

US EPA ARCHIVE DOCUMENT

Study Title

Gas Chromatographic Determination of HOE-039866
[Ammonium-DL-homoalanin-4-YL(methyl)-phosphinate]
and Its Metabolite HOE-061517
[3-methylphosphinico-propionic acid]
as Residues in Apples, Grapes, Soybeans, Corn, and Tree Nuts

Data Requirement

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Study Submitted By

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Herbicide for Use on Soybeans, Field Corn, Tree Nuts,
Apples, and Grapes

Submission Volume

Volume 2 of 2

STATEMENT OF NO FIFRA SECTION 10 DATA CONFIDENTIALITY CLAIM

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Good Laboratory Practices Statement

The laboratory work reported herein is not subject to the 40 CFR 160 Good Laboratory Practice standards.

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1.0 Introduction:

The methodology described in HRAV-5A determines HOE-039866 [ammonium-DL-homoalanin-4-yl (methyl)phosphinate] and HOE-061517 [3-methylphosphinico-propionic acid] in:

- i. apples (fruit),
- ii. grapes (fruit),
- iii. soybeans (seed),
- iv. corn (grain, forage, fodder),
and
- v. tree nuts (almonds, almond hulls, pecans and walnuts).

Both HOE-039866 (parent compound) and HOE-061517 (metabolite compound) are highly polar, ionizable substances. These properties present a formidable challenge to the development and implementation of rugged residue methodology, considering the various biological matrices of interest.

Following a thorough evaluation of a number of strategies (including HPLC), the optimum approach was determined to be a multi-step procedure involving water extraction, anion exchange column clean-up, derivatization, silica gel column clean-up, and gas chromatography analysis using flame photometric detection. This method quantifies both parent and metabolite at levels ≥ 0.05 ppm (with the exception of almond hull, ≥ 0.5 ppm).

For background and insight, major considerations with regard to the developed analytical methodology are summarized below:

1.1 Extraction:

Batch extraction using distilled water solubilizes the compounds of interest. Although simple and straightforward, this procedure has the potential for co-extracting biological matrix components which also have an affinity for the solvent (water). The effects of such background components are minimized by using additional clean-up steps followed by GC/FPD analysis in the phosphorous selective mode.

1.2 Intermediate Clean-Up Steps:

Depending on the sample matrix type, a number of intermediate clean-up procedures have been investigated. These procedures include centrifugation, filtration, precipitation, solvent extraction and/or ion exchange column chromatography. In practice, one or more of the above techniques are generally useful in reducing the level of matrix background components, thereby improving the efficiency of subsequent derivatization/measurement steps. (1) Anion exchange column clean-up of the filtered sample extract has been found to be the most effective procedure for reducing background matrix effects (interferences) prior to the derivatization step for all sample matrices tested. Thus, this procedure is specified in the HRAV-5A methodology for all cases.

1.0 Introduction (Continued):1.3 Derivatization:

Trimethylorthoacetate/acetic acid was found to be a suitable derivatization reagent. This reagent esterifies the phosphinic and carboxylic acid functional groups of both HOE-039866 and HOE-061517 and also acetylates the basic amino group of HOE-039866. This one-step technique is relatively insensitive to any residual matrix components (following anion exchange) and requires four to five hours of reflux. On the other hand, methylation with diazomethane or methanol/HCl followed by acetylation with acetic anhydride or acetylchloride leads to poor conversion and interfering by-products.

1.4 Final Clean-Up:

Silica gel column chromatography provides the final sample clean-up prior to GC/FPD determination. Either user-prepared mini columns (packed with four-percent water deactivated silica gel) or commercially available silica gel cartridges are useful. For each matrix type, characterization of the silica gel adsorbent to be used is required. This ensures quantitative recovery of both derivatized parent and metabolite compounds using the conditions described in the method. Typical (or expected) solvent composition and elution volume are described in the method with consideration that laboratory optimization may be required based on the actual compound recovery results and/or system specific GC/FPD sensitivity constraints.

1.5 GC/FPD Determination:

The derivatives of both the parent and metabolite compounds are still highly polar and require appropriate attention to the maintenance and operation of the gas chromatography system. Both glass packed column chromatography and fused silica (wide bore) capillary chromatography have been successfully utilized for this application. (1) However, wide bore capillary columns coated with a (crosslinked) Carbowax^R phase have proven to be the most rugged columns for all sample applications. Despite the use of glass (or fused silica) injection liners and other "inert" connections/surfaces, it is necessary to "condition" the GC system by making two to three injections of a prepared sample prior to the actual analytical sequence. This technique minimizes the influence of system active sites which otherwise may cause erratic results during the analytical GC determination of trace-level polar compounds. (2)

The flame photometric detector (FPD) provides both sensitive and selective detection of the derivatized compounds (each containing a phosphorous atom). Furthermore, the FPD response is linear and adequately stable in the presence of non-phosphorous containing background components.

1.0 Introduction (Continued):

Quantitation is achieved by measurement of the calibrated FPD response of the derivatized parent and metabolite compounds. The FPD response is calibrated (externally) using analytical test standards. Peak height measurements are utilized and provide acceptably consistent quantitative data.

Peak area measurements are not used for calibration/quantitation because of potential background-induced solvent effects within the chromatography process. (3)

1.6 Other Considerations:

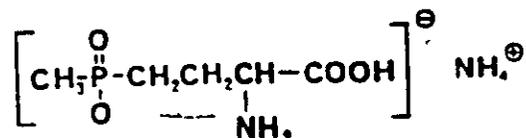
As mentioned above, because of the non-volatile and highly polar properties of both HOE-039866 and HOE-061517, utilization of HPLC technology was also examined. Unfortunately, the absence of strong chromophoric groups in these compounds precludes direct measurement via UV or fluorescence HPLC detectors (for residue analysis applications).

Derivatizing reagents which involve the amino function of HOE-039866 did not provide adequate conversion and/or selectivity in the presence of co-extracted matrix components. In addition, the metabolite does not contain the amino function. This presents even greater difficulties with derivatization/HPLC quantitation. It is therefore unlikely that an HPLC application can provide a viable and rugged residue method for both parent and metabolite determination.

2.0 Chemical Information:2.1 HOE-039866 (Parent):

Chemical Name: Ammonium-DL-homoalanin-4-yl(methyl)-phosphinate (IUPAC, English).

Structure:



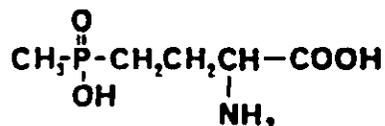
Molecular Formula: $\text{C}_5\text{H}_{15}\text{N}_2\text{O}_4\text{P}$

Molecular Weight: 198.2 g/mole

2.2 HOE-035956 (Free Acid of HOE-039866):

Chemical Name: DL-homoalanin-4-yl(methyl)-phosphinic acid (IUPAC, English)

Structure:



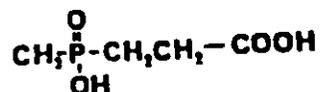
Molecular Formula: $\text{C}_5\text{H}_{12}\text{NO}_4\text{P}$

Molecular Weight: 181.1 g/mole

2.0 Chemical Information (Continued):2.3 HOE-061517 (Metabolite of HOE-039866):

Chemical Name: 3-Methylphosphinico-propionic acid
(IUPAC, English)

Structure:



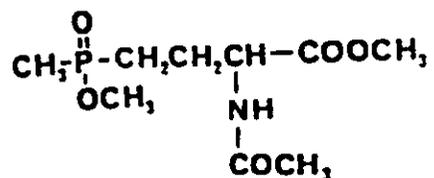
Molecular Formula: $\text{C}_4\text{H}_9\text{O}_4\text{P}$

Molecular Weight: 152.1 g/mole

2.4 HOE-064706 (GC Derivative of HOE-039866/HOE-035956):

Chemical Name: Methyl-4-(methoxymethyl)-phosphinoyl-2-acetamido-butyrates (IUPAC English)

Structure:



Molecular Formula: $\text{C}_9\text{H}_{18}\text{NO}_5\text{P}$

Molecular Weight: 251.3 g/mole

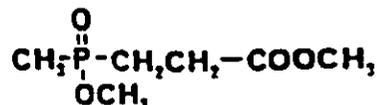
2.0

Chemical Information (Continued):

2.5 HOE-070951 (GC Derivative of HOE-061517):

Chemical Name: Methyl-3-(methoxymethyl)-phosphinoyl-propionate (IUPAC English)

Structure:

Molecular Formula: $\text{C}_6\text{H}_{13}\text{O}_4\text{P}$

Molecular Weight: 180.2 g/mole

3.0

Principle of the Method:

Residues of HOE-039866 [Ammonium-DL-homoalanin-4-yl (methyl) phosphinate] and its principal metabolite [HOE-061517: 3-Methylphosphinico-propionic acid] are extracted from finely ground and/or homogenized sample material into distilled water (pH 4-8). The aqueous extract is filtered to remove undissolved solids.

An aliquot of the filtrate is passed through anion exchange resin (hydroxide form) to remove potentially interfering co-extracted sample matrix components. These unbound or weakly bound components are water washed through the column and discarded. The parent and metabolite compounds are eluted from the column using a formic acid solution. The column eluate containing HOE-039866 and HOE-061517 is then evaporated to dryness using a rotary evaporator.

At this point, both the parent and metabolite residues are derivatized under reflux for about 4 hours using trimethylorthoacetate in glacial acetic acid. After a solvent exchange procedure, the solution containing the derivatives, HOE-064706 [Methyl-4-(methoxymethyl)-phosphinoyl-2-acetamido-butyrates] for HOE-039866 and HOE-070951 [Methyl-3-(methoxymethyl)-phosphinoyl propionate] for HOE-061517, is passed through a silica gel column for additional clean up. A final solvent exchange (into pure methyl acetate) is carried out prior to GC analysis.

Quantitative determination of both HOE-064706 and HOE-070951 is accomplished using gas chromatography with flame photometric detection (P-mode).

Residues are expressed as HOE-035956 (free acid) equivalents.

4.0 Equipment:

- 1a. Food Processor: Moulinex SR Blender, or equivalent.
- 1b. Sample Homogenizer: Brinkmann Model PT 10/35, equipped with PT 6 36/50 generator, or equivalent.
2. Balances: Mettler Model PC 2000, or equivalent (for sample preparation); Mettler Model AE 160, or equivalent (for standard preparation).
3. Microliter Syringes: 10, 100, 250, 500 microliter (uL), Hamilton, or equivalent.
4. Round Bottom Flask: 250 mL capacity, [Fisher Scientific: 10-067E] or equivalent.
5. Reflux Condenser: High efficiency, 300 mm, equipped with a 24/40 ground glass joint. [Fisher Scientific: 07-735A], or equivalent. If necessary, connect a drying tube filled with anhydrous CaCl_2 to the top of the condenser [See Section 7.4].
6. Heating Mantles: Glas-Col; 500 mL [Fisher Scientific: 11-472-10F].
7. Erlenmeyer Flasks: 500 mL [VWR: 29140566], or equivalent.
8. Collection Tubes, Glass: 16mm X 100 mm, for collection of silica gel column fractions [Fisher Scientific:14-961-29], or equivalent.
9. Volumetric Pipets, Glass: Class A; 0.5, 1.0, 2.0, 25.0, 50.0 mL capacity.
10. Luer Stopcocks: Analytichem International [No. A16078], or equivalent; used with SPE silica gel cartridges.
11. Ultrasonic Bath: Branson [VWR: 21810-304], or equivalent.
12. Rotary Flash Evaporator: Buchi, RE-120 or equivalent [Fisher (09-548-151C)], with a water bath at 45-50°C.
13. Millex SR Filters: Millipore No. SIR 025NS.
14. Vacuum Flask: 500 mL [Fisher 10-180E], or equivalent.
15. Vortex: Lab Line Instruments, Super Mixer Model 1290.
16. Disposable Pasteur Pipettes, Glass: 9" x 0.7 mm O.D. [VWR: 14672-380].
17. Glass Wool: DMCS treated [Alltech Associates: 4037]; used for packing the mini silica gel columns.
18. Graduated Centrifuge Tubes: 10-15 mL [Fisher Scientific: 05-538-35B].

