GAS CHROMATOGRAPHIC DETERMINATION OF FENOXAPROP-ETHYL [ETHYL-2-(4-(6-CHLORO-2-BENZOXAZOLYLOXY)PHENOXY)PROPANOATE] AND ITS METABOLITES 2-(4-(6-CHLORO-2-BENZOXAZOLYLOXY)PHENOXY)PROPANOIC ACID AND 6-CHLORO-2,3-DIHYDROBENZOXAZOL-2-ONE AS RESIDUES IN WHEAT AND BARLEY GRAIN, STRAW, AND MILLED PRODUCTS

HRAV Analytical Method: HRAV-4B

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Amended to include barley grain, straw, and milled products:

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Developed by: Hoechst Aktiengesellschaft, Analytical Laboratory
6230 Frankfurt (M) 80
Postfach 80 03 20

Dr. H. Idstein, Hoechst AG
Mr. H. J. Werner, Hoechst AG

Revised by: J. J. Czarnecki, Ph.D.
Hoechst-Roussel Agri-Vet Company

Submitted by: Hoechst Celanese Corporation
Route 202-206
PO Box 2500
Somerville, NJ 08876-1258

J. J. Czarnecki, Ph.D.
Manager, Analytical Chemistry
Hoechst-Roussel Agri-Vet Company
(908) 231-2887

Approved by: J. S. O’Grodnick, Ph.D.
Director, Scientific Affairs
Hoechst-Roussel Agri-Vet Company

DATA REQUIREMENT

Guideline 171-4(c)

[60 Pages]
1.0 Chemical Information:

A. Parent:

Common Name: Fenoxaprop-ethyl

Code No.: HOE-033171 (racemic mixture of d and l isomers)
           HOE-046360 (single d isomer)

Chemical Name: ethyl-2-(4-(6-chloro-2-benzoxazolyl)oxy)propanoate (IUPAC, English).

CAS No: 66441-23-4

Structure:  

-CH-\text{COOH}

Molecular Formula: C_{16}H_{16}ClNO_{3}

Molecular Weight: 361.8 g/mole

Solubility: Nearly insoluble in water; soluble in ethanol, cyclohexane, n-hexane; extremely soluble in acetone, toluene, ethyl acetate.

B. Metabolite:

Common Name: Fenoxaprop (free acid)

Code No.: HOE-053022 (racemic mixture of d and l isomers)
           HOE-088406 (single d isomer)

Chemical Name: 2-(4-(6-chloro-2-benzoxazolyl)oxy)propanoic acid.

Structure:  

-CH-\text{COOH}

Molecular Formula: C_{16}H_{16}ClNO_{3}

Molecular Weight: 333.7 g/mole
1.0 Chemical Information: (continued)

C. Metabolite:

Common Name (Code No.): HOE-054014

Chemical Name: 6-chloro-2,3-dihydrobenzoxazol-2-one.

Structure:

\[
\begin{array}{c}
\text{H} \\
\text{Cl} \\
\text{N} \\
\text{O} \\
\end{array}
\]

Molecular Formula: \(\text{C}_7\text{H}_4\text{ClNO}_2\)
Molecular Weight: 169.6 g/mole

D. Chemical Derivative:

Common Name (Code No.): HOE-083312

Chemical Name: 3-acetyl-6-chloro-2,3-dihydrobenzoxazol-2-one.

Structure:

\[
\begin{array}{c}
\text{CH}_3 \\
\text{C} = \text{O} \\
\text{Cl} \\
\text{N} \\
\text{O} \\
\end{array}
\]

Molecular Formula: \(\text{C}_9\text{H}_6\text{ClNO}_3\)
Molecular Weight: 211.6 g/mole
2.0 Principle of the Method:

Residues of fenoxaprop-ethyl and its major metabolites are removed from the matrices by refluxing the sample in a mixture of acetonitrile:HCl:water (90:10:50) for 6 hours.

During this process, fenoxaprop-ethyl \( \text{[ethyl-2-(4-(6-chloro-2-benzoxazolyloxy)phenoxy)propanoate]} \) and fenoxaprop \( \text{[2-(4-(6-chloro-2-benzoxazolyloxy)phenoxy)propanoic acid]} \) are converted to HOE-054014 \( \text{[6-chloro-2,3-dihydrobenzoxazol-2-one]} \).

The mixture is then diluted with distilled water to obtain a solution containing approximately 50 percent acetonitrile. Any undissolved material is removed by filtration. An aliquot of the filtrate is added to an Extrelut column. Coextracted substances are then eluted with hexane(s). The hydrolysis product \( \text{[HOE-054014: 6-chloro-2,3-dihydrobenzoxazol-2-one]} \) resulting from the reflux procedure is subsequently eluted with a 20% solution of diethyl ether in hexane(s).

The eluate containing HOE-054014 is concentrated to dryness and dissolved in ethyl acetate. An aliquot is evaporated to dryness and derivatized using acetic anhydride/pyridine mixture (pyridine serving as a catalyst). Derivatization is accomplished over a three-hour period at 130°C.

To prepare for quantitation, the resultant derivative \( \text{[HOE-083312: 3-acetyl-6-chloro-2,3-dihydrobenzoxazol-2-one]} \) is cleaned up using a combination of reverse phase (C-18 SEP PAK) and silica gel chromatography.

Final gas chromatographic determination of fenoxaprop-ethyl residues is carried out using electron capture detection of the derivative, HOE-083312.

Residues are measured as combined residues of fenoxaprop-ethyl, fenoxaprop free acid, and HOE-054014 and are ultimately expressed as fenoxaprop-ethyl equivalents. The limit of quantitation of the method is 0.05 ppm.

3.0 Equipment:

1. **Food Processing or Milling Device**: (e.g.) Hobart Food Processor, Retsch Mill, Laboratory (Waring) Blender, or functional equivalent.

2. **Balances**: Mettler Model PC 2000, or equivalent (for sample preparation); Mettler Model AE 160, or equivalent (for standard preparation).

3. **Microliter Syringes**: 100, 250, 500 microliter (uL), Hamilton, or equivalent.

4. **Round Bottom Flask**: 500 mL capacity with a 29/42 ground glass joint [Ace Scientific: 6887-407]; 500 mL capacity with a 24/40 ground glass joint [Fisher Scientific: 10-0676].
3.0 **Equipment:** (continued)

5. **Reflux Condenser:** 12" (typical), equipped with a 29/42 ground glass joint [Ace Scientific: 5955-34].

6. **Heating Mantles:** Glas-Col; 500 mL [Fisher Scientific: 11-472-10F] or equivalent device (e.g., hot plate/stirrer).

7. **Glass Funnels:** Pyrex, 10 cm diameter [Fisher Scientific: 10-329 D].

8. **Fluted Filter Paper:** 24 cm, fast [Whatman Reeve Angel: 802].

9. **Volumetric Pipets:** Glass; 1.0, 5.0, 10.0 and 20.0 mL capacity.

10. **Extrelut OE Columns:** 20-mL capacity [VWR: EM-901020-1]. Alternately, Extrelut 20 columns may be used [Merck-11737; VWR: EM-11737-1].

11. **Volumetric Flasks:** Glass; 250 mL capacity. [Fisher Scientific: 10-210E].

12. **Rotary Flash Evaporator:** Buchi, R110 or equivalent, with a water bath capable of operating at 45-50°C.

13. **Culture Tubes:** 16 x 125 mm, with screw caps (Teflon lined) [Scientific Products: T 1358-I]; used for the derivatization reaction.

   [Note: It is essential that the reaction vials (culture tubes) remain completely sealed during the derivatization reaction. Also, the heating device (block) must cover the reaction vials approximately up to the screw cap of the vial. No more than 10 mm of glass tube surface should remain exposed above the heating block. See also Item 14 below.]

14. **Tube Heater:** Kontes Model K 720000; used for the derivatization reaction. A 4-inch deep heating block is required to satisfy the criteria noted above in Item 13 if 125 mm culture tubes are used.

15. **N-Evap Analytical Concentrator:** Organamation Associates, Inc. or equivalent.

16. **Vortex:** Lab Line Instruments, Super Mixer Model 1290.

17. **C-18 SEP PAK Cartridges:** Waters Associates [51910] or equivalent.

18. **Disposable Pasteur Pipette:** Glass; 9" x 0.7 mm O.D. [VWR: 14672-380].

19. **Glass Wool:** DMCS treated [Alltech Associates: 4037]; used for packing the mini-silica gel columns.

20. ** Graduated Centrifuge Tube:** 10-15 mL [Fisher Scientific: 05-538-35B].
3.0 Equipment: (continued)

21. **Syringes**: Glass, 10 mL capacity, equipped with Luerlock fitting [Fisher Scientific: 14-823-15C].

22. **Rheostat**: Staco; Input 120V, Output 0-120/140 V.

23. **Syringe Needles**: Stainless steel, 100 mm [Organonetics: 11305].

24. **Wide Bore Luer Connector**: Special goose-neck syringe needle, see Figure 3.

