

Study Title

Determination of HOE-033171 [Fenoxaprop-Ethyl:2-(4-(6-Chloro-2-Benzoxazolyloxy)Phenoxy) Propanoate] and Its Metabolites [HOE-053022 (Fenoxaprop): 2-(4-6-Chloro-2-Benzoxazolyloxy)-Phenoxy)Propionic Acid and HOE-054014: 6-Chloro-2,3-Dihydrobenzoxazol-2-One]] in Wheat Grain, Wheat Forage, and Wheat Straw (Winter and Spring Wheat) and Wheat Grain Process Fractions

Data Requirement

Residue Chemistry Data Requirements: Section D
40 CFR 158.125
Magnitude of the Residue: Crop Field Trials
Guidelines Reference Number
171-4

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Report Dates

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Laboratory Project ID Numbers

Report Nos. ADC-2, 4, 5, 6, 7, 11, 12, and HRV-9R, 10M

Study Submitted By

Hoechst Celanese Corporation
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Purpose of Submission

Application for Registration and Petition for Tolerance for Use
of Whip 1EC Herbicide on Cotton, Wheat, and Peanuts

Submission Volume

Volume 19 of 22

STUDY #

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Appendix I - Enforcement Procedure

HRAV Analytical Method: HRAV-4

GAS CHROMATOGRAPHIC DETERMINATION OF FENOXAPROP-ETHYL [HOE-033171:
ETHYL-2-(4-(6-CHLORO-2-BENZOXAZOLYLOXY)PHENOXY)PROPANOATE]
AND ITS
METABOLITES [HOE-053022; 2-(4-(6-CHLORO-2-BENZOXAZOLYLOXY)PHENOXY)PROPANOIC
ACID AND HOE-054014: 6-CHLORO-2,3-DIHYDROBENZOXAZOL-2-ONE]
AS RESIDUES IN WHEAT GRAIN, WHEAT STRAW AND MILLED PRODUCTS

GAS CHROMATOGRAPHIC DETERMINATION OF FENOXAPROP-ETHYL [HOE-033171:
ETHYL-2-(4-(6-CHLORO-2-BENZOXAZOLYLOXY)PHENOXY)PROPANOATE]
AND ITS
METABOLITES [HOE-053022: 2-(4-(6-CHLORO-2-BENZOXAZOLYLOXY)PHENOXY) PROPANOIC
ACID AND HOE-054014: 6-CHLORO-2,3-DIHYDROBENZOXAZOL-2-ONE]
AS RESIDUES IN WHEAT GRAIN, WHEAT STRAW, AND MILLED PRODUCTS

HRAV Analytical Method: HRAV-4

Issued: October 15, 1987

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DATA REQUIREMENT

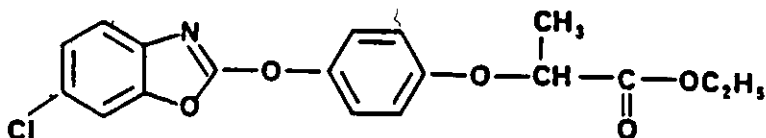
Guideline 171-4

1.0 Chemical Information:A. Parent, HOE-033171:

Common Name (Proposed): Fenoxaprop-ethyl, HOE-033171

Chemical Name: ethyl-2-(4-(6-chloro-2-benzoxazolyloxy)phenoxy)propanoate (IUPAC, english).

Structure:

Molecular Formula: $C_{18}H_{16}ClNO_5$

Molecular Weight: 361.8 g/mole

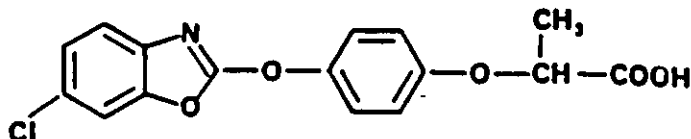
Solubility: Nearly insoluble in water; soluble in ethanol, cyclohexane, n-hexane; extremely soluble in acetone, toluene, ethyl acetate.

B. Metabolite, HOE-053022:

Common Name (Proposed): Fenoxaprop, HOE-053022

Chemical Name: 2-(4-(6-chloro-2-benzoxazolyloxy)phenoxy)propanoic acid.

Structure:

Molecular Formula: $C_{16}H_{12}ClNO_5$

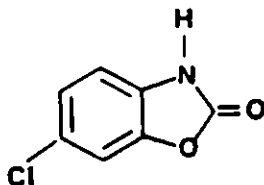
Molecular Weight: 333.7 g/mole

C. Metabolite, HOE-054014:

Common Name: HOE-054014 .

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

Structure:

Molecular Formula: $C_7H_4ClNO_2$

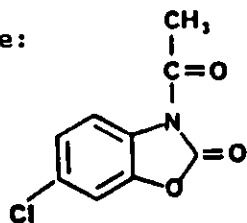
Molecular Weight: 169.6 g/mole

D. Derivative, HOE-083312:

Common Name: HOE-083312

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

Structure:

Molecular Formula: $C_9H_6ClNO_3$

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 [HOE-033171: ethyl-2-(4-(6-chloro-2-benzoxazolylloxy)phenoxy)propanoate] and fenoxaprop [HOE-053022: 2-(4-(6-chloro-2-benzoxazolylloxy)phenoxy)propanoic acid] are converted to HOE-054014 [6-chloro-2,3-dihydrobenzoxazol-2-one].

2.0 Principle of the Method (Continued):

The refluxate is then diluted with distilled water to achieve a solution containing 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 n-hexane. The hydrolysis product resulting from the reflux [HOE-054014: 6-chloro-2,3-dihydrobenzoxazol-2-one] is subsequently eluted with a 20% solution of diethyl ether in n-hexane.

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

To prepare for quantitation, the resultant derivative [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 accomplished using electron capture detection of the derivative, HOE-083312 [3-acetyl-6-chloro-2,3-dihydrobenzoxazol-2-one].

Residues are ultimately expressed as fenoxaprop-ethyl equivalents. Limit of detection for this method is 0.05 ppm.

