VOLUME / OF /

CHEVRON CHEMICAL COMPANY
AGRICULTURAL CHEMICALS DIVISION
15049 SAN PABLO AVENUE
RICHMOND, CALIFORNIA 94806

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:
Confirmatory Method for the Determination of Clethodim and Clethodim Metabolites in Crops, Animal Tissues, Milk and Eggs
Method: EPA-RM-26D-1

Supplemental to: Confirmatory Method for the Determination of Clethodim and Clethodim Metabolites in Crops, Animal Tissues, Milk, and Eggs
Method: RM-26D-1; B. Ho; August 23, 1990
MRID #41623402

DATA REQUIREMENT:
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40 CFR 158.240 - Guideline Reference No. 171-4

AUTHOR:
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Original Study: B. Ho

STUDY COMPLETED ON:
Supplemental Study: December 14, 1990
Original Study: August 23, 1990

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LABORATORY PROJECT ID:
EPA-RM-26D-1

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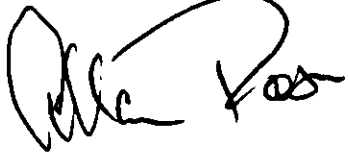
STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

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

COMPANY: Chevron Chemical Company

COMPANY AGENT: Allan F. Rose

TITLE: Project Manager

SIGNATURE: 

DATE: 14 Dec 90

GOOD LABORATORY PRACTICE STATEMENT

This report is 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.

J.C. Lai

J.C. Lai, Study Director
Chevron Chemical Company
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12-14-90
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Allan F. Rose

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CHEVRON CHEMICAL COMPANY
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CONFIRMATORY METHOD FOR THE DETERMINATION OF CLETHODIM AND CLETHODIM METABOLITES IN CROPS, ANIMAL TISSUES, MILK, AND EGGS
METHOD: EPA-RM-26D-1

FILE NO: 740.01/SELECT
REWRITE DATE: DECEMBER 13, 1990

INTRODUCTION

The confirmatory method described here is specific for the determination of the metabolites of clethodim and thus can be used to distinguish clethodim residues from residues of similar herbicides (i.e., sethoxydim). The method includes procedures for the specific determination of the clethodim residues in crops, animal tissues, eggs, and milk.

In crop materials, this method can be used for the analysis of the major plant metabolites of clethodim: clethodim sulfoxide, clethodim sulfone, 5-hydroxyclethodim sulfoxide, and 5-hydroxyclethodim sulfone. Briefly, this method involves the extraction of residues from crop with methanol/water followed by an alkaline precipitation clean-up step. An internal standard, cloproxydim sulfoxide, is added to aid in quantitation. After partitioning with methylene chloride, the residue is methylated with diazomethane, oxidized with meta-chloroperbenzoic acid, and cleaned-up with silica Sep-Pak. Analysis for the analytes, the methylated clethodim sulfone, the methylated 5-hydroxyclethodim sulfone, and the methylated cloproxydim sulfone, is conducted by HPLC on a C-18 column with UV detection. This method can be used for the analysis of the major metabolites of clethodim, clethodim sulfoxide and clethodim sulfone, in animal tissues, eggs, and milk. EPA-RM-26D-1 is prepared to fulfill requests from EPA: changing when the internal standard is added for the crop procedure and listing more detailed information on solvents and procedures.

REAGENTS

Acetone - EM Omnisolv®, Catalog No. AX0116-1 or equivalent

Acetonitrile - EM Omnisolv®, Catalog No. AX0142-1 or equivalent

Calcium Hydroxide - Powder, BAKER ANALYZED® Reagent, Catalog No. 1372-1 or equivalent

Celite 545 - BAKER ANALYZED® Reagent, Catalog No. 3371-1 or equivalent

m-Chloroperbenzoic acid - Kodak Chemical, practical

Clethodim sulfoxide (RE-45924) - Prepare 1 mg/mL acetone stock solutions; prepare 50 μg/mL and 100 μg/mL acetone standard solutions from the stock solution.

Cloproxydim sulfoxide (RE-41988) - Prepare 1 mg/mL acetone stock solutions; prepare 10 μg/mL and 50 μg/mL acetone standard solutions from the stock solution.

Diazomethane (ether solution) - Prepared according to Aldrichimica Acta, 16, 3 (1983); Aldrich Bulletin No. AL-113. See Appendix I for the justification for use of diazomethane as the methylating reagent.