25. **Male Luer Connector**: Luer to 1/8 inch tube [Rainin Instrument Co., Inc.: 47-ML4]. This may be used in place of item No. 24, see Figure 3a.

26. **Magnetic Stirrer**: Mini Stirrer; [VWR: 58950-158] or equivalent.

27. **Magnetic Stir Bars**: TFE, 1/2 inch diameter x 1 inch long [VWR: 58949-196] or equivalent.

28. **Vacuum Manifold Apparatus**: J & W SPE Vacuum Manifold [No. 600-4000]; for use with silica gel solid phase extraction (SPE) cartridges. Note: A functionally equivalent apparatus or technique which is compatible with the SPE cartridge system may be utilized to provide a uniform and controllable cartridge elution (drip) rate.

29. **Silica Gel SPE Cartridges**: 500 mg cartridge with reservoir, [Varian: No. AI-121130-36 (formerly branded Analytichem International), or functional equivalent].

30. **Collection Tubes, Glass**: 16 mm X 100 mm (Fisher Scientific 14-961-29), or equivalent; for collection of SPE column fractions (typically used in conjunction with vacuum box assembly).

31. **Luer Fittings/Stopcocks**: J & W Scientific [Nos. 811-4008/700-4022], or equivalent; for connection/control of SPE cartridges.

32. **Centrifuge**: Centra 7 (VWR No. 20673-503), or equivalent apparatus capable of generating 3000 RPM (with 150 mL centrifuge tubes) as per the requirements of Section 6.1.1d (see below).

33. **Centrifuge Tubes**: 150 mL with round bottom (Corning No. 1256), or equivalent.

4.0 Reagents/Chemical Supplies:

a) **Acetic Anhydride**: Aldrich (99 + %, No. 32,010-2), or equivalent.

b) **Acetonitrile**: Pesticide quality or equivalent (Mallinkrodt).

c) **Diethyl Ether**: Pesticide quality or equivalent (Mallinkrodt).

d) **Ethyl Acetate**: Pesticide quality or equivalent (Mallinkrodt).
4.0 Reagents/Chemical Supplies: (continued)

e) **Glacial Acetic Acid**: ACS reagent grade or equivalent (Mallinkrodt).

f) **n-Hexane**: Pesticide quality or equivalent (Mallinkrodt: 4159) or hexanes [Mallinkrodt Nanograde (No. 4159)], or equivalent.

g) **Hydrochloric Acid**: Concentrated, ACS reagent grade.

h) **Methanol**: Pesticide quality or equivalent (Mallinkrodt: 5160)

i) **Pyridine, Anhydrous**: Aldrich Gold Label (99+ %, No. 27,097-0), or equivalent.

j) **Toluene**: Pesticide quality or equivalent (EM Merck, OmniSolv: TX 0737-1).

k) **Reflux Solution**: Acetonitrile:Concentrated HCL:Distilled Water, 90:10:50 (v/v/v)

l) **20% Diethyl Ether in Hexane(s)**: Prepared by adding 20% (v/v) diethyl ether to hexane(s).

m) **Acetylation Solution**: Prepared by adding 1 mL pyridine to 5 mL acetic anhydride. [The solution is to be made fresh every day.]

n) **Silica Gel**: Merck 7734, dried for minimum of 5 hrs. at 130°C.

o) **Water Deactivated Silica Gel**: The silica gel is deactivated to 5% water content with distilled water.

Five (5) grams of distilled water are added to 95 grams of oven-dried (5 hr) silica gel. The mixture is then allowed to equilibrate for 24 hours on a roller mill or other equivalent equipment.

The elution pattern of HOE-083312 on the silica gel should be determined as each batch is prepared (See Section 8.0, Analytical Notes).

p) **Anhydrous Sodium Sulfate**: Granular (Kodak:842) or equivalent.

q) **Gas Chromatography Columns**: 1.5 um DB-1 phase, 15 meters x 0.53 mm megabore fused silica capillary column (J&W Scientific: 125-1012) or 1.5 um DB-5 phase, megabore fused silica capillary column, 15 meters X 0.53 mm ID megabore column (J & W Scientific No. 125-5012), or 1.0 um DB-5 phase, 20 meters x 0.32 mm ID widebore fused silica capillary column (J & W Scientific No. 123-5063, e.g., reduced from 60 to 20 meters), or a functionally equivalent fused silica capillary column or 10 ft. x 2 mm id, 3% SP-2100 on Supelcoport (100/120 MESH) packed column.
4.0 Reagents/Chemical Supplies: (continued)

r) Fenoxaprop-ethyl (HOE-033171 or HOE-046360):
   ethyl-2-(4-(6-chloro-2-benzoxazolyl)ethoxy)propanoate; analytical standard:
   Hoechst-Roussel Agri-Vet Company, Somerville, NJ 08876-1258

s) Fenoxaprop (HOE-053022 or HOE-088406):
   2-(4-(6-chloro-2-benzoxazolyl)ethoxy)propanoic acid; analytical standard:
   Hoechst-Roussel Agri-Vet Company, Somerville, NJ 08876-1258

t) HOE-054014: 6-chloro-2,3-dihydrobenzoxazol-2-one;
   analytical standard: Hoechst-Roussel Agri-Vet Company, Somerville, NJ 08876-1258

u) HOE-083312: 3-acetyl-6-chloro-2,3-dihydrobenzoxazol-2-one; analytical standard: Hoechst-
   Roussel Agri-Vet Company, Somerville, NJ 08876-1258

v) Water: Acetic Acid (5:1 v/v): Mix 25 mL of glacial acetic acid and 125 ml of distilled
   water.

5.0 Preparation of Standard Solutions:

HOE-083312: [For GC/ECD Calibration]

Weigh 100.0 mg HOE-083312 into a 100 mL volumetric flask. Dilute to volume with toluene. This
solution (Stock Solution A) contains 1.0 mg HOE-083312/mL. This stock solution should be
prepared fresh every three months, or as necessary. Transfer 5.0 mL of Stock Solution A to a 100
mL volumetric flask. Dilute to volume with toluene. This solution (Solution B) contains 50.0 ug
HOE-083312/mL. This solution should be prepared fresh every month, or as necessary.

Transfer 5.0 mL of Solution B to a 100 mL volumetric flask. Dilute to volume with toluene. This
solution (Solution C) contains 2.5 ug HOE-083312/mL. This solution should be prepared fresh
every month, or as necessary.

Transfer 4.0 mL of Solution C to a 100 mL volumetric flask. Dilute to volume with toluene. This
solution (Solution D) contains 0.10 ug HOE-083312/mL. This solution should be prepared fresh
every month, or as necessary.
5.0 Preparation of Standard Solutions: (continued)

Make dilutions of Solution D (or C) every month or more frequently (as needed) for calibration of the gas chromatograph. A typical set of dilutions is shown in Table I. [Note: The actual concentrations of GC calibration standards which are prepared and used must be compatible with the achievable sensitivity of the GC/ECD instrumentation and with the particular analyte level(s) of interest.]

<table>
<thead>
<tr>
<th>Amount (mL)</th>
<th>Solution</th>
<th>Final Volume (mL)</th>
<th>Concentration of Injection Standard (ng/μL)</th>
<th>Concentration of Injection Standard (pg/μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>D</td>
<td>100.0</td>
<td>0.0020</td>
<td>2.0</td>
</tr>
<tr>
<td>4.0</td>
<td>D</td>
<td>100.0</td>
<td>0.0040</td>
<td>4.0</td>
</tr>
<tr>
<td>6.0</td>
<td>D</td>
<td>100.0</td>
<td>0.0060</td>
<td>6.0</td>
</tr>
<tr>
<td>10.0</td>
<td>D</td>
<td>100.0</td>
<td>0.0100</td>
<td>10.0</td>
</tr>
<tr>
<td>1.0</td>
<td>C</td>
<td>100.0</td>
<td>0.0250</td>
<td>25.0</td>
</tr>
</tbody>
</table>

Fenoxaprop-Ethyl: [For Sample Fortification]

[Note: For determination of procedural recovery, fenoxaprop-ethyl may be fortified as either HOE-033171 (racemic mixture) or HOE-046360 (single d isomer). The methodology will effectively determine both the d and l isomer forms of fenoxaprop-ethyl.]

HOE-033171

Weigh 100.0 mg of pure HOE-033171 into a 100 mL volumetric flask. Dilute to volume with ethyl acetate. This solution (Stock Solution E) contains 1.0 mg/mL of fenoxaprop-ethyl. This stock solution should be prepared fresh every month, or as necessary.

Transfer 5.0 mL of Stock Solution E to a 100 mL volumetric flask. Dilute to volume with ethyl acetate. This solution (Solution F) contains 50.0 μg/mL of fenoxaprop-ethyl. This solution should be prepared fresh every two weeks, or as necessary.

