1. INTRODUCTION AND SUMMARY

1.1 Scope and Source of the Method

1.1.1 Scope

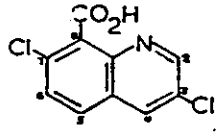
The method is used for the determination of quinclorac in soil. The method contains an alkaline hydrolytic extraction step which liberatas chemically bound residues. Following extraction into an organic phase, the active ingredient is methylated and determined by GC with column switching and electron capture detection.

1.1.2 Source

This method was developed at the BASF Agricultural Research Center in Research Triangle Park, North Carolina. The initial extraction procedure is identical to other methods (Ref. 1,2) used for the determination of quinclorac in soil. This method is also capable of achieving lower detection limits.

1.2 Description of Test and Reference Substances

Active Ingredient and Fortification Standard

Chemical Name:	3,7-Dichloro-8-quinolinecarboxylic acid
Common Name:	Quinclorac
Laboratory number:	150 732
BASF Number:	BAS 514 H
Lot Number:	CH 40/113-1
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. INTRODUCTION AND SUMMARY

1.2 Description of Test and Reference Substances (Continued)

Methyl Ester Derivative Used as External Standard for GC Quantitation

Chemical Name: Methyl-3,7-Dichloro-8-quinolinecarboxylate
Common Name: Quinclorac Methyl Ester
Lot Number: CH L33/65

Quinclorac Metabolite, Fortification Standard[1]

Chemical Name: 3-chloro-8-quinolinecarboxylic acid
Common Name: Quinclorac Metabolite
BASF Number: BH 514-1
Lot Number: L33/67

1.3 Principle of the Method

Soil is extracted with a 0.1 N NaOH solution under reflux conditions. The extract is acidified and quinclorac is partitioned into dichloromethane. Quinclorac is methylated with diazomethane and determined by GC using a column switching technique and electron capture detection.

Limit of quantitation: 0.01 mg/kg

2. MATERIALS AND METHODS

2.1 Equipment

	Suggested Sizes/Manufacturer
Bottles, amber, screw cap	4 oz
Buchner funnels	12 cm i.d.
Screw top test tubes	15 mL
Regular test tubes with 2-holed rubber stoppers	15 mL
Vacuum filter flasks	500 mL
Flat bottom flasks with standard ground joint	300 mL, 1 Liter

[1]This standard was used as a fortification standard to validate Method A9002. Method A9001 was not used to determine recovery of this compound.

2. MATERIALS AND METHODS (Continued)

2.1 Equipment	Suggested Sizes/Manufacture
Screw top Erlenmeyer flask	125 mL
Funnels, plastic	8 cm i.d.
Plastic tubing	
Magnetic stirring bars	2.5 cm
Volumetric flasks	25.0 mL, 100.0 mL
Disposable pipets	0.5 mL, 2 mL, 10 mL
Volumetric pipets	1.0 mL, 2.0 mL, 4.0 mL, 5.0 mL, 10 mL, 20.0 mL, 25.0 mL, 50.0 mL, 100.0 mL
Pasteur pipets	23 cm long, disposable
Capillary Gas Chromatograph with ^{63}Ni ECD	Varian 6000 or 6500 or equivalent
Capillary GC columns DB5, DB Wax	J&W Scientific Rancho Cordova, CA
N-evap (Nitrogen Stream Evaporator)	Organomation Association Northborough, MA
Rotary evaporators	Büchi or equivalent
Ultrasonic bath	Branson 1200 or equivalent
Reflux condensers with standard glass joint	
Stirring hot plates	Corning PC-351
Separatory funnels	250 mL
Delivery head	30 mL
Vortex mixer	American Scientific Products McGraw Park, IL
Spatula or small scoop	
Filter aid e.g., Celite type 545	Fisher Scientific Fairlawn, NJ

