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ANALYTICAL METHOD

SANDOZ AGRO, INC.	Method Number AM-0691B-0593-3
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DETERMINATION OF DICAMBA AND 5-HYDROXY DICAMBA RESIDUES IN BARLEY, CORN, COTTON, COTTON PROCESSED FRACTIONS, PASTURE GRASS, PEANUT, SORGHUM, SOYBEAN, SUGAR CANE, TOMATO, TOMATO PROCESSED FRACTIONS, WHEAT AND WHEAT PROCESSED FRACTIONS (GC)

1. SUMMARY

1.1 Scope

This method is a revision of the original Sandoz Crop Protection Corporation's analytical method AM-0691B, "Determination of Dicamba and 5-Hydroxy Dicamba Residues in Barley, Corn, Cotton, Cotton Processing Fractions, Pasture Grass, Peanut, Sorghum, Soybean, Sugar Cane, Tomato, Tomato Processed Fractions, Wheat and Wheat Processed Fractions (GC)". The revision consists of a more detailed step by step description of the procedures, GC-MS confirmatory tests and additional recovery data. The analytical method has not been changed from what was presented in AM-0691B. The limit of detection for each compound is 0.01 ppm.

1.2 Principle

1.2.1 A known weight of sample is treated with 1N HCl and hydrolyzed for 1.5 hours at 95°C in a water bath.

1.2.2 The hydrolysate is adjusted to pH equal or greater than 8 and a 50 mL aliquot taken for analysis.

1.2.3 The aliquot is acidified to pH less than 1 and extracted twice with ethyl ether.

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1.2.4 The combined ether extracts are concentrated and the residues methylated using diazomethane.

1.2.5 The sample is cleaned up by silica gel chromatography.

1.2.6 Dicamba and 5-hydroxy dicamba are quantitated by gas chromatography using a ^{63}Ni electron capture detector (GC-ECD).

1.2.7 Confirmatory analysis is conducted by gas chromatography using a mass selective detector (GC-MSD).

2. SAFETY

2.1 The acute oral LD_{50} of technical dicamba in rats is 1707 mg/kg. The acute oral LD_{50} of dicamba methyl ester in rats is 2.7 g/kg. The acute oral LD_{50} for 5-hydroxy dicamba in mice is > 4.6 g/kg.

2.2 Personal protective equipment including safety glasses, disposable gloves, and laboratory coats should be used when handling samples.

2.3 Ethyl ether and pentane are flammable and should be used in well vented laboratories and away from open flames and sources of sparks.

2.4 Solutions of 1 and 6N HCl and 4N KOH are corrosive and should be handled with care. Acid and base solutions are especially harmful to eyes and protective glasses or shields must be worn when working with these solutions.

2.5 Diazald is a suspected carcinogen, gloves must be worn when working with this reagent.

2.6 Diazomethane is extremely toxic and should only be used in hoods with face velocities > 150 LFM. Diazomethane gas may explode when in contact with ground glass or cracked or chipped glass, therefore, do not use ground glass connections when working with this compound. Wearing a respirator with chemical cartridge filters is recommended. The diazomethane charged extracts must be properly stoppered before removal from hood (teflon stopcocks are suitable stoppers for this purpose).

2.7 Disposal of samples, extracts and standards must be done in compliance with on-site safety policies and procedures.

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3. MATERIALS AND METHODS

3.1 Apparatus

3.1.1 Balances, analytical and top load.

3.1.2 Bath, hot water, 40°C, 65°C and 95°C.

3.1.3 Blender, Waring, Waring Products Corp., New York, NY.

3.1.4 Bottles, glass with poly-seal screw cap, 2 oz and 8 oz, VWR Scientific, Chicago, IL.

3.1.5 Centrifuge, 510G (1500 RPM), model CU-5000, Damon/IEC Division.

3.1.6 Chromatographs, GC-EC and GC-MS (see sections 3.9.1 and 3.11.1.

3.1.7 Columns, capillary for GC-EC and GC-MS

GC-EC
DB-210; 50% trifluoropropyl methyl siloxane, 30 m x 0.53 mm x 1.0 µm film thickness, part #125-0232, J & W Scientific.

HP-1; crosslinked 100% dimethyl polysiloxane, 30 m x 0.53 mm x 0.88 µm film thickness, part #19095z-023, Hewlett-Packard.

RTX-5; crossbonded 95% dimethyl 5% diphenyl polysiloxane, 30 m x 0.53 mm x 1.5 µm film thickness, cat. #10270, column # 38819, Baxter.

GC-MS
HP-1; 100% dimethyl polysiloxane, 12 m x 0.2 mm x 0.33 µm film thickness, part #19091J-101, Hewlett-Packard.

3.1.8 Columns, chromatographic, water jacketed, 450 mm x 15 mm (cat. #5821-15) with nylon coupling (cat. #5840-10) and adapter with teflon stopcock (cat. #5835B-11), Ace Glass Incorp., Vineland, N.J.

3.1.9 Column, distillation, Vigreaux, 24/40, 150 mm, cat. #503500-0221, Kontes, Vineland, NJ.

3.1.10 Column, C_{18} mega bond elut, 5 g, 12 cc, cat. #1225-6015, Chrom Tech, Apply Valley, MN.

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3.1.11 Concentrator, evaporative, Kuderna-Danish (KD), 24/40 top, 14/20 bottom, cat. #570011-0125, Kontes, Vineland, N.J.

3.1.12 Cylinders, graduated, 50 mL and 250 mL.

3.1.13 Esterification apparatus, see Figure 2.

3.1.14 Evaporator, nitrogen with 40°C water bath, model #'s 106 & 112, Organomation Associates, South Berlin, MA.

3.1.15 Evaporators, rotary, Model #'s R, RE and RE-111, Buchi/Brinkmann, Westbury, NY.

3.1.16 Flask, volumetric, 100 mL.

3.1.17 Flask, round-bottom, 250-mL.

3.1.18 Food cutter, model #84181D, Hobart, Troy, OH.

3.1.19 Funnel, filter, 60°, 75 mm.

3.1.20 Funnel, separatory with teflon stopcock, 125 mL and 500 mL.

3.1.21 Glass wool.

3.1.22 Manifold, Visiprep solid phase extraction, cat. #5-7030, Supelco, Inc., Bellefonte, PA.

3.1.23 Oven, 250°C.

3.1.24 Paper, pH, range 1-14.

3.1.25 Pipets, 0.10 mL, 0.50 mL, 1.0 mL, 2.0 mL, 5.0 mL, 10.0 mL and pasteur, 9 inch, disposable.

3.1.26 Receiver, distillation, graduated 12 mL (KD tube) 14/20, cat. #288251-0000, Kontes, Vineland, N.J.

3.1.27 Reservoir, 250 mL preparative funnel (cat. #5824-05) with nylon coupling (cat. #5842-05), Ace Glass Incorp., Vineland, NJ.

3.1.28 Shaker, platform, Eberbach Co., Ann Arbor, MI.

3.1.29 Stopcock, teflon.

3.1.30 Stopper, flat head, solid, cat. # LG10380-100, Lab Glass, Kingsport, TN.

3.1.31 Test tube, 20 x 150 mm.

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3.2 Reagents

3.2.1 Acetone*

3.2.2 Boiling chips, 10 mesh, Hengar Company, Philadelphia, PA.

3.2.3 Carbitol, 2-(2-ethoxyethoxy) ethanol, Aldrich Chemical Co., Milwaukee, WI.

3.2.4 Deionized water, Milli-Q Water Purification System, Millipore Corporation, Bedford, MA.

3.2.5 Diazald, Aldrich Chemical Co., Milwaukee, WI.

3.2.6 Ethyl ether, 2% ethanol preservative.*

3.2.7 Ethanol, absolute, 200 proof.

3.2.8 Hexane*

3.2.9 Hydrochloric acid, reagent grade.

3.2.10 Methanol*

3.2.11 Pentane*

3.2.12 Potassium Hydroxide, pellets, reagent grade.

3.2.13 Silica Gel 60, 70-230 mesh, EM Science, Cherry Hill, NJ.

3.2.14 Sodium Chloride, crystals, analytical grade.

3.2.15 Sodium Sulfate, anhydrous granular.

* "High Purity Solvent", Burdick and Jackson, Muskegon, MI/Baker Resi-Analyzed, J. T. Baker Chemical Co., Phillipsburg, N.J.

3.3 Preparation of Standard Solutions

3.3.1 Sandoz Agro, Inc. Analytical Reference Standards (See Figure 1 for Chemical Structures).

Dicamba: 2-methoxy-3,6-dichlorobenzoic acid

5-Hydroxy Dicamba: 5-hydroxy-2-methoxy-3,6-dichlorobenzoic acid

3.3.2 Weigh 100 mg each of dicamba and 5-hydroxy dicamba analytical reference standards in separate 100-mL volumetric flasks. Dissolve the standards with 10 mL methanol and dilute to the mark with methanol. The concentration

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of each stock solution is 1 X 10^{-4}g/uL, (1 mg/mL). The stock solutions should be made using at least 10 mg of standard and 10 mL of solvent. The stock solutions are to be made in duplicate. The concentrations of the solutions are determined by analysis and should not vary by more than ± 3% from the mean concentration.

3.3.3 Transfer 1.0 mL from each of the stock solutions from 3.3.2 to a single 100-mL volumetric flask and dilute to the mark with methanol. The concentration of this solution is 1 X 10^{-5} g/uL (10 µg/mL) each compound. This solution is used for fortifying control samples for recovery determination.

3.3.4 Prepare diluted fortifying standard solutions. Perform the serial dilutions described below in 100-mL volumetric flasks and dilute to the mark with methanol.

Concentration of Initial Standard Solution	Volume of Aliquot	Concentration of Final Standard Solution
1 X 10^{-5}g/uL	10.0 mL	1 X 10^{-6}g/uL
1 X 10^{-6}g/uL	1.0 mL	1 X 10^{-10}g/uL

3.3.5 Transfer 1.0 mL from each of the stock solutions from 3.3.2 to a 12-mL KD tube. Concentrate to approximately 0.5 mL under a stream of nitrogen in a well-ventilated hood. Methylate as described in section 3.7.

3.3.6 Transfer the methylated compounds to a 100 mL volumetric flask and dilute to the mark with hexane. The concentration of this solution is 1 X 10^{-5}g/uL (10 µg/mL) equivalents of dicamba and 5-hydroxy dicamba. This is used for the preparation of GC standard solutions.

3.3.7 Prepare a set of GC standard solutions ranging from 5 X 10^{-12}g/uL to 1 X 10^{-10}g/uL using the methylated standard from section 3.3.6. Perform the serial dilutions described below in 100-mL volumetric flasks and dilute to the mark with hexane.

Concentration of Initial Standard Solution	Volume of Aliquot	Concentration of Final Standard Solution
1 X 10^{-5}g/uL	10.0 mL	1 X 10^{-9}g/uL
1 X 10^{-6}g/uL	1.0 mL	1 X 10^{-10}g/uL
1 X 10^{-9}g/uL	5.0 mL	5 X 10^{-11}g/uL
1 X 10^{-9}g/uL	2.0 mL	2 X 10^{-11}g/uL

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1×10^{-9}g/uL	1.0 mL	1×10^{-11}g/uL
1×10^{-10}g/uL	5.0 mL	5×10^{-12}g/uL

3.4 Preparation of Silica Gel

3.4.1 Activate approximately 250 g of silica gel at 250°C for 2½ hours.

3.4.2 Transfer to a 32-oz bottle, cap tightly and cool by shaking for ½ hour on a mechanical platform shaker.

3.4.3 Weigh the silica gel and deactivate with distilled water (194 g silica gel, 6 g water).

3.4.4 Shake continuously for 15 hours. Store the silica gel at room temperature in a 32-oz bottle with polyseal screw cap. The silica gel is good for two weeks.

3.5 Acid Hydrolysis and Extraction Procedures

3.5.1 Plant Materials, Processed Fractions and Grain Samples

Plant materials are chopped using the Hobart food cutter and grain samples are pulverized using the Waring Blender.

3.5.1.1 Weigh 5-10 g chopped plant material, processed fraction or pulverized grain sample into an 8-oz bottle.

3.5.1.2 If fortifying for recovery determination, add an apppropriate volume of standard solution (sections 3.3.3-3.3.4) to the sample. Adding 0.10 mL of the 1×10^{-9}g/uL or 1×10^{-8}g/uL standard solution would give a 0.01 ppm or 0.10 ppm fortification level, respectively for a 10 g sample. Allow 10 minutes for the fortification solvent to evaporate.

3.5.1.3 Add 150 mL of 1N HCl to the sample.

3.5.1.4 Tighten the cap on the 8-oz bottle and place the bottle in a 95°C water bath. Loosen the cap by half a turn to allow pressure to escape but also prevent evaporation. Hydrolyze for 1.5 hours and preferably, swirl the mixture occasionally during the hydrolysis.

3.5.1.5 Cool hydrolysate to room temperature. For faster cooling, let the sample sit

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on the bench top for 5 minutes, then transfer to a cold water bath.

3.5.1.6 Slowly add 50 mL of 4N KOH to the cooled hydrolysate. Caution: The mixture will get warm because of heat of neutralization. Allow the sample again to cool to room temperature.

3.5.1.7 Shake the mixture vigorously by hand for 1 minute and check the pH with a pH paper. The pH must be equal or greater than 8, if not, add more base.

3.5.1.8 Centrifuge at 510G (1500 RPM) for 5 minutes.

3.5.1.9 Proceed to section 3.6.

3.5.2 Crude or Refined Oil

3.5.2.1 Weigh 5 g of oil into a 2-oz bottle.

3.5.2.2 If fortifying for recovery determination, add an appropriate volume of standard solution (section 3.3.4) to the sample. Adding 0.50 mL of 1 X 10^{-10}g/uL or 0.50 mL of 1 X 10^{-9}g/uL standard solution would give a 0.01 ppm or 0.10 ppm fortification level, respectively. Allow 10 minutes for the fortification solvent to evaporate.

3.5.2.3 Add 10 mL of 80% ethanol in 1N HCl to the oil and swirl in 65°C water bath for 1 min.

3.5.2.4 Centrifuge the mixture for 5 minutes to allow the phases to separate.

3.5.2.5 Very carefully transfer the aqueous ethanol phase with a disposable pasteur pipet, to a 250-mL round-bottom flask.

3.5.2.6 Repeat steps 3.5.2.3 - 3.5.2.5 two more times, combining all 3 aqueous ethanol phases in the same round-bottom flask.

