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ATTACHMENT 2

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106406

Study Title

An Analytical Method for the Determination of FOE 5043 Residues in Plant Matrices

Data Requirement

EPA Ref: 171-4(c), Residue Analytical Method - Plants

Authors

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Completion Date

May 11, 1995

Perfonned by

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(formerly Miles Inc)
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Bayer Study Number

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Data Confidentiality Statement

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA #10(d)(1)(A), (B) or (C).

Company: Bayer Corporation
Agriculture Division
Research and Development Department
Environmental Research Section

Company Agent: D.R. Flint Date: 5/11/95
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These data are the property of the Agriculture Division of Bayer and, as such, are considered to be confidential for all purposes other than compliance with FIFRA 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any other country.

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Certification of Good Laboratory Practice

The study described in this document meets the requirements of 40 CFR Part 160. A quality assurance statement is presented on page 4 of this report.

Submitter: Bayer Corporation
Agriculture Division
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Date: 5/11/95

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Date: 5/11/95

Study Director:

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Senior Research Chemist

Date: 5/11/95

Certification of Availability of Raw Data

It is hereby certified that the registrant possesses or has access to the raw data identified in Appendix 1 of this report.

Company Agent:

T. J. Olson
Quality Assurance

Date: 5/11/95

106406

Quality Assurance Statement

Study Title: An Analytical Method for the Determination of FOE 5043 Residues in Plant Matrices.

Bayer Study Number: F3121601

Audits of this study were conducted as required by the Good Laboratory Practice regulations of FIFRA, Part 160, August 17, 1989. The audits are listed below.

Inspection Date	Phase Inspected	Date Reported to Study Director	Date Reported to Management
05/20/93	Protocol	05/27/93	05/28/93
05/24/93	Standard Hydrolysis	05/28/93	05/28/93
11/16/93	Sample Set Extraction, Recoveries	11/18/93	11/18/93
05/02/95	Final Report (Report No. 106406)	05/05/95	05/11/95

Based on the audits described above, it is concluded that the results presented in this report accurately describe the methods and standard procedures followed and reflect the raw data generated during the conduct of the study.

Company Agent: _____ Date: 5/11/95

T. J. Olson
Quality Assurance

Certification of Authenticity

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Revisions

(This page will be used to indicate any modifications of the analytical method made after the original completion date.)

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An Analytical Method for the Determination of FOE 5043 Residues in Plant Matrices

1.0 Summary

An analytical method was developed to measure the residues of FOE 5043 and its metabolites in crops and processed products. The residues were briefly oxidized with potassium permanganate for 5 min or monoperoxyphthalic acid (magnesium salt) for 30 min and hydrolyzed to 4-fluoro-*N*-methylethyl benzenamine (fluoroaniline) by digesting the crop mixture with 47% sulfuric acid for 24 hours. The fluoroaniline was separated from the crop matrix by steam distillation after making the crop digest basic with 50% sodium hydroxide. The fluoroaniline was extracted from the steam distillate and derivatized. The derivative, 4-fluoro-*N*-methylethyl benzenamine trifluoroacetamide (trifluoroacetamide derivative), was measured by gas chromatography/mass spectroscopy-selected ion monitoring (gc/ms-sim). Recoveries from various crops fortified at 0.1 ppm with FOE 5043 or its plant metabolites ranged from 70% to 114%. Recoveries from various crops fortified at 0.05 ppm with FOE 5043 or its plant metabolites ranged from 65% to 91%.

2.0 Introduction

The plant metabolism of FOE 5043, a preemergent herbicide, was well defined by studies conducted with corn[1] and soybeans.[2] No parent compound was detected in the mature crop matrices. The plants contained three major metabolites, FOE oxalate, FOE sulfonic acid, and FOE thioglycolate sulfoxide, and two minor metabolites, FOE methyl sulfoxide and FOE methyl sulfone. Corn matrices also contained a fourth major metabolite, FOE thiolactate sulfoxide. The metabolic pathway of FOE 5043 in plants is delineated in Figure 1. The distribution of metabolites in corn and soybean matrices is listed in Table 1.

Propachlor and alachlor, herbicides which are structurally similar to FOE 5043, have been registered by the EPA. Analytical residue methods[3,4] were published for these compounds in the Pesticide Analytical Manual Vol. II. In these methods, the parent herbicide and metabolites were hydrolyzed to a common moiety. The common moiety was steam distilled from the hydrolysis mixture and measured by gas chromatography (gc). Conversion of metabolites to a common chemical moiety is a recommended analysis method in the Pesticide Assessment Guidelines.[5]

A similar method, with appropriate modifications, was developed for the analysis of FOE 5043 residues and is presented in this report.

3.0 Experimental

3.0.1 Location

This study was conducted between May, 1993 and May, 1995 at the Bayer Research Park near Stilwell, KS. Raw data and the final report are archived at Bayer, Kansas City, MO.

3.1 Materials

3.1.1 Apparatus

Assorted clamps and clamp holders.

Assorted laboratory glassware (including, but not limited to)
- 470 mm Allihn condenser with 24/40 ground glass joints (Kontes Glass Co., Vineland, NJ, #431000-2430 or equivalent).
- 1000-ml flat bottom flask with a 24/40 ground glass joint.
- 500-ml flat bottom flask with a 24/40 ground glass joint.
- 13 ml graduated centrifuge tubes (Kontes #410550-0013 or equivalent).
- 5-ml graduated centrifuge tube (Kontes #410550-0005 or equivalent).
- 500-ml separatory funnel.
- Short path distillation head with 24/40 ground glass joints (Kontes #513750-0000 or equivalent) and a ground glass stopper to fit the top of the distillation head and Teflon sleeves to fit 24/40 ground glass joints (Aldrich Chemical Company, Inc. Milwaukee, WI, #Z10,488-4 or equivalent).
- 35 to 50 mm Teflon jacketed magnetic stirring bar.
- Volumetric pipets and flasks.

Autosampler vials and septa (to fit the autosampler of the gc/ms).

Gas chromatograph/mass spectrometer (Hewlett Packard Company, Wilmington, DE, model HP 5890, HP 5995, or equivalent) capable of capillary column chromatography and equipped with an autosampler, a mass selective detector with appropriate data collection hardware and software, and a fused silica capillary column: 0.20 mm i.d. x 12 m, methyl silicone, 0.33 µm film thickness (Hewlett Packard, Ultra-1 or equivalent).

Gastight microliter syringes, 100 µl, 250 µl, and 500 µl (Hamilton, Inc. Reno, NV, #1700 or equivalent).

Ice bucket, about 4 liter capacity.

N-EVAP analytical evaporator (Organomation Associates Inc., South Berlin, MA, Model N-EVAP or equivalent).

Stirrer/hot plate (Corning Inc., Corning, NY, model PC-351, PC-320, or equivalent).

Vacuum manifold for processing solid phase extraction cartridges (J. T. Baker Inc., Philipsburg, NJ, Baker spe-12 or equivalent).

Waring Laboratory Blendor and 1-liter Blendor jar (Waring Products Division, New York, NY, model 700G or equivalent).

3.1.2 Reagents/Supplies

Antifoam A (Dow Corning, Midland, MI) or equivalent (ie. Antifoam A Concentrate, Sigma #A 5633).

Crushed ice.

Deionized water.

4-Dimethylaminopyridine (DMAP), 99+% (Aldrich, #33,245-3 or equivalent).

Glass wool.

Granular anhydrous sodium sulfate (Mallinckrodt Speciality Chemicals Co., Paris, KN, AR grade, #8024 or equivalent).

Hydrochloric acid, 37% aqueous solution (Mallinckrodt, AR grade, #2062, or equivalent).

Monoperoxyphthalic acid, magnesium salt (MMPP, technical grade, Aldrich #28,320-7 or equivalent).

Octadecyl solid phase extraction cartridges, 3.0 ml volume, 0.50 g resin capacity (C-18 spe) (J. T. Baker, #7020-03 or equivalent).

Potassium permanganate, A.C.S. reagent grade (J. T. Baker #3227-01 or equivalent).

Pyridine, A.C.S. reagent grade (Aldrich #36,057-0 or equivalent).

Sodium bisulfite, A.C.S. reagent grade (Mallinckrodt, AR grade, #7448 or equivalent).

Sodium hydroxide, 50% (w/w) aqueous solution (Fisher Scientific, Fair Lawn, NJ, #SS254-1 or solution equivalent to 19*M*).

Solvents: methylene chloride, dimethylformamide, methyl *tert*-butyl ether, and acetonitrile (Burdick and Jackson Division, Baxter Healthcare Corporation, Muskegon, MI, pesticide grade).

96% Sulfuric acid, (Mallinckrodt, AR grade, #2468 or equivalent)

Sulfuric acid, 1 *N* solution in water.

Trifluoroacetic anhydride (TFAA), 99.9% (Aldrich, #10,623-2 or equivalent). Caution: This reagent is toxic and very hygroscopic. Buy the reagent in small quantities and use within 2 months of opening the bottle. Handle with care in an adequate fume hood to protect the analyst.

3.1.3 Standards Required

The analytical standard 4-fluoro-*N*-methylethyl benzenamine trifluoroacetamide may be obtained from Bayer Corporation, Agriculture Division, Environmental Research, Bayer Research Park, 17745 S. Metcalf Ave., Stilwell, KS 66085. Alternatively, the standard may be prepared as outlined in Appendix 2. Analytical standards of FOE 5043, FOE oxalate, FOE sulfonic acid, sodium salt, monohydrate, and FOE thioglycolate sulfoxide may be obtained from Bayer.

3.1.3.1 Trifluoroacetamide Derivative Standards

Primary Standard: Using a balance accurate to 0.1 mg, weigh 0.0171 g of 4-fluoro-*N*-methylethyl benzenamine trifluoroacetamide into a 100-ml volumetric flask. Dilute the chemical to volume with methyl *tert*-butyl ether. This solution is equivalent to 250 ppm of FOE 5043 in a 10 gram sample after processing through the method.

Secondary Standards: Prepare additional solutions from the primary standard as follows:

(A) 25.0 ppm — Pipet 10.0 ml of the primary standard into a 100-ml volumetric flask, and dilute the solution to volume with methyl *tert*-butyl ether.

(B) 2.50 ppm — Pipet 1.00 ml of the primary standard into a 100-ml volumetric flask, and dilute the solution to volume with methyl *tert*-butyl ether.

(C) 1.00 ppm	Pipet 4.00 ml of the 25.0 ppm secondary standard into a 100-ml volumetric flask, and dilute the solution to volume with methyl *tert*-butyl ether.
(D) 0.50 ppm	Pipet 2.00 ml of the 25.0 ppm secondary standard into a 100-ml volumetric flask, and dilute the solution to volume with methyl *tert*-butyl ether.
(E) 0.25 ppm	Pipet 1.00 ml of the 25.0 ppm secondary standard into a 100-ml volumetric flask, and dilute the solution to volume with methyl *tert*-butyl ether.
(F) 0.10 ppm	Pipet 0.400 ml of the 25.0 ppm secondary standard into a 100-ml volumetric flask, and dilute the solution to volume with methyl *tert*-butyl ether.
(G) 0.05 ppm	Pipet 2.00 ml of the 2.50 ppm secondary standard into a 100-ml volumetric flask, and dilute the solution to volume with methyl *tert*-butyl ether.
(H) 0.025 ppm	Pipet 1.00 ml of the 25.0 ppm secondary standard into a 100-ml volumetric flask, and dilute the solution to volume with methyl *tert*-butyl ether.

Store the standards under refrigerated conditions (0±3°C); under these conditions the standard solutions are stable for at least 3 months.

3.1.3.2 Method Validation Standards

Primary Standards: Using a balance accurate to 0.1 mg, weigh 0.0100 g of FOE 5043 into a 10-ml volumetric flask. Dilute the chemical to volume with methanol.

Secondary Standards: Prepare a 5.00 µg/ml solution from the primary standard by pipeting 0.50 ml of primary standard into a 100-ml volumetric flask. Dilute the solution to volume with methanol.

Standard solutions of FOE oxalate; FOE sulfonic acid, sodium salt, monohydrate; and FOE thioglycolate sulfoxide should be prepared in the same manner.

Store the standards under refrigerated conditions (0±3°C); under these conditions, the standard solutions are stable for at least 3 months.

3.2 Analytical Method

3.2.1 General Instructions

3.2.1.1 Evaporations

All evaporations are done with an N-EVAP using a gentle stream of nitrogen and a room temperature (20±5°C) water bath. Remove the sample immediately after the solvent has evaporated.

3.2.1.2 Measurements

Unless otherwise indicated, all volumes should be measured with a graduated cylinder or a pipet, whichever is more convenient. Do not use micropipetors (ie. Rainen Pipetman). Unless otherwise indicated, weights should be measured on a balance capable of accuracy to 10 mg.

3.2.2 Detailed Procedure

3.2.2.1 Sample Preparation

3.2.2.1.1 Corn Forage, Corn Fodder, Corn Grain, Soybean Forage, Spinach Tops, Wheat Grain, or Wheat Straw

1. Add approximately 100 g of dry ice to a Waring Blendor jar. Add about an equal portion (100 g) of the raw agricultural commodity (RAC) to the jar in small portions. Blend the contents of the jar after each addition until a homogeneous mixture is obtained.

2. Pour the contents of the jar into doubled plastic bags, and store the open bags at -20±3°C until the last traces of dry ice have sublimed.

3. Seal and label the bags appropriately.

4. Maintain the homogenized RAC under freezer conditions, -20±3°C.

3.2.2.1.2 Peanut Nutmeat, Soybean Seed, Sunflower Seed, or Turnip Roots

1. Add approximately 100 g of the RAC to a Waring Blendor jar, and blend the contents until a homogeneous mixture is obtained.

2. Pour or scrape the contents of the jar into doubled plastic bags. Seal and label the bags appropriately.

3. Maintain the homogenized RAC under freezer conditions, -20±3°C.

3.2.2.1.3 Corn Oil, Soapstock, or other Processed Commodities

1. Maintain the processed RAC under freezer conditions, -20±3°C.

3.2.2.2 Extraction

1. Weigh 10.0 g of the frozen sample from 3.2.2.1 into a 1000-ml flat bottomed boiling flask. Use a balance accurate to 0.01 g.

   Note: Begin recovery samples at this point. Fortify control tissue matrix samples as described in 3.3.

2. Add a magnetic stirring bar and 75 ml of water to the flask. Stir the mixture for 1 hour.

3. Add 10 ml of 1.0 *N* sulfuric acid solution, and stir the mixture well for 1 to 2 min.

4a. For low moisture matrices, except corn oil, add 2.0 g of potassium permanganate to the flask, and mix the contents well, such that the entire mixture takes on a purple color from the permanganate. Stir the mixture for 5 min.

4b. For high moisture matrices (ie. soybean forage), add 1.0 g of potassium permanganate to the flask, and mix the contents well, such that the entire mixture takes on a purple color from the permanganate. Stir the mixture for 5 min.

4c. For corn oil, add 2.5 g of monoperoxyphthalic acid, magnesium salt to the flask, and mix the contents well. Stir the mixture 30 min.

5. Add 2.0 g of sodium bisulfite. Stir and swirl the flask to dissolve the bisulfite. Permanganate treated mixtures will lose any remaining purple color.

6. Attach an Allihn reflux condenser to the boiling flask. Pour 50 ml of concentrated sulfuric acid down the condenser into the matrix mixture. Rinse down the condenser with 2 to 3 ml of water. Heat the mixture to reflux with stirring (bring to reflux within about 20 to 30 min) on a stirring hot plate.

   Note: Maintain adequate water flow in the condenser to ensure complete condensation of the refluxing vapors. As the reflux will be conducted overnight, be sure water lines are firmly attached and wired to both the condenser and the water tap to prevent leaks.

7. Continue refluxing the sample for 24 hours.

8. Remove the sample from the heat, and allow the mixture to cool for about 10 to 15 min. Carefully add 350 ml of deionized water to the flask through the condenser, and then remove the condenser from the flask.

9. Cool the flask in an ice bucket half full of a crushed ice/water slurry for 15 min.

10. Slowly add (in 10 ml portions) 100 ml of 50% (w/w) sodium hydroxide solution to the flask. Stir the mixture throughout the addition. Allow time (3 to 4 min) between additions for the mixture to cool.

    Note: The 50% (w/w) NaOH solution was approximately 19 *M*. If a more dilute solution is used, calculate the appropriate volume of solution.

11. Check the pH of the mixture with pH paper. If the pH is <12, add an additional 5 ml of 50% sodium hydroxide solution to the mixture. Recheck the pH, and, if necessary, add additional 50% sodium hydroxide to achieve a pH ≥12.

12. Add 0.5 ml of antifoaming compound to the flask. Remove the flask from the ice bath.

13. Attach a short path distillation head to the 1000-ml boiling flask containing the alkaline sample.

14. Add 2.0 ml of 37% hydrochloric acid solution to a 500-ml boiling flask. Attach the 500-ml boiling flask to the distillation head as a distillation receiver. Figure 2 illustrates the distillation apparatus.

    Note: Wrap the necks of the flask and the short path distillation head with aluminum foil to increase the rate of distillation.

15. Heat the contents of the 1000-ml boiling flask, with stirring, until distillation begins (within 45 to 60 min).

    Note: Maintain adequate water flow in the distillation head to ensure complete condensation of the distillate. Incomplete condensation may lead to poor recoveries.

16. Distill the mixture at a rate of about 2.0 ml of distillate/min for 2.0 to 2.5 hours (about 250 to 300 ml of distillate should be collected).

17. Remove the heat source, and allow the apparatus to cool for about 10 to 15 min. Remove the 500-ml receiver flask.

    Note: Allow the distillation flask to cool completely before disassembling the remainder of the distillation apparatus. If necessary, the distillate can be stored overnight before partitioning. See 3.2.2.6 for disposal recommendations.

3.2.2.3 Partitioning

Note: Perform the entire partitioning procedure without interruptions.

1. Transfer the distillate from the receiver flask, 3.2.2.2 step 17, to a 500-ml separatory funnel. Rinse the receiver flask with two, 10-ml portions of water, and add the rinses to the separatory funnel.

2. Check the pH of the distillate with pH paper. The solution should be pH 2 or less. If a pH >2 is indicated, add 1.0 ml of 37% hydrochloric acid to the solution. Recheck the pH, and if necessary add additional 37% hydrochloric acid to the solution to achieve pH ≤2.

3. Add 10 ml of methylene chloride to the separatory funnel, and shake the funnel for 30 sec. Allow the two phases to separate. Drain off and discard the bottom phase (methylene chloride).

4. Repeat step 3 once with a fresh 10-ml portion of methylene chloride.

5. Add 2.5 ml of 50% (w/w) sodium hydroxide solution to the separatory funnel. Check the pH of the solution in the funnel with pH paper. The pH should be 10 or greater. If a pH of <10 is indicated, add 1.0 ml of 50% sodium hydroxide solution to the funnel, and recheck the pH. If necessary, add additional 50% sodium hydroxide to achieve pH ≥10.

6. Add 5.0 ml of methylene chloride to the separatory funnel, and shake the funnel for 30 sec. Allow the two phases to separate. Drain off the bottom layer (methylene chloride) into a 13-ml graduated centrifuge tube or a 20-ml glass vial.

   Note: This 5 ml of extract contains the majority of the analyte. Loss of a small portion of the extract will significantly affect the recovery. Use care in handling the extract.

7. Repeat step 6 two times with 2.0-ml portions of fresh methylene chloride. Combine all the methylene chloride extracts. After the second 2 ml extraction, discard the aqueous phase.

8. Transfer the methylene chloride solution from the centrifuge tube or vial to a drying tube with a disposable Pasteur pipet (a diagram of the drying tube is shown in Figure 3). Try not to transfer any water into the drying tube. Allow the methylene chloride solution to percolate through the drying tube and into a 13-ml graduated centrifuge tube.

9. Rinse the centrifuge tube or vial two times with 0.50-ml portions of fresh methylene chloride, and transfer the rinses into the drying tube. Allow the rinses to percolate through the tube and into the 13-ml centrifuge tube. Expel the last traces of methylene chloride into the 13-ml centrifuge tube by using pressure from a pipet bulb. Rinse the drying tube with 0.50 ml of fresh methylene chloride, and once again expel the last of the solvent from the drying tube into the 13-ml centrifuge tube.

10. Dilute the solution in the 13-ml centrifuge tube to 10.0 ml with methylene chloride.

    Note: If necessary, the solution can be stored in the refrigerator (0±5°C) before derivatization.

3.2.2.4 Derivatization with Trifluoroacetic Anhydride

Note: Once begun, complete the entire derivatization process without delay.

1. Transfer a 5.0-ml aliquot of the methylene chloride solution from 3.2.2.3 step 10 to a 13-ml graduated centrifuge tube. Using gas tight syringes, add 10.0 µl of concentrated sulfuric acid and 250 µl of dimethylformamide (DMF) to the centrifuge tube. Mix the contents of the tube well. Evaporate the methylene chloride solvent under a gentle stream of nitrogen gas until the sample volume is <200 µl. Add DMF to the sample to bring the total volume to 300 µl.

   Note: Be sure the methylene chloride solvent is completely evaporated. Residual methylene chloride may cause salt formation and possibly incomplete derivatization.

2. Place the centrifuge tube in a beaker of water at room temperature.

3. Add 100 µl of a solution of 0.2% (w/v) DMAP in pyridine to the tube with a gas tight syringe. Cap and remove the tube from the beaker of water. Mix the contents very thoroughly. Rinse the solution up the sides of the centrifuge tube. Replace the tube into the beaker of water.

   Note: Prepare the 0.2% DMAP in pyridine by adding 0.050 g of DMAP to a 25-ml volumetric flask. Dissolve the DMAP in pyridine and dilute to 25 ml. Handle the pyridine solution only in an adequate fume hood. Occasionally, a precipitate forms at the addition of the pyridine mixture to the sample. The precipitate should dissolve on addition of the TFAA in the next step. If not, the sample should be repeated with the other 5.0 ml of methylene chloride solution.

4. Slowly add 300 µl of TFAA dropwise, using a gas tight syringe, to the centrifuge tube with gentle agitation. Cap and remove the tube from the beaker of water. Mix the contents well. Rinse the solution up the sides of the centrifuge tube. A clear yellow solution should be obtained.

   Note: Handle the TFAA only in an adequate fume hood. This is a hazardous reagent; handle with care! After addition of the TFAA, the reaction mixture may form a small amount of precipitate on standing. This should not affect the results.

5. Replace the tube into the beaker of water. Allow the mixture to stand for about 15 min.

6. Mount a 3-ml, C-18 spe cartridge onto a vacuum manifold. Wash the cartridge twice with 2.5-ml portions of methyl *tert*-butyl ether and twice with 2.5-ml portions of acetonitrile, and dispose of the combined eluates. Wash the cartridge twice with 2.5-ml portions of water. Elute each solvent until the liquid level reaches the top of the sorbent bed before adding the next wash solvent. (The solvents should be eluted at a vacuum of about -2 kPa./-1.5 inches Hg) Discard the wash solvents after elution.

7. Carefully add 3.0 ml of deionized water dropwise to the centrifuge tube with gentle shaking. Dilute the solution with deionized water to 8.0 ml total volume. Cap and remove the tube from the beaker of water; mix the contents well.

8. Add the solution from step 7 to the C-18 spe cartridge in several portions. Pull the solution through the cartridge by applying a gentle vacuum (about -2 kPa/-1.5 inches Hg) to the manifold. Rinse the centrifuge tube twice with 1.0-ml portions of water, and add the rinses to the cartridge.

9. Rinse the cartridge twice with 2.5-ml portions of water. Discard the combined water eluates from the cartridge.

10. Dry the cartridge by using the vacuum manifold to draw air through the sorbent bed for 15 min (about -25 kPa/-7 inches Hg vacuum).

11. Elute the cartridge twice with 2.0-ml portions of methyl *tert*-butyl ether. The methyl *tert*-butyl ether should be pushed through the cartridge with a gentle positive pressure from a syringe or a pressure manifold. Attempt to elute the cartridge at a flow rate of about 15 to 20 drops/min (0.5 to 0.7 ml/min). Collect the eluate into a clean 13-ml graduated centrifuge tube. A small quantity of water will probably be observed in the bottom of the tube.

    Note: Do not use vacuum to elute the methyl tert-butyl ether through the cartridge.

12. Transfer the eluate from the centrifuge tube to a drying tube with a disposable Pasteur pipet (see Figure 3). Try not to transfer the water from the bottom of the tube. Allow the methyl *tert*-butyl ether solution to percolate through the drying tube and into a 5-ml graduated centrifuge tube.

13. Rinse the 13-ml centrifuge tube twice with 0.50-ml portions of fresh methyl *tert*-butyl ether. Transfer the rinses to the drying tube. Allow the rinses to percolate through the drying tube and into a 5-ml graduated centrifuge

tube. Expel the last traces of solvent from the drying tube into the 5-ml graduated centrifuge tube with a pipet bulb. Rinse the drying tube with 0.50 ml of fresh methyl *tert*-butyl ether, and once again expel the last of the solvent from the drying tube into the 5-ml centrifuge tube.

14. Dilute the solution in the 5-ml centrifuge tube to 5.0 ml with methyl *tert*-butyl ether.

Note: This solution can be refrigerated (4°C ± 3°C) for 2 weeks before analysis. Dilute the solution to 5.0 ml with methyl *tert*-butyl ether before analysis. Because of the volitility of the methyl *tert*-butyl ether, add the solution to the autosampler vials just prior to gc/ms analysis.

15. Place 0.5-ml to 0.7-ml aliquots of the solution from step 14 into gc autosampler vials. Seal the vials with suitable septa. Label one vial repetition 1 and the other repetition 2.

3.2.2.5 Analysis by Gas Chromatography/Mass Spectroscopy (gc/ms-sim)

3.2.2.5.1 Standard Procedure

A. Instrument Conditions:

Injector: Splitless mode, 200°C, purge off time 0.75 min.

Column: Fused silica capillary columns: 0.20 mm i.d. x 12 m, methyl silicone, 0.33 µm film thickness.

Carrier gas: Helium, 8 psi (33.5 cm/sec flow rate).

Temperatures: Hold at 55°C for 1.7 min,
Ramp at 15°C/min to 150°C.
Ramp at 25°C/min to 250°C.

Detector: Mass selective detector in selected ion mode. Data are acquired for ions of m/z 238, 207, and 249. These data are summed to give a total ion chromatogram (TIC). The TIC data are used for calculations. Data are processed using Hewlett Packard software.

B. Procedure:

1. Place 0.5-ml to 0.7-ml aliquots of the 0.50 ppm standard (see 3.1.3.1) into gc autosampler vials. Seal the vials with suitable septa.

2. Inject 1.0 µl from the first vial of the 0.50 ppm standard.

3. Inject 1.0 µl of the derivatized sample (see 3.2.2.4 step 15) from the vial labeled repetition 1

4. Inject 1.0 µl from the second vial of the 0.50 ppm standard.

5. Inject 1.0 µl of the derivatized sample (see 3.2.2.4 step 15) from the vial labeled repetition 2.

6. Inject 1.0 µl from the third vial of the 0.50 ppm standard.

7. Inject 1.0 µl of methyl *tert*-butyl ether as a blank.

8. Compare the peak area of the sample to those of the 0.50 ppm standards on either side. If the peak area for the sample is greater than the peak areas for the 0.50 ppm standards, then prepare new aliquots of the sample (see 3.2.2.4 step 15), and repeat steps 1 through 7 using the 2.50 ppm standard (see 3.1.3.1) instead of the 0.50 ppm standard. If the peak area for the sample is greater than the peak areas for the 2.50 ppm standards, repeat steps 1 through 7 with the 25.0 ppm standard (see 3.1.3.1).

9. Compare the peak areas of the standards injected before and after both repetitions of the sample. If the peak areas of the standards vary by >20%, prepare two new aliquots of the sample (see 3.2.2.4 step 15), and repeat steps 1 through 8. If there is <20% variation, proceed to part C.

C. Calculations:

1. Compare the gc retention times with those of the 0.50 ppm standards on either side. If the gc retention time for the sample is within ±0.05 min of either standard, then proceed to step 2.

2. Calculate ppm of FOE 5043 equivalents in each sample by comparing the detector response (peak areas) for the sample to the average response to the standards injected before and after the sample.

$$\text{Sample ppm} = \frac{\text{sample response}}{\text{average standard response}} \times \text{standard concentration}$$

D. Detector Linearity Curves:

1. To demonstrate detector response linearity to the trifluoroacetamide derivative in solvent, sequentially inject 1.0 µl of each of the 0.10 ppm, 0.25 ppm, 0.50 ppm, 1.00 ppm, and 2.50 ppm standards (see 3.1.3.1).

2. To demonstrate detector response linearity to the trifluoroacetamide derivative in the presence of matrix, prepare and analyze fortified matrix controls. Using a 500-µl gas tight syringe, add 0.50 ml aliquots of control matrix sample (obtained by processing a control tissue sample through the method to 3.2.2.4 step 14) to each of five auto sampler vials. Label one each of the vials 0.10 ppm, 0.25 ppm, 0.50 ppm, 1.00 ppm, and 2.50 ppm, respectively. In the same order, fortify the five vials with 2.0 µl, 5.0 µl, 10.2 µl, 21.2 µl, and 55.5 µl, respectively, of the 25.0 ppm standard (see 3.1.3.1). These fortifications represent final concentrations of 0.10 ppm, 0.25 ppm, 0.50 ppm, 1.00 ppm, and 2.50 ppm, respectively). Inject 1.0 µl of each fortified matrix sample.

3. Plot the TIC mass selective detector response as a function of the standard concentration (ppm) for the data from steps 1 and 2. Assess the linearity of each curve by a least squares determination (such a determination is described by Aiken *et al.*[9]). To be considered linear, each curve should have a correlation coefficient (r) of ≥0.99.

4. Assess the two curves plotted in step 4. Determine detector response values from each curve at 0.10 ppm, 0.25 ppm, 0.50 ppm, and 1.00 ppm. At each of these points, the detector response value for the fortified matrix curve should be within 20% of the same point on the solvent only sample curve for the curves to be sufficiently comparable.

5. If the linearity of the two curves is not sufficient, repeat steps 1 to 5 after evaluating the performance of the gc/ms system.

Note: If standards greater than 0.50 ppm were necessary in step 3.2.2.5.1 B-8, use correspondingly higher linearity curves (ie. 1.00 ppm, 2.50 ppm, 5.00 ppm, 10.0 ppm, and 25.0 ppm with 1.00 ppm recoveries and fortify the control matrix with 250 ppm standard). For recoveries lower than 0.10, use a correspondingly lower linearity curve.

3.2.2.5.2 Confirmatory Procedure

1. Individually integrate the single ion chromatograms for 138, 207, and 249 amu.

2. Compare the ratio of peak areas for 138 amu to 207 amu and 249 amu to 207 amu for the standards and the samples.

$$138 \text{ Ion Ratio} = \frac{\text{Integration for 138 amu}}{\text{Integration for 207 amu}}$$

$$249 \text{ Ion Ratio} = \frac{\text{Integration for 249 amu}}{\text{Integration for 207 amu}}$$

3. If the ion ratios for the sample are similar (±15%) to the average ion ratios of the standards injected before and after the sample, the presence of FOE 5043 residue, measured as the trifluoroacetamide derivative, is confirmed. See Appendix 3 for sample calculations.

3.2.2.6 Disposal of Solutions

1. All organic solvent waste (methylene chloride, acetonitrile, methyl *tert*-butyl ether, and the aqueous elutes from C-18 cartridge cleanup) should be disposed of in approved hazardous waste containers.

2. The aqueous wastes (the aqueous phase from partitioning) should be disposed into an approved water waste system.

3. Pot residues from the steam distillation should be diluted with water (about 300 ml), neutralized to about pH 7 with 37% hydrochloric acid, and emptied into an approved water waste system. Flush drain lines with a copious quantity of water.