2. MATERIALS AND METHODS (Continued)

2.1 Equipment	Suggested Sizes/Manufacture
Filter paper (Whatman No. 2 and 4) 11 cm i.d.	Whatman Limited, England Fisher Scientific, Fairlawn, NJ
Phase separating filter, 1PS, 15 cm	Whatman Limited, England Fisher Scientific, Fairlawn, NJ
Universal pH indicator sticks or paper, pH 0-2.5	EM Science Cherry Hill, NJ
2.2 Reagents and Chemicals	**Recommended Manufacturer**
Acetic acid, glacial, reagent ACS	J.T. Baker Phillipsburg, NJ CAS 64-19-7
Acetone, distilled, high purity	Burdick & Jackson Muskegon, MI CAS 67-64-1
Carbitol (diethylene glycol monoethyl ether)	Fisher Scientific, Fairlawn, NJ CAS 111-90-0
Dichloromethane, distilled, high purity	Burdick & Jackson Muskegon, MI CAS 75-09-2
Diethyl ether, reagent ACS	Fisher Scientific Fairlawn, NJ CAS 60-29-7
Methanol, distilled, high purity	Burdick & Jackson Muskegon, MI CAS 67-56-1
N-Methyl-N-nitroso-p-toluenesulfonamide (Diazald)	Aldrich, Milwaukee, WI CAS 80-11-5
Potassium hydroxide analytical reagent - 50% in deionized water	Mallinckrodt Paris, KY
Sodium hydroxide pellets, reagent ACS	Kodak, Rochester, NY
Sodium hydroxide solution, 0.1N(0.1 mol/L) in deionized water	
Sulfuric acid, concentrated, analytical grade	J.T. Baker, Phillipsburg, NJ CAS 7664-93-9
Water, deionized	

2. MATERIALS AND METHODS (Continued)

2.2.1 Preparation of Diazomethane Solution

(All operations with diazomethane are performed in a well-ventilated hood!)

Assemble two test tubes, each fitted with a 2-hole rubber stopper and connected in series with plastic tubing. Add a few milliliters of diethyl ether to test tube A. Add approximately 100 mL of diethyl ether to a 125 mL Erlenmeyer flask. To test tube B, add 2.0 mL of carbitol with a disposable pipet, 2.0 mL of 50% potassium hydroxide in deionized water with a disposable pipet, approximately 1.0 g of Diazald, and 2.0 mL of diethyl ether with a disposable pipet. Use the ether solution to rinse the walls of the test tube. Slowly bubble nitrogen into the solution in test tube A, from test tube A into the solution in test tube B, and from test tube B into the solution in the Erlenmeyer flask. Allow gas to escape from the Erlenmeyer flask into the hood. Continue bubbling nitrogen until the yellow color in test tube B dissipates and a deep yellow color persists in the Erlenmeyer flask with no further intensification of the color. The level of diazomethane in the Erlenmeyer flask should be adequate for a set of 10 samples. Quench any remaining diazomethane solution in test tube B with acetic acid. The yellow color will disappear.

2.3 Standard Substances and Solutions

Quinclorac (BAS 514 H)	lot number CH 40/113-1 >99.0%
3,7-Dichloro-8-quinolinecarboxylic acid methylester (BAS 514 methylester)	lot number CH L33/65 >99.0%

Store these standards in a freezer.

(both standards supplied by: Dr. Josef Pawliczek, APS/UP
BASF Aktiengesellschaft
Landwirtschaftliche Versuchsstation
D-6703 Limburgerhof, West Germany
Telephone: 06236/68-2422

2. MATERIALS AND METHODS (Continued)

2.3.1 Standard Solutions for Fortifications

Quinclorac (BAS 514 H): 1000, 50.0, 10.0, 1.0, and 0.2 µg/mL in acetone.

Prepare a 1.00 mg/mL BAS 514 H stock solution by weighing an appropriate amount of BAS 514 H into a volumetric flask. Dissolve with acetone and dilute to the mark: For example, to prepare a 25 mL stock solution, dissolve 25.0 mg of BAS 514 H in a 25 mL volumetric flask.

Prepare a 50.0 µg/mL BAS 514 H standard solution by transferring an appropriate amount of the 1.00 mg/mL stock solution with a volumetric pipet to a volumetric flask (typically 5 mL of the 1.00 mg/mL stock solution in a 100 mL volumetric flask). Dilute to the mark with acetone.

Prepare a 10.0 µg/mL BAS 514 H standard solution by transferring an appropriate amount of the 50.0 µg/mL standard solution with a volumetric pipet to a volumetric flask (typically 20 mL of the 50.0 µg/mL stock solution in a 100 mL volumetric flask). Dilute to the mark with acetone.

