US EPA ARCHIVE DOCUMENT

AM-0691

THE GROWING WORLD OF
VELSICOL
ANALYTICAL RESEARCH DIVISION
CHICAGO, ILLINOIS 60611

ANALYTICAL METHOD

Determination of Dicamba and 5-Hydroxy Dicamba Residues in Vegetables, Forage Crops, Legumes, Cottonseed and Cottonseed Fractions and Grains.

1. Summary

1.1 The method has been used for the determination of dicamba and 5-hydroxy dicamba in field corn ensilage, kernels and foliage, tomatoes, cucumbers, alfalfa, sugar beet roots and foliage, pinto beans, sunflower seeds, cottonseeds, cottonseed hulls, crude and refined cottonseed oil, cottonseed soapstock, wheat grain, wheat straw, and cured tobacco.

1.2 A known weight of crop sample is blended with ethyl ether and 20% aqueous sulfuric acid.

1.3 An aliquot of the ether extract is transferred to a separatory funnel and partitioned with 2% sodium bicarbonate solution.

1.4 Cottonseed oil fractions and soapstock are weighed directly into a separatory funnel, dissolved in ether and partitioned with 2% sodium bicarbonate solution.

1.5 The organic phase is discarded, and the aqueous phase is acidified to pH $\angle$ 1 with 6 N HCl.

1.6 The aqueous phase is then extracted with two 50 ml portions of ethyl ether. The extracts are concentrated in a water bath and the residues methylated using diazomethane gas.

1.7 The derivatized extract is cleaned up on a 20 g silica gel column using 5 and 10% ether in pentane eluents.

1.8 The residues are detected by GC/EC using a ^{63}Ni electron capture detector.

1.9 Quantitation is based on a standard curve prepared by plotting peak height versus ng of parent compound injected.

1.10 The limits of detection range from 0.005 to 0.01 ppm for dicamba and 0.01 ppm for 5-hydroxy dicamba. Recoveries are generally greater than 80% for dicamba and 70% for 5-hydroxy dicamba at 0.1 ppm fortification.

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2. Apparatus

2.1 Bath, hot water, 65°C.

2.2 Blender, Virtis 23, The Virtis Co., Route 28, Gardner, NY 12525.

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

2.4 Bottles, Glass, 8 and 32 oz. with Poly-seal screw cap.

2.5 Columns, Chromatographic, 15 mm (id) x 45 cm, teflon stopcock, fitted with water jacket, Lab-Crest Scientific Division, 1531 County Line Road, Warminster, PA. 18974.

2.6 Cylinders, Graduated, 50 and 250 ml.

2.7 Esterification Apparatus, see diagram section 5.3.

2.8 Filter Paper, glass fiber, Whatman, GF/A, 7.0 cm.

2.9 Flask, Erlenmeyer with sidearm, 250 ml.

2.10 Flask, Kuderna Danish, 125 ml with 12 ml graduated distillation receiver tubes and Vigreaux column.

2.11 Flask, Virtis, 500 ml.

2.12 Funnel, Buchner, 7 cm (id).

2.13 Funnel, Filter, 60°, 75 mm.

2.14 Funnel, Separatory with teflon stopcock, 125 ml.

2.15 Gas Chromatograph, equipped with ^{63}Ni electron capture detector.

2.16 Glass Wool.

2.17 Nitrogen Evaporator, N-Evap, Organomation Associates, Worcester, Mass.

2.18 Oven, 250°C.

2.19 Paper, pH, range 1-11.

2.20 Platform Shaker, Eberbach Co., Ann Arbor, MI.

3. Reagents

3.1 Carbitol, 2-(2-Ethoxyethoxy) ethanol, Aldrich Chemical Co., Milwaukee, WI.

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

3.3 Ethanol, Rossville Gold Shield, 200 proof, International Minerals and Chemical Corp., Terre Haute, IN 47807.

3.4 Ethyl Ether, 2% ethanol preservative, "Distilled in Glass", Burdick and Jackson, Muskegon, MI 49442.

3.5 Hexane, "Distilled in Glass", Burdick and Jackson, Muskegon, MI 49442.

3.6 Hydrochloric Acid, reagent.

3.7 Methanol, Nanograde®, Mallinckrodt, Inc., St. Louis MO 63147.

3.8 Pentane, "Distilled in Glass", Burdick and Jackson, Muskegon, MI 49442.

3.9 Potassium Hydroxide, reagent.

3.10 Silica Gel 60, 70-200 mesh, EM Reagents, MC/B Manufacturing Chemists, Inc., 2909 Highland Avenue, Cincinnati, Ohio 45212.

