B 99(88-93)

ATTACHMENT 4

VOLUME 111 OF 136 OF SUBMISSION

CGA-184927 & CGA-185072

443992-11

TITLE

DETERMINATION OF RESIDUES OF PARENT COMPOUNDS BY LIQUID CHROMATOGRAPHY (HPLC) [WHEAT]

DATA REQUIREMENT

GUIDELINE NO. 171-4 (C)
(NEW GUIDELINE NO. 860-1340)

STUDY DIRECTOR

DR. E. ALTENBURGER

DATE COMPLETED

March 19, 1990

PERFORMING LABORATORIES

Agro Division /Residue Analysis
Novartis Crop Protection AG (Formerly Ciba -Geigy Limited)
Basle, Switzerland

LABORATORY PROJECT IDENTIFICATION

ANALYTICAL METHOD REM 138.01
NEXUS STUDY NUMBER 357-90

Novartis Crop Protection, Inc.
Post Office Box 18300
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VOLUME 1 OF 1 OF REPORT

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

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

Company Novartis Crop Protection, Inc

Company Representative Karen S Stumpf

Title Senior Regulatory Manager

Karen S. Stumpf
Signature

9/8/97
Date

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STATEMENT CONCERNING GOOD LABORATORY PRACTICES

The document in this submission, is an analytical method and is not considered a final report because validation of the method is not included Therefore, certification of compliance with Environmental Protection Agency's Good Laboratory Practice Standards (40 CFR Part 160, October 16, 1989) is not required

Robert K Williams
Agent of Submitter/Sponsor
And Manager, Residue Chemistry
Human Safety Department

Date 9/5/97

CGA 184927 (HERBICIDE)
CGA 185072 (SAFENER)

DETERMINATION OF RESIDUES OF PARENT COMPOUNDS BY LIQUID CHROMATOGRAPHY	PLANT MATERIAL SOIL	March 19, AG 2.53/AB

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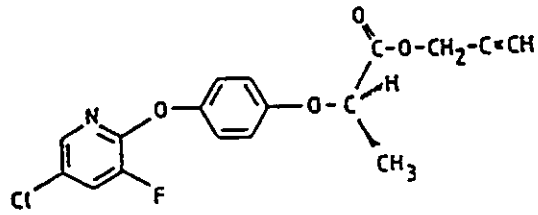
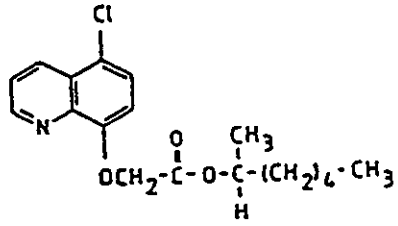
CHEMICAL STRUCTURES

CGA 184927



CGA 185072



1. INTRODUCTION

1.1 Scope of the Method

CGA 184927 is an experimental herbicide, applied postemergence to weeds. It provides excellent control of annual grasses in cereals and barley).
CGA 185072 is an experimental safener compound, being developed to full crop tolerance with CGA 184927.
This method allows residues of CGA 184927 and CGA 185072 to be det and quantitated at lower practical levels of 0.02 mg/kg in plant ma and 0.05 mg/kg in soil.

1.2 Principle of the Method

PLANT MATERIAL: homogenized samples are extracted with acetonitrile. Fa coextracts are removed by partitioning into hexane. The analytes are cle up subsequently by solid phase extraction on a C-18 cartridge, reextracti into hexane-diethyl ether and a second solid phase extraction step on a si ca cartridge. CGA 184927 and CGA 185072 are eluted in separate fractions and determined separately on a 2-column switch HPLC system with UV-detection. ~~SOIL: deactivated samples are extracted with acetone and - from the diluted extract - the analytes are partitioned into hexane-diethyl ether. Determination is done without further cleanup by HPLC with UV-detection using a single column system.~~

2. MATERIALS AND METHODS

Standard laboratory equipment is not listed. All equipment and chemicals mentioned herein can be substituted by suitable products of any origin. Prove suitability of reagents by analyzing reagent blanks.

2.1 Equipment

2.1.1 Sample concentrator DB-3 (Techne Ltd., Duxford, Cambridge, England)
2.1.2 Laboratory Shaker, Type KL-2 (E. Bühler, Tübingen, FRG)
2.1.3 High speed homogenizer, type Ultra-Turrax (Janke & Kunkel, Staufen i. Br., FRG)
2.1.4 Vacuum manifold to accommodate solid phase extraction cartridges (build in-house)

2.2 Reagents

Main suppliers' addresses: - Fluka Chemie AG, CH-9470 Buchs.
- E. Merck AG, D-6100 Darmstadt.

2.2.1 Acetone, analytical grade (Merck # 14)
2.2.2 Acetonitrile, HPLC grade (Fluka, # 692)
2.2.3 Diethyl ether, analytical grade (Merck, # 921)
2.2.4 Ethanol, Uvasol (Merck, # 980)
2.2.5 n-Hexane, analytical grade, (Merck, # 4371)
2.2.6 Methanol, analytical grade, (Merck, # 6009)
2.2.7 Sodium chloride, analytical grade (Merck, # 6404), saturated aqueous solution
2.2.8 Tetrahydrofuran, for HPLC, (Fluka, # 87367)
2.2.9 Water, deionized grade (prepared inhouse)
2.2.10 C-18 Bond Elut solid phase extraction cartridge, 500 mg/3 mL; N° 607303 (Analytichem Int. Co., Harbor City, CA 90710, USA)
2.2.11 Silica Sep-Pak solid phase extraction cartridge, No. 51900; Waters/ Millipore Co., Milford, MA 01757, USA)

2.2.12 CGA 184927 reference substance for standardisation :
Analytical Master Standard, 99.5 % (CIBA-GEIGY).
CGA 185072 reference substance for standardisation a
Analytical Master Standard, 99.4 % (CIBA-GEIGY).

2.2.13 Standard Solutions

Prepare stock solutions (200 µg/mL) of CGA 184927 and CG
in acetonitrile.