Prepare a 1.00 µg/mL BAS 514 H standard solution by transferring an appropriate amount of the 10.0 µg/mL standard solution with a volumetric pipet to a volumetric flask (typically 10 mL of the 10.0 µg/mL standard solution in a 100 mL volumetric flask). Dilute to the mark with acetone.

Prepare a 0.200 µg/mL BAS 514 H standard solution by transferring an appropriate amount of the 1.0 µg/mL standard solution with a volumetric pipet to a volumetric flask (typically 20 mL of the 1.0 µg/mL standard solution in a 100 mL volumetric flask). Dilute to the mark with acetone.

Transfer each stock and standard solution to an amber bottle fitted with a plastic lined screw cap and store at room temperature. Replace stock solutions 90 days after preparation. Replace standard solutions 30 days after preparation.

2.3.2 Standard Solutions for GC Analysis

3,7-Dichloro-8-quinolinecarboxylic acid methylester (BAS 514 methylester): 10, 20, 30, 40, 200 ng/mL; 2, 50, 1000 µg/mL in acetone.

2. MATERIALS AND METHODS

2.3.2 Standard Solutions for GC Analysis (Continued)

Prepare a 1.00 mg/mL BAS 514 methylester stock solution by weighing an appropriate amount of BAS 514 methylester into a volumetric flask. Dissolve with acetone and dilute to the mark. For example, to prepare a 25 mL stock solution, dissolve 25.0 mg of BAS 514 H methylester in a 25 mL volumetric flask.

Prepare a 50.0 µg/mL BAS 514 methylester standard solution by transferring an appropriate amount of the 1.00 mg/mL stock solution with a volumetric pipet to a volumetric flask (typically 5 mL of the 1.00 mg/mL stock solution in a 100 mL volumetric flask). Dilute to the mark with acetone.

Prepare a 2.00 µg/mL BAS 514 methylester standard solution by transferring an appropriate amount of the 50.0 µg/mL standard solution with a volumetric pipet to a volumetric flask (typically 4 mL of the 50.0 µg/mL standard solution in a 100 mL volumetric flask). Dilute to the mark with acetone.

Prepare a 200 ng/mL BAS 514 methylester standard solution by transferring an appropriate amount of the 2.00 µg/mL standard solution with a volumetric pipet to a volumetric flask (typically 10 mL of the 2.00 µg/mL standard solution in a 100 mL volumetric flask). Dilute to the mark with acetone.

Prepare a 40.0 ng/mL BAS 514 methylester standard solution by transferring an appropriate amount of the 200 ng/mL standard solution with a volumetric pipet to a volumetric flask (typically 20 mL of the 200 ng/mL standard solution in a 100 mL volumetric flask). Dilute to the mark with acetone.

Prepare a 30.0 ng/mL BAS 514 methylester standard solution by transferring an appropriate amount of the 200 ng/mL standard solution with a volumetric pipet to a volumetric flask (typically 15 mL of the 200 ng/ml standard solution in a 100 mL volumetric flask). Dilute to the mark with acetone.

Prepare a 20.0 ng/mL BAS 514 methylester standard solution by transferring an appropriate amount of the 200 ng/mL standard solution with a volumetric pipet to a volumetric flask (typically 10 mL of the 200 ng/mL standard solution in a 100 mL volumetric flask). Dilute to the mark with acetone.

2. MATERIALS AND METHODS

2.3.2 Standard Solutions for GC Analysis (Continued)

Prepare a 10.0 ng/mL BAS 514 methylester standard solution by transferring an appropriate amount of the 200 ng/mL standard solution with a volumetric pipet to a volumetric flask (typically 5 mL of the 200 ng/mL standard solution in a 100 mL volumetric flask). Dilute to the mark with acetone.

Transfer each stock and standard BAS 514 methylester solution to an amber bottle fitted with a plastic lined screw cap. Store stock solutions in a refrigerator and store standard solutions at room-temperature. Replace stock solutions 90 days after preparation. Replace standard solutions 30 days after preparation.

2.3.3 Stability of Standard Solutions (Ref. 3)

Storage Days	Room Temperature Daylight	4° Refrigerator
	Quinclorac (BAS 514 H) 200 µg/mL in acetone	
5	100%	100%
71	100%	100%
	Quincloraoc Methyl Ester (BAS 514 ME) 0.2 µg/mL in acetone	
7	99.7%	103.8%
35	100%	100.8%
106		101.1%

3. ANALYTICAL PROCEDURE

3.1 Extract Preparation

3.1.1 Preparation of Samples

The samples are air dried, ground in a small mill, or passed through a 10 mesh screen and then stored at less than -5°C until analysis.

