

1 INTRODUCTION AND SUMMARY

1.1 Scope and Source of Method

1.1.1 Scope

Quinclorac (BAS 514 H) is an experimental herbicide being developed by BASF Corporation for use in rice and turf. It is currently being formulated as a 50% wettable powder. It is generally applied pre or post emergence to rice at 0.5 lb ai/A either under flooded or nonflooded conditions to control barnyard grass and certain broadleaf weeds. For turf, BAS 514 H is applied post emergence at 1 lb ai/A to control certain broad leaf weeds and crabgrass. Quinclorac, 3,7-dichloro-8-quinoline-carboxylic acid, is metabolized by dechlorination to 3-chloro-8-quinoline-carboxylic acid (BH 514-1). The analytical method was developed to measure both BAS 514 H and BH 514-1 in turf and aquatic soils.

1.1.2 Source

This method was developed by Dr. Victor Winkler at the Agricultural Research Center in Research Triangle Park, N.C. to measure quinclorac residues in a confined field dissipation study (Protocol M8813).

1.1.3 Principle of Method

The residues are extracted by refluxing in dilute alkali and partitioned into ethyl acetate at pH 1. The residues are derivatized to methyl esters using diazomethane and purified by preparative TLC. Total sample work up time is about 4 hours. The lower limit of quantitation ranges between 0.01 and 0.05 ppm depending upon the soil type and equipment.

2 MATERIALS AND METHOD

2.1 Equipment

- Erlenmeyer flasks with standard ground joint: 500 mL
- Volumetric pipettes: 1 mL; 2 mL; 10 mL
- Round bottom flasks: 250 mL
- Graduated cylinders: 100 mL
- Powder funnels: 10 cm
- Separatory funnels: 250 mL
- Snyder column (condenser): 3 ball
- Stirrer hot plates: standard
- Magnetized stir bars: standard
- Rotary evaporator: standard
- TLC developing chamber: 20 x 20 plates
- Balances: analytical and top load
- Gas Chromatograph and mass spectrometer: Varian Model 3300, or equivalent, interfaced into Nermag P-10-10 Mass Spectrometer, or equivalent, and a 5 m x 0.25 mm DB-5 column (J&W, Inc.). Operating conditions: Temperatures (°C) - Column, 100 to 240 @ 50/min and isothermal for 3 min to clean column; injection 230, flow 2 mL/min helium; SIM at m/z 190 (BH 514-1 ME) and 224 (BAS 514 H); integration time at 100 milliseconds.
- Tenivent vector 2 data system, or equivalent.
- Sliding needle injector, Chrompack Model 8992, or equivalent (optional for microsyringe injection).
- Centrifuge Beckman Model J-21C equipped with J-14 rotor, or equivalent.

2.2 Reagents and Chemicals

Solvents must be pesticide grade or equivalent. Suitable products are available from Burdick and Jackson Laboratories, Inc., and other manufacturers.

- Ether, anhydrous
- Ethyl acetate (distilled in glass)
- Methanol
- Hexane
- Carbitol
- Potassium hydroxide solution, 60% v/w in water
- Sodium hydroxide solution, 0.1 N in water
- Hydrochloric acid solution, 1 N in water
- Acetic acid solution, 10% water
- Sodium Sulfate, anhydrous
- N-methyl-N-nitroso-p-toluenesulfonamide (Diazald<sup>TM</sup>, Aldrich Chemical Co.)
- Universal pH indicator sticks, pH 0-14
- Cotton or glass wool
- TLC Plates: 20 x 20 cm, 250 µm Silica gel 60 with a pre-adsorbent strip (Whatman LK6F)
- Microfilters compatible with methanol: 0.45 µm (Acrodisk CR<sup>R</sup>, Gelman Sciences)
- Syringes: 5 mL to fit microfilters (Scientific Products)

2.2.1 Preparation of Diazomethane Solution

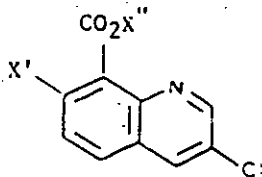
Preparation of diazomethane solution (in a hood): Add a few milliliters of diethyl ether to test tube A. Add 50 mL of diethyl ether and 12.5 mL of methanol to a 100 mL screw cap volumetric flask. Add 2.0 mL of carbitol, 2.0 g of diazald, and 2.0 mL of diethyl ether to test tube B. Use the ether solution 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 volumetric flask. Allow gas to escape from the volumetric flask into a 10% acetic acid trap. Continue bubbling nitrogen until the yellow color in test tube B dissipates and a deep yellow color forms in the solution in the volumetric flask. The level of diazomethane in the volumetric flask is adequate for the set of 8 samples. Quench any remaining diazomethane solution in test tube B with 10% acetic acid. The yellow color will disappear.

