

US EPA ARCHIVE DOCUMENT

VOLUME 1 of 5

416234-01

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SUBMITTED TO SUPPORT THE REGISTRATION OF:

Clethodim Technical (EPA File Symbol 59639-E)  
CHEVRON Clethodim Technical (EPA File Symbol 62499-GL)  
SELECT Herbicide (EPA File Symbol 59639-G)

STUDY TITLE:

The Determination of Clethodim Residues in Crops,  
Chicken and Beef Tissues, Milk, and Eggs  
Method: RM-26B-2

Supplemental to: Analytical Method for the Determination  
of Clethodim Residues: G.O. Breault; December 21, 1988.  
MRID #41030141

The Determination of Clethodim Residues in Crops,  
Chicken and Beef Tissues, Milk, and Eggs; B. HO; February 9, 1990.  
Method: RM-26B-1 (Revised) MRID #41396001

DATA REQUIREMENT:

Residue Chemistry - Residue Analytical Method  
40 CFR 158.240 - Guideline Reference No. 171-4

AUTHOR:

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STUDY COMPLETED ON:

August 15, 1990

PERFORMING LABORATORY:

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LABORATORY PROJECT ID:

RM-26B-2

TOTAL PAGES

17

GOOD LABORATORY PRACTICE STATEMENT

This report is simply a modification of a previously submitted report. The original study submitted to the Agency was conducted according to EPA Good Laboratory Practice Standards (40 CFR 160), and the original report contained a GLP statement verifying compliance with 40 CFR 160. The information in this submission contains no new data and is not subject to GLP compliance. Therefore a GLP statement is not required with this submission.

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THE DETERMINATION OF CLETHODIM  
RESIDUES IN CROPS, CHICKEN AND  
BEEF TISSUES, MILK, AND EGGS  
METHOD: RM-26B-2

FILE NO: 740.01/SELECT  
DATE: JANUARY 30, 1990  
REVISION DATE: AUGUST 23, 1990

## INTRODUCTION

This analytical method determines the residues of clethodim and its metabolites containing the 2-cyclohexen-1-one moiety. The total residue is expressed in clethodim equivalents. Briefly, the method involves extraction with methanol and/or water, followed by cleanup with alkaline precipitation and acidic back extraction, oxidation to a dicarboxylic acid, derivatization to the dimethyl ester, further cleanup by liquid-liquid partitioning and measurement of the pentanedioic acid dimethyl esters by gas chromatography using a flame photometric detector in the sulfur mode. Quantification is made from a standard curve.

This method modifies RM-26A-1 to include procedures in the Pesticide Analytical Manual, Volume II, Pesticide Reg. Sec. 180.412 for the analysis of animal tissues, milk, and eggs. It includes modifications such as the analysis of the animal metabolite, S-methyl-clethodim sulfoxide, the use of concentrated hydrochloric acid instead of HCL gas to prepare the methylating solution and a second temperature program for the gas chromatograph. A specific calculation procedure for determining the concentration (ppm) found as clethodim is also described. As requested by the EPA, this revision provides clarifications for procedures.

## REAGENTS

Acetone - Pesticide Quality

Hexane - Pesticide Quality

Methyl Alcohol - Pesticide Quality

Dichloromethane - Pesticide Quality

Water - Deionized

Acetic Acid - Glacial, ACS reagent grade.

Barium Hydroxide - Add 10 g barium hydroxide octahydrate to 1 L deionized water. Heat with stirring until solid is dissolved. Gravity-filter solution through a 10-cm filter funnel lined with Whatman 2v filter paper into 100 ml graduated cylinder while solution is still hot. Prepared fresh each day.

