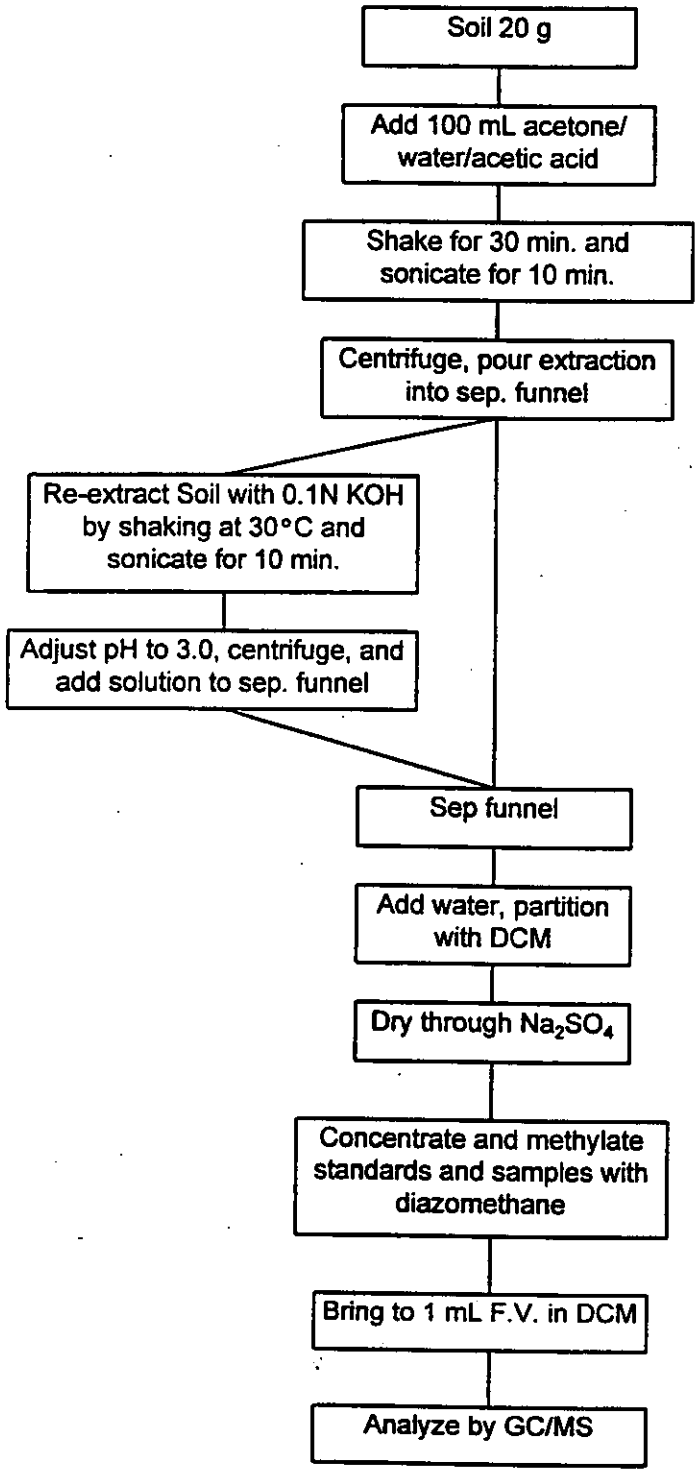
SUMMARY FLOWCHART OF ANALYTICAL METHOD



1.0 INTRODUCTION

1.1 Scope

This method sets forth the procedure for determining the residues of CGA 184927 and CGA 193469 in soil. The method is based on an in-house method developed at Enviro-Test Laboratories (Edmonton, Alberta).

1.2 Principle

An analytical method is described for the determination of residues of CGA 184927 and CGA 193469 in soil. Residues of target analytes are extracted from soil by shaking and sonication using acidic acetone and basic aqueous solutions. The acid metabolite CGA 193469 is methylated with diazomethane and residue analysis is done by GC/MS on a DB-1701 column. Quantitation of results is based on a comparison of peak areas with those of known standards or quantitation can be done using a linearity curve.

1.3 Method Limits

The limit of detection (LOD) and minimum limit of quantitation (LOQ) was different for each compound. They are listed below and are based on a 20 g sample and a 1.0 final volume.