For preparation of standard solutions calculate the lowest
concentration as follows:

$$C = \frac{L \times W \times Va}{Ve \times Vf} \quad [\mu g/mL]$$

L is lower practical level [
W is weight of subsample
Va is volume of aliquot cleaned up [
Ve is volume of extract solution [m
Vf is final volume for determination [mL

Examples are
Cereal (grain): C = 0.02 x 25 x 10 / 250 x 2 = 0.01 µg/mL.
Cereal (straw): C = 0.05 x 10 x 10 / 250 x 2 = 0.01 µg/mL.
~~Seed~~ Forage : C = 0.05 x 25 x 10 / 250 x 4 = 0.0125 µg/mL.
10 x 10/250 x 2 =

Prepare at least four standard solutions of different concentrations of either compound by diluting the stock solutions with mobile phase (section 2.4.1). Evaporate acetonitrile of the stock solution prior to dilution (in air stream at ~ 40 °C). Select the concentrations as required. Typical values are: 0.01, 0.02, 0.05, 0.1, 0.2 µg/mL.

2.3 Analytical Procedure

2.3.1 Preparation of Samples and Subsamples

Prepare a representative, homogeneous laboratory sample from the field treated samples. Store the laboratory sample at freezer temperature until analysis.
GRAIN: weigh a subsample of 25 g into a 250 mL wide-mouth flask.
STRAW: weigh a subsample of 10 g into a 250 mL wide-mouth flask.
~~SOIL~~ : weigh a subsample of 25 g into a 250 mL wide-mouth flask.

2.3.2 Fortification

To regularly check the performance of the method, analyze also two fortified control samples with each series of analyses. To prepare these samples, add known amounts of CGA 184927 and CGA 185072 to uncontaminated control samples prior to extraction. Select the fortification levels to be either two- and ten- to twenty-times the lower practical level of determination, or in the range of the expected residues.

Prepare the following fortification solutions of CGA 184927 and CGA 185072, each, by appropriate dilution of the stock solution (section 2.2.13) with acetonitrile:

[1] ---> 5.0 ug/mL; [2] ---> 0.5 ug/mL

Fortify untreated control subsamples by adding the following amount of fortification solutions of either compound to each subsample:

GRAIN: 2.0 mL of [1] for 0.4 mg/kg recovery
2.0 mL of [2] for 0.04 mg/kg recovery
STRAW: 1.0 mL of [1] for 0.5 mg/kg recovery
2.0 mL of [2] for 0.1 mg/kg recovery
SOIL : 5.0 mL of [1] for 1.0 mg/kg recovery
5.0 mL of [2] for 0.1 mg/kg recovery

2.3.3 Extraction

GRAIN, STRAW: Add 100 mL of acetonitrile to the subsample (section 2.3.1). Mazerate the suspension with a high speed homogeniser for 2 minutes or shake for 1 hour at about 300 cycles/min.
Filter the suspension through a Buchner funnel into a 250 mL round bottom flask, using slight suction. Wash filter cake and funnel twice with 25 mL portions of acetonitrile. Transfer the combined filtrate and washings to a 250 mL volumetric flask and adjust the volume to the mark by adding acetonitrile. Transfer an extract aliquot of 10 mL (corresponding to 1 g of crop) to a 25 mL graduated test tube and proceed with cleanup according to section 2.3.4.

SOIL: Add 25 mL of water to the subsample (section 2.3.1) for desactivation. Add 100 mL of acetone to the subsample. Shake for 1 hour at about 300 cycles/min.
Filter the suspension through a Buchner funnel into a 250 mL round bottom flask, using slight suction. Wash filter cake and funnel with 25 mL of acetone. Transfer the filter cake back to the flask, add 25 mL of acetone, and shake again for 30 minutes. Filter again through the funnel, wash the cake with 25 mL of acetone and transfer the combined filtrate and washings to a 250 mL volumetric flask. Adjust to the mark with acetone. Transfer an extract aliquot of 10 mL (corresponding to 1 g of soil) to a 25 mL graduated test tube and proceed with cleanup according to section 2.3.6.

2.3.4 Cleanup by Hexane Partitioning (for Removing Fatty Coextracts)

Use this step for PLANT MATERIAL samples.
Add 3 mL of hexane to the test tube with the acetonitrile extract aliquot (see section 2.3.3). Shake thoroughly, pipette off and discard the supernatant hexane phase. Repeat this extraction step with a second 3 mL portion of hexane. Discard this hexane, too. Reduce the remaining acetonitrile solution to 5 mL in a gentle stream of air at ~ 40°C. Apply a further cleanup step according to section 2.3.5.

2.3.5 Cleanup by Solid-Phase Extraction on C-18 Cartridge

Use this step for PLANT MATERIAL samples.
Condition a C-18 solid phase extraction cartridge first with 2 mL of methanol, then with 2 mL of water-acetonitrile (75+25; v/v). Discard both eluates.
To the extract solution of the previous extraction step (section 2.3.4) in the 25 mL test tube add water up to 20 mL mark. Transfer the solution to the cartridge and let it pass dropwise through the cartridge. Discard the eluate. Elute both analytes with 2 mL of acetone. Collect this eluate in a 25 mL test tube and proceed with the next cleanup step according to section 2.3.6.

2.3.6 Cleanup by Reextraction

Use this step for PLANT MATERIAL and SOIL.
To the solution from the C-18 partition (see section 2.3.5; plant material) or from soil extraction (section 2.3.3) in a 25 mL test tube add water up to the 16 mL-mark. Add 4 mL of sodium chloride solution. Add 3 mL of a mixture of hexane-diethyl ether (9+1; v/v) and extract the analytes into the organic solvent by vigorous shaking. Transfer the supernatant organic phase into a 10 mL graduated test tube. Repeat the extraction procedure twice with 3 mL of the same solvent mixture. Combine the organic phases in the test tube and proceed as follows:

- PLANT MATERIAL: continue with cleanup according to section 2.3.7.

- SOIL: adjust the solvent volume to 10 mL with hexane. Transfer twice an aliquot of 4 mL (corresponding to 0.4 g soil) to 5 mL test tubes a) and b), for subsequent separate determination of CGA 184927 and CGA 185072. Evaporate the solvent in tubes a) and b) in a gentle stream of air (at ~ 40 °C) and redissolve the residue in 2 mL of the appropriate mobile phase (section 2.4.1) for final quantitation. Use tube a) for determination of CGA 184927 and tube b) for determination of CGA 185072 in soil.