4.0 Equipment (Continued)

19. Syringes: Polypropylene, 10 mL capacity, equipped with a Luer tip [Aldrich Chemical Co.].
20. Rheostat: Staco, or equivalent; Input 120V, Output 0-120/140V.
21. Syringe Needles: Stainless steel, 100 mm [Organization: 11305].
22. Hypodermic Needles: 20G x 1 1/2 inch length [WWR BD 5176], or equivalent.
23. Magnetic Stirrer: [WWR: 0558954-016], or equivalent.
24. Magnetic Stir Bars: TFE, [WWR: 58948150], or equivalent.
25. N-Evap Analytical Concentrator: Organization Associates, or equivalent.
26. Gas Chromatograph: Hewlett Packard 5880A GC equipped with a Flame Photometric Detector operating in the Phosphorous mode, or equivalent instrumentation. [Note: According to the application, the GC system is configured for wide-bore capillary column operation, with heated (flash) injection.] Use of an autosampler device is optional but recommended for optimum reproducibility and efficiency.
27. Gas Chromatography Column: 1.0 μ m DB-WAX, fused silica, 8 meter x 0.53 mm wide bore column with crosslinked (Carbowax 20M) stationary phase, [J & W Scientific: 125-7012]. Note: The commercially available 15 meter column may be divided into two shorter column segments. Typically, a column of 7 to 10 meter length is adequate for these applications.
28. Vacuum Box Apparatus: Vac Elut SPS 24 Vacuum Box [Analytichem International: No. A16500-2]; for use with silica gel SPE cartridges. Note: Any equivalent apparatus which is compatible with the SPE cartridge system may be utilized to provide a uniform and controllable cartridge elution (drip) rate.
29. Glass Column: with stopcock and reservoir, 10.5 mm id., 250 mm length; [Lab Glass: IG-4565-T-100], or equivalent; to be used for anion exchange column chromatography.
30. Silica Gel SPE Cartridge: 50 mg cartridge with reservoir, [Analytichem International: No. IR01304], or equivalent.
31. Buchner Type Funnel: for vacuum filtration [Fisher Scientific: 10-356C], or equivalent.
32. Filter Paper: Whatman No. 1 [e.g., Fisher Scientific: 09-805C]; Whatman No. GF-C [e.g., Fisher Scientific: 09-804-90A], sized to fit the Buchner funnel (Item 31).

5.0 Reagents/Chemical Supplies:

- a) Formic Acid: Fisher Scientific (88% w/w), ACS Reagent Grade or equivalent.
- b) Methyl Acetate: Aldrich Gold Label (99+%), or equivalent.
- c) Ethyl Acetate: Mallinckrodt Nanograde, pesticide quality, or equivalent.
- d) Glacial Acetic Acid: Mallinckrodt AR, or equivalent.
- e) Trimethylorthoacetate: Kodak (114 9202), or equivalent grade.
- f) Methanol: Mallinckrodt Nanograde, pesticide quality, or equivalent (Mallinckrodt: 5160).
- g) Toluene: Mallinckrodt Nanograde, pesticide quality, or equivalent
- h) Hydrochloric Acid, Concentrated: ACS Reagent Grade, or equivalent.
- i) 50% Methanol in Methyl Acetate: Mix pure reagents to a 1:1 volume ratio of Methanol:Methyl Acetate.
- j) 50% Toluene in Methyl Acetate: Mix pure reagents to a 1:1 volume ratio of Toluene:Methyl Acetate.
- k) Silica Gel: Merck 7734 (Kieselgel 60), dried for minimum of 5 hours at 130 °C.
- l) Water Deactivated Silica Gel:

The silica gel is deactivated to 4% water content with distilled water.

Four (4) grams of distilled water are added to 96 grams of oven-dried (5 hr) silica gel. The mixture is then equilibrated for no less than 48 hours on a roller mill or other equivalent equipment. Store the prepared silica gel in an air-tight container.

The elution pattern of the silica gel is determined for each batch prepared (see also Section 7.6.1, Preparation of the Mini Silica Gel Column).

- m) Formic Acid (50% w/w): Dilute 570 mL of Formic Acid reagent (88%) to 1 L (final volume) with distilled water. Always add the concentrated acid to 300-400 mL of water before diluting to final volume.
- n) Ammonium Hydroxide (0.015 M): Dilute 1 mL of ammonia solution [Riedel de Haen No. 30851 (25% NH₃)] to 100 mL with distilled water.

5.0 Reagents/Chemical Supplies (Continued):

- o) HOE-039866: Ammonium DL-homoalanin-4-yl (methyl)phosphinate, analytical standard.
- p) HOE-035956: DL-homoalanin-4-yl (methyl)phosphinic acid, analytical standard.
- q) HOE-061517: 3-Methylphosphinico-propionic acid, analytical standard.
- r) HOE-064706: Methyl-4-(methoxymethyl)phosphinoyl-2-acetamido-butyrate, analytical standard.
- s) HOE-070951: Methyl-3(methoxymethyl)phosphinoyl propionate, analytical standard.
- t) Calcium Chloride (anhydrous): Fisher Scientific (C614), or equivalent.
- u) Anion Exchange Resin: Aldrich (21,741-7) Dowex 1X8-100 ion exchange resin, 8% crosslinking, 50-100 dry mesh.
- v) Sodium Hydroxide (1M): Dilute 51.5 mL of 50% Sodium Hydroxide [Mallinckrodt (50% w/w)] with distilled water to a final volume of 1 liter.
- w) Preparation of Anion Exchange Resin: Mix 100 g of Anion Exchange Resin (u) with 1 L of 1M Sodium Hydroxide (v) in a 1-2 L beaker and stir using a magnetic stir bar for approximately 30 minutes or until resin solution is pH 10-11 (as indicated using pH paper). The solution must be stirred slowly to avoid crushing the resin. Filter the resin through Whatman No. 1 filter paper/Buchner funnel apparatus (to remove excess Sodium Hydroxide) and continue to wash the resin with distilled water until the pH of the filtrate is no longer basic (pH 5.5-7.5). Typically, for 100 g of resin, about 3-5 liters of distilled water are required.

[NOTE: Analytical Standards 5.0(o), 5.0(p), 5.0(q), 5.0(r), and 5.0(s) are available from Hoechst AG, Frankfurt (M), FRG or Hoechst-Roussel Agri-Vet Company, Somerville, NJ.]

6.0 Preparation of Standard Solutions:

(Note: Store all solutions in a refrigerator).

6.1 Calibration Solutions:HOE-064706 and HOE-070951:

Weigh 139 (+1) mg of HOE-064706 and 100 (+1) mg of HOE-070951 into separate 100 mL volumetric flasks. Dilute each to volume with pure methyl acetate. These solutions (Stock Solutions A1 and A2) contain 1.0 mg of HOE-064706/mL and 1.0 mg of HOE-070951/mL, respectively, expressed as HOE 035956 equivalents. They must be prepared fresh every six months.

Transfer a 5.0 mL aliquot from both Stock Solution A1 and Stock Solution A2 to the same 100 mL volumetric flask. Dilute to volume with pure methyl acetate. This solution (Solution B) contains 50.0 ug of HOE-064706 and HOE-070951/mL expressed as HOE-035956 equivalents. It must be prepared fresh every six months.

Transfer 2.0 mL of Solution B to a 100 mL volumetric flask. Dilute to volume with pure methyl acetate. This solution (Solution C) contains 1.0 ug of HOE-064706 and HOE-070951/mL expressed as HOE-035956 equivalents. It must be prepared fresh every month (or as needed).

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

TABLE I

Typical Series of GC Calibration Standards

<u>AMOUNT</u> <u>(mL)</u>	<u>SOLUTION</u>	<u>FINAL DILUTION</u> <u>VOLUME (mL)</u>	<u>FINAL CONCENTRATION OF</u> <u>GC CALIBRATION STANDARD*</u> <u>(ng/uL)</u>
2.0	C	100.0	0.020
4.0	C	100.0	0.040
5.0	C	100.0	0.050
6.0	C	100.0	0.060
8.0	C	100.0	0.080
10.0	C	100.0	0.100

*) Concentrations are expressed as HOE-035956 equivalents.

6.0 Preparation of Standard Solutions (Continued):

6.2 Standard Solutions for Fortification Studies:

HOE-039866:

Weigh 109 (± 1) mg of HOE-039866 into a 100 mL volumetric flask. Dilute to volume with 0.015M ammonium hydroxide. This solution (Stock Solution D) contains 1.0 mg of HOE-039866/mL expressed as HOE-035956 equivalents. It must be prepared fresh every six months.

HOE-061517:

Weigh 84 (± 1) mg of HOE-061517 into a 100 mL volumetric flask. Dilute to volume with 0.015 M ammonium hydroxide. This solution (Stock Solution E) contains 1.0 mg of HOE-061517/mL expressed as HOE-035956 equivalents. It must be prepared fresh every six months.

Combined Standards:

Transfer a 5.0 mL aliquot of both Stock Solutions D and E to the same 100 mL volumetric flask. Dilute to volume with 0.015 M ammonium hydroxide. This solution (Stock Solution F) contains 50.0 ug of HOE-039866 and HOE-061517/mL expressed as HOE-035956 equivalents. It must be prepared fresh every six months.

Transfer a 5.0 mL aliquot of Stock Solution F to a 100 mL volumetric flask. Dilute to volume with 0.015 M ammonium hydroxide. This solution (Stock Solution G) contains 2.5 ug of HOE-039866 and HOE-061517/mL expressed as HOE-035956 equivalents. It must be prepared fresh every six months (or as needed).

7.0 Analytical Procedure:

7.1 Sample Preparation:

Analytical samples are finely ground (or homogenized) with dry ice (as necessary) using a food processor or equivalent apparatus. It is critical that the laboratory sample be finely ground (e.g., to a particle size ≤ 2 mm diameter) or homogenized before subjecting it to the analysis procedures described below.

7.0 Analytical Procedure (Continued):7.2 Extraction:

Into a 500 mL Erlenmeyer flask, weigh 25 (± 0.1) g (W_s) of the analytical sample prepared as described in Section 7.1. Add 200 mL of distilled water (pH 4-8) and a magnetic stirring bar. [Total sample volume (V_1) is considered to be 200 (± 1) mL at this point for all sample matrices except grapes. Due to the very high water content of grape samples, the total sample volume is considered to be 225 (± 1) mL in this case.]

Place the flask containing the sample and distilled water onto a magnetic stirring plate. Stir vigorously for a minimum of 1/2 hour.

Suction filter the mixture through Whatman GF-C (glass fiber) filter paper contained in a Buchner funnel, collecting the filtrate in a clean 500 mL filtering flask.

7.3 Extract Clean Up Using Anion Exchange:

Slurry pack a glass chromatography column (10.5 mm id) with the amount of anion exchange resin specified below (wet weight, ± 0.5 g), after conditioning the resin as described in the Reagents Section (5.0). Use distilled water to assist in transferring and packing the resin into the column as needed.

<u>Matrix</u>	<u>Amount of Anion Exchange Resin</u>
Apples	6 g
Grapes	10 g
Corn Grain	6 g
Corn Forage	10 g
Corn Fodder	10 g
Almond Nutmeat	10 g
Almond Hulls	10 g
Pecans	6 g
Walnuts	10 g
Soybeans	15 g

7.0 Analytical Procedure (Continued):

Load the column with 20.0 ± 0.1 mL of the filtered aqueous extract. Allow this solution to pass through the resin (gravity flow). At this point, wash the resin with 100 ± 10 mL of distilled water. Regulate the flow of column effluent as required using the column stopcock valve such that a flow rate of approximately one to two drops per second is attained. Discard the effluents from both the loading and water wash steps which contain only residual sample matrix.

Elute both the parent (HOE-039866) and metabolite (HOE-061517) compounds using 100 ± 10 mL of 50% Formic Acid, collecting the eluate directly into a 250 mL round bottom flask. Also use a drip rate of about one to two drops per second.

