



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

OFFICE OF  
PESTICIDES AND TOXIC  
SUBSTANCES

January 18, 2000

SUBJECT: Acibenzolar (Actigard) Method Evaluation  
PC Code: 061402  
DP Barcode: None

FROM: Sid Abel, Environmental Scientist *S. Abel 1/18/00*  
Fate and Monitoring Branch  
Environmental Fate and Effects Division (7507C)

THRU: Betsy Behl, Chief *Betsy Behl 1/18/2000*  
Fate and Monitoring Branch  
Environmental Fate and Effects Division (7507C)

TO: Luis Suguiyama, Chief  
Fungicide Branch  
Registration Division (7505C)

The Environmental Fate and Effects Division (EFED) requested and received an evaluation for the determination of residues of acibenzolar (Actigard) in soil using a method submitted by Novartis Crop Protection. Attached is a copy of the method evaluation provided by the Environmental Chemistry Laboratory (ECL).

The EFED concludes, along with the ECL, that this method is suitable for monitoring residues of acibenzolar (Actigard) in soil at or above the suggested analytical limits. The estimated limit of detection (LOD) was determined to be 7 ppb and the estimated limit of quantitation (LOQ) to be 20 ppb. The following problems were noted during method review and lab evaluation. Steps should be taken to correct and address these problems in future submissions using this method:

The chromatograms which were supplied at the LOQ and 10 times the LOQ were poorly presented with no retention times supplied.

During the extraction step, a silica SPE cartridge stopped



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flowing during final elution, which resulted in loss of those extracts. The cause may have been due to moisture on the column, but the lab was not certain. Leaving the packed cartridges in a dessicator for a few days prior to using them appeared to solve the problem.

If you have any questions concerning this matter, please feel free to contact Sid Abel.

Attachment



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Actigard (CGA 245704) Method Evaluation -  
Report No. ECM0147S1

FROM: *me* Aubry E. Dupuy, Jr., Branch Chief *Douglas W. Zeller*  
BEAD/Environmental Chemistry Laboratory

THRU: Donald A. Marlow, Acting Associate Director  
Biological and Economic Analysis Division (7503C)

THRU: Elizabeth Leovey, Acting Associate Director  
Environmental Effects and Fate Division (7507C)

TO: Betsy Behl, Branch Chief  
EFED/Fate and Monitoring Branch

The EFED/Environmental Fate and Effects Division has requested an Environmental Chemistry Method Evaluation (ECME) on Actigard (CGA 245704) in soil using the Novartis Crop Protection method, "Determination of Residues of Parent Compound by High Performance Liquid Chromatography (HPLC)"

The attached method evaluation report includes three parts:

Part I: Summary and Conclusions

In this section any problems encountered with the method and how they were handled are discussed. ECL's opinion of how well the method performed is also presented.

Part II: Analytical Results

In this section the individual results of each sample at each spiking level for each matrix are listed. The relative standard deviation (RSD) for each spiking level is also presented here.

### Part III: Experimental Details

In this section any modification(s) that were made to the method, instrument parameters, spiking levels, explanation of instrument calibration, representative sample and standard chromatograms and standard curves are listed and/or discussed.

If you have any questions concerning this report, please contact Elizabeth Flynt at (228) 688-2410 or me at (228) 688-3212.

#### Attachments

cc: Dr. Christian Byrne, QA Officer  
BEAD/Environmental Chemistry Laboratory

Elizabeth C. Flynt  
BEAD/ECL

Environmental Chemistry Method Evaluation Report No. ECM0147S1

Actigard Determination in Soil

October 13, 1999

Environmental Chemistry Laboratory  
Biological and Economic Analysis Division

Prepared by: Elizabeth C. Flynt, Chemist Elizabeth Flynt 10/13/99  
Signature Date

Reviewed by: Dr. Christian Byrne, QAO Christian Byrne 10/14/99  
Signature Date

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Part I

Summary and Conclusions

We have completed a laboratory evaluation of the Environmental Chemistry Method (MRID No.445370-44) for the detection of Actigard (CGA 245704) in soil. The method was submitted by Novartis Crop Protection, Inc. in accordance with the registration of Actigard (CGA 245704).

We feel that this method, "Determination of Parent Compound by High Performance Liquid Chromatography (HPLC)" can be used to monitor soil for the presence of Actigard (CGA 245704). ||

We estimated ECL's method detection limit to be 0.007 ppm (7 ppb) for Actigard, with an LOQ of 0.02 ppm, which is the same as the "Lower Practical Level" reported by the registrant.

The precision and accuracy results of the controls fortified at the LOQ with Actigard (CGA 245704) were acceptable, with a mean recovery of 81.0% (i.e. > 70% recovery and < 120% recovery) and an RSD of 13.0 (≤20). The mean recovery at the 10 X LOQ level was also within the target range at 71.7% with an RSD of 7.68.

Problems discovered during the method review of the ECM associated with the registration studies for Actigard (CGA 245704) in soil.

- The chromatograms which were supplied at the LOQ and 10 X LOQ were poorly presented with no retention times supplied. It appears that there is a peak in the matrix blank that is close to the retention time of CGA 245704 but it's difficult to tell due to the lack of a time scale on the chromatogram.

Problems discovered during the lab evaluation of the ECM associated with the registration studies for Actigard in soil.

There were no major problems discovered with this ECM during lab evaluation. There was a minor problem with the SPE cartridges. Twice during the extraction, a silica SPE cartridge stopped flowing during the final elution step, which resulted in the loss of those extracts. [We are not certain of the cause, but found that leaving the packed cartridges in a dessicator for a few days prior to using them appeared to solve the problem.] ||

# ACTIGARD ANALYTICAL RESULTS

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Method: Determination of Actigard (CGA-245704) in Soil

## Fortification Conc. 0.007 ppm = MDL

Sample	Found ppm
Matrix Blank	ND
Spike A	0.0040
Spike B	0.0040
Spike C	0.0020
Spike D	ND

## Fortification Conc. 0.020 ppm = LOQ

Sample	Found ppm	% Recovery
Matrix Blank	ND	ND
Spike A	0.0160	80.0
Spike B	0.0139	69.5
Spike C	0.0159	79.5
Spike D	0.0190	95.0
	<b>Mean Recovery</b>	<b>81.0</b>
	<b>SD</b>	<b>10.51</b>
	<b>RSD</b>	<b>12.98</b>

## Fortification Conc. 0.2 ppm =10 x LOQ

Sample	Found ppm	% Recovery
Matrix Blank	ND	ND
Spike A	0.156	78.0
Spike B	0.136	68.0
Spike C	0.138	69.0
Spike D	(LOST DURING EXTRACTION)	
	<b>Mean Recovery</b>	<b>71.7</b>
	<b>SD</b>	<b>5.51</b>
	<b>RSD</b>	<b>7.68</b>



Part III

Experimental Details

General description of method:

CGA 245704 is extracted by shaking a homogenized 10 g soil subsample with 20 ml of water and 80 ml of acetonitrile and then filtering the mixture through filter paper. A 20 ml aliquot of the 100 ml extract is cleaned up by passage through a C<sub>18</sub> solid phase extraction (SPE) cartridge and elution with a solution of acetonitrile and water (60:40). The eluate containing the CGA 245704 is diluted with a saturated sodium chloride:water solution and then partitioned into n-hexane. This extract is then cleaned-up by passage through a silica gel SPE cartridge, eluted with 10 ml of n-hexane. The concentration of CGA 245704 is determined by HPLC with UV-detection.