3.11 Sodium Bicarbonate, reagent.

3.12 Sodium Sulfate, anhydrous granular, reagent.

3.13 Sulfuric Acid, reagent.

4. Standards

4.1 Velsicol Analytical Reference Standards

4.1.1 Dicamba, 2 methoxy-3,6-dichlorobenzoic acid.

4.1.2 5-Hydroxy dicamba, 2-methoxy-3,6-dichloro-5-hydroxybenzoic acid.

5. Procedure

5.1 Extraction (For cottonseed oil and soapstock, see 5.2.2).

5.1.1 Chop 200-500 g of the sample in a Hobart Chopper. Seed samples may be processed beginning with 5.1.2.

5.1.2 Weigh 20 g of the chopped or seed sample (use 10 g straw) into a Virtis blender. A Waring blender may be used for grain and seeds which are difficult to blend.

5.1.3 Add 150 ml of ethyl ether, 5 ml of 20% H_2SO_4, and 5 ml of ethanol.

5.1.4 Secure the cap and blend at high speed for 5 minutes.

5.1.5 Filter the solution through a Buchner funnel and rinse cup and pulp with approximately 50 ml of ether. When filtering corn kernel and cucumber extracts cover filter paper with 1 cm layer of anhydrous granular sodium sulfate.

5.1.6 Transfer filtrate to a 250 ml graduate cylinder and adjust the volume of the ether layer to 200 ml with ether (200 ml of ether in addition to any aqueous phase).

5.2 Partition

5.2.1 Transfer a 5 g equivalent of the sample extract to a 125 ml separatory funnel.

5.2.2 For cottonseed oil and soapstock, weigh 5 and 1 g respectively into a separatory funnel and dissolve in 50 ml of ethyl ether.

5.2.3 Add 25 ml of a 2% $NaHCO_3$ solution and shake funnel well. Discard the ether layer.

5.2.4 Extract the aqueous phase with another 50 ml of ether and discard the ether.

5.2.5 Acidify the aqueous phase with 6 N HCl to a pH $\leq$ 1.

5.2.6 Extract the acidic aqueous layer with two 50 ml portions of ether. Filter each ether extract through anhydrous Na_2SO_4 into a 125 ml Kuderna-Danish concentrator.

5.2.7 Rinse Na_2SO_4 with about 25 ml of ether, attach K-D set up to vigreaux column, and concentrate the combined ether phases to approximately 0.5 ml in the water bath (65°C).

5.2.8 Concentrate to near dryness with a gentle stream of nitrogen on the N-Evap (50°C water bath).

5.2.9 Add 3 ml of ether and proceed with methylation.

5.3 Derivatization

5.3.1 Esterification Reference Schlenk and Gellerman, Anal. Chem. 32, 1412 (1960).

5.3.2 Set up tubes as in the following diagram. Tube A serves to saturate the nitrogen flow with ether which transmits diazomethane from B to C. Tube D is used as an indicator for excess diazomethane when the sample extract is colored.