3.0 Equipment :

1. High Speed Blender: Waring Model 31 BL 46, with one (1) quart containers.
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].
5. Reflux Condenser: High efficiency, 12", equipped with a $\$$ 29/42 ground glass joint [Ace Scientific: 5955-34].

3.0 Equipment (Continued):

6. Heating Mantles: Glas-Col; 500 mL [Fisher Scientific: 11-472-10F].
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, 10 and 20 mL capacity.
10. Extrelut 20 Columns: Merck-11737 [VWR: EM-11737-1]; refill packages are also available without columns [Merck-11738].
11. Volumetric Flasks: Glass; 250 mL capacity. [Fisher Scientific: 10-210E].
12. Rotary Flash Evaporator: Buchi, RE-120 or equivalent, with a water bath at 45-50°C.
13. Culture Tubes: 16 x 123mm, screw cap (Teflon lined) [Scientific Products: T-1358-1]; used for the derivatization reaction.
14. Tube Heater: Kontes Model K 720000; used for the derivatization reaction.
15. N-Evap Analytical Concentrator: Organomation Associates, Inc. or equivalent.
16. Vortex: Lab Line Instruments, Super Mixer Model 1290.
17. C-18 SEP PAK Cartridges: Waters Associates [51910]
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 Tubes: 10-15 mL [Fisher Scientific: 05-538-35B].
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 [Organomation: 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.

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4.0 Reagents/Chemical Supplies (Continued):

- r) Fenoxaprop-ethyl [HOE-033171:
ethyl-2-(4-(6-chloro-2-benzoxazolyloxy)phenoxy)propanoate];
analytical standard:
Hoechst-Roussel Agri-Vet Company, Somerville, NJ 08876
- s) Fenoxaprop [HOE-053022:
2-(4-(6-chloro-2-benzoxazolyloxy)phenoxy)propanoic acid];
analytical standard:
Hoechst-Roussel Agri-Vet Company, Somerville, NJ 08876
- t) HOE-054014: 6-chloro-2,3-dihydrobenzoxazol-2-one;
analytical standard:
Hoechst-Roussel Agri-Vet Company, Somerville, NJ 08876
- u) HOE-083312: 3-acetyl-6-chloro-2,3-dihydrobenzoxazol-2-one;
analytical standard:
Hoechst-Roussel Agri-Vet Company, Somerville, NJ 08876
- v) HOE-S-1728 - Emulsifier: ATA TH-1 Hoechst Aktiengesellschaft;
Hoechst-Roussel Agri-Vet Company, Somerville, NJ 08876
- w) Emulsifier Solution:
1% HOE-S-1728 in distilled water. Prepared by dissolving 10.0 grams of HOE-S-1728 in 1000 mL distilled water. The 1% emulsifier solution often needs purification. This is accomplished by partitioning 500 mL of the emulsifier solution with 3 x 100 mL n-hexane. The resultant aqueous solution is placed on a rotary evaporator with a water bath at 45-50°C until all traces of hexane are removed.

5.0 Preparation of Standard Solutions:

(store all solutions in a refrigerator).

HOE-083312:

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.

5.0 Preparation of Standard Solutions (Continued):
HOE-083312:

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.

Make dilutions of Solution D every month or as needed for calibration of the gas chromatograph. A typical set of dilutions is shown in Table I.

Table I: TYPICAL DILUTIONS FOR CALIBRATION OF GAS CHROMATOGRAPH.

<u>Amount</u> <u>(mL)</u>	<u>Solution</u>	<u>Final</u> <u>Dilution</u> <u>Volume</u> <u>(mL)</u>	<u>Final Concentration of</u> <u>Injection Standard</u>	
			<u>(ng/uL)</u>	<u>(pg/uL)</u>
2.0	D	100.0	0.0020	2.0
4.0	D	100.0	0.0040	4.0
6.0	D	100.0	0.0060	6.0
10.0	D	100.0	0.0100	10.0
1.0	C	100.0	0.0250	25.0

Fenoxaprop-Ethyl (HOE-033171):

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

Transfer 5.0 mL of Stock Solution E to a 100 mL volumetric flask. Dilute to volume with toluene. This solution (Solution F) contains 50.0 ug HOE-033171/mL. This solution should be prepared fresh every month, or as necessary.

5.0 Preparation of Standard Solutions (Continued):
Fenoxaprop-Ethyl (HOE-033171):

Transfer 5.0 mL of Solution F to a 100 mL volumetric flask. Dilute to volume with toluene. This solution (Solution G) contains 2.5 ug HOE-033171/mL. This solution should be prepared fresh every month, or as necessary.

Fenoxaprop (HOE-053022):

Weigh 100.0 mg HOE-053022 into a 100 mL volumetric flask. Dilute to volume with ethyl acetate. This solution (Stock Solution H) contains 1.0 mg HOE-053022/mL. This stock solution should be prepared fresh every three months, 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-053022/mL. This solution should be prepared fresh every month, 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-053022/mL. This solution should be prepared fresh every month, or as necessary.

HOE-054014:

Weigh 100.0 mg HOE-054014 into a 100 mL volumetric flask. Dilute to volume with ethyl acetate. This solution (Stock Solution K) 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 K to a 100 mL volumetric flask. Dilute to volume with ethyl acetate. This solution (Solution L) contains 50.0 ug HOE-054014/mL. This solution should be prepared fresh every month, or as necessary.

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 HOE-054014/mL. This solution should be prepared fresh every month, or as necessary.

SOLUTIONS G, J, AND M ARE USED IN FORTIFICATION STUDIES.

5.1 Sample Preparation:

A homogeneous, finely ground, analytical laboratory sample must be prepared from the field sample. It is critical that the laboratory sample be finely ground before subjecting it to the analysis procedures described in Section 6.0.

6.0 General Procedure: (See Flow Diagram - Figure 1; see Analytical Notes for Variations)

6.1 Extraction:

6.1.1. 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 mixture (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 in a heating mantle directly over a stirring plate. At the end of the reflux period, add 50 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 225 mL (equivalent to 200 mL of extraction solvent + 25g of analytical sample: V_1). Following filtration, proceed with the Extrelut column clean-up step (see section 6.2).