2.3.7 Cleanup by Solid Phase Extraction on Silica Cartridge

Use this step for PLANT MATERIAL samples.
Transfer the solution from the reextraction step (see section 2.3.6) to a silica Sep-Pak cartridge. Allow it to pass dropwise through the cartridge. Collect the eluate. Continue elution with 9 mL of hexane-diethyl ether (9+1,v/v). Collect and combine this eluate with the pre-eluate to form eluate I (containing CGA 184927).
Continue elution with 6 mL of hexane-acetone (8+2;v/v); collect this portion as eluate II (containing CGA 185072).
Evaporate eluates I and II in a gentle stream of air (at ~ 40 °C).
Redissolve the residues in 2 mL of the appropriate mobile phase for quantitation according to section 2.4.1.

2.4 Instrumentation

2.4.1 High Performance Liquid Chromatographic System

2.4.1.1 For DETERMINATION of CGA 184927 and CGA 185072 in SOIL SAMPLES use a HPLC single-column system with UV-detector, pump and autosampler-injector as follows or with suitable equivalents.

Detector:	Kratos Spectroflow 773 UV/VIS Detector (Applied Biosystems, Ramsey, NJ 07446, USA)
Pump:	Dual piston pump model 420 (KONTRON Instruments AG)
Sampling system:	Automatic Sampling System MSI 660 (Kontron Instruments AG, CH-8010 Zürich, Switzerland) with Rheodyne injection valve model 7010
Recorder:	Dual channel recorder (ABB Goerz AG, SE 120, A-1101 Wien); sensitivity set to 10 mV full scale; chart speed: 0.5 cm/min

Optional (for system automation): control and data collection unit, HP 3357 Laboratory Automation System, from Hewlett-Packard model 7010

For CGA 184927 --> Column: Stainless steel, 25 cm length, 4.6 mm id. packed with Nucleosil-NH2, particle size 5 um (Machery-Nagel AG, CH-4702 Oensingen, Switzerland)
Mobile phase: n-hexane-tetrahydrofuran (90+10; v/v)
Flow rate: 1.0 mL/min
Detector wave length: 226 nm
Sensitivity: 0.004 aufs
Retention time: ~ 8 minutes
Volume injected: 100 - 200 µL

For CGA 185072 --> Column: Stainless steel, 25 cm length, 4.6 mm id. packed with Nucleosil-NH2, particle size 5 um (Machery-Nagel AG, CH-4702 Oensingen, Switzerland)
Mobile phase: n-hexane-ethanol (100+2; v/v)
Flow rate: 0.9 mL/min
Detector wave length: 244 nm
Sensitivity: 0.005 aufs
Retention time: ~ 7.5 minutes
Volume injected: 100 - 200 µL

2.4.1.2. For DETERMINATION of CGA 184927 and CGA 185072 in PLANT MATERIAL SAMPLES use a HPLC dual-column switch-system with UV-detector, two pumps and automated sampling/injection/switching unit as follows or with suitable equivalents.

Detector: Kratos Spectroflow 773, UV/VIS Detector (Applied Biosystems, Ramsey, NJ 07446, USA)

Sampling system: Liquid sample injector/column switching unit made in-house, or, e.g. KONTRON autosampler/injector Mod 360 (KONTRON Instruments AG, CH-8010 Zürich, Switzerland) with 2 low-dead volume valves for LC-injection and column switching, model C6W (VICI AG, CH-6214 Schenkon, Switzerland).

Pumps 1 + 2: Dual piston pump model 420 (KONTRON Instruments AG)

Recorder and option for automation: as above

For CGA 184927 --> Column 1: Stainless steel, 25 cm length, 4.6 mm id. packed with Nucleosil-NH2, particle size 5 um (Machery-Nagel AG CH-4702 Oensingen, Switzerland)
Column 2: do. packed with Nucleosil 50, particle size 5 um (Machery-Nagel AG)
Mobile phases 1 + 2: n-hexane-tetrahydrofuran (9+1; v/v)
Flow rates pump 1 + 2: 0.8 mL/min
Detector wave length: 226 nm
Sensitivity: 0.006 aufs
Retention time: ~ 18 minutes
Volume injected: 100 - 200 µL

For CGA 185072 --> Column 1: Stainless steel, 25 cm length, 4 6 mm id. packed with Nucleosil-NH2, particle size 5 um (Machery-Nagel AG CH-4702 Oensingen, Switzerland)
Column 2: do. packed with Nucleosil 50, particle size 5 um (Machery-Nagel AG)
Mobile phases 1 + 2: n-hexane-ethanol (100+2; v/v)

Flow rate pump 1:	0.9 mL/min
Flow rate pump 2:	1,1 mL/min
Detector wave length:	244 nm
Sensitivity:	0.010 aufs
Retention time:	~ 18 minutes
Volume injected:	100 - 200 uL

Operation: Determine actual switching times each time before starting a series of sample analyses. Connect the outlet of column 1 directly to the UV-detector. Inject 100 or 200 µL of the 0.01 µg/mL standard solution (section 2.2.13) and determine beginning and end of the elution (transfer time) of the respective compound from column 1.
Typical switching times are:

CGA 184927	begin of cut: ~ 10 min	end of cut: ~ 11 min
CGA 185072	begin of cut: ~ 7.5 min	end of cut: ~ 8.5 min

Before starting a series of sample analyses, mount column 2 between column 1 and the detector and switch the eluate portion of column 1 containing the compound onto column 2. Determine the retention time of the compound after column 2, i.e. the overall retention time. For a retention time of about 18 min set the total run time to about 21 min. The setup of the column switching system pictured in section 5.2.

2.4.2 Quantitation of Residues

Standardise the chromatographic system each time a series of samples is to be quantitated. The range of the concentrations is depending on the range of residues to be determined, in particular, the lowest standard concentration is depending on the lower practical level. For calculation of the lowest standard concentration see section 2.2.13.

Measure the response of the analytes at the characteristic retention times and calculate response function and residues as detailed in section 2.9.

2.5 Interferences

Using a single-column system may result in interfering peaks in cereal grain or straw samples. Applying a 2-column switch system avoids this problem - and thus the need for further cleanup.

2.6 Confirmatory Techniques

None developed.

2.7 Time Required for Analysis

A series of 10 samples can be processed during two working days. Automated HPLC chromatographic analysis can be performed overnight. The run time per injection is about 21 minutes.

2.8 Modifications and Potential Problems

Acetone might be replaced as extracting solvent for soil samples by acetonitrile. This has not been proved, however.

Determining residues in soil samples, a single-column system is sufficient for 0.05 mg/kg as a lower level of quantitation. For lower levels such as 0.01 mg/kg, a dual-column system may have to be applied.