Transfer 5.0 mL of solution F to a 100 mL volumetric flask. Dilute to volume with ethyl acetate. This solution (Solution G) contains 2.5 μg/mL of fenoxaprop-ethyl. This solution should be prepared fresh every two weeks, or as necessary.
5.0 Preparation of Standard Solutions: (continued)

**HOE-046360**

Weigh 100.0 mg of pure HOE-046360 into a 100 mL volumetric flask. Dilute to volume with ethyl acetate. This solution (Stock Solution H) contains 1.0 mg HOE-046360/mL. This stock solution should be prepared fresh every month, or as necessary.

Transfer 5.0 mL of Stock Solution H to a 100 mL volumetric flask. Dilute to volume with ethyl acetate. This solution (Solution I) contains 50.0 ug HOE-046360/mL. This solution should be prepared fresh every two weeks, or as necessary.

Transfer 5.0 mL of solution I to a 100 mL volumetric flask. Dilute to volume with ethyl acetate. This solution (Solution J) contains 2.5 ug HOE-046360/mL. This solution should be prepared fresh every two weeks, or as necessary.

**Fenoxaprop: [For Sample Fortification]**

[Note: For determination of procedural recovery, fenoxaprop (free acid) may be fortified as either HOE-053022 (racemic mixture) or HOE-088406 (single d isomer). The methodology will effectively determine both the d and l isomer forms of fenoxaprop.]

**HOE-053022**: Weigh 100.0 mg of pure HOE-053022 (or HOE-088406) into a 100 mL volumetric flask. Dilute to volume with ethyl acetate. This solution (Stock Solution K) contains 1.0 mg/mL of fenoxaprop free acid. This stock solution should be prepared fresh every month, or as necessary.

Transfer 5.0 mL of Stock Solution K to a 100 mL volumetric flask. Dilute to volume with ethyl acetate. This solution (Solution L) contains 50.0 ug/mL of fenoxaprop free acid.

Transfer 5.0 mL of Solution L to a 100 mL volumetric flask. Dilute to volume with ethyl acetate. This solution (Solution M) contains 2.5 ug/mL of fenoxaprop free acid. Solutions L and M should be prepared fresh every month, or as necessary.

**HOE-088406**: Weigh 100.0 mg HOE-088406 into a 100 mL volumetric flask. Dilute to volume with ethyl acetate. This solution (Stock Solution N) contains 1.0 mg HOE-088406/mL. This stock solution should be prepared fresh every month, or as necessary.

Transfer 5.0 mL of Stock Solution N to a 100 mL volumetric flask. Dilute to volume with ethyl acetate. This solution (Solution O) contains 50.0 ug HOE-088406/mL.

Transfer 5.0 mL of Solution O to a 100 mL volumetric flask. Dilute to volume with ethyl acetate. This solution (Solution P) contains 2.5 ug HOE-088406/mL. Solutions O and P should be prepared fresh every month, or as necessary.
5.0 Preparation of Standard Solutions: (continued)

**HOE-054014: [For Sample Fortification]**

Weigh 100.0 mg of pure HOE-054014 into a 100 mL volumetric flask. Dilute to volume with ethyl acetate. This solution (Stock Solution Q) contains 1.0 mg HOE-054014/mL. This stock solution should be prepared fresh every three months, or as necessary.

Transfer 5.0 mL of Stock Solution Q to a 100 mL volumetric flask. Dilute to volume with ethyl acetate. This solution (Solution R) contains 50.0 ug HOE-054014/mL.

Transfer 5.0 mL of Solution R to a 100 mL volumetric flask. Dilute to volume with ethyl acetate. This solution (Solution S) contains 2.5 ug HOE-054014/mL. Solutions R and S should be prepared fresh every month, or as necessary.

SOLUTIONS G, J, M, P, AND S ARE TYPICALLY USED TO FORTIFY SAMPLES FOR METHOD RECOVERY VERIFICATION. FORTIFICATION SOLUTION LEVEL(S) AND/OR VOLUMES MAY BE ADJUSTED TO ACHIEVE THE NECESSARY FORTIFICATION LEVEL(S). SEE ALSO SECTION 7.1.

**Note:**

The amount of analytical standard material specified for preparation of the stock solutions is adjusted to account for standard material purity (e.g., use 101 mg of certified 99% pure standard to obtain a 100-mg equivalent of pure standard).

5.1 Sample Preparation:

A homogeneous, finely ground, analytical laboratory sample must be prepared from the field sample (i.e., grain and straw). [Ref: Pesticide Analytical Manual Volume I, Sections 141 and 142 as applicable.] It is important that the laboratory sample be finely ground before subjecting it to the analysis procedures described in Section 6.0. For example, as required, the analytical sample should be ground to approximately 20 mesh (or until reduced to a particle size of about 2 mm or less).

6.0 General Procedure: (See Flow Diagram - Figure 1)

6.1 Extraction:

6.1.1. Barley and Wheat Grain:

a. Weigh a 25.0 gram (W) subsample (described in Section 5.1) into a 500 mL round bottom flask equipped with a 29/42 ground glass joint.

b. Add 150 mL of the reflux solution (acetonitrile:concentrated hydrochloric acid:distilled water; 90:10:50) and a magnetic stir bar.

c. Attach the sample flask to a reflux condenser and gently reflux with stirring for 6 hours using (e.g.) a heating mantle directly over a stirring plate.

[Note: For increased laboratory efficiency, electrical timers would allow automatic termination of the reflux. Thus, samples may undergo the 6-hour reflux overnight, with appropriate laboratory coverage. The completion of reflux also corresponds to a convenient stopping point in the method.]
6.1 Extraction: (continued)

6.1.1. Barley and Wheat Grain: (continued)

d. At the end of the reflux period (or upon resuming the procedure), add 50 mL distilled water through the top of the condenser. The volume of the extract at this point is considered to be 203 mL (equivalent to 200 mL of extraction solvent + 3.0 mL of sample moisture: V1). See also Reference 1 and Section 8.4.2. Centrifuge approximately 125 mL of the hydrolyzed wheat grain extract for 15-20 minutes (3000 rpm).

e. Filter the supernatant through fluted filter paper without rinsing. Following filtration, proceed with the Extrelut column clean-up step (see Section 6.2).

6.1.2. Barley and Wheat Straw and Milled Products:

a. Weigh a 10.0 gram subsample (described in Section 5.1) into a 500 mL round bottom flask equipped with a 29/42 ground glass joint.

b. Add 150 mL of the reflux solution (acetonitrile:concentrated hydrochloric acid:distilled water; 90:10:50) and a magnetic stir bar.

c. Attach the sample flask to a reflux condenser and gently reflux with stirring for 6 hours using (e.g.) a heating mantle directly over a stirring plate. [See Note in Section 6.1.1(c) above.]

At the end of the reflux period, add 40 mL distilled water through the top of the condenser.

d. Filter the warm solution through fluted filter paper without rinsing. The volume of the extract at this point is considered to be 191 mL (equivalent to 190 mL of extraction solvent + 1.0 mL of sample moisture: V1). See also Reference 1 and Section 8.4.2. Following filtration, proceed with the Extrelut column clean-up step (see Section 6.2).

6.2 Extrelut Column Clean-Up:

Following extraction and filtration, a 20 mL (T1) aliquot of the filtered extract is added to an Extrelut column. [The Extrelut column is used as received connected to a 22 G x 1.25 inch needle.] The filtered extract is allowed to equilibrate with the column matrix for thirty (30) minutes. After equilibration, the column is eluted with 50 mL hexane(s). The hexane(s) eluate is discarded. [Elution with more than 50 mL hexane(s) has been found to partially elute the compound of interest]. The hydrolysis product (HOE-054014) is eluted from the column with 250 mL of a solution containing 8:2 (v/v) hexane(s):diethyl ether. This eluate is collected in a 500 mL round bottom flask equipped with a 24/40 ground glass joint. [It is efficient in this elution step to use an inverted 250 mL volumetric flask containing the elution solvent. The volumetric flask is filled with the solvent and inverted onto the top of the Extrelut column. Because the Extrelut column has no reservoir, this technique is used to ensure continuous flow of elution solvent and permits unattended operation. This also corresponds to a possible stopping point in the method, i.e., allowing unattended elution to occur (e.g.) overnight.]
6.2 Extrelut Column Clean-Up: (continued)

The Extrelut column eluate is evaporated to dryness using a rotary flash evaporator with a water bath maintained at 45-50°C. Residue adhering to the flask wall is rinsed with 20 mL of ethyl acetate and evaporated to dryness as described above. Rotation of the flask should be at a sufficiently slow speed to keep the residue at the bottom. Remove the flask from the rotary flash evaporator and cool to room temperature. For grain samples, proceed to Section 6.2.1; for straw and milled products, proceed to Section 6.2.2.

6.2.1 Grain Samples:

Residue in the flask is dissolved with 10.0 mL ethyl acetate and mixed. A 5.0 mL aliquot is transferred to a screw-capped culture tube; proceed to Section 6.3: Derivatization.

