DC Code 125501

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

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Method Validation for the Determination of Clofentezine in Soil.
Report No. RESID/81/39 MRID No. 105941

FROM: Kevin Poff, Chemist
Environmental Fate and Ground Water Branch
Environmental Fate and Effects Division (H7507C)

THRU: Henry Jacoby, Chief
Environmental Fate and Ground Water Branch
Environmental Fate and Effects Division (H7507C)

TO: Dennis Edwards
Product Manager #19
Registration Division (H7505C)

The Environmental Chemistry Section of BEAD/ACB has completed the validation of the analytical method entitled "Analytical Method for Residues NC 21 314 in Soil" The method appeared to perform to the point of not needing revisions.

The report was classified as FIFRA Confidential Business Information. Please contact the registrant to remove the CBI classification from their format and have the registrant send the unclassified method to the Laboratory Chief, USEPA/CS/ECL, Building 1105, SSC, Ms, 39529-6000, so that they may provide the method to the states.

The soil method appears to be suitable to gather residue data for Clofentezine at levels at or greater than 0.015 ppm. The Minimum detection limit (MDL) for Clofentezine in soil was estimated at 0.005 ppm and the Limit of Quantitation (LOQ) was at 0.015 ppm.

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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

SEP 12 1995

MEMORANDUM

SUBJECT: Environmental Chemistry Method Evaluation Report - Clofentezine (NC 21 314) in Soil (MRID105941) Report No. ECM0025S1

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

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

THRU: Donald A. Marlow, Chief DAM
BEAD/Analytical Chemistry Branch (7503W)

The Environmental Fate and Ground Water Branch has requested an Environmental Chemistry Method Evaluation on Clofentezine in soil. The analytical method is included in a report "Analytical Method For Residues NC 21 314 in Soil," authored by P.J. Snowdon, dated 31st July 1981, FBC Limited, Report No. RESID/81/39 (MRID No. 105941). This report has been classified as FIFRA Confidential Business Information. Please ask the technical reviewer to contact the Information Service Branch in PMSD and/or the registrant to remove the CBI classification on this method in order for us to publish the reformatted method in the new ECM manual.

Four replicate analyses were performed on soil samples at fortification levels of 0.007, 0.02 and 0.20 parts per million (ppm) and on a sample matrix blank. The attached Lab Evaluation Analysis Report, No. ECM0025S1, contains a summary, analysis results and an experimental section, including copies of representative chromatograms, calibration curve and an example calculation. Also, attached is a copy of the completed checklist evaluation for this method.

If you have any questions concerning this report, please contact Han Tai at 601 688-3252 or Aubry Dupuy at 601 688-3212.

Attachment

cc: Han Tai, Chemist ECS
Danny McDaniel, QA Coordinator, ECS

ENVIRONMENTAL CHEMISTRY METHOD EVALUATION REPORT

CLOFENTEZINE IN SOIL

ECM0025S1

ENVIRONMENTAL CHEMISTRY SECTION

ANALYTICAL CHEMISTRY BRANCH

BIOLOGICAL AND ECONOMIC ANALYSIS DIVISION

PREPARED BY: HAN TAI, Chemist — Han Tai (Signature) — Sept. 6, 1995 (Date)

REVIEWED BY: DANNY MCDANIEL, Acting QAC — Danny McDaniel (Signature) — Sep 6, 1995 (Date)

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PART I

SUMMARY AND CONCLUSION

An Environmental Chemistry Method Evaluation has been performed on the analysis of Clofentezine, Code NC 21 314, in soil. The analytical method is described in a report "Analytical Method For Residues of NC 21 314 in Soil," by P.J. Snowdon, dated 31st July, 1981, FBC Limited, Report No. RESID/81/39. MRID No. 105941. The report is classified as Confidential Business Information.

The soil sample is extracted under reflux with a 1:9 mixture of methanol: dichloromethane. The extract is cleaned-up by partitioning with water, then through a sep-pak silica cartridge. Reverse phase high pressure liquid chromatography (HPLC) is used to determine Clofentezine, on a C-18 column by UV detection at 268 nm. A compound 2,2'-Difluorobiphenyl is used as an HPLC internal standard.

Four replicate analyses have been done at each fortification level. The fortification levels, the mean percent recoveries and relative standard deviations are, respectively, 0.007 parts per million (ppm), 94.8%, 2.8%; 0.02 ppm, 94.6%, 3.0%; 0.20 ppm, 86.8%, 2.7%. The Minimum Detection Limit (MDL) is 0.005 ppm. The Limit of Quantitation (LOQ) is 0.015 ppm.

The results appear to be acceptable. No difficulties were encountered during the present laboratory work.

PART II

ANALYSIS RESULTS

Method: "Analytical Method For Residues of NC 21 314 In Soil. "P.J. Snowdon, 31st July, 1981, FBC Limited. Report No. RESID/81/39, MRID 105941

Results(1):

ppm Added	ppm Found	% Recovery	$\bar{X}$(2)	SD	RSD
0.007	0.0065	92.5			
"	0.0065	92.5			
"	0.0068	97.1			
"	0.0068	97.1	94.8	2.7	2.8
0.02	0.0192	96.0			
"	0.0195	97.5			
"	0.0188	94.0			
"	0.0182	91.0	94.6	2.8	3.0
0.20	0.1800	90.0			
	0.1700	85.0			
	0.1700	85.0			
	0.1750	87.0	86.8	2.4	2.7
0.00 (Blank)	ND (3)	___			
"	ND	___			
"	ND	___			
"	ND	___			

NOTES:

(1) Four separate analyses at each fortification level including fortification, extraction, clean-up and HPLC.

(2) $\bar{X}$ = Mean
SD = Standard Deviation
RSD = Relative Standard Deviation, (SD/x) x 100

(3) ND = Not detected
Minimun Detection Limit (MDL) - 0.005 ppm
Limit of Quantitation (LOQ) - 0.015 ppm

Calculations of MDL and LOQ are based on sample size of 20.0 gm, final extract volume 0.5 ml, marker solution concentrations 10 ug/ml, injection volume 20 ul, noise 2 mm.
MDL is 3 x noise; LOQ is 10 x noise.