Silica Sep-Pak cartridge may be replaced by Bondelut silica cartridge

2.9 Methods of Calculation

2.9.1 Standardise the chromatographic system as outlined in sections 2.2.13 and 2.4.2.

2.9.2 Determine response (peak height or area; prefer height) of standard injections either electronically or with a ruler.

2.9.3 Plot the standard curve (amount vs. response) in a double logarithmic form or - preferably - calculate the response function: response = f (amount), by linear regression.

Note: In case of calculation take into account the fact, that random errors in chromatographic systems predominantly are relative to the amount, and therefore an adequate calculation procedure has to be used, e.g. linear regression by minimizing the sum of the squares of the relative deviations of all measured points from the curve.

With the amount expressed as ng, the response function will have the form:

$$\text{response} = a\,(ng) + b$$

2.9.4 From the response function calculate its inverse function, the analytical function:

$$\text{amount} = f'\,(\text{response})$$

or

$$ng = [(\text{response}) - b] / a$$

2.9.5 Calculate also the relative deviations from the curve of all standard points and the variance of all these deviations. Take this variance for an estimate of the variance of the chromatographic system.

2.9.6 Consider the range of definition of the analytical function to be limited by the two points (ng_{min}, $peak_{min}$) and (ng_{max}, $peak_{max}$); ng_{min} and ng_{max} being the smallest and the largest standard amount, respectively; $peak_{min}$ and $peak_{max}$ being the response calculated from the response function with ng_{min} or ng_{max}, respectively.

2.9.7 Consider $peak_{min}$ to represent the most probable estimate of the response and $peak_{min}$ minus twice the standard deviation of the chromatographic system (calculated from the variance) to be the minimum response at the 97.5% limit of confidence.

2.9.8 Divide ng_{min} by milligrams sample injected (cf. section 2.9.10) to determine the lower practical level. Make sure that the performance of the chromatographic system is sufficient to guarantee this lowest residue to be always above or equal to the statistically defined absolute limit of determination (which is about five times the instrument noise from peak to peak).

2.9.9 Measure the response of analyte - if present - at the characteristic retention time. If the response found is within the range of definition of the analytical function (cf. section 2.9.6) determine ng found by comparing the response with the plotted standard curve or by computing with the analytical function.

2.9.10 Calculate the subsample portion injected for determination as follows:

$$\frac{W \times Va \times Vi}{Ve \times Vf} \quad [mg]$$

Calculate the residue of a sample by dividing the amount [ng] of analyte found by the subsample portion [mg] injected:

$$\text{residue found [mg/kg]} = \frac{N \times Ve \times Vf}{W \times Va \times Vi}$$

		[values used in method]
W : weight of the subsample extracted	[g]	-> 25 g/10 g
N : amount analyte found and determined	[ng]	-> 1 ng (lower limit)
Vf: final volume for determination	[mL]	-> 4 mL
Va: volume of aliquot cleaned up	[mL]	-> 10 mL
Ve: volume of extract solution	[mL]	-> 250 mL
Vi: volume injected for determination	[µL]	-> 100 - 200 µL

2.9.11 If the response found is outside the range of definition of the analytical function do not extrapolate, but proceed as described in the following three sections.

2.9.12 If the response is above the upper limit of the range (i.e. above peak $_{max}$), repeat the final determination either with a more diluted sample solution or with more concentrated standard solutions.

2.9.13 If the response is below the lower limit of the range (i.e. below peak $_{min}$), proceed as follows:
Subtract the double standard deviation of the chromatographic system (calculated from the variance, cf. section 2.9.5) from the lower limit of the range; take 80% of this value as the lower threshold.

If the response under consideration is above this lower threshold take the difference to the lower limit for a random and/or systematic error; therefore round up ng calculated to ng $_{min}$, and report residue to correspond to the lower practical level. This is to make sure that the probability of falsely reporting a residue to be below the lower practical level (false negatives) is as low as 2.5% even if the recovery value is only 80%.

2.9.14 If the response is below the lower threshold, report the residue to be below the lower practical level.

Note: This procedure is intended to be followed for registration purposes In the case of enforcement analyses the procedure has to be changed in such a way as to make sure that the chance of false positives is small.

2.9.15 Find an example how to calculate and interpret the standardisation and the quantitation of residues in Table 1.

2.9.16 Calculate recovery values from the recovery samples (cf. section 2.3.2) as

$$\% \text{ recovery} = \frac{\text{mg/kg found}}{\text{mg/kg added}} \times 100$$

Consider the analysis of the whole series to have failed if recoveries are outside the acceptable range (cf. Appendix 7.2).

3. RESULTS AND DISCUSSION

3.1 Accuracy and Precision

Average recoveries are as follows (status as of March 1990, see section 7.2):

0.04, 0.1, 0.4 and 1.0 mg/kg CGA 184927: 94.4 % s_{abs}= 4.7% (n=14)
0.04, 0.1, 0.5 and 1.0 mg/kg CGA 185072: 90.9 % s_{abs}= 10.4% (n=14)

3.2 Limit of Determination

The lowest limit of quantitative determination by this method was not established. So far, residue experiments have been analysed at a lower practical level of 0.02 mg/kg for grain and whole plant and of 0.05 mg/kg for straw and soil.

3.3 Ruggedness

Not particularly tested. The recovery experiments summarized in section 7.2 were conducted by different technicians at different times.

3.4 Limitations

None known.

3.5 Method Validation

The performance of the method was checked by performing recovery experiments with each series of sample quantitation (section 7.2).

4. CERTIFICATION

The experimental results included in this method are certified to be authentic accounts of the experiments.

Method developed by:

Ph.Litzler

issued by:

Dr.E.R.Altenburger
CIBA-GEIGY, AG 2.53
CH-4002 Basel

5. TABLES AND FIGURES

5.1 Table 1 EXAMPLE OF CALCULATION AND OF INTERPRETATION OF RESULTS

Note: These examples do not actually represent results generated by this method; they are general ones intended to illustrate the calculation procedure.