- Calcium Hydroxide - Powder, reagent grade.
- Catalase - Suspension from bovine liver (Boehringer Mannheim Biochemicals Cat. No. 106828). Analyze each lot of catalase for activity (65,000 U/mg for complete reduction of hydrogen peroxide) by manufacturer's procedure.
- Celite 545 - Suitable for pesticide analysis.
- Filter paper - Whatman No. 1 or 4.
- Glass wool - Pyrex (Dow Corning).
- Hydrochloric Acid - Concentrated, ACS reagent grade.
- Hydrogen Peroxide - Reagent grade, 30% stabilized solution. (Concentration of the hydrogen peroxide must be no less than 29% to ensure complete oxidation.)
- Potassium Iodide-starch Test Paper or Ether-peroxide Test Paper - Scientific Products, or equivalent.
- Potassium Metabisulfite or Pyrosulfite - ACS reagent grade, certified.
- Silica Gel - Chromatographic grade, E. Merck, A. G. Darmstadt (Germany), 70-230 mesh, or equivalent.
- Sodium Bicarbonate Solution - Saturated: Add 50 g sodium bicarbonate to 500 mL deionized water and stir vigorously for 15 minutes.
- Sodium Chloride - Certified ACS grade.
- Sodium Hydroxide Solution - 2 N: Dilute 10 mL 50% solution to 100 mL with deionized water or prepare from pellets.
- Sodium Sulfate - Anhydrous, granular.
- C18 Sep Pak - Cartridges (Cat. #51910) available from Waters, Associates, Milford, MA
- Clethodim reference standard: For recovery purposes. Prepare 10  $\mu\text{g}/\text{mL}$  (or other concentration) acetone solution by diluting 1 mg/mL acetone stock solution. Check diluted solutions at monthly intervals to verify stability.
- Clethodim metabolite reference standards: For recovery purposes. Prepare individual 10  $\mu\text{g}/\text{mL}$  (or other concentration) acetone solutions by diluting 1 mg/mL acetone stock solutions. Check diluted solutions at monthly intervals to verify stability.

DME reference standard: For GC standard solutions. Prepare 1 mg/mL acetone stock solution.

DME-OH reference standard: For GC standard solutions. Prepare 1 mg/mL acetone stock solution.

S-methyl-DME reference standard: For GC standard solutions. Prepare 1 mg/mL acetone stock solution.

GC standards: Using the above reference standards, prepare GC standards containing all three of the above standards (DME, DME-OH, and S-methyl-DME) at 1.0, 2.5, 5.0, and 10.0  $\mu\text{g/mL}$  acetone. If the analysis of S-methyl-DME is not required, the GC standard will contain only DME and DME-OH at 1.0, 2.5, 5.0, and 10.0  $\mu\text{g/mL}$  acetone.

#### EQUIPMENT

Waring Blenders or equivalent.

Hobart Food Chopper and meat grinder or equivalent.

Wiley Mill.

Liquid Chromatography Columns - 400 x 25 mm i.d. with Teflon stopcock plugs.

Magnetic Stirrers.

Heating Mantles - Suitable for 1 L round-bottomed flasks.

Powerstats

Oxidation Glassware - See Figure 1.

Reflux Condensers - 500 mm jacket, with 24/40  $\text{S}$  inner and outer ground glass joints.

Rotary Evaporators fitted with water bath capable of being heated to 80°C.

pH Meter.

Ultrasonic bath.

Buchner Funnels.

Filter Funnels.

Suction Flasks.

Round-bottom Flasks - 500 mL and 1000 mL capacity with 24/40  $\overline{3}$  ground glass joint.

