Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Quinclorac

MRID #: 410635-69

Matrix: Soil

Analysis: HPLC/UV

HPLC Method for Residue Determinations of Quinclorac

(3,7-Dichloro-8-quinolinecarboxylic acid)

and its Metabolite BH 514-1

(3-Chloro-8-quinolinecarboxylic acid)

in Soil

Method No. A8903

Date Issued: March 1989

Study Performed by: BASF Corporation Chemicals Division
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QUALITY ASSURANCE STATEMENT

We, the undersigned, hereby declare that this work was perfomed under our supervision according to the procedure described herein, and that this report provides a true and accurate record of the results obtained.

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Quality Assurance Unit
Date: March 21, 1989

SIGNATURES

We, the undersigned, have participated in various phases of this work. The procedures described and the results reported herein are correct and accurate, to the best of our knowledge.

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Date: 3/20/89

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Date: 3/20/89

Method No. A8921

BASF CORPORATION CHEMICALS DIVISION

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STATEMENT

of the Quality Assurance Unit

Method Number: A8903

Name/Number of test substance: Quinclorac; BH 514-1

Type of Study: Residue Analytical Method

The quality assurance unit of the testing facility at the ARC has audited this report and reported its findings to the study director and to management.

Date of inspection	Report to study director and to management
March 21, 1989	March 21, 1989

William T. [illegible]

Signature of QAU

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1 INTRODUCTION AND SUMMARY

1.1 Scope and Source of the Method

1.1.1 Scope

Metabolism investigations (Ref. 1) showed that Quinclorac residues in soil can be degraded to a dechlorinated metabolite 3-chloro-8-quinolinecarboxylic acid (BH 514-1) under certain conditions. Thus, this method was developed in order to include this metabolite in the determination of Quinclorac residues in soil. It contains an alkaline hydrolytic extraction step which liberates chemically bound residues. Active ingredient and metabolite are determined simultaneously by HPLC using column switching and UV detection.

1.1.2 Source

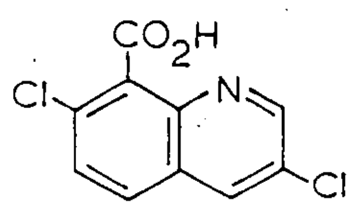
This method is a revision of Method 280 (Ref. 2), developed by Dr. Frank Mayer in the BASF laboratory in Limburgerhof, Germany, with subsequent modifications made by Dr. David McAleese, Robert Eswein and Dr. Frank Mayer in the BASF laboratory in North Carolina. The method was revised to make it compatible with materials and soils available in the US.

1.2 Substances

1.2.1 Active Ingredient

Proposed common name:	Quinclorac
Laboratory number:	150 732
BASF developmental number:	BAS 514 .. H (.. = These digits specify the formulation)
Chemical name:	3,7-Dichloro-8-quinoline-carboxylic acid

Structural formula:



Empirical formula:	$C_{10}H_5Cl_2NO_2$
Molecular weight:	242.1
Melting point:	Above 237 °C decomposition
Appearance:	Crystalline, colorless
Odor:	Weak

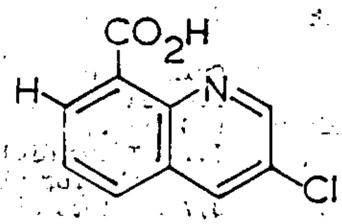
Solubility: (g substance in 100 g solvent at 20 °C)

Water	6.2×10^{-3}
Ethanol	0.2
Acetonitrile	<0.1
Acetone	0.2
Ethylacetate	0.1
Dichloromethane	<0.1
Diethylether	0.1
Toluene	<0.1
n-Hexane	<0.1
Olive oil	<0.1

1.2.2 Metabolite

Metabolite code:	BH 514-1
Laboratory number:	195 540
Chemical name:	3-Chloro-8-quinoline-carboxylic acid

Structural formula:



Empirical formula:	$C_{10}H_6ClNO_2$
Molecular weight:	207.6
Melting point:	195 °C
Appearance:	Crystalline, colorless
Odor:	Odorless

Solubility: (g substance in 100 g solvent at 20 °C)

Water	3.8×10^{-3}
Ethanol	0.3
Acetonitrile	1.2
Acetone	1.4
Ethylacetate	0.6
Dichloromethane	2.9
Diethylether	0.1
Toluene	0.5
n-Hexane	<0.1
Olive oil	<0.1

1.3 Principle of the Method

The soil is extracted by refluxing with sodium hydroxide solution. The extract is cleaned up by three water/dichloromethane partitions at various pH values. Final determination of the free acids by HPLC uses column switching and UV detection.

Limit of quantitation: 0.05 mg/kg.

2 MATERIALS AND METHODS

Equipment and reagents in the following lists are examples and can be replaced by equivalent ones.

2.1 Equipment

Flat bottom flask with standard ground glass joint	1 Liter, 125 mL
Funnels	4 cm, 10 cm i.d.
Volumetric pipettes	0.5 mL, 2.0 mL, 10.0 mL, 20.0 mL
Magnetic stirring bars	2.5 cm

Reflux condensor with standard ground glass joint	
Stirring hot plate	Corning PC-351
Plastic centrifuge bottle	250 mL
Centrifuge	Damon/IEC
Volumetric flask	500 mL
Graduated cylinder	100 mL
Spatula or small scoop	
Eppendorf pipet	50 uL
pH sticks (0 - 14; 0 - 2.5)	EM Science, Cherry Hill, NJ #9580, 9590
Rotary Evaporator	Buchi
Separatory funnel	125 mL
Pasteur pipets	23 cm long, disposable
Whatman #1PS phase separation filter paper	Whatman Limited, England, via Fisher Scientific Delmar, Newark, DE # 2200110
Glass centrifuge tube	50 ml
Ultrasonic bath	Branson 1200
Vortex mixer	American Scientific Products McGaw Parlell
Acrodisc LC-13 syringe filter	0.45 um Gelman Sciences, # 4450
Plastic Syringe	3 cc. Becton Dickenson, Rutherford, NJ
N-EVAP (nitrogen stream evaporator)	Organomation Assoc., Northborough, MA
Millipore filter GV 0.22 μm	Millipore Corp. Bedford, MA #GVWP 04700

2.2 Reagents and Chemicals

Acetone, high purity solvent	Burdick & Jackson
Dichloromethane, distilled	Burdick & Jackson
Water, deionized	
Sodium hydroxide pellets, reagent ACS	Kodak, Rochester, NY Cat 137 6466
Sodium hydroxide solution 0.1 N (0.1 mol/l) in water	
Calcium chloride dihydrate, cert. ACS	Fisher Scientific, Fairlawn, NJ
Phosphoric acid, meets ACS specs	J.T. Baker, Phillipsburg, NJ
3 % calcium chloride + 1.5 % phosphoric acid aqueous solution (w/w/v)	
Sulfuric acid, concentrated analytical grade	J.T. Baker Phillipsburg, NJ
Sodium Bicarbonate powder, analytical grade	Fisher Scientific Fairlawn, NJ
Saturated aqueous sodium bicarbonate solution	
50 % acetone + 1 % acetic acid in water (v/v/v)	
Acetic acid, glacial, equivalent to USP specifications	EM Science, Cherry Hill, NJ # AX0072-1
Water, high purity solvent	Burdick & Jackson Product 365
Acetonitrile UV	Burdick & Jackson Product 015

2.3 Standard Substances and Solutions

2.3.1. Standard Substances

Quinclorac (structure 1.2.1)	>99.5 %
BH 514-1 (structure 1.2.2)	>99.5 %

(both standards supplied by: Dr. Pawliczek, APE/CP
BASF Aktiengesellschaft
Agricultural Research
Center
D-6703 Limburgerhof
West Germany
Phone: 06236/68-2422)

Store standard substances in a freezer. Store standard solutions of Quinclorac and BH 514-1 in an amber bottle with a plastic lined screw cap and refrigerate.

