Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Clethodim

MRID #: 410302-09

Matrix: Soil

Analysis: GC/FPD

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FIELD DISSIPATION STUDY WITH CLETHODIM
ON SOYBEAN IN NORTH CAROLINA - T7229

APPENDIX III

ANALYTICAL METHODS

CHEVRON CHEMICAL COMPANY
AGRICULTURAL CHEMICALS DIVISION
RESIDUE CHEMISTRY LABORATORY
RICHMOND, CALIFORNIA

DETERMINATION OF CLETHODIM AND METABOLITES IN SOIL
METHOD: RAM-26-S-1

FILE NO.: 721.07/SELECT
DATE: JULY 25, 1983

INTRODUCTION

This method describes the determination of clethodim and its metabolites (clethodim sulfoxide, clethodim sulfone, oxazole sulfoxide and oxazole sulfone) in soil. Briefly, this method involves the extraction of soil with methanol/water, partitioning into hexane and methylene chloride, derivatization with diazomethane, base wash and silica Sep-Pak cleanup; metabolite analysis is performed by HPLC at 254 nm with a C-1 column and clethodim analysis is performed with a C-18 column.

REAGENTS

Methanol - Pesticide grade

Water - Deionized

Hexane - Pesticide grade

Methylene Chloride - Pesticide grade

Hydrochloric Acid - Concentrated reagent grade

Sodium Chloride - Reagent

Sodium Sulfate - Reagent, anhydrous granular

Diazomethane (ether solution) - Prepared according to Aldrichimica Acta, 16, 3 (1983) or Aldrich Bulletin No. AL-113.

Acetone - Pesticide grade

Sodium hydroxide - 0.2 N solution

Silica Sep-Pak - Waters Associates

APPARATUS

Omni-Mixer with adapters for 1-pt. Mason jars.

Rotary vacuum evaporators with a water bath (maintain below 30° C).

High Performance Liquid Chromatograph

Hewlett-Packard 1090 (or equivalent) equipped with an autosampler and UV (254 nm) detector.

PROCEDURE

Weigh 20 grams of the soil sample into 1-pint Mason jars. For recovery purposes, a 20-gram untreated soil sample should be fortified with 100 µL of an acetone solution containing 20 µg each of clethodim sulfoxide, clethodim sulfone, oxazole sulfoxide and oxazole sulfone; another 20-gram untreated soil sample should be fortified with 100 µL of an acetone solution of clethodim (20 µg/mL). Add 150 mL of 80/20 methanol/water to the soil and mix the sample for 5 minutes on the Omni-Mixer. Vacuum filter the extract through #42 filter paper in a Buchner funnel into a 500-mL vacuum flask. Place the filter disk back into the Mason jar and gently mix again with 150 mL of 80/20 methanol/water (do not mix vigorously with the Omni-Mixer during this second extraction); vacuum filter the extract again, wash the filter with 50 mL of 80/20 methanol and combine the extracts and wash into a 1-liter separatory funnel. Add 300 mL water, 1 mL concentrated hydrochloric acid, and 10 g sodium chloride and extract the mixture by shaking with 100 mL hexane for 1 minute; drain the bottom aqueous layer into another container and transfer the hexane extract through sodium sulfate into a 500-mL roundbottom flask. Repeat the hexane extraction with another 100 mL of hexane, combine the hexane extracts and evaporate to dryness for clethodim analysis. Extract the aqueous layer with 3 x 100 mL methylene chloride, filter the extracts through sodium sulfate into a roundbottom flask and evaporate to dryness for Metabolite analysis.

Metabolites

Transfer the residue from the above methylene chloride extraction with 2 + 2 + 2 mL of acetone to a 10-mL Reactivial; add approximately 3 mg of silica gel to the vial. Quickly add 1 mL of diazomethane (ether solution) to the vial; cap the vial, shake and allow to sit for 15 minutes at room temperature with occasional shaking. Evaporate the solution under a stream of nitrogen to a volume of approximately 3 mL; decant the solution (care is taken to leave silica gel behind) into a 50-mL roundbottom flask. Rinse the vial with three 5-mL portions of acetone, decant the acetone into the roundbottom flask; evaporate (rotary) the solution to dryness.

