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DETERMINATION OF CLETHODIM
RESIDUES IN SOIL
METHOD RM-26SA

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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.

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Reviewed by G. O. Breault

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