5.3.3 To tube B (18 x 145 mm test tube), add 2 ml 9 N KOH, 1.4 ml Carbitol, and 1.4 ml ether.

5.3.4 To tube C which contains the sample extract, add 0.2 to 0.5 ml methanol.

5.3.5 To tube D add 3 ml of ethyl ether.

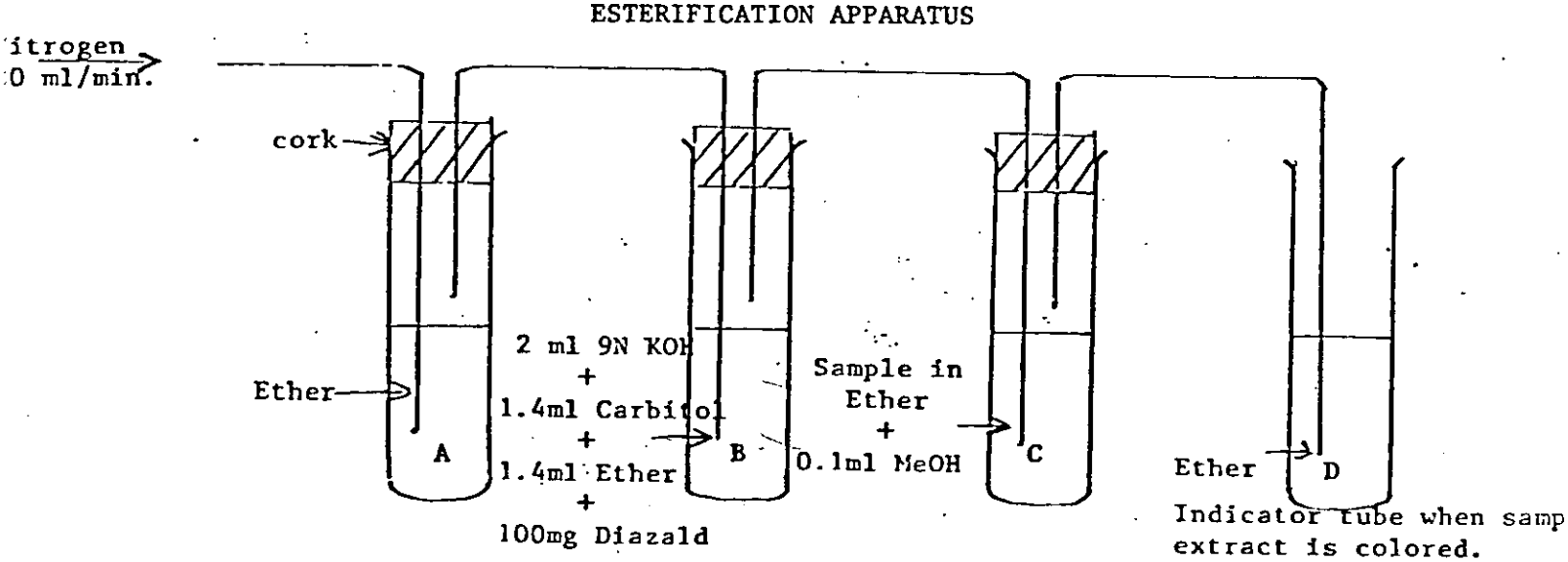
5.3.6 Adjust nitrogen flow rate to 30 ml/min. Add approximately 100 mg of Diazald to tube B and seat stoppers in tubes A, B and C. Remove tube C when a yellow color is obtained. For colored extracts, observe the yellow color in tube D. Note- Diazald is a carcinogen. In addition the diazomethane gas generated is extremely toxic and should only be used in hoods which are operating efficiently ($\geq$ 100 lfm). Do not use ground glass connections when working with diazomethane.

5.3.7 Rinse the diazomethane delivery tube with ether before derivatizing the next sample.

5.3.8 Cap the K-D for 15 minutes. If the solution remains yellow throughout this interval, then evaporate the sample to near dryness under a gentle nitrogen stream in a ventilated hood. Do not use heat for this procedure.

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

ESTERIFICATION APPARATUS



5.4 Column Cleanup of Derivatized Sample

5.4.1 Set up the jacketed columns. Fit each with a glass wool plug to contain the silica gel, and open the stopcock.

5.4.2 To a 250 ml separatory funnel add 70 ml of 5% ethyl ether in pentane and 20 g of 3% water deactivated silica gel. (See 5.4.10 for silica gel preparation).

5.4.3 Shake well and quickly transfer slurry to the column.

5.4.4 Rinse the separatory funnel with 10 ml of the 5% eluent and add to the column.

5.4.5 Cover the silica gel bed with 1 cm granular Na_2SO_4 and drain the solvent just to the Na_2SO_4 layer.

5.4.6 Add 5 ml of the 5% eluent to the methylated sample and transfer to the silica gel column.

5.4.7 Rinse the KD with two 5 ml portions of the eluent and allow each rinse to penetrate to the top of the bed.

5.4.8 Pass another 55 ml of the 5% eluent through the column and discard.

5.4.9 Elute and collect the methylated dicamba and 5-hydroxy residues with 150 ml of 10% ether in pentane. In wheat fractions (from grain and straw), 150 ml of 15% ether in pentane is necessary to elute methylated 5-hydroxy dicamba quantitatively. If only dicamba residues are to be determined, elute with 85 ml of the 5% eluent.

5.4.10 Silica gel preparation

5.4.10.1 Activate the silica gel at 250°C for 2½ hours.

5.4.10.2 Transfer to 32 oz bottles and shake for ½ hour.

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

5.4.10.4 Shake continuously for 15 hours. Silica gel is good for two weeks.

5.5 Preparation of Standard Solutions

5.5.1 Weigh 10 mg each of dicamba and 5-hydroxy dicamba into separate 100 ml volumetric flasks. Dilute to the mark with methanol. The concentration of each stock solution is 1×10^{-7} g/µl.