3.3 Method Validation

3.3.1 Requirements

1. Duplicate recoveries of 70 to 120% in all plant matrices and processed products at 0.10 ppm for FOE 5043, FOE oxalate, FOE sulfonic acid, and FOE thioglycolate sulfoxide are required.

2. Each gc/ms measurement of a 0.10 ppm recovery is compared to a 0.50 ppm standard to determine the ppm level of FOE 5043 equivalents of residue. (0.25 ppm standard should be used for 0.05 ppm recoveries.)

3. Each sample is analyzed by the confirmatory method.

4. Calculate recoveries by the following equation:

$$\text{Recovery} = \frac{\text{Ppm found}}{\text{Ppm fortification level}} \times 100\%$$

3.3.2 Procedure for 0.10 ppm recoveries

1. Using a 250 µl gas tight syringe, add 0.200 ml of FOE 5043 standard solution (5.00 µg/ml in methanol, see 3.1.3.2); 0.124 ml of FOE oxalate (5.00 µg/ml in methanol, see 3.1.3.2); 0.173 ml of FOE sulfonic acid, sodium salt, monohydrate (5.00 µg/ml in methanol, see 3.1.3.2); or 0.166 ml of FOE thioglycolate sulfoxide (5.00 µg/ml in methanol, see 3.1.3.2) to the 1000-ml flask (3.2.2.2 step 1).

   Note: If the FOE sulfonic acid is in the form of the free acid, use 0.151 ml of 5.00 µg/ml solution. For 0.05 ppm recoveries, use half the volumes of fortification solution.

2. Run duplicate recoveries for each chemical in each matrix. Run duplicates sample for each matrix. Run a control sample with each matrix set.

3. Perform the method as written without modifications.

4.0 Results and Discussion

Hydrolysis of FOE 5043 and its plant metabolites yields a common chemical fragment, fluoroaniline (see Appendix 4). Thus, a common residue method was developed. A flow diagram of the analytical residue method is presented in Figure 4.

4.1 Sample Oxidation and Hydrolysis

A 24-hour hydrolysis with refluxing 47% sulfuric acid completely converted FOE 5043, FOE oxalate, FOE sulfonic acid, FOE methyl sulfone, and FOE thioglycolate sulfone to fluoroaniline. Lower concentrations of acid or shorter reflux times did not hydrolyze all of the plant metabolites to the fluoroaniline. Higher concentrations of acid (>60%) degraded the fluoroaniline. Alkaline hydrolysis was also attempted, but a significant fraction of the highly volatile fluoroaniline was lost during the hydrolysis.

Under the acidic conditions, FOE thioglycolate sulfoxide and FOE methyl sulfoxide did not hydrolyze completely to the fluoroaniline, so the residue samples were oxidized in

order to covert the sulfoxide metabolites to the corresponding sulfones prior to the acid hydrolysis. The oxidation conditions proved to be matrix dependent. Most samples were best oxidized with mildly acidic potassium permanganate. For some high moisture matrices (i.e. soybean forage), 1.0 g of potassium permanganate was sufficient to oxidize the sulfoxides. However, most matrices required treatment with 2.0 g of potassium permanganate to ensure adequete recoveries of FOE thioglycolate sulfoxide. Oil samples were best oxidized with an oxidizing agent soluble in both oil and water, monoperoxyphtalic acid magnesium salt. After oxidation, all samples were treated with sodium bisulfite to destroy excess oxidant before the addition of concentrated sulfuric acid. The subsequent addition of acid generates gas, therefore these procedures should be done in a fume hood.

The use of excess permanganate may lead to low recoveries, particularly of the FOE oxalate metabolite. Therefore, matrices should be initially validated with 2.0 g of potassium permanganate. If FOE oxalate recoveries are low (<70%), the matrix should be revalidated with only 1.0 g of potassium permanaganate, and FOE thioglycolate sulfoxide recoveries should be examined. If the FOE thioglycolate sulfoxide recoveries are also low (<70%), the use of 1.5 g of potassium permanganate and/or 2.5 g of monoperoxyphtalic acid, magnesium salt should be attempted.

The vigorous acidic hydrolysis conditions used in this method have three advantages. First, the high concentration of sulfuric acid increases the hydrolysis rates by providing a large proton concentration and a high reflux temperature (115°C) in the hydrolysis mixture. Second, the fluoroaniline is converted to a non-volatile acid salt. Third, the acid hydrolysis is less prone to uncontrolled foaming. A summary of the hydrolysis rates of FOE 5043 and its plant metabolites is presented in Appendix 4.

The oxidation and the 24-hour acidic reflux were necessary parts of this residue method and could not be circumvented. Because no flammable organic solvents are involved in the reflux, the overnight acid hydrolysis is not a hazard.

4.2 Sample Extraction and Partitioning

After the acid hydrolysis, the matrix mixture was cooled, diluted with water, and made alkaline with 50% (w/w) sodium hydroxide. Subsequent steam distillation of the mixture into hydrochloric acid solution efficiently extracted the fluoroaniline. Antifoam A was added to the hydrolysis mixture to prevent excess foaming during the distillation. The fluoroaniline was then partitioned from the steam distillate with methylene chloride. While other organic solvents could be used for this purpose, methylene chloride had the best combination of properties, including partition coefficient, low water solubility, and ease of handling during the extraction.

4.3 Sample Derivatization

Direct gc/ms-sim analysis of the fluoroaniline solution was desirable. However, initial analyses of standards showed non-linear detector response to low levels of the fluoroaniline. Therefore, the fluoroaniline was derivatized prior to gc/ms-sim analysis.

Investigation of various potential derivatives showed the derivative 4-fluoro-*N*-methylethyl benzenamine trifluoroacetamide (trifluoroacetamide derivative) to be the best.[7] The trifluoroacetamide had three strong ions suitable for selected ion monitoring, as well as a linear gc/ms-sim detector response. Ei mass spectra are shown for fluoroaniline and the trifluoroacetamide in Figure 5. Derivatization of the fluoroaniline in methylene chloride solution was unsuccessful.

The fluoroaniline was concentrated into DMF for derivatization. Concentrated sulfuric acid was added to the methylene chloride solution to convert the extremely volatile fluoroaniline to a non-volatile salt. DMF was added to produce a homogeneous solution and to act as a reaction solvent. The methylene chloride was then evaporated from the sample with a stream of nitrogen gas. The DMF solution was amended with excess pyridine containing DMAP (0.2% w/v). Addition of trifluoroacetic anhydride (TFAA)to the DMF solution yielded the trifluoroacetamide derivative. While initial experiments were performed with 100 µl of TFAA, the Independent Laboratory Validation[8] (see Appendix 5) indicated 300 µl of TFAA gave more consistent results. This change was adopted in the final method. The excess reagents were quenched after 15 min by cautious addition of water.

If methylene chloride remained in the sample or too much sulfuric acid was added to the sample, salt formation occurred during derivatization, which sometimes led to low recoveries. Complete removal of methylene chloride, derivatization at room temperature, and careful measurement of the sulfuric acid avoided this problem.

The trifluoroacetamide derivative was extracted from the aqueous reaction mixture by solid phase extraction (spe). A C-18 spe cartridge was charged with the reaction mixture, washed with water to remove pyridine, trifluoroacetic acid, and DMF, and then briefly air dried to remove most of the water. The trifluoroacetamide derivative was then eluted from the C-18 spe cartridge with methyl *tert*-butyl ether. The resultant solution was analyzed by gc/ms-sim.

4.4 Gc/ms-sim of 4-Fluorophenyl-*N*-Methylethyl Benzenamine Trifluoroacetate

For each analysis, selected ion monitoring data were collected and summed for ions with m/z of 138, 207, and 249 to yield a total ion chromatogram (TIC). The TIC was used to calculate the FOE 5043 residue levels due to its greater signal to noise ratio than the individual ion chromatograms. Figure 6 shows a typical gc/ms-sim

chromatogram for the trifluoroacetamide derivative standard. The chromatography yielded a Gaussian shaped peak with slight asymmetry and a retention time of approximately 6 min. Figure 7 shows gc/ms-sim chromatograms for typical control and recovery samples from corn forage. The control sample showed a very slight matrix interference. The recovery sample again showed a peak with a retention time of about 6 min. Figures 8 to 19 show gc/ms-sim chromatograms for typical control and recovery samples from various plant tissues. The control samples showed little or no matrix interference. The recovery samples showed Gaussian peaks with retention times of about 6 min in all matrices. Figure 20 shows a gc/ms-sim chromatogram of a methyl *tert*-butyl ether solvent blank.

4.5 Quantitation of Residue Levels

The TIC detector response for each recovery sample was inspected for a peak falling within ±0.05 min of the trifluoroacetamide derivative standards injected before and after the sample (braketing standards). If a peak was found, the TIC detector response of the peak was compared to the average TIC detector response for the bracketing standards. All residue levels were calculated as ppm of FOE 5043 equivalents. Example calculations for the determination of FOE 5043 residue levels are shown in Appendix 3.

4.6 Confirmation of Analyte Identity

The identity of the analyte in each recovery sample was confirmed by the determination of ion ratios. This technique has been described by Sphon[9] and Wilson.[10] The 138 amu, 207 amu, and 249 amu single ion chromatograms were integrated for samples and bracketing standards. The ratio of the detector response for the 138 amu ion to the detector response for the 207 amu ion was calculated, as was the ratio of the 249 amu ion to the 207 amu ion. To confirm the identity of the analyte, the ion ratios for the sample and the average of the bracketing standards must be within ±15%. The three single-ion chromatograms for the trifluoroacetamide derivative standard are shown in Figure 6B. Typical single-ion chromatograms for controls and recoveries from various plant tissues are shown in Figures 7 through 19. Example calculations for the determination of ion ratios are shown in Appendix 3.

4.7 Recovery of FOE 5043 and Related Plant Metabolites from Crop Samples

Table 2 lists recoveries of FOE 5043 and its plant metabolites from various matrices. The recovery values were corrected for the small interferences seen in some control matrix samples.

4.9 Additional Validation Studies

An independent laboratory validation (PR Notice 88-5) of the analytical method was performed on the first attempt[8], indicating that the method could be satisfactorily run by following the written procedure. The performing lab suggested increasing the amount of TFAA used in the derivatization step of the method. This change was incorporated into the method (Appendix 5).

A competitor product interference study[11] indicated that no compounds having tolerances in for corn, cotton, peanut, soybean, sunflower, wheat interfered with the analysis for FOE residues (Appendix 19). A total of 179 compounds were tested with plant matrix samples (corn forage).

A validation study[12] using aged radioactive residues in corn fodder, soybean seed, and soybean forage demonstrated the extraction efficiency of the method. The FOE 5043 residues were effectively extracted, converted to fluoroaniline, and derivatized to the trifluoroacetamide derivative (Appendix 20).

5.0 Conclusions

An analytical method for measuring the residues of FOE 5043 and its plant metabolites in crop matrices was developed. This method showed recoveries of 67% to 116% of FOE 5043 and its metabolites at the 0.10 ppm level and 65% to 91% at the 0.05 ppm level. The limit of quantitation was 0.05 to 0.1 ppm of FOE 5043 equivalents, depending on the plant matrix. The minimum detectable level was 0.01 to 0.05 ppm FOE 5043 equivalents, depending on the plant matrix.

6.0 Bibliography

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11. Gould, T. J. and K. L. Zoloty. 1995. A registered product interference study for the analytical method for determining FOE 5043 residues in plant matrices. Bayer Ag Div Report No. 106906.

12. Gould, T. J. 1995. Extraction efficiency of the analytical method for determining FOE 5043 residues in plant matrices. Bayer Ag Div Report No. 106927.

Table 1. Distribution of FOE 5043 metabolites in plant matrices from crops grown in soil treated with [^{14}C] FOE 5043. (Values in percent of total radioactive FOE 5043 residue).

	FOE 5043 Metabolites					
Crop Matrix	Oxalate	Sulfonic Acid	Thioglycolate Sulfoxide	Methyl Sulfone	Methyl Sulfoxide	Thiolactate Sulfoxide
Corn[1]						
Forage	44	7	10	1	1	10
Fodder	42	6	11	1	1	9
Soybeans						
Forage	15	40	19	5	2	0
Fresh Beans	0	0	39	4	2	0
Hay	16	40	17	5	4	0
Dry Beans	5	8	39	6	5	0

[1] The residues in corn kernels were too low (0.012 ppm) for identification of individual metabolites.

Table 2. Recovery of FOE 5043 and its metabolites from plant matrices.

Crop Matrix	Compound	Ppm Fortification	Sample Recovery (%)
Corn Forage[1]	FOE 5043	0.10	83/ 83
	FOE oxalate	0.10	72/ 78
	FOE sulfonic acid	0.10	75/ 64
	FOE thioglycolate sulfoxide	0.10	80/ 78
Corn Forage[1]	FOE 5043	0.05	91
	FOE oxalate	0.05	79
	FOE sulfonic acid	0.05	65
	FOE thioglycolate sulfoxide	0.05	73
Corn Grain[2]	FOE 5043	0.10	112/ 116
	FOE oxalate	0.10	79/ 82
	FOE sulfonic acid	0.10	78/ 91
	FOE thioglycolate sulfoxide	0.10	86/ 98
Corn Fodder[3]	Mixed standard	0.10	83/ 90
Corn Oil[4]	FOE 5043	0.10	83/ 86
	FOE oxalate	0.10	78/ 72
	FOE sulfonic acid	0.10	67/ 74
	FOE thioglycolate sulfoxide	0.10	76/ 77

[1] For summaries of the raw data and chromatograms, see Appendix 6. Control samples had residues of <0.05 ppm.

[2] For summaries of the raw data and chromatograms, see Appendix 7. Control samples had residues of <0.10 ppm.

[3] For summaries of the raw data and chromatograms, see Appendix 8. Control samples had residues of <0.10 ppm.

[4] For summaries of the raw data and chromatograms, see Appendix 9. Control samples had residues of <0.10 ppm.

Table 2. (cont.)

Crop Matrix	Compound	Ppm Fortification	Sample Recovery (%)
Peanut Nutmeat[5]	Mixed Standard	0.10	78/ 72
Spinach Tops[6]	Mixed Standard	0.05	71/ 72
Soybean Seed[7]	FOE 5043	0.10	89/ 91
	FOE oxalate	0.10	89/ 80
	FOE sulfonic acid	0.10	74/ 65
	FOE thioglycolate sulfoxide	0.10	86/ 82
Soybean Forage[8]	FOE 5043	0.10	89/ 85
	FOE oxalate	0.10	85/ 83
	FOE sulfonic acid	0.10	74/ 74
	FOE thioglycolate sulfoxide	0.10	79/ 80
Soybean Soapstock[9]	Mixed standard	0.10	83/ 85
Sunflower Seed[10]	FOE 5043	0.10	94/ 95
	FOE oxalate	0.10	92/ 87
	FOE sulfonic acid	0.10	70/ 70
	FOE thioglycolate sulfoxide	0.10	93/ 75

[5] For summaries of the raw data and chromatograms, see Appendix 10. Control samples had residues of <0.10 ppm.

[6] For summaries of the raw data and chromatograms, see Appendix 11. Control samples had residues of <0.05 ppm.

[7] For summaries of the raw data and chromatograms, see Appendix 12. Control samples had residues of <0.10 ppm.

[8] For summaries of the raw data and chromatograms, see Appendix 13. Control samples had residues of <0.10 ppm.

[9] For summaries of the raw data and chromatograms, see Appendix 14. Control samples had residues of <0.10 ppm.

[10] For summaries of the raw data and chromatograms, see Appendix 15. Control samples had residues of <0.10 ppm.

Table 2 (cont.)

Crop Matrix	Compound	Ppm Fortification	Sample Recovery (%)
Turnip Roots[11]	Mixed standard	0.05	71/ 72
Wheat Grain[12]	FOE 5043	0.10	88/ 86
	FOE oxalate	0.10	87/ 85
	FOE sulfonic acid	0.10	73/ 65
	FOE thioglycolate sulfoxide	0.10	80/ 77
Wheat Grain[12]	Mixed standard	0.05	78/ 79
Wheat Straw[13]	Mixed standard	0.10	80/ 85

[11] For summaries of the raw data and chromatograms, see Appendix 16. Control samples had residues of <0.05 ppm.

[12] For summaries of the raw data and chromatograms, see Appendix 17. Control samples had residues of <0.05 ppm.

[13] For summaries of the raw data and chromatograms, see Appendix 18. Control samples had residues of <0.10 ppm.

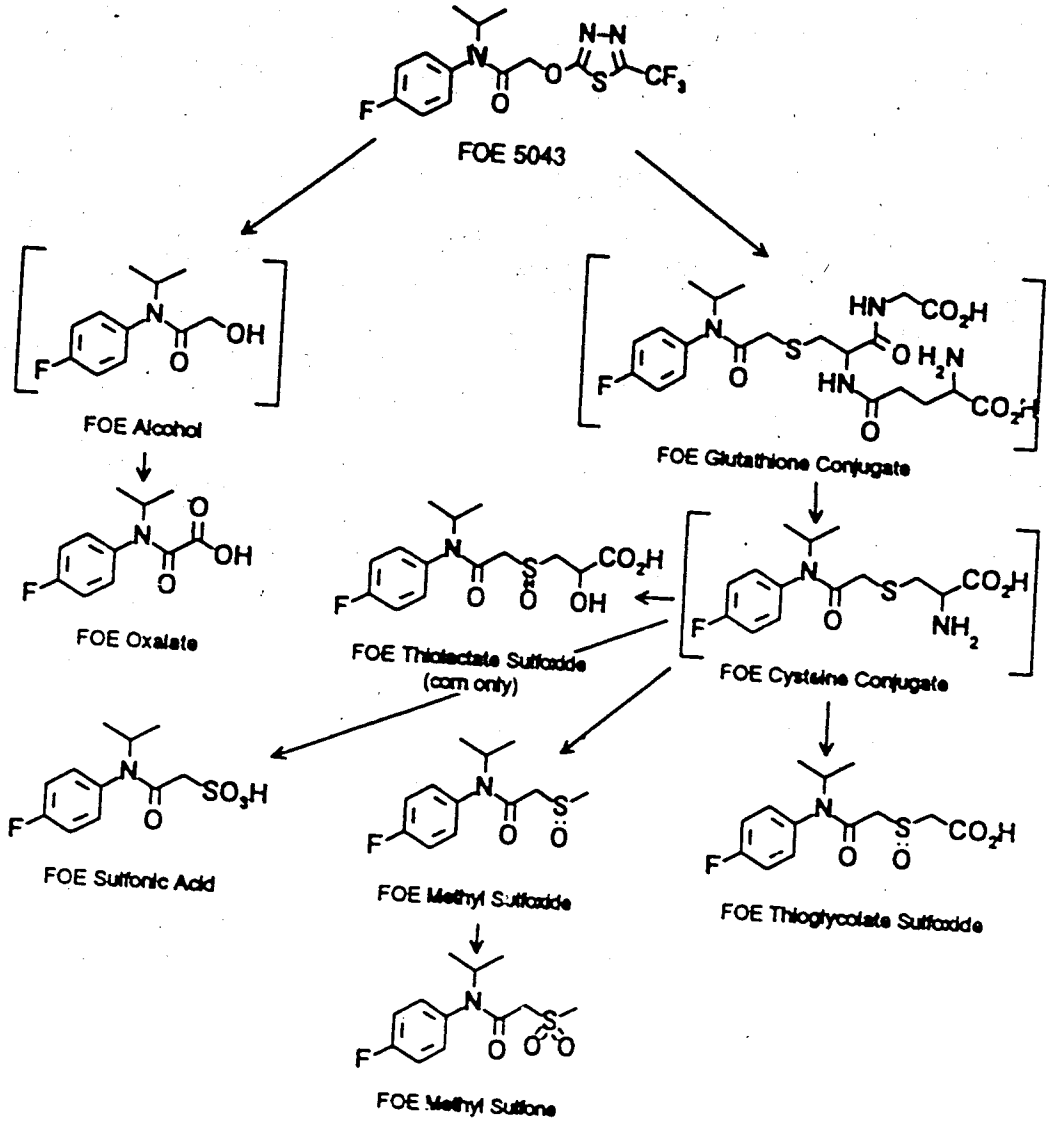


Figure 1. Plant metabolism of FOE 5043.

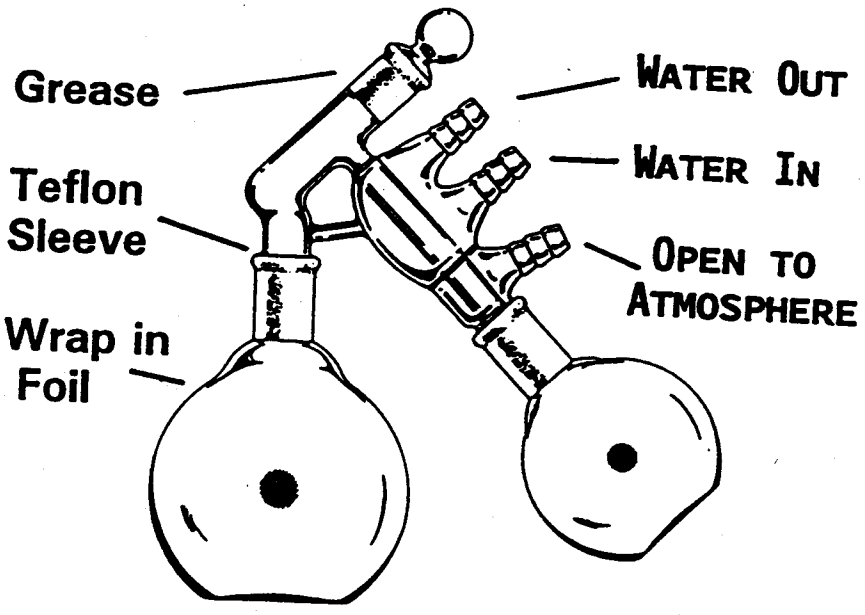


Figure 2. Short path distillation setup for steam distillation of the acid digest.

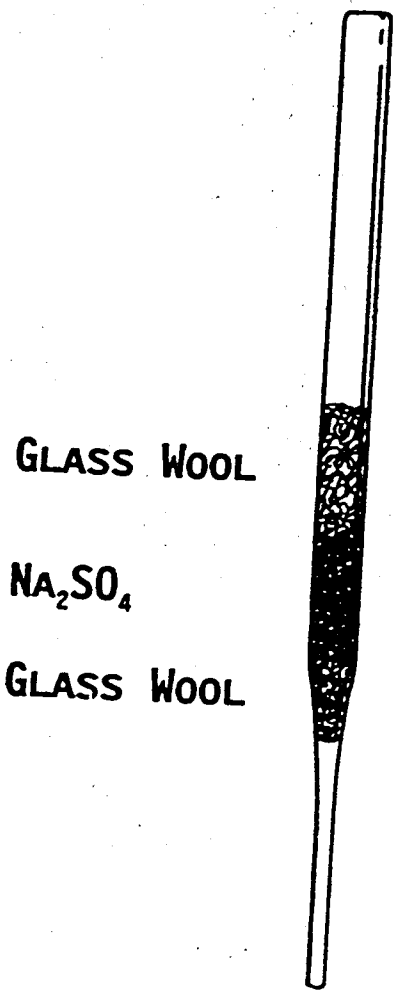


Figure 3. Drying tube setup for the drying of organic solutions. Preparation: Add a small plug of glass wool to a disposable Pasteur pipet. Add about 0.5 g of anhydrous sodium sulfate to the pipet. Add a loose plug of glass wool about 2 cm long to the pipet.

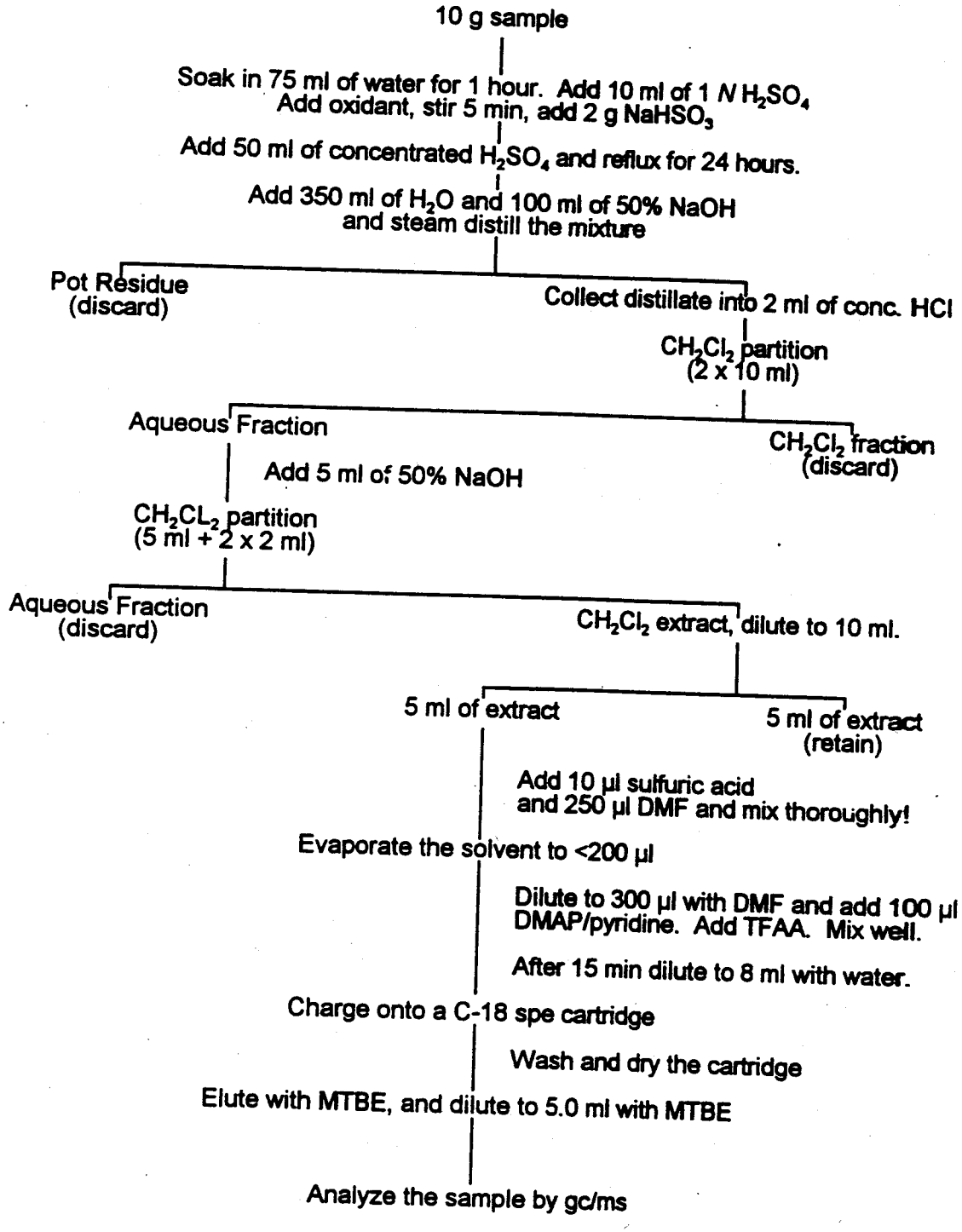
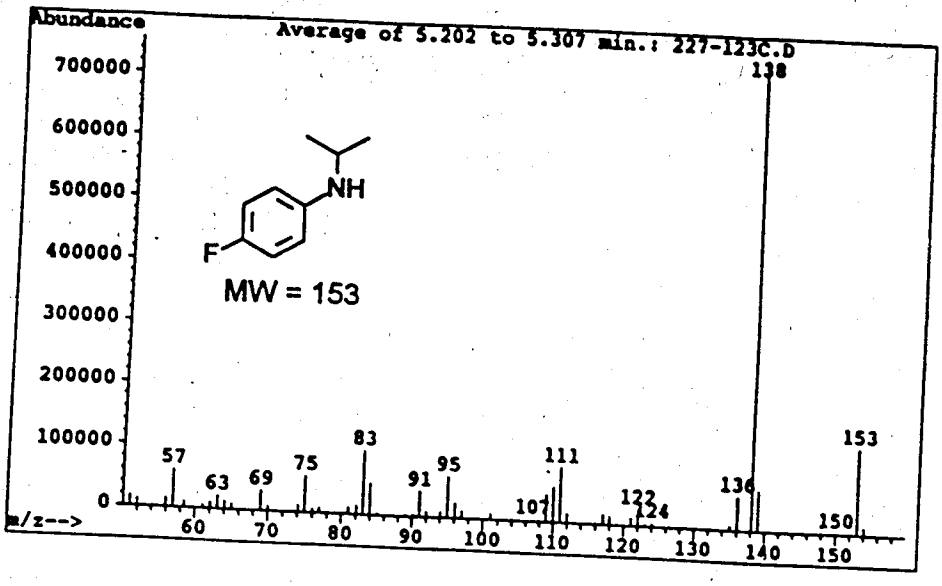


Figure 4. Flow diagram of the analytical residue method.

4-Fluoro-*N*-methylethyl benzenamine.



4-fluoro-*N*-methylethyl benzenamine trifluoroacetamide.

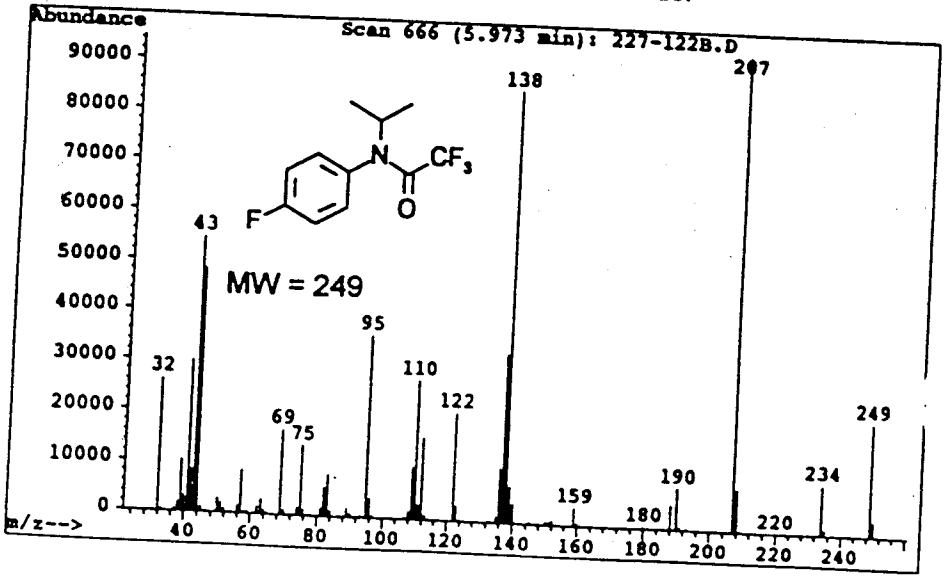
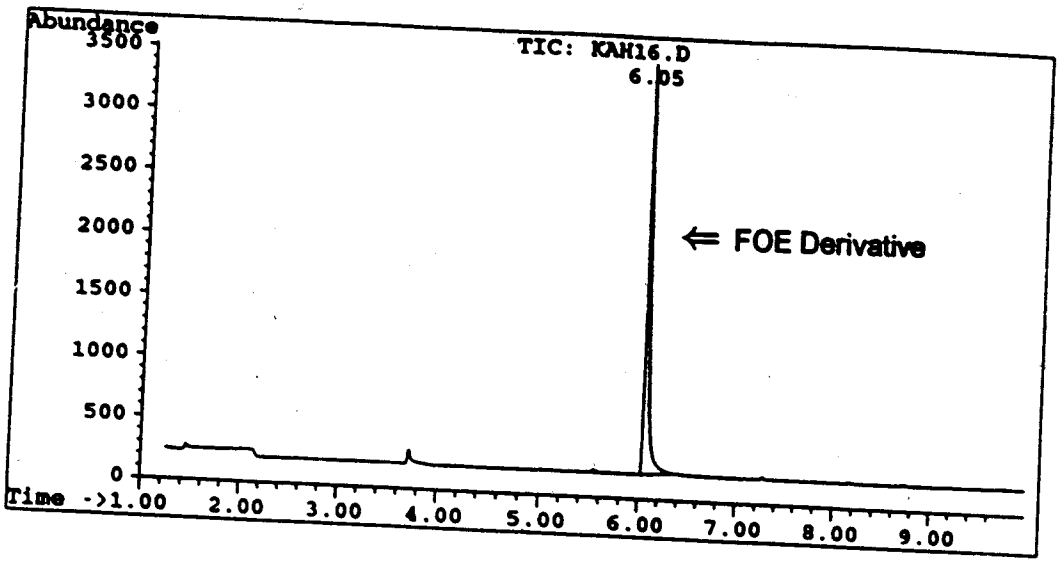


Figure 5. Ei mass spectra of fluoroaniline and the trifluoroacetamide derivative.