3.5.2.7 Add a boiling chip and concentrate the combined aqueous ethanol extracts to approximately 6 mL, using a rotary evaporator with a 40°C water bath.

3.5.2.8 Transfer the concentrate, with a disposable pasteur pipet, to a 250-mL graduated cylinder.

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3.5.2.9 Rinse the round-bottom flask with 3 X 10 mL of 1N HCl. Transfer all rinses to the 250-mL graduated cylinder.

3.5.2.10 Adjust the volume to 150-mL with 1N HCl and transfer the solution to an 8-oz bottle.

3.5.2.11 Follow the hydrolysis procedure starting at step 3.5.1.4.

3.6 Partition Procedure

3.6.1 Transfer a 50-mL aliquot (2.5 g equivalents) of the basic hydrolyzed solution from 3.5.1.8 to another 8-oz bottle.

3.6.2 Add approximately 15 g of NaCl crystals and 5 mL of 6N HCl. Swirl the mixture and check the pH with a pH paper. The pH must be less than 1, if not, add more acid.

3.6.3 Add 50 mL of ethyl ether, tighten the cap and shake for 10 minutes on a mechanical platform shaker.
Note: The analysis may be interrupted here and resumed the next day.

3.6.4 Centrifuge at 510G (1500 RPM) for 5 minutes or longer if needed, to break the emulsion.

3.6.5 Pour contents of the bottle into a 125-mL separatory funnel. Allow the phases to separate.

3.6.6 Drain the aqueous phase (lower layer) back into the same 8-oz bottle in step 3.6.5.

3.6.7 Plug a filter funnel with glass wool, then fill it with anhydrous sodium sulfate. Place the filter funnel on top of a KD tube-concentrator set-up. Pass the organic layer through anhydrous sodium sulfate into a KD set-up.

3.6.8 Repeat steps 3.6.3 - 3.6.7 with the aqueous phase combining the second organic phase with the first.

3.6.9 Rinse the sodium sulfate with 10-15 mL of ethyl ether.

3.6.10 Add 0.5 mL of hexane and a boiling chip to the combined ethyl ether extracts. Connect a Vigreaux distillation column to the KD set-up and concentrate the combined extracts to approximately 0.5 mL using a 65°C water bath.

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Additional use of a nitrogen evaporator and a 40°C hot water bath may be necessary to reach a 0.5 mL volume.

3.6.11 Transfer the concentrated extract to a test tube (20 x 150 mm) using a disposable pasteur pipet. Rinse the KD tube with 3 x 1 mL of ethyl ether. Transfer all rinses to the test tube with the sample extract, adding another 1 mL of ethyl ether.

3.7 Derivatization (Methylation) Procedure

3.7.1 Set up tubes (20 x 150 mm test tubes) using glass tubing connections as in Figure 2. Tube A serves to saturate the nitrogen flow with ether which transmits diazomethane from Tubes B to C. Tube D is used as an indicator for excess diazomethane when the sample extract in Tube C is colored.

3.7.2 To tube B, add 2 ml of 9 N KOH solution and 3 mL of 1:1 carbitol/ ethyl ether.

3.7.3 To tube C, which contains the sample extract, add 0.5 mL methanol.

3.7.4 To tube D, add 3 mL of ethyl ether.

3.7.5 Adjust the nitrogen flow rate to 30 mL/min. Add approximately 100 mg of Diazald with a spatula to tube B and seat stoppers in tubes A, B and C. Methylate for about 2 minutes. Remove tube C when a yellow color is obtained. For colored extracts, observe the yellow color in tube D.

Note: Diazald is a carcinogen. In addition, the diazomethane generated is extremely toxic and should only be used in hoods which are operating efficiently (≥ 150 LFM). Diazomethane gas may explode when heated to 100°C or when in contact with ground glass or cracked and chipped glass. Alkaline metals may cause diazomethane to explode.

3.7.6 Derivatize samples in order of increasing concentration of the compound, if this is known, to prevent cross-contamination of sample. Rinse the diazomethane delivery tube with ether before derivatizing the next sample. When contamination is suspected, replace tube C with another test tube containing ethyl ether and bubble the diazomethane gas for 1 min. Do this before derivatizing each sample or change the delivery tube.

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3.7.7 The diazomethane generating solution should be changed after every two samples.

3.7.8 Cap the KD for 15 minutes (teflon stopcocks are useful for this purpose). If the solution remains yellow throughout this interval, evaporate the sample to 1-mL using a gentle nitrogen stream in a hood with a face velocity ≥ 150 LFM. Do not use heat for this procedure.

3.7.9 If, however, the yellow color disappears before 15 minutes, recharge the sample extract with diazomethane.

3.8 Column Cleanup Procedure

3.8.1 Set up the water-jacketed columns in a well-ventilated hood. Fit each column with a glass wool plug to contain the silica gel. Open the stopcock. Put a waste container under the column.

3.8.2 To a 500-mL separatory funnel, add 70 mL of 5% ethyl ether in pentane. Then add 20 g of 3% water deactivated silica gel with a filter funnel.

3.8.3 Shake well and quickly transfer the slurry to the column.

3.8.4 Rinse the separatory funnel with the remaining 5% eluant and add to the column.

3.8.5 Cover the silica gel bed with 1 cm of granular sodium sulfate and drain the solvent just to the top of the sodium sulfate layer.

3.8.6 Measure 70 mL of the 5% ethyl ether in pentane eluant with a 100-mL graduated cylinder.

3.8.7 Pass the 70 mL of 5% eluant through the column and discard to a waste container as follows:

3.8.7.1 Add 5 mL of the 5% eluant to the methylated sample. Slightly tap the bottom side of the KD tube with your fingers to mix the sample. Then transfer the sample carefully to the silica gel column. Do not let the solvent level go below the top of the sodium sulfate.

3.8.7.2 While the eluant is penetrating the silica gel, rinse the KD tube by adding

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another 5 mL of the 5% eluant. Again, slightly tap the bottom side of the KD tube to dissolve the residues.

3.8.7.3 When the sample solution reaches the top of the sodium sulfate layer, add the rinse, carefully, to the column.

3.8.7.4 Repeat steps 3.8.7.2 - 3.8.7.3. Do not let the solvent level go below the top of the sodium sulfate.

3.8.7.5 Connect the reservoir to the top of the column.

3.8.7.6 When the second rinse reaches the top of the sodium sulfate layer, transfer the remaining eluant (~ 55 ml) to the reservoir and pass it through the column. All solvent passed through the column up to this point is discarded.

3.8.8 Measure 150 mL of the 10% ethyl ether in pentane eluant with a 250 mL graduated cylinder.

3.8.9 When the 5% eluant reaches the top of the sodium sulfate layer, close the stopcock, transfer the 10% eluant to the empty reservoir, replace the waste container under the column with the KD tube-concentrator set-up by raising the column and, open the stopcock to collect the 10% eluant in the KD set-up.

3.8.10 The 10% ethyl ether in pentane will elute the methylated dicamba and 5-hydroxy dicamba residues.

<u>Note:</u> Use 150 mL of 15% ethyl ether in pentane to elute quantitatively the methylated 5-hydroxy dicamba residue in wheat (grain and straw), soybean (forage) and cottonseed fractions. Use 150 mL Of 25% ethyl ether in pentane to elute quantitatively the methylated 5-hydroxy dicamba residue in soybean (straw) and pasture (hay).

3.8.11 Add 0.5 mL hexane and a boiling chip to the collected 10% ethyl ether/pentane. Connect a Vigreaux distillation column to the KD set-up and concentrate the solution to approximately 0.5 mL on a 65° water bath. Additional use of a nitrogen evaporator and a 40°C hot water bath may be necessary to reach a 0.5 mL volume.

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3.8.12 Dilute the concentrated sample with hexane to a volume of 5 ml for a 2.5 g equivalents or to 2.5 mL for a 1.25 g equivalents. Cover the KD tube with a stopper and mix by hand. This sample is now ready for quantitation.

3.9 Gas Chromatographic Analysis (^{63}Ni electron capture detector)

The following instruments and conditions are suitable for the analysis of dicamba and 5-hydroxy dicamba methyl esters in the title matrices. Other instruments and conditions (section 3.12.1.2) may be used provided that the subject compounds are separated from sample interferences, the detector response is linear over the desired range, and the retention time is stable on a daily basis. This is demonstrated by injecting standard solutions prior to the analysis of samples and after each 2-4 samples during analysis.

3.9.1 Instrument:
Gas Chromatograph; Hewlett-Packard model 5890 with ^{63}Ni electron capture detector, equipped with HP 7673A autosampler and HP 3393A integrator. The GC is interfaced with the Waters 860 networking computer system or equivalent, for data collection.

3.9.2 Column:
DB-210; 50% trifluoropropyl methyl siloxane, 30 m x 0.53 mm x 1.0 µm film thickness, cat. #125-0232, J & W Scientific.

3.9.3 GC Conditions:

Oven Temperature -	4 min. at 140°C then ramp to 180°C at 5°C/min.
Post Temperature -	Ramp to 220°C at 30°C/min and hold for 3 min.
Injector Temperature -	250°C
Detector Temperature -	350°C
Carrier Gas -	Helium at 30 mL/min.
Makeup Gas -	5% Argon/Methane at 30 mL/min.
Retention Times -	Dicamba M.E. = 3.3 min.
	5-OH Dicamba M.E. = 8.7 min.

3.10 Quantitation

The sample is fortified with the dicamba and 5-OH dicamba acids. The acids are converted to their methyl

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esters during the extraction procedures for GC quantitation. The gas chromatography reference standard solutions are also acids which have been methylated (section 3.3.5), therefore, there is no need for a conversion factor to quantitate dicamba and 5-OH dicamba.

3.10.1 Prepare standard curves for dicamba and 5-hydroxy dicamba methyl esters by injecting a fixed volume (2 uL or 5 uL) of each of a series of standards of known concentrations from section 3.3.7 and plotting the corresponding peak height versus the amount of standard injected.

3.10.2 Determine the concentration of dicamba and 5-hydroxy dicamba methyl esters in a sample by injecting the same volume of sample extract and interpolating the concentration using the peak height in the sample and the standard curve. Baseline corrected chromatographic peak heights of standards and samples are collected by a "Waters Chromatographic Data System". These peak heights are processed into sample concentrations using a linear least squares analysis program (RS/1, Software, BBN Software Products Corporation, Cambridge, MA 02238) to generate a standard curve from 3.10.1 and to interpolate sample concen-trations from the standard curve (section 3.10.2). This program is run on a VAX 6220 computer (Digital Equipment Corporation).

3.10.3 The concentrations of dicamba and 5-hydroxy dicamba methyl esters can also be determined manually. Plot peak heights versus concen-trations of injected standards (ng/uL) from section 3.10.1, on a log-log paper. Determine the concentration of the compound in the injected aliquot from section 3.10.2 by comparing the peak height of the compound to those of the standards in the standard curve.

3.10.4 Calculate the concentration of each compound in the sample using the following expression:

$$ppm = \frac{C\ (ng/uL) \times V\ (mL)}{W\ (g)}$$

where:

ppm= Concentration of the compound/ion in the sample in parts per million (ug/g).

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C= Concentration of compound/ion in the injected aliquot as determined from the standard curve (ng/uL).

V= Final volume of the sample extract taking into account all dilutions (mL).

W= Weight of sample taken for analysis (g).

3.11 Confirmatory Test (GC-MSD)

The mass selective detector (MSD) in the single ion monitoring mode (SIM) is used to confirm the methyl esters of dicamba and 5-hydroxy dicamba by monitoring three ions for each compound. The three ions for dicamba methyl ester are 203, 205 and 234. The three ions for 5-hydroxy dicamba are 233, 235 and 266. Confirmation of a chromatographic peak as dicamba methyl ester or 5-hydroxy methyl ester requires that the following criteria are met for each compound. First, the peak should be present at the retention time set for the compound within 1%. Second, the ratio of the three ions monitored should be within 20% of the average ratio of the three ions from the injected analytical standards. Once the peak is confirmed, the compound is quantitated as described in section 3.10 of this method. The results for all the three ions are averaged to get a single value for the residue level. The methyl ester of dicamba in the sample extract can often be detected at 0.01 ppm. The methyl ester of 5-hydroxy dicamba in the sample extract cannot be detected at 0.01 ppm because sample co-extractives contribute to the response of one and more ions giving different ratios for the three ions.
The following instruments and conditions are suitable for the confirmation of dicamba and 5-hydroxy dicamba methyl esters. Other instruments and conditions maybe used provided that the subject compounds are separated from sample interferences.

3.11.1 Instrument:
Gas Chromatograph; Hewlett-Packard, model 5890 with 5971A mass selective detector, equipped with HP 7673 autosampler and HP Vectra QS/20 Computer.

3.11.2 Column:
HP-1; 100% dimethyl polysiloxane, 12 m x 0.2 mm x 0.33 µm film thickness, part #19091J-101, Hewlett-Packard.

3.11.3 GC Conditions:
Oven Temperature - 0.5 min at 50°C then ramp to 200°C at 70°C/min and hold

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	for 2 min, then ramp to 225°C at 70°C/min.
Post Temperature -	Hold at 225°C for 3 min.
Injector Temperature -	230°C
Interface Temperature -	280°C
Carrier Gas -	Helium at 7 mL/min.
MSD Mode -	Selected Ion Monitoring
Dicamba Methyl Ester Ions -	m/e 203, 205, 234
5-OH Dicamba Methyl Ester Ions -	m/e 233, 235, 266
Retention Times -	Dicamba M.E.= 3.6 min. 5-OH Dicamba M.E = 4.6 min.

3.12 Interferences

3.12.1 Sample Matrices
Matrix interference can be minimized by performing one or more of the following steps:

3.12.1.1 The extract can be cleaned up by an additional column clean up before methylation using a C_{18} column.

3.12.1.1.1 Attach a 12cc C_{18} Bond Elut cartridge to a vacuum manifold and a vacuum source.

3.12.1.1.2 Condition the column with 2 column volumes of methanol. Do not allow sorbent to dry. Follow with 1 volume of deionized water. Leave 1 mL of water on top of the sorbent.

3.12.1.1.3 Attach a reservoir with an adaptor to the C_{18} cartridge. Transfer 50 mL of the sample extract from section 3.5.1.8

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into the reservoir and through the column. Collect the eluate in a 2- oz bottle. Do not allow sorbent to dry.