2.3.2 Standard Solutions for Fortifications

Quinclorac plus BH 514-1 (both in one solution):
25.0 and 2.5 µg/ml in acetone

Prepare a 1.00 mg/mL Quinclorac plus BH 514-1 stock solution by weighing 25.0 mg of Quinclorac and 25.0 mg of BH 514-1 into a 25 mL volumetric flask. Dissolve with acetone and dilute to the mark.

Prepare a 25.0 µg/mL Quinclorac plus BH 514-1 standard solution by transferring 5 mL of the 1.00 mg/mL stock solution with a volumetric pipet to a 200 mL volumetric flask. Dilute to the mark with acetone.

Prepare a 2.5 µg/mL Quinclorac plus BH 514-1 standard solution by transferring 10 mL of the 25.0 µg/mL Quinclorac plus BH 514-1 solution with a volumetric pipet to a 100 mL volumetric flask. Dilute to the mark with acetone.

2.3.2 Standard Solutions for HPLC Analysis

Quinclorac plus BH 514-1 (both in one solution):
12.5; 25.0; 50.0; 100.0 ng/mL in acetone / acetic acid/ water solvent.

Prepare the acetone/acetic acid/water solvent in a volumetric flask. Place 50% (volume) acetone and 1% (volume) acetic acid into the flask and dilute to the mark with water.

Prepare a 100 ng/mL Quinclorac plus BH 514-1 solution by transferring 4 mL of the 2.5 ug/mL acetonic solution with a volumetric pipet to a 100 mL volumetric flask. Evaporate to dryness using a N-EVAP. Dissolve and dilute to the mark with the acetone/ acetic acid/ water solvent.

Prepare a 50 ng/mL Quinclorac plus BH 514-1 solution by transferring 2 mL of the 2.5 ug/mL acetonic solution with a volumetric pipet to a 100 mL volumetric flask. Evaporate to dryness using an N-EVAP. Dissolve and dilute to the mark with the acetone/ acetic acid/ water solvent.

Prepare a 25 ng/mL Quinclorac plus BH 514-1 solution by transferring 1 mL of the 2.5 ug/mL acetonic solution with a volumetric pipet to a 100 mL volumetric flask. Evaporate to dryness using an N-EVAP. Dissolve and dilute to the mark with the acetone/ acetic acid/ water solvent.

Prepare a 12.5 ng/mL Quinclorac plus BH 514-1 solution by transferring 0.5 mL of the 2.5 ug/mL acetonic solution with a volumetric pipet to a 100 mL volumetric flask. Evaporate to dryness using an N-EVAP. Dissolve and dilute to the mark with the acetone/ acetic acid/ water solvent.

2.3.3 Stability of Standard Solutions (Ref. 2)

Storage Days	Room temperature Daylight	4 °C Refrigerator
	Quinclorac 200 µg/mL in acetone	
71	100 %	100 %
	BH 514-1 1 µg/mL in acetone	
19	101.3 %	101.3 %
71	99.3 %	100.2 %
118	97.8 %	105.3 %

3 ANALYTICAL PROCEDURE

3.1 Extract Preparation

3.1.1 Preparation of Samples

The samples are air dried, ground in a small mill, and then stored at <-5°C until analysis.

3.1.2 Extraction and Fortification

Weigh 25.0 g of soil sample into a 1 liter flat bottom flask equipped with a plastic funnel. For fortification samples, pipet 0.5 mL of Quinclorac and BH 514-1 in acetone with a volumetric pipet onto the soil sample. Do not use a larger volume of the acetone solution for fortification; refluxing with additional acetone may extract substances from the soil that interfere with the analytes in the HPLC chromatogram. At least two fortifications and one untreated sample (control) are run with each set of samples. The amount of Quinclorac and BH 514-1 for fortification trials should be in the range of the expected residue.

Add a magnetic stirring bar. Add 200 mL of 0.1 M NaOH to the flat bottom flask and rinse the funnel. Rinse the reflux condenser with approximately 10 mL of water before use. Connect the flat bottom flask to the condenser and reflux for one hour while stirring. Allow the flat bottom flask to cool. Use a water bath if needed. After cooling, rinse the condenser with 10 mL of water.

3.1.3 Centrifugation

Swirl the contents of the flat bottom flask and transfer into a 250 mL plastic centrifuge bottle. Centrifuge for 10 minutes at 2000 rpm or faster. Pour the supernatant into a 500 mL volumetric flask equipped with a plastic funnel. Rinse the 1 liter flat bottom flask with 100 mL of a wash solution prepared by mixing equal volumes of acetone and an aqueous solution containing 3% calcium chloride and 1.5% phosphoric acid. Swirl the contents of the flat bottom flask and pour onto the leftover soil in the centrifuge bottle. Stir the soil with a spatula and sonicate for 1 minute if necessary. Centrifuge again for 10 minutes at 2000 rpm or faster. Transfer the supernatant to the 500 mL volumetric flask. Repeat rinsing with another 100 mL of the wash solution, stir, sonicate and centrifuge as above. Dilute to the 500 mL mark with acetone. Shake well enough to achieve a homogeneous solution. Do not sonicate. Let the precipitate settle. Part of the precipitate may remain right under the surface of the solution. Wait until a clear layer in the middle of the flask has formed.

3.1.4 Dichloromethane Extraction

Aliquot 20 mL of the clear layer in the middle of the flask with a volumetric pipet into a 125 mL flat bottom rotovap flask. Save a portion of the solution left in the 500 mL volumetric flask for reanalysis if necessary. Add 50 µL or more of concentrated sulfuric acid with an Eppendorf pipet to the 125 mL flat bottom rotovap flask until the pH is 1.3-1.6. Measure the pH with pH sticks. Remove the acetone on a rotary evaporator with the bath temperature set at 40°C. Stop when water condenses inside the condenser. Transfer the acidic extract to a 125 mL separatory funnel. Rinse the rotovap flask with 10 mL of water and transfer to the separatory funnel. Rinse the rotovap flask with 25 mL of DCM (dichloromethane) and transfer to the separatory funnel. Ensure that the pH of the aqueous phase is between 1.3 and 1.6 with pH sticks. Shake and vent the solution for 30 seconds. Drain the bottom DCM layer (do not take emulsions) into another 125 mL separatory funnel (second funnel) with the stopcock closed. Add 25 mL of DCM to the aqueous phase left in the first separatory funnel. Shake and vent as before for 30 seconds. Drain the bottom DCM layer (do not take emulsions) into the second separatory funnel as before. Pour the contents of the first separatory funnel to waste. Rinse the first separatory funnel with acetone and let drip dry.

3.1.5 Dichloromethane/Sodium Bicarbonate Partition

Add 25 mL of saturated sodium bicarbonate solution to the DCM extract in the second separatory funnel. Shake and vent for 30 seconds. Close the stopcock on the first separatory funnel. Drain the bottom DCM layer into the first 125 mL separatory funnel. Let the phase boundary pass through the stop cock before closing it. Add 25 mL of saturated sodium bicarbonate solution to the DCM extract in the first separatory funnel. Shake and vent for 30 seconds. Drain the bottom DCM layer and the phase boundary to waste and pour the top layer into the second 125 mL separatory funnel.

