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ENVIRONMENTAL CHEMISTRY SECTION
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September 30, 1992

MEMORANDUM

SUBJECT: Avermectin Method Validation - Report No. ECM-0001

FROM: Han Tai, Chemist
BEAD/ACB/Environmental Chemistry Section

TO: Dan Rieder
EFED/Ecological Effects Branch (H7507C)

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

THRU: Donald A. Marlow, Chief
BEAD/Analytical Chemistry Branch (H7503W)

THRU: Douglas Urban, Chief
(EFED/Ecological Effects Branch (H7507C)

The EFED/Ecological Effects Branch has requested an Environmental Chemistry Method Validation (ECMV) on Avermectin in soil using the Merck, Sharp, and Dohme Company analytical Method 8003 "HPLC Fluorescence Determination for Avermectin B1 and its Delta 8, 9 Isomer in Soil." The method validation request has specified triplicate analyses on two types of soils (low and high organic content) and reagent blanks at fortification levels of 0, 1, 2, 10 and 20 parts per billion (ppb).

The attached method validation 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. ECS'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 standard deviation (SD) and 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, example calculations, representative sample and standard chromatograms and standard curves are listed and/or discussed.

If you have questions concerning this report, please contact Han Tai at (601) 688-3252.

Attachments

cc: Danny McDaniel, QA Officer
BEAD/ACB/Environmental Chemistry Section

Environmental Chemistry Method Validation Report Number ECM-0001

Avermectin B1 and Avermectin Delta 8,9 Isomer

Environmental Chemistry Section
Analytical Chemistry Branch
Biological and Economical Analysis Division

Prepared by: Han Tai
Reviewed by: Danny McDaniel
Approved by: Aubry E. Dupuy, Jr.
Donald A. Marlow

Date: Feb. 20, 1992 (1st draft)
Sept.30, 1992 (Final draft)

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Part I
Summary and Conclusions

We have completed an Environmental Chemistry Method Validation (ECMV) on Avermectin for two types of soil The method appears to be suitable to gather residue data for Avermectin and its Delta - 8,9 isomer from 1 ppb to at least 20 ppb in both types of soil we tested if some modifications are utilized. This indicates that the data gathered by the registrant using the method as it was written should also be acceptable for the soil types they tested.

The analytical method involves Soxhlet extraction of the matrix (soil) with acetonitrile/water and subsequent clean up by solid phase and liquid-liquid extraction. The resultant extract is derivatized and analyzed by High Performance Liquid Chromatography (HPLC) with fluorescence detection. The fortification levels were at 0 (sample blank), 1.0, 2.0, 10.0 and 20.0 parts per billion (ppb), with triplicate analyses at each level.

ECS's Method Limit of Detection (LOD) was 0.6 ppb and the Limit of Quantitation (LOQ) was 1.0 ppb in both soil types which are comparable to those claimed in the method. Recoveries for Avermectin B1a in soil ranged from 62% to 98% with a mean of 78.5% and relative standard deviation (RSD) of 11.0% (total 24 analyses). Recoveries for Avermectin Delta 8,9 isomer in soil ranged from 70% to 105% with a mean of 83.2% and RSD of 11.2% (total 24 analyses).

ECS estimates that it takes three (3) working days to start and finish one (1) set of five (5) samples with appropriate blanks and standards.

As a whole, the validation verified the methodology, but there were a few problems that had to be overcome. The problems and their solutions are as follows:

1 Problem: The Kratos Model FS950 fluorescence detector specified in Method 8003 is no longer commercially available.

Solution: The Waters Model 420-AC fluorescence detector was used for the present work.

2 Problem: The sensitivity of the Waters detector was approximately one tenth of that claimed in the method for the Kratos detector.

Solution: The step 14 of the method specified to split half of the extract and keep as a reserve. In the present work, the entire extract was used without splitting. The volumes of final extract for HPLC analysis (step 25) was 2.0 ml for the 1.0 and 2.0 ppb spiking levels and 5.0 ml for the 10.0 and 20.0 ppb spiking levels.

3 Problem: Shifting retention times of the analytical peak.

Solution: The short liquid chromatograph columns are very sensitive to temperature changes. During the day our HPLC lab would warm up slightly and the retention times would decrease. We intermingled the standard runs in with the sample runs to keep track of the slight decrease in retention times.

The slight shifting of retention times we encountered is not a major problem for analyzing laboratory spiked soils or field trial soil samples; however, if the method is to be used to detect inadvertent residues or for monitoring studies, the temperature of the room containing the HPLC instrument and/or the temperature of the HPLC column must be closely regulated. We also recommend this method be used by experienced chemists.

Part II
Analytical Results

Method: HPLC-Fluorescence Determination for Avermectin B1 and its Delta-8, 9 Isomer in Soil, Method No. 8003. Merck, Sharp,and Dohme Research Laboratories, October 12, 1988.

Results:

1. Avermectin B1a

Added (ppb)	Found (ppb)			% Recovery					
	Run A	Run B (2)	Run C	Run A	Run B	Run C	X (3)	SD	RSD
(a) L.O. Soil(4)									
0	ND (1)	ND	ND	--	--	--	--	--	--
1.0	0.663	0.727	0.622	66.3	72.7	62.2	67.1	5.3	7.9
2.0	1.669	1.683	1.450	83.4	84.2	72.5	80.0	6.5	8.2
10.0	8.643	7.589	9.036	86.4	75.9	90.4	84.2	7.5	8.9
20.0	14.426	13.820	14.360	72.1	69.1	71.8	71.0	1.6	2.3
(b) H.O. Soil(4)									
0	ND	ND	ND	--	--	--	--	--	--
1.0	0.800	0.844	0.726	80.0	84.4	72.6	79.0	6.0	7.6
2.0	1.966	1.527	1.590	98.3	76.4	79.5	84.7	11.9	14.0
10.0	8.949	9.010	7.982	89.5	90.1	79.8	86.5	5.8	6.7
20.0	15.897	14.862	14.386	79.5	74.3	71.9	75.2	3.9	5.2
(c) Reagent(5)									
0	ND	ND	ND	--	--	--	--	--	--
1.0	1.031	0.954	0.979	103.1	95.4	97.9	98.8	3.9	4.0
2.0	1.963	2.038	1.743	98.2	101.9	87.2	95.8	7.6	7.9
10.0	8.697	8.657	8.996	87.0	86.6	90.0	87.9	1.9	2.2
20.0	19.474	18.648	18.546	97.4	93.2	92.7	94.4	2.6	2.8

Results Con't

2. Avermectin Delta - 8, 9 Isomer[6]

	Added (ppb)	Found (ppb)[2]			% Recovery					
		Run A	Run B	Run C	Run A	Run B	Run C	X[3]	SD	RSD
(a) L.O. Soil[4]										
	1.0	0.726	1.014	0.788	72.6	101.4	78.8	84.3	15.2	18.0
	2.0	1.547	1.549	1.450	77.4	77.4	72.5	75.8	2.8	3.7
	10.0	8.687	9.433	8.138	86.9	94.3	81.4	87.5	6.5	7.4
	20.0	14.585	19.993	15.877	72.9	100.0	79.4	84.1	14.2	16.9
(b) H.O. Soil[4]										
	1.0	0.696	0.843	0.756	69.6	84.3	75.6	76.5	7.4	9.7
	2.0	1.815	1.693	1.580	90.8	84.6	79.0	84.8	5.9	7.0
	10.0	8.051	8.126	8.323	80.5	81.2	83.2	81.6	1.4	1.7
	20.0	20.917	16.858	16.814	104.6	84.3	84.1	91.0	11.8	13.0
(c) Reagent[5]										
	1.0	0.906	0.968	0.849	90.6	96.8	84.9	90.8	6.0	6.6
	2.0	1.882	1.913	1.716	94.1	95.6	85.8	91.8	5.3	5.8
	10.0	9.345	8.443	8.515	93.4	84.4	85.2	87.7	5.0	5.7
	20.0	19.256	17.839	17.020	96.3	89.2	85.1	90.2	5.7	6.3

Notes:

(1) ND - not detected

Limit of Detection (LOD) 0.3 ng on HPLC column, equivalent to 0.6 ng/gm or 0.6 ppb in sample

Limit of Quantitation (LOQ) 0.5 ng on HPLC column, equivalent to 1.0 ng/gm or 1.0 ppb in sample

The above LOD and LOQ apply to Avermectin Bla and Avermectin Delta 8, 9 isomer. (Signal to noise ratio used to calculate the LOD and LOQ were 3:1 and 5:1 respectively)

(2) Run A, B, C are three separate analysis runs (extraction, cleanup, and HPLC determination) in the same set.

(3) X - mean; SD - Standard Deviation; RSD - % Relative Standard Deviation

(4) L.O. soil - Low organic soil; H.O. soil - High organic soil

(5) Reagent blank - Fortifications were made assuming a sample size of 25 gm, but no actual soil sample was present.

(6) The analytical procedure for Avermectin Delta 8, 9 Isomer is identical to that of Avermectin B1. Due to the limited supply of characterized soil sample, fortification at 0 ppb (sample blank) was not repeated for the Delta 8, 9 Isomer.

Part III

Experimental Details

General description of method:

The Monsanto method lists 25 steps in the analysis procedure. The 25.0 gm soil sample is extracted in a Soxhlet extractor for 2 hours with 200 ml of 1:1 acetonitrile:water. The sample is cooled and diluted with 250 ml water (steps 1 thru 5). The aqueous solution is passed through a C-8 column which is eluted with 20 ml acetonitrile. The eluant is concentrated to 1 ml and diluted to 5 ml with water (steps 6 thru 10). This solution is partitioned with hexane (step 11). The hexane extract is then passed through an aminopropyl column and washed successively with hexane, toluene and methylene chloride. The column is then eluted with 50% acetone in methylene chloride into a silylated glass tube. The eluant is then evaporated to dryness (steps 12 thru 16). The dried residue is derivatized with 1- methyl imidazole and trifluoroacetic anhydride in dimethyl formamide, 30°C for 1 hour, followed by hydrolysis with methanolic ammonia, 30°C for 30 minutes (step 17 thru 19). The mixture is then dissolved in 4 ml chloroform and passed through a silica column to remove the excess unreacted derivatizing reagents. The chloroform eluant is collected and the column is washed twice with 2 ml followed by 5 ml of chloroform. The combined chloroform eluant is evaporated and reconstituted with methanol to the appropriate volume for HPLC analysis (steps 20 thru 25).