3.12.1.1.4 Elute the column with 15 mL of 10% methanol in water collecting in the same 2-oz bottle.

3.12.1.1.5 Transfer the combined eluates into another 8-oz bottle.

3.1.2.1.1.6 Proceed to section 3.6.2.

3.12.1.2 Changing GC columns from DB-210 to HP-1 or RTX-5 for quantitation will help resolve the analyte peaks from the interfering peaks.

3.12.1.2.1 HP-1; crosslinked 100% dimethyl polysiloxane, 30 m x 0.53 mm x 0.88 µm film thickness, part #19095Z-023, Hewlett-Packard.

Conditions:
Oven Temperature - 0.5 min at 100°C then ramp to 165°C at 35°C/min. and hold for 1.5 min.
Post Temperature - Ramp to 250°C at 70°C/min and hold for 2 min.
Injector Temperature - 250°C
Detector Temperature - 350°C
Carrier Gas - Helium at 16 mL/min.
Makeup Gas - 5% Argon/ Methane at 44 mL/min.
Retention Times - Dicamba M.E. = 2.6 min.
- 5-OH Dicamba M.E. = 4.1 min.

3.12.1.2.2 RTX-5; crossbonded 95% dimethyl-5% diphenyl polysiloxane, 30 m x 0.53 mm x 1.5 µm film thickness, cat. #10270, column #38819, Baxter.

Conditions:
Oven Temperature -200°C for 5 min.
Post Temperature - Ramp to 250°C at 30°C/min and hold for 5 min.
Injector Temperature - 250°C

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Detector Temperature - 350°C
Carrier Gas - Helium at 8 mL/min.
Makeup Gas - 5% Argon/ Methane at 50 mL/min.
Retention Times - Dicamba M.E. = 2.5 min.
- 5-OH Dicamba M.E = 4.9 min.

3.12.2 Glassware
Glassware must be thoroughly cleaned by washing with hot water and detergents followed by rinsing with tap water and deionized water. Then glassware must be dried at room temperature or in a 120°C oven and finally rinsed with acetone.

3.12.3 Solvents and Reagents
Solvents and reagents must be of high purity grade.

3.12.4 Sample Cross-Contaminations
Derivatize samples in order of increasing concentration of the compound if this is known. Rinse the diazomethane delivery tube with ethyl ether before derivatizing the next sample. When contamination is suspected, replace tube C with another test tube containing ethyl ether and bubble the diazomethane gas. Do this before derivatizing each sample or change the delivery tube. During GC quantitation, there should be a between-sample rinsing of the syringe with hexane and then with the next sample extract.

3.13 Time Required for Analysis

A single sample can be extracted and cleaned up for gas chromatographic analysis in eight hours. A set of six samples requires 16 hours for complete analysis.

4. RESULTS AND DISCUSSIONS

4.1 Accuracy

The recoveries of dicamba and its 5-hydroxy metabolite from fortified control samples are listed in Table 1. The average recoveries (x ± sd) for dicamba and 5-hydroxy dicamba are 98% ± 8 and 90% ± 10, respectively.

4.2 Precision

The coefficient of variation for the recoveries in Table 1 are from 0.0% to 21% for dicamba and 3% to 23% for 5-hydroxy dicamba.

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4.3 Limit of Detection

The limit of detection is 0.01 ppm for dicamba and 5-hydroxy dicamba for all matrices tested.

5. CONCLUSIONS

This method was developed by Sandoz Agro, Inc. for the determination of dicamba and 5-hydroxy dicamba residues in the title substrates. It detects and quantitates residues at or over the limit of detection of 0.01 ppm. The recoveries of the compounds from fortified control samples are within the acceptable range of 70-120%.

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6. CERTIFICATE OF AUTHENTICITY

I hereby state that the description of this method and the supporting data (recoveries) are accurate and correct to the best of my knowledge.

Nancy C. Jimenez, Sr. Scientist II (author) — Date 7/30/93

Andrea Clouser, Scientist II — Date 7/30/93

Kevin Adlaf, Associate Scientist — Date 7-30/93

Lois Marquardt, Scientist I — Date 7/30/93

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TABLES AND FIGURES

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Table 1. Recoveries of Dicamba and 5-Hydroxy Dicamba from Fortified Control Samples.

Sample No.	Description	Fort. Level (PPM)	Percent Recoveries	
			Dicamba	5-OH Dicamba
857-12368	Barley (grain)	0.05	100	84
847-04308	"	0.10	98	60
-04308	"	0.10	118	115
-02943	"	0.10	91	66
-02943	"	0.10	96	94
827-05630	"	0.10	111	86
857-12693	"	0.50	96	84
		$\bar{x}$ ± sd	101 ± 10	84 ± 18
847-04303	Barley (straw)	0.10	107	85
827-05621	"	0.10	109	102
857-12689	"	1.00	90	86
		$\bar{x}$ ± sd	102 ± 10	91 ± 10
0109231A/ 01-001-1	Corn (forage)	0.01	110	80
0109231A/ 01-001-1	"	0.01	100	70
0109231A/ 01-001-1	"	0.10	92	91
		$\bar{x}$ ± sd	100 ± 9	80 ± 10
0109208A/ 001-03-2	Corn (grain)	0.01	110	70
847-04579	"	0.05	96	96
0109208A/ 001-03-2	"	0.10	120	103
807-43188	"	0.50	98	90
		$\bar{x}$ ± sd	106 ± 11	90 ± 14

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Table 1. (continued)
Recoveries of Dicamba and 5-Hydroxy Dicamba from Fortified Control Samples.

Sample No.	Description	Fort. Level (PPM)	Percent Recoveries	
			Dicamba	5-OH Dicamba
0109322A/ 01-002-1	Corn (silage)	0.01	100	100
0109322A/ 01-002-1	"	0.10	94	102
847-02847	"	0.50	90	86
847-03470	"	1.00	96	94
807-43192	"	5.00	1.00	90
		$\bar{x} \pm sd$	96 ± 4	94 ± 7
0109317A/ 01-004-2	Corn (fodder)	0.01	110	70
0109317A/ 01-004-2	"	0.10	75	73
847-02862	"	0.10	90	86
847-04567	"	0.50	90	83
		$\bar{x} \pm sd$	91 ± 14	78 ± 8
847-04197	Corn (stalks)	0.50	98	94
807-43190	"	5.00	99	98
		$\bar{x} \pm sd$	98 ± 1	96 ± 3
807-44231	Cotton (seed)	0.10	98	86
807-06982	"	0.20	123	125
-07898	"	0.20	98	115
-02476	"	0.20	88	100
-04175	"	0.20	90	100
807-42003	"	0.20	95	112
-41907	"	0.20	92	112
		$\bar{x} \pm sd$	98 ± 12	107 ± 13

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Table 1. (continued)
Recoveries of Dicamba and 5-Hydroxy Dicamba from Fortified Control Samples.

Sample No.	Description	Fort. Level (PPM)	Percent Recoveries	
			Dicamba	5-OH Dicamba
807-44232	Cotton (trash)	0.10	98	100
817-01907	"	0.20	109	121
		$\bar{x}$ ± sd	104 ± 8	110 ± 15
854-15616	Cotton (hulls)	0.20	85	78
-15616	"	0.20	85	82
-15616	"	0.20	85	77
		$\bar{x}$ ± sd	85 ± 0.0	79 ± 3
854-16204	Cotton (meal)	0.20	88	78
-16204	"	0.20	82	74
-16204	"	0.20	82	73
		$\bar{x}$ ± sd	84 ± 4	75 ± 3
231118A/ 01-001-01	Cotton (refined oil)	0.01	120	80
231118A/ 01-001-01	"	0.10	70	70
854-16206	"	0.10	110	96
-16206	"	0.10	85	76
-16206	"	0.10	93	86
		$\bar{x}$ ± sd	96 ± 20	82 ± 10
854-16205	Cotton (crude oil)	0.10	100	91
-16205	"	0.10	91	83
-16205	"	0.10	98	91
		$\bar{x}$ ± sd	96 ± 5	88 ± 5

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Table 1. (continued) Recoveries of Dicamba and 5-Hydroxy Dicamba from Fortified Control Samples.

Sample No.	Description	Fort. Level (PPM)	Percent Recoveries	
			Dicamba	5-OH Dicamba
827-00161	Pasture (grass)	20	95	--
-00161	"	10	--	105
-00086	"	50	96	--
-00086		20	--	95
		$\bar{x}$ ± sd	96 ± 1	100 ± 7
2422117B/ 01-002-1-A	Pasture (hay)	0.01	80	70
2422117B/ 01-002-1-A	"	0.10	74	75
827-00195	"	100	94	--
-00195	"	50	--	97
		$\bar{x}$ ± sd	83 ± 10	81 ± 14
854-15398	Peanut (green hay)	0.10	94	88
-15398	"	0.10	93	86
-15398	"	0.10	94	82
		$\bar{x}$ ± sd	94 ± 1	85 ± 3
2111172A/ 01-012-1	Sorghum (grain)	0.01	110	80
2111172A/ 01-012-1	"	0.10	80	75
857-13020	"	0.10	92	92
		$\bar{x}$ ± sd	94 ± 15	82 ± 9
2111172A/ 01-013-1	Sorghum (silage)	0.01	110	90
2111172A/0 1-013-1	"	0.10	85	79
857-13017	"	0.50	91	82
		$\bar{x}$ ± sd	95 ± 13	84 ± 6

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Table 1. (continued)
Recoveries of Dicamba and 5-Hydroxy Dicamba from Fortified Control Samples.

Sample No.	Description	Fort. Level (PPM)	Percent Recoveries	
			Dicamba	5-OH Dicamba
857-13154	Soybean (grain)	0.50	118	122
-13154	"	0.10	120	80
807-27741	"	0.05	100	88
		$\bar{x}$ ± sd	113 ± 11	97 ± 22
857-13151	Soybean (forage)	0.50	120	120
-13151	"	0.10	120	90
		$\bar{x}$ ± sd	120 ± 0.0	105 ± 21
807-27742	Soybean (stalks)	0.05	110	110
857-13157	Soybean (straw)	0.50	116	102
857-14740	Sugar Cane (leaves)	0.50	94	88
1/	Sugar Cane (stalks)	0.01	100	70
"	"	0.10	88	90
857-14746	"	0.50	96	88
		$\bar{x}$ ± sd	95 ± 6	83 ± 11
1/	Tomato (fruit)	0.01	70	90
"	"	0.10	73	82
857-12129	"	0.10	94	98
-12129	"	0.10	99	83
-12129	"	0.50	100	96
-12129	"	0.50	106	91
		$\bar{x}$ ± sd	90 ± 15	90 ± 6

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Table 1. (continued)
Recoveries of Dicamba and 5-Hydroxy Dicamba from Fortified Control Samples.

Sample No.	Description	Fort. Level (PPM)	Percent Recoveries	
			Dicamba	5-OH Dicamba
857-11988	Tomato (juice)	0.10	102	102
-11988	"	0.10	98	101
-11988	"	0.50	78	71
-11988	"	0.50	104	89
		$\bar{x} \pm sd$	95 ± 12	91 ± 14
857-11989	Tomato (pomace)	0.10	82	69
-11989	"	0.10	98	110
-11989	"	0.50	80	84
-11989	"	0.50	102	88
		$\bar{x} \pm sd$	90 ± 11	88 ± 17
857-11990	Tomato (sauce)	0.10	108	110
-11990	"	0.10	97	90
-11990	"	0.50	102	96
-11990	"	0.50	113	88
		$\bar{x} \pm sd$	105 ± 7	96 ± 10
857-11377	Wheat (grain)	0.02	100	90
-12970	"	0.05	96	84
827-05737	"	0.10	130	100
-04118	"	0.10	105	131
847-02899	"	0.10	115	97
-02899	"	0.10	105	72
-04297	"	0.10	100	72
-02963	"	0.10	87	97
-02963	"	0.20	92	92
-02963	"	1.00	96	80

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Table 1. (continued)
Recoveries of Dicamba and 5-Hydroxy Dicamba from Fortified Control Samples.

Sample No.	Description	Fort. Level (PPM)	Percent Recoveries	
			Dicamba	5-OH Dicamba
847-02963	Wheat (grain)	1.00	98	88
		$\bar{x}$ ± sd	102 ± 12	91 ± 16
857-13816	Wheat (silage)	0.50	95	90
847-04300	"	0.10	90	58
		$\bar{x}$ ± sd	92 ± 4	74 ± 23
857-13822	Wheat (straw)	0.50	88	88
-12651	"	0.50	82	84
827-04117	"	0.50	80	88
847-02920	"	0.10	112	105
		$\bar{x}$ ± sd	90 ± 15	91 ± 9
857-11126	Wheat (bran)	1.00	108	78
-11126	"	0.20	94	89
-11126	"	0.20	100	90
-11126	"	0.10	88	105
-11126	"	0.10	107	105
		$\bar{x}$ ± sd	99 ± 10	94 ± 13
857-11128	Wheat (germ)	1.00	102	92
-11128	"	1.00	98	74
-11128	"	0.10	84	92
-11128	"	0.10	94	84
-11128	"	0.10	95	101
		$\bar{x}$ ± sd	95 ± 7	89 ± 10

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Table 1. (continued)
Recoveries of Dicamba and 5-Hydroxy Dicamba from Fortified Control Samples.

Sample No.	Description	Fort. Level (PPM)	Percent Recoveries	
			Dicamba	5-OH Dicamba
857-11130	Wheat (flour)	1.00	118	101
-11130	"	1.00	112	100
-11130	"	0.10	102	108
-11130	"	0.10	76	65
-11130	"	0.10	92	106
		$\bar{x} \pm sd$	100 ± 17	96 ± 18

1/ Samples were obtained from commercial sources.

2/ Recoveries were not determined.