PART III EXPERIMENTAL

General Description of Method:

1. Fortification - On air-dried soil matrix, 20.0 gm.

2. Extraction - reflux, 30 minutes with 100 ml 9: 1 dichloromethane/methanol, repeat extraction and combine extracts.

3. Clean-up, solvent partitioning - with 50 ml. water, twice. Discard water, concentrate in Kuderna-Danish Evaporator to 5 ml, evaporate to dryness (under nitrogen stream), dissolve residue in 2 ml hexane.

4. Clean-up, Sep-pak silica cartridge-
   First fraction - Elute with 10 ml 1:4 dichloromethane/hexane. Discard.
   Second fraction - Elute with 7 ml 3:2 dichloromethane/hexane. Evaporate to dryness. Dissolve in marker solution, 0.5 - 2.0 ml. The marker solution contains 2,2'- Difluorobiphenyl at a concentration of 10 ug/ml.

5. Calibration standards - 5 standards (0.25 - 2.0 ug/ml) Evaporate appropriate volume of stock solution to dryness, dissolve in 10.0 ml. marker solution.

6. HPLC, See below

HPLC Instrumentation

Column:	Varian micro-pak MCH-10, C-18, 4.6 x 300 mm. Serial #T-70378-18 Catalog #03-91251-44, Installed in Waters Temperature Control System at 30.0°C
Mobile phase:	75/25, v/v, methanol/water, isocratic. flow rate 1.5 ml/min
Pump:	Waters Model 590 Solvent Delivery System
Injector:	Waters Model U6K Manual Injector
Injection Volume:	20 ul for all standards and samples
Detector:	Waters Model 490 Multi-Wavelength Detector Absorbance mode: uv 268 nm; Absorbance Unit Full Scale (AUFS) 0.01; Time Constant 5.0
Recorder:	Kipp-Zonan Model BD-41 Full scale 10 mv; chart speed 5 mm/min

Retention Time: Marker, 2,2'-Difluorobiphenyl 7.0 - 7.2 minutes
Analyte, Clofentezine 11.5 - 11.7 minutes

Source of Analytical Standards

Clofentezine - EPA RTP Repository, Research Triangle Park, N.C. 27709
03877, Lot FZB2, Purity 99.5%

2.2'-Difluorobiphenyl - Chem Service, 660 Tower Lane, West Chester PA 19381
F-400. Lot 140-106A, Purity 99.0%

Source of Sample Matrix

Soil file sample, obtained from Agricultural Experimental Station, Auburn University AL. The soil is classed as silt loam, with composition of 23.75% sand, 58.75% silt, 17.5% clay, 1.5% organic matter. The soil was kept in a freezer at 0°C.

Comments

1. Fortification:

   The fortification levels used in the ECS lab evaluation were: 0.007 ppm (ECS estimated MDL), 0.02 ppm (registrant claimed LOQ), and 0.2 ppm (10 x LOQ).

2. Retention Time:

   The Varian MCH-10 column used in this evaluation produced longer retention times than the ones listed in the method dated in 1981 (7.1 vs 4.4 minutes and 11.5 vs 6.5 minutes for marker and analyte, respectively). This may indicate possible higher column efficiency (more theoretical plates) for the present column, reflecting the changing column packing technology during the past decade, even though they carry the same column model number.

3. Processing one sample from extraction to final extract solution required about 3 hours. One set of 4 samples and 5 standards requires about 24 working hours (3 regular working days) for completion, including extraction, cleanup, HPLC analysis and calculation of results.

Chromatograms

1. Standards

   1-1 0.25 ug/ml

   1-2 0.50 ug/ml

   1-3 1.00 ug/ml

   1-4 1.50 ug/ml

   1-5 2.00 ug/ml

   1-6 Calibration Curve

2. Samples

   2-1 Soil, blank

   2-2 Soil, fortified 0.007 ppm

   2-3 Soil, fortified 0.02 ppm

   2-4 Soil, fortified 0.20 ppm

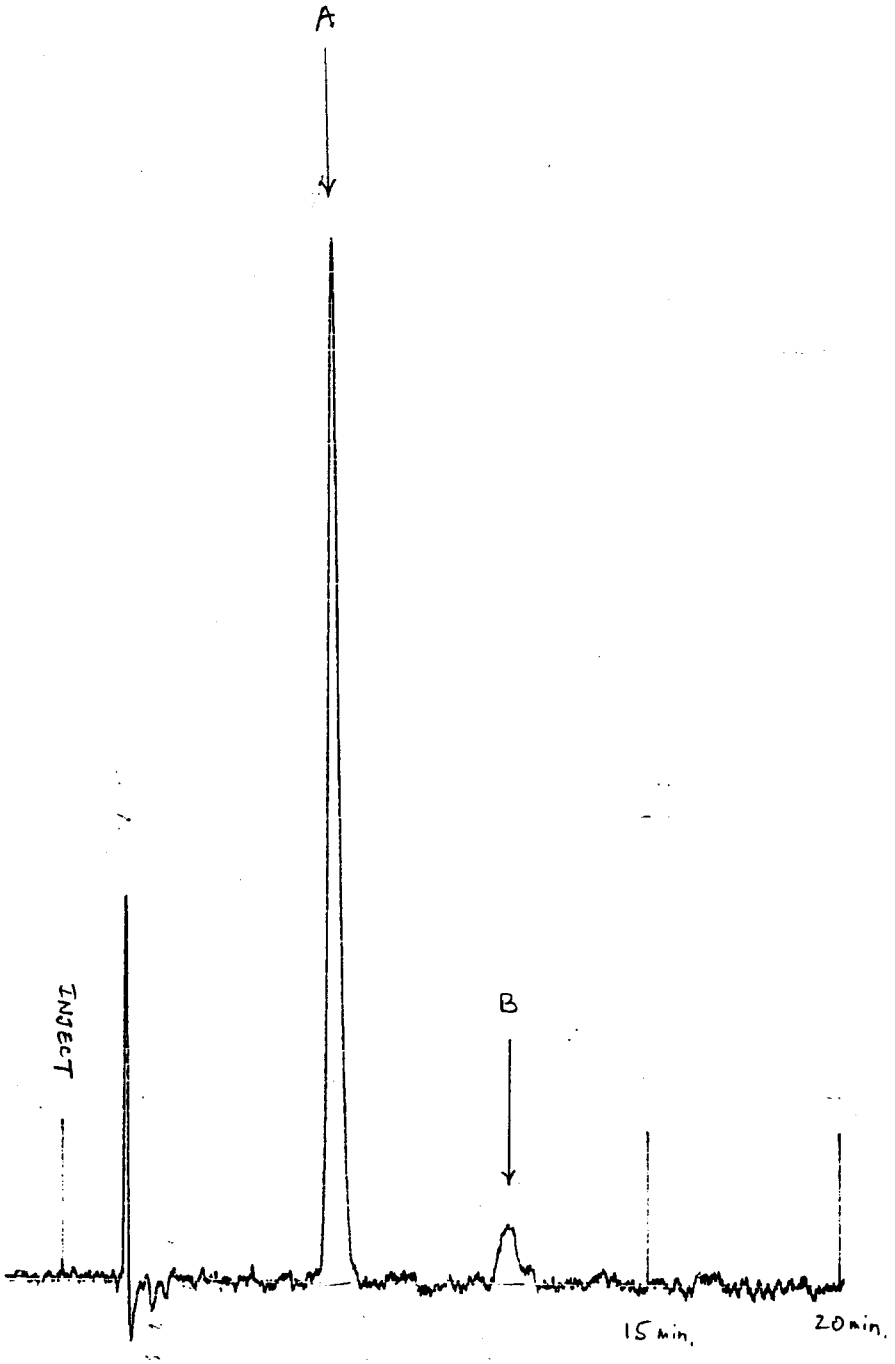
   Note: Arrows indicate position of HPLC peak