3.1.6 Sulfuric Acid/Dichloromethane Partition

To the basic solution from Section 3.1.5 very carefully add concentrated sulfuric acid dropwise with a Pasteur pipet. Let the mixture settle and very carefully mix. Add acid until the pH is between 1.3 and 1.6. Approximately 2.6 mL of sulfuric acid is necessary. Check the pH with pH sticks. (If the acid is added too quickly, the solution will bubble out of the top of the separatory funnel). Shake and vent the funnel carefully several times until the CO_2 evolution has diminished. Add 25 mL of DCM to the separatory funnel. Shake and vent for 30 seconds. Drain the bottom DCM layer through Whatman 1PS phase separation filter paper into a 50 mL centrifuge tube. Repeat the extraction and phase separation. N-evap with nitrogen to dryness with heat (30 - 40 °C). Remove the centrifuge tube from the N-EVAP immediately after drying. Bring up to an appropriate final volume using the acetone/ acetic acid/ water solvent (Final volume for the limit of quantitation is 2 mL.) Sonicate and vortex until the sample solution is clear. Filter the solution through a 0.45 µm Acrodisc LC13 syringe-end filter into the HPLC autosampler vial. Inject 50 µL of the solution into the HPLC. Dilute further with the acetone/acetic acid/water solvent if necessary. Use a volumetric pipet for all dilutions.

3.2 Instrumentation

Equipment and conditions in the following lists are examples and may be replaced by equivalent ones.

3.2.1 Principle of HPLC Separation

The separation is achieved on C_{18} reversed phase material with column switching between a precolumn and a main column (see figure 1 for a schematic diagram). The mobile phase of the precolumn is solvent mixture I with a low acetonitrile content (see section 3.2.2). The active ingredient elutes more than 5 minutes after the dead volume. The active ingredient and the metabolite have very different retention times.

Only the peaks of interest are switched onto the main column. These are then reconcentrated and separated further by a stepwise gradient. Both low and high pressure gradient mixing procedures are possible.

Important:
Before running a set of samples, check the retention times of Quinclorac and BH 514-1 on the precolumn and adjust the switching times, if necessary.

3.2.2 Description of Equipment

Pump with low pressure gradient mixer (for main column)	Varian Model 5000 Liquid Chromatograph
Pump (for precolumn)	Beckman Model 110-A HPLC pump
Autosampler	Varian 8000 Series Autosampler
Switching valve	Rheodyne No. 7000
Pneumatical unit	Rheodyne No. 7001
System computer	Varian Vista 402 Chromatography Data Station
UV detector	Varian 2550 UV Detector
Columns	Stainless steel
	Precolumn: 50 mm x 4.6 mm Main Column: 250 mm x 4.6 mm
Stationary phase	Nucleosil 100-5-C_{18} Alltech Associates
Guard column	Waters Guard-Pak Precolumn Module with Resolve C_{18} Cartridge

3.2.2 Operating Conditions

Injection volume	50 µL	
Wavelength	230 nm	
Recorder chart speed	0.5 cm/min	
Flow rate	1 mL/min for both pumps	
Switch times	Quinclorac	BH 514-1
	7 - 10 min	22 - 27 min
Retention times (Precolumn + main column)	19.1 min	38.4 min
Mobile phases	Acetonitrile/ water/ acetic acid	

<u>Mobile Phase I:</u>

Prepare the acetonitrile/acetic acid/water mobile phases in a 4L volumetric flask. For mobile phase I (precolumn), add 17% (volume) acetonitrile to the flask using a graduated cylinder. Add 0.25% (volume) acetic acid to the flask with a volumetric pipet and dilute to the mark with water.

Mobile Phase II:

For mobile phase II (elutes the parent from the main column), add 37% (volume) acetonitrile to a 4L volumetric flask using a graduated cylinder. Add 0.25% (volume) acetic acid to the flask with a volumetric pipet, and dilute to the mark with water.

Mobile Phase III:

For mobile phase III (elutes the metabolite from the main column), add 45% (volume) acetonitrile to a 4L volumetric flask using a graduated cylinder. Add 0.25% (volume) acetic acid to the flask with a volumetric pipet, and dilute to the mark with water.

Filter the mobile phases using a Millipore filtering apparatus equipped with a GV 0.22 μm membrane. This vacuum filtration may be sufficient for degassing. If not, degas the mobile phases for 30 minutes using a slight stream of helium. The system control program is shown below. After a given number of samples, a stop program can be used to terminate the run. The pump for the precolumn (mobile phase I) is not controlled by the program.

HPLC-Program:

Line	Time	Event	Value	Description
1	0.0	Flow	1.0	Flow rate 1.0 mL/min
2	0.0	Reservoirs	AB	Selection of solvent reservoirs
3	0.0	%A	100	Mobile phase II
4	0.0	%B	0	on main column
5	0.1	Relay		Detector
6	0.2	Relay		autozero
7	7.0	Relay		Connects precolumn with main column
8	10.0	Relay		Disconnects columns
9	16.9	Relay		Detector
10	17.0	Relay		autozero
11	20.0	%A	100	Gradient from mobile
12	20.0	%B	0	phase II to mobile
13	20.1	%A	0	phase III on main
14	20.1	%B	100	column
15	22.0	Relay		Connects precolumn with main column
16	27.0	Relay		Disconnects columns
17	35.9	Relay		Detector
18	36.0	Relay		autozero
19	39.9	%B	100	Gradient from mobile
20	39.9	%A	0	phase III to mobile
21	40.0	%B	0	phase II on main
22	40.0	%A	100	column

3.2.4 Calibration Procedures

Calculation of results is based on peak height measurements using a calibration curve. To obtain this standard curve inject 50 µL from solutions that contain 12.5, 25, 50, 100 ng/mL Quinclorac and BH 514-1 into the HPLC system. Plot peak height (mm) versus amount (ng) of injected standard (absolute amount).

3.2.5 Sample Analysis

Inject 50 uL of each sample and each standard into the HPLC system for analysis. Do not use a larger injection volume. For each set of samples, inject each standard at least in triplicate and inject each sample at least once. Bracket the sample injections with standard injections. Inject standards every 2 - 3 samples.

3.3 Interferences

3.3.1 Sample Matrices

If interfering peaks occur in the chromatogram, analyze another aliquot of the extract in the 500 mL flask in 2.3.2 using GC/MS as final determination as described in BASF Analytical Method Number A8901 (Ref. 3).

3.3.2 Other Sources

Other Pesticides: None known to date.

Solvents: Impurities in the acetic acid used in mobile phase I may be concentrated on the main column and cause ghost peaks in the chromatogram which interfere with analyte peaks. This can be checked by running the system with different concentrations of acetic acid in mobile phase I or longer/shorter peak switching time periods. If the interferences change their peak height accordingly, a better quality of acetic acid must be used.

Labware: None known to date.

3.4 Confirmatory Techniques

If UV determination fails because of interferences or peak identity is doubtful, determination can be made by GC/MS as described in method No. A8901 (Ref. 3).

3.5 Time Required for Analysis

Extract preparation for a set of 6 samples, 2 recoveries and 1 control requires 8 hours. HPLC injection can be done automatically over night. Evaluation and report take approximately 2 hours. This time schedule is valid if no special problems arise, such as matrix interferences. Larger sets of samples or continuous flow of analyses take less time per sample depending on available equipment, personnel and organization of work.

3.6 Potential Problems

Window shifting may occur during long runs. If the peak height of the standard decreased significantly over more than two injections, the retention times on the precolumn should be checked and the windows adjusted accordingly. The use of a guard column minimizes this potential problem.

4 METHODS OF CALCULATION

4.1 Calibration

Measure the peak heights of the standards. Construct linear least squares working curves for parent and metabolite in the form y=ax + b from the standards by plotting peak height versus nanograms of standard injected.

