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:

\[
\begin{align*}
\text{Cl} & \quad \text{CO}_2\text{H} \\
\text{Cl} & \quad \text{Cl} \\
\text{N} & \\
\end{align*}
\]
Method No. A8903

Empirical formula: \( C_{10}H_8ClNO_2 \)

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: \( 6.2 \times 10^{-1} \)
- 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:

![Structural formula image]

Empirical formula: \( C_{10}H_8ClNO_2 \)

Molecular weight: 207.6

Melting point: 195 °C

Appearance: Crystalline, colorless

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

<table>
<thead>
<tr>
<th>Substance</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>3.8 x 10^{-3}</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.3</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>1.2</td>
</tr>
<tr>
<td>Acetone</td>
<td>1.4</td>
</tr>
<tr>
<td>Ethylacetate</td>
<td>0.6</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>2.9</td>
</tr>
<tr>
<td>Diethylether</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Toluene</td>
<td>0.5</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Olive oil</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

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
- Funnel
- Volumetric pipettes
- Magnetic stirring bars

1 Liter, 125 mL
4 cm, 10 cm i.d.
0.5 mL, 2.0 mL, 10.0 mL, 20.0 mL
2.5 cm
Reflex condensor with standard ground glass joint
Stirring hot plate
Plastic centrifuge bottle 250 mL
Centrifuge
Volumetric flask 500 mL
Graduated cylinder 100 mL
Spatula or small scoop
Eppendorf pipet 50 µL
pH sticks (0 - 14; 0 - 2.5)
Rotary Evaporator Buchi
Separatory funnel 125 mL
Pasteur pipets 23 cm long, disposable
Whatman #1FS phase separation filter paper
Glass centrifuge tube 50 mL
Ultrasonic bath Branson 1200
Vortex mixer American Scientific Products McGaw Parrell
Acrodisc LC-13 syringe filter 0.45 µm Gelman Sciences, # 4450
Plastic Syringe 3 cc Becton Dickenson, Rutherford, NJ
N-EVAP Millipore filter
(nitrogen stream evaporator) Millipore Corp.
GV 0.22 µm Bedford, MA
#GWP 04700

89/501- 0013
2.2 Reagents and Chemicals

Acetone, high purity solvent
Dichloromethane, distilled
Water, deionized
Sodium hydroxide pellets, reagent ACS
Sodium hydroxide solution 0.1 N (0.1 mol/l) in water
Calcium chloride dihydrate, cert. ACS
Phosphoric acid, meets ACS specs
3 % calcium chloride + 1.5 % phosphoric acid aqueous solution (w/w/v)
Sulfuric acid, concentrated analytical grade
Sodium Bicarbonate powder, analytical grade
Saturated aqueous sodium bicarbonate solution
50 % acetone + 1 % acetic acid in water (v/v/v)
Acetic acid, glacial, equivalent to USP specifications
Water, high purity solvent
Acetonitrile UV

Burdick & Jackson
Burdick & Jackson
Kodak, Rochester, NY Cat 137 6466
Fisher Scientific, Fairlawn, NJ
J.T. Baker, Phillipsburg, NJ
J.T. Baker, Phillipsburg, NJ
Fisher Scientific Fairlawn, NJ
EM Science, Cherry Hill, NJ # AX0072-1
Burdick & Jackson Product 365
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)

<table>
<thead>
<tr>
<th>Storage Days</th>
<th>Room Temperature Daylight</th>
<th>4 °C Refrigerator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinclorac 200 µg/mL in acetone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>71</td>
<td>100 %</td>
<td>100 %</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BH 514-1 1 µg/mL in acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
</tr>
<tr>
<td>71</td>
</tr>
<tr>
<td>118</td>
</tr>
</tbody>
</table>

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 50 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 CO₂ 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 C₁₈ 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-C₁₈
- Alltech Associates
- Guard column: Waters Guard-Pak Precolumn Module with Resolve C₁₈ 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

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:

<table>
<thead>
<tr>
<th>Line</th>
<th>Time</th>
<th>Event</th>
<th>Value</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0.0</td>
<td>Flow</td>
<td>1.0</td>
<td>Flow rate 1.0 mL/min</td>
</tr>
<tr>
<td>2</td>
<td>0.0</td>
<td>Reservoirs</td>
<td>AB</td>
<td>Selection of solvent reservoirs</td>
</tr>
<tr>
<td>3</td>
<td>0.0</td>
<td>%A</td>
<td>100</td>
<td>Mobile phase II on main column</td>
</tr>
<tr>
<td>4</td>
<td>0.0</td>
<td>%B</td>
<td>0</td>
<td>Mobile phase II on main column</td>
</tr>
<tr>
<td>5</td>
<td>0.1</td>
<td>Relay</td>
<td></td>
<td>Detector autozero</td>
</tr>
<tr>
<td>6</td>
<td>0.2</td>
<td>Relay</td>
<td></td>
<td>Detector autozero</td>
</tr>
<tr>
<td>7</td>
<td>1.0</td>
<td>Relay</td>
<td></td>
<td>Connects precolumn with main column</td>
</tr>
<tr>
<td>8</td>
<td>1.0</td>
<td>Relay</td>
<td></td>
<td>Disconnects columns</td>
</tr>
<tr>
<td>9</td>
<td>16.9</td>
<td>Relay</td>
<td></td>
<td>Detector autozero</td>
</tr>
<tr>
<td>10</td>
<td>17.0</td>
<td>Relay</td>
<td></td>
<td>Detector autozero</td>
</tr>
<tr>
<td>11</td>
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<td>100</td>
<td>Gradient from mobile phase II to mobile phase III on main column</td>
</tr>
<tr>
<td>12</td>
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<td>0</td>
<td>Gradient from mobile phase II to mobile phase III on main column</td>
</tr>
<tr>
<td>13</td>
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<td>Gradient from mobile phase II to mobile phase III on main column</td>
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<td>14</td>
<td>20.1</td>
<td>%B</td>
<td>100</td>
<td>Gradient from mobile phase II to mobile phase III on main column</td>
</tr>
<tr>
<td>15</td>
<td>22.0</td>
<td>Relay</td>
<td></td>
<td>Connects precolumn with main column</td>
</tr>
<tr>
<td>16</td>
<td>27.0</td>
<td>Relay</td>
<td></td>
<td>Disconnects columns</td>
</tr>
<tr>
<td>17</td>
<td>35.9</td>
<td>Relay</td>
<td></td>
<td>Detector autozero</td>
</tr>
<tr>
<td>18</td>
<td>36.0</td>
<td>Relay</td>
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<td>Detector autozero</td>
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<tr>
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<td>%B</td>
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<td>Gradient from mobile phase III to mobile phase II on main column</td>
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<tr>
<td>20</td>
<td>39.9</td>
<td>%A</td>
<td>0</td>
<td>Gradient from mobile phase III to mobile phase II on main column</td>
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<tr>
<td>21</td>
<td>40.0</td>
<td>%B</td>
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<td>Gradient from mobile phase III to mobile phase II on main column</td>
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<tr>
<td>22</td>
<td>40.0</td>
<td>%A</td>
<td>100</td>
<td>Gradient from mobile phase III to mobile phase II on main column</td>
</tr>
</tbody>
</table>
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 µL 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 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 Quinlorac and the BH 514-1 peaks in the samples. From the least squares working curves, determine the nanograms of Quinlorac 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 Quinlorac and its metabolite BH 514-1 expressed as Quinlorac equivalents are calculated as follows:

\[
\text{ppm} = \frac{V_e \cdot W_A \cdot U - 100}{G \cdot V_e \cdot A}
\]

- \( G \) = Weight in (g) of sample extracted
- \( V_e \) = Final volume after all dilution steps (mL)
- \( V_i \) = \( \mu \)L injected from \( V_e \)
- \( W_A \) = Amount of determined substance read from calibration curve in \( \mu \)g
- \( A \) = Aliquot in %, taken during sample extract processing
- \( U \) = Conversion factor (for determination of metabolite only; converts determined metabolite residues to Quinlorac equivalents).

\[
U = \frac{\text{Molecular weight of Quinlorac}}{\text{Molecular weight of BH 514-1}} = \frac{242.1}{207.6} = 1.166
\]

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

4.3 Calculation of Recoveries

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

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 Quinlorac equivalents.
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 % CaCl₂/1.5 % H₃PO₄/acetone

Marx Extract

Discard
- Take 4% aliquot, acidify with 50 µL of conc. H₂SO₄
- Evaporate acetone, extract twice with 25 mL of DCM

Aqueous layer  DCM layer I

Discard
- Extract twice with 25 mL of saturated NaHCO₃ solution

DCM layer  NaHCO₃ layer

Discard
- Acidify with conc. H₂SO₄
- Extract 2x with 25 mL of DCM

Aqueous layer  DCM layer II

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

HPLC/UV
REFERENCES

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


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