Evaporate the formic acid eluate to dryness using a rotary evaporator operating at $60-65^{\circ}\text{C}$. Operate the rotary evaporator at a speed which avoids excessive dispersion of the sample inside of the round bottom flask. Add five to ten milliliters of ethyl acetate to the dried sample extract and again evaporate to dryness as above. Repeat this procedure an additional 2-3 times to remove all residual water. Proceed to Section 7.4.

7.4 Derivatization:

Add 3 mL of glacial acetic acid to the round bottom and swirl the flask. Add 12 mL of trimethylorthoacetate and a few glass beads. Cap the flask and suspend in an ultrasonic bath (operating at room temperature) for approximately 15 minutes (or until all visible sample residue is dissolved and/or dislodged from the wall of the flask). When mixing is complete, reflux the reaction mixture for 4-5 hours.

After the reflux period, allow the sample to cool to room temperature. Disconnect the flask from the reflux condenser. Add 15 mL of toluene. The procedure may be stopped at this point (capping the flask for storage at room temperature). Otherwise, proceed to Section 7.5.

7.5 Reconstitution:

The cooled derivatized sample mixture is reconstituted as follows. Evaporate the contents of the round bottom flask to a final volume of approximately 2 mL (using the rotary evaporator operating with a water bath temperature of 45°C). Add successive 15 mL portions of toluene and continue to repeat the evaporation procedure. At a minimum, three 15 mL portions of toluene are added and evaporated to a final volume of approximately 2 mL to remove all traces of the derivatization solution. It is important that the solution is not evaporated to dryness during the reconstitution procedure. Both the derivatized parent and metabolite test substances may be lost should this occur.

7.0 Analytical Procedure (Continued):

7.6 Post Derivatization Clean Up:

The derivatized sample extract requires additional clean up prior to GC analysis. Clean up may be carried out using mini silica gel columns which are prepared as described in Section 7.6.1. The mini silica gel clean up procedure is described in Section 7.6.2.

Alternately, commercially available silica gel solid phase extraction (SPE) cartridges can be used. This procedure is described in Section 7.6.3.

Either clean up technique requires characterization/verification of the retention and elution conditions of the silica gel material(s) utilized. The characterization procedure is described in Section 7.6.4.

7.6.1 Preparation of the Mini Silica Gel Column:

The mini-silica gel columns are prepared as follows. Weigh 0.60 (± 0.01) g of deactivated silica gel [prepared as described in Section 5.0 (1)] into a 9 inch Pasteur pipette, the bottom of which is closed by means of a small glass wool plug (inserted to the point of taper).

Condition the column with 50% toluene in methyl acetate while gently tapping to remove air bubbles. The column should appear uniform and somewhat opaque. This indicates a suitable packing condition. Columns must be prepared just prior to use. See Section 7.6.4.

7.6.2 Silica Gel Column Clean Up:

Attach a 100 mm syringe needle to a 10 mL disposable syringe and draw the reconstituted sample from section 7.5 (in toluene) into the syringe. Adjust the volume in the syringe to 3 mL using pure toluene. Rinse the round bottom flask with 5 mL of methyl acetate and draw this rinse solution into the syringe. The total volume in the syringe is now 8 mL. Reserve the round bottom flask.

Invert the syringe (plunger end down), remove the 100 mm needle, and attach a Millex SR disposable filter to the tip of the syringe. Vortex the contents of the syringe. Keeping the filter end up (after the vortex procedure), attach a 20G x 1-1/2 inch disposable hypodermic needle to the exit end of the filter. [Note: Before attaching the needle, carefully bend the hypodermic needle (forming a U-shape) such that it can conveniently be hung from the top rim of the mini silica gel column.] Having done this, allow one to two minutes for precipitates to settle out in the barrel of the (still) inverted syringe.

7.0 Analytical Procedure (Continued):

Load the sample onto the mini silica gel column using the inverted syringe/filter/needle apparatus as set up above. This apparatus is illustrated in Figure 1. Loading must be done in incremental fashion, allowing for the drip rate of the silica gel column. After loading, wash the round bottom flask with an additional 10 mL of pure methyl acetate. Draw this final wash solution into the syringe via the 100 mm syringe needle (filter removed).

Reconnect the filter/hypodermic needle assembly to the syringe and apply the wash solution to the silica gel column. Discard the eluates.

Elute the ~~HOE-064706 and HOE-070951~~ with (1/1) methanol/methyl acetate solvent. Collect the first 5.0 (± 0.1) mL of eluate in a small measuring flask such as a 10-15 mL graduated centrifuge tube.

7.6.3 Silica Gel SPE Cartridge Clean Up:

Silica Gel SPE cartridges are typically used in conjunction with a vacuum box assembly as described in the Equipment Section (4.0). In addition, refer to the manufacturer's operating instructions for the particular device utilized. See also Section 7.6.4.

Immediately prior to use, the SPE cartridges are conditioned by passing 10 mL of methanol reagent followed by 10 mL of 50:50 methyl acetate:toluene through each cartridge. Do not allow the cartridge to become dry after this point.

To load the SPE cartridge, attach a 100 mm syringe needle to a 10 mL disposable syringe and draw the reconstituted sample (in toluene) from the round bottom flask into the syringe. Adjust the volume in the syringe to 3 mL using pure toluene. Rinse the round bottom flask with 5 mL of methyl acetate and draw this rinse solution into the syringe. The total volume in the syringe is now 8 mL. Reserve the round bottom flask.

Invert the syringe (plunger end down), remove the 100 mm needle, and attach a Millex SR disposable filter to the tip of the syringe. Vortex the contents of the syringe.

Load the sample directly onto the SPE cartridge through the syringe/disposable filter apparatus. After loading, wash the (reserved) round bottom flask with an additional 10 mL of pure methyl acetate. Draw this final wash solution into the syringe via the 100 mm syringe needle (filter removed).

7.0 Analytical Procedure (Continued):

Reconnect the filter assembly to the syringe and apply the wash solution to the cartridge. Discard the eluates.

Elute the HOE-064706 and HOE-070951 with 5.0 (± 0.1) mL of (1/1) methanol/methyl acetate solvent. Collect the eluate in a glass collection tube which is typically contained in the vacuum box assembly. Approximately 5 mL of eluate should be collected.

7.6.4 Column Characterization:

Before analyses are conducted, the mini silica gel columns or SPE cartridges must be characterized. Such characterization is necessary to account for possible variability in silica gel raw materials as well as to verify the packing/conditioning procedures described above. To characterize column performance, add HOE-070951 and HOE-064706 standard solutions to a control sample which has undergone analytical work-up through the derivatization step (Section 7.4) such that a representative level of test compound is obtained (i.e., 200-250 ng in 5.0 mL final volume). Continue the analytical procedure and observe the "recovery" of the test compounds after GC/FPD analysis (see below). The methanol:methyl acetate solvent strength and/or elution volume may need to be optimized to maximize recovery (90-100%) for both test substances. If this is necessary, reconstitute and adjust the final eluate volume to obtain (typically) 5.0 mL of methyl acetate solvent (to match the solvent composition of the analytical standards).

7.6.5 Solvent Exchange:

Using an N-Evap Analytical Concentrator operating at 50°C or a rotary evaporator at 40°C (or equivalent apparatus), reconstitute the silica gel column eluate in pure methyl acetate by successive evaporation and addition of 4-5 mL portions of methyl acetate. [Note: Do not evaporate to a volume < 0.5 mL at any point.]

The above procedure is repeated four times to adequately remove the methanol solvent. The final volume (V3) is typically adjusted to 5.0 (± 0.1) mL for GC analysis.

[Note: The volume of the solution for GC analysis may be reduced to compensate for particular GC/FPD sensitivity limitations. However, evaporation/reconstitution to volumes ≥ 0.5 mL is required to minimize evaporative losses of derivatized compounds. The final GC solution solvent must be methyl acetate to match the analytical calibration standards.]

8.0 Determination by Gas Chromatography:

Both the derivatized parent (HOE-064706) and metabolite (HOE-070951) are determined using gas chromatography with flame photometric detection (P-mode). A wide bore (0.53 mm id) fused silica capillary column application is used. Note the final volume (V3) of the GC test solution, typically 5.0 (+0.1) mL in methyl acetate solvent.

8.1 Gas Chromatography Instrumentation:

A Hewlett Packard Model 5880A Gas Chromatograph equipped with a Flame Photometric Detector operating in the phosphorous selective mode is adequate for determination of 0.05 ppm levels of the test compounds. Other GC/FPD instrumentation systems which have equivalent performance may be utilized. For method validation, data acquisition was carried out using a Nelson Analytical Turbochrom Data System. In addition, the output of the HP printer/plotter was used as a back-up GC signal recording device. Both data reduction techniques provided acceptable and equivalent quantitative measurements.

8.1.1 Electrometer/Chart Recorder:

Operate in the linear mode (for phosphorous); attenuate as required to obtain peak heights ≥ 10 mm (S/N >3) for a level corresponding to 0.05 ppm of each test compound as HOE 035956 equivalents.

8.1.2 Digital Data Acquisition Rate:

Using the Nelson Analytical Data System, the data acquisition rate was typically 3 points per second. The minimum acceptable data acquisition rate is determined according to the frequency characteristics of the test component GC peak. A GC peak must be defined by a minimum of twenty digital data points (signals).

8.1.3 Detection Limit (Minimum):

0.05 ppm of each test compound (quantified as HOE-035956 equivalents).

8.0 Determination by Gas Chromatography: (Continued)8.2 DB-Wax Wide Bore Capillary Column Chromatography:

Column: 8 meter x 0.53 mm id fused silica (wide bore) column with 1.0 micron DB-Wax stationary phase (J&W Scientific).

Temperatures:

Inlet: 225°C
 Detector: 225°C
 Column: Temperature Program:
 Initial Temp....110°C
 Initial Time....2 min
 Rate.....30°C/min
 Final Temp.....220°C
 Final Time.....10 min

Gas Flows:

Carrier: 25 mL/min (Helium)
 Detector: 10-15 mL/min (Oxygen)
 100-200 mL/min (Hydrogen)
 70-120 mL/min (Air)

[Detector make-up gas is optional, typically 15-30 mL/min. Also, see discussion of specific operating conditions below.]

Injection Volume: 2-5 uL (Typically 5 uL using HP autosampler)

Approximate Retention Time: 3 - 4 min (HOE-070951)
 7 - 9 min (HOE-064706)

The component retention times are estimated for a DB-Wax GC column length of 7-10 meters which is operated as described above. Other operating conditions may be required according to the specifications of alternative instrumentation and/or laboratory variables (including GC column length, supplier, etc.). For example, oxygen may not be required for operation of other types of FPD instrumentation and is not considered essential for the above applications.

Alternative operating conditions must, of course, produce adequate performance (i.e., reproducible retention times, sensitivity, and component resolution). However, the use of capillary GC column lengths > 15 meters is not recommended.

Representative chromatograms are included in Appendices I through X.

8.0 Determination by Gas Chromatography: (Continued)

8.3 GC/FPD Calibration:

The GC/FPD response is calibrated as follows using the GC/FPD operating conditions outlined in Section 8.0.

Determine the GC/FPD response in peak height units for HOE-064706 (parent compound derivative) and HOE-070951 (metabolite compound derivative) for a series of analytical standards prepared as described in Section 6.1. The lowest level analytical standard must correspond to a sample residue level of at least 0.04 ppm. In addition, the detector response of this standard must have a signal to noise ratio >3 (also, peak height >10mm if a chart recorder is used).

For example, assuming a 25.0 g sample ($V_1 = 200$ mL), a sample aliquot of 20.0 mL (V_2), a final volume (V_3) of 5.0 mL, and a 5 μ L injection volume, successful GC/FPD analysis of a 0.025 ng/ μ L standard is required to approximate 0.05 ppm of test compound. [All concentration levels are expressed as HOE-035956 equivalents.]