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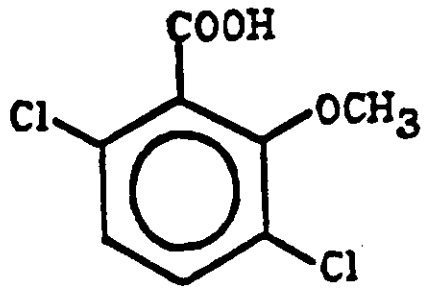
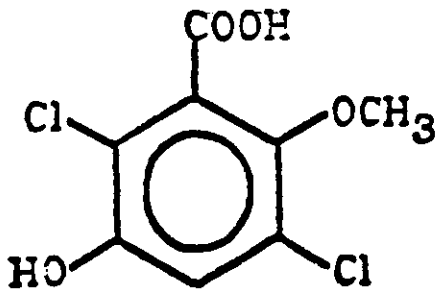
FIGURE 1. Chemical Structures



DICAMBA; 2-methoxy-3,6-dichlorobenzoic acid, or
3,6-dichloro-o-anisic acid



5-HYDROXY DICAMBA; 2-methoxy-3,6-dichloro-5-
hydroxybenzoic acid

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FIGURE 2. Derivatization Set-up

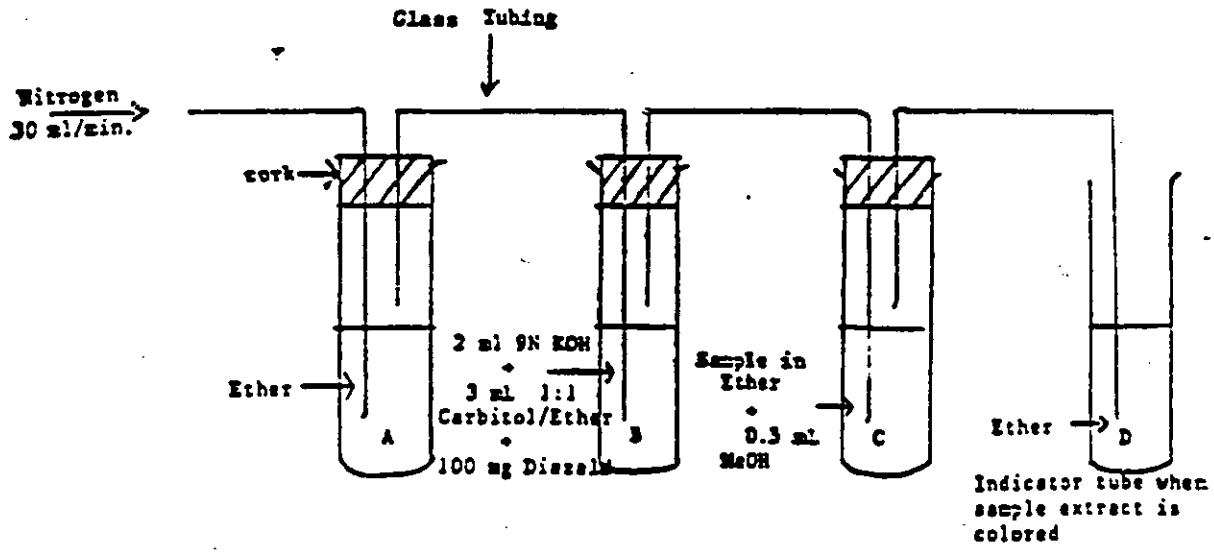

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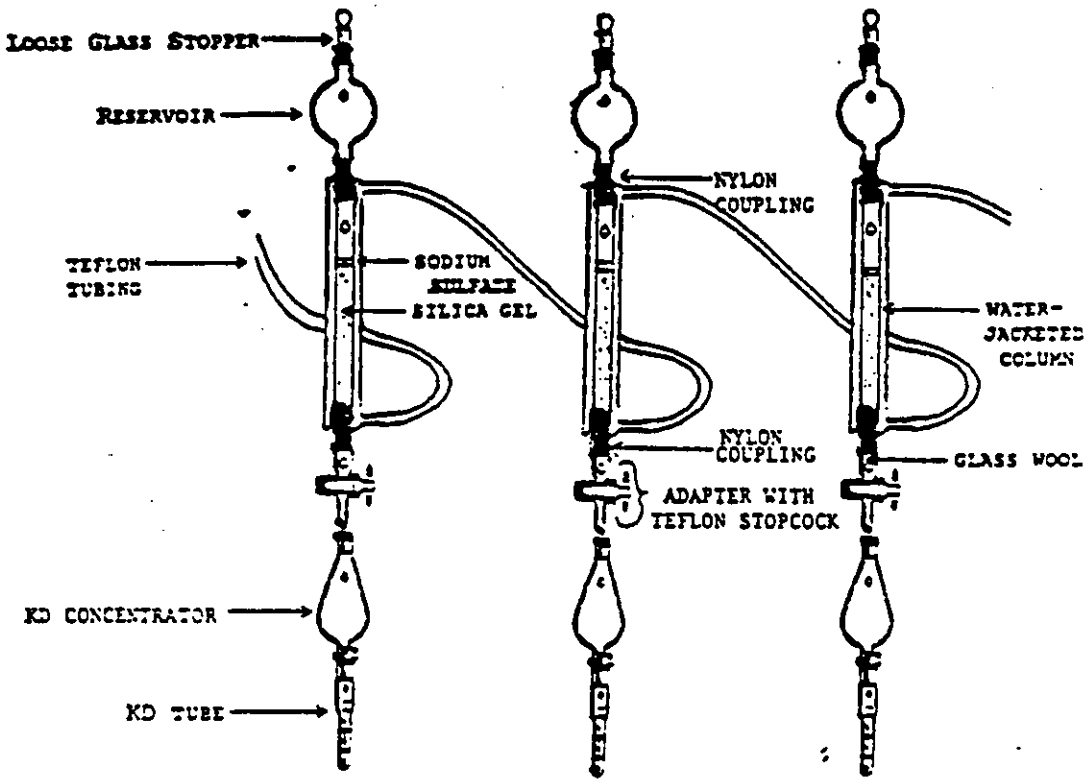
FIGURE 3. Column Cleanup Set-up



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CURVES AND CHROMATOGRAMS

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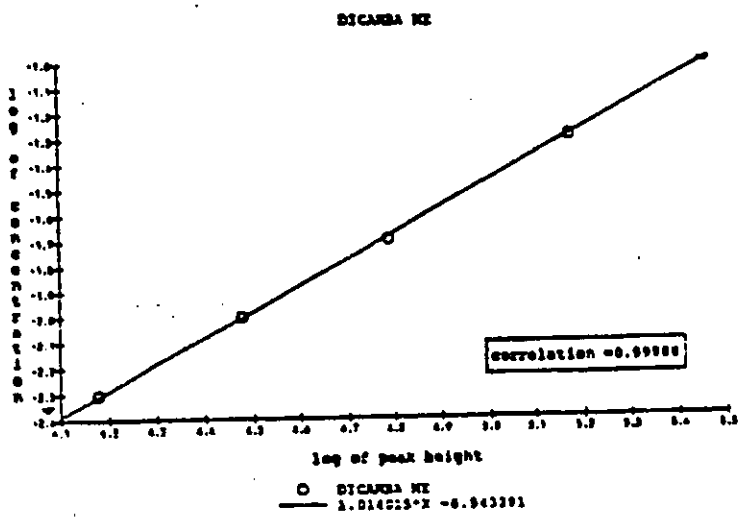

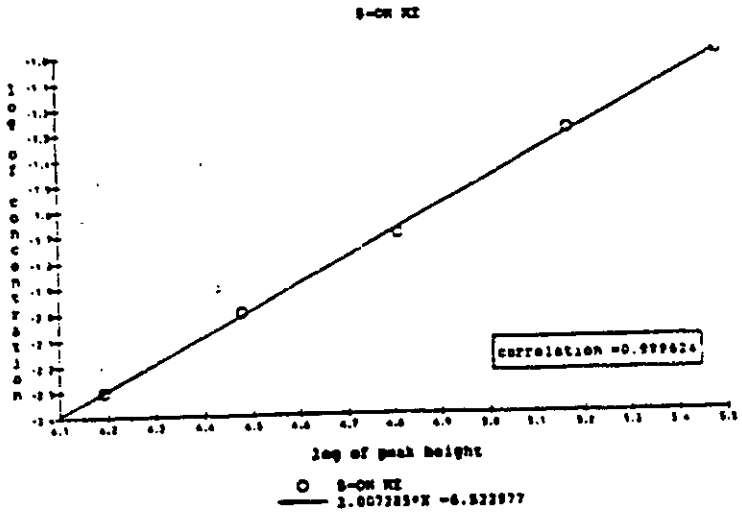

Figure 1. Representative Computer-Generated GC-ECD Standard Curves of Dicamba Methyl Ester and 5-Hydroxy (5-OH) Dicamba Methyl Ester (M.E.). Concentrations of 0.10, 0.05, 0.02, 0.01 and 0.005 ng/uL acid equivalents for each compound.

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Millipore Corporation, Waters Chromatography Division
34 Maple Street, Milford, Massachusetts 01757

Version: 860/V3.0 Printed: 17-Mar-1993 at 14:14:53 Page 1
Node: CHISTC GC Project: DIC_SOH_CROP User: CLOUSER
Waters GC Report Using Multi-method AC031793B Line 4

0317STD5E12B 17-Mar-1993 11:24:55

Header:

Acquisition method	[illegible]_B	Processing method	AM0691_B
Units	PPM	System number	4
Channel	1	Manual Injector Vial	0
Injection	1	Total injections	1
Run time	10.00 min	Sample rate	20.00 per sec
Injection volume	2 uL	Mode	Calibration
Acquisition version	LAC/E/V3.0	Processing version	V3.0

Description:

Node:
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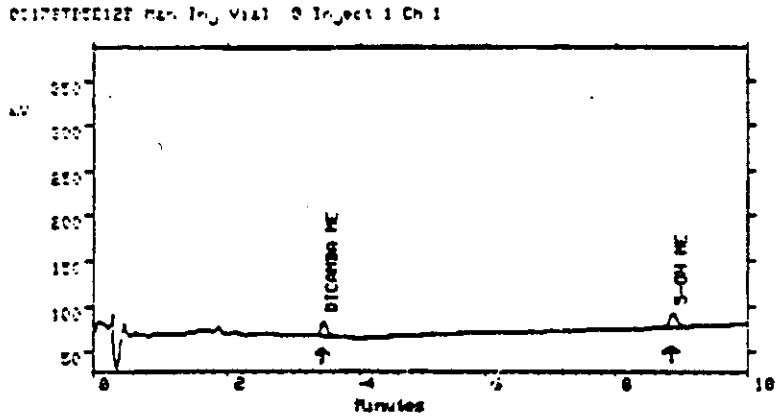
First Plot:



GC Results:

Peak Name	Ret Time	Area	Height
DICAMBA ME	3.448	99014	15107
5-OH ME	8.873	125132	15586

Figure 2. Representative GC-ECD chromatogram of Dicamba M.E. and 5-OH Dicamba M.E. Reference Standards, 0.005 ng/uL acid equivalent for each compound.

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Version: 860/V3.0 Printed: 17-Mar-1993 at 14:[illegible]5:14 Page 1
Node: CMISTC GC Project: DIC_5OH_CROP User: CLOUSER
Waters GC Report Using Multi-method AC031793B Line 7

0 3 1 7 S T D 1 E 1 0 B 1 7 - M a r - 1 9 9 3 1 2 : 1 6 : 2 1

Header:

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Injection	1	Total injections	1
Run time	10.00 min	Sample rate	20.00 per sec
Injection volume	2 uL	Mode	Calibration
Acquisition version	LAC/E/V3.0	Processing version	V3.0

Description:

Node:
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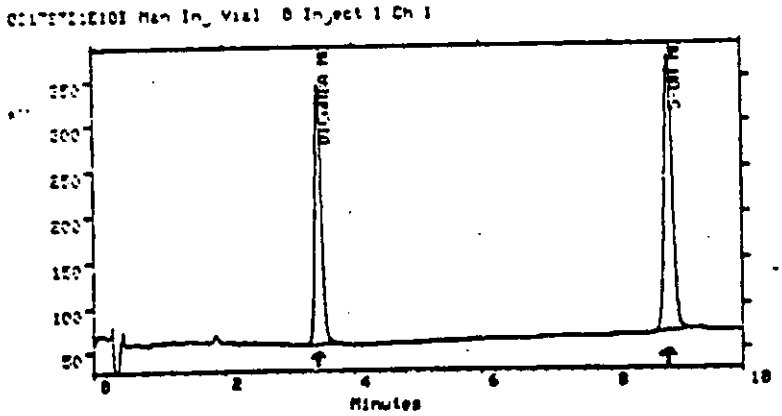
First Plot:



GC Results:

Peak Name	Ret Time	Area	Height
DICAMBA ME	3.448	1995520	287183
5-OH ME	8.873	2619931	305619

Figure 3. Representative GC-ECD chromatogram of Dicamba M.E. and 5-OH Dicamba M.E. Reference Standards, 0.10 ng/uL acid equivalent for each compound.

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Version: 860/V3.0 Printed: 20-Apr-1993 at 15:13:32 Page 1
Node: CHISTC GC Project: DIC_SOH_CROP User: ADLAF

K A F O R A G E C K 2 0 - A p r - 1 9 9 3 1 3 : 5 0 : 4 6

Header:

Acquisition method	AM0691_B	Processing method	AM0691_B
Units	PPM	System number	4
Channel	1	Manual Injector Vial	0
Injection	1	Total injections	1
Run time	10.00 min	Sample rate	20.00 per sec
Injection volume	2 uL	Mode	Analysis
Acquisition version	LAC/I/V3.0	Processing version	V3.0

Description:

Mode:
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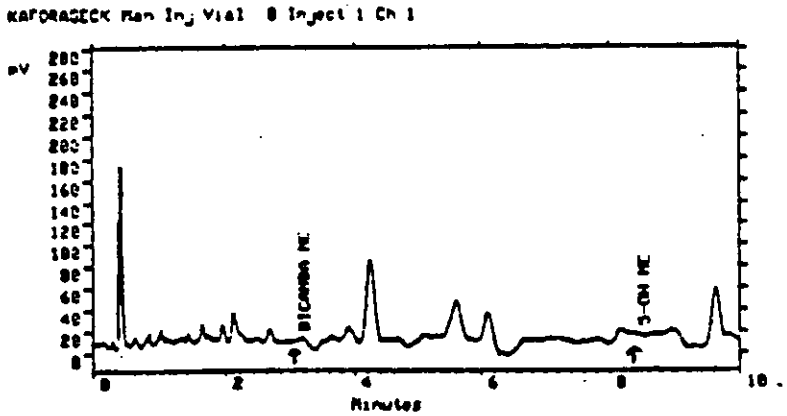
First Plot:



GC Results:

Peak Name	Ret Time	Area	Height
DICAMBA ME	3.083	33	283
5-OH ME	8.437	-	-

Figure 4. Representative GC-ECD chromatogram of Corn Forage Control Sample, 0.50 mg equivalent/uL injected, <0.005 ng/uL acid equivalent (<0.01 ppm) for each Dicamba M.E.