6.2.2 Straw and Milled Products:

Residue in the flask is dissolved with small amounts of ethyl acetate (< 10 mL total) and quantitatively transferred to a screw-capped culture tube; proceed to Section 6.3: Derivatization.

6.3 Derivatization:

Evaporate the ethyl acetate to dryness with a stream of nitrogen using an N-Evap analytical concentrator with a water bath maintained at 45-50°C.

Add exactly 1.0 mL of the acetylation mixture [freshly prepared acetic anhydride: pyridine 5:1 (v/v)] to the residue in the culture tube. The tube is tightly closed with a teflon-lined screw cap and mixed thoroughly. The tube is then placed in the tube heater maintained at 130°C for three (3) hours. [Note: It is important that nearly the entire glass portion of the tube be within the heated area, while the cap and teflon liner be outside the heated area. For example, no more than 10 mm of the glass tube portion should be exposed above the heating block. An air-tight seal must be maintained throughout the heating procedure, using a suitable teflon-lined cap. Teflon-rubber cap liners must not be used. See also Section 3.0 above (Item nos. 13 and 14).]

This reaction quantitatively converts HOE-054014 [6-chloro-2,3-dihydro-benzoxazol-2-one] to HOE-083312 [3-acetyl-6-chloro-2,3-dihydro-benzoxazol-2-one].

Following derivatization, the capped tubes are removed and allowed to cool to room temperature. For increased laboratory efficiency, the use of electrical timers in conjunction with the heating block would allow automatic termination of the derivatization reaction. This corresponds to the third possible stopping point in the method. Do not quench the reaction until the procedure is resumed (see below). The procedure should be resumed within a 24-hour period.

Once cool (or upon resuming the procedure at this point), the derivatized mixture is cleaned up using the C-18 SEP-PAK (see Section 6.4).
6.4 C-18 SEP-PAK Clean-Up:

6.4.1 Prepare the C-18 cartridge immediately prior to use with 5.0 mL methanol eluted in short pulses (the cartridge should appear opaque with no air pockets). Follow with 10.0 mL distilled water. The top of the cartridge should contain water (not air).

6.4.2 Add 5 mL of water:acetic acid solution (5:1 v/v) to the culture tube. Cap the culture tube and vortex vigorously for 15 seconds. This quenching process destroys residual acetic anhydride.

[Note: It is important to minimize the time between quenching of the derivatization reaction (6.4.2) and the loading of the derivatized analyte onto the C-18 cartridge (6.4.3).]

6.4.3 Attach a 150 mm stainless steel Luer needle to a 10 mL glass syringe and draw up the mixture. Rinse the culture tube walls with 1.0 mL distilled water and draw this into the syringe. To the short end of the conditioned C-18 cartridge, add 1 to 2 drops of solution from the syringe (to prevent air from entering). Connect the syringe and slowly press the solution through; discard eluate. Connect an empty 10 mL syringe (without the plunger) to the short end of C-18 cartridge and (under vacuum) wash with 10 mL distilled water. This technique is shown in Figure 2A. [House vacuum is normally sufficient; water aspiration typically is not.] After drying for five (5) minutes, the cartridge is tapped on a flat surface to remove any residual water droplets. [When residual water is minimized, the next steps are easier to accomplish.]

The HOE-083312 [3-acetyl-6-chloro-2,3-dihydrobenzoxazol-2-one] is eluted from the C-18 SEP-PAK directly onto the silica gel clean-up column. However, prior to this step it is important to eliminate as much residual water as possible. Proceed to the Silica Gel Column Clean Up (Section 6.5).

6.5 Silica Gel Column Clean-Up*:

Two alternative procedures are described below for the final clean-up step. Section 6.5.1 describes the preparation of mini silica gel columns. Section 6.5.2 describes the conditioning step for the commercially available SPE cartridge. Follow either Section 6.5.1 or Section 6.5.2 according to the laboratory operating procedure selected.

6.5.1 Preparation of Mini Silica Gel Column:

Place a silanized glass wool plug in the bottom of a 9" Pasteur pipette (see Fig. 3). Add 0.3 g of the 5% water deactivated silica gel [prepared as in Section 4(o)]. The column is preconditioned by washing with 5 mL hexane(s). [It is important that all air bubbles are removed. This can be accomplished by gentle tapping. The column will also appear opaque at this point.]

While hexane(s) solvent is still present above the surface of the column, add approximately 3 mm of anhydrous sodium sulfate. The column is then rinsed with another small portion (approximately 3 mL) of hexane(s) and allowed to drain. [It is not critical that the column continually contains hexane(s). However, do not allow the column to stand for more than one hour.] Proceed to Section 6.5.3.

a) Before analyses are conducted, the elution profile of the Silica Gel must be characterized; see Analytical Notes Section 8.0
6.5.2 Silica Gel Solid Phase Extraction (SPE) Cartridges:

Silica gel SPE cartridges are typically used in conjunction with a vacuum box assembly. If used, refer to the manufacturer’s operating instructions for the particular device utilized.

**Conditioning Step:** Anhydrous sodium sulfate is added to the SPE silica gel cartridge reservoir to a depth of approximately 5 mm. Condition the cartridge by passing 10 mL of hexane(s) through the column. Elute in a drop-wise fashion until the hexane(s) reach the top of the sodium sulfate layer. Discard the hexane(s) eluate. Proceed to Section 6.5.4.

6.5.3 Elution From C-18 SEP-PAK to Mini Silica Gel Column:

Draw 7.0 mL hexane(s) into a 10 mL glass syringe equipped with a Luer fitting. The short end of the C-18 SEP-PAK cartridge is attached to the syringe. The syringe is held pointing straight down and the hexane(s) is pressed gently through the SEP-PAK.

As the hexane(s) moves through the cartridge, the remaining water is forced through. The first drops of eluate are typically two or three drops of water and should be blotted onto filter paper. [Either the water is observed as a much darker liquid or as a meniscus at the exit end of the cartridge.] The hexane(s) is then eluted directly onto the silica gel column (Section 6.5). The special technique described in Figure 3 or 3a should be followed.

After the last of the hexane(s) solution passes through the sodium sulfate layer, the column is rinsed with 4 column volumes of hexane(s). All hexane(s) fractions are discarded.

After the last hexane(s) rinse has eluted through the column, all of the hexane(s) is removed with a brief application of vacuum. An apparatus similar to that used to dry the C-18 SEP-PAK (Section 6.4, see Figure 2) has been successful. [Remove all hexane(s) before beginning the elution step.] Proceed to Section 6.5.5.

6.5.4 Elution From C-18 SEP-PAK to SPE Cartridge:

Load the sample onto the silica gel SPE cartridge as described above (6.5.3) using hexane(s) reagent. As the sample in hexane(s) reaches the sodium sulfate layer, add 10 mL of hexane(s) and elute to dryness in a drop-wise fashion (under mild vacuum). Discard the hexane(s) eluate. Proceed to Section 6.5.6.

6.5.5 Mini Silica Gel Column Elution:

HOE-083312 [(3-acetyl-6-chloro-2,3-dihydrobenzoxazol-2-one)] is eluted from the column using toluene. The first 3.0 mL of toluene (or appropriate volume as determined by column characterization) are collected in a 10 mL graduated centrifuge tube. The eluate is then brought to a suitable final volume with toluene. Typically 10.0 mL is appropriate for 0.05 ppm fortifications. [This depends, of course, upon the sensitivity of the GC/ECD equipment used.]
6.5.6 Silica Gel SPE Cartridge Elution:

HOE-083312 [(3-acetyl-6-chloro-2,3-dihydrobenzoxazol-2-one)] is eluted from the SPE cartridge using 5.0 mL of toluene and collected in a 16 mm X 100 mm glass tube. Quantitatively transfer the toluene eluate to a 10 mL or 15 mL graduated centrifuge tube and adjust to volume with toluene. Typically, a 10.0 mL final volume provides adequate sensitivity for determination of 0.05 ppm residue levels. [Alternately, the SPE cartridge may be eluted directly into a graduated centrifuge tube if compatible with the particular SPE (e.g., vacuum box) assembly used.]

6.6 Gas Chromatographic Analysis:

The sample residue is injected into a gas chromatograph equipped with a 63Ni Electron Capture Detector.

Gas Chromatography separations are made using a 15 meter DB-1 (or DB-5) megabore column or a 10 ft. x 2 mm id glass column packed with 3% SP2100. If so equipped, megabore column applications are recommended.

6.6.1 Gas Chromatography Conditions for the Determination of HOE-083312

-PACKED COLUMN CHROMATOGRAPHY-

Gas Chromatograph: Hewlett Packard Model 5880A, equipped with a 63Ni Electron Capture Detector.

Column: 10 ft. x 2 mm id, 3% SP-2100 on Supelcoport (100/120 mesh).