Source of analytical reference standards:

CGA 245704, Lot# 595-1833, purity 98.6%, expiration date 3/2000

Novartis Crop Protection  
410 Swing Road  
Greensboro, NC 27409

Source of sample matrices:

The soil sample was received from the University of Iowa on 05/07/97. The soil characterization is in Appendix A of this document.

Instrumentation for quantitation (listed only if different from that listed in method)

HPLC: Waters™ 2690 Separation Module  
HPLC Detector: Waters™ 490E Multi Wavelength UV Detector  
HPLC Data-Capture System: Waters™ Millennium<sup>32</sup> v3.05.01  
Software System

Instrument for confirmation: HPLC/UV

Notes on Analytical Procedure:

*Extraction* - This method was straightforward and short. The extraction could be done in approximately 6 hours followed by an overnight analysis with an automatic injector.

[The only difficulty we experienced was a minor one with the silica SPE cartridges. Twice during the extractions, a silica SPE cartridge stopped flowing during the final elution step. We are not certain of the cause but suspect it may be related to moisture on the column and found that leaving the packed cartridges in a dessicator for a few days prior to using them appeared to solve the problem.]

Instrument Calibration (listed only if different from that listed in method)

The registrant used a 25 ul injection, the Independent Lab used a 100 ul, and we used a 75 ul injection. These adjustments compensated for differences in instrument sensitivities among laboratories. ( )

**Actigard Calibration**

Calibration standards were prepared at 0.003 ug/ml, 0.01 ug/ml, 0.1 ug/ml and 0.15 ug/ml, which is equivalent to 0.006 ppm through 0.3 ppm of Actigard in soil and analyzed along with each sample set. The Waters Millenium software calculated the linear regression curve formula and automatically calculated the sample concentrations in ug/ml based on that curve.

To convert the concentration of the extract in ug/ml to ppm in the soil sample the following formula was used:

$$\frac{\text{Actigard in Sample Ext (ug/ml)} \times \text{Final Volume (ml)} \times \text{Extract Volume (ml)}}{\text{Weight of Sample (g)} \times \text{Extraction Aliquot Used (ml)}} = \text{ppm}$$

To calculate the percent recovery of the sample, the following formula was used:

$$\frac{\text{ppm of Actigard (actual) in sample} \times 100}{\text{ppm of Actigard (fortified)}} = \text{Percent Recovery in sample}$$

Example for Actigard - (Spike A @0.2 ppm)

The concentration in the extract from the soil fortified at 0.2 ppm Actigard was 0.078 ug/ml as determined by the Millenium software as described above.

$$\frac{0.078 \text{ ug/ml} \times 4 \text{ ml} \times 100 \text{ ml}}{10 \text{ g} \times 20 \text{ ml}} = 0.156 \text{ ug/g} = 0.156 \text{ ppm}$$

To solve for percent recovery,

$$\frac{0.156 \text{ ppm} \times 100}{0.2 \text{ ppm}} = 78\% \text{ Recovery}$$

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**Chromatograms and Calibration Curves:**

- A. HPLC Calibration Curves
  - A-1. Actigard (CGA 245704) Calibration Curve
- B. HPLC Calibration Standard Chromatograms for Actigard
  - B-1. 75  $\mu$ l @ 0.003 ug/ml (equiv. to 0.006 ppm)
  - B-2. 75  $\mu$ l @ 0.01 ug/ml (equiv. to 0.02 ppm)
  - B-3. 75  $\mu$ l @ 0.1 ug/ml (equiv. to 0.2 ppm)
  - B-4. 75  $\mu$ l @ 0.15 ug/ml (equiv. to 0.3 ppm)
- C. HPLC Chromatograms at the MDL (0.007 ppm) Fortification Level
  - C-1. Matrix Blank for 0.007 ppm level
  - C-2. Soil Fortified at 0.007 ppm level
- D. HPLC Chromatograms at the LOQ (0.02 ppm) Fortification Level
  - D-1. Matrix Blank for 0.02 ppm level
  - D-2. Soil Fortified at 0.02 ppm level
- E. HPLC Chromatograms at 0.2 ppm (10 x LOQ) Fortification Level
  - E-1. Matrix Blank for 0.2 ppm level
  - E-2. Soil Fortified at 0.2 ppm level

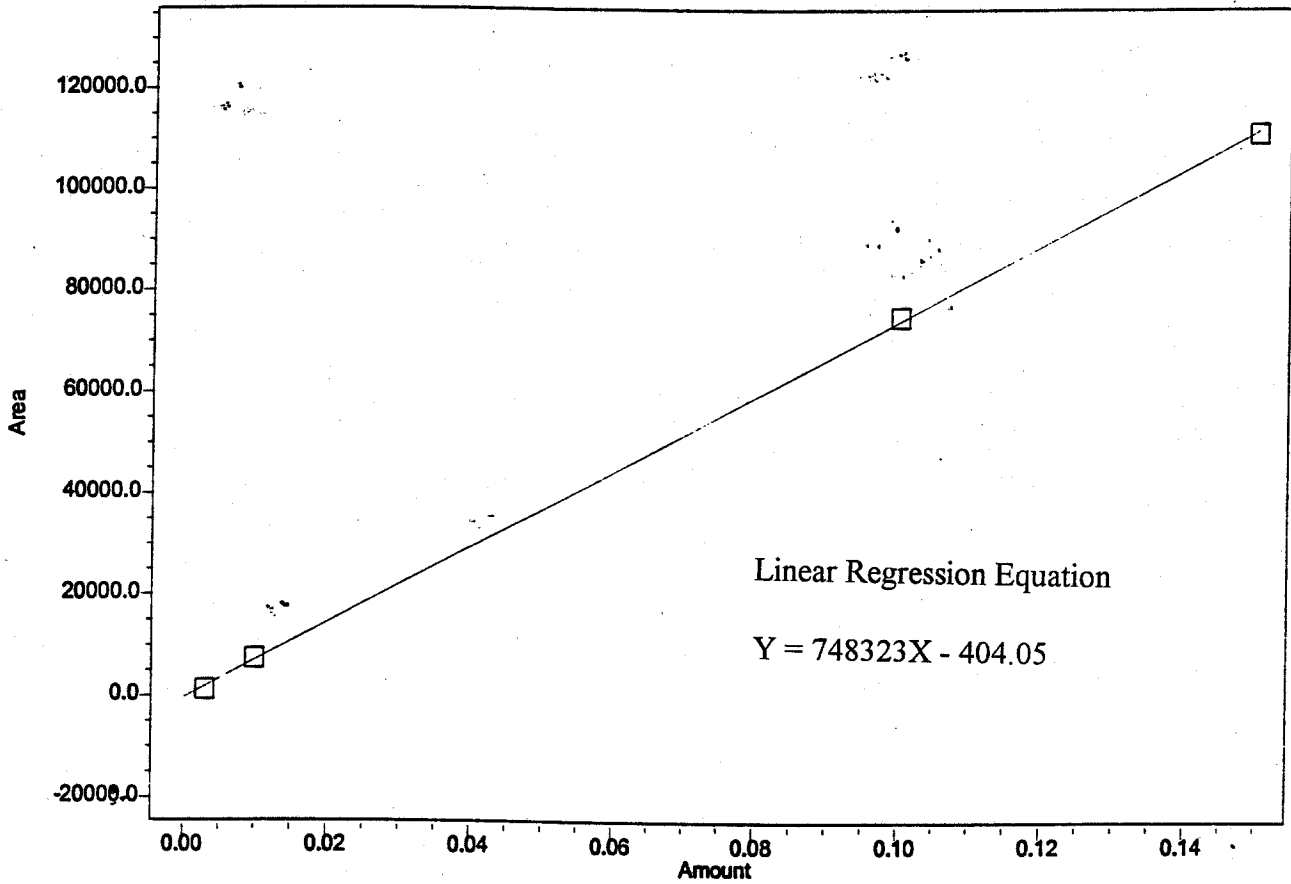
A-1. Actigard (CGA 245704) Calibration Curve