For verification of stable GC/FPD response, construct a standard curve which includes the levels of interest. [See Note below]. Typical calibration curves are shown in Figures 2 and 3 for HOE-064706 and HOE-070951, respectively. Every 2 to 3 sample injections within an analytical sequence are to be bracketed with an analytical standard. This practice serves as an ongoing "quality control" check of detector sensitivity/drift and component retention time (column) stability.

Residue results must not be determined by extrapolation of calibration data outside of the concentration range of the analyzed test standards.

[Note: Before standards and/or samples are analyzed, three or more sample injections into the GC/FPD are required. These preliminary "analyses" are carried out to condition the system (e.g., to mask active sites). Instrument response must be stabilized such that the GC/FPD peak heights from replicate injections agree to within $\pm 10\%$ relative].

8.4 Sample Analysis:

Inject an appropriate aliquot (2-5 μ L) of the prepared sample as obtained from Section 7.6. Note the injection volume (V_{inj}). For convenience, the use of equivalent injection volumes for standards and samples is recommended.

8.0 Determination by Gas Chromatography (Continued):8.4 Sample Analysis (Continued):

Measure the peak heights obtained for both HOE-064706 and HOE-070951 in the chromatogram. Compare the component peak height value with the test substance amount found on the corresponding calibration curve. Both samples and standards must be analyzed under stabilized GC conditions and within the same time frame (analytical sequence).

For calculation purposes, express the amount of test substance found as nanograms per microliter injected, ng/uL (Mt). This is equivalent to: (total nanograms found)/Vinj.

9.0 Calculation of the Residue:

Following the GC/FPD analysis described in Section 8.4, determine the residue of test compound in the sample:

$$\text{PPM of HOE-035956 (free acid) equivalents} = \frac{V1 \times V3 \times Mt}{Ws \times V2}$$

where:

V1 = initial total volume from batch extraction (mL)

V2 = sample aliquot from batch extraction (mL)

V3 = final volume for GC analysis (mL)

Ws = initial sample weight (g)

Mt = amount of test compound from GC/FPD analysis (ng/uL), expressed as HOE-035956 equivalents.

9.0 Calculation of the Residue (Continued):

Using Apple Fruit as an example:

for HOE-064706:

$$\begin{aligned} \text{PPM of HOE-035956 equivalents} &= \frac{200 \text{ mL} \times 5.0 \text{ mL} \times Mt}{25.0 \text{ g} \times 20.0 \text{ mL}} \\ &= 2.0 \times Mt \end{aligned}$$

for HOE-070951:

$$\begin{aligned} \text{PPM of HOE-035956 equivalents} &= \frac{200 \text{ mL} \times 5.0 \text{ mL} \times Mt}{25.0 \text{ g} \times 20.0 \text{ mL}} \\ &= 2.0 \times Mt \end{aligned}$$

10.0 Quality Assurance Procedures:

10.1 Laboratory Fortifications:

To assure the quality of the laboratory data, laboratory fortifications 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 fortifications. Spiking of quality control samples should be directly onto the analytical sample prior to extraction. Preparation of fortification standards are described in Section 6.2. Spiking volumes may range from 500 uL to 2 mL. The concentration of the spiking solution should be adjusted accordingly.

For example, 1.0 mL of Stock Solution G (2.5 ug/mL HOE-035956 equivalents) added to 25.0 g of (control) sample corresponds to 0.10 ppm of HOE-035956 (free acid) equivalents in the form of each test compound, HOE-039866 and HOE-061517.

10.2 Sample Storage:

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

11.0 Confirmatory Techniques:

At least two sources of information provide confidence in the identification the GC/FPD peaks assigned to the derivatized parent and metabolite test compounds. First, analysis crosschecks carried out using four different GC columns provided results consistent with the assigned GC peaks. (1)

Secondly, capillary GC/MS analysis yields characteristic EI mass spectra for both the derivatized parent and metabolite compounds. The respective capillary GC/MS retention times of the verified test compounds are also in line with GC/FPD chromatography retention times. In addition, GC/MS operation in the Selected Ion Monitoring mode provides the opportunity for verifying characteristic relative mass abundance ratios using the following ions: m/z 149 and 165 (for HOE-070951) and m/z 150 and 192 (for HOE-064706). A Hewlett Packard 5890A GC interfaced to a Model 5970 Mass Selective Detector has been utilized successfully for such confirmatory analytics. (4)

12.0 Modifications and Potential Problem Areas:

Derivatization:

Variable and/or low derivatization yields may be the result of spurious water contamination of the derivatization mixture. All glassware used to carry out the derivatization reaction must be dry. It is helpful to connect a drying tube (e.g., filled with anhydrous CaCl_2) to the top of the condenser to prevent the possible build-up of water condensate within the condenser.

Chromatography:

Because analyses are carried out in the presence of some residual matrix components, a gradual deterioration of GC column performance may be experienced. For wide bore capillary GC columns, performance may often be restored by breaking off and discarding the first six to twelve inches of column from the inlet end.

13.0 Safety:

Derivatization reactions (Section 7.4) must be carried out in a fume hood or high efficiency condensers must be used (to prevent escape of reagent fumes into the laboratory).

14.0 Results and Discussion:

The methodology described above determines residues of HOE-039866 (parent compound) and HOE-061517 (metabolite compound) in a number of biological matrices. For purposes of residue analysis, a limit of detection of 0.05 ppm must be demonstrated for these test substances in all matrices except almond hull (0.5 ppm). Furthermore, the GC/FPD signals which provide the quantitative measurement at this detection limit must be greater than 10 mm (in chart recorder peak height) and/or have a signal-to-noise ratio > 3 . These criteria are generally attainable using the commercially available instrumentation and techniques/test procedures described in the above report. To further compensate for specific laboratory (instrumentation) characteristics, procedural flexibilities are included which provide opportunities to overcome potential sensitivity limitations. These include adjustment of the final analytical sample volume, GC injection volume, and alternative silica gel clean-up procedure.

14.0 Results and Discussion (Continued):

A summary of experimental method recovery values for a variety of test samples is provided in Table II. Typical recoveries fall within the benchmark recovery range of 70 to 120% of the fortified test substance.

Because of the need for water extraction of the highly polar residue compounds, "complete" sample clean-up appears beyond the scope of rugged, straightforward methodology. It is important that the GC/FPD analyses be carried out with this potential limitation in mind. For example, preconditioning of the GC system with injected sample solutions is an essential element of the analytical GC/FPD determination (see also Ref. 2).

To test ruggedness, the methodology as described above was carried out without anion exchange clean up for apple samples by two independent, non-affiliated laboratories. These laboratories had no previous experience with the residue methodology and worked without HRAV supervision. Thus, simulated MTO activities were performed. Results of the method trials are included in Reference 1. Overall combined average recoveries as obtained by both independent laboratories were 96% (n = 36) and 85% (n = 36) for parent and metabolite compounds, respectively.

Representative chromatograms of both control matrices and corresponding fortified control matrices are provided in Appendices I through X. As noted in Section 8.0, optimized GC operating conditions may vary according to the individual performing laboratory. However, these chromatograms illustrate actual GC/FPD results obtained using the basic methodology described in Section 8.0 of this report.

15.0 References

1. HRAV-5 Analytical Method, submitted to EPA, MRID No.: 40501005
2. Froberg, J. E. and Doose, G. M. (1986) In "Analytical Methods for Pesticides and Plant Growth Regulators, Volume XIV", (G. Zweig and J. Sherma. eds.), pp55-56, Academic Press, Inc., New York.
3. Cretney, W. J., McLaughlin, F. A., and Fowler, B. R., Journal of High Resolution Chromatography and Chromatography Communications, 10, (1987) 428.
4. Hoechst Aktiengesellschaft, Analytical Laboratory, 6230 Frankfurt M 80, West Germany.

TABLE II
SUMMARY OF EXPERIMENTAL METHOD RECOVERY VALUES

<u>SAMPLE MATRIX</u>	<u>PERCENT RECOVERY</u>	
	<u>PARENT COMPOUND</u> <u>(HOE-039866)</u>	<u>METABOLITE COMPOUND</u> <u>(HOE-061517)</u>
APPLES	93 ± 9 (n=5)	84 ± 5 (n=5)
GRAPES	90 ± 8 (n=12)	78 ± 4 (n=12)
ALMOND NUTMEAT	81 ± 7 (n=9)	88 ± 9 (n=9)
ALMOND HULLS	84 (n=2)	82 (n=2)
WALNUT MEAT	91 ± 9 (n=7)	90 ± 7 (n=7)
CORN FODDER	82 ± 4 (n=3)	98 ± 4 (n=3)
CORN FORAGE	85 ± 2 (n=4)	85 ± 3 (n=4)
CORN GRAIN	98 ± 6 (n=6)	94 ± 6 (n=6)
SOYBEAN SEED	97 ± 7 (n=12)	97 ± 7 (n=12)
PECAN NUTMEAT	94 ± 6 (n=18)	98 ± 10 (n=18)

NOTES

1. For all samples except almond hulls, fortification levels ranged from 0.05 to 0.2 ppm of each compound (as HOE-035956 equivalents). For almond hulls, fortification levels were 0.5 ppm of both parent compound and metabolite compound (as HOE-035956 equivalents). In this case, samples for analysis were diluted by a factor of 5 prior to GC injection.
2. PERFORMING LABORATORY: Hoechst-Roussel Agri-Vet Company, Analytical Chemistry Laboratory, Rt. 202-206, Somerville, NJ 08876-1258.