Glass Wool - Pyrex® (Dow Corning)

Hydrochloric Acid - 36.5-38%, BAKER ANALYZED® Reagent, Catalog No. 9535-2 or equivalent

5-Hydroxyclethodim sulfoxide (RE- 51229) - Prepare 1 mg/mL acetone stock solutions; prepare 100 μg/mL acetone standard solution from the stock solution.

Fortifying solution for crops (50 μg/mL) - Combine 5 mL of the 1 mg/mL clethodim sulfoxide stock solution with 5 mL of the 1 mg/mL 5-hydroxyclethodim sulfoxide stock solution and dilute with acetone to 100 mL to produce the 50 μg/mL fortifying solution.

Methanol - EM Omnisolv®, Catalog No. MX0488-1 or equivalent

Methylene Chloride - EM Omnisolv®, Catalog No. DX0831-1 or equivalent

Sodium chloride - Crystal, BAKER ANALYZED® Reagent, Catalog No. 3624-07 or equivalent

Sodium hydroxide - 50% Solution, BAKER ANALYZED® Reagent, Catalog No. 3727-01 or equivalent

Sodium sulfate - Anhydrous, granular, BAKER ANALYZED® Reagent, Catalog No. 3375-07 or equivalent

Sodium thiosulfate - Anhydrous, granular, BAKER ANALYZED® Reagent, Catalog No. 3954-1 or equivalent

Water - EM Omnisolv®, Catalog No. WX0004-1 or equivalent

EQUIPMENT

Buchner funnels - Coors® 6024 Series

Centrifuge bottles, 500-mL - Polypropylene, Beckman Catalog No. 355665 or equivalent

Disposable centrifugal microfilter - 0.45 μm, nylon membrane, 1.5 or 5.0 mL tube, Centrex 01260 or equivalent

Filter flasks - 1000-mL, Pyrex® or equivalent

HPLC Column - Hypersil ODS, 3 μm, 150 x 4.6 mm (Shandon, now Keystone Part No. 59874301 or equivalent)

Liquid chromatograph - Hewlett-Packard Model 1090 equipped with diode-array or filter photometric detector or equivalent

Low-speed centrifuge - Beckman Model GPKR or equivalent

Magnetic stir plates - Corning Model PC-351 or equivalent

Mixer or Homogenizer - Omni International Model 17105 or equivalent

Reacti-Vials®, 10-mL - Pierce Catalog No. 13225 or equivalent

Reciprocating shaker - Eberbach Model 6010 or equivalent

Rotary evaporators with water bath - Buchi Model ROT-M or equivalent

Round-bottom flasks - 50-mL, 100-mL, 250-mL, and 500-mL with 24/40 ground glass joint, Pyrex® or equivalent

Separatory funnels - 125-mL and 1000-mL, Pyrex® or equivalent

Syringe equipped with Luer-lok - B-D Multifit #2152, 10 mL capacity, or equivalent

Silica Sep-Pak® cartridges - Waters Associates #51900

PROCEDURE - CROP MATERIALS

EXTRACTION

Weigh approximately 50 grams of well-mixed sample into a 1-pint mason jar. For recovery purposes, fortify an untreated sample at an appropriate level with clethodim sulfoxide and 5-hydroxyclethodim sulfoxide. Fortify all samples with 50 μg of the internal standard, cloproxydim sulfoxide (1 mL of 50 μg/mL acetone solution). For soybean seed, add 150 mL of methanol/water (80/20, v/v); for cotton fuzzy seed, add 200 mL methanol/water (80/20, v/v). Blend for five minutes (on Omni-mixer) then vacuum filter the extract through a 126 mm diameter Buchner funnel and filter paper (Whatman #42) into a 1-liter filter flask. Re-extract the filter cake with another 150 mL of methanol/water (80/20, v/v) and vacuum filter into the filter flask (for cotton fuzzy seed, use 200 mL methanol/water (80/20, v/v) for each extraction). Rinse the filter cake with 50 mL of methanol/water (80/20, v/v) and discard the filter cake. Immediately take the combined methanol/water filtrates and rinse to ALKALINE PRECIPITATION.

ALKALINE PRECIPITATION

Add 300 mL of water, 2 grams of calcium hydroxide, and 3 grams of Celite to the filtrate, swirl and vacuum filter through a 126 mm diameter Buchner funnel and filter paper (Whatman #42) into a 1-liter filter flask. Carefully (possible heat and gas formation although only slight gas formation has actually been observed) add 5 mL of concentrated hydrochloric acid to the filtrate and mix well with adequate venting. Rinse flask with an additional 50 mL water; filter into filter flask. This procedure should be completed in less than 10 minutes to avoid the loss of metabolites at alkaline pH. Proceed to LIQUID-LIQUID PARTITIONING.