6.1.2. 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 29/42 ground glass joint.
- b. Add 150 mL of the reflux mixture (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 in a heating mantle directly over a stirring plate. 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 200 mL (equivalent to 190 mL of extraction solvent + 10 grams of analytical sample V_1). 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 1/4 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 n-hexane. The n-hexane eluate is discarded [Elution with more than 50 mL hexane has been

6.2 Extrelut Column Clean-Up (continued)

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) n-hexane:diethyl ether. This eluate is collected in a 500 mL round bottom flask equipped with a 24/40 S ground glass joint. [It is helpful 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.]

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 walls is rinsed with 20mL of ethyl acetate and evaporated to dryness as described above. Rotation of the flask should be at slow speed to keep the residue at the bottom. Remove the flask from the rotary flash evaporator and cool to room temperature. For wheat grain, proceed to Section 6.2.1; for wheat straw and milled products, proceed to Section 6.2.2.

6.2.1. Wheat Grain:

Residue in the flask is dissolved with 10 mL ethyl acetate and mixed. A 5 mL aliquot is transferred to a screw-capped culture tube; proceed to Section 6.3: Derivatization

6.2.2. Wheat 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 [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. [It is important that the entire tube be within the heated area, while the cap and teflon liner be outside the heated area].

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. Once cool, 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) followed with 10.0 mL distilled water. The top of the cartridge should contain water (not air).
- 6.4.2. Add to the culture tube 5 mL of emulsifier solution (1% HOE-S-1728 in distilled water) and 0.5 mL glacial acetic acid. Cap the culture tube and vortex vigorously for 15 seconds. This process destroys any residual acetic anhydride. 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 mini-silica gel column. However, prior to this step it is essential to eliminate as much residual water as possible. Proceed with Mini Silica Gel Column Clean-Up (Section 6.5).

6.5 Mini Silica Gel Column Clean-Up:

6.5.1. Preparation of Mini Silica Gel Column¹:

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 n-hexane. [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 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 and allowed to drain. [It is not critical that the column continually contain hexane. However, do not allow the column to stand for more than one hour.]

¹ Before analyses are conducted, the elution profile of the Silica Gel must be characterized; see Analytical Notes Section 8.0.

6.5.2. Sample Elution From C-18 SEP-PAK onto Mini Silica Gel Column:

This is accomplished by drawing 7.0 mL n-hexane 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 is pressed gently through the SEP-PAK.

As the hexane moves through the cartridge, the remaining water is forced through. The first drops of eluate (the amount depends on prior water removal, typically two or three drops remain) are 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 is then eluted directly onto the mini-silica gel column (Section 6.5) using the special technique described in Figure 3 or 3a.

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

After the last hexane rinse has eluted through the column, all of the hexane 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 before beginning the next elution step.]

6.5.3. 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 [or appropriate volume determined by column standardization] of toluene are collected in a 10 mL graduated centrifuge tube. The eluate is then brought to a suitable final volume using toluene. Typically 5.0 mL is appropriate for 0.05 ppm HOE-033171 fortifications, while 10.0 mL is appropriate for 0.05 ppm HOE-054014 fortifications. (This depends, of course, upon the sensitivity of the GC-ECD equipment 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 megabore column or a 10 ft. x 2mm id glass, 3% SP2100 packed column.

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
-MEGABORE COLUMN CHROMATOGRAPHY-

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

Column: 1.5 um DB-1, 15 Meter x.53 mm i.d.

Temperatures:

Inlet: 200°C

Detector: 325°C

Column: initial temperature 130°C for 13 minute, program at
30°C/minute to 170°C, hold 3 minutes; time.

Gas Flows:

Carrier: 10 mL/minute - Helium

Make-up: 30 mL/minute - Argon/Methane (95:5)

Injection Volume: 1.0 ul

Attenuation: 2 x 3

Chart Speed: 0.5 cm/minute

Minimum Detection Limit: 2.0pg HOE-083312 injected

Approximate Retention Time: 9.4 minutes for HOE-083312

6.7 Calibration of the ⁶³Ni/Electron Capture Detector:

Inject an appropriate microliter amount (1.0-5.0 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 4-6. Typical megabore column chromatograms are provided in Figures 7-9.

Determine the peak height or area for each standard (The lowest standard should not be less than 10mm 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 aliquot (1.0-5.0 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.

A standard curve must be prepared for every analytical series. An initial standard curve is prepared once 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. Residue results should not be extrapolated above or below the level of standards injected.

6.9 Calculation of Residue (Final Determination):6.9.1 Standard Curve:

If a standard curve is used, determine the residue result in terms of fenoxaprop-ethyl equivalents in the sample by:

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

A = value extrapolated from standard curve, expressed as nanograms or picograms HOE-083312.

B = mg or ug of sample injected

$$= \frac{\text{grams of initial sample (W)} \times \text{aliquot volume (T}_1\text{)} \times \text{microliters injected}}{\text{initial total volume (V}_1\text{)} \times \text{milliliters final volume}}$$

F = molecular weight correction factor

$$= \frac{\text{Gram Molecular Weight of HOE-033171}}{\text{Gram Molecular Weight of HOE-083312}} = \frac{361.8}{211.6} = 1.71$$

(See section 6.10)

Using Wheat Grain as an example:

$$B = \frac{25 \text{ grams (W)}}{225 \text{ mL initial volume (V}_1\text{)}} \times \frac{20 \text{ mL aliquot (T}_1\text{)}}{2^*} \times \frac{5.0 \text{ uL}}{10.0 \text{ mL}}$$

$$= 0.555 \text{ mg of sample injected}$$

Continuing, the final residue using this example:

$$\text{PPM fenoxaprop-ethyl equivalents} = \frac{A(\text{ng}) \times 1.71}{0.555}$$

* Represents one-half of sample taken for derivatization, 25 gram samples only.