Compound	LOD (ppm)	LOQ (ppm)
CGA 184927	0.010	0.030
CGA 193469	0.0030	0.010

2.0 MATERIALS

2.1 Reagents/Solvents (Equivalent/better grade reagents/solvents may be substituted.)

Acetone - Pesticide grade (B & J)
Acetic Acid - (BDH, Analar Analytical Reagent)
Benzyl Chloroformate - Silanizing Reagent (Aldrich, Technical grade)
Diazomethane - (PSOP3.01 - Appendix 2)

2.1 Reagents/Solvents cont'd (Equivalent/better grade reagents/solvents may be substituted.)

Dichloromethane (DCM) - (OmniSolv®)
Potassium Hydroxide Pellets - (Fisher Scientific)
Sulfuric Acid - (Analar Analytical Reagent)
Sodium Sulphate - (BDH, Analytical Reagent)(GSOP10.01 - Appendix 2)
Acid washed: Bake at 400°C for at least 4 hours, cool, transfer to a 1 L flask and add enough ether to cover a ~2.0 mL of concentrated H_2SO_4. Mix, remove ether and dry at room temperature.
Water, deionized - Millipore Purification System

2.2 Equipment and Supplies (Equivalent equipment may be substituted.)

Bottles, centrifuge, polypropylene, 250 mL - (Baxter/CanLab)
Centrifuge - Sorvall®, RC2-B with 250 mL rotor head, (DuPont Instruments)
Flasks, round bottom - 500 mL
Funnels, separatory - 250 mL and 500 mL
Funnels, polypropylene - (Baxter/CanLab)
Incubator Shaker, Controlled Environment - (New Brunswick Scientific Co.Inc.)
N-Evap-Organomation - Model 111 (Meyer)
pH Paper, wide range - Colorphast® Range 0-14 (Chemonics Scientific)
Rotary Evaporator - Janke & Hunkel (IKA Labortechnik, Cincinnati, OH 45241)
Sonicator, Solid State/Ultrasonic FS-28 - (Fisher Scientific)

2.3 Solutions

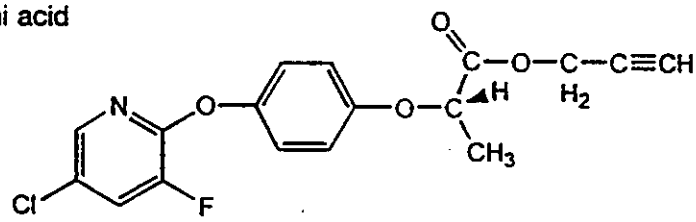
2.3.1 Water/Acetone/Acetic acid (20:79:1) Percent by volume ie. Mix 200 mL water, 790 mL acetone and 10 mL of acetic acid.

2.3.2 0.1N KOH: Weigh out 8.4 g of KOH pellets and dissolve by bringing it to 1.5 L with water.

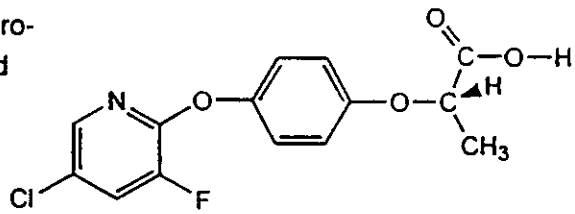
2.3.3 6N H_2SO_4: Take 160 mL of concentrated H_2SO_4 and bring to 1 L with water.

2.4 Analytical Standard and Chemical Structure:

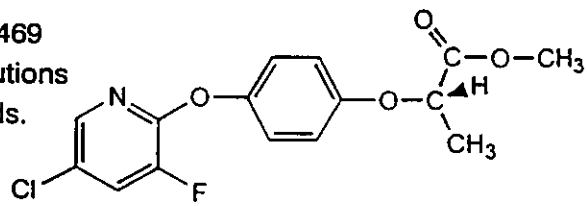
CGA 184927
Chemical Name: 2-[4-(5-Chloro-3-fluoro-pyridin-2-yloxy)-phenoxy]-propioni acid prop-2-ynyl ester
Lot #: OP111005
% Purity: 97.2%
Source: Novartis Crop Protection



CGA 193469
Chemical Name: 2-[4-(5-Chloro-3-fluoro-pyridin-2-yloxy)-phenoxy]-propioni acid
Lot #: SC 3944\B
% Purity: 99.4%
Source: Novartis Crop Protection



Methyl ester of CGA 193469
A 10 ppm procedural methylated CGA 193469 standard is prepared with each set and dilutions of this are done for the calibration standards.



3.0 FORTIFICATION AND CALIBRATION STANDARD SOLUTIONS

3.1 Preparation

All the standard solutions must be stored in glass at or below 10°C when not in use. Solutions should be allowed to warm to room temperature prior to use. The following is an example procedure for preparing a standard solution. Alternate or additional standards of appropriate weight and volume may be prepared as needed. The "~" symbol indicates approximately.

3.1 Preparation cont'd

3.1.1 Weigh ~ 0.010 g (corrected for purity) and record the exact amount of the analyte of interest and then place into a 10 mL volumetric flask and dilute to the mark with acetonitrile. Cap and mix by inversion. The concentration of these stock standards is ~1000 µg/mL.