4.2 Analyte in Sample

Calculation of results is based on peak height measurements. Measure the peak heights of the Quinclorac and the BH 514-1 peaks in the samples. From the least squares working curves, determine the nanograms of Quinclorac and BH 514-1 in the samples. Determine recovery factors from the fortification experiments. Do not correct sample residues for either control residues or procedural recovery.

The residues in mg/kg (ppm) of Quinclorac and its metabolite BH 514-1 expressed as Quinclorac equivalents are calculated as follows:

$$ppm = \frac{V_E \cdot W_A \cdot U \cdot 100}{G \cdot V_I \cdot A}$$

G = Weight in (g) of sample extracted
V_E = Final volume after all dilution steps (mL)
V_I = µL injected from V_E
W_A = Amount of determined substance read from calibration curve in ng
A = Aliquot in %, taken during sample extract processing
U = Conversion factor (for determination of metabolite only; converts determined metabolite residues to Quinclorac equivalents).

$$U = \frac{\text{Molecular weight of Quinclorac}}{\text{Molecular weight of BH 514-1}} = \frac{242.1}{207.6} = 1.166$$

Calculate parent (Quinclorac) and metabolite (BH 514-1) residues separately. Add them to get the total residue.

4.3 Calculation of Recoveries

$$\%\ Recovery = \frac{(\text{ppm in fortified control} - \text{ppm in control}) \cdot 100}{\text{ppm analyte added}}$$

Analyte can be either parent or metabolite. For calculation of metabolite recoveries, all ppm values in the formula must be in the same format, either metabolite amounts or Quinclorac equivalents.

5. RESULTS AND DISCUSSION

5.1 Accuracy and Precision

The following recovery results were obtained along with the analyses of residue samples from a soil dissipation study and a rotational crop study analyzed in February 1989. The soils were fortified at 0.05 ppm and at 0.5 ppm. Details can be found in the analytical reports to these studies as referenced.

Residue Control Number	Reference	Analyte	Recovery %	Average recovery %
87101	4	Quinclorac	91, 78, 79, 85, 90	85 ± 6 (N = 5)
		BH-514-1	67, 63, 65, 79, 87	72 ± 10 (N = 5)
87127	5	Quinclorac	84, 103, 86, 77, 89, 97, 71, 74, 92, 66, 89, 86	85 ± 11 (N = 12)
		BH-514-1	66, 74, 98, 65, 71, 74, 75, 74, 95, 62, 72, 56	74 ± 12 (N = 12)
87125	6	Quinclorac	90, 78, 86, 78, 73, 83	81 ± 6 (N = 6)
		BH-514-1	78, 75, 65, 65, 69, 65	70 ± 6 (N = 6)
87098	7	Quinclorac	70, 70, 71, 66, 79, 83, 72, 85, 81, 82	76 ± 7 (N = 10)
		BH-514-1	61, 58, 54, 56, 63, 61, 54, 66	59 ± 4 (N = 8)

5.2 Determination Limit

The limit of quantitation for Quinclorac and BH 514-1 residues in soil is 0.05 mg/kg. This is the lowest amount which is supported by recovery data.

5.3 Ruggedness Testing

This method has been satisfactorily used in the BASF laboratories.

5.4 Limitations

None known to date.

6 CONCLUSIONS

The analytical procedure is applicable for measuring residues of Quinclorac and its metabolite BH 514-1 in soil.

7 QUALITY ASSURANCE PROCEDURES

Location of raw data:

Raw data is stored in the BASF archives.

8 FLOW CHART OF ANALYTICAL PROCEDURE

25 g Soil

- Reflux with 200 mL of 0.1 N NaOH for 1 hour
- Centrifuge, wash twice with 3 % $CaCl_2$/1.5 % H_3PO_4/acetone

Marc → Discard

Extract

- Take 4% aliquot, acidify with 50 µL of conc. H_2SO_4
- Evaporate acetone, extract twice with 25 mL of DCM

Aqueous layer → Discard

DCM layer I

- Extract twice with 25 mL of saturated $NaHCO_3$ solution

DCM layer → Discard

$NaHCO_3$ layer

- Acidify with conc. H_2SO_4
- Extract 2x with 25 mL of DCM

Aqueous layer → Discard

DCM layer II

- Evaporate to dryness
- Dissolve in acetone/ acetic acid/water

HPLC/UV

9 REFERENCES

1. Clark, J. "BAS 514 H - ^{14}C Laboratory Soil Metabolism Study: Aerobic Aquatic System", BASF Report No. 8716, June 1987 (MRID No. 40320817)

2. Mayer, F., "HPLC Method for Determinations of Quinclorac and its Metabolite BH 514-1 in Soil", BASF Residue Method No. 280, July 1988

3. Winkler, V. "The Determination of Quinclorac (BAS 514 H) and its Metabolite (BH 514-1) Residues in Soils by GC/MS Techniques", BASF Method No. A8901, February 1989

4. Single, Y.H. "Aquatic Dissipation of the Residues of Quinclorac (BAS 514 H) and its Metabolite (BH 514-1) in California (RCN 87101) Rice Paddy Soil", BASF Report No. A8915, March 1989

5. Single, Y.H. "Dissipation of the Residues of Quinclorac (BAS 514 H) and its Metabolite (BH 514-1) in Mississippi (RCN 87127) Rice Paddy Soil", BASF Report No. A8916, March 1989

6. Single, Y.H. "Magnitude of the Residues of Quinclorac (BAS 514 H) and its Metabolite (BH 514-1) in Soils from a California (RCN 87125) Rotational Crop Study", BASF Report No. A8913, March 1989

7. Single, Y.H. "Magnitude of the Residues of Quinclorac (BAS 514 H) and its Metabolite (BH 514-1) in Soils from a Mississippi (RCN 87098) Rotational Crop Study", BASF Report No. A8912, March 1989

10 FIGURES

1. Sketch of HPLC column switching
2. Typical chromatogram of a control soil sample
3.,4. Typical chromatograms from recovery trials
5.-8. Typical calibration standard chromatograms
9.,10. Typical calibration curves