6.10 Calculation of Fortifications of Fenoxaprop-ethyl (HOE-033171), Fenoxaprop (HOE-53022) and HOE-054014:

Due to molecular weight differences and the fact that all residues are quantitated against the derivative HOE-083312, the percent recovery obtained from fortifications using fenoxaprop-ethyl (HOE-033171), fenoxaprop (HOE-053022) and HOE-054014 must reflect this difference. Results are corrected according to the following:

$$\text{for fenoxaprop ethyl: } \frac{\text{GMW fenoxaprop-ethyl}}{\text{GMW HOE-083312}} = \frac{361.8}{211.6} = 1.71$$

$$\text{for fenoxaprop: } \frac{\text{GMW fenoxaprop}}{\text{GMW HOE-083312}} = \frac{333.7}{211.6} = 1.58$$

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6.10 Calculation of Fortifications of Fenoxaprop-ethyl (HOE-033171), Fenoxaprop (HOE-53022) and HOE-054014 (continued):

for HOE-054014:
$$\frac{\text{GMW HOE-054014}}{\text{GMW HOE-083312}} = \frac{169.6}{211.6} = 0.80$$

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 Assurance Procedures

1 Laboratory Spiking

To assure 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 assurance samples should be directly onto the analytical sample prior to extraction, allowing enough time for the spiking solvent to evaporate. 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 and III.

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 notified in writing by the Study Director.

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 maximum recovery (90-100%) are to be attained. The toluene elution volume can be expected to be between 2.5 and 3.0 mL.

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

PACKED COLUMN CHROMATOGRAPHY:

WHEAT GRAIN

<u>FORTIFICATION LEVEL (PPM)</u>	<u>PERCENTAGE RECOVERED</u>		
	<u>HOE-033171</u>	<u>HOE-053022</u>	<u>HOE-054014</u>
0.05	70, 92	-	68
	122, 104	66	66
	70, 98	-	72
0.10	72	64	81
	110	70	89
	86	82	95
	86	-	-
Average (+ S.D.)	<u>91</u> ± 18	<u>71</u> ± 8	<u>79</u> ± 12

MEGABORE COLUMN CHROMATOGRAPHY:

WHEAT STRAW

0.05	100	73	70
	66	73	74
	90	63	67
0.10	94	63	72
	99	62	69
	94	65	83
	94	-	-
Average (+ S.D.)	<u>91</u> ± 13	<u>67</u> ± 5	<u>73</u> ± 6

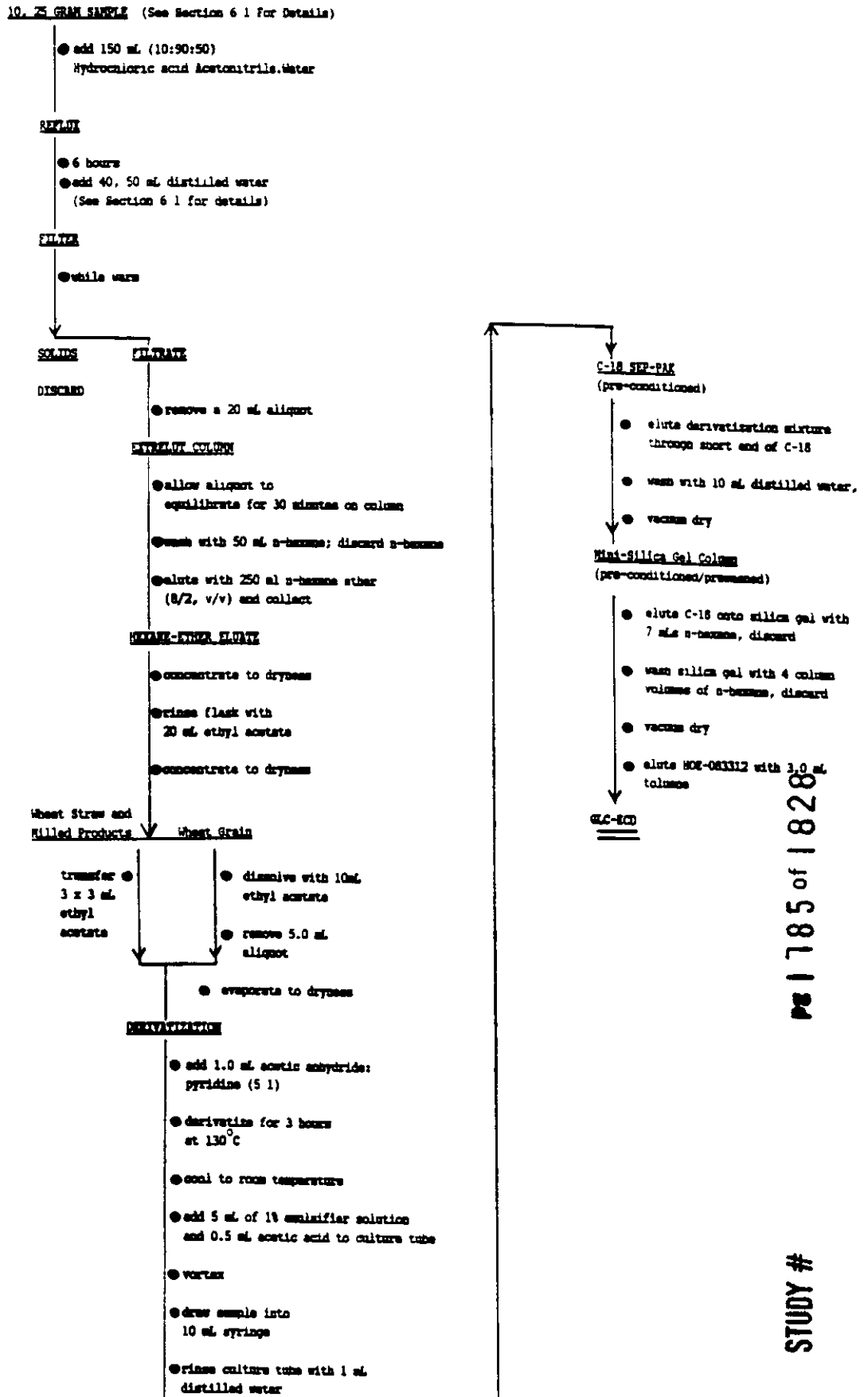
TABLE III: SUMMARY OF THE RECOVERY OF FENOXAPROP-ETHYL AND ITS METABOLITES FROM MILLED PRODUCTS