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Version: 860/V3.0 Printed: 20-Apr-1993 at 15:15:28 Page 1
Node: CHISTC GC Project: DIC_SOH_CROP User: ADLAF

X A F O R A G E F E 20-Apr-1993 13:07:40

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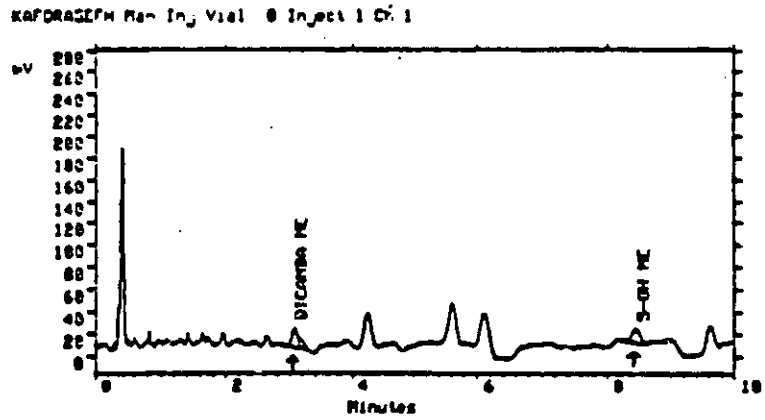
Injection	1	Total injections	1
Run time	10.00 min	Sample rate	20.00 per sec
Injection volume	2 uL	Mode	Analysis
Acquisition version	LAC/E/V3.0	Processing version	V3.0

Description:

Mode:
Acquired on node LACR02 system 4 for DIC_SOH_CROP

First Plot:



GC Results:

Peak Name	Ret Time	Area	Height
DICAMBA ME	3.067	95005	15951
5-OH ME	8.440	94161	11812

Figure 5. Representative GC-ECD chromatogram of Corn Forage Control Sample Fortified at 0.01 ppm each Dicamba and 5-OH Dicamba acids, 0.50 mg equivalent/uL injected, 0.005 ng/uL acid equivalent (0.01 ppm) Dicamba M.E. and 0.0035 ng/uL acid equivalent (0.007 ppm) 5-OH Dicamba M.E.; 100% Dicamba M.E. and 70% 5-OH Dicamba M.E. recoveries.

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Version: 860/V3.0 Printed: 4-May-1993 at 10:39:49 Page 1
Node: CHISTC GC Project: DIC_SOH_CROP User: ADLAF

A C C H S I S C K 17-Mar-1993 10:50:33

Header:

Acquisition method	AM0691_B	Processing method	AM0691_B
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Channel	1	Manual Injector Vial	0
Injection	1	Total injections	1
Run time	10.00 min	Sample rate	20.00 per sec
Injection volume	2 uL	Mode	Analysis
Acquisition version	LAC/E/V3.0	Processing version	V3.0

Description:

Node:
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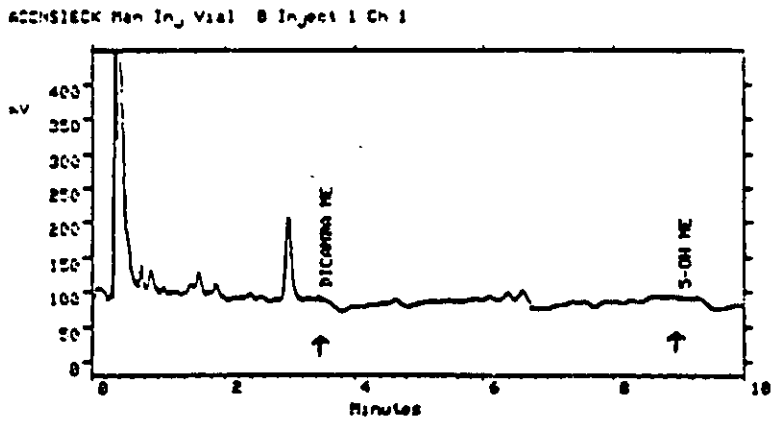
First Plot:



GC Results:

Peak Name	Ret Time	Area	Height
DICAMBA ME	3.450	21160	4548
5-OH ME	8.983	9450	1511

Figure 6. Representative GC-ECD chromatogram of Corn Silage Control Sample, 0.50 mg equivalent/uL injected, <0.005 ng/uL acid equivalent (<0.01 ppm) for each Dicamba M.E. and 5-OH Dicamba M.E. detected.

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Version: 860/V3.0 Printed: 4-May-1993 at 10:57:16 Page 1
Node: CHISTC GC Project: DIC_SOH_CROP User: ADLAF

A C C N S I S F N 17-Mar-1993 11:07:42

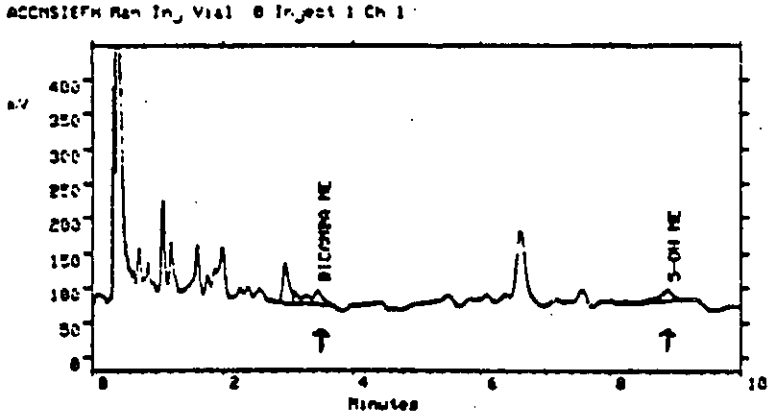
Header:

Acquisition method	AM0691_B	Processing method	AM0691_B
Units	PPM	System number	4
Channel	1	Manual Injector Vial	0
Injection	1	Total injections	1
Run time	10.00 min	Sample rate	20.00 per sec
Injection volume	2 uL	Mode	Analysis
Acquisition version	LAC/E/V3.0	Processing version	V3.0

Description:

Node:
Acquired on node LACR02 system 4 for DIC_SOH_CROP

First Plot:



GC Results:

Peak Name	Ret Time	Area	Height
DICAMBA ME	3.452	165167	19103
5-OH ME	8.878	175927	16409

Figure 7. Representative GC-ECD chromatogram of Corn Silage Control Sample Fortified at 0.01 ppm each Dicamba and 5-OH Dicamba acids, 0.50 mg equivalent/uL injected, 0.005 ng/uL acid equivalent (0.01 ppm) Dicamba M.E. and 0.005 ng/uL acid equivalent (0.01 ppm) 5-OH Dicamba M.E.; 100% Dicamba M.E. and 100% 5-OH Dicamba M.E. recoveries.

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Version: 860/V3.0 Printed: 28-Apr-1993 at 10:..:32 Page 1
Node: CMISTC GC Project: DIC_SOH_CROP User: ADLAF

K J A G R A I N C K 28-Apr-1993 8:56:31

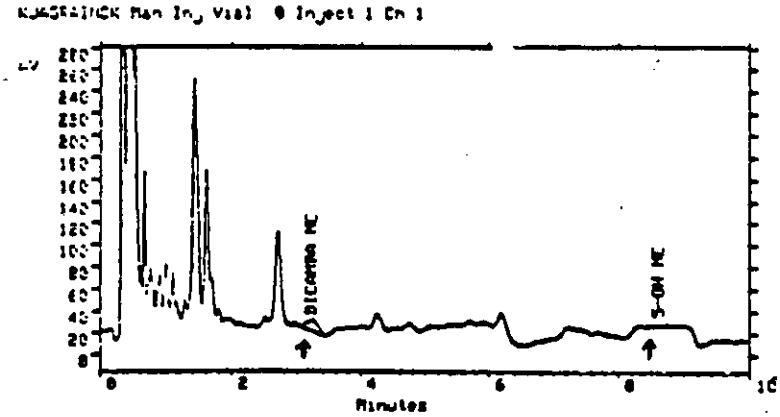
Header:

Acquisition method	AM0691_B	Processing method	AM0691_B
Units	PPM	System number	4
Channel	1	Manual Injector Vial	0
Injection	1	Total injections	1
Run time	10.00 min	Sample rate	20.00 per sec
Injection volume	2 uL	Mode	Analysis
Acquisition version	LAC/E/V3.0	Processing version	V3.0

Description:

Node:
Acquired on node LACR02 system 4 for DIC_SOH_CROP

First Plot:



GC Results:

Peak Name	Ret Time	Area	Height
DICAMBA ME	3.100	-	-
5-OH ME	8.500	-	-

Figure 8. Representative GC-ECD chromatogram of Corn Grain Control Sample, 0.50 mg/equivalent/uL injected, <0.005 ng/uL acid equivalent (<0.01 ppm) for each Dicamba M.E. and 5-OH Dicamba M.E. detected.
Note: The above residue levels were confirmed by GC-MSD (see Figure 25).

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Version: 860/V3.0 Printed: 28-Apr-1993 at 12:19:06 Page 1
Node: CHISTC GC Project: DIC_SOH_CROP User: ADLAF

K J A G R A I N F E 28-Apr-1993 9:41:16

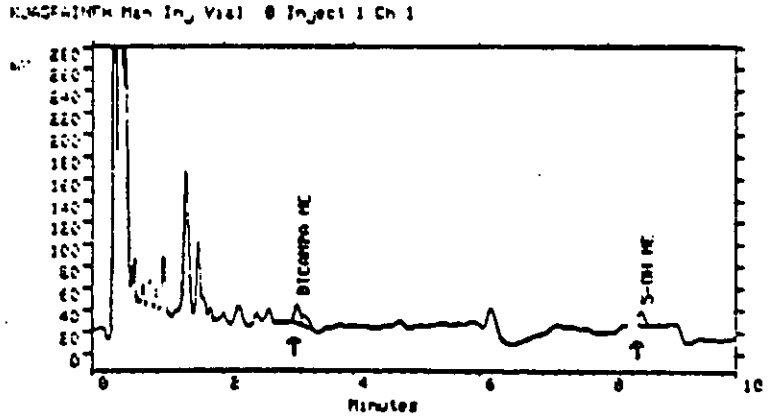
Header:

Acquisition method	AM0691_B	Processing method	AM0691_B
Units	PPM	System number	4
Channel	1	Manual Injector Vial	0
Injection	1	Total injections	1
Run time	10.00 min	Sample rate	30.00 per sec
Injection volume	2 uL	Mode	Analysis
Acquisition version	LAC/E/V3.0	Processing version	V3.0

Description:

Node:
Acquired on node LACR02 system 4 for DIC_SOH_CROP

First Plot:



GC Results:

Peak Name	Ret Time	Area	Height
DICAMBA ME	3.128	99692	17672
5-OH ME	8.512	94984	12091

Figure 9. Representative GC-ECD chromatogram of Corn Grain Control Sample Fortified at 0.01 ppm each Dicamba and 5-OH Dicamba acids, 0.50 mg equivalent/uL injected, 0.0055 ng/uL acid equivalent (0.011 ppm) Dicamba M.E. and 0.0035 ng/uL acid equivalent (0.007 ppm) 5-OH Dicamba M.E.; 110% Dicamba M.E. and 70% 5-OH Dicamba M.E. recoveries.

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Version: 860/V3.0 Printed: 28-Apr-1993 at 12:30:46 Page 1
Node: CHISTC GC Project: DIC_SOH_CROP User: ADLAF

K J A G R A I N F 1 28-Apr-1993 10:11:10

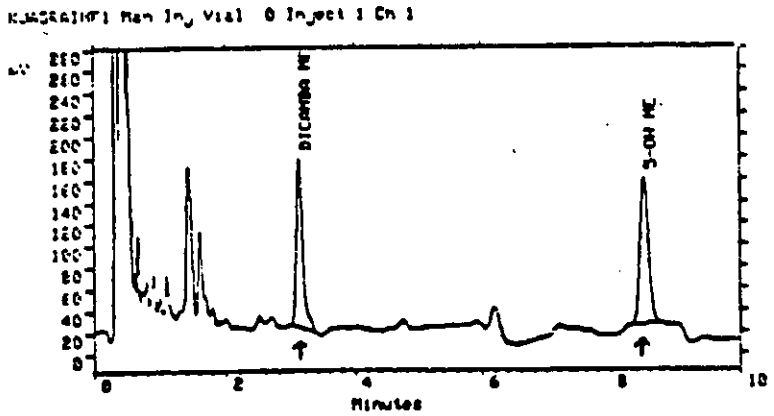
Header:

Acquisition method	AM0691_B	Processing method	AM0691_B
Units	PPM	System number	4
Channel	1	Manual Injector Vial	0
Injection	1	Total injections	1
Run time	10.00 min	Sample rate	20.00 per sec
Injection volume	2 uL	Mode	Analysis
Acquisition version	LAC/E/V3.0	Processing version	V3.0

Description:

Node:
Acquired on node LACR02 system 4 for DIC_SOH_CROP

First Plot:



GC Results:

Peak Name	Ret Time	Area	Height
DICAMBA ME	3.123	1061911	154366
5-OH ME	8.528	1226485	133808

Figure 10. Representative GC-ECD chromatogram of Corn Grain Control Sample Fortified at 0.10 ppm each Dicamba and 5-OH Dicamba acids, 0.50 mg equivalent/uL injected, 0.060 ng/uL acid equivalent (0.120 ppm) Dicamba M.E. and 0.0515 ng/uL acid equivalent (0.103 ppm) 5-OH Dicamba M.E.; 120% Dicamba M.E. and 103% 5-OH Dicamba M.E. recoveries.
Note: The above residue levels were confirmed by GC-MSD (see Figure 26).