3.1.2 For the preparation of calibration and fortification standards transfer 10 mL of the ~1000 µg/mL standard via volumetric class "A" pipettes, to a 100 mL volumetric flask. Dilute to mark with acetonitrile. Cap and mix by inversion. The concentration of this standard is ~100 µg/mL. Prepare a similar fortification standard of 10 ppm by serial dilution in DCM.

3.1.3 Mixed Calibration Standards of the methyl ester of CGA 193469 and CGA 184927 are prepared by combining 200 µL of the 100 ppm fortification standards of CGA 193469 and CGA 184927 with 1 mL of diazomethane. The reaction is allowed to react for 5 minutes, ensuring the yellow color persists. If not, 0.5 mL of diazomethane is added. The diazomethane is then evaporated using an N-evaporator in the fumehood to ~ 0.5 mL then made up to a 5 mL final volume in DCM. This give a concentration of 4 ppm. Other calibration standards (eg. at 2.0, 1.0 and 0.03 ppm) are made by serial dilution in DCM.

3.2 Stability

To evaluate the stability, the following formula has been used:

$$\% \ Stability = \left(1 - \frac{old\ standard\ solution}{new\ standard\ solution}\right) \times 100$$

The old standard solution should give detector responses of 10% or less of those of the new standard solution in order for the given standard solution to be considered stable under the storage conditions.

4.0 METHOD PROCEDURES

4.1 General Notes

4.1.1 The "◆" symbol indicates an optional stopping point after completing the indicated step. Samples may be stored overnight in a refrigerator (at or below 10°C).

4.1.2 The "~" symbol indicates approximately.

4.2 Soil Analysis

(Analysis of CGA 184927 and CGA 193469)

◆ 4.2.1 Weigh 20 g of a prepared* subsample of soil into a 250 mL polypropylene centrifuge bottle. Untreated control samples may be fortified at this point for determination of recovery. 100-200 µL of the appropriate fortificaiton standard (see 3.1.2) are added to the soil, mixed and let equilibrate for about 15 min.

* Subsample may be prepared by mixing with dry ice using a Hobart food chopper. These samples must be free-flowing and homogenous prior to subsampling.

4.2.2 Add 100 mL of 20:79:1 Water/acetone/acetic acid solution to the soil, shake using a platform shaker for ~30 min.

◆ 4.2.3 Remove and place in a sonic bath for ~10 minutes.

4.2.4 Centrifuge the sample at ~5000 RPM for at least 5 minutes or until separated and decant into a 500 mL separatory funnel.

4.2.5 Add 100 mL of 0.1N KOH and shake for 30 minutes at about 30°C.

4.2.6 Sonicate and adjust the pH to 3±0.5 (using a pH meter) using 6N H_2SO_4.

4.2.7 Centrifuge and combine with previous extraction solution into the 500 mL sep. funnel.

4.2 Soil Analysis cont'd

4.2.8 Add 300 mL of water to the sep. funnel.

4.2.9 Partition 2 times with 100 mL of dichloromethane (DCM) and dry through acid washed Na_2SO_4.

4.2.10 Concentrate to a low final volume and use diazomethane to transfer to a 3.5 mL vial. Let stand for ~5 minutes, making sure the yellow color remains. Procedural standards of methylated CGA 193469 are prepared at this time as well.

4.2.11 Using a nitrogen evaporator adjust final volume to 2.0 mL in DCM. This final volume can range from 1.0 to 5.0 mL, depending on the sensitivity of the GC/MS.

4.2.12 Analysis is done by GC/MS using selective ion monitoring (see Section 5.0). These extracts can be stored at -20°C until analyzed by GC/MS.

5.0 INSTRUMENTATION GC/MS

(Equivalent instrumentation can be used)

5.1 GC/MS Conditions:

Instruments used:
HP 5890 Gas Chromatograph coupled with a HP 5971 Series Mass Selective Detector
HP 7673A Automatic Sampler
HP LaserJet Series II Printer
G1030A MS Chemstation (DOS Series)

Conditions:
Column - DB1701, J & W, 30m × 0.25mm, 0.25μm
Temp. Program: 100°C 0 min. to 280°C @ 10°/min, hold @ 280°C for 7 min.
Injector Temp. - 250°C, splitless injection mode
Detector Temp. (Interface) - 280°C
Carrier Gas - Helium
Flow rate - 60 mL/min.
Sample Inj. Volume - 2μL

5.1 GC/MS Conditions: cont'd

Approximate Retention Times and Masses		
	m/z*	R.T. (min.)
CGA 184927	349,266,238	18.80
CGA 193469	325,266,238	17.27

Retention times may vary from those present above.