2.3 Standard Substances and Solutions

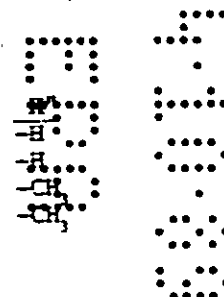
2.3.1 Standards

- 3,7-dichloro-8-carboxylic acid (BAS 514 H) standard: 99+% pure (BASF, Limburgerhof, FRG)
- 3-chloro-8-carboxylic acid (BAS 514-1) standard: 99+% pure (BASF, Limburgerhof, FRG)
- 3,7-dichloro-8-carboxylic acid methyl ester (BAS 514 ME): 99+% pure (BASF, Limburgerhof, FRG)
- 3-chloro-8-carboxylic acid methyl ester (BH 514-1 ME): 99+% pure (BASF, Limburgerhof, FRG)

2.3.2 Structures



	X'
BAS 514 H	-Cl
BH 514-1	-H
BAS 514 ME	-Cl
BH 514-1 ME	-H



2.3.3 Preparation of Solutions

- (a) BAS 514 H stock solution: 1 mg/mL. Dissolve 50 mg BAS 514 H in methanol and dilute to 50 mL with same solvent.
- (b) BH 514-1 stock solution: 1 mg/mL. Same as above, using BH 514-1.

- (c) BAS 514 ME stock solution: 1 mg/mL. Same as above, using BAS 514 ME.
- (d) BH 514-1 ME stock solution: 1 mg/mL. Same as above, using BH 514-1 ME.
- (e) BAS 514 H plus BH 514-1 working solution A: 10 mcg/mL admixture. Mix 1 mL each of (a) BAS 514 H and (b) BH 514-1 1 mg/mL stock solutions and dilute to 100 mL with methanol.
- (f) BAS 514 H plus BH 514-1 spiking solution: 1 mcg/mL admixture. Dilute 10 mL (e) working solution A to 100 mL with methanol.
- (g) BAS 514 ME plus BH 514-1 ME working solution B: 10 mcg/mL admixture. Mix 1 mL each of (c) BAS 514 ME and (d) BH 514-1 ME 1 mg/mL stock solution to 100 mL with methanol.
- (h) BAS 514 ME plus BH 514-1 ME working solution C: 1 mcg/mL admixture. Dilute 10 mL (g) working solution B to 100 mL with methanol.
- (i) BAS 514 ME plus BH 514-1 ME standard solution A: 100 ng/mL admixture. Dilute 10 mL (h) working solution C to 100 mL with methanol.
- (j) BAS 514 ME plus BH 514-1 ME standard solution B: 50 ng/mL admixture. Dilute 5 mL (h) working solution C to 100 mL with methanol.
- (k) BAS 514 ME plus BH 514-1 ME standard solution C: 5 ng/mL (5 pg/mL) admixture. Dilute 10 mL (j) standard solution B to 100 mL with methanol.

2.3.4 Stability of Standard Solutions (BASF Residue No. 266)

Storage days	Room Temperature Daylight	4°C Refrigerator
	(3,7-Dichloro-8-quinolinecarboxylic acid methyl ester) 0.2 g/mL in acetone.	
7	99.7%	103.8%
35	100.1%	100.8%
106	-	101.1%

2.3.5 Preparation of Standard Curves

The following standard curve solutions are prepared to cover a soil residue range of 0.025 to 0.10 ppm for final equivalent sample concentrations of 0.5 g/mL. The lowest standard curve concentration should be at 50% of the lowest spiked control

sample used for recovery analysis. If the soil sample matrix causes peak enhancement than standard curves solutions must be prepared using 1:1 v/v admixtures of standard solutions and control soil extracts.

2.3.5.1 Sliding Needle Technique

To calibrate the GC/MS using a sliding needle injection technique various aliquots are taken from the standard solution C and applied to the injection needle as shown below.