LIQUID-LIQUID PARTITIONING

Transfer filtrate to a 1-liter separatory funnel; add 10-15 grams of sodium chloride, and 100 mL methylene chloride. Shake the separatory funnel for about 30 seconds, allow layers to separate, drain the bottom organic phase through a bed of sodium sulfate (ca 150 gm) in a 10-cm filter funnel plugged with glass wool into a 500-mL round-bottom flask. Repeat the extraction with two 100-mL portions of methylene chloride, draining the lower phase into the same flask through the sodium sulfate bed each time. Evaporate the combined organic extracts to dryness using a vacuum rotary evaporator and a 20-25°C water bath. Transfer the residue to a 100-mL round-bottom flask with three 10-mL portions of acetone and evaporate to dryness using a vacuum rotary evaporator and a 20-25°C water bath. Proceed to METHYLATION.

METHYLATION

Transfer the residue from the above extraction with three 2-mL portions of acetone to a 10-mL Reacti-vial; add approximately 5 - 50 mg of silica gel to the vial. Quickly add 1 mL of diazomethane (ether solution) to the vial; cap the vial, shake on reciprocating shaker (low speed) for 15 minutes at room temperature.

Warning: Use appropriate precautions with diazomethane. Diazomethane is not only toxic, but its solutions have been known to explode unaccountably. All work with diazomethane should be carried out behind a safety shield in an efficient hood. Never allow diazomethane solutions to contact ground-glass joints or sharp glassware edges. See Aldrichimica Acta, Vol. 16 3 (1983). See Appendix I for the justification for use of diazomethane as the methylating reagent and additional safety precautions.

Evaporate the solution under a gentle stream of nitrogen at room temperature to a volume of approximately 3 mL; decant the solution (care is taken to leave the silica gel behind) into a 100-mL round-bottom flask. Rinse the Reacti-vial with three 5-mL portions of acetone, decant the acetone into the round-bottom flask; evaporate the solution to dryness using a vacuum rotary evaporator and a 20-25°C water bath. Proceed to BASE WASH.

BASE WASH

Dissolve the residue in 25 mL methylene chloride and transfer into a 125-mL separatory funnel. Rinse the flask with three 5-mL portions of methylene chloride and combine the rinses in the separatory funnel. Wash the methylene chloride solution with 10 mL 0.1 N sodium hydroxide (containing 10% w/v sodium chloride). Drain the lower organic layer into another 125-mL separatory funnel. Add 10 mL water to the organic fraction, shake, and allow layers to separate. Drain the lower organic layer through sodium sulfate (ca 75 g) in a 7.5-cm filter funnel plugged with glass wool into a 100-mL round-bottom flask and evaporate to dryness using a vacuum rotary evaporator and a 20-25°C water bath. Discard the aqueous phases. Proceed to OXIDATION.

OXIDATION

Add 30 mL methylene chloride to the 100-mL flask. Weigh approximately 250 mg of m-chloroperbenzoic acid into a 100-mL beaker and dissolve it in 10 mL methylene chloride; add 1 mL of this m-chloroperbenzoic acid solution to the extract in the flask, swirl, and allow to stand at room temperature for approximately 15 minutes for optimum conversion. Immediately transfer the oxidized solution to a 125-mL separatory funnel containing a 25 mL solution of 2% (w/v) sodium thiosulfate and 10% (w/v) sodium chloride in water. Shake vigorously for 30 seconds, allow layers to separate and drain the lower organic phase through a sodium sulfate bed, as described previously, into a 250-mL round-bottom flask. Rinse the 100-mL round-bottom flask with 25 mL of methylene chloride, use this rinse to re-extract the aqueous phase; repeat with another 25 mL of methylene chloride. Combine the rinses in the 250-mL round-bottom flask and evaporate to dryness using a vacuum rotary evaporator and a 20-25°C water bath. Proceed to SILICA SEP-PAK.

SILICA SEP-PAK CARTRIDGE

NOTE: After preconditioning the cartridge, do not allow cartridge to go dry. All elution is by gravity flow.

Attach a silica Sep-Pak cartridge to a 10 mL capacity Luer-lok syringe. Precondition the cartridge by rinsing with 10mL acetone followed by 10 mL methylene chloride.