* Different ions may be selected if matrix interferences are present.

At the beginning of each GC/MS sequence, condition the inlet system by making 4-6 control soil extract injections.

Example chromatograms are attached (Appendix 1). Note that the retention times may vary from system to system and may require optimization.

5.2 Performance Criteria

First Criterion:
Run a calibration standard on GC/MS at the LOQ of 0.030 ppm for CGA 193469 and at 0.10 ppm for CGA 184927 to meet sensitivity requirements (S/N>3:1).

If this criteria cannot be met, optimize instrument operating parameters or change instrument method parameters such as multiplier voltage, replace the column guide or pre-column and clean the inlet.

Second Criterion:
Run a set of standards of four or more concentration levels, from 0.03 ppm to the highest concentration level to be included in the analysis. Generate a calibration curve for each analyte and obtain a linear regression with a correlation coefficient of at least 0.98 for each analyte. If this criterion is met, the samples may be run with standards interspersed with at least one standard at the beginning and one at the end of each sequence.

6.0 CALCULATIONS

6.1 Response Factor (R.F.):

$$R.F. = \frac{Concentration\ of\ Standard\ (ppm)}{Peak\ Area\ of\ Standard}$$

6.2 Concentration of Analyte in Soil (ppm):

$$Conc.\ (ppm) = \frac{(Peak\ Area \times R.F.) \times F.V.}{g.Extracted}$$

Where: Conc.(ppm) = Concentration of analyte in µg/g(ppm)
F.V. = Final sample volume (mL)
g.Extracted = initial grams of sample extracted

6.3 % Recovery:

$$\%\ Recovery = \frac{Recovery\ Level\ (ppm)}{Fortification\ Level\ (ppm)} \times 100$$

Recovery Level = Residue in the spiked control

7.0 SAFETY

Appropriate MSDS's should be available to the study personnel during the conduct of the study. General laboratory safety precautions should be taken. This method does not present any specific risks.

8.0 DETECTION LIMITS:

The LOQ for CGA 184927 and CGA 193469 is 0.030 ppm and 0.010 ppm respectively. The LOD was 0.010 ppm and 0.0030 ppm respectively based on a S/N of >3:1 of analyte to control soil background.

ETL

EFFECTIVE DATE: July 17, 1995
REVIEW DATE: 2 YEARS

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STANDARD OPERATING PROCEDURE
REGULAR BAKED AND ACIDIFIED Na_2SO_4

1. Purpose: When performing solvent extractions, the solvent layer is dried by passing it through a layer of Na_2SO_4. Regular baked and acidified Na_2SO_4 are used in base-neutral and acid extraction procedures (respectively).

2. Safety: When handling concentrated H_2SO_4, special care must be taken as this acid is highly corrosive and causes severe burns. Gloves, safety glasses and protective clothing must be worn and all work must be done in a fume hood.

3. Apparatus: crucible, 250 mL
1000 mL round- bottom flask
filtering flask, 2 - litre
Büchner funnel, large (15 cm)
filtervac, rubber
filter paper, Whatman No. 4, 15.0 cm
stirring rod, glass, large
graduated cylinder, 10 mL
aluminum pan
glass jars, 2 - litre, with Teflon lined caps
Na_2SO_4, ACS grade, granular, anhydrous
H_2SO_4, Fisher, ACS grade
ethyl ether, BDH, distilled in glass

4. Procedure: A) Regular Baked Na_2SO_4

1. Fill a crucible with Na_2SO_4 and bake at 500°C for at least 4 hours in a muffle oven.
2. Take out of oven and cover until cool.
3. Once cooled, store in a glass jar.

B) Acidified Na_2SO_4

1. Fill a 1000 mL round bottom 2/3 full with regular baked Na_2SO_4.
2. Add ethyl ether until the surface of the Na_2SO_4 is covered.

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B) Acidified Na_2SO_4 ... continued

3. Using a Pasteur pipet, slowly add 1-2 mL of concentrated H_2SO_4 to the round bottom.
4. Stopper the round bottom with ground glass stopper and invert several times to mix.
5. Set up filtering apparatus and connect to an aspirator (not vacuum line) in fume hood.
6. Pour acidified Na_2SO_4/ether mixture into Büchner funnel and allow the ether to filter off for 5 minutes.
7. Transfer the acidified Na_2SO_4 to an aluminum pan and allow to air-dry in the fume hood until free flowing (~2 hours).
8. Once dried, transfer to a glass jar and label ("Acidified Na_2SO_4", analyst's initials, date of preparation).

Prepared: Heather Gordon Date: 07/18/95

Approved: Doug Johnson Date: 07/19/95

Enviro - Test Laboratories