A. Total ion chromatogram of the FOE trifluoroacetamide derivative.



B. Single ion chromatograms for m/z of 138, 207, and 249.

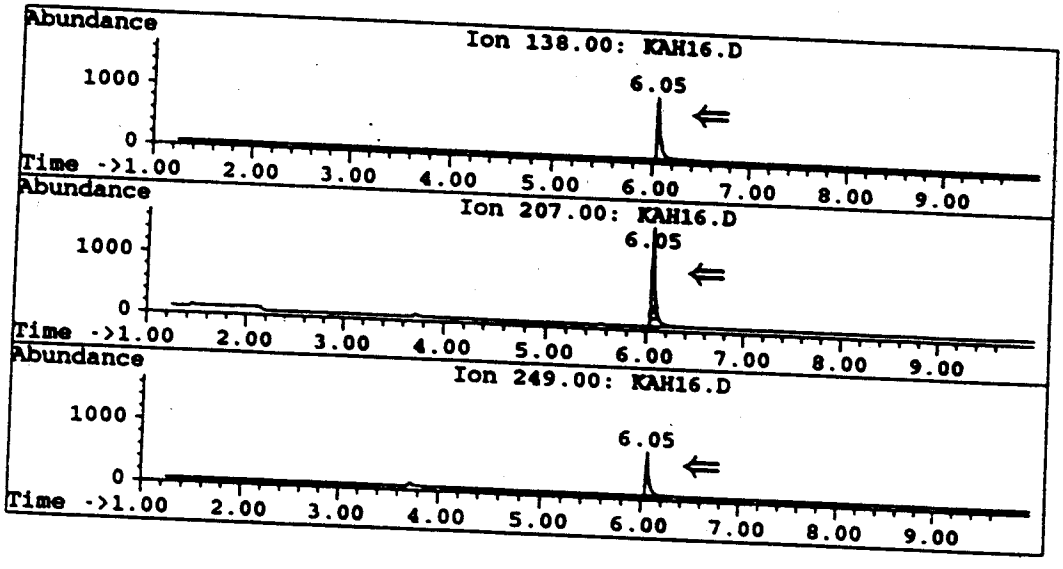
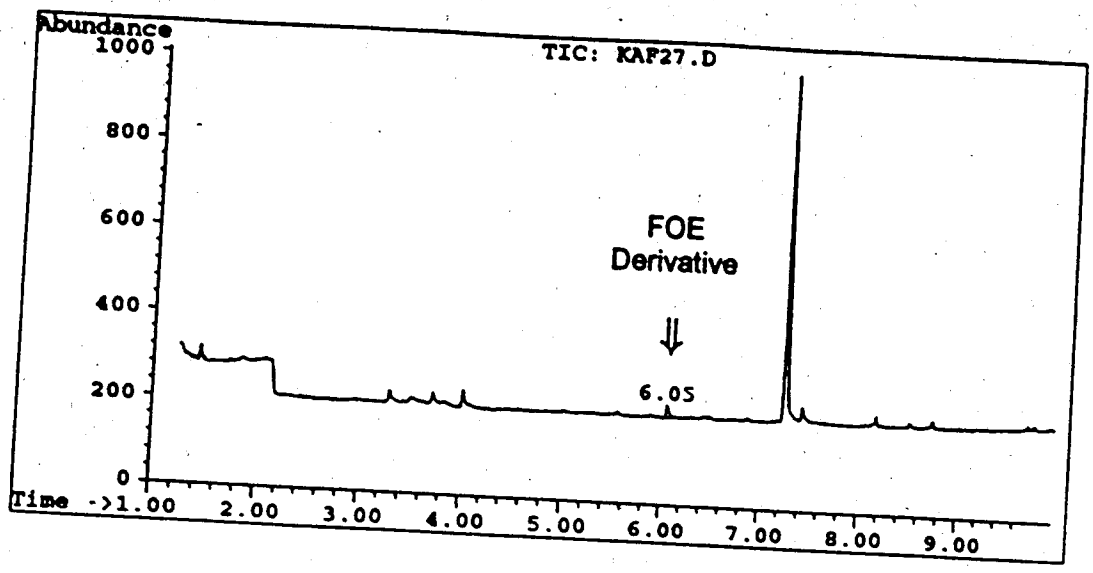


Figure 6. Gc/ms chromatogram of a 4-fluoro-*N*-methylethyl benzenamine trifluoroacetamide standard sample.

A. Total ion chromatogram of a corn forage control sample.



B. Single ion chromatograms for m/z of 138, 207, and 249.

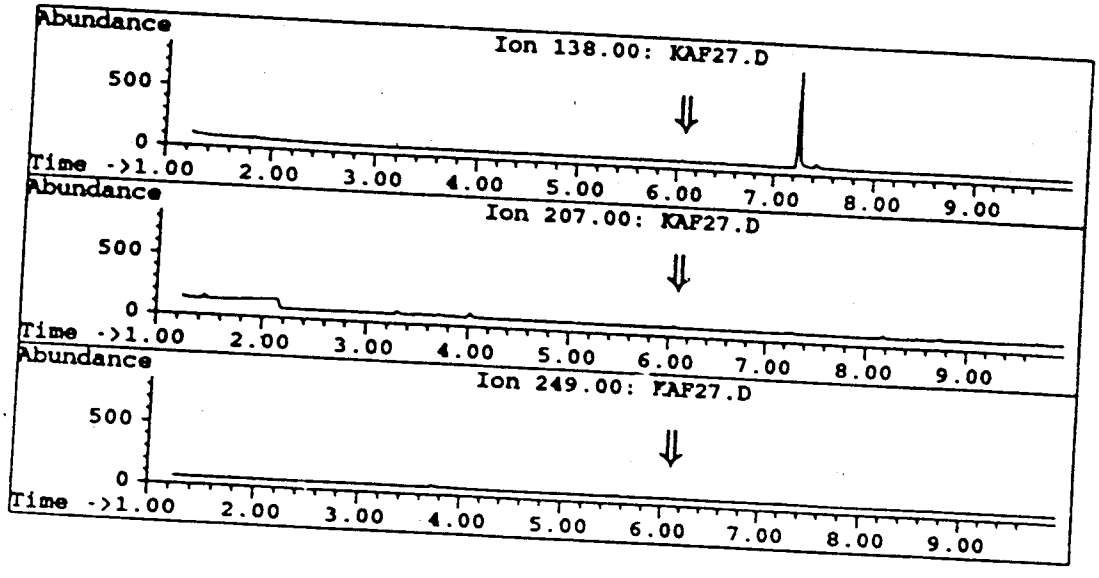
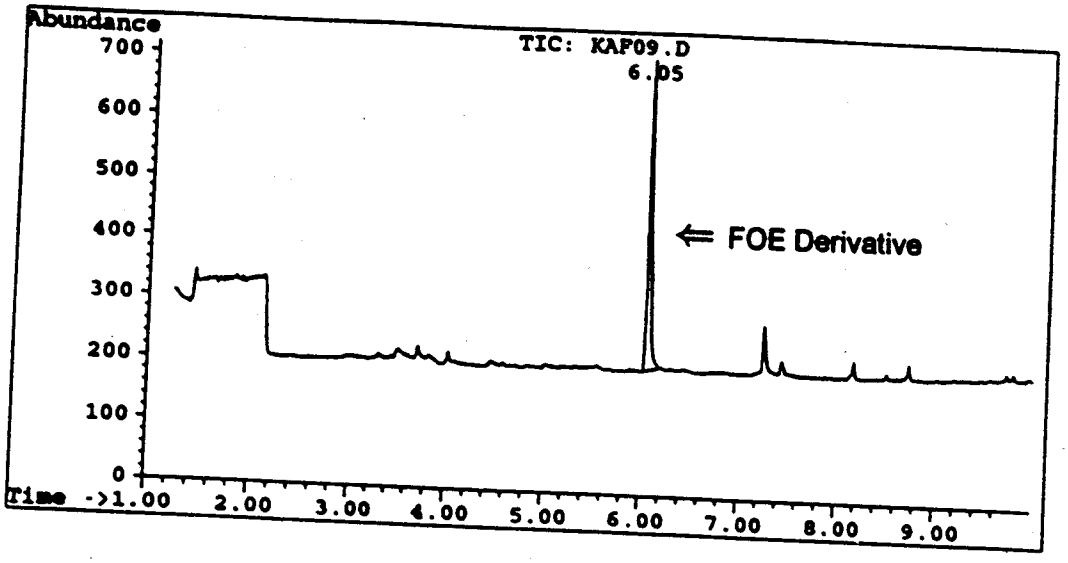


Figure 7. Gc/ms chromatograms of typical corn forage control and recovery samples.
A and B: Corn forage control sample.

C. Total ion chromatogram of a corn forage recovery sample.



D. Single ion chromatograms for m/z of 138, 207, and 249.

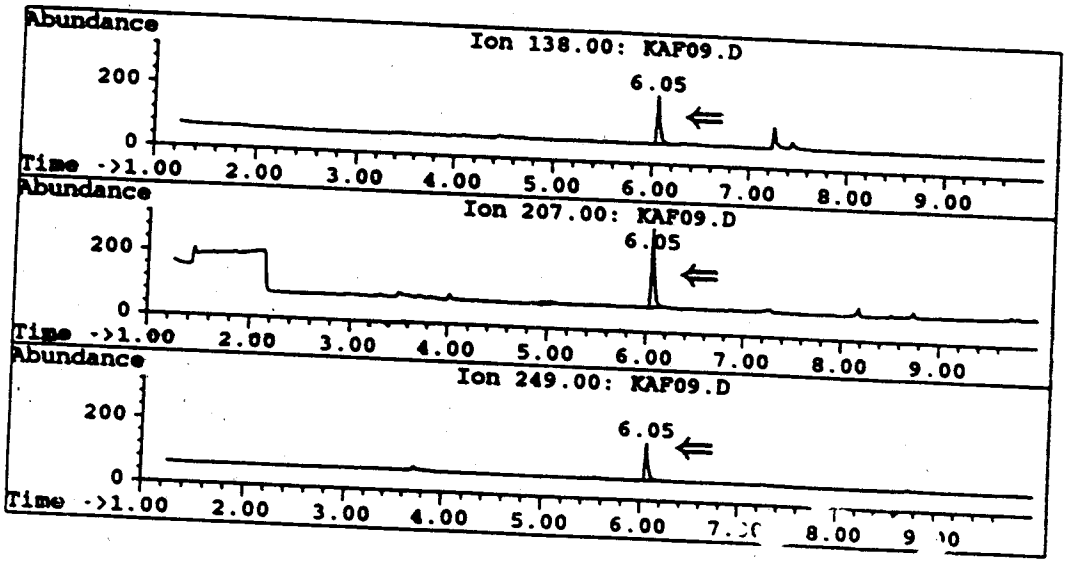
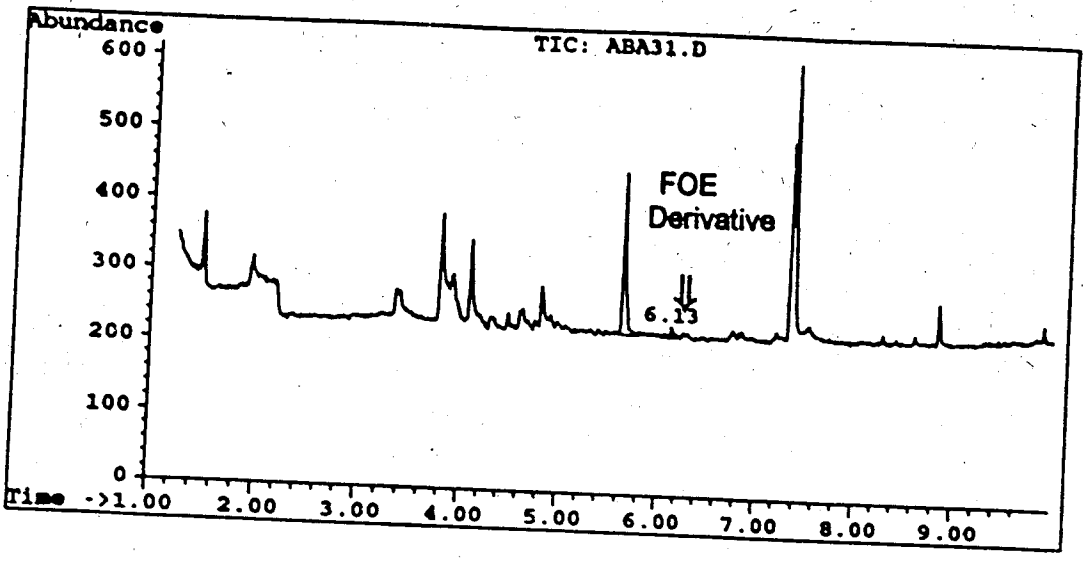


Figure 7 (cont). C and D: Corn forage sample amended with 0.10 ppm of FOE oxalate.

A. Total ion chromatogram of a corn grain control sample.



B. Single ion chromatograms for m/z of 138, 207, and 249.

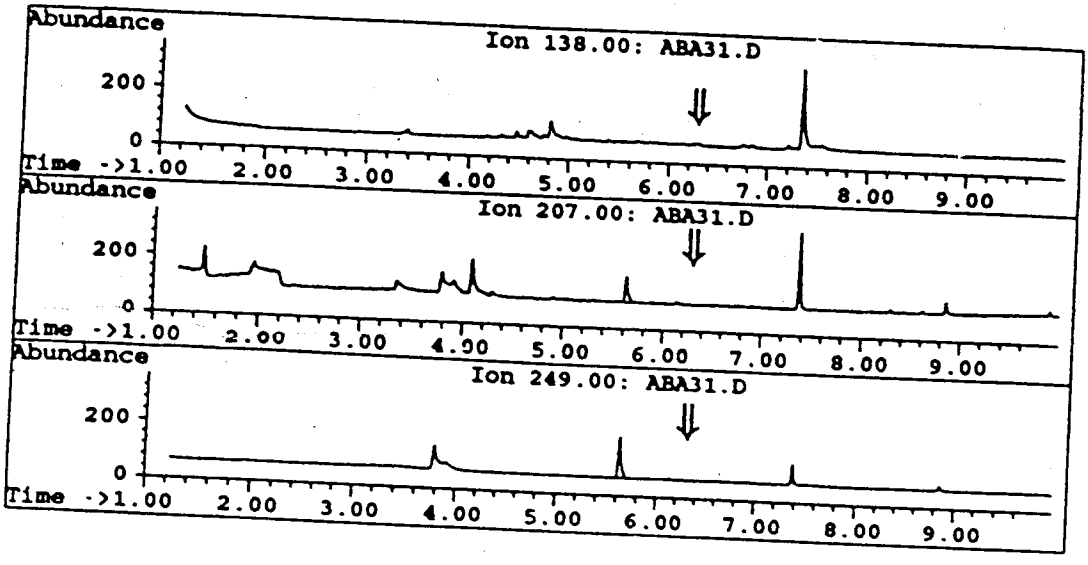
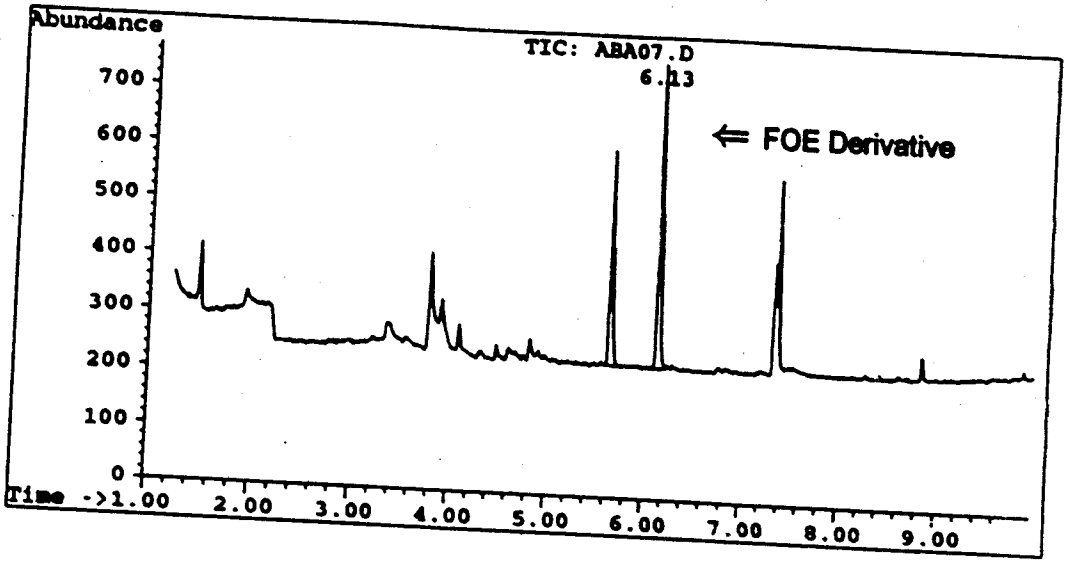


Figure 8. Gc/ms chromatograms of typical corn grain control and recovery samples.
A and B: Corn grain control sample.

C. Total ion chromatogram of a corn grain recovery sample.



D. Single ion chromatograms for m/z of 138, 207, and 249.

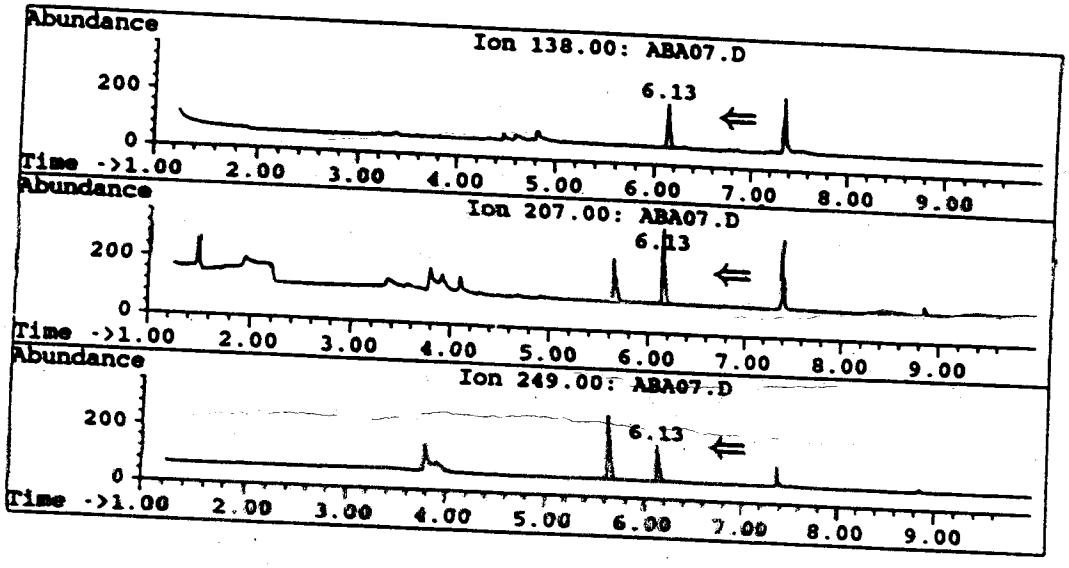
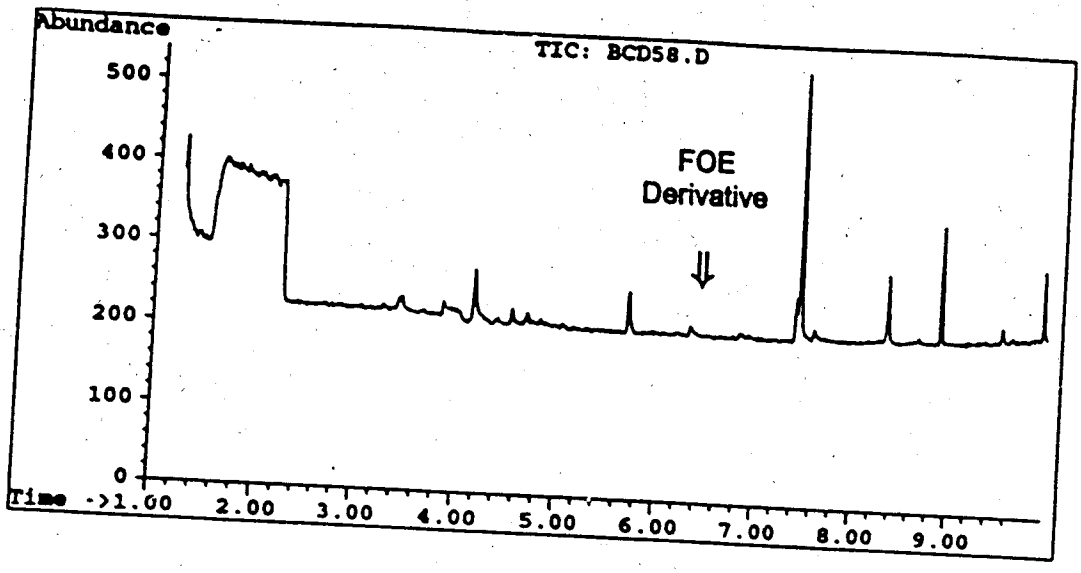


Figure 8 (cont). C and D: Corn grain sample amended with 0.10 ppm of FOE 5043.

A. Total ion chromatogram of a corn fodder control sample.



B. Single ion chromatograms for m/z of 138, 207, and 249.

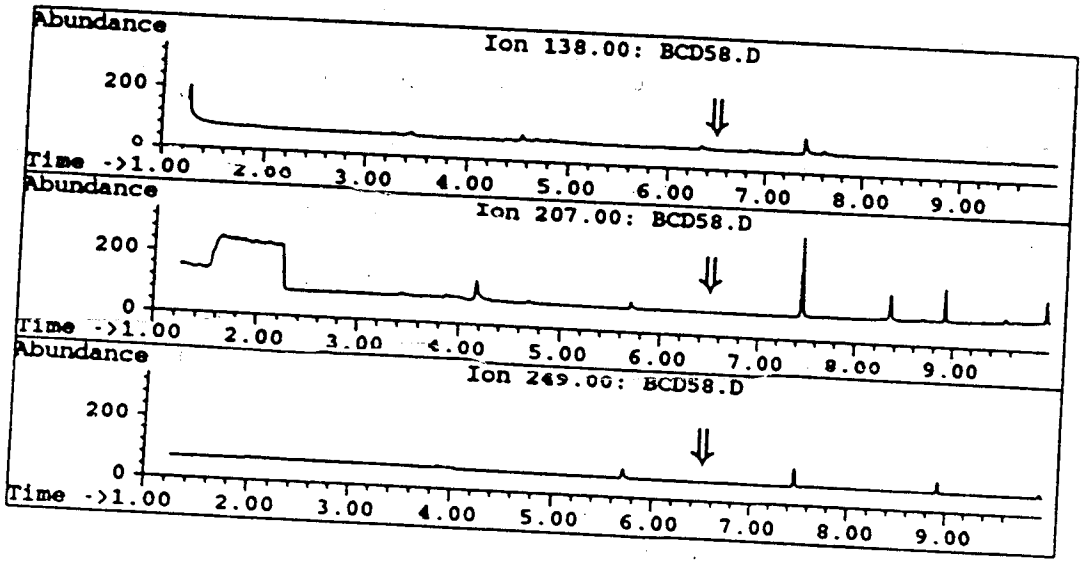
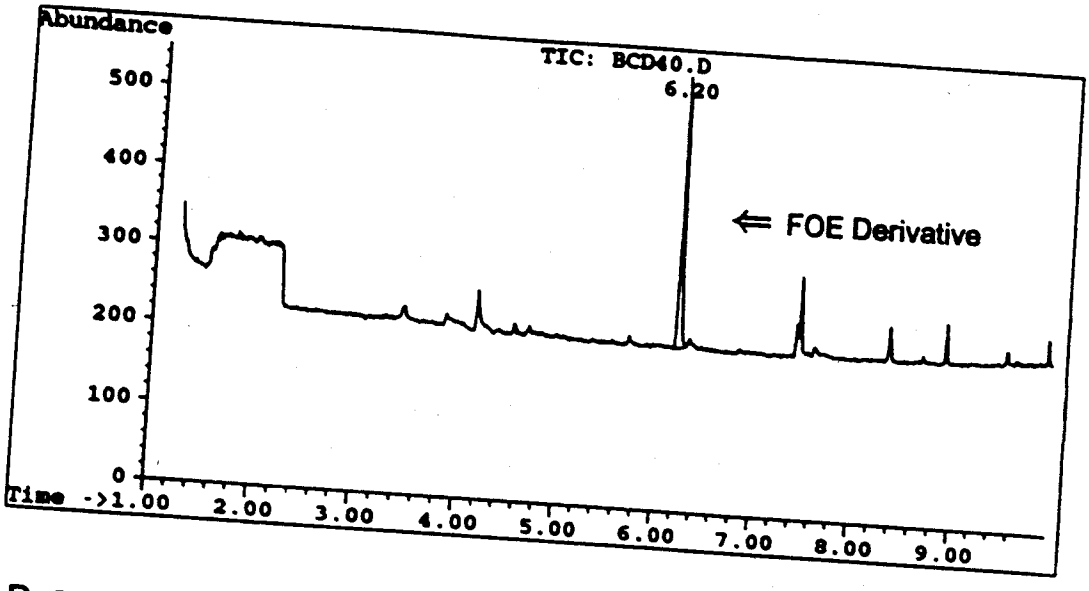


Figure 9. Gc/ms chromatograms of typical corn fodder control and recovery samples.
A and B: Corn fodder control sample.

C. Total ion chromatogram of a corn fodder recovery sample.



D. Single ion chromatograms for m/z of 138, 207, and 249.

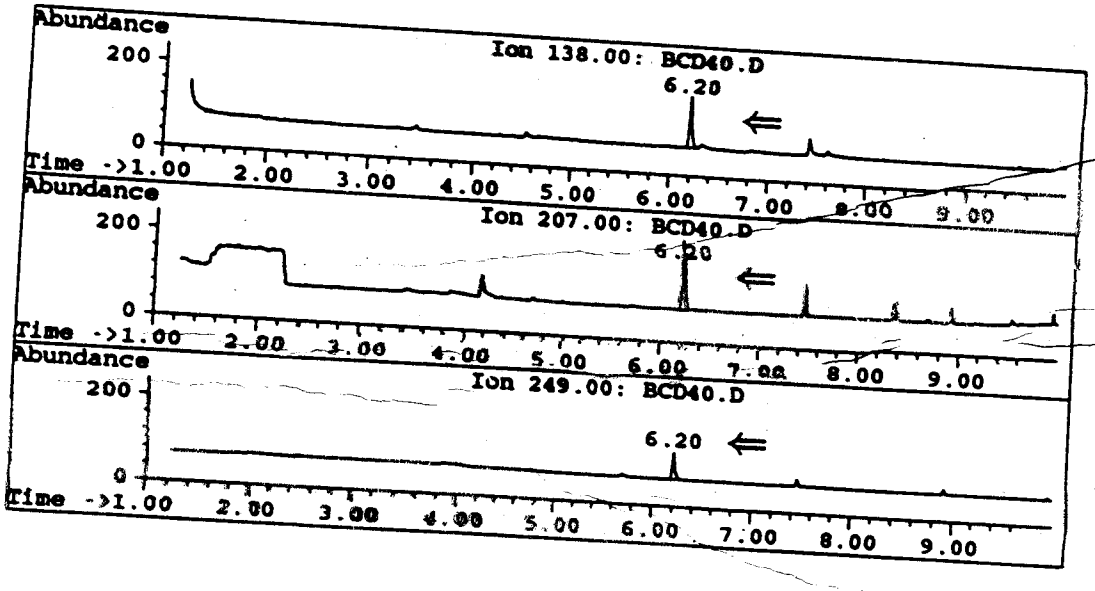
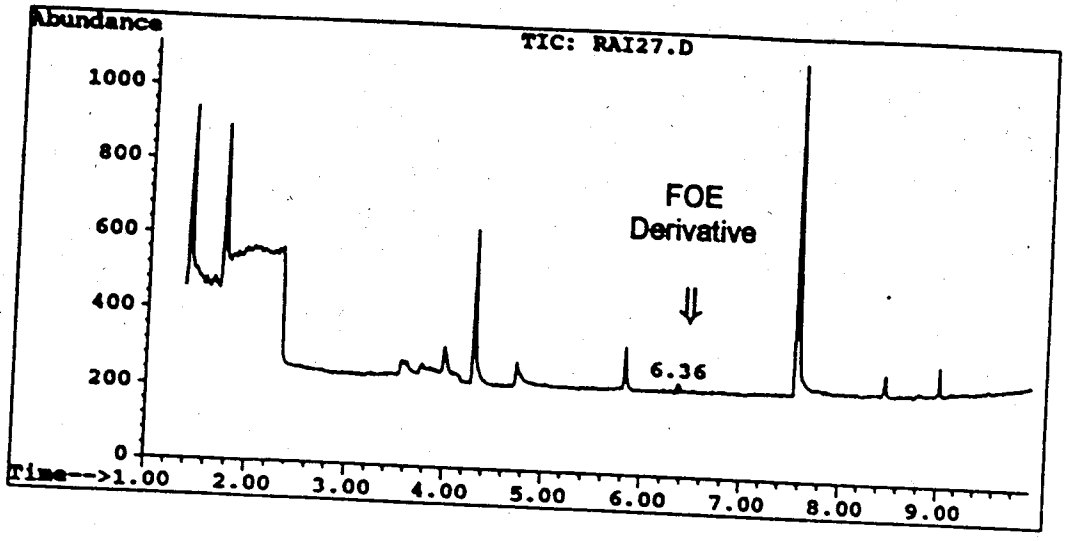


Figure 9 (cont). C and D: Corn fodder sample amended with 0.10 ppm of mixed standards.