Dissolve the residue from the above OXIDATION in 5 mL methylene chloride and transfer the solution onto the preconditioned silica Sep-Pak; rinse the round-bottom flask with 5 mL methylene chloride and transfer the rinse to the Sep-Pak. Rinse the round-bottom flask with another 2 mL methylene chloride and transfer the rinse to the Sep-Pak. Allow the methylene chloride to elute and discard the eluate. Elute the Sep-Pak with exactly 10 mL 10% (v/v) acetone/methylene chloride; discard eluate. Elute the desired products into a 50-mL round-bottom flask with 10 mL 20% (v/v) acetone/methylene chloride. Evaporate the Sep-Pak eluent to dryness using a vacuum rotary evaporator and a 20-25°C water bath. Reconstitute in 10 mL acetone. Transfer 1.0 mL of this solution to a 50-mL round-bottom flask, evaporate to dryness using a vacuum rotary evaporator and a 20-25°C water bath; reconstitute in 1.0 mL 20% (v/v) acetonitrile/water for HPLC measurement. If necessary to obtain lower detection limit, the remaining 9.0 mL of acetone solution can be evaporated and reconstituted in 1.0 mL 20% (v/v) acetonitrile/water for HPLC measurement. (If analyzing samples of known concentration, the Sep-Pak eluent can be evaporated and reconstituted directly in 1 or 10 mL 20% (v/v) acetonitrile/water.) Transfer the final solution to HPLC injection vials for HPLC analysis. See HPLC CONDITIONS.

STANDARDS

Place an appropriate volume (equivalent to amount used for fortification) of 50 μg/mL acetone fortifying solution containing clethodim sulfoxide and 5-hydroxyclethodim sulfoxide in a Reacti-vial with 1 mL of 50 μg/mL acetone solution of cloproxydim sulfoxide (the internal standard); add 5-50 mg silica gel. Follow the above METHYLATION and OXIDATION steps, skipping the BASE WASH. Reconstitute the product in 1.0 or 10.0 mL 20% (v/v) acetonitrile/water to obtain shooting standards for HPLC. See Figure 1 for typical HPLC chromatogram.

LINEARITY

Linearity of the instrument should be verified weekly. For example, linearity solutions can be prepared by treating metabolite standards (e.g., 400 μg; 8 mL x 50 μg/mL acetone) with diazomethane followed by oxidation (follow the above METHYLATION and OXIDATION procedures, skipping the BASE WASH); an appropriate amount of internal standard (e.g., 300 μg; 6 mL x 50 μg/mL acetone) should also be treated with diazomethane followed by oxidation. The derivatized internal standard should be taken up in 6 mL acetone to give 50 μg/mL solutions. The derivatized metabolite standard is diluted with acetone to give solutions of 1.0, 2.5, 25, 50, 100 μg/mL.

One mL of the diluted derivatized metabolite standards is combined with 1.0 mL of the 50 μg/mL derivatized internal standard in a 50 mL round-bottom flask, the combined solutions are evaporated to dryness and reconstituted with 1 mL of 20% (v/v) acetonitrile/water for HPLC analysis.

HPLC CONDITIONS (Suggested) - Crop Extracts

Column: Hypersil ODS, 3 μm, 150 x 4.6 mm

Wavelength: 270 nm (recommended); (254 nm - alternate)

Solvent: A = Water; B = Acetonitrile (CH_3CN)

Flow: 1.0 mL/min

Gradient:	t =	B =
	0 min	15%
	10	15
	15	35
	30	35
	32	45
	35	45

Stop Time: 40 min

Post Time: 5 min

Injection Volume: 20 µL

CALCULATIONS

Clethodim equivalents (ppm) =

$$\left[\left(\frac{CSSR}{CSTR}\right) \times \text{reference std.} \times \text{final volume} \times \text{dilution factor} \div \text{weight of sample}\right] \times .96$$

$$\left[\left(\frac{5\text{-OHSR}}{5\text{-OHSTR}}\right) \times \text{reference std.} \times \text{final volume} \times \text{dilution factor} \div \text{weight of sample}\right] \times .92$$

CSSR = clethodim sulfone sample ratio = (peak units clethodim sulfone in sample) + (peak units I.S. in sample)
CSTR = clethodim sulfone standard ratio = (peak units clethodim sulfone in standard) + (peak units I.S. in standard)
5-OHSR = 5-hydroxyclethodim sulfone sample ratio = (peak units 5-hydroxyclethodim sulfone in sample) + (peak units I.S. in sample)
5-OHSTR = 5-hydroxyclethodim sulfone standard ratio = (peak units 5-hydroxyclethodim sulfone in standard) + (peak units I.S. in standard)
Reference std. = concentration of reference standard
.96 = molecular weight correction factor (360 + 376)
.92 = molecular weight correction factor (360 + 392)