A. STANDARDISATION

amount (ng)	response (counts)	response calculated	relative deviation
0.5 (= ng_{min})	1199	1389 (= $peak_{min}$)	-0.137
0.5	1431	1389	0.030
1.0	2942	2580	0.140
1.0	2832	2580	0.098
2.0	5124	4961	0.033
2.0	4998	4961	0.007
6.0	12933	14487	-0.107
6.0	13647	14487	-0.058
15.0	35437	35919	-0.013
15.0 (= ng_{max})	36161	35919 (= $peak_{max}$)	0.007

y = response	response function	y = 2381 x + 199
x = amount	analytical function	x = (y - 199) / 2381
relative deviations:	average	0.000
	variance	0.0072
	standard deviation	0.085 (= 8.5%)
range of definition:	between (0.5, 1389) and (15.0, 35919)	

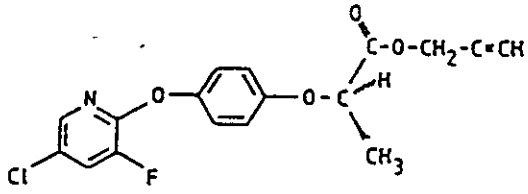
B. QUANTITATION OF RESIDUES

Injected portion corresponds to extract of 25 mg sample, hence lower practical level is 0.5 ng / 25 mg = 0.02 mg/kg.

- Response 27301 counts (i.e. within the range of definition): Calculation from the analytical function gives 11.4 ng, which corresponds to 0.46 mg/kg.
- Response 43411 counts (i.e. range of definition exceeded): Report residue to be >0.60 mg/kg and proceed as outlined in section 2.9.12.
- Response 968 counts (i.e. outside the range of definition): Since response is above 80% of $peak_{min}$ (1389) minus twice the standard deviation (236), i.e. above 922 counts (= lower threshold), round up residue and report 0.02 mg/kg.
- Response 817 counts (i.e. outside the range of definition): Since response is smaller than 922 counts (the lower threshold as calculated above), report residue to be <0.02 mg/kg.

5.2 FIGURES

Figure 1: Structure and Chemical Name of CGA 184927



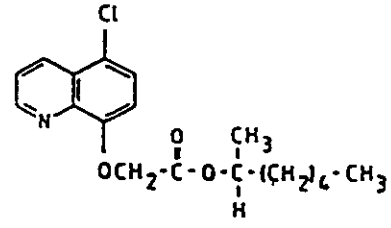
$C_{17}H_{13}ClFNO_4$

molecular mass: 349.75

IUPAC: 2-propynyl-(R)-2-[4-(5-chloro-3-fluoro-2-pyridinyloxy)-phenoxy]-propionate

CA: 2-[4-(5-chloro-3-fluoro-2-pyridinyloxy)-phenoxy]-propanoic acid-2-propynylester

Figure 2: Structure and Chemical Name of CGA 185072.



$C_{18}H_{22}ClN\ O_3$

molecular mass: 335.83

5-chloro-8-quinolinoacetic acid-1-methylhexylester

Figure 3: Procedure Flow Diagram

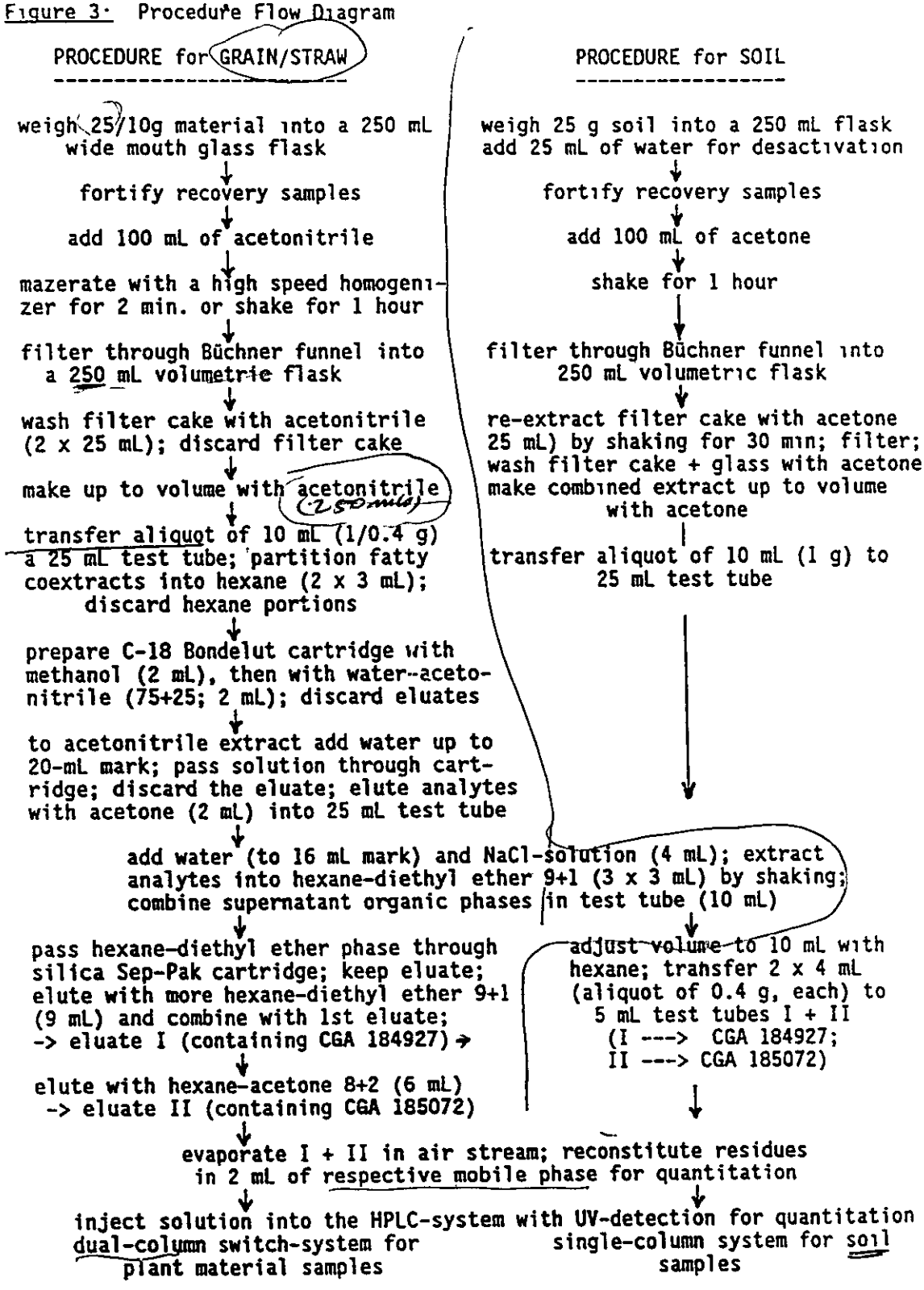
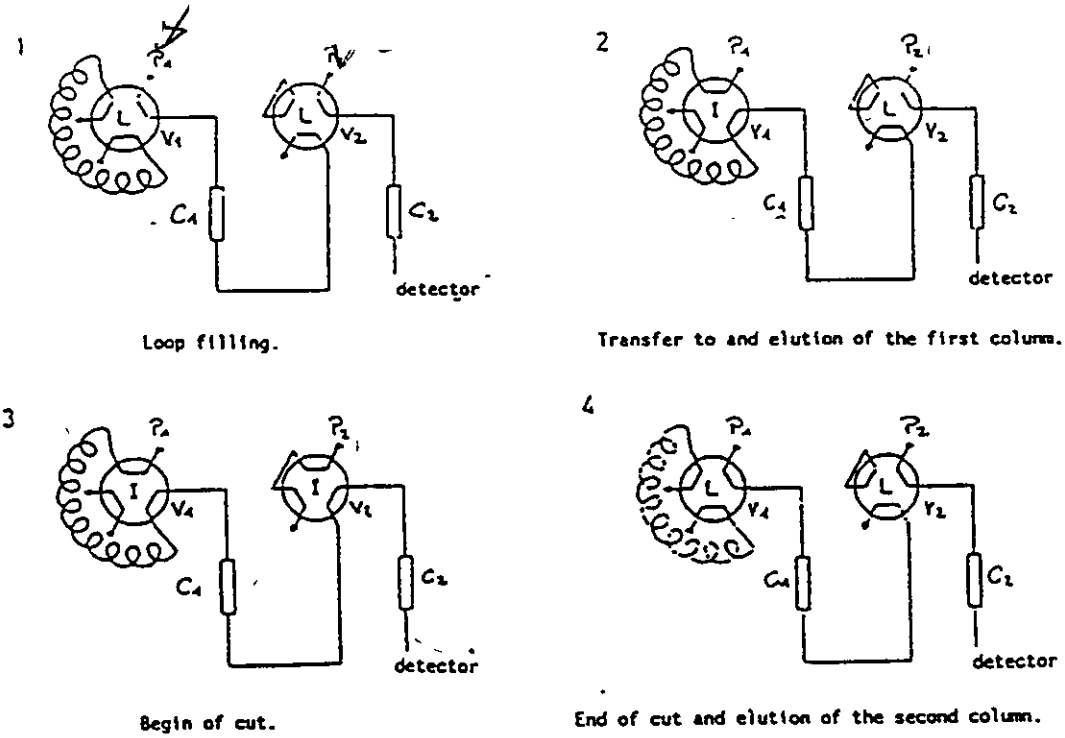