Gas Chromatograph, FPD in the sulfur mode:

Hewlett-Packard 5890 (or equivalent) equipped with FPD in the sulfur mode, an autosampler and the following suggested parameters:

Column: 0.53 mm I.D. x 10 m fused silica coated with methyl silicone (HP-1) or 50% phenyl (HP-17)

Flow Rates: Carrier gas (Nitrogen)	-	6	mL/min
Make-up gas (Nitrogen)	-	100	mL/min
Hydrogen	-	55	mL/min
Air	-	50	mL/min
Oxygen	-	20	mL/min

Injector Temperature: 350° C

Detector Temperature: 250° C

Column Oven Temperature: 200° C (Isothermal Run)

Retention Times - 4 min (S-methyl-DME)  
 5.3 min (DME) [See Figure 2]  
 6.4 min (DME-OH)

Column Oven Temperature: (Programmed Run #1)

Initial	-	180° C, hold 5 min
Rate	-	10° C/min
Final	-	225° C

Retention Times - 7 min (S-methyl-DME)  
 8.3 min (DME) [See Figure 3]  
 9.0 min (DME-OH)

Column Oven Temperature: (Programmed Run #2)

Initial	-	200° C, hold 2 min.
Rate	-	10° C/min
Final	-	225° C

Retention Times - 4 min (S-methyl-DME)  
 4.3 min (DME) [See Figure 4]  
 4.8 min (DME-OH)

## ANALYTICAL PROCEDURE

### SAMPLING

(Sample weights will vary according to crop type and desired method limit of detection.)

Oily crops (i.e., Cottonseed): Weigh 20 gm of cottonseed macerate into blender vessel. Proceed to EXTRACTION.

Dry crops (i.e., Soybean seed, forage, hay, meal, hulls): Weigh 10 gm of macerated or ground crop into blender vessel. Proceed to EXTRACTION.

Oils: Weigh 50 gm sample of well mixed sample into beaker. (For recovery purposes, fortify a control sample with an aliquot of a solution of clethodim and/or clethodim metabolites.) Quantitatively transfer oil from beaker into 250-mL separatory funnel. Rinse beaker with 50 mL hexane and transfer to separatory funnel. Mix well. Partition hexane and oil mixture with six 100-mL portions of acetonitrile. Drain acetonitrile (Note: acetonitrile is normally the lower layer but with some oil types, the phases are reversed, check layer with the addition of a drop of hexane) layer into clean 1-liter round bottom flask. Evaporate the combined acetonitrile extracts to dryness on a vacuum rotary evaporator using a 50°C water bath. Add 100 mL methyl alcohol and 200 mL distilled water to residue. Mix well to ensure that any residue adhering to flask is dissolved. Proceed to PRECIPITATION CLEANUP.

Soapstock: Weigh 5 gm of a well mixed sample into blender vessel. Proceed to EXTRACTION.

Beef and Chicken tissues, milk and eggs: Cut tissue into small pieces. Grind sample to fine consistency with meat grinder. Mix manually and weigh exactly 25 grams (tissues) or 50 grams (milk and eggs) for extraction. (For recovery purposes, fortify a control sample with an aliquot of a solution of clethodim and/or clethodim metabolites.) Add 500 mL (milk and eggs) or 300 mL (tissues) methanol and blend for 5 min, add 20 g Celite 545 and blend for 2 sec. Vacuum filter the extract into a filter flask through a Buchner funnel containing a 1-cm layer of Celite on Whatman #1 or #4 filter paper. Rinse pad with 2 x 50 mL methanol, transfer extract and washes to a 1-liter roundbottom flask and rotovap to dryness in 50 - 55° water bath. Add 100 mL methanol and 200 mL distilled water to dissolve residue. Proceed to PRECIPITATION CLEANUP.

### EXTRACTION

(For recovery purposes, fortify a control sample with an aliquot of a solution of clethodim and/or clethodim metabolites.) Add 100 mL water and soak sample for 1 hour. Add 300 mL (100 mL for soapstock only) methanol and blend for 5 minutes. During the last 30 seconds of blending add 5 g Celite to the extract. Vacuum filter the extract into a filter flask through a Buchner funnel containing a 1-cm layer of Celite on a Whatman #1 or #4 filter paper. Rinse pad with 25 mL methanol twice. Transfer the extract to a 1-liter roundbottom flask and evaporate to approximately 180 mL volume using a rotary evaporator and a 30°

water bath. Adjust volume to 200 mL with methanol in a 250 mL graduated cylinder, return to the 1-liter roundbottom flask and add 100 mL deionized water. (Extract composition should be approximately 2 parts water to one part methanol.) Proceed to PRECIPITATION CLEANUP