3. ANALYTICAL PROCEDURE (Continued)

3.1.2 Fortification and Extraction

Weigh 20.0 g of soil into a 1 liter flat bottom flask equipped with a plastic funnel. Record the sample weight to one decimal place. At least one fortification and one untreated sample (control) are run with each set of samples.

For each fortification, pipet an appropriate amount of standard BAS 514 H solution with a volumetric pipet onto the control soil sample. Evaporate the acetone fortification solution from the fortified samples using a rotary evaporator (without vacuum) for 5 minutes with a water bath at 35°C - 40°C.

Place a magnetic stirring bar into the flask. Add 200 mL of 0.1 N NaOH with a volumetric dispensing device into the flat bottom flask and rinse the funnel. Rinse the reflux condenser with acetone and allow to dry completely before use. Connect the flat bottom flask to the condenser and reflux for 1 hour while stirring. Allow the flat bottom flask to cool, using a water bath if needed.

3.1.3 Filtration

Allow the contents of the flat bottom flask to settle for 5 minutes. Place a sheet of Whatman filter paper in a Buchner funnel connected to a 500 mL vacuum filter flask. Typically Whatman #4 is used unless a smaller mesh is needed to separate out particulate matter. Wet the filter paper completely by pouring a small amount of the extract into the Buchner funnel. Add approximately 20 g of Celite filter aid to the Buchner funnel. Carefully pour all of the extract into the Buchner funnel. Turn on the aspirator and filter. The filtration process may take 10 to 15 minutes.

3.1.4. Dichloromethane Partition

Remove a 50 mL aliquot of the filtrate with a volumetric pipet and transfer to a 250 mL separatory funnel. In case a repeat analysis is necessary, transfer the remaining extract to an amber bottle.

3. ANALYTICAL PROCEDURE

3.1.4. Dichloromethane Partition (Continued)

Using a Pasteur pipet, adjust to pH 1.3 - 1.6 with concentrated sulfuric acid. Check the pH value with pH indicator strips (pH 0 - 2.5).

Using a delivery head, add 30 mL of dichloromethane to the separatory funnel. Shake the solution gently for a few seconds and vent the flask. Shake and vent a few more times and allow the phases to separate. Warning: An emulsion may form if the flask is shaken too vigorously. Small emulsions can be broken by stirring with a Pasteur pipet. If an emulsion cannot be broken, discard the sample, repeat the first paragraph of 3.1.4 with a 25 mL aliquot of the filtrate, plus 30 mL of 0.1 N NaOH, and shake the flask more gently during the next dichloromethane partition. Drain the lower dichloromethane layer (do not take emulsions) through a funnel containing Whatman 1PS phase separation filter paper into a 300 mL flat bottom flask. Repeat this extraction procedure three more times each with 30 mL of dichloromethane.

3.1.5. Concentration

After the four dichloromethane extracts have been combined in the 300 mL flat bottom flask, concentrate the solution to about 1 mL using a rotary evaporator with a water bath at 35°C - 40°C. Do not allow the residue to dry completely during rotary evaporation.

Using a Pasteur pipet, quantitatively transfer the sample to a 15 mL screw cap test tube with four successive 2-3 mL washes of dichloromethane.

Evaporate the solution to dryness using an N-evap (Nitrogen Stream Evaporator) with a water bath at room-temperature. Do not heat the water bath.

3.1.6. Methylation

Add approximately 2.0 mL of methanol to the sample. Rinse the walls of the test tube during the addition of methanol. Sonicate until the sample is redissolved.

3. ANALYTICAL PROCEDURE

3.1.6. Methylation (Continued)

Using a disposable pipet, add about 10 mL of ethereal diazomethane (see preparation of diazomethane in 2.2.1) to each sample. Note: All operations with diazomethane should be conducted in the hood, and the pipet should be fire polished. Cap the test tube and allow to stand for 1 hour. Add more ethereal diazomethane to the sample if the yellow color disappears in less than 30 minutes.

Evaporate the solution to dryness using an N-evap with a water bath at room temperature. Do not heat the water bath. Remove the sample from the N-evap immediately after evaporation.