FIGURE 1

ILLUSTRATION OF SILICA GEL COLUMN LOADING

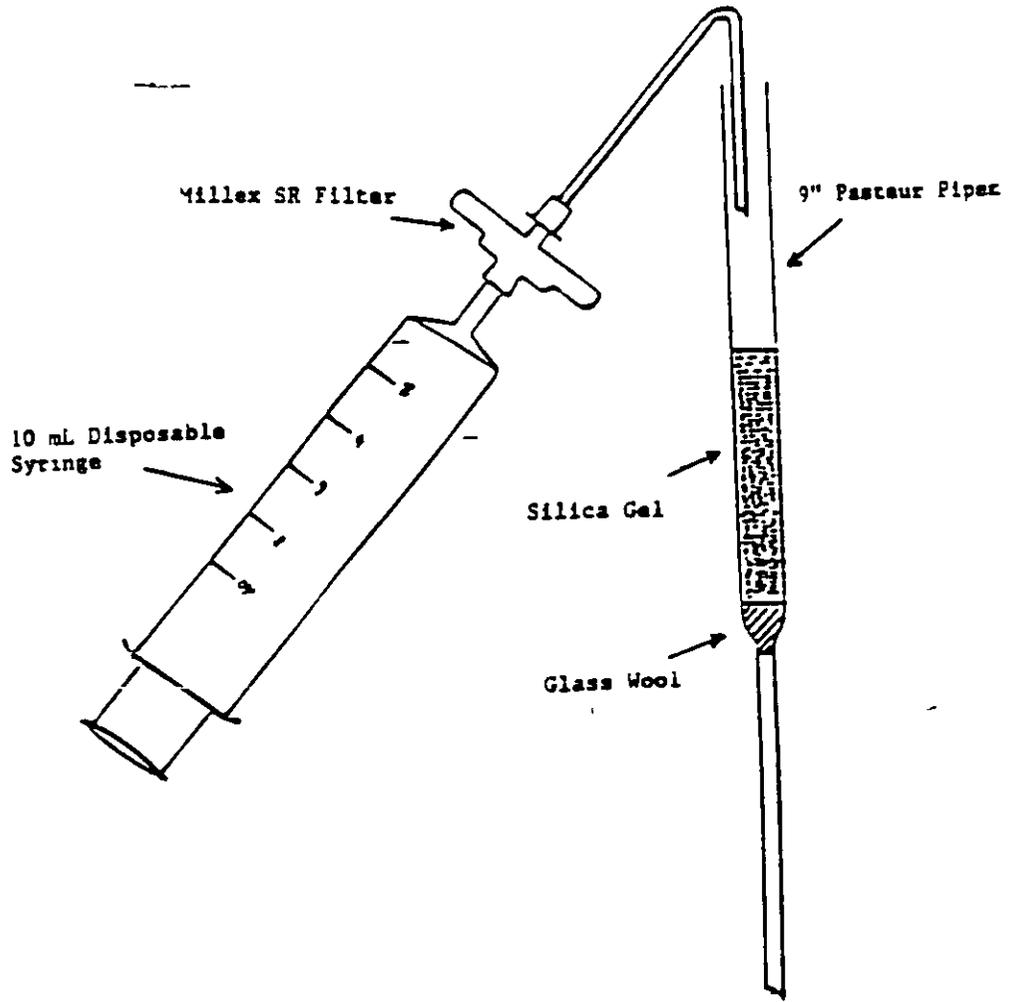


FIGURE 2

GC/FPD Calibration Curve

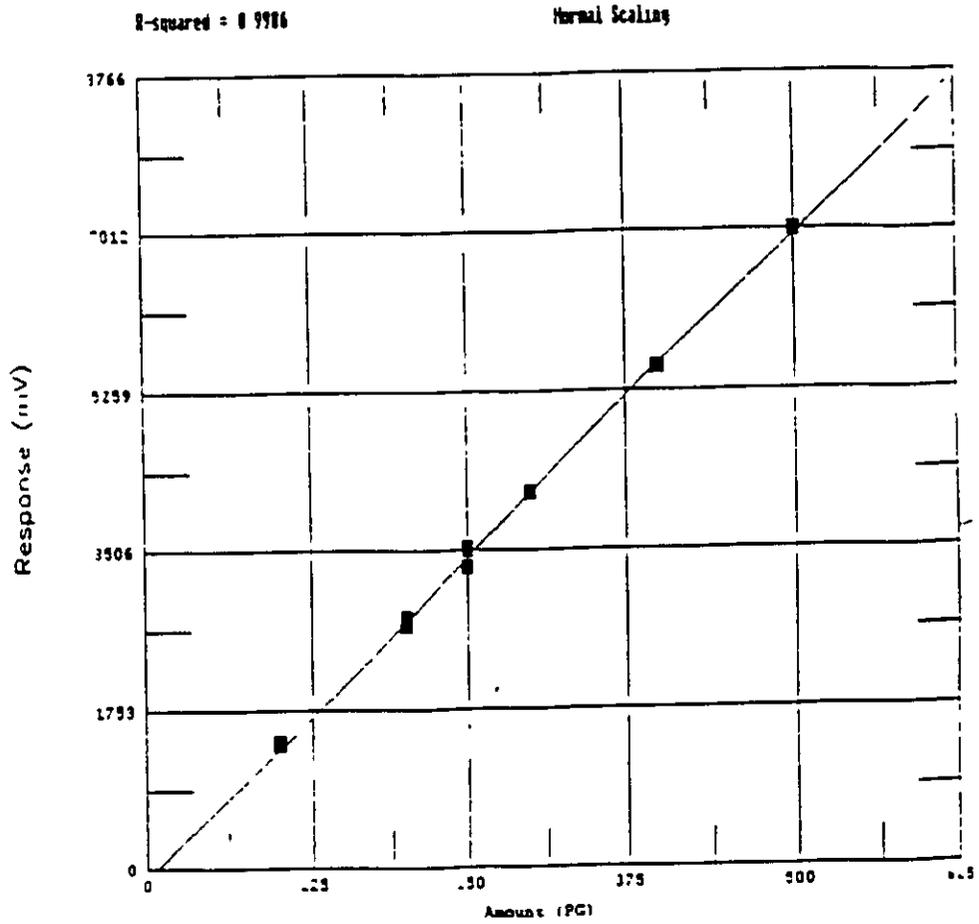
Sample: Grapes

Component: HOE-064706 (derivative of HOE-039866)

Sample File - c:\2700\data\GPE.SMP

First Order Fit of HOE-064706

c0 = -98.238
c1 = 14.119



DK-8

FIGURE 3

GC/FPD Calibration Curve

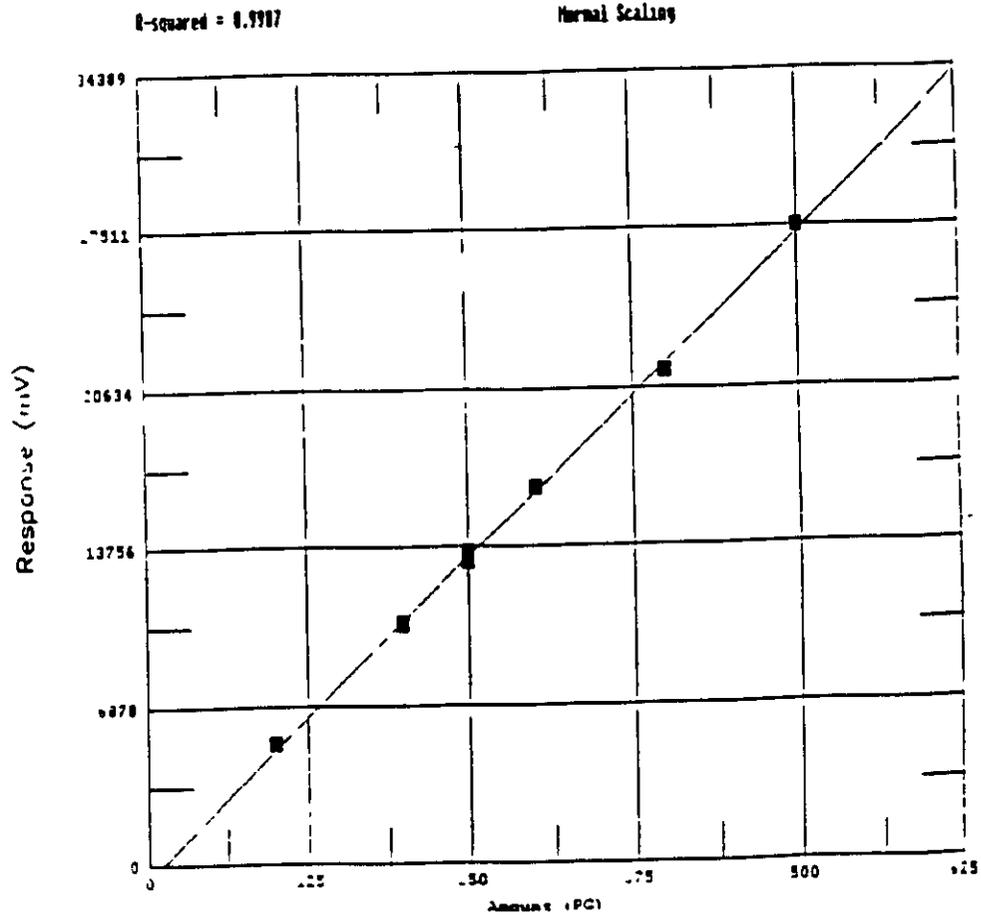
Samples: Grapes

Component: HDE-070951 (derivative of HDE-061517)

Sample file - c:\2700\data\AQPE.SMP

First Order Fit of HDE-070951

c0 = -503.309
c1 = 55.673



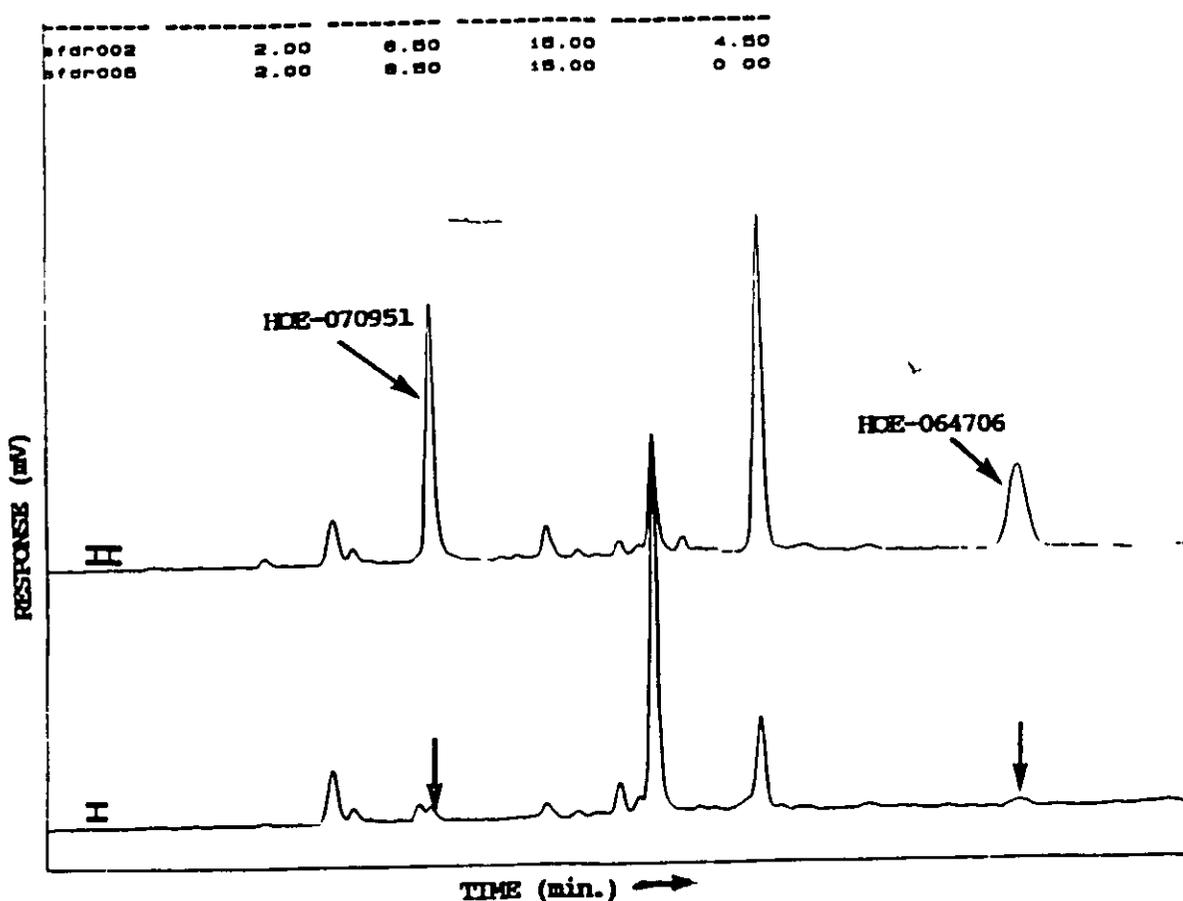
DK-9

APPENDIX I

SAMPLE MATRIX: CORN FODDER

CHROMATOGRAM I: CONTROL SAMPLE

CHROMATOGRAM II: FORTIFIED SAMPLE
(0.05 PPM OF EACH COMPOUND, AS
HOE-035956 EQUIVALENTS)



INJECTION VOLUME: 5 μ L (corresponds to 2.5mg of sample)