Reverse phase HPLC is used for quantitation. The column is a Zorbax ODS column, 150 x 4.6 mm. The mobile phase is 10/90 water/methanol (v/v) at a flow rate of 1.5 ml/min. The fluorescence detection uses the 365 nm excitation line (Mercury lamp and 365 nm bandpass filter) and 400 nm cutoff filter for emission. The retention time for the present work is about 18 minutes for Avermectin B1a and its delta 8, 9 isomer, and about 15 minutes for Avermectin B1b.

The structural formulas of Avermectin B1 and the fluorescence product are shown in appendix 1.

Modifications to method: (Please see "Comments" section of this report for discussions)

1. The Fluorescence Detector, Kratos Instrument Model FS 950, as listed in the method, was no longer commercially available. The Waters Fluorescence Detector Model 420 was used for the present work.

2. The Fluorescence Detector, Waters Model 420, showed a Limit of Quantitation (LOQ) at 0.5 ng of Avermectin B1a on the HPLC column. Five standard solutions at concentrations of 10, 20, 40, 80 and 100 ng/ml were prepared for injection volume of 50 ul. The Merck method specified concentrations of 1, 3, 5, 7, 10 ng/ml which were below the LOQ of the Waters detector.

3. In order to maintain a sufficient concentration of Avermectin B1a in the final extract to reach an LOQ of 1 ppb, the eluant from step 14 was not split for a reserve sample. The step 15 was omitted.

4. Due to the limited supply of soil, a sample size of 12.5 gm was used for the third set (Run C) of analysis. The Merck method specified a sample size of 25.0 gm.

Sourcee of analytical reference standards:

Two standards were received from Pesticides and Industrial Chemical Repository (MD-8), EPA, Research Triangle Park, NC 27709.

1. Avermectin B1, F-774, Lot FT6-3, about 0.5 ml - Merck L 676,863, B-68-038A002; B1a 0.956%. B1b 0.071% in glycerol

2. Avermectin Delta - 8, 9 Isomer, F-941, Lot ASA-1, about 0.5 ml Merck L 652,580, 002T001; 0.38% in glycerol.

Source of sample matrix:

Two soil samples, one low organic matter (0.7%), one high organic matter (1.5%), were prepared by: Mr. James M. Bartos, Soil Testing Laboratory of Auburn University, Auburn University, Alabama 36849-5411. Dr. Willard L. Douglas, Sverdrup Technology Inc., (NASA On-Site-Service Contractor) Stennis Space Center, MS, made arrangements for this supply. We gratefully acknowledge their assistance.

A copy of a letter from Mr. Bartos, and a report on the soil texture and % organic matter are attached as Appendix 2.

Instrumentation for quantitation:

High Performance Liquid Chromatography (HPLC) --

Column: Dupont Zorbax ODS C-18, 4.6 x 150 mm, stainless steel column (#883952.202, G-9864)

Injector: Waters Model U6K Manual Injector

Detector: Waters Model 420-AC Fluorescence detector

Recorder: Omni Scribe Recorder

Instrumentation for confirmation: Not applicable

Instrument parameters for the present evaluation that differ from original method:

HPLC Fluorescence Detector - Waters Model 420 AC

Light Source: Aflatoxin Lamp, 74TS/BL, wavelength 365 nm (#78409)

Excitation Filter: 365 nm Bandpass Filter (#78228)

Emmission Filter: 400 nm Longpass Filter (#78229)

Span: Set to Max (fully clockwise)

Gain: Set at 16

Blank: Adjust (coarse or fine) to set baselines (zero adjust before each injection)

HPLC Parameters:

Moble phase: 90/10 (v/v) methanol/water, isocratic, flow rate 1.5 mL/min

Recorder: 10 mv full scale, 0.25 cm/min chart speed

Injection Volume: 50 ul for standards and sample extracts, manual injection using Hamilton syringe

Retention Time: Avermectin Bla and Delta - 8, 9 isomer - about 18 minutes

Avermectin Blb - about 15 minutes

A Stopwatch was used for timing.

Notes on analytical procedures:

1. Step 1 - Fortification of samples:

   a. Fortifications of soil samples were performed as described in the method.

   b. Fortifications of reagent blanks were made assuming a sample size of 25.0 gm. The bottom of the extraction thimble was lined with several glass beads. Measured amounts of standard solutions were introduced directly on the glass beads, usually less than 0.5 ml by a microsyringe or a pipet. The thimble was then placed in the Soxhet extraction tube without touching any other surface. The extraction was carried out according to procedure.

2. Step 7 - The use of Analytichem SPS-24 apparatus for C-8 column Solid Phase Extraction:

   The SPS-24 apparatus was found to be convenient to use and facilitated the column clean-up operations. This apparatus could accommodate 24 cartridges. However, the simultaneous handling of more than 5 samples and 5 standards on the manifold would likely cause confusion and affect an orderly operation.

3. Step 11 - Solvent partition of acetonitrile eluant (from C-8 column) with hexane in the centrifuge tube:

   At this step, it was necessary to lift the stopper to release the initial build-up of solvent pressure after 10 seconds of shaking the centrifuge tube. The subsequent shaking (about 1 minute) did not need the release of pressure. The ground glass stopper should be securely placed in the ground glass joint of the tube, otherwise a slight leak might cause loss of liquid during shaking.

4. Step 17 - Use of derivatizing reagents:

   The reagents, imidazole and trifluoroacetic anhydride, should be handled with care (in fume hood). The bottles were tightly capped (with leak-proof cap) after each use and stored individually in a desiccator to avoid exposure to moisture.

5. Step 20 - Incomplete derivatization reaction:

   The possible incomplete derivatization of the fluorescence product can easily be detected when chloroform was added to the mixture after the methanolic ammonia hydrolysis. The sample should appear white and cloudy after the addition of chloroform (step 20). If not, a low yield due to incomplete derivatization might have occured. If this happened on one of the five standards, this sample could be discarded and the calibration curve (or regression line) could be constructed

from the remaining four standards. For soil extracts, however, the samples should be redone. The Monsanto method clearly explained this point in step 20 (p.0737) and in a statement in second paragraph on p.0742.

Such incomplete derivatization occured twice in the preparation of standard solutions. Data points corresponding to these solutions were discarded.

6. Step 22 - Silica cartridge:

   The silica cartridge could accommodate 5 ml of liquid above the solid packing. Therefore, the solution was poured directly into the cartridge without using a Pasteur pipet (to save one extra step on transfer).

7. Step 25 - Final volume of the sample extract:

   For the 25 gm samples, the final volumes were 2.0 ml methanol for spiking levels at 1.0 and 2.0 ppb, and 5.0 ml for spiking levels at 10.0 and 20.0 ppb. For 12.5 gm samples, the volumes were 1.0 ml and 2.5 ml respectively. The final volumes for all standards were 5.0 ml methanol.

8. The "Gain" Control of Model 420 Detector :

   The "gain" function of the Waters model 420 detector could only be mannually set step-wise from 1 to 128, doubling the gain with each advance of the knob position. To reach a compromise between sensitivity and noise, the gain was set at position 16, beyond which noise became excessive.

Comments:

1. HPLC Fluorescence Detector:

The fluorescence detection of the Avermectin derivative was first tried on the Analytical Bio-system (formerly Kratos) Model 980 Spectro fluorometer. No HPLC analytical peak was detected at concentrations specified in the Merck Method at an excitation wavelength of 365 nm. Since the light source of the Model 980 was a deuterium lamp, its low spectral energy at 365 nm was thought to be the cause of a "not detected" response. Excitation at 280 nm did show an Avermectin peak from standard solutions at concentrations of 3, 5, 7 and 10 ng/ml with a 50 ul injection volume. The plot of peak heights vs concentrations gave a straight line. However, the use of a different excitation line would be a serious deviation from the given method so it was decided not to proceed this way. A mercury lamp for 365 nm is not available for the model 980 detector.

The Kratos Model FS950 Fluorescence Detector, used in the Merck Method, has been discontinued by the manufacturer and is no longer commercially available. Dr. Teresa Wehner, Merck Company, Phone (201) 594-6261, indicated that their contractor has used a Shimadzu instrument equipped with a Xenon lamp.

The Waters Model 420 Detector was obtained as it was equipped with UV source lamps at a reasonable cost. The Aflatoxin lamp, a mercury lamp, specific for the 365 nm line, detected Avermectin at a level of 0.5 ng on column. Although it was only one tenth as sensitive as the Kratos FSA110 mercury lamp as indicated in the Merck Method, quantitative responses were obtained for standard solutions at concentrations of 10 ng/ml to 100 ng/ml of Avermectin B1a. The method limit of quantitation (LOQ) at 1 ppb could be reached by adjusting the final volumes of the sample extracts. This detector, however, exhibited excessive noise at a gain setting beyond 16 when the Aflatoxin lamp was used.

2. The ingredient Avermectin B1b

The relative concentration of B1b in the Avermectin standard was about 7.4% of that of B1a. (The primary standard contained 0.956% B1a and 0.071% B1b in glycerol). The presence of B1b was not detected by the Waters model 420 detector in the standard solutions at concentrations of 10, 20, 40 ng/ml, and was barely detectable at 80 and 100 ng/ml. At the highest fortification level of 20 ppb, the peak height of the B1b peak was still below the limit of quantitation. Therefore, quantitative measurements of B1b were not feasible using the Waters model 420 detector at the requested fortification levels.

3. Time (approximate) required for completing one set of 5 samples and 5 standards:

   Soxhlet extraction, steps 1 to 5 - 3 hours

   C-8 colums, steps 6 to 9 - 4 hours

   Solvent partition, steps 10, 11 - 2 hours

   Aminopropyl column, steps 12, 13 - 2 hours

   Derivatization, Silica Column, steps 16 to 25 - 5 hours

   HPLC, manual injection - 6 hours

   Suggested overnight stopping points were steps 9, 14 (or 15), and 25. The present operator at times could reach step 11 on the first day and found that keeping the hexane extract overnight (in refrigerator) did not seem to affect the results.