A. Total ion chromatogram of a corn oil control sample.



B. Single ion chromatograms for m/z of 138, 207, and 249.

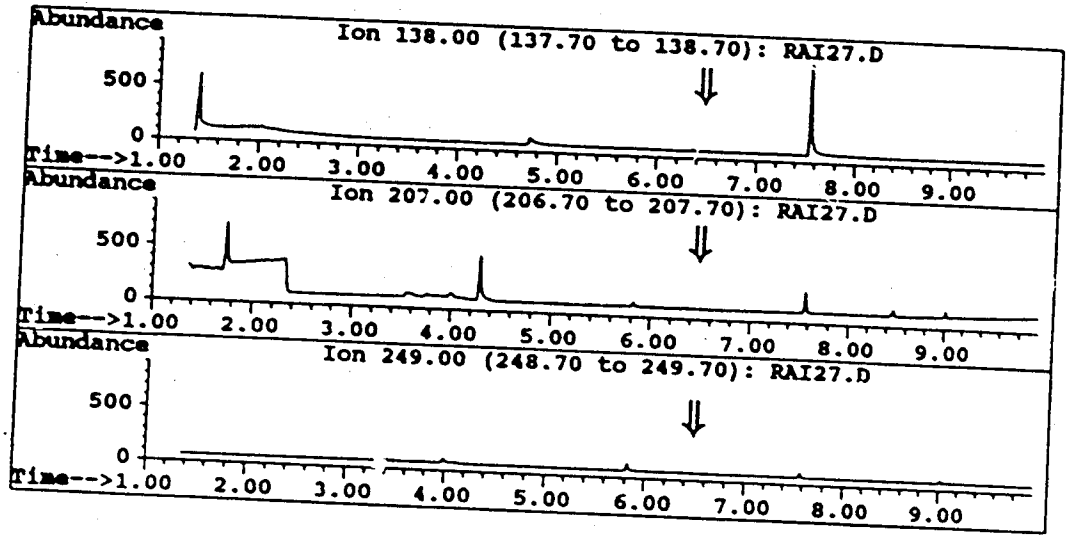
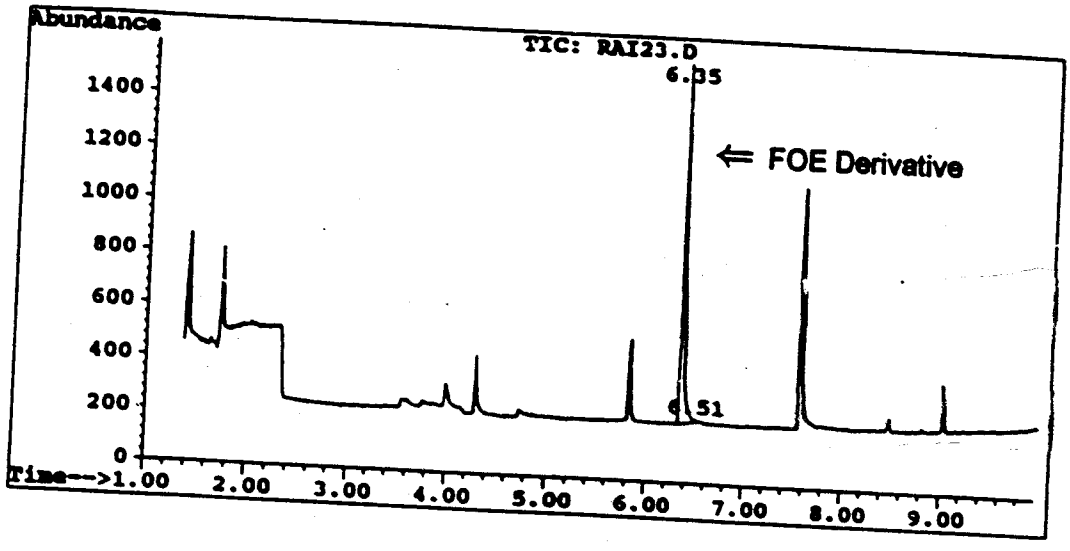


Figure 10. Gc/ms chromatograms of typical corn oil control and recovery samples. A and B: Corn oil control sample.

C. Total ion chromatogram of a corn oil recovery sample.



D. Single ion chromatograms for m/z of 138, 207, and 249.

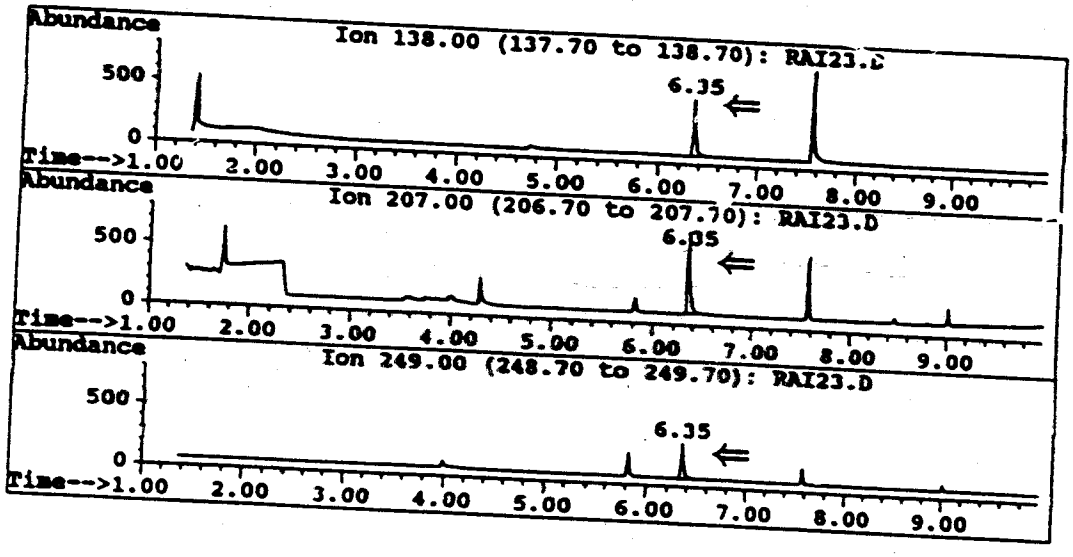
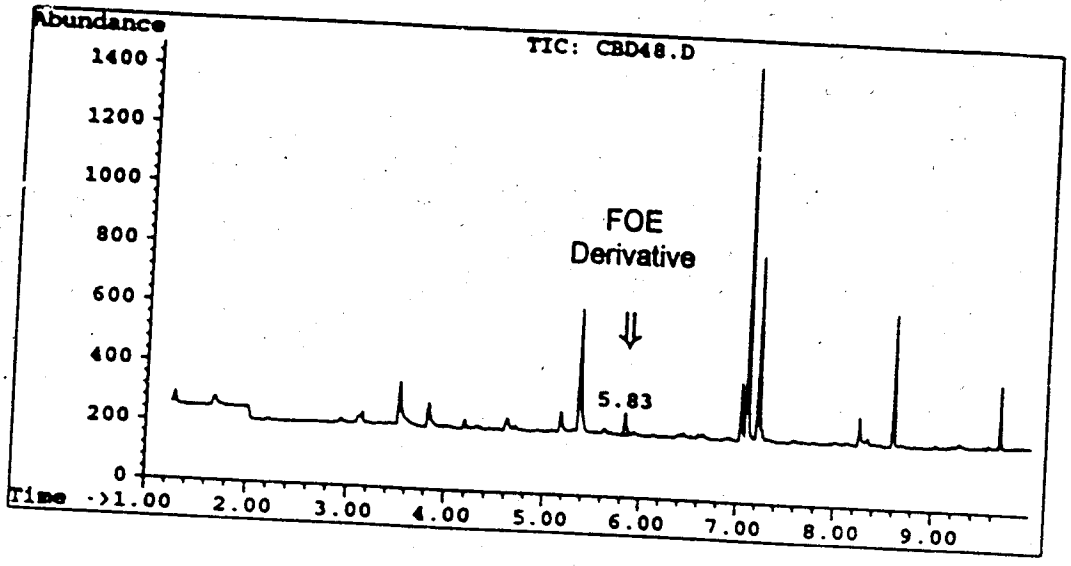


Figure 10 (cont). C and D: Corn oil sample amended with 0.10 ppm of FOE thioglycolate sulfoxide.

A. Total ion chromatogram of a peanut nutmeat control sample.



B. Single ion chromatograms for m/z of 138, 207, and 249.

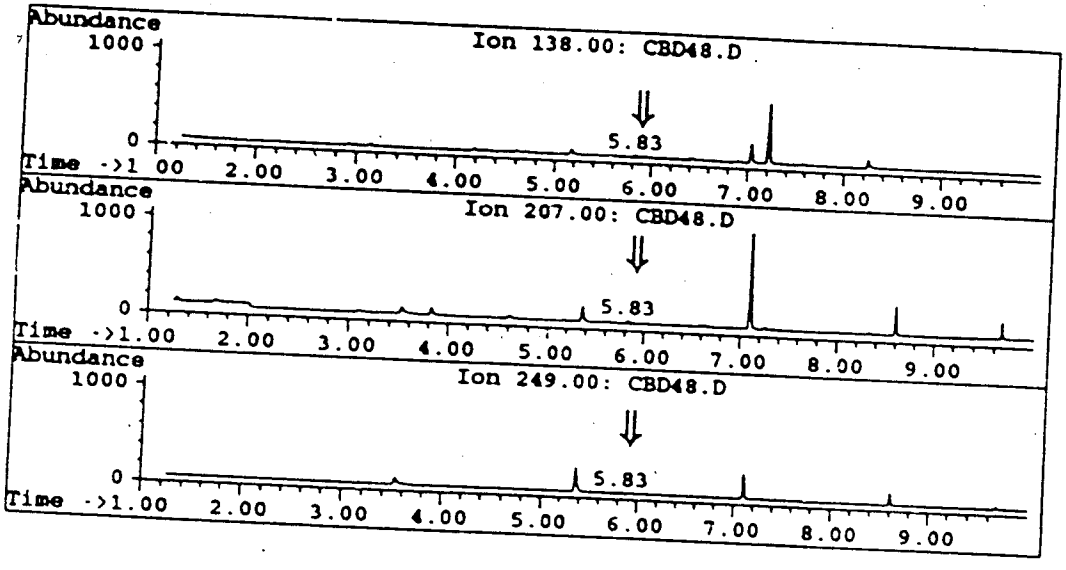
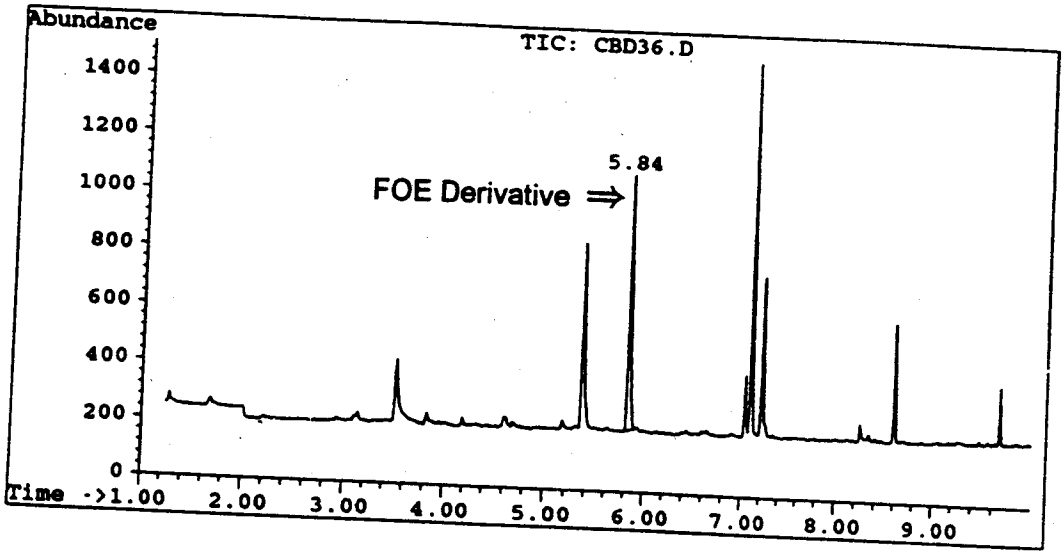


Figure 11. Gc/ms chromatograms of typical peanut nutmeat control and recovery samples.
A and B: Peanut · ıtmeat control sample.

C. Total ion chromatogram of a peanut nutmeat recovery sample.



D. Single ion chromatograms for m/z of 138, 207, and 249.

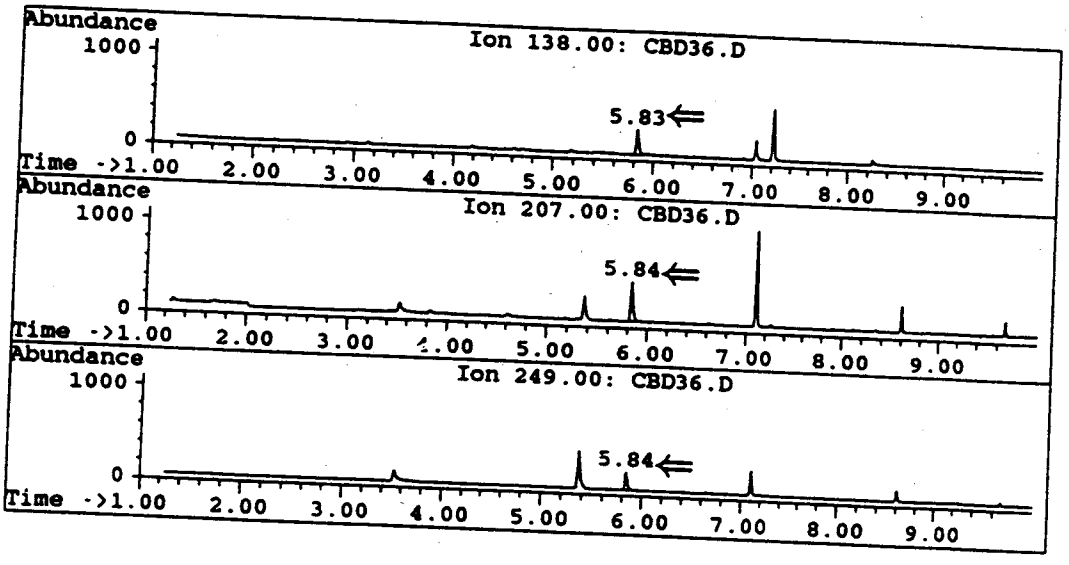
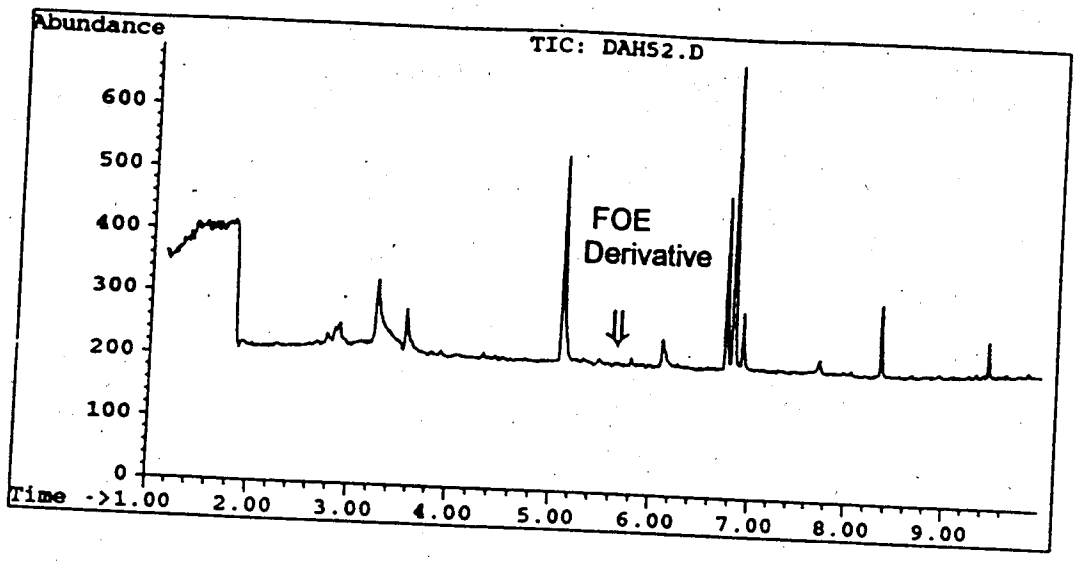


Figure 11 (cont). C and D: Peanut nutmeat sample amended with 0.10 ppm of mixed standards.

A. Total ion chromatogram of a spinach tops control sample.



B. Single ion chromatograms for m/z of 138, 207, and 249.

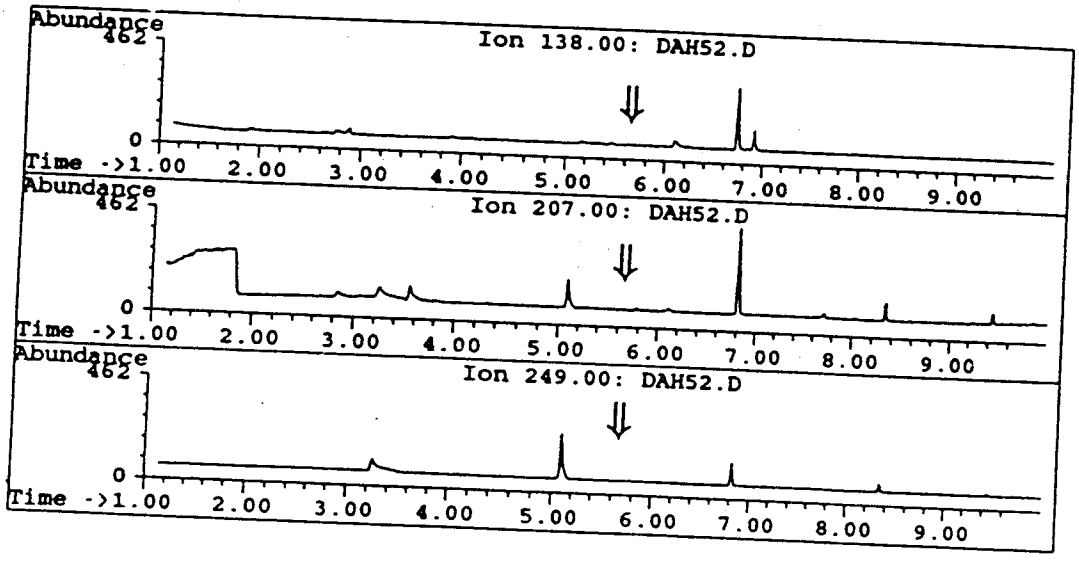
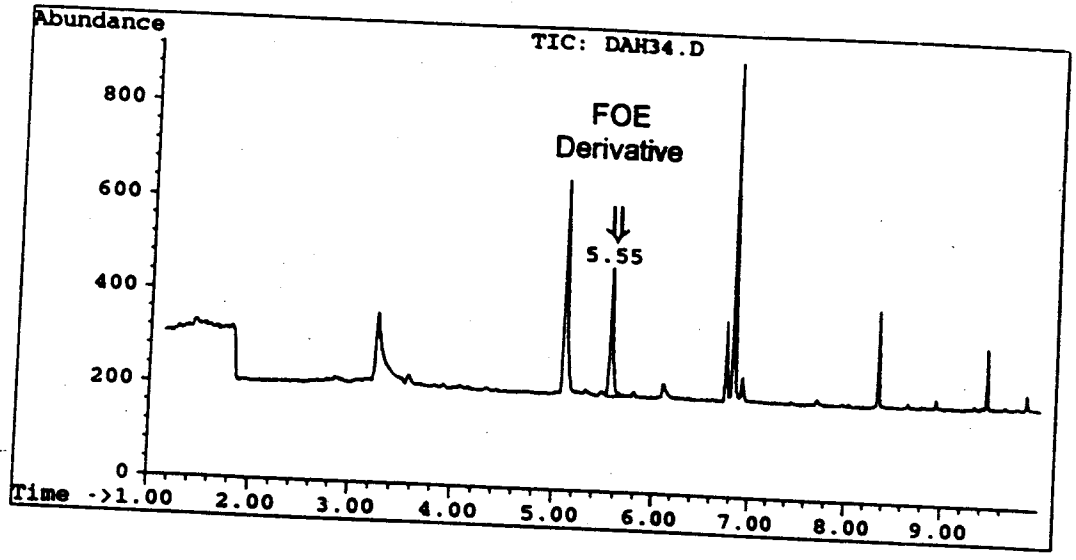


Figure 12. Gc/ms chromatograms of typical spinach tops control and recovery samples.
A and B: Spinach tops control sample.

C. Total ion chromatogram of a spinach tops recovery sample.



D. Single ion chromatograms for m/z of 138, 207, and 249.

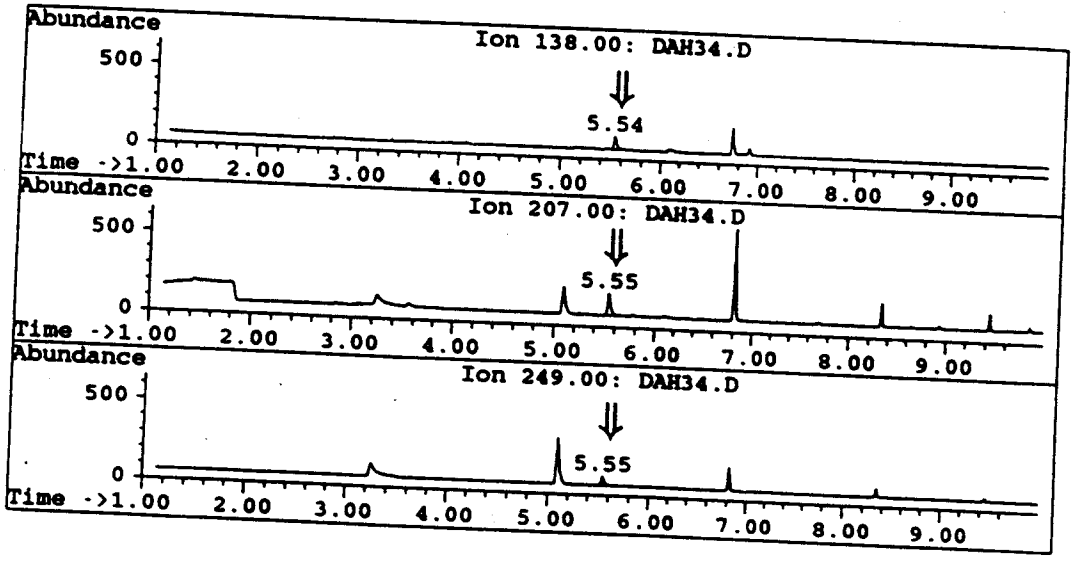
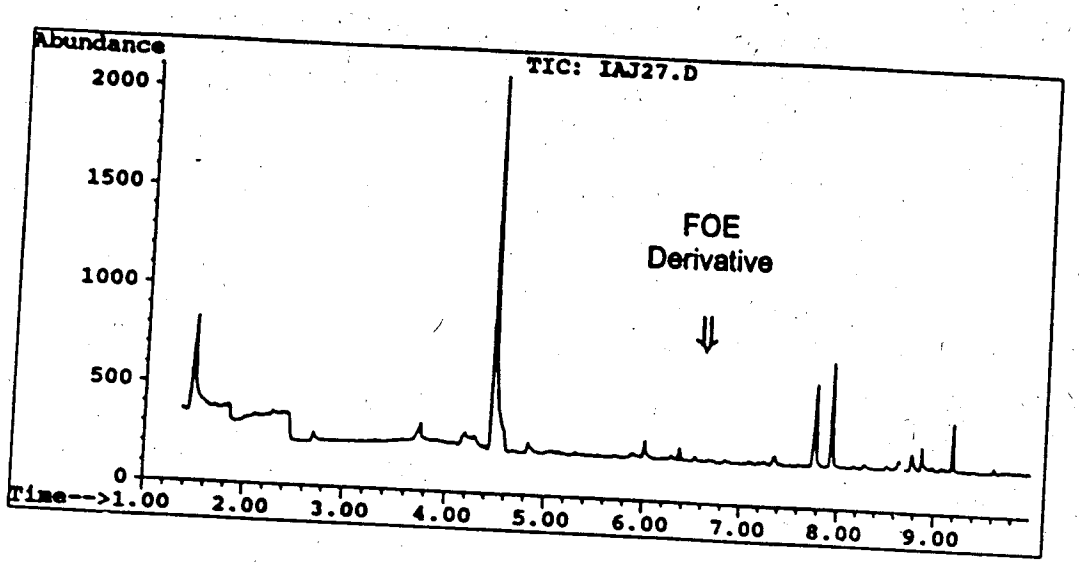


Figure 12 (cont). C and D: Spinach tops sample amended with 0.05 ppm of mixed standards.

A. Total ion chromatogram of a soybean forage control sample.



B. Single ion chromatograms for m/z of 138, 207, and 249.

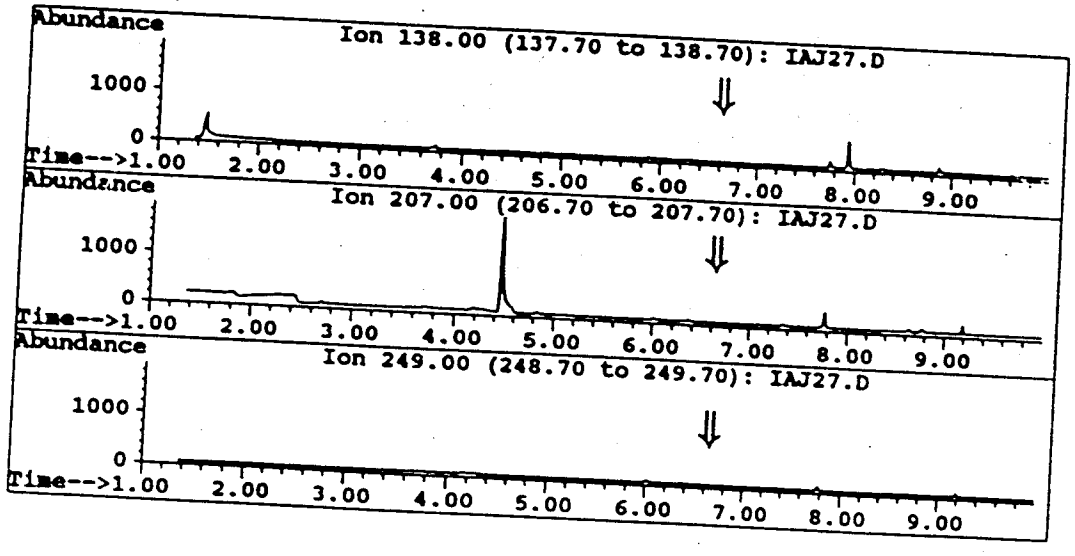
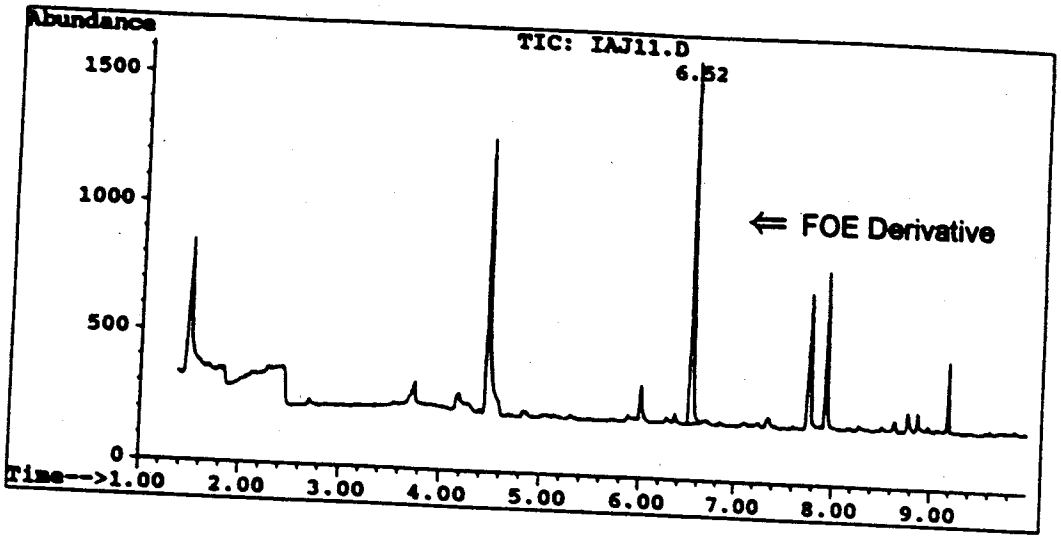


Figure 13. Gc/ms chromatograms of typical soybean forage control and recovery samples.
A and B: Soybean forage control sample.

C. Total ion chromatogram of a soybean forage recovery sample.



D. Single ion chromatograms for m/z of 138, 207, and 249.

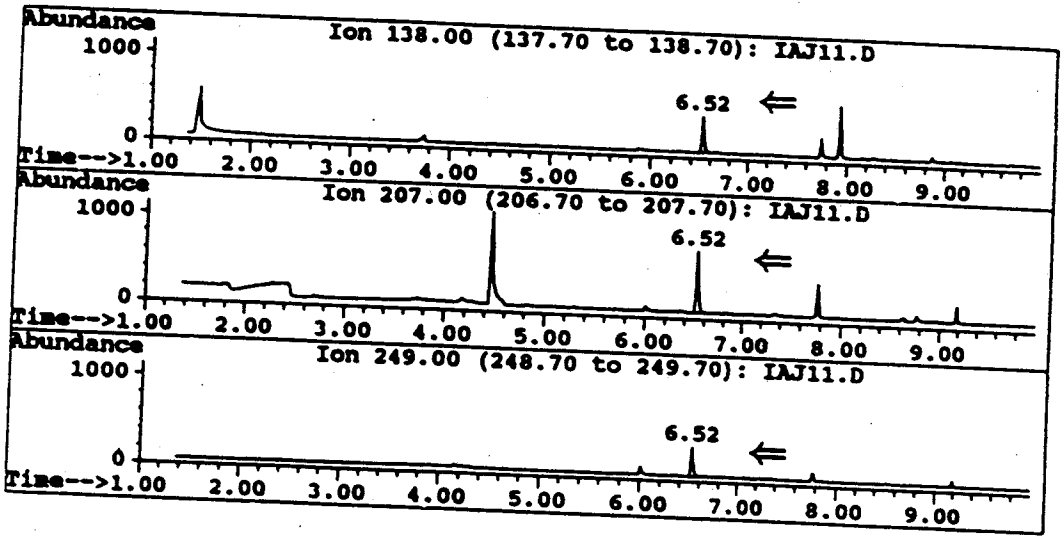
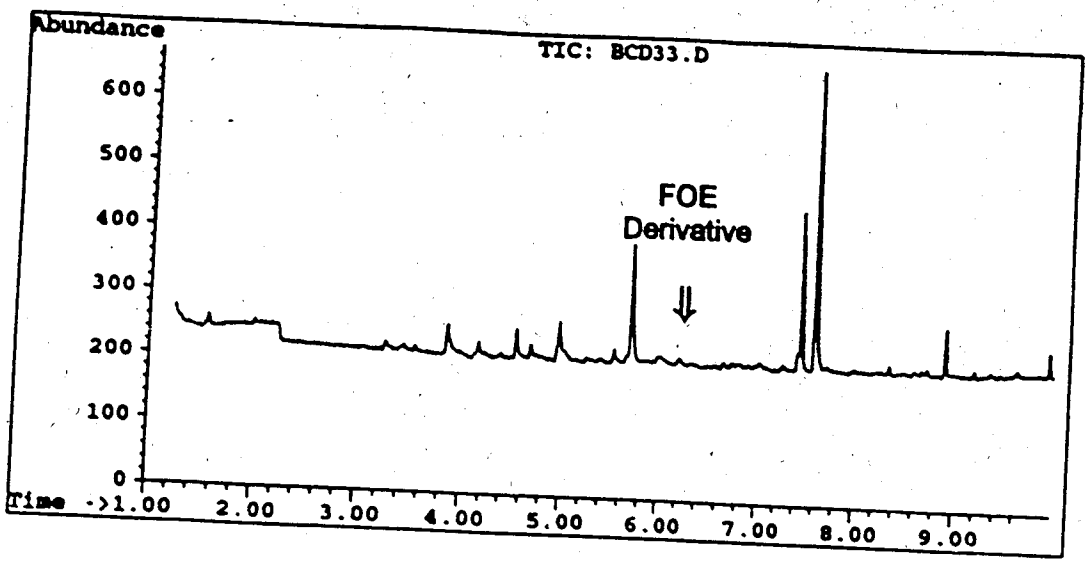


Figure 13 (cont). C and D: Soybean forage sample amended with 0.10 ppm of FOE oxalate.