### PRECIPITATION CLEANUP

Add 1 gm (2 gm for soapstock and oil samples) of calcium hydroxide per 10 gram sample to extract in the 1-liter roundbottom flask. Mix well and let stand for 30 minutes. Vacuum filter through Buchner funnel containing Whatman #1 filter paper and a 1-cm layer of Celite. Rinse pad twice with 25 mL of 2:1 water:methyl alcohol. Proceed to PARTITION, then follow remaining steps sequentially as listed.

### PARTITION

Acidify solution with 5 mL concentrated hydrochloric acid. Add enough sodium chloride to saturate (ca 100 gm) the solutions. Mix vigorously for 15 - 30 minutes using a magnetic stirrer. Transfer sample to a 1-liter separatory funnel. Rinse the 1-liter roundbottom flask with 100 mL dichloromethane and decant rinse into separatory funnel. Shake separatory funnel for one minute and let layers separate. Drain organic (lower) layer into a 1-liter round-bottom flask. Partition the aqueous layer with three additional 100 mL portions of dichloromethane, collecting and combining each organic layer into the round-bottom flask. Discard the aqueous layer. Evaporate the dichloromethane extract to dryness using a rotary evaporator and a 50°C water bath.

### OXIDATION

(See Figure 1 for oxidation set-up)

Add 100 mL 1% aqueous barium hydroxide solution (freshly prepared and filtered before use) and a magnetic stirring bar to round-bottom flask from partitioning step. Place flask in heating mantle, which is on top of a magnetic stirrer. Attach flask to reflux condenser and begin stirring sample. Turn on power to powerstat controlling heating mantle. Powerstat is set at mid-point range for medium heating. Once the sample begins to reflux, immediately begin the slow addition of 10 mL 30% hydrogen peroxide through Bantam-Ware separatory funnel. Let mixture reflux for 10 minutes. Add another 10 mL 30% hydrogen peroxide to mixture via the attached separatory funnel and reflux for an additional 15 minutes. Allow mixture to cool to room temperature before removing reflux condenser. An ice bath may be used for cooling the sample.

### EXCESS HYDROGEN PEROXIDE REMOVAL

Add 1 mL concentrated HCl to flask and mix. Loosen residue on flask using an ultrasonic bath. Use 2 N sodium hydroxide and 2 N hydrochloric acid to adjust the solution to about pH 7. Add 50  $\mu$ L catalase suspension. Caution: oxygen evolution. After oxygen has evolved, add potassium pyrosulfite crystals until pH of 4.0-4.5 is obtained. Test with potassium iodide-starch indicator paper to determine if oxidant has been completely destroyed (Blue color indicates

remaining oxidant). If oxidant is still present, repeat adjustment to pH 7, catalase addition and pH 4-4.5 adjustment.

Add 5 mL glacial acetic acid. Evaporate sample to dryness using a rotary evaporator and a 70°C water bath.

### METHYLATION

Dissolve residue in solution by adding 90 mL anhydrous methanol and mixing well; loosen any residue with aid of ultrasonic bath. Add 10 mL of concentrated hydrochloric acid. Place flask in heating mantle, attach flask to reflux condenser, begin stirring sample and apply heat to heating mantles. Reflux for 30 minutes.

Cool mixture to room temperature before removing reflux condenser. Carefully add 150 mL saturated sodium bicarbonate solution to contents of round-bottom flask. (Sample will bubble from evolving carbon dioxide gas during addition of sodium bicarbonate.) Check pH with pH paper to ensure that solution is neutral or weakly alkaline. If pH is not  $\geq 7$ , add saturated bicarbonate solution until pH is  $\geq 7$ . Transfer contents of flask to 500 mL separatory funnel.