5.5.2 Combine 5 ml of each stock solution in a 50 ml volumetric flask and dilute with methanol to the mark. The concentration of each compound is 1×10^{-8} g/µl.

5.5.3 Methylate 1 ml of the 1×10^{-8} g/µl solution as described in section 5.2. Concentrate under a stream of nitrogen in a well-ventilated hood to approximately 1 ml.

5.5.4 Transfer the methylated compounds to a 100 ml volumetric flask and adjust volume to the mark with hexane. The concentration of GC standard is 1×10^{-10} g/µl each compound.

6. Analysis

6.1 Gas Chromatographic Conditions

6.1.1 Instrument: Hewlett-Packard, Model 5710A with ^{63}Ni electron capture detector; Tracor Instrument Co., Model 220 with ^{63}Ni electron capture detector.

6.1.2 Column: 1.2 m x 4 mm (id) Pyrex with 3% SE-30 on Gas Chrom Q, 80-100 mesh. Other liquid phases have been shown to be suitable for the GC/EC determination of dicamba and 5-hydroxy dicamba. They are 5-10% OV-101 at 150-170°C and 1.5% OV-17 + 1.95% QF-1 at 155°C.

6.1.3 Temperatures: Oven: 135°C.
Injection port: 250°C.
Detector: 280°C.

6.1.4 Carrier Gas: Hewlett Packard, 5% methane in argon, 30 ml/min; Tracor, nitrogen, 20 ml/min.

6.1.5 Attenuation: Hewlett-Packard, x128; Tracor x16.

6.2 Quantitation

6.2.1 Prepare a standard curve for each compound by plotting on log log paper the peak heights versus the respective quantity (nanograms) of each compound injected.

6.2.2 Read the quantity of each compound in the injected aliquot of the sample extract from the standard curve.

6.2.3 Calculate the concentration of the residue in the sample using the following expression:

$$ppm = \frac{ng \times Vs}{\mu l \times W}$$

where:

ppm = concentration of the compound in the sample in parts per million.

ng = quantity of compound in injected aliquot as read from the standard curve in nanograms.

Vs = volume of sample extract in milliliters from which GC aliquot was taken, allowing for all dilutions.

µl = volume of sample extract injected into gas chromatograph in microliters.

W = weight of the sample taken for analysis in grams.

6.3 Recovery

The following recovery data were determined according to the above analytical method. Nontreated crop samples (checks) were fortified with each compound at 0.1 ppm. A typical standard curve is shown in figure 1 and representative gas chromatograms of these determinations are presented in figures 2 and 3.

Sample Type	Percent Recovery Dicamba	Percent Recovery 5-Hydroxy Dicamba
Field Corn		
Ensilage	92	78
Kernels	98	73
	100	71
Foliage	96	79
Tomatoes	104	95
	98	83
	110	78
Cucumbers	110	83
	98	90
	82	84
Alfalfa	90	69
	98	71
	110	75
	86	78
Sugar Beets		
Foliage	87	76
	96	76
	81	79
Roots	91	84
	92	92
	84	74
Pinto Beans	83	82
	88	71
	93	84
Cottonseed		
Seeds	84[1]	72
	90[1]	98
Hulls	83[1]	86
	105[1]	84
Crude Oil	102	98
	96	98
Soapstock	82[1]	68
	80[1]	72

Percent Recovery (cont'd)

Sample Type	Dicamba	5-Hydroxy Dicamba
Wheat		
Soft Dough	87	82
	84	82
	82	80
	85	101
Grain	80	86
	82	100
	80	93
	86	86
Straw	72	68
	71	73
	81	77
Sunflowers	115	92
Tobacco (cured)	87[1/]	75

1/ Recovery values corrected for average of check sample values.