Take up the residue in 25 mL of methylene chloride and transfer to a 125-mL sep funnel; rinse the roundbottom flask with several 5 mL portions of methylene chloride into the sep funnel. Extract the methylene chloride solution with 10 mL of 0.1 N sodium hydroxide solution followed with 10 mL of water; discard the aqueous portions, filter the organic layer through sodium sulfate into a 50-mL roundbottom flask and evaporate to dryness.

Take up the residue in 5 mL methylene chloride and transfer onto silica gel Sep-Pak (previously washed with 5 mL acetone, 10 mL methylene chloride); rinse the roundbottom flask with 2 mL methlyene chloride which is also transferred to the Sep-Pak. Elute the Sep-Pak with exactly 5 mL 10% acetone/methylene chloride (discard) and then elute the desired products into a 50-mL roundbottom flask with 5 + 5 mL of acetone. Evaporate the eluate to dryness and dissolve the residue in 1.0 mL of 30% acetonitrile/water for HPLC measurement.

Clethodim

Follow above METABOLITE procedure with residue from hexane extraction, but skip paragraph 2 of METABOLITE procedure. Follow above Sep-Pak procedure except skip elution with 10% acetone/methylene chloride. Take acetone eluent to dryness, reconstitute in 1 mL 30% acetonitrile/water, and split the sample into two autosampler vials for HPLC measurement.

Standards

Place 1 or 2 mL of 20 µg/mL acetone solution of clethodim (fortifying solution) in a 10-mL Reactivial and 1 or 2 mL of an acetone solution containing 20 µg each of clethodim sulfoxide, clethodim sulfone, oxazole sulfoxide and oxazole sulfone (fortifying solution) in another Reactivial; 4 mL acetone and 3 mg silica gel is added to each vial. Follow above METABOLITE Procedure at the diazomethane addition step, but, skip paragraph 2 of the METABOLITE procedure. For the metabolite standards, follow above Sep-Pak procedure and take the acetone eluent to dryness. For the clethodim standard, follow the Sep-Pak procedure except skip the elution with 10% acetone/methylene chloride and then take the acetone eluent to dryness. Reconstitute the products in an appropriate volume of 30% acetonitrile/water to produce the required linearity and shooting standards.

HPLC CONDITIONS

Metabolites (Conditions may vary to optimize resolution)

Column: Hypersil 3 µm, SAS, 150 x 4.6 mm or Hypersil 5µm, Butyl, 150 x 4.6 mm

Wavelength: 254 nm

Solvent: A - water; B - acetonitrile

Flow: 1.0 mL/min.

Gradient: t = 0 min B = 20%

5	20
12	50
13	50
16	80
22	80

Stop Time: 23 min.

Post Time: 5 min.

Injection Volume: 20 µL

Clethodim (Conditions may vary to optimize resolution)

Column: Hypersil ODS, 3 µm, 250 x 2 mm
Wavelength: 254 nm
Solvent: A - water; B - acetonitrile
Flow: 0.3 mL/min.
Gradient: t = 0 min B = 35%

5	35
15	60
25	60
26	100
36	100

Stop Time: 36 min.
Post Time: 10 min.
Injection Volume: 20 µL

MEASUREMENT

Transfer the solutions to be measured to auto sampler vials and load the sample tray as follows:

Metabolite Analysis: Metabolite standard, metabolite standard, sample, sample, etc., - a minimum of 5 metabolite standards should be interspersed throughout the run (standards are a concentration of 2 µg/mL). The linearity of the measurement should be verified at least weekly using metabolite standards at concentrations of 0.2, 0.5, 1.0, 2.0 and 5.0 µg/mL (as prepared above). The response factor (response equivalent to 1 µg/mL) should have a coefficient of variation ± 10% or less.

Clethodim Analysis: Clethodim standard, clethodim standard, sample, sample, etc., - a minimum of 5 clethodim standards should be interspersed throughout the run (standards are a concentration of 2 µg/mL). The linearity of the measurement should be verified at least weekly using clethodim standards at concentrations of 0.2, 0.5, 1.0, 2.0 and 5.0 µg/mL (as prepared above). The response factor (response equivalent to 1 µg/mL) should have a coefficient of variation ± 10% or less.