Name Actigard  
System 2690 490E  
Channel 490 Ch1  
Fit Type Linear

A -4.040511e+002  
B 7.483229e+005  
C 0.000000e+000  
D 0.000000e+000  
R<sup>2</sup> 0.999854

Calibration Id 1334  
Date Calibrated 09/13/1999 9:33:21 A  
Time 5.408  
Processing Method Actigard\_Proc

Calibration Plot



Peak Actigard

	Name	Level	X Value	Response	Calc. Value	% Deviation	Manual	Ignore
1	Actigard		0.003000	1255.840000	0.002218	-26.062	No	No
2	Actigard		0.010000	7432.880952	0.010473	4.727	No	No
3	Actigard		0.100000	75158.000000	0.100975	0.975	No	No
4	Actigard		0.150000	111346.000000	0.149334	-0.444	No	No

## B. HPLC Calibration Standard Chromatograms for Actigard

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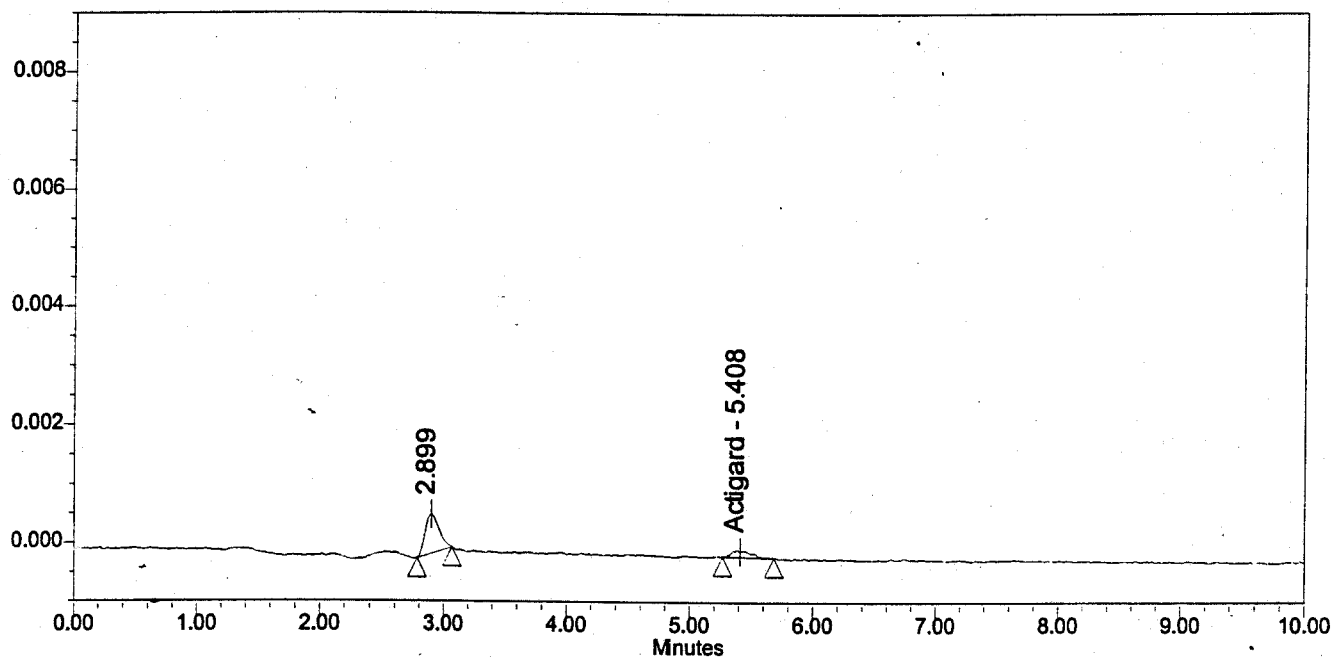
**B-1. 75 ul @ 0.003 ug/ml (equiv. to .006 ppm)**

### Sample Information

SampleName Actigard Std. 0.003 ug/m  
Vial 2  
Injection 1  
Injection Volume 75.00 ul  
Channel 490 Ch1  
Run Time 10.0 Minutes

Sample Type Standard  
Date Acquired 09/09/1999 3:44:31 PM  
Acq Method Set Actigard\_Met\_Set  
Processing Method Actigard\_Proc  
Date Processed 09/13/1999 9:29:25 AM