   At least two working days (assuming 8-hr working day) would be needed to complete processing (3 days following the suggested stopping points), and one working day for HPLC.

   Soxhlet extraction could be started when derivatization (step 16) for the previous set was in progress. Such overlapping could speed up the processing, but would keep the operator very busy on the bench. Since the method involves many steps, and each step must be handled in an orderly sequence, it seemed desirable to have a single operator carry out analysis throughout each set, because splitting steps between operators might cause confusion.

4. This procedure involves Soxhlet extraction, several solid phase extraction (SPE) column clean-up steps,solvent partition in a centrifuge tube, as well as a derivatization reaction. It was by no means a simple operation and required careful manipulation. However, the clean-up procedures seemed to work well. The final extracts exhibited well-defined analytical peaks for Avermectin B1a and its Delta 8, 9 isomer without any apparent interferences. There were several early peaks (not identified), but they were far removed from the analytical peaks.

5. Since the fluorescence detector as specified in the Merck Method, the Kratos Model FS950, is no longer commercially available, are there any supporting data obtained from other detectors which Merck could provide?

Chromatograms and Calibration Curves:

A. Avermectin Standards

A-1: 10 ng/ml

A-2: 20 ng/ml

A-3: 40 ng/ml

A-4: 80 ng/ml

A-5: 100 ng/ml

A-6: Calibration Curve

B. Avermectin Fortification, Low Organic Soil

B-1: Avermectin fortified 1.0 ppb

B-2: Avermectin fortified 2.0 ppb

B-3: Avermectin fortified 10.0 ppb

B-4: Avermectin fortified 20.0 ppb

C. Avermectin Fortification, High Organic Soil

C-1: Avermectin fortified 1.0 ppb

C-2: Avermectin fortified 2.0 ppb

C-3: Avermectin fortified 10.0 ppb

C-4: Avermectin fortified 20.0 ppb

D. Avermectin Fortification, Reagents

D-1: Avermectin fortified 1.0 ppb

D-2: Avermectin fortified 2.0 ppb

D-3: Avermectin fortified 10.0 ppb

D-4: Avermectin fortified 20.0 ppb

E. Avermectin Delta- 8, 9 Isomer Standards