TIME AXIS: 2.0 to 8.5 minutes

RESPONSE AXIS: 15 mV full scale

RECOVERY (%) : Hoe-070951 = 103%
Hoe-064706 = 86%

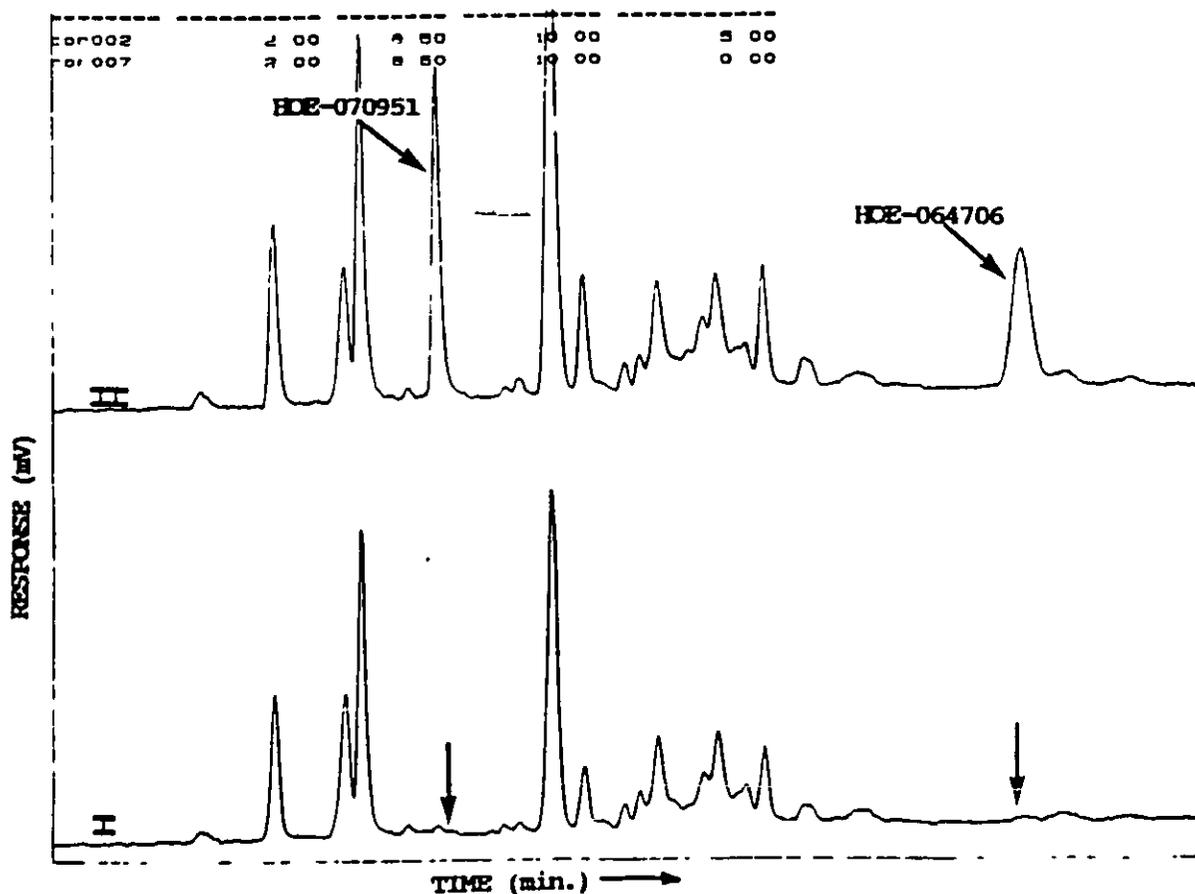
DK-356

APPENDIX II

SAMPLE MATRIX: CORN GRAIN

CHROMATOGRAM I: CONTROL SAMPLE

CHROMATOGRAM II: FORTIFIED SAMPLE
(0.05 PPM OF EACH COMPOUND, AS
HOE-035956 EQUIVALENTS)



INJECTION VOLUME: 5uL (corresponds to 2.5mg of sample)

TIME AXIS: 2.0 to 8.5 minutes

RESPONSE AXIS: 15 mV full scale

RECOVERY (%) : Hoe-070951 = 105%
Hoe-064706 = 106%

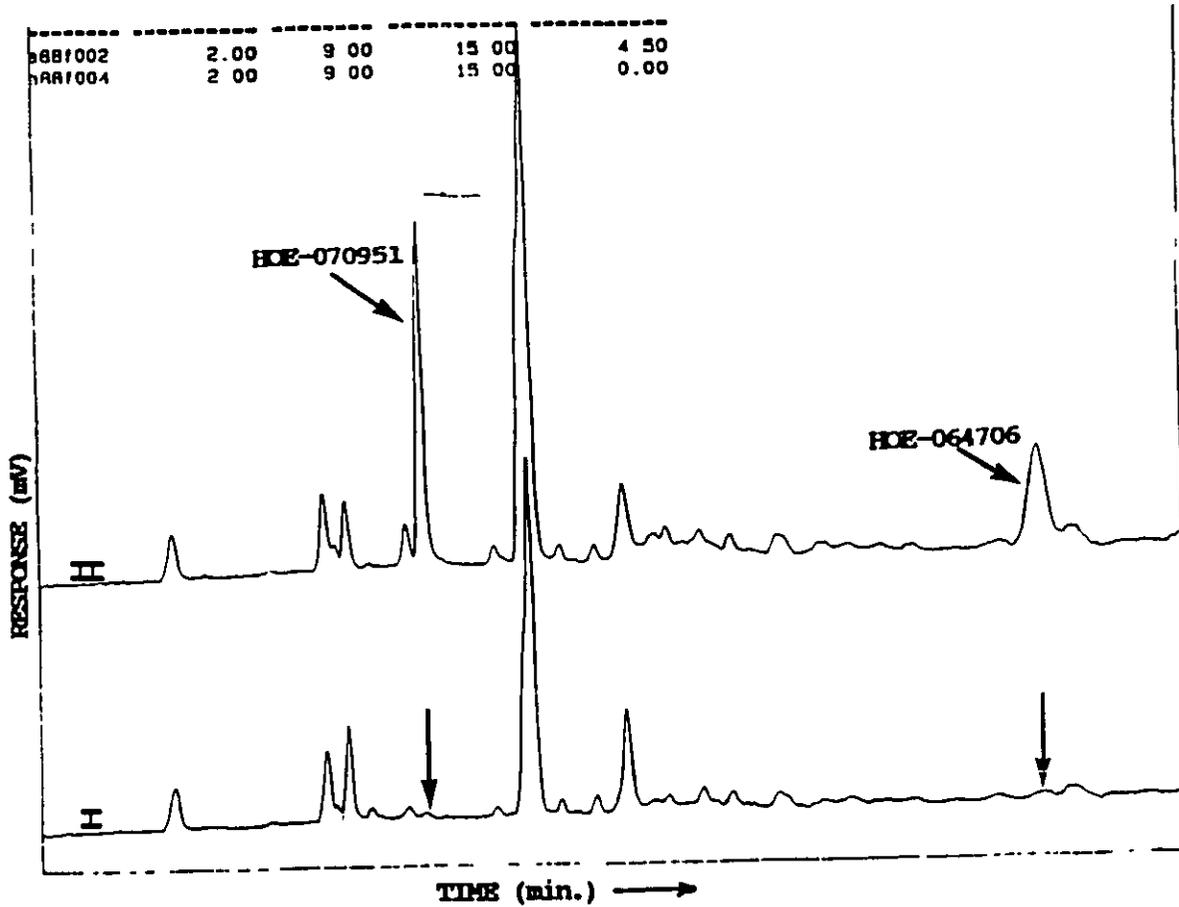
DK-357

APPENDIX III

SAMPLE MATRIX: CORN FORAGE

CHROMATOGRAM I: CONTROL SAMPLE

CHROMATOGRAM II: FORTIFIED SAMPLE
(0.05 PPM OF EACH COMPOUND, AS
HOE-035956 EQUIVALENTS)



INJECTION VOLUME: 5uL (corresponds to 2.5mg of sample)

TIME AXIS: 2.0 to 9.0 minutes

RESPONSE AXIS: 15 mV full scale

RECOVERY (%) : Hoe-070951 = 87%
Hoe-064706 = 87%

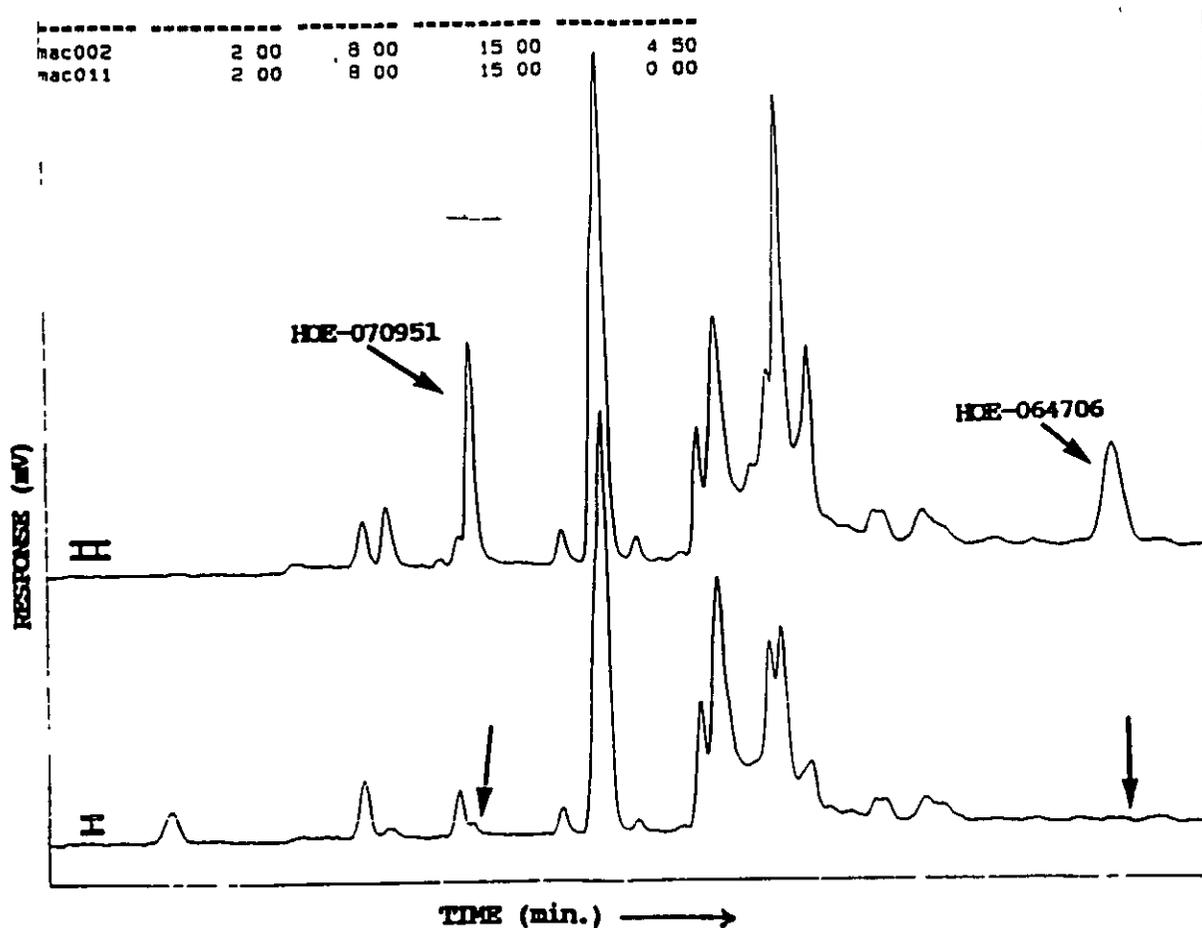
DK-358

APPENDIX IV

SAMPLE MATRIX: PECANS

CHROMATOGRAM I: CONTROL SAMPLE

CHROMATOGRAM II: FORTIFIED SAMPLE
.05 PPM OF EACH COMPOUND, AS
HOE-035956 EQUIVALENTS)



INJECTION VOLUME: 5uL (corresponds to 2.5mg of sample)