### Auto-Scaled Chromatogram



### Peak Results

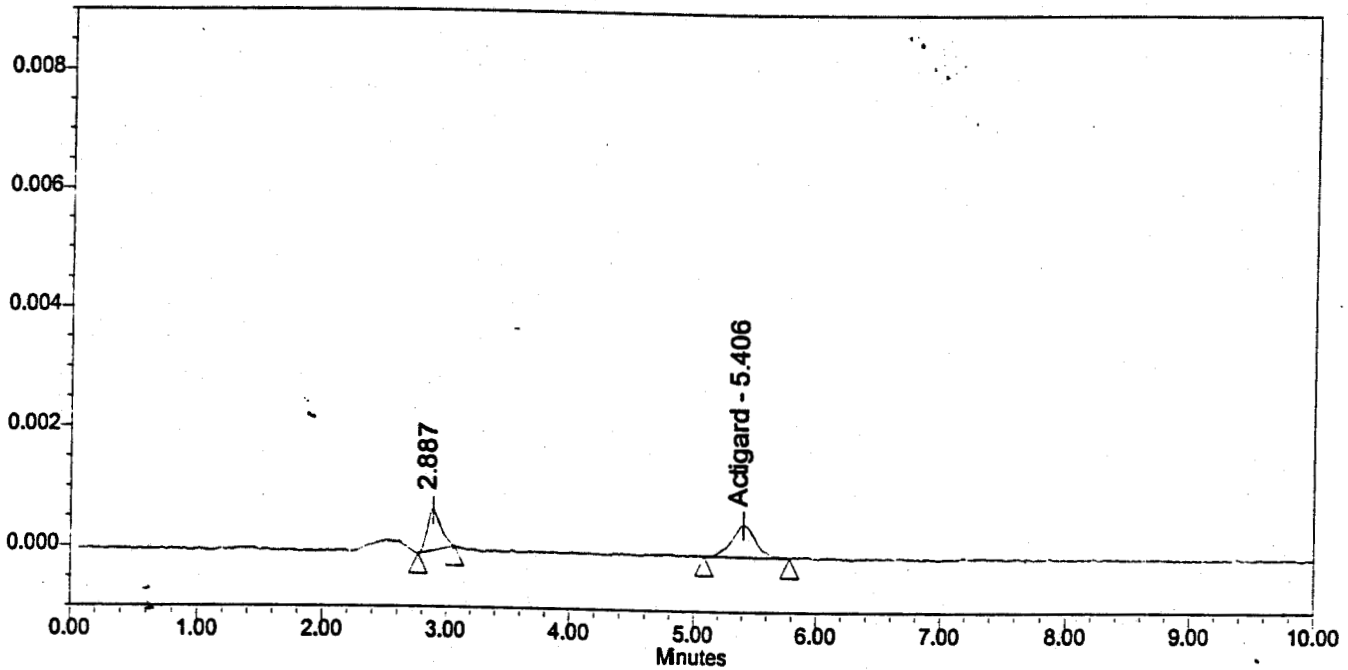
Name	RT	Area	Height	Amount	Units
1	2.899	5285	671		
2 Actigard	5.408	1256	104	0.003	ug/ml

**Sample Information**

SampleName Actigard Std. 0.01 ug/ml  
Vial 3  
Injection 1  
Injection Volume 75.00 ul  
Channel 490 Ch1  
Run Time 10.0 Minutes

Sample Type Standard  
Date Acquired 09/09/1999 3:55:43 PM  
Acq Method Set Actigard\_Met\_Set  
Processing Method Actigard\_Proc  
Date Processed 09/13/1999 9:32:02 AM

**Auto-Scaled Chromatogram**



**Peak Results**

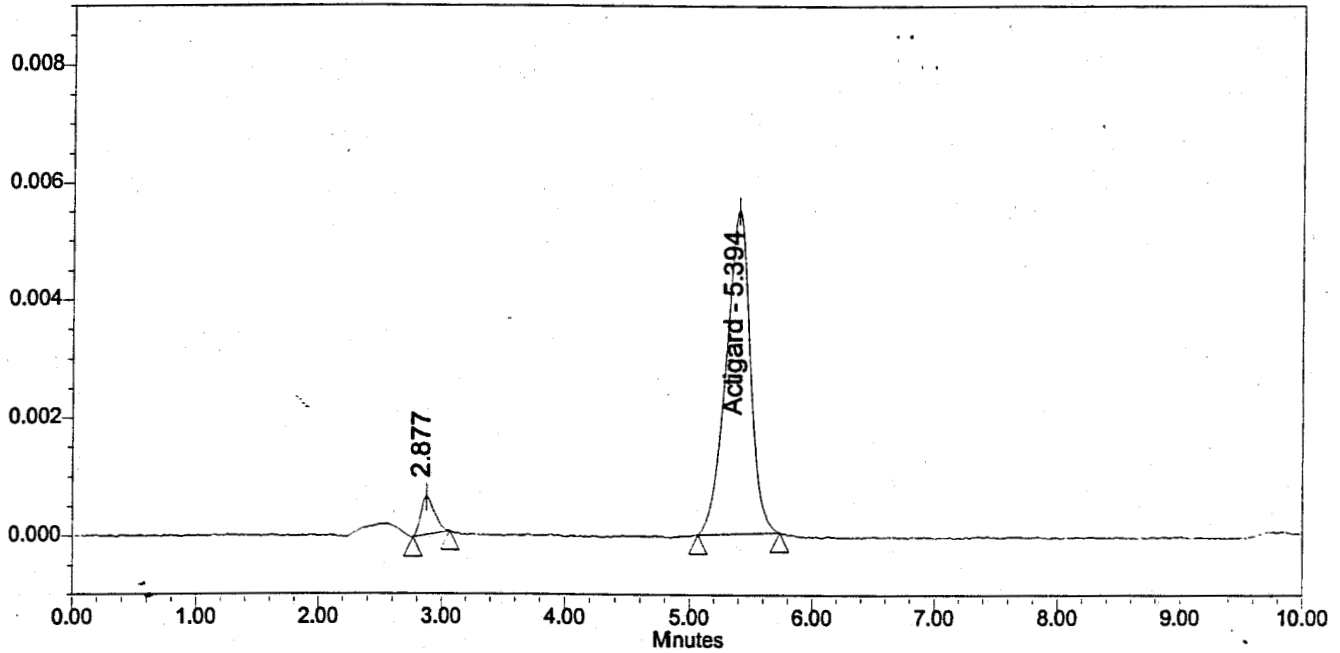
Peak #	Name	RT	Area	Height	Amount	Units
1		2.887	5265	686		
2	Actigard	5.406	7433	531	0.010	ug/ml

**Sample Information**

SampleName Actigard Std. 0.1 ug/ml  
Vial 4  
Injection 1  
Injection Volume 75.00 ul  
Channel 490 Ch1  
Run Time 10.0 Minutes

Sample Type Standard  
Date Acquired 09/09/1999 4:06:55 PM  
Acq Method Set Actigard\_Met\_Set  
Processing Method Actigard\_Proc  
Date Processed 09/13/1999 9:33:02 AM