PROCEDURE - ANIMAL TISSUES, MILK, EGGS

EXTRACTION

Weigh approximately 50 grams of ground animal tissue or milk or approximately 20 grams of eggs (shelled and blended) into a 1-pint mason jar. Add internal standard, cloproxydim sulfoxide (1 mL of 10 µg/mL acetone solution), to all samples immediately after weighing. For purposes of recovery, fortify untreated samples as necessary with clethodim sulfoxide. Add 100 mL methanol and blend for five minutes, transfer contents to centrifuge bottles, and then centrifuge for five minutes at approximately 3000 rpm. Vacuum filter the supernatant through a 25 g bed of celite in a 126 mm diameter Buchner funnel lined with filter paper (Whatman #54) into a 1-liter filter flask. Re-extract the marc from the centrifuge bottle with another 100 mL of methanol and vacuum filter into the filter flask. Rinse the filter cake with 50 mL of methanol and discard the filter cake. Immediately take the combined methanol filtrates and (rinse) to ALKALINE PRECIPITATION.

ALKALINE PRECIPITATION

Add 350 mL of water, 2 grams of calcium hydroxide, and 23 grams of Celite to the filtrate. Swirl to mix and vacuum filter through a Buchner funnel and filter paper (Whatman #54) into a 1-liter filter flask containing 5 mL of concentrated hydrochloric acid and mix well. Rinse flask with an additional 50 mL water; filter into filter flask. This procedure should be completed in less than 10 minutes to avoid the loss of metabolites at alkaline pH. Proceed to LIQUID-LIQUID PARTITIONING.

LIQUID-LIQUID PARTITIONING

Add approximately 100 g sodium chloride and a magnetic stir bar to the filtrate in the filter flask and stir on a magnetic stir plate for 5-10 minutes. Transfer filtrate to a 1-liter separatory funnel and partition with 200 mL methylene chloride; allow layers to separate. Drain the bottom organic phase through a bed of sodium sulfate (ca 150 g) in a 10-cm filter funnel plugged with glass wool into a 500-mL round-bottom flask. Repeat the partitioning with another 100-mL methylene chloride; drain the lower organic phase through the sodium sulfate bed into the 500-mL round-bottom flask. Rinse the sodium sulfate with 50 mL methylene chloride, evaporate the combined organic extracts to dryness using a vacuum rotary evaporator and a 20-25°C water bath. Transfer the residue to a 100-mL round-bottom flask with three 10-mL portions of acetone; evaporate to dryness using a vacuum rotary evaporator and a 20-25°C water bath. Proceed to METHYLATION.

METHYLATION

Follow the METHYLATION procedure described above for crops. Skip the BASE WASH step. Proceed directly to OXIDATION.

OXIDATION

Add 30 mL methylene chloride to the 100-mL round-bottom flask. Weigh approximately 250 mg of m-chloroperbenzoic acid into a 100-mL beaker and dissolve in 10 mL methylene chloride; add 1 mL of this m-chloroperbenzoic acid solution to the extract in the flask, swirl, and allow to stand at room temperature for approximately 15 minutes (for optimum conversion). Immediately transfer the oxidized solution to a 125-mL separatory funnel containing a 25 mL solution of 2% (w/v) sodium thiosulfate and 10% (w/v) sodium chloride in water. Shake vigorously for 30 seconds, allow layers to separate and drain the lower organic phase through sodium sulfate (ca 75 g) in a 7.5-cm filter funnel plugged with glass wool into a 100-mL round-bottom flask. Rinse the first 100-mL round-bottom flask with 25 mL of methylene chloride, use this rinse to re-extract the aqueous phase in the separatory funnel. Combine the methylene chloride fractions by draining the lower organic phase thorugh the same bed of sodium sulfate into the 100-mL round-bottom flask and evaporate to dryness using a vacuum rotary evaporator and a 20-25°C water bath. Reconstitute in 1 or 5 mL 20% (v/v) acetonitrile/water for HPLC analysis; place sample in Centrex centrifugal microfilter and centrifuge for five minutes at approximately 2000 rpm. Transfer sample to HPLC injection vials for HPLC analysis. See HPLC CONDITIONS.