Figure 4: Chromatographic system with column switching option



Loop filling.

Transfer to and elution of the first column.

Begin of cut.

End of cut and elution of the second column.

P_1 ---> pump 1 V_1 ---> valve 1
P_2 ---> pump 2 V_2 ---> valve 2

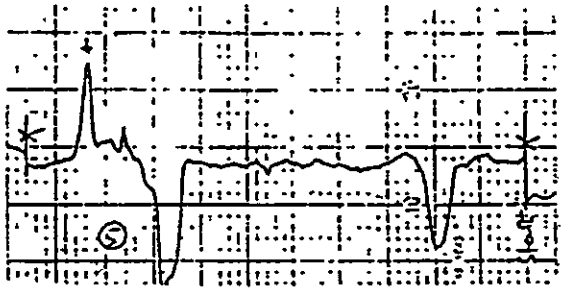
C_1 ---> column 1 L ---> Load position
C_2 ---> column 2 I ---> Inject position

6. REFERENCES

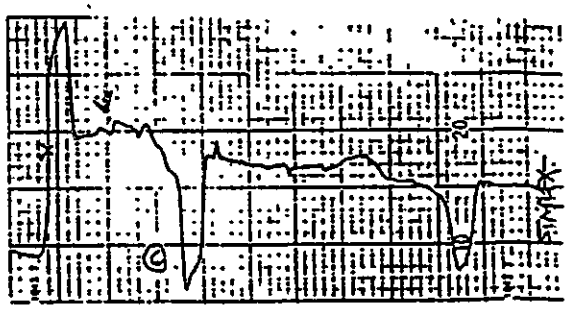
None

7. APPENDICES

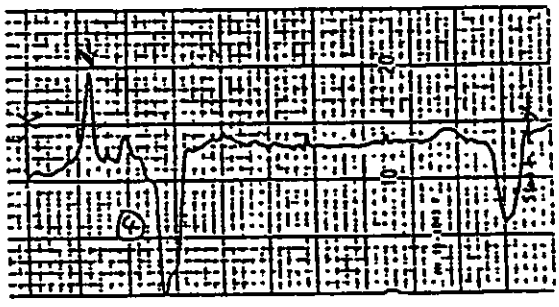
7.1. Representative Chromatograms of CGA 184927 in WHEAT (Grain)



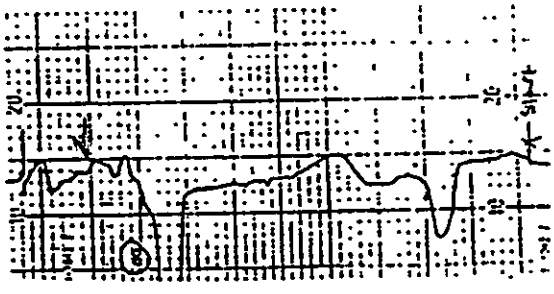
Standard CGA 184927
4.0 ng
200 µL injected



WHEAT (grain) #3017/87
untreated field sample
extract of 100 mg inj.
residue found (CGA 184927)
<0.02 mg/kg

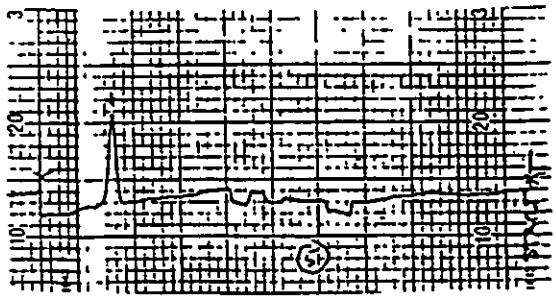


WHEAT (grain) untreated
fortified with
CGA 184927 (0.04 mg/kg)
extract of 100 mg injected
recovery found: 93 %