**Auto-Scaled Chromatogram**



**Peak Results**

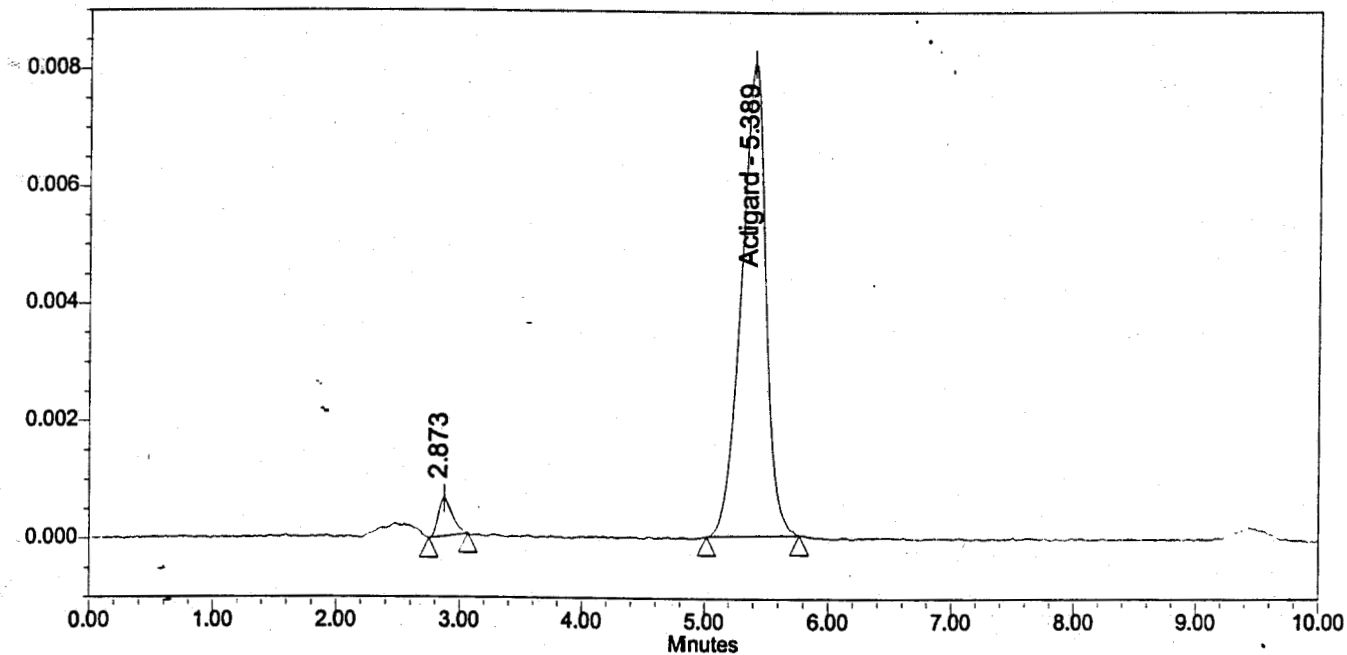
PK	Name	RT	Area	Height	Amount	Units
1		2.877	4992	654		
2	Actigard	5.394	75158	5504	0.100	ug/ml

**Sample Information**

SampleName Actigard Std. 0.15 ug/ml  
 Vial 5  
 Injection 1  
 Injection Volume 75.00 ul  
 Channel 490 Ch1  
 Run Time 10.0 Minutes

Sample Type Standard  
 Date Acquired 09/09/1999 4:18:08 PM  
 Acq Method Set Actigard\_Met\_Set  
 Processing Method Actigard\_Proc  
 Date Processed 09/13/1999 9:33:21 AM

**Auto-Scaled Chromatogram**



**Peak Results**

PK	Name	RT	Area	Height	Amount	Units
1		2.873	5110	654		
2	Actigard	5.389	111346	8081	0.150	ug/ml



# C-1. HPLC Chromatograms at the MDL (0.003 ppm) Fortification Level

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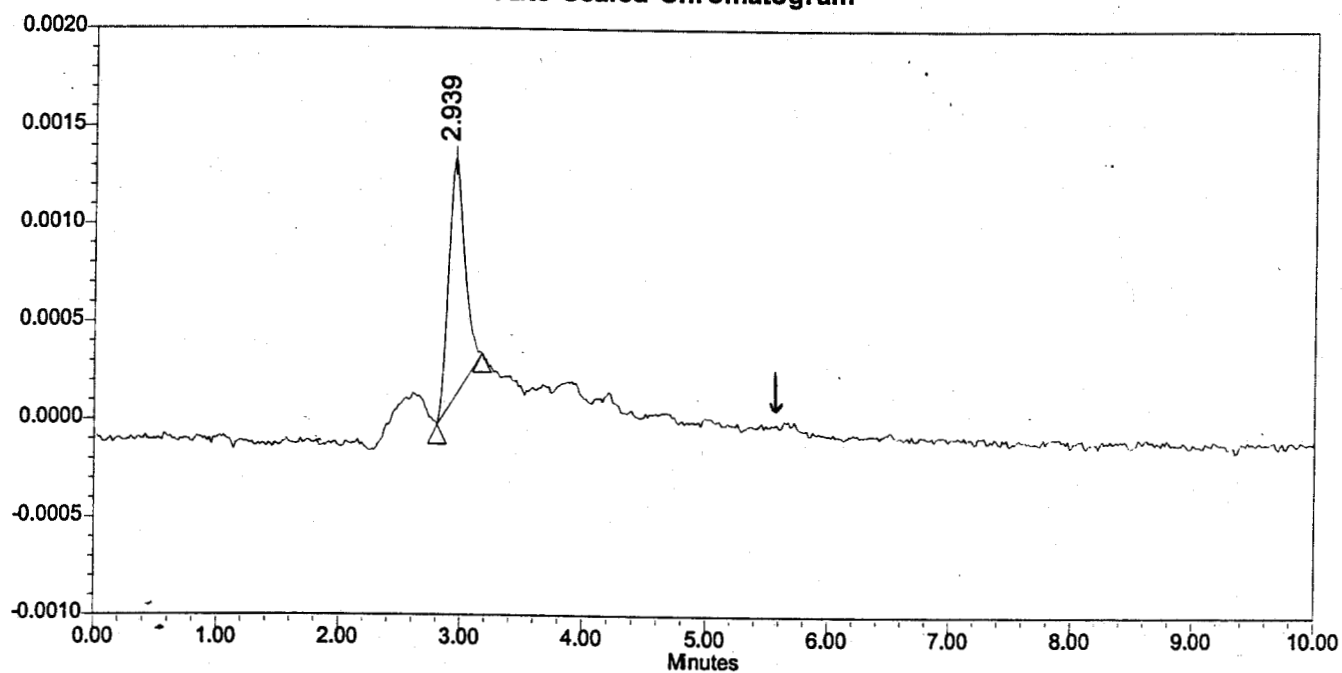
## C-1. Matrix Blank for 0.003 ppm level

### Sample Information

SampleName Matrix Blk. Set # 7  
Vial 7  
Injection 1  
Injection Volume 75.00 ul  
Channel 490 Ch1  
Run Time 10.0 Minutes

Sample Type Unknown  
Date Acquired 09/21/1999 8:52:33 AM  
Acq Method Set Actigard\_Met\_Set  
Processing Method Actigard\_Proc  
Date Processed 09/21/1999 10:53:56 AM