3.1.7. Preparation of Final Solution

For GC/ECD determination, dissolve each sample with an appropriate amount of acetone added with a volumetric pipet (typically 2 mL for control and 0.01 ppm fortifications; 10 mL for 0.05 ppm fortifications; 100 mL for 0.5 ppm fortifications). Use an ultrasonic bath and a vortex mixer to ensure complete dissolution of sample. A flow diagram for the method is depicted in Figure 1.

3.2 Instrumentation

3.2.1. Description of Equipment

Gas Chromatograph	Varian 6000 or 6500 or equivalent equipped with ^{63}Ni-ECD and column switching valves (Figure 2).
Capillary Columns	WCOT fused silica, 30 m x 0.25 mm, 1 µm film thickness, DB5 or equivalent.
	WCOT fused silica, 30 m x 0.25 mm, 0.25 µm film thickness, DBWax or equivalent.

3.2.2. Typical Operating Conditions

Injection Temperature	250°C
Injector Configuration	Splitless, 4 mm I.D. fritted insert
Septum Purge	5.0 mL/min
Injection Volume	2 µL
Split Valve	Opened 30 seconds after injection, flow rate 100 mL/min

3. ANALYTICAL PROCEDURE

3.2.2 Typical Operating Conditions (Continued)

Oven Temperature	Temperature Programming[1]
Carrier Gas	Helium, 35 psi for each column
Detector Temperature	300°C
Make-up Gas	Nitrogen, 30 mL/min
Detector Settings	Range 10, Attenuation 8
Column Switching Window	9.5 - 12.5 minutes[1]
Retention Time	17.8 minutes
Run Time	20.0 minutes

3.2.3 Column Switching Conditions

At the start of each run, the DB5 and DBWax columns should not be connected to each other; the DBWax column should be connected to the detector, and the DB5 column should run to vent as shown in Figure 2(C). The sample is then injected onto the DB5 column. Shortly before the compound elutes from the DB5 column, the DB5 and DBWax columns should be connected in series using the value configuration shown in Figure 2(D). BAS 514 methylester will then switch onto the DBWax column. After the compound has had time to completely elute from the DB5 column, the valves should be switched back to the configuration shown in Figure 2(C) for the remainder of the run.

To establish the proper column switching window, it will be necessary to connect the DB5 column directly to the detector to determine the retention time of BAS 514 methylester on the DB5 column. This configuration is shown in Figure 2(A). An example of the instrument control settings for column switching using the varian vista 402 is shown in Figure 3.

3.2.4 Calibration Procedures

Calculation of results is based on peak height measurements using a calibration curve. To obtain this standard curve inject e.g. 20, 40, 60, 80 pg of BAS 514 methylester into the gas chromatograph. Plot peak height versus amount of injected standard.

[1]Temperature programming and the column switching window may be varied, depending on required peak resolution. Suggested conditions: initial temperature of 150°C and hold for 1 min, increase to 230°C at 35°C/min and hold for 7 min, increase to 240°C at 10°C/min and hold for 9 min. With these temperature conditions and the column switching window set from 9.5 to 12.5 minutes, a 17.8 min retention time was observed for BAS 514 methylester.

3. ANALYTICAL PROCEDURE (Continued)

3.2.5 Sample Analysis

Inject 2 µL of each sample and each BAS 514 methylester standard into the gas chromatograph for analysis. Use a larger injection volume if there are sensitivity problems; however, inject the same volume for all standards and samples. For each set of samples, it is recommended that each standard be injected at least in triplicate. Bracket the sample injections with standard injections. If it is necessary, each sample injection can be immediately followed by an injection of acetone to clear the column of slowly eluting compounds.

3.3 Interferences

3.3.1 Sample Matrices

If interfering peaks occur in the chromatogram, change GC operating conditions (see 3.2.2), analyze another aliquot of the filtrate (see 3.1.4.), or re-extract the sample (see 3.1.2). Other types of GC capillary columns could also be utilized.

3.3.2. Other Sources

Other Pesticides:	None known to date.
Solvents:	None known to date.
Labware:	None known to date.

3.4 Confirmatory Techniques

If ECD determination fails because of interferences or peak identity is doubtful, determination can be made by GC/MS using multiple ion detection (MID) on the ions m/e = 224, 226, 255, and 257 (Ref. 2). Choose one or more of these ions that is/are free from interferences.