PACKED COLUMN CHROMATOGRAPHY:

<u>SAMPLE</u> ⁽¹⁾	<u>PERCENTAGE RECOVERED</u>		
	<u>HOE-033171</u>	<u>HOE-053022</u>	<u>HOE-054014</u>
Whole Wheat	70	71	---
Wheat Husk	79	---	74
Wheat Bran	73	67	---
Wheat Shorts and Germ	68	---	59
Low Grade Flour	72	67	---
Patent Flour	<u>83</u>	<u>---</u>	<u>77</u>
Average (<u>±</u> S.D.):	74 <u>±</u> 6	68 <u>±</u> 2	70 <u>±</u> 10

(1) All samples were fortified at the 0.05 ppm level.

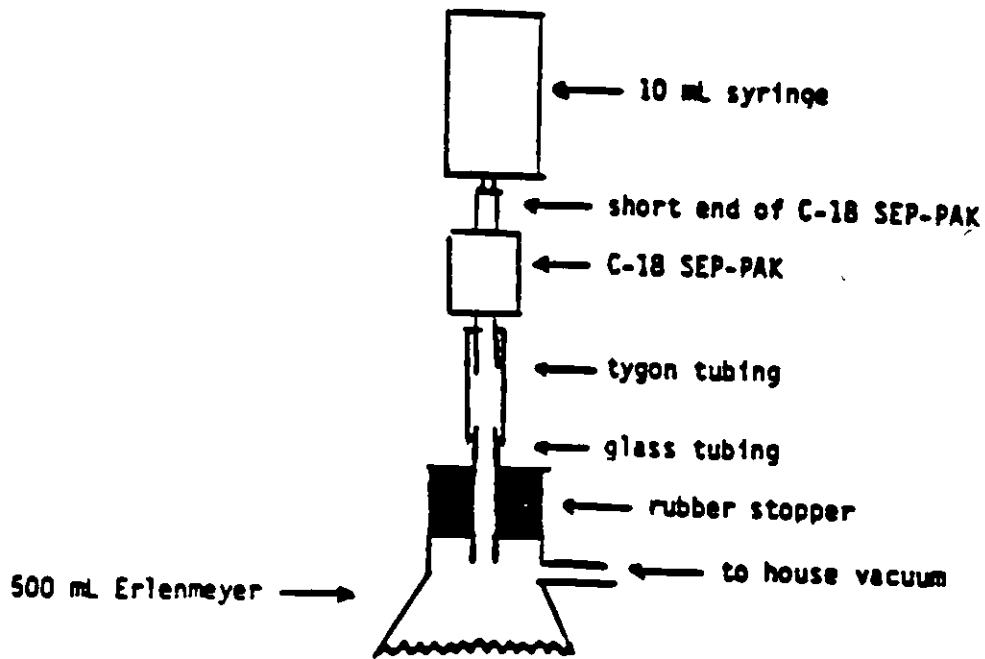
PHOSPHOPRO-ETHYL AND ITS MAJOR METABOLITE RESIDUES



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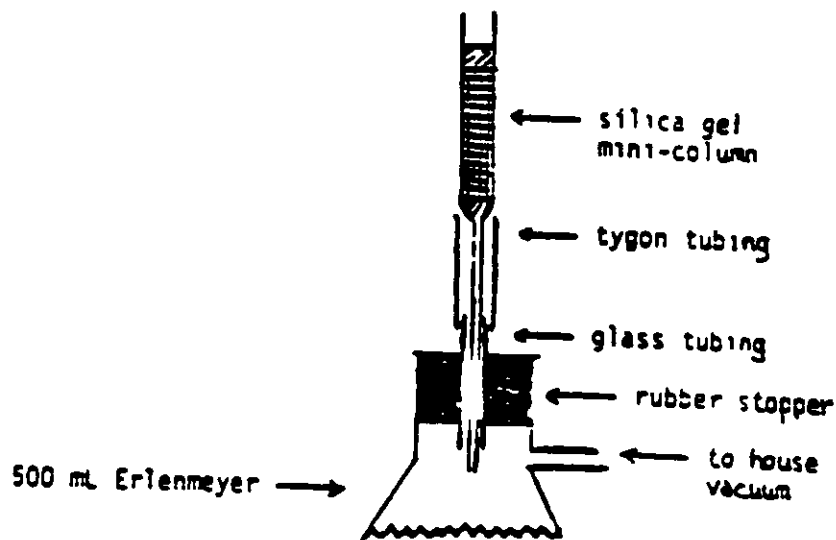
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Figure 2: Specialized Apparatus