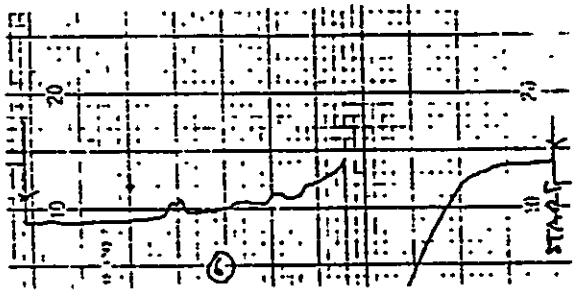


WHEAT (grain) #3017/87
field treated sample W1
extract of 100 mg injected
residue found: <0.02 mg/kg
(as CGA 184927)

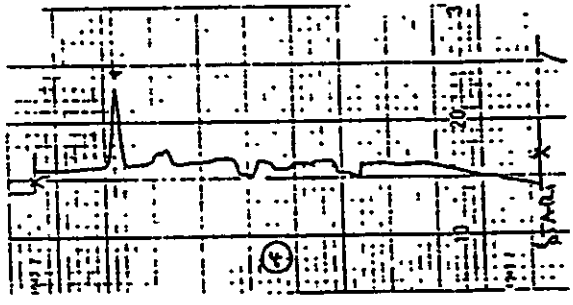
Representative Chromatograms of CGA 185072 in WHEAT (Grain)



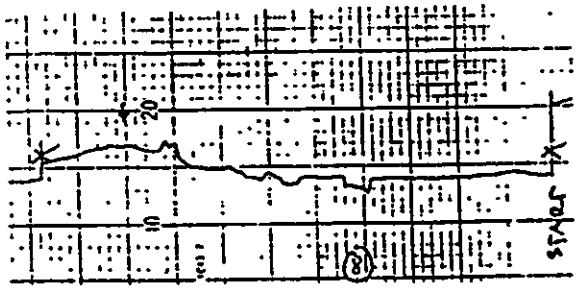
Standard CGA 185072
2.0 ng
200 µL injected



WHEAT (grain)
untreated control
extract of 100 mg inj.
residue found (CGA 185072)
<0.02 mg/kg

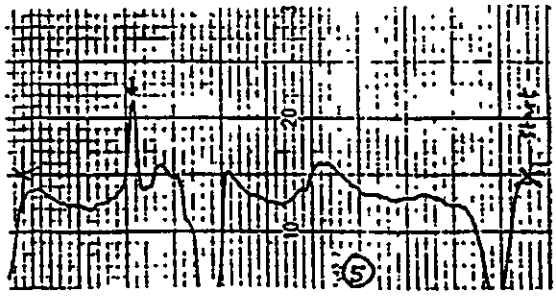


WHEAT (grain)
fortified with
CGA 185072 (0.04 mg/kg)
extract of 100 mg injected
recovery found: 80 %

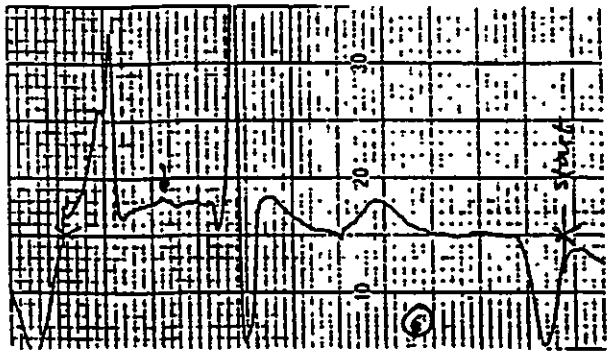


WHEAT (grain) #3047/87-01
field treated sample,
extract of 100 mg injected
residue found: <0.02 mg/kg
(as CGA 185072)

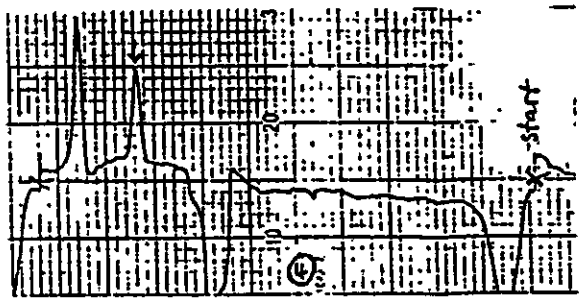
Representative Chromatograms of CGA 184927 in WHEAT (STRAW)



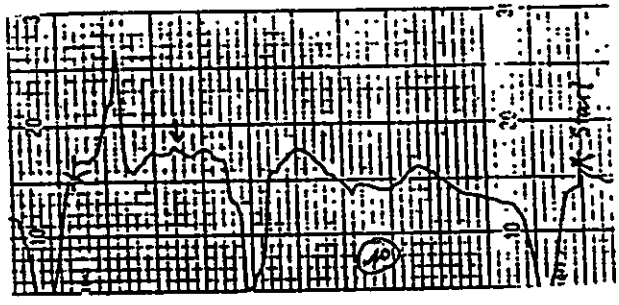
Standard CGA 184927
4.0 ng
200 µL injected



WHEAT (STRAW)
untreated control
extract of 40 mg inj.
residue found (CGA 184927)
<0.05 mg/kg

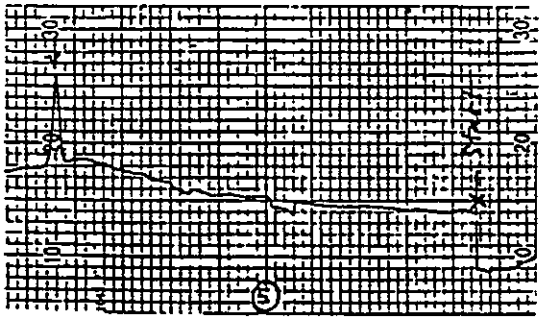


WHEAT (STRAW)
fortified with
CGA 184927 (0.10 mg/kg)
extract of 40 mg injected
recovery found: 98 %