### Auto-Scaled Chromatogram



### Peak Results

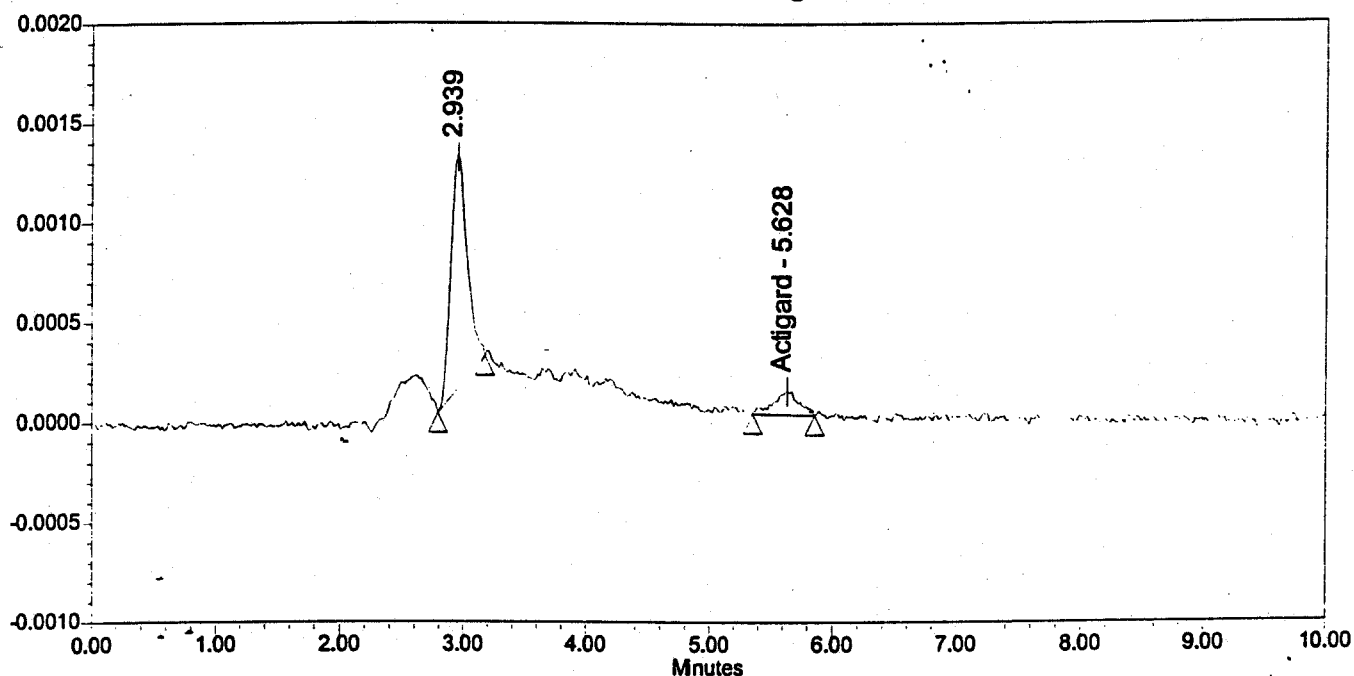
Peak #	Name	RT	Area	Height	Amount	Units
1		2.939	11026	1229		
2	Actigard	5.700				

### C-2. Soil Fortified at 0.003 ppm level

### Sample Information

SampleName	Spike A Set #7	Sample Type	Unknown
Vial	9	Date Acquired	09/21/1999 9:15:00 AM
Injection	1	Acq Method Set	Actigard_Met_Set
Injection Volume	75.00 ul	Processing Method	Actigard_Proc
Channel	490 Ch1	Date Processed	09/21/1999 10:55:21 AM
Run Time	10.0 Minutes		

Auto-Scaled Chromatogram



Peak Results

39	Name	RT	Area	Height	Amount	Units
1		2.939	10892	1185		
2	Actigard	5.628	1747	117	0.002	ug/ml

# D. HPLC Chromatograms at the LOQ (0.02 ppm) Fortification Level

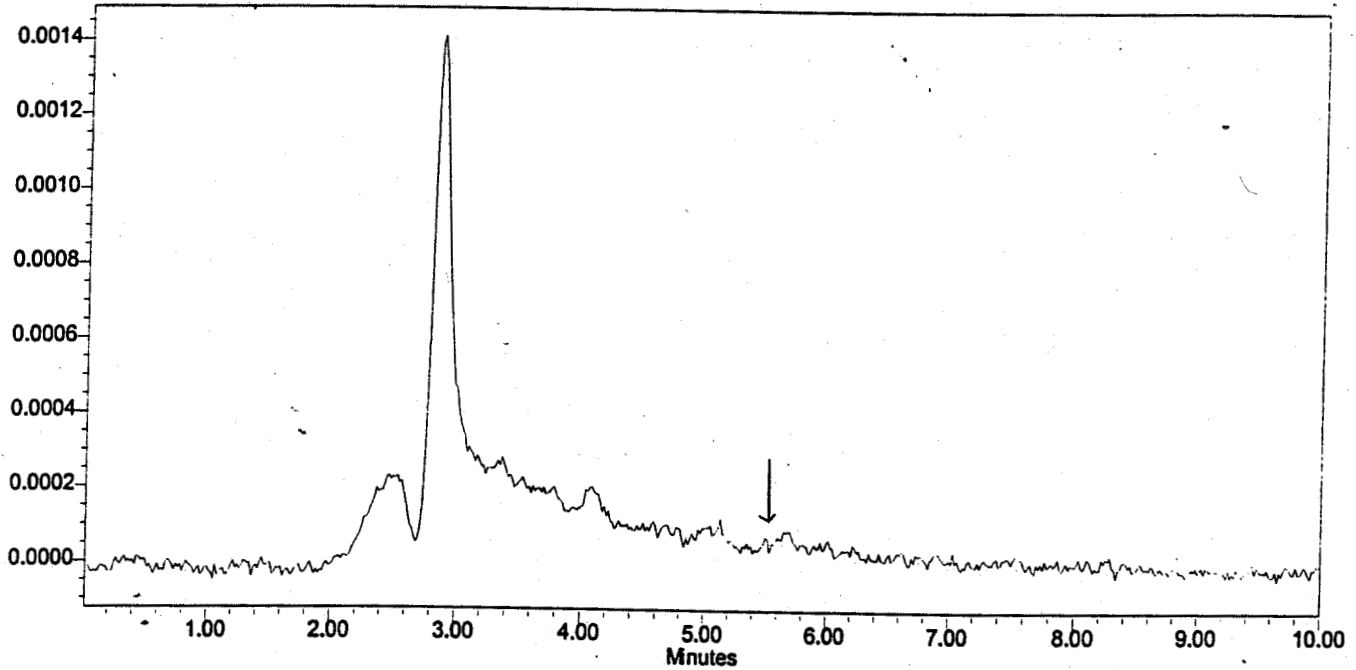
## D-1. Matrix Blank for 0.02 ppm level

### Sample Information

SampleName Matrix Blank  
Vial 7  
Injection 1  
Injection Volume 75.00 ul  
Channel 490 Ch1  
Run Time 10.0 Minutes

Sample Type Unknown  
Date Acquired 08/17/1999 6:01:28 PM  
Acq Method Set acti\_set  
Processing Method ActiLOQ2  
Date Processed 08/20/1999 1:51:59 PM