A. Washing of C-18 SEP-PAK (Section 6.4)

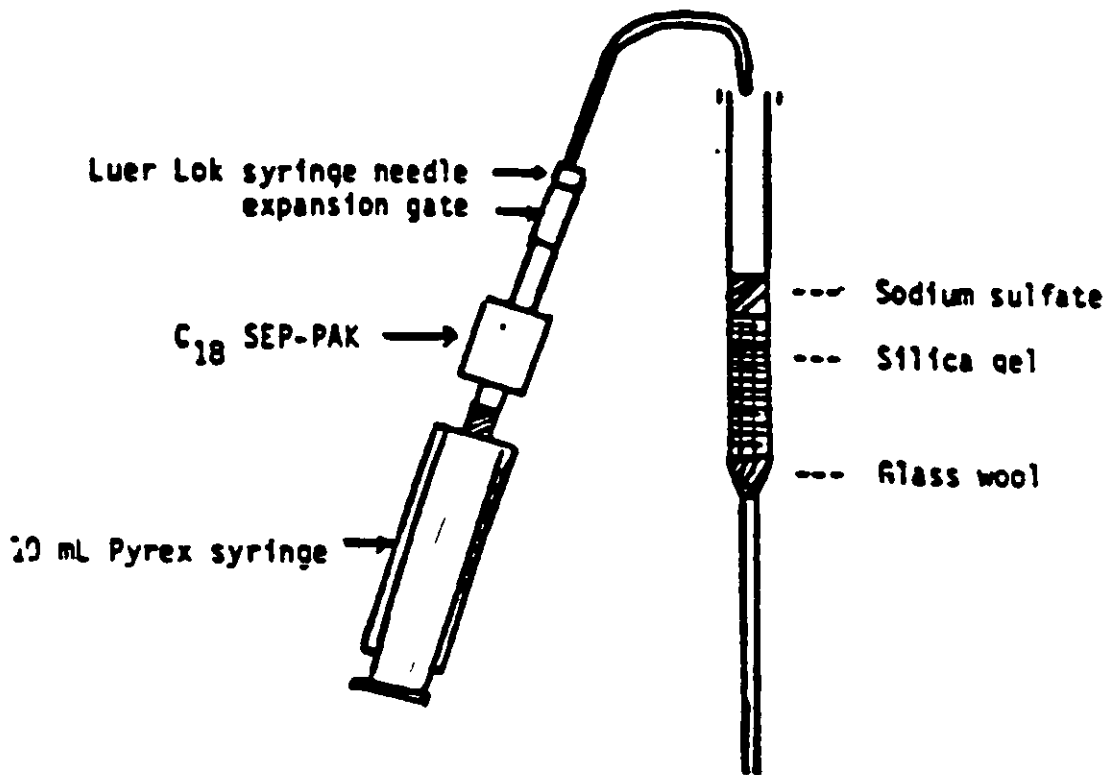
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B. Removal of hexane from mini silica gel column (Section 6.5)

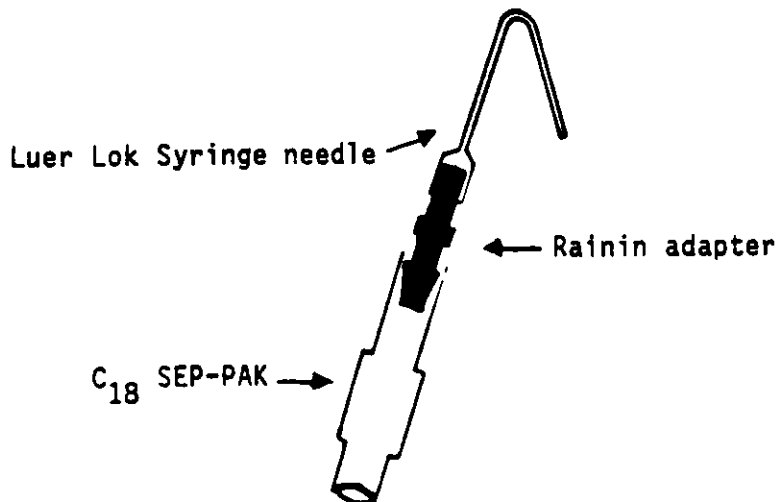
STUDY #

Figure 3: Assembly for transferring the SEP-PAK C₁₈ elute to the mini silica gel column.



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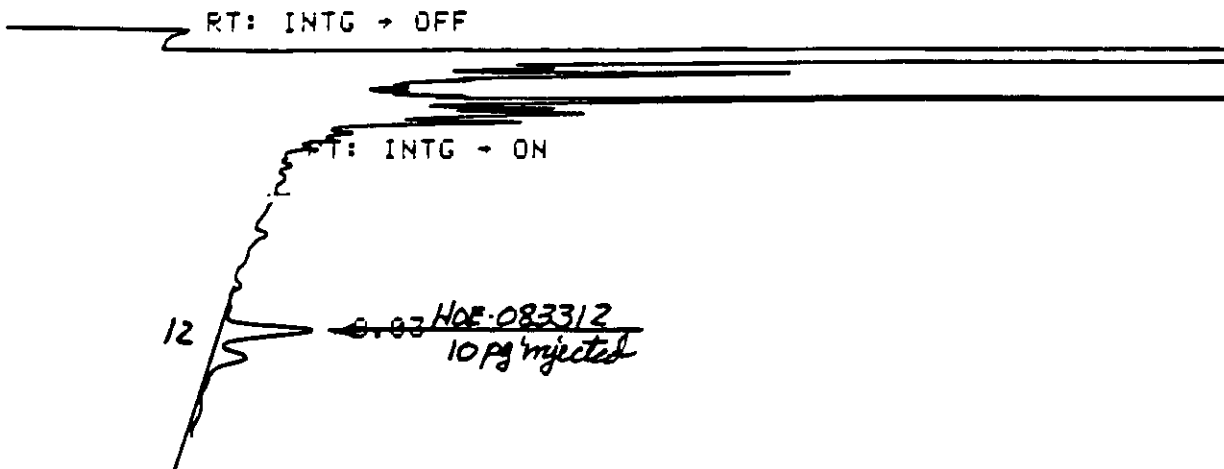
Figure 3a: Alternate assembly using Rainin adapter and Luer Lok syringe needle without expansion gate.