A. Total ion chromatogram of a soybean seed control sample.



B. Single ion chromatograms for m/z of 138, 207, and 249.

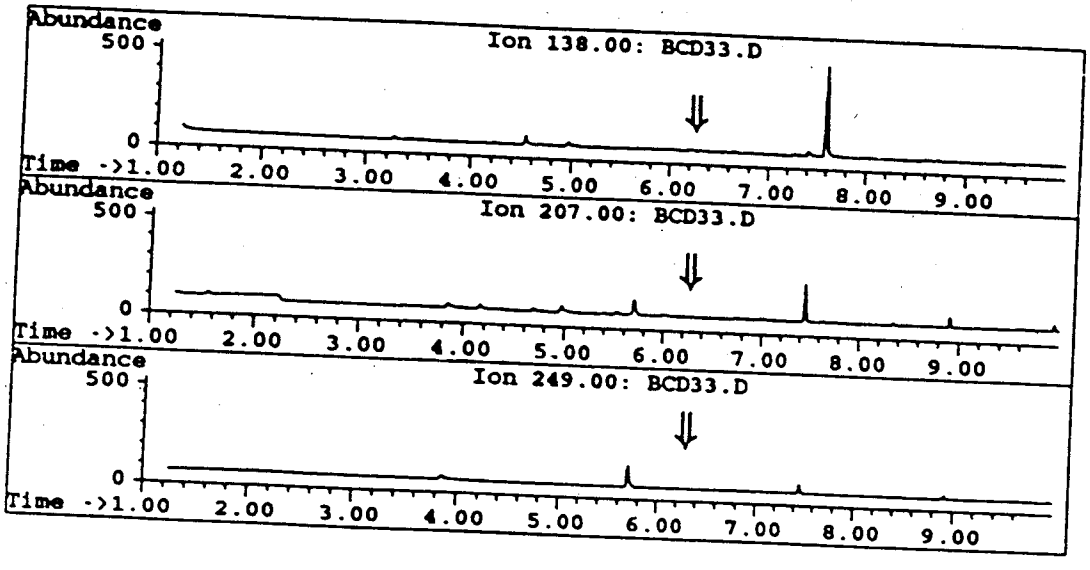
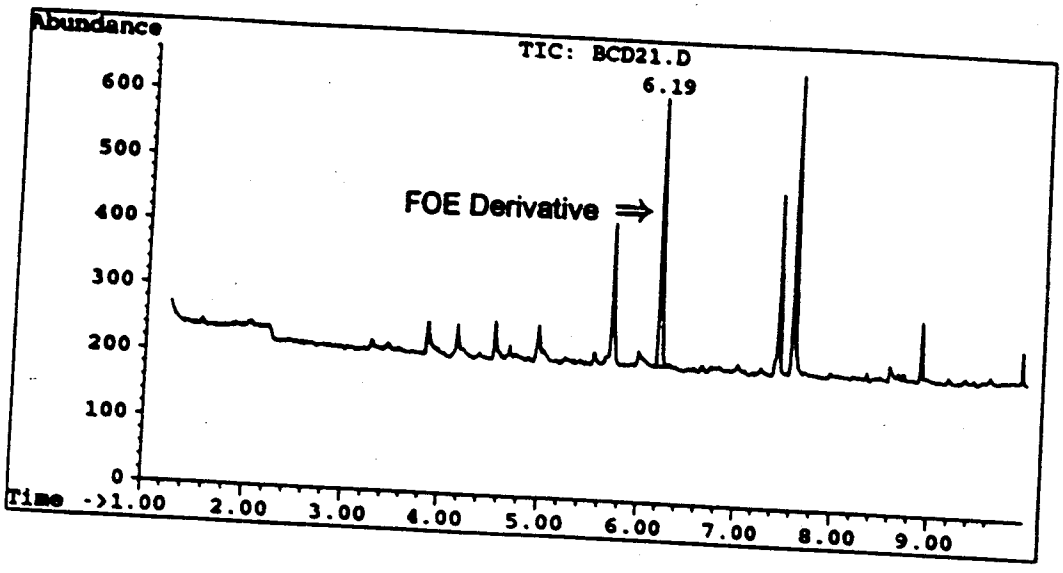


Figure 14. Gc/ms chromatograms of typical soybean seed control and recovery samples.
A and B: Soybean seed control sample.

C. Total ion chromatogram of a soybean seed recovery sample.



D. Single ion chromatograms for m/z of 138, 207, and 249.

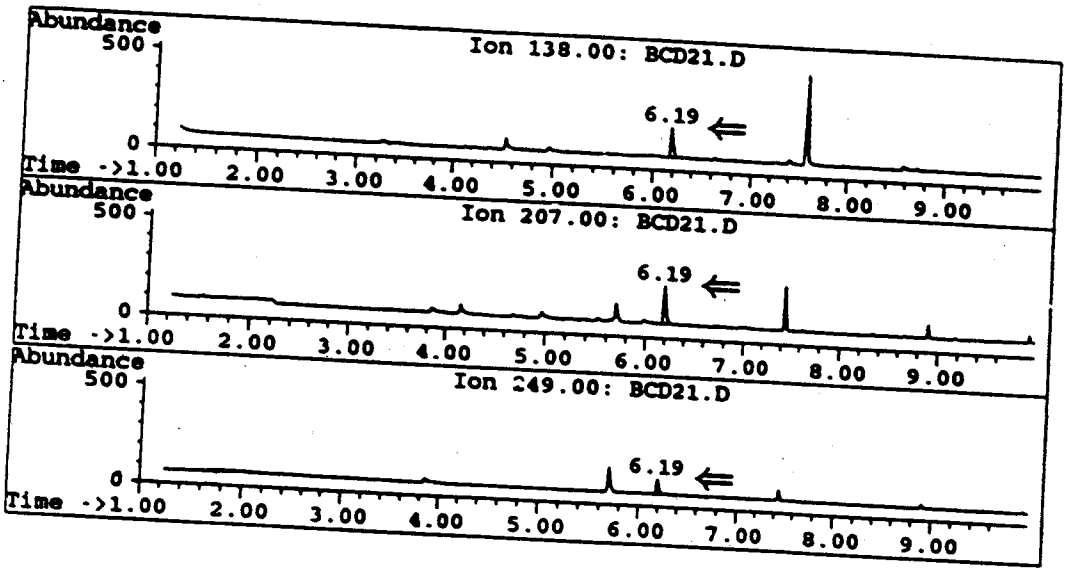
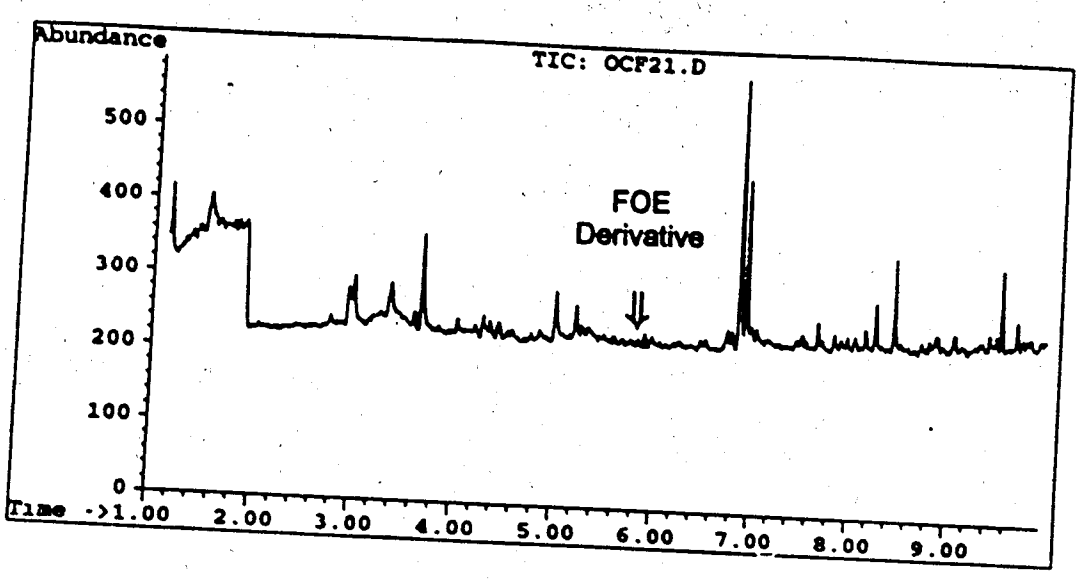


Figure 14 (cont). C and D: Soybean seed sample amended with 0.10 ppm of FOE sulfonic acid.

A. Total ion chromatogram of a soybean soapstock control sample.



B. Single ion chromatograms for m/z of 138, 207, and 249.

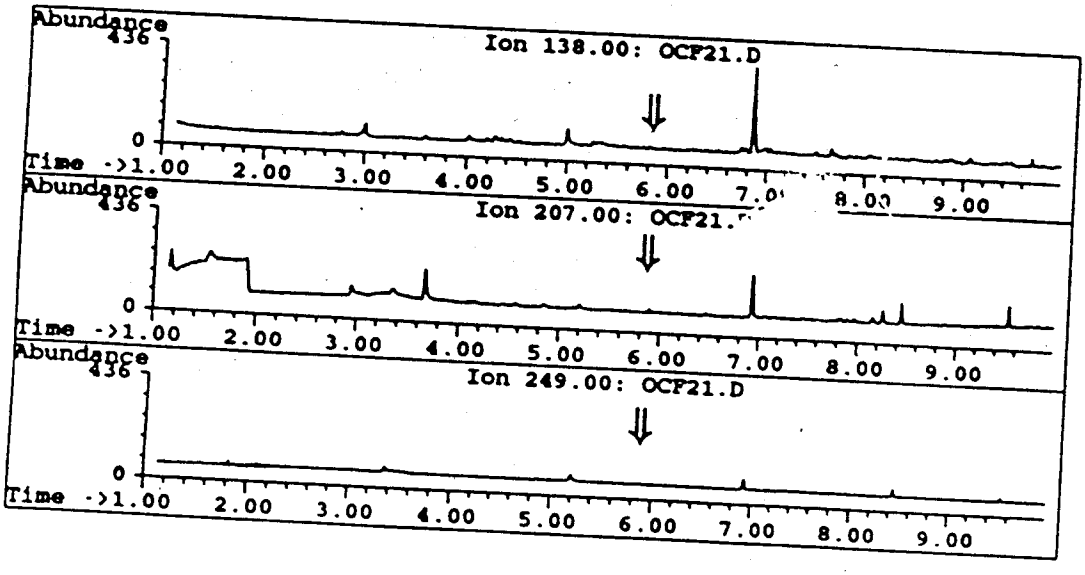
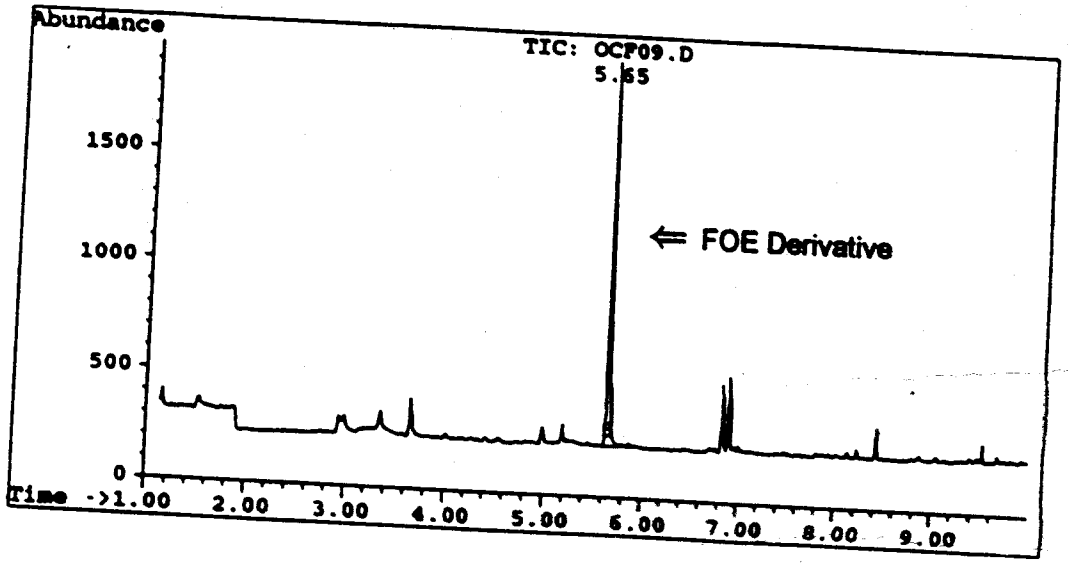


Figure 15. Gc/ms chromatograms of typical soybean soapstock control and recovery samples.
A and B: Soybean soapstock control sample.

C. Total ion chromatogram of a soybean soapstock recovery sample.



D. Single ion chromatograms for m/z of 138, 207, and 249.

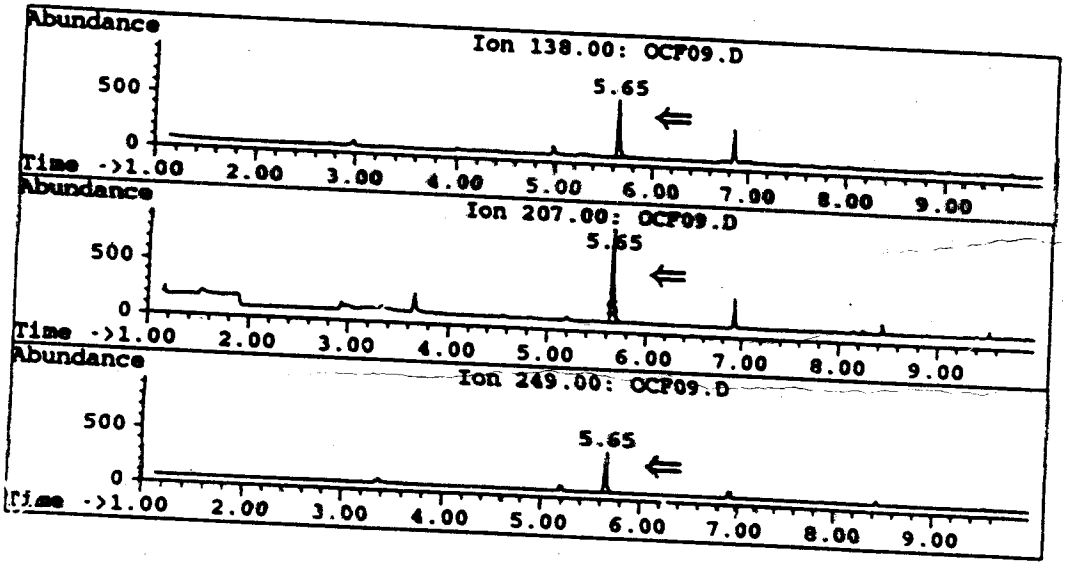
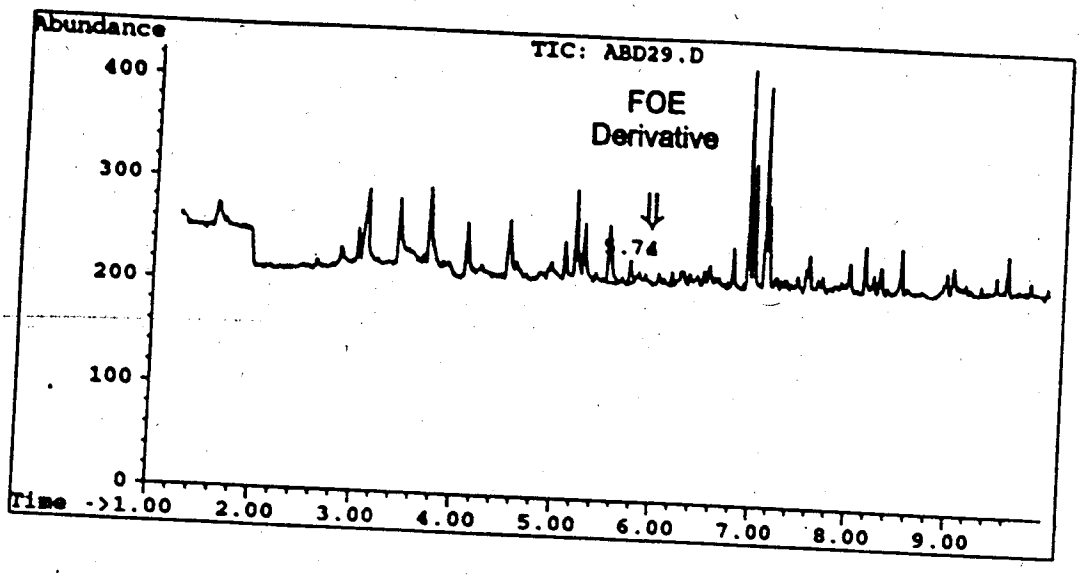


Figure 15 (cont). C and D: Soybean soapstock sample amended with 0.10 ppm of mixed standards.

A. Total ion chromatogram of a suflower seed control sample.



B. Single ion chromatograms for m/z of 138, 207, and 249.

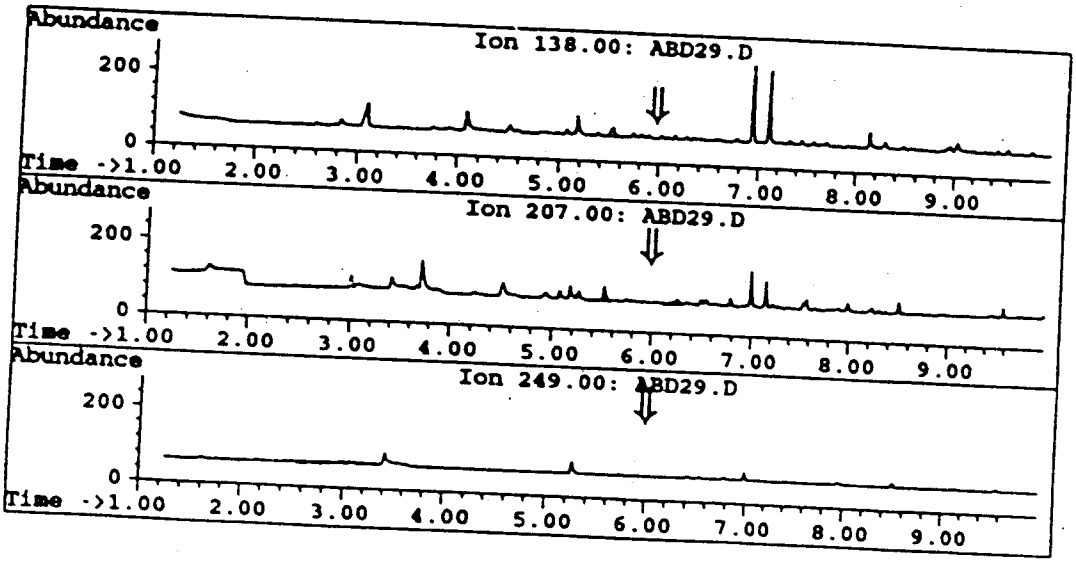
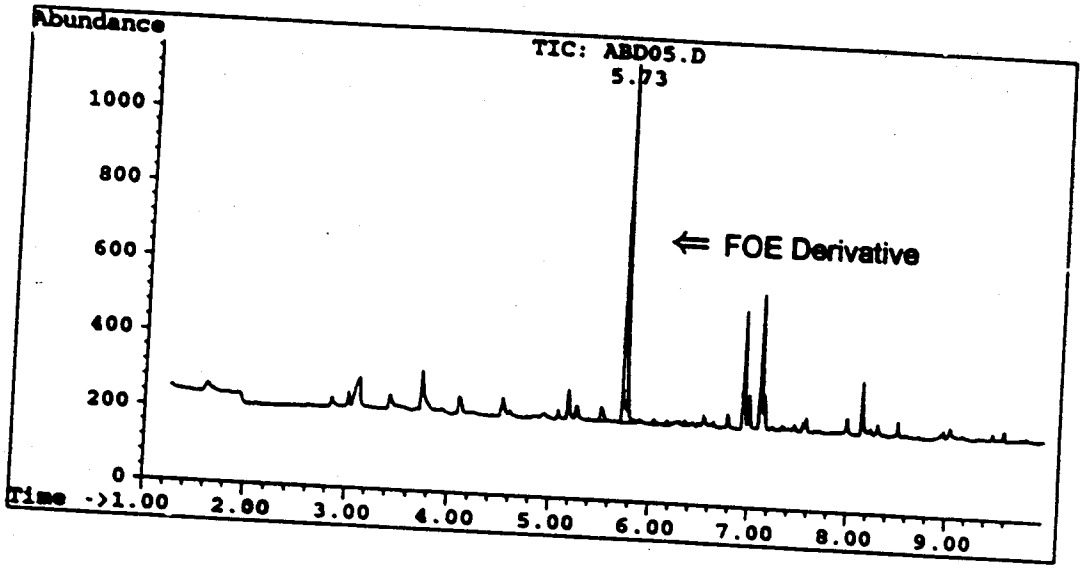


Figure 16. Gc/ms chromatograms of typical sunflower seed control and recovery samples.
A and B: Sunflower seed control sample.

C. Total ion chromatogram of a sunflower seed recovery sample.



D. Single ion chromatograms for m/z of 138, 207, and 249.

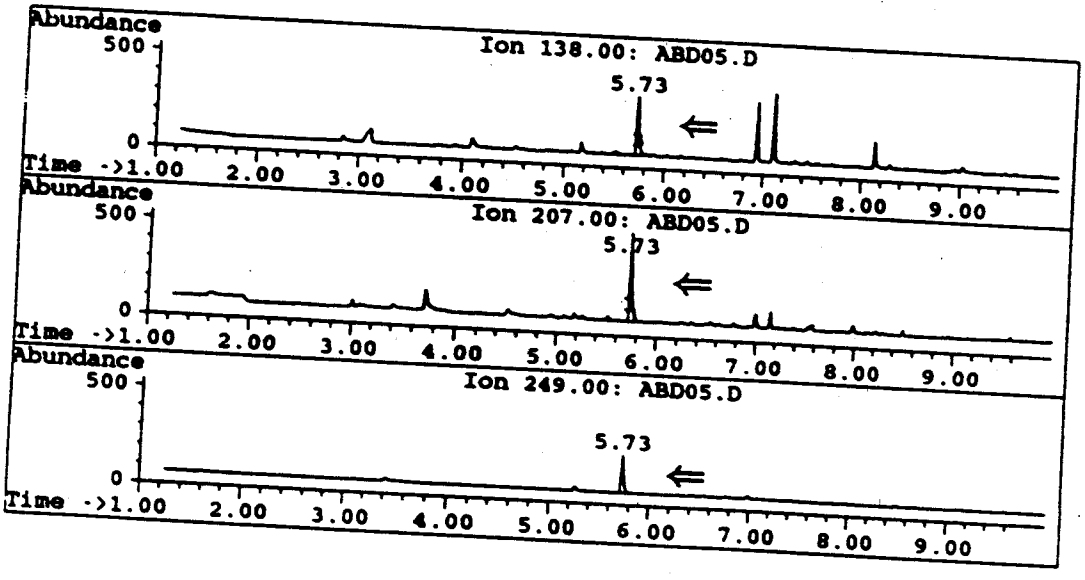
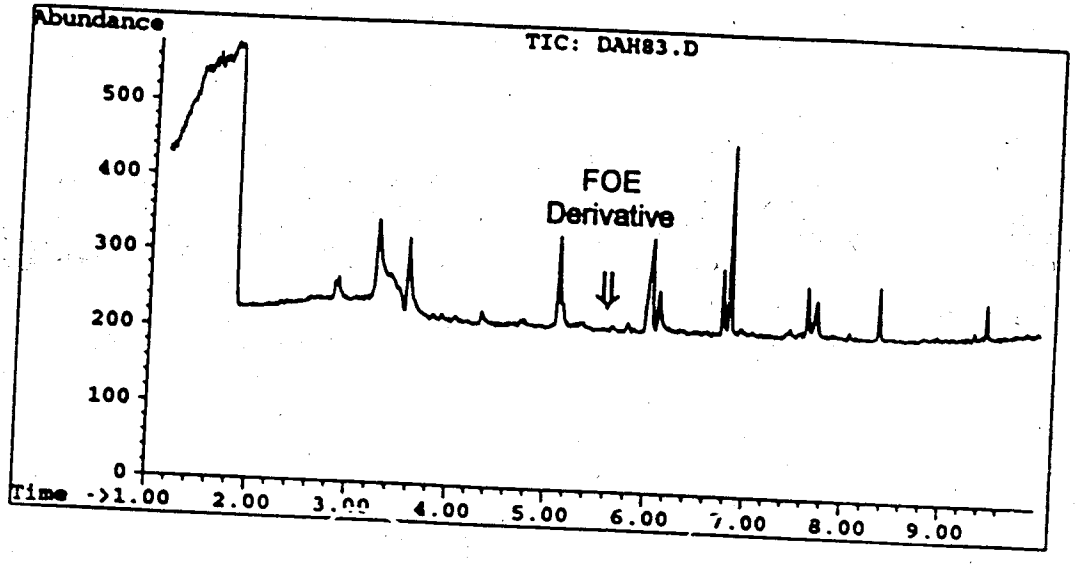


Figure 16 (cont). C and D: Sunflower seed sample amended with 0.10 ppm of FOE 5043.

A. Total ion chromatogram of a turnip roots control sample.



B. Single ion chromatograms for m/z of 136, 207, and 249.

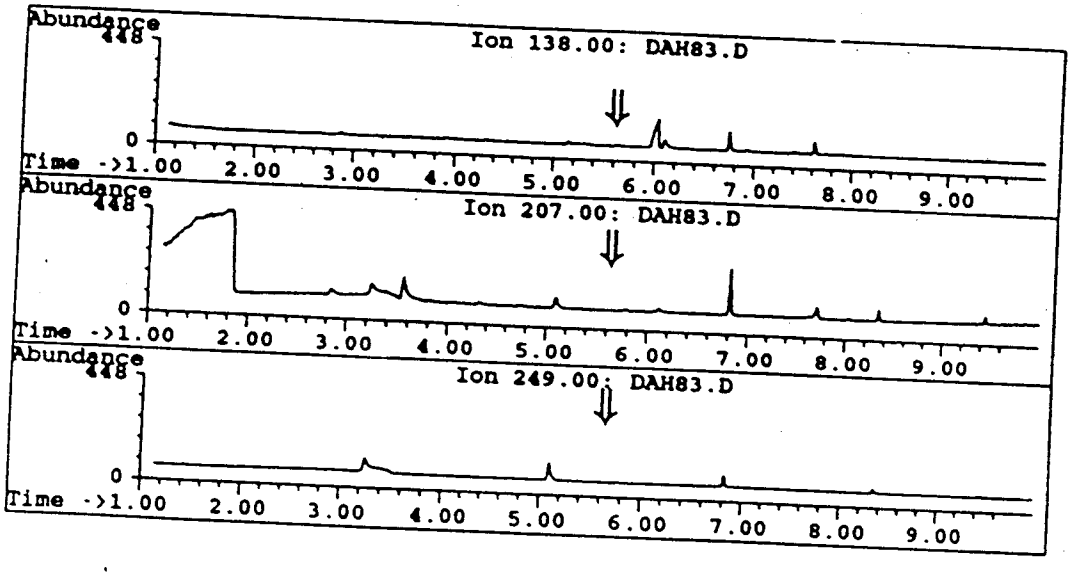
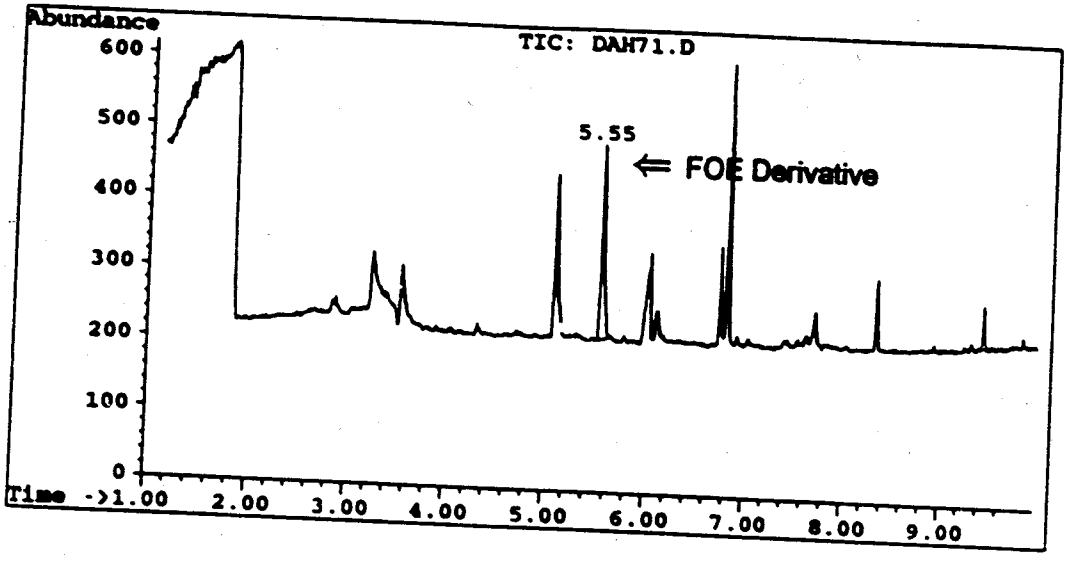


Figure 17. Gc/ms chromatograms of typical turnip roots control and recovery samples.
A and B: Turnip roots control sample.

C. Total ion chromatogram of a turnip roots recovery sample.



D. Single ion chromatograms for m/z of 138, 207, and 249.

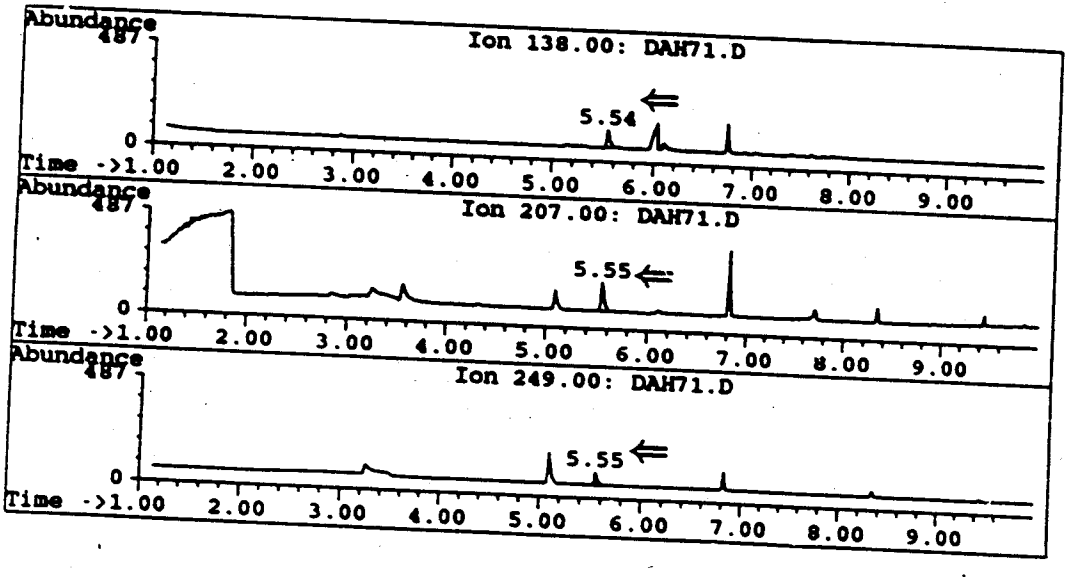
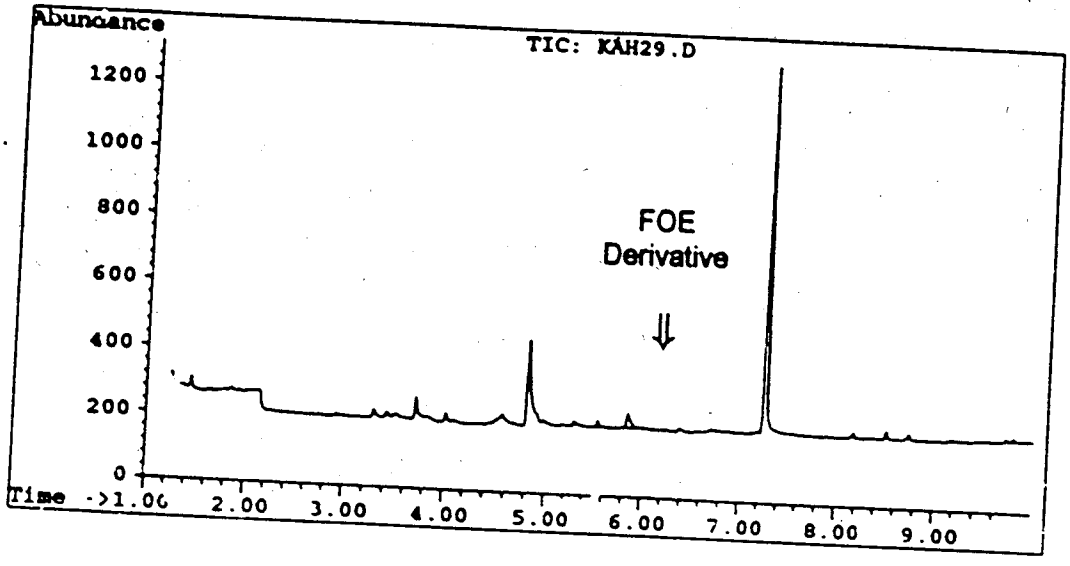


Figure 17 (cont). C and D: Turnip roots sample amended with 0.05 ppm of mixed standards.