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Version: 860/V3.0 Printed: 4-May-1993 at 12:18:55 Page 1
Node: CHISTC GC Project: DIC_SOH_CROP User: ADLAF

A C C N F D S C X 17-Mar-1993 11:42:03

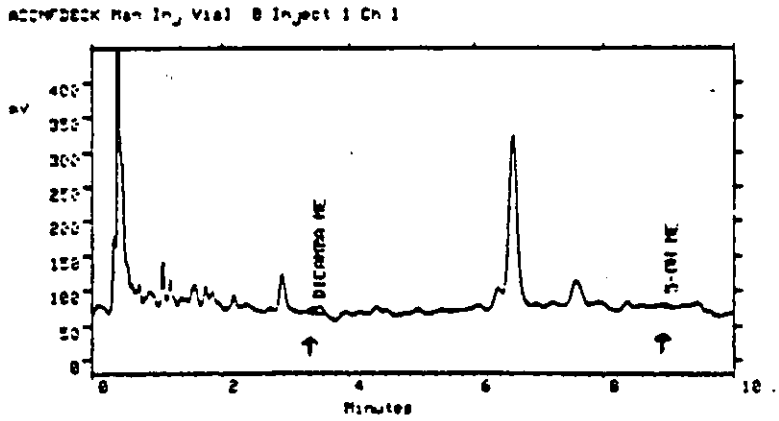
Header:

Acquisition method	AM0691_B	Processing method	AM0691_B
Units	PPM	System number	4
Channel	1	Manual Injector Vial	0
Injection	1	Total injections	1
Run time	10.00 min	Sample rate	20.00 per sec
Injection volume	2 uL	Mode	Analysis
Acquisition version	LAC/E/V3.0	Processing version	V3.0

Description:

Node:
Acquired on node LACR02 system 4 for DIC_SOH_CROP

First Plot:



GC Results:

Peak Name	Ret Time	Area	Height
DICAMBA ME	3.442	38742	6356
5-OH ME	8.945	34040	4872

Figure 11. Representative GC-ECD chromatogram of Corn Fodder Control Sample, 0.50 mg equivalent/uL injected, <0.005 ng/uL acid equivalent (<0.01 ppm) for each Dicamba M.E. and 5-OH Dicamba M.E. detected.

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Version: 860/V3.0 Printed: 4-May-1993 at 12:27:34 Page 1
Node: CHISTC GC Project: DIC_SOH_CROP User: ADLAF

A C C N F D S F N 17-Mar-1993 11:59:10

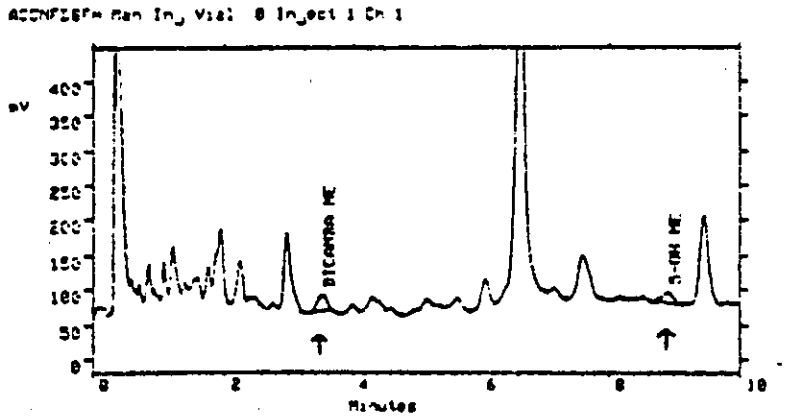
Header:

Acquisition method	AM0691_B	Processing method	AM0691_B
Units	PPM	System number	4
Channel	1	Manual Injector Vial	0
Injection	1	Total injections	1
Run time	10.00 min	Sample rate	20.00 per sec
Injection volume	2 uL	Mode	Analysis
Acquisition version	LAC/E/V3.0	Processing version	V3.0

Description:

Node:
Acquired on node LACR02 system 4 for DIC_SOH_CROP

First Plot:



GC Results:

Peak Name	Ret Time	Area	Height
DICAMBA ME	3.503	239681	22366
5-OH ME	8.905	178538	15216

Figure 12. Representative GC-ECD chromatogram of Corn Fodder Control Sample Fortified at 0.01 ppm each Dicamba and 5-OH Dicamba acids, 0.50 mg equivalent/uL injected, 0.0055 ng/uL acid equivalent (0.011 ppm) Dicamba M.E. and 0.0035 ng/uL acid equivalent (0.007 ppm) 5-OH Dicamba M.E.; 110% Dicamba M.E. and 70% 5-OH Dicamba M.E. recoveries.

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Version: 860/V3.0 Printed: 4-May-1993 at 12:36:07 Page 1
Node: CHISTC GC Project: DIC_5OH_CROP User: ADLAF

D B 2 1 0 T O M A C K 1 5 - M a r - 1 9 9 3 1 3 : 1 4 : 4 9

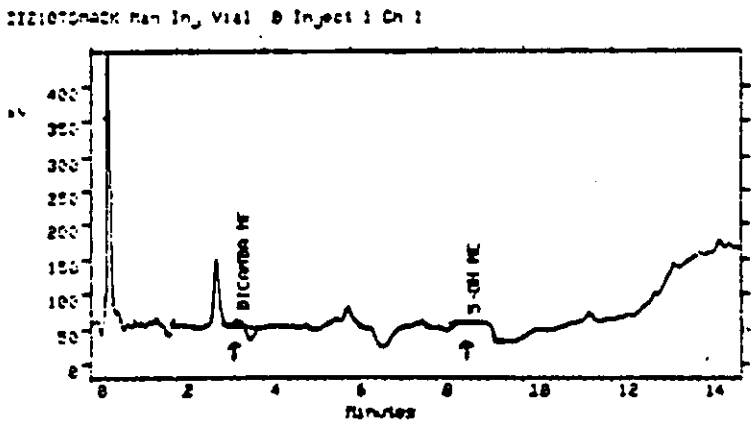
Header:

Acquisition method	AM0691_B	Processing method	AM0691_B
Units	ppm	System number	4
Channel	1	Manual Injector Vial	0
Injection	1	Total injections	1
Run time	20.00 min	Sample rate	20.00 per sec
Injection volume	2 uL	Mode	Analysis
Acquisition version	LAC/E/V3.0	Processing version	V3.0

Description:

Node:
Acquired on node LACR02 system 4 for DIC_5OH_CROP

First Plot:



GC Results:

Peak Name	Ret Time	Area	Height
DICAMBA ME	3.335	53871	8430
5-OH ME	8.710	-	-

Figure 13. Representative GC-ECD chromatogram of Whole Tomato Control Sample, 0.50 mg equivalent/uL injected, <0.005 ng/uL acid equivalent (<0.01 ppm) for each Dicamba M.E. and 5-OH Dicamba M.E. detected.

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Version: 860/V3.0 Printed: 4-May-1993 at 13:39:30 Page 1
Node: CHISTC GC Project: DIC_SOH_CROP User: ADLAF

D B 2 1 0 T O M A T O 18-Mar-1993 14:23:00

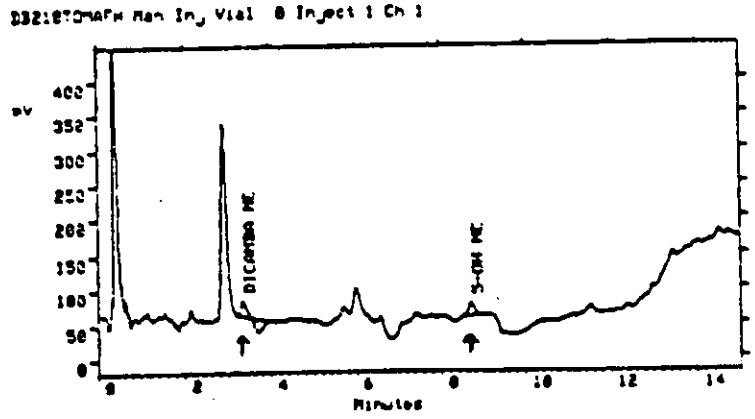
Header:

Acquisition method	AM0691_B	Processing method	AM0691_B
Units	ppm	System number	4
Channel	1	Manual Injector Vial	0
Injection	1	Total injections	1
Run time	20.00 min	Sample rate	20.00 per sec
Injection volume	3 uL	Mode	Analysis
Acquisition version	LAC/E/V3.0	Processing version	V3.0

Description:

Node:
Acquired on node LACR02 system 4 for DIC_SOH_CROP

First Plot:



GC Results:

Peak Name	Ret Time	Area	Height
DICAMBA ME	3.342	191194	21670
5-OH ME	8.698	165854	18508

Figure 14. Representative GC-ECD chromatogram of Whole Tomato Control Sample Fortified at 0.01 ppm each Dicamba and 5-OH Dicamba acids, 0.50 mg equivalent/uL injected, 0.0035 ng/uL acid equivalent (0.007 ppm) Dicamba M.E. and 0.0045 ng/uL acid equivalent (0.009 ppm) 5-OH Dicamba M.E.; 70% Dicamba M.E. and 90% 5-OH Dicamba M.E. recoveries.

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Version: 860/V3.0 Printed: 23-Mar-1993 at 13:12:04 Page 1
Node: CHISTC GC Project: DIC_SOH_CROP User: CLOUSER

D B 2 1 0 S U C A N C R 2 3 - M a r - 1 9 9 3 9 : 3 8 : 1 8

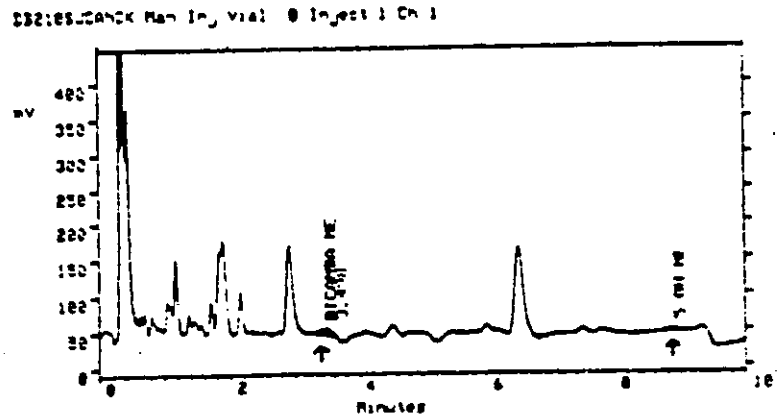
Header:

Acquisition method	AM0691_B	Processing method	AM0691_B
Units	PPM	System number	4
Channel	1	Manual Injector Vial	0
Injection	1	Total injections	1
Run time	10.00 min	Sample rate	20.00 per sec
Injection volume	2 uL	Mode	Analysis
Acquisition version	LAC/E/V3.0	Processing version	V3.0

Description:

Node:
Acquired on node LACRO2 system 4 for DIC_SOH_CROP

First Plot:



GC Results:

Peak Name	Ret Time	Area	Height
DICAMBA ME	3.438	41389	7498
5-OH ME	8.878	17217	2628

Figure 15. Representative GC-ECD chromatogram of Sugar Cane Stalks Control Sample, 0.50 mg equivalent/uL injected, <0.005 ng/uL acid equivalent (<0.01 ppm) for each Dicamba M.E. and 5-OH Dicamba M.E. detected.

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Version: 860/V3.0 Printed: 23-Mar-1993 at 13:14:00 Page 3
Node: CHISTC GC Project: DIC_SOH_CROP User: CLOUSER

D B 2 1 0 S U C A N F 1 23-Mar-1993 10:29:23

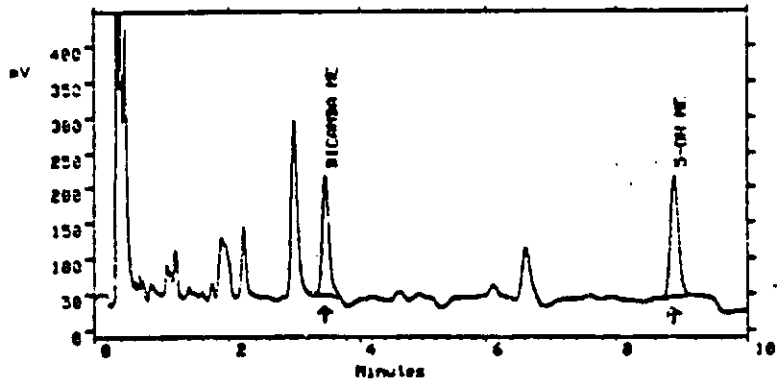
Header:

Acquisition method	AM0691_B	Processing method	AM0691_B
Units	PPM	System number	4
Channel	1	Manual Injector Vial	0
Injection	1	Total injections	1
Run time	10.00 min	Sample rate	20.00 per sec
Injection volume	2 uL	Mode	Analysis
Acquisition version	LAC/E/V3.0	Processing version	V3.0

Description:

Mode:
Acquired on node LACR02 system 4 for DIC_SOH_CROP

First Plot:



GC Results:

Peak Name	Ret Time	Area	Height
DICAMBA ME	3.465	1214470	168669
5-OH ME	8.895	1550579	170228

Figure 16. Representative GC-ECD chromatogram of Sugar Cane Stalks Control Sample Fortified at 0.10 ppm each Dicamba and 5-OH Dicamba acids, 0.50 mg equivalent/uL injected, 0.044 ng/uL acid equivalent (0.088 ppm) Dicamba M.E. and 0.045 ng/uL acid equivalent (0.090 ppm) 5-OH Dicamba M.E.; 88% Dicamba M.E. and 90% 5-OH Dicamba M.E. recoveries.

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Version: 860/V3.0 Printed: 12-Apr-1993 at 09:03:38 Page 1
Mode: CNISTC GC Project: DIC_5OH_CROP User: ADLAF

KJAGRASS2CK 9-Apr-1993 14:06:59

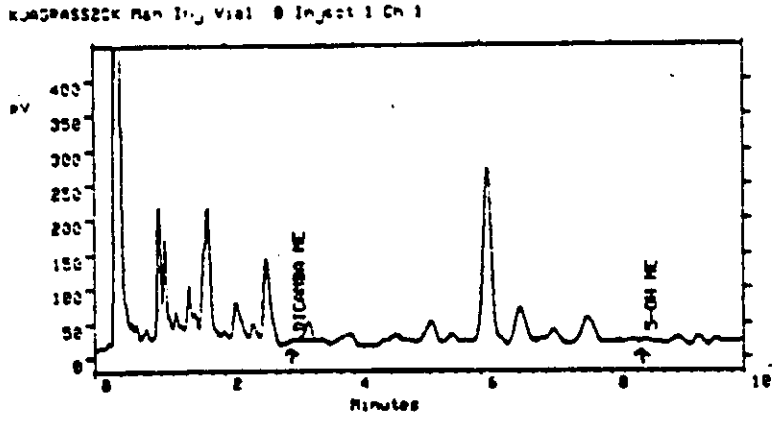
Header:

Acquisition method	AM0691_B	Processing method	AM0691_B
Units	PPM	System number	4
Channel	1	Manual Injector Vial	0
Injection	1	Total injections	1
Run time	10.00 min	Sample rate	20.00 per sec
Injection volume	2 uL	Mode	Analysis
Acquisition version	LAC/E/V3.0	Processing version	V3.0

Description:

Mode:
Acquired on node LACR02 system 4 for DIC_5OH_CROP

First Plot:



GC Results:

Peak Name	Ret Time	Area	Height	Int
DICAMBA ME	3.050	-	-	NF
5-OH ME	8.500	-	-	NF

Total Area 0 Total Amount 0.000 Total Height 0

Figure 17. Representative GC-ECD chromatogram of Pasture Hay Control Sample, 0.50 mg equivalent/uL injected, <0.005 ng/uL acid equivalent (<0.01 ppm) for each Dicamba M.E. and 5-OH Dicamba M.E. detected.