Partition with two 100-mL portions of dichloromethane, filtering the lower dichloromethane layer through sodium sulfate in a 10-cm funnel plugged with glass wool into a 500-mL round-bottom flask. Rinse filter pad with another 50 mL dichloromethane, combining rinse with filtrate in flask. Evaporate to dryness using a rotary evaporator and a 50°C water bath. Transfer sample to 50-mL roundbottom flask with three 5-mL portions of acetone and evaporate to dryness using a rotary evaporator and a 50°C water bath. Redissolve residue in 1.0 mL or 2.0 mL acetone, depending on the desired limit of detection. Proceed to MEASUREMENT. If, upon initial GC analysis, the sample causes major sensitivity loss such that the limit of detection cannot be reached, the sample may be evaporated to dryness using a rotary evaporator and a 50°C water bath and redissolved in hexane (this procedure may be necessary for fat samples). If GC analysis shows matrix peaks which interfere with measurement, another aliquot of the residue sample should be extracted and the SILICA GEL COLUMN CLEANUP step should be utilized after the METHYLATION step.

### MEASUREMENT

NOTE: Frequent replacement of the silanized glass wool in the injector liner is highly recommended to assure optimum sensitivity.

Transfer the solutions to be measured to vials for use on the automatic liquid sampler. Load the sample tray in a specified order, such as follows: conditioning shot, conditioning shot, standard, standard, fortified sample, control sample, standard, sample, sample, standard,..... Set the syringe to deliver from 3 to 4  $\mu\text{L}$ . The standard vials contain reference standards containing 10.0, 5.0, 2.5 or 1.0  $\mu\text{g}/\text{mL}$  concentrations of DME and DME-OH (and S-methyl-DME, if desired) in 1:1(:1) ratio. The standards are interspersed throughout the run. Dilute sample with acetone (or hexane for fat samples) if

area is not within the range of standard concentrations used for the standard curve.

#### SILICA GEL COLUMN CLEANUP (Optional - See Note 1)

(This cleanup step is provided in the event additional cleanup is necessary.)

Place glass wool plug at bottom of 400 x 25 mm i.d. glass chromatographic column. Add 10 mL 15% (v/v) acetone in hexane. Slurry 8 g silica gel in 25 mL 15% acetone in hexane and quantitatively transfer silica gel to column with small rinses of 15% acetone in hexane. Tap the column gently to release air bubbles from the silica gel and let column stand for 5 minutes. Let solvent drain to just above top of column packing.

If required, evaporate the sample to dryness in a 50-mL roundbottom flask and redissolve in 10 mL dichloromethane. Add 2.0 g silica gel to round-bottom flask. Evaporate the silica gel-dichloromethane mixture to dryness on a 50°C water bath. Transfer dry silica gel to top of silica gel column. Let silica gel settle for about 5 minutes. Then gently tap sides of column to release any trapped air bubbles. Rinse flask containing sample residue with two 10 mL portions of 15% acetone in hexane, transferring each rinse to column just when previous rinse is about 2-3 cm above silica gel surface. Wash column with an additional 110 mL 15% acetone in hexane. Elute DME and DME-OH with 200 mL methanol-acetone-hexane (5+10+85, v/v/v) into a 500 mL roundbottom flask. Evaporate the eluate to dryness using a rotary evaporator and a 50°C water bath. Transfer sample to a 50-mL roundbottom flask with three 5-mL portions of acetone and evaporate to dryness using a rotary evaporator and a 50°C water bath. Redissolve the residue in 1.0 mL or 2.0 mL acetone, depending on the desired limit of detection. Proceed to MEASUREMENT. If GC analysis shows the interference is still present, the sample extracts should be combined in a 50-mL roundbottom flask and evaporated to dryness using a rotary evaporator and a 50°C water bath; proceed to C18 CARTRIDGE CLEANUP.