A. Total ion chromatogram of a wheat grain control sample.



B. Single ion chromatograms for m/z of 138, 207, and 249.

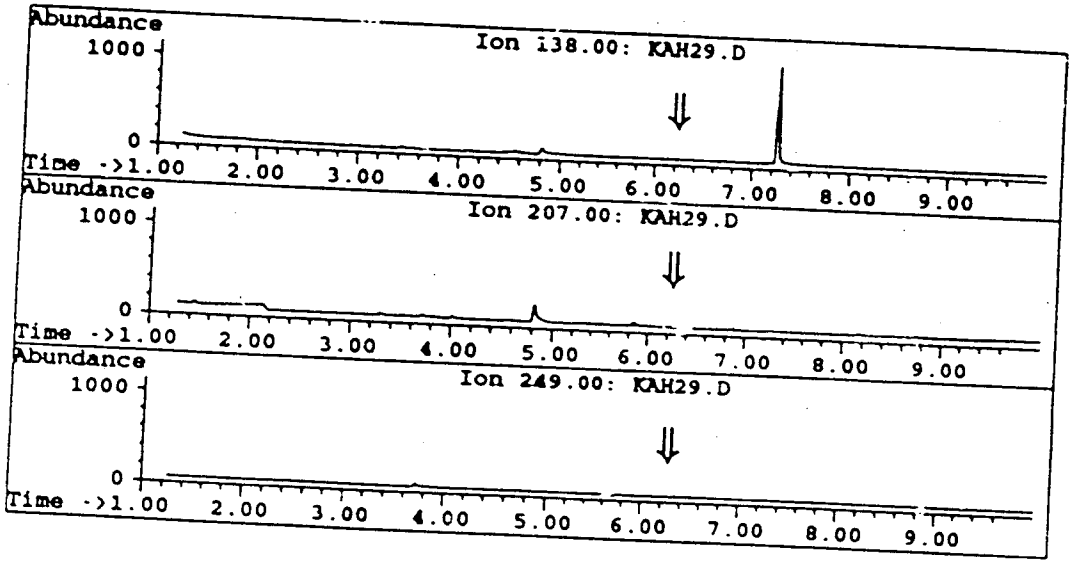
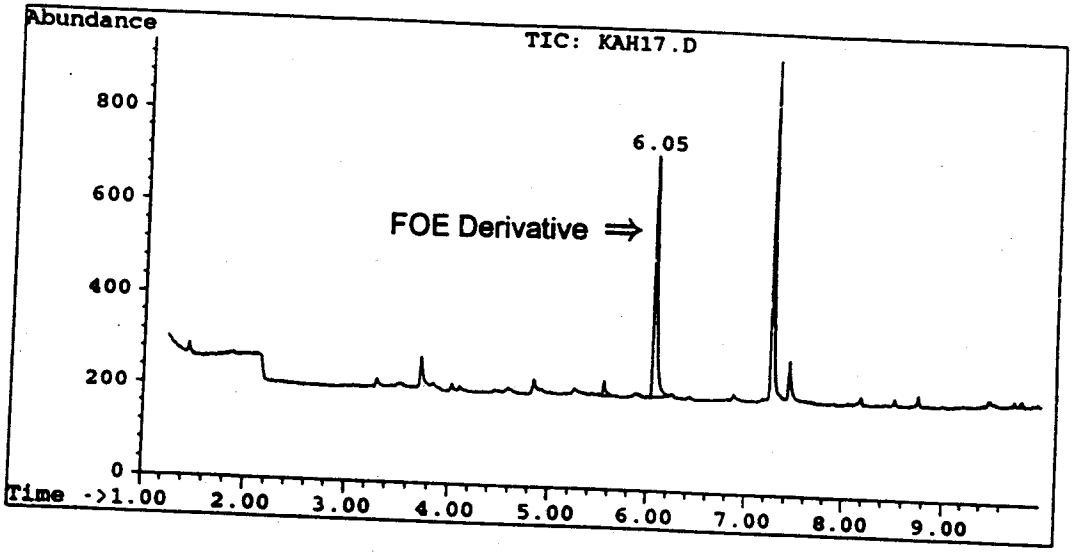


Figure 18. Gc/ms chromatograms of typical wheat grain control and recovery samples.
A and B: Wheat grain control sample

C. Total ion chromatogram of a wheat grain recovery sample.



D. Single ion chromatograms for m/z of 138, 207, and 249.

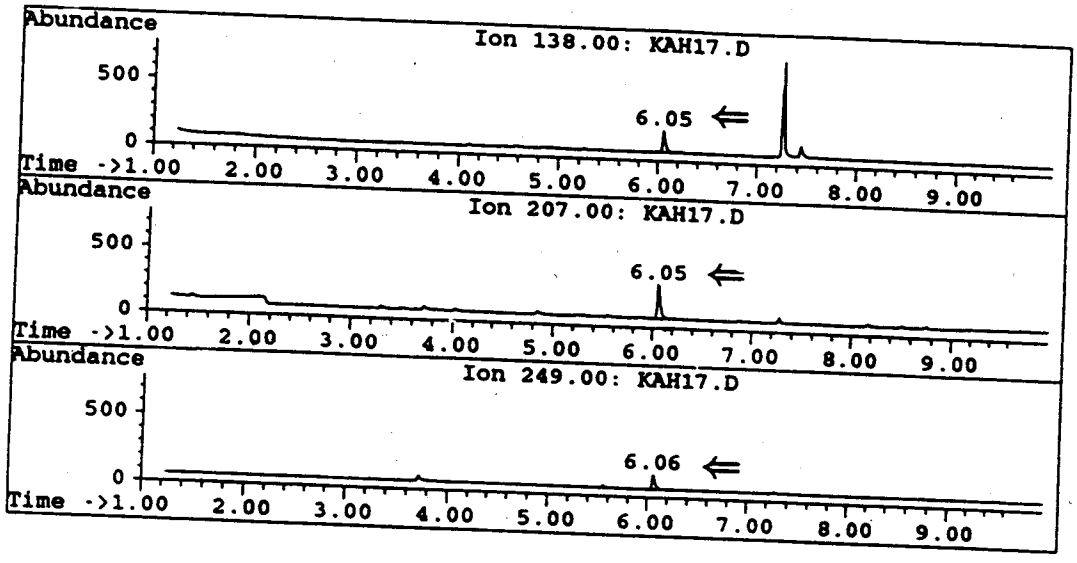
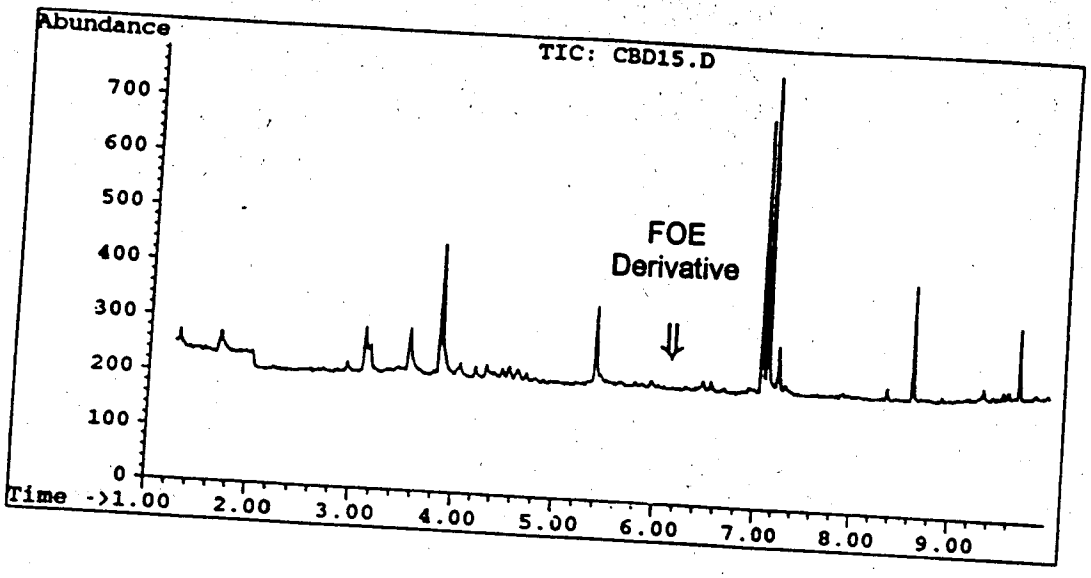


Figure 18 (cont). C and D: Wheat grain sample amended with 0.10 ppm of FOE sulfonic acid.

A. Total ion chromatogram of a wheat straw control sample.



B. Single ion chromatograms for m/z of 138, 207, and 249.

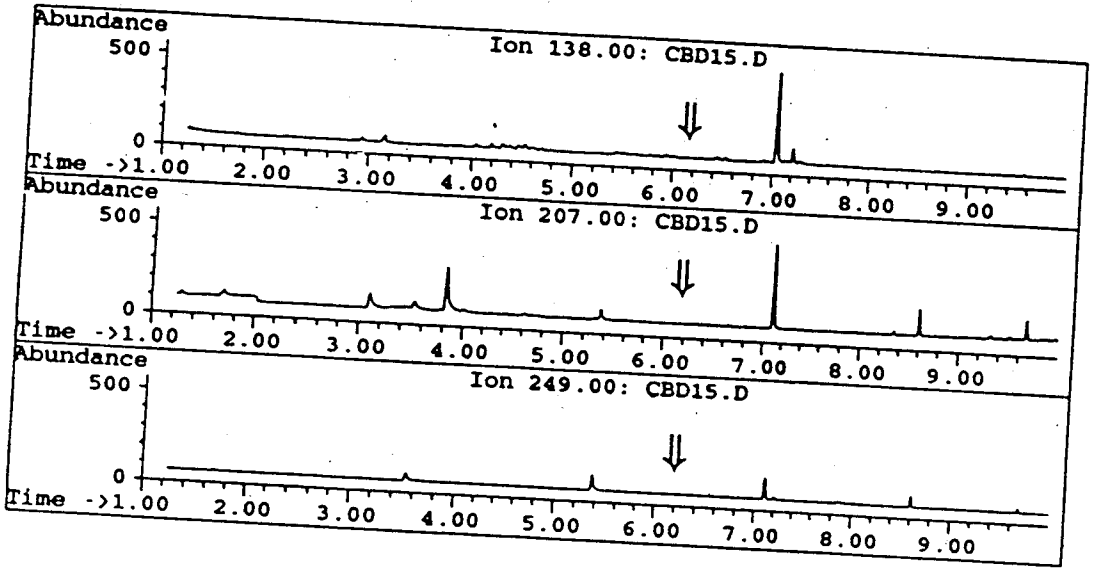
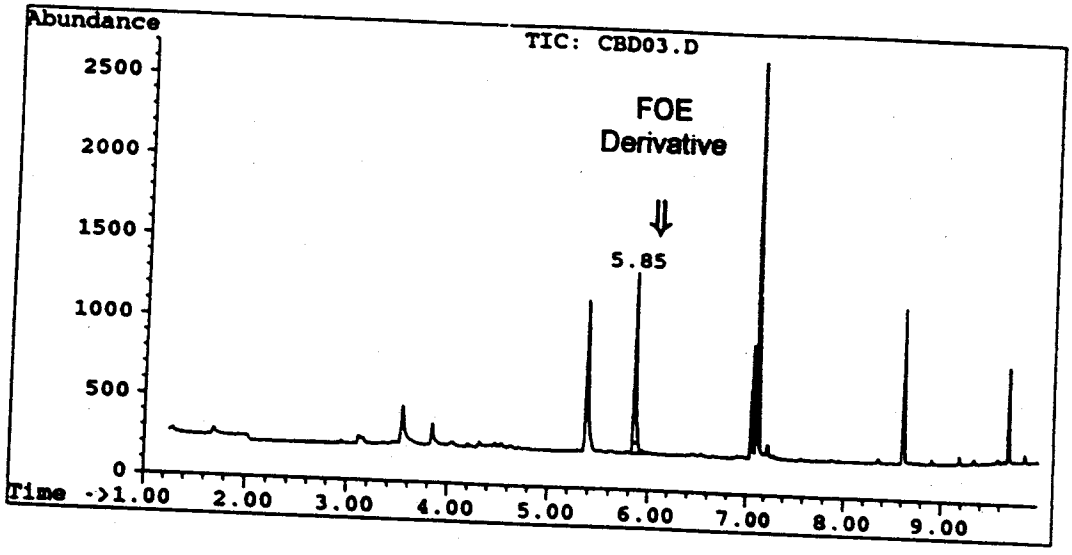


Figure 19. Gc/ms chromatograms of typical wheat straw control and recovery samples.
A and B: Wheat straw control sample.

C. Total ion chromatogram of a wheat straw recovery sample.



D. Single ion chromatograms for m/z of 138, 207, and 249.

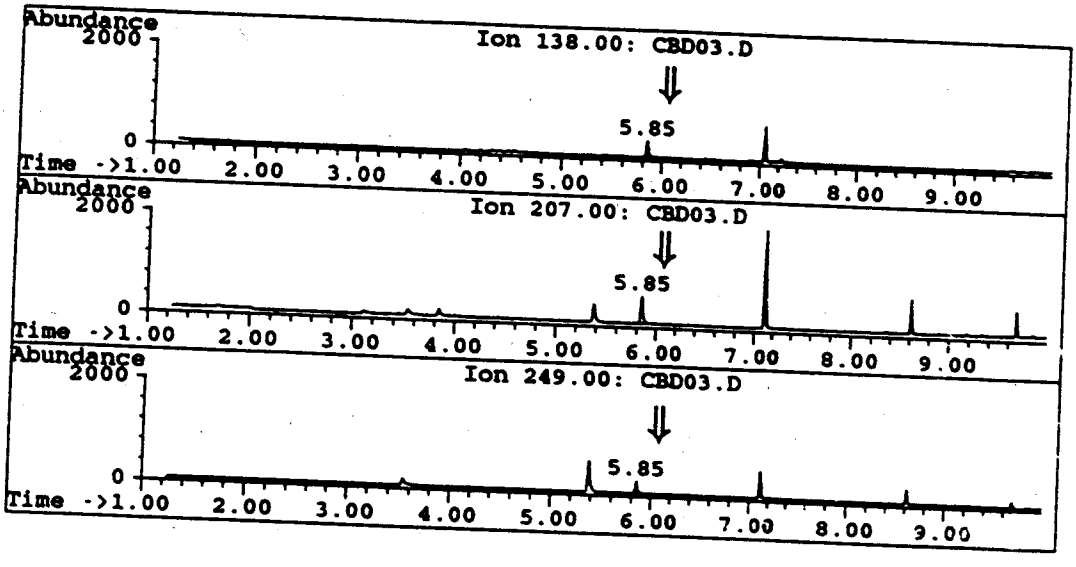
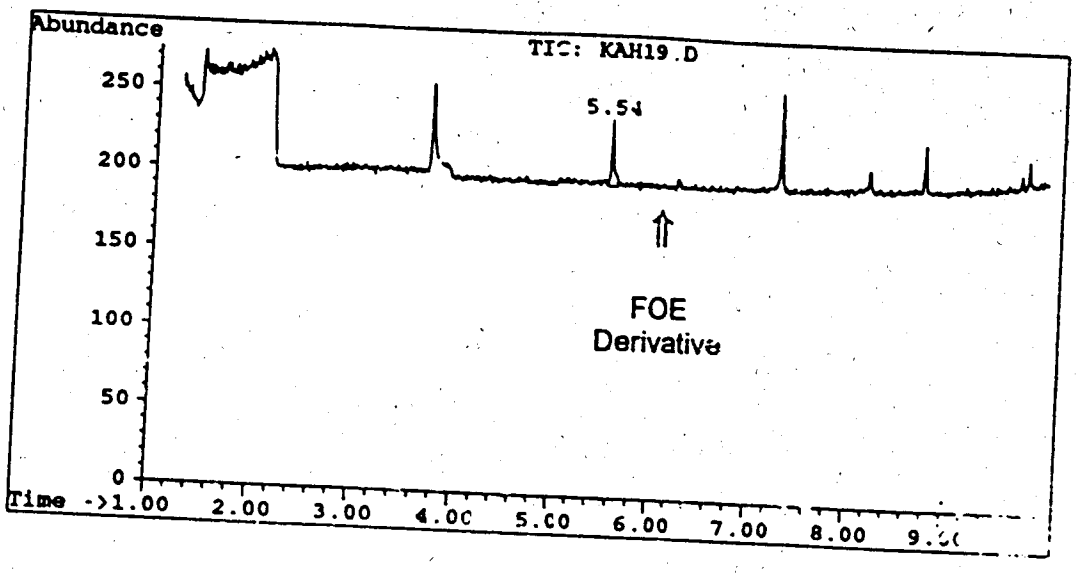


Figure 19 (cont). C and D: Wheat straw sample amended with 0.10 ppm of mixed standards.

A. Total ion chromatogram of a methyl *tert*-butyl ether solvent blank.



B. Single ion chromatograms for m/z of 138, 207, and 249.

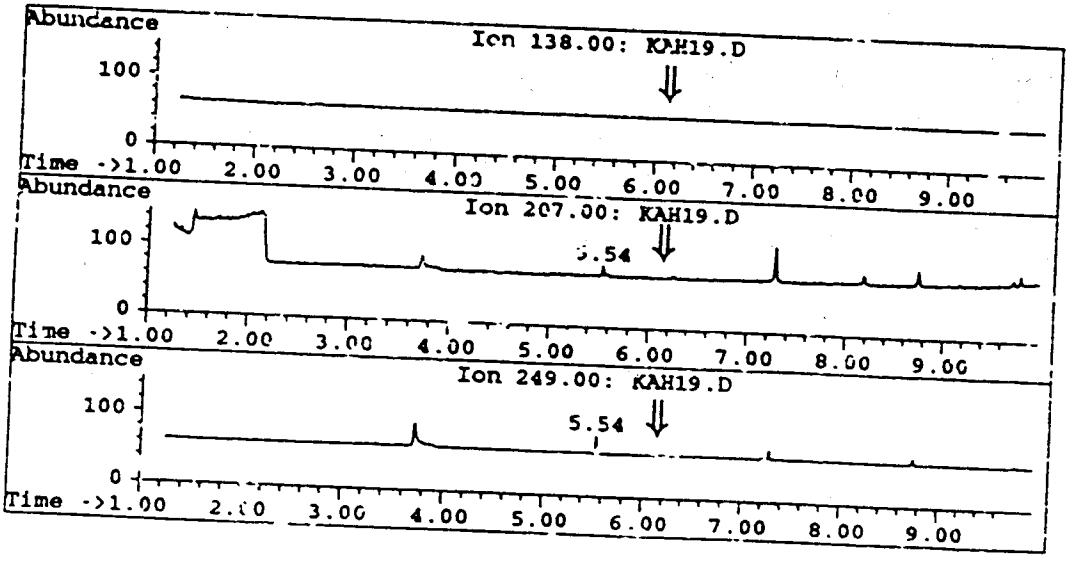
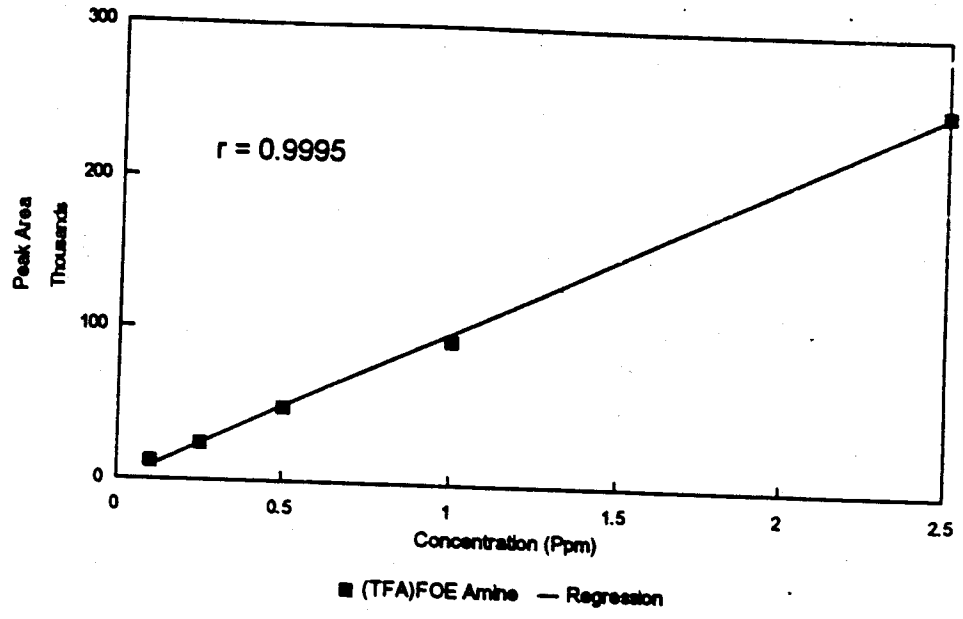


Figure 20. Gc/ms chromatograms of a methyl *tert*-butyl ether solvent blank.

A.



B.

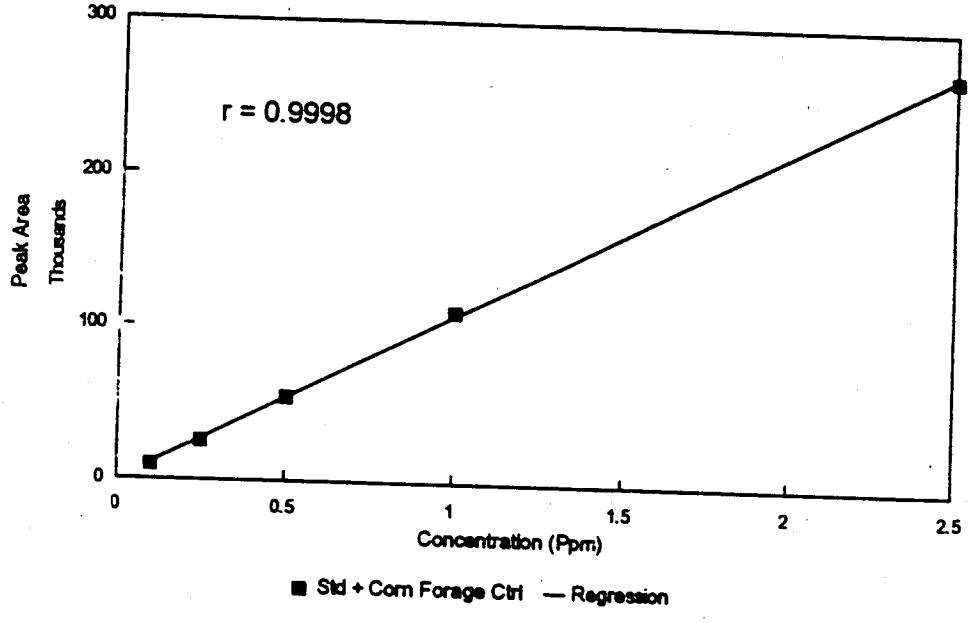
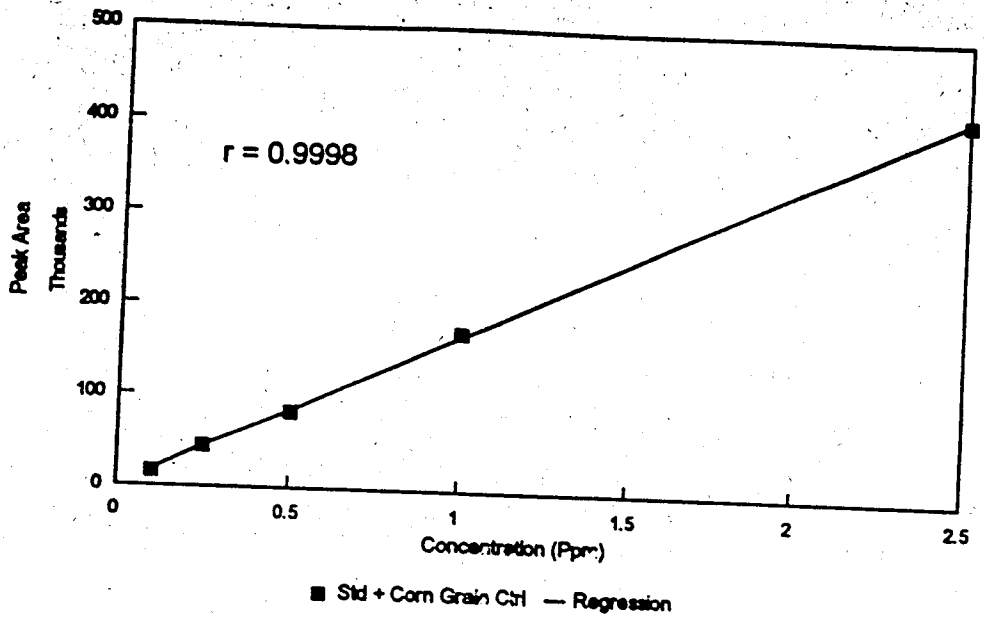


Figure 21. Linearity curves for 4-fluoro-*N*-methylethyl benzenamine trifluoroacetamide.
A. In methyl *tert*-butyl ether.
B. In the presence of corn forage matrix.

C.



D.

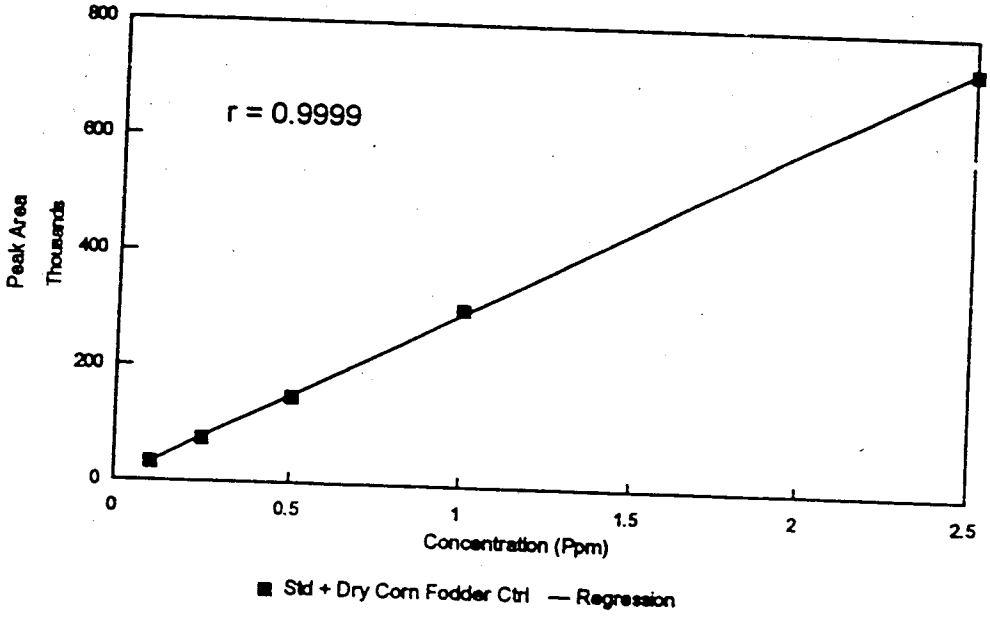
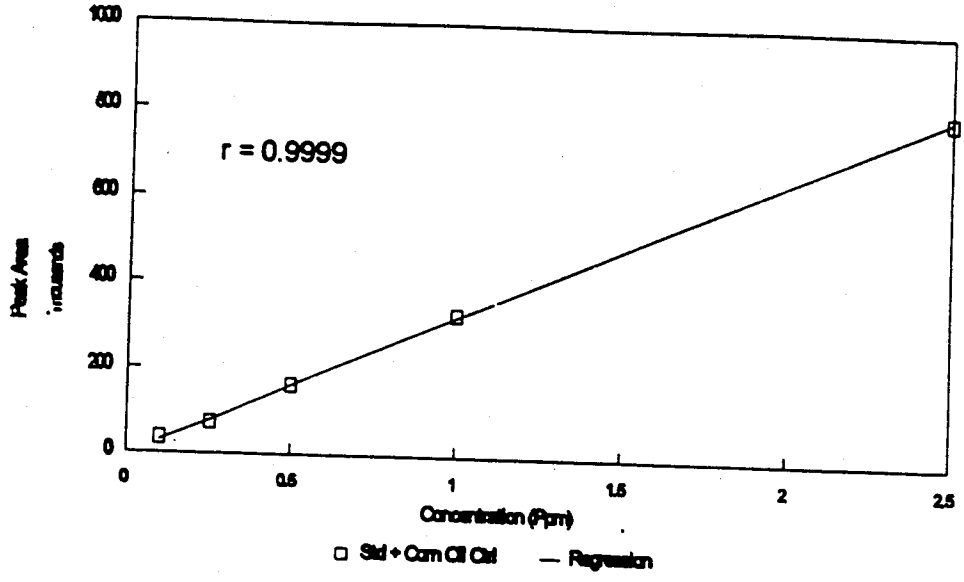


Figure 21 (cont) C. In the presence of corn grain matrix.
D. In the presence of corn fodder matrix.

E.



F.