   A - Internal Standard (Marker), 2,2'-Difluorobiphenyl

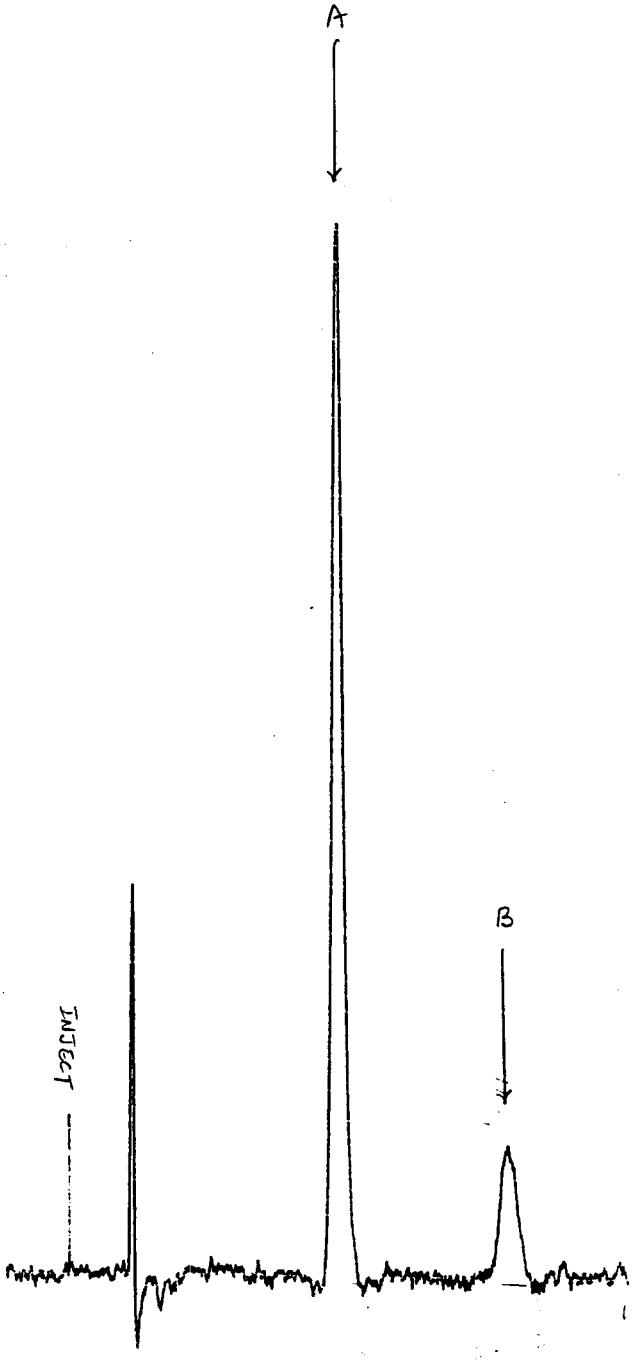
   B - Analyte, Clofentezine

1. STANDARDS

1-1: 0.25 µg/ml

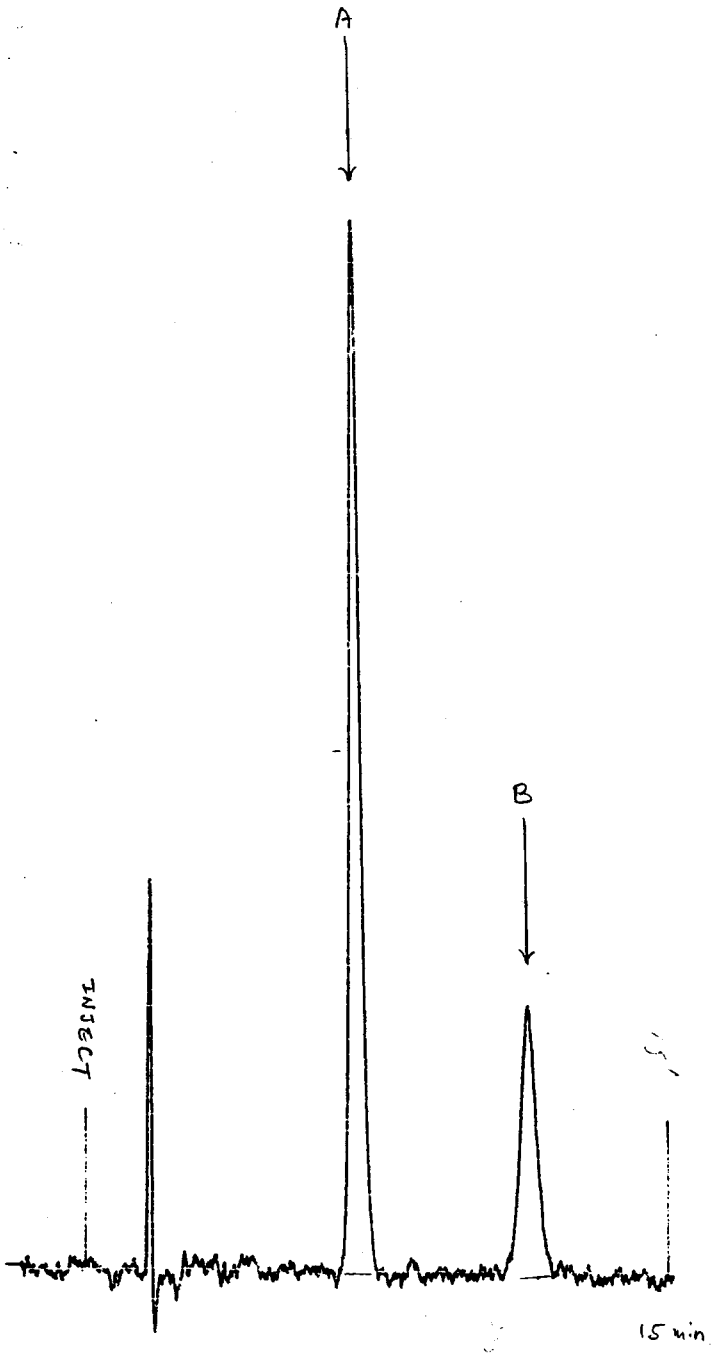


1-2: 0.50 µg/ml

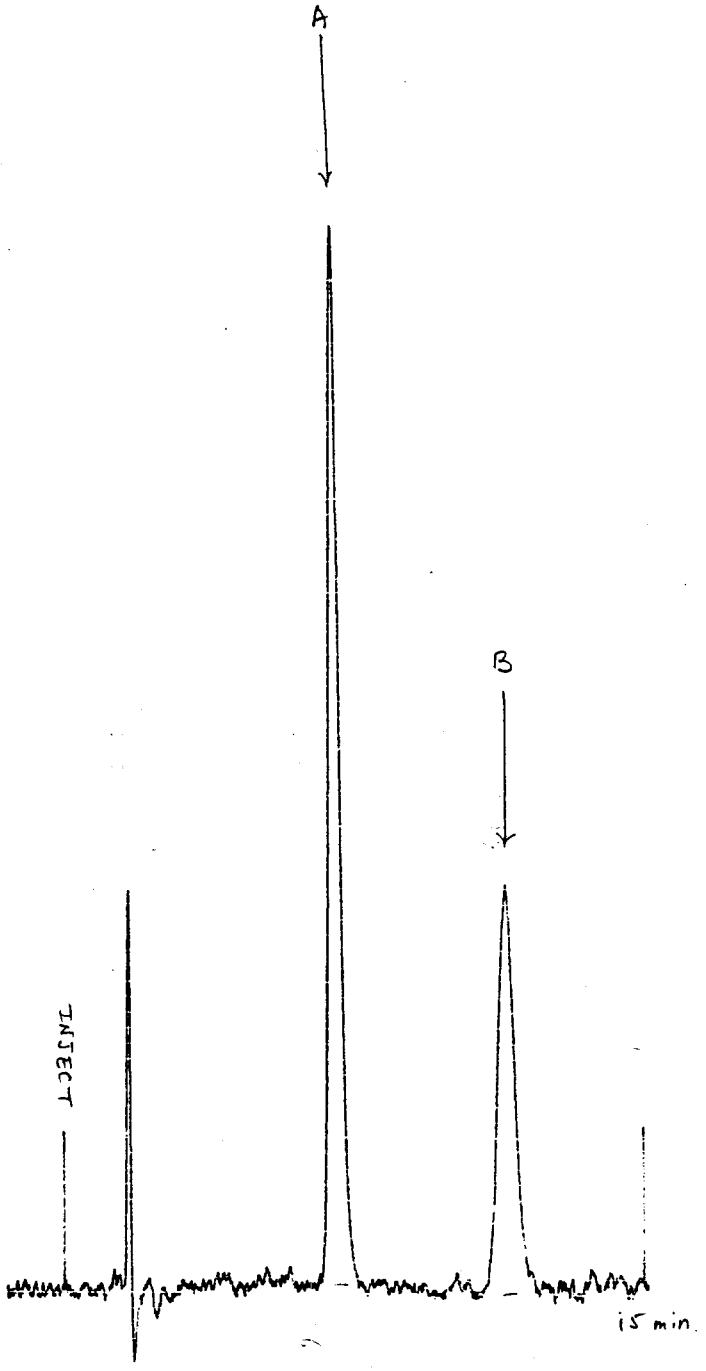


1. STANDARDS

1-3: 1.00 µg/ml

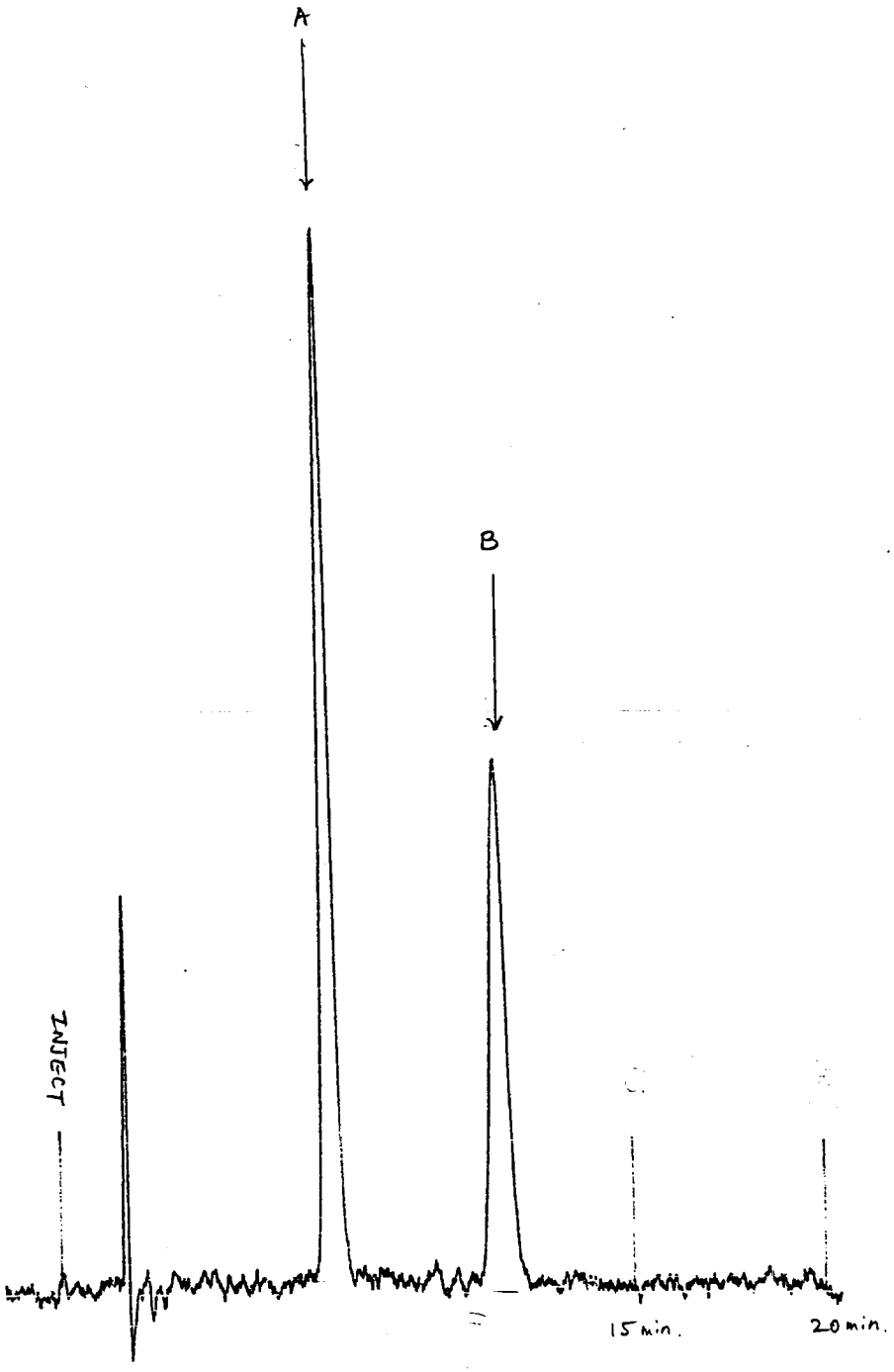


1-4: 1.50 µg/ml

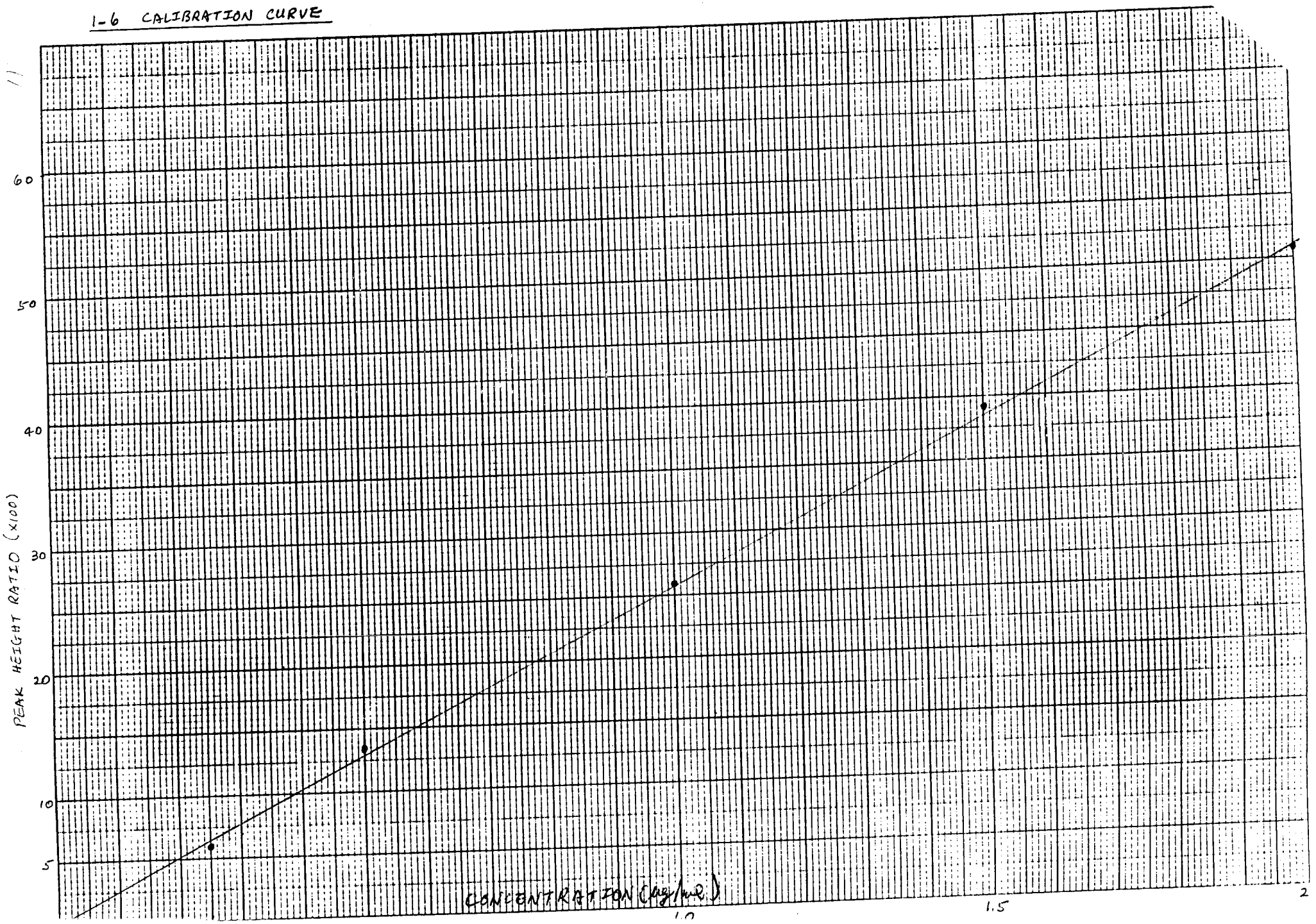


1. STANDARDS

1-5: 2.00 µg/ml



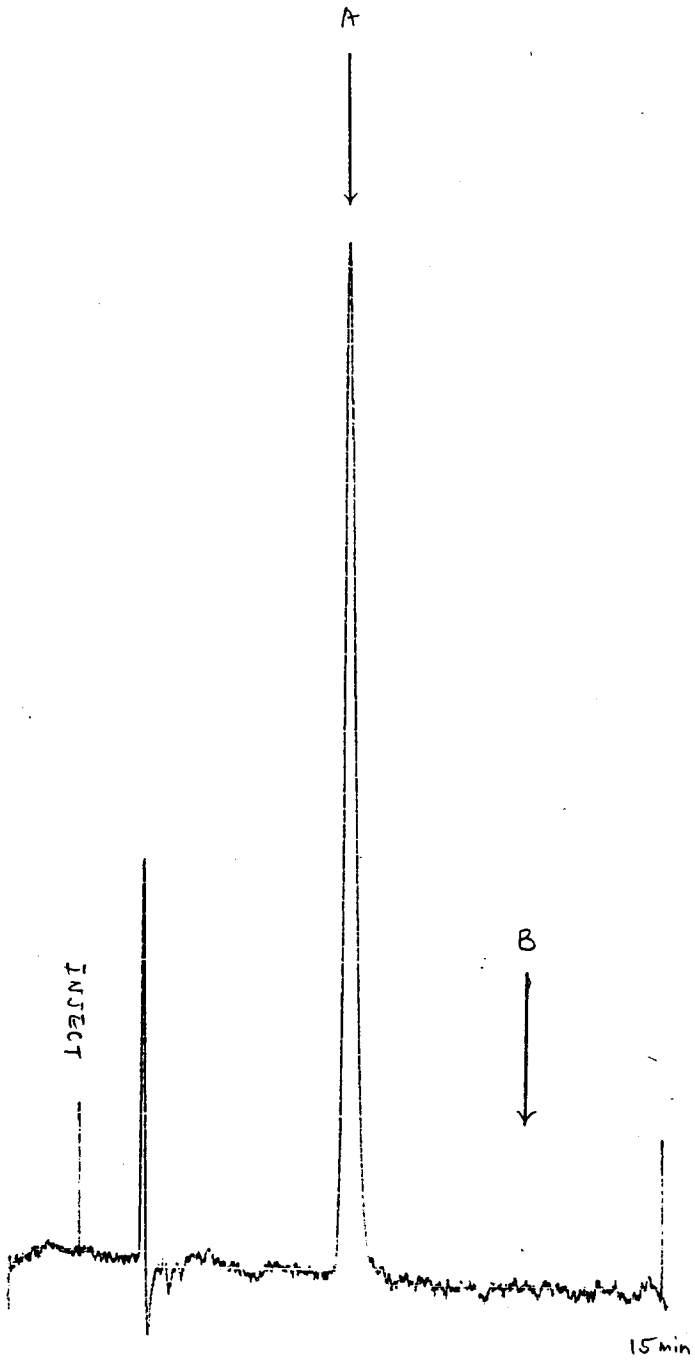
1-6 CALIBRATION CURVE



2 SOIL SAMPLES

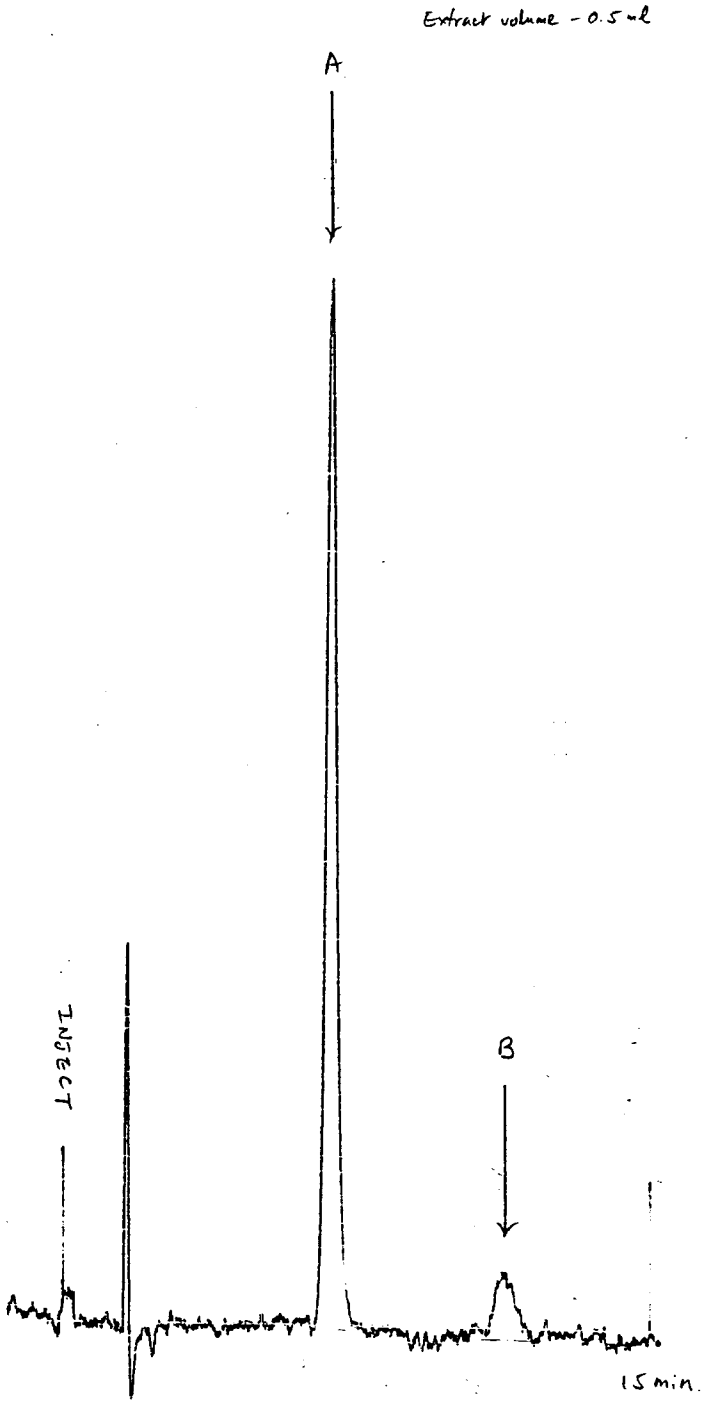
2-1: BLANK

Extract volume - 0.5 ml



2-2: FORTIFIED 0.007 ppm

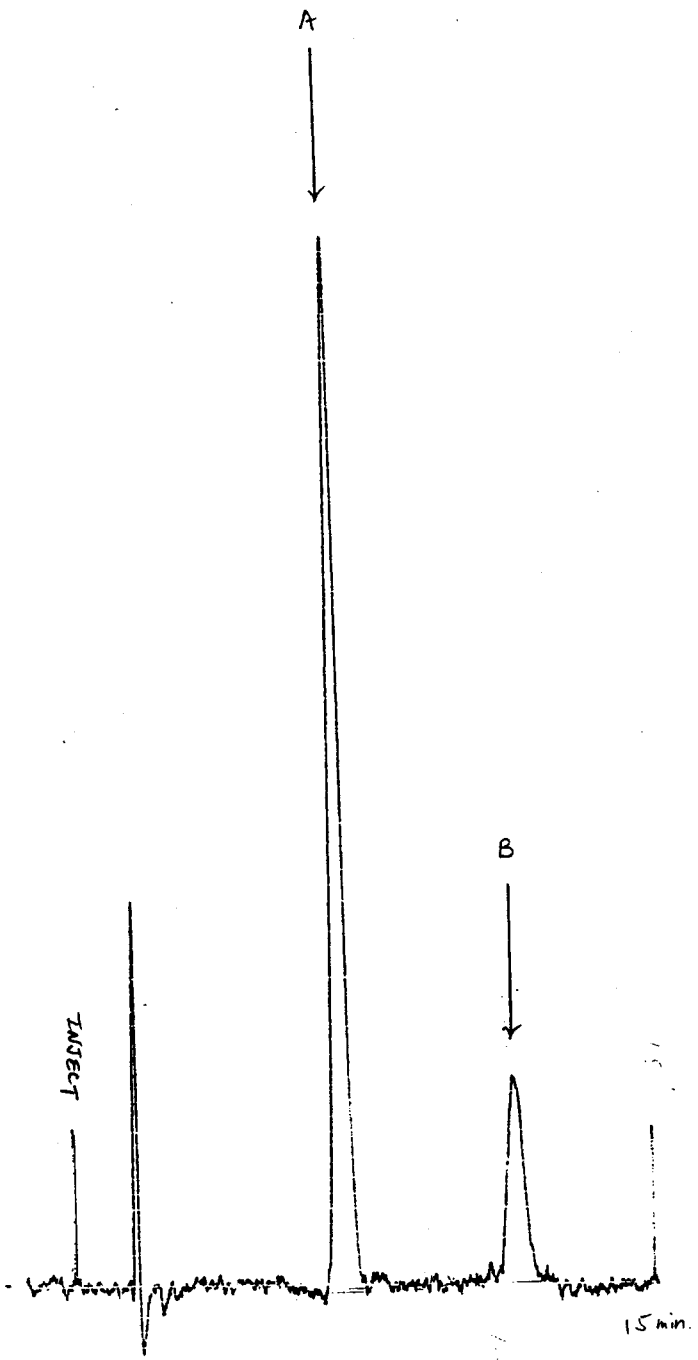
Extract volume - 0.5 ml



2 SOIL SAMPLES

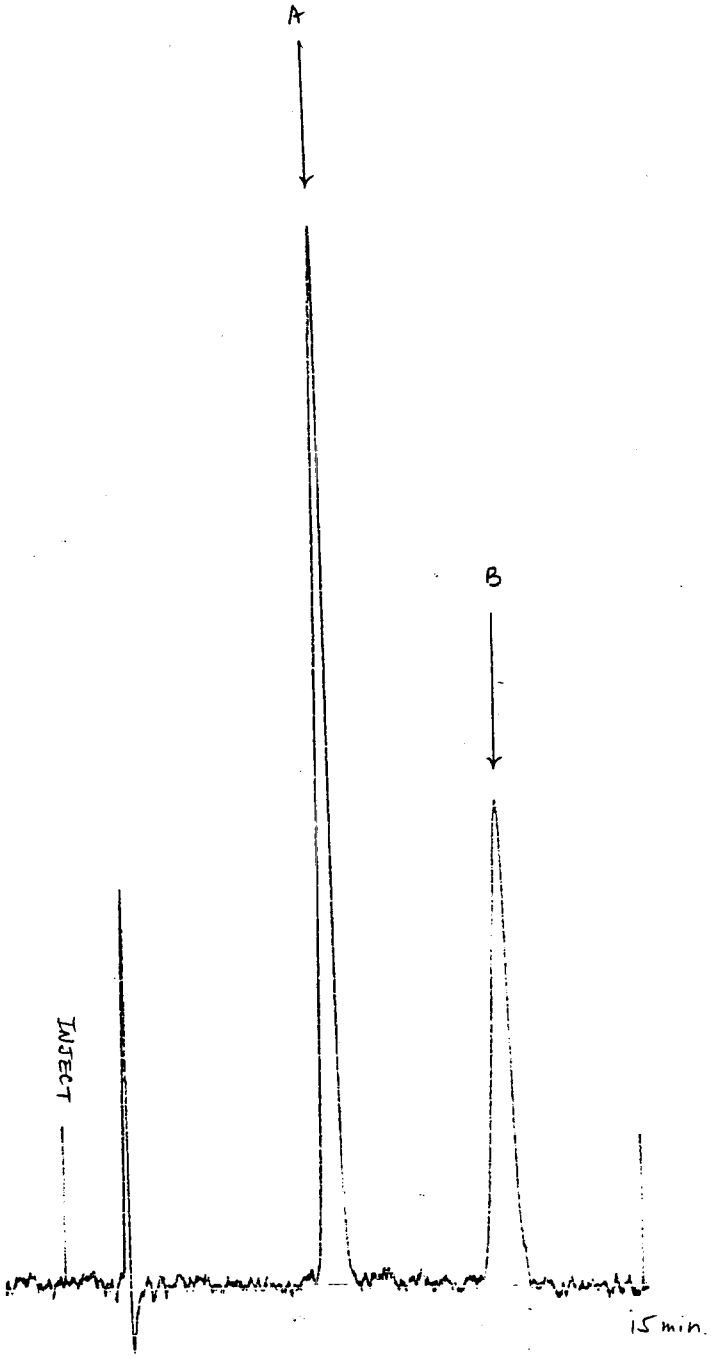
2-3: FORTIFIED 0.02 ppm

Extract volume 0.5 ml