E-1: 10 ng/ml

E-2: 20 ng/ml

E-3: 40 ng/ml

E-4: 80 ng/ml

E-5: 100 ng/ml

E-6: Calibration Curve

F. Avermectin Delta- 8, 9 Isomer Fortification, Low Organic Soil

F-1: Avermectin Delta- 8, 9 Isomer fortified 1.0 ppb

F-2: Avermectin Delta- 8, 9 Isomer fortified 2.0 ppb

F-3: Avermectin Delta- 8, 9 Isomer fortified 10.0 ppb

F-4: Avermectin Delta- 8, 9 Isomer fortified 20.0 ppb

G. Avermectin Delta- 8, 9 Isomer Fortification, High Organic Soil

G-1: Avermectin Delta- 8, 9 Isomer fortified 1.0 ppb

G-2: Avermectin Delta- 8, 9 Isomer forgified 2.0 ppb

G-3: Avermectin Delta- 8, 9 Isomer fortified 10.0 ppb

G-4: Avermectin Delta- 8, 9 Isomer fortified 20.0 ppb

H. Avermectin Delta, 8, 9 Isomer Fortification Reagents

H-1: Avermectin Delta- 8, 9 Isomer fortified 1.0 ppb

H-2: Avermectin Delta- 8, 9 Isomer fortified 2.0 ppb

H-3: Avermectin Delta- 8, 9 Isomer fortified 10.0 ppb

H-4: Avermectin Delta- 8, 9 Isomer fortified 20.0 ppb

I. Blanks (no fortification)

I-1: Low Organic Soil

I-2: High Organic Soil

I-3: Reagent

Note: 1. Injection volume for all chromatograms is 50 ul

2. An arrow indicates the position of HPLC peak of the analyte

3. Notations on the chromatogram

Inject - Injection of sample

min - Run time in minutes

Bla - Avermectin Bla

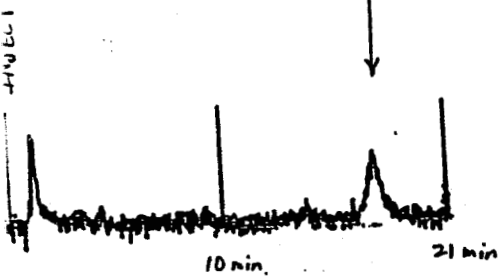
Blb - Avermectin Blb

Δ 8, 9 - Avermectin Delta- 8, 9 Isomer

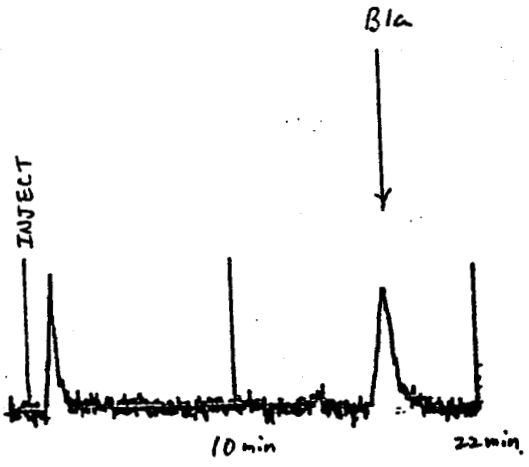
A. AVERMECTIN STADARDS

INJECT VOLUME : 50 µl

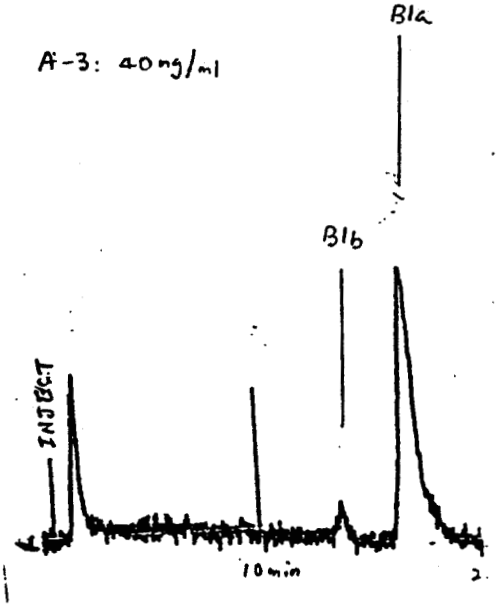
A-1 : 10 ng/ml



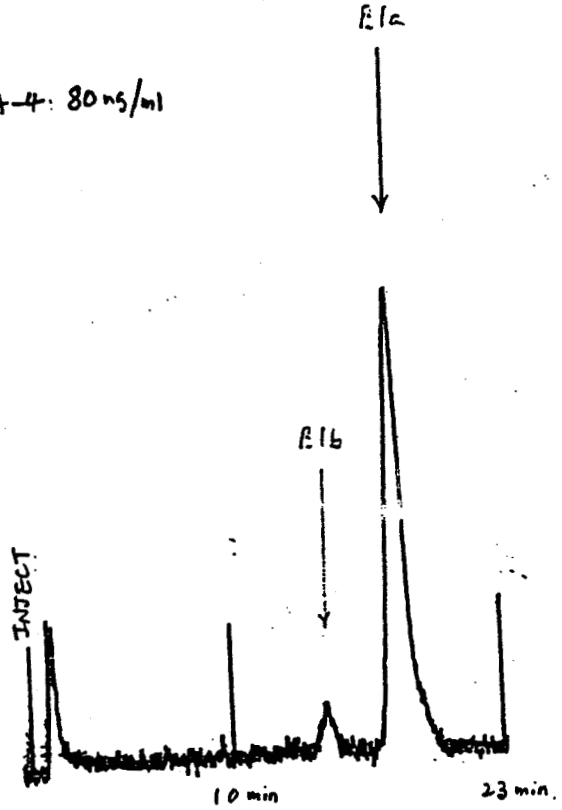
A-2 : 20 ng/ml



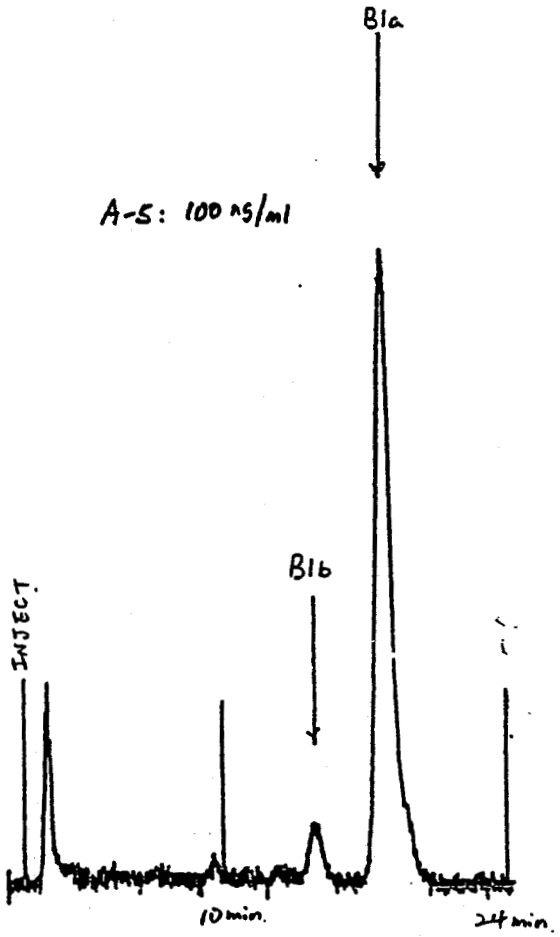
A-3: 40 ng/ml



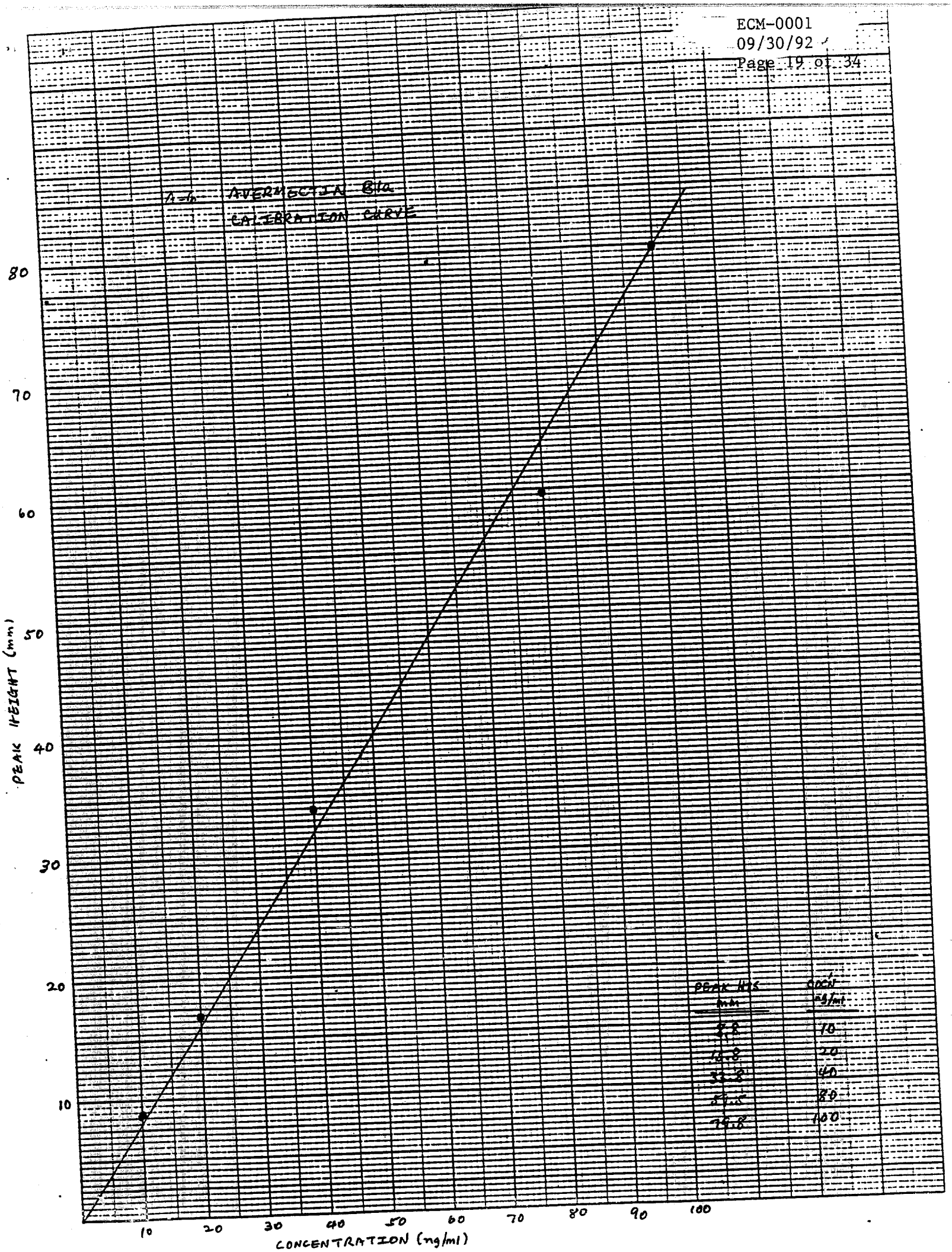
A-4: 80 ng/ml



A-5: 100 ng/ml



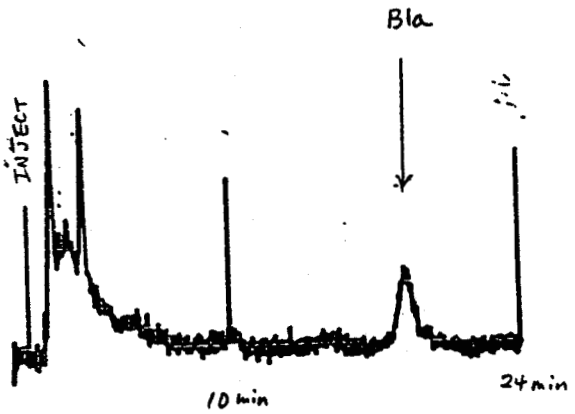
A-6 AVERMECTIN B1a
CALIBRATION CURVE



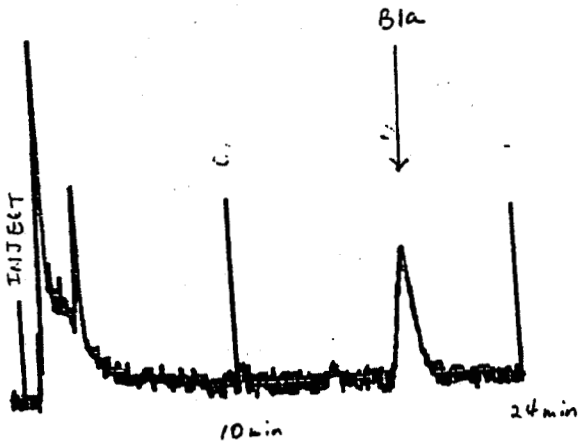
B. AVERMECTIN FORTIFICATION – LOW ORGANIC SOIL

INJECT VOLUME 50 µl

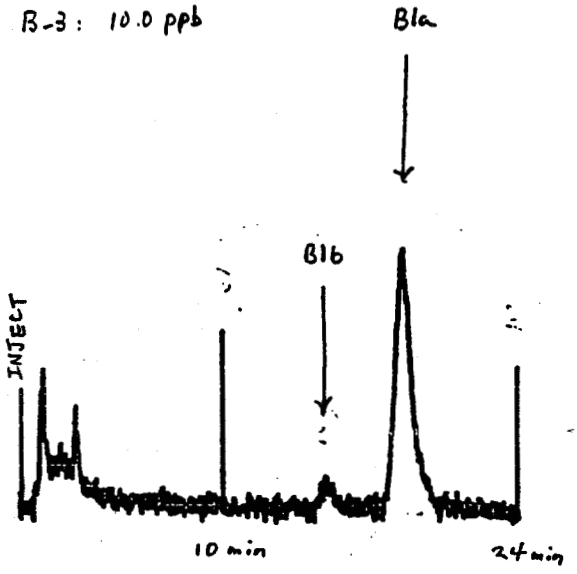
B-1: 1.0 ppb



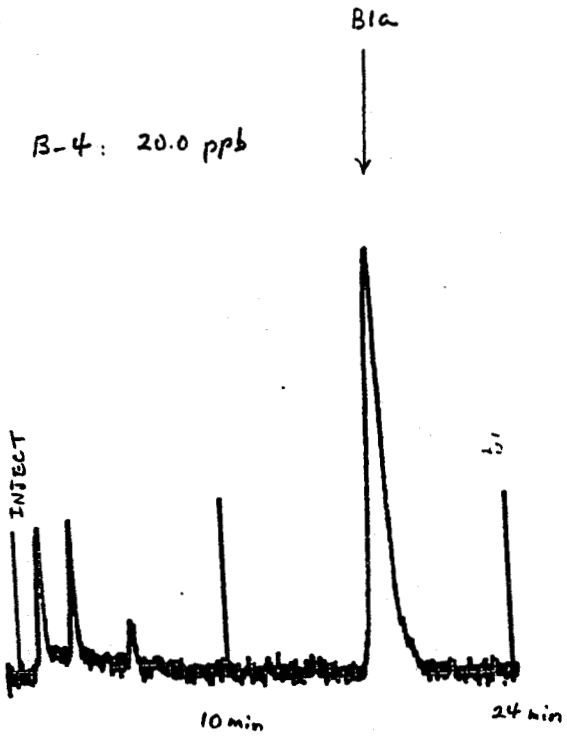
B-2 2.0 ppt



B-3: 10.0 ppb



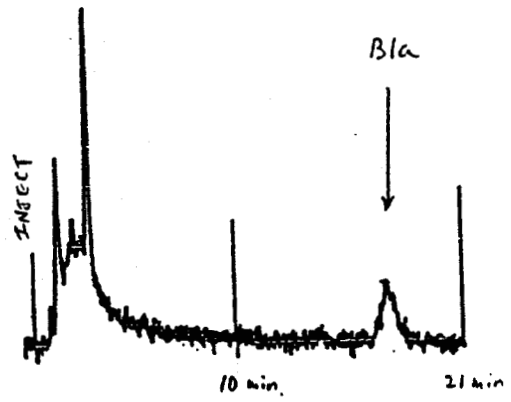
B-4: 20.0 ppb



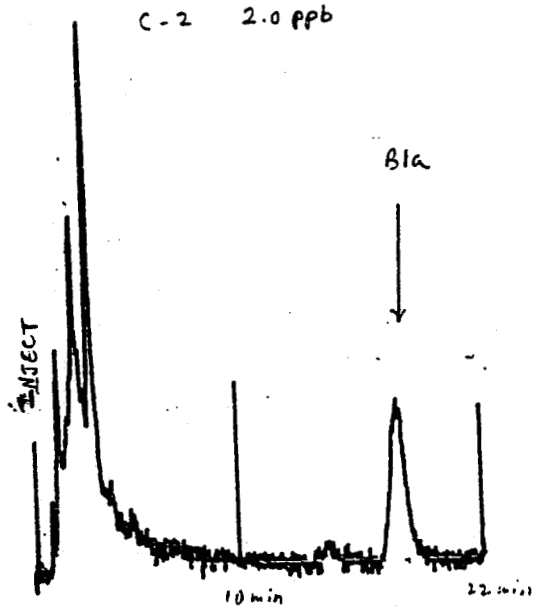
C AVERMECTIN FORTIFICATION — HIGH ORGANIC SOIL