For the analysis of clethodim sulfoxide in the hexane (clethodim) fraction, half of the clethodim extracts are analyzed under the metabolite analysis conditions. Load the clethodim samples onto the metabolite analysis instrument in sequence following the metabolite samples: clethodim standard, metabolite standard, samples, clethodim standard, metabolite standard, samples, etc. - a minimum of 5 clethodim standards should be interspersed throughout the run (standards are at a concentration of 2 µg/mL).

The coefficient of variation for the reproducibility of reference standard peak heights/areas should be ± 10% or less.

Note: After the above methylation procedure, the compounds actually measured by HPLC are: the O-methylethers of clethodim, clethodim sulfoxide and clethodim sulfone; oxazole sulfoxide and oxazole sulfone. For simplicity, we do not refer to the derivative names in this method. Figures 1 and 2 show typical HPLC chromatograms of clethodim and metabolites (80 ng of each component on column).

CALCULATION

Metabolites:

$$ppm = \frac{\text{Peak Height (Sample)}}{\text{Average Peak Height (Standard)}} \times 2\,\mu g \times \frac{1}{20\,g}$$

Clethodim:

ppm = ppm clethodim (hexane fraction) + (0.96 x ppm clethodim sulfoxide (hexane fraction))

LIMIT OF DETECTION

The limit of detection is 0.01 ppm for clethodim and metabolites from 20 grams of soil.

ACKNOWLEDGEMENT

G. H. Fujie, J. W. Pensyl and A. L. Hamada contributed to the development of this method.

B. HO

Reviewed by:

BH:gt

cc: G. O. Breault
R & D Files
Residue Files

CALCULATIONS FOR CLETHODIM EQUIVALENTS

SO = clethodim sulfoxide
SEL = clethodim
SOSTD = clethodim sulfoxide standard
SELSTD = clethodim standard

using peak heights (or areas):

1. (SO [in SELSTD] / SO [in SOSTD]) x 2.0 = ug of SO in SELSTD
2. 2.0 - (ug of SO in SELSTD) = ug of SEL in SELSTD
3. (SEL [in sample]/SEL [in SELSTD]) x (ug of SEL in SELSTD) = ug SEL in sample
4. (SO [in sample]/SG [in SOSTD]) x 2.0 = ug SO in sample
5. ug SEL in sample + 1.04(ug SO in sample) = ug clethodim equivalents

CHEVRON CHEMICAL COMPANY

RESIDUE LABORATORY

ANALYTICAL METHOD NO. RM 26 S-1, METABOLITES **VALIDATION REPORT**

S.O.P. – 2.312 and 2.313	Reproducibility	Reproducibility (aged)	L.O.D. = 0.01
Sample Matrix	SOIL		
Level:	0.1 ppm		FORTIFY @ 0.02
$\bar{X}$ =	OXAZOLE SULFOXIDE = 70%, 3.2% CV		70%, 0.8 %CV
% of Nominal =	OXAZOLE SULFONE = 79%, 7.3% CV		68.76%, 3.8% CV
C.V. =	CLETHODIM SULFOXIDE = 85%, 12.2% CV		81.70%, 15.5% CV
	CLETHODIM SULFONE = 81%, 13.3% CV		80.73%, 9.7% CV
n =	6		N = 3
Notebook Reference:	9920-41		10180-07
	AH 10-11-84		AJ. 10-11-84
	Analyst(s)		Date
	Approved		10/11/84 Date

Comments:

S.O.P. – 2.314 Efficacy of Extraction Procedure

Procedure used:

Notebook Reference:

Analyst(s) Date

Approved Date

Comments:

Reviewed by: S C Brandt

CHEVRON CHEMICAL COMPANY

RESIDUE LABORATORY

ANALYTICAL METHOD NO. RM 26 S - 1 CLETHODIM VALIDATION REPORT

S.O.P. - 2.312 and 2.313

	Reproducibility	Reproducibility (aged)	L.O.D. = 0.01
Sample Matrix	SOIL	/	
Level:	0.1 ppm	/	FORTIFY @ 0.02
$\bar{X}$ =			
% of Nominal =	92 %		107 %
C.V. =	8.1 %		5.3 %
n =	6		3
Notebook Reference:	10180-01		10170-07
Analyst(s)	J. J.		J. J.
Date	10-11-88		10-11-88
Approved	[illegible]		
Date	10/11/88		

Comments:

S.O.P. - 2.314 Efficacy of Extraction Procedure

Procedure used:

Notebook Reference:

Analyst(s) Date

Approved Date

Comments:

Reviewed by: [signature]

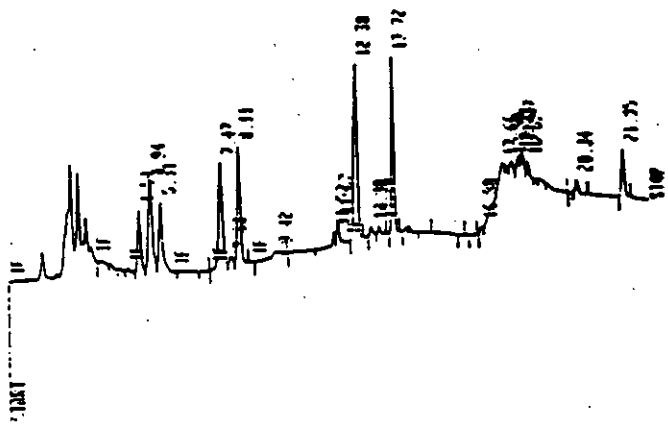

Figure 1. HPLC Chromatogram of Clethodim Metabolites.
Oxazole Sulfoxide = 4.54 min + 4.94 min + 5.31 min
Oxazole Sulfone = 7.47 min + 8.11 min
Clethodim Sulfoxide = 12.38 min
Clethodim Sulfone = 13.72 min
(retention times may vary)

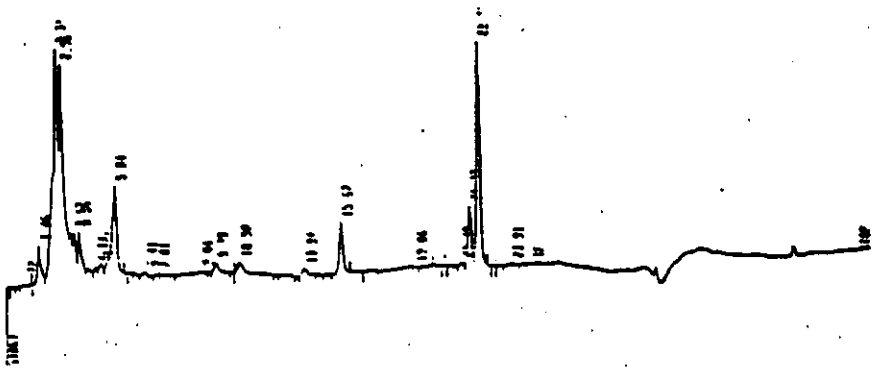

Figure 2. HPLC Chromatogram of Clethodim.
Clethodim = 22.7 min
(retention times may vary)

CALCULATIONS FOR TOTAL RESIDUES

Because the residue values obtained in analysis of samples are expressed in ppm, a concentration term, these values cannot be simply added to give a sum of total residues across several depth increments. For example,

Depth (cm)	ppm (ug/g)
0 - 5	0.04
5 - 10	0.04

If the residue values were simply summed, one would arrive at 0.08 ppm for the concentration of residues in the 0 - 10 cm horizon. However, it is clear that if equal portions of these two samples were mixed and analyzed, the result would be 0.04 ppm not 0.08 ppm. Because "ppm" is a concentration term, the volume or weight of sample must be considered in any mathematical manipulation. Using the same example, assume that each cm is equivalent to one gram (as long as this relationship is constant, it really does not matter what the actual value is):

Depth (cm)			ppm (ug/g)		ug of residue
0 - 5	5.0 g	x	0.04	=	0.2
5 - 10	5.0 g	x	0.04	=	0.2
					0.4 ug total residue