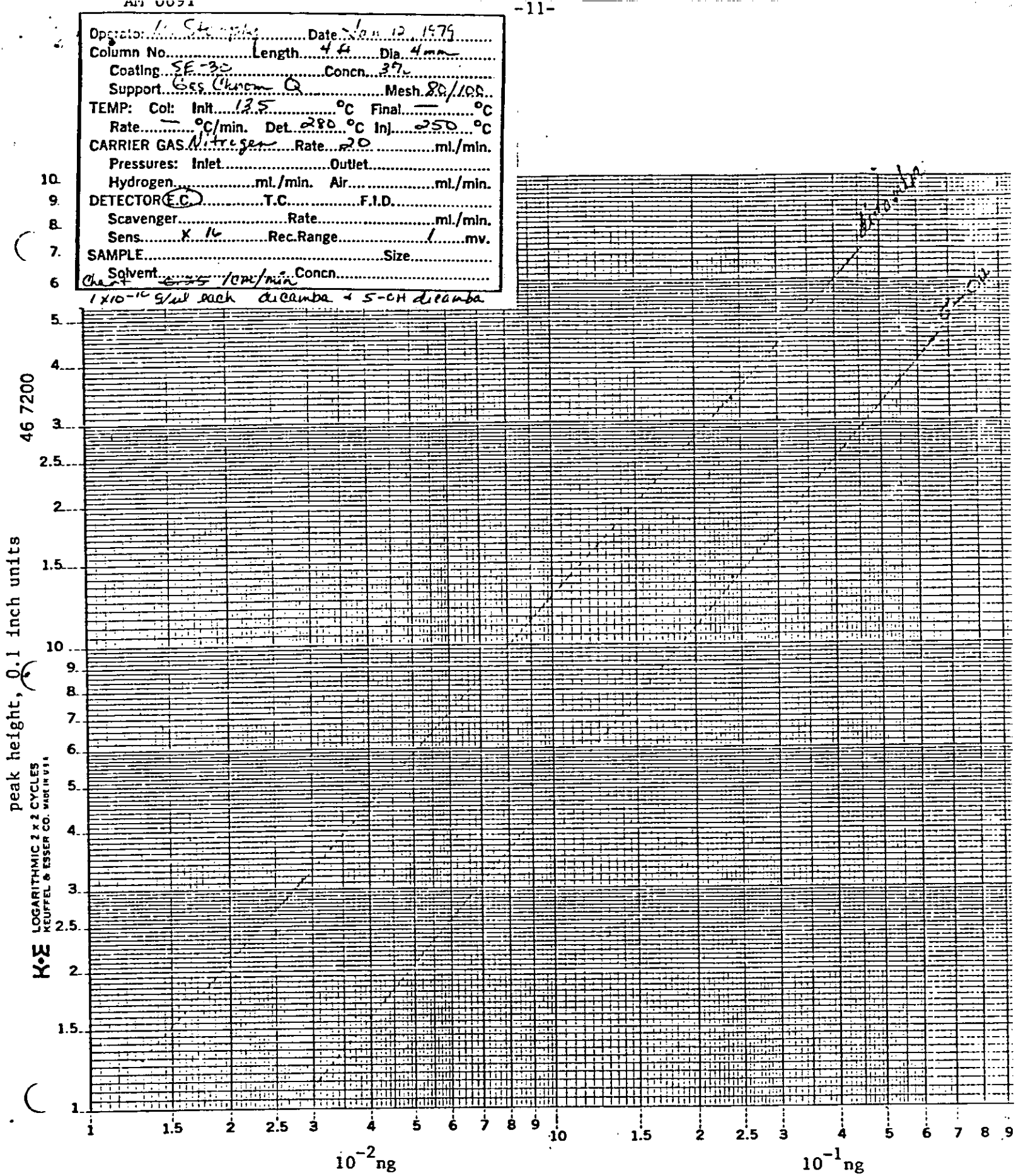


Figure 1. Representative standard curves for EC-GC quantitation of Dicamba and 5-Hydroxy dicamba.