#### C18 CARTRIDGE CLEANUP (Optional - See Note 1)

NOTE: After preconditioning the cartridge, do not allow cartridge to go dry. Elution is allowed to proceed by gravity.

Dissolve the residue in 0.5 mL methyl alcohol; add 9.5 mL of water and mix well. Pass the solution through a preconditioned Sep Pak® C18 cartridge. The cartridge is preconditioned by rinsing with 10 mL of 5% methyl alcohol in water.

After passing the sample through the cartridge, rinse the sample flask with another 10 mL of 5% methyl alcohol in water, which is also passed through the cartridge. Rinse with 8 mL 10% methyl alcohol in water. Discard all eluates to this point. Elute the DME and DME-OH with two 5-mL portions of 30% methyl alcohol in water, collecting eluate into a small beaker.

Transfer eluates to a 125 mL separatory funnel. Partition with 10 mL ethyl acetate. Repeat with two additional 10 mL portions of ethyl acetate. Combine the ethyl acetate extracts into a 100-mL roundbottom flask. Evaporate to dryness

using a rotary evaporator and a 50°C water bath. Redissolve the residue in 1.0 mL or 2.0 mL acetone depending on the desired limit of detection. Proceed to MEASUREMENT.

CONCENTRATION DETERMINATION

Generate a standard curve correlating the concentration of the standards with their respective measured average area units.

In the PAM procedure a non computer generated calculation is described. The formula is as follows:

$$\mu\text{g/mL} = L(\text{conc}) + \frac{[(\text{sample R} - \text{LR}) (\text{Hconc} - \text{Lconc}) / (\text{HR} - \text{LR})]}{\text{where R is peak height}}$$

L is standard with lower response than sample and  
 H is standard with higher response than sample.

Results can also be obtained by first generating a standard curve fitted to the functional form  $Y = AX^B$ , where Y (average area) is predicted by values of X (concentration) with initial values of 10 for parameter A and 1 for parameter B. After the curve has been fitted, the concentration of DME, DME-OH, or S-methyl-DME is calculated using the formula:

$$\mu\text{g/mL} = \left( \frac{\text{area}}{A} \right)^{1/B}$$

PPM CALCULATION

After the concentration of each extract is determined, the results for the samples are calculated in clethodim equivalents by the following formula:

$$\text{ppm} = \frac{\text{conc. DME} \times \text{vol.} \times 1.22}{\text{wt. of sample}} \quad \text{or} \quad \frac{\text{conc. DME-OH} \times \text{vol.} \times 1.16}{\text{wt. of sample}}$$

$$\text{or} \quad \frac{\text{conc. S-methyl-DME} \times \text{vol.} \times 1.29}{\text{wt. of sample}}$$

- where vol. = total volume, including dilution factors, if any,
- conc. =  $\mu\text{g/mL}$  calculated from calibration curve.
- 1.22 = factor to convert DME to clethodim units.
- 1.16 = factor to convert DME-OH to clethodim units.
- 1.29 = factor to convert S-methyl-DME to clethodim units.

If other metabolites are used for fortification purposes, the factor used to determine recoveries is calculated by dividing the molecular weight of the metabolite by the molecular weight of the corresponding analyte. For example, 1.32 is the factor to convert DME-OH to 5-OH clethodim sulfone.

**LIMIT OF DETECTION**

The limit of detection (LOD) is determined by the following calculation:

$$\text{LOD (in ppm)} = \frac{1 \mu\text{g/mL} \times \text{final volume of untreated sample}}{\text{sample weight}}$$



**B. HO**

Reviewed by: Y. U. Fujie

BH:kw

cc: Technical Records  
Residue Files

Note 1. The Silica Gel Column Cleanup and C18 Cartridge Cleanup are optional steps which are normally not required for routine analysis.