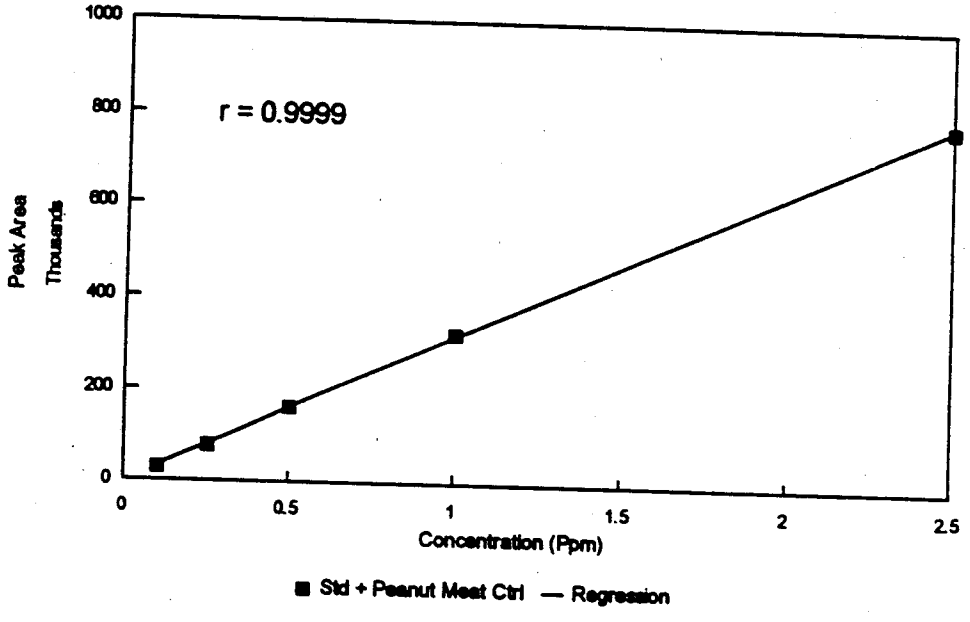
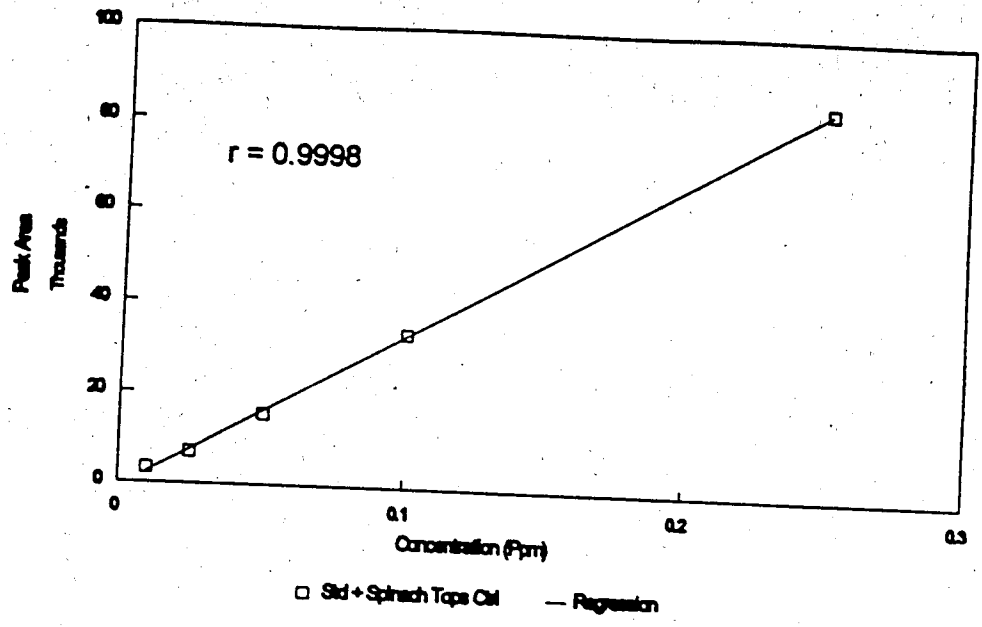


Figure 21 (cont) E. In the presence of corn oil matrix.
F. In the presence of peanut nutmeat matrix.

G.



H.

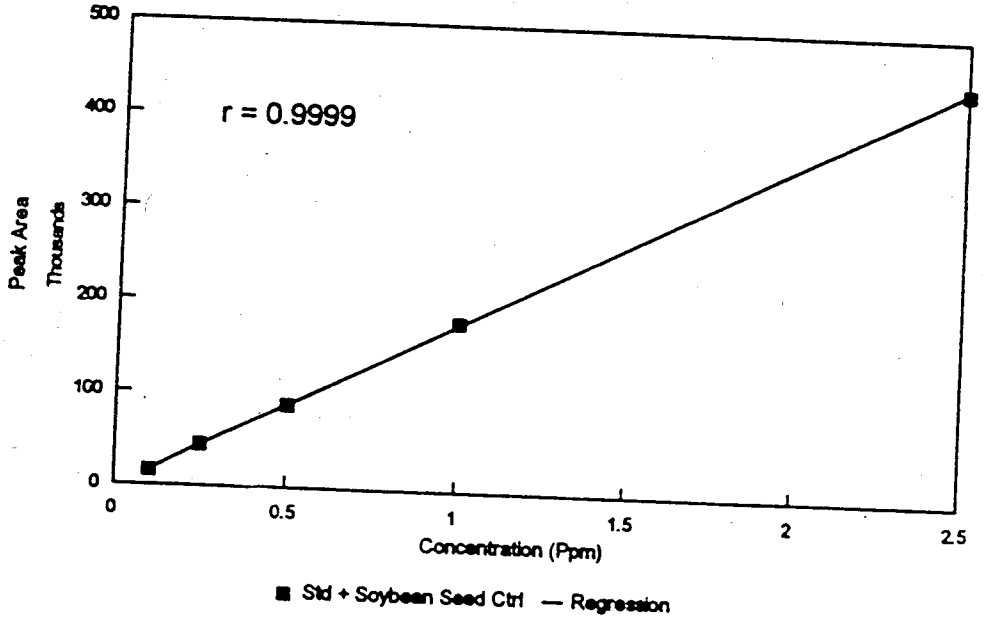
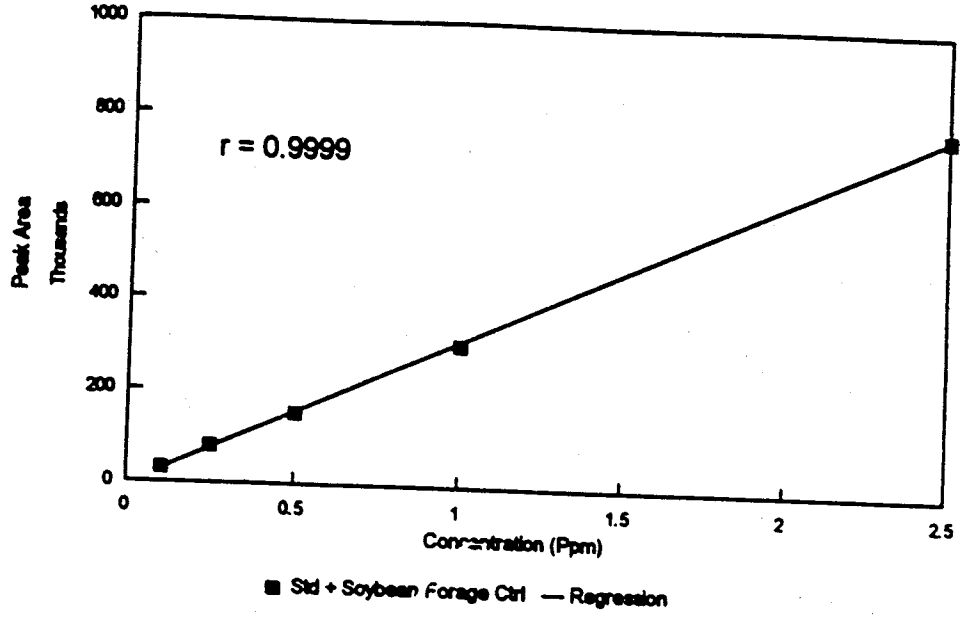


Figure 21 (cont) G. In the presence of spinach tops matrix.
H. In the presence of soybean seed matrix.

I.



J.

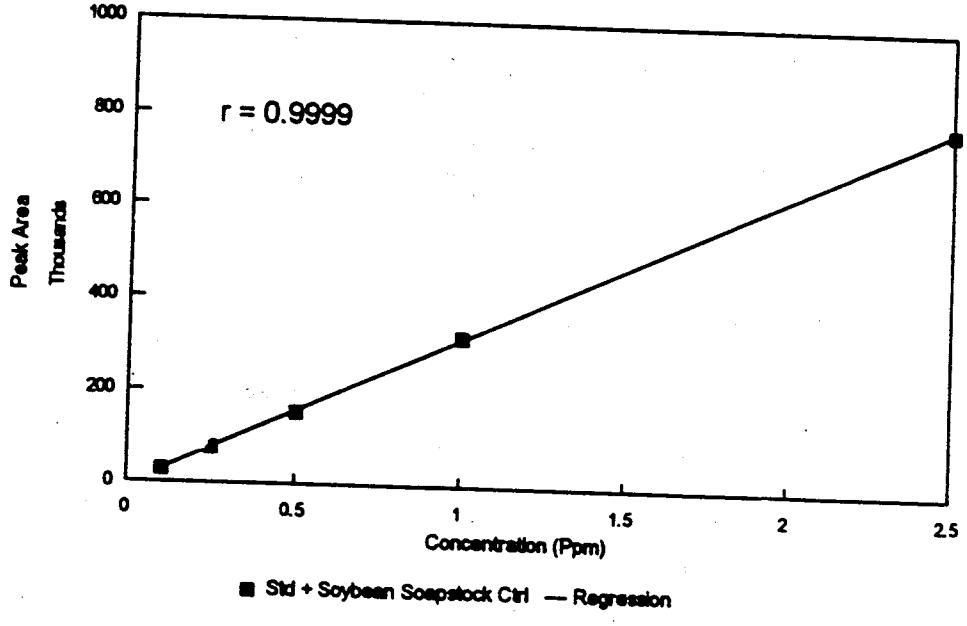


Figure 21 (cont) I. In the presence of soybean forage matrix.
J. In the presence of soybean soapstock matrix.

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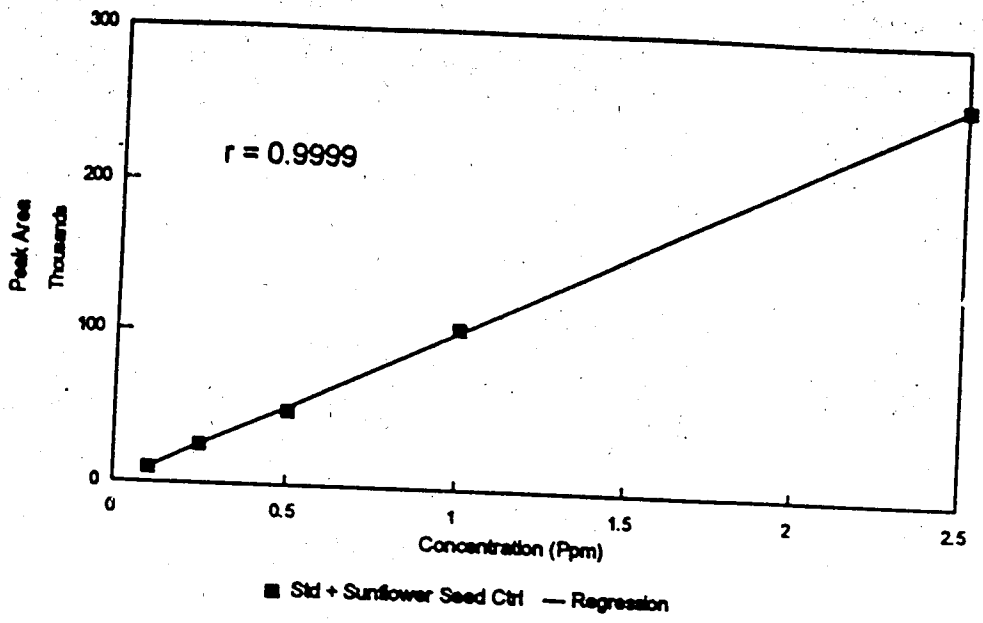
K.



L.

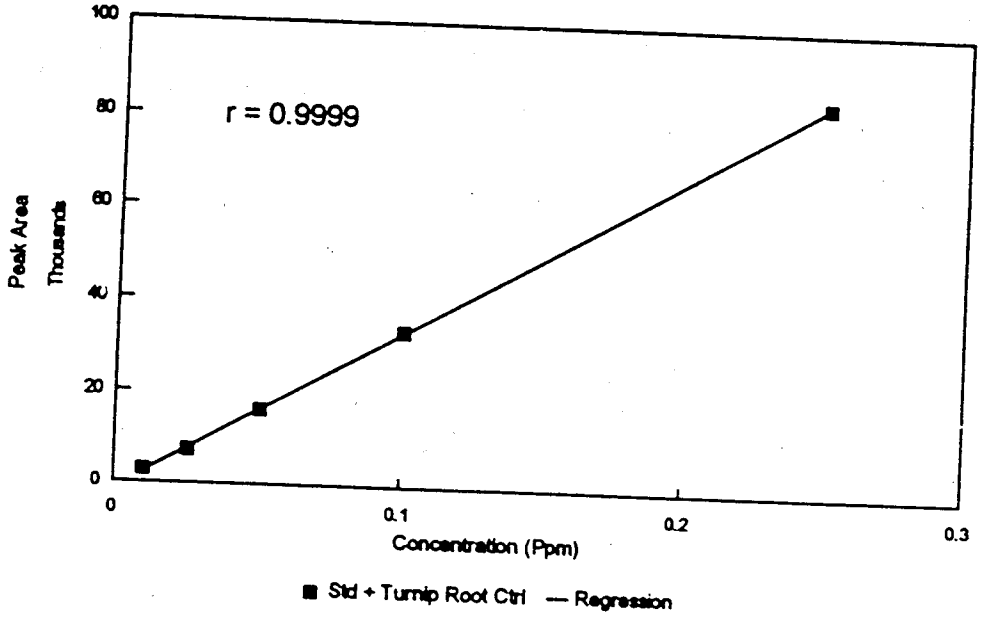
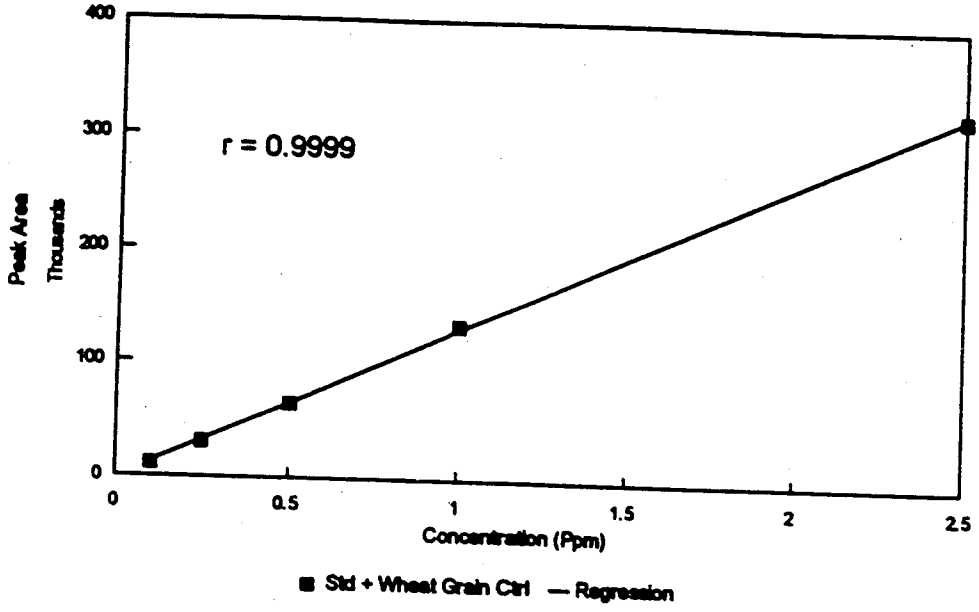


Figure 21 (cont) K. In the presence of sunflower seed matrix.
L. In the presence of turnip root matrix.

M.



N.

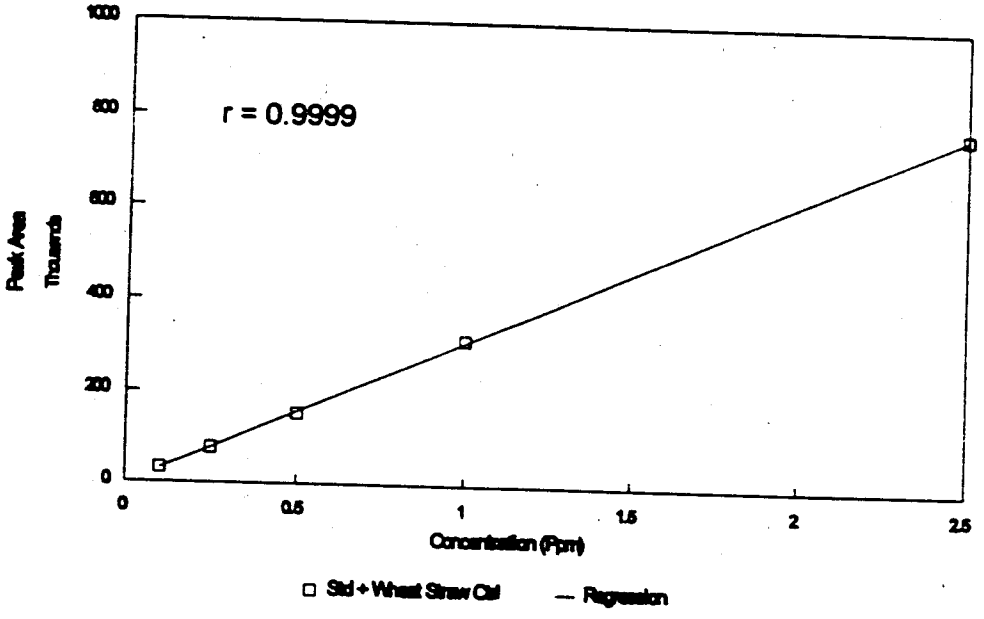


Figure 21 (cont) M. In the presence of wheat grain matrix.
N. In the presence of wheat straw matrix.

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Appendix 1. Archive listing of notebook references and project personnel.

Notebook Reference

Notebook Number	Name	Year Issued	Page Numbers
92-B-145	V. J. Lemke T. J. Gould	1992	all pages
92-B-151	V. J. Lemke T. J. Gould K. L. Zoloty	1993	all pages
89-R-148	J. Morgan	1989	222, 271
93-B-8	J. Morgan	1993	172

Project Personnel

Name	Duties
T. J. Gould	Study director, generation and maintenence of raw data, extraction and preparation of tissues, chromatography, synthesis of trifluoroacetamide derivative, preparation of final report.
V. J. Lemke	Generation and maintenance of raw data, extraction and preparation of tissues, chromatography, generation of mass spectra, preparation of final report.
K. L. Zoloty	Generation of raw data, extraction and preparation of tissues.
J. Morgan	Synthesis of reference standards.

Appendix 2. Synthesis of 4-fluoro-*N*-methylethyl benzenamine trifluoroacetamide.

Procedure: To a solution of 4-fluoro-*N*-methylethyl benzenamine (10.0 g, 65.4 mmol) in methylene chloride (100 ml) contained in a 250 ml flask was added, with stirring, 0.2% (w/w) DMAP/pyridine solution (10.6 ml, 2.0 equiv.). The flask was cooled in an ice bath. After about 5 min, trifluoroacetic anhydride (10.2 ml, 1.1 equiv.) was added dropwise over a 15 min period. The ice was allowed to melt, and the mixture was stirred overnight at room temperature. The mixture was diluted with methylene chloride (150 ml), washed with 4 *N* HCl solution (2 x 50 ml), and then washed with saturated sodium bicarbonate solution (2 x 100 ml). The methylene chloride solution was filtered through a plug of glass wool and dryed over anhydrous magnesium sulfate.

The methylene chloride solution of 4-fluoro-*N*-methylethyl benzenamine trifluoroacetamide was percolated through a bed of silica gel (6.5 cm x 3.0 cm) supported on a sintered glass frit (6.5 cm d:a.). The silica gel was washed with methylene chloride (150 ml). The methylene chloride solvent was removed on the rotory vacuum evaporator, and the residual oil was distilled at reduced pressure (160-163°C at 75 torr) to give 22.7 g (91%) of a pale oil.

Spectral data: ^{1}H-NMR (300 MHz) δ 7.15-7.05 (m, 4H), 4.86 (sept, J = 6.8 Hz, 1H), 1.08 (d, J = 6.8 Hz, 6H); ^{13}C-NMR (75.4 MHz) δ 162.84 (d, J = 250 Hz), 156.47 (q, J = 34.7 Hz), 132.28 (d, J = 8.5 Hz), 130.76, 116.40 (q, J = 288.5 Hz), 115.80 (d, J = 23.2 Hz), 49.30, 20.20. ^{19}F-NMR δ (282.2 MHz) 126.63, 82.55.

Appendix 3. Sample calculations.

Calculation of standard solution concentrations.

The concentrations of the trifluoroacetamide derivative in standard solutions were calculated on the following basis:

A 1.00 ppm standard represents 1.00 µg of FOE 5043 per g of initial matrix sample.

One gram of initial matrix sample is represented in each ml of final solution. Thus, a 1.00 ppm standard represents 1.00 µg of FOE 5043 per ml of final solution.

Molecular weight of FOE 5043 = 363.4 amu.

Molecular weight of the trifluoroacetamide derivative = 249 amu.

On a molar basis 1.00 µg FOE 5043 ÷ 363.4 x 249 = 0.685 µg of trifluoroacetamide.

A 1.00 ppm standard = 0.685 µg of trifluoroacetamide derivative per ml of final solution.

Trifluoroacetamide derivative in standards = 0.685 µg/ml x ppm desired.

For example, a 0.50 ppm standard = 0.685 µg x 0.50 ppm = 0.343 µg/ml.

Calculation of the residue level in sample.

Two measurements were made for each sample and averaged. The individually measured residue levels were determined by comparing the gc/ms detector response for each repetition to the response for bracketing trifluoroacetamide derivative standards as follows:

$$\text{Ppm} = \frac{\text{sample response}}{\text{average standard response}} \times \text{standard concentration}$$

Therefore, for a corn forage FOE 5043 recovery sample compared to 0.50 ppm standards the calculations were:

First repetition

$$\text{Ppm} = \frac{16123}{(96766 + 95564) \div 2} \times 0.50 \text{ ppm} = 0.0838 \text{ ppm}$$

Second repetition

$$\text{Ppm} = \frac{15736}{(95564 + 96395) \div 2} \times 0.50 \text{ ppm} = 0.0820 \text{ ppm}$$

Appendix 3 (cont).

The two repetitions were averaged to give a residue level for the sample:

(0.0838 + 0.0820) ÷ 2 = 0.083 ppm

Calculation of percent recovery.

Percent recovery was the measured residue level of the sample divided by the fortification level of the sample. For the corn forage example above this calculation was:

0.083 ppm ÷ 0.10 ppm X 100 = 83%

Calculation of ion ratios and confirmation of analyte identity.

The ion ratios for braketing standards and individual sample repetitions were calculated from the following equations:

$$138 \text{ Ion Ratio} = \frac{\text{Integration for 138 amu}}{\text{Integration for 207 amu}}$$

$$249 \text{ Ion Ratio} = \frac{\text{Integration for 249 amu}}{\text{Integration for 207 amu}}$$

Thus, for the corn forage example shown above, the ion ratios were:

Standard:	138 ion ratio	= 28518 ÷ 47237 = 0.604
	249 ion ratio	= 20152 ÷ 47237 = 0.427
Repetition 1	138 ion ratio	= 4770 ÷ 7900 = 0.604
	249 ion ratio	= 3449 ÷ 7900 = 0.437
Standard:	138 ion ratio	= 27983 ÷ 46266 = 0.605
	249 ion ratio	= 19920 ÷ 46266 = 0.431
Repetition 2	138 ion ratio	= 4682 ÷ 7678 = 0.610
	249 ion ratio	= 3377 ÷ 7678 = 0.440
Standard:	138 ion ratio	= 28235 ÷ 47144 = 0.599
	249 ion ratio	= 20189 ÷ 47144 = 0.428

These values all fall within 15% of each other, thus the identity of the analyte was confirmed.

Appendix 4. Hydrolysis rates of FOE 5043 and its plant metabolites.

App4-1.0 Introduction and Summary

In order to determine conditions for the hydrolysis of FOE 5043 and its plant metabolites to fluoroaniline, a hydrolysis rate study was performed. Hydrolysis using 47% (w/w) sulfuric acid solution at 115° C was found to hydrolyze FOE 5043 and most of its plant metabolites completely to fluoroaniline within 24 hours.

App4-2.0 Experimental

App4-2.1 Chemicals

[Phenyl-UL-^{14}C] labeled standards were obtained from the Environmental Research Section of Bayer, except FOE thioglycolate sulfone. Stock solutions of the standards were prepared in methanol. FOE thioglycolate sulfone was prepared by overnight mCPBA oxidation of the FOE thioglycolate sulfoxide methanol stock solution and was used without purification. The specific activities, radiochemical purities, stock solution concentrations, and hplc retention times for the standards are listed in Table A3-1.

App4-2.2 Measurement of the Reflux Temperature of 47% (w/w) H_2SO_4

A 47% (w/w) solution of H_2SO_4 was prepared by adding 50 ml of H_2SO_4 (92 g; 96.1% pure) to 100 ml of water in a 250-ml flask. A magnetic stirring bar was added to the flask, and a reflux condenser was attached. The solution was heated to reflux, and a thermometer was suspended down the condenser until the thermometer bulb was immersed in the boiling liquid. The temperature of the refluxing liquid was noted.

App4-2.3 General Hydrolysis Conditions

App4-2.3.1 Hydrolysis of FOE 5043 and FOE Plant Metabolites with 2 *N* NaOH

Sample sets of the FOE 5043, FOE oxalate, and FOE sulfonic acid standards were hydrolysed by 2 *N* NaOH. Aliquots (100 µl) of the stock solution of each standard were added to four 5-ml Reactivials (eight vials in the case of FOE 5043). A 2-ml aliquot of 2 *N* NaOH was added to each vial, the vials were capped, and the vials were heated at 100°C in a temperature-regulated heat block. One vial from each sample set was removed from the heat block at the time points of 1 hour, 2 hours, 4 hours, and 6 hours. Additional samples at 15 min, 30 min, 3 hours, and 5 hours were taken for FOE 5043. After being removed from the heat, each vial was cooled in an ice bath for at least 15 min, and the contents were neutralized to about pH 7 with concentrated HCl. The resultant solution was filtered through a 0.45 µm Acrodisc filter (Model LC 13, Gelman Sciences, Ann Arbor, MI) and radioassayed. A 1-ml aliquot from each vial was analyzed by hplc.

Appendix 4. (cont).

App4-2.3 Hydrolysis of FOE 5043 and FOE Plant Metabolites with 47% H_2SO_4

Sample sets of FOE sulfonic acid, FOE thioglycolate sulfone, and FOE methyl sulfone standards were hydrolysed by 47% H_2SO_4. Aliquots (100-µl) of the stock solution of the standard were added to four 5-ml Reactivials. The methanol solvent was removed under a stream of nitrogen gas. A 2-ml aliquot of 47% H_2SO_4 was added to each vial, the vials were capped, and the vials were heated at 115°C in a temperature-regulated heat block. One vial from each sample set was removed from the heat block at the time points of 2 hours, 4 hours, 8 hours, and 24 hours. After being removed from the heat, each vial was cooled in an ice bath for at least 15 min. The contents of the vial were transferred to a 13-ml centrifuge tube. The solution was diluted with about 5 ml of water and was neutralized to about pH 7 with 50% NaOH. Additional water was added as needed to dissolve precipitated salts. The final volume was noted, and the solution was refrigerated. An aliquot of each sample was analysed by hplc.

Single 100-µl aliquots of the FOE 5043, FOE oxalate, FOE thioglycolate sulfoxide, and FOE methyl sulfoxide standards were hydrolysed with 47% H_2SO_4 at 115°C for 24 hours. The samples were neutralized and analyzed as described above.

App4-2.4 Determination of Hydrolysis Rates

The hydrolysis rates of FOE 5043 and its plant metabolites were determined by monitoring the disappearance of the starting standard and any initial non-fluoroaniline hydrolysis product(s) by hplc. For each time point, the combined integrated area (in cpm) of starting standard and non-fluoroaniline hydrolysis product(s) was determined (Tables App4-2 and App4-3). The natural log of the integrated area (ln cpm) was plotted versus time to determine the reaction kinetics.[1] No attempt was made to determine an absolute rate constant. In the case of apparent first order reactions, the half life[2] ($t_{½}$) for the hydrolysis of the standard was approximated from the plot. A hydrolysis was considered complete when only fluoroaniline was detected by hplc.

App4-2.5 High Performance Liquid Chromatography (hplc)

All hplc was performed using a Beckman Model 345 Chromatographic System (Fullerton, CA). The system was comprised of two model 112 solvent pumps, a model 421 controller, a model 340 solvent mixing module, and a model 210 injector equipped with a 2-ml loop. The chromatographic system was connected to a Ramona D radioactivity detector (Raytest, Pittsburgh, PA) equipped with a lithium glass scintillation cell. The detector response for FOE 5043 and its plant metabolites was found to be linear. However, the detector response for fluoroaniline was not linear. All data were collected and analyzed using an IBM PC AT running Ramona software (Raytest, Version 10.7).

Appendix 4. (cont).

The chromatography system was attached to a PRP-1, 300 mm x 7.0 mm id reverse phase column (Hamilton Co., Reno, NV, serial #79426/780) equipped with a Spheri-10, RP-18, 10 µ, 30 mm x 4.6 mm id reverse phase precolumn (Applied Biosystems, Foster City, CA). Acetonitrile and 0.5% aqueous acetic acid were used as mobile phases. Two solvent gradient programs were used as follows: Program 1; Flow rate of 2.0 ml/min; 40% acetonitrile in 0.5% aqueous acetic acid for 3 min, ramped to 60% acetonitrile over 25 min, ramped to 90% acetonitrile over 7 min, and finally held at 90% acetonitrile for 10 min; Program 2; Flow rate of 1.0 ml/min; 0% acetonitrile in 0.5% aqueous acetic acid for 5 min, ramped to 90% acetonitrile in 0.5% aqueous acetic acid over 45 min, and finally held at 90% acetonitrile for 10 min. The column was equilibrated with the starting solvent mixture before each analysis. Hplc retention times for the standards are listed in Table App4-1.

For each standard in Table App4-1, an hplc mixture was prepared. A 2-ml portion of 2 *N* NaOH was added to a 5-ml Reactivial and neutralized to about pH 7 with concentrated HCl. The resultant salt solution was amended with FOE amine and the standard. A 1-ml aliquot of the sample was analysed by hplc.

App4-2.6 Radioassay

Liquid samples were radioassayed by scintillation counting in a Beckman Model LS 9000 liquid scintillation counter (Beckman, Irvine, CA) after admixture with 15 ml of ScintiVerse™ (Fisher Scientific, FairLawn, NJ). The data were processed with Beckman data reduction software.

App4-3.0 Results and Discussion

Basic hydrolysis of FOE 5043 by 2 *N* NaOH yielded a mixture of mostly FOE alcohol and some fluoroaniline in less than 15 min. The FOE alcohol was further hydrolysed to fluoroaniline in about 4 hours more (Figure App4-1). The $t_{½}$ for the hydrolysis of FOE alcohol was about 1.5 hours. Basic hydrolysis of FOE oxalate and FOE sulfonic acid, however, was slow (Figures App4-2 and App4-3) The $t_{½}$'s for these reactions were approximately 24 hours and 48 hours, respectively.

In addition to the slow reaction rates for the plant metabolites, the basic hydrolyses gave variable recoveries of fluoroaniline (Figure App4-1, note the different peak heights for the fluoroaniline). The fluoroaniline was highly volatile and probably was lost from the reaction vessel.

Similar experiments with 2 *N* H_2SO_4 and 8 *N* H_2SO_4 indicated the FOE methyl sulfone and FOE sulfonic acid were the two slowest hydrolysing metabolites (data not shown). Therefore, hydrolyses with 47% (w/w) H_2SO_4 were performed. These vigorous

Appendix 4. (cont).

conditions had three benefits. First, the proton concentration was very high. Second, the reflux temperature increased. Both of these factors increased the hydrolysis rate. Third, the fluoroaniline would be converted to a non-volatile salt under the acidic conditions.