STANDARDS

Place an appropriate volume (equivalent to amount used for fortification) of the acetone fortifying solution containing clethodim sulfoxide in a Reacti-vial containing 1 mL of 10 μg/mL acetone solution of cloproxydim sulfoxide (internal standard); add 5 - 50 mg silica gel. Follow the METHYLATION and OXIDATION procedures described above for animal tissues. Reconstitute the product in 1 or 5 mL 20% acetonitrile/water to obtain shooting standards for HPLC. See Figure 2 for typical HPLC chromatogram.

LINEARITY

Linearity of the instrument should be verified weekly. For example, linearity solutions can be prepared by treating metabolite standards (e.g., 200 μg; 2 mL x 100 μg/mL acetone) with diazomethane followed by oxidation (follow the above METHYLATION and OXIDATION procedures, skipping the BASE WASH); an appropriate amount of internal standard (e.g., 80 μg; 8 mL x 10 μg/mL acetone) should also be treated with diazomethane followed by oxidation. The derivatized internal standard should be taken up in 8 mL acetone to give 10 μg/mL solutions. The derivatized metabolite standard is diluted with acetone to give solutions of 1.0, 2.5, 5, 10, 20 μg/mL.

One mL of the diluted derivatized metabolite standards is combined with 1.0 mL of the 10 μg/mL derivatized internal standard in a 50 mL round-bottom flask, the combined solutions are evaporated to dryness using a vacuum rotary evaporator and a 20-25°C water bath and reconstituted with 1 mL of 20% (v/v) acetonitrile/water for HPLC analysis.

HPLC CONDITIONS (Suggested) - Animal Extracts

Column:Hypersil ODS, 3 μm, 150 x 4.6 mm

Wavelength:254 nm

Solvent:A = Water; B = Acetonitrile

Flow:1.0 mL/min

Gradient:	t =	B =
	0 min	31%
	20	31
	21	35
	30	35
	31	50

Stop Time: 35 min

Post Time: 5 min

Injection Volume: 20 μL

CALCULATIONS

Clethodim equivalents (ppm) =

$$\left[\left(\frac{CSSR}{CSTR}\right) \times \text{reference std.} \times \text{final volume} \times \text{dilution factor} \times \text{weight of sample}\right] \times .96$$

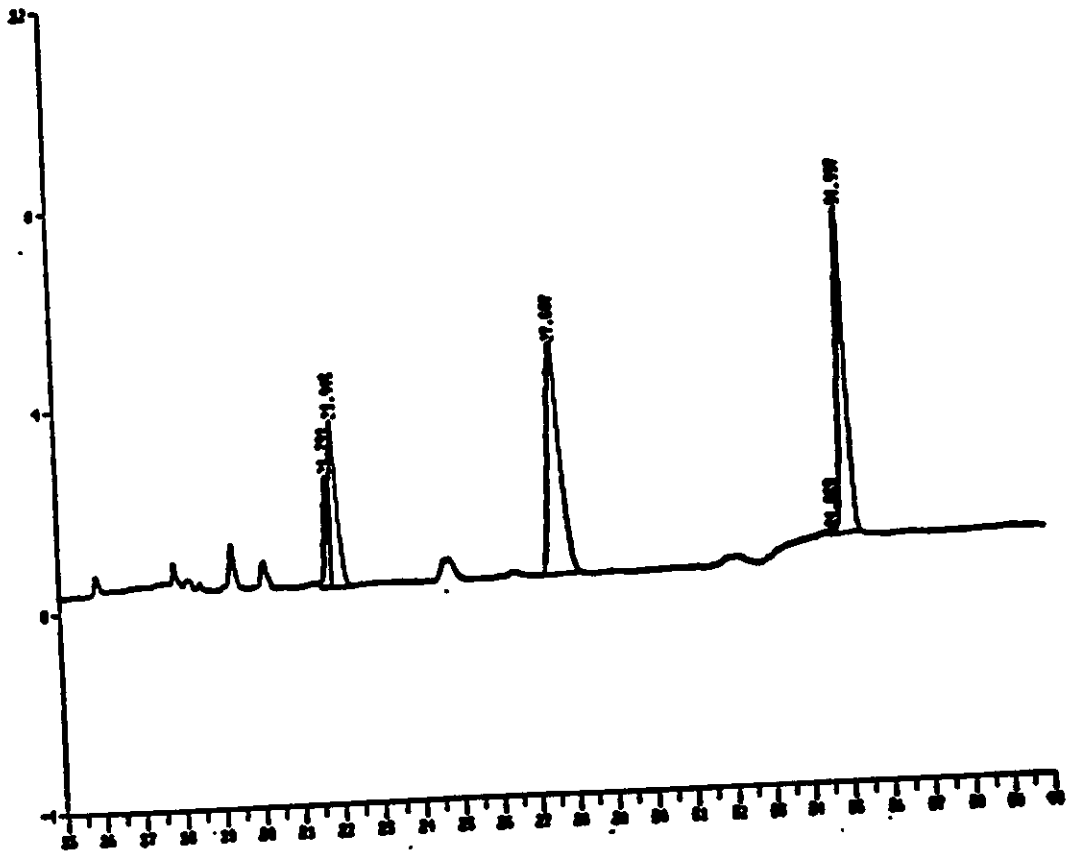
CSSR = clethodim sulfone sample ratio = (peak units clethodim sulfone in sample) + (peak units I.S. in sample)
CSTR = clethodim sulfone standard ratio = (peak units clethodim sulfone in standard) + (peak units I.S. in standard)
Reference std. = concentration of reference standard
.96 = molecular weight correction factor (360 ÷ 376)