Figure 1

Oxidation Glassware

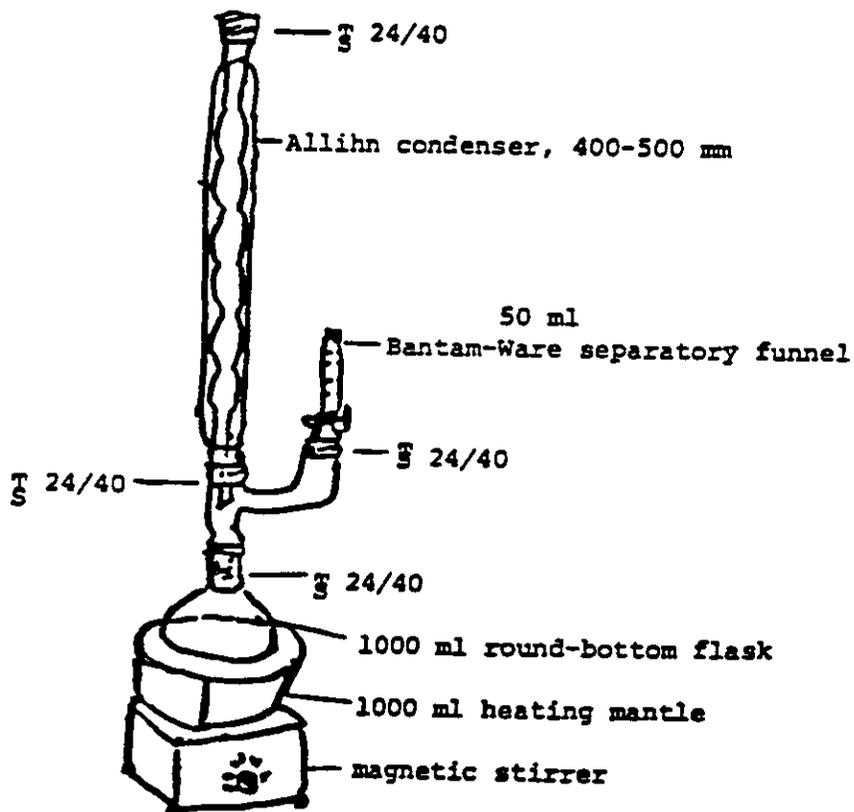
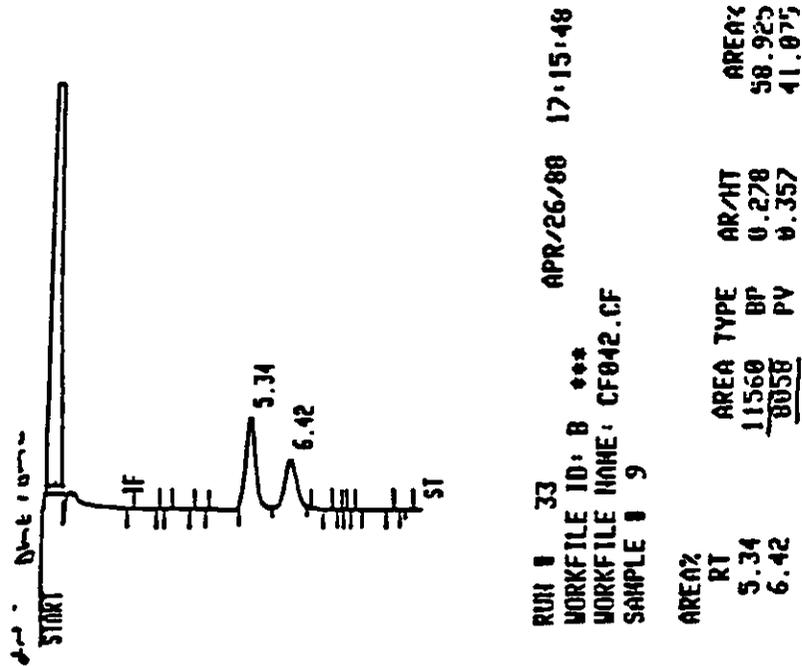