The total residue is 0.4 ug in the 0 - 10 cm horizon; the concentration of residue in the 0 - 10 cm horizon is:

0.4 ug / 10 g = 0.04 ug/g = 0.04 ppm

The same holds true for unequal depth intervals:

Depth (cm)			ppm (ug/g)		ug of residue
0 - 5	5.0 g	x	0.04	=	0.2
5 - 10	5.0 g	x	0.04	=	0.2
10 - 20	10.0 g	x	0.02	=	0.2
	20.0 g				0.6 ug total residue

0.6 ug / 20 g = 0.03 ug/g = 0.03 ppm

Results in this report which refer to total residues across two or more depth horizons were calculated in this manner. A value of "0.0" was used in these calculations where data values of less than 0.02 ppm, the limit of quantitation, were indicated.

CHEVRON CHEMICAL COMPANY
ORTHO AGRICULTURAL CHEMICALS DIVISION
CHEMISTRY SERVICES DEPARTMENT
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DETERMINATION OF CLETHODIM
RESIDUES IN SOIL
METHOD RM-26SA

FILE NO.: 721.11/SELECT
DATE: MAY 2, 1988

INTRODUCTION

Clethodim, also referred to as SELECT and RE-45601, (E,E)-2-[1-([(3-chloro-2-propenoxyl)oxy]imino)propyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one) is a herbicide currently in development by Chevron Chemical Company. Since clethodim has been found to rapidly degrade to yield a wide variety of metabolites, a common moiety method for determining residues is necessary. The following procedure describes the determination in soil for all clethodim residues which contain the ethyl-sulfonyl-propyl-pentanedioic acid moiety. In addition, the method will also measure certain hydroxylated metabolites. This procedure, with slight modifications, is described in the Pesticide Analytical Manual, Volume II, Section 180.412, 1984.

Briefly, the method involves extraction with a methanol water mixture, optional cleanup by alkaline precipitation, partition with dichloromethane from a saturated salt solution, oxidation to a dicarboxylic acid, derivatization to the dimethyl ester, partition into dichloromethane, optional cleanup with silica gel chromatography and measurement of the pentanedioic acid dimethyl esters by gas chromatography using a flame photometric detector in the sulfur mode. The total residue found is expressed in clethodim equivalents.

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 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.

2N HCL in anhydrous methanol - Prepared by bubbling HCl gas through anhydrous methanol (144 g HCl gas diluted to 2 L with methanol).

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.

Sodium Sulfate - Anhydrous, granular.

Clethodim reference standard: For recovery purposes.

Clethodim metabolite reference standards: For recovery purposes.

DME reference standard: For GLC standard solutions.

DME-OH reference standard: For GLC standard solutions.

EQUIPMENT

Waring Blendors 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 T 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.

Suction Flasks.

Round-bottom Flasks - 500 ml and 1000 ml capacity with 24/40 T ground glass joint.

Gas Chromatograph, FPD in the sulfur mode:

Hewlett-Packard 5780 equipped with FPD in the sulfur mode, an autosampler and the following parameters:

Column: 0.53 mm I.D. x 10 m fused silica coated with methyl silicone (HP series 530 u column)

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 - 5.3 min (DME)
6.4 min (DME-OH)

Column Oven Temperature: (Programmed Run)

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

Retention Times - 8.3 min (DME)
9.0 min (DME-OH)

ANALYTICAL METHOD

EXTRACTION

Weigh 50 g sample of soil into Waring Blendor container. (For recovery purposes, fortify a control sample with an aliquot of an acetone solution of clethodim.) Add 100 ml water and soak sample for 1 hour. Add 100 ml methanol and blend for 5 minutes. During the last 30 seconds of blending add 5 g Celite to the extract. Filter the extract into a suction 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. Refilter if extract appears to contain soil or Celite particles. Adjust volume to about 300 ml using water. (Extract composition should be approximately 2 parts water to one part methanol. Excess methanol should be evaporated off prior to partitioning.) Transfer extract to a flat-bottom vessel.

PRECIPITATION CLEANUP FOR HIGH ORGANIC CONTENT SOIL

(The following cleanup step is used for soils which tend to emulsify during the initial extraction step.)