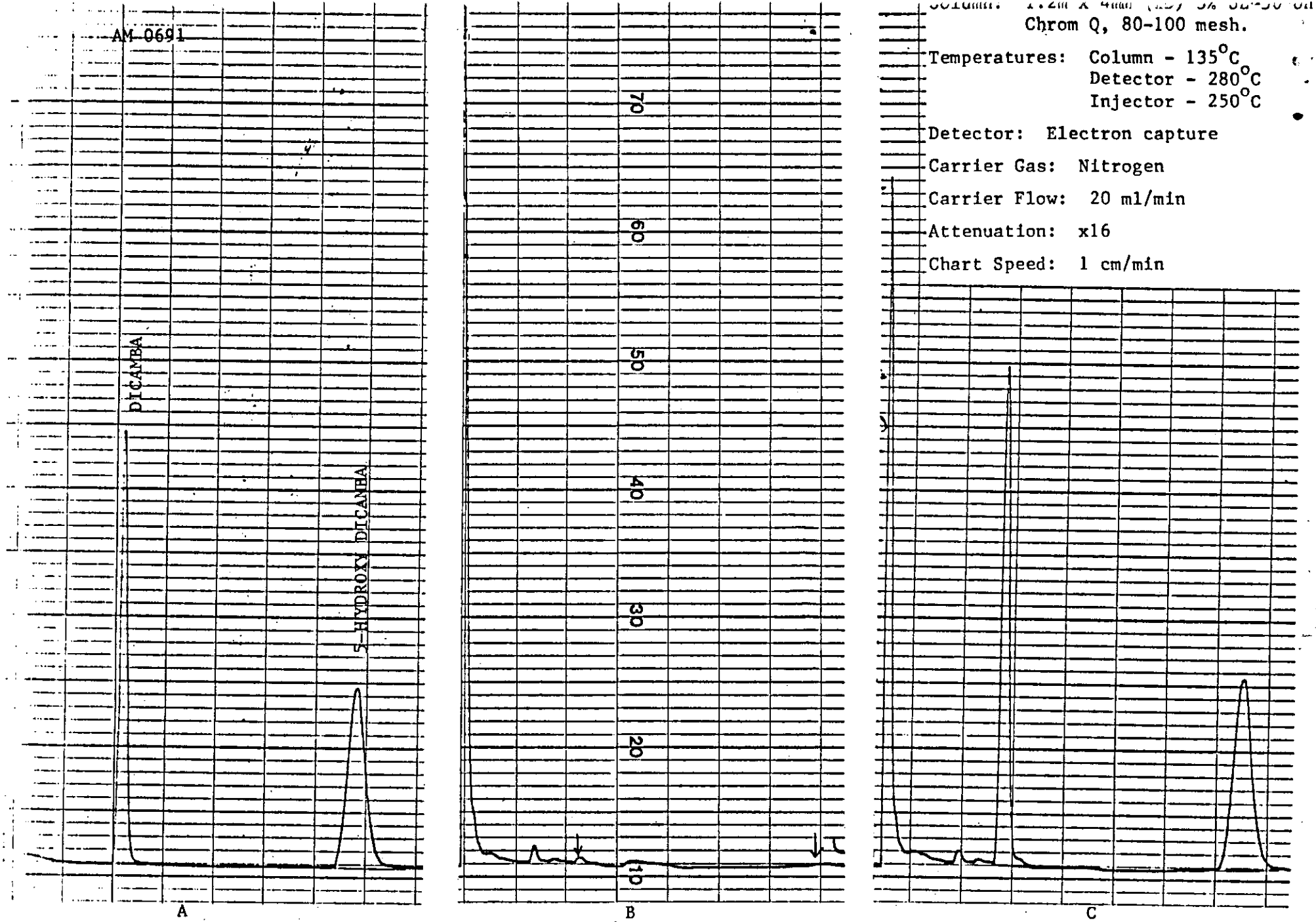


Figure 2. Representative chromatograms of Dicamba and 5-Hydroxy dicamba standards, a tomato check sample, and fortified check sample.