Temperatures:

Inlet: 250°C
Detector: 350°C
Column: 145°C (isothermal)

Gas Flows:
Carrier: 30 mL/min - Argon/Methane (95:5)

Injection Volume: 5.0 uL
Attention: 2 x 4
Chart Speed: 0.5 cm/minute
Minimum Detection Limit: 10.0 pg HOE-083312 injected
Approximate Retention Time: 8.1 minutes for HOE-083312
6.6.2 Gas Chromatography Conditions for the Determination of HOE-083312

-CAPILLARY COLUMN CHROMATOGRAPHY-

Gas Chromatograph: Hewlett Packard Model 5880A or Varian Vista 6000 equipped with a 63Ni Electron Capture Detector

Megabore Column Application: 1.5 um DB-1 (or DB-5), 15 Meters x 0.53 mm i.d.

Typical Operating Parameters (HP 5880A):

Temperatures:
- Inlet (flash): 225°C
- Detector: 325°C
- Column: Initial temperature: 130°C (DB-1) or 140°C (DB-5) for 13 minutes (isothermal); post analysis temperature 225°C (typical) for 10 minutes (typical). [Note: The post analysis hold time should be optimized according to sample requirements. Sufficient column bake-out is to be achieved using minimum hold time between samples.]

Gas Flows:
- Carrier: 10 mL/minute - Helium
- Make-up: 30 mL/minute - Argon/Methane (95:5)

Injection Volume: 1.0 uL or 2 uL (typical)

Attenuation: 2 x 3

Chart Speed: 0.5 cm/minute

Limit of Quantitation: 0.05 ppm Fenoxaprop-ethyl equivalents

Approximate Retention Time: 9 minutes for HOE-083312 (DB-1)
- 6 minutes for HOE-083312 (DB-5)

Widebore Column Application: 1.0 um DB-5, 20 Meters x 0.32 mm i.d. (coupled to approx. 30 cm of 0.53 mm i.d. fused silica (DB-5) which is located in the GC inlet, allowing on-column injection)

Typical Operating Parameters (Vista 6000):

Temperatures:
- Inlet (on column): 180°C
- Detector: 380°C
- Column: Initial temperature 120°C for 0.5 minutes, program to 140°C at 2°C/min, then program to 220°C at 30°C/min. [Note: A post analysis hold time may be required and should be optimized according to sample requirements. Sufficient column bake-out is to be achieved using minimum hold time between samples.]
6.6.2 Gas Chromatography Conditions for the Determination of HOE-083312 (continued)

-CAPILLARY COLUMN CHROMATOGRAPHY-
(continued)

Gas Flows:

Carrier: 5 mL/minute - Helium
Make-up: 25 mL/minute - Nitrogen

Injection Volume: 2 uL (typical)

Attenuation: 64

Chart Speed: 0.50 cm/minute, then 1.0 cm/minute (after 6 min.)

Limit of Quantitation: 0.05 ppm Fenoxaprop-ethyl equivalents

Approximate Retention Time: 8 minutes (HOE-083312)

6.7 Calibration of the 63-Ni/Electron Capture Detector:

Inject an appropriate volume (1 or 2 uL) of the injection standards containing HOE-083312 listed in Section 5.0 Table I into the Gas Chromatograph operating at the conditions shown in Section 6.6.1 or 6.6.2.

Typical packed column chromatograms are provided in Figures 4 through 6. Typical capillary column chromatograms are provided in Figures 7 through 12 for wheat and Figures 13 through 34 for barley samples.

Determine the peak height or area for each standard (the lowest standard should not be less than 10 mm in peak height.)

Construct a standard curve by plotting detector response (peak height or area) versus picograms of standard injected.

6.8 Final Determination:

Inject an appropriate volume (1 or 2 uL) of the sample from Section 6.5.3 into the gas chromatograph and determine the peak height or peak area for HOE-083312.

Directly compare the peak heights or areas of unknown samples injected with the appropriate standard curve and determine the amount of HOE-083312 in the injected sample aliquot.
6.8 **Final Determination:** (continued)

A standard curve must be prepared for every analytical series. An initial standard curve is prepared after the instrument response is stable. Subsequently, every 3 to 4 sample injections are to be bracketed with an analytical standard in order to maintain a continual check for shifts in sensitivity and retention time. The analytical series should end with analysis of a standard solution. Residue results should not be extrapolated above or below the level of standards injected.

6.9 **Calculation of Residue (Final Determination):**

Using the standard curve, determine the residue result in terms of fenoxaprop-ethyl equivalents in the sample by:

\[
\text{PPM fenoxaprop-ethyl equivalents} = \frac{A \times F}{B},
\]

where

- \(A\) = value extrapolated from standard curve, expressed as nanograms or picograms HOE-083312.
- \(B\) = mg or ug of sample (equivalents) injected
  
  \[
  \text{mg or ug of sample} = \frac{\text{initial sample wt (W, g) \times aliquot volume (T, mL) \times uL injected}}{\text{initial total volume (V, mL) \times 2} \times \text{final volume (mL)}}
  \]
- \(F\) = mol. weight correction factor (for residues expressed as HOE-033171)
  
  \[
  \text{Gram Molecular Weight of HOE-033171} = 361.8 \times 1.71 = 1.71
  \]
  
  \[
  \text{Gram Molecular Weight of HOE-083312} = 211.6
  \]

[See Section 6.10 for determination of other "fortification" compounds.]

**Using Wheat Grain as an example:**

\[
B = \frac{25 \text{ grams (W)} \times 20 \text{ mL (T)} \times 5.0 \text{ uL}}{203 \text{ mL (V)} \times 2 \times 10.0 \text{ mL}} = 0.616 \text{ mg of sample (equivalents) injected}
\]

Continuing, the final residue using this example:

\[
\text{PPM fenoxaprop-ethyl equivalents} = \frac{A(\text{ng}) \times 1.71}{0.616 \text{ (mg)}}
\]

*) This divisor is used for 25-g grain samples only, accounting for one-half of the sample aliquot which is derivatized.
6.10 Calculation of Fortifications of Fenoxaprop-ethyl, Fenoxaprop, and HOE-054014:

Due to molecular weight differences and the fact that all residues are quantified against the derivative HOE-083312, the percent recovery obtained from fortifications using fenoxaprop-ethyl, fenoxaprop, and HOE-054014 must reflect this difference. Results are corrected using the following factors (F) as indicated above:

For fenoxaprop-ethyl: \[
\frac{GMW \text{ fenoxaprop-ethyl}}{GMW \text{ HOE-083312}} = \frac{361.8}{211.6} = 1.71
\]

For fenoxaprop: \[
\frac{GMW \text{ fenoxaprop}}{GMW \text{ HOE-083312}} = \frac{333.7}{211.6} = 1.58
\]

For HOE-054014: \[
\frac{GMW \text{ HOE-054014}}{GMW \text{ HOE-083312}} = \frac{169.6}{211.6} = 0.80
\]

Therefore:

To determine ng fenoxaprop-ethyl, multiply ng HOE-083312 found by 1.71; to determine ng fenoxaprop, multiply ng HOE-083312 found by 1.58; to determine ng HOE-054014, multiply ng HOE-083312 found by 0.80.

7.0 Quality Control Procedures

7.1 Laboratory Spiking

To verify the quality of the analytical data, laboratory spikes are to be run along with each set of residue samples. These spiked samples should cover the range of expected residues in the set and at least 10% of the set must be laboratory spikes. Spiking of quality control samples should be directly onto the analytical sample prior to extraction (Section 6.1.1a), allowing enough time for the spiking solvent to evaporate (typically 30 minutes in a fume hood). Spiking volume may range from 100 uL to 2 mL. The concentration of the spiking solution should be adjusted accordingly. Examples of experimental laboratory recovery data for fenoxaprop-ethyl and its metabolites are provided in Tables II through V.

7.2 Sample Storage

All residue samples should be stored frozen until analysis. After analysis, the remaining sample should be re-frozen and stored until authorized for disposal.
8.0 Analytical Notes:

8.1 Safety

To prevent the escape of hydrochloric acid fumes into the laboratory, the reflux equipment must be in
a fume hood or high efficiency condensers must be used.

8.2 Characterization of the Mini Silica Gel Column

Before analyses are conducted, the elution profile of HOE-083312 on the silica gel column must be
characterized completely. This is accomplished by adding 0.40 ug HOE-083312 (dissolved in hexane)
and observing the elution pattern. Effective clean-up and quantitative recovery are to be attained.
The toluene elution volume can be expected to be between 2.5 and 3.0 mL for mini silica gel columns
and 4.0 and 6.0 mL for the silica gel SPE columns.

8.3 Analysis Time Considerations

Given the sample reflux and derivatization time requirements, a typical analytical series which may
include six samples and at least three standards should require approximately two (8 hr.) working
days (requiring operator attention) for completion. Recommended stopping points during the analysis
are i) after completing the extraction procedure and/or ii) after Extrelut column elution and/or iii)
after the derivatization procedure (prior to quenching of the reaction). For efficiency, the use of
electrical timers to automatically terminate reflux after extraction and/or derivatization and the use of
a GC autosampler are also recommended.