Figure 2  
(Isothermal Run)

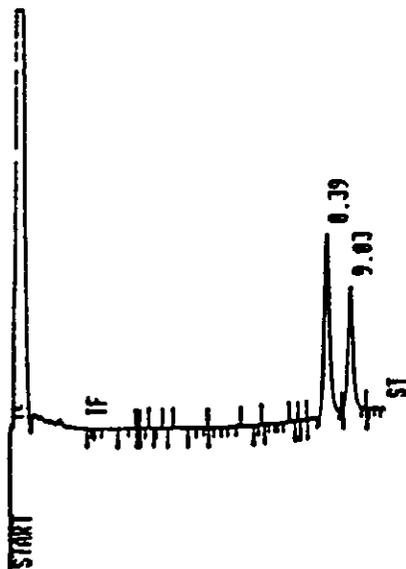


LIST: LIST  
PEAK CAPACITY: 995  
ZERO = 20. -0.65  
ATT 21 = -1  
CHI SP = 0.5  
PK WD = 0.10  
THRSH = 0  
AR REJ = 0

LIST: TIME @  
0.01 INTG # = 9  
2.00 INTG # = 9  
10.00 STOP

8.75 ng DME & 8.75 ng DME-OH

Figure 3  
(Programmed Run #1)



APR/22/88 09:04:42

RUN # 620  
WORKFILE ID: C1  
WORKFILE NAME:  
SAMPLE # 1

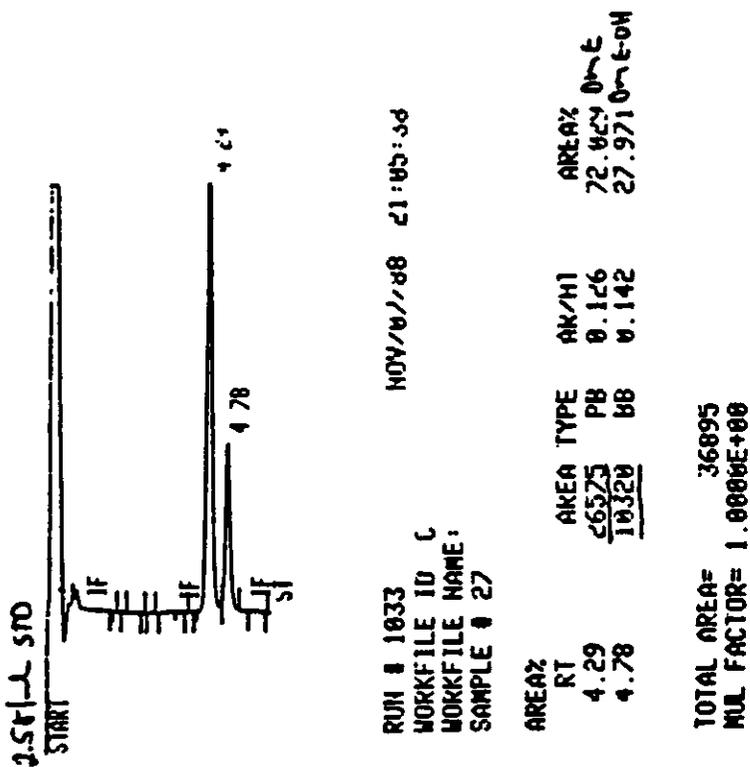
AREA%	RT	AREA	TYPE	AR/HT	AREA%
	8.39	13383	PV	0.163	58.987
	9.03	9303	BV	0.167	41.013

PEAK CAPACITY: 975

ZERO - = 29, -0.69  
ATT 2† = -1  
CHT SP = 0.5  
PK WD = 0.10  
THRSH = -1  
AR REJ = 0

8.75 ng DME & 8.75 ng DME-OH

Figure 4  
(Programmed Run #2)



7.5 ng DME & 7.5 ng DME-OH