A. 0.24 ng each of dicamba and 5-hydroxy dicamba standard.

B. Tomato check 787-02246 2.7 mg equivalent injected, $<$ 0.005 ppm dicamba and $<$ 0.01 ppm 5-hydroxy

C. Tomato check 787-02246 fortified 0.1 ppm each dicamba and 5-hydroxy dicamba, 2.6 mg equivalent injected

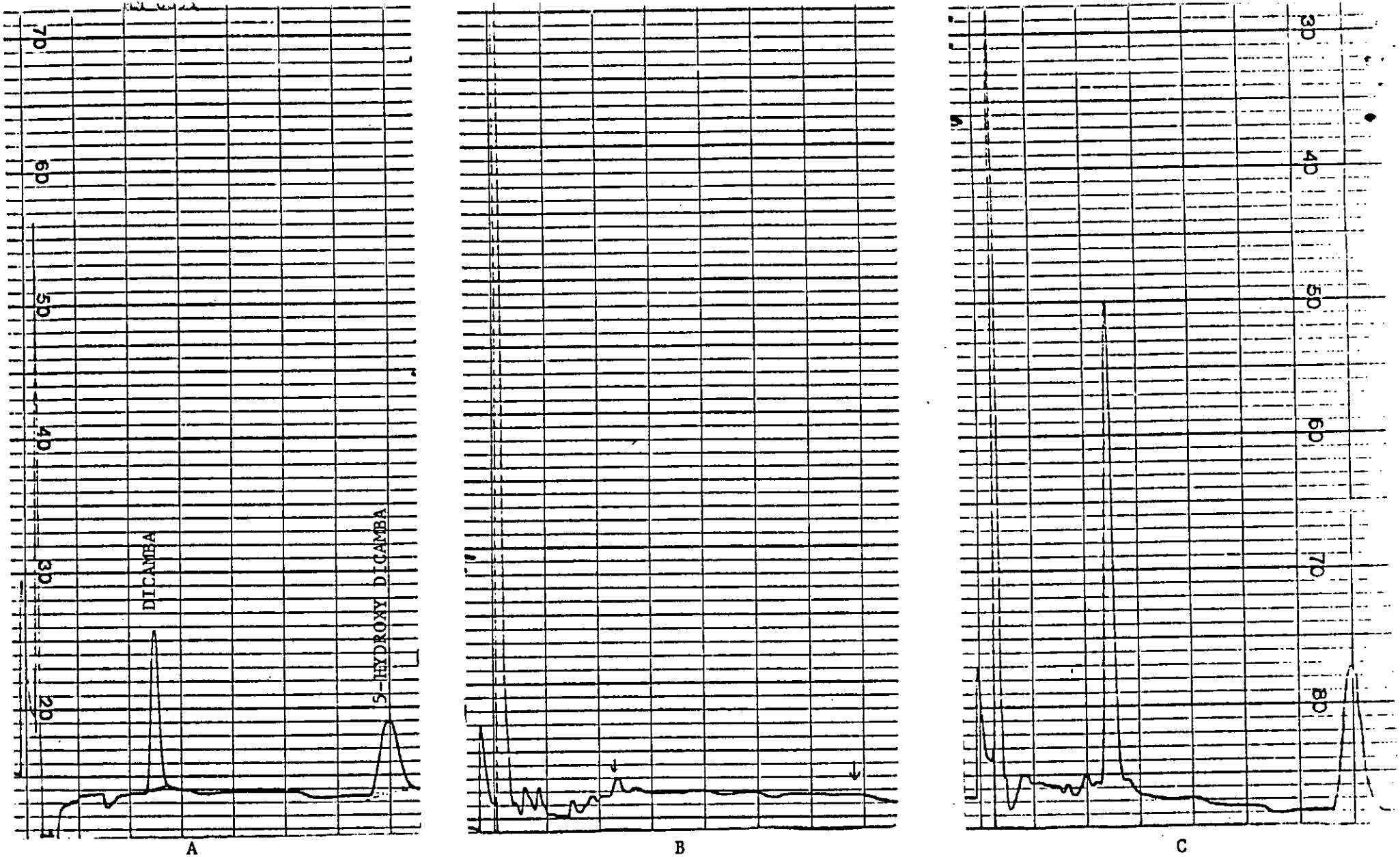


Figure 3. Representative chromatograms of Dicamba and 5-OH dicamba standards, cucumber check, and fortified check sample.

A. 0.084 ng each of dicamba and 5-hydroxy dicamba standard.

B. Cucumber check 787-02892, 2.7 mg equivalent injected. ∠0.005 ppm dicamba, ∠0.01 ppm 5-hydroxy dicamba.

C. Cucumber check 787-02892 fortified 0.1 ppm each dicamba and 5-hydroxy dicamba, 2.7 mg equivalent injected, 93% dicamba and 70% 5-hydroxy dicamba