Standard Solution C @ 5 pg/mL		pg/Injection
2.5	mL	12.5
5	mL	25
10	mL	50

2.3.5.2 Microsyringe Technique

To calibrate the GC/MS using a microsyringe technique prepare the following standard curve solutions by diluting the 1 mcg/mL working solution C with methanol as shown below:

Std. Curve Solution No.	Working Sol. C mL @ 1 mcg/mL	Methanol mL Dilution	pg/1 mL Injection
1	1.25	98.75	12.5
2	2.5	97.5	25
3	5.0	95.0	50

3 ANALYTICAL PROCEDURE

3.1 Preparation of Sample

- (1) Soil cores are sectioned into predesignated lengths (defined by study protocol).
- (2) Replicate sections are air dried to a consistency which is suitable for pulverizing (typically between 2 and 8% moisture).
- (3) The air dried soil cores are pulverized either by milling or by mortar and pestle to approximately 10 mesh and mixed thoroughly before sampling for analysis.
- (4) Aliquots of the soil samples are assayed for moisture content either by loss-on-oven drying or Karl Fisher techniques.

3.2 Extraction of Sample

- (1) Each sample is assigned a discrete identification number with each extraction which stays with it throughout the analysis and is included with the final analytical result.

- (2) Each soil sample of known weight (20 g) is refluxed in 0.1 N NaOH (200 mL) for one hour and allowed to settle. Control Soils are spiked to 0.01, 0.05 and 0.10 ppm by adding 0.2, 1 and 2 mL of the spiking solution (f) for each analytical batch.
- (3) Sufficient solution is centrifuged at a speed to obtain a known aliquot (50 mL) of moderate clarity (e.g., 5,000 rpm x 10 min).
- (4) The aliquot (50 mL) is acidified to ca pH 1 with 1 N HCl and extracted 3 times with an equivalent volume of ethyl acetate (3x30 mL). Methanol can be used to break emulsions.
- (5) Each ethyl acetate extract is passed through anhydrous  $\text{Na}_2\text{SO}_4$  into a 250 mL R.B. flask which is followed by a final ethyl acetate wash (25 mL) of the  $\text{Na}_2\text{SO}_4$ .
- (6) The ethyl acetate extract is taken just to dryness by rotary evaporation at 45°C and reacted in a hood with 10 mL of fresh dark yellow diazomethane solution in ether:methanol (8:2) for 30 min to 1 hour. If solution does not remain yellow, add more diazomethane.
- (7) The reaction mixture is taken just to dryness by rotary evaporation at 45°C and resuspended in 2 mL methanol. Excessive drying of the methyl ester derivatives may result in sublimation and cause low recoveries.
- (8) A 1 mL aliquot of the resuspended residue is pipetted onto the preabsorbant zone of a 20x20 Whatman LK6F 250 micron TLC plate. Hot air drying between passes is optional.
- (9) The plate has ca a 2 cm runway scored on one side for developing ca 5 mcg of the methyl ester reference standards, BAS 514 ME and BH 514-1 ME. Use a mixture (1:1 v/v) of the 1 mg/mL stock solutions (c) and (d).
- (10) The TLC plate is developed to 10 cm with hexane:ethyl acetate (1:1) and allowed to air dry.
- (11) The sample residue zone is located under 254 nm UV light, using the BAS 514 ME and BH 514-1 ME spots as the upper and lower reference points (A typical developed TLC plate is shown in Figure 1).
- (12) The sample residue zone (ca 2 cm) is scraped off the plate and transferred into vials containing a known volume of MeOH (5 mL).
- (13) The sample is mixed (ca 15 seconds) and an aliquot (ca 2 mL) is withdrawn into a 5 mL syringe and filtered through a 0.45 micron filter (Acrodisk<sup>TM</sup>) to clarify the solution for GC/MS(SIM) analysis.

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(14) Weights and volumes taken for the total process are recorded for each sample to obtain equivalent sample weights as shown in the example below:

$$\frac{20 \text{ g sample}}{200 \text{ mL (alk ext.)}} \times \frac{50 \text{ mL (partition aliquot)}}{2 \text{ mL (MeOH resuspension)}} \times \frac{1 \text{ mL (MeOH for TLC)}}{5 \text{ mL (MeOH resuspension)}}$$

= 0.5 g equivalent sample wt per mL in the final solution.

(15) If necessary dilute samples with methanol to keep peak responses within the standard curve range.