8.4 Optimization/Troubleshooting

8.4.1 GC Peak Measurement: On a case by case basis, manual peak height measurement of the analyte
signal (from the GC/ECD) may be required to obtain/verify accurate and consistent quantitation (e.g.,
vs. electronic integration). The use of electronic integrators and/or data systems for analyte signal
measurement must be verified by the responsible analyst vis-a-vis the applicability-validity of the
measurement algorithm (e.g., the position of the electronic "baseline" relative to the GC peak apex).
Typically, these considerations are most significant in the case of GC/ECD determinations near the
limit of quantitation.

8.4.2 Sample Moisture: Field sample moisture may vary from sample to sample. If excessively moist
samples require analysis (i.e., > > 10% moisture), the actual moisture content of the sample should
be accounted for in the calculation of total extract volume (V1).

9.0 Reference

**TABLE II**

SUMMARY OF THE RECOVERY OF FENOXAPROP-ETHYL AND ITS METABOLITES FROM WHEAT GRAIN AND STRAW

Packed Column Chromatography: Wheat Grain

<table>
<thead>
<tr>
<th>Fortification (ppm)</th>
<th>HOE-033171</th>
<th>HOE-053022</th>
<th>HOE-054014</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>70, 92</td>
<td>----</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>122, 104</td>
<td>66</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>70, 98</td>
<td>----</td>
<td>72</td>
</tr>
<tr>
<td>0.10</td>
<td>72</td>
<td>64</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>110</td>
<td>70</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>86</td>
<td>82</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>86</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Average (± SD)</td>
<td>91 ± 18</td>
<td>71 ± 8</td>
<td>79 ± 12</td>
</tr>
</tbody>
</table>

Megabore Column Chromatography (DB-1): Wheat Straw

<table>
<thead>
<tr>
<th>Fortification (ppm)</th>
<th>HOE-033171</th>
<th>HOE-053022</th>
<th>HOE-054014</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>100</td>
<td>73</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>73</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>63</td>
<td>67</td>
</tr>
<tr>
<td>0.10</td>
<td>94</td>
<td>63</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>99</td>
<td>62</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>94</td>
<td>65</td>
<td>83</td>
</tr>
<tr>
<td>Average (± SD)</td>
<td>91 ± 13</td>
<td>67 ± 5</td>
<td>73 ± 6</td>
</tr>
</tbody>
</table>
### TABLE III

SUMMARY OF THE RECOVERY OF FENOXAPROP-ETHYL AND ITS METABOLITES FROM REPRESENTATIVE WHEAT MILLED PRODUCTS

(Packed Column Chromatography)

<table>
<thead>
<tr>
<th>WHEAT FRACTION</th>
<th>PERCENT RECOVERY</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HOE-033171</td>
<td>HOE-053022</td>
<td>HOE-054014</td>
</tr>
<tr>
<td>Bran</td>
<td>73</td>
<td>67</td>
<td>----</td>
</tr>
<tr>
<td>Husk</td>
<td>79</td>
<td>----</td>
<td>74</td>
</tr>
<tr>
<td>Shorts &amp; Germ</td>
<td>68</td>
<td>----</td>
<td>59</td>
</tr>
<tr>
<td>Low Grade Flour</td>
<td>72</td>
<td>67</td>
<td>----</td>
</tr>
<tr>
<td>Patent Flour</td>
<td>83</td>
<td>----</td>
<td>77</td>
</tr>
</tbody>
</table>
## TABLE IV

**SUMMARY OF THE RECOVERY OF FENOXAPROP-ETHYL FROM BARLEY GRAIN AND BARLEY STRAW**

<table>
<thead>
<tr>
<th>SAMPLE TYPE</th>
<th>ANALYTE</th>
<th>FORTIFICATION LEVEL (ppm)</th>
<th>PERCENT RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley Grain</td>
<td>HOE-033171</td>
<td>0.05</td>
<td>98.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.10</td>
<td>77.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.20</td>
<td>88.1</td>
</tr>
<tr>
<td>Barley Grain</td>
<td>HOE-053022</td>
<td>0.05</td>
<td>92.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.10</td>
<td>96.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.20</td>
<td>87.7</td>
</tr>
<tr>
<td>Barley Grain</td>
<td>HOE-054014</td>
<td>0.05</td>
<td>88.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.10</td>
<td>100.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.20</td>
<td>92.6</td>
</tr>
<tr>
<td>Barley Straw</td>
<td>HOE-033171</td>
<td>0.05</td>
<td>86.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.10</td>
<td>99.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.20</td>
<td>73.8</td>
</tr>
<tr>
<td>Barley Straw</td>
<td>HOE-053022</td>
<td>0.05</td>
<td>81.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.10</td>
<td>88.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.20</td>
<td>88.0</td>
</tr>
<tr>
<td>Barley Straw</td>
<td>HOE-054014</td>
<td>0.05</td>
<td>77.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.10</td>
<td>85.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.20</td>
<td>86.5</td>
</tr>
</tbody>
</table>

Source: Colorado Analytical R & D Laboratory Report: Hoechst 1123 [MRID No: 416982-01]

Chromatography: Megabore capillary (1.5 um DD-5 phase, 15 meters x 0.53 mm i.d.)
# TABLE V

**SUMMARY OF THE RECOVERY OF FENOXAPROP-ETHYL FROM REPRESENTATIVE BARLEY MILLED PRODUCTS**

<table>
<thead>
<tr>
<th>BARLEY FRACTION</th>
<th>FORTIFICATION LEVEL (ppm)</th>
<th>PERCENT RECOVERY</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HOE-033171</td>
<td>HOE-053022</td>
</tr>
<tr>
<td>Bran</td>
<td>0.05</td>
<td>-----</td>
</tr>
<tr>
<td>Shorts &amp; Germ</td>
<td>0.10</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td>-----</td>
<td>0.05</td>
</tr>
<tr>
<td>Pearled Barley</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Low Grade Flour</td>
<td>0.50</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td>-----</td>
<td>0.05</td>
</tr>
<tr>
<td>Patent Flour</td>
<td>-----</td>
<td>-----</td>
</tr>
</tbody>
</table>

Source: Xenos Laboratories Report: OU89-09 [MRID No: 416888-02]

Chromatography: Widebore capillary (1.0 um DB-5 phase, 20 meters x 0.32 mm i.d.)
Flow Diagram For The Determination Of Penoxaprop-ethyl And Its Major Metabolite Residues

10 or 25 GRAM SAMPLE (See Section 6.1 For Details)
- add 150 mL (10:90:50)
- Hydrochloric acid:Acetonitrile:Water

REFLUX
- 6 hours
- add 40 or 50 mL distilled water
- (See Section 6.1 for details)

FILTER
- while warm

SULLUS <-- FILTRATE
- DISCARD
- remove 20 mL aliquot

EXTRACT COLUMN
- allow aliquot to equilibrate for 30 min. on column
- wash with 50 mL hexane; discard hexane
- elute with 250 mL hexane:ether (8/2, v/v) and collect

HEXANE:ETHER ELUATE
- concentrate to dryness
- rinse flask with 20 mL ethyl acetate
- concentrate to dryness

Straw and Milled Products
- transfer 3 X 3 mL ethyl acetate

Grain
- dissolve with 10.0 mL ethyl acetate
- remove 5.0 mL aliquot
- evaporate to dryness

DERIVATIZATION (continue on next page)
FIGURE 1 (continued)

Flow Diagram For The Determination Of Fenoxaprop-ethyl And Its Major Metabolite Residues

DERIVATIZATION
- add 1.0 mL acetic anhydride:pyridine (5:1)
- derivatize for 3 hours at 130°C
- cool to room temperature
- add 5 mL 1% emulsifier solution and 0.5 mL acetic acid to culture tube
- vortex
- draw sample into 10 mL syringe
- rinse culture tube with 1 mL distilled water
- draw water into syringe

C-18 SPE-PAK (pre-conditioned)
- elute derivatization mixture through short end of C-18 cartridge
- wash with 10 mL distilled water
- vacuum dry

MINI-SILICA GEL COLUMN² (pre-conditioned/pre-washed)
- elute C-18 onto silica gel with 7 mL hexane, discard
- wash silica gel with 4 column volumes of hexane, discard
- vacuum dry
- elute HDE-083312 with 3.0 mL toluene (typical)

GC-ECD

Note

a) Alternately, a silica gel SPE cartridge may be used. Refer to Section 6.5.
A. Washing of C-18 SEP-PAK (Section 6.4)

B. Removal of hexane from mini silica gel column (Section 6.5)
Assembly for transferring the SEP-PAK C\textsubscript{18} eluate to the mini silica gel column.