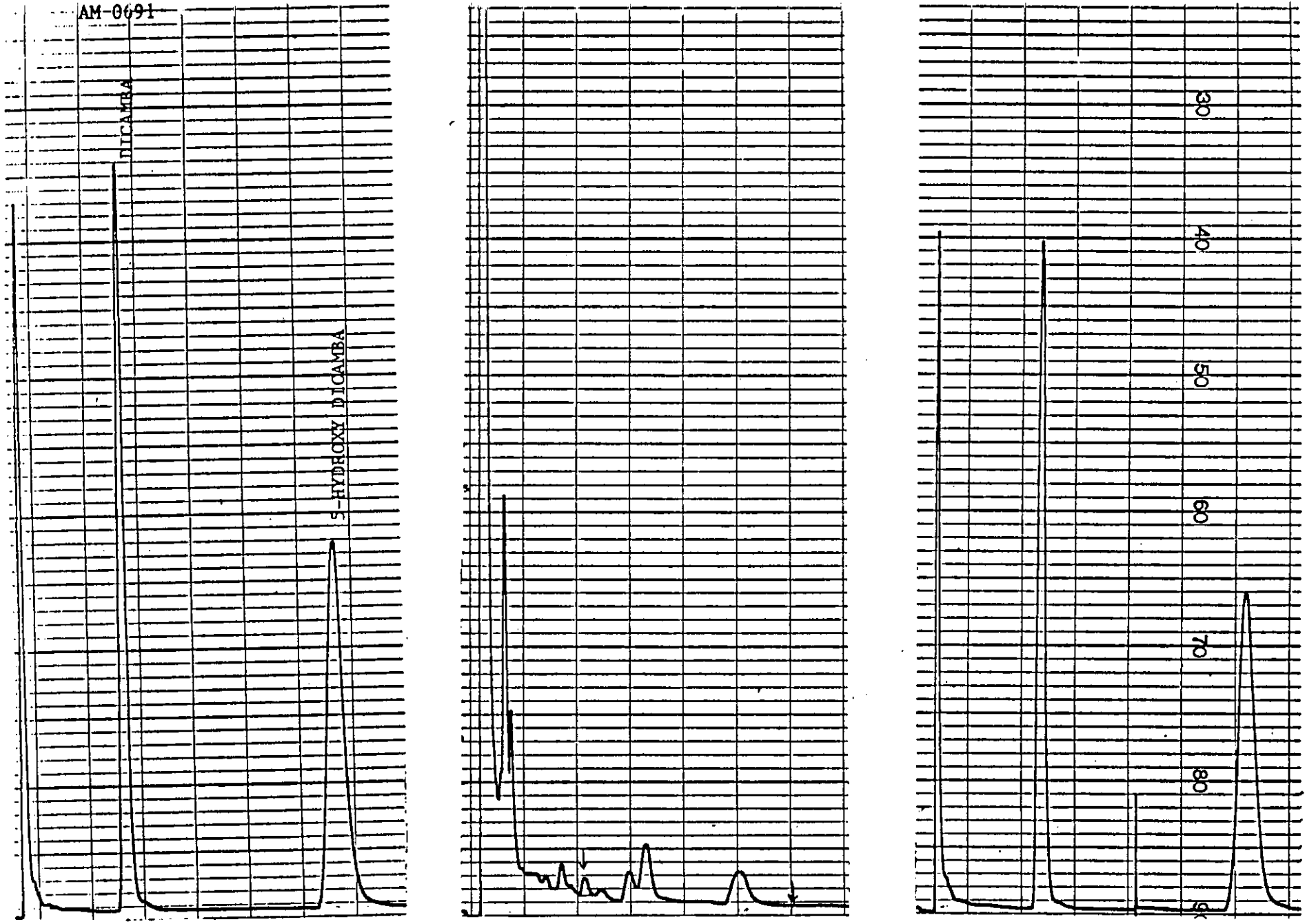


Figure 4. Representative chromatograms of Dicamba and 5-Hydroxy dicamba standards, sugar beet check, and fortified check sample.

A. 0.27 ng each of dicamba and 5-hydroxy dicamba standard.

B. Sugar Beet Check 787-02759 (leaves), 3.2 mg equivalent injected, ∠ 0.005 ppm dicamba and ∠ 0.01 ppm 5-hydroxy dicamba.

C. Sugar Beet Check 787-02759 (Leaves fortified 0.1 ppm each dicamba and 5-hydroxy dicamba, 3.5 mg equivale injected, 96% dicamba and 76% 5-hydroxy dicamba

ANALYTICAL METHOD

VELSICOL CHEMICAL CORPORATION	Method Number AM-0691
Location: ⊗341 E. OHIO ST. CHICAGO, IL 60611	Addendum
	Supercedes
	DATE October 21, 1982
⊗RESEARCH ○QUALITY CONTROL	Approved

Determination of Dicamba and 5- Hydroxy dicamba Residue in Palm Oil

Recovery of dicamba and 5-hydroxy dicamba from fortified palm oil[1/] samples (0.10 ppm each compound) were determined according to the procedure for cottonseed oil described in AM-0691.[2/] The limit of detection of the method was 0.01 ppm each compound. The recoveries are presented as follows:

Replicate	Percent Recovery Dicamba	5-OH
1	90	87
2	107	87
3	93	90
4	108	86
5	96	78
6	84	74
7	104	92
$\bar{X} \pm SD$	97 ± 9.2	85 ± 6.5

1/ Palm oil used in analyses:
African Maid Palm Oil
Cooking Oil 100% Pure
La Preferida Inc, Bronx, NY 10473
Product of Liberia, Africa

2/ In addition to the columns listed under gas chromatographic conditions in AM-0691, a 1.8m X 2mm (i.d.) Pyrex column with 3% OV-1 an Gas Chrom Q, 100-120 mesh may be used in the determination of dicamba and 5-hydroxy dicamba residues.