### 3.3 Instrumentation

#### 3.3.1 Description

Location of Use	BASF	CompuChem
Gas Chromatograph	Varian 3300	Perkin Elmer Sigma 3B
Mass Spectrometer	Nermag P-10-10	Finnigan OWA
Capillary Column	DB5; 5m x 0.05mm	DB5; 20m x 0.32mm thick film
Injection Technique	Sliding Needle	Microsyringe

#### 3.3.2 Operating Conditions

Location of Use	BASF	CompuChem
Injection Temp	230°C	250°C
Oven Temperature	100°C to 240°C ± 50°C/min	130°C to 310°C at 18°/min
Retention Time		
BAS 514 ME	1.8 min	8.3 min
BH 514-1	1.5 min	7.3 min
Carrier Gas	He @ 2 mL/min	He @ 1 mL/min
Injection Volume	1 µL	3 µL
Internal Standard	none	0.5 µg/mL D <sub>10</sub> Phenanthrene and D <sub>10</sub> Acenaphthene

#### 3.3.3 Calibration Procedures

The GC/MS conditions are adjusted to give a response for the lower limit of quantitation to be ca 10 times background. This is typically in the 5 to 25 pg range, which is at 0.01 to 0.05 ppm residue levels for a 0.5 mg/mL sample equivalent weight final solution. Typically, the strongest ions for monitoring are m/z 224 for BAS 514 H ME and m/z 190 for BH 514-1 ME.

Standard curves are prepared by plotting peak area as the independent variable versus pg standard injected as the dependent variable. Linearity and zero y-intercept response must be established prior to conducting sample analysis if calculations are to be done by proportionality equations. If there is a peak response enhancement by a sample matrix effect, then the standard curve solutions must be prepared using 1:1 v/v admixtures of standard solutions and control soil extracts.

If linearity can not be established (i.e., coef corr,  $R > 0.95$ ), then a nonlinear but consistent standard curve must be established (i.e., relative std dev,  $CV < 10\%$ ).

**3.4 Sample Analysis**

After linearity (or at least a consistent standard curve response) has been established by injecting various amounts of standard solution, (e.g., 12.5, 25 and 50 pg BAS 514 ME + BH 514-1 ME) and graphing the corresponding integrated counts, known amounts of samples are injected and the BAS 514 ME and BH 514-1 ME peak response recorded. If necessary more or less sample material is injected for peak responses to correspond to the range of the standard curves. The calibrations must be checked by injecting a reference standard at least between every 10 samples or every twelve hours, whichever comes first. If the calibration has change all analysis following the previous calibration are invalid.

**3.5 Interferences**

**3.5.1 Sample Matrices**

If interfering peaks occurs at m/z 190 for BH 514-1 ME or m/z 224 for BAS 514 ME reassy using alternative ions (See Figure 2).

**3.5.2 Other Pesticides**

None known to date.

**3.5.3 Solvents**

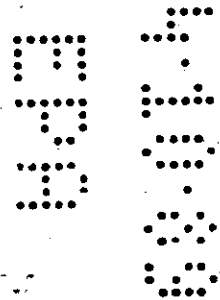
None known to date.

**3.5.4 Labware**

None known to date.

**3.6 Confirmatory Techniques**

Multiple ion peak matching:





### 3.7 Time Required for Analysis

The time required for a set of 6 samples, 2 recoveries and 1 control is about 8 hours. This includes sample preparation GC/MS analysis and the data report provided that no special problems arise.

### 3.8 Potential Problems

Low recoveries can possibly result from sublimation of the methyl ester derivatives if rotary evaporation drying is excessive.

### 3.9 Methods of Calculation

#### 3.9.1 Calibration

Prepare standard curves by plotting the integrated peak areas as the dependent variable versus the pg standard injected as the independent variable.

Use linear regression to test for linearity and the y-intercept. Proportionality equations can be used if there is 95% or greater correlation ( $R > 0.95$ ) and the y-intercept is close enough to zero so that any systematic error is less than 10%. Otherwise, concentrations of the sample solutions must be obtained directly from the standard curve provided the reproducibility of triplicate injections is within a 10% relative coefficient of variation.

#### 3.9.2 Analyte in Sample

The residues (=R) in mg/kg of Quinclorac and its metabolite BH 514-1 can be calculated as follows:

$$R = \frac{V_E * W_A * D}{G * V_I * A}$$

G = Weight in (g)

$V_E$  = Final volume after all dilution steps (mL)

$V_I$  = mL injected from  $V_E$

$W_A$  = Amount of determined substance read from calibration curve in ng

A = Aliquot in % (i.e., 12.5% for [(20 g/200 mL)(500 mL/2 mL) (1 mL/5 mL)])

D = Derivatization factor.

For Quinclorac:

$$D = \frac{\text{MW Quinclorac}}{\text{MW Quinclorac Methyl Ester}} = \frac{242}{246} = 0.945.$$

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For BH 514-1:

$$D = \frac{\text{MW BH 514-1} \cdot 707.6}{\text{MW BH 514-1 Methyl Ester} \cdot 721.6} = 0.937$$

If residues are to be reported as quinclorac equivalents multiply mg/kg BH 514-1 by the conversion factor, 1.166 (i.e., MW BAS 514/MW BH 514-1).