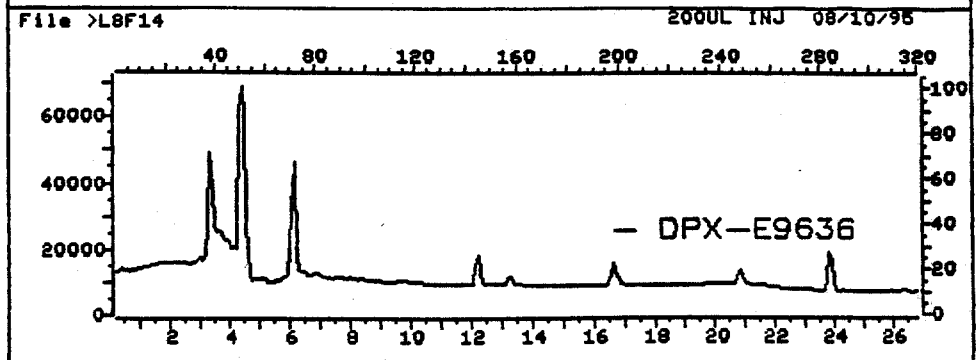
$m/z = 325$



$m/z = 156 + 199 + 230 + 247$

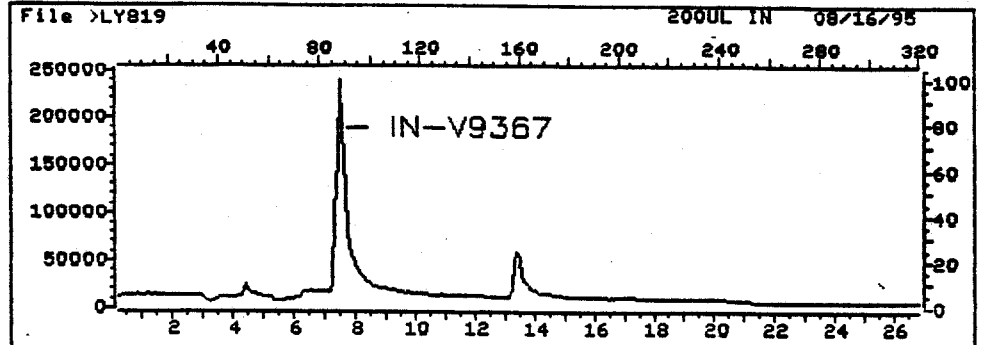


$m/z = 156 + 199 + 325$

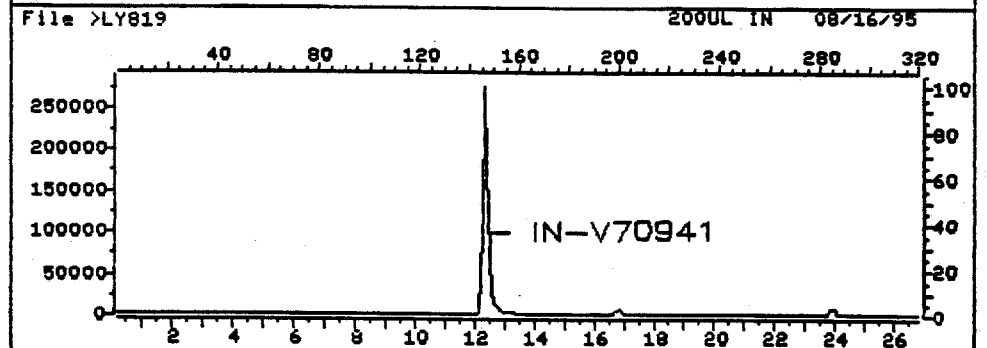


Soil at 10XLOQ
0.2 mg/Kg
1 mL final volume
200 μ L injection volume

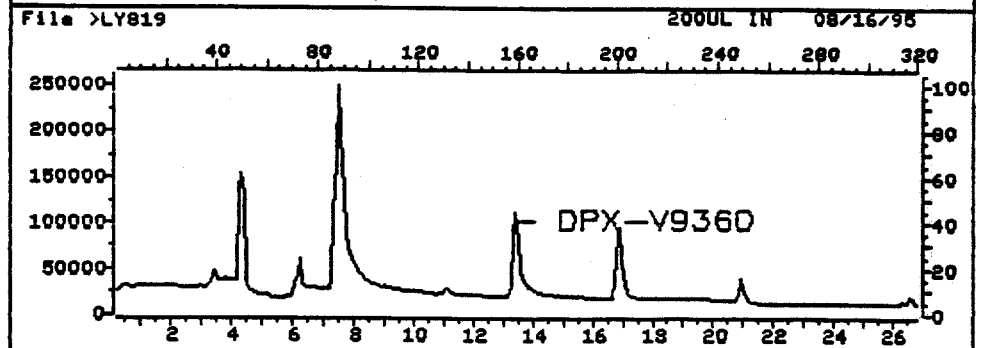
$m/z = 230 + 247$



$m/z = 325$



$m/z = 156 + 199 + 230 + 247$



$m/z = 156 + 199 + 325$