TIME AXIS: 2.0 to 8.0 minutes

RESPONSE AXIS: 15 mV full scale

RECOVERY (%) : Hoe-070951 = 84%
Hoe-064706 = 100%

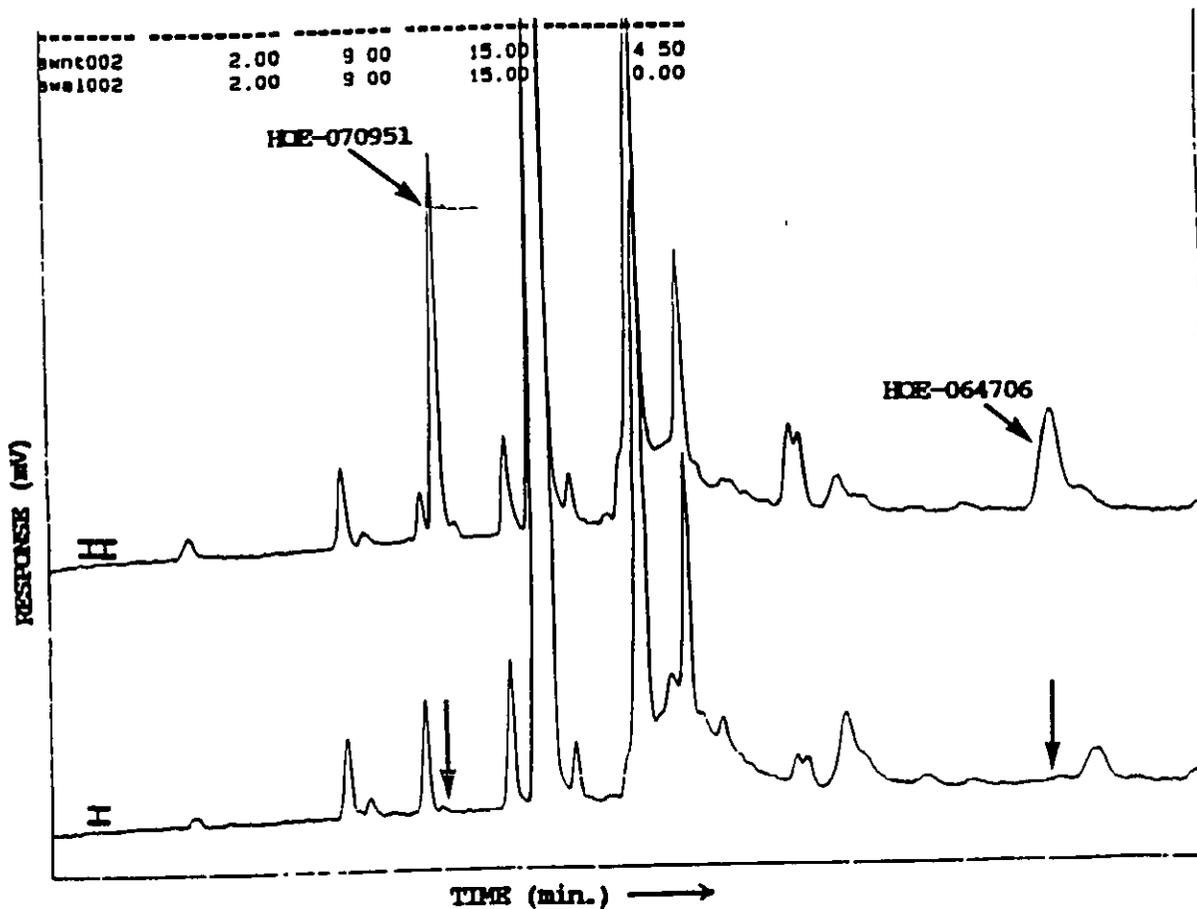
DK-359

APPENDIX V

SAMPLE MATRIX: WALNUTS

CHROMATOGRAM I. CONTROL SAMPLE

CHROMATOGRAM II: FORTIFIED SAMPLE
(0.05 PPM OF EACH COMPOUND, AS
HOE-035956 EQUIVALENTS)



INJECTION VOLUME: 5 μ L (corresponds to 2.5mg of sample)

TIME AXIS: 2.0 to 9.0 minutes

RESPONSE AXIS: 15 mV full scale

RECOVERY (%) . Hoe-070951 = 94%
Hoe-064706 = 98%

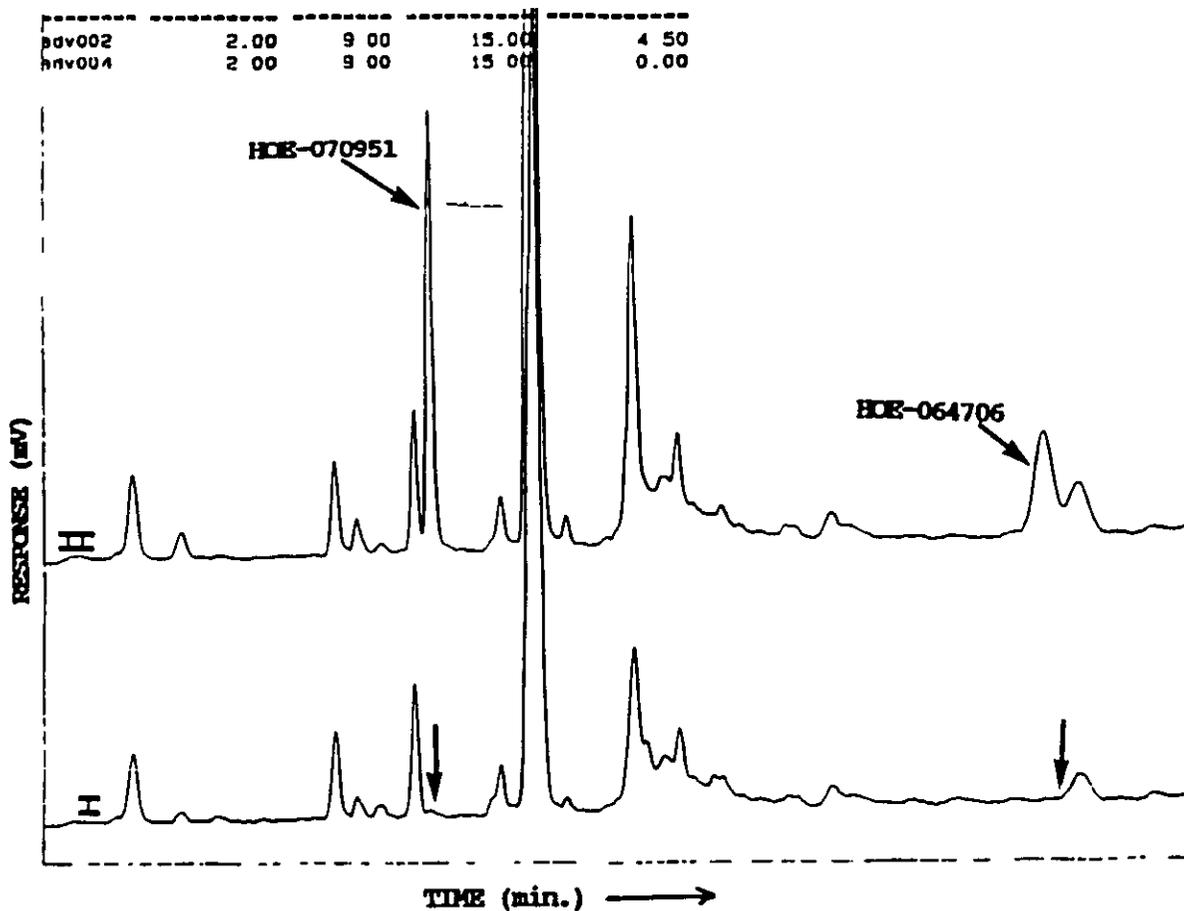
DK-360

APPENDIX VI

SAMPLE MATRIX: ALMOND MEAT

CHROMATOGRAM I: CONTROL SAMPLE

CHROMATOGRAM II. FORTIFIED SAMPLE
(0.05 PPM OF EACH COMPOUND, AS
HOE-035956 EQUIVALENTS)



INJECTION VOLUME: 5uL (corresponds to 2.5mg of sample)

TIME AXIS: 2.0 to 9.0 minutes

RESPONSE AXIS: 15 mV full scale

RECOVERY (%) · Hoe-070951 = 95%
Hoe-064706 = 83%

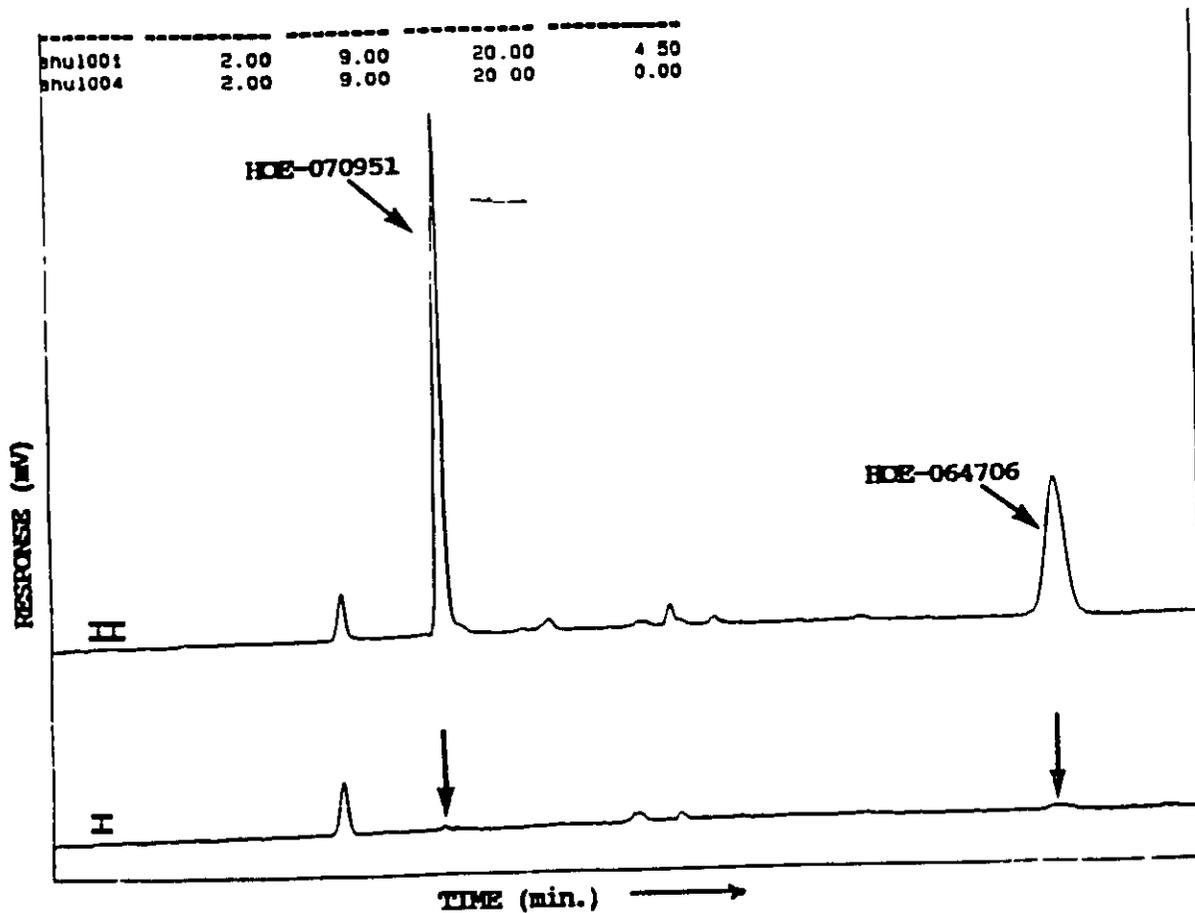
DK-361

APPENDIX VII

SAMPLE MATRIX. ALMOND HULLS

CHROMATOGRAM I CONTROL SAMPLE

CHROMATOGRAM II: FORTIFIED SAMPLE
(0.5 PPM OF EACH COMPOUND, DILUTED
5-FOLD BEFORE INJECTION, AS Hoe-035956
EQUIVALENTS)



INJECTION VOLUME: 5 μ L (corresponds to 2.5mg of sample)