INJECT VOLUME 50µl

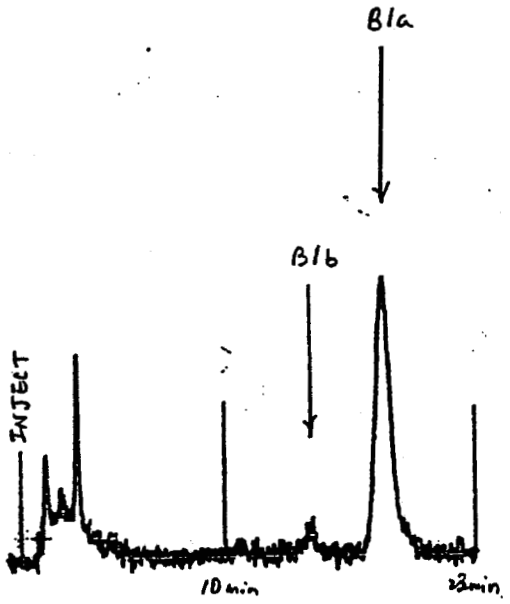
C-1: 1.0 ppb



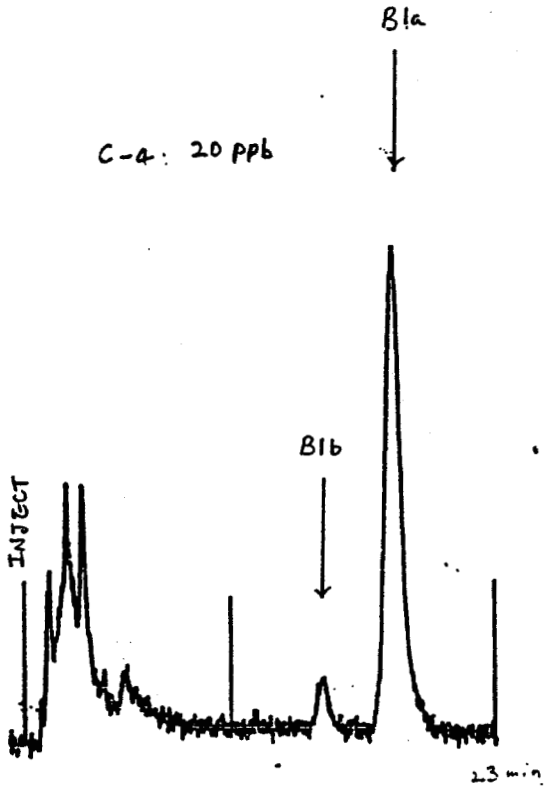
C-2 2.0 ppb



C-3: 10 ppb



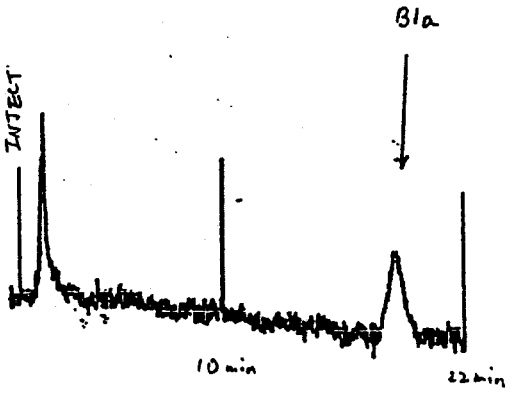
C-4: 20 ppb



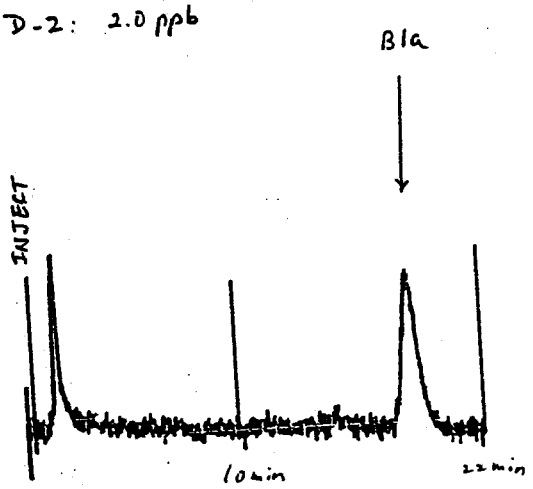
D AVERMECTIN FORTIFICATION — REAGENTS

INJECT VOLUME 50 µl

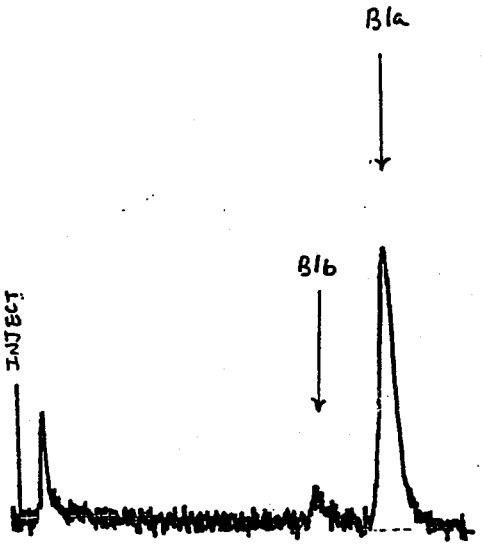
D-1: 1.0 ppb



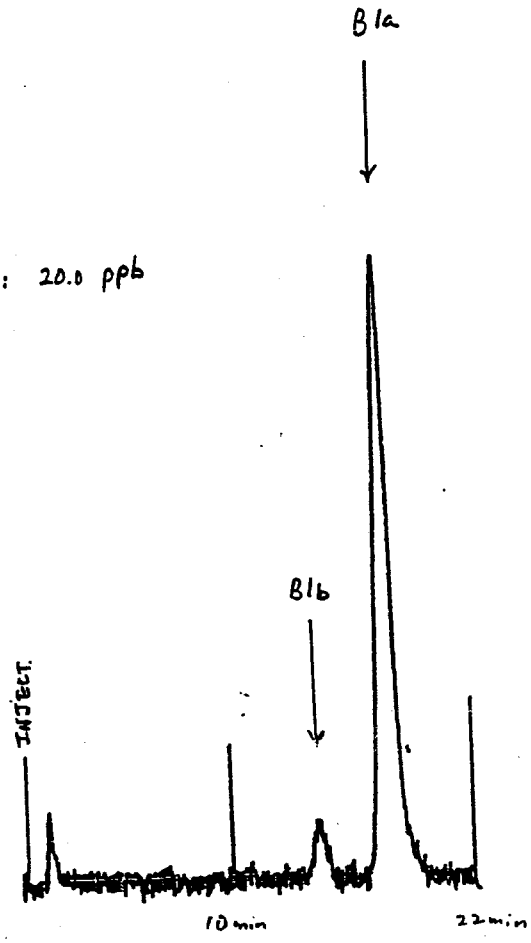
D-2: 2.0 ppb



D-3: 10.0 ppb



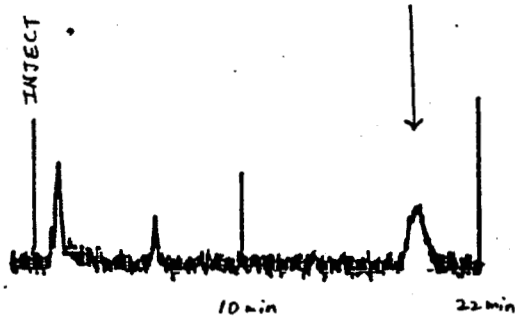
D-4: 20.0 ppb



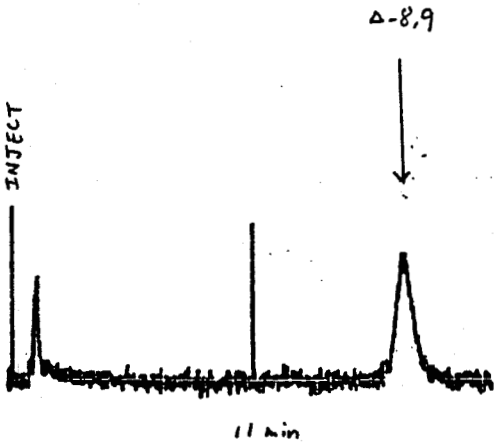
E. AVERMECTIN DELTA-8,9 ISOMER STANDARDS