2-4: FORTIFIED 0.20 ppm

Extract volume 2.0 ml



Example of Calculation

1. Peak Height - The distance, in millimeters (mm), measured from the apex to the baseline of the HPLC peak. Adjustment for injection volume is not needed because the volume was kept constant at 20 ul for all standards and samples.

2. Calculation Formula (Section 4.6, p.40):

   a. Peak height ratio = (B/A) x 100

      A = Peak height of the marker, mm.

      B = Peak height of the analyte, mm.

   b. Standard Calibration Curve - plot ratio vs concentration (ug/ml) of standards.

   c. Sample concentration (C) - read from Calibration Curve.

   d. PPM Found = (C)(V)/(W)

      C - Concentration (ug/ml)

      V - Volume of final extract, ml.

      W - Weight of soil sample, gm.

   e. % Recovery = (ppm Found) x 100/(ppm added)

3. Example

a. Standards Calibration Curve (Fig. 1-6)

Concentration (ug/ul)	A (mm) marker	B (mm) Analyte	(B/A) x 100	chromatogram
0.25	135	8	5.9	1-1
0.50	136	18	13.2	1-2
1.00	135	35	25.9	1-3
1.50	136	53	39.0	1-4
2.00	138	70	50.7	1-5

b. Sample fortified at 0.20 ppm (chromatogram 2-4)

(1) Peak height A = 137 mm; B = 63 mm

(2) Ratio (B/A) x 100 = (63/137) x 100

= 46.0

(3) Concentration from Calibration Curve (Fig 1-6),

C = 1.80 ug/ml

Final extract Volume(V) = 2.0 ml; Sample weight W = 20.0 gm

(4) ppm Found = (C)(V)/(W) = (1.80)(2.0)/(20.0) = 0.180 ppm

ppm added = 0.20 ppm

(5) % Recovery = (0.180)(100)/(0.20) = 90.0

Clofentezin

ATTACHMENT 1

164-1 **ENVIRONMENTAL CHEMISTRY METHODS (ECMs) PROGRAM**
STANDARD EVALUATION PROCEDURE (SEP) CHECKLIST