Rewrite by J. C. LAI

Reviewed by 12-13-90

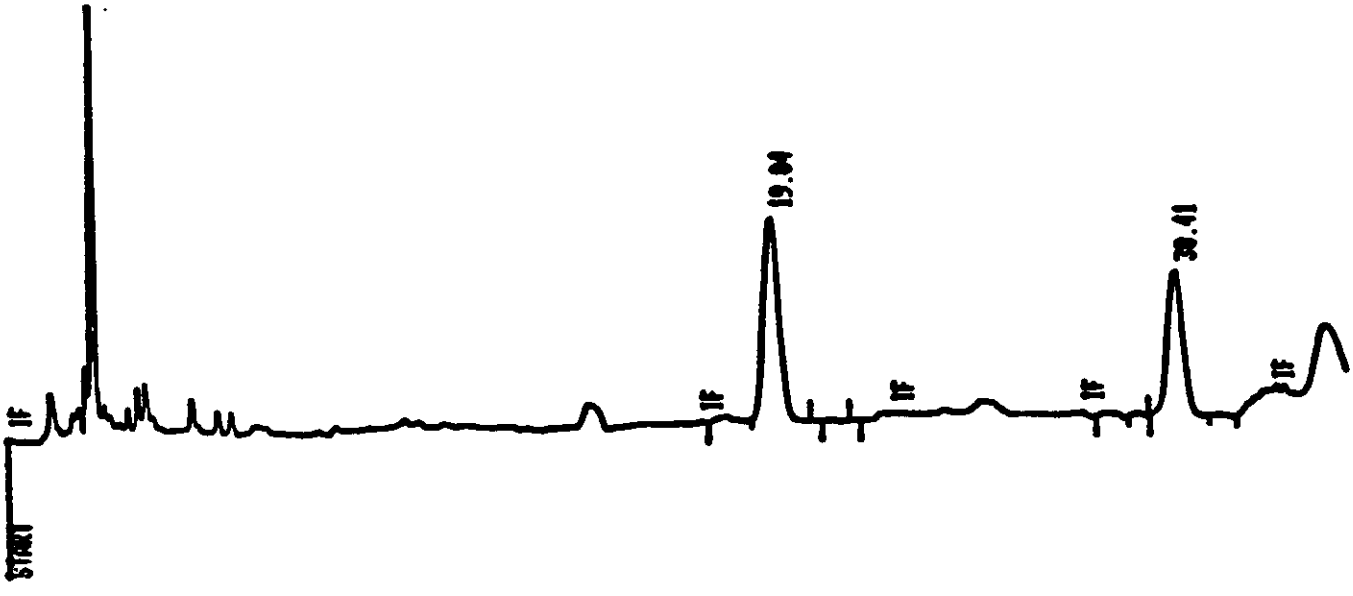
cc: Tech Records
Residue Lab

Figure 1



HPLC chromatogram (crop HPLC conditions) of methylated 5-hydroxyclethodim sulfone (21.8, 21.9 min.), methylated clethodim sulfone (27.6 min.), and methylated cloproxydim sulfone (35.0 min.).

Figure 2



HPLC chromatogram (animal extract HPLC conditions) of methylated clethodim sulfone (19.8 min.) and methylated cloproxydim sulfone (30.4 min.).