Add 1 gm calcium hydroxide per 10 gram soil sample to extract in flat-bottom vessel. Mix well and let stand for 30 minutes. Vacuum filter through Buchner funnel containing a Whatman filter paper and a 1 cm layer of Celite. Rinse pad twice with 25 ml of a 2:1 water:methyl alcohol.

PARTITION

Acidify solution with 5 ml concentrated hydrochloric acid. Add enough sodium chloride to saturate (ca 100 gm) and a stirring bar. Mix vigorously for at least 15 minutes using a magnetic stirrer. Transfer sample to separatory funnel. Rinse flat-bottom 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 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 aqueous layer. Evaporate the dichloromethane extract to dryness using a 50°C water bath. Proceed to oxidation step.

OXIDATION

(See Figure 1 for oxidation set-up)

Add 100 ml 1% aqueous barium hydroxide solution (freshly prepared and filtered before use) and 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. When sample begins to reflux, slowly add 10 ml 30% hydrogen peroxide solution through Bantam-Ware separatory funnel. Let mixture reflux for 10 minutes. Add another 10 ml 30% hydrogen peroxide to mixture 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

Loosen residue on flask using an ultrasonic bath. Add 1 ml concentrated HCl to flask and mix. Use 2 N sodium hydroxide and 2 N hydrochloric acid to adjust the solution to about pH 7. Add 50 ul catalase suspension. 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 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 on a 70° C water bath. Proceed to methylation step.

METHYLATION

Add 50 ml 2 N HCl in anhydrous methanol and 50 ml anhydrous methanol to residue. Loosen any residue with aid of ultrasonic bath. 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 130 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 into a round-bottom flask. Rinse filter pad with another 50 ml dichloromethane, combining rinse with filtrate in flask. Evaporate to dryness on a 50° C water bath.

SILICA GEL COLUMN CLEANUP (Optional)

(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.

Evaporate the sample to dryness in a round bottom 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) collecting eluate in round bottom flask.

MEASUREMENT

Evaporate filtrate or eluate to dryness using a 50° C water bath. Redissolve residue in 2.0 ml acetone.

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 ul. The standard vials contain reference standards containing 10.0, 5.0, 2.5 or 1.0 ug/ml concentrations of DME and DME-OH in 1:1 ratio. The standards are interspersed throughout the run. Dilute samples with acetone if area is not within the range of standard concentrations used for the standard curve.

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:

ug/ml = Lconc. + [(sampleR - LR)(Hconc - Lconc)/(HR - LR)]

where R is peak height
L is standard with lower response than sample and
H is standard with higher response than sample.

CALCULATION

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

$$ppm = \frac{\text{conc. DME x vol. x 1.22}}{W} + \frac{\text{conc. DME-OH x vol. x I.16}}{W}$$

where vol. = total volume, including dilution factors, if any.
W = weight of sample (50 g).
conc. = ug/ml calculated from calibration curve.
1.22 = factor for converting DME to clethodim units.
1.16 = factor for converting DME-OH to clethodim units.