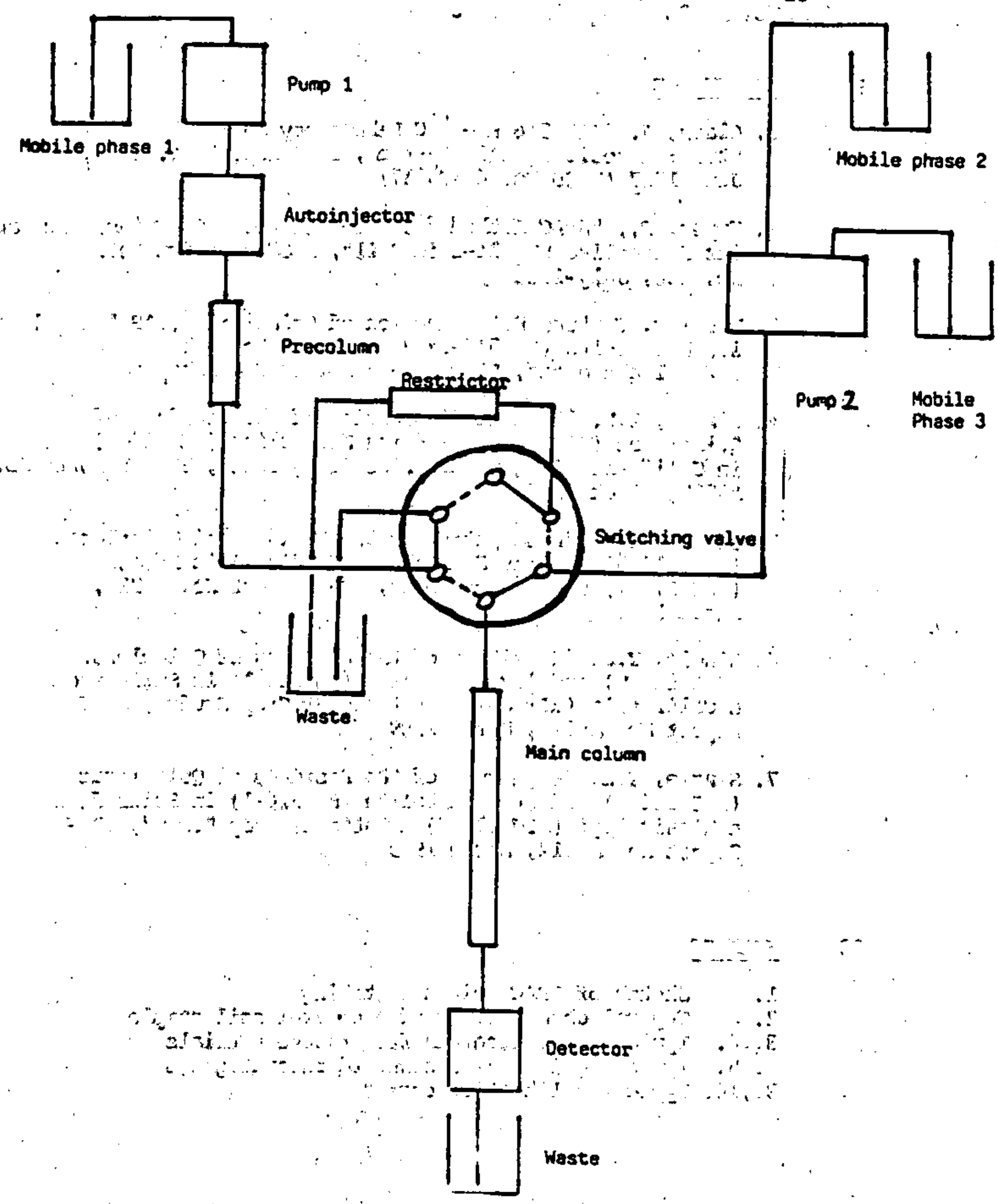


Figure 1: Sketch of HPLC column switching

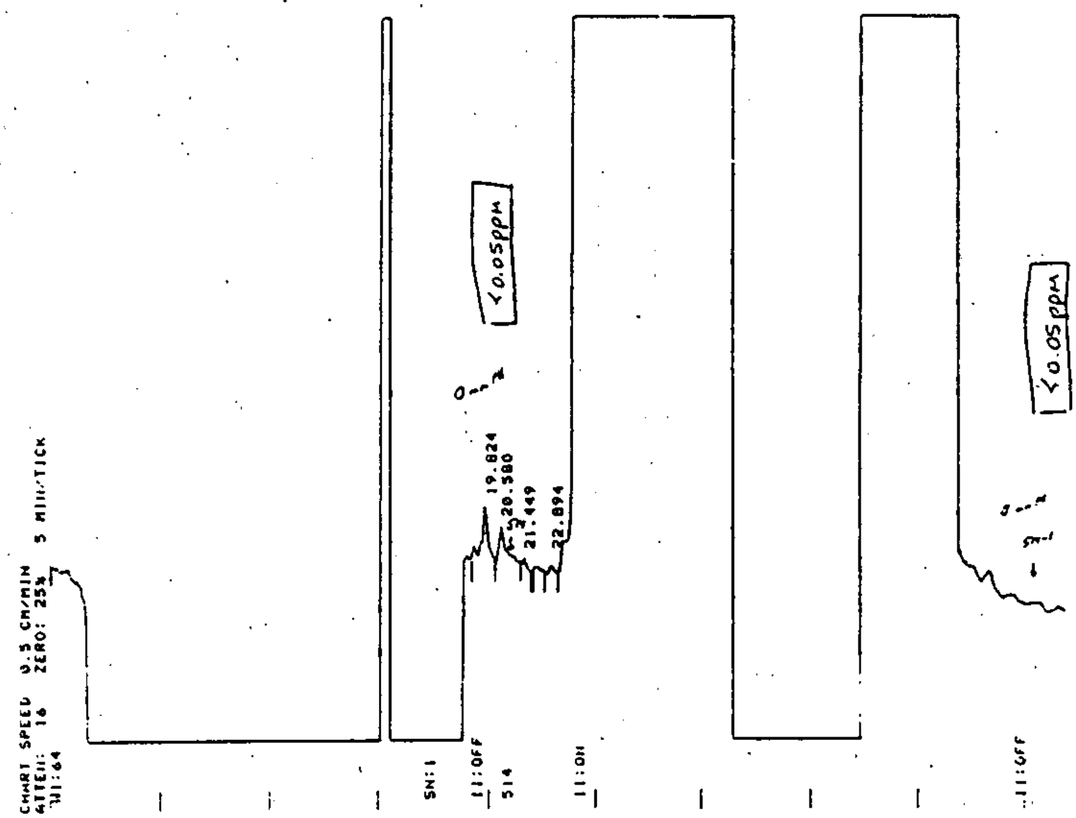


Figure 2: Typical chromatogram of a control soil sample
Mississippi soil; Residue Sample # 87127-277

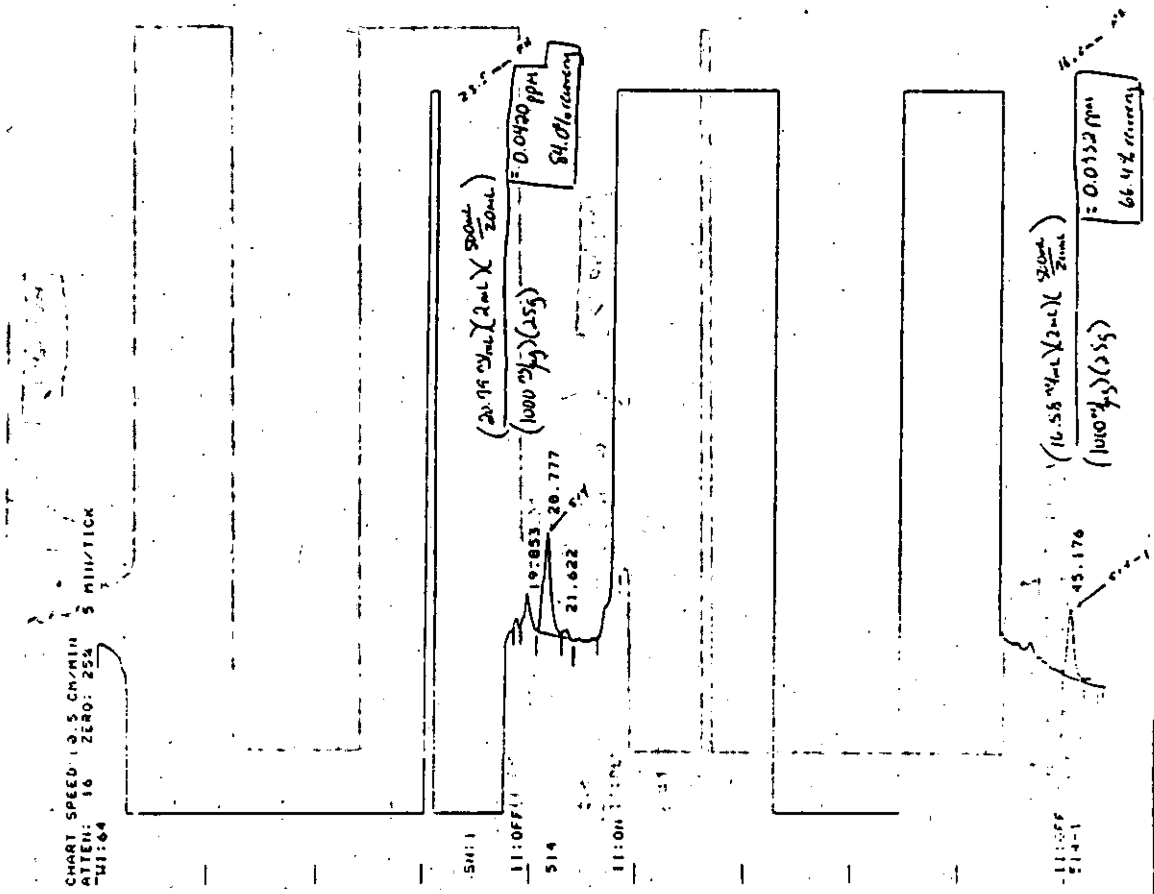


Figure 3: Typical chromatogram from a fortified sample 0.05 ppm
Residue Sample # 87127-277