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Version: 860/V3.0 Printed: 12-Apr-1993 at 09:11:15 Page 1
Node: CHISTC GC Project: DIC_SOH_CROP User: ADLAF

K J A G R A S S 2 F 1 9 - A p r - 1 9 9 3 1 4 : 2 4 : 0 0

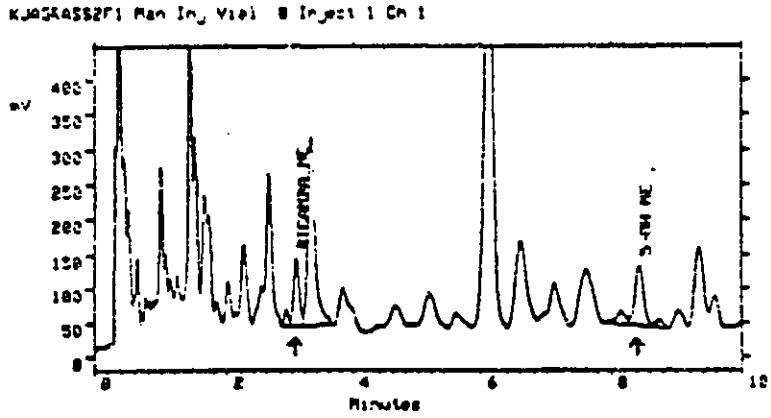
Header:

Acquisition method	AM0691_B	Processing method	AM0691_B
Units	PPM	System number	4
Channel	1	Manual Injector Vial	0
Injection	1	Total injections	1
Run time	10.00 min	Sample rate	20.00 per sec
Injection volume	2 uL	Mode	Analysis
Acquisition version	LAC/E/V3.0	Processing version	V3.0

Description:

Node:
Acquired on node LACR02 system 4 for DIC_SOH_CROP

First Plot:



GC Results:

Peak Name	Ret Time	Area	Height	Int
DICAMBA ME	3.062	615267	99273	**
5-OH ME	8.435	817465	89678	**

Total Area 1432732 Total Amount 0.166 Total Height 188951

Figure 18. Representative GC-ECD chromatogram of Pasture Hay Control Sample Fortified at 0.10 ppm each Dicamba and 5-OH Dicamba acids, 0.50 mg equivalent/uL injected, 0.037 ng/uL acid equivalent (0.074 ppm) Dicamba M.E. and 0.0375 ng/uL acid equivalent (0.075 ppm) 5-OH Dicamba M.E.; 74% Dicamba M.E. and 75% 5-OH Dicamba M.E. recoveries.

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Version: 860/V3.0 Printed: 5-Apr-1993 at 14:18:28 Page 1
Node: CHISTC GC Project: DIC_SOH_CROP User: CLOUIER

A C C E R O _ C X 5 - A p r - 1 9 9 3 1 0 : 0 3 : 0 8

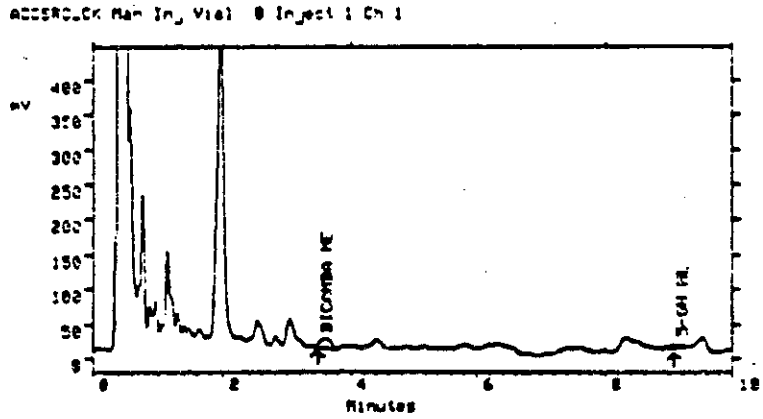
Header:

Acquisition method	AM0691_B	Processing method	AM0691_B
Units	PPM	System number	4
Channel	1	Manual Injector Vial	0
Injection	1	Total injections	1
Run time	10.00 min	Sample rate	20.00 per sec
Injection volume	2 uL	Mode	Analysis
Acquisition version	LAC/E/V3.0	Processing version	V3.0

Description:

Node:
Acquired on node LACR02 system 4 for DIC_SOH_CROP

First Plot:



GC Results:

Peak Name	Ret Time	Area	Height
DICAMBA ME	3.502	-	-
5-OH ME	9.083	30331	3647

Figure 19. Representative GC-ECD chromatogram of Refined Cottonseed Oil Control Sample, 0.50 mg equivalent/uL injected, <0.005 ng/uL acid equivalent (<0.01 ppm) for each Dicamba M.E. and 5-OH Dicamba M.E. detected.

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Version 860/V3.0 Printed: 5-Apr-1993 at 14:12:32 Page 1
Node: CHISTC GC Project: DIC_SOH_CROP User: CLOUSER

A C C S R O _ F 1 5 - A p r - 1 9 9 3 1 1 : 1 1 : 2 8

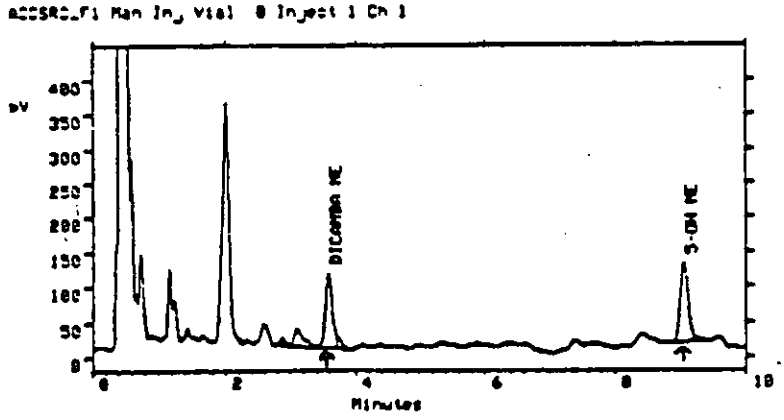
Header:

Acquisition method	AM0691_B	Processing method	AM0691_B
Units	PPM	System number	4
Channel	1	Manual Injector Vial	0
Injection	1	Total injections	1
Run time	10.00 min	Sample rate	20.00 per sec
Injection volume	2 uL	Mode	Analysis
Acquisition version	LAC/E/V3.0	Processing version	V3.0

Description:

Mode:
Acquired on node LACR02 system 4 for DIC_SOH_CROP

First Plot:



GC Results:

Peak Name	Ret Time	Area	Height
DICAMBA ME	3.578	790688	105102
5-OH ME	9.068	969762	111812

Figure 20. Representative GC-ECD chromatogram of Refined Cottonseed Oil Control Sample Fortified at 0.10 ppm each Dicamba and 5-OH Dicamba acids, 0.50 mg equivalent/uL injected, 0.035 ng/uL acid equivalent (0.070 ppm) Dicamba M.E. and 0.035 ng/uL acid equivalent (0.070 ppm) 5-OH Dicamba M.E.; 70% Dicamba M.E. and 70% 5-OH Dicamba M.E. recoveries.

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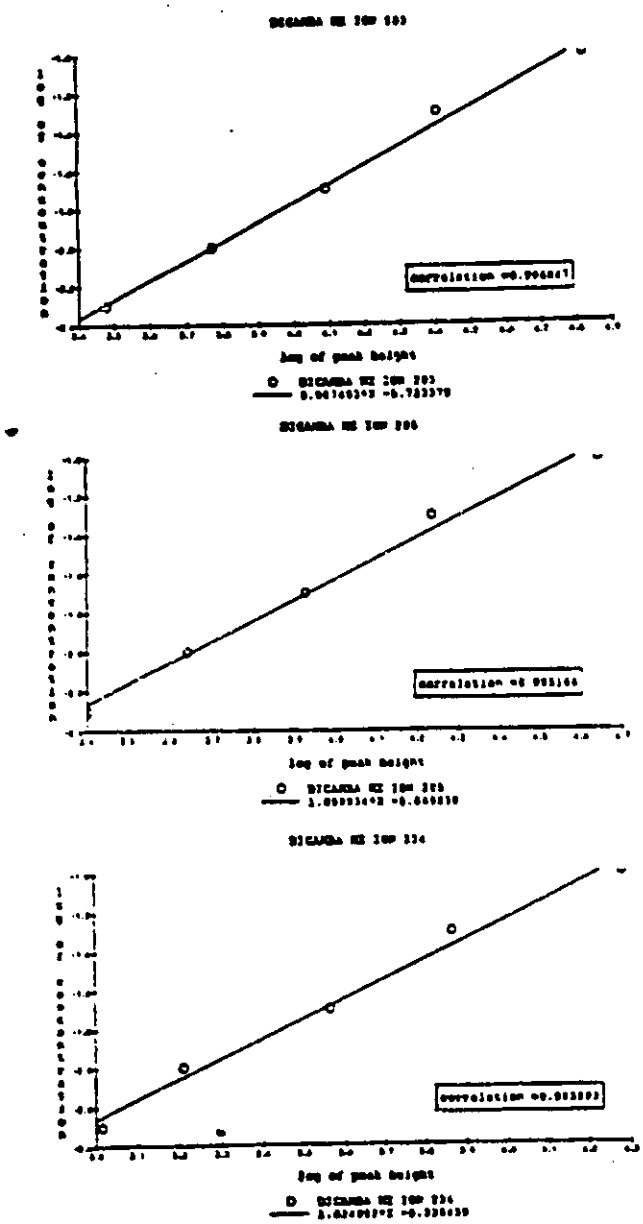

Figure 21. Representative Computer-Generated GC-MSD Standard Curves of Dicamba, Methyl Ester (Ions 203, 205, and 234). Concentrations of 0.10, 0.05, 0.02, 0.01 nd 0.005 ng/uL acid equivalents.

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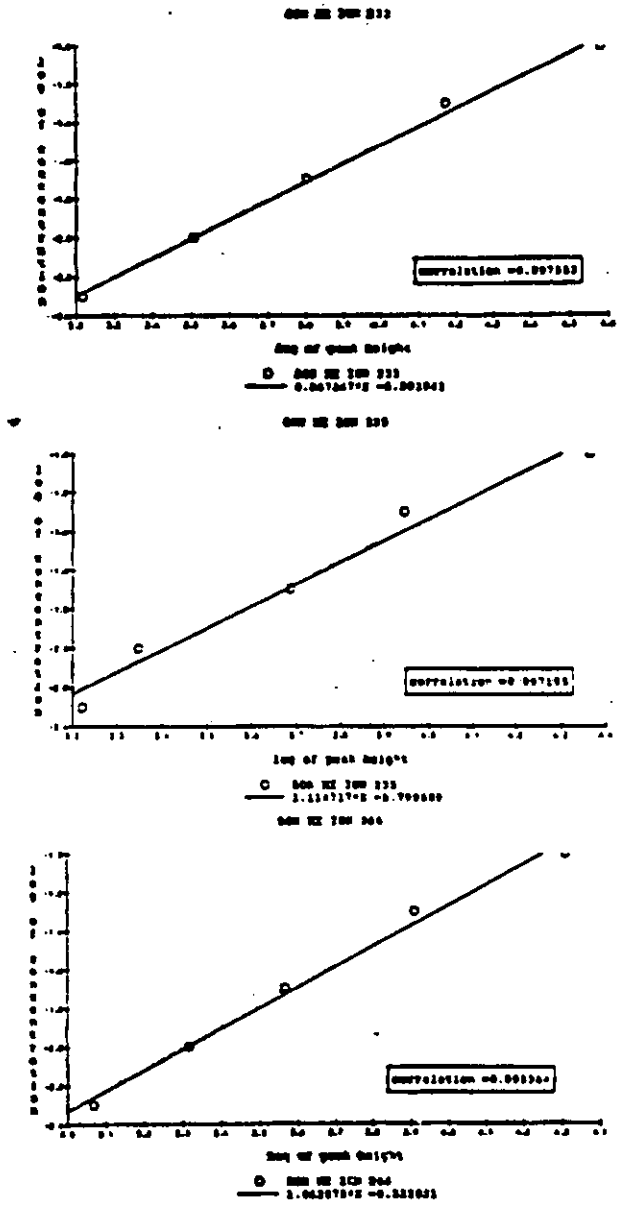

Figure 22. Representative Computer-Generated GC-MSD Standard Curves of 5-Hydroxy Dicamba Methyl Ester (Ions 233, 235 and 266). Concentrations of 0.10, 0.05, 0.02, 0.01 and 0.005 ng/uL acid equivalents.