### Auto-Scaled Chromatogram



### Peak Results

66	Name	RT	Area	Height	Amount	Units
1						

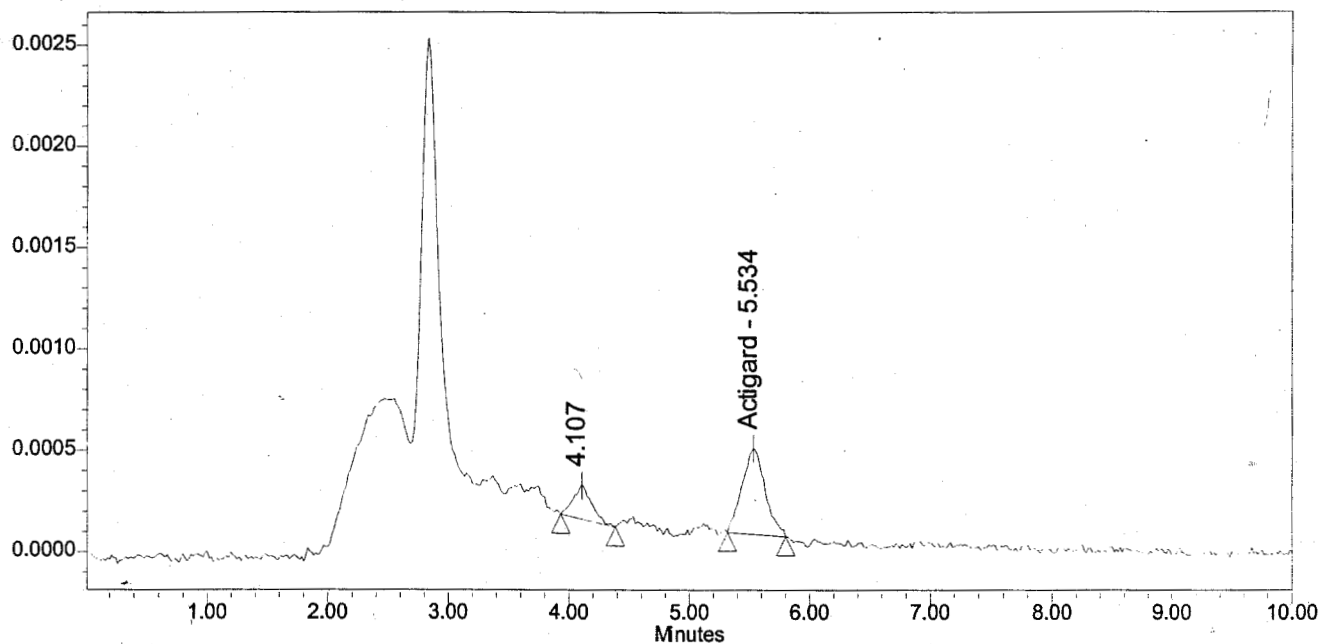
## D-2. Soil Fortified at 0.02 ppm

### Sample Information

SampleName Spike A Set #7  
 Vial 8  
 Injection 1  
 Injection Volume 75.00 ul  
 Channel 490 Ch1  
 Run Time 10.0 Minutes

Sample Type Unknown  
 Date Acquired 08/17/1999 6:23:55 PM  
 Acq Method Set acti\_set  
 Processing Method ActiLOQ2  
 Date Processed 08/20/1999 1:51:59 PM

### Auto-Scaled Chromatogram



### Peak Results

RT	Name	RT	Area	Height	Amount	Units
4.107		4.107	1769	165		
5.534	Actigard	5.534	5665	420	0.008	ug/ml

# E. HPLC Chromatograms at the 10 x LOQ (0.2 ppm) Fortification Level

ECM0147S1

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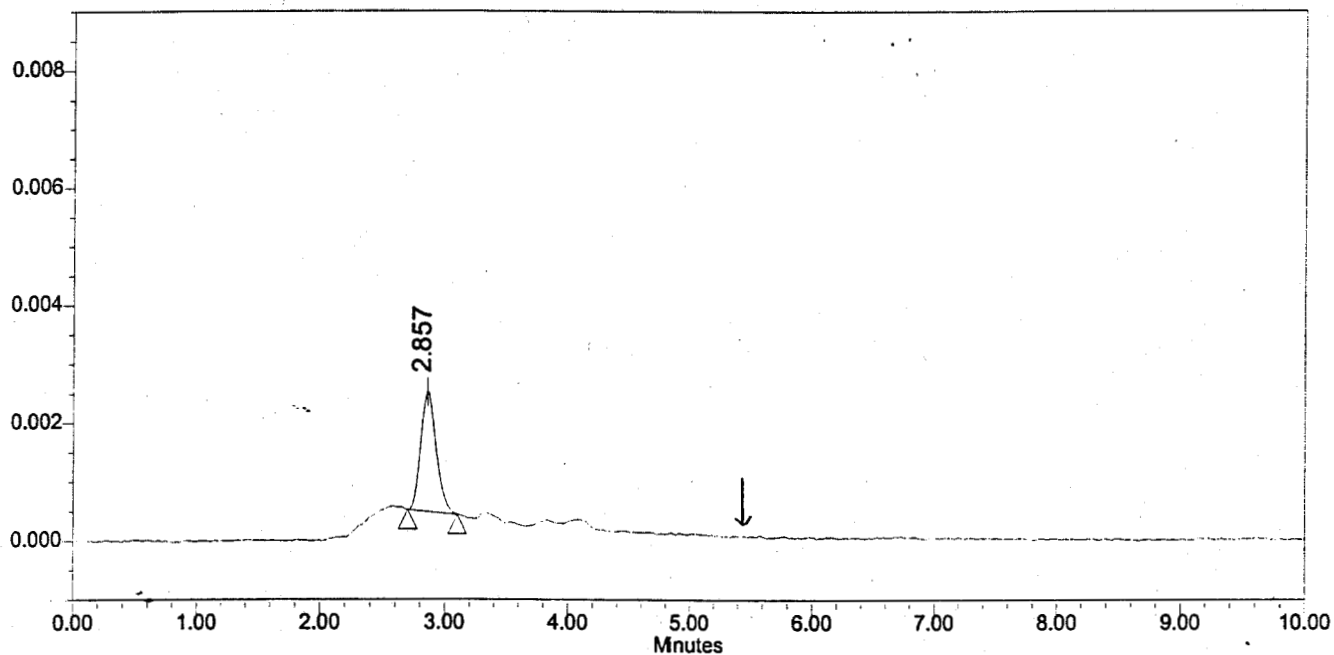
## E-1. Matrix Blank for 0.2 ppm level

### Sample Information

SampleName Set #5 Matrix Blk  
Vial 7  
Injection 1  
Injection Volume 75.00 ul  
Channel 490 Ch1  
Run Time 10.0 Minutes