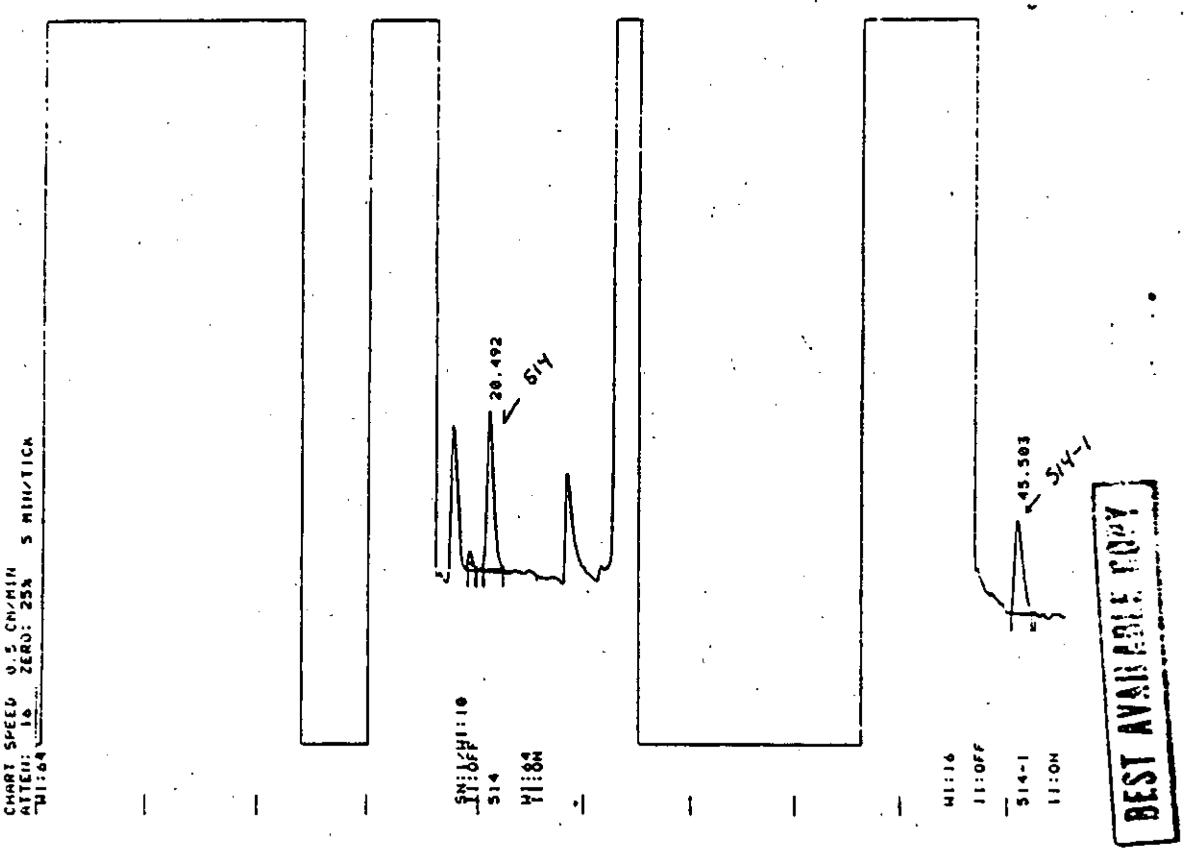


Figure 4: Typical chromatogram from a fortified sample 0.5 ppm
Residue Sample # 87127-277

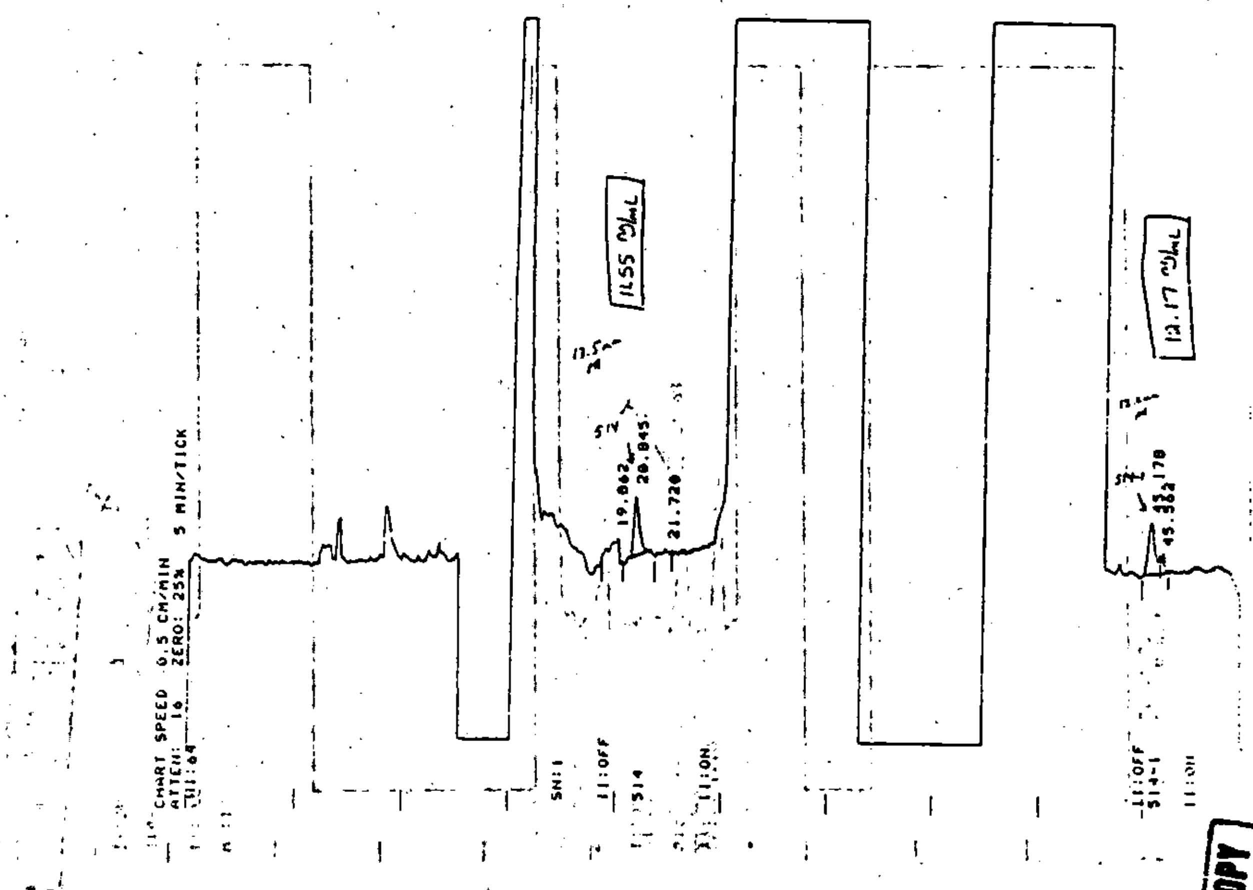


Figure 5: Typical chromatogram of a calibration standard: 12.5 ng/mL (0.625 ng standard)