INJECT VOLUME 50µl

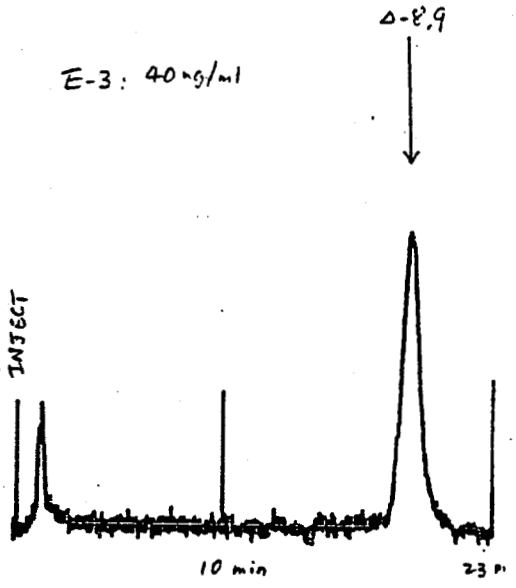
E-1: 10 ng/ml



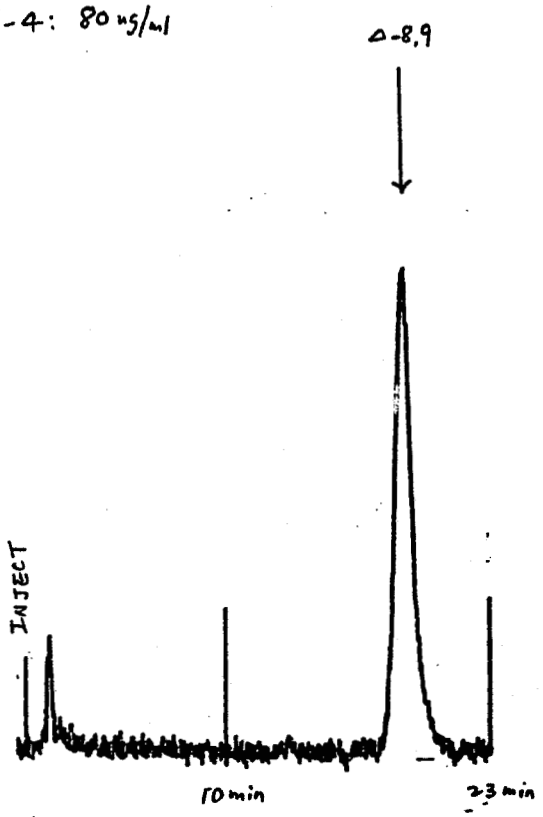
E-2: 20 ng/ml



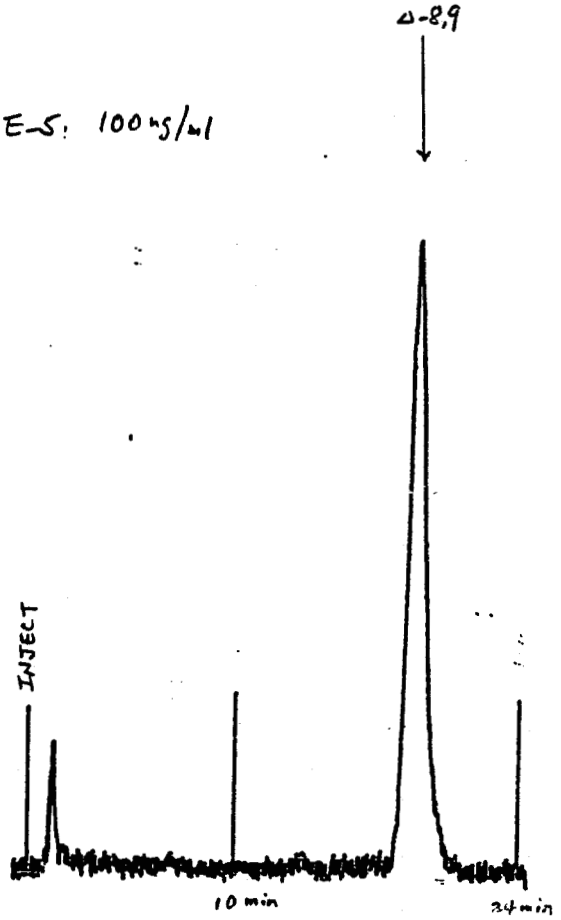
E-3: 40 ng/ml

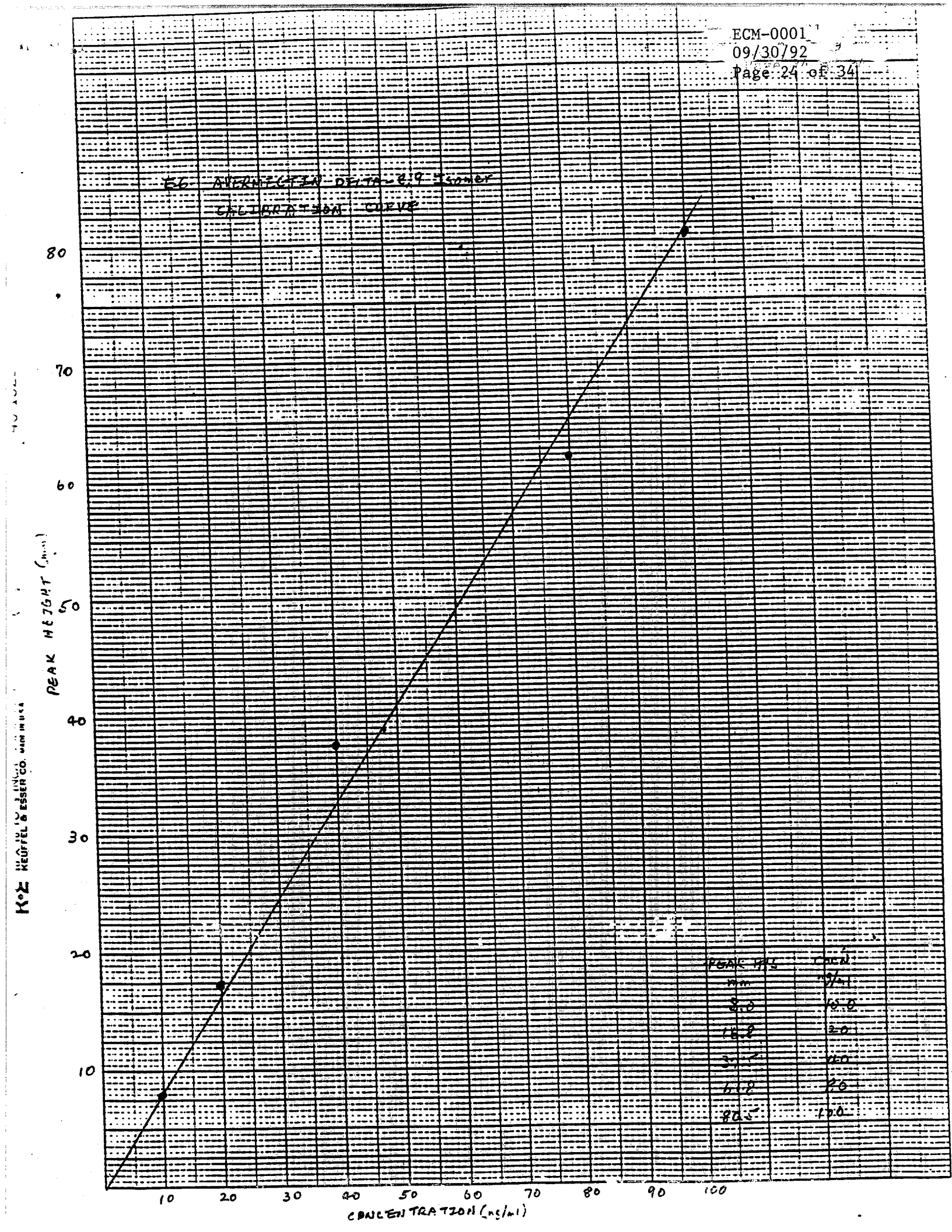


E-4: 80 ng/ml



E-5: 100 ng/ml

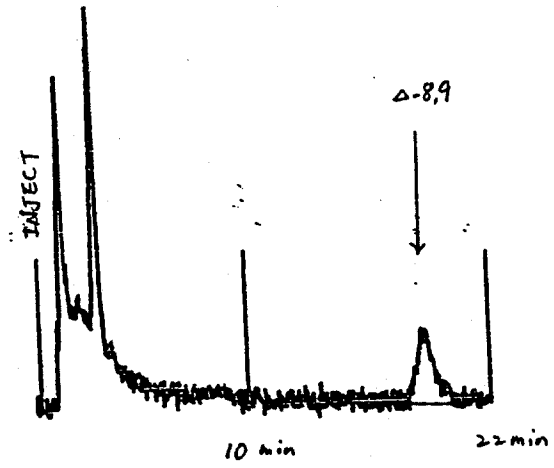



EC AVERMECTIN DELTA-8,9 ISOMER
CALIBRATION CURVE
PEAK HEIGHT (mm)
CONCENTRATION (ng/ml)
10
20
30
40
50
60
70
80
90
100
PEAK HTS
mm
8.0
16.8
31.2
61.2
80.5
CONC
ng/ml
10.0
20
40
80
100
KEUFFEL & ESSER CO. MADE IN U.S.A.