**Figure 3a:** Alternate assembly using Rainin adapter and Luer Lok syringe needle without expansion gate.
Figure 4: Typical Packed Column Chromatography

Multiplier = 1

RT: INTC - OFF

RT: INTC - ON

[HP] 5388A Sampler Injection @ 11:09 Nov 5, 1986
Sample #: ID Code:
2 2 pg/ul std

Area %:

RT Area Type Area %:
8.33 681.35 HH 100.000

Total Area = 681.35
Multiplier = 1

Standard: Equivalent to 10.0 pg HOE-083312 (5.0 ul x 2.0 pg/ul)

Reference: Section 6.6.1
Figure 5: TYPICAL PACKED COLUMN CHROMATOGRAPHY

RT: INTG + OFF

5.51 11.46 BP 0.297
7.08 237.74 VV 6.154
8.88 787.57 PP 20.386
9.97 444.59 BB 11.506
12.08 2382.03 BB 61.658

TOTAL AREA = 3863.29
MULTIPLIER = 1

WHEAT GRAIN CONTROL: 5.0 uL injected (equivalent to 0.555 mg)

REFERENCE: Section 6.6.1
**Figure 6: TYPICAL PACKED COLUMN CHROMATOGRAPHY**

RT: INTG - OFF

RT: STOP RUN  OV: STOP RUN

![Calibration Curve Graph]

- 3.30 - 13.9 ppm found, from calibration curve
- 9.38
- 12.09

**[HP] 5330A SAMPLER INJECTION @ 12:17 NOV 6, 1986**

<table>
<thead>
<tr>
<th>AREA %</th>
<th>RT</th>
<th>AREA</th>
<th>TYPE</th>
<th>AREA %</th>
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<tbody>
<tr>
<td>5.48</td>
<td>13.20</td>
<td>BP</td>
<td>0.212</td>
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<tr>
<td>6.22</td>
<td>95.91</td>
<td>PV</td>
<td>1.537</td>
<td></td>
</tr>
<tr>
<td>6.71</td>
<td>141.51</td>
<td>VV</td>
<td>2.267</td>
<td></td>
</tr>
<tr>
<td>7.09</td>
<td>387.05</td>
<td>VV</td>
<td>6.202</td>
<td></td>
</tr>
<tr>
<td>8.06</td>
<td>620.78</td>
<td>VV</td>
<td>9.946</td>
<td></td>
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<tr>
<td>8.80</td>
<td>1002.73</td>
<td>VV</td>
<td>16.066</td>
<td></td>
</tr>
<tr>
<td>9.88</td>
<td>701.39</td>
<td>VV</td>
<td>11.238</td>
<td></td>
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<tr>
<td>12.09</td>
<td>3278.60</td>
<td>BB</td>
<td>52.532</td>
<td></td>
</tr>
</tbody>
</table>

**TOTAL AREA = 6241.17**

**MULTIPLIER = 1**

**WHEAT GRAIN CK+0.05 ppm HOE-33171: 5.0 uL injected (equivalent 0.555 mg)**

70% recovered

**REFERENCE: Section 6.6.1**
**Figure 7**

**TYPICAL MEGABORE COLUMN CHROMATOGRAPHY**

---

**[HPLC 5390A] SAMPLER INJECTION 16:33 FEB 19, 1987**

**SAMPLE #:** 16:33

**ID CODE:** 2

**REFERENCE:** Section 6.6.2

**AREA %:** COMPENSATED ANALYSIS

<table>
<thead>
<tr>
<th>RT</th>
<th>AREA</th>
<th>TYPE</th>
<th>AREA %</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.47</td>
<td>146.38</td>
<td>3B</td>
<td>100.000</td>
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</tbody>
</table>

**TOTAL AREA = 146.38**

**MULTIPLIER = 1**

**STANDARD:** EQUIVALENT TO 2.0 pg HOE-083312 (1.0 UL x 2.0 pg/UL)
Figure 8: TYPICAL MEGABORE COLUMN CHROMATOGRAPHY

WHEAT STRAW CONTROL: 1.0 µL injected (equivalent to 0.100 mg)

REFERENCE: Section 6.6.2
Figure 9: TYPICAL MEGABORE COLUMN CHROMATOGRAPHY

[Image of a chromatography chart with peaks labeled]

**[HP] 330A SAMPLER INJECTION @ 13:55 FEB 19, 1987**

**SAMPLE #: ID CODE: **

9 QA417-4

**AREA %: COMPENSATED ANALYSIS**

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<thead>
<tr>
<th>RT</th>
<th>AREA</th>
<th>TYPE</th>
<th>AREA %</th>
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<tbody>
<tr>
<td>7.66</td>
<td>1023.55</td>
<td>BP</td>
<td>42.365</td>
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<td>9.17</td>
<td>713.52</td>
<td>BV</td>
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<tr>
<td>8.49</td>
<td>214.70</td>
<td>VB</td>
<td>2.526</td>
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<td>8.93</td>
<td>111.51</td>
<td>BH</td>
<td>4.415</td>
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<tr>
<td>9.34</td>
<td>352.74</td>
<td>HH</td>
<td>14.600</td>
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</tbody>
</table>

**TOTAL AREA = 2416.02**

**MULTIPLIER = 1**

**WHEAT STRAW CK+0.05 ppm HOE-033171: 1.0 uL injected (equivalent 0.100 mg)**

**90% recovered**

**REFERENCE: Section 6.6.2**

Page 35
Figure 10
TYPICAL MEGABORE COLUMN CHROMATOGRAPHY

Analytical Standard: HOE-083312

Column: DB-5 (Ref. Section 6.6.2); 140°C (isothermal)

Analytical Standard Concentration: 2 pg (injected)

Reference: Colorado Analytical Project: Hoechst 1123
Figure 11
TYPICAL MEGABORE COLUMN CHROMATOGRAPHY

Wheat Straw

Column: DB-5 (Ref. Section 6.6.2); 140°C (isothermal)
Reference: Colorado Analytical Project: Hoechst 1123

- Control Wheat Straw, <0.05 ppm Found
  (NOTE: Oven temperature 140°C)

Chromatograms reduced to 70% of original size by photocopy.

Fortification compound HOE-046360 corresponds to the single
isomer chemical equivalent of HOE-033171.

Notes:
1. Chromatograms reduced to 70% of original size by photocopy.
2. Fortification compound HOE-046360 corresponds to the single
   isomer chemical equivalent of HOE-033171.
Figure 12

TYPICAL MEGABORE COLUMN CHROMATOGRAPHY

Wheat Grain

Column: DB-5 (Ref. Section 6.6.2); 140°C (isothermal)
Reference: Colorado Analytical Project: Hoechst 1123

Control

Fortified: 0.05 ppm HOE-053022; Recovery: 95%

Notes:
1. Chromatograms reduced to 70% of original size by photocopy.
Figure 13
HOE083312 STANDARD: 2ul x 0.004 ng/ul injected

CHROMATOGRAPHY: Widebore capillary (1.0 um DB-5 phase, 20 meters x 0.32 mm i.d.)

Note: Chromatogram has been reduced by photocopy for illustration purposes.
Figure 14
Hoe083312 STANDARD: 2μl x 0.010 ng/μl injected

Table: 12-11-89 10:14:16  CH = "A" FS = 1.
FILE 4.  METHOD 5.  FUn 141  INDEX 1

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<th>NAME</th>
<th>NG</th>
<th>RT</th>
<th>AREA BC</th>
<th>RF</th>
<th>RRT</th>
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</thead>
<tbody>
<tr>
<td>Hoe083312</td>
<td>0.021</td>
<td>7.64</td>
<td>34045</td>
<td>0.143245</td>
<td>1.</td>
</tr>
<tr>
<td>TOTALS</td>
<td>0.021</td>
<td></td>
<td>34045</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: Xenos Laboratories Report: 0U89-09
MRID No: 416888-02
Chromatography: Widebore capillary (1.0 um DB-5 phase, 20 meters x 0.32 mm i.d.)

Note: Chromatogram has been reduced by photocopy for illustration purposes.
PEARLED BARLEY CONTROL: 2ul of 5ml injected

CHANNEL A | INJECT 21-11-89 13:46:38

Note: Chromatogram has been reduced by photocopy for illustration purposes.
Figure: 16

PEARLED BARLEY CONTROL FORTIFIED AT 0.10PPM AS HOE054014

2uL of 10mL injected

CHANNEL A INJECT 21-11-89 13:19:52

Source: Xenos Laboratories Report: 0089-09
MRID No: 416888-02
Chromatography: Widebore capillary (1.0 um DB-5 phase, 20 meters x 0.32 mm i.d.)

Note: Chromatogram has been reduced by photocopy for illustration purposes.
Figure: 17

BRAN CONTROL: 2uL of 5mL injected

Source: Xenos Laboratories Report: OU89-09
MRID No: 416888-02
Chromatography: Widebore capillary (1.0 um DB-5 phase, 20 meters x 0.32 mm i.d.)

Note: Chromatogram has been reduced by photocopy for illustration purposes.
Figure: 18

BRAN CONTROL FORTIFIED AT 0.05PPM AS HOEO33171
2uL of 5mL injected

CHANNEL A /\ INJECT 11-11-89 15:11:29

CS 1
5.69
7.60
8.02

TILLER
21-11-89 15:11:29 CH= "A" PS= 1

Source: Xenos Laboratories Report: 0U89-09
MRID No: 416888-02
Chromatography: Widebore capillary (1.0 um DB-5 phase, 20 meters x 0.32 mm i.d.)