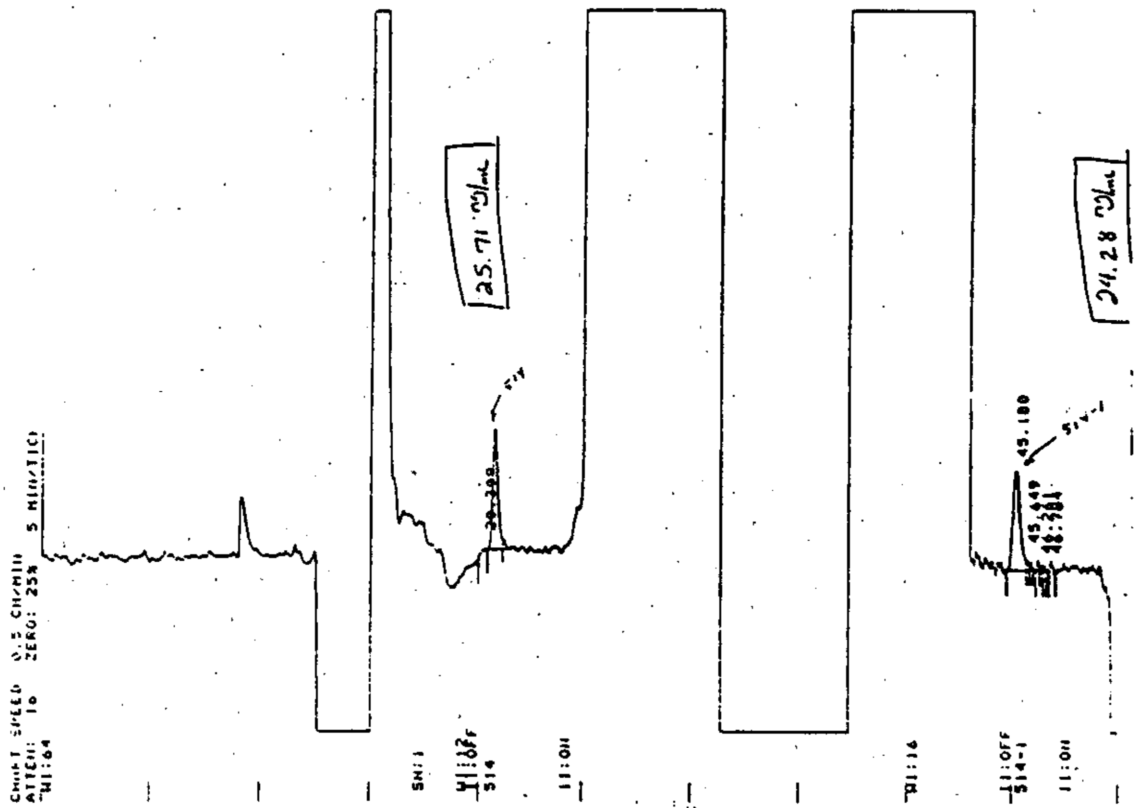


Figure 6: Typical chromatogram of a calibration standard: 25 ng/mL (1.25 ng standard)

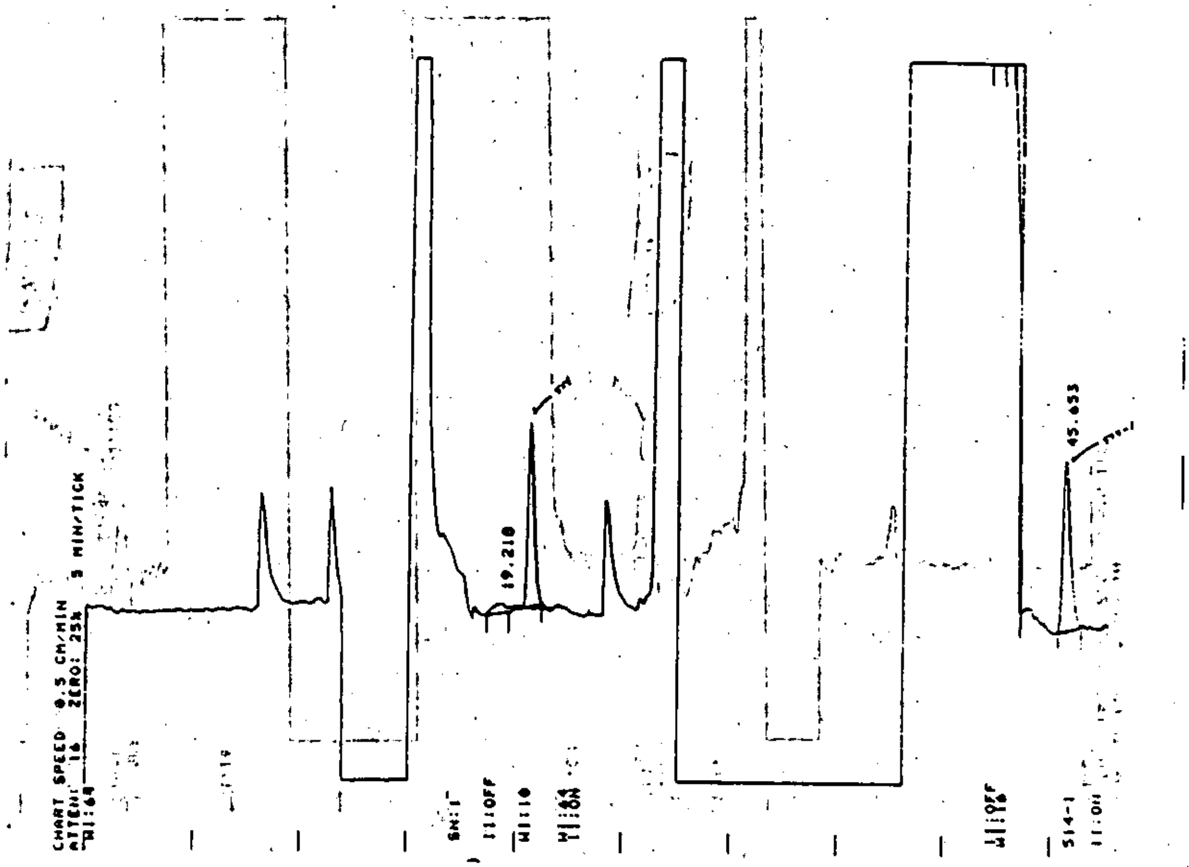


Figure 7: Typical chromatogram of a calibration standard: 50 ng/mL (2.5 ng standard)