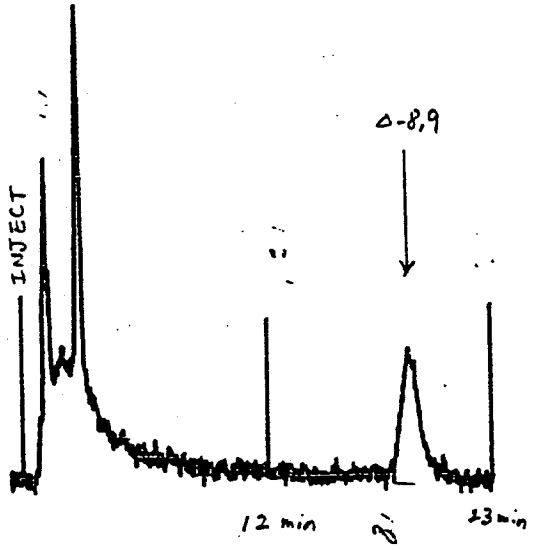
F. AVERMECTIN DELTA-8,9 ISOMER FORTIFICATION — LOW ORGANIC SOIL

INJECT VOLUME 50µl

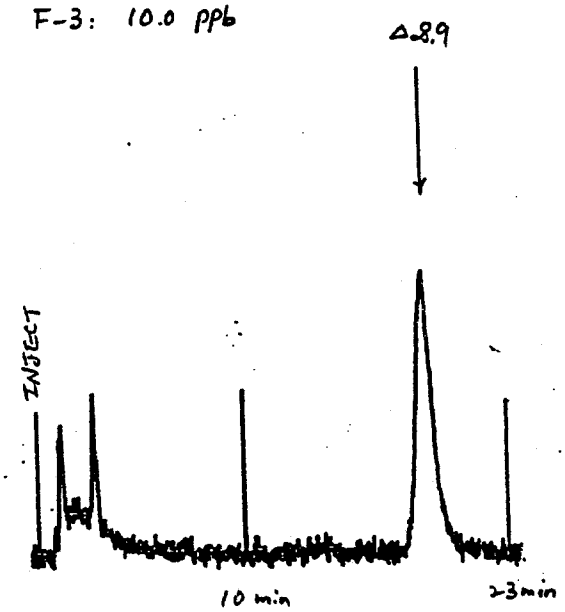
F-1: 1.0 ppb



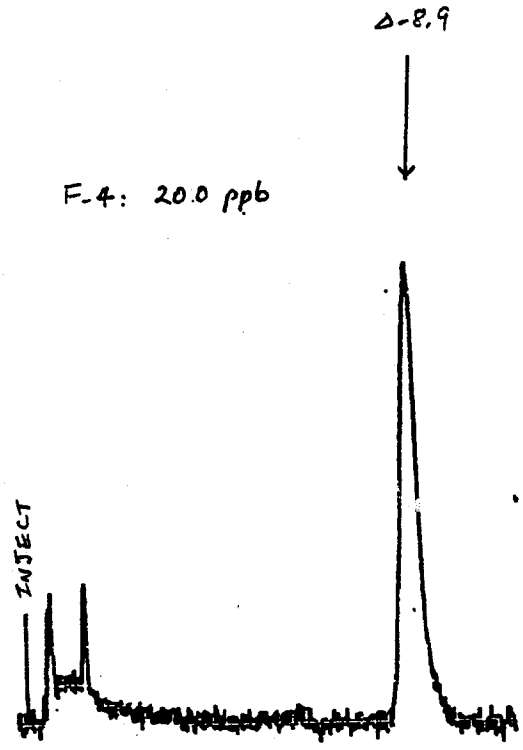
F-2: 2.0 ppb



F-3: 10.0 ppb



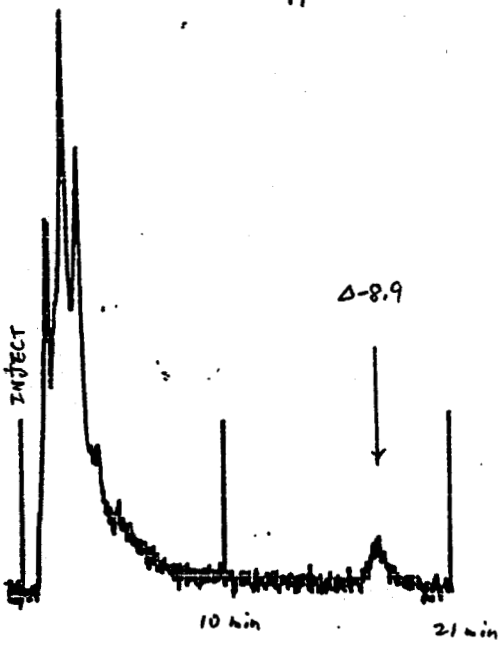
F-4: 20.0 ppb



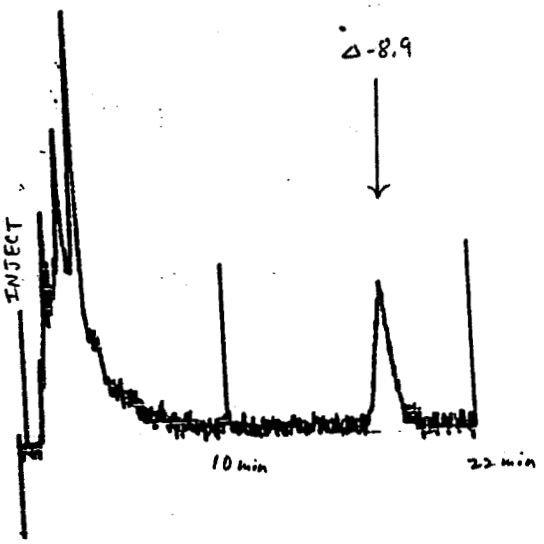
G. AVERMECTIN DELTA-8,9 ISOMER FORTIFICATION — HIGH ORGANIC SOIL

INJECT VOLUME 50µl

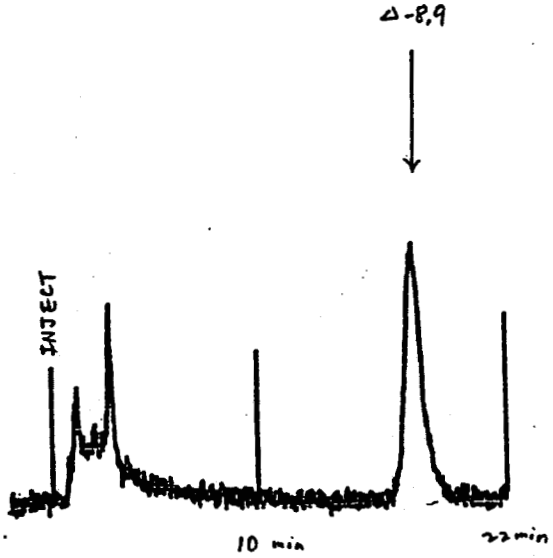
G-1: 1.0 ppb



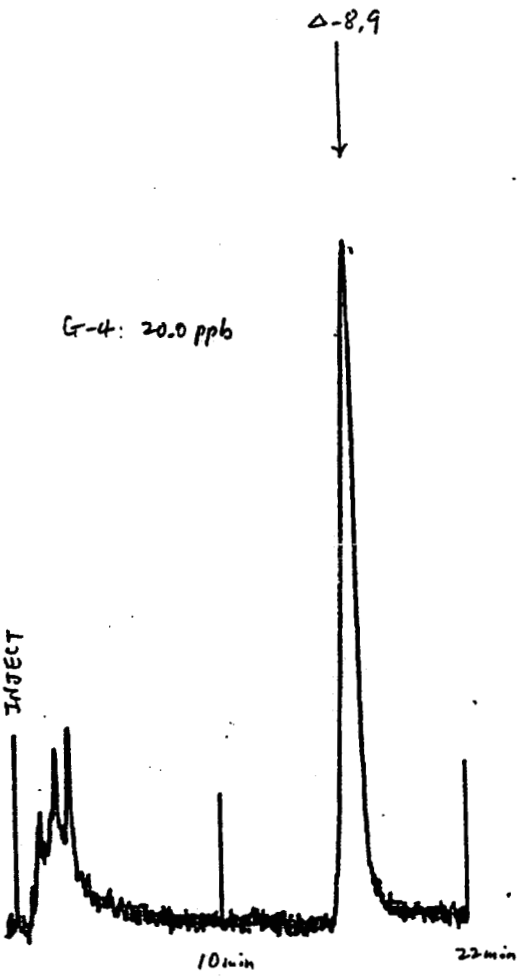
G-2: 2.0 ppb



G-3: 10.0 ppb



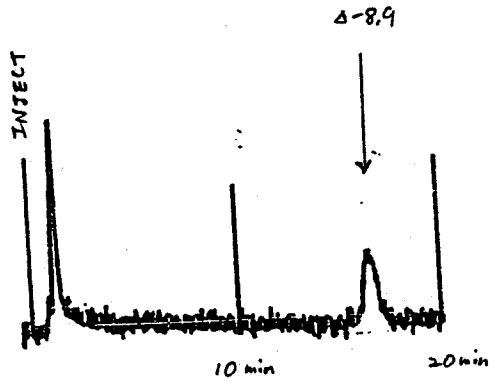
G-4: 20.0 ppb



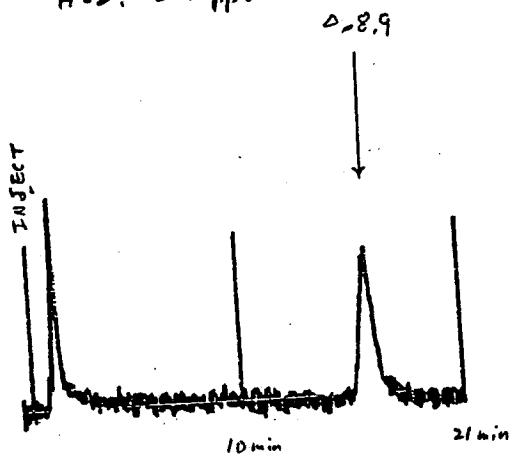
H. AVERMECTIN DELTA-8.9 ISOMER FORTIFICATION — REAGENTS

INJECT VOLUME 50µl

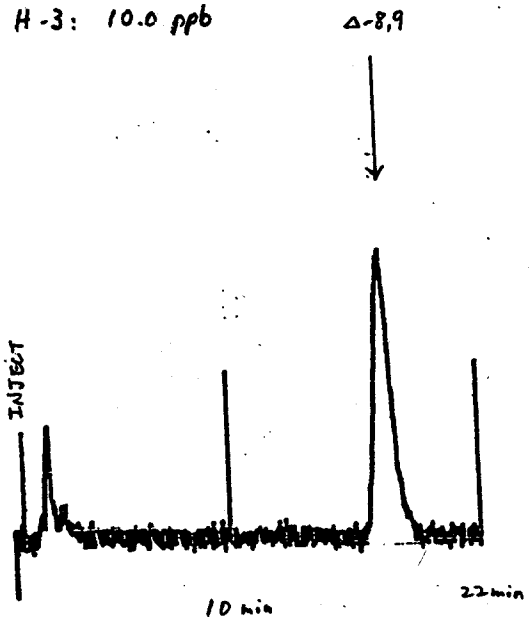
H-1: 1.0 ppb



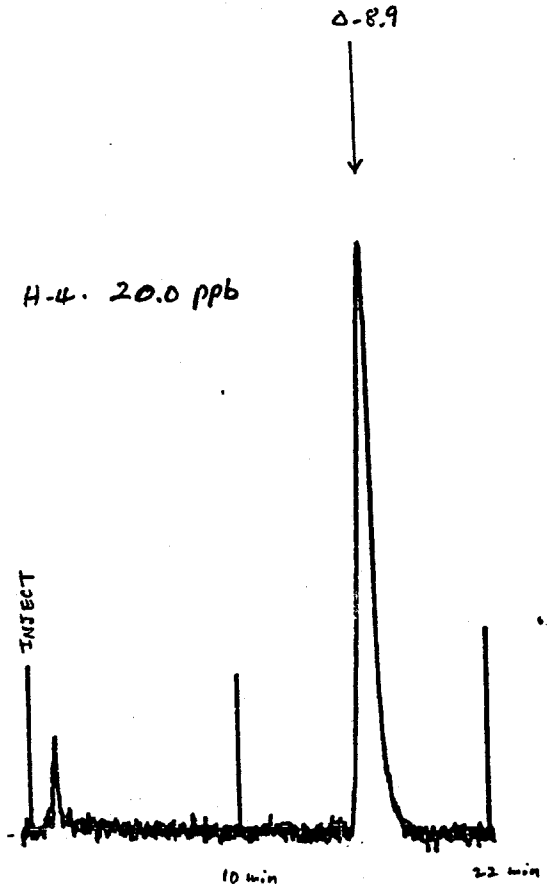
H-2: 2.0 ppb



H-3: 10.0 ppb



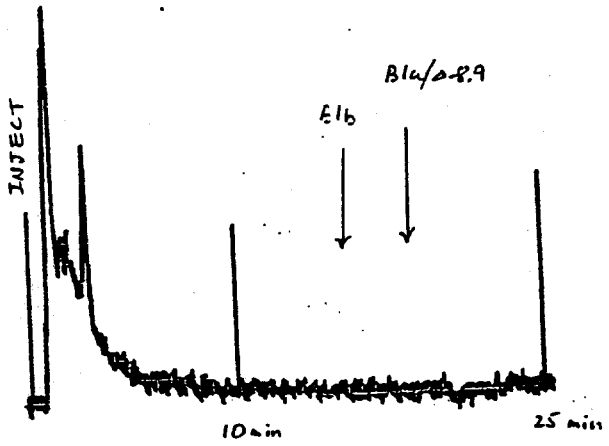
H-4. 20.0 ppb



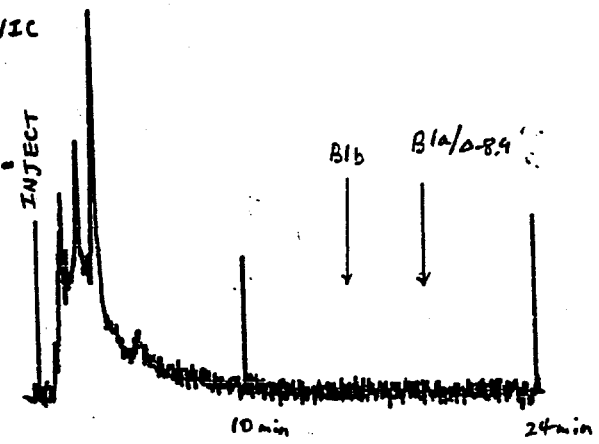
I. BLANKS

INJECT VOLUME 50µl

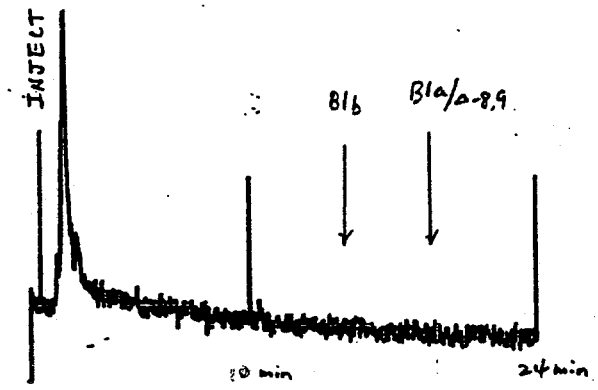
I-1: LOW ORGANIC SOIL



I-2: HIGH ORGANIC SOIL



I-3: REAGENTS



Examples of Calculations:

1. HPLC Peak Intensity:

The peak intensity is expressed in terms of peak height. The peak height, in millimeters (mm), is measured manually from the apex of the peak to the baseline drawn across the bottom of the peak.

A set of 5 standards and 5 samples were derivatized and analyzed by HPLC in the same batch. Injection volume for all standards and samples was 50 ul, therefore, no injection volume adjustment is necessary in calculations.

2. Calculation Formulas:

a. Linear regression line of standards:

$T = s\,C + k$

$C = (T - k)/(s)$

C = concentration, ng/ml
T = peak height, mm
s, k = regression line coefficients
s = slope
k = T-axis intercept

A Hewlett-Packard Model 41C calculator, with STAT PAK module, was used for obtaining s, k and R^2 (coefficient of determination) of the regression fitting.

b. Total nanograms of Avermectin B1a (or delta 8,9 isomer):

$ng = CV$

C = concentration, ng/ml, from (a) above
V = final volume of sample extract, ml

c. ppb found:

$ppb\ found = (ng)/W$

ng = total ng, from (b) above
W = sample weight, gm

d. % recovery:

$$\%\ recovery = \frac{ppb\ found}{ppb\ added} \times 100$$

3. Example - Avermectin B1a, high organic soil, fortification 20.0 ppb (Run A)

a. Standards - Chromatograms A-1, A-2. A-3, A-4, A-5

C, concentration (ng/ml)	T, peak height (mm)
10	8.8
20	15.8
40	33.8
80	59.5
100	79.8

Regression line: $T = (0.7685)C + 1.1150$ $R^2 = 0.9952$

$C = (T - 1.1150)/(0.7685)$

Sample - Chromatogram C-4

$T = 62.2$ mm

$C = (62.2 - 1.1150)/(0.7685)$

$= 79.4860$ ng/ml

b. Total nanograms - $V = 5.0$ ml

$ng = CV$

$= (79.4860)(5.0) = 397.43$ ng

c. ppb found - $W = 25.0$ gm

ppb found $= (ng)/W$

$= (397.43)/(25.0) = 15.897$

ppb added - 20.0 ppb