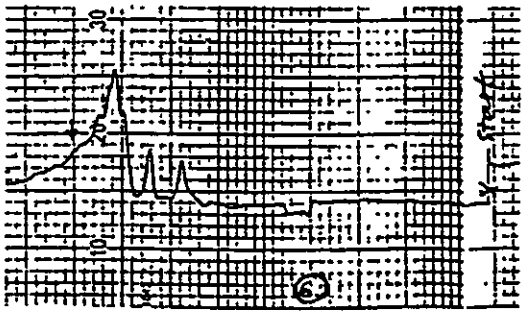


WHEAT (STRAW) #3017/87
field treated sample W2
extract of 40 mg injected
residue found: <0.05 mg/kg
(as CGA 184927)

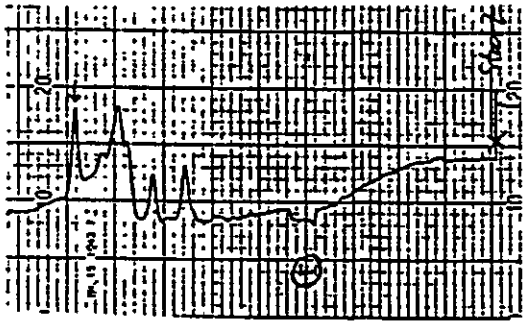
Representative Chromatograms of CGA 185072 in WHEAT (STRAW)



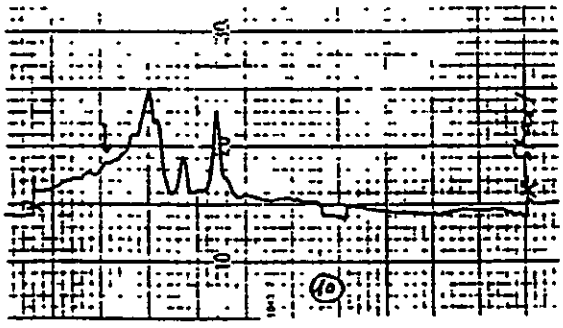
Standard CGA 185072
4.0 ng
200 µL injected



WHEAT (STRAW)
untreated control
extract of 40 mg inj.
residue found (CGA 185072)
<0.05 mg/kg

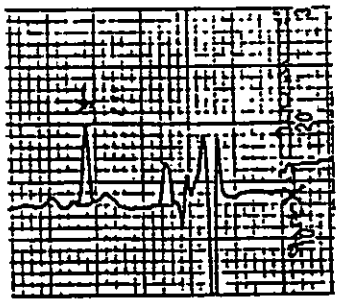


WHEAT (STRAW)
fortified with
CGA 185072 (0.10 mg/kg)
extract of 40 mg injected
recovery found: 87 %

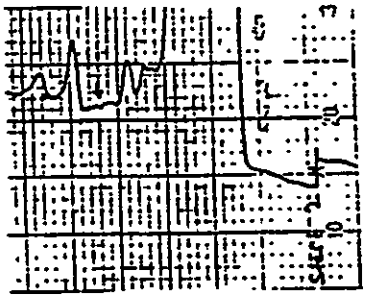


WHEAT (STRAW) #3017/87
field treated sample W2
extract of 40 mg injected
residue found: <0.05 mg/kg
(as CGA 185072)

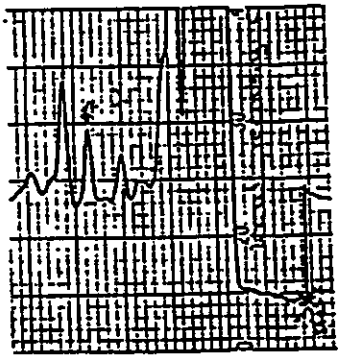
Representative Chromatograms of CGA 184927 in SOIL



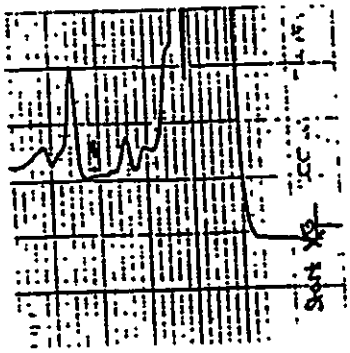
Standard CGA 184927
4.0 ng
200 µL injected



SOIL, untreated control
3017/87 (0 - 10 cm)
extract of 20 mg inj.
residue found (CGA 184927)
<0.05 mg/kg

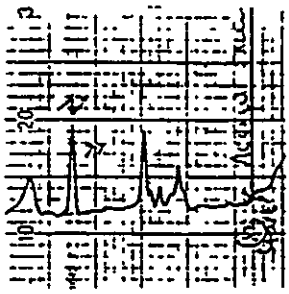


SOIL, untreated control
fortified with
CGA 184927 (0.1 mg/kg)
extract of 20 mg injected
recovery found: 88 %

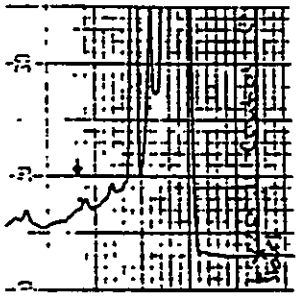


SOIL field treated sample
3017/87 W1 (0 - 10 cm)
extract of 20 mg injected
residue found: <0.05 mg/kg
(as CGA 184927)

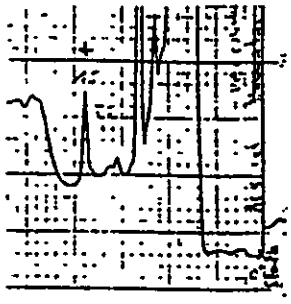
Representative Chromatograms of CGA 185072 in SOIL



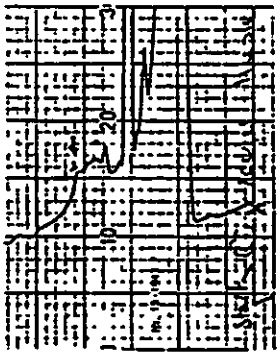
Standard CGA 185072
2.0 ng
100 µl injected



SOIL, untreated control
3017/87 (0 - 10 cm)
extract of 20 mg inj.
residue found (CGA 185072)
<0.05 mg/kg



SOIL, untreated control
fortified with
CGA 185072 (0.1 mg/kg)
extract of 20 mg injected
recovery found: 98 %



SOIL field treated sample
3017/87 W1 (0 - 10 cm)
extract of 20 mg injected
residue found: <0.05 mg/kg
(as CGA 185072)