Sample Type Unknown  
Date Acquired 09/09/1999 4:40:34 PM  
Acq Method Set Actigard\_Met\_Set  
Processing Method Actigard\_Proc  
Date Processed 09/13/1999 9:35:39 AM

### Auto-Scaled Chromatogram



### Peak Results

66	Name	RT	Area	Height	Amount	Units
1		2.857	17676	2040		
2	Actigard	5.408				

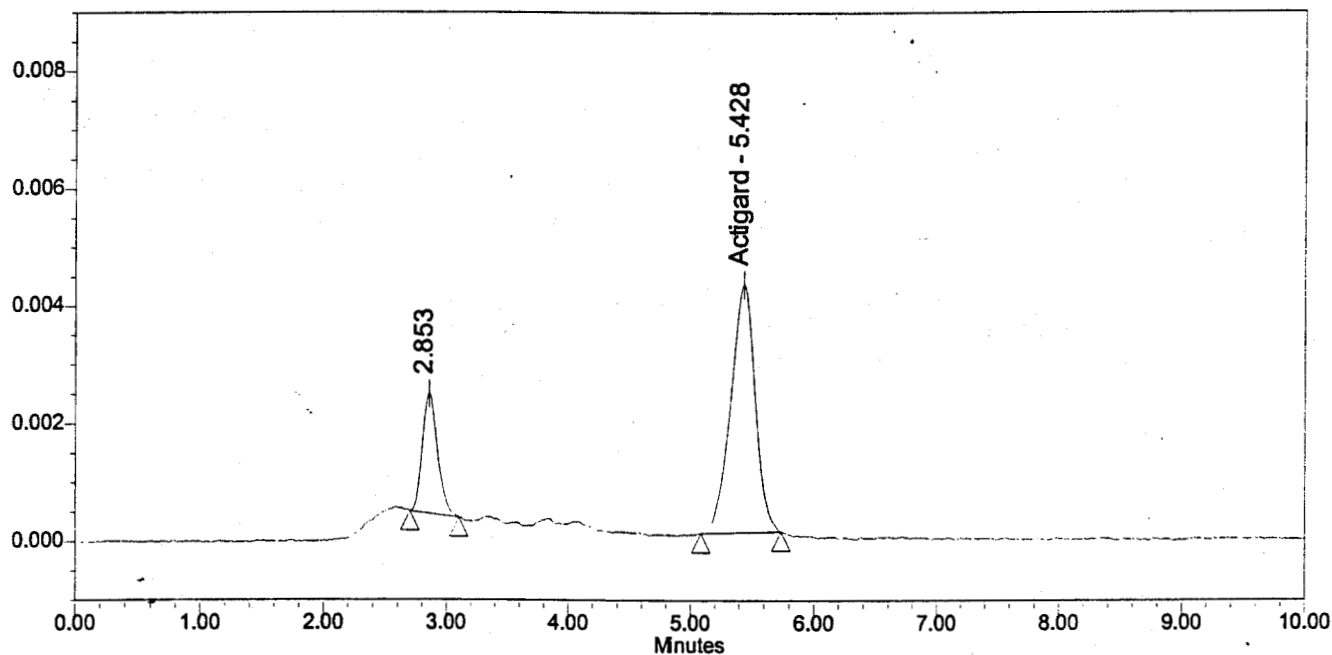
E-2. Soil Fortified at 0.2 ppm

**Sample Information**

SampleName Set #5 Spike A  
 Vial 9  
 Injection 1  
 Injection Volume 75.00 ul  
 Channel 490 Ch1  
 Run Time 10.0 Minutes

Sample Type Unknown  
 Date Acquired 09/09/1999 5:02:59 PM  
 Acq Method Set Actigard\_Met\_Set  
 Processing Method Actigard\_Proc  
 Date Processed 09/13/1999 9:36:32 AM

**Auto-Scaled Chromatogram**

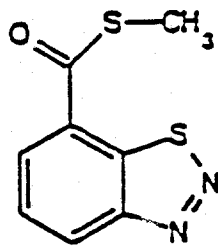


**Peak Results**

Name	RT	Area	Height	Amount	Units
1	2.853	17653	2049		
2 Actigard	5.428	58038	4227	0.078	ug/ml

Appendix A: Actigard Chemical Structure

CGA 245704



$C_8H_6N_2OS_2$

Molecular mass: 210.28

Benzo[1,2,3]thiadiazole-7-carbothioic acid S-methyl ester

Figure 2: Procedure flow diagram

REPORT NUMBER  
97-143-0524 PAGE 1  
ACCOUNT NUMBER  
15028

# A&L Analytical Laboratories, Inc.

411 North Third Street • Memphis, TN 38105 • (901) 527-2780 • FAX (901) 526-1031



SEND  
TO:

U.S. EPA/ECS  
BLDG. 1105  
STENNIS SPACE CNTR, MS 39529

CLIENT:US EPA/ECS

## REPORT OF ANALYSIS

REPORT DATE: 06/04/97  
DATE RECEIVED: 05/25/97

LAB NO	SAMPLE IDENTIFICATION	PERCENT SAND	PERCENT SILT	PERCENT CLAY	TEXTURAL CLASSIFICATION
03589	IDWA BATCH 3A	52	28	20	SANDY CLAY LOI

Appendix B: Control Soil Characterization

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DP BARCODE: D262452

DATA PACKAGE RECORD  
BEAN SHEET

DATE: 01/18/00  
Page 1 of 1

\* \* \* FREE STANDING DATA PACKAGE \* \* \*

THERE IS NO CASE OR SUBMISSION DATA

\* \* \* DATA PACKAGE INFORMATION \* \* \*

DP BARCODE: 262452 EXPEDITE: N DATE SENT: 01/18/00 DATE RET.: / /  
CHEMICAL: 061402 1,2,3-Benzothiadiazole-7-carbothioic acid, S-methyl ester(9  
DP TYPE: 999

ASSIGNED TO	CSF: Y	DATE	IN	DATE	OUT	ADMIN DUE DATE:	/	/
DIV : RD		/	/	/	/	NEGOT DATE:	/	/
BRAN: FB		/	/	/	/	PROJ DATE:	/	/
SECT: IO		/	/	/	/			
REVR :		/	/	/	/			
CONTR:		/	/	/	/			

\* \* \* DATA REVIEW INSTRUCTIONS \* \* \*

THIS SUB-BEAN WAS CREATED IN EFED AS A INSTRUMENT FOR  
FORAWRDING AND TRACKING OF THE ATTACHED DOCUMENT ON  
ACIBENZOLAR(ACTIGARD) : METHOD EVALUATION BY ECL/BEAD AND  
EFED/FMB. SENT TO LUIS SUGUIYAMA, CHIEF/FB/RD BY SID  
ABEL/BETSY BEHL OF FMB/EFED

\* \* \* DATA PACKAGE EVALUATION \* \* \*

No evaluation is written for this data package

THERE ARE NO ADDITIONAL DATA PACKAGES