Note: Chromatogram has been reduced by photocopy for illustration purposes.
Figure: 19

SHORTS AND GERMS CONTROL: 2ul of 5mL injected

Source: Xenos Laboratories Report: 0U89-09
MRID No: 416688-02
Chromatography: Widebore capillary (1.0 um DB-5 phase, 20 meters x 0.32 mm i.d.)

Note: Chromatogram has been reduced by photocopy for illustration purposes.
Figure: 20

SHORTS AND GERMS CONTROL FORTIFIED AT 0.10PPM
AS HOE033171

2µL of 5mL injected

Source: Xenos Laboratories Report: OU89-09
MRID No: 416888-02
Chromatography: Widebore capillary (1.0 um DB-5 phase, 20 meters x 0.32 mm i.d.)

Note: Chromatogram has been reduced by photocopy for illustration purposes.
Figure: 21

LOW GRADE FLOUR CONTROL: 2ul of 5ml injected

Source: Xenos Laboratories Report: OU89-09
MRID No: 416888-02
Chromatography: Widebore capillary (1.0 um DB-5 phase, 20 meters x 0.32 mm i.d.)

Note: Chromatogram has been reduced by photocopy for illustration purposes.
Figure: 22

LOW GRADE FLOUR CONTROL FORTIFIED AT 0.08PPM AS HOE093022
2uL OF 3mL INJECTED

CHANNEL A INJECT 24-11-99 16:22:29

Source: Xenos Laboratories Report: OU89-09
MRID No: 416888-02
Chromatography: Widebore capillary (1.0 um DB-5 phase, 20 meters x 0.32 mm i.d.)

Note: Chromatogram has been reduced by photocopy for illustration purposes.
Figure: 23

PENT FLOUR CONTROL: 2u1 of 5mL injected

Source: Xenos Laboratories Report: 0U89-09
MRID No: 416888-02
Chromatography: Widebore capillary (1.0 um DB-5 phase, 20 meters x 0.32 mm i.d.)

Note: Chromatogram has been reduced by photocopy for illustration purposes.
Figure: 24

PATENT FLOUR CONTROL FORTIFIED AT 0.10PPM AHOE054014

2ul of 25ml injected

Source: Xenos Laboratories Report: 0U89-09
MRID No: 416888-02
Chromatography: Widebore capillary (1.0 um DB-5 phase, 20 meters x 0.32 mm i.d.)

Note: Chromatogram has been reduced by photocopy for illustration purposes.
Figure: 25

Representative Chromatogram of Control Barley Grain

Date Sample Injected: Nov. 8, 1989
Amount Injected: 2 uL

Analyte (HDE-083312) Retention Time: 7.6 minutes
Injection Number: 10

Sample ID: CA01-10

Fortification Level: N/A

Percent Recovery: N/A (< 0.05 ppm)

Note: Chromatogram corresponds to photocopy reduction to 95% of original size.

Source: Xenos Laboratories Method Validation
MRID No: 41688801
Chromatography: Widebore capillary (1.0 um DB-5 phase, 20 meters x 0.32 mm i.d.)
Figure: 26
Representative Chromatogram of Fortified Barley Grain

Test Compound: HDE-033171

Date Sample Injected: Nov. 6, 1989
Amount Injected: 2 uL

Analyte (HDE-083312) Retention Time: 7.6 minutes
Injection Number: 16

Sample ID: CA01-11
Fortification Level: 0.05 ppm
Percent Recovery: 83.4%

Note: Chromatogram corresponds to photocopy reduction to 95% of original size.

Source: Xenos Laboratories Method Validation
MRID No: 41688801
Chromatography: Widebore capillary (1.0 um DB-5 phase, 20 meters x 0.32 mm i.d.)
Figure: 27

Representative Chromatogram of Fortified Barley Grain

Test Compound: HDE-053022

Data Sample Injected: Nov. 6, 1989
Amount Injected: 2 uL

Analyte (HDE-083312) Retention Time: 7.6 minutes

Sample ID: CA01-11
Injection Number: 5

Fortification Level: 0.10 ppm
Percent Recovery: 93.9%

Note: Chromatogram corresponds to photocopy reduction to 95% of original size.

Source: Xenos Laboratories Method Validation
MRID No: 41688801
Chromatography: Widebore capillary (1.0 um DB-5 phase, 20 meters x 0.32 mm i.d.)
Figure: 28

Representative Chromatogram of Fortified Barley Grain

Test Compound: HDE-054014

Date Sample Injected: Nov. 1, 1989
Amount Injected: 2 uL

Analyte (HDE-083312) Retention Time: 7.6 minutes
Injection Number: 16

Sample ID: CA01-11

Fortification Level: 0.05 ppm

Percent Recovery: 89.0%

Note: Chromatogram corresponds to photocopy reduction to 95% of original size.

Source: Xenos Laboratories Method Validation
MRTD No: 41688801
Chromatography: Widebore capillary (1.0 um DB-5 phase, 20 meters x 0.32 mm i.d.)
Figure: 29
Representative Chromatogram of Control Barley Straw

Date Sample Injected: Nov. 8, 1989
Amount Injected: 2 μL

Analyte (HDE-083312) Retention Time: 7.6 minutes
Sample ID: CA01-18
Injection Number: 7

Fortification Level: N/A
Percent Recovery: N/A (< 0.05 ppm)

Note: Chromatogram corresponds to photocopy reduction to 95% of original size.

Source: Xenos Laboratories Method Validation
MRID No: 41688801
Chromatography: Widebore capillary (1.0 um DB-5 phase, 20 meters x 0.32 mm i.d.)
**Figure: 30**

Representative Chromatogram of Fortified Barley Straw

**Test Compound:** HDE-033171

Date Sample Injected: Nov. 6, 1989

Amount Injected: 2 μL

Analyte (HDE-083312) Retention Time: 7.6 minutes

Sample ID: CA01-13

Injection Number: 13

Fortification Level: 0.05 ppm

Percent Recovery: 93.5%

**Note:** Chromatogram corresponds to photocopy reduction to 95% of original size.

Source: Xenos Laboratories Method Validation

MRID No: 41688801

Chromatography: Widebore capillary (1.0 um DB-5 phase, 20 meters x 0.32 mm i.d.)
Figure: 31

Representative Chromatogram of Fortified Barley Straw

Test Compound: HDE-053022

Date Sample Injected: Nov. 2, 1989
Amount Injected: 2 uL

Analyte (HDE-083312) Retention Time: 7.6 minutes

Sample ID: CA01-13
Injection Number: 14
Fortification Level: 0.10 ppm
Percent Recovery: 82.4%

Note: Chromatogram corresponds to photocopy reduction to 95% of original size.

Source: Xenos Laboratories Method Validation
MRID No: 41688801
Chromatography: Widebore capillary (1.0 um DB-5 phase, 20 meters x 0.32 mm i.d.)
Figure: 32

Representative Chromatogram of Fortified Barley Straw

Test Compound: HDE-054014

Date Sample Injected: Oct 31, 1989
Amount Injected: 2 uL

Analyte (HDE-083313) Retention Time: 7.6 minutes

Sample ID: CA01-13
Injection Number: 12

Fortification Level: 0.20 ppm
Dilution Factor: 

Percent Recovery: 80.4%

Note: Chromatogram corresponds to photocopy reduction to 95% of original size.

Source: Xenos Laboratories Method Validation
MRID No: 41688801
Chromatography: Widebore capillary (1.0 um DB-5 phase, 20 meters x 0.32 mm i.d.)
TYPICAL GAS CHROMATOGRAMS

Control Barley Grain, <0.05 ppm Found

(NOTE: Injection to 8.5 minutes was not recorded by use of a
high attenuation run time program. Oven temperature 130°C)

Control Barley Grain + 0.05 ppm HOE-053022
92.0% Recovery

Source: Colorado Analytical R & D Laboratory Report: Hoechst 1123
MRID No: 416982-01
Chromatography: Megabore capillary (1.5 um DB-5 phase, 15 meters x 0.53 mm i.d.)

Note: Chromatogram has been reduced by photocopy for illustration purposes.
Figure: 34

TYPICAL GAS CHROMATOGRAMS

Control Barley Straw, <0.05 ppm Found
(NOTE: Injection to 8.5 minutes was not recorded by use of a high attenuation run time program. Oven temperature 130°C)

Control Barley Straw + 0.05 ppm HOE-033171
86.2% Recovery

Source: Colorado Analytical R & D Laboratory Report: Hoechst 1123
MRID No: 416982-01
Chromatography: Megabore capillary (1.5 um DB-5 phase, 15 meters x 0.53 mm i.d.)

Note: Chromatogram has been reduced by photocopy for illustration purposes.