TIME AXIS: 2.0 to 9.0 minutes

RESPONSE AXIS: 15 mV full scale

RECOVERY (%) : Hoe-070951 = 80%
Hoe-064706 = 84%

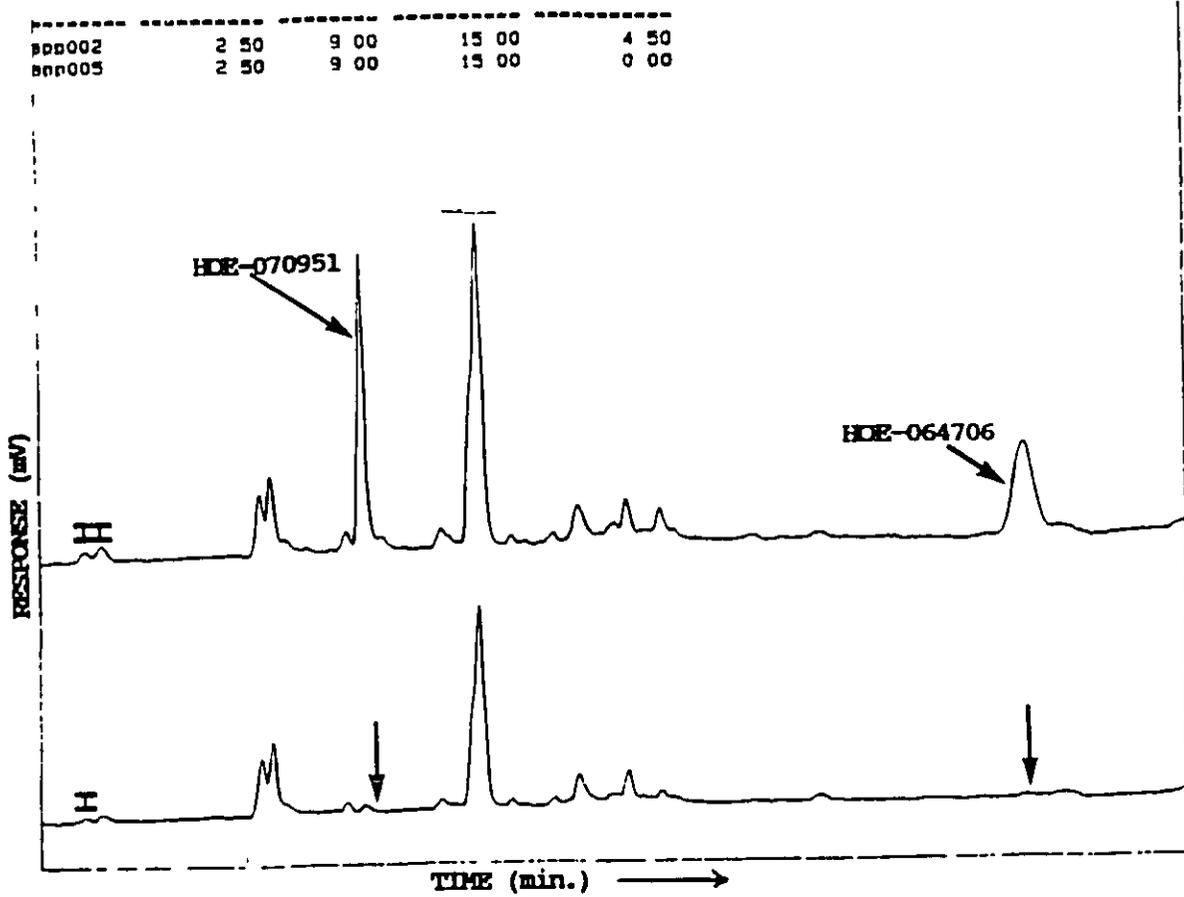
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APPENDIX VIII

SAMPLE MATRIX. APPLES

CHROMATOGRAM I. CONTROL SAMPLE

CHROMATOGRAM II: FORTIFIED SAMPLE
(0.05 PPM OF EACH COMPOUND, AS
HOE-035956 EQUIVALENTS)



INJECTION VOLUME: 5uL (corresponds to 2.5mg of sample)

TIME AXIS: 2.5 to 9.0 minutes

RESPONSE AXIS: 15 mV full scale

RECOVERY (%) : Hoe-070951 = 86Z
Hoe-064706 = 105Z

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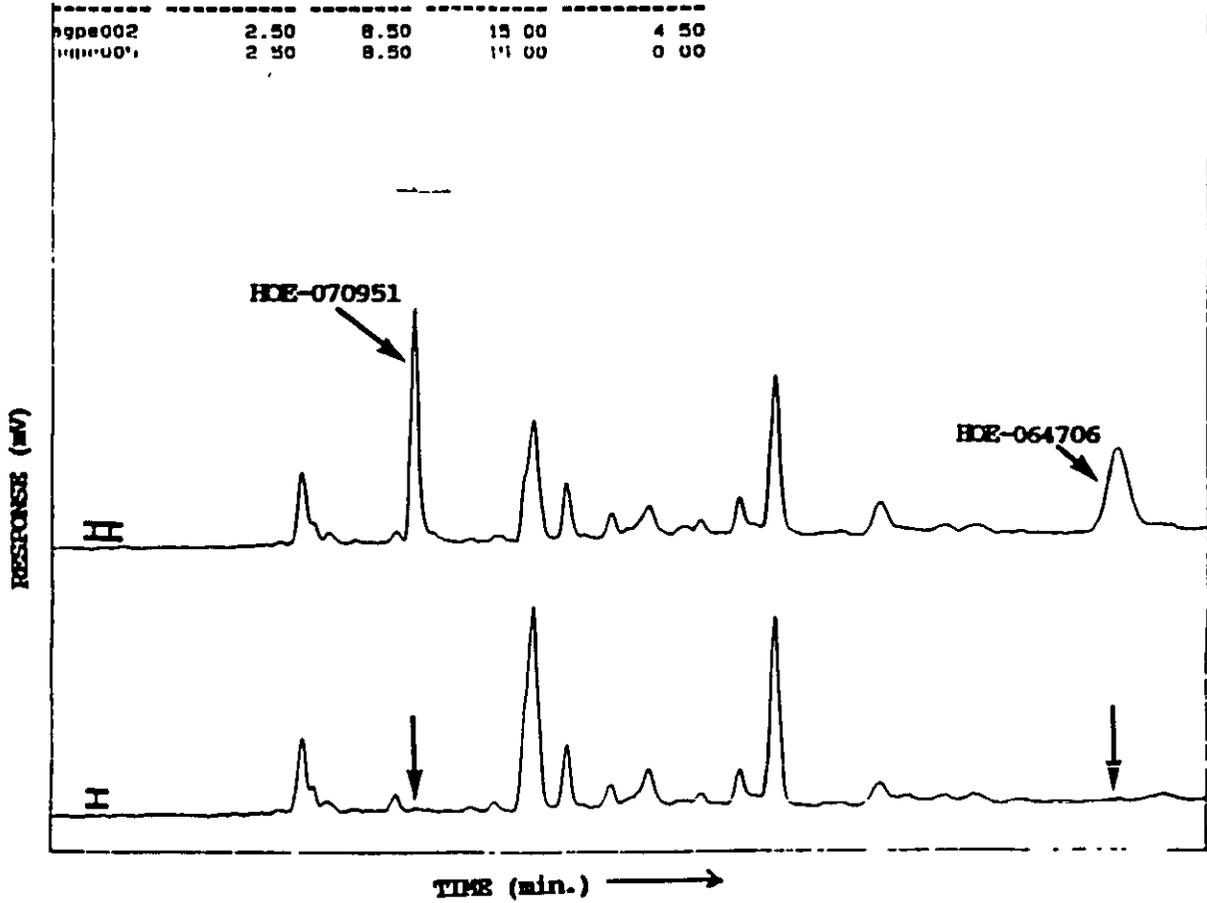
44

APPENDIX IX

SAMPLE MATRIX: GRAPES

CHROMATOGRAM I: CONTROL SAMPLE

CHROMATOGRAM II: FORTIFIED SAMPLE
(0.05 PPM OF EACH COMPOUND, AS
HOE-035956 EQUIVALENTS)



INJECTION VOLUME: 5uL (corresponds to 2.22mg of sample)

TIME AXIS: 2.5 to 8.5 minutes

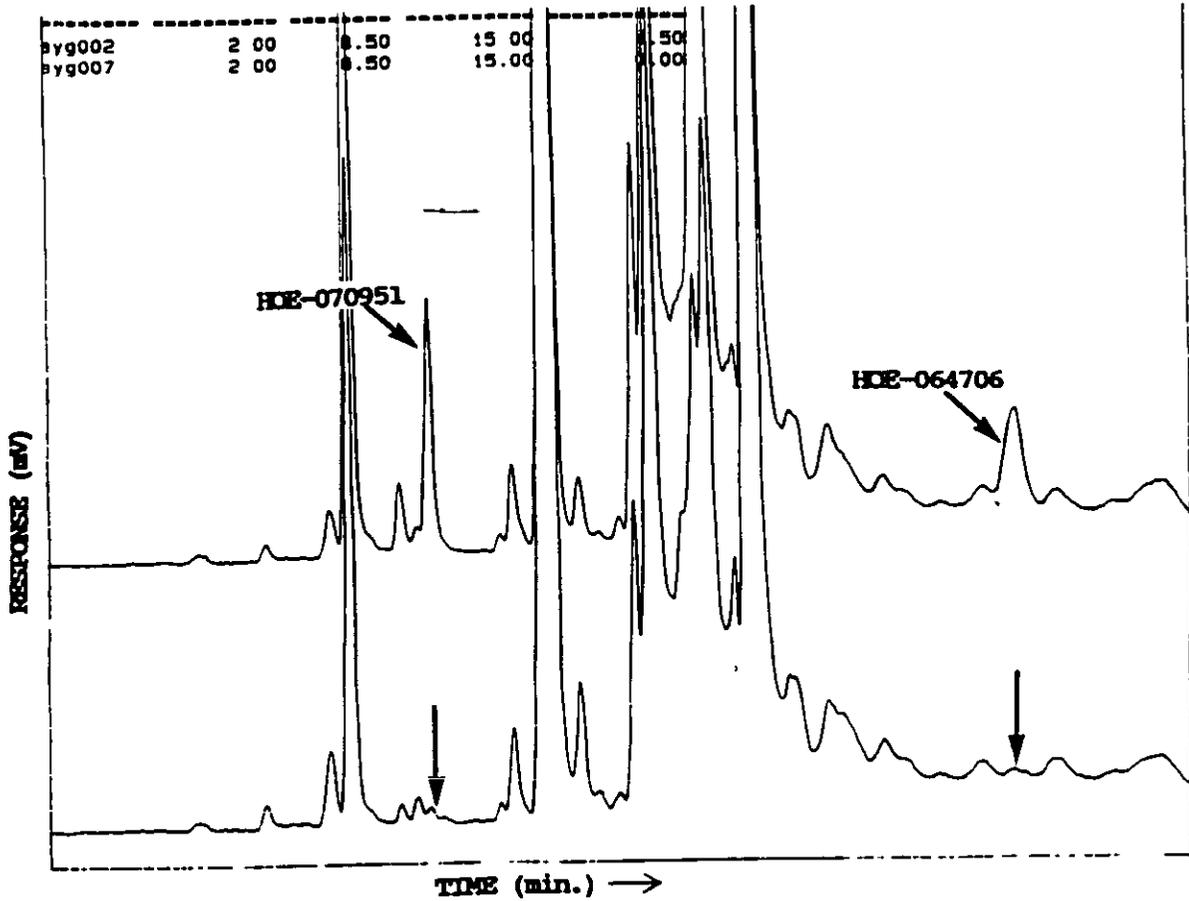
RESPONSE AXIS: 15 mV full scale

RECOVERY (%) : Hoe-070951 = 77%
Hoe-064706 = 98%

DK-364

APPENDIX X

SAMPLE MATRIX. SOYBEAN
CHROMATOGRAM I. CONTROL SAMPLE
CHROMATOGRAM II. FORTIFIED SAMPLE
(0.05 PPM OF EACH COMPOUND, AS
HOE-035956 EQUIVALENTS)



INJECTION VOLUME: 5uL (corresponds to 2.5mg of sample)

TIME AXIS: 2.0 to 8.5 minutes

RESPONSE AXIS: 15 mV full scale

RECOVERY (%) : Hoe-070951 = 107%
Hoe-064706 = 103%

DK-365