APPENDIX I

Diazomethane Use Justification

Chevron Chemical Company
15049 San Pablo Avenue, Richmond, California
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Chevron AgChem Technical Center
Agricultural Chemicals Division

MEMORANDUM

Richmond, CA
December 10, 1990

Clethodim Confirmatory Method RM-26D-1
Diazomethane Use Justification

TO WHOM IT MAY CONCERN:

A memorandum (May 1, 1990 from M. J. Nelson of DEB to J. Miller) requested that Chevron/Valent submit a revised compound-specific confirmatory method that replaces diazomethane with a safer derivatizing agent or provide supporting documentation for the necessity of diazomethane. We should like to point out that all alkylating agents are toxic and potentially carcinogenic and that both caution and good laboratory procedures are mandatory when working with them. We tried to replace diazomethane with either dimethyl sulfate or methyl iodide. A summary of our replacement experiments is presented.

Justification for Diazomethane Use in EPA-RM-26D-1.

EPA-RM-26D-1 is not the primary residue enforcement method: it is to be used rarely to differentiate clethodim residues from sethoxydim residues.

Methylation of the sample is necessary because residues containing the 3-hydroxy-2-cyclohexene-1-one moiety will not chromatograph using the described conditions. Methylation of the 3-hydroxy-2-cyclohexene-1-one moiety with diazomethane is advantageous because it reacts rapidly under very mild (room temperature or below) conditions, is volatile and therefore easy to remove by evaporation after the reaction period and leaves no residual reagent or impurities to interfere with the analysis. Experiments with alternate methylating reagents (dimethyl sulfate and methyl iodide) proved that they were unsatisfactory because they didn't give high or reproducible yields of the required enol methyl ether. When clethodim was allowed to react with excess dimethyl sulfate for 4 hours, the yield of enol methyl ether varied from 59% to 73% with about 15% unidentified reaction product(s). Likewise, when clethodim was allowed to react with excess methyl iodide for 4 hours, the yield of required enol methyl ether was only about 33%. Here the amount of unidentified reaction products increased to about 25%. These results were unsatisfactory for an analytical derivatization scheme.

Clethodim is thermally unstable: it undergoes an acid-catalyzed, heat accelerated Beckmann Rearrangement to form a dihydrobenzoxazolone (RE-47356).[1] For this reason, it is unlikely that increasing the reaction temperature would result in an increased yield of enol methyl ether. The Beckmann Rearrangement reaction itself cannot be use in the analytical derivatization scheme because significant and variable amounts of unidentified, tarry material formed.

Diazomethane is toxic[2] and prone to cause development of specific sensitivity; in addition, it is potentially explosive. However, it can be used safely if several precautions are followed. Gloves and safety glasses (or goggles) should be worn while using it and work should be done behind a safety shield or a hood door with safety glass in a well-ventilated hood. Also, it is recommended that ground glass joints and sharp surfaces be avoided. Thus all glass tubes should be carefully fire-polished and the use of scratched, chipped or broken glassware should be avoided. Do not expose diazomethane solutions to direct sunlight or place them near strong artificial light, because light is thought to have been responsible for some explosions reported with diazomethane.

Proper precautionary measures can reduce the risk due to the toxic and explosive potential of diazomethane. Glassware used to prepare dilute solutions of diazomethane is commercially available. Glassware kits for the preparation of various quantities of the reagent are available from Aldrich Chemical Company. Avoid preparation of excess diazomethane solutions; interim storage should be near 0°C in an ice and water bath. Do not allow the solution to freeze as the resulting crystals with sharp edges may increase the potential for explosion. Dispose excess reagent safely. One method is to add carefully the excess ethereal diazomethane solution to a cold (0°C), dilute solution of acetic acid in diethyl ether (diazomethane reacts with acetic acid to form methyl acetate). Diazomethane is intensely yellow colored and the disappearance of the yellow color suggests that it has completely reacted. Discard the resulting solution according to local waste regulations.

Several reagents are commercially available for the preparation of diazomethane.[3]

Please let me know if you require additional information.

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[1] P.A. Lipton, *Synthesis and Certification of Analytical Reference Standards of Clethodim-Related Compounds*, 1988. MRID #88280-41.

[2] I. Bretherick, "Hazards in the Chemical Laboratory," 4th ed, The Royal Society of Chemistry, London, 1986, p 272.

[3] T. Howard Black, *The Preparation and Reactions of Diazomethane*, Aldrichimica Acta 16 (1) (1983).