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Figure 4: TYPICAL PACKED COLUMN CHROMATOGRAPHY

MULTIPLIER = 1



HP 5890A SAMPLER INJECTION @ 11:09 NOV 6 1986
SAMPLE # : ID CODE :
2 2 PG/UL STD

AREA %

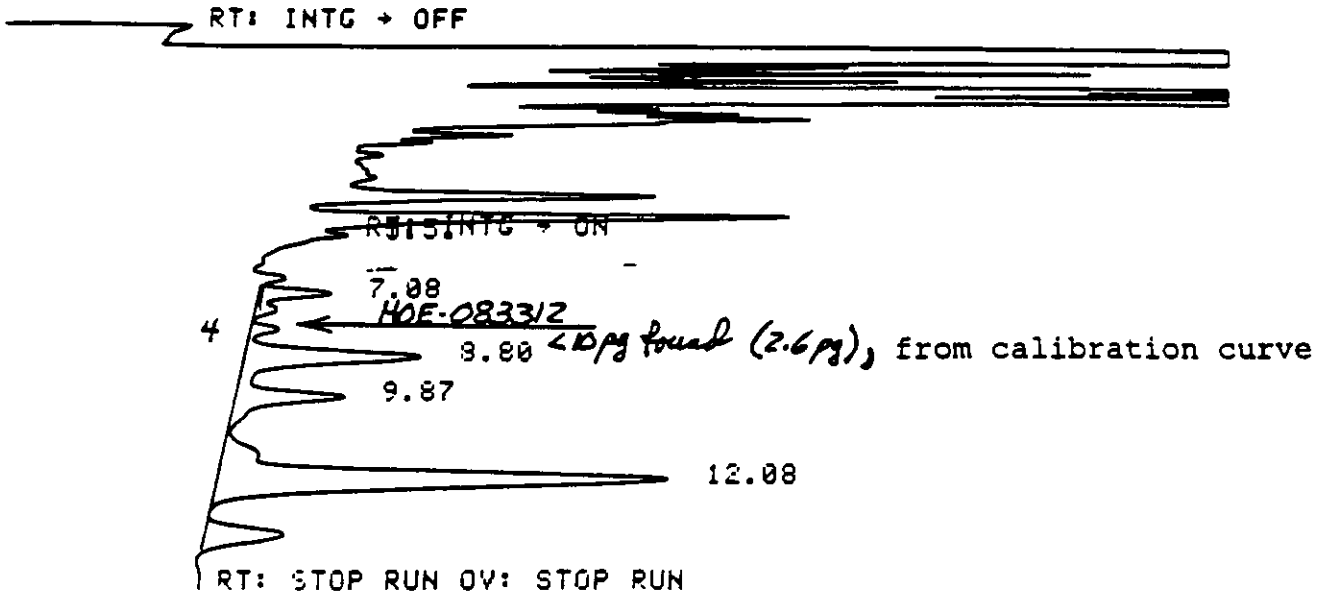
RT	AREA	TYPE	AREA %
8.03	681.85	HH	100.000

TOTAL AREA = 681.85
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



HP 5880A SAMPLER INJECTION @ 12:00 NOV 6, 1986

SAMPLE # : ID CODE :

6 09395-1.1:2

AREA %

RT	AREA	TYPE	AREA %
5.51	11.46	BP	0.297
7.08	237.74	VV	6.154
8.80	787.57	PP	20.386
9.87	444.50	BB	11.506
12.08	2382.03	BB	61.658

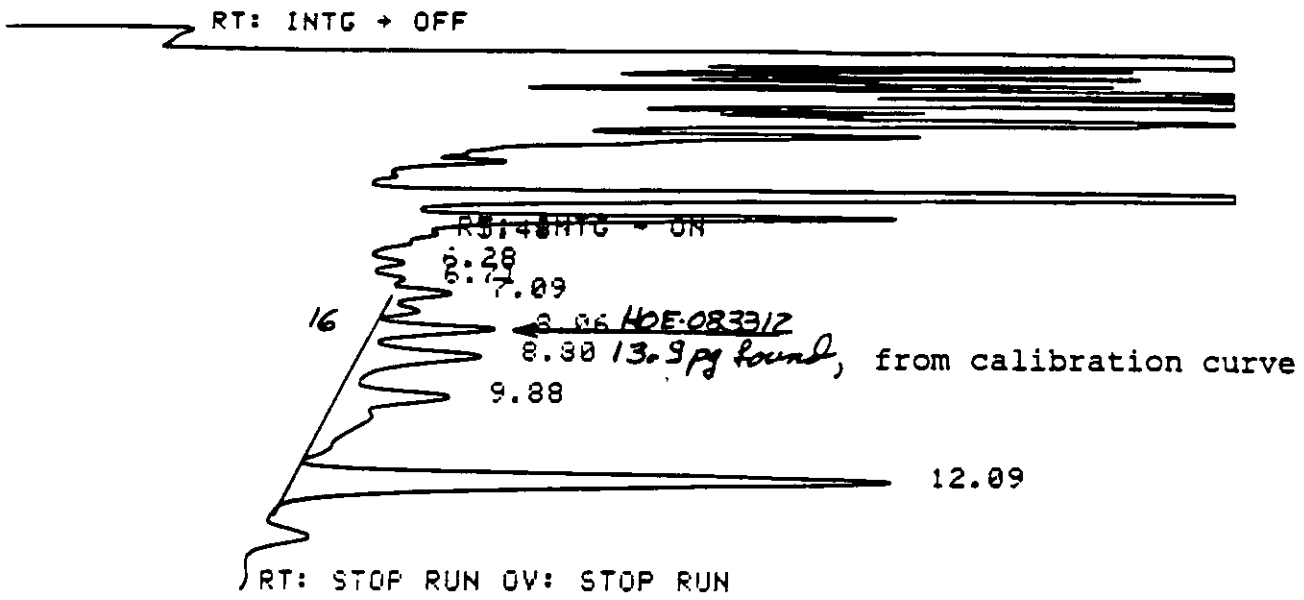
TOTAL AREA = 3863.29

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



HP 5880A SAMPLER INJECTION @ 12:17 NOV 6, 1986

SAMPLE # : ID CODE :

7 QA395-2 1:2

AREA %

RT	AREA	TYPE	AREA %
5.48	13.30	BP	0.212
6.28	95.91	PV	1.537
6.71	141.51	VV	2.267
7.09	387.05	VV	6.202
8.06	620.78	VV	9.946
8.80	1002.73	VV	16.066
9.88	701.39	VV	11.238
12.09	3278.60	BB	52.532