3.5 Time Required for Analysis

The time required for a set of 7 samples, 2 fortifications, and 1 blank (= control) is 12 working hours. This includes the analytical procedure, overnight automated GC-injection (unattended), evaluation and report, provided that no special problems arise, such as matrix interferences.

3.6 Potential Problems

No problems have been encountered to date.

The column switching window may have to be adjusted at some point. If the peak height of the standards decrease significantly, the retention time on the DB5 column (Figure 2(A) valve configuration) should be checked and the switching window adjusted accordingly.

4. METHODS OF CALCULATION

4.1 Calibration

Measure the peak heights of the standards. Construct a linear least squares working curve in the form $y=ax+b$ or a polynomial least squares working curve in the form $y=ax^2 + bx + c$ from the standards by plotting peak height versus picograms of standard injected.

4.2 Analyte in Sample

Calculation of results is based on peak height measurements. Measure the peak height of the BAS 514 methylester peak in the samples. From the least squares working curve, determine the picograms of BAS 514 methylester in the samples.

The residues in mg/kg (ppm) of BAS 514 H are calculated as follows:

$$ppm = \frac{V_E \cdot W_A \cdot D}{G \cdot V_I \cdot A \cdot 1000}$$

- 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 BAS 514 methylester read from calibration curve in pg
- A = Aliquot in % as a decimal, taken during sample extract processing
- D = Derivatization factor = 0.945:

$$D = \frac{\text{Molecular weight quinclorac}}{\text{Molecular weight derivative}} = \frac{242}{256} = 0.945$$

4.3 Calculation of Recoveries

Correct fortification results for residues found in the control sample as follows:

ppm (corrected) = ppm in fortified control - ppm in control.

Determine percent recovery from the fortification experiments as follows:

$$\% \text{ Recovery} = \frac{\text{ppm (corrected)} \cdot 100}{\text{ppm BAS 514 H added}}$$

Do not correct treated sample results for either control residues or recovery.

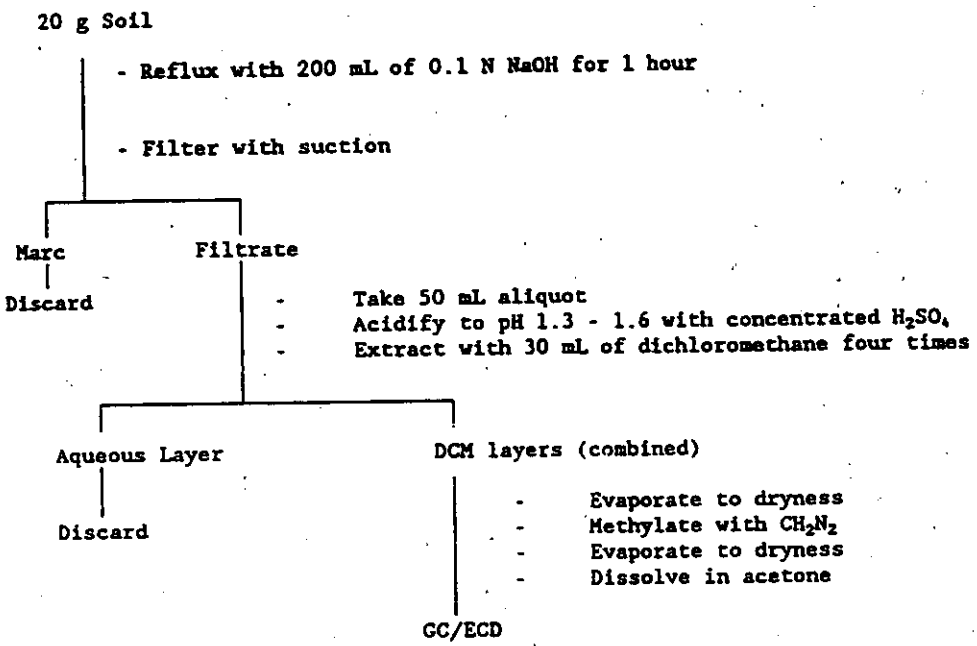


Figure 1. Flow Diagram for BASF Analytical Method Number A9001 as Applied to Soil.