The reflux temperature of 47% H_2SO_4 was measured to be 115°C. The hydrolysis of FOE sulfonic acid, FOE thioglycolate sulfone, and FOE methyl sulfone by 47% H_2SO_4 at 115°C was clean and complete (Figures App4-4, App4-5, and App4-6). The $t_{½}$'s for the hydrolyses were approximately 6 hours for all three.

Single aliquots of FOE 5043, the FOE oxalate, FOE methyl sulfoxide, and FOE thioglycolate sulfoxide were hydrolysed by 47% H_2SO_4 at 115°C for 24 hours (Figure App4-6). Two of the four reactions proceeded with >95% conversion to fluoroaniline. Hydrolysis of FOE methyl sulfoxide and FOE thioglycolate sulfoxide under these conditions yielded fluoroaniline and other products. However, both FOE methyl sulfone and FOE thioglycolate sulfone hydrolysed cleanly and completely to the fluoroaniline.

App4-4.0 Conclusion

Conditions were found which hydrolyse FOE 5043, three of the five plant metabolites, and FOE thioglycolate sulfone to fluoroaniline in 24 hours or less. As the hydrolyses of both FOE methyl sulfoxide and FOE thioglycolate sulfoxide were not satisfactory, oxidation of these two metabolites to the corresponding sulfones prior to hydrolysis was required.

App4-5.0 Footnotes

1. Because the concentration of acid or base effectively does not change, the integrated area for the analyte of interest (cpm) is directly proportional to the concentration of the standard, and the reverse reaction is negligible, the rate expression simplifies to:

$$\frac{-d(cpm)}{dt} = k(cpm)^n$$

where n is the order of the reaction. For n = 1, a first order reaction, the rate expression will integrate to:

$$-\ln(cpm) = kt$$

and a plot of ln (cpm) versus time yields a straight line.

2. The $t_{½}$ is the time required for the concentration of the standard to decrease by one-half. This interval was estimated by the time necessary for the curve to drop 0.69 (ln 2) units on the y-axis.

Appendix 4. (cont).

Table App4-1. Specific activities, purities, stock solution concentrations, and hplc retention times of FOE 5043 and related standards.

Compound	Specific Activity (dpm/µg)	Radiochemical Purity (%)	Stock Solution Concentration (µg/ml in MeOH)	Approximate Retention Times (hplc)
FOE 5043	406,000	99	3.50	37'02"[1]
FOE Oxalate	182,000	99	6.43	5'14"[1]
FOE Sulfonic Acid	171,000	99	7.35	5'07"[1]
FOE Thioglycolate Sulfoxide	156,000	99	8.18	5'21"[1] 34'19"[2]
FOE Thioglycolate Sulfone	156,000	99	8.18	38'59"[2]
FOE Methyl Sulfoxide	183,000	99	3.79	7'13"[1]
FOE Methyl Sulfone	173,000	99	13.08	15'12"[1]
FOE Alcohol	1,219,780	>90	0.50	17'07"[1]
FOE Amine	283,027	98	2.40	8'47"[1] 36'13"[2]

[1] Chromatographed on a PRP-1, 300 mm x 7.0 mm id reverse phase column with 0.5% aqueous acetic acid/acetonitrile at a flow rate of 2.0 ml/min by program 1.

[2] Chromatographed on a PRP-1, 300 mm x 7.0 mm id reverse phase column with 0.5% aqueous acetic acid/acetonitrile at a flow rate of 2.0 ml/min by program 2.

Appendix 4. (cont).

Table App4-2. Summary of the alkaline hydrolysis data for FOE 5043 and plant metabolites.

Basic Hydrolyses

Compound (dpm/samp)	Reaction Time (hr)	Cpm of FOE 5043[1]	Ln cpm FOE 5043	Cpm of FOE amine
FOE 5043	0.0	3,480	8.15	0
	0.25	2,993	8.00	967
(94,400 dpm)	0.5	2,842	7.95	1,390
	1.0	2,823	7.94	10,890
	2.0	1,154	7.05	18,980
	3.0	612	6.42	21,204
	4.0	242	5.49	7,934
	5.0	—	—	24,439
	6.0	—	—	8,084

[1] Includes both FOE 5043 and FOE alcohol.

Compound (dpm/samp)	Reaction Time (hr)	Cpm of FOE Oxalate	Ln cpm FOE Oxalate	Cpm of FOE amine
FOE Oxalate	0.0	4,530	8.42	0
	1.0	4,698	8.45	—
(117,100 dpm)	2.0	4,322	8.37	402
	4.0	4,170	8.34	304
	6.0	3,705	8.22	1,232

Compound (dpm/samp)	Reaction Time (hr)	Cpm of FOE Sul Ac	Ln cpm FOE Sul Ac	Cpm of FOE amine
FOE Sulfonic Acid	0.0	5,899	8.68	0
	1.0	5,242	8.56	83
(125,600 dpm)	2.0	5,389	8.59	301
	4.0	5,190	8.55	572
	6.0	5,140	8.54	191

Appendix 4. (cont).

Table App4-3 Summary of the acidic hydrolysis data for FOE 5043 and plant metabolites.

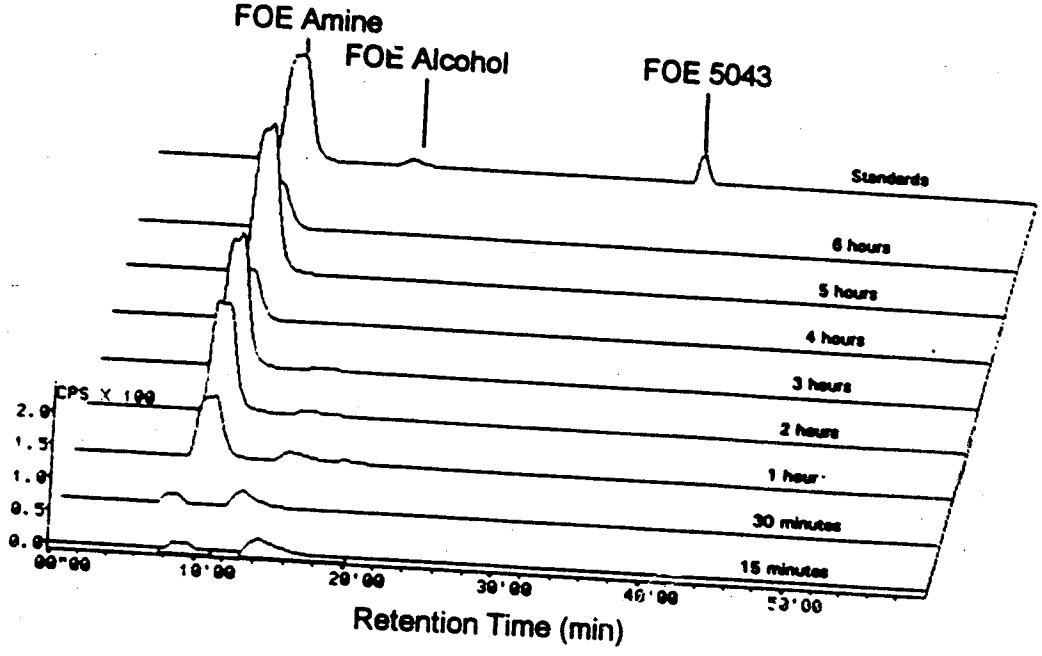
Acidic Hydrolyses

Compound (dpm/samp)	Reaction Time (hr)	Final Volume (ml)	Cpm of FOE Sul Ac[1]	Ln cpm FOE Sul Ac
FOE Sulfonic Acid (125,600 dpm)	0.0	2.4	5,653	8.64
	2.0	8.1	4,485	8.41
	4.0	9.9	3,312	8.11
	8.0	10.0	2,417	7.79
	24.0	11.0	—	—
Compound (dpm/samp)	Reaction Time (hr)	Final Volume (ml)	Cpm of FOE $TGSO_2$[1]	Ln cpm FOE $TGSO_2$
FOE Thioglycolate Sulfone (127,600 dpm)	0.0	1.6	10,873	9.29
	2.0	10.0	8,154	9.01
	4.0	10.0	7,002	8.85
	8.0	10.0	4,325	8.37
	24.0	10.0	414	6.03
Compound (dpm/samp)	Reaction Time (hr)	Final Volume (ml)	Cpm of FOE $MeSO_2$[1]	Ln cpm FOE $MeSO_2$
FOE Methyl Sulfone (226,600 dpm)	0.0	2.4	10,859	9.29
	2.0	8.3	7,328	8.90
	4.0	8.0	5,797	8.67
	8.0	10.5	3,815	8.25
	24.0	12.1	—	—

[1] Values corrected to be equivalent to 2.4 ml final volume.

Appendix 4. (cont).

A.



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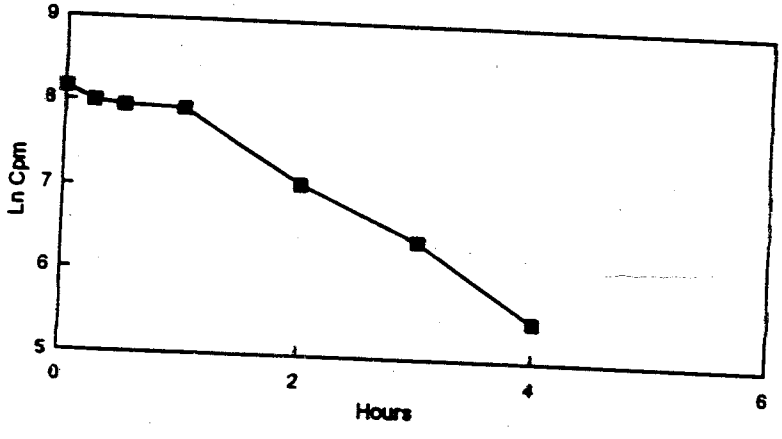
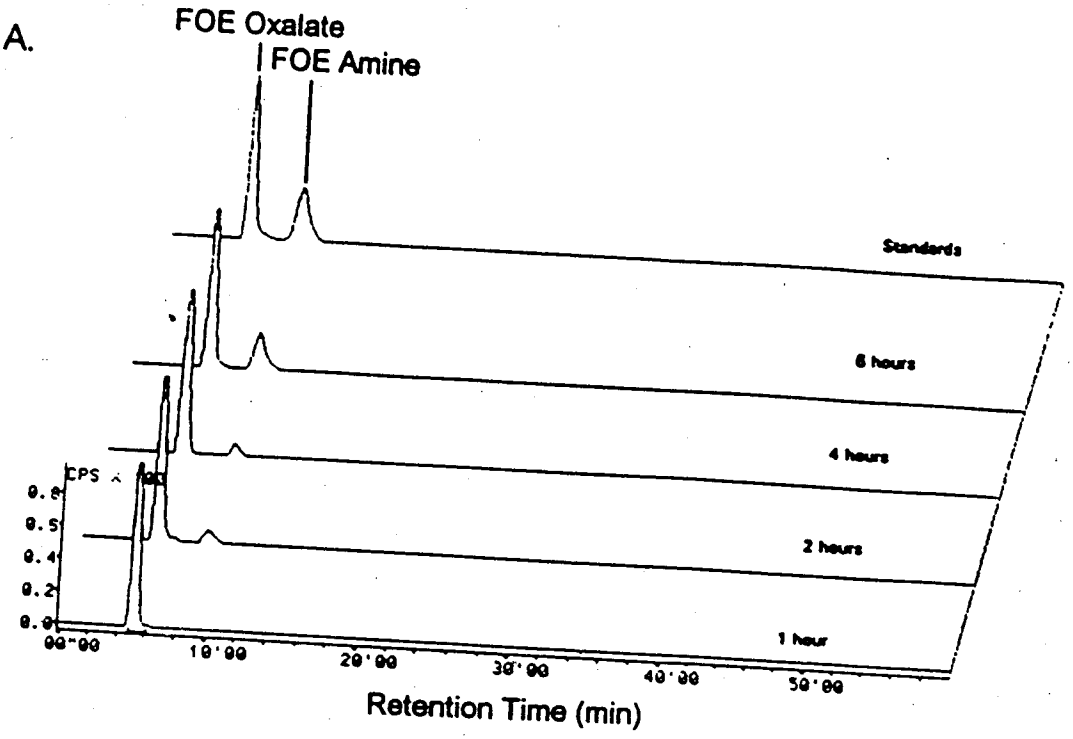


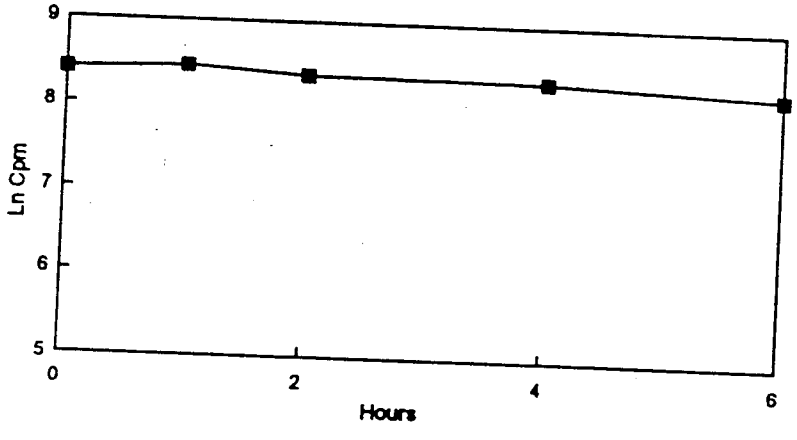
igure App4-1. A. Stacked plot of hplc analyses of FOE 5043 2 *N* NaOH hydrolysis mixtures. Hamilton PRP-1 column; 0.5% aqueous acetic acid/ acetonitrile mobile phase, program 1.
B. Plot of ln (cpm) of FOE alcohol versus reaction time.

Appendix 4. (cont).

A.



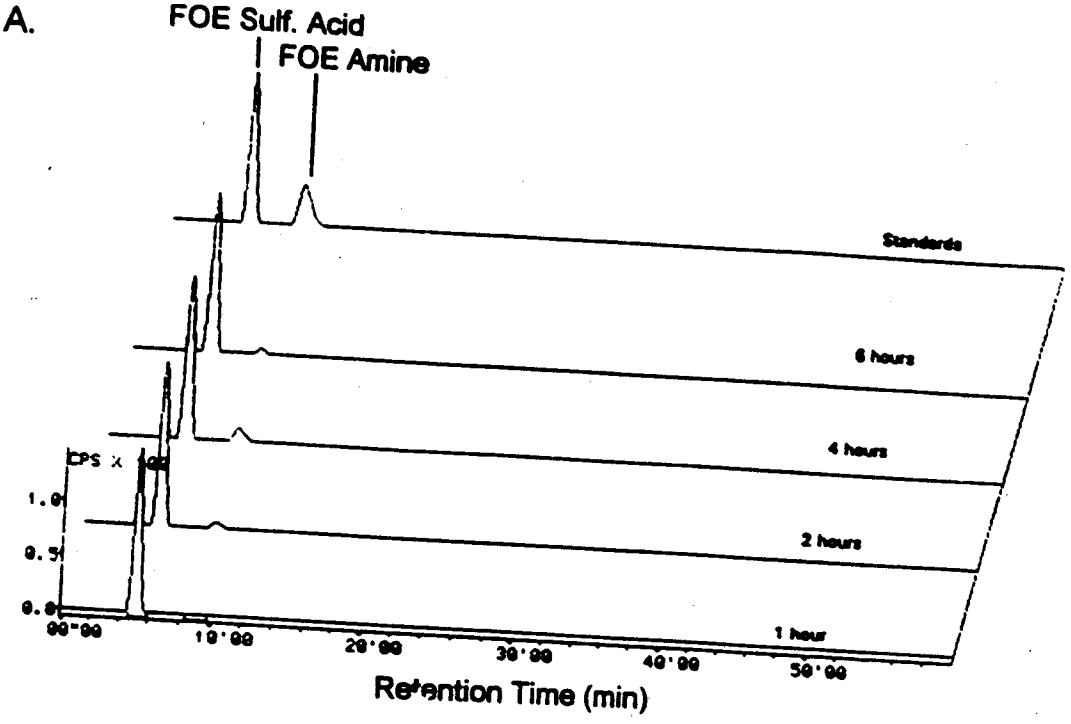
B.



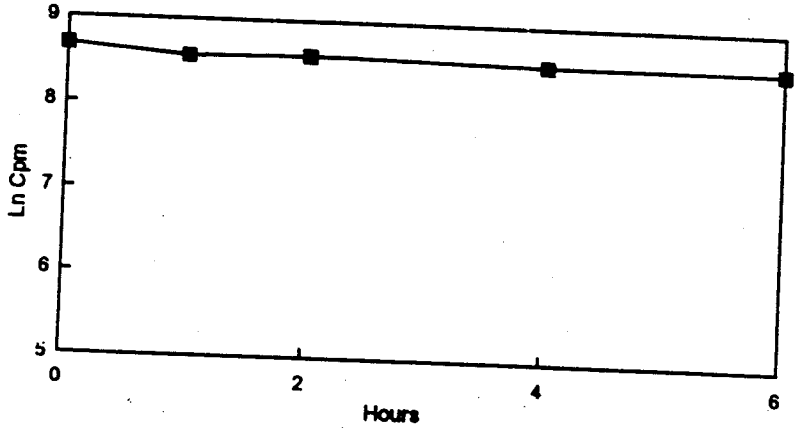
igure App4-2. A. Stacked plot of hplc analyses of FOE oxalate 2 *N* NaOH hydrolysis mixtures. Hamilton PRP-1 column; 0.5% aqueous acetic acid/acetonitrile mobile phase, program 1.
B. Plot of ln (cpm) of FOE oxalate versus reaction time.

Appendix 4. (cont).

A.



3.



igure App4-3. A. Stacked plot of hplc analyses of FOE sulfonic acid 2 *N* NaOH hydrolysis mixtures. Hamilton PRP-1 column; 0.5% aqueous acetic acid/acetonitrile mobile phase, program 1.
B. Plot of ln (cpm) of FOE sulfonic acid versus reaction time.

Appendix 4. (cont).

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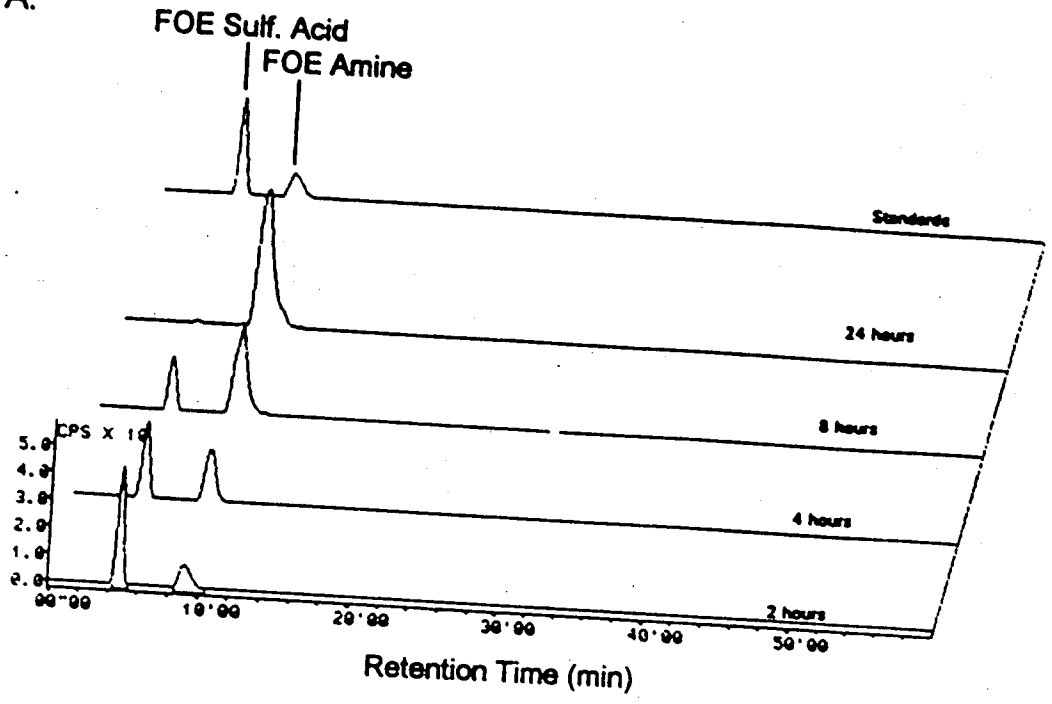
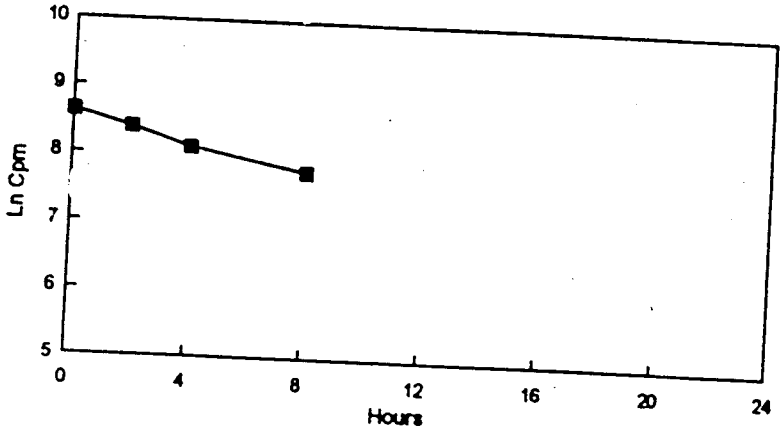
A.



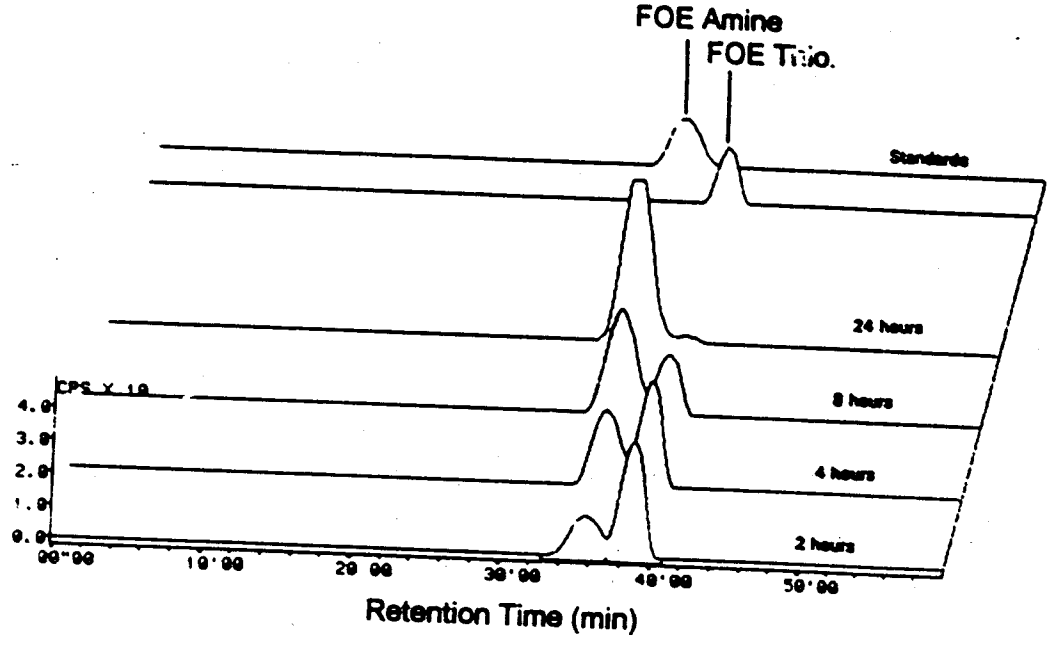
B.



gure App4-4. A. Stacked plot of hplc analyses of FOE sulfonic acid 47% H_2SO_4 hydrolysis mixtures. Hamilton PRP-1 column; 0.5% aqueous acetic acid/acetonitrile mobile phase, program 1.
B. Plot of ln (cpm) of FOE sulfonic acid versus reaction time.

Appendix 4. (cont).

A.



B.

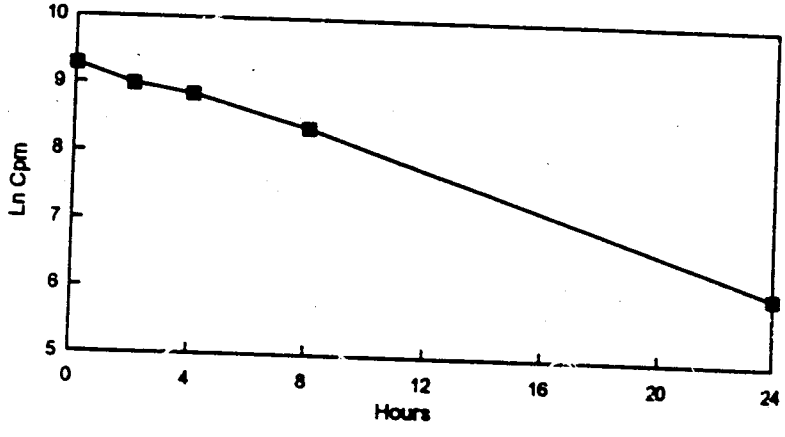
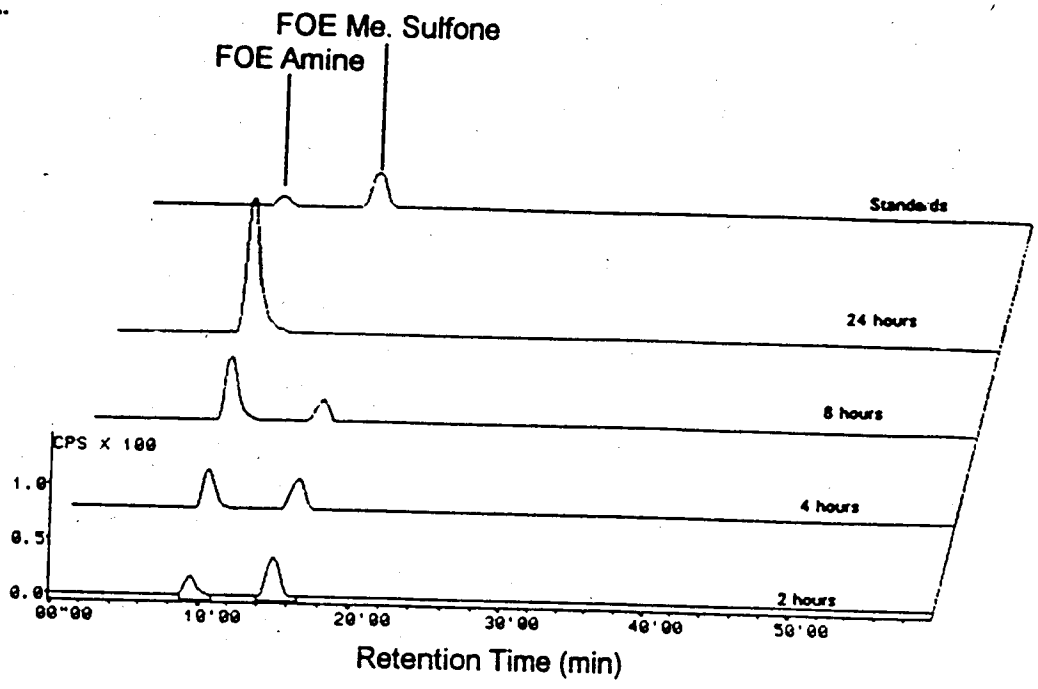


Figure App4-5. A. Stacked plot of hplc analyses of FOE thioglycolate sulfone 47% H_2SO_4 hydrolysis mixtures. Hamilton PRP-1 column; 0.5% aqueous acetic acid/acetonitrile mobile phase, program 2.
B. Plot of ln (cpm) of FOE thioglycolate sulfone versus reaction time

Appendix 4. (cont).

A.



B.

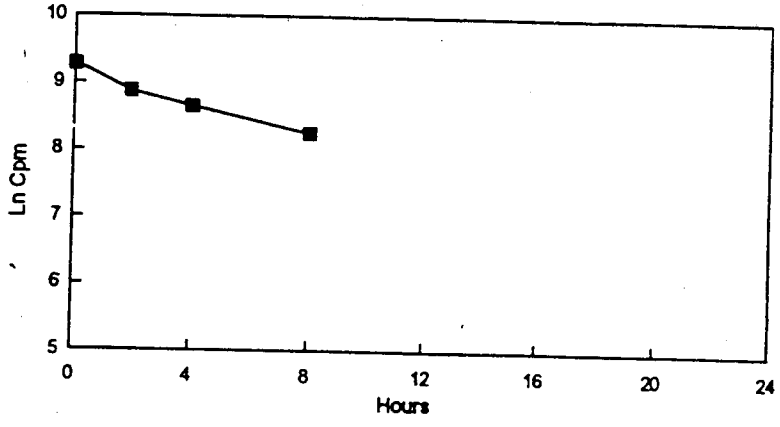


Figure App4-6. A. Stacked plot of hplc analyses of FOE methyl sulfone 47% H_2SO_4 hydrolysis mixtures. Hamilton PRP-1 column; 0.5% aqueous acetic acid/acetonitrile mobile phase, program 1.
B. Plot of ln (cpm) of FOE methyl sulfone versus reaction time.

Appendix 4. (cont).

A.

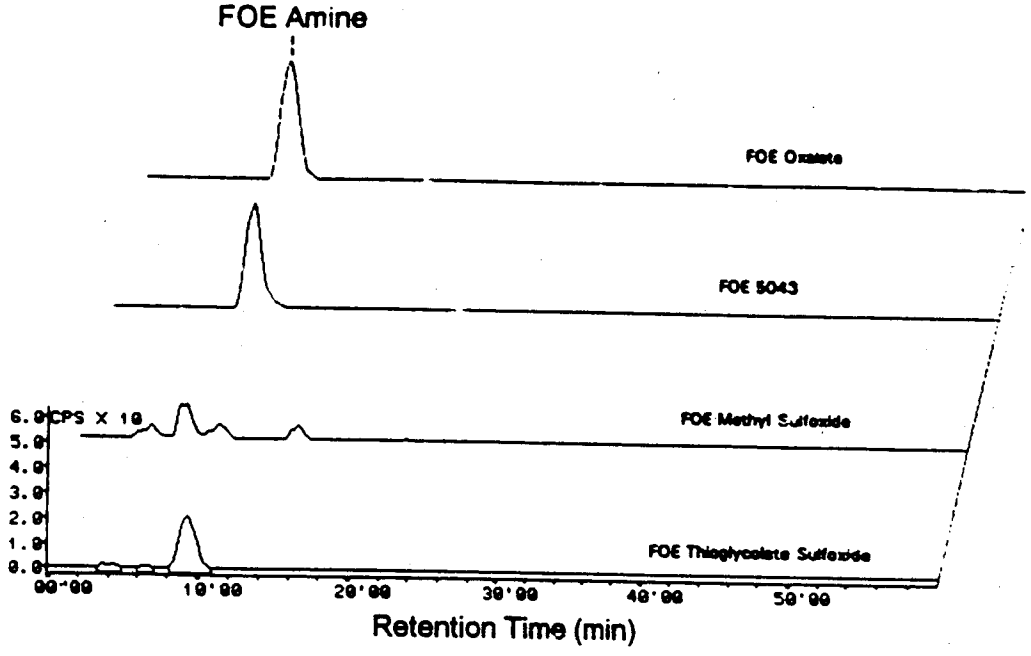


Figure App4-7. Stacked plot of hplc analyses of FOE 5043 and FOE plant metabolite hydrolysis mixtures after reaction with 47% H_2SO_4 solution at 115°C for 24 hours. Hamilton PRP-1 column; 0.5% aqueous acetic acid/acetonitrile mobile phase, program 1.