d. % recovery - $(15.897)(100)/(20.0) = 79.5$

4. Example - Avermectin delta 8,9 isomer, low organic soil, fortification 10.0 ppb (Run A)

a. Standards - Chromatograms E-1, E-2, E-3, E-4, E-5

C, concentration (ng/ml)	T, peak heught (mm)
10	8.0
20	16.8
40	37.5
80	61.8
100	80.5

Regression line - $T = (0.7800)C + 1.9200$ $R^2 = 0.9920$

$C = (T - 1.9200)/(0.7800)$

Sample - Chromatogram F-3

$T = 35.8$ mm

$C = (35.8 - 1.9200)/(0.7800)$

$= 43.4359$ ng/ml

b. Total nanograms -

$V = 5.0$ ml

$ng = CV$

$= (43.4359)(5.0) = 217.180$ ng

c. ppb found -

$W = 25.0$ gm

ppb found $= (ng)/W$

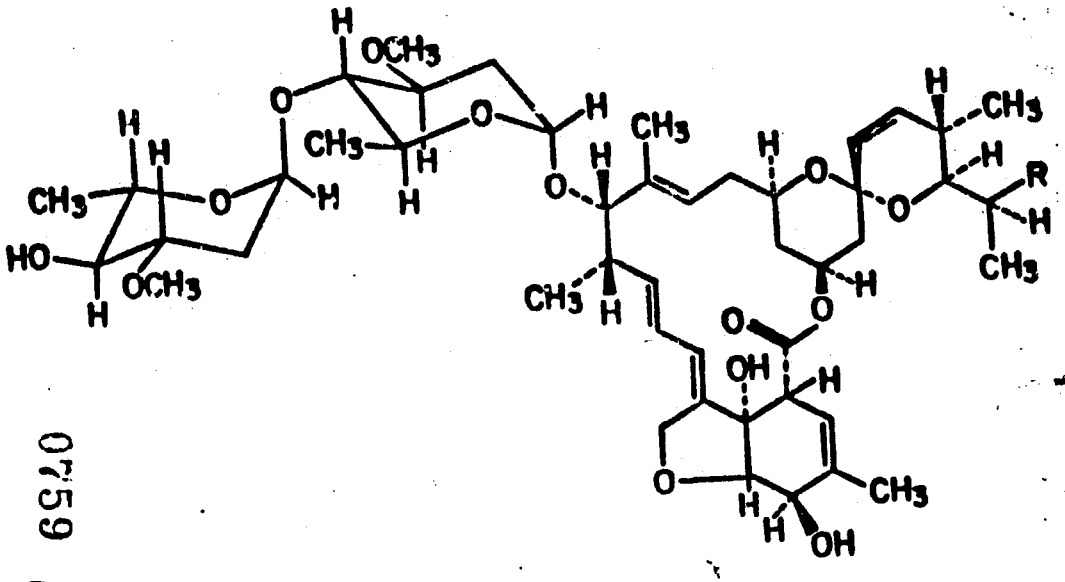
$= (217.180)/(25.0) = 8.687$

ppb added - 10.0 ppb

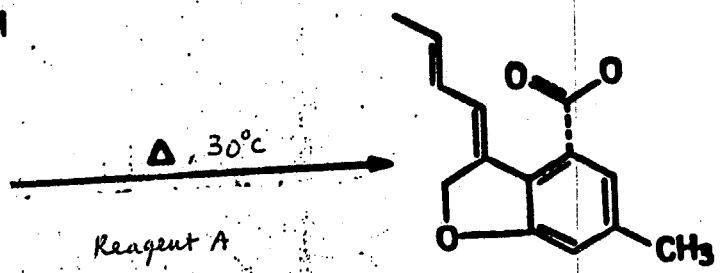
d. % recovery - $(8.687)(100)/(10.0) = 86.9$

Appendix 1

Structure of Avermectin B1 and Derivatization Reaction



R=C_2H_5 for B_{1a}
R=CH_3 for B_{1b}



Reagent A

The Fluorescing Derivative for HPLC

Reagent A :

(1) 1-Methylimidazole / Trifluoroacetic anhydride / Dimethyl formamide

(2) Methanol / Ammonium Hydroxide

Auburn University
Auburn University, Alabama 36849-5411
College of Agriculture and
Agricultural Experiment Station System

Department of Agronomy and Soils
Telephone: 844-3958
Area Code 205

MARCH 19, 1991

Reply: Soil Testing Laboratory
Auburn University
Auburn University, AL 36849-5411

Mr. Han Tai
Environmental Protection Agency
Chemistry Lab Building 1105
Stennis Space Center, MS 39529

Dear Mr. Man Tai,

Please find the two soil samples requested, and their accompanying results enclosed. Our apologies for the extended delay in answering your request, but it came at an inopportune time. This is our busiest time of the year, with many people eagerly anticipating spring planting. Also, we do not have reference soils, so we had to do analysis on a trial basis until we came up with something close to your request. The samples are identified "Low O.M. (Organic Matter)" and "High O.M.", however, you may choose to identify them differently.

We hope you will find the enclosed soils helpful. Please contact us if you have any questions regarding these soils, or if we can be of further assistance.

Sincerely,

James M. Bartos

James M. Bartos
Graduate Research Assistant

Auburn University
Auburn University, Alabama 36849-5411
College of Agriculture and
Agricultural Experiment Station System

Department of Agronomy and Soils
Telephone: 844-3958
Area Code 205

Reply: Soil Testing Laboratory
Auburn University
Auburn University, AL 36849-5411

HAN TAI
EPA CHEM. LAB BLDG. 1105
STENNIS SPACE CENTER
MS 39529

DATE OF REPORT: 3/19/91

SAMPLE ID	PARTICLE SIZE SAND	SILT	CLAY	TEXTURAL CLASS	H2O AVAIL. CM/CM
	<----------%---------->				
LOW O.M.	68.75	21.25	10	SANDY LOAM	.07
HIGH O.M.	23.75	58.75	17.5	SILT LOAM	.19

ADDTIONAL ANALYSIS REQUESTED:

SAMPLE	% ORGANIC MATTER
LOW O.M.	0.7%
HIGH O.M.	1.5%