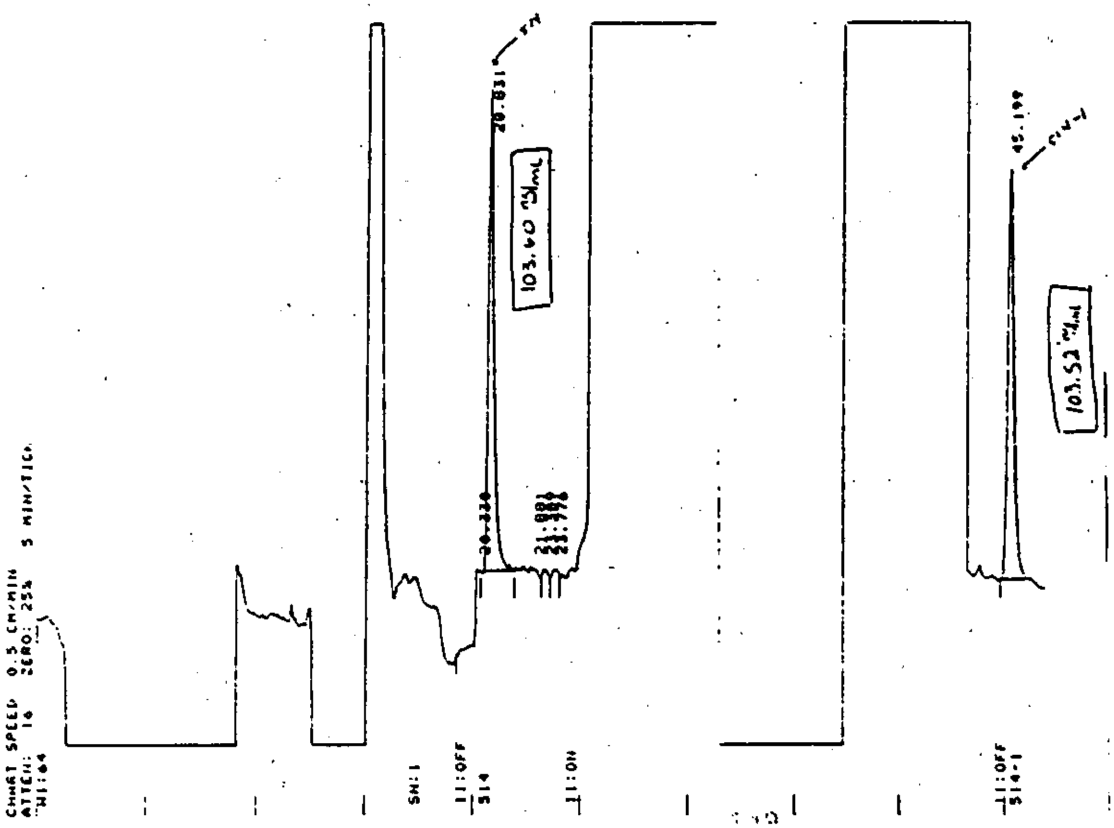


Figure 8: Typical chromatogram of a calibration standard:
100 ng/mL (5 ng standard)

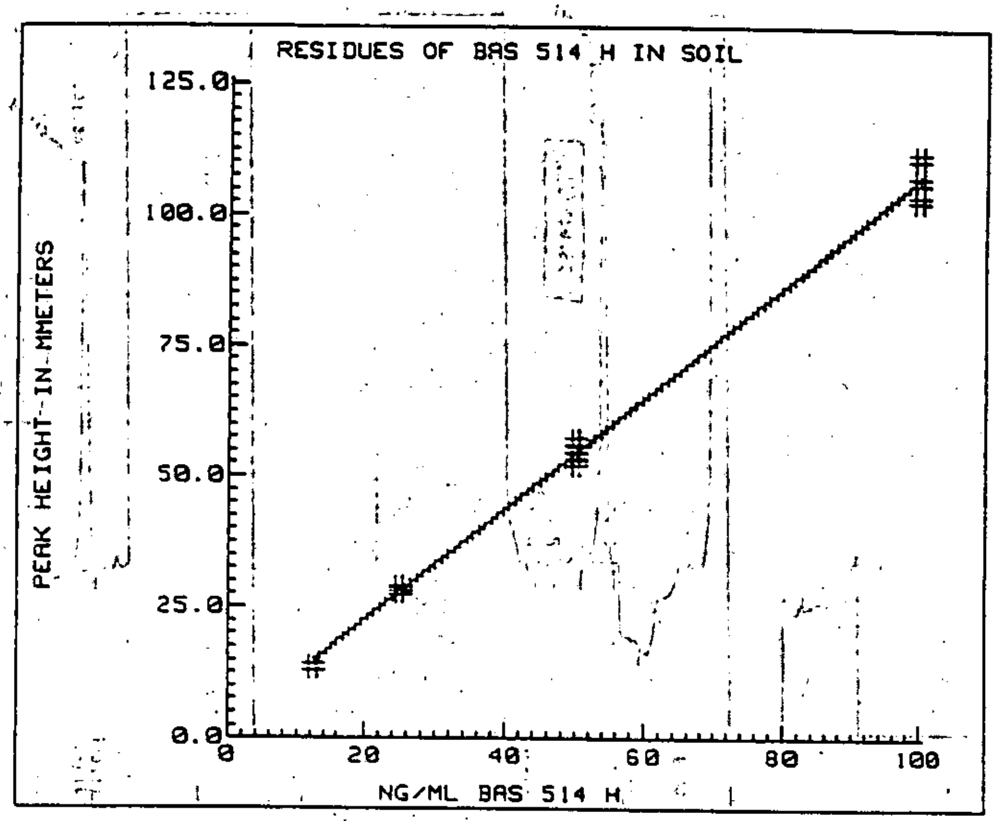


Figure 9: Typical calibration curve for Quinclorac

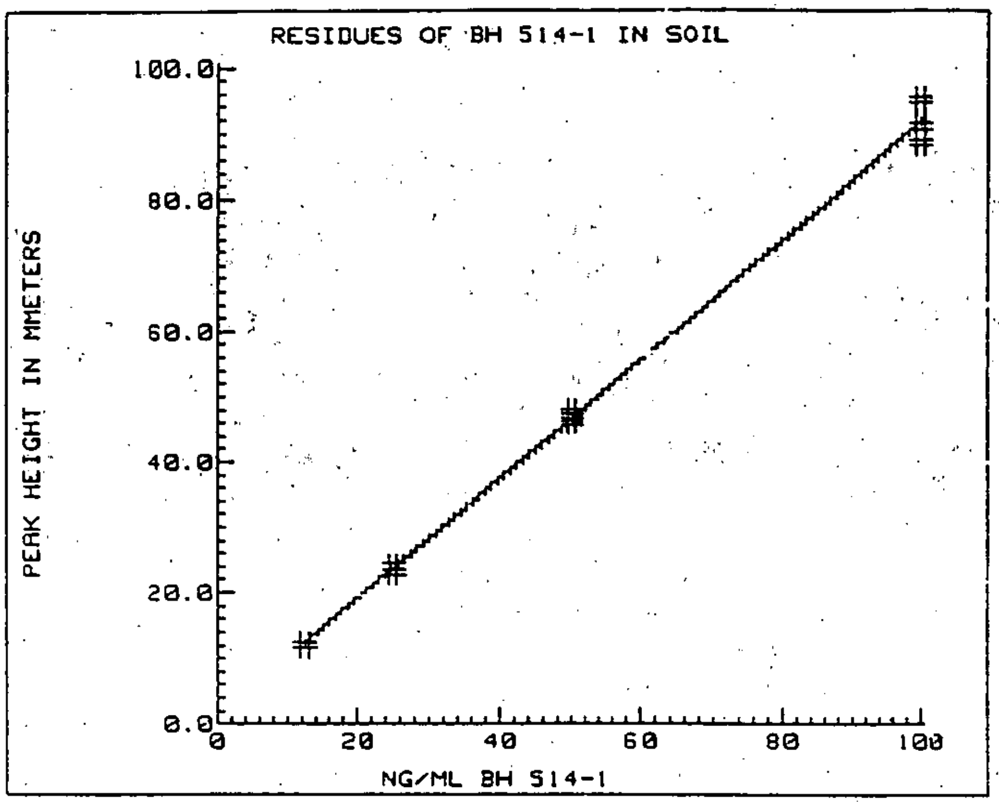


Figure 10: Typical calibration curve for BH 514-1

END