1980-81 **BACKGROUND AND INITIAL REVIEW INFORMATION**

I. Background Information

A. **Title of Method** Residue Decline in Soil Following Application of NC 21 314 To Bare Plots in Texas, 1980

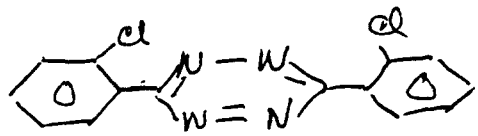
B. ~~**ECS No.**~~

C. **MRID or TRID No.** 105 941

D. **Matrix(es)** Soil

E. **Analyte(s) detected** Clofentezine

Sponsor: FBC Ltd.



Limit of Determination

P 28 Sec 1.8 2. ~~Method Detection Limit (MDL)~~ Claimed in Method _____ Estimated _____

H. Recovery ~~(Accuracy) Data~~

P33 Sec 5.1 Limit of Determination 0.006 mg/kg. in clean chromatogram. Generally set at 0.01 or 0.02 mg/kg.

P 28 Sec. 1.6 P34 Recovery 79.9% n = 37

P28 Sec 1.7 P34 I. Precision Data 14.0%

IV. Detailed Information about the Method	Yes	No	Review Further
A. Is the method marked CONFIDENTIAL?	✓		
B. Is it the most up-to-date method?			✓
C. Does the method require spiking with the analyte(s) of interest?	✓		
D. If the method requires explosive or carcinogenic reagents, are proper precautions explained?			✓
E. Is the following information supplied?			
1. Detailed stepwise description of			
a. The sample preparation procedure	✓		
b. The sample spiking procedure	✓		
c. The extraction procedure	✓		
d. The derivatization procedure		N/A	
e. The cleanup procedure	✓		

		Yes	No	Review Further
	8. Description and/or explanation of			
P29 Sec 3	a. Areas where problems may be encountered?	✓		
	b. Steps that are critical?	✓		
	c. Interferences that may be encountered?			✓
P3	9. Characterization of the matrix(es)	✓		
V.	Representative Chromatograms			
	A. Are there representative chromatograms for			
P35 ✓	1. Analyte(s) in each matrix at the MDL, LOQ, and 10 x LOQ?		✓	
37	2. Method blanks?		✓	
	3. Matrix blanks?		✓	
	4. Standard curves?		✓	
	5. Standards that can be used to recalculate some of the values for analyte(s) in the sample chromatograms?		✓	
	B. Can the responses of the analyte(s) in the chromatograms of the lowest spiking level be accurately measured?		✓	
VI.	Good Laboratory Practice Standards (GLP)			
	A. Is there a statement of adherence to the FIFRA/GLP? Too early		✓	
VII.	Completeness			
	A. Has enough information been supplied to do a proper review?	✓		
	B. Has enough information been supplied to do a laboratory evaluation, if requested?	✓		
	C. Are there any steps in the method that are not scientifically sound?		✓	
	D. Is a confirmatory method or technique provided?		✓	