J. C. LAI

Reviewed by G. O. Breault

cc: G. O. Breault
Files

Figure 1

Oxidation Glassware

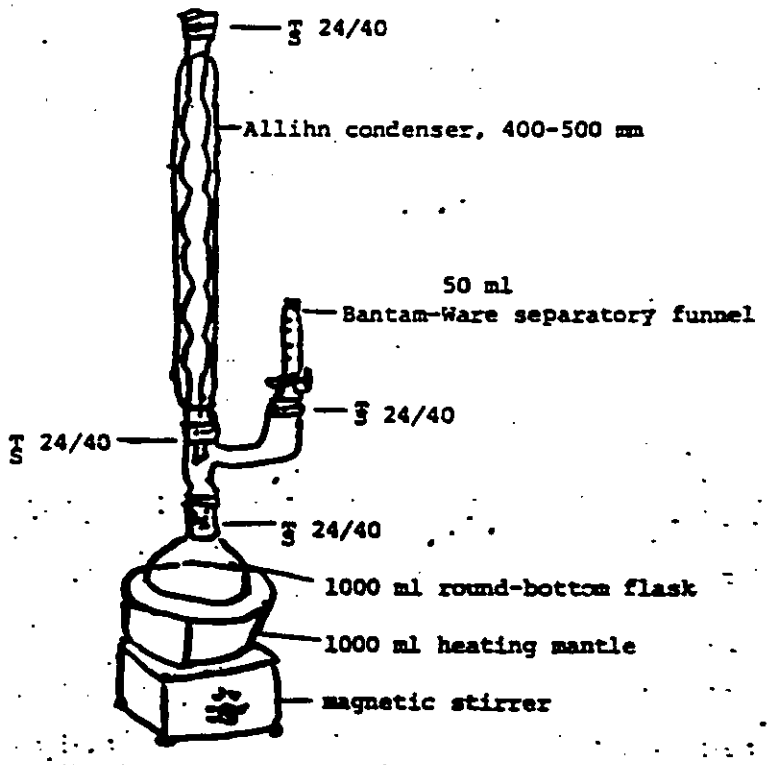
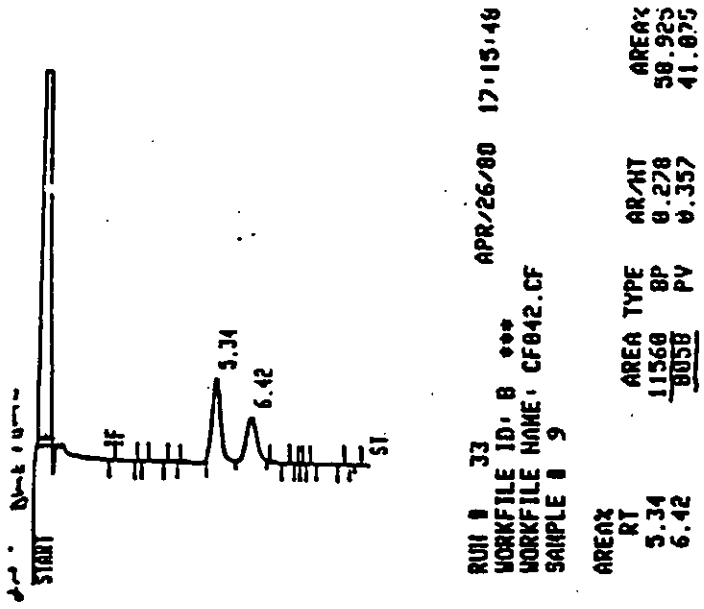


Figure 2
(Isothermal Run)



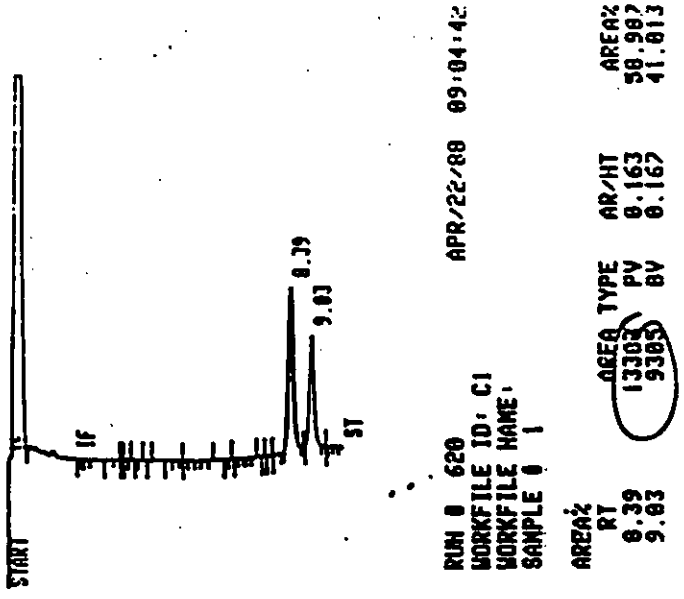
LIST: LIST
PEAK CAPACITY: 995

ZERO = 20,-0.65
ATT 2↑ = -1
CHT 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)



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

METHOD VALIDATION AND RECOVERY STUDIES