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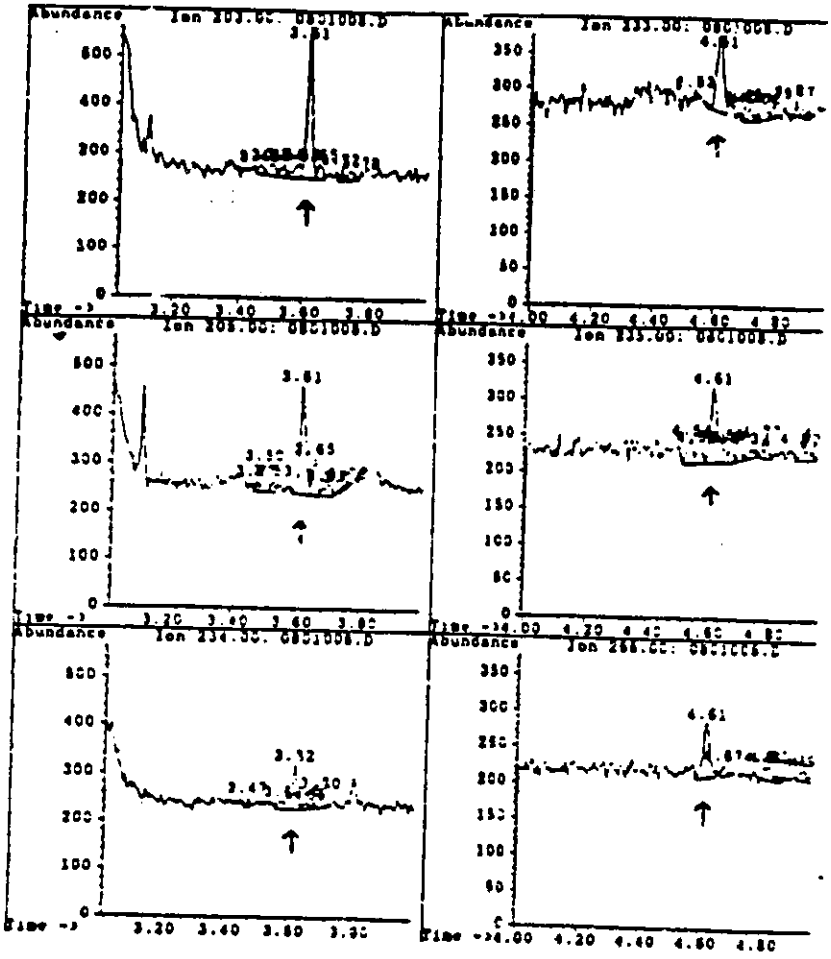
File: D:\CHEMPC\DATA\EJA05C3\0601008.D
Operator:
Date Acquired: 2 May 93 6:01 am
Method File Name: DICSOM.M
Sample Name: 0503STD5E12
Misc Info:
Bottle Number: 8



Ret Time	Signal Descr	Area	% Pk	% LPk
- 3.614	203.00 amu	3017	45.849	100.000
- 3.615	205.00 amu	2524	38.354	83.654
- 3.618	234.00 amu	1039	15.786	34.453
- 4.611	235.00 amu	1666	37.128	100.000
- 4.612	233.00 amu	1651	36.807	99.135
- 4.614	266.00 amu	1169	26.064	70.201

Figure 23. Representative GC-MSD Chromatogram of Dicamba M.E. (Ions 203,205 and 234) and 5-OH Dicamba M.E. (Ions 233, 235 and 266) Reference Standards, 0.005 ng/uL acid equivalent for each compound.

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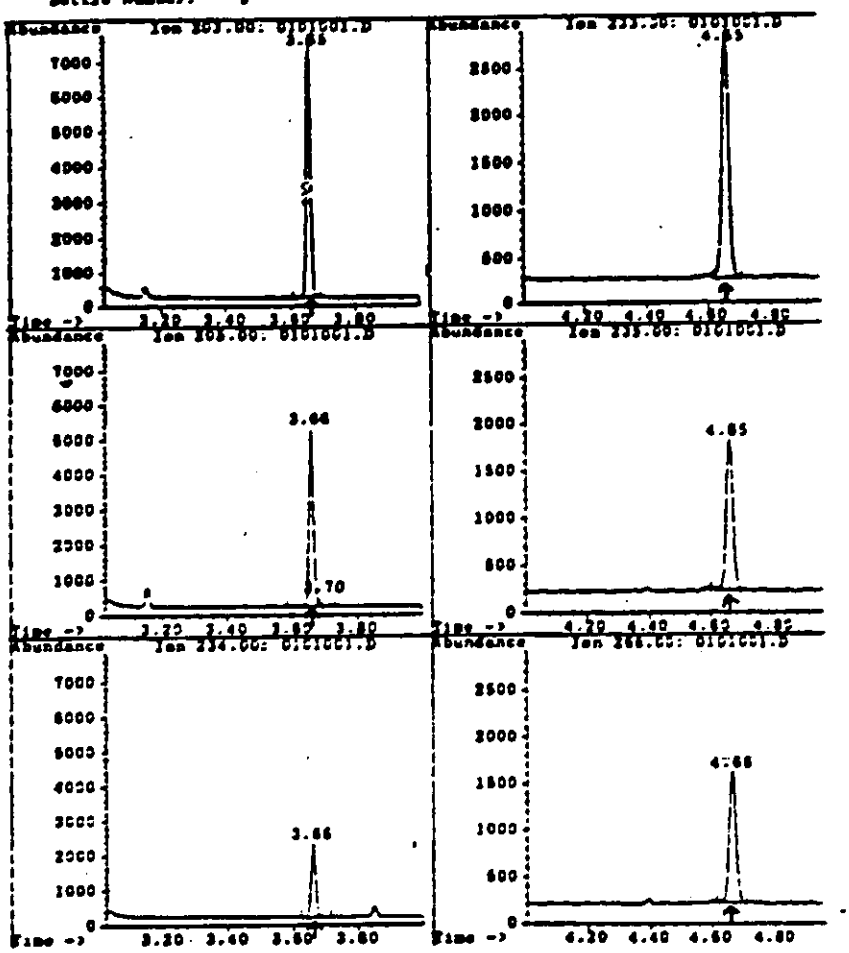
File: D:\CHEMPC\DATA\EJA0503\0101001.D
Operator:
Date Acquired: 2 May 03 3:24 am
Method File Name: DICSON.M
Sample Name: 0503STD1E0
Misc Info:
Bottle Number: 1



Ret Time	Signal Descr	Area	% Pk	% LPk
- 3.654	203.00 amu	66817	51.232	100.000
- 3.656	205.00 amu	44425	34.063	66.488
- 3.657	234.00 amu	19179	14.705	28.704
3.700	205.00 amu	2418	100.000	100.000
- 4.652	233.00 amu	28393	46.818	100.000
- 4.654	235.00 amu	23242	28.342	60.537
- 4.657	266.00 amu	20270	24.840	53.057

Figure 24. Representative GC-MSD Chromatogram of Dicamba M.E. (Ions 203, 205 and 234) and 5-OH Dicamba M.E. (Ions 233, 235 and 266) Reference Standards, 0.10 ng/uL acid equivalent for each compound.

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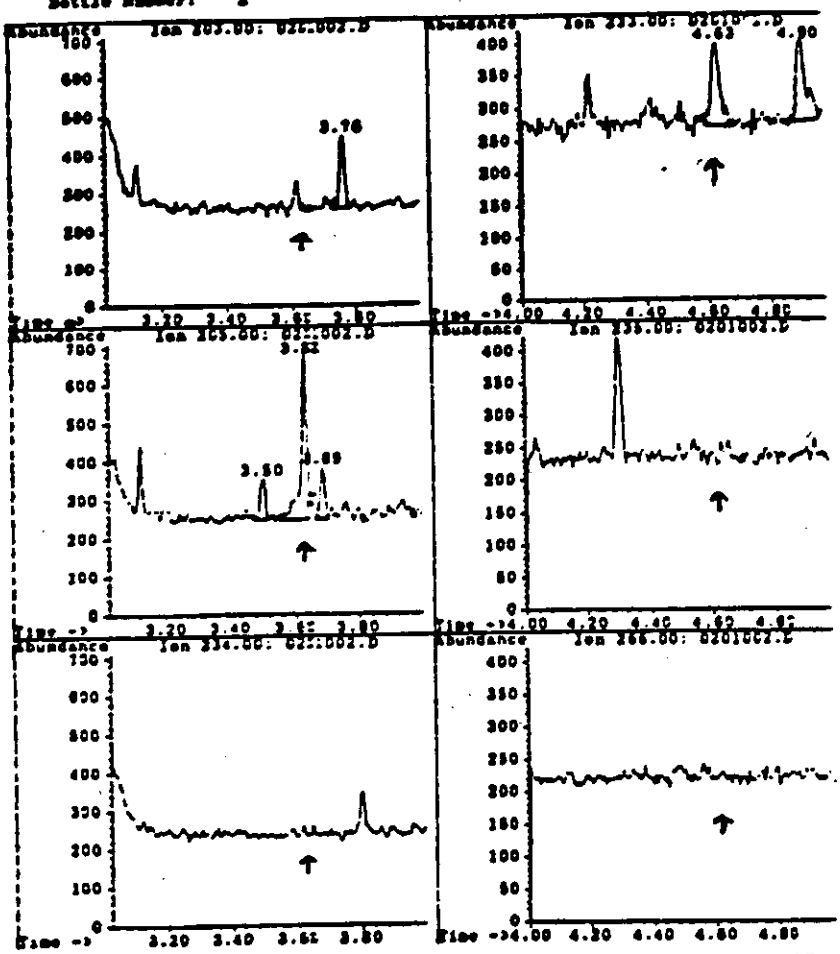
File: D:\CHEMPC\DATA\EJA0503\0201002.D
Operator:
Date Acquired: 2 May 93 3:38 am
Method File Name: DIC60M.M
Sample Name: EJAGRAINCK
Misc Info:
Bottle Number: 2



Ret Time	Signal Descr	Area	% Pk	% LPk
3.498	203.00 amu	1132	100.000	100.000
3.524	203.00 amu	5451	100.000	100.000
3.888	203.00 amu	1246	100.000	100.000
3.762	203.00 amu	2062	100.000	100.000
4.626	233.00 amu	2422	100.000	100.000

Figure 25. Representative GC-MSD chromatogram of Corn Grain Control Sample, 0.50 mg equivalent/uL injected, <0.005 ng/uL acid equivalent (<0.01 ppm) for each Dicamba M.E. and 5-OH Dicamba M.E. detected.

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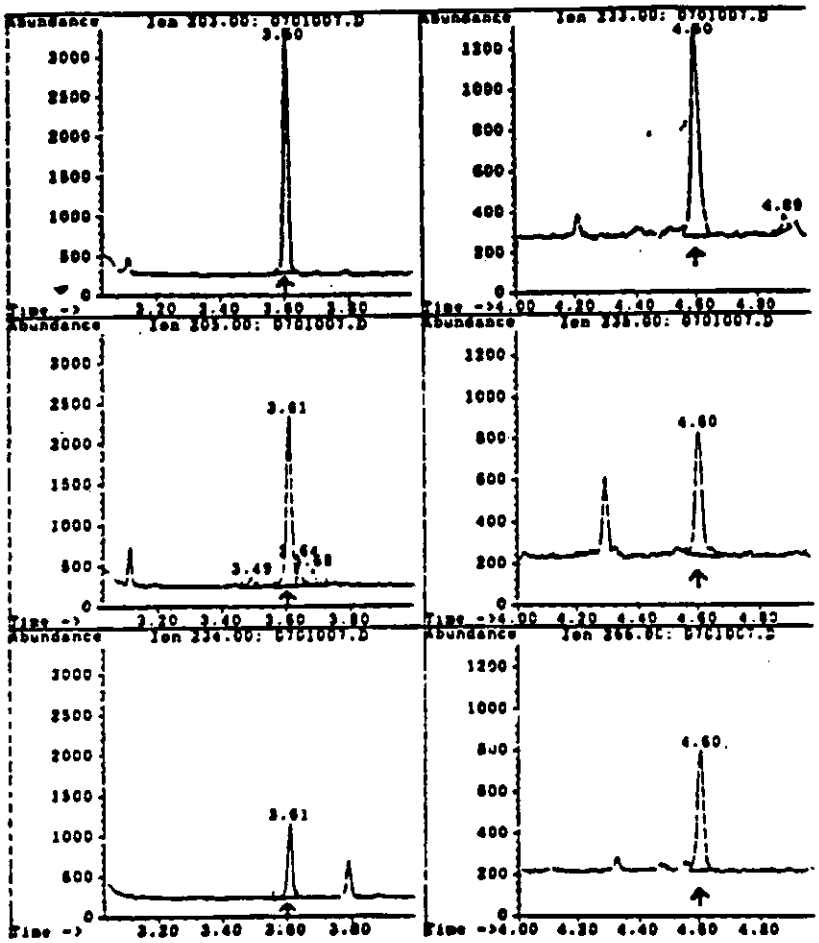
File: D:\CHEMPC\DATA\EJA0503\0701007.D
Operator:
Date Acquired: 2 May 93 4:47 am
Method File Name: DICSON.M
Sample Name: EAGRAINF1
Misc Info:
Bottle Number: 7



Ret Time	Signal Descr	Area	% Pk	% LPk
3.602	203.00 amu	29030	47.174	100.000
3.605	205.00 amu	23802	38.678	81.991
3.606	234.00 amu	8708	14.147	29.980
4.597	233.00 amu	17942	49.201	100.000
4.597	235.00 amu	9942	27.263	55.412
4.602	266.00 amu	8583	23.538	47.835

Figure 26. Representative GC-MSD chromatogram of Corn Grain Control Sample Fortified at 0.01 ppm each Dicamba and 5-OH Dicamba acids, 0.50 mg equivalent/uL injected, 0.0045 ng/uL acid equivalent (0.009 ppm) Dicamba M.E. and 0.0052 ng/uL acid equivalent (0.0104 ppm) 5-OH Dicamba M.E.; 90% Dicamba M.E. and 104% 5-OH Dicamba M.E. recoveries.

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Appendix VI

Method Used for Calculating the PPM Values

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To determine the concentration of Dicamba M.E. or 5-OH M.E. a standard curve for each is prepared by injecting a fixed volume of each of a series of standards of known concentrations and plotting the corresponding peak height versus the amount of standard injected. During this run, the same volume of sample extracts are also injected and the concentration is interpolated by using the peak height of the sample and the standard curve obtained.

$$\text{ppm (ng/mg)} = \frac{C_e \text{ (ng/uL)} \times V_s \text{ (uL)}}{W_s \text{ (mg)}}$$

Where:

ppm = Concentration of ion of the analyte in the sample in parts per million.

Ce = Concentration of residue in extract determined from the standard curve (ng/uL).

V_s = Volume of final sample extract taking into account all dilutions and/or aliquots used (uL).

W_s = Weight of sample taken for analysis (mg).

A sample calculation is shown below.

V_s = 1 mL (1,000 uL)

W_s = 1 gm (1,000 mg)

C_e = 0.01 ng/uL

$$\text{ppm} = 0.01 \text{ ng/uL} \times \frac{1{,}000 \text{ uL}}{1{,}000 \text{ mg}}$$

ppm = 0.01

Baseline corrected chromatographic peak height of standards and samples are collected by a "Waters Chromatographic Data System". These peak heights are processed into sample concentrations using a linear least squares analysis program (RS/1 Software, BBN Software Products Corporation, Cambridge, MA 02238) to generate a standard curve and interpolate sample concentration from the standard curve. This program is run on a VAX 6220 computer.

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