TOTAL AREA = 6241.17

MULTIPLIER = 1

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

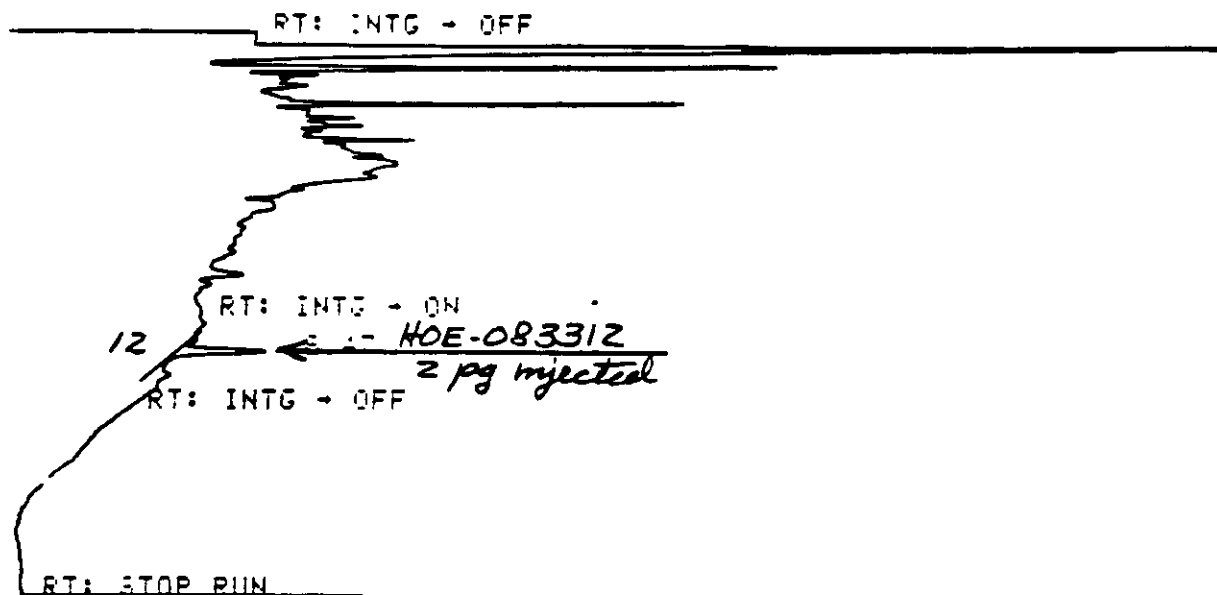
70% recovered

REFERENCE: Section 6.6.1

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Figure 7 TYPICAL MEGABORE COLUMN CHROMATOGRAPHY



HP 5880A SAMPLE INJECTION @ 16:33 FEB 19, 1987

SAMPLE # : ID CODE :

2 2 PG/UL STD

AREA % COMPENSATED ANALYSIS

RT	AREA	TYPE	AREA %
8.47	146.38	BB	100.000

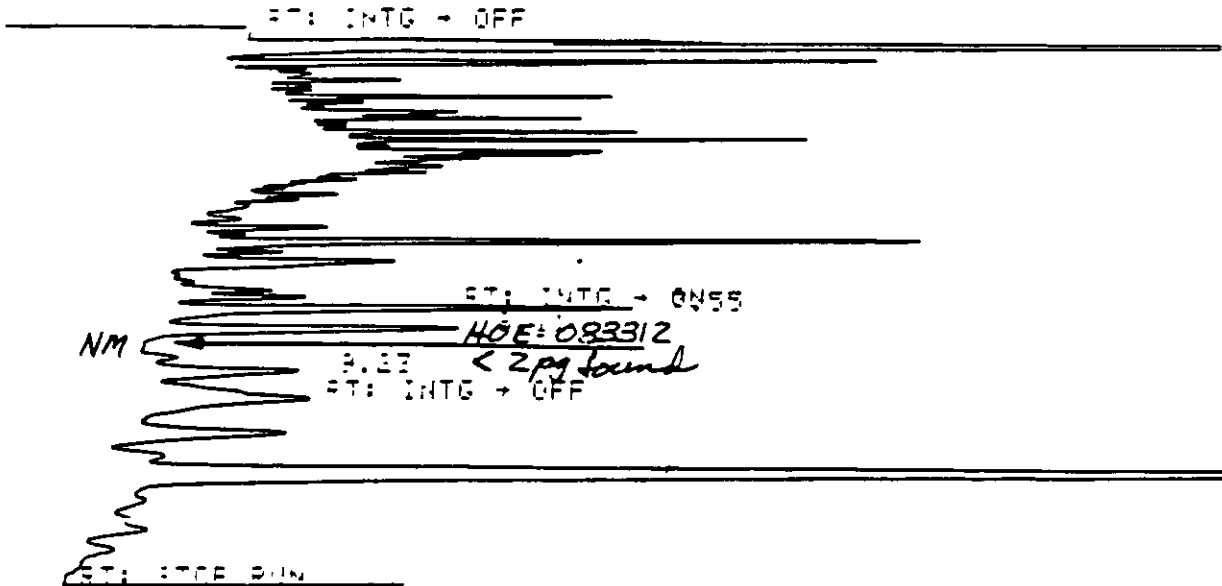
TOTAL AREA = 146.38

MULTIPLIER = 1

STANDARD: EQUIVALENT TO 2.0 pg HOE-083312 (1.0 uL x 2.0 pg/uL)

REFERENCE: Section 6.6.2

Figure 8: TYPICAL MEGABORE COLUMN CHROMATOGRAPHY



【OP】 5980A SAMPLER INJECTION @ 17:30 FEB 19, 1987

SAMPLE # : ID CODE :

4 08417-1

AREA % COMPENSATED ANALYSIS

RT	AREA	TYPE	AREA %
7.55	506.49	BP	32.092
8.11	617.61	PB	39.133
9.23	454.13	HH	28.775

TOTAL AREA = 1578.23

MULTIPLIER = 1

